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Review

Spectrins: A structural platform for stabilization and activation of membrane channels, receptors and transporters ☆

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ABSTRACT

This review focuses on structure and functions of spectrin as a major component of the membrane skeleton. Recent advances on spectrin function as an interface for signal transduction mediation and a number of data concerning interaction of spectrin with membrane channels, adhesion molecules, receptors and transporters draw a picture of multifaceted protein. Here, we attempted to show the current depiction of multitask role of spectrin in cell physiology. This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters. Guest Editor: Jean Claude Hervé.

Contents

Abbreviations: PH, pleckstrin homology domain; ABD, actin binding domain; SH3, SRC homology 3 domain; EF-hand domain, calcium-binding domain; PIP2, Phosphatidylinositol 4,5-bisphosphate; CH1, calponin homology domain; PKA, Protein Kinase-A; cAMP, 3′-5′-cyclic adenosine monophosphate; GPC, glycophorin C; Rh, Rhesus protein; RhAG, Rh-associated glycoprotein; CD47, integrin-associated protein; LW, (after Landsteiner and Wiener) blood group antigen glycoprotein; GPB, glycophorin B; AE1, Anion exchange protein 1 and Band 3 anion transport protein; MPP1, membrane protein, palmitoylated 1; GLUT1, glucose transporter protein 1; Lu/B-CAM, Lutheran blood group and basal cell adhesion molecule; DRM, detergent-resistant membrane; NgCAM, Neuron-Glia Cell Adhesion Molecule)-Related Cell Adhesion Molecule; NrCAM, Neuronal cell adhesion molecule; ERM, ezrin–radixin–moesin family; PKCβ$_2$, protein kinase C β2; PSDs, perforated postsynaptic densities; CHL1, Neural cell adhesion molecule L1-like protein; NMDA, (N-methyl-D-aspartate) receptor; EAAT4, excitatory amino acid transporters 4; SCA5, Spinocerebellar Ataxia Type 5; Arp1, actin-related protein 1; GABA, γ-Aminobutyric acid; VGSC, voltage-gated sodium channels; VASP, Vasodilator-stimulated phosphoprotein; EVL, Enabled/VASP (vasodilator-stimulated phosphoprotein)-like protein; FA, Fanconi anemia; TGFβ, Transforming growth factor beta; TCR, T cell receptor

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1. Introduction

Plasma membranes of multicellular animals are organized into highly specialized domains which confer to the cell the capacity to participate in diverse and appropriate physiological functions. Cells have to segregate functionally related membrane proteins within the same domains, but also maintain the topographical organization of such domains. During evolution, a set of proteins emerged to address these functions: they are organized in a scaffold known as the spectrin-based membrane skeleton, which is located at the inner surface of the plasma membranes, linked to a number of integral membrane proteins (for review see [1]). Such a structure was first identified in mammalian red blood cells [2] (Fig. 1). Components of this membrane skeleton have been further characterized in all animal tissues that have been examined so far, indicating their likely presence in all metazoan cells. Existence of the membrane skeleton is thought also to be responsible for the membrane integrity and its mechanical properties, i.e. very high linear elasticity but negligible extensibility. Recent progress demonstrates that spectrins cooperate in both the establishment and the maintenance of a diverse specialized plasma membrane domain.

2. Spectrin, a major component of the membrane skeleton

Membrane skeleton, a dense proteinaceous network, is thought to be responsible for the remarkable mechanical properties of the erythrocyte membrane, which permit it to withstand and respond to very strong mechanical stresses the red cell experiences during its 120-day life-span within the circulation. These include high shear stress during the high speed flow in the circulation and multiple cycles of shape changes due to the passage of the 8-μm-diameter biconcave disk through 2-μm capillaries. It is very well known that an erythrocyte membrane even partially devoid of a spectrin-actin network no longer exists as a ghost, but starts to fragment and form a small, approx. 50 nm inverted vesicle. The importance of this structure is highlighted by the fact that mutations in genes encoding or regulating the expression of particular skeletal components underlie the molecular mechanism of hereditary disorders of which the best known examples are hemolytic anemias [3]. The unique arrangement of spectrin, F-actin, protein 4.1 and ankyrin, with direct and indirect connections to the membrane bilayer with integral proteins immersed in it, creates a filamentous network crucial for maintaining erythrocyte shape and elasticity. A major component of this network is spectrin, which in erythrocytes is a tetrameric or higher oligomeric protein composed of antiparallel filamentous heterodimers, of α (280 kDa) and β (240 kDa) subunits, each with high α-helical content.

2.1. Spectrin molecule

Spectrin was first described in erythrocytes in 1968 by Marchesi and Steers [4]. In mammals, two major spectrin isoforms of alpha subunits are encoded by separate genes, *SPTA1* and *SPTAN1* [5,6]. Alternative processing of the primary transcript of the *SPTAN1* gene results in at least 4 (possibly up to 8) αII spectrin isoforms [7]. Four "conventional" β genes, *SPTB*, *SPTBN1*, *SPTBN2*, *SPTBN4*, coding for the βI–βIV spectrins, respectively, and one gene, *SPTBN5*, coding for one large βV-spectrin

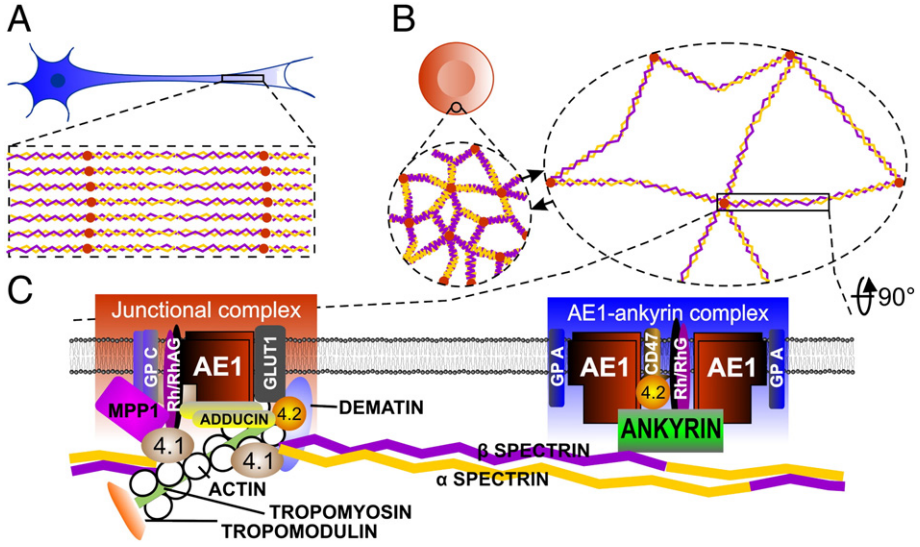

Fig. 1. The membrane skeleton. (A) Periodicity of membrane skeleton in neuronal axons, where spectrin heterotetramers (α-yellow strands and β-purple strands) are connected to junctional complexes (red dots) and the latter are spaced along the axon with periodicity of approximately 190 nm [139]. (B) In erythrocytes, spectrin dimers transiently self-associate halfway between the junctional complexes to form not only tetramers, but also hexamers and octamers; upon expansion of the skeleton (as seen on electron microscopy images of shear stressed membranes), lower surface density of spectrin dimers favors fully extended tetrameric state of the protein; the dynamics of the spectrin skeleton allow the cell to experience fully reversible deformations in response to shear force [36]. (C) The network of the spectrin skeleton is anchored to the plasma membrane of the erythrocyte via two major membrane skeleton macrocomplexes and through direct interactions with lipids (cross section through the membrane); AE1—anion exchanger 1, GLUT1—glucose transporter 1, GP A—glycophorin A, GP C—glycophorin C, Rh/Rh AG—rhesus factor/antigen, MPP1—membrane protein palmitoylated 1; see the main text for details.

(β-heavy) [8–11], are present in the mammalian genome. The expression of the diverse isoforms is regulated in a complex tissue- and time-specific manner. *SPTB*, *SPTBN1*, *SPTBN2*, *SPTBN4* genes are all differentially spliced. In particular, carboxyl-terminal regions of βI, βII, and βIV spectrins are subject to differential mRNA splicing to generate "short" or "long" carboxyl-terminal regions [8,12,13]. The long carboxyl-terminal region includes a pleckstrin homology (PH) domain. More details of the spectrin structure are given below in Sections 2.2 and 3.

The diversity of the gene products can be correlated to the structural complexity of the nucleated cell. These multiple protein isoforms are distributed to various intracellular locations besides the plasma membrane, such as the Golgi apparatus, vesicles, the endoplasmic reticulum and the nucleus. These diverse cell locations indicate that the spectrins fulfill a variety of functions at multiple sites in nucleated cells.

Invertebrates have a smaller repertoire of spectrin genes. The *Caenorhabditis elegans* and *Drosophila melanogaster* genomes include a single gene coding for an α-spectrin similar to the mammalian αII-spectrin [spc-1 and I(3)dre3, respectively] [14] and two genes coding for β-spectrin; one codes for a β_G protein resembling the mammalian βII-spectrin referred to as "conventional β-spectrin" (Unc-70/bgs-1 and b-Spc), and the other (sma 1 in *C. elegans* and karst in *D. melanogaster*) encodes βH-spectrin (β-heavy, similar to mammalian βV) [15].

Sequence analyses suggest that the erythroid spectrin genes arose during vertebrate evolution, and some of the sequence changes may correspond to neo-functionalization of the erythroid spectrin genes [16–18].

Moreover, annotation of the genome of the choanoflagellate *Monosiga brevicollis* [17] reveals spectrin genes. These appear to be very similar to human spectrins [17]. Spectrin evolved before ankyrin or protein 4.1 and must have functions independent of those proteins.

The spectrin–ankyrin–protein 4.1 complexes presumably arose after (or simultaneously with) the appearance of tissues [19]. Since such complexes are required for the life of *Eumetazoa*, it is tempting to speculate that the appearance of ankyrin and protein 4.1 was indispensable for the evolution of tissues [17,20].

2.2. Spectrin structure

2.2.1. Spectrin repeat

The core structural element of spectrins and a few other proteins, including spectrin family proteins (e.g. α-actinin, dystrophin), is a helical repeating unit referred to as a spectrin repeat (see Fig. 2). Typically, 20 complete repeats can be found in α-spectrin, while β-spectrins contain 16 (or 29 in the case of β-heavy isoforms). Full sequence analysis of both spectrin subunits confirmed that the size of such segments varies in the range between 99 and 114 amino acid residues and the sequence homology between them does not exceed 30% [21]. Nevertheless, there is a common structural fold shared by all the spectrin repeats consisting of three helices, of which A and C are parallel and B is antiparallel [22]. The building block is approximately 50 Å in length and 20 Å in diameter, as was first determined for the crystal structure of the 14th repeat of *D. melanogaster* α-spectrin [23], and further confirmed via NMR of the 16th segment of chicken αII spectrin [24]. Remarkably, helix B appeared to be longer than the others and the conserved proline residue in its middle makes the helix slightly kinked. The three helices are mildly curved and wrap around each other in a left-handed supercoil [25]. The length and exact position of loops connecting helices seem to be an individual feature of each of the spectrin repeats [26]. Consecutively arranged repeats are connected through the junction of helix C and helix A of the following repeat in an uninterrupted helical structure [26,27].

The stable triple-helical structure of a spectrin repeat is one of the consequences of a regular heptad (a–g) amino acid residue arrangement of helices within a bundle, where positions a and d are occupied by conservative hydrophobic residues forming a core of each repeat further stabilized by salt bridges between residues at positions e and g. This is true for all the helices except linkers between repeats, which leads to discontinuity between individual modules. Conserved tryptophan residues are of particular importance in those arrangements [28], although the core of some of the spectrin repeats could be stabilized differently, e.g. via water-mediated hydrogen bonds in the case of the 9th segment of erythroid β-spectrin [26]. The variances in number and type of interhelical interactions within a repeat are the reason for the diversity in the folding landscape of the individual domains [29]. Some of the repeats appeared to be largely unfolded even at physiological temperatures, but their stability may be modulated by energetic coupling with neighboring domains [30]. Strikingly, nonerythroid spectrin repeats appeared to be remarkably more stable than the erythroid equivalents, which may account for the higher rigidity of brain spectrin [31].

2.2.2. Elasticity

The above-mentioned data support the spectrin flexibility model, according to which spectrin repeats may respond to the shearing forces by at least partially unfolding and acting as a molecular spring. This is in accordance with the results from forced unfolding extension curves of atomic force microscopy, where spectrin repeats have been demonstrated to reversibly unfold and refold when subjected to forces up to 20 pN [32]. In the case of some triple helical repeats the loss of resistance to pulling at physiological temperatures could be observed [30]. It is also worth mentioning that the helical linkers interconnecting repeats cooperatively propagate the forced unfolding [33]. Various spectrin conformations may be achieved by changes in relative orientation of two repeats at the linker region with a variety of twist, tilt and roll angles without losing its helical structure [27], and the interaction between loops flanked by individual helices of the neighboring coiled coils and linker were also suggested to control the bending flexibility of consecutive spectrin repeats [34]. The array of flexibility is further broadened by potential conformational rearrangement of helices within a spectrin repeat, mostly associated with translocation of the B–C loop, without any net change in its secondary structure [25]. Most recently, the shear-induced unfolding of spectrin was examined within red blood cells [35]. It appeared that shielded cysteine residues at various positions within repeats of both α and β spectrin became increasingly accessible as a function of shear stress and time, which strongly suggests that forced unfolding of specific domains takes place. Taken together, the evidence suggests that at least three reversible stages of the spectrin network should be considered as the origin of reversible deformation of red cells during their passage though the thinnest capillaries. In the resting membrane skeleton the average contour length of spectrin tetramers is much smaller than that of fully extended ones, known from images of a shear-experienced erythrocyte membrane adsorbed to a support [36]. With increased shear force further straightening of spectrin occurs, which could be followed by partial unfolding of the coiled coils of some of the repeats and eventually reversible interruption of dimer–dimer contacts. It should be stressed that the observed broad range of oligomeric states of spectrin in native erythrocytes is a dynamic determinant of the global flexibility of the erythrocyte membrane.

2.2.3. Domains in spectrin

Apart from the actin-binding domain (ABD) described below (Section 3.2), other protein modules play an important role in formation of membrane skeleton and stabilization of membrane transporters, channels and receptors. Ankyrin-binding and oligomerization domains belong to spectrin-repeat based motifs, while others, similarly as ABD, are non-spectrin-repeat structural motifs (see Fig. 2).

2.2.3.1. SH3. Within repeat 9 of the α-subunit between helix B and C, an SH3 domain is inserted [37]. It is present both in erythroid and nonerythroid spectrins of vertebrates. The SH3 domain is a common structural motif often found in proteins involved in signal

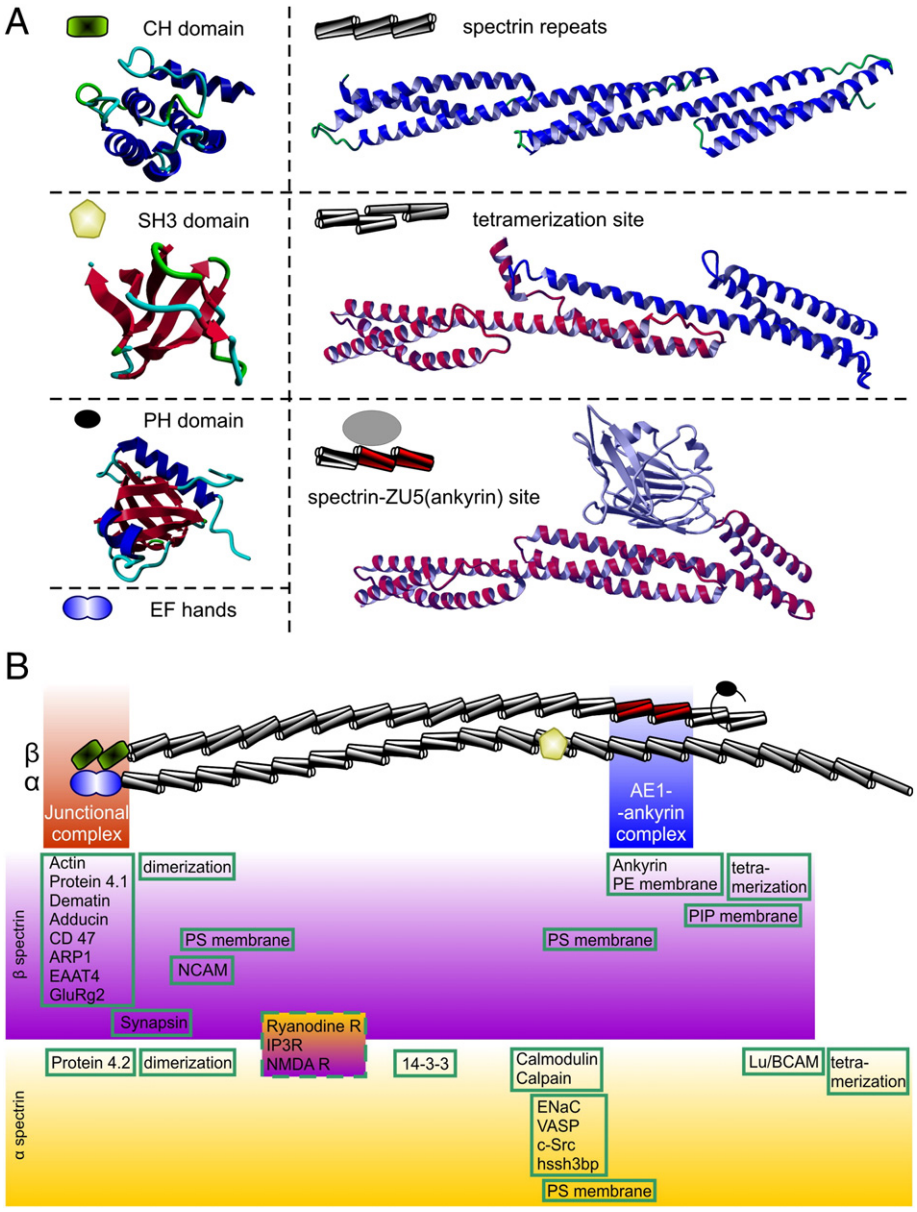

Fig. 2. Structural and functional features of the spectrin dimer. (A) The domains of spectrin are: CH domain (PDB ID: 1BKR), SH3 domain (PDB ID: 1U06), PH domain—except βI isoform (PDB ID: 1WJM), EF hands; major structural elements are spectrin repeats (structure of repeats 15, 16 and 17 of brain alpha spectrin PDB ID: 1U4Q; structure of erythroid spectrin tetramerization complex PDB ID: 3LBX; structure of repeats 13, 14 and 15 of erythroid beta spectrin in complex with ZU5 domain of erythroid ankyrin; ribbon representations of helices of repeats of beta spectrin are in red and of alpha spectrin are blue). (B) Schematic representation of the domain structure of spectrin heterodimer together with examples of binding partners for each of the spectrin subunits ascribed to certain parts of the molecules (solid rectangles); double color dashed rectangles represent binding partners of unassigned specificity to a certain part of a dimer.

transduction. This structure consists of five antiparallel β-strands and a short 3_{10}-helix. The β-strands form two β-sheets that are almost perpendicular to each other in a sandwich structure. SH3 domains interact with proline-rich segments (minimal consensus sequence PXXP [38]) of other proteins through a hydrophobic patch rich in aromatic residues. The ligand-binding site is located opposite to the region where the SH3 domain is connected to the rest of the protein [39]. Although the overall native structure appears well conserved, especially in the so-called core region, the amino acid sequence can vary considerably among SH3 domains of different proteins. The structural differences are found mainly in the loops connecting the five β-strands (Fig. 2A). Possible roles of this domain are mentioned below in Section 7.

2.2.3.2. EF-hand domain. The EF domain is structurally similar to calmodulin and has four EF-hands. The amino-terminal EF-hands are functional and bind Ca^{2+} with affinities in the 0.5 mM range. The carboxyl-terminal pair does not bind Ca^{2+}. Like calmodulin, Ca^{2+} binding causes the proximal EF-hands in brain, (αIIβII)spectrin, to change from a "closed" to an "open" state [40]. The EF-hand domain is critical for skeletal integrity, by binding protein 4.2 in a calcium and calmodulin-dependent manner (see below) and stabilizing CH2 domain-F-actin interactions [41]. According to these authors, the EF-hand domain of α-spectrin modulates the ABD function by binding the linker that connects the actin-binding domain to the first β-spectrin segment (see also Section 3.2).

2.2.3.3. CCC region. αII-Spectrin differs from αI-spectrin by a 36-residue insert in the α10 repeat (named CCC), which bears a Ca^{2+}-dependent binding site for calmodulin and cleavage sites for both caspases (2, 7 and 3) and for m and μ calpain [42–45]. Calpain and caspase cleavage is regulated by Ca^{2+}/calmodulin [45] and also by phosphorylation of

Y1176 located in the calpain recognition site [46,47]. The physiological importance of this site remains to be solved.

2.2.3.4. PH domain. Beta spectrins in their "long" carboxyl end isoforms in the COOH-terminal region possess a pleckstrin homology (PH) domain [48,49]. The region of homology named the pleckstrin homology (or PH) domain, described first by Haslam et al. [50] and by others [51,52], is a 100–120 residue stretch of amino acid sequence similarity in many proteins involved in cellular signaling, cytoskeletal organization, and other processes, appearing as an internally repeated motif in the hematopoietic protein pleckstrin. PH domains are now known to occur in a very large number of proteins, from yeast to mammals (review: [53]). The physiological role of phosphoinositide binding has not been thoroughly explored to date, while the structure of this domain was solved to the atomic level and the inositide part of the PIP_2 molecule was found to be responsible for this binding. An attempt towards solving the role of the PH domain was undertaken by Das et al., who found that in *Drosophila* the PH domain is involved in two of the three mechanisms of targeting of spectrin to the membrane: 1. either ankyrin or PH-domain driven, 2. PH-domain driven, and 3. C-terminal region of the β-spectrin independent mechanism. Namely, only the presence of the PH domain is required for membrane targeting of spectrin in midgut copper cells [54].

2.2.3.5. Oligomerization site. The lateral interactions between the first two repeats of β spectrin and most C-terminal repeats of α spectrin bring together the two spectrin molecules to form the spectrin filament heterodimers [55,56]. The functional spectrin tetramer is formed by head-to-head dimer interactions. These α–β interactions lead to the reconstitution of a complete triple helical repeat as present along the spectrin molecule [57–60]. Most recently, a crystal structure of the tetramerization site was obtained, proving that the interaction of the approx. 30 amino acid residue N-terminus of α spectrin forming a single helix and approx. 70 residue C-terminal double-helical domain of β spectrin (β-17) results in formation of a triple-helix bundle [61]. Such structure strikingly resembles a fully folded spectrin repeat, and is stabilized predominantly by hydrophobic contacts. In the absence of β spectrin, the linker region following the first helix in erythroid α spectrin seems to be unstructured and adopts helical conformation only after binding of β spectrin, while in the case of the brain isoform of spectrin it is fully folded in both situations [62]. These findings suggest that the hydrogen bond networks contribute to structural domain stability, and thus rigidity, in αII [63]. The lack of such hydrogen bond networks in αI leads to its flexibility, an important difference for alpha-spectrin association with beta-spectrin in forming tetramers. This may underlie the difference in the affinity of the αIβI association into the tetramer (K_D ~800 nM [58]) compared to K_D ~10 nM [64], and is related to a higher degree of elasticity of the erythroid membrane skeleton.

By using a yeast two-hybrid system, the oligomerization domain of either αII or βII spectrins was found to bind several proteins. Some of them were found to bind αII in the presence of βII fragments and some bound βII in the presence of αII. The syntaxin binding protein 1 fragment abolished N-terminal αII-C-terminal βII interaction, suggesting a role of this protein in non-erythroid spectrin tetramer formation [65,66]. The details of these interactions and their role should be explored further.

2.2.3.6. Ankyrin-binding site. The issue of having sufficient specificity within a regular repetitive structure has been most thoroughly explored for the ankyrin-binding site located on the β chain of spectrin. Although highly conserved, the region within the 14th and 15th repeats [67,68] was found to have the general features of tandem triple-helical repeats both in its crystal structure [69–71] and in solution [72]. The conservative patch of anionic amino acid residues on helix C of the 14th repeat together with a few residues within the linker and loop flanked by helices B and C of repeat 15 is the major docking site for ankyrin. The highly conserved motif within the loop seems to play a key role in maintaining the appropriate tilt angle between the two repeats due to interactions with the linker. Further structural data showed that the recognition of ankyrin by spectrin requires shape complementarity, but no induced fit takes place [61]. It is worth stressing that the distinctive inter-repeat kink seems to be important for ankyrin-spectrin recognition [73]. The important issue of how ankyrin maintains its interaction with spectrin while exerting mechanical stress on the membrane skeleton has also been recently discussed [74].

As mentioned above, ankyrin(s)-mediated interactions with the vast majority of spectrin ligands – various transmembrane proteins such as channels, transporters and receptors – play an important role in normal cell/organism function and also underlie the molecular mechanism(s) of several pathological processes requiring separate reviews.

3. Erythrocyte membrane skeleton: a well-known example of a membrane supporting protein scaffold

3.1. Lateral network

The lateral structure of the membrane skeletal network has been studied since the mid-1980s when the negative staining electron microscopic observations of Byers and Branton [75] and Shen et al. [76] were published. These observations suggested a network of fully extended spectrin tetramers which were approx. 200 nm long, five to six of which were attached to a short, 37-nm-long actin filament composed of 12–14 actin molecules. It should be noted that although these studies provided the first high resolution details on the molecular structure of membrane skeleton, they were based on observations of shear force stressed membrane skeletons, so the real density of the skeletal network might be much higher. Further observations by using quick freeze, deep rotary replication (QFDERR) technique [77–79] on intact skeletons revealed the presence of 30–50 nm long filaments which were suggested to represent spectrin tetramers. Three or four of such filaments instead of 5 to 6 were found attached to each junctional complex. Additionally, observations by atomic force microscopy (AFM) [80–83] also support the proposition that the end-to-end distance of spectrin tetramers in the cell in the equilibrium state is much shorter than the contour length of the extended molecule and that substantial rearrangements of the spectrin-actin network occur when it is expanded by low ionic strength extraction from the cell. Recent data obtained via cryo-electron tomography of unexpanded mouse erythrocyte skeletons frozen in physiological buffer also revealed an average spectrin tetramer length in this range (46 nm), which is much less than the length of the fully extended molecule, approx. 190 nm. It is also worth mentioning that the thickness and density of the skeletal network were highest at the center of the cell and gradually decreased towards the edge of the cell. The number of spectrin molecules at each junctional complex was 3 to 5. Moreover, the authors observed the prevalence of higher spectrin oligomers (hexamers and octamers) in almost every junctional complex of the network [36]. This observation is somewhat surprising, although in agreement with some of the earlier suggestions [84,85]. At the same time, it is rather contradictory to conventional models of the membrane skeleton (for a recent review see, e.g. [86]). On the basis of comparison of the electron tomographs of free floating erythrocyte skeletons and skeletons from erythrocytes subjected to shear stress, Nans et al. [36] suggest that higher order oligomers are converted into tetramers or even into unassociated heterodimers, allowing the local regions of the network to dynamically change their mechanical properties, thus facilitating reversible deformations that the erythrocyte undergoes in the circulation (Fig. 1).

3.2. Horizontal interactions

Horizontal interactions responsible for the formation of the above-described network are mainly those related to tetramer formation,

whose molecular mechanism is best understood, and/or higher order oligomerization. Each end of the spectrin tetramer is engaged in interaction with a 37 nm long actin protofilament consisting of 12–14 monomers [75]. This interaction is possible due to the presence of the N-terminal domain of the β-subunit, which is called the actin-binding domain (ABD, or 2CH domain) and requires the presence of other proteins, first of all protein 4.1 (erythroid isoform), whose presence is necessary for spectrin-actin complex formation [87] and also plays a role in vertical skeleton-membrane bilayer interactions (see below).

The F-actin binding site in βI spectrin was determined by Goodman's laboratory back in 1991 to be located between residues 47 and 186 [88], forming a highly conserved region within several actin-binding proteins, such as α-actinin, dystrophin (similar to spectrin in other structural features such as a triple helical "spectrin repeat" and EF-hand domains) or otherwise unrelated proteins, such as filamin and ABP120 (actin binding protein 120) or fimbrin [89]. This region, later called CH1 (residues 51–156) due to its calponin homology, together with a neighboring region of the same homology called CH2 (residues 171–282), is thought to form the so-called actin-binding domain [90]. Crystal structure of CH domains, up to reasonably high resolution (1.1 A), was determined [91,92]. Further studies revealed that removal of the first 20 amino acid residues from the CH2 region exposes an actin-binding activity. Moreover, it appeared that both CH domains bind protein 4.1 and removal of the first 20 residues of the CH2 domain markedly enhances this binding [93]. The actin-binding domain contains a PIP_2 binding site highly homologous to the one found in α-actinin [94]. Experiments carried out using recombinant β-spectrin actin-binding domain or it fragments showed that in the presence of 11 μM PIP_2 binding of protein 4.1 was markedly enhanced as K_D reached 25 nM [93]. As these authors suggest, the 20-residue helix which is suggested to uncover the protein 4.1 and F-actin binding by CH2, is not responsible for the effect of PIP_2, as CH2 binding to protein 4.1 remained insensitive to the presence of PIP_2. A still unanswered question concerns the molecular mechanism of enhancing the affinity of spectrin to F-actin upon the presence of protein 4.1. It should be mentioned that the above-cited study, in contrast to the data for α-actinin ABD [94], shows that PIP_2 does not affect F-actin binding by β spectrin ABD. This implies that more extensive conformational changes occur upon protein 4.1 binding. Recent data on spectrin (erythroid and nonerythroid) could support such a suggestion, as digestion of β-spectrin by caspase-8 at residue 470, so distant from "proper" actin-binding domain, could occur only in the presence of cellular proteins or purified protein 4.1 [95]. Further studies on a larger N-terminal fragment of β-spectrin or on a heterodimer of an N-terminal fragment of the β- and C-terminal part of α-spectrins should be carried out. Some light on the mechanism of CH domain-F-actin interaction comes from recent studies which show that the tandem CH domains of alpha-actinin bind F-actin in an open conformation, suggesting that the opening of these domains may be one of the main regulatory mechanisms for binding of actin by the ABD domain [96].

Another member of the spectrin-actin junctional complex is adducin, discovered by Gardner and Bennett [97], a protein kinase C substrate [98,99]. Three genes (*Add-1*, *-2* and *-3*) encode three types of subunits (α, β and γ) sharing a substantial sequence homology. Adducins form heterodimers and heterotetramers via interactions at their globular heads located at their N-terminal ends and their C-terminal "tail" MARCKS (myristoylated alanine rich C-kinase substrate) domains. Adducin, interacting with the plus end of an actin filament, caps it and recruits spectrin tetramers to bind to the actin filament [99–102].

Erythrocyte tropomyosin, which forms dimers of length close to that of the actin protofilament, is thought to determine the unique length of actin filament of the erythrocyte membrane skeleton [103]. Tropomyosin forms a ternary complex with tropomodulin, which binds to the minus end of the actin filament [104].

Experimental data [105,106] indicate that dematin, a trimeric protein which belongs to the villin protein family known to bind and bundle actin filaments [106,107], has a role in erythrocyte membrane stability. Dematin, but not its PKA phosphorylated form, binds to the tail region of the spectrin dimer (N-terminal part of β-spectrin including actin-binding domain and segments 1 and 2 and C-terminal part of α-spectrin including EF-hands domain and segments 20 and 21) [108] with an equilibrium dissociation constant of 15 nM and moderate cooperativity (Hill coefficient ~1.8). This binding facilitated spectrin-actin binding in co-sedimentation assays. This is in accordance with the finding that activation of PKA by endogenous cAMP reduced mechanical stability of the erythrocyte membrane. A still unanswered question concerns the relationship between at least three proteins (adducin, dematin and protein 4.1R) activating interactions of spectrin with F-actin in the membrane skeleton and the role of their phosphorylation by protein kinases A and/or C. Some light on this relationship comes from studies on 4.1R knockout mice ($4.1R^{-/-}$) which have elliptocytotic erythrocytes and anemia [109]. Largely decreased actin content accompanied by marked loss of membrane skeletal lattice structure and formation of bare areas of the membrane [110] was probably responsible for the elliptocytotic phenotype. This is in agreement with previous data on GPC-deficient erythrocytes with a secondary protein 4.1R deficiency in which the spectrin-actin-binding domain of protein 4.1R restored mechanical stability [111]. On the other hand, erythrocytes of α-adducin homozygous null mice in which β and γ-adducin were also absent display features characteristic for hereditary spherocytosis (erythrocytes with a loss of surface area, decreased mean corpuscular/cell volume, cell dehydration and osmotic fragility), which is known to be related to loss of the vertical junction of membrane skeleton to the integral proteins of plasma membrane. Later studies confirmed engagement of adducin in the complex with the cytoplasmic domain of AE1 protein [112] (see below). The relationship between the role of adducin and dematin was addressed by obtaining double knock-out mice with a combined deletion of dematin headpiece and β-adducin [113]. While deletions of each gene separately result in a rather moderate phenotype resembling hereditary spherocytosis [105,114–116], knock-out of both genes resulted in a marked decrease in erythrocyte membrane stability and dramatic shape change, suggesting functional redundancy of the two proteins at the junctional complex.

3.3. Vertical interactions

Another important factor in creating the structural and mechanical properties of the erythrocyte membrane is the connection of the membrane skeleton to the membrane bilayer containing integral membrane proteins. From the mid-1980s until 2003 the picture of vertical connections between spectrin and the membrane bilayer seemed to be reasonably clear and well understood. It was based on two major interaction sites: the ankyrin-band 3 junction and protein 4.1R-p55 (now called MPP1)-glycophorin C junction. The ankyrin-binding site was found in the C-terminal region of the β subunit (therefore two sites located near the middle of the spectrin heterotetramer), while the protein 4.1R binding site was thought to be located near or within the N-terminal part of the β subunit, namely the actin-binding domain (see below). Ankyrin binding in erythrocyte membrane as well as in nonerythroid cell membranes has been very well characterized by many laboratories, first of all by Vann Bennett and his colleagues (some aspects of the studies are discussed below, but for the details see the excellent reviews of Vann Bennett and his colleagues, e.g. [117]).

Involvement of band 4.2 protein in spectrin membrane bilayer interactions was suggested by many cases of HS [3]. In 2003 several reports were published indicating involvement of several other components of the erythrocyte membrane, in particular Rh, RhAG, CD47, LW and GPB, in the interaction of membrane through protein 4.2 and ankyrin R with spectrin [46,118–120]. These findings based on

research on mouse Band3$^{-/-}$, CD47$^{-/-}$, 4.2$^{-/-}$, Rh$_{null}$ human erythrocytes and *in vitro* experiments led to the concept of an AE1-based macromolecular complex. Bruce et al. suggested another function of this complex (metabolon), namely as a gas exchange (CO_2/O_2) channel [118]. Indeed at least CO_2 transport activity (in addition to NH_3 transport) was experimentally confirmed [121]. Aquaporin 1 (AQP-1) also shows CO_2 transport activity. So far experimental data on possible O_2 transport activity of RhAG (or other proteins of the AE1 macrocomplex) are not available from the literature.

Protein 4.1R binding by spectrin is thought to play a double role. One, mentioned above, refers to activation of F-actin binding [87,122] and the other is participation in the "vertical" junction of the membrane skeleton with the membrane bilayer.

As mentioned above, protein 4.1R was thought to be responsible for a second "vertical" membrane skeleton–membrane bilayer link in the erythrocyte membrane. This linkage is formed by simultaneous binding of MPP1 and glycophorin C [123,124]: a 39-residue long segment of MPP1 called the D5 domain [125] binds the 31-amino acid sequence located near the C-terminus of the exon 10 encoded 51-amino acid part of the FERM domain of protein 4.1R [126]. Protein 4.1R was found to bind directly to glycophorin C and to AE1 protein, but with 100 times lower affinity [124]. Careful study of the red cell membrane of protein 4.1R$^{-/-}$ mice [110] revealed that similarly as in the case of ankyrin (AE1)-based macrocomplex also in the case of protein 4.1R-based junction the multicomponent macrocomplex is observed. This macrocomplex, apart from MPP1 and glycophorin C, includes AE1 dimer and several transmembrane proteins which are probably present only in certain macrocomplexes as they are present in a smaller copy number than glycophorin C and protein 4.1R. Moreover, this macrocomplex seems to be highly enriched in adducin, which forms a bridge between the F-actin protofilament-spectrin complex and AE1 protein dimer [112].

Adducin-AE1 dimer binding is, according to these authors, a very important factor in stabilization of the red blood cell membrane, and it explains the presence of Rh and 4.2 proteins in the "protein 4.1R macrocomplex". Moreover, an explanation could be found for the fact that membrane mechanical properties remain normal after breakage of the glycophorin C–protein 4.1R bridge [127,128] and that reconstitution of the SAB (spectrin-actin binding) domain of protein 4.1 into 4.1/glycophorin C-deficient membranes restores membrane mechanical properties without re-establishing the glycophorin C–protein 4.1R bridge [128]. As mentioned above, another, well-known member of the junctional complex, dematin, which was found to activate spectrin–actin interaction, has been shown to form a vertical bridge between the spectrin–actin complex and membrane bilayer by interaction with GLUT1 (glucose transporter protein 1) with a K_D of approx. 400 nM. In addition, GLUT1 was shown to form a complex with β-adducin [106].

Another bi- or multifunctional protein that belongs to the membrane skeleton is protein 4.2, an enzymatically inactive member of the transglutaminase protein family, all of which is thought to be bound to AE1 protein and at the same time binds ankyrin [129], and CD47 that is associated with the Rh complex [46,118] (see above) was recently found to directly associate with spectrin [130–132]. This binding, which occurs at moderate affinity (K_D ~0.30 μM), did not interfere with 4.2–AE1 protein interaction, was significantly amplified in the presence of ~100 μM Ca^{2+} and was inhibited in the presence of Ca^{2+}-calmodulin. The protein 4.2 binding site in spectrin was mapped to the carboxyl terminal EF-hand domain in the α-spectrin molecule (α1 2262–2418) and removal of 13 C-terminal residues completely abolished this binding [130]. Dependence of this binding on such high Ca^{2+} concentrations makes the physiological importance of this binding questionable.

Published data indicate that spectrin can interact directly or indirectly with proteins involved in adhesion to extracellular matrix. In erythroid cells, αI-spectrin interacts with the cytoplasmic tail of Lu/B-CAM [133] and regulates adhesive activity of this adhesion glycoprotein with a K_D of 3.4 μM for Lu and 2.7 μM for Lu(v13). Disruption of this interaction is linked to enhanced adhesion of the red blood cell to laminin [134] and occurs particularly in red blood cells from patients with hereditary spherocytosis [135]. Moreover, contact between nonerythroid αII-spectrin and Lu/B-CAM in epithelial and endothelial cells mediates actin reorganization during cell adhesion and spreading on laminin 511/521 [136].

The red cell membrane skeleton is relatively well understood and schematic models of it have been presented in the recent literature. However, for nonerythroid cells no universal model has been presented, although there are well-known membrane skeletal structures which are connected to cell-cell junctions that form specific membrane domains (for review see [137]). Due to the differences in the stability of the tetramer (a rather substantial difference in the affinity of dimer–dimer association) the flexibility of the membrane skeletal structure should be lower.

The question whether the same kind of lateral membrane skeletal network exists in nonerythroid cells is just starting to be elucidated. For example, in *Drosophila* motoneuron axons near the neuromuscular junctions spectrin seems to be organized into an erythrocyte skeleton like structure [138]. Recent data concerning the axonal membrane skeleton obtained by stochastic optical reconstruction microscopy indicate that actin, spectrin and associated proteins form a highly organized, periodic lattice. Actin and adducin are organized in circumferential rings under the axonal membrane which are evenly spaced (180–190 nm) probably by spectrin tetramers which form straight filaments between actin/adducin rings (Fig. 1A). Sodium channels in axons (which in axon initial segments are ankyrin G and spectrin βIV dependent) show similar periodic distribution. On the other hand, dendrites contained long actin filaments running along the dendritic shaft [139]. The molecular mechanisms of the particular organization of the membrane skeleton remain unknown and seem not to be dependent on a particular isoform of spectrin or ankyrin.

4. Interactions of spectrins with membrane phospholipids

The first reports on the interaction of spectrin molecules with phospholipid membranes appeared even before ankyrin and other membrane receptors for that protein were discovered [140]. Over the decades, it has been discovered that both erythroid and nonerythroid isoforms of spectrin possess a general ability to interact with phospholipids in model and natural membranes, as well as with various hydrophobic compounds [141,142]. This could be mostly attributed to the character of spectrin repeat motifs [143]. However, most recently high affinity lipid-binding sites have been recognized in particular regions of the molecule. These include not only the PH domain within some β isoforms, which is highly specific towards PIP_2, but also some structural elements within spectrin repeats specifically recognizing phosphatidylserine-containing membranes [144–146] or phosphatidylethanolamine-enriched lipid mono- and bilayers [147–150]. The most remarkable fact is that the site interacting with the latter is located at the N-terminal part of the β-spectrin ankyrin-binding domain, and the phospholipid-binding activity is inhibited by ankyrin. The structure and the mechanism of lipid binding of this domain have been revealed in great detail [72,151,152]. This site has been ascribed mostly to helix C of the 14th repeat of β-spectrin, the hallmark of which is high amphipathicity. Intriguingly, the usual triple-helical repeat structure switches to "open" conformation upon lipid binding, and the hydrophobic residues become capable of penetrating the core of a lipid membrane. Keeping in mind that phosphatidylethanolamine has a smaller head group, which gives the lipid a cone shape, PE-rich membranes are more prone to penetration via amphipathic lipid packing sensors [153]. Indeed, the aforementioned helix (site) could be classified as this type of peripheral membrane protein, since the affinity of spectrin to PE/PC monolayers increased with decrease of the initial surface pressure [148], and the isolated domain

induced a decrease in the order parameter of PE/PC membranes [149]. Thus, one can speculate on the role of spectrin in regulation of fluidity of the inner lipid leaflet of the plasma membrane.

Recently, some new functional properties of the lipid binding domain of β spectrin have emerged. Namely, the lipid binding part of the ankyrin-binding domain seems to be involved in preventing the aggregation of the spectrin tetramers together with ankyrin-bound transmembrane proteins such as Na^+/K^+-ATPase, IP3 (Inositol trisphosphate) receptor protein and L1 CAM (L1 cell adhesion molecule), while the localization of transmembrane proteins independent of ankyrin such as cadherin E or N remained unchanged [152,154]. Our hypothesis is that this part of spectrin may play a key role in keeping an appropriate distance between individual ankyrin–transmembrane protein complexes, as the number of ankyrin molecules equals the number of spectrin tetramers in an erythrocyte, while each of the latter comprises two ankyrin-binding domains. Such a scenario could be reflected in maintaining the mechanical and barrier properties of the membrane, where appropriate lateral distribution of integral proteins linked to the membrane skeleton is preserved.

A very interesting issue is the presence of spectrin in detergent-resistant membranes (DRMs), a fraction of cholesterol- and glycosphingolipid-enriched cell membranes considered as a representative of lipid rafts. The fact that spectrin in the proteome of DRMs [155–157] is not accompanied by the usual anchors of the membrane skeleton to the membrane raises the question of how spectrin is associated with lipid rafts. Although raft anchorage via direct spectrin-lipid interaction cannot be excluded, protein-protein interactions are more probable in light of the obtained results. An illustrative example could be found in neurons, where neural cell adhesion molecules (NCAMs) bind to spectrin and promote incorporation of the latter into DRMs or, conversely, CHL1 (cell adhesion molecule with homology to L1CAM (close homolog of L1)) dissociation from the complex with βII spectrin to be incorporated into rafts [158,159] (see below). On the other hand, in leukocytes the DRM proteome inspired a more complex picture of spectrin association with lipid rafts [156]. In that model, spectrin may be attached to DRMs indirectly via the cytoskeletal network including supervillin, α-actinin and myosins.

5. Spectrins present numerous binding sites for a broad diversity of partners

Spectrins appear as large platforms of interactions exhibiting numerous binding sites given by the non-homologous sequences specific to the different isoforms but also by the spectrin repeats themselves. Nowadays, spectrin repeats are regarded not only as structural and mechanical elements of filamentous proteins, but also as a docking surface for cytoskeletal, signal transduction and membrane integral proteins [22].

5.1. Immunoglobulin superfamily cell adhesion molecules

Different spectrin repeats serve as binding sites for cell adhesion molecules, ion channels and receptors in many tissues including nervous tissue. Both α- and β-spectrins are required during nervous system development. The immunoglobulin superfamily cell adhesion molecules L1 and other L1 family members, such as CHL1, neurofascin, NgCAM, NrCAM, and neuroglian, mediate various interactions between cells and with the extracellular matrix in the developing and mature brain, and are thus intimately involved in the regulation of brain development and function in the adult brain [160]. They were found to be associated with membrane skeleton. Most known interactions take place through ankyrin (ankyrin G) binding to the conserved motif (which contains a tyrosine residue, phosphorylation of which abolishes this binding) within the cytoplasmic domain of the CAM molecule (review [160]). Some of them (CHL1) have also been shown to interact with the ezrin–radixin–moesin (ERM) family of proteins [161–163].

β-Spectrin interacts directly with the neural cell adhesion molecule (NCAM), a synaptic adhesion molecule involved in mechanical stabilization of neuronal contacts [164,165]. The earliest information concerns NCAM-180, the largest major isoform of NCAM, which interacts with spectrin [166]. Later on the same authors found that in hippocampal neurons and transfected CHO cells, αIβI spectrin binds to the cytosolic domain of NCAM-180, and isolated spectrin subunits bind to both NCAM-180 and NCAM-140, as does the βI spectrin fragment encompassing second and third spectrin repeats (βI_{2-3}) [167]. In NCAM-120-transfected cells, βI spectrin is detectable predominantly in lipid rafts. Treatment of cells with methyl-β-cyclodextrin disrupts the NCAM-120–spectrin complex, implicating lipid rafts as a platform linking NCAM-120 and spectrin. Furthermore, transfection with βI_{2-3} inhibits NCAM-induced neurite outgrowth, showing that formation of the NCAM–spectrin–PKCβ_2 complex is necessary for NCAM-mediated neurite outgrowth. The same group showed that neural cell adhesion molecule-deficient ($NCAM^{-/-}$) hippocampal neurons have an abnormally high percentage of synapses with perforated postsynaptic densities (PSDs). The proportion of synapses with perforated PSDs is also increased in wild-type neurons after the disruption of the NCAM/spectrin complex, suggesting that the NCAM-assembled spectrin skeleton maintains the structural integrity of PSDs. In $NCAM^{-/-}$ or wild-type neurons with a dissociated spectrin meshwork, AMPAR (a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) endocytosis is enhanced under conditions of basal activity [168]. Moreover, genetic variations of NCAM are considered a risk factor in bipolar affective disease and schizophrenia [165].

CHL1, another protein that is a close homolog of L1 and is highly expressed in neurons, but is also detectable in astrocytes, oligodendrocytes and Schwann cells [169], interacts with and recruits to the cell surface plasma membrane skeleton-linker proteins such as ankyrin and the ERM family of proteins [162,163]. This protein was recently found by Tian et al. [158] to directly associate with βII spectrin. This ligand-induced clustering of CHL1 induced palmitoylation of CHL1 and membrane raft-dependent remodeling of the CHL1/βII spectrin complex, accompanied by CHL1 endocytosis, which is required for CHL1-dependent neurite outgrowth.

Interesting observations concerning interactions of βH spectrin of *Drosophila* with the IgG CAM protein Roughest were published by Thomas et al. [170,171], characterizing the effect of the βH33 fragment in the developing eye. They showed that βH interacts with Roughest to maintain its wild-type distribution. They found that βH33 expression preferentially results in the loss of interommatidial cells associated with fragmentation of the zonula adherens (ZA) and disruption of the Ig-CAM Roughest. The authors suggest that the activity of βH serves to coordinate the ZA and Roughest/Ig-CAM adhesion systems. It is not known, however, whether the interaction of Roughest with βH33 occurs via direct or indirect interactions.

5.2. NMDA receptors

Spectrin is a key point of signal convergence between tyrosine kinase/phosphatase and Ca^{2+}-mediated signal cascades. This kind of control may be particularly important in vesicle trafficking, endocytosis, neurite outgrowth and NMDA (N-methyl-D-aspartate) receptor activation (for a review see, e.g. [86]). NMDA receptors, a class of glutamate-gated cation channels with high Ca^{2+} conductance, mediate the fast transmission and plasticity of the central nervous system excitatory synapses. As mentioned above, the α-spectrin Tyr^{1176} phosphorylation state could modulate spectrin cleavage by calpain [47,172]. Spectrin phosphorylation in particular controls protection of the integrity of the glutamate-gated NMDA receptor complex. Spectrin associates with the NR2 cytosolic subunit of the NMDA receptor. The activity of the NR2 subunit is regulated by tyrosine phosphorylation. Calpain

proteolysis of NR2 disrupts its association with spectrin, and conversely its phosphorylation by c-Src protects it from calpain cleavage. The highly regulated linkage of NMDA to spectrin may underlie the morphological changes that occur in neuronal dendrites concurrently with synaptic activity and plasticity [137,159,173].

5.3. Glutamate EAAT4 transporter

The multifunctional spectrin-based skeleton participates in the complexes linking various structures or organelles to the motors involved in microtubule-directed transport, and in the facilitation of membrane protein transport via the secretory and endocytic pathways [174]. βIII-Spectrin is highly expressed in Purkinje cells [10,175], where it participates in the membrane stabilization of the glutamate transporter EAAT4 [176]. βIII-Spectrin defects are associated with mislocation of this transporter from the surface of the plasma membrane in Purkinje cells. Cell fractionation studies suggest that the mutant βIII-spectrin (with a 39-bp deletion) affects the synaptosomal plasma membrane localization of both EAAT4 and GluRg2. Co-expression of GFP-EAAT4 with either wild type or 39-bp βIII-spectrin deletion mutant showed that wild type spectrin stabilized EAAT4 at the membrane [176,177]. Several studies have suggested the possible role of EAAT4 in ataxia [178–180]: loss of EAAT4 and GluRg2 at the plasma membrane could lead to glutamate signaling abnormalities that, over time, could cause Purkinje cell death in autosomal dominant spinocerebellar ataxia type 5 (SCA5). Mutations in the *SPTBN2* gene, coding for βIII-spectrin (2390 residues), have been recently recognized as the cause of SCA5 and neurodegenerative disease [10,181–183]. Moreover, βIII-spectrin is present in the Golgi and vesicle membranes [10], and binds to the dynactin subunit Arp1, suggesting a possible role of βIII-spectrin in vesicular transport [184]. In patients exhibiting SCA5, a mutation found in the calponin homology domain (CH) alters the interaction of βIII-spectrin with Arp1 and consequently affects the stabilization of membrane protein, or may cause alterations in EAAT4 transport by disrupting binding to the Arp1 and dynein motor complex. Cell culture studies reveal that the L253P mutant of βIII-spectrin, instead of being found at the cell membrane, appears trapped in the cytoplasm associated with the Golgi apparatus. Moreover, L253P mutation of βIII-spectrin prevents correct localization of wild-type βIII-spectrin and stops EAAT4 from reaching the plasma membrane. These data provide evidence for a dominant-negative effect of SCA5 mutation and show that trafficking of both βIII-spectrin and EAAT4 from the Golgi is disrupted through failure of the L253P mutant βIII-spectrin to interact with Arp1 [185].

5.4. Other neuronal proteins

Brain spectrin, mostly $(\alpha II\beta II)_2$ was found to interact with small synaptic vesicles via synapsin I, the major phosphoprotein on the membrane of small synaptic vesicles [186,187]. The synapsin I attachment site in the βSpIIΣI spectrin molecule comprises a ~25 amino acid residue segment (211 to 235) and interaction of these two proteins is essential for the process of neurotransmission [188,189] as the injection of anti-synapsin I-binding domain IgG inhibited neurotransmission in cultured hippocampal neurons.

5.5. Sodium channels and βIV-spectrin

βIV-spectrin acts as a multifunctional regulatory platform for sodium channels, and has important roles in maintaining the structure and stability of excitable membranes in heart and brain, targeting critical structural and regulatory proteins. The loss of βIV-spectrin observed in quivering mice with progressive ataxia exhibiting tremors and hearing defect is associated with mislocation of voltage-gated channels at the axon initial segment and node of Ranvier [190,191]. Alterations in the location of sodium and potassium channels in myelinated nerves slow propagation and desynchronize action potentials.

Deletion of β-spectrin repeats may compromise the formation of the tetramer (as observed in hereditary elliptocytosis), reducing its ability to anchor proteins including ion channels at specialized subcellular domains. Characterization of βIV-spectrin knockout mice [191] demonstrated that βIV-spectrin acts as a multifunctional regulatory platform for sodium channels and has important roles in the structure and stability of excitable membranes in the heart and brain, targeting critical structural and regulatory proteins. Ankyrin-G and voltage-gated sodium channels, which normally cocluster with βIV-spectrin at nodes of Ranvier and axon initial segments [8], were mislocalized in the mutants. Conversely, βIV-spectrin was mislocated in ankyrin-G knockout mice, suggesting mutually dependent targeting of these two proteins. Voltage gate dependent sodium channels, neurofascin (an L1 family cell adhesion molecule), and βIV-spectrin (all of which interact with ankyrin *in vitro*) were mislocated in ankyrin-G knockout mice [192,193]. In a similar way, ankyrinB (−/−) mice exhibited a severe phenotype that partially overlapped the phenotype of human patients with L1 mutations [194]. Besides the role in the formation of functional domains (targeting and stabilization of proteins), βIV-spectrin might be involved in a regulatory mechanism for Na^+ channels (Nav1.5), via direct phosphorylation by βIV-spectrin targeted calcium/calmodulin-dependent kinase II [195]. These findings provide evidence for an unexpected yet commanding molecular platform involving spectrin that determines vertebrate membrane excitability.

5.6. α-Catenin

α-Catenin, an adaptor protein (which in addition to β- and p-120 catenins) of the adherens junction of epithelial cells linking cadherin E to cytoskeletal and intracellular trafficking machinery [196,197], can bind directly to the region of the first 313 residues of βII spectrin with a K_D of ~20–100 nM [198]. According to these authors, this interaction seems responsible for recruitment of spectrin to regions of direct cell–cell contacts. Pradhan et al. suggest that the binding of spectrin to the cadherin-based adhesion complex stabilizes it and facilitates lateral adhesion by linking adjacent adhesion complexes into macromolecular membrane domains. This event although explored well by the initial study awaits further studies as there are many signs in the literature indicating the biological importance of membrane skeleton as an emerging regulator of epithelial junction integrity and dynamic remodeling [199,200].

6. Examples of diseases associated with mutations of nonerythroid spectrins

βII-spectrin mutations induce destabilization of the membrane structure and mislocation of membrane receptors and channels, often leading to serious diseases, such as spinocerebellar ataxia and neurodegenerative diseases. Recent data have revealed that αII-spectrin mutations are associated with the rare epileptic disease West syndrome (also known as infantile spasms) and epileptic encephalopathy [201,202]. It is conjectured that it is a defective neurotransmitter function, or more precisely, a defect in the regulation of the GABA transmission process. Mutations causing WS are located within the spectrin repeats involved at the initial nucleation site between α- and β-spectrins and they were predicted to affect formation of αβ-spectrin heterodimers. They cause early-onset WS with spastic quadriplegia, poor visual attention, and severe developmental delay.

βII- and βIII-spectrins have been shown to participate in stabilization of membrane proteins and axonal transport [183,184]; their defects could disturb clustering of ankyrin G and voltage-gated sodium channels (VGSC) at the axon initial segment, together with an elevated action potential threshold. In metazoans, the coordinated activities of voltage-gated sodium channels underlie cellular excitability and control

neuronal communication, cardiac excitation–contraction coupling, and skeletal muscle function. Sodium channel dysfunction is associated with arrhythmia, epilepsy, and myotonia. In myelinated nerve fibers, action potential initiation and propagation requires that voltage-gated ion channels be clustered at high density in the axon initial segments and nodes of Ranvier.

Previous results indicate that αII-spectrin is enriched at the paranodes which flank the node of Ranvier, notably in nodes and paranodes at early stages of development, as observed in zebrafish [203,204]. The nodal expression diminishes as nodes mature in zebrafish; αII-spectrin mutants harboring a nonsense mutation destabilize nascent clusters of sodium channels (VGSC) and affect normal development of mature nodes of Ranvier. The mutants also showed impaired myelination in motor nerves and in the dorsal spinal cord, suggesting that αII-spectrin plays important roles in maintaining the integrity of myelinated axons. These findings revealed essential roles of an αII-spectrin in human brain development and suggest that abnormal axon initial segments are possibly involved in pathogenesis of infantile epilepsy.

7. Spectrin in cell signaling

The occurrence of a variety of spectrin isoforms in different nucleated cells indicates that its functions may vary among different cells as a result of their specializations.

In the last few years, more and more reports providing new data concerning the previously unrecognized role of α-spectrins in signaling pathways have appeared (for review see [86]). Recent data pointed out the role of the SH3 domain of αII-spectrin in transmission of signals leading to Rac activation, adhesion, lamellipodia extension, and cell spreading through several ligands and partners regulating actin skeleton organization and dynamics [205–207].

The SH3 domain of erythroid α-spectrin interacts with a tyrosine kinase-binding protein, hssh3bp1/e3B1/Abi1 (Abelson interactor 1) [208], and that of nonerythroid spectrin with a low-molecular weight phosphotyrosine phosphatase [172], c-Src (a tyrosine kinase) [47], Na^+/H^+ exchangers [209] and Na^+ channels (ENaC) in epithelial cells [210]. αII-Spectrin binds to the vasodilator-stimulated phosphoprotein (VASP) triple GP_5 motif via its SH3 domain. This binding is inhibited upon phosphorylation of VASP at Ser157 by cAMP-dependent protein kinase. αII-Spectrin colocalizes with nonphosphorylated VASP at cell–cell junctions in confluent cells. In contrast to the effect of VASP, knock-out ectopic expression of the αII-spectrin SH3 domain in endothelial cells translocates VASP, initiates cortical actin cytoskeleton formation, stabilizes cell–cell contacts, and decreases endothelial permeability [207]. αII-Spectrin interacts with two F-actin-binding proteins, Tes (a potential tumor suppressor) and EVL (Ena/VASP-like), located at the cell contacts [211,212]. Tes interacts with EVL and this interaction prevents the interaction between spectrin and Tes. The above-mentioned data could represent a link between the spectrin-based skeleton and the actin polymerization machinery.

αII-Spectrin is also present in nuclei of human cells and through its SH3 domain could play an important role in the repair of DNA interstrand cross-links [213,214]. It is absent from cells of patients with the genetic disorder Fanconi anemia [215]. Sridharan et al. [216] found five groups of nuclear proteins interacting with αII-spectrin: structural proteins, proteins involved in DNA repair, chromatin remodeling proteins, FA proteins, and transcription and RNA processing factors. Moreover, when αII-spectrin was knocked down in normal cells using siRNA, a number of defects observed in FA cells, such as chromosome instability and a deficiency in cross-link repair, were observed [217]. Therefore the question remains, what are the functions of interactions of the other nuclear proteins with spectrin?

Spectrins are involved in the cell cycle by regulating the expression of membrane receptors. The loss of β-spectrin results in defective TGFβ (transforming growth factor β receptor-1) signaling by mislocation of the proteins smads 3 and 4 which modulate the activity of TGFβ [218,219]. In a mouse model, downregulation of ELF expression (an isoform of human βII-spectrin) confers susceptibility to tumorigenesis. βII-$Sp^{+/-}$ mutant mice develop frequent tumors associated with deregulation of cell cycle control at the G1/S transition and defective TGFβ signaling [220–223]. Moreover, these βII-$Sp^{+/-}$ mice are born with many phenotypic characteristics observed in Beckwith–Wiedemann syndrome (BWS), a hereditary stem cell cancer syndrome. These include dramatic visceromegaly, followed in later months by the development of multiple cancers, including carcinomas of the gastrointestinal tract, as well as renal and adrenal adenocarcinomas. Epigenetic silencing of βII-spectrin expression in human BWS could be a potential causal factor in this stem cell disorder [224].

Spectrins are also engaged in different pathways of cell transduction and signaling in lymphocyte activation. It has been clearly demonstrated that the spectrin-based skeleton via its two major proteins, spectrin and ankyrin, directly binds CD45 in lymphocytes [225,226]. Moreover, spectrin and PKCθ (Protein kinase C theta) were observed in aggregates in lymphocytes and in a large signaling complex at the site of T cell receptor clustering in immunological synapses. These facts may be related phenomena [227]. Spectrin aggregation in lymphocytes may also be associated with early cellular apoptotic events preceding a loss of membrane aminophospholipid asymmetry [228–230].

8. Is spectrin a scaffold protein or mere physical platform for membrane channels, receptors and adhesion molecules?

According to the recent view [231], scaffold proteins are multidomain proteins that organize signaling modules into particular domains carrying specific signaling pathways. It is known that signaling pathways depend not only on the specificity and affinity of the members of the signaling pathway but also on when and where these signaling pathway elements are organized into higher order units in response to the stimuli that activate the pathway. To achieve this, cells have evolved a class of multidomain proteins, termed “scaffold proteins” that: (a) recruit several proteins to a specific locality, (b) organize them into a higher order macromolecular complex, (c) facilitate the interaction and fine-tune the activity and crosstalk among the proteins within the entire assembly, and (d) coordinate functions of different molecular assemblies in different parts or “microdomains” of the cell.

Spectrins in essence fulfill the criterion of multidomain structure and the condition of recruiting several proteins to a particular location within the cell. Formation of the membrane skeleton is the best known example of such activity. The consequence of this is the organization of many skeletal/membrane and nonskeletal proteins into a higher order macromolecular complex. An example of the latter is the aforementioned direct interaction of Abi1 or VASP or EVL proteins with the SH3 domain. All of these proteins are involved in rearrangement of the actin skeleton [232–234]. At this point, the analogy between professional “scaffold” proteins such as KSR, paxillin or beta arrestin and many others ends. Apart from the data mentioned above concerning particular membrane and signaling proteins there are no data on spectrin interacting with several participants of a particular pathway organizing it into a chain of signaling events.

In the light of available data, spectrins could be considered as simpler, modular devices such as the adaptor or docker proteins. According to the above-mentioned authors, an adaptor protein simply bridges two proteins together, and in some instances dockers can undergo certain modifications such as phosphorylation for the purpose of localization or recruitment of target proteins. In general, adaptors and dockers do not directly affect the properties of their target proteins. There are many examples suggesting that spectrins fulfill these criteria; however, they still possess the domains and motifs which are present in scaffold proteins involved in signaling pathway organization. It seems that apart from several examples (ELF), these signal receiving modules such as SH3, but first of all PH and other phosphoinositide binding

motifs, are engaged mostly in the regulation of the membrane skeletal function of spectrin. So in view of the current knowledge (data) canonical spectrins could be considered as adaptor or docker proteins, although further studies such as studies on involvement of spectrins in cell cycle regulation [206] or in apoptosis [230] may change this classification.

9. Conclusion

The above description of the structure and interactions of spectrins is far from complete. However our intention was to summarize important issues and studies, particularly those undertaken relatively recently. We believe that the emerging picture supports the view that spectrin is a mechanical scaffold which supports the membrane bilayer and controls the mobility and perhaps the activity of membrane integral proteins, which are membrane channels, transporters and receptors. Moreover, it is well known that this scaffold by itself is dynamic and on top of that contains domains such as SH3, PH and others including proteolytic or phosphorylation sites which function as interfaces for receiving signals from the signal transduction system. Taken together a picture of a system which functions like a microchip remote controlled storehouse is emerging. In spite of more than 40 years of the history of spectrin/membrane skeleton research, there are still several issues to be elucidated to facilitate understanding of the basic mechanisms of its functions. Among others, the following are important: 1. molecular mechanism(s) underlying physiological roles of particular isoforms; 2. mechanisms of membrane domain formation; 3. formation of signaling assemblies; 4. whether the interactions with spectrin affect the activity/function of particular membrane proteins.

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