

Segregation of Two Spectrin Isoforms: Polarized Membrane-binding Sites Direct Polarized Membrane Skeleton Assembly

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Submitted May 15, 1997; Accepted July 14, 1997
Monitoring Editor: Masatoshi Takeichi

Spectrin isoforms are often segregated within specialized plasma membrane subdomains where they are thought to contribute to the development of cell surface polarity. It was previously shown that ankyrin and β spectrin are recruited to sites of cell–cell contact in *Drosophila* S2 cells expressing the homophilic adhesion molecule neuroglian. Here, we show that neuroglian has no apparent effect on a second spectrin isoform ($\alpha\beta_H$), which is constitutively associated with the plasma membrane in S2 cells. Another membrane marker, the Na,K-ATPase, codistributes with ankyrin and $\alpha\beta$ spectrin at sites of neuroglian-mediated contact. The distributions of these markers in epithelial cells in vivo are consistent with the order of events observed in S2 cells. Neuroglian, ankyrin, $\alpha\beta$ spectrin, and the Na,K-ATPase colocalize at the lateral domain of salivary gland cells. In contrast, $\alpha\beta_H$ spectrin is sorted to the apical domain of salivary gland and somatic follicle cells. Thus, the two spectrin isoforms respond independently to positional cues at the cell surface: in one case an apically sorted receptor and in the other case a locally activated cell–cell adhesion molecule. The results support a model in which the membrane skeleton behaves as a transducer of positional information within cells.

INTRODUCTION

The spectrin-based membrane skeleton is a nearly ubiquitous structural component of the plasma membrane in eukaryotic cells. It forms a proteinaceous network at the cytoplasmic face of the membrane where it interacts with integral membrane proteins and other molecules of the cytoskeleton (reviewed by Bennett and Gilligan, 1993; Lux and Palek, 1995). Genetic studies have established essential roles for spectrin and related proteins in cell shape and tissue integrity. Defects in human erythrocyte spectrin cause abnormal cell shape, altered membrane deformability, and consequent anemia (reviewed by Lux and Palek, 1995). Defects in a nonerythroid spectrin from *Drosophila* are lethal and result in abnormal cell shape and aberrant epithelial organization (Lee *et al.*, 1993). Defects in the spectrin-like molecule dystrophin are the

cause of Duchenne muscular dystrophy (Anderson and Kunkel, 1992; Campbell, 1995). In each case, the membrane skeleton defect is thought to disrupt the mechanical properties of the plasma membrane.

Most of our knowledge of the structure and biochemistry of the membrane skeleton has come from studies of erythrocyte spectrin. Spectrin is a rod-shaped ~ 200 -nm-long heterotetramer composed of α and β subunits (Bennett and Gilligan, 1993; Lux and Palek, 1995). An actin-binding site is found at each end of the spectrin tetramer, enabling spectrin and actin to associate in a long-range two-dimensional network beneath the plasma membrane. In the erythrocyte, ~ 6 spectrin molecules converge at junctional complexes containing a single actin filament to yield a geodesic structure beneath the lipid bilayer (Byers and Branton, 1985; Liu *et al.*, 1987). Direct structural evidence is lacking, but the conserved protein interactions of nonerythroid spectrins suggest that they are also arranged in a protein network beneath the plasma membrane.

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Studies of the membrane skeleton in nonerythroid cells demonstrated that spectrin and ankyrin are often segregated within specialized domains of the plasma membrane (Lazarides and Nelson, 1983; Drenckhahn *et al.*, 1985; Flucher and Daniels, 1989; reviewed by Bennett and Gilligan, 1993). In some cases there are multiple membrane skeleton domains composed of distinct spectrin and ankyrin isoforms within a single cell (Lazarides *et al.*, 1984; Nelson and Lazarides, 1984; Goodman *et al.*, 1995). Therefore, sorting mechanisms must exist to correctly target the membrane skeleton to a particular plasma membrane domain and to segregate spectrin and ankyrin isoforms in the plane of the membrane. However, it is difficult to identify the cues that target the membrane skeleton, because spectrin and ankyrin are already associated with the plasma membrane in most cells that have been examined and also because of the complexity of possible attachments between spectrin and the plasma membrane.

There are two major classes of interaction between spectrin and the plasma membrane. The first identified and best characterized link is mediated by ankyrin (Bennett, 1992). Ankyrin is a peripheral membrane protein that interacts with the β subunit of spectrin and with the cytoplasmic domains of several integral membrane proteins such as the erythrocyte anion exchanger, the Na,K-ATPase, sodium channels, and cell adhesion molecules (Bennett and Gilligan, 1993). A second class of interactions involves the ankyrin-independent sites on spectrin which are thought to directly bind integral membrane proteins (Steiner and Bennett, 1988; Lombardo *et al.*, 1994). Spectrin is known to interact with epithelial sodium channels (Rotin *et al.*, 1994) and CD45 (Iida *et al.*, 1994) by this mechanism. Additional indirect connections between spectrin and the plasma membrane have been described in the human erythrocyte (e.g., via protein 4.1; reviewed by Lux and Palek, 1995), but the significance of these sites in nonerythroid cells is not known.

The interactions between the membrane and membrane skeleton can also be grouped according to their downstream effects. In some cases, membrane proteins appear to target the assembly of the membrane skeleton. For example, spectrin and ankyrin are recruited to sites of cell-cell contact by a direct interaction between ankyrin and the cytoplasmic domains of *Drosophila* neuroglian and human L1 (Dubreuil *et al.*, 1996; Hortsch *et al.*, 1997). Spectrin and ankyrin are also recruited to sites of E-cadherin-mediated adhesion (McNeill *et al.*, 1990), perhaps via an association between spectrin and α catenin (Devarajan and Morrow, 1996). In other cases, the membrane skeleton appears to influence the composition of a plasma membrane domain by stabilizing interacting membrane proteins. One example is the Na,K-ATPase,

which codistributes with spectrin and ankyrin at sites of cell contact in E-cadherin-expressing cells (McNeill *et al.*, 1990). Na,K-ATPase molecules are short-lived at the cell surface in the absence of spectrin and ankyrin (Hammerton *et al.*, 1991). Another example is the loss of dystrophin-associated glycoproteins in Duchenne muscular dystrophy patients. A complement of sarcolemmal glycoproteins copurifies with dystrophin from normal muscle, but these proteins are virtually absent from the sarcolemma of dystrophic muscle (Ervasti *et al.*, 1990; Matsumura and Campbell, 1994).

Drosophila S2 tissue culture cells provide a unique model system in which to study the cues for membrane skeleton assembly and its contribution to plasma membrane domain organization. Ankyrin and the $\alpha\beta$ isoform of spectrin are not detectably associated with the plasma membrane in the absence of a suitable membrane anchor. However, spectrin and ankyrin are selectively recruited to sites of cell-cell contact in transfected S2 cells that express the cell adhesion molecule neuroglian (Dubreuil *et al.*, 1996). Neuroglian is the *Drosophila* homologue of the L1-neurofascin-NrCam family of vertebrate cell adhesion molecules which are known to interact with ankyrin *in vitro* (Davis and Bennett, 1994). By manipulating neuroglian expression, it is possible to study the steps of membrane skeleton assembly under controlled conditions in S2 cells. Here, we examine three parameters of membrane skeleton function and organization. First, we describe an isoform of spectrin in S2 cells ($\alpha\beta_H$) that behaves independently of $\alpha\beta$ spectrin and ankyrin. Second, we examine the distribution of the Na,K-ATPase as a marker for the effect of the membrane skeleton on interacting membrane proteins. Third, we describe the distribution of all of these proteins in dissected *Drosophila* tissues and we propose that mechanisms identified in S2 cells also contribute to the segregation of membrane skeleton domains during the development of polarized cells *in vivo*.

MATERIALS AND METHODS

Antibodies

The specificities of affinity-purified rabbit anti-*Drosophila* ankyrin antibody (5 $\mu\text{g}/\text{ml}$; Dubreuil and Yu, 1994; Dubreuil *et al.*, 1996), mouse monoclonal α spectrin antibody (1:10 culture supernatant; Dubreuil *et al.*, 1987), and rabbit anti-*Drosophila* β spectrin antibody (1:250–1:500 serum; Byers *et al.*, 1989) were described previously. The mouse antichickens Na,K-ATPase antibody $\alpha 5$ was previously shown to cross-react with the α subunit of the *Drosophila* Na,K-ATPase (1:500 ascites fluid; Lebovitz *et al.* 1989; Schubiger *et al.*, 1994). The monoclonal 1B7 antibody was previously shown to react specifically with *Drosophila* neuroglian (1:500 ascites fluid; Bieber *et al.*, 1989). Affinity-purified fluorescein isothiocyanate- (FITC) conjugated goat anti-rabbit antibody (Sigma Chemical Co., St. Louis, MO) and affinity-purified Texas Red-conjugated goat anti-mouse antibody (Jackson Immunoresearch Laboratories, West Grove, PA) were used at 1:250–1:500. Alkaline phosphatase-conjugated secondary

goat anti-rabbit antibody (Zymed Laboratories, South San Francisco, CA) was used at 1:1000.

A β_H spectrin-specific antibody was produced against a recombinant β_H spectrin fragment expressed in bacteria. A 1.8-kb fragment (*Bam*HI-*Kpn*I) of β_H spectrin cDNA from the amino terminal coding region (Dubreuil *et al.*, 1990) was expressed in pBluescript (Stratagene, La Jolla, CA), and the protein product was purified by its insolubility after cell lysis in the presence of neutral detergent. The protein was electroeluted from polyacrylamide gels and injected into a rabbit, as previously described for production of ankyrin antibody (Dubreuil and Yu, 1994). Immune serum was affinity purified using standard methods, and the antibody was diluted to 0.48 μ g/ml-0.96 μ g/ml for immunolocalizations and 0.24 μ g/ml for Western blots.

Cell Lines and Immunofluorescent Staining

Drosophila S2 tissue culture cells were grown under standard conditions in Schneiders *Drosophila* medium with 10% fetal calf serum (both from Life Technologies, Gaithersburg, MD) and 50 U/ml penicillin-50 μ g/ml streptomycin (Sigma) at 25°C. Stably transfected cells were induced to express a neuroglian transgene (180-kDa isoform) under control of the metallothionein promoter (Hortsch *et al.*, 1995) by addition of 0.7 mM copper chloride to the growth medium. Cells were incubated for 18 to 24 h to allow neuroglian expression and formation of cell aggregates before attaching them to Alcian blue-coated microscope slides. Cells were fixed with 2% freshly prepared formaldehyde in phosphate-buffered saline and then permeabilized with 0.1% Triton X-100 in buffered saline before staining as previously described (Dubreuil *et al.*, 1996).

Cells were viewed and photographed with an ausJena microscope (Jena, Germany) using a 50 \times or 25 \times Plan Apo objective. Photographs were taken on either TMax400 or Ektachrome 400 film. Transparencies were digitized with a Polaroid SprintScan scanner.

Isolation and Staining of *Drosophila* Tissues

Salivary glands were dissected from late third instar larvae in fresh 2% formaldehyde in phosphate-buffered saline, pH 7.1. Glands were fixed for an additional 10 min at room temperature, rinsed for 10 min in *Drosophila* Ringer's solution, and then permeabilized for 30 min in Tris-buffered saline (pH 7.5) containing 0.1% Tween 20 and 1% Triton X-100. Staining of glands was carried out as previously described for S2 cells (Dubreuil *et al.*, 1996), except that tissues were kept in suspension, primary antibody incubations were carried out overnight at 4°C, and secondary antibody incubations were carried out for 3 h at room temperature. Ovaries were dissected from adult female flies and processed as above except that formaldehyde fixation was carried out on ice rather than at room temperature.

Western Blots

Gel electrophoresis, electrophoretic transfer to nitrocellulose, and reaction with antibodies were carried out as described previously (Dubreuil *et al.*, 1987).

RESULTS

We previously used ankyrin and β spectrin as markers of membrane skeleton assembly in neuroglian-expressing S2 cells (Dubreuil *et al.*, 1996). Upon expression of neuroglian and formation of cell aggregates, ankyrin (Figure 1B) and β spectrin (Figure 3B) were selectively recruited to sites of cell-cell contact. In Figure 1, the distribution of the α subunit of spectrin

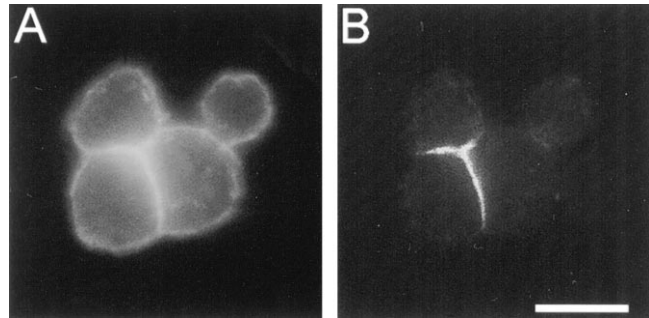


Figure 1. Spectrin and ankyrin have distinct, partially overlapping distributions in neuroglian-expressing *Drosophila* tissue culture cells. Cell aggregates, formed upon expression of neuroglian, were double labeled with mouse anti- α spectrin (A) and rabbit anti-ankyrin (B) primary antibodies, followed by Texas red- and FITC-conjugated secondary antibodies, respectively. Bar, 10 μ m.

was compared with ankyrin using a previously characterized monoclonal antibody (Dubreuil *et al.*, 1987; Figure 2, lane 1). Unlike ankyrin, α spectrin was constitutively associated with the plasma membrane of single S2 cells and neuroglian-expressing S2 cell aggregates (Figure 1A). The lack of ankyrin colocalization with α spectrin at nonadherent regions of the plasma membrane suggested that there is a population of spectrin in S2 cells that associates with the plasma membrane independently of ankyrin.

Two isoforms of spectrin have been described in *Drosophila* tissue culture cells: $\alpha\beta$ and $\alpha\beta_H$ (Dubreuil *et al.*, 1990). There is a single known α spectrin gene in *Drosophila* (Byers *et al.*, 1987), and its product associates with either the β or the β_H subunit (products of



Figure 2. Western blot analysis of α spectrin and isoform-specific β spectrin antibodies. Total S2 cell proteins were reacted with monoclonal anti- α spectrin antibody (lane 1), polyclonal anti- β spectrin (lane 2), or affinity-purified anti- β_H spectrin antibodies (lane 3) and stained with alkaline phosphatase-conjugated secondary antibody.

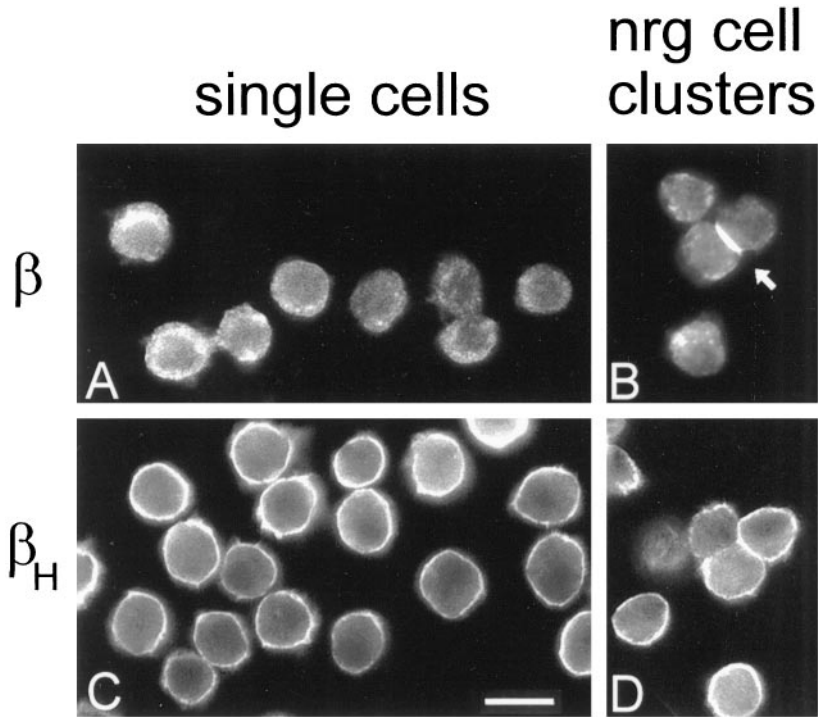


Figure 3. Distinct distributions of two β spectrin isoforms in *Drosophila* tissue culture cells before and after expression of neuroglial and formation of cell–cell contacts. Control S2 cells (A and C) and neuroglial-expressing S2 cell aggregates (B and D) were attached to microscope slides and stained with antibodies against β spectrin (A and B; 1:500 serum) or β_H spectrin (C and D; 5 $\mu\text{g}/\text{ml}$) followed by FITC-conjugated secondary antibody. Arrowhead marks the accumulation of β spectrin at a cell–cell contact. Bar, 10 μm .

distinct genes; Byers *et al.*, 1989; Dubreuil *et al.*, 1990) to form spectrin heterotetramers. The specificity of polyclonal antibodies against the two β spectrin isoforms was demonstrated in Western blots of total S2 cell proteins (Figure 2). The anti- β spectrin antibody (Byers *et al.*, 1989) reacted with its 265-kDa antigen (Figure 2, lane 2) and the anti- β_H spectrin antibody reacted with its 430-kDa antigen (Figure 2, lane 3) with no detectable cross-reactions. Despite its unusually large size, *Drosophila* β_H spectrin is a bona fide β subunit that forms spectrin tetramers resembling conventional spectrins (Dubreuil, 1996; see DISCUSSION).

The isoform-specific β spectrin antibodies revealed two distinct spectrin distributions in control S2 cells and neuroglial-expressing S2 cell clusters. The sum of their staining patterns corresponded to the broad distribution of α spectrin (Figure 1A). β spectrin was not detectably associated with the plasma membrane of control cells (Figure 3A) but it was recruited to the plasma membrane at sites of cell–cell contact in neuroglial-expressing cells (Figure 3B). In contrast, the β_H -specific antibody stained the plasma membrane of all cells (Figure 3C). β_H spectrin appeared to be uniformly distributed along the plasma membrane, although some variations in staining intensity were apparent (presumably because of the topology of the cell surface). Neuroglial-expressing S2 cell clusters exhibited the same uniform distribution of β_H spectrin at the plasma membrane, with no apparent concentra-

tion of this isoform at sites of neuroglial-mediated cell–cell contact (Figure 3D).

Previous studies demonstrated that the Na,K-ATPase interacts directly with ankyrin (Nelson and Veshnock, 1987) and that it colocalizes with spectrin and ankyrin at sites of E-cadherin-mediated cell–cell contact in mammalian cells (McNeill *et al.*, 1990). We examined the distribution of the Na,K-ATPase in neuroglial-expressing S2 cells using a monoclonal antibody against the α subunit of the chicken Na,K-ATPase that was previously shown to react with the *Drosophila* Na,K-ATPase (Lebovitz *et al.*, 1989; Schubiger *et al.*, 1994). Antibody staining revealed colocalization of the Na,K-ATPase with ankyrin at sites of cell–cell contact (Figure 4). Of the cell contacts that stained detectably with ankyrin antibody, 67% also stained with the Na,K-ATPase antibody ($n = 610$). The Na,K-ATPase was weakly stained in a punctate pattern at the plasma membrane of nonadherent cells or at noncontact sites of adherent cells, presumably because of its low abundance in S2 cells relative to other *Drosophila* cell types that have been studied (e.g., salivary gland, Figure 5).

We next examined the distribution of α , β , and β_H spectrin, ankyrin, neuroglial, and the Na,K-ATPase in vivo. The epithelial cells of the larval salivary gland abundantly expressed neuroglial at lateral regions of cell–cell contact (Figure 5A). Ankyrin colocalized with neuroglial at these sites (Figure 5B), as observed in S2 cells. A similar staining pattern was detected with

antibodies against α and β spectrin (our unpublished results). The Na,K-ATPase (Figure 5C) codistributed with ankyrin (Figure 5D) at lateral sites of cell–cell contact. Ankyrin and the Na,K-ATPase were also detected at the basal surface of cells (Figure 5, E and F), but neither protein was detected at the apical surface, facing the gland lumen. Whereas β_H spectrin was uniformly distributed at the surface of cultured S2 cells, it was found almost exclusively at the apical domain of the salivary gland epithelium (Figure 5H, thick arrow). In the same field, the Na,K-ATPase was concentrated at lateral sites of cell–cell contact (Figure 5G, thin arrow). Thus, the two spectrin isoforms are segregated in a nonoverlapping distribution in the salivary gland epithelium, in contrast to their overlapping distribution in neuroglian-expressing S2 cells.

Salivary glands from third instar larvae typically exhibited a thin epithelial cell layer surrounding a large gland lumen (Figure 5). The β_H spectrin staining pattern in glands from first and second instar larvae (our unpublished results) and from embryos (Thomas and Kiehart, 1994) defined a relatively small lumen and a proportionately thicker epithelium. However, regardless of the stage examined, the distributions of $\alpha\beta_H$ spectrin at the apical surface and $\alpha\beta$ spectrin/ankyrin at the lateral surface remained segregated.

Ankyrin was detected at sites of cell–cell contact in a segment of the fat body that usually remains attached to the salivary glands after dissection (Figure 5F). However, the Na,K-ATPase (Figure 5E) and neuroglian (our unpublished results) were not detectable in the fat body.

Lee *et al.* (1997) recently described the polarized distributions of $\alpha\beta$ and $\alpha\beta_H$ spectrins in the somatic follicle epithelium of the adult ovary. Here, we examined the distribution of neuroglian in relation to the follicle cell membrane skeleton. Neuroglian staining was concentrated along the lateral membranes of the epithelium (Figure 6D) along with ankyrin (Figure 6, A and C) and β spectrin (our unpublished results). In contrast, β_H spectrin was restricted to the apical surface facing the enclosed nurse cells and oocyte (Figure 6B). Thus, in follicle cells as in the salivary gland, the two spectrin isoforms appear to respond independently to their respective positional cues at the cell surface.

DISCUSSION

The membrane skeleton forms a protein scaffold that contributes to the shape and stability of the plasma membrane. Because of its polarized distribution in many cell types, the membrane skeleton is also thought to contribute to the development and maintenance of specialized membrane domains (reviewed by Beck and Nelson, 1996; Devarajan and Morrow, 1996; Lambert and Bennett, 1996). Moreover, individ-

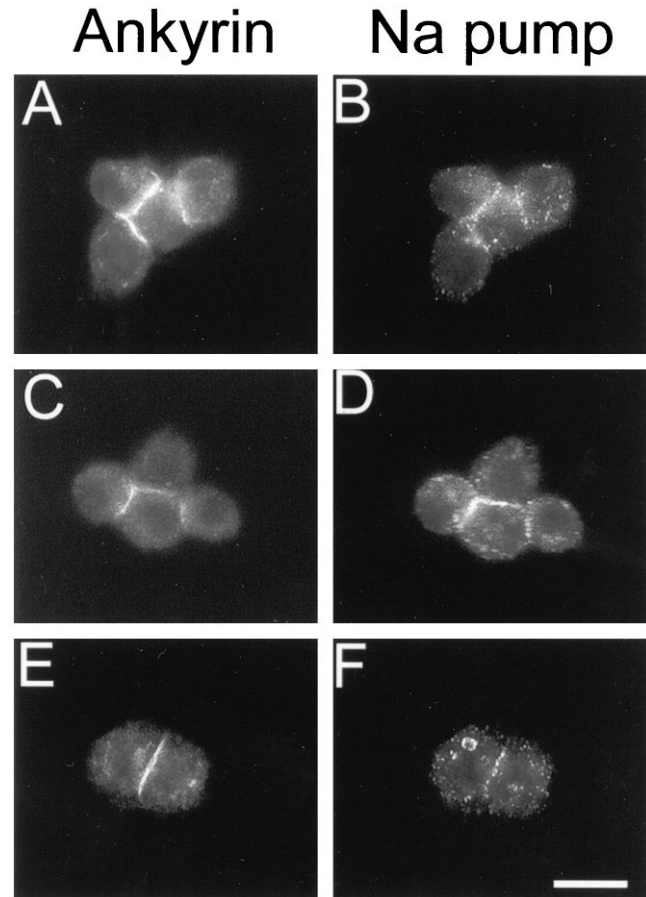


Figure 4. The Na,K-ATPase codistributes with ankyrin at sites of neuroglian-mediated cell–cell contact. Neuroglian-expressing S2 cells were double labeled with mouse monoclonal anti-Na,K-ATPase antibody and rabbit anti-ankyrin antibody and secondary antibodies as in Figure 1. Bar, 10 μ m.

ual cells can segregate multiple spectrin isoforms to divide the plasma membrane into multiple unique domains. Here, we have used *Drosophila* S2 cells to analyze the mechanisms behind spectrin isoform segregation and the response of an interacting membrane marker, the Na,K-ATPase.

The distribution of segregated membrane skeleton domains in S2 cells is summarized schematically in Figure 7A. One spectrin isoform, $\alpha\beta_H$, is uniformly associated with the plasma membrane (submembrane shading), whether or not neuroglian is present. In contrast, ankyrin and $\alpha\beta$ spectrin are recruited from the cytoplasm to sites of cell–cell adhesion upon expression of neuroglian. Interestingly, the recruitment of $\alpha\beta$ spectrin and ankyrin coincides with sites of adhesion, and not with the distribution of total neuroglian, implying that neuroglian is somehow activated to recruit membrane skeleton assembly (Dubreuil *et al.*, 1996). The Na,K-ATPase also becomes

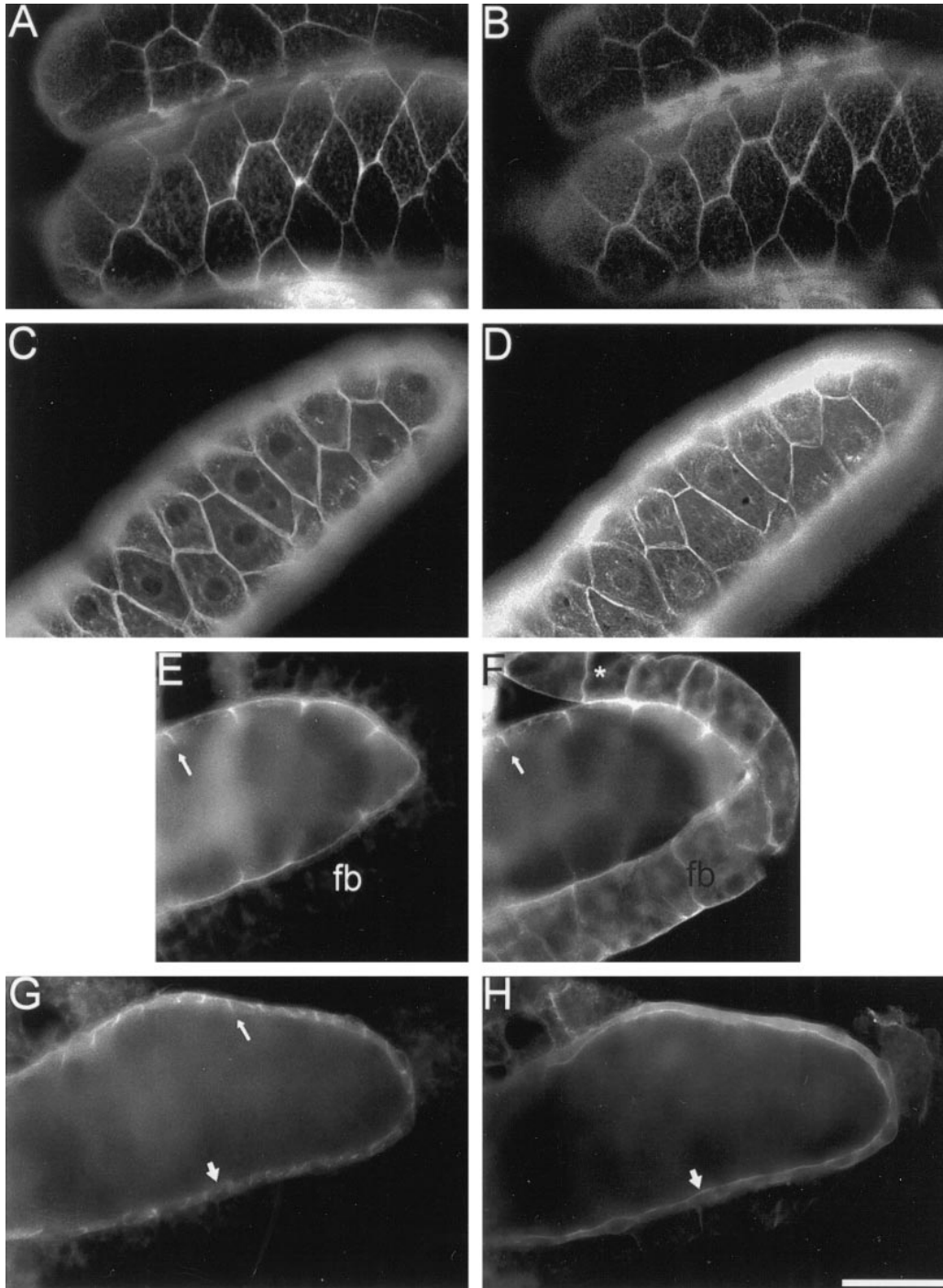


Figure 5. Distribution of neuroglian, ankyrin, Na,K-ATPase, and β_H spectrin in the larval salivary gland epithelium. Salivary glands were dissected from late third instar wild-type larvae, fixed, permeabilized and double labeled with mouse antineuroglian (A), rabbit anti-*Drosophila* ankyrin (B, D, and F), mouse anti-Na,K-ATPase (C, E, and G); and rabbit anti-*Drosophila* β_H spectrin (H). Mouse antibodies were detected with Texas Red-conjugated secondary antibodies (A, C, E, and G) and rabbit antibodies were detected with FITC-conjugated secondary antibodies (B, D, F, and H). Neuroglian, ankyrin, and Na,K-ATPase colocalize at lateral sites of cell-cell contact (A–D). Ankyrin and the Na,K-ATPase colocalize at the lateral margin of salivary gland cells (arrow, E and F). Ankyrin is also concentrated at sites of cell adhesion (*, F) in the fat body (fb). Although the Na,K-ATPase was concentrated at lateral sites of cell contact in the salivary gland (thin arrow, G), the β_H subunit of spectrin was concentrated at the apical cell surface (wide arrow, H). Bars: A–D and G–H, 100 μM ; E and F, 67 μm .

concentrated at sites of neuroglial-mediated cell adhesion, presumably through a direct interaction with ankyrin (Nelson and Veshnock, 1987).

The segregated distribution of membrane markers in salivary glands from third instar *Drosophila* larvae is consistent with the mechanism of segregation found in S2 cells (Figure 7B). Neuroglial and the Na,K-ATPase are concentrated at lateral cell-cell contacts. Spectrin and ankyrin are also concentrated at lateral sites of cell contact, as expected if they serve as a bridge between sites of cell adhesion and the Na,K-ATPase. Since neuroglial is likely to be activated by cell-cell adhesion in the lateral domain of follicle cells and salivary gland cells, it is a likely candidate to provide the signal that recruits ankyrin and $\alpha\beta$ spectrin in these epithelia. Although $\alpha\beta_{\text{H}}$ spectrin is uniformly associated with the plasma membrane in S2 cells, it is restricted to the apical domains of salivary gland cells, somatic follicle cells of the adult ovary (Lee *et al.*, 1997 and present study), and a subset of cells in the developing *Drosophila* embryo (Thomas and Kiehart, 1994). The results imply that a sorting mechanism acts on $\alpha\beta_{\text{H}}$ spectrin to produce its apical distribution in polarized cells.

An ankyrin-independent membrane-binding site is likely to be responsible for targeting $\alpha\beta_{\text{H}}$ spectrin to the plasma membrane. The present results reveal that in S2 cells, larval salivary glands, and adult ovaries, ankyrin, and β_{H} spectrin have strikingly different distributions. There appears to be a single ankyrin gene in *Drosophila* with a single protein product (Dubreuil and Yu, 1994). Even though the evidence is negative, it is significant that degenerate polymerase chain reaction, low stringency hybridizations with evolutionarily conserved regions of the ankyrin gene, and the reactivities of three independent polyclonal antibodies have all failed to detect additional ankyrin products (our unpublished observations). If there is only one ankyrin, and that ankyrin codistributes with $\alpha\beta$ spectrin, then that ankyrin cannot be the membrane attachment site for $\alpha\beta_{\text{H}}$ spectrin. Membrane-targeting activity is not likely to reside in the α subunit of spectrin either, since the two isoforms described here share the same α subunit (Dubreuil *et al.*, 1990) yet they acquire distinct subcellular distributions.

Drosophila β_{H} spectrin resembles TW260, the avian terminal web-associated β spectrin. Based on their similarities, we suggest that *Drosophila* $\alpha\beta_{\text{H}}$ spectrin and vertebrate terminal web spectrins constitute a distinct class of apically directed spectrin isoforms. Both proteins are unusually large compared with other known β spectrin subunits and both utilize an α spectrin subunit found in other spectrin isoforms (Coleman *et al.*, 1989; Dubreuil *et al.*, 1990). In addition, both proteins are found in the apical region of epithelial cells. A terminal web spectrin has been identified in mammals, although its exact subunit composition is

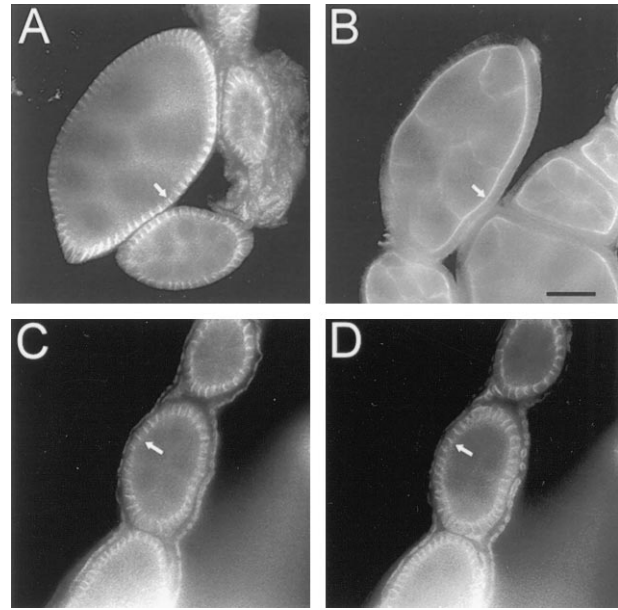


Figure 6. Distribution of ankyrin (A and C), β_{H} spectrin (B), and neuroglial (D) in the somatic follicle epithelium of the adult ovary. Ovaries were dissected from adult *Drosophila* females, fixed, permeabilized, and stained as in Figure 5. Arrows mark staining of ankyrin at lateral cell contacts (A and C), neuroglial at cell contacts (D), and β_{H} spectrin at the apical surface (B). Bar, 20 μm .

not known (Hirokawa *et al.*, 1983). The apical receptor for the vertebrate terminal web spectrins is not known, but it does not appear to be ankyrin (Howe *et al.*, 1985). The nature of the binding site on the spectrin molecule that responds to an ankyrin-independent, apical targeting cue is also unknown. Based on its association with the plasma membrane of S2 cells, where there is no terminal web, we suggest that targeting of $\alpha\beta_{\text{H}}$ spectrin occurs via its direct or indirect association with an integral membrane receptor.

Differential use of ankyrin-dependent and ankyrin-independent membrane-binding sites by two spectrin isoforms provides a sorting mechanism that segregates spectrin isoforms in the plane of the membrane. Just as $\alpha\beta_{\text{H}}$ spectrin appears to lack an ankyrin-binding site, $\alpha\beta$ spectrin lacks a binding site for the $\alpha\beta_{\text{H}}$ spectrin receptor. As a result, the two spectrin isoforms are able to respond independently to positional information specified by their respective membrane anchors. The segregated pattern of membrane skeleton domains found in a cell is thus likely to depend on which receptor proteins that cell expresses and on the positional cues received by the cell during development.

The codistribution of the Na,K-ATPase with $\alpha\beta$ spectrin and ankyrin at sites of neuroglial-mediated cell-cell adhesion suggests that the link between cell adhesion and Na,K-ATPase polarity is a general one. The α subunit of the *Drosophila* Na,K-ATPase is $\sim 80\%$

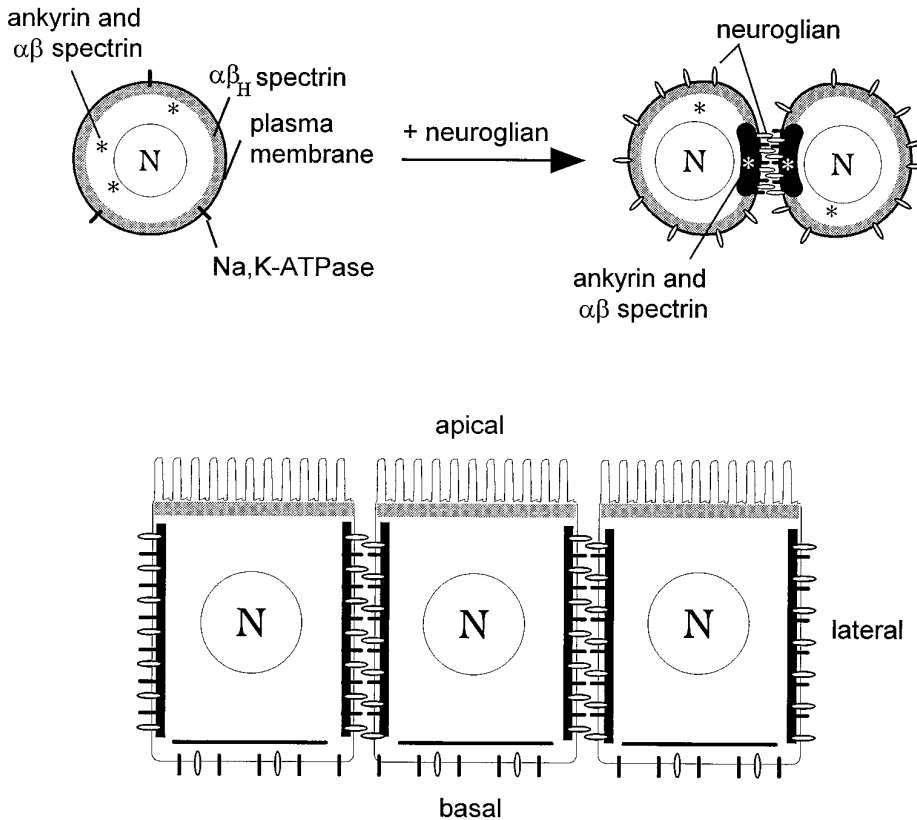


Figure 7. Segregation of spectrin isoforms in response to positional cues at the cell surface. (A) The uniform distribution of $\alpha\beta_H$ spectrin (submembrane shading) at the plasma membrane of S2 cells (left) implies the presence of a constitutive β_H -specific receptor that is uniformly distributed at the cell surface. In contrast, ankyrin and $\alpha\beta$ spectrin are found in the cytoplasm of control S2 cells (*, left), but they are recruited to sites of cell–cell contact in response to neuroglian-mediated cell adhesion (right). The Na,K-ATPase also becomes concentrated at cell contacts in response to neuroglian expression and ankyrin/ $\alpha\beta$ spectrin assembly. (B) Neuroglian, ankyrin, $\alpha\beta$ spectrin, and Na,K-ATPase are concentrated at sites of cell–cell contact in the larval salivary gland epithelium, in agreement with the order of events observed in S2 cells. However, the putative $\alpha\beta_H$ spectrin receptor is sorted apically in these polarized cells. Despite their common features (including an identical α subunit), the two spectrin isoforms respond independently to their respective membrane receptors to form segregated membrane skeleton domains.

identical to the vertebrate Na,K-ATPase (Lebovitz *et al.*, 1989) and is 84% identical within a region implicated in the Na,K-ATPase interaction with ankyrin (Jordan *et al.*, 1995). The membrane-binding domain of *Drosophila* ankyrin is also conserved in amino acid sequence (Dubreuil and Yu, 1994) and function. For example, *Drosophila* ankyrin is recruited to sites of cell–cell contact by L1, the human homologue of neuroglian (Hortsch *et al.*, 1997). The striking sequence conservation between these molecules in mammals and *Drosophila* suggests that the interaction between ankyrin and the Na,K-ATPase is conserved as well. It appears that, regardless of the source of positional information (E-cadherin or neuroglian), polarized assembly of ankyrin and the membrane skeleton can produce a codistribution of the Na,K-ATPase within the same membrane domain. The lack of an effect of an α spectrin mutation on the distribution of Na,K-ATPase molecules *in vivo* (Lee *et al.*, 1993; Lee *et al.*, 1997) is puzzling. However, there is evidence to suggest that residual ankyrin and β spectrin function in these mutants (Dubreuil and Yu, 1994) could explain the maintenance of Na,K-ATPase polarity in salivary glands and midgut epithelium. An important next step will be to examine the effects of ankyrin and β spectrin mutations on Na,K-ATPase polarity in these cells.

The results reported here illustrate mechanisms that govern the two-way flow of information between the plasma membrane and membrane skeleton. There are positional cues that direct polarized membrane skeleton assembly (neuroglian and the β_H spectrin receptor), which in turn confers polarity on an interacting membrane marker (the Na,K-ATPase). An important future goal will be to classify other interacting membrane proteins as either inducers (that provide positional information for the membrane skeleton) or responders (that conform to membrane skeleton polarity). Many of the proteins that associate with the membrane skeleton are physiologically important factors whose position within the cell is likely to be critical to their function [e.g., sodium channels (Srinivasan *et al.*, 1988; Rotin *et al.*, 1994), IP₃ receptor (Joseph and Samanta, 1993), H,K-ATPase (Mercier *et al.*, 1989), and anion exchangers (Morgans and Kopito, 1993)]. Their interactions with the membrane skeleton may have evolved as a mechanism that provides access to positional information within the cell.

ACKNOWLEDGMENTS

We thank Stacey Asem, Jason Frankel, and Runna Moussa for technical assistance, Chris Schonbaum for help with ovary dissections, and Jerry Lorenz for computer assistance. We thank Nava

Segev and Ted Steck for comments on the manuscript. We also thank Dr. Michael Hortsch for providing neuroglial antibody and Dr. Doug Fambrough for providing $\alpha 5$ Na,K-ATPase antibody. Supported by National Institutes of Health grant GM-49301 and the American Heart Association Chicago Affiliate.

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