INTRODUCTION

The many functions of an epithelium depend on the polarized nature of its constituent cells. A hallmark of such polarity is the division of the plasma membrane into apical and basolateral domains, to which proteins are differentially sorted. The stable, asymmetric distribution of proteins at the apical and basolateral surfaces confers distinct functions on these two domains, and facilitates such processes as vectorial transport of solutes across the epithelium. A key step in the development of a polarized cell phenotype is thus the generation of membrane asymmetry, beginning with small ‘targeting patches’ to permit the differential delivery of proteins, and their subsequent elaboration into distinct functional domains (Drubin and Nelson, 1996; Yeaman et al., 1999).

The origins of epithelial cell polarity have been examined in tissue culture models as well as in epithelium formation in embryonic development. In Madin-Darby canine kidney (MDCK) cell cultures, cell polarization emerges as cultures grow to confluence. Initial asymmetry at the membrane is provided by cadherin-mediated cell-cell contact (McNeill et al., 1990). This cue recruits the Sec6/8 complex, which permits the sorting of proteins to the basolateral domain (Grindstaff et al., 1998). The spectrin-based membrane skeleton (SBMS) is also an early recruit to initial sites of cell-cell contact and has been suggested to play a role in reinforcing the initial polarizing cue, initiating the assembly of an asymmetric membrane domain (reviewed in Drubin and Nelson, 1996; Yeaman et al., 1999).

The SBMS exhibits many properties that are consistent with a role in the generation of membrane subdomains. This structure comprises a branching network of spectrin tetramers crosslinking F-actin at the membrane. Each tetramer comprises two α and two β chains and contains several binding sites for other proteins. Some of these proteins probably act as receptors to recruit and/or retain the SBMS to the membrane, while binding to spectrin in turn serves to stabilize other proteins at the membrane, and to act as a scaffold for protein complex assembly. Such activities, plus the observation that different spectrin isoforms tend to reside in...
mutually exclusive membrane domains, particularly in neuronal tissues, has reinforced the notion that spectrin is involved in establishing and/or maintaining cell polarity at the membrane (reviewed in Bennett and Gilligan, 1993; DeMatiase and Morrow, 1998; Veil and Branton, 1996; Yeaman et al., 1999).

In embryonic models it is clear that factor(s) residing in the plasma membrane or the adjacent cortex are again key players in the generation of polarity in the primary epithelium of several organisms. Surface labeling and cell transplantation experiments in Xenopus indicate that only cells retaining part of the original egg membrane can become polarized (Müller and Hausen, 1995). Cells that were generated by divisions where the cleavage furrow did not bisect the domain of residual egg membrane are unable to become polar even when placed in an epithelial environment. In the mouse, overt surface polarity appears at compaction with the appearance of junctional complexes. However, this polarity is stable to dissociation of the blastomeres (Reeve and Ziomek, 1981) and remains stable during subsequent mitosis in the dissociated state (Johnson et al., 1988). Such data has led to the suggestion that a ‘cortical memory’ is established during compaction and is responsible for directing overall cell polarity at these early cleavage stages (Johnson et al., 1988).

In Drosophila, primary epithelium formation begins at cellularization and is complete by mid to late gastrulation. A period of syncytial nuclear division results in a layer of approximately 6000 nuclei at the surface of the embryo at syncytial blastoderm. At cellularization membranes plunge inward to encapsulate each nucleus in a cell, forming the cellular blastoderm (reviewed in Foe et al., 1993). Cellularization proceeds in two distinct phases: an initial slow phase where membrane ingression is dependent on the microtubule cytoskeleton, and a later, fast phase that is dependent on the actin cytoskeleton. At cellularization membranes plunge inward to encapsulate each nucleus in a cell, forming the cellular blastoderm (reviewed in Foe et al., 1993). Cellularization on the growing lateral membranes of the cells as the completion of the zonula adherens (ZA; Tepass and Hartenstein, 1994). Adherens junctions first appear during compaction and are responsible for directing overall cell polarity. The results presented here indicate that the basolateral membrane skeleton is added to a membrane that initially has an apical membrane skeleton. We have called this process ‘basolateral interpolation’, and suggest that this may constitute a common step in the generation of primary epithelial polarity in many organisms. The two β-spectrin isoforms initially colocalize, but later become segregated into a mosaic structure associated with the assembling ZA. This mosaic is ultimately resolved as the ZA becomes fully established. At this time the apical SBMS is associated with the ZA, and the basolateral SBMS spreads to cover the basolateral domain. Thus, in Drosophila, as in the MDCK model, apico-basal membrane polarity emerges in concert with the development of cadherin-mediated adhesion. Our data also indicate that βH is specifically associated with a concentration of DE-cadherin at the furrow canal that may be involved in the maintenance of close membrane apposition in the face of force generated by the contractile rings in the neighboring domain at the base of the forming cells. This further reinforces the close relationship between βH and DE-cadherin (Thomas et al., 1998).

**MATERIALS AND METHODS**

**Antibodies**

Immunolocalizations used the following antibodies and concentrations: for βH-spectrin we used rabbit serum #89 at 1:100 or the monoclonal mouse serum DUM2 at 1:200 (Thomas and Kiehart, 1994); for β-spectrin we used rabbit serum #89 at 1:200 (Byers et al., 1989). We have previously verified that these two antibodies are specific for their respective isoforms (Thomas and Kiehart, 1994). For DE-cadherin we used the monoclonal antibody DCAD2 at 1:20 (Oda et al., 1994). Secondary antibodies generated in goats and conjugated with FITC, Cy3 or Cy5 were obtained from Jackson ImmunoResearch Inc. (West Grove, PA), reconstituted according to the manufacturer's instructions and used at a concentration of 1:100. All secondary antibodies were routinely preadsorbed against fixed embryos.

**Embryo fixation and staining**

Embryo fixation with glutaraldehyde-doped heptane (HEPG) and methanol was as previously described (Thomas and Kiehart, 1994). Brieﬂy, dechorionated and washed D. melanogaster embryos were transferred to 20 ml of a 1:1 mixture of HEPG:methanol and gently tumbled for 30 minutes to ﬁx and remove the vitelline membrane. Embryos that sank were washed for 5 minutes in methanol, rehydrated and stained as previously described (Thomas and Kiehart, 1994). Dual

of either α-spectrin (Lee et al., 1997) or βH-spectrin (βH; Thomas et al., 1998; D. C. Zarnescu and G. H. T., submitted for publication) produces defects in epithelial tissues in the adult fly. Mutations in the gene encoding the homologue of βH in Caenorhabditis elegans similarly produce defects in epithelial morphogenesis (McKeown et al., 1998). In order to understand the sequence of steps leading to the development of apico-basal polarity, as well as to the steps leading to the assembly and maturation of the ZA, it is clearly critical to understand the domain structure of the plasma membrane in the early epithelium. The availability of antibodies to both β-spectrin and βH provides a unique opportunity to bring together concepts developed in both tissue culture and developmental models by examining the relationship between emerging SBMS polarity, apico-basal polarity and the assembly of the ZA during the formation of the primary epithelium in Drosophila.
fixation for $\beta_H$ and DE-cadherin has been challenging (Thomas et al., 1998). During cellularization and early gastrulation (Fig. 7A-R), our best results were obtained with the optimum $\beta_H$ fixation just described. At later stages (Fig. 7S-U), PLP fixative was used (McLean and Nakane, 1974). Nuclei were stained with 1-5 $\mu$g/ml propidium iodide, 2 $\mu$g/ml RNase A for 30 minutes during the first wash after the secondary antibody incubation, and mounted in 80% glycerol, 100 mM Tris-Cl, pH 8.5, 2% n-propyl gallate for observation.

Imaging of stained embryos was done using a BioRad MRC1024 confocal microscope (BioRad, Hercules, CA). Images were processed using Adobe Photoshop v4.0 (Adobe Systems, San Jose, CA). Image channels containing protein distributions were contrast-stretched, in some instances following subtraction of excessive bleed-through of signal from the propidium iodide nuclear stain. The channel containing the DNA stain was processed to best exemplify nuclear shape and position. Figures were assembled and annotated in Adobe Illustrator v6.0.

**RESULTS**

**Nomenclature**

In this paper, we characterize the development of the apical and basolateral membrane domains in cells of the primary epithelium in *Drosophila* by following the subcellular localization of two spectrin isoforms, $\beta_{\text{Heavy}}$-spectrin ($\beta_H$) and $\beta$-spectrin. In mature epithelia, these two proteins are apically and basolaterally restricted, respectively. Our results indicate that membrane polarity does not become fully established until mid to late gastrulation and that at intermediate stages the apical and basolateral membrane skeleton domains are not cleanly segregated along the developing apico-basal axis. This makes discussion of the subcellular locations of these incipient domains with reference to the ultimate apico-basal axis cumbersome and confusing. In this paper, we use a nomenclature that simply numbers the different membrane domains defined by distinct distributions of the proteins that we and other laboratories have identified during cell formation. This numbering system is outlined in Fig. 1.

**Immunolocalization of the $\beta_H$ and $\beta$-spectrin membrane skeletons**

**Pole cell formation**

The pole cells which give rise to the germline are the first cells to form at syncytial blastoderm. Several nuclei bud off at the posterior pole and divide to produce 34-40 pole cells (Turner and Mahowald, 1976). Double immunofluorescence localization of $\beta$-spectrin and $\beta_H$ has shown that at this time only $\beta_H$ is detectable on the membrane of the embryo (data not shown) and so the pole cell spectrin-based membrane skeleton (SBMS) exclusively comprises $\alpha$/$\beta_H$-spectrin (see Fig. 6F).

Until nuclear cycle 13, the $\beta_H$ distribution is often more concentrated on the most posterior hemisphere of each pole cell (Fig. 2A), but becomes more irregular and tends to be restricted to regions of pole cell-pole cell contact as the embryo progresses through nuclear cycle 14 (Fig. 2B-D). This resembles the pattern described for $\alpha$-spectrin (Pesacreta et al., 1989). During this period the pole cell membranes become progressively more active and the removal of the SBMS was speculated to be a factor in this increase in membrane flexibility (Pesacreta et al., 1989).

**Pseudocleavage furrows**

During the first 10 nuclear divisions of fly development the nuclei progressively migrate outwards to the egg cortex where they undergo a final three rounds of mitosis prior to cellularization. These last three divisions are segregated from one another by the transient invagination of the surface membrane during mitosis (pseudocleavage furrows),

![Fig. 2. The distribution of $\beta_H$-spectrin in the pole cells. (A) Cycle 13; (B) early cellularization; (C) at the slow/fast transition; (D) late cellularization. Bar, 30 $\mu$m.](Image)
preventing the occurrence of multipolar spindles (see Foe et al., 1993 for review). As each furrow is formed β-spectrin is strongly recruited to the invaginating membranes, where it colocalizes with βH (Fig. 3A-F). βH and β-spectrin often show reciprocal changes in level along the membranes, suggesting that a mosaic of SBMS is present at this time (Fig. 3D-I). In tangential confocal sections it is apparent that β-spectrin is not present at uniform levels around the cell periphery, but is somewhat particulate (Fig. 3I). At the end of the mitotic cycle, β-spectrin is lost from the membrane as the pseudocleavage furrows withdraw.

Cellularization

During cellularization βH and β-spectrin exhibit dynamic and sometimes overlapping distributions. We identify the following sequence of events: (1) βH is present at the cortex prior to cellularization while there is little or no detectable β-spectrin (data not shown; see also Fig. 7A). (2) Very rapidly, during the earliest phases of cellularization, βH moves to the sites of membrane invagination where it is joined by β-spectrin, and the two proteins initially colocalize in a manner similar to the pseudocleavage furrows (Fig. 4A-F). In tangential confocal sections this colocalization is most prominent in regions of close membrane apposition (Fig. 5A-C). Again, there is reciprocity in the level of the two isoforms, suggesting that a mosaic of domains is present. (3) As the membranes progress to the top of the nuclei, a region containing only βH emerges near the bottom of the invaginating membranes at the top of the furrow canal, defining domain 3 (Figs 4G-I, 5D-F). We have previously shown that this domain is distinct from the contractile apparatus containing cytoplasmic myosin II in domain 4 (Thomas and Kiehart, 1994). Above domain 3, in domain 2, the two spectrins continue to colocalize, although the βH signal is decreasing (Fig. 4G-I). In domain 1 the βH signal has become very weak. (4) During the remainder of the slow phase βH becomes even less prominent throughout domain 2. Concomitantly, β-spectrin fades in domain 2b but remains evident in domain 2a (Figs 4J-L, 5G-I). (5) During the fast phase of cellularization βH is recruited back to, or stabilized in, domain 2a, and β-spectrin becomes increasingly concentrated in this same region. At this stage both proteins exhibit an overlapping distribution of patches in domain 2a that often appear to exclude one another, suggesting that there is a mosaic of membrane domains during this period (Figs 4M-O, 5J-L). (6) Finally, during late fast phase/early gastrulation the βH staining in domain 3 disappears. The distributions of βH and β-spectrin in domain 2a remain mosaic for some time with βH becoming progressively more apical to β-spectrin (Figs 4P-R, 5M-O). Eventually the two isoforms resolve from one another, βH colocalizing with DE-cadherin at the zonula adherens (see Fig. 7) and β-spectrin spreading to cover the basolateral domain (Fig. 6).

Coimmunolocalization of DE-cadherin and βH

The time course with which βH becomes resolved from the mixed membrane skeleton to lie at the ZA strongly resembles that seen for the coalescence of spot adherens junctions into the mature ZA (Tepass and Hartenstein, 1994). Moreover, we have previously shown that βH generally colocalizes with the ZA in mature epithelia (Thomas et al., 1998; D. C. Zarnescu and G. H. T., unpublished observations). To determine whether βH also colocalizes with adherens junction proteins during cellularization, we performed double immunofluorescence localization of βH and DE-cadherin.

βH and DE-cadherin are both present on the precellularization membrane (Oda et al., 1998; Thomas and Kiehart, 1994; Fig. 7A,D,G). During pseudocleavage furrow formation DE-cadherin localizes to the invaginating membranes and also becomes concentrated in the immediately adjacent cytoplasm (data not shown). At the onset of cellularization DE-cadherin becomes concentrated at the forming furrows along with βH (Fig. 7B,C,E,F,H,I). As cellularization progresses, DE-cadherin is concentrated in domain 3 along with βH (Fig. 7J-O). Within this domain DE-cadherin is most concentrated in
regions of closest membrane apposition in the upper part of domain 3 and away from the vertices between three adjacent cells (Fig. 7N,O). The concentration of DE-cadherin in the region of membrane contact may function as an adhesive reinforcement at the top of the furrow canal to counteract the lateral pull generated by the acto-myosin rings in domain 4. At the end of cellularization the patches of $\beta_H$ in domain 2a appear to colocalize with the most prominent spot adherens junctions (Fig. 7P-R). However, the interdigitated distribution of the two spectrin isoforms at this time makes it difficult to say that they exclusively localize to the $\beta_H$ patches. In the mature epithelium the $\beta_H$ distribution can be seen to include the ZA (Fig. 7S-U).

**DISCUSSION**

Spectrins have long been speculated to have a role in establishing and/or maintaining the fundamental division of the epithelial plasma membrane into an apical and basolateral domain (reviewed in Drubin and Nelson, 1996; Yeaman et al., 1999). In this paper we show that the syncytial *Drosophila* embryo has an apical membrane skeleton (defined by the presence of $\beta_{\text{Heavy}}$-spectrin; $\beta_H$), into which the incipient basolateral membrane skeleton (defined by the presence of $\beta$-spectrin) is added. We have named this process ‘basolateral interpolation’. Our results further indicate that while the

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**Fig. 4.** The distribution of $\beta$-spectrin isoforms during cellularization: sagittal confocal sections. Each row of panels was imaged from an embryo at successive stages of cellularization from the start of membrane ingression (A-C) and progressing through to early gastrulation (P-R). Left panels show the pattern of $\beta_H$ staining; right panels show the pattern of $\beta$-spectrin staining; center panels are a merged pseudocolor images with $\beta_H$ in red, $\beta$-spectrin in green and nuclear position in blue (not shown in isolation). The insets in H and N indicate the extent and location of domains 2(a/b) and 3 as defined in Fig. 1. Bar, 30 $\mu$m.
incipient apico-basal axis is anticipated by the orientation of cytoplasmic structures early in the formation of the epithelium (Foe et al., 1993; Müller and Wieschaus, 1996), membrane polarity does not become fully established until mid to late gastrulation. This time course is similar if not identical to the assembly of the zonula adherens (ZA; Tepass and Hartenstein, 1994) and the ability to restrict proteins to the basolateral domain (Müller and Wieschaus, 1996). Furthermore, at intermediate stages the apical and basolateral membrane skeleton domains are not cleanly segregated along the incipient apico-basal axis of the cell. The dynamic redistribution of apical β₁ is consistent with a role at specific cadherin-mediated adhesion complexes. The appearance and ultimate expansion of the β-spectrin distribution to cover the basolateral domain is consistent with its proposed role in establishing and/or maintaining cell polarity (Yeaman et al., 1999). Our observations thus indicate that the apical and basolateral SBMS have distinct rather than analogous roles within their respective domains during primary epithelium formation.

The membrane at the start of development has an apical membrane skeleton that is maternally provided (Thomas and Kiehart, 1994), suggesting that the egg membrane is predisposed to be the apical pole even before cleavage begins. The addition of the basolateral β-spectrin membrane skeleton first seen during the formation of the pseudocleavage furrows, is reversed as the membranes withdraw and begins again.
Membrane polarity during cellularization during early cellularization (Figs 3, 4). The invagination at cellularization is rapidly distinguished from the pseudocleavage furrows by the time the membranes have reached the nucleus with the emergence of domain 3, containing only \( \beta_H \), at the top of the furrow canal (Fig. 4; see Fig. 1 for domain nomenclature). The \( \beta_H \) signal in domain 2 decreases throughout the slow phase of cellularization. Concomitantly, \( \beta \)-spectrin selectively disappears from domain 2b while remaining in 2a. During the fast phase the \( \beta_H \) signal increases again in domain 2a and the distribution of the two isoforms becomes a patchy mosaic in this region. The two isoforms completely segregate during gastrulation as the epithelium matures and the zonula adherens (ZA) forms. \( \beta_H \) ultimately colocalizes with the ZA while the \( \beta \)-spectrin distribution expands to cover the basolateral domain of the cell.

These results agree well with the prior description of \( \alpha \)-spectrin during cellularization (Pesacreta et al., 1989) where that protein’s distribution represents the sum of the distributions of \( \beta_H \) and \( \beta \)-spectrins that we describe. The colocalization of \( \beta \)- and \( \beta_H \)-spectrins in the pseudocleavage furrows and during the initial phases of cellularization was unexpected since the two isoforms are believed to be recruited to the membrane by distinct mechanisms (Dubreuil et al., 1998b; Lee et al., 1997; Thomas et al., 1997). Since we observe reciprocal changes in the intensity of staining for each isoform within many confocal sections, we believe that these proteins are present in distinct microdomains rather than in a truly comingled network. The reversibility of this phenomenon on retraction of the pseudocleavage furrows suggests that this structure arises from the activation of latent \( \beta \)-spectrin receptors at the membrane brought on by membrane invagination. However, we cannot yet exclude the possibility that new membrane is being delivered along with this isoform. While the former mechanism might result in a mixed network, the latter should result in a mosaic structure of patches with differing properties. At present, the origin, site and timing of insertion of new membrane during cellularization is unknown. The embryonic membrane is highly folded at the end of syncytial blastoderm, and becomes smooth as cellularization progresses suggesting that membrane is drawn in to contribute to the formation of the new cells (Fullilove and Jacobsen, 1971; Turner and Mahowald, 1976). However, it is clear that there is insufficient membrane present at the onset of cellularization to account for the entire surface area at cellular blastoderm (Fullilove and Jacobson, 1971), and the requirement for proteins such as the t-SNARE syntaxin (Burgess et al., 1997) and dynamin (Chen et al., 1991; Swanson and Poodry, 1981; van der Bliek and Meyerowitz, 1991) during cellularization, suggests that membrane growth and/or recycling play a crucial role in cell formation.

The rapid redistribution of \( \beta_H \) to the membrane invagination sites at the beginning of cellularization occurs as the outer
membrane greatly increases its surface area and becomes highly folded (Turner and Mahowald, 1976). This might occur by active recruitment of $\beta_H$ to sites of invagination or by displacement of the original $\beta_H$-associated membrane to the invaginations by rapid membrane growth in this domain and the progression of pre-existing membrane at the furrows. $\beta$-spectrin also rapidly recruited to these incipient furrows where the two isoforms colocalize (see Discussion above). The subsequent decrease in the $\beta_H$ signal in domain 2 during the slow phase of cellularization could occur in one of three ways: (1) $\beta_H$ may be actively removed from the membrane; (2) it may be displaced by $\beta$-spectrin; (3) the addition of new membrane (associated with $\beta$-spectrin) might ‘dilute’ the $\beta_H$-associated membrane.

Shortly after cellularization initiates, membrane domain 3, containing only $\beta_H$, emerges near the furrow canals where it colocalizes with $DE$-cadherin. This region is distinct from domain 4, which contains cytoplasmic myosin II (Thomas and Kiehart, 1994; Young et al., 1991). Domain 3 also contains the other adherens junction components $\alpha$- and $\beta$-catenin (Müller and Wieschaus, 1996; Tepass, 1996; data not shown). $\beta_H$ levels vary in close correspondence with those of $DE$-cadherin at the ZA in mature epithelia (Thomas et al., 1998) and these junctions are disrupted in mutants lacking $\beta_H$ (D. C. Zarnescu and G. H. T., submitted for publication), suggesting that $\beta_H$ is closely associated with the complex of proteins necessary for proper $DE$-cadherin-based cell adhesion. We therefore speculate that $\beta_H$ in domain 3 is part of a region of adhesive reinforcement at the top of the furrow canal, and is used to prevent lateral membrane separation due to the contractile forces generated by the acto-myosin contractile ring in domain 4. Alternatively, the furrow canal is a region of close association between microtubules and the membrane undercoat (Katoh and Ishikawa, 1989) and spectrins have been postulated to function at the interface between the actin and microtubule cytoskeletons (e.g. Holleran et al., 1996; Ishikawa et al., 1983). Thus, $\beta_H$ might be functioning to tether microtubules or associated proteins to the membrane in this region.

Whatever the mechanism by which the basolateral membrane skeleton is recruited, the colocalization of the apical and basolateral structures is clearly transient and they segregate by the time a mature epithelium is formed. The appearance of a patchy $\beta_H$ distribution in domain 2a late in cellularization and the subsequent segregation of this isoform from $\beta$-spectrin occurs with a time course that is similar to the appearance and coalescence of spot adherens junctions into the ZA (Tepass and Hartenstein, 1994). While spot adherens junctions are found throughout domains 2a and 2b during cellularization (Müller and Wieschaus, 1996; Tepass and Hartenstein, 1994), $\beta_H$ patches are confined to domain 2a. A distinct class of spot adherens junction (type I) has been identified in this region that is associated with a denser undercoat than the type II junctions in domain 2b (Tepass and Hartenstein, 1994). Given the close relationship between $\beta_H$ and the ZA, we suggest a model in which $\beta_H$ is specifically recruited to type 1 spot adherens junctions where it forms part of this dense undercoat and that these junctions specifically go on to form the ZA. While we cannot be confident at this resolution that $\beta$-spectrin is not associating with some of the spot adherens junctions at later stages, $\beta$-spectrin can be seen to be well segregated from $\beta_H$ in regions where the ZA has already matured (e.g. the posterior midgut invagination; Tepass and Hartenstein, 1994; Fig. 6D-F). These results suggest a role for $\beta_H$ specifically in the structure of large-scale adherens junctions such as the ZA. Spectrin has been previously suggested to be part of an E-cadherin-containing complex (Nelson et al., 1990) and has more recently been identified as an $\alpha$-catenin binding protein (Lombardo et al., 1994). Moreover, spectrin and
the spectrin-associated Na+/K+-ATPase (Nelson and Veshnock, 1987) are recruited to the basolateral SBMS in an E-cadherin-dependent manner during the late morula stage of mouse embryogenesis (Watson et al., 1990; Sobel and Goldstein; 1988; Sobel et al., 1988; Watson and Kidder, 1988).

Several mechanisms could result in the resolution of the mosaic SBMS that we observe in domain 2a at the end of cellularization. The coalescence of spot adherens junctions into a mature adhesion belt requires the protein Crumbs, which appears at the apical boundary of the ZA during gastrulation (Grawe et al., 1996; Tepass, 1996). However, it remains unknown if this protein defines an active boundary against which the final coalescence of the junction occurs (Tepass, 1996), or whether it ‘directs’ aggregation in the neighboring membrane domain by some undefined pathway after the spot adherens junctions are gathered together by some other molecule. A similar coalescence of focal adherens junctions called ‘puncta’ has been described during MDCK cell polarization (Adams et al., 1998). It is possible that the SBMS could play a direct role in coalescence in the fly: homotypic fusion of SBMS patches associated with spot adherens junctions that come into close proximity might promote ZA coalescence and simultaneously segregate the two spectrin isoforms. Indeed, we have observed visible disruption of the ZA in karst flies (D. C. Zamescu and G. H. T., submitted for publication). However, the inverse model where membrane skeleton segregation is driven by ZA formation is perhaps more likely since the zygotic phenotype associated with mutants lacking β4 does not include a gross breakdown in apico-basal polarity (D. C. Zamescu and G. H. T., submitted for publication).

This paper represents the first characterization of distinct apical and basolateral SBMS during early development. Previous studies of spectrin distributions during primary epithelium formation in several organisms were all done before the field had a true appreciation of the diversity of β-spectrin isoforms that has since emerged. Moreover, neither the α-spectrin-specific nor the αβ dual specificity antibodies used would necessarily have revealed the type of membrane dynamics described in this paper, since we now know that α-spectrins can dimerize with more than one β-isofrom. However, reexamination of a series of independent studies on α-spectrin localization during early mouse development reveals one intriguing parallel. Antibodies directed against mouse erythroid α-spectrin stain the entire cell cortex after the two-cell stage, but become confined to the basolateral domain after primary polarity is established (Sobel and Alliegro, 1985). In contrast, antibodies directed against non-erythroid α-spectrin reveal a contact-dependent distribution throughout cleavage that becomes concentrated at the apicolateral junctional regions on compaction (Reima and Lehtonen, 1986; Schatten et al., 1986). Thus, it would appear that primary epithelium formation during the mouse might well involve the functional segregation of two SBMS in a manner analogous to the fly.

Other, indirect, data on the origins of polarity in the primary epithelium during mouse and Xenopus development also suggest an important role for the membrane skeleton in a manner that is consistent with basolateral interolation. First, both embryos have apical characteristics prior to polarization. The membranes of the mouse embryo are uniformly covered in microvilli (Reeve and Ziomek, 1981), while there is active removal of basolateral markers from the Xenopus egg during maturation (Gawantka et al., 1992; Müller et al., 1992). Second, a basolateral domain is generated by both organisms during cleavage and/or polarization. In the mouse E-cadherin becomes concentrated at, and microvilli are excluded from, the blastomere interfaces at compaction (Vestweber et al., 1987; Reeve and Ziomek, 1981), while the basolateral marker β1-integrin is added along with new membrane at cytokinesis throughout Xenopus cleavage (Gawantka et al., 1992; Singal and Sanders, 1974).

Since β1 has not yet been identified in either frogs or mice it remains unknown exactly how spectrin might play a role in establishing the apical and basolateral domains in either of these organisms. However, the stability of these domains in the disaggregated state in both organisms (Müller and Hausen, 1995; Johnson et al., 1988; Reeve and Ziomek, 1981) strongly suggests the presence of stabilizing cytoskeletal elements underlying the membrane: a role that has long been attributed to spectrins. We speculate that the prominence of the apical membrane domain during primary epithelial polarization (e.g. Müller and Hausen, 1995; Tepass, 1996; Wiley et al., 1990) results from the fact that the egg membrane in all these cases is apical in character and is stabilized by an apical SBMS; and that one requirement for stable membrane asymmetry is the addition of a basolateral SBMS. It is likely that there is a vertebrate equivalent to β1: this isoform has an early evolutionary origin (Thomas et al., 1997); it has recently been cloned from C. elegans (McKeown et al., 1998); and there is a morphologically similar molecule (TW260) that forms actin crosslinking tetramers with α-spectrin below the apical membrane of chicken enterocytes (Glenney Jr. et al., 1982, 1983). Finally, the parallels in the generation of apico-basal polarity in the primary epithelia of Drosophila and the systems discussed above suggest that basolateral interolation may be a conserved step in the polarization pathway of these widely separated phyla, despite the undoubted specializations required for cellularization.

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