The Spread of Spectrin in Ataxia and Neurodegenerative Disease

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Abstract

Experimental and hereditary defects in the ubiquitous scaffolding proteins of the spectrin gene family cause an array of neuropathologies. Most recognized are ataxias caused by missense, deletions, or truncations in the SPTBN2 gene that encodes beta III spectrin. Such mutations disrupt the organization of post-synaptic receptors, their active transport through the secretory pathway, and the organization and dynamics of the actin-based neuronal skeleton. Similar mutations in SPTAN1 that encodes alpha II spectrin cause severe and usually lethal neurodevelopmental defects including one form of early infantile epileptic encephalopathy type 5 (West syndrome). Defects in these and other spectrins are implicated in degenerative and psychiatric conditions. In recent published work, we describe in mice a novel variant of alpha II spectrin that results in a progressive ataxia with widespread neurodegenerative change. The action of this variant is distinct, in that rather than disrupting a constitutive ligand-binding function of spectrin, the mutation alters its response to calcium and calmodulin-regulated signaling pathways including its response to calpain activation. As such, it represents a novel spectrinopathy that targets a key regulatory pathway where calcium and tyrosine kinase signals converge. Here we briefly discuss the various roles of spectrin in neuronal processes and calcium activated regulatory inputs that control its participation in neuronal growth, organization, and remodeling. We hypothesize that damage to the neuronal spectrin scaffold may be a common final pathway in many neurodegenerative disorders. Targeting the pathways that regulate spectrin function may thus offer novel avenues for therapeutic intervention.

Keywords

Membrane-associated periodic skeleton; Calpain; Proteolysis; SPTB; SPTBN1; SPTBN2; SPTAN1; Alzheimer's; SCA5; Parkinson's; Neurodegeneration; Proteostasis; RTK signaling; Calcium signaling; HIPPO/YAP signaling

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Introduction

Since its recognition five decades ago as a major component of the erythrocyte's cortical membrane skeleton, our understanding of spectrin has evolved to include recognition of its ubiquitous presence in probably all animal cells and its role in surprisingly diverse biological functions. This is perhaps most apparent in the nervous system. Seven genes encode the mammalian spectrins (Figure 1). All but one (αI spectrin) are expressed in various neuronal and neurosensory cells. Classically, spectrin is recognized as an actin filament cross-linking protein that also binds directly and through adapter proteins (e.g. ankyrin) to biologic membranes and membrane lipids. In neurons and glia, spectrin forms a caricature of the erythrocyte skeleton, termed the membrane-associated periodic skeleton (MPS) [1]. Beyond its canonical role as an actin binding and membrane-linking protein, the spectrins also serve other roles: i) linkage to motors of intracellular transport, myosin, dynactin and kinesin [2–5]; ii) linkage to the axonal transport of lipid and protein laden vesicles [3,4] iii) stabilization of the Golgi and endoplasmic reticulum [6–9]; iv) trafficking of selected proteins in the secretory and endocytic pathways [8,10–12]; v) upstream regulation of the HIPPO/YAP signaling pathway that guides many aspects of neuronal development and remodeling [13–16]; vi) a multivalent protein-protein interaction scaffold that organizes membrane-associated signaling ensembles [17]; and vii) a target of multiple post-translational modifications that regulate its various functions. The richness of spectrin's direct and indirect interactions with many biologic pathways can be appreciated in genome wide interaction diagrams of any spectrin; two examples for human βIII spectrin and αII spectrin are shown in Figure 2.

Spectrinopathies

Reflecting their diverse roles, spectrin deficiencies or defects lead to diverse neuropathology. Most studied have been the beta spectrins. Disorders in βI spectrin (SPTB) have been linked genetically to autism, learning difficulties, and spinal cord disease [18–20]. Genetic deletion of βII spectrin is embryonic lethal with loss of neural stem cells in the subventricular zone [21,22]; heterozygotes appear neurologically normal, but are prone to develop liver and gastrointestinal cancers putatively due to alterations in TGF-β/SMAD signaling [23]. Spectrin is also linked genetically to late-onset Parkinson's disease and Lewy-body pathology [24,25] as well as other neurodevelopmental syndromes [26]. βIII spectrinopathies include spinocerebellar ataxia type 5 (SCA5) as found in afflicted decedents of Abraham Lincoln [27] and now recognized in several other pedigrees [28]. Other variants in SPTBN2 show cognitive impairment as well as ataxia (spectrin-associated autosomal recessive cerebellar ataxia type 1, SPARCA1) [29,30]. CpG hypomethylation of SPTBN2 links to attention deficits in children [31]. Animal models with genetic deletion of βIII spectrin recapitulate these conditions, with demonstrative disruption of the ER and Golgi architecture and selective mis-localization of postsynaptic proteins and excitatory amino acid transporters [11,32]. Defects in βIV spectrin disrupt axonal organization at the nodes of Ranvier and subsequently neurotransmission, and are associated with congenital myopathies and deafness [25,33]. Finally, spectrin βV is required to link myosin VIIA to trafficking vesicles; failure of this linkage leads to progressive hearing loss and blindness in Usher syndrome Type I [34] along with impairment of innervation of the organ of Corti [35].

Defects in αII spectrin also lead to neurologic pathology. Mice lacking αII spectrin are embryonic lethal due to cardiac and nervous system malformations [36]. Mice without αII spectrin in the peripheral nervous system suffer impaired neuronal excitability and axonal defects [37,38]. Human mutations in αII spectrin (*SPTAN1*) link to early infantile epileptic encephalopathy (EIEE) type 5 (West Syndrome), characterized by refractory seizures, intellectual disability, agenesis of the corpus callosum and hypomyelination [38–41]. Other SPTAN1 neurological disorders include juvenile onset hereditary motor neuropathy and hereditary spastic paraplegia [28,42–46].

In recent published work, we describe a novel variant of the murine Sptan1 gene (αII spectrin) with a substitution of Gln for Arg at codon 1098. In heterozygotes this substitution causes a progressive age-dependent ataxia with widespread neurodegeneration [47]. The action of this variant is distinct from other αII spectrin neuropathologic mutations, in that rather than directly disrupting a constitutive ligand-binding or protein-protein interaction (e.g. heterodimer formation, tetramer formation, or actin binding), the mutation alters spectrin's susceptibility to calcium and calmodulin activated calpain proteolysis, with secondary consequences for its overall function. Beyond the pathways that modulate calpain activity [48], two factors control spectrin's susceptibility at the substrate level to activated calpain: the calcium-calmodulin dependent exposure of its Y-G residues at position 1176–1177 [49,50], and whether Y1176 is phosphorylated [51,52]. The R1098Q variant spectrin thus represents a novel spectrinopathy that targets a key regulatory site where calcium and tyrosine kinase signal pathways converge to alter spectrin's function. Beyond the novelty of the R1098Q mutation, the implications of this pathway for understanding other neurodegenerative disorders are significant.

Impaired Spectrin Homeostasis: A Unifying Concept of Neuronal Injury

Inappropriate calcium signaling and activation of calcium activated neutral proteases (calpain) is implicated in a variety of neurologic or degenerative disorders. These include Alzheimer's and Parkinson's disease [48,53–55], aging [56,57], and traumatic brain injury [58,59]. The literature is replete with putative calpain targets, and a case can be made that many of these contribute to any given pathology. However, αII spectrin is a major target of calpain attack in all of these conditions, and its cleavage has been widely used as a sensitive measure of neuronal remodeling or neurodegeneration or neurotoxicity. It is an early event in the generation of dark Purkinje cells [60], and calpain-generated breakdown products of spectrin appear in association with amyloid-beta (Aβ deposits and neurofibrillary tangles in Alzheimer's disease patients [61,62]. Spectrin is also a component of the Lewy bodies found in Parkinson's disease patients, although its state of proteolytic cleavage in the Lewy bodies is undetermined [63]. While the association of spectrin breakdown with disease could simply reflect the end-stage consequences of neuronal injury, we believe this is unlikely based on the global involvement of spectrin in so many cellular processes, and particularly based on our findings with the αII spectrin R1098Q variant [47]. This variant informs us that up-regulation alone of spectrin's sensitivity to calpain cleavage is sufficient to induce widespread neurodegenerative change and lethal cell injury. It is important to emphasize, this effect is mediated by an enhancement of spectrin's intrinsic sensitivity to cleavage, not by a global activation of calpain or enhanced Ca^{++} signaling. While mutations in spectrin

remain a rare cause of neurologic disease, processes that perturb intracellular calcium homeostasis and calpain activity are not rare, and accompany many neurodegenerative and other disorders as noted above. It is thus likely that enhanced cellular calpain activity alone, acting on wild-type αII spectrin, will phenocopy the consequences of the R1098Q variant. It will be important in the future to confirm this by determining that a reduction in calpain activity can rescue the R1098Q phenotype. Regardless, the totality of this data suggests that disruption of the spectrin scaffold in neuronal or neurosensory cells may be a common final pathway of neurodegeneration or malfunction.

A Path to Therapy

To the extent that disruption of the neuronal spectrin scaffold is an important factor in the progression of neurologic disease, strategies designed to ameliorate spectrin dysfunction may offer new routes for therapeutic intervention. Inhibition of calpain activity has long been recognized as a potential therapeutic target, and pharmacological calpain inhibitors [64] or over-expression of the natural calpain inhibitor calpastatin [65] prevents or reduces neurodegeneration in murine models. However, recognition that the effects of calpain on the spectrin scaffold can also be regulated at the substrate level opens the door to novel and possibly more specific interventions. One strategy might focus on the phosphorylation of the tyrosine at codon 1176 (Y1176) by a Src family kinase, a post-translational modification that also blocks the calpain cleavage of αII spectrin [51,52]. Designing a therapeutic strategy that either activates such a kinase or inhibits a relevant phosphatase could offer benefit and greater specificity than global calpain suppression. Recently, one study found that Trodusquemine, an inhibitor of tyrosine phosphatase PTB1B that is currently in phase 1–2 clinical trials for obesity, restored synaptic plasticity and improved cognitive function in a murine model of Alzheimer's disease [66,67]. While spectrin was not identified as a target of PTP1B in this study, the enzyme is abundant in brain and any blockage of calpain processing of spectrin by tyrosine phosphorylation at residue 1176 would also impair synaptic plasticity [68,69].

Beyond the action of calpain, the spectrin scaffold as a major organizing and structural hub offers many other putative targets for therapeutic intervention. In Parkinson's disease models, phosphorylated α-synuclein binds preferentially to spectrin, which removes potentially toxic α-synuclein aggregates [70]. However, the binding of monomeric or oligomeric α-synuclein to spectrin disrupts spectrin-actin dynamics and the membrane-associated periodic skeleton. As seen in most pedigrees with spectrin mutations related to its actin-binding function, disruption of the MPS alone is significantly pathologic. It is interesting to speculate that the end-stage injury in Parkinson's disease could be related as much to α-synuclein induced damage to the MPS as to the accumulation of α-synuclein aggregates. Since α-synuclein's affinity for spectrin is enhanced in the Drosophila model by its phosphorylation at serine 129, therapies that limit this phosphorylation might stabilize the MPS and enhance neuronal survival.

There are many other regulated interactions of the spectrin scaffold with its various partners, and more no doubt await discovery. Some examples include complex allosteric interactions that link membrane binding to its self-association properties [71,72], post-translational

regulation controlling its interaction with calmodulin-regulated kinases (CaMKII) [73–75], its control of calcium-regulated exocytosis [76], phosphorylation control of its stabilization of specific organelles [6,7], and its interaction with long non-coding RNA's (lncRNA) responsible for activity-dependent synaptic plasticity in hippocampal neurons [77]. If indeed as we have postulated that damage to the spectrin scaffold is a crucial final pathway in a broad spectrum of neuronal pathologies, then strategies designed to stabilize this structure may offer truly new approaches to their treatment.

Conclusion

From its humble beginnings as a cytoskeletal protein originally thought to be unique to the red cell, the spectrin scaffold has emerged as a central component of diverse signaling systems and a crucial member of pathways that control cellular organization, size, and function. This is most apparent in the nervous system. Defects in the spectrin scaffold lead to diverse neurodevelopmental and acquired disorders. The recent identification of an unusual αII spectrin defect in a murine model that renders it uniquely hypersensitive to calcium-mediated calpain processing indicates that damage to the spectrin skeleton alone is sufficient to generate a severe ataxic phenotype with widespread neurodegenerative change [47]. Increasing evidence indicates that diverse neurodegenerative and acquired conditions including Alzheimer's disease, Parkinson's disease, several ataxias, and traumatic brain injury all involve damage to the spectrin scaffold. Thus, an attractive hypothesis is that disruption of the neuronal spectrin scaffold represents a common end-stage event in such disorders. Therapeutic strategies focused on preservation of spectrin's function may thus offer novel approaches to the treatment of many neurodegenerative conditions.

Acknowledgements

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| Spectrin	Gene	Locus	Isoforms	Disposition in neuronal/neurosensory cells
α I	SPTA1	1q23.1	1	not expressed
α II	SPTAN1	9q34.11	5	ubiquitous
β I	SPTB	14q23.3	2	PSD of cerebellar granule cells, cortical layers II and V, interneurons, trafficking vesicles
β II	SPTBN1	2p16.2	4	neuronal skeleton, axons, pre-synaptic terminals
β III	SPTBN2	11q13.2	1	PSD of Purkinje and cortical neurons layers I, III, IV, and VI, apical dendrites & spines
β IV	SPTBN4	19q13.2	6	Nodes of Ranvier & axonal initial segment
β V	SPTBN5	15q15.1	1	Photoreceptors & spiral ganglion inner ear
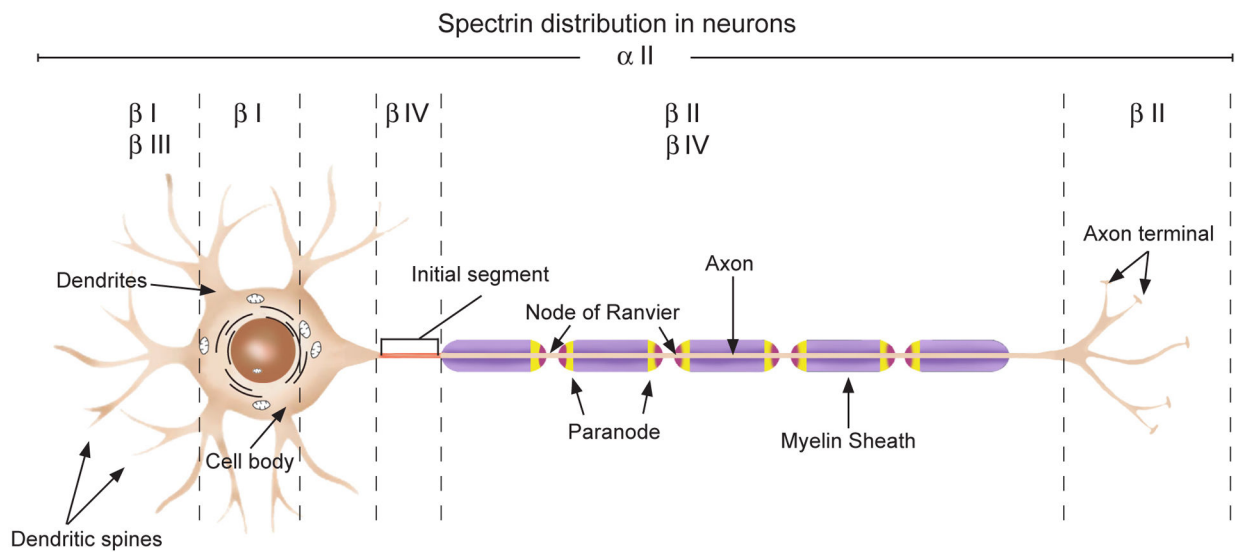
Figure 1: Disposition of the spectrin gene family in neuronal and neurosensory cells and tissues.

Figure 2: Genome-wide Interaction map of spectrin in humans.
(A) Interaction map of βIII spectrin. **(B)** Interaction map of αII spectrin. The fifty
interacting genes with the highest confidence score (>0.9) are represented in each
diagram. The edges represent known protein-protein interactions (not necessarily directly
bound). Nodes are the respective proteins centered on each spectrin. Edge colors are:
purple, experimentally determined; light blue, curated databases; green, gene neighborhood
associations. Note the significant interactions of spectrin with cytoskeletal elements, motors
of intracellular transport; many ion channels and transporters; components of the Golgi
apparatus and the secretory and endocytic pathways; and various receptor tyrosine kinases
and adapter proteins. Generated by Strings V11 [78].