Minireview

Spectrin's chimeric E2/E3 enzymatic activity

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Abstract

In this minireview, we cover the discovery of the human erythrocyte α spectrin E2/E3 ubiquitin conjugating/ligating enzymatic activity and the specific cysteines involved. We then discuss the consequences when this activity is partially inhibited in sickle cell disease and the possibility that the same attenuation is occurring in multiple organ dysfunction syndrome. We finish by discussing the reasons for believing that nonerythroid α spectrin isoforms (I and II) also have this activity and the importance of testing this hypothesis. If correct, this would suggest that the nonerythroid spectrin isoforms play a major role in protein ubiquitination in all cell types. This would open new fields in experimental biology focused on uncovering the impact that this enzymatic activity has upon protein–protein interactions, protein turnover, cellular signaling, and many other functions impacted by spectrin, including DNA repair.

Keywords: Spectrin, proteome, red blood cell, ubiquitin, cell signaling, multiorgan organ dysfunction syndrome

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The erythrocyte spectrin membrane skeleton

The erythrocyte, or red blood cell (RBC), travels the circulatory system for 120 days in people who are not anemic. During this four-month journey, this 8 μm, biconcave disc must pass through the circulatory system, which narrows to 2 μm in the smallest venules. To accomplish this feat, the RBC must be reversibly deformable and elastic, but at the same time have membrane properties that maintain the cell's structural integrity despite the shear forces that it encounters. The spectrin membrane skeleton is a two-dimensional meshwork of proteins that spans the cytoplasmic membrane surface of the RBC, and provides it with these properties as well as maintaining its biconcave shape (Figure 1). A sampling of important prior reviews is provided.[1–6]

The membrane skeleton, visualized by negative staining and electron microscopy, is primarily a hexagonal lattice. The lattice contains central actin protofilaments interconnected by spectrin tetramers.[7] Spectrin in its simplest form is an antiparallel $\alpha\beta$ heterodimer, which *in vivo* forms an $(\alpha\beta)_2$ tetramer by head-to-head linkage of two heterodimers.[8,9] The α and β spectrin subunits contain a primary repeating unit of ~106 amino acids called the spectrin repeat. Complete amino acid sequence deduced for the spectrin subunits demonstrated multiple spectrin repeats in both the α (~20) and β (~16), which are numbered beginning at the N terminus by convention.[10–12] Karinch et al.[13] demonstrated that the tail ends of spectrin tetramers, which do not contain the spectrin repeats, attach to actin protofilaments associating with residues 47 through 186 at the N-terminus of the β subunit. To reinforce this spectrin–actin interaction, protein 4.1 binds to the tails of β spectrin creating a spectrin–4.1–f-actin ternary complex.[14–17] Another peripheral membrane protein named adducin binds to both β spectrin and the barbed fast-growing end of F-actin.[18–20] Attachment of the membrane skeleton to the membrane occurs in at least two ways. Protein 4.1 that binds to β spectrin, close to the N-terminal actin-binding domain also binds glycophorin C.[21,22] Ankyrin binds β spectrin[15,23] to the anion transport channel, Band 3.[24,25]

Discovery of spectrin's chimeric E2/E3 ubiquitin conjugating/ligating activity

From its earliest description in 1968,[26] to its appreciation as a major component of the membrane skeleton in the early 1980s,[1] spectrin was thought to play the structural role as indicated in Figure 1. In 2001, the understanding of spectrin function was extended when Kakhniashvili et al.[27] demonstrated that spectrin also had an enzymatic activity that allowed it to ubiquitinate itself. The story of this discovery was preceded by a bit of serendipity. A rabbit autoantibody stained control RBC membrane skeletons brightly, but showed very weak staining when indirect immunofloures-cence (IF) was performed on homozygous (SS) sickle cell anemia (SCA) RBC membrane skeletons. Interestingly, when running sodium dodecyl sulfate polyacrylamide gel

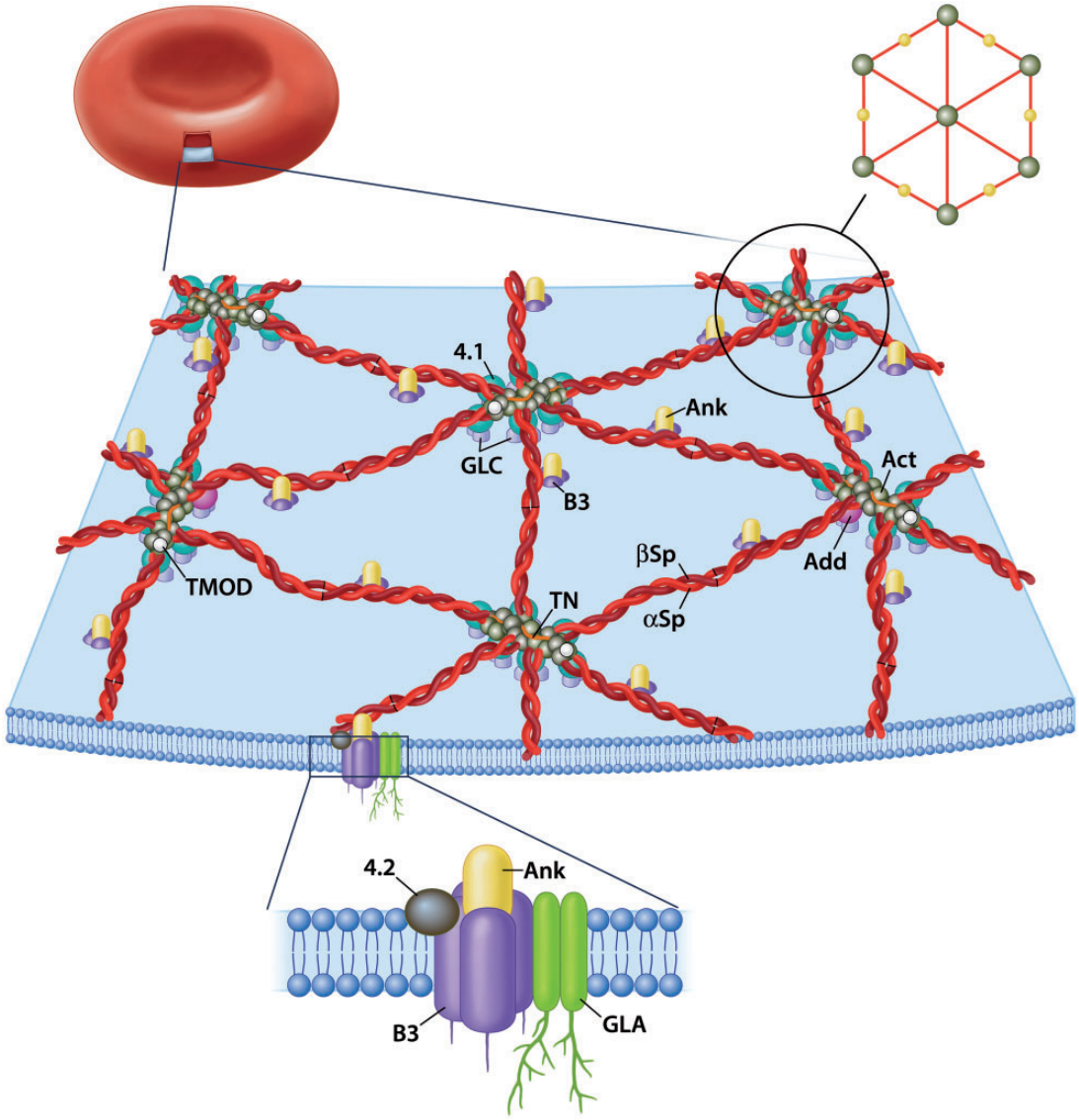

Figure 1 The RBC spectrin membrane skeleton. The RBC is a biconcave disc (upper left) which on its cytoplasmic surface (light blue) contains a network called the spectrin membrane skeleton. The detailed protein interactions constituting this membrane skeleton are shown in the center. In the upper right-hand corner is a diagram showing that actin assemblies, including protein 4.1 or adducin troponin and tropomodulin, form a hexameric array that is crosslinked by spectrin tetramers. The drawing below gives a transbilayer view of the protein assembly, which attaches spectrin, via ankyrin, to the band 3 tetramer (with associated 4.2 and GLA). ⊥αSp: alpha spectrin; βSp: beta spectrin; B3: band 3; Ank: ankyrin; TMOD: tropomodulin; TN: troponin; GLA: glycophorin A; 4.1: protein 4.1; GLC: glycophorin C; and 4.2: protein 4.2

electrophoresis (SDS-PAGE) of RBC membranes, *without reducing agent*, a prominent band was observed above α spectrin in the case of control RBCs, which was substantially diminished in RBC membranes derived from a patient with SCA (but not a person with sickle cell trait). This band of interest was stained by the autoantibody and anti-α-spectrin (but not anti-β-spectrin) antibody on Western blots. Thus, it was named it $\alpha^{/}$ spectrin. When SDS PAGE was run in the presence of reducing agent, the $\alpha^{/}$ spectrin was diminished in the Coomassie blue-stained gel and the western blots. Now we had an interesting mystery. What was this dithiothreitol (DTT) reducing agent sensitive $\alpha^{/}$ spectrin?

The journey from this unexplained finding, to an understanding of spectrin's enzymatic function, began with an article by Kakhniashvili et al.[27] In this study, it was first demonstrated that the modification was ubiquitin. Next was the demonstration that erythrocyte α-spectrin is an E2 ubiquitin-conjugating enzyme that is able to target itself.[27] The DTT-sensitive adduct, $\alpha^{/}$ spectrin, was ubiquitin linked to α-spectrin via a thioester bond. E1 ubiquitin-activating enzyme and ATP were required to form both the α-spectrin–ubiquitin adduct and conjugate. Using computer programs (COMPARE and PEPTIDE STRUCTURE) and a structural prediction program (PROPSEARCH), we analyzed the α-spectrin sequence.[27] Analyses indicated that a segment within α-spectrin repeat 20 could be responsible for E2 ubiquitin-conjugating activity. Cysteine residue 2071 was surrounded by a sequence that shared ~70% identity (11 of 16 residues) with the active site consensus sequence critical for all known E2 enzymes. It had not yet determined

whether an additional E3 ubiquitin-ligating enzyme was necessary for the ubiquitination involving α spectrin, or alternatively that spectrin had both E2 and E3 activity. However, a potential E3 site that conformed to the cleft structure surrounding the active site residues of E3 HECT domain enzymes was identified with this analysis. This potential E3 site surrounded Cys2100, which was also located in α-spectrin repeat 20. Furthermore, there was a region of sequence that contained a lysine cluster within repeat 21 (2199-KRKQKEIQAMK-2209) that could possibly contain a lysine acceptor site(s) for ubiquitin attachment.[27] Therefore, Kakhniashvili et al. proposed an initial hypothesis where ubiquitin was transferred from an E1 enzyme to cysteine 2071, then from this cysteine to cysteine 2100, and then finally to the lysine-rich region of α spectrin repeat 21.[27,28] This hypothesis was subsequently proven to be partially correct, as we discuss below.

The hypothesis could be tested utilizing a recombinant peptide representing residue 2005 to the C-terminus of α spectrin. Hsu et al.[29] cloned this segment into a glutathione S-transferase (GST) vector and demonstrated that this C-terminal α spectrin recombinant had the ubiquitin-conjugating and ligating activity and could transfer ubiquitin to itself. This finding proved that the activity was inherent in the α spectrin structure, rather than a copurifying RBC protein. By testing the C-terminal recombinant protein, GST-fusion α spectrin (2005-2415), using an *in vitro* ubiquitination assay, these studies demonstrated that both cysteine 2071 and cysteine 2100 are capable of receiving ubiquitin from an E1-activating enzyme and directly transferring ubiquitin to a target lysine within this α-spectrin C-terminal recombinant peptide. Site-specific mutational analyses using the GST-fusion C-terminal human α-spectrin recombinant was employed to examine this activity. Wild-type recombinant protein, α-spectrin (2005–2415) has six cysteines. Mut1 to Mut13 had different combinations of cysteine(s) mutated into alanine(s) (Table 1). Single mutations, such as C2071A or C2100A, were created and the spectrin ubiquitination activity was unaffected by these mutants. Only recombinant peptides containing the C2071A/C2100A double mutant demonstrated loss of activity (Table 1).[29,30] Based on these results, the model shown in Figure 2[29,30] was proposed. In this model, cysteines 2071 and 2100 both have chimeric E2/E3 activity in human RBCs. In mice, and species other than human, residue 2100 is converted to a tyrosine or glutamine suggesting that the conserved cysteine 2071 is the primary E2/E3 site and cysteine 2100 serves a back up function in humans.[29,30]

In summary, Goodman and colleagues had demonstrated that RBC spectrin has a chimeric E2/E3 ubiquitin-conjugating/ligating activity, which is capable of ubiquitinating itself. Later it was shown that it could also ubiquitinate other membrane skeleton-associated target proteins.[31,32] The other known target proteins were ankyrin,

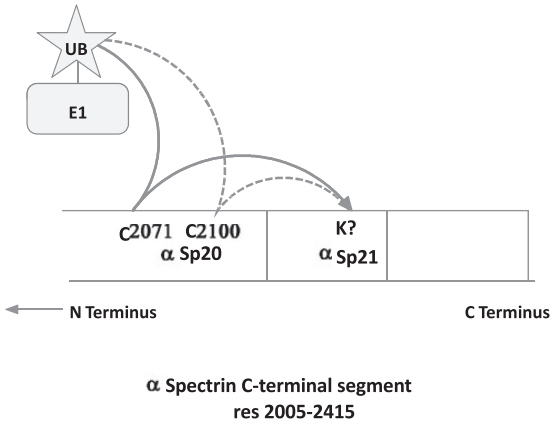

Figure 2 Model of human α-spectrin (2005–2415) ubiquitination enzymatic sites and acceptor sites. Human α spectrin cysteines 2071 and 2100 can both accept ubuiquitin from an E1-activating enzyme and transfer it directly to cysteine(s) with the a21 repeat unit

Table 1 The use of mutational analysis of recombinant peptides representing the C-terminus of α spectrin to define the critical chimeric E2/E3 active site cysteines

Clone	Amino acids						Activity
WT	C2071,	C2100,	C2158,	C2387,	C2298,	C2058	+
Mut1	**C2071A**,	C2100,	C2158,	C2387,	C2298,	C2058	+
Mut2	**C2071A,**	**C2100A**,	C2158,	C2387,	C2298,	C2058	–
Mut3	**C2071A,**	**C2100A,**	**C2158A**,	C2387,	C2298,	C2058	–
Mut4	**C2071A,**	**C2100A,**	**C2158A,**	**C2387A**,	C2298,	C2058	–
Mut5	**C2071A,**	**C2100A,**	**C2158A,**	**C2387A,**	**C2298A**,	C2058	–
Mut6	**C2071A,**	**C2100A,**	**C2158A,**	**C2387A,**	**C2298A,**	**C2058A**	–
Mut7	C2071,	**C2100A**,	C2158,	C2387,	C2298,	C2058	+
Mut8	C2071,	C2100,	C2158,	C2387,	C2298,	**C2058A**	+
Mut9	C2071,	C2100,	**C2158A**,	C2387,	C2298,	C2058	+
Mut10	**C2071A**,	C2100,	C2158,	C2387,	C2298,	**C2058A**	+
Mut11	**C2071A**,	C2100,	**C2158A**,	C2387,	C2298,	C2058	+
Mut12	C2071,	**C2100A**,	C2158,	C2387,	C2298,	**C2058A**	+
Mut13	C2071,	**C2100A,**	**C2158A**,	C2387,	C2298,	C2058	+

Source: Modified from Hsu et al.[29]

protein 4.1, protein 4.2, the anion transport channel, and a protein of currently unknown function (gi 13278939).[31,32]

Previously analyzed proteins that also fell into this chimeric E2/E3 category were E2-230K[33] and BRUCE.[34] E2-230K is required for remodeling of erythroid cells during differentiation;[33] and BRUCE is a 528-KDa endomembrane-associated protein.[34] Corsi et al.[35] were the first to demonstrate that RBC spectrin was ubiquitinated and Galluzzi et al.[36] demonstrated two ubiquitination sites within α spectrin. The two sites were localized to α spectrin repeat 17 and repeats 20/21 (in agreement with our findings). The lysine involved in ubiquitin linkage to α spectrin repeat 17 is residue 1709.[36] There is no currently known function for the repeat 17 ubiquitination site.

Role of the spectrin chimeric E2/E3 activity in sickle cell disease

SCA is the major form of hemoglobinopathies, which result in RBC sickling. This family of diseases is collectively called sickle cell disease (SCD), and SCA was the first described, affects the most people, and is the most severe. The hallmarks of SCA are anemia, vasoocclusion (leading to sickle cell crises), oxidative stress, inflammation, organ damage, and, in many cases, a shortened life span. Within the circulation of a SCA patient, most of the RBCs can convert back and forth from the sickle to the biconcave shape depending on whether hemoglobin-S (HbS) is deoxygenated, with formation of 14 stranded polymers, or oxygenated where the polymers are converted back to HbS monomers. These are referred to as reversibly sickled cells (RSCs). There are other RBCs that are irreversibly sickled cells (ISCs), and highly elongated and dehydrated. The ISCs remain sickled even when HbS is well oxygenated and depolymerized, and account for 2–40% of the RBCs in the circulation of a SCA patient. Previous reviews on the topic will expand upon this discussion.[3,37,38] For much of the 20th century, the molecular basis of ISC formation eluded the research community.

Lux et al.[39] demonstrated that most RBC membranes and triton skeletons isolated from ISCs remained sickled. This was not the case for RSC and control membranes and triton skeletons. We defined the molecular defects, within the membrane skeleton, which cause the ISC to be ''locked'' into an irreversibly sickled shape.[40–46] Shartava et al. demonstrated that core skeletons isolated from ISCs dissociate more slowly than skeletons derived from RSC or control RBCs.[40,41] We then demonstrated that spectrin–4.1–actin ternary complexes, created *in vitro* from proteins isolated from SCA ISCs verus control RBCs, also dissociate more slowly at 37°C.[40] Shartava et al.[40] demonstrated that β actin and spectrin were responsible for the slow dissociation. The defect in ISC β-actin, due to increased oxidative stress, is a disulfide bridge between Cys 284 and Cys 373, which is found at very low levels in RSC and control β-actin.[40,41,44] This post-translational modification in ISC β-actin caused slower and incomplete depolymerization than observed with RSC and control β-actin.[43] It did not impact ISC β-actin binding to spectrin.[43]

SCA RBC α spectrin demonstrates diminished ubiquitination (50–90% reduced) when compared to control RBC spectrin.[28,45,47] This is caused by diminished spectrin E2/E3 activity, which we believe to be caused by the conversion of active site cysteine thiolates (C2071 and C2100) into cysteine oxiforms, which cause loss of function.[47] As nonubiquitinated α spectrin participates in a more tightly associated spectrin–4.1–actin and spectrin–adducin–actin ternary complex than ubiquitinated spectrin,[45,46] the rate of SCA ternary complex dissociation is far slower than the control ternary complex dissociation rate.[40] Therefore, the molecular basis of the formation of the ISC is a membrane skeleton that disassembles and reassembles slowly leading to a cell ''locked'' into the sickled shape. These experiments therefore defined the molecular basis of the ISC being a disulfide bridge in β-actin and diminished ubiquitination of a spectrin, both contributing to a ''locked'' membrane skeleton and cell (Figure 3).[37,38]

In SCA patients, there are circulating RSCs and dense ISCs, and both play roles in vasoocclusion.[37,38] We have demonstrated that n-acetyl cysteine (NAC) blocks the formation of ISCs *in vitro*.[48–50] NACs inhibition of ISC formation correlated with reduction of the ISC β-actin disulfide bridge.[48] Pace et al. performed a phase II human trial to determine the efficacy of NAC in reducing dense cell and ISC levels, increasing intracellular GSH, and reducing acute vasoocclusive crises (VOC) episodes in SCD patients. At 2400 mg per day, NAC reduced dense cells significantly by 37%, doubled intracellular GSH, and lowered the relative risk of VOC episodes by 61%.[51] Percentage ISCs showed a downward trend at all doses tested (600, 1200, and 2400 mg). The findings of our phase II clinical trial have been confirmed by Nur et al.,[52] and phase III trials are anticipated.

Erythrocyte spectrin ubiquitination and multiple organ dysfunction syndrome (MODS)

Caprio et al.[53] recognized the potential relationship between the role of diminished α^{I} or ubiquitinated α spectrin in ISC formation and the rigidity observed in RBCs in MODS. MODS is a major potential clinical problem faced postsurgery. They tested this relationship and demonstrated that the decreased RBC deformability observed in a rat model of trauma and hemorrhagic shock was correlated with diminished α^{I}/α spectrin ratio.[53] They concluded that ''the fact that α-spectrin, in addition to being a structural membrane protein, has important enzymatic activity that helps regulate the association of the various RBC membrane proteins to the actin cytoskeleton makes it an attractive target for further study and possible therapy'' related to MODS.[53] We would suggest that this may be true for many disorders in which RBCs are less deformable and misshapen.

Spectrin isoforms in nonerythroid cells

In 1981, Goodman et al.[54] demonstrated that spectrin and spectrin-related proteins could be found in nonerythroid cells and tissues. This article led to a flurry of articles in the 1980s demonstrating the ubiquity of spectrin and close relatives in mammalian and avian cells.[1,55–62] It became appreciated that spectrin was widely found in eukaryotic

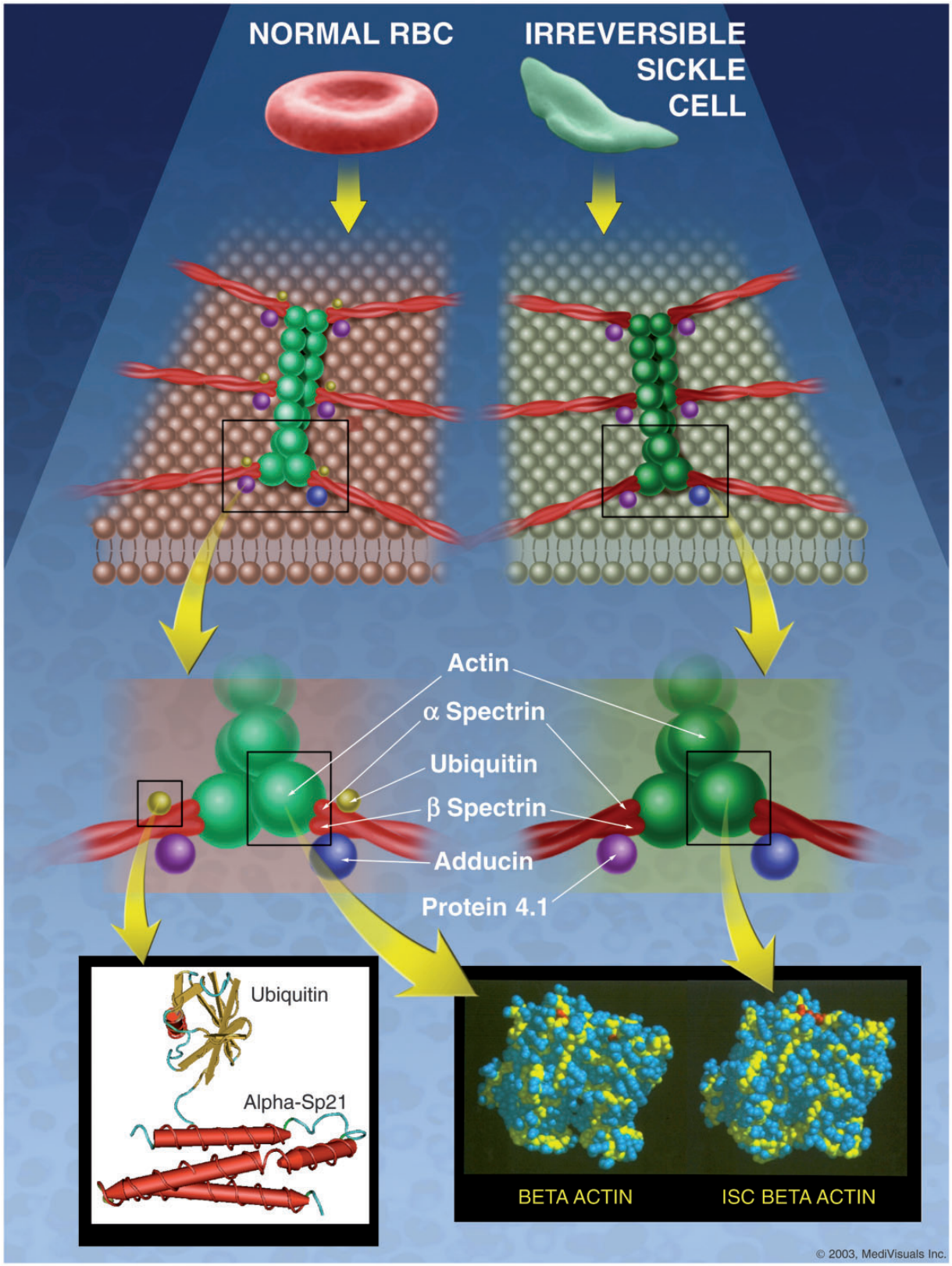

Figure 3 The molecular basis of the ISC. Taken from Goodman[37] with permission of the publisher

cells. Much attention was focused on brain spectrin, because of its very high content in neural tissue (2–3% of the total protein content) and the intrinsic interest in uncovering its neural functions. A large number of comprehensive review articles are available on brain spectrin structure, function, and location,[63–67] which will be briefly covered here. Riederer et al. made the important discovery of multiple isoforms of spectrin in neurons.[68,69] We described an

erythroid isoform composed of α subunits, identical to RBC α spectrin, associated with β subunits, which were an alternately spliced forms of RBC β-spectrin, with an extended C-terminus.[68–70] This form, originally called brain spectrin 240/235E and now called αSpI/βSpIΣ2, was found in the soma, dendrites, and postsynaptic densities of all neurons.[68–70] There was a second major form of spectrin in neurons, which had α and β subunits, which were distinct gene products from RBC spectrin but shared ~60% sequence identity with the RBC spectrin subunits, increasing in sequence identity within the functional domains.[68–70] This isoform, originally called brain spectrin (240/235) and now called αSpIIΣI/βSpIIΣ1, was found to be primarily located in the axons and presynaptic terminals of all neurons.[68–70] Brain spectrin isoforms are $(\alpha\beta)_2$ tetramers.[71,72] αSpI/βSpIΣ2 had binding sites for erythroid ankyrin, protein 4.1, and actin, which are colocalized in the neuronal soma, dendrites, and postsynaptic densities.[64–66,73] In the case of αSpIIΣI/βSpIIΣ1, the tetramer had binding sites for nonerythroid isoforms of protein 4.1 and ankyrin, but also had association sites for calmodulin and synapsin.[64–66,74,75]

An immunoelectron microscopy study from Zagon et al.[76] led to the interesting conclusion that the spectrin isoforms were located on the cytoplasmic surface of not only the plasma membrane but also all organelle membranes, and within the nucleus. They were also cross-linking cytoskeletal structures to each other and membrane surfaces. The location of spectrin isoforms in brain is summarized in Figure 4. Of great interest in this immunoelectron microscopy study was the finding of αSpIIΣI/βSpIIΣ1 associating with the cytoplasmic surface of small spherical synaptic vesicles. Lambert and colleagues have demonstrated that αSpII can be found within the nucleus where it plays an important role in DNA repair.[77–81]

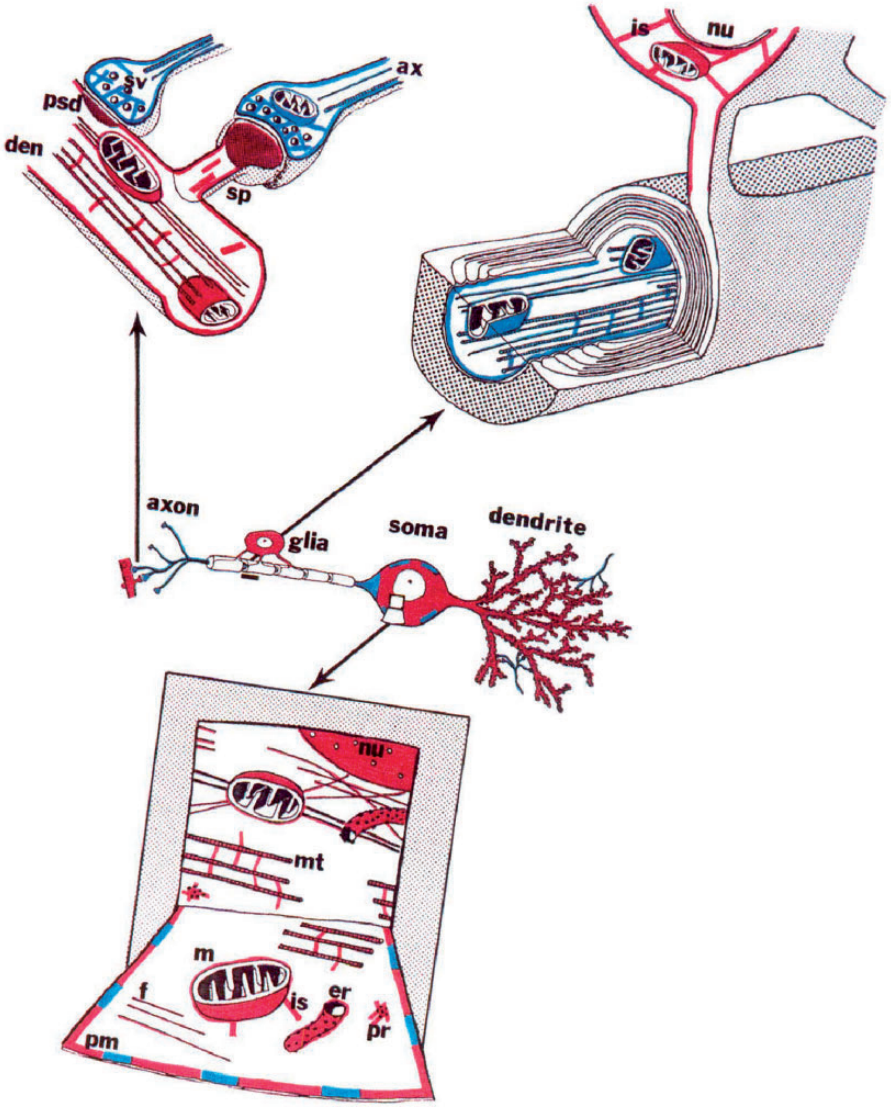

Figure 4 Location of spectrin isoforms. Red indicates the position of spectrin αI/βIΣ2 and blue αII/βII in neurons. Taken from Zagon et al.[76] with permission of the publisher

Table 2 Spectrin isoform nomenclature and properties.

Spectrin isoform	Previous names	Molecular weight	mRNA size	Genomic locus	Chromosome	
					Mouse	Human
αSpIΣ1	α Erythrocyte spectrin	280 kDa	8 kb	*Spna1*	1	1
αSpIIΣ1	Nonerythroid	280 kDa	7.8 kb	*Spna2*	2	9
Σ2	α Spectrin					
Σ3	α Fodrin					
Σ4						
βSpIΣ1	β Erythrocyte	246 kDa	6/8 kb	*Spnb1*	12	14
βSpIΣ2	β Erythrocyte spectrin	268 kDa	11 kb	*Spnb1*	12	14
βSpIIΣ1	Nonerythroid	275 kDa	9 kb	*Spnb2*	11	2
Σ2	β Spectrin					
	β fodrin					
βSpIIIΣ1	–	271 kDa	8–9 kb	*Spnb3*	19	11
βSpIVΣ1	–	288 kDa	9 kb	*Spnb4*	7	19
Σ2						
Σ3						
Σ4						
βSpVΣ1	–	417 kDa	11–12 kb	*Spnb5*	–	15

It has been demonstrated that the connection between spectrin and the small spherical synaptic vesicles was end-on via synapsin.[74,82,83] The Goodman lab also demonstrated that the brain spectrin–synaptic vesicle interaction is not regulated by phosphorylation of synapsin by Cam Kinase II or A-Kinase,[84] but is directly regulated by free Ca^{2+}.[66] Ma et al.[85] cloned and sequenced βSpIIΣ1 spectrin from mouse, which demonstrated that βSpIIΣ1 had 2363 residues that had 59% identity with βSpIΣ1. This sequence identity rose to 89% in the actin-binding domain, and to 87% in residues 207–445, which was predicted to be the synapsin-binding domain. The suggestion by Ma et al.[85] proved to be correct based on microinjection of peptide-specific antibodies against this region of βSpIIΣ1 into paired hippocampal neurons.[86] Importantly, Sikorski et al.[86] demonstrated that when these antibodies were injected into the presynaptic neuron, this inhibited excitatory postsynaptic currents (EPSCs) in the postsynaptic neuron. Peptide-specific antibodies against flanking sequences had no effect upon EPSCs. The attachment site for synapsin on βSpII was mapped to L211–Q235 by Zimmer et al.[87] Based on these, and other studies, we concluded that αSpII/βSpII serves an essential role as a docking protein for Ca2+-regulated exocytosis of neurotransmitter at the active zone of the nerve terminal.[66]

Potential regulatory role for spectrin in nonerythroid cells

While αSpIIΣI/βSpIIΣ1 spectrin is found in all cell types except RBCs, αSpI/βSpIΣ1 or 2 are found only in RBCs, neurons, skeletal muscle, and cardiac muscle (Table 2).[54,66,68,70,88–91] In human, there are two α spectrin genes: SPTA1, SPTAN1-encoding αSpI and αSpIIΣI, respectively, and five genes encoding β spectrins: SPTB, SPTBN1, SPTBN2, SPTBN4, and SPTBN5. We have been discussing the gene products of SPTB, SPTBN1 (βSpIΣ2 and βSpIIΣ1, respectively), which are the major isoforms found in brain. Clark et al.[70] demonstrated that hybrid tetramers of spectrin subunits also exist in the brain. These hybrid species are less abundant than tetramers consisting solely of erythroid subunits $(\alpha SpI/\beta SpI\Sigma 2)_2$ or nonerythroid subunits $(\alpha SpII\Sigma 1/\beta SpII\Sigma 1)_2$. The hybrid tetramers provide an explanation of how two α spectrin isoforms (αSpI and αSpII) can couple with five different β spectrin isoforms (βSpI-V).

We anticipate that the αSpI subunit found in neurons, skeletal, and cardiac muscle[54,66,68,70,88–91] would have the same chimeric E2/E3 activity as this subunit found in RBCs, but with a broader range of targets. While logical this has not yet been tested. In RBCs, spectrin's targets include ankyrin, protein 4.1, protein 4.2, adducin, the anion transport channel, and protein gi13278939. Therefore, it would be pertinent to examine whether spectrin possesses ubiquitination activity with these proteins or their analogs in cardiac and skeletal muscle cells as well. αSpI and αSpII expressed in cardiac muscle cells are found on the plasma membrane and within contractile fibers near the Z-disc and intercalated disc.[89,92] In mice, it has also been shown that protein 4.1 products (4.1R,G,N,B) occur with spectrins at many subcellular locations in the heart, along with ankyrins (AnkB, AnkR, AnkG) and actins.[93] The significance of the spectrin membrane cytoskeleton and the ubiquitin-proteasome system in maintenance of myocyte integrity is illustrated by linkage of cardiac pathologies with mutations in these cellular components.[94,95] Thus, examining spectrin chimeric ubiquitin ligase activity in heart cells will promote a better understanding of its contribution to cardiac protein turnover, protein–protein interactions, and cellular signaling in cardiac pathophysiology.

In the case of αSpIIΣI, we know that it shares sequence homology with αSpI in the region surrounding a cysteine

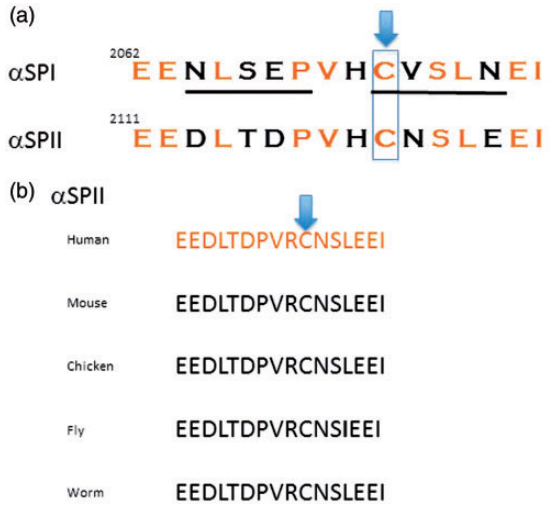

Figure 5 E2/E3 chimeric active sites. Conservation of sequence around the active site 2071Cys of human αSPI. In (a), the sequence surrounding 2071Cys is shown in the upper line with the amino acids conforming to E2 ubiquitin-conjugating enzyme active sites are underlined. The region of the nonerythroid αSpII that demonstrates a ~63% identity to αSpI is denoted in the lower line with the sequence identical to αSpI shown in red. Cys2071 is shown by the arrow. In (b), the region of human aSpII containing the putative E2 site was used to search databases using tBlastn and Tfasa programs. This analysis demonstrated a very high identity of the region conserved in sequence and location within the various spectrin molecules. Illustrated here are the sequences for mouse (P16546), chicken (X02593.1), Drosophila (XM_002026155.1), and *Caenorhabditis elegans* (LK928133.1). All are identical to the human aSpII except for Drosophila with an isoleucine substituted for a leucine. The arrow denotes the potential active site Cys for these proteins

equivalent to Cys 2071 (Figure 5). We also know that both αSpI and αSpIIΣI are ubiquitinated in the hippocampal neurons.[96–98] αSpIIΣI shares sequence homology with αSpI in the region surrounding a cysteine equivalent to Cys 2071 (Figure 5). Moreover, there is an absolute conservation of the Cys 2071 sequence domain in eukaryotes throughout evolution, highly suggestive of conservation of a functional domain within the αSpII molecule. There have been no reported studies on whether αSpIIΣI can serve as an E2/E3 chimeric enzyme in nonerythroid cell types. This is an extremely important question as spectrin makes up ~2–3% of the total protein in nonerythroid cells. We would predict that it will have such chimeric E2/E3 enzymatic activity, with far greater number of target proteins, for several reasons: (1) It is found on the cytoplasmic surface of both organelle and plasma membranes,[76,99] and within the nucleus.[77–81] (2) αSpII is directly associated with many cytoskeletal and membrane skeletal components,[2,5,6,63–67,100–102] and cell adhesion proteins (e.g. NCAM180).[2,5,6,63–67,103] (3) It has indirect interaction, via protein 4.1 and ankyrin, to a myriad of ion channels and transporters.[2,5,6,44,63–67,104,105] (4) αSpII is expressed throughout all mammalian developmental stages.[106–108] (5) The RBC proteomic and interactomic analyses to date have indicated 2289 unique proteins, with αSpI/βSpI having moderate connectivity.[109–112] The RBC has no internal organelles except 20S proteasomes.[113] The proteome of nucleated nonerythroid cells, with a complete complement of organelles, will have at least 10 times the number of unique proteins, as compared with RBCs, with far greater connectivity of αSpII/βSpII spectrin. Further study of the E2/E3 activity of α spectrin isoforms in nonerythroid cells is of great importance to our understanding the ubiquitination of many proteins and the cellular functional impact of this posttranslational modification.

AUTHORS' CONTRIBUTIONS

All authors participated in the writing and editing of the manuscript. SRG and WEZ serve as co-corresponding authors on the final manuscript.

CONFLICT OF INTEREST

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REFERENCES

1. Goodman SR, Shiffer K. The spectrin membrane skeleton of normal and abnormal human erythrocytes: a review. *Am J Physiol* 1983;**244**:C121–41
2. Goodman SR, Krebs KE, Whitfield CF, Riederer BM, Zagon IS. Spectrin and related molecules. *CRC Crit Rev Biochem* 1988;**23**:171–234
3. Goodman SR, Kurdia A, Ammann L, Kakhniashvili D, Daescu O. The human red blood cell proteome and interactome. *Exp Biol Med* 2007;**232**:1391–408
4. Goodman SR, Daescu O, Kakhniashvili DG, Zivanic M. The proteomics and interactomics of human erythrocytes. *Exp Biol Med* 2013;**238**:509–18
5. Baines AJ. The spectrin-ankyrin-4.1-adducin membrane skeleton: adapting eukaryotic cells to the demands of animal life. *Protoplasma* 2010;**244**:99–131
6. Machnicka B, Czogalla A, Hryniewicz-Jankowska A, Boguslawska DM, Grochowalska R, Heger E, Sikorski AF. Spectrins: a structural platform for stabilization and activation of membrane channels, receptors and transporters. *Biochimica et Biophysica Acta* 2014;**1838**:620–34
7. Liu SC, Derick LH, Palek J. Visualization of the hexagonal lattice in the erythrocyte membrane skeleton. *J Cell Biol* 1987;**104**:527–36
8. Shotton DM, Burke BE, Branton D. The molecular structure of human erythrocyte spectrin. Biophysical and electron microscopic studies. *J Mol Biol* 1979;**131**:303–29
9. Goodman SR, Weidner SA. Binding of spectrin alpha 2-beta 2 tetramers to human erythrocyte membranes. *J Biol Chem* 1980;**255**:8082–6
10. Cioe L, Laurila P, Meo P, Krebs K, Goodman S, Curtis PJ. Cloning and nucleotide sequence of a mouse erythrocyte beta-spectrin cDNA. *Blood* 1987;**70**:915–20
11. Sahr KE, Laurila P, Kotula L, Scarpa AL, Coupal E, Leto TL, Linnenbach AJ, Winkelman JC, Speicher DW, Marchesi VT, Curtis PJ, Forget BG. The complete cDNA and polypeptide sequences of human erythroid alpha-spectrin. *J Biol Chem* 1990;**265**:4434–43
12. Winkelmann JC, Chang JG, Tse WT, Scarpa AL, Marchesi VT, Forget BG. Full-length sequence of the cDNA for human erythroid beta-spectrin. *J Biol Chem* 1990;**265**:11827–32
13. Karinch AM, Zimmer WE, Goodman SR. The identification and sequence of the actin-binding domain of human red blood cell beta-spectrin. *J Biol Chem* 1990;**265**:11833–40
14. Fowler V, Taylor DL. Spectrin plus band 4.1 cross-link actin. Regulation by micromolar calcium. *J Cell Biol* 1980;**85**:361–76
15. Tyler JM, Hargreaves WR, Branton D. Purification of two spectrin-binding proteins: biochemical and electron microscopic evidence for

site-specific reassociation between spectrin and bands 2.1 and 4.1. *Proc Natl Acad Sci USA* 1979;**76**:5192–6
16. Ungewickell E, Bennett PM, Calvert R, Ohanian V, Gratzer WB. In vitro formation of a complex between cytoskeletal proteins of the human erythrocyte. *Nature* 1979;**280**:811–4
17. Goodman SR, Yu J, Whitfield CF, Culp EN, Posnak EJ. Erythrocyte membrane skeletal protein bands 4.1 a and b are sequence-related phosphoproteins. *J Biol Chem* 1982;**257**:4564–9
18. Gardner K, Bennett V. Modulation of spectrin-actin assembly by erythrocyte adducin. *Nature* 1987;**328**:359–62
19. Li X, Bennett V. Identification of the spectrin subunit and domains required for formation of spectrin/adducin/actin complexes. *J Biol Chem* 1996;**271**:15695–702
20. Mische SM, Mooseker MS, Morrow JS. Erythrocyte adducin: a calmodulin-regulated actin-bundling protein that stimulates spectrin-actin binding. *J Cell Biol* 1987;**105**:2837–45
21. Shiffer KA, Goodman SR. Protein 4.1: its association with the human erythrocyte membrane. *Proc Natl Acad Sci USA* 1984;**81**:4404–8
22. Mueller TJ, Morrison M. Glycoconnectin (PAS 2), a membrane attachment site for the human erythrocyte cytoskeleton. *Prog Clin Biol Res* 1981;**56**:95–9116
23. Yu J, Goodman SR. Syndeins: the spectrin-binding protein(s) of the human erythrocyte membrane. *Proc Natl Acad Sci USA* 1979;**76**:2340–4
24. Wallin R, Culp EN, Coleman DB, Goodman SR. A structural model of human erythrocyte band 2.1: alignment of chemical and functional domains. *Proc Natl Acad Sci USA* 1984;**81**:4095–9
25. Bennett V, Stenbuck PJ. Association between ankyrin and the cytoplasmic domain of band 3 isolated from the human erythrocyte membrane. *J Biol Chem* 1980;**255**:6424–32
26. Marchesi VT, Steers E. Selective solubilization of a protein component of the red cell membrane. *Science* 1968;**159**:203–4
27. Kakhniashvili DG, Chaudhary T, Zimmer WE, Bencsath FA, Jardine I, Goodman SR. Erythrocyte spectrin is an E2 ubiquitin conjugating enzyme. *Biochemistry* 2001;**40**:11630–42
28. Monteiro CA, Gibson X, Shartava A, Goodman SR. Preliminary characterization of a structural defect in homozygous sickled cell alpha spectrin demonstrated by a rabbit autoantibody. *Am J Hematol* 1998;**58**:200–5
29. Hsu YJ, Zimmer WE, Goodman SR. Erythrocyte spectrin's chimeric E2/E3 ubiquitin conjugating/ligating activity. *Cell Mol Biol* 2005;**51**:187–93
30. Hsu YJ, Goodman SR. Spectrin and ubiquitination: a review. *Cell Mol Biol* 2005;**51**(Suppl 51):OL801–7
31. Chang TL, Cubillos FF, Kakhniashvili DG, Goodman SR. Ankyrin is a target of spectrin's E2/E3 ubiquitin-conjugating/ligating activity. *Cell Mol Biol* 2004;**50**:59–66
32. Chang TL, Cubillos FF, Kakhniashvili DG, Goodman SR. Band 3 is a target protein of spectrin's E2/E3 activity: implication for sickle cell disease and normal red blood cell aging. *Cell Mol Biol* 2004;**50**:171–7
33. Berleth ES, Pickart CM. Mechanism of ubiquitin conjugating enzyme E2-230K: catalysis involving a thiol relay? *Biochemistry* 1996;**35**:1664–71
34. Bartke T, Pohl C, Pyrowolakis G, Jentsch S. Dual role of BRUCE as an antiapoptotic IAP and a chimeric E2/E3 ubiquitin ligase. *Mol Cell* 2004;**14**:801–11
35. Corsi D, Galluzzi L, Crinelli R, Magnani M. Ubiquitin is conjugated to the cytoskeletal protein alpha-spectrin in mature erythrocytes. *J Biol Chem* 1995;**270**:8928–35
36. Galluzzi L, Paiardini M, Lecomte MC, Magnani M. Identification of the main ubiquitination site in human erythroid alpha-spectrin. *FEBS Lett* 2001;**489**:254–8
37. Goodman SR. The role of the membrane skeleton in formation of the irreversibly sickled cell: a review. *Cell Mol Biol Lett* 1996;**01**(1):3–14
38. Goodman SR. The irreversibly sickled cell: a perspective. *Cell Mol Biol* 2004;**50**:53–8
39. Lux SE, John KM, Karnovsky MJ. Irreversible deformation of the spectrin-actin lattice in irreversibly sickled cells. *J Clin Invest* 1976;**58**:955–63
40. Shartava A, Monteiro CA, Bencsath FA, Schneider K, Chait BT, Gussio R, Casoria-Scott LA, Shah AK, Heurerman CA, Goodman SR. A post-translational modification of beta-actin contributes to the slow dissociation of the spectrin-protein 4.1-actin complex of irreversibly sickled cells. *J Cell Biol* 1995;**128**(5):805–18
41. Bencsath FA, Shartava A, Monteiro CA, Goodman SR. Identification of the disulfide-linked peptide in irreversibly sickled cell beta-actin. *Biochemistry* 1996;**35**:4403–8
42. Shartava A, Miranda P, Williams KN, Shah A, Monteiro CA, Goodman SR. High density sickle cell erythrocyte core membrane skeletons demonstrate slow temperature dependent dissociation. *Am J Hemat* 1996;**51**:214–9
43. Shartava A, Korn W, Shah AK, Goodman SR. Irreversibly sickled cell beta-actin: defective filament formation. *Am J Hematol* 1997;**55**:97–103
44. Abraham A, Bencsath FA, Shartava A, Kakhniashvili DG, Goodman SR. Preparation of irreversibly sickled cell beta-actin from normal red blood cell beta-actin. *Biochemistry* 2002;**41**:292–6
45. Ghatpande SS. *Effect of spectrin ubiquitination on the dissociation of the spectrin-protein 4.1-actin ternary complex in erythrocytes*. Dallas: University of Texas, 2003
46. Mishra R, Goodman SR. Ubiquitination of erythrocyte spectrin regulates the dissociation of the spectrin-adducin-f-actin ternary complex in vitro. *Cell Mol Biol* 2004;**50**:75–80
47. Chang TL, Kakhniashvili DG, Goodman SR. Spectrin's E2/E3 ubiquitin conjugating/ligating activity is diminished in sickle cells. *Am J Hematol* 2005;**79**:89–96
48. Gibson XA, Shartava A, McIntyre J, Monteiro CA, Zhang Y, Shah A, Campbell NF, Goodman SR. The efficacy of reducing agents or anti-oxidants in blocking the formation of dense cells and irreversibly sickled cells in vitro. *Blood* 1998;**91**:4373–8
49. Shartava A, McIntyre J, Shah AK, Goodman SR. The Gardos channel is responsible for CDNB-induced dense sickle cell formation. *Am J Hematol* 2000;**64**:184–9
50. Shartava A, Shah AK, Goodman SR. N-acetylcysteine and clotrimazole inhibit sickle erythrocyte dehydration induced by 1-chloro-2,4-dinitrobenzene. *Am J Hematol* 1999;**62**:19–24
51. Pace BS, Shartava A, Pack-Mabien A, Mulekar M, Ardia A, Goodman SR. Effects of N-acetylcysteine on dense cell formation in sickle cell disease. *Am J Hematol* 2003;**73**:26–32
52. Nur E, Biemond BJ, Otten H-M, Brandjes DP, Schnog J-JB. Oxidative stress in sickle cell disease; pathophysiology and potential implications for disease management. *Am J Hematol* 2011;**86**:484–9
53. Caprio K, Condon MR, Deitch EA, Xu DZ, Feketova E, Machiedo GW. Alteration of alpha-spectrin ubiquitination after hemorrhagic shock. *Am J Surg* 2008;**196**:663–9
54. Goodman SR, Zagon IS, Kulikowski RR. Identification of a spectrin-like protein in nonerythroid cells. *Proc Natl Acad Sci USA* 1981;**78**:7570–4
55. Glenney JR, Glenney P, Weber K. F-actin-binding and cross-linking properties of porcine brain fodrin, a spectrin-related molecule. *J Biol Chem* 1982;**257**:9781–7
56. Repasky EA, Granger BL, Lazarides E. Widespread occurrence of avian spectrin in nonerythroid cells. *Cell* 1982;**29**:821–33
57. Bennett V, Davis J, Fowler WE. Brain spectrin, a membrane-associated protein related in structure and function to erythrocyte spectrin. *Nature* 1982;**299**:126–31
58. Goodman SR, Zagon IS, Whitfield CF, Casoria LA, McLaughlin PJ, Laskiewicz TL. A spectrin-like protein from mouse brain membranes: immunological and structural correlations with erythrocyte spectrin. *Cell Motil* 1983;**3**:635–47
59. Borland K, Osawa S, Kew D, Coleman DB, Goodman SR, Hall PF. Identification of a spectrin-like protein in Sertoli cells. *Biol Reprod* 1985;**32**:1143–56
60. Osawa S, Kew D, Borland K, Krebs KE, Coleman DB, Goodman SR, Hall PF. Occurrence of spectrin-like protein in Y-1 adrenal tumor cells. *Endocrinology* 1986;**118**:2458–63
61. Isayama T, Goodman SR, Zagon IS. Spectrin isoforms in the mammalian retina. *J Neurosci: Off J Soc Neurosci* 1991;**11**:3531–8
62. Goodman SR, Zagon IS, Coleman DB, McLaughlin PJ. Spectrin expression in neuroblastoma cells. *Brain Res Bull* 1986;**16**:597–602
63. Goodman SR, Zagon IS. Brain spectrin: a review. *Brain Res Bull* 1984;**13**:813–32

64. Goodman SR, Zagon IS. The neural cell spectrin skeleton: a review. *Am J Physiol* 1986;**250**:C347–60
65. Goodman SR, Riederer BM, Zagon IS. Spectrin subtypes in mammalian brain. *BioEssays: News Rev Mol Cell Develop Biol* 1986;**5**:25–9
66. Goodman SR, Zimmer WE, Clark MB, Zagon IS, Barker JE, Bloom ML. Brain spectrin: of mice and men. *Brain Res Bull* 1995;**36**:593–606
67. Goodman SR. Discovery of nonerythroid spectrin to the demonstration of its key role in synaptic transmission. *Brain Res Bull* 1999;**50**:345–6
68. Riederer BM, Zagon IS, Goodman SR. Brain spectrin(240/235) and brain spectrin(240/235E): two distinct spectrin subtypes with different locations within mammalian neural cells. *J Cell Biol* 1986;**102**:2088–97
69. Goodman SR, Zagon IS, Riederer BM. Spectrin isoforms in mammalian brain. *Brain Res Bull* 1987;**18**:787–92
70. Clark MB, Ma Y, Bloom ML, Barker JE, Zagon IS, Zimmer WE, Goodman SR. Brain alpha erythroid spectrin: identification, compartmentalization, and beta spectrin associations. *Brain Res* 1994;**663**:223–36
71. Goodman SR, Zagon IS, Whitfield CF, Casoria LA, Shohet SB, Bernstein SE, McLaughlin PJ, Laskiewicz TL. A spectrin-like protein from mouse brain membranes: phosphorylation of the 235,000-dalton subunit. *Am J Physiol* 1984;**247**:C61–73
72. Riederer BM, Lopresti LL, Krebs KE, Zagon IS, Goodman SR. Brain spectrin(240/235) and brain spectrin(240/235E): conservation of structure and location within mammalian neural tissue. *Brain Res Bull* 1988;**21**:607–16
73. Goodman SR, Casoria LA, Coleman DB, Zagon IS. Identification and location of brain protein 4.1. *Science* 1984;**224**:1433–6
74. Krebs KE, Prouty SM, Zagon IS, Goodman SR. Structural and functional relationship of red blood cell protein 4.1 to synapsin I. *Am J Physiol* 1987;**253**:C500–5
75. Zimmer WE, Zagon IS, Casoria LA, Goodman SR. Identification of an amelin isoform located in axons. *Brain Res* 1992;**582**:94–9100.
76. Zagon IS, Higbee R, Riederer BM, Goodman SR. Spectrin subtypes in mammalian brain: an immunoelectron microscopic study. *J Neurosci: Off J Soc Neurosci* 1986;**6**:2977–86
77. McMahon LW, Sangerman J, Goodman SR, Kumaresan K, Lambert MW. Human alpha spectrin II and the FANCA, FANCC, and FANCG proteins bind to DNA containing psoralen interstrand cross-links. *Biochemistry* 2001;**40**:7025–34
78. Sridharan D, Brown M, Lambert WC, McMahon LW, Lambert MW. Nonerythroid alphaII spectrin is required for recruitment of FANCA and XPF to nuclear foci induced by DNA interstrand cross-links. *J Cell Sci* 2003;**116**:823–35
79. McMahon LW, Zhang P, Sridharan DM, Lefferts JA, Lambert MW. Knockdown of alphaII spectrin in normal human cells by siRNA leads to chromosomal instability and decreased DNA interstrand cross-link repair. *Biochem Biophys Res Commun* 2009;**381**:288–93
80. Lefferts JA, Wang C, Sridharan D, Baralt M, Lambert MW. The SH3 domain of alphaII spectrin is a target for the Fanconi anemia protein, FANCG. *Biochemistry* 2009;**48**:254–63
81. Sridharan DM, McMahon LW, Lambert MW. alphaII-Spectrin interacts with five groups of functionally important proteins in the nucleus. *Cell Biol Int* 2006;**30**:866–78
82. Sikorski AF, Terlecki G, Zagon IS, Goodman SR. Synapsin I-mediated interaction of brain spectrin with synaptic vesicles. *J Cell Biol* 1991;**114**:313–8
83. Sikorski A. The role of spectrin and synapsin I in synaptic transmission. *Biophys Memb Trans* 1990;**27**(2):129–37
84. Sikorski AF, Goodman SR. The effect of synapsin I phosphorylation upon binding of synaptic vesicles to spectrin. *Brain Res Bull* 1991;**27**:195–8
85. Ma Y, Zimmer WE, Riederer BM, Bloom ML, Barker JE, Goodman SM, Goodman SR. The complete amino acid sequence for brain beta spectrin (beta fodrin): relationship to globin sequences. *Brain Res Mol Brain Res* 1993;**18**:87–99
86. Sikorski AF, Sangerman J, Goodman SR, Critz SD. Spectrin (betaSpIIsigma1) is an essential component of synaptic transmission. *Brain Res* 2000;**852**:161–6
87. Zimmer WE, Zhao Y, Sikorski AF, Critz SD, Sangerman J, Elferink LA, Xu XS, Goodman SR. The domain of brain beta-spectrin responsible for synaptic vesicle association is essential for synaptic transmission. *Brain Res* 2000;**881**:18–27
88. Zimmer WE. Tissue distribution of brain β-spectrin mRNAs. *Br Res Bul* 1991;**27**:187–93
89. Isayama T, Goodman SR, Zagon IS. Localization of spectrin isoforms in the adult mouse heart. *Cell Tiss Res* 1993;**274**:127–33
90. Zhou D, Ursitti JA, Bloch RJ. Developmental expression of spectrins in rat skeletal muscle. *Mol Biol Cell* 1998;**9**:47–61
91. Bennett PM, Baines AJ, Lecomte MC, Maggs AM, Pinder JC. Not just a plasma membrane protein: in cardiac muscle cells alpha-II spectrin also shows a close association with myofibrils. *J Muscle Res Cell Motil* 2004;**25**:119–26
92. Hund TJ, Mohler PJ. Cardiac spectrins: alternative splicing encodes functional diversity. *J Mol Cell Cardiol* 2010;**48**:1031–2
93. Baines AJ, Pinder JC. The spectrin-associated cytoskeleton in mammalian heart. *Front Biosci: J Virt Libr* 2005;**10**:3020–33
94. Smith SA, Sturm AC, Curran J, Kline CF, Little SC, Bonilla IM, Long VP, Makara M, Polina I, Hughes LD, Webb TR, Wei Z, Wright P, Voigt N, Bhakta D, Spoonamore KG, Zhang C, Weiss R, Brinkley PF, Janssen PM, Kilic A, Higgins RS, Sun M, Ma J, Dobrev D, Zhang M, Carnes CA, Vatta M, Rasband MN, Hund TJ, Mohler PJ. Dysfunction in the βII spectrin-dependent cytoskeleton underlies human arrhythmia. *Circulation* 2015;**131**(8):695–708
95. Willis MS, Bevilacqua A, Pulinilkunnil T, Kienesberger P, Tannu M, Patterson C. The role of ubiquitin ligases in cardiac disease. *J Mol Cell Cardiol* 2014;**71**:43–53
96. Sangerman J, Gard AL, Shah A, Goodman SR. Synthesis, assembly, and turnover of alpha and beta-erythroid and nonerythroid spectrins in rat hippocampal neurons. *Brain Res* 1999;**849**:128–38
97. Sangerman J, Kakhniashvili D, Brown A, Shartava A, Goodman SR. Spectrin ubiquitination and oxidative stress: potential roles in blood and neurological disorders. *Cell Mol Biol Lett* 2001;**6**:607–36
98. Sangerman J, Killilea A, Chronister R, Pappolla M, Goodman SR. Alpha-spectrins are major ubiquitinated proteins in rat hippocampal neurons and components of ubiquitinated inclusions in neurodegenerative disorders. *Brain Res Bull* 2001;**54**:405–11
99. Zagon IS, McLaughlin PJ, Goodman SR. Localization of spectrin in mammalian brain. *J Neurosci: Off J Soc Neurosci* 1984;**4**: 3089–100
100. Koteliansky VE, Gneushev GN, Shartava AS, Shirinsky VP, Glukhova MA, Goodman SR. The regulation by vinculin of filamin, alpha-actinin, and spectrin tetramer-induced actin sol-gel transformation. *FEBS Lett* 1983;**151**:206–10
101. Weisenberg RC, Flynn J, Gao BC, Awodi S, Skee F, Goodman SR, Riederer BM. Microtubule gelation-contraction: essential components and relation to slow axonal transport. *Science* 1987;**238**:1119–22
102. Riederer BM, Goodman SR. Association of brain spectrin isoforms with microtubules. *FEBS Lett* 1990;**277**:49–52
103. Pollerberg GE, Burridge K, Krebs KE, Goodman SR, Schachner M. The 180-kD component of the neural cell adhesion molecule N-CAM is involved in cell-cell contacts and cytoskeleton-membrane interactions. *Cell Tiss Res* 1987;**250**:227–36
104. Cioffi DL, Wu S, Alexeyev M, Goodman SR, Zhu MX, Stevens T. Activation of the endothelial store-operated ISOC Ca2+ channel requires interaction of protein 4.1 with TRPC4. *Circ Res* 2005;**97**:1164–72
105. Wu S, Sangerman J, Li M, Brough GH, Goodman SR, Stevens T. Essential control of an endothelial cell ISOC by the spectrin membrane skeleton. *J Cell Biol* 2001;**154**:1225–33
106. Riederer BM, Zagon IS, Goodman SR. Brain spectrin(240/235) and brain spectrin(240/235E): differential expression during mouse brain development. *J Neurosci: Off J Soc Neurosci* 1987;**7**:864–74
107. Zagon IS, Riederer BM, Goodman SR. Spectrin expression during mammalian brain ontogeny. *Brain Res Bull* 1987;**18**:799–807
108. Zimmer WE, Ma Y, Zagon IS, Goodman SR. Developmental expression of brain beta-spectrin isoform messenger RNAs. *Brain Res* 1992;**594**:75–83

109. Kakhniashvili DG, Bulla LA Jr, Goodman SR. The human erythrocyte proteome: analysis by ion trap mass spectrometry. *Mol Cell Proteom: MCP* 2004;**3**:501–9
110. Kurdia A, Daescu O, Ammann L, Kakhniashvili D, Goodman SR, editors. Centrality measures for the human red blood cell interactome. In: Engineering in Medicine and Biology Workshop, 2007 IEEE Dallas, 2007, 11–12 November 2007, pp.98–101
111. Ammann LP, Goodman SR. Cluster analysis for the impact of sickle cell disease on the human erythrocyte protein interactome. *Exp Biol Med* 2009;**234**:703–11
112. Zivanic M, Daescu O, Kurdia A, Goodman SR. The Voronoi diagram for graphs and its application in the Sickle Cell Disease research. *J Comput Sci* 2012;**3**:335–43
113. Neelam S, Kakhniashvili DG, Wilkens S, Levene SD, Goodman SR. Functional 20S proteasomes in mature human red blood cells. *Exp Biol Med* 2011;**236**:580–91
114. Hsu YJ. Identification of erythrocyte spectrin's E2/E3 ubiquitin conjugating/ligating sites. PhD [dissertation]. Dallas, Texas: University of Texas at Dallas, 2004