REVIEW ARTICLE

Erythroid and Nonerythroid Spectrins

By John C. Winkelmann and Bernard G. Forget

In recent years, knowledge of the red blood cell (RBC) membrane skeleton has greatly increased. This progress has been spurred, in large part, by the application of the techniques of molecular biology to the analysis of the structure of normal and mutant membrane skeleton proteins. This review focuses on spectrin, the most abundant protein of the erythrocyte membrane skeleton. Molecular studies of other constituents, such as ankyrin, the anion channel (band 3), protein 4.1, glycoporphin, and others, have also contributed importantly to the current understanding of the RBC membrane skeleton.1,4

Spectrin is a heterodimer, composed of nonidentical α and β subunits with calculated molecular weights (Mr) of 280 and 246 Kd, respectively. The Mr estimated from electrophoretic mobility are 240 and 220 Kd, respectively. Analysis of spectrin primary structure led to the discovery that spectrin subunits exhibit an internal 106 amino acid repeating motif.3 On theoretical grounds, most investigators believe that each repeating segment folds into three α helices.5-8 The spectrin subunits associate side-to-side in antiparallel fashion into a 100-nm flexible rod-like dimer that serves as the basic structural element of the RBC membrane skeleton. Recent data indicate that this side-to-side dimer assembly is dependent on an essential nucleation site near the actin binding end of the molecule (see below).9 Spectrin dimers self-assemble head-to-head into tetramers and, at least in vitro, oligomers. Multiple spectrin tetramers, in turn, associate with a single short actin filament (fewer than 12 actin monomers10) via interaction with their "tails." This interaction is augmented by protein 4.1. The resulting spectrin lattice is attached to the RBC membrane via spectrin binding to ankyrin, a protein that associates with the erythrocyte anion channel (band 3), an integral membrane protein. Another site of membrane attachment is mediated by the association of protein 4.1 with glycoporphin C, a transmembraneialoglycoprotein. The details of these interactions have been summarized elsewhere.11-13 Techniques have evolved to visualize the spectrin-based RBC membrane skeleton by electron microscopy, confirming and extending the biochemically derived understanding of its organization.14-18 The goal of this review is to discuss recent developments in the structural analysis of normal and mutant spectrins and what they show about the normal function of spectrin. Early reports pertaining to the spectrin genes have been previously reviewed.19

The Structure of Erythroid α Spectrin

Molecular clones have been isolated for the erythrocyte α spectrin gene of chicken, mouse, and human.20-23 The α spectrin gene has been mapped to chromosome 1 in mouse and human.24 The full-length human α spectrin cDNA and derived polypeptide sequence have been determined,25 as well as the genomic organization of the α spectrin gene.26,27 α Spectrin is a 2429 amino acid polypeptide with a calculated Mr of 280 Kd, substantially greater than the Mr estimated from electrophoretic mobility. The structure of α spectrin is summarized schematically in Fig 1A. The first repeating segment of α spectrin near the N-terminus, begins with "helix 3" of the hypothetical triple helical structure.5 There are 22 segments, most of which are typical 106 amino acid homologous spectrin repeats.

A striking deviation from the spectrin repeat pattern is evident in segment 10. This region lacks homology to typical spectrin repeats and is considerably shorter. It is highly conserved between spectrin and the major nonerythroid α spectrin homolog (see below). Surprisingly, there is substantial homology between segment 10 and the modulatory, nonkinase (SH3) domain of the src protein family.28,29 This SH3 motif has also been identified in several diverse proteins, including phospholipase C, myosin Iβ, and cdc25,29,30 SH3 domain homology has been observed in proteins from lower organisms including yeast and Caenorhabditis elegans.31,32 The function of this highly conserved motif is uncertain, although it may mediate association of diverse molecules to specific sites on membrane proteins.33,34 Interestingly, each of the proteins exhibiting this homology, like spectrin, associates with the inside of the cell membrane. The recent description of a ligand protein for the abl SH3 domain that has homology to bcr and GAP-rho raises the possibility that some SH3 domains are involved in the control of small ras-like guanine nucleotide-binding proteins.35,36 Other data support the view that SH3 domains might be part of a highly conserved signalling pathway involving ras.37 The relevance of this exciting hypothesis to the biology of erythrocyte spectrin is unknown.

Segments 20 through 22 also exhibit atypical features. There are insertions of several amino acids into the spectrin repeat motif of segments 20 and 21. Segment 22 has reduced homology to a typical spectrin repeat. Interestingly, segments 20 through 22, together with the nonrepeat C-terminus, are highly homologous with the C-terminus of α actinin, a homodimeric actin-binding protein.38

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3173
The C-terminus of α spectrin differs completely from spectrin repeat structure.²⁵ Like segments 20 through 22, this region is homologous to the C-terminus of α actinin. This domain of α actinin contains two EF hand structures.²⁹,³⁰ Similar structures are present in erythroid and nonerythroid α spectrin.²⁵,²⁸,⁴¹ EF hands, present in such molecules as calmodulin, tropomyosin C, and myosin light chain, are conserved calcium-binding elements. The EF hand of α actinin, which can be alternatively spliced, is thought to influence the binding of actin by the nonmuscle α actinin homodimer, via direct interaction with calcium.²⁹,⁴² The influence of calcium on the function of the RBC membrane skeleton is complex. Recent evidence suggests that the primary calcium effects are mediated by calmodulin interacting with protein 4.1.⁴³,⁴⁴ There is little evidence that erythroid spectrin directly binds calcium or that spectrin-calmodulin binding is biologically important. Therefore, the function of the EF hands in erythrocyte spectrin remains uncertain. Perhaps they are vestigial. However, the C-terminal region of erythrocyte α spectrin is probably important because isolated, individual spectrin subunits do not exhibit protein 4.1-stimulated actin binding.⁴⁵ Therefore, the α spectrin C-terminus may be required to maintain the functional conformation of the juxtaposed actin binding domain of β spectrin (see below).

The putative structure/function relationships within the α spectrin molecule are summarized in Fig 1A. The N-terminus is involved in spectrin dimer self-association into tetramers. The C-terminus is involved, perhaps indirectly, in spectrin-actin binding. As discussed above, the function of the conserved segment 10 (SH3 domain) is unknown. With the structural information now available, it is likely that the next several years will see further progress in the analysis of α spectrin functional domains.

**THE STRUCTURE OF ERYTHROID β SPECTRIN**

Molecular clones for the β spectrin gene of chicken, mouse, Drosophila, and human have been isolated.²⁰,²⁶,⁴⁶ The gene has been assigned to human chromosome 14.²⁸,⁴⁶ At band q23-24.²⁶ and mouse chromosome 12.²⁵ The full-length β spectrin cDNA and derived polypeptide sequences have been reported for human and Drosophila.²⁶,²⁹ The human protein is 2137 amino acids, with a calculated Mr of 246 Kd. The structure of β spectrin is summarized schematically in Fig 1B. There are several interesting structural features.

The N-terminal region does not conform to spectrin repeat structure and exhibits strong homology to an extended family of actin-binding proteins, including α actinin, dystrophin, gelation factor (ABP 120), filamin (ABP 280), actin, and fimbrin.²⁹,³⁰,⁴⁷,⁵²,⁵⁴,⁶² Interestingly, the conserved region is also at the N-terminus of each homolog. Nonerythroid spectrins (see below), α actinins, and dystrophins share with spectrin other structural features, including a hypothetical α helical repeat structure.⁷,⁶⁴ Filamin and gelation factor, in contrast, have a repeating motif that is composed primarily of β sheet structure. Recently, functional data have confirmed, for spectrin, α actinin, dystrophin, and gelation factor, that the capacity to bind actin resides in the conserved N-terminal domain.⁶⁵-⁷² A 27-residue segment within the conserved actin-binding domain is the actin-binding site of gelation factor.⁷³ Presumably, the same site is used by spectrin and other proteins with homologous structure. Actin binding by β spectrin may require the α subunit and is enhanced 10- to 100-fold by protein 4.1.⁴⁵ The site of 4.1 binding has been determined to reside near the N-terminus, but is not precisely mapped.⁷⁴

β Spectrin has 17 fairly typical spectrin repeats with some variations in sequence. Segments 1 and 2, like segments 20 through 22 of α spectrin, are somewhat atypical and exhibit homology with α actinin.⁷⁵,⁷⁶ Therefore, the homology of β spectrin with α actinin extends considerably beyond the highly conserved N-terminal actin-binding domain shared by diverse molecules such as dystrophin and filamin.⁷⁷,⁷⁸

Recent experiments have localized the ankyrin binding site of β spectrin.⁷⁹ Artificial deletion mutants of human RBC β spectrin cDNA were expressed in Escherichia coli as glutathione-S-transferase fusion proteins and purified. The ankyrin-binding activity of the fusion proteins was measured by competition with radiolabeled spectrin for binding to erythrocyte vesicles stripped of spectrin and actin. In this manner, the ankyrin-binding site was localized to a 15.3-Kd peptide including segment 15 and a small portion of segment 16. The primary sequence of the second half of segment 15 is atypical.⁸⁰ A β sheet structure has been proposed for this region that may be important in ankyrin binding by β spectrin.⁷⁵

The β subunit of spectrin is phosphorylated in intact erythrocytes. The sites of phosphorylation are known to reside near the C-terminus.⁷⁶,⁷⁷ The phosphorylation sites fall within the C-terminal nonrepeat domain of β spectrin. A single consensus casein kinase substrate sequence has been identified,⁶⁹ but several sites of phosphate addition have been shown. The functional significance of β spectrin phosphorylation remains unclear, but recent data imply that phosphorylation may regulate αβ subunit interactions.⁸⁰

The C-terminal domain of β spectrin is, together with N-terminal α spectrin, involved in spectrin dimer self-association. A recently proposed model predicts that, in self-association, α spectrin contributes a single α helix from its N-terminus to two α helices from repeat 17 of C-terminal β spectrin to form a triple helical structure folded in the manner of a typical spectrin repeat (Fig 1C, inset). Data from spectrin mutants that disrupt subunit self-association are consistent with this model (see below).

**MEMBRANE SKELETON ASSEMBLY**

The functional unit of erythrocyte spectrin is the αβ heterodimer. As discussed above, spectrin subunits associate into dimers; these self-associate into tetramers; tetramers assemble with numerous other proteins to form the mature erythrocyte membrane skeleton. Understanding the details of the assembly process and its regulation promises to provide insight into the biology of the membrane skeleton and the phenotypic expression of mutations affecting membrane skeleton constituents. Recent progress concerning assembly of the mammalian erythrocyte membrane skeleton has been reviewed.⁸⁰ The avian RBC is the subject of an
earlier review. Spectrin synthesis begins early in erythropoiesis, before the colony-forming unit-erythroid (CFU-E) stage of RBC maturation. Initially, spectrin, along with protein 4.1 and ankyrin, is synthesized and degraded with little assembly into a stable membrane skeleton complex. With further differentiation, band 3 synthesis is induced, whereas spectrin and ankyrin synthesis declines. Band 3 synthesis appears to initiate the stable assembly of spectrin and ankyrin into the membrane skeleton. Protein 4.1 synthesis similarly increases with terminal differentiation; it is late to assemble into the skeleton complex. Interestingly, α and β spectrin synthetic rates are unequal. α Spectrin is synthesized at two to three times the rate of β spectrin, whereas their incorporation into the membrane skeleton is equimolar. This observation suggests that the synthesis of β spectrin limits the overall production of spectrin dimers. Interestingly, erythropoietin stimulates β spectrin synthesis in murine erythroblasts transformed with the anemia-producing strain of Friend virus, increasing the overall rate of dimer assembly into the membrane skeleton. The molecular basis of this regulation is unknown, although nuclear run-off experiments suggest control of β spectrin gene transcription directly or indirectly by erythropoietin.

**MUTATIONS OF ERYTHROID SPECTRIN**

Defects of spectrin have been implicated in the pathogenesis of inherited hemolytic anemias in mice and humans, including hereditary elliptocytosis, hereditary pyropoikilocytosis, and some cases of hereditary spherocytosis. These disorders have been recently reviewed. Several pathogenic mutations of human erythroid spectrin have been identified at the molecular level. Some were initially described at the level of amino acid sequence. Many have now been identified at the level of gene structure. Spectrin mutations have been identified in several racial and ethnic groups. Some mutations appear to be confined largely to a single group, whereas others occur in more than one. Most of the α spectrin mutations identified so far involve nucleotide and amino acid substitution. Examples have also been found of exon skipping, alternative splice site
selection, codon deletion, and codon insertion. Exon skipping, short deletion, and frameshift mutations have been identified in \( \beta \) spectrin mutants that result in a truncated \( \beta \) spectrin subunit.

Haplotype analysis of \( \alpha \) spectrin polymorphisms (in the \( aI1 \) domain) shows that all genes studied (5 of 5) bearing one known mutation have the same polymorphic background. A second mutation is identified only in genes with another, constant polymorphic haplotype (4 of 4). Therefore, the two mutations studied exhibit the genetic founder effect, rather than evidence that the mutations arose multiple separate times.

Many of the mutations were initially detected and classified by their effect on the pattern of digestion of \( \alpha \) spectrin by the protease trypsin. Many individual, abnormal tryptic peptides of the 80-Kd "\( \alpha I \)" domain from the N-terminus of \( \alpha \) spectrin have been described in cases of hereditary elliptocytosis and/or pyropoikilocytosis. One remarkable observation is that different genetic lesions are identified that generate indistinguishable pathologic tryptic peptides. Most surprising is the fact that mutations of \( \beta \) spectrin can result in abnormal \( \alpha \) spectrin digestion, suggesting a direct interaction between the corresponding areas of \( \alpha \) and \( \beta \) subunits in spectrin dimer self-association.\(^9\)

The location of each mutation within the spectrin dimer is shown in Fig 2. Mutations occur in proposed helical regions of the spectrin repeats and, frequently, near the putative connecting regions between triple helices. Several of the C-terminal \( \beta \) spectrin mutations result in truncation of the peptide. Hereditary elliptocytosis (HE) and pyropoikilocytosis (HPP). Most of the known spectrin mutations cause HE and/or HPP. It should be noted that spectrin mutations are not the only cause of HE. Abnormalities of protein 4.1 and other membrane skeleton components have been shown to result in this extremely heterogeneous disorder.\(^1\) The spectrin mutations cluster around the N-terminus of \( \alpha \) spectrin and the C-terminus of \( \beta \) spectrin (Fig 2). The functional consequence of mutations in this region is defective spectrin dimer self-association. An exception to this rule is Spectrin Jendoubah, an elliptogenic \( \alpha \) spectrin mutation with normal spectrin self-association.\(^12\) Impairment of dimer self-association leads to reduced mechanical stability of the RBC membrane and grossly altered membrane skeleton ultrastructure.\(^124,125\) There is mounting evidence that the HPP phenotype occurs in individuals inheriting either two elliptocytogenic \( \alpha \) spectrin mutations (that may be the same or different) or one elliptocytogenic mutation together with a defect in \( \alpha \) spectrin synthesis in the opposite allele.\(^126\) Defective \( \alpha \) spectrin synthesis is reflected in decreased mRNA transcripts from the affected allele in some cases.\(^127,128\) It has recently been shown that a "low production" \( \alpha \) spectrin allele is due to skipping of exon 46.\(^116\) This allele is found in association with a polymorphism characterized by an abnormal tryptic cleavage product (\( \alpha V^{41} \)) resulting from a point mutation/amino acid substitution some distance from the skipped exon.\(^116,129\) Elucidation of the cause for the exon skipping will provide insight into the molecular mechanism of \( \alpha \) spectrin synthetic defects that may play a role in HPP.

Hereditary spherocytosis. Autosomal recessive hereditary spherocytosis (rHS) is characterized by severe hemolytic anemia with poikilocytosis and spherocytosis, marked spectrin deficiency, and phenotypically normal parents of affected patients.\(^130,131\) An \( \alpha \) spectrin gene abnormality has been associated with many cases of autosomal recessive spherocytosis.\(^106,122,133\) The position of the mutation is indicated by the open triangle in Fig 2. It is associated with an alteration of the isoelectric point of the \( \alpha I1 \) tryptic peptide, termed \( \alpha I1a \). An atypical inheritance pattern of \( \alpha I1a \) in some kindreds with apparent recessive spherocytosis has cast doubt on the original hypothesis that this mutation is pathogenic (unpublished data). Perhaps \( \alpha I1a \) is a rare polymorphism that is in linkage disequilibrium with a separate pathogenic mutation that has yet to be defined. Whatever the pathogenic mutation, virtually nothing is known about

Fig 2. The location of mutations in the human erythroid spectrin dimer. Mutations causing hereditary elliptocytosis are indicated by arrows. Triangles designate mutations associated with HS. The solid triangle points to a dominant HS mutation.\(^133\) The open triangle shows the location of a polymorphism/mutation associated with some cases of recessive HS.\(^106,122,133\) An asterisk shows the location of a polymorphism associated with a "low expression" \( \alpha \) spectrin allele. The "low expression" phenotype is apparently due to exon skipping 3' to the polymorphism, in the region encoding segment 21 (double asterisk).\(^118\)
the mechanism by which marked spectrin deficiency occurs in rHS. Perhaps the mutation perturbs ankyrin-binding by spectrin, or diminishes spectrin subunit stability, or interchain binding. Recent descriptions of rHS with normal spectrin content\(^1\) and HS with a profound defect of \(\alpha\) spectrin synthesis\(^2\) expand the spectrum of this disease entity.

A \(\beta\) spectrin mutation is associated, in a single family, with autosomal dominant HS (dHS).\(^3\) This rare mutation results in an amino acid substitution in the conserved N-terminus of \(\beta\) spectrin (solid triangle in Fig 2) and leads to diminished binding of spectrin to protein 4.1.\(^4\) If this substitution identifies the site of direct binding of protein 4.1 to spectrin, then one might predict that other protein species with conserved sequence in this domain would bind to protein 4.1 or its homolog. It is interesting that protein 4.1 deficiencies and mutations cause elliptocytosis, whereas defective spectrin/4.1 binding, as a result of the N-terminal domain substitution, causes spherocytosis. Recently, dHS has been linked to the gene for erythrocyte ankyrin in a large kindred with typical disease.\(^5\) Other reports implicate band 3.\(^3\)\(^8\)-\(^1\) D Ankyrin gene deletion has been implicated in cases of spherocytosis with abnormalities of chromosome 8.\(^1\)^\(^3\)\(^4\)\(^6\)\(^7\) It is not yet clear what fraction of cases with dHS have spectrin mutations, as opposed to mutations affecting other membrane skeleton proteins. Because most cases of dHS have no demonstrable abnormality of spectrin peptides, aside from a mild quantitative deficiency, it seems likely that the majority are not defects of spectrin. However, a large multifamily study of dHS found an association of the disease with polymorphisms of the Ig heavy chain that is, like \(\beta\) spectrin, encoded on chromosome 14q.\(^1\)\(^5\) Furthermore, a recent report describes an unstable \(\beta\) spectrin in two families with HS.\(^1\)\(^6\)

Several other pathogenic defects in human erythrocyte spectrin have been identified in mice and humans but have yet to be solved at the molecular level. The details of such mutations will shed additional light on structure/function relationships within the spectrin molecule.

CONVENTIONS IN NUMBERING AND NAMING SPECTRIN MUTATIONS

In many early published reports of spectrin mutations, numbering of \(\alpha\) spectrin codons begins at the N-terminus of the 80-Kd \(\alpha\) tryptic peptide, actually codon 6. This convention is superseded by knowledge of the cDNA sequence. We recommend that, in future reports, the translation initiation codon is assigned the number +1. Conceivably, pathogenic mutations might exist in codons 1 through 5 that would be difficult to describe using the earlier convention.

Also, there is some confusion of nomenclature describing spectrin mutations. Because of the extreme genetic heterogeneity underlying clinical phenotypes and pathologic tryptic peptides, these properties are no longer useful for naming individual mutations, although they remain important for detection and classification of mutations. We propose that spectrin mutations be named for their city of origin, in conformity with the convention used in the genetics of, for example, hemoglobin and glucose-6-phosphate dehydrogenase (G6PD). Several mutations have already been so named.

THE STRUCTURE OF NONERYTHROID SPECTRINS

Nomenclature. The existence of spectrin-like proteins in nonerythroid tissues has been appreciated for several years.\(^1\)\(^7\)-\(^1\)\(^5\) Spectrins have been described in lower organisms, including Drosophila,\(^1\)\(^2\) sea urchin,\(^1\)\(^3\)\(^1\)\(^4\) Amoeba,\(^1\)\(^5\) and even plants.\(^1\)\(^6\) Molecular cloning of cDNAs encoding spectrins from a variety of cell types and species has provided insight into the structural basis of the observed diversity in nonerythroid spectrins. In humans, four spectrin genes have been identified, which include erythroid \(\alpha\) and \(\beta\) spectrin genes (see above). Genes for nonerythroid homologs of \(\alpha\) and \(\beta\) spectrin have also been described that encode proteins that are approximately 60% identical to their erythroid counterparts.\(^4\)\(^1\)\(^7\)-\(^1\)\(^6\) The protein products of these genes may be altered by alternative processing of pre-mRNA, leading to even greater diversity of spectrin subunits. Therefore, we propose a systematic nomenclature for human spectrin proteins that accounts for these structural features (Table 1). Our proposal is based on the nomenclature of Ma et al\(^1\)\(^5\) and Zimmer et al\(^6\) combined with that of S.P. Kennedy and J.S. Morrow (personal communication). The spectrins are classified as \(\alpha\) or \(\beta\) and numbered with Roman numerals, in the order of their characterization. Isoforms of each spectrin gene generated by alternative pre-mRNA processing (or, theoretically, alternative promoter use) are given Arabic numbers after the symbol \(\Sigma\) (to denote subtype), in order of their description. As additional human spectrins are cloned and sequenced and as more isoforms of known spectrins are characterized, they can be readily named using this nomenclature. Examples of human/mammalian spectrin species characterized immunologically, but not yet cloned, are given at the bottom of Table 1.\(^1\)\(^6\)\(^3\)-\(^1\)\(^6\)

The major advantage of this system is that it reduces the ambiguity that is now widespread in the literature. The terms “spectrin,” “fodrin,” and other synonyms (Table 1) are frequently used without reference to the genes encoding the protein discussed or the specific isoform. With the structural information now available, it is desirable to eliminate, to the degree possible, this unfortunate ambiguity. Our nomenclature is based on the human spectrins. We suggest that nonhuman spectrins be referenced to human spectrins by homology. High molecular weight spectrins, such as avian TW260\(^1\)\(^4\)\(^8\)-\(^1\)\(^6\) and Drosophila \(\delta\)_H,\(^1\)\(^6\) have, as yet, no human counterparts.

The functional unit of spectrin is usually an \(\alpha\)\(\beta\) heterodimer. Another source of nonerythroid spectrin diversity, in addition to subunit variety, is combinatorial diversity. The proposed nomenclature allows combinatorial forms of spectrin subunits to be described precisely. Erythroid spectrin can be designated \(\alpha\)\(\Sigma\)\(\beta\)\(\Sigma\)\(1\). The common or general nonerythroid spectrin (\(\delta\)odrin) is \(\alpha\)\(\Sigma\)\(1\)\(\beta\)\(\Sigma\)\(1\). If the specific splicing isoform under discussion is uncertain or unknown, it can be formally designated using the symbol \(\star\) (eg, \(\alpha\)\(\Sigma\)\(\ast\)\(\beta\)\(\Sigma\) for nonerythroid spectrin). Combinatorial forms can be readily specified, such as spectrin (found in
muscle and brain) that evidently includes nonerythroid \( \alpha \) spectrin and the alternatively spliced isoform of erythroid \( \beta \) spectrin (\( \alpha 122^* / \beta 122^* \)).

**Erythroid** spectrin in nonerythroid tissues. The expression of erythroid \( \alpha \) spectrin (\( \alpha 1 \)) is apparently limited to RBCs. In contrast, the detection of \( \beta \) spectrin species in brain and muscle that antigenically and structurally resemble RBC \( \beta \) spectrin raised the possibility that the erythroid \( \beta \) spectrin gene is expressed in other cell types. This idea was supported by the observation that hybridization to erythroid spectrin cDNA showed mRNA transcripts in nonerythroid tissues.\(^{3,4,6,16,17,18,19}\)

Nonerythroid expression of \( \beta \) spectrin has been confirmed by the isolation and characterization of human skeletal muscle \( \beta \) spectrin cDNAs.\(^{16}\) Interestingly, muscle \( \beta \) spectrin cDNA differs from RBC \( \beta \) spectrin at the 3’ end. The result is a \( \beta \) spectrin isoform, \( \beta 122^2 \), with a unique C-terminus (Fig 3B). The muscle C-terminus adds 21.4 Kd to the Mr of \( \beta \) spectrin, assuming that the N-terminal region of the muscle subunit is identical to that of RBC \( \beta \) spectrin. The ankyrin-binding site is intact, suggesting that erythroid ankyrin mediates \( \beta 122^2 \) attachment to muscle membrane proteins. Interestingly, *Drosophila* \( \beta \) spectrin is homologous (49% amino acid identity) to the human \( \beta 122^2,5,13 \)

Analysis of the 3’ \( \beta \) spectrin genomic DNA structure shows that \( \beta 122^2 \) is generated by differential processing of \( \beta \) spectrin pre-mRNA.\(^{16} \) Furthermore, analysis of brain and cardiac muscle RNA and cDNA indicates that these tissue also express \( \beta \) spectrin I and use the same alternative splice site as skeletal muscle. Gene transfer studies in which exons of the 3’ human \( \beta \) spectrin I gene are expressed in murine cell lines show that only erythroid-lineage cells are able to process \( \beta \) spectrin I pre-mRNA in the erythroid manner.\(^{17} \) Developing RBCs acquire this novel processing capability early in erythropoiesis.\(^{17,18} \)

The question arises as to whether \( \beta \) spectrin \( 122^2 \) repre-

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**Table 1. Proposed Nomenclature for Human Spectrin Subunits**

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<th>Proposed Name</th>
<th>Current Name(s)</th>
<th>Chromosome</th>
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<td>Nonerythroid ( \alpha ) spectrin, ( \alpha _{f} ) fodrin, brain ( \alpha ) spectrin, calspectrin, ( \alpha _{p} ) spectrin</td>
<td>9</td>
</tr>
<tr>
<td>( \beta ) Spectrin II</td>
<td>Isoforms of ( \alpha ) spectrin resulting from alternative splicing of pre-mRNA</td>
<td>9</td>
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<td>14</td>
</tr>
<tr>
<td>( \beta ) Spectrin II</td>
<td>Spectrin 235E, isoform of erythroid ( \beta ) spectrin resulting from alternative splicing of pre-mRNA, 270-Kd ( \beta _{p} ) spectrin, ( \beta _{p} ) spectrin</td>
<td>14</td>
</tr>
<tr>
<td>( \beta ) Spectrin II</td>
<td>Nonerythroid ( \beta ) spectrin, ( \beta _{f} ) fodrin, brain ( \beta ) spectrin, general isoform of ( \beta ) spectrin, ( \beta _{p} ) spectrin, ( \beta _{p} ) spectrin</td>
<td>2</td>
</tr>
<tr>
<td>( \beta ) Spectrin II</td>
<td>Isoforms of ( \beta ) spectrin resulting from alternative splicing of pre-mRNA</td>
<td>2</td>
</tr>
<tr>
<td>Unnamed spectrins (await molecular cloning)</td>
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<tr>
<td>Cardiac muscle-specific 255-Kd ( \beta ) spectrin</td>
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**Fig 3.** The structure of human nonerythroid spectrin subunits. (A) Schematic representation of nonerythroid \( \alpha \) spectrin (\( \alpha 121^* \)).\(^{15} \) Homologous segments are numbered. Domains not conforming to typical repeats are indicated by rectangles. Notable structural and functional features are shown. (B) Schematic representation of the nonerythroid isoform of erythroid \( \beta \) spectrin (\( \beta 122^2 \)). Homologous segments are numbered. Domains not conforming to typical repeats are indicated by rectangles. Notable structural and functional features are shown. (C) Schematic representation of nonerythroid \( \beta \) spectrin (\( \beta 121^2 \)). Homologous segments are numbered. Domains not conforming to typical repeats are indicated by rectangles. The region showing no homology to spectrin \( \beta 122^2 \) is indicated in black. Pre-mRNA processing variants, affecting the C-terminus of this spectrin species, have been detected (unpublished data).
SPECTRIN

sent a known spectrin species in muscle and brain. Immunochemical analysis using antibodies raised to a synthetic peptide or a fusion protein derived from the muscle-specific C-terminus shows that the $\beta_{122}$ species is identical in mobility to the previously described $\beta$ spectrin 1.235E.161,164,172-174 $\beta_{122}$ spectrin may also be homologous to avian $\beta$ spectrin.175-177 $\beta_{122}$ spectrin colocalizes with dystrophin in a subsarcolemmal lattice that includes costameres, transverse elements overlying the M line, and occasional longitudinal elements.178

Several fundamental questions concerning spectrin $\beta_{122}$ remain: (1) What is the $\alpha$ subunit with which this $\beta$ spectrin associates? It is likely to be $\alpha$ spectrin II, because $\alpha$ spectrin I has never been clearly identified outside the RBC. (2) What is the function of the unique muscle spectrin C-terminus? The amino acid sequence of this region exhibits homology with nonerythroid $\beta$ spectrin ($\beta_{II2}$).163 Because the C-terminus of RBC $\beta$ spectrin is involved in spectrin dimer-dimer self-association, it is predicted that the replacement of the C-terminus in muscle cells affects this function. Nonerythroid spectrin ($\alpha_{II2}^*/\beta_{II2}^*$) forms more stable tetramers than does erythroid spectrin ($\alpha/\beta_{II1}$).1 Perhaps the spectrin $\beta_{122}$ C-terminus includes a tetramer stabilization sequence. (3) Are there inherited defects of the $\beta$ spectrin I gene that affect both RBCs and muscle? None of the known mutations of $\beta$ spectrin I are known to result in abnormal muscle structure or function. The RBC phenotype is evident in the heterozygous state; perhaps only homozygotes would have affected muscle. Possibly, other molecules, such as $\beta$ spectrin II, $\alpha$ actinin, or dystrophin, are capable of complementing the function of spectrin $\beta_{II2}$ in muscle.

$\alpha$ Spectrin II. Nonerythroid cells share a common $\alpha$ spectrin subunit we refer to as $\alpha$ spectrin II (often called $\alpha$ fodrin; Table 1).165,179 Therefore, most studies of nonerythroid spectrin structure, function, and localization describe properties of spectrin $\alpha_{II2}^*/\beta_{II2}^*$ ($\alpha$ fodrin), the ubiquitous nonerythroid species. This spectrin is similar to RBC spectrin in some respects, including immunoochemical cross-reactivity, rod-like appearance on electron microscopy, tetramer formation, and ability to bind actin, protein 4.1, and ankyrin.180 However, there are several qualitative differences between spectrins $\alpha/\beta_{II1}$ (erythroid spectrin) and $\alpha_{II2}^*/\beta_{II2}^*$ (nonerythroid spectrin). $\alpha_{II2}^*/\beta_{II2}^*$ exhibits high-affinity calmodulin binding, increased tetramer stability, different affinities for associated proteins, and unique peptide maps.180,181 Analysis of molecular clones encoding $\alpha$ and $\beta$ spectrin II has helped to explain some of the observed differences. It should be noted that, unlike mammalian RBCs, avian erythrocytes do not have an erythroid-specific $\alpha$ spectrin. Rather, avian erythrocyte spectrin is composed of homologs of human $\alpha$ spectrin II and $\beta$ spectrin I.

The entire primary structure of human $\alpha$ spectrin II has been deduced from the cDNA.182 Homologous nonerythroid $\alpha$ spectrins have been cloned from chicken and Drosophila.28,182 The $\alpha$ spectrin II gene is on human chromosome 9.183 Its structure is depicted in Fig 3A. The derived peptide exhibits a high degree of homology (58% amino acid identity) with $\alpha$ spectrin I. The most notable difference is a 36 amino acid sequence at the end of segment 11 of $\alpha$ spectrin II that is absent in erythroid $\alpha$ spectrin. Functional studies have shown that this segment confers high-affinity binding of calmodulin to $\alpha$ spectrin II.184-187 Interestingly, this segment and the corresponding activity are lacking in the Drosophila nonerythroid $\alpha$ spectrin homolog. There is also a 20 amino acid insertion at the junction of segment 10 and 11 that has no counterpart in erythroid $\alpha$ spectrin and is variably expressed in different $\alpha$ spectrin II molecules in the same cell type.41,188 An additional 6 amino acid insertion occurs in segment 21 of most $\alpha$ spectrin II transcripts. Presumably, these variably expressed sequences result from alternative pre-mRNA splicing. The functional significance of these sequence variations, $\alpha_{II2}^*/\beta_{II2}^*$, etc, in our nomenclature, is unknown.

Unlike the EF hands of $\alpha$ spectrin I that are possibly vestigial (see above), there is evidence that the C-terminal domain EF hands of $\alpha$ spectrin II can directly bind to calcium.189 Such an interaction may be involved in the modulation by calcium of sea urchin spectrin binding to actin.190 The relationship between direct effects of calcium on spectrin II and those mediated by calmodulin remain to be precisely defined. There is also a postulated role for calcium-dependent protease, in addition to calmodulin, in control of actin binding by nonerythroid spectrin ($\alpha_{II2}^*/\beta_{II2}^*$).191

$\beta$ Spectrin II. $\beta$ Spectrin II cDNA has also been cloned from mouse157,158 and human.159-161 The gene for $\beta$ spectrin II resides on human chromosome 2p12192 and mouse chromosome 11.192 The derived amino acid sequence of $\beta$ spectrin II is 60% identical to that of $\beta$ spectrin I. A calculated Mr of 274.5 Kd is obtained. Its structural features are shown in Fig 3C. The carboxyl terminus is homologous to that of the nonerythroid isoform of $\beta$ spectrin I ($\beta_{II2}$), except for 92 amino acids (indicated by black in Fig 3C).161 Some $\beta$ spectrin II cDNA sequences exhibit variation (unpublished data). These variations affect predicted protein structure and suggest that alternative pre-mRNA processing produces isoforms designated spectrin $\beta_{II2}^*2,3$, etc ($\beta_{II2}$ refers to the isoform reported in full length).161

Human nonerythroid $\beta$ spectrin ($\beta_{II2}^*2$) is apparently coexpressed with spectrin $\beta_{II2}$ in muscle164,165,174,175 and brain,172,173 albeit with different cellular distribution and developmental expression. It is unknown whether these proteins can complement each other’s function. Little is known of their associated molecules. Spectrin $\beta_{II2}$ may be homologous to avian $\gamma$ spectrin.175,176

The function of nonerythroid spectrin. The current understanding of the function of nonerythroid spectrins is incomplete. Their biologic importance is suggested indirectly by their degree of sequence conservation across wide evolutionary distances, and directly by the observation that a homozygous mutation of the Drosophila $\alpha$ spectrin II homolog is lethal.193 Like erythroid spectrin ($\alpha/\beta_{II1}$), nonerythroid spectrin ($\alpha_{II2}^*/\beta_{II2}^*$) cross-links actin filaments. Unlike RBC spectrin, it is not uniformly distributed along the cell membrane. For example, spectrin $\alpha_{II2}^*/\beta_{II2}^*$ distributes asymmetrically in polarized epithelium.194 Most likely, nonerythroid spectrins maintain the spatial organization of specialized membrane proteins and mediate their

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attachment to the actin cytoskeleton. Nonerythroid spectrin have been implicated in the structure and function of various blood cell membranes, including platelets, neutrophils, lymphocytes, and salivary gland Na+ channels at the neuromuscular junction, and voltage-dependent Na+ channels in brain. Nonerythroid spectrin is also found in a complex with ankyrin and the cell adhesion molecule uvomorulin.

Most investigations of nonerythroid spectrin function have studied α spectrin II/β spectrin II, yet it is unclear in many published studies which of the several spectrins is actually being investigated. In the future, investigators must understand and specify which spectrin subunit species and spliced isoforms are present in each experimental system.

SUMMARY

Recent developments have contributed important information to understanding the role of spectrins in the RBC membrane skeleton and nonerythroid cells. Many questions can now be framed, informed by structural knowledge of various spectrin subunit types and alternatively spliced variants, that previously could not have been addressed. Their solution in the coming years will likely lead to further advances with direct relevance to biology and medicine.

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Erythroid and nonerythroid spectrins

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