**Drosophila β Spectrin Functions Independently of α Spectrin to Polarize the Na,K ATPase in Epithelial Cells**

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**Abstract.** Spectrin has been proposed to function as a sorting machine that concentrates interacting proteins such as the Na,K ATPase within specialized plasma membrane domains of polarized cells. However, little direct evidence to support this model has been obtained. Here we used a genetic approach to directly test the requirement for the β subunit of the αβ spectrin molecule in morphogenesis and function of epithelial cells in Drosophila. β Spectrin mutations were lethal during late embryonic/early larval development and they produced subtle defects in midgut morphology and stomach acid secretion. The polarized distributions of αβ, spectrin and ankyrin were not significantly altered in β spectrin mutants, indicating that the two isoforms of Drosophila spectrin assemble independently of one another, and that ankyrin is upstream of αβ spectrin in the spectrin assembly pathway. In contrast, β spectrin mutations had a striking effect on the basolateral accumulation of the Na,K ATPase. The results establish a role for β spectrin in determining the subcellular distribution of the Na,K ATPase and, unexpectedly, this role is independent of α spectrin.

Key words: cell polarity • cytoskeleton • Drosophila melanogaster • plasma membrane • ankyrins

**Introduction**

Two different mechanisms have been proposed to explain the enrichment of Na,K ATPase within the basolateral membrane domain of polarized epithelial cells. One relies on sorting events in the secretory pathway that separate newly synthesized proteins into transport vesicles destined for either the basolateral or apical membrane domain. This model accounts for the observation that newly synthesized Na,K ATPase molecules are directly delivered to the basolateral domain (Caplan et al., 1986; Gottardi and Caplan, 1993; Zurzolo and Rodriguez-Boulan, 1993). The second relies on selective stabilization of Na,K ATPase molecules once they have arrived at the basolateral domain, whether or not the protein is sorted in the secretory pathway. This model was based on a series of observations: (a) the Na,K ATPase colocalized with sites of polarized spectrin/ankyrin assembly at the cytoplasmic face of the plasma membrane (Nelson and Veshnock, 1986; McNeill et al., 1990); (b) Na,K ATPase was shown to physically interact with ankyrin in vitro (Nelson and Veshnock, 1987); and (c) basolateral accumulation of Na,K ATPase was observed in an MDCK cell line that did not sort this protein in the secretory pathway (Hammerton et al., 1991; Mays et al., 1995). Direct evidence demonstrating that either of these mechanisms is sufficient to explain the steady-state distribution of the Na,K ATPase is lacking.

Genetic methods in Drosophila provide an opportunity to directly test these models. Virtually all of the known properties and protein interactions of spectrin are conserved in Drosophila relative to the vertebrate systems in which they were first characterized (for reviews see Bennett and Gilligan, 1993; Dubreuil, 1996). The native spectrin molecule consists of high molecular weight α and β subunits that assemble as elongated heterotetramers. Spectrin tetramers associate with ankyrin, which in turn associates with the cell adhesion molecule neuroglian and presumably other integral plasma membrane proteins. The sequence of the ankyrin binding site of the Na,K ATPase is conserved in Drosophila (Zhang et al., 1998), and the Na,K ATPase codistributes with ankyrin and spectrin in polarized fly cells (Baumann et al., 1994; Dubreuil et al., 1997). Yet, despite these conserved features, Na,K ATPase polarity was not detectably altered in epithelial cells from null α spectrin mutants (Lee et al., 1993, 1997). These
results led to the conclusion that basolateral accumulation of the Na,K ATPase in Drosophila epithelia did not require a stabilizing interaction with the spectrin membrane skeleton.

Here we describe the production and initial characterization of lethal mutations in the Drosophila β spectrin gene. These mutations provide an opportunity to use a genetic approach to directly test the proposed functions of the conventional β spectrin isoform that is broadly expressed in nonerythroid cells. The phenotypes of mutations in the β subunit of spectrin in Drosophila (Thomas et al., 1998) and Caenorhabditis elegans (M.C. Ewton et al., 1998) have already been described. β subunit is an unusual spectrin isoform found in invertebrates that appears to have specialized functions that are distinct from conventional β spectrins (Dubreuil and Grushko, 1998). We focused attention on midgut copper cells because of their unique form and function, which make them well-suited for studies of the role of the spectrin membrane skeleton in the development and function of polarized cells (Lee et al., 1993; Dubreuil et al., 1998). The results provide the first direct evidence that spectrin contributes to the polarized distribution of the Na,K ATPase in epithelial cells and, unexpectedly, that the β subunit of spectrin carries out this role independently of α spectrin.

Materials and Methods

Fly Stocks and Genetic Screen

A lethal deficiency in the β spectrin region of the X chromosome, Df(1)S10D, was produced by γ-ray mutagenesis of flies carrying the γ-R P-element insertion S5.9-11 (Wakimoto et al., 1986). In brief, adult males were exposed to 3,300 rads from a Cs⁺ source, crossed to γR γR γR γR females, then scored for γ-R female progeny. A total of 32 γ-R revertant females were recovered from 145,000 females screened, and three hemizygous lethal lines were obtained. Df(1)S10D was the only cytologically visible deficiency (16A-16D-E) that lacked the β spectrin gene by polytene hybridization. Duplications of the β spectrin region on chromosome 3 were produced by γ-ray mutagenesis of the stock Tp(1;3)B53, which is marked with the Bar-super dominant eye phenotype. Tp(1;3)B53 males and females were irradiated with 4,000 rad, then crossed to Oregan R females, Bar-eye male progeny retaining the duplication were mated with w/yw/yw females, and crosses were scored for reversion of the male sterile phenotype. Screening of 36,000 progeny yielded three male-fertile duplications that are chromosomal and do not recombine with the X chromosome.

Identification and Characterization of β Spectrin Mutants

Chemically-induced X-linked lethal mutations in the region of the β spectrin gene were identified in a genetic screen for rescue by Dp(1;3)B310D (a duplication that

Abbreviation used in this paper: GFP, green fluorescent protein.
spans the β spectrin locus; Fig. 1). Mutant alleles from six independent complementation groups met the criterion of survival only in the presence of the duplication. Four complementation groups were rescued by the smaller duplication Dp(1;3)B28bD2, which defines interval II. None of the mutations were rescued by the smallest duplication, Dp(1;3)B28bD1. The complementation groups in interval II failed to complement the deficiency Df(1)SD10, which spans the β spectrin locus. The mutations in interval II were further tested for their ability to be rescued by a β spectrin transgene, P[β-specT3I], composed of the cDNA coding sequence for β spectrin under control of the Drosophila ubiquitin promoter. Using this strategy, complementation group 2 from interval II was identified as the β spectrin locus. Hereafter, these four mutant alleles are referred to as β-specem6, β-specem12, β-specem15, and β-specem21. None of the other mutations in interval II or III were rescued by the β spectrin transgene.

Initial examination of the β spectrin mutants indicated that they were embryonic lethal. To further characterize them, balanced stocks of each allele over a GFP-marked FM7 balancer chromosome were generated. Heterozygous mutant females in these stocks produced mutant male progeny that were identified by their lack of GFP expression. Using this strategy, it was found that β spectrin mutant males developed to the point of hatching (Table I). However, most of the mutants died before hatching, even though they appeared to be fully developed and motile. The β-specem6 and β-specem15 alleles had the lowest hatching rates (~10%), and the β-specem12 and β-specem21 alleles had a somewhat higher hatching rate (~25%) relative to their wild-type siblings. Whether or not they hatched, the mutant larvae survived for 1–3 d. A null mutation in Drosophila α spectrin (α-spec941) was characterized previously as a first instar larval lethal (Lee et al., 1993). Here we quantified the hatching rate of α-spec941 (Table I) and found that it was on average approximately fivefold greater than the strongest β spectrin allele (β-specem6).

The β spectrin mutants that hatched were generally similar in appearance and behavior to null α spectrin mutants. Newly hatched larvae initially crawled, ate, and responded to touch, but they grew increasingly sluggish over time, failed to grow, and eventually died.

The same FM7[Kr-GFP]-based strategy was used to isolate mutant embryos for Western blot analysis using a rabbit anti-β spectrin antibody. Staining of wild-type embryo revealed the expected 273-kD full-length β spectrin polypeptide (Fig. 2, lanes 1, 3, 5, and 7) as well as an ~150-kD proteolytic fragment. The β-specem6 (Fig. 2, lane 2) and β-specem15 (Fig. 2, lane 6) mutants exhibited no significant reactivity with the antibody, indicating that all of the proteins detected in wild-type siblings are encoded by the β spectrin gene. Truncated immunoreactive β spectrin products were observed in β-specem12 and β-specem21 (Fig. 2, lanes 4 and 6, asterisks), with estimated mobilities of 190 kD and 170 kD, respectively. The ~150-kD proteolytic fragment of β spectrin was observed in the latter two mutants as well. The truncated fragments were faintly visible in wild-type siblings (Fig. 2, lanes 3 and 7), but were under-represented here because the embryo collection included homozygotes for the β-spec+ balancer chromosome as well as β-spec+/FM7[Kr-GFP] heterozygotes.

Based on the size of the truncated products in β-specem12 and β-specem21, a PCR-based strategy was developed to an-
analyze the genomic sequence of each mutant allele. A ≈2-kb fragment of genomic DNA from each mutant was amplified, cloned in duplicate from independent PCR reactions, and sequenced on both DNA strands. The nonmutagenized parent chromosome was used as a control in these reactions. The sequence of the parent chromosome was identical in this region to the previously published \( \beta \)-spectrin cDNA sequence (Byers et al., 1992), except for several silent third base substitutions. The \( \beta \)-spec\textsuperscript{em12} and \( \beta \)-spec\textsuperscript{em21} sequences revealed nonsense stop codons (TGA) at the position of the highly conserved tyrosine TGG codon found within the spectrin repeat domain (Fig. 2 B). The nonsense mutations occurred near the start of repeat 12 in \( \beta \)-spec\textsuperscript{em12} and near the start of repeat 13 in \( \beta \)-spec\textsuperscript{em21}. The predicted molecular masses of these truncated products (175 kD and 188 kD, respectively) agreed well with the estimated sizes of the truncated products observed in Western blots. Both of these truncations deleted the ankyrin binding site of \( \beta \) spectrin (Kennedy et al., 1991).

Role of \( \beta \) Spectrin in Epithelial Differentiation

The cellular consequences of the \( \beta \) spectrin mutations were analyzed in epithelial cells of the larval middle midgut. The copper cells in particular were shown previously to require \( \alpha \) spectrin for their normal differentiation and function in stomach acid secretion (Lee et al., 1993; Dubreuil et al., 1998). These cells have a peculiar invaginated morphology in which the apical cell surface is tucked within the cell body (Fig. 3 A). The invagination is connected to the gut lumen through a pore formed by neighboring interstitial cells. Smooth septate junctions (Dubreuil, R.R., T. Grushko, O. Baumann, manuscript submitted for publication) occupy the apicolateral contact region between copper cells and interstitial cells, forming a collar that surrounds the pore. Despite their unusual morphology, copper cells exhibit many of the properties of conventional epithelia. The apical surface, extending inward from the collar, displays densely packed microvilli toward the gut lumen. The basolateral domain, including the apicolateral collar, is the site of contact with neighboring cells in the epithelial sheet. All plasma membrane markers that have been examined so far are segregated within either the apical or the basolateral domain.

Double-label immunofluorescent staining was used to compare the relative distributions of ankyrin and \( \beta \) spectrin within the basolateral membrane domain of copper cells. \( \beta \) spectrin, encoded here by an epitope-tagged transgene, was detected throughout the basolateral region in first instar larvae (Fig. 3 B, arrow), and was especially concentrated in the apicolateral collar (Fig. 3 B, arrowhead). A nkyrin was also concentrated at the collar, with only faint staining visible in the rest of the basolateral domain. A nkyrin staining appeared as comma shapes on either side of the entrance to the apical invagination in favorable optical sections (Fig. 3 C, arrowhead). As larvae grew and copper cells increased in size, ankyrin staining became visible throughout the basolateral domain (Fig. 3 D, arrow), although ankyrin remained relatively concentrated at the apicolateral contacts (Fig. 3 D, arrowhead). These results are consistent with a role for ankyrin in attaching \( \alpha \)\( \beta \) spectrin to the plasma membrane, both at the apicolateral contact region and throughout the rest of the basolateral domain of copper cells. However, ankyrin staining outside of the apicolateral collar was relatively weak and near the threshold of detection in first instar larvae.

A nkyrin staining was used to monitor the effect of \( \beta \) spectrin mutations on cell pattern in the middle midgut epithelium. The en face pattern of ankyrin staining in the first instar middle midgut provides a convenient map of cell outlines in the epithelial sheet (Fig. 4 A) (Dubreuil et
The apicolateral contacts between wild-type copper cells and interstitial cells appeared as small rings (Fig. 4 A, arrow) interconnected by lines that represent contacts between adjacent interstitial cells (Fig. 4 A, arrowhead). Ankyrin staining revealed the same overall pattern of cell contacts in β-spectrin mutants (Fig. 4, B and C) and β-spectrin transgene (not shown) male first instar larvae, indicating that development of the cell pattern was normal in the mutants and that the association of ankyrin with the plasma membrane was independent of β-spectrin. However, whereas the ring-shaped profiles were consistently small in the posterior region of the wild-type middle midgut (Fig. 4 A), the rings from β-spectrin mutants were large and irregular. In some cases, the diameter of the pore remained relatively small, whereas the zone of ankyrin staining was broadened into a wide collar (Fig. 4 B). In other cases, the thickness of the ring of ankyrin staining remained narrow, but the pore size was expanded (Fig. 4 C) as in most anterior copper cells of the wild-type middle midgut (Dubreuil et al., 1998). Thus, the size and shape of the apicolateral contact between copper cells and interstitial cells was dependent on β-spectrin function.

The effects of β-spectrin mutations on α and βH spectrin assembly were also examined by immunofluorescence. The α subunit of spectrin was associated with both the apical and basolateral domains of the wild-type copper cell plasma membrane (Fig. 5 A) (Lee et al., 1993). The βH subunit, in contrast, was exclusively associated with the apical invagination (Fig. 5 B) where it colocalized with 30% of pixels in the α-spectrin channel. The spacing and organization of cells in the wild-type midgut was remarkably consistent. In contrast, α and βH spectrin staining patterns in the mutants were highly irregular and it was difficult to find fields of cells that were all in the same plane of focus. There was a conspicuous shift in the α-spectrin staining pattern of β-spectrin copper cells (Fig. 5 D). Much of the α-spectrin signal in the mutants colocalized with βH spectrin in the apical invagination with 78% pixel overlap of βH spectrin (Fig. 5 E) with α-spectrin. There was also conspicuous cytoplasmic staining of α-spectrin in the mutants that was not typically observed in wild-type. Some residual α-spectrin staining of the basolateral membrane observed in β-spectrin mutants (data not shown). Most consistent feature observed with both antibodies was staining of the apical invagination. Identical results were obtained with β-spectrin mutants (data not shown). Thus, β-spectrin appeared to be required for efficient basolateral targeting of the α subunit, but not for the apical assembly of αβH spectrin.

The effect of β-spectrin mutations on plasma membrane polarity was monitored by staining for the Na,K ATPase, which is normally concentrated in the basolateral membrane domain of copper cells (Lee et al., 1993). In wild-type larvae, the en face Na,K ATPase pattern appeared as rings representing the copper cell basolateral domain (Fig. 6 A). Optical sections through the central region of the gut revealed that basolateral staining of copper cells extended...
up to the point of apicolateral contact with interstitial cells (Fig. 6 B). A fine reticular pattern of cytoplasmic staining was also observed, but most of the signal was associated with the plasma membrane. A striking change in the distribution of Na,K ATPase staining was observed in β-spec<sup>em6</sup> mutants (Fig. 6, C and D). Identical results were observed in β-spec<sup>em15</sup> mutants (data not shown). The nature of the change was dependent on the region of the gut examined. The most anterior copper cells (arrowheads) exhibited occasional plasma membrane staining, although in most cells the Na,K ATPase was associated with intracellular compartments. In the most posterior cells, Na,K ATPase staining was typically punctate and irregular (arrows). The large puncta of staining were often closely apposed to the nucleus, indicating that the Na,K ATPase was intracellular rather than clumped at the plasma membrane. Copper cells in between these two regions exhibited very weak staining that was not obviously associated with the plasma membrane. Thus, it appears that there are different fates of the Na,K ATPase within copper cell subpopulations in the β spectrin mutants. However, in all cases, the normal accumulation of Na,K ATPase at the plasma membrane was severely perturbed by the loss of β spectrin function.

**Role of β Spectrin in Epithelial Function**

The physiological role of copper cells is to secrete stomach acid (Dubreuil et al., 1998). A cid secretion is easily monitored by feeding larvae with yeast paste containing bromphenol blue. The dye changes from a brilliant blue color (pH > 4) to a bright yellow color (pH < 2.35) in the copper cell region of wild-type larvae. In between these pH ranges, the dye exhibits a variable green color. α Spectrin mutants were previously found to lack detectable midgut acidification, presumably because of defects within the apical or basolateral domain, or both, of copper cells (Dubreuil et al., 1998). Results of bromphenol blue feeding experiments with β spectrin mutants and their wild-type sibs are summarized in Fig. 7. As expected, most yw control larvae and larvae carrying the β-spec<sup>-FM7[Kr-GFP] balancer chromosome exhibited strong (pH < 2.3) midgut acidification. A significant fraction of the β-spec<sup>em6</sup> and β-spec<sup>em15</sup> mutant larvae also exhibited acidification below pH 2.3. Interestingly, the β-spec<sup>em12</sup> and β-spec<sup>em21</sup> mutants, which express large truncated fragments of β spectrin, were less efficient in acid secretion than the mutants that altogether lack detectable β spectrin. Nevertheless, the effect on midgut acidification in β spectrin mutants was small compared with the previously described null α spectrin mutants (Dubreuil et al., 1998). Based on these results we conclude that the β spectrin mutations had little effect on plasma membrane integrity or the ac-
Spectrin Functions Independently of α Spectrin

Discussion

The results of this study provide a number of novel insights into αβ spectrin assembly and function. (a) The most striking new observation was the discovery that the basolateral distribution of the Na,K ATPase in larval copper cells was dependent upon β spectrin function, but not on α spectrin. (b) β Spectrin is essential: mutations in the β spectrin gene were lethal early in development. (c) β Spectrin is downstream of ankyrin in the membrane skeleton assembly pathway, since ankyrin remained associated with the plasma membrane in β spectrin mutants. (d) Spectrin isoforms assemble independently of one another. The αβH isoform of spectrin remained associated with the apical membrane domain of epithelial cells in β spectrin mutants. (e) β Spectrin mutations produced a relatively modest effect on the acid secretion activity of midgut copper cells, in contrast to the severe defect observed in α spectrin mutants. Thus, the latter phenotype appears largely attributable to loss of αβH spectrin function from the apical domain of copper cells.

Essential Function of β Spectrin

β Spectrin mutants complete much of embryonic development before they ultimately die as fully formed larvae. The broad expression pattern of αβ spectrin in the developing embryo (Pesacreta et al., 1989) makes it likely that many different tissues are affected by the mutations, any of which could be responsible for lethality. The β spectrin lethal phenotype is remarkably similar to mutations in genes that affect nervous system function, such as synaptotagmin (DiAntonio et al., 1993). Indeed, spectrin is a major structural protein in neurons, comprising ~2.4% of total protein in mammalian brain homogenates (Davis and Bennett, 1983). Future studies of the Drosophila nervous system in α and β spectrin mutants are therefore likely to provide valuable insights into the function of spectrin in the brain.

The lethality of the β spectrin mutations reflects an essential requirement for αβ spectrin function. Mutations in the α subunit affect both spectrin isoforms and they result in death early in larval development (Lee et al., 1993). In contrast, mutations in βH spectrin produce a more subtle phenotype (Thomas et al., 1998). Occasionally mutants altogether lacking βH spectrin survive to adulthood and reproduce, although many die as larvae. The early larval-lethal phenotypes of α and β spectrin mutations suggest a stringent requirement for αβ spectrin function early in development, with a less stringent requirement for αβH spectrin at later stages.

The β spectrin mutants die shortly before larval hatching, whereas α spectrin mutants generally die after hatch-
ing. Survival through embryonic development in both cases is thought to depend on maternally derived protein. There are two possible explanations for the observed differences in lethal phase. There may be functions of β spectrin that are independent of α spectrin, including a role in larval hatching. The differential effects of α and β spectrin mutations on Na,K ATPase polarity (discussed below) support this interpretation. Or there may simply be a larger pool of maternally contributed α spectrin that outlasts the β spectrin pool, allowing α spectrin mutants to survive longer. Further experiments will be necessary to distinguish between these possibilities.

Spectrin in Copper Cell Differentiation and Function

A though mutations in the α and β subunits of spectrin were both lethal, they produced remarkably different phenotypes in the larval midgut epithelium. It was previously shown that α spectrin mutants had severe defects in the apical morphology and acid secretion activity of copper cells, but there was no apparent effect on basolateral accumulation of the Na,K ATPase (Lee et al., 1993, 1997; Dubreuil et al., 1998). Thus, it appears that the α subunit is critical to the function of αβH spectrin in the apical domain of copper cells. In contrast, the finding that Na,K ATPase accumulation was perturbed in β spectrin mutants, but not in α spectrin mutants, suggests that β spectrin functions independently of α spectrin in the basolateral region of copper cells. β Spectrin mutations also affected morphogenesis of the apical domain of copper cells. The severity of the apical defect in α spectrin mutants precludes analysis of whether or not this structural role of β spectrin is also independent of α spectrin.

Copper cells differentiate in response to extracellular gradients of wingless and decapentaplegic during embryonic development, which in turn activate expression of the homeotic gene labial within copper cell progenitors (Hoppler and Bienz, 1994, 1995). The opening of the apical invagination of copper cells to the gut lumen is constricted into a narrow pore in the most posterior cells, which are closest to the source of the wingless signal. The opening gradually broadens toward the anterior, as cells receive a weaker wingless signal and express less labial protein (Hoppler and Bienz, 1994, 1995; Dubreuil et al., 1998). Ankyrin and β spectrin are both highly enriched in the apical domain of copper cells. We speculate that spectrin and ankyrin couple these components to a cytoplasmic contractile mechanism that determines the diameter of the apical pore as well as the size and shape of the surrounding sepalate junction. Interestingly, the phenotype of a weak labial mutant allele in adult Drosophila copper cells includes a defect in apical domain pattern that is remarkably similar to the β-spectrom phenotype described here (Dubreuil, R.R., T. Grushko, O. Baumann, manuscript submitted for publication).

Spectrin as a Determinant of Plasma Membrane Polarity

The quantity and distribution of the Na,K ATPase at the basolateral membrane of copper cells was significantly altered in β spectrin mutants. Based on the known protein interactions of the spectrin membrane skeleton in vertebrates and its conserved properties in Drosophila, this effect is likely to involve disruption of a ternary complex between the Na,K ATPase, ankyrin, and spectrin. This population of spectrin and ankyrin appears to be distinct from the apicolateral population described above, since the Na,K ATPase was excluded from the apicolateral region of copper cells (Fig. 6 B).

The proposed role of spectrin in the development of plasma membrane polarity is based on its capacity to transduce subcellular positional information. The term positional information is traditionally used to describe the cues that govern tissue patterning in developing organisms. Virtually the same problem of establishing and interpreting spatial coordinates is faced during the differentiation of polarized cells (Drubin and Nelson, 1996). The αβ isoform of spectrin assembles in response to the positional cue of cell adhesion during the differentiation of many polarized epithelial cells. Once targeted to a discrete plasma membrane domain, the spectrin membrane skeleton is thought to capture and stabilize additional interacting membrane proteins, thereby altering the composition and function of that domain (Drubin and Nelson, 1996; Dubreuil, 1996).

The model requires that spectrin simultaneously receive and transmit subcellular positional information. That requirement is potentially met by the spectrin tetramer, which includes two ankyrin binding sites (Fig. 8 a). One ankyrin molecule may interact with a source of positional information such as a cell adhesion molecule, whereas a second ankyrin molecule transmits positional information to the Na,K ATPase, resulting in its stable accumulation within the basolateral domain. The positional cue responsible for ankyrin and αβ spectrin assembly at the basolateral domain of copper cells is not known. By analogy to other systems, it is likely to be a cell adhesion molecule.

Biochemical studies have shown that ankyrin has the capacity to simultaneously interact with multiple integral membrane proteins such as the cell adhesion molecule neurofascin and the anion exchanger (Michaely and Bennett, 1995). From this result, one might predict that ankyrin functions as a transmitter of positional information independently of spectrin (Fig. 8 b). However, the current results demonstrate that the Na,K ATPase also relies on β spectrin to acquire its polarized distribution. One possible explanation of this requirement is that the membrane binding activity of ankyrin is dependent on its physical association with β spectrin, perhaps through an allosteric mechanism. It has also been suggested that ankyrin-independent membrane binding sites convey positional information to β spectrin, which then transmits the information to ankyrin and the Na,K ATPase (Dvarajan and Morrow, 1996). This latter alternative seems unlikely, since the current results demonstrate that ankyrin can acquire positional information in the absence of β spectrin. A nother possible explanation is that β spectrin may be required to cross-link ankyrin molecules that independently associate with the source of positional information and the Na,K ATPase (Fig. 8 c). For example, spectrin-ankyrin complexes could be linked together through the actin binding activity of β spectrin. Similar models have been
proposed to explain the autonomous biochemical properties of β-spectrin in vertebrate muscle (Böloch and Morrow, 1989; Porter et al., 1997). Further genetic studies aimed at identifying the minimal functional unit of β-spectrin that can support polarized accumulation of Na,K-ATPase will provide a powerful experimental approach with which to distinguish between these possibilities.

The exact fate of the Na,K-ATPase in the β-spectrin mutants is not yet known. Indeed, there appear to be multiple fates that correlate with the morphology, and perhaps the physiology, of the affected cell. The anterior-most copper cells often exhibited some detectable plasma membrane staining of the Na,K-ATPase, whereas the more posterior copper cells exhibited either complete loss of staining or dense aggregates. There may be differences between copper cell populations in the timing of their differentiation during development, or perhaps different rates of degeneration in the absence of β-spectrin. Interestingly, overexpression of the ankyrin binding domain of β-spectrin in mammalian Caco-2 cells also caused the disappearance of Na,K-ATPase staining at the plasma membrane along with the appearance of diffuse cytoplasmic staining and cytoplasmic aggregates (Hu et al., 1995). Recent studies of an ankyrin knock-out mouse demonstrated that two other ankyrin-associated membrane proteins, the voltage-dependent sodium channel and neurofascin, rely on the spectrin membrane skeleton for their normal accumulation at the axon initial segment of Purkinje neurons (Zhou et al., 1998). These observations are consistent with a role for the spectrin membrane skeleton in stabilizing membrane activities and/or preventing their endocytosis after delivery to the plasma membrane. However, based on the demonstration that β-spectrin interacts with the Na,K-ATPase within the secretory pathway (Devarajan et al., 1997), it will be important in future studies to address the possibility that the β-spectrin mutants described here affect the accumulation of the Na,K-ATPase before its arrival at the cell surface.

We thank Dr. Tom Kornberg (University of California San Francisco, San Francisco, CA) for providing the GFP-marked FM7 balancer chromosome, and Dr. Doug Fambrough (Johns Hopkins University, Baltimore, MD) for providing the v5 antibody against the chicken Na,K-ATPase. We thank Tanya Grushko and Grete Linder for technical assistance with antibody labeling experiments, Jenny Xu for assistance with mutagenesis, and Dr. Anthony Mahowald for comments on the manuscript. Special thanks to Dr. Vytas Bindokas for expert assistance with confocal microscopy and image analysis, and to Susan M. Lundy, M.A.M.S., for artwork. We also gratefully acknowledge Dr. Daniel Branton for support and encouragement during the initial stages of this work.

Supported by National Institutes of Health grants GM 49301 and DK 42086 to R.R. Dubreuil. L.S.B. Goldstein is an investigator of the Howard Hughes Medical Institute.

Submitted: 1 October 1999
R evised: 20 March 2000
A ccepted: 23 March 2000

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