Published in final edited form as:
J Mol Cell Cardiol. 2010 June ; 48(6): 1031–1032. doi:10.1016/j.yjmcc.2010.02.002.

Cardiac spectrins: Alternative splicing encodes functional diversity

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Normal cardiac physiology relies on the precise localization and function of ion channels, receptors, regulatory molecules, and structural proteins at distinct locations in the cell. Spectrins are actin-associated proteins that provide structural support to the cell membrane and play critical roles in compartmentalization of subcellular microdomains. Importantly, abnormalities in spectrin and spectrin-associated proteins (e.g. ankyrins, obscurin, protein 4.1) have been linked with disease including congenital and acquired arrhythmia syndromes and myopathy [1-10]. The unique structure of spectrin not only allows it to provide mechanical integrity to the cell membrane, but also organize macromolecular complexes at well-defined subcellular domains. Thus, increasing our understanding of spectrin function in the heart may illuminate exciting new pathways for altering cardiac excitability and function.

Spectrin is a flexible hetero-tetrameric molecule formed from anti-parallel heterodimers of α- and β-subunits. Originally discovered in the erythrocyte, spectrins are now known to be widely expressed in mammalian tissues. α–spectrin contains 22 functional domains comprised of 21 triple helical spectrin repeats responsible for binding associated proteins, and a C-terminal calmodulin-like domain (Figure 1). β-spectrin consists of an N-terminal actin-binding domain, 17 spectrin repeats, and a C-terminal domain containing a pleckstrin homology domain [11]. α–spectrin binds to β-spectrin at repeat 17 on β–spectrin and repeat one on α–spectrin [12-15]. Importantly, ankyrins bind β–spectrin at repeat 15 [16]. Thus, each spectrin hetero-tetramer contains two binding sites for ankyrin allowing for construction of diverse macromolecular complexes.

To date, two α– isoforms and five β–isoforms have been identified. αI- and βI-spectrin are the predominant isoforms found in erythrocytes while other spectrin gene products are more broadly expressed. In the heart, αII-spectrin is found with βII-spectrin at Z-line and sarcoplasmic reticulum (SR) membranes while both αI- and αII-spectrin are found with βI-spectrin at the plasma membrane [1]. Importantly, alternative splicing of both α– and β–isoforms provides further functional diversity. Specifically, several splicing events have been described for αII-spectrin resulting in small (6-20 residue) insertions in different spectrin

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Disclosures None

repeats [17,18]. C-terminal regions of βI-, βII-, and βIV-spectrin are also alternatively spliced to produce “long” and “short” isoforms [11,19,20]. The “long” β-spectrin isoforms contain a pleckstrin homology (PH) domain, a roughly 100-residue domain with roles in ligand binding and intracellular signaling [11]. Greatly truncated forms of both βII- and βIV-spectrin are also generated through splicing events [19,21,22]. While the functional consequences for these splicing events remain unclear, they likely impact the localization and function of spectrin [20].

The importance of spectrin and associated proteins for normal human physiology is illustrated by the strong association between dysfunction in these proteins and human disease. Gene mutations in α- and β- spectrin have been linked to hereditary spherocytosis and hemolytic anemia in humans and mice, although the most common cause of the disease are mutations in *ANK1*, the gene encoding the spectrin-associated protein ankyrin-R [23,24]. Spectrin mutations have also been identified as a cause of human spinocerebellar ataxia, a progressive neurological disorder characterized by loss of coordination. Specifically, several loss-of-function mutations in βIII-spectrin have been linked to spinocerebellar ataxia type 5 [25]. Recently, a genome-wide association study identified linkage between variability in the human *ANK3* locus (encodes spectrin-associated ankyrin-G) and bipolar disorder [26]. Finally, dysfunction in ankyrins has been associated with potentially lethal cardiac arrhythmia syndromes. Human gene mutations in *ANK2* (encodes ankyrin-B) cause a complex arrhythmia syndrome characterized by sinus node dysfunction, atrial fibrillation, ventricular arrhythmias, and increased likelihood for sudden death [3,5,7,8,27]. Similarly, a human mutation that disrupts ankyrin-G/Nav1.5 interaction results in Brugada Syndrome, characterized by abnormal electrocardiograms and ventricular arrhythmia [4,9].

In this issue of *Journal of Molecular and Cellular Cardiology*, Ursitti and colleagues add to our understanding of the spectrin superfamily by identifying a novel splice variant of αII-spectrin in heart [28]. This new variant, termed cardi+ (named for its predominant expression in heart), accounts for almost 30% of total αII-spectrin in rat hearts at embryonic day 16, but only 6% after three weeks of age suggesting a possible role for αII-spectrin cardi+ in development. The authors also identify two new αII-spectrin isoforms, ∑9 and ∑10, containing the αII-spectrin cardi+ splice variant. Interestingly, the cardi+ insertion decreases the binding affinity of αII-spectrin for β-spectrin. Notably, αII-spectrin cardi+ fusion proteins containing the two terminal spectrin repeats were highly insoluble compared with αII-spectrin cardi-. As noted by the authors, these data suggest that the novel insertion may sequester the αII-spectrin cardi+ population from αII-spectrin cardi- polypeptides. However, the most significant finding from the study is the effect of the cardi+ sequence on cardiomyocyte cellular shape and organization. Specifically, neonatal cardiomyocytes positive for expression of GFP-αII-spectrin cardi+ display aggregates of the fusion protein throughout the cell. In contrast, GFP-αII-spectrin cardi- is diffusely spread throughout the myocyte cytoplasm. Moreover, GFP-αII-spectrin cardi+ expressing myocytes display pronounced processes with significant gaps between the membrane and the myofibrils. These processes are not observed in either control or GFP-αII-spectrin cardi- expressing myocytes.

In summary, the new findings or Ursitti, Bloch, and colleagues add important new understanding regarding the role of the spectrin-associated network in heart. However, these findings also illustrate how little we still understand about these critical molecules. For example, are specific spectrin isoforms restricted to unique excitable cell types (i.e. ventricular, atrial, sinus node)? Moreover, do C-terminal splicing events confer novel protein interaction sites or alter normal regulatory pathways (i.e. add/subtract phosphorylation sites)? Finally, do C-terminal splicing events alter the subcellular distribution and function of specific spectrin gene products? While much remains unknown about the function of different spectrin isoforms

in heart, this important new study adds to our growing appreciation for the diversity of spectrin polypeptides in excitable tissues.

Acknowledgments

We acknowledge support from the NIH (HL084583, HL083422 to PJM; HL096805 to TJH) and Pew Scholars Trust (PJM).

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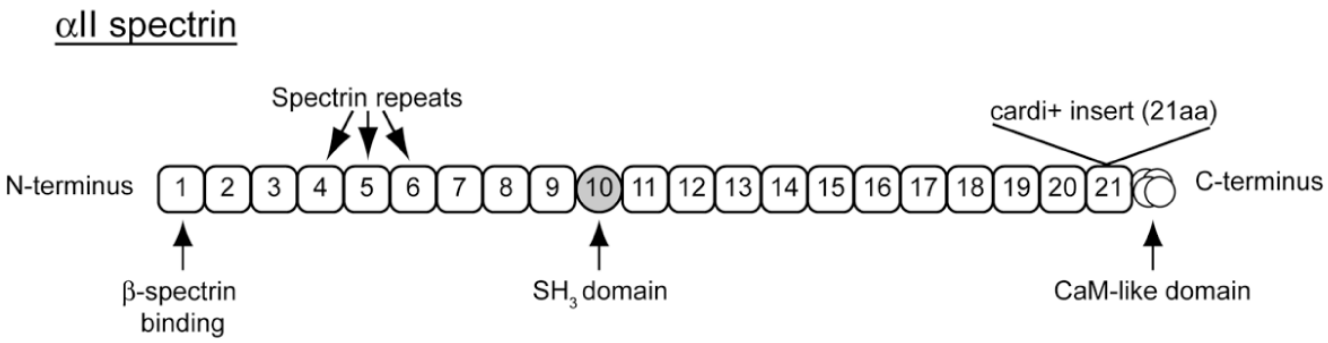

Figure 1. Structure of αII-spectrin and location of cardi+ insert

αII-spectrin is comprised of 21 triple helical spectrin repeats and a C-terminal calmodulin-like domain. Binding to β-spectrin occurs in repeat 1. Repeat 10 contains an SH3 domain commonly found in cell signaling proteins. The newly identified cardi+ splice variant results in a 21 amino acid insert in repeat 21 that affects binding affinity for β-spectrin and may regulate cardiomyocyte growth [28].