J. Membrane Biol. 211, 151–161 (2006)
DOI: 10.1007/s00232-006-0863-y

The Journal of
Membrane
Biology

Topical Review

Functional Links between Membrane Transport and the Spectrin Cytoskeleton

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Received: 5 June 2006/Revised: 10 July 2006

Abstract. Membrane transporters precisely regulate which molecules cross the plasma membrane and when they can cross. In many cases it is also important to regulate where substances can cross the plasma membrane. Consequently, cells have evolved mechanisms to confine and stabilize membrane transport proteins within specific subdomains of the plasma membrane. A number of different transporters (including ion pumps, channels and exchangers) are known to physically associate with the spectrin cytoskeleton, a submembrane complex of spectrin and ankyrin. These proteins form a protein scaffold that assembles within discrete subdomains of the plasma membrane in polarized cells. Recent genetic studies in humans and model organisms have provided the opportunity to test the hypothesis that the spectrin cytoskeleton has a direct role in restricting transporters to specialized domains. Remarkably, genetic defects in spectrin and ankyrin can produce effects on cell physiology that are comparable to knockouts of the transporters themselves.

Key words: Spectrin — Ankyrin — Sodium channel — Sodium pump — Potassium channel — *Drosophila* — Genetics

Introduction

The relationship between membrane transport and the spectrin cytoskeleton can be traced back to studies of the human erythrocyte membrane. A fundamental principle that emerged from the erythrocyte is that the structure and organization of the plasma membrane is determined in part by an underlying network of peripheral membrane proteins known as the spectrin cytoskeleton (reviewed by Lux & Palek, 1995). Defects in major components of the network including spectrin, ankyrin and protein 4.1 are associated with abnormal cell shape and membrane fragility (Tse & Lux, 1999). In addition to its structural properties, the submembrane network also acts locally to restrict the lateral mobility of the major membrane transporter in erythrocytes, the anion exchanger band 3 (Fowler & Branton, 1977; Golan & Veach, 1980; Nigg & Cherry, 1980). That effect on band 3 was the first glimpse of a widely occurring phenomenon. We now know that homologs of spectrin and ankyrin are present in many cell types throughout the animal kingdom and they are associated with a number of different membrane transport activities (reviewed by Bennett & Baines, 2001).

Several other proteins that were first identified as components of the spectrin cytoskeleton in erythrocytes are also members of broadly expressed gene families. For example, protein 4.1 belongs to a large superfamily of proteins with a conserved FERM domain (Bretscher et al., 2002). Likewise, the protein p55 belongs to the large family of PDZ domain-containing proteins (Funke et al., 2005). Both of these proteins are associated with the junctions between spectrin and actin that mediate network formation. But, even though these proteins are all part of one supramolecular complex in the erythrocyte, their relatives in other cell types often appear to function independently of spectrin. In contrast, interactions between spectrin and ankyrin are generally conserved among most isoforms and they will be considered here as a functional unit. However, this operational definition comes with the caveat that there are likely to be other interacting scaffold proteins that contribute to spectrin and ankyrin function in non-erythroid cells.

This review will focus on a series of recent studies that shed light on the relationships between the spectrin cytoskeleton and interacting membrane transport activities. In particular, I will summarize results of several genetic studies establishing that the behavior of specific membrane transporters is

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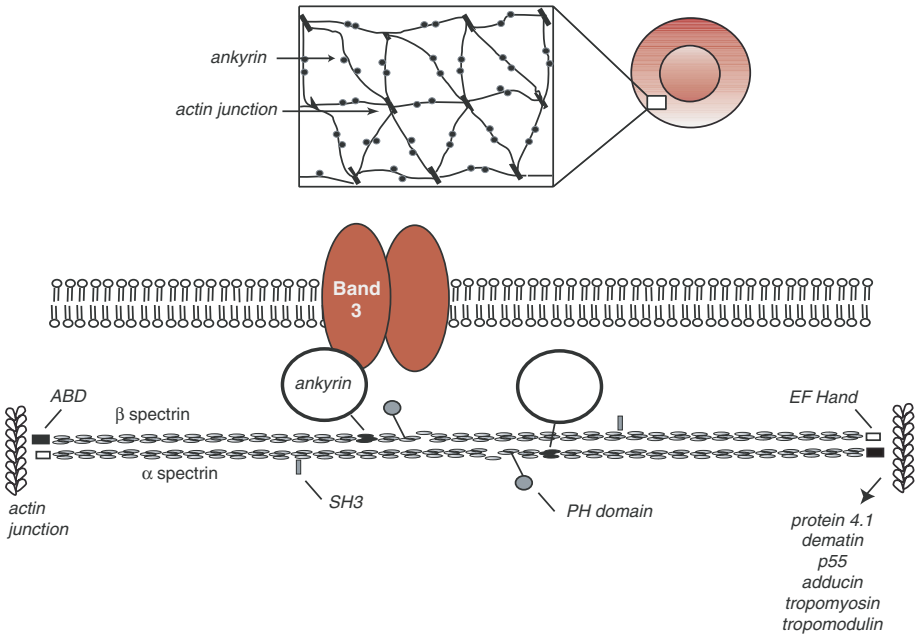

Fig. 1. The spectrin cytoskeleton forms a two-dimensional submembrane network in human erythrocytes (*top*). Spectrin molecules form junctional complexes with short actin filaments at their termini to generate a hexagonal pattern. A number of proteins (e.g., protein 4.1, p55) are associated with spectrin-actin junctions in erythrocytes, but they are not known to have a functional relationship to spectrin in other cells (*bottom*). Each spectrin molecule is a tetramer composed of α and β subunits (*bottom*). Much of the protein consists of three-barrel degenerate repeats. Partial repeats from the ends of α and β spectrin join together to produce a complete three-barrel structural repeat at the tetramer formation site near the center of the molecule. Non-repetitive sequences of spectrin include the N-terminal actin binding domain (ABD) on β, and a C-terminal EF-hand domain and an SH3 domain on α spectrin. A carboxy terminal pleckstrin homology (PH) domain is not usually present in the major splice variant of erythroid β spectrin, but it is commonly present in other spectrin isoforms. Segment 16 of β spectrin (*shaded*) includes an ankyrin binding site which can provide two potential membrane attachment sites per spectrin tetramer. Ankyrin attaches the spectrin scaffold to the anion exchanger band 3 in human erythrocytes, and to a host of different membrane transport proteins in other cell types.

dependent on the presence of an intact spectrin cytoskeleton. I will consider those cases where a direct link between transporters and the spectrin cytoskeleton has been established and where function has been directly tested by genetic analysis. The reader is referred to excellent previous reviews of the extensive spectrin literature and the interactions of spectrin and ankyrin with other classes of membrane proteins (e.g., Lux & Palek, 1995, Bennett & Baines, 2001).

Spectrin Cytoskeleton Structure

The spectrin cytoskeleton is a two-dimensional submembrane protein network in human erythrocytes. Spectrin molecules are a major structural component of the network, forming junctional complexes with short actin filaments at their termini to generate a hexagonal pattern (Fig. 1, boxed region; Byers & Branton, 1985). The developmental origin of the hexagonal pattern is an interesting problem, as it does not appear to be an inherent property of spectrin and actin. There may be contributions from interacting proteins found at the spectrin-actin junction of mature erythrocytes (such as protein 4.1, adducin, p55, etc.), or from other proteins transiently expressed during erythrocyte development (Bennett, 1989).

Each spectrin molecule is a tetramer composed of α and β subunits and most of each subunit consists of degenerate sequence repeats (~106 amino acids long) which are folded into three-barrel α-helical structures (Speicher & Marchesi, 1984; Yan et al., 1993). Tetramer formation depends on head-to-head interactions between α and β subunits near the center of the tetramer (Tse et al., 1990), and on lateral interactions between subunits at the tail ends (Harper et al., 2001). Actin binding activity resides at the amino terminus of the β subunit (Banuelos et al., 1998). Segment 16 of β spectrin (also referred to as the 15th repeat) includes an ankyrin binding site which provides two potential membrane attachment sites per spectrin tetramer (Kennedy et al., 1991). The membrane attachment site for ankyrin in the erythrocyte is a dimer of the anion exchanger band 3 (Bennett & Baines, 2001). Non-repetitive domains of α spectrin include a C-terminal EF-hand calcium-binding

domain and an SH3 motif within repetitive segment 9 (Wasenius et al., 1989). The pleckstrin homology (PH) domain shown at the C-terminus of β spectrin is not usually present in the major splice variant of erythroid β spectrin, but it is present in most other β spectrin isoforms (Tse et al., 2001; Pradhan et al., 2004).

Characteristics of the Spectrin Cytoskeleton in Non-erythroid Cells

The α and β spectrins and ankyrins are all members of multigene families (Bennett & Baines, 2001). In humans there are five β spectrin genes, two α spectrin genes and three ankyrin genes, with further diversity arising through alternative splicing of transcripts. Invertebrates such as *C. elegans* and *Drosophila* have a smaller repertoire with one α spectrin gene, two β spectrin genes and one or two ankyrin genes (respectively). The ankyrins exhibit greater diversity than spectrins, both between genes and by alternative splicing (Bennett & Chen, 2001). However, the adapter function of ankyrin (attachment of spectrin to membrane proteins) appears to be generally conserved among the plasma membrane-associated isoforms. Interactions with membrane proteins are mediated by an amino-terminal domain composed of 24 copies of a 33-amino acid ANK repeat. The ankyrin interaction with spectrin occurs within a ~150-amino acid domain near the center of the molecule (Mohler et al., 2004b).

All of the α and β spectrin genes have a characteristic domain organization which allows them to form structurally similar tetramers. The genes encoding mammalian erythrocyte α and β spectrin probably arose during vertebrate evolution and their sequence divergence has been ascribed to neofunctionalization (Salomao et al., 2006). Specialized high molecular weight β spectrins (βV in mammals and β_H in invertebrates) have a slightly different domain structure with a larger number of repeats than conventional β spectrins (Dubreuil et al., 1990; McKeown et al., 1998; Stabach & Morrow, 2000). These spectrins form tetramers with conventional α spectrin subunits, but the tetramers are unusually long and appear to associate with membranes independently of ankyrin. The remaining β spectrins (βII, βIII, and βIV in vertebrates and invertebrate β) exhibit substantial sequence similarity to one another throughout most of their length except for a region found between the last repeat and the PH domain (Tse et al., 2001). The functional significance of the sequence differences between β spectrin isoforms is not yet known.

Many of the functional similarities apparent in sequence comparisons of spectrins and ankyrins have also been directly tested. For example, non-erythroid spectrins share the ability to bind to actin filaments in vitro (Levine & Willard, 1981; Bennett et al., 1982; Glenney et al., 1982; Dubreuil et al., 1990). However, while actin seems certain to be a component of the spectrin cytoskeleton in non-erythroid cells, its structural organization and its contribution to function have yet to be characterized. Non-erythroid spectrins also bind to ankyrin (Davis & Bennett, 1984; Dubreuil & Yu, 1994) and they form a long-lived submembrane complex in polarized epithelial cells (Nelson & Veshnock, 1987a).

Two important differences were also immediately apparent in comparisons of the erythroid and non-erythroid spectrins and ankryins. First, whereas the spectrin cytoskeleton of erythrocytes was uniformly distributed beneath the plasma membrane, spectrin and ankyrin were found to be compartmentalized within specific subdomains of the plasma membrane in polarized cells such as neurons (Lazarides & Nelson, 1983), kidney cells (Drenckhahn et al., 1985; Nelson & Veshnock, 1986), and muscle (Craig & Pardo, 1983). Second, spectrin was attached via ankyrin to a single species of transmembrane protein in erythrocytes (Band *3*; Fig. 1). In contrast, non-erythroid ankyrins and spectrins have been shown to interact with a host of different membrane proteins (many of them transporters; Bennett & Baines, 2001). These observations formed the basis for the hypothesis that spectrin and ankyrin have a role in determining the composition and function of specialized regions of the plasma membrane (reviewed by Drubin & Nelson, 1996; Bennett & Chen, 2001).

It can be technically difficult to demonstrate an interaction between an integral membrane protein and a cytoskeletal protein. Spectrin, ankyrin and integral membrane proteins are all insoluble in vivo, and if anything they are even more insoluble when they interact (i.e., membrane proteins form detergent-resistant complexes with the cytoskeleton). Naturally, steps that render these proteins soluble, making them accessible to the techniques used to detect protein interactions, tend to disrupt the very interactions that one would like to detect. Fortuitously, the transporter that binds to ankyrin happens to be the most abundant integral membrane protein in human erythrocytes (Bennett & Baines, 2001). In other systems, membrane attachment sites are usually less abundant and exist in a background that is vastly more complex.

Despite these potential complications, a remarkable number of transporter interactions with the spectrin cytoskeleton have been described. The codistribution of ankyrin with the Na,K ATPase in epithelial cells suggested a molecular association that was ultimately reconstituted from purified proteins in vitro (Nelson & Veshnock, 1987b). Another one of the first interactions detected was based on the finding that ankyrin co-purified with voltage-dependent

sodium channels from rat brain (Srinivasan et al., 1988). Other candidate transporters had properties that suggested an interaction with the cytoskeleton and were found to associate with labeled ankyrin in direct binding assays in vitro. Thus, interactions between purified ankyrin and the Na/Ca exchanger from cardiac muscle (Li et al., 1993) and T lymphocyte IP_3 receptors (Bourguignon et al., 1993) were demonstrated in vitro. Some of these interactions were recently verified by co-immunoprecipitation of ankyrin with antibodies directed against the Na,K ATPase, the IP_3 receptor, and the Na/Ca exchanger (Mohler et al., 2003). The human erythrocyte Rh protein, an ammonium transporter, interacts with erythrocyte ankyrin in yeast 2-hybrid analysis (Nicolas et al., 2003). An ankyrin-binding site was also recently mapped to the cytoplasmic domain of the non-erythroid ammonium transporter RhBG and mutations in that site caused the protein to accumulate intracellularly, rather than at the basolateral membrane of MDCK cells (Lopez et al., 2005).

In addition to the ankyrin-dependent interactions, there are also direct interactions between spectrin and membrane transporters, including NMDA receptors (Wechsler & Teichberg, 1998), cGMP-gated cation channels (Molday et al., 1990), and the epithelial sodium channel ENaC (Rotin et al., 1994). The site in ENaC that interacts with spectrin was originally proposed to contribute to subcellular sorting of the channel. However, subsequent studies have shown that targeting information resides elsewhere in the molecule (Hanwell et al., 2002). Spectrin also codistributes with proton pumps in mammalian gastric parietal cells (Mercier et al., 1989) and recently an apical H^+ V-ATPase was found to be mislocalized in *Drosophila* β_H spectrin mutants (Philips & Thomas, 2006). However, a direct interaction between spectrin and proton pumps remains to be demonstrated. Finally, as described in greater detail below, a functional interaction between βIII spectrin and the glutamate transporter EAAT4 has recently been described (Ikeda et al., 2006). There are interactions between the spectrin cytoskeleton and proteins other than transporters such as CD44 (Kalomiris & Bourguignon, 1988), L1 family members (Davis et al., 1993), and CD45 (Pradhan & Morrow, 2002). But clearly, interactions with membrane transporters account for the majority of the known links between the spectrin cytoskeleton and cell membranes.

Genetic Studies of the Spectrin Cytoskeleton

Genetic analyses can proceed by many routes. Forward genetic approaches may begin with an unusual phenotype such as a human disease state, or a model system phenotype identified in a genetic screen. One may work backward through a candidate gene approach to directly associate the phenotype with a specific gene's defect. In the reverse genetic approach a model organism is used to knock out the gene of interest. From there a number of approaches can be taken, depending on the initial phenotype observed. If the gene defect leads to lethality it is often possible to perform cause of death studies to determine what kind of role the protein has under normal circumstances. In some cases, gene knockouts may produce only subtle defects, making it difficult to evaluate function (presumably because of other compensating or redundant gene activities). That has not been the case in studies of spectrin. Both forward and reverse genetic strategies have been employed to uncover dramatic phenotypic consequences arising from mutations in either spectrin or ankyrin.

Ankyrin-G and Voltage-Dependent Channels in Neurons

Ankyrin-G is one of three different ankyrin genes that are expressed in diverse mammalian cell types (reviewed by Bennett & Baines, 2001). Ankyrin-R, the founding member of the ankyrin family, was first identified in erythrocytes, but it is also known to be expressed in mammalian brain. In addition to anemia, loss of ankyrin-R function has been associated with a neurological syndrome that is not observed with other types of anemia (Peters et al., 1991). Ankyrin-B was originally identified in brain, but it is now known to be more broadly expressed. Ankyrin-B knockouts have a severe phenotype in brain that resembles loss of L1 function (Scotland et al., 1998) and they produce phenotypes in the thymus and in muscle (Tuvia et al., 1999; discussed further below). Ankyrin-G was initially discovered in brain as a component of the node of Ranvier and axon initial segment (Kordeli et al., 1995), and it was simultaneously identified as an epithelial ankyrin (Peters et al., 1995). The functional differences between these ankyrin isoforms were not immediately apparent from their sequences or biochemical properties. However, the pattern emerging from genetic studies is that mutations in each of the ankyrins produces a signature phenotype, even though more than one isoform may be expressed in a given cell type (Mohler & Bennett, 2005). Thus, despite their conserved features, it appears that the ankyrins have non-redundant functions in vivo.

Studies of ankyrin-G knockout mice established the functional significance of the interaction between ankyrin and voltage-dependent sodium channels. Targeted disruption of the ankyrin-G gene was achieved using an exon that is uniquely expressed in the cerebellum, thereby producing a tissue-specific effect (Zhou et al., 1998). Heterozygotes were indistinguishable from wild-type littermates. However,

when homozygous, the mutation led to a pronounced age-dependent ataxia in the cerebellum. Voltage-dependent sodium channels (Na_v), which normally localized to the axon initial segment of cerebellar granule cells, were mislocalized in the mutants and a similar reduction was presumed to occur in the Purkinje cells. These observations were consistent with the finding by electrophysiogical recordings that it was difficult to propagate an action potential in the Purkinje cells. Thus the normal accumulation of functioning sodium channels was compromised in the absence of ankyrin-G in cerebellum.

Recently KCNQ potassium channels were found to have a similar distribution to Na_v channels at the axon initial segment and the node of Ranvier in mammalian neurons (Pan et al., 2006; Chung et al., 2006). These studies revealed that KCNQ channels also interact with ankyrin-G and disruption of that interaction, either by knocking out ankyrin-G or by mutating the cytoplasmic domain of the channel, blocks KCNQ targeting. The ankyrin-G binding site in KCNQ was mapped to a region with remarkable sequence similarity to the corresponding binding site in the Na_V channel (LeMaillet et al., 2003; Garrido et al., 2003). Interestingly, phylogenetic analysis of these two channels revealed that while they are both conserved among vertebrates and invertebrates, the ankyrin binding function only appears to be present in vertebrates (Pan et al., 2006). Thus the interaction of these two types of voltage-dependent ion channels with ankyrin appears to be the product of convergent evolution after the split between vertebrates and invertebrates

βIV Spectrin and Voltage-dependent Sodium Channels

Further studies in the mouse established that the βIV subunit of spectrin codistributed with ankyrin-G at the node of Ranvier and at the axon initial segment (Berghs et al., 2000). Soon thereafter, truncations in mouse βIV spectrin were shown to be responsible for the mutant phenotype in *quivering* mice, which exhibit ataxia, tremor and deafness (Parkinson et al., 2001). In their study of βIV knockout mice, Komada and Soriano (2002) demonstrated that βIV spectrin interacts with ankyrin-G and that neither ankyrin-G nor voltage-gated sodium channels were correctly targeted to the axon initial segment or node of Ranvier in the mutants. Interestingly, these authors also demonstrated that βIV spectrin was mislocalized in ankyrin-G mutant mice, indicating that the targeting of ankyrin and spectrin was mutually dependent. Komada and Soriano (2002) suggested that the phenotype of quivering mice was caused by the effect of the βIV spectrin mutation on voltage-dependent sodium-channel targeting or stability. These observations suggest that βIV spectrin and ankyrin exert their effects on interacting membrane proteins in a coordinated fashion.

Several other interesting insights have emerged during characterization of βIV spectrin mutant mice. For example, βIV spectrin appears to be a major structural component of the node membrane, since the membrane undercoat visible by electron microscopy in control mice was absent from the node membrane of the mutants (Lacas-Gervais et al., 2004; Yang et al., 2004). In addition, the size and shape of the node were altered and there were conspicuous evaginations or swellings of the node membrane that were never observed in controls. These alterations were visible in both the central nervous system and in the peripheral nervous system, although they were more pronounced in the former. Interestingly, these studies detected several isoforms of βIV spectrin. In fact, the ''conventionally''- sized βIV product (288 kD) was a relatively minor component relative to an unusual variant (~150 kD) in which most of the amino terminal half (including the actin-binding domain and 10 repeats) was absent (Berghs et al., 2000). Yet, the node phenotype appeared similar whether knockouts affected all βIV spectrin isoforms (Komada & Soriano, 2002) or just the largest isoform (βIVE1; Lacas-Gervais et al., 2004). These observations raise the possibility that the novel small isoform may have unique functions that are distinct from conventional β spectrins.

Human βIII Spectrin and the Glutamate Transporter EAAT4

Sequencing of candidate mutations revealed that defects in the βIII spectrin gene are responsible for human spinocerebellar ataxia type 5 (Ikeda et al., 2006). Three mutations were identified in three independent lineages. Two affected the third spectrin repeat domain downstream of the actin-binding domain and the other affected the actin-binding domain (*see* Fig. 1). Interestingly, one of the mutations was traced through the pedigree of Abraham Lincoln and the authors suggest that the former US president may have suffered from this disease. βIII Spectrin was originally described as a novel spectrin isoform associated with intracellular vesicles and the Golgi apparatus in tissue culture cells (Stankewich et al., 1998; Siddhanta et al., 2003). However, less is known about the subcellular distribution of the protein in vivo. A yeast-2-hybrid study detected βIII spectrin in a screen for proteins that interact with the glutamate transporter EAAT4 (Jackson et al., 2001). The effects of the spinocerebellar ataxia mutations on EAAT4 behavior were assayed biochemically and in a tissue culture cell assay for protein mobility in the plasma membrane (Ikeda et al., 2006). The βIII spectrin mutations did not appear to affect the quantity of EAAT4 in autopsy

samples of brain tissue, but the extraction properties of EAAT4 were altered suggesting a possible change in its subcellular distribution or perhaps altered interactions with other brain proteins. The mobility of glutamate transporters in the plasma membrane of HEK293 cells was greatly reduced with co-expression of wild-type βIII spectrin, but not with the mutant version of spectrin. These results suggest that βIII spectrin has effects on interacting membrane activities at the plasma membrane, but they do not exclude the possibility that spectrin has additional effects within intracellular compartments (de Mattheis & Morrow, 2000).

Ankyrin-B and -G and Mammalian Cardiomyocyte Transporters

Sequencing of candidate mutations also led to the identification of human ankyrin-B defects as a cause of sudden cardiac death and cardiac arrhythmia (Mohler et al., 2003). The exact mechanism of this disease is not yet known, but several intriguing observations have been made in studies of Ank-B mutant mice, which serve as a valuable model. Initial characterization of homozygous Ank-B mutant mice revealed an abnormal localization of SR Ca ATPase and ryanodine receptors (Tuvia et al., 1999) in cardiomyocytes. However, a somewhat different picture emerged in studies of heterozygous Ank-B cardiomyocytes. There was an ~50% reduction in the amount of Ank-B detected in western blots of cardiac tissue, along with a selective loss of staining at T tubules and Z lines (Mohler et al., 2003). As expected from previous biochemical studies (Bennett & Baines, 2001), ankyrin-B co-immunoprecipitated with Na,K ATPase, InsP3 receptors and Na/Ca exchangers. Subsequent work has demonstrated that one ankyrin-B molecule can simultaneously bind to two of these molecules at once (and possibly three), thus enabling ankyrin-B to coordinate their transport activities in cardiac muscle (Mohler et al., 2005). The abundance of each of these proteins in mutant cardiac tissues was also reduced, as detected in binding studies and by immunolocalization (Mohler et al., 2003). It was tentatively concluded that the reduction in activity of the Na,K ATPase in response to the ankyrin-B mutation was likely to have a key role in the disease process because of its likely effects on calcium homeostasis.

Independent evidence for the importance of ankyrin-G in cardiac function came from studies of a Brugada syndrome patient (Mohler et al., 2004a). Brugada syndrome shares physiological properties with the ankyrin-B induced arrythmias described above, including sudden cardiac death. About 20% of cases of Brugada syndrome, including the one described here, can be attributed to voltage-dependent sodium-channel defects. The single amino acid change in a cytoplasmic loop of Nav1.5 (E1053K) found in this study coincides precisely with the ankyrin-G-binding activity of the sodium channel (Lemaillet et al., 2003; Garrido et al., 2003). An interaction between the ankyrin-G isoform expressed in cardiomyocytes and Nav1.5 was demonstrated in pulldown assays and by co-immunoprecipitation. That interaction was disrupted by the E1053K mutation (Mohler et al., 2004a). There were some subtle effects of the mutation on channel function when expressed in HEK293 cells, although the mutation did not affect its targeting to the plasma membrane or its ability to function as a sodium channel. However, the mutation did affect the ability of the protein to be stably expressed on the surface of cardiomyocytes. Thus the functional interaction between voltage-dependent sodium channels and ankyrin G first observed in neurons appears to also have important consequences for the behavior of the sodium channel in heart.

Drosophila β Spectrin and the Na,K ATPase

Invertebrate model systems have a number of potential advantages over mammalian systems in studies of protein function. Genetic approaches are usually more streamlined and often gene families in invertebrates are less complex than their vertebrate counterparts, making it easier to evaluate the outcome. Thus one would expect that *Drosophila* or *C. elegans* should be useful experimental systems in which to study the effects of the spectrin cytoskeleton on interacting membrane transporters. Yet there are also potential complications that can affect progress when studying specific protein interactions. First, not all protein interactions found in vertebrate systems are conserved in invertebrates. For example *para*, the *Drosophila* homolog of voltage-dependent sodium channel α subunits, lacks the conserved ankyrin-binding site found in vertebrate family members. Thus, as discussed above, the functional interaction between sodium channels and ankyrin may be a product of vertebrate evolution (Pan et al., 2006). Second, for those protein interactions that are conserved, suitable probes (e.g., antibodies) are required to study the effect of one gene on the fate of another gene (e.g., spectrin and sodium channels). Most of the probes that have been characterized in mammalian systems do not cross-react with their invertebrate counterparts. Consequently, work done in model organisms so far has followed a somewhat different course from the studies in vertebrate systems described above.

An effort has been made to develop and characterize reagents that allow the study of interactions between the spectrin cytoskeleton and membrane transporters in *Drosophila*. Three relevant interactions have been examined so far. First, there is a conserved interaction between ankyrin and L1-family

cell-adhesion molecules (Dubreuil et al., 1996; Hortsch et al., 1998). While interactions with cell adhesion molecules are beyond the scope of this review, it is worth noting them here as an example of functional conservation between vertebrate and invertebrate ankyrins. Second, we recently characterized a *Drosophila* homolog of the erythroid anion exchanger band 3 (Das et al., 2003). The goal was to develop another useful marker with which to evaluate the effect of spectrin mutations on interacting membrane proteins. However, despite significant sequence conservation relative to mammalian anion exchangers, antibody localization experiments failed to detect any significant codistribution of the anion exchanger with spectrin or ankyrin in *Drosophila* epithelial cells (*our unpublished observations*). Thus it appears that the well-characterized interaction between the anion exchanger (AE1) and the spectrin cytoskeleton in mammalian erythrocytes is not conserved in *Drosophila*. Third, an antibody raised against a chicken α subunit of the Na,K ATPase (Lebovitz et al., 1989) was used to monitor its fate in spectrin mutants. Staining experiments with that antibody have demonstrated a conserved functional interaction between Na,K ATPase and the spectrin cytoskeleton in *Drosophila* (Dubreuil et al., 2000).

The Na,K ATPase was one of the first membrane transport activities proposed to be functionally connected to the spectrin cytoskeleton in non-erythroid cells. It was found to codistribute and cofractionate with spectrin and ankyrin in MDCK cells (Nelson & Veshnock, 1986; Nelson & Hammerton, 1989) and it was induced to redistribute to cell contacts along with spectrin and ankyrin in fibroblasts expressing the adhesion molecule E-cadherin (McNeill et al., 1990). A direct interaction between Na,K ATPase and ankyrin has been demonstrated in a number of studies (Nelson & Veshnock, 1987b; Devarajan et al., 1994; Zhang et al., 1998). These observations were incorporated into a model in which the Na,K ATPase is stabilized at the plasma membrane by its direct interaction with the spectrin cytoskeleton through ankyrin (Drubin & Nelson, 1996).

The identification of spectrin mutants in *Drosophila* provided the first opportunity to directly test the hypothesis that the behavior of the Na,K ATPase in polarized cells was linked to the spectrin cytoskeleton. A null mutation in the sole *Drosophila* α spectrin gene was shown to be lethal, causing death early in larval development (Lee et al., 1993). Yet, despite a number of phenotypic consequences of the spectrin mutation, there did not appear to be any significant effect on either the accumulating level of the Na,K ATPase or its polarized distribution at the basolateral membrane domain of copper cells in the midgut epithelium. Based on that data it was concluded that the fate of the Na,K ATPase was independent of spectrin function.

Subsequent studies of lethal mutations in the conventional β spectrin gene of *Drosophila* detected a significant effect on the behavior of the Na,K ATPase in copper cells (Dubreuil et al., 2000). The prominent basolateral staining observed in the wild type was significantly reduced in quantity and was often shifted to a speckled pattern in the cytoplasm. In light of the β spectrin phenotype, we recently re-examined the fate of the Na,K ATPase in copper cells from α spectrin mutants and determined that there is a dramatic phenotype, but it is incompletely penetrant (*our unpublished observations*). One can find cells that have a relatively normal Na,K ATPase distribution, as originally described (Lee et al., 1993). However, most cells exhibit a loss of Na,K ATPase staining at the plasma membrane that is at least as severe as the phenotype observed in β spectrin mutants (Dubreuil et al., 2000). Thus the behavior of the Na,K ATPase in *Drosophila* copper cells depends on both α and β spectrin.

The interaction of the Na,K ATPase with the spectrin cytoskeleton in *Drosophila* seems likely to occur through ankyrin, as in mammalian systems. The sequences of the fly α subunit of Na,K ATPase and the human α1 Na,K ATPase are 74% identical, including 23/26 identical residues in the proposed minimal active peptide sequence of the ankyrin binding site (Lebovitz et al., 1989; Zhang et al., 1998). An ankyrin-1-GFP reporter expressed in copper cells codistributes with the Na,K ATPase throughout the basolateral domain (*unpublished observation*). But, while all of these observations are consistent with an interaction between fly ankyrin and the Na,K ATPase α subunit, that interaction remains to be directly demonstrated.

Implications and Insights

The work summarized here shows that the spectrin cytoskeleton has a role in modulating the behavior of interacting membrane transporters. Consequently, the spectrin cytoskeleton exerts an important influence on cell physiology, since both the subcellular location and the relative abundance of transporters are integral to their function. However, while the effects of the spectrin cytoskeleton are becoming clear, there are many remaining questions about the underlying mechanisms. In fact, some surprising differences are apparent when the systems that have been analyzed so far are compared:

1. A Central Role for Ankyrin. There is evidence from knockouts in *C elegans* and in mouse to suggest that ankyrin has a central role, carrying out its functions upstream of spectrin (Bennett & Chen, 2001) and in some cases even operating independently of spectrin (Mohler et al., 2004b). Cultured neonatal cardiomyocytes from heterozygous Ank-B

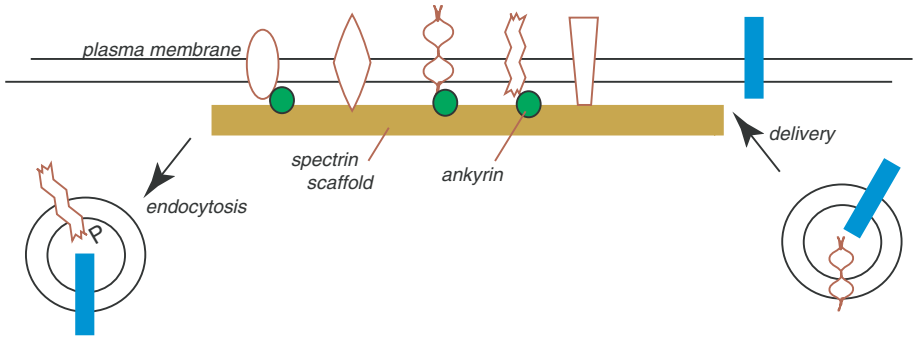

Fig. 2. The spectrin cytoskeleton is often found within specialized plasma membrane domains of polarized cells. A number of membrane transport proteins (depicted here by different shapes) bind to ankyrin through their cytoplasmic domains, enabling them to interact with the spectrin scaffold. Other transporters appear to interact directly with spectrin. An interaction with the spectrin cytoskeleton may block internalization by the endocytic pathway or lateral diffusion within the plane of the membrane, thereby promoting stable accumulation within a discrete plasma membrane domain. Some ankyrin interactions with the plasma membrane can be regulated by phosphorylation, which provides a potential mechanism to modify the composition of a specialized membrane domain.

(+/−) mutant mice exhibit defects in contractility and IP_3 receptor localization that can be rescued by expression of a wild-type Ank-B transgene. The phenotypes can also be rescued by expression of a defective transgene that lacks β-2 spectrin binding activity, indicating that the functions measured in this study did not require a direct interaction between ankyrin and spectrin (Mohler et al., 2004b).

2. A Central Role for Spectrin. There is also evidence that spectrin may function upstream and in some cases independently of ankyrin in *Drosophila* (Das et al., 2006). The lethal phenotype of a β spectrin mutation can be efficiently rescued by expression of a wild-type transgene (Dubreuil et al., 2000). A mutant transgene product that lacks ankyrin-binding activity can also rescue lethality ~20% of the time and its targeting to the plasma membrane is indistinguishable from the wild type (Das et al., 2006). Therefore, in this system there is a contribution of spectrin to viability that is independent of its ability to bind ankyrin, essentially the opposite of the effect described above. And both of these examples are distinctly different from the observation that the function and targeting of ankyrin-G and βIV spectrin in mammalian brain are interdependent (Komada & Soriano, 2002).

3. Secretory Pathway Effects. There is general agreement that spectrins and ankyrins form a cytoskeletal scaffold at the plasma membrane of most cells. But there is also a body of evidence implicating spectrin and ankyrin function in the secretory pathway of at least some cells (deMatteis & Morrow, 2000). Spectrin and ankyrin are envisioned as having broad effects on both structure and motility of secretory organelles with concomitant effects on delivery of interacting proteins to the plasma membrane. However, many basic details of the model have yet to be worked out. With regard to the issues discussed here, βIII spectrin was originally described as a component of a Golgi-associated skeleton (Stankewich et al., 1998). Yet the mutations in βIII spectrin that cause spinocerebellar ataxia affect the lateral mobility of EAAT4 at the plasma membrane rather than its delivery to the plasma membrane (Ikeda et al., 2006). That effect seems more consistent with a scaffold function at the plasma membrane than with a secretory defect. These issues (and others, too) are likely to be clarified once the differences that distinguish Golgi and plasma membrane spectrins and ankyrins have been more precisely defined.

4. Membrane Domain Formation. Another intriguing recent finding is that knockdown of ankyrin-G expression in cultured human bronchial epithelial cells blocked their ability to form a lateral plasma membrane domain (Kizhatil and Bennett, 2004). As a result, the morphology of the differentiated culture switched from columnar to a squamous-like epithelium. Before this work, most studies had focused on the ability of spectrin and ankyrin to influence the composition of a defined membrane domain. But in this case, ankyrin appears to function at the earliest step in domain formation. Further work will be necessary to determine how broadly this mechanism applies to the differentiation of other polarized cells.

These mechanistic differences between systems were unexpected and they raise new and interesting questions about the requirements for spectrin and ankyrin function. What determines sites of assembly? When and where in the cell does assembly occur? How can spectrin function independently of ankyrin, or ankyrin function independently of spectrin? Are there other proteins that can take either one's place? It seems likely at this point that the answers will depend to some extent on which system is studied. But, on the other hand, there is no *a priori* reason that all of the effects of the spectrin cytoskeleton on interacting transporters should rely

on exactly the same mechanism. Differences between systems may simply indicate that the interactions between the spectrin cytoskeleton and individual transporters arose through a series of independent steps.

In any case, the effects of spectrin and ankyrin mutations on transporter behavior can still be reconciled with the long-standing model of the spectrin cytoskeleton as a submembrane scaffold. In the model (Fig. 2), spectrin and ankyrin assemble within discrete membrane subdomains, usually at the plasma membrane. Different membrane transporters (represented here by different shapes) may become tethered within a membrane subdomain through interactions with either ankyrin or spectrin. Conversely, a molecule that does not interact with the spectrin cytoskeleton (shaded rectangle) would not be restricted to the region defined by the spectrin cytoskeleton and, in the absence of other stabilizing interactions, would be susceptible to removal from the plasma membrane by endocytosis. Fine-tuning of the composition of a plasma membrane domain could be achieved by regulating the interaction of transporters with the scaffold. For example, the interaction between ankyrin and L1-family cell adhesion molecules can be regulated by phosphorylation of their ankyrin-binding site (Garver et al., 1997). Phosphorylation of comparable sites in interacting transporters (*P*) could potentially regulate their association with and stabilization by the spectrin cytoskeleton.

In their transporter-centric description of protein-sorting in polarized cells, Muth & Caplan (2003) made the interesting (and admittedly untestable) suggestion that the mechanisms that generate and maintain cell polarity arose, in large measure, to produce asymmetrical membrane transport systems. They emphasized polarized membrane traffic and PDZ domain-containing scaffold proteins as two important mechanisms that contribute to transporter asymmetry. In light of the recent advances described here, the spectrin cytoskeleton appears to be another important effector of transporter asymmetry. Further characterization of the mechanisms of spectrin and ankyrin function is expected to provide important insights into human disease processes and also to enhance our understanding of the basic biology of polarized cells.

This work was supported by NIH GM49301.

References

Banuelos, S., Saraste, M., Carugo, K.D. 1998. Structural comparisons of calponin homology domains: implications for actin binding. *Structure* **6:**1419–1431

Bennett, V 1989. The spectrin-actin junction of erythrocyte membrane skeletons. *Biochim. Biophys. Acta* **988:**107–121

Bennett, V., Baines, A.J. 2001. Spectrin and ankyrin-based pathways: Metazoan inventions for integrating cells into tissues. *Physiol. Rev.* **81:**1353–1388

Bennett, V., Chen, L. 2001. Ankyrins and cellular targeting of diverse membrane proteins to physiological sites. *Curr. Op. Cell Biol.* **13:**61–67

Bennett, V., Davis, J., Fowler, W.E. 1982. Brain spectrin, a membrane-associated protein related in structure and function to human erythrocyte spectrin. *Nature* **299:**126–131

Berghs, S., Aggujaro, D., Dirkx, R., Maksimova, E., Stabach, P., Mermel, J.-M., Zhang, M.-P., Philbrick, W., Slepnev, V., Ort, T., Solimena, M. 2000. βIV spectrin, a new spectrin localized at axon initial segments and nodes of ranvier in the central and peripheral nervous system. *J. Cell Biol.* **151:**985–1001

Bourguignon, L.Y.W., Jin, H., Iida, N., Brandt, N.R., Zhang, S.H. 1993. The involvement of ankyrin in the regulation of inositol 1,4,5-triphosphate receptor-mediated internal Ca^{2+} release from Ca^{2+} storage vesicles in mouse T-lymphoma cells. *J. Biol. Chem.* **268:**7290–7297

Bretscher, A., Edwards, K., Fehon, R.G. 2002. ERM proteins and merlin: integrators at the cell cortex. *Nat Rev Mol Cell Biol* **3:**586–599

Byers, T.J., Branton, D. 1985. Visualization of the protein associations in the erythrocyte membrane skeleton. *Proc. Natl. Acad. Sci. USA* **82:**6153–6157

Craig, S.W., Pardo, J.V. 1983. Gamma actin, spectrin, and intermediate filament proteins colocalize with vinculin at costameres, myofibril-to-sarcolemma attachment sites. *Cell Motil.* **3:**449–462

Chung, H. J., Jan, Y. N., Jan, L.Y. 2006. Polarized axonal surface expression of neuronal KCNQ channels is mediated by multiple signals in the KCNQ2 and KCNQ3 C-erminal domains. *Proc. Natl Acad. Sci. USA* **103:**8870–8875

Das, A., Srinivasan, S., Base, C., Ng, P., Pruden, D., Dubreuil, R.R. 2003. Characterization of a new *Drosophila* anion exchanger (DAE) and its dependence on the spectrin cytoskeleton. *Mol. Biol.Cell* **14:**194a

Das, A., Base, C., Dhulipala, S., Dubreuil, R.R. 2006. Spectrin functions upstream of ankyrin in a spectrin cytoskeleton assembly pathway. J. Cell Biol., in press

Davis, J., Bennett, V. 1984. Brain ankyrin. *J. Biol. Chem.* **259:**13550–13559

Davis, J.Q., McLaughlin, T., Bennett, V. 1993. Ankyrin-binding proteins related to nervous system cell adhesion molecules: Candidates to provide transmembrane and intercellular connections in adult brain. *J. Cell Biol.* **121:**121–133

Devarajan, P., Scaramuzzino, D.A., Morrow, J.S. 1994. Ankyrin binds to two distinct cytoplasmic domains of Na,K-ATPase α subunit. *Proc. Natl. Acad. Sci. USA.* **91:**2965–2969

Drenckhahn, D., Schluter, K., Allen, D.P., Bennett, V. 1985. Colocalization of band 3 with ankyrin and spectrin at the basal membrane of intercalated cells in the rat kidney. *Science.* **230:**1287–1289

Drubin, D.G., Nelson, W.J. 1996. Origins of Cell Polarity. *Cell.* **84:**335–344

Dubreuil, R.R., Byers, T.J., Stewart, C.T., Kiehart, D.P. 1990. A β spectrin isoform from *Drosophila* (β_H) is similar in size to vertebrate dystrophin. *J. Cell Biol.* **111:**1849–1858

Dubreuil, R.R., MacVicar, G.R., Dissanayake, S., Liu, C., Homer, D., Hortsch, M. 1996. Neuroglian-mediated adhesion induces assembly of the membrane skeleton at cell contact sites. *J. Cell Biol.* **133:**647–655

Dubreuil, R.R., Wang, P., Dahl, S.C., Lee, J.K., Goldstein, L.S.B. 2000. *Drosophila* βspectrin functions independently of α spectrin to polarize the Na,K ATPase in epithelial cells. *J. Cell Biol.* **149:**647–656

Dubreuil, R.R., Yu, J. 1994. Ankyrin and β spectrin accumulate independently of α spectrin in *Drosophila*. *Proc. Natl. Acad. Sci. USA*. **91:**10285–10289

Fowler, V., Branton, D. 1977. Lateral mobility of human erythrocyte integral membrane proteins. *Nature* **268:**23–26

Funke, L., Dakoji, S., Bredt, D.S. 2005. Membrane-associated guanylate kinases regulate adhesion and plasticity at cell junctions. *Ann. Rev. Biochem.* **74:**219–245

Garrido, J.J., Giraud, P., Carlier, E., Fernandes, F., Moussif, A., Fache, M.-P., Debanne, D., Dargent, B. 2003. A targeting motif involved in sodium channel clustering at the axonal initial segment. *Science*. **300:**2091–2094

Garver, T.D., Ren, Q., Tuvia, S., Bennett, V. 1997. Tyrosine phosphorylation at a site highly conserved in the L1 family of cell adhesion molecules abolishes ankyrin binding and increases lateral mobility of neurofascin. *J. Cell Biol.* **137:**703–714

Glenney, J.R., Glenney, P., Osborn, M., Weber, K. 1982. An F-actin and calmodulin-binding protein from isolated intestinal brush borders has a morphology related to spectrin. *Cell.* **28:**843–854

Golan, D. E., Veatch, W. 1980. Lateral mobility of band 3 in the human erythrocyte membrane studied by fluorescence photobleaching recovery: Evidence for control by cytoskeletal interactions. *Proc. Natl Acad. Sci. USA* **77:**2537–2541

Hanwell, D., Ishikawa, T., Saleki, R., Rotin, D. 2002. Trafficking and cell surface stability of the epithelial Na^+ channel expressed in epithelial madin-Darby canine kidney cells. *J. Biol. Chem.* **277:**9772–9779

Harper, S.L., Begg, G.E., Speicher, D.W. 2001. Role of terminal nonhomologous domains in initiation of human red cell spectrin dimerization. *Biochemistry* **40:**9935–9943

Hortsch, M., O'Shea, K.S., Zhao, G., Kim, F., Vallejo, Y., Dubreuil, R.R. 1998. A conserved role for L1 as a transmembrane link between neuronal adhesion and membrane cytoskleton assembly. *Cell Adhesion & Communication.* **5:**61–73

Ikeda, Y., Dick, K.A., Westherspoon, M.R., Gincel, D., et al. 2006. Spectrin mutations cause spinocerebellar ataxia type 5. *Nat. Genetics*. **38:**184–190

Jackson, M., Song, W., Liu, M.-Y., Jin, L., Dykes-Hoberg, M., Lin, C.-L.G., Bowers, W.J., Federoff, H.J., Sternweis, P.C., Rothstein, J.D. 2001. Modulation of the neuronal glutamate transporter EAAT4 by two interacting proteins. *Nature*. **410:**89–93

Kalomiris, E.L., Bourguignon, L.Y.W. 1988. Mouse T lymphoma cells contain a transmembrane glycoprotein (gp85) that binds ankyrin. *J. Cell Biol.* **106:**319–327

Kennedy, S.P., Warren, S.L., Forget, B.G., Morrow, J.S. 1991. Ankyrin binds to the 15th repetitive unit of erythroid and nonerythroid β spectrin. *J. Cell Biol.* **114:**267–277

Kizhatil, K., Bennett, V. 2004. Lateral membrane biogenesis in human bronchial epithelial cells requires 190-kDa ankyrin-G. *J. Biol. Chem.* **279:**16706–16714

Komada, M., Soriano, P. 2002. BIV-spectrin regulates sodium channel clustering through ankyrin-G at axon initial segments and nodes of Ranvier. *J. Cell Biol.* **156:**337–348

Kordeli, E., Lambert, S., Bennett, V. 1995. Ankyrin-G. *J. Biol. Chem.* **270:**2352–2359

Lacas-Gervais, S., Guo, J., Strenzke, N., Scarfone, E., Kolpe, M., Jahkel, M., DeCamilli, P., Moser, T., Rasband, M.N., Solimena, M. 2004. BIVE1 spectrin stabilizes the nodes of Ranvier and axon initial segments. *J. Cell Biol.* **166:**983–990

Lazarides, E., Nelson, W.J. 1983. Erythrocyte and brain forms of spectrin in cerebellum: Distinct membrane-cytoskeleton domains in neurons. *Science* **220:**1295–1297

Lebovitz, R.M., Takeyasu, K., Fambrough, D.M. 1989. Molecular characterization and expression of the (Na+ + K+)-ATPase α-subunit in *Drosophila* melanogaster. *EMBO J.* **8:**193–202

Lee, J., Coyne, R., Dubreuil, R.R., Goldstein, L.S.B., Branton, D. 1993. Cell shape and interaction defects in α-spectrin mutants of *Drosophila* melanogaster. *J. Cell Biol.* **123:**1797–1809

Lemaillet, G., Walker, B., Lambert, S. 2003. Identification of a conserved ankyrin-binding motif in the family of sodium channel α subunits. *J. Biol. Chem.* **278:**27333–27339

Levine, J., Willard, M. 1981. Fodrin: Axonally transported polypeptides associated with the internal periphery of many cells. *J. Cell Biol.* **90:**631–643

Li, Z., Burke, E.P., Frank, J.S., Bennett, V., Phillipson, K.D. 1993. The cardiac Na+-Ca+ exchanger binds to the cytoskeletal protein ankyrin. *J. Biol. Chem.* **268:**11489–11491

Lopez, C., Metral, S., Eladari, D., Drevensek, S., Gane, P., Chambrey, R., Bennett, V., Cartron, J.-P., LeVanKim, C., Colin, Y. 2005. The ammonium transporter RhBG. *J. Biol. Chem.* **280:**8221–8228

Lux, S.E., J. Palek, 1995. Disorders of the Red Cell Membrane. In: Blood: Principles and practice of hematology. R.I. Handin, S.E. Lux, T.P. Stossel editors. J.B. Lippincott Co., Philadelphia. 1701–1818

Matteis, M.A.D., Morrow, J.S. 2000. Spectrin tethers and mesh in the biosynthetic pathway. *J. Cell Sci.* **113:**2331–2343

McKeown, C., Praitis, V., Austin, J. 1998. sma-1 encodes a β_H-spectrin homolog required for Caenorhabditis elegans morphogenesis. *Development* **125:**2087–2098

McNeill, H., Ozawa, M., Kemler, R., Nelson, W.J. 1990. Novel function of the cell adhesion molecule uvomorulin as an inducer of cell surface polarity. *Cell* **62:**309–316

Mercier, F., Teggio, H., Deviliers, G., Bataille, D., Mangeat, P. 1989. Membrane-cytoskeleton dynamics in rat parietal cells: Mobilization of actin and spectrin upon stimulation of gastric acid secretion. *J. Cell Biol.* **108:**441–453

Mohler, P.J., Bennett, V. 2005. Ankyrin-based cardiac arrhythmias: A new class of channelopathies due to loss of cellular targeting. *Curr. Op. Cardiol.* **20:**189–193

Mohler, P. J., Davis, J. Q., Bennett, V. 2005. Ankyrin-B coordinates the Na/K ATPase, Na/Ca exchanger, and InsP3 receptor in a cardiac T-tubule/SR microdomain. *PloS Biology* **3:**e423

Mohler, P.J., Rivolta, I., Napolitano, C., LeMaillet, G., Lambert, S., Priori, S.G., Bennett, V. 2004a. Nav1.5 E1053K mutation causing Brugada syndrome blocks binding to ankyrin-G and expression of nav1.5 on the surface of cardiomyocytes. *Proc. Natl. Acad. Sci. USA* **101:**17533–17538

Mohler, P.J., Schott, J.-J., Gramolini, A.O., Dilly, K.W., Guatimoisim, S., duBell, W.H., Song, L.-S., Haurogne, K., Kyndt, F., Ali, M.E., Rogers, T.B., Lederer, W.J., Escande, D., Marec, H.L., Bennett, V. 2003. Ankyrin-B mutations causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. *Nature* 634–639

Mohler, P.J., Yoon, W., Bennett, V. 2004b. Ankyrin-B targets B2-spectrin to an intracellular compartment in neonatal cardiomyocytes. *J. Biol. Chem.* **279:**40185–40193

Molday, L.L., Cook, M.J., Kaupp, U.B., Molday, R.S. 1990. The cGMP-gated cation-channel of bovine rod photoreceptor cells is associated with a 240 kDa protein exhibiting immunochemical cross-reactivity with spectrin. *J. Biol. Chem.* **265:**18690–18695

Nelson, W.J., Hammerton, R.W. 1989. A membrane-cytoskeletal complex containing Na^+, K^+-ATPase, ankyrin, and fodrin in Madin-Darby canine kidney (MDCK) cells: Implications for the biogenesis of epithelial cell polarity. *J. Cell Biol.* **108:**893–902

Nelson, W.J., Veshnock, P.J. 1986. Dynamics of membrane skeleton (fodrin) organization during development of polarity in Madin-Darby Canine Kidney epithelial cells. *J. Cell Biol.* **103:**1751–1765

Nelson, W.J., Veshnock, P.J. 1987a. Modulation of fodrin (membrane skeleton) stability by cell-cell contact in Madin-Darby Canine Kidney epithelial cells. *J. Cell Biol.* **104:**1527–1537

Nelson, W.J., Veshnock, P.J. 1987b. Ankyrin binding to (Na^+ & K^+) ATPase and implications for the organization of membrane domains in polarized cells. *Nature*. **328:**533–536

Nicolas, V., Kim, C.L.V., Gane, P., Birkenmeier, C., Cartron, J.-P., Colin, Y., Mouro-Chanteloup, I. 2003. Rh-RhAG/Ankyrin-R, a new interaction site between the membrane bilayer and the red cell skeleton, is impaired by Rhnull-associated mutation. *J. Biol. Chem.* **278:**25526–25533

Nigg, E. A., Cherry, R. J. 1980. Anchorage of a band 3 population at the erythrocyte cytoplasmic membrane surface: Protein rotational diffusion measurements. *Proc. Natl Acad. Sci. USA* **77:**4702–4706

Pan, Z., Kao, T., Horvath, Z., Lemos, J., Sul, J.-Y., Cranstoun, S.D., Bennett, V., Scherer, S.S., Cooper, E.C. 2006. A common ankyrin-G-based mechanism retains KCNQ and Nav channels at electrically active domains of the axon. *J. Neurosci.* **26:**2599–2613

Parkinson, N.J., Olsson, C.L., Hallows, J.L., McKee-Johnson, J., Keogh, B.P., Noben-Trauth, K., Kujawa, S.G., Tempel, B.L. 2001. Mutant β-spectrin 4 causes auditory and motor neuropathies in quivering mice. *Nature Gen.* **29:**61–65

Peters, L.L., Birkenmeier, C.S., Bronson, R.T., White, R.A., Lux, S.E., Otto, E., Bennett, V., Higgins, A., Barker, J.E. 1991. Purkinje cell degeneration associated with erythroid ankyrin deficiency in nb/nb mice. *J. Cell Biol.* **114:**1233–1241

Peters, L.L., John, K.M., Lu, F.M., Eicher, E.M., Higgins, A., Yialamas, M., Turtzo, L.C., Otsuka, A.J., Lux, S.E. 1995. Ank3 (epithelial ankyrin), a widely distributed new member of the ankyrin gene family and the major ankyrin in kidney, is expressed in alternatively spliced forms, including forms that lack the repeat domain. *J. Cell Biol.* **130:**313–330

Phillips, M.D., Thomas, G.H. 2006. Brush border spectrin is required for early endosome recycling in *Drosophila*. *J. Cell Sci.* **119:**1361–1370

Pradhan, D., Morrow, J.S. 2002. The spectrin-ankyrin skeleton controls CD45 surface display and interleukin-2 production. *Immunity* **17:**303–315

Pradhan, D., Tseng, K., Cianci, C.D., Morrow, J.S. 2004. Antibodies to β a I E2 spectrin identify in-homogeneities in the erythrocyte membrane skeleton. *Blood Cells, Molecules, & Diseases* **32:**408–410

Rotin, D., Bar-Sagi, D., O'Brodovich, H., Merilainen, J., Lehto, V.P., Canessa, C.M., Rossier, B.C., Downey, G.P. 1994. An SH3 binding region in the epithelial Na+ channel (alpharENaC) mediates its localization at the apical membrane. *EMBO J.* **13:**4440–4450

Salomao, M., An, X., Guo, X., Gratzer, W.B., Mohandas, N., Baines, A.J. 2006. Mammalian αI spectrin is a neofunctionalized polypeptide adapted to small highly deformable erythrocytes. *Proc. Natl Acad. Sci. USA* **103:**643–648

Scotland, P., Zhou, D., Benveniste, H., Bennett, V. 1998. Nervous system defects of ankyrin B (-/-) mice suggest functional overlap between the cell adhesion molecule L1 and 440 kD ankyrin B in premyelinated axons. *J. Cell Biol.* **143:**1305–1315

Siddhanta, A., Radulescu, A., Stankewich, M.C., Morrow, J.S., Shields, D. 2003. Fragmentation of the Golgi apparatus. *J. Biol. Chem.* **278:**1957–1965

Speicher, D.W., Marchesi, V.T. 1984. Erythrocyte spectrin is comprised of many homologous triple helical segments. *Nature* **311:**177–180

Srinivasan, Y., Elmer, L., Davis, J., Bennett, V., Angelides, K. 1988. Ankyrin and spectrin associate with voltage-dependent sodium channels in brain. *Nature* **333:**177–180

Stabach, P.R., Morrow, J.S. 2000. Identification and characterization of βV spectrin, a mammalian ortholog of *Drosophila* β_H spectrin. *J. Biol. Chem.* **275:**21385–21395

Stankewich, M.C., Tse, W.T., Peters, L.L., Ch'ng, Y., John, K.M., Stabach, P.R., Devarajan, P., Morrow, J.S., Lux, S.E. 1998. A widely expressed βIII spectrin associated with Golgi and cytoplasmic vesicles. *Proc. Natl. Acad. Sci. USA* **95:** 14158–14163

Tse, W.T., Lecomte, M.C., Costa, F.F., Garbarz, M., Feo, C., Boivin, P., Dhermy, D., Forget, B.G. 1990. A point mutation in the β-spectrin gene associated with α-I/74 hereditary elliptocytosis - implications for the mechanism of spectrin dimer self association. *J. Clin. Invest.* **86:**909–916

Tse, W.T., Lux, S.E. 1999. Red Blood cell membrane disorders. *Br. J. Hematol.* **104:**2–13

Tse, W.T., Tang, J., Jin, O., Korsgren, C., John, K.M., Kung, A.L., Gwynn, B., Peters, L.L., Lux, S.E. 2001. A new spectrin β4, has a major truncated isoform that associates with promyelocytic leukemia protein nuclear bodies and the nuclear matrix. *J. Biol. Chem.* **276:**23974–23985

Tuvia, S., Buhusi, M., Davis, L., Reedy, M., Bennett, V. 1999. Ankyrin-B is required for intracellular sorting of structurally diverse Ca^{2+}-homeostasis proteins. *J. Cell Biol.* **147:**995–1007

Wasenius, V.-M., Saraste, M., Salven, P., Eramaa, M., Holm, L., Lehto, V.-P. 1989. Primary structure of the brain -spectrin. *J. Cell Biol.* **108:**79–93

Wechsler, A., Teichberg, V.I. 1998. Brain spectrin binding to the NMDA receptor is regulated by phosphorylation, calcium and calmodulin. *EMBO J.* **17:**3931–3939

Yan, Y., Winograd, E., Viel, A., Cronin, T., Harrison, S.C., Branton, D. 1993. Crystal structure of the repetitive segments of spectrin. *Science* **262:**2027–2030

Yang, Y., Lacas-Gervais, S., Morest, D.K., Solimena, M., Rasband, M.N. 2004. βIV spectrins are essential for membrane stability and the molecular organization of nodes of Ranvier. *J. Neurosci.* **24:**7230–7240

Zhang, Z., Devarajan, P., Dorfman, A.L., Morrow, J.S. 1998. Structure of the ankyrin-binding domain of -Na,K-ATPase. *J. Biol. Chem.* **273:**18681–18684

Zhou, D., Lambert, S., Malen, P.L., Carpenter, S., Boland, L.M., Bennett, V. 1998. Ankyrin G is required for clustering of voltage-gated Na channels at axon initial segments and for normal action potential firing. *J. Cell Biol.* **143:**1295–1304