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EPITHELIAL CELL POLARITY AND CELL JUNCTIONS IN *DROSOPHILA*

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■ **Abstract** The polarized architecture of epithelial cells and tissues is a fundamental determinant of animal anatomy and physiology. Recent progress made in the genetic and molecular analysis of epithelial polarity and cellular junctions in *Drosophila* has led to the most detailed understanding of these processes in a whole animal model system to date. Asymmetry of the plasma membrane and the differentiation of membrane domains and cellular junctions are controlled by protein complexes that assemble around transmembrane proteins such as DE-cadherin, Crumbs, and Neurexin IV, or other cytoplasmic protein complexes that associate with the plasma membrane. Much remains to be learned of how these complexes assemble, establish their polarized distribution, and contribute to the asymmetric organization of epithelial cells.

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INTRODUCTION

Epithelial tissues have emerged early during animal evolution, and their ability to form different shapes and to subdivide the body into physiologically distinct compartments is fundamental for the evolution of complex animal body plans. The plasma membrane of epithelial cells is subdivided into regions or domains that fulfill specialized roles in cell organization and physiology. The main subdivisions of the plasma membrane are the apical domain, which faces the external environment and the basolateral domain, which is in contact with the interstitial space of the body. These domains are segregated by a circumferential junctional complex (CJC) that binds adjacent epithelial cells together and forms a semipermeable barrier to the diffusion of solutes through the intercellular space (38). The movement of ions and molecules across an epithelial layer therefore requires regulated transport mechanisms that shuttle solutes from apical to basolateral, or vice versa, and allow epithelia to control the physiological composition of body compartments. In addition to the apical/basolateral distinction, membrane domains of epithelial cells are further regionalized. The basolateral membrane, for example, is subdivided into a basal domain characterized by cell-substrate adhesion and a lateral domain distinguished by cell-cell adhesion. Further, the lateral domain is partitioned into the apical CJC and a region basal to it (121).

The mechanisms that establish and maintain an asymmetric distribution of lipid and protein components of the plasma membrane of epithelial cells have been intensively studied in mammalian cell culture (100). Early work in this system led to a model suggesting that the sorting of plasma membrane components in the Trans-Golgi Network (TGN) into apical and basolateral transport vesicles and the subsequent polarized delivery to the appropriate surface domain are the key mechanisms by which epithelial polarity is maintained (130). However, this model failed to explain how apical and basolateral domains are established initially, and how the two main surface domains are further regionalized. Moreover, it was recognized that apical and basolateral transport vesicles are also formed in nonpolarized cells in which the components of such vesicles show overlapping distributions in the plasma membrane (98, 186).

Analysis of the role of cell adhesion and its consequences on cellular organization led to the current conceptual framework of epithelial polarity (30, 100).

External cues mediated by cell-cell or cell-substrate adhesion generate asymmetries within the plasma membrane that are elaborated by the formation of a local specific membrane cytoskeleton. Retention of transmembrane and cytoplasmic proteins that associate with this local actin/spectrin cytoskeleton emphasize regional differences (92). These differences are further elaborated by adhesion-dependent reorganization of the microtubule cytoskeleton that is necessary for vesicle traffic, and the formation of targeting patches at the lateral membrane that preferentially attract basolateral transport vesicles (51, 185). This model of membrane domain formation integrates several interdependent polarization mechanisms, the concerted activity of which is triggered by adhesive interactions that provide positional cues for cell polarization. The formation of the apical domain is thus viewed as a default pathway in which the plasma membrane assumes apical character wherever no adhesive interactions take place.

Using *Drosophila* as a genetic model to study epithelial polarization offers the opportunity to complement and expand on the mammalian cell culture studies by placing the mechanisms that control epithelial differentiation into a developmental context. Genetic screens have identified a number of factors essential for epithelial polarity that are either integral to, or associated with, the plasma membrane, but did not reveal components of the TGN that contribute to the formation of apical or basolateral transport vesicles. These findings are consistent with a predominant role of extrinsic cues mediated by transmembrane adhesion receptors and cytocortical factors in epithelial polarization. Work using *Drosophila* also revealed a number of regulators of epithelial polarity that are associated with the apical membrane, suggesting that the formation of the apical domain is not a default pathway but instead requires a specific molecular machinery. Finally, studies on *Drosophila* are beginning to reveal an unforeseen complexity in the mechanisms controlling epithelial polarization that may vary from tissue to tissue and over time in the same tissue. This review gives an overview of epithelial development in *Drosophila*, with emphasis on recent studies that have provided novel insights into polarity and the differentiation and function of cellular junctions in epithelial cells.

EPITHELIAL DIFFERENTIATION IN *DROSOPHILA*: AN OVERVIEW

Development of Primary and Secondary Epithelia

The first epithelium that emerges during *Drosophila* development is the blastoderm. It forms by a process known as cellularization, a modified form of cytokinesis discussed in detail below. Many epithelia in *Drosophila* derive from the blastoderm epithelium without a non-epithelial intermediate. Such primary epithelia, all derived from the ectoderm, include the larval and adult epidermis as well as the foregut, hindgut, Malphigian tubules, tracheae, and salivary glands.

In contrast, secondary epithelia arise by mesenchymal-epithelial transitions later in development. Embryonic secondary epithelia are the midgut epithelium, glia sheets that form the blood-nerve barrier, and the dorsal vessel (heart). In addition to their mode of formation, primary and secondary epithelia differ in structure and mechanisms used for cell polarization (150, 154).

Epithelial development can be subdivided roughly into three phases in which (I) the initial establishment of polarity is (II) followed by the consolidation and elaboration of surface domains and cytoplasmic asymmetries, and finally, (III) the terminal differentiation and specialization of surface domains. For primary epithelia, phase I occurs at cellularization during which distinct membrane domains are established. Phase II extends from gastrulation throughout organogenesis and includes the formation of a CJC. Groups of epithelia behave uniformly during this phase; for example, the ectoderm and its epithelial derivatives such as the epidermis and the tracheae, all establish the same CJC. During phase III, individual epithelia undergo sometimes dramatic specializations to accommodate their anatomical or physiological function, and tissue-specific control mechanisms for epithelial differentiation become apparent. For example, the Zinc-finger transcription factor Hindsight is required to maintain the integrity of the tracheal epithelium and controls the differentiation of a specialized tracheal cuticle, which contains a characteristic spiral-shaped superstructure, the taenidium, that prevents the tracheal lumen from collapsing (177). The mechanisms that control the terminal differentiation of specialized epithelia in *Drosophila* remain largely elusive. In addition, because little progress has been made in understanding the polarization mechanisms of embryonic secondary epithelia since we last reviewed the subject (150), this topic is not covered here.

Models for epithelial differentiation in postembryonic development of *Drosophila* include the imaginal discs and the ovarian follicular epithelium (96). The larval imaginal discs are fully polarized epithelial sheets with a well-developed junctional complex comparable to the epidermis of mid-embryonic stages from which they derive. Imaginal disc epithelia lend themselves to the analysis of epithelial maintenance and terminal differentiation and specialization of epithelial surface domains. In contrast, the follicular epithelium renews itself constantly as follicle cells originate from stem cells, and allows the analysis of the full range of phases in epithelial differentiation, including epithelial formation by mesenchymal-epithelial transition and the successive assembly of a CJC. The simplicity and accessibility of the follicular epithelium together with the large number of genes known that effect its epithelial integrity make the follicular epithelium a favored genetic system to study epithelial development (96, 147).

Cellular Junctions in *Drosophila* Epithelia

The complement of cellular junctions in *Drosophila* epithelia comprises spot adherens junctions (SAJs), the zonula adherens (ZA), pleated and smooth septate junctions (SJs), gap junctions, and hemiadherens junctions (HAJs) (154). Early

during development, epithelia acquire a ZA, which assembles from the coalescence of individual SAJs. HAJs and SJs form only later during epithelial differentiation. Basal HAJs (elsewhere called focal contacts) are integrin-based and connect epithelial cells to basement membranes or specialize into muscle-tendon junctions (20, 118). Apical HAJs are seen in cuticle-secreting epithelia connecting the apical membrane to the cuticle. SJs act as the trans-epithelial barrier in most epithelia of non-chordate animals, and thus functionally substitute for the chordate tight junction. The CJC in primary epithelia and in the follicular epithelium is composed of the ZA and the SJ. Embryonic secondary epithelia such as glia sheets and the midgut epithelium lack a ZA but contain the SJ. Desmosomes and hemidesmosomes as well as tight junctions are not seen in *Drosophila* epithelia. Absence of desmosomes and hemidesmosomes is corroborated by the lack of cytoplasmic intermediate filaments in *Drosophila* (3), and the phylogenic analysis of the cadherin superfamily that suggests that desmosomal cadherins have evolved from classic cadherins within the chordate lineage (158). Gap junctions in *Drosophila* and other invertebrates are formed by innexins that appear unrelated in sequence to vertebrate connexins but perform similar functions (116). From gastrulation onwards, gap junctions are ubiquitous components of epithelia (154) but their role in epithelial differentiation is currently not understood.

EPITHELIUM FORMATION: CELLULARIZATION

The formation of an epithelial sheet is typically achieved through the reorganization of a cluster of mesenchymal cells into a monolayer of tightly adhering polarized cells. Such mesenchymal-epithelial transitions are seen many times during development but are best studied in early vertebrate embryos in which a cluster of mesenchymal blastomeres forms the blastula, a hollow ball, that is bound by a blastoderm epithelium. In contrast, the formation of the blastoderm in *Drosophila* embryos, and most other insects takes a different route to establish an epithelium. The fertilized *Drosophila* egg undergoes 13 nuclear divisions that are not followed by cytokinesis. At the end of the 13^{th} cell cycle $\sim$5000 nuclei form a monolayer just beneath the egg membrane. Invaginations of the egg membrane surround each nucleus and associated cytoplasm during the 14^{th} cell cycle, cellularizing the blastoderm and establishing an epithelium of highly columnar cells (42–44, 126). As cytokinesis and epithelium formation go hand in hand, the exploration of epithelial polarization of the *Drosophila* blastoderm provides some unique challenges.

Cellularization is initiated by the formation of the furrow canal that remains at the leading edge of the invaginating membrane (Figure 1). As cellularization proceeds, SAJs are assembled next to the furrow canal in the emerging lateral membrane (55, 97, 149, 154). These "basal junctions" (55) remain associated with the furrow canal during cellularization but resolve as cellularization is completed. Additional SAJs form as the lateral membrane grows, and concentrate apically where they will form the ZA during gastrulation (97, 149). A number of proteins

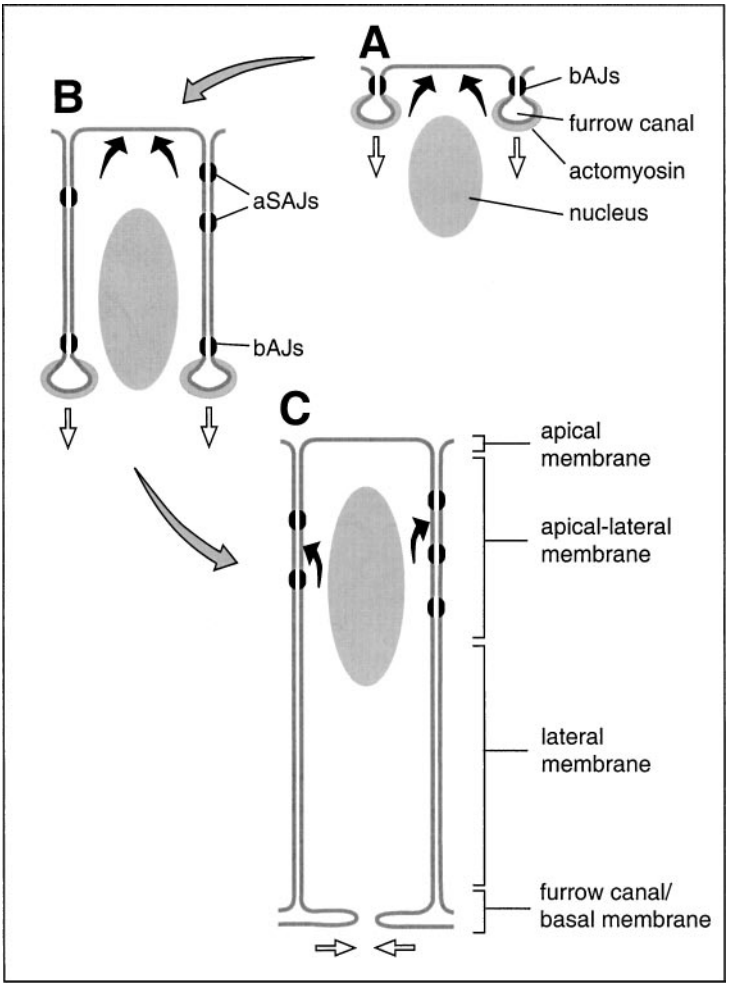


Figure 1 Cellularization forms the blastoderm epithelium in *Drosophila*. Three stages at early (*A*), mid- (*B*) and late- (*C*) cellularization are illustrated. The open arrows indicate the direction of plasma membrane movement and the black arrows point to the main membrane insertion sites as identified in (72). (*A*) Invaginations of the egg membrane surround each of ~5000 nuclei and form the furrow canals. The basal adherens junctions (bAJs) remain closely linked with the furrow canals during cellularization. (*B*) Apical spot adherens junctions (aSAJs) form at midcellularization, increase in number and are retained in the apical one third of the lateral membrane. These SAJs will form the ZA during gastrulation. (*C*) Four membrane domains, indicated to the right, have formed at late-cellularization. The bAJs and the actomyosin ring resolve at this time and the furrow canals expand to form the basal membrane.

show an asymmetric distribution in the forming lateral membranes during cellularization (see below). These include the cadherin-catenin-complex (CCC) as part of the basal and the more apical SAJs (27, 55, 97, 149). Taken together, these observations suggest that a polarized lateral membrane domain is established during cellularization.

Cellularization is a modified form of cytokinesis, in which the furrow canal represents the leading edge of the cleavage furrows. As in other cells, the contractile ring of Actin and Myosin II at the furrow canal associates with Septins and Anillin during cytokinesis (2, 41, 43). The actomyosin rings of all 5000 blastoderm cells form an interlocking hexagonal array that plays an important role in cellularization (42, 189). Mutations in a number of genes have been described that disrupt the actomyosin array and compromise cellularization. These genes encode factors that co-localize with the actomyosin array such as Peanut, a septin (2), Bottleneck (125), Serendipity-α (127), Discontinuous Actin Hexagon (191, 192), the Formin-homology protein Diaphanous (4), and possibly the small GTPases Rho1 and Cdc42 (28). Others factors that contribute to the organization or function of the actomyosin array but act at a distance include Nullo, which localizes to the basal junction (55, 117, 131), the transcriptional regulator Lilliputian, which controls the expression of Serendipity-α (148), and Nuclear Fallout, a centrosome-associated protein required for recruitment of Actin and Discontinuous Actin Hexagon to the furrow canal (123, 124). Also Discs Lost (Dlt) localizes to the furrow canal during cellularization and, if disrupted, causes cuboidal rather than highly columnar cells to form (11).

Data from a number of systems point to a prominent role of forces generated by the insertion of new membrane in cytokinesis (53). In this model, contraction of the actomyosin ring is not the main driving force of cytokinesis but, instead, forms an elastic and tensile structure that orients and synchronizes membrane movement. For cellularization, it has now been established that at least the bulk of the membrane material required for the ingrowth of the plasma membrane is derived from the biosynthetic pathway (21, 72, 132) and does not come from unfolding of the egg membrane, as had been proposed previously (44, 166). The t-SNARE Syntaxin1 (21) and the Golgi-associated protein Lava Lamp (132) are required for cellularization, and the progression of the furrow canal is blocked in response to injection of Brefeldin A, an inhibitor of Golgi-derived vesicle transport (132). The movements of vesicles and Golgi bodies from a reservoir below the nuclei into the apical cytoplasm depends on (−) end-directed microtubule transport. This vesicle movement contributes to membrane growth in particular during early cellularization (42, 43, 132, 172).

One contentious issue is the location of the site of new membrane insertion. The close association of Golgi bodies with the furrow canal (132) and the alignment of vesicles in front of the progressing furrow canal (80) suggest that the furrow canal is the primary site of membrane insertion. This scenario implies that membrane turnover in the furrow canal is rapid, with new membrane being "exported" to the growing lateral membrane. However, a recent study that traces membrane flow

during cellularization arrives at a different conclusion (72). Labeling of glycoproteins with fluorescent wheat germ agglutinin (WGA) in live embryos shortly before or early during cellularization revealed that the successive formation of membrane domains starts with the furrow canal. The main insertion site during early cellularization is the apical membrane. WGA remains with the furrow canals as they progress inwards, and apically inserted membrane moves basally to form lateral membrane. Late during cellularization the membrane insertion site shifts to the apical part of the lateral membrane. Membrane mixing appears rather limited as WGA-labeled membrane areas remain coherent and the label does not diffuse into other membrane domains. These findings suggest a sequential establishment of membrane domains during cellularization during which the furrow canal forms first followed by the basal part of lateral membrane, the apical membrane, and finally, the apical part of lateral membrane (Figure 1). The notion that the lateral membrane is subdivided into an apical and a basal region is further supported by the asymmetric distribution of molecular markers such as Neurotactin and Spectrin (72, 161).

We are only beginning to unravel the mechanisms that act to establish epithelial polarity during cellularization. Distinct apical and basolateral vesicle targeting mechanisms may not contribute to the formation of the blastoderm epithelium as bulk membrane insertion from the biosynthetic pathway appears to take place first at the apical membrane and later at the apical lateral membrane (72). Cadherin-based adhesive interactions may facilitate lateral membrane formation as adherens junctions are formed as soon as lateral membranes appear. Cellularization has so far not been studied in the complete absence of the CCC. However, recent analysis of Nullo indicates that it localizes to the basal adherens junctions and is required for their formation (55). This observation suggests that this junction plays an important role as cellularization in *nullo* mutant embryos is highly irregular (131). In wild-type embryos, Nullo degrades prior to the formation of the apical SAJs, and prolonged expression of Nullo blocks their assembly, causing abnormalities in epithelial morphology at gastrulation (55). Taken together, these findings suggest that the novel protein Nullo differentiates between the apical and the basal part of the lateral membrane and between the adherens junctions residing in these regions. These results also indicate that the adherens junctions that form during early cellularization play an important role in the formation of the blastoderm epithelium.

PROTEIN COMPLEXES INVOLVED IN SPECIFICATION AND REGIONALIZATION OF EPITHELIAL SURFACE DOMAINS

The exploration of epithelial differentiation in *Drosophila* has now led to the characterization of protein complexes that regulate polarity and junctional differentiation. Pioneering genetic work has identified several genes that are required for epithelial differentiation in *Drosophila*, including *crumbs* (*crb*), *shotgun*

(*shg*), *bazooka* (*baz*), *stardust* (*sdt*), *lethal giant larvae* (*lgl*), and *discs large* (*dlg*) (19, 45, 61, 102, 175). The products of these genes have now been characterized, and biochemical and/or genetic data suggest that these and other proteins form complexes that associate with the plasma membrane and show a polarized distribution. These complexes give essential cues that govern the polarized organization of epithelial cells. Aside from wondering about the molecular composition and mutual interactions within each complex, we need to ask how the polarized localization of these complexes is achieved, and how the activity of each complex affects cellular organization. Figure 2 illustrates the position of cellular junctions and the distribution of protein complexes important for epithelial differentiation.

Adherens Junctions and the Cadherin-Catenin Complex

The first event after cellularization that indicates the further elaboration of the epithelial cell surface is the formation of the ZA that occurs as cellularization nears completion and gastrulation proceeds. ZA formation has been characterized as a three-step process. First, SAJs form in the lateral membrane during cellularization. Second, at the onset of gastrulation these SAJs move toward the apicolateral edge of the cells. Third, SAJs fuse into a circumferential belt, the ZA, during gastrulation (97, 149, 150, 154). At late cellularization, apical markers such as Baz and β_{Heavy}-Spectrin (β_{H}-Spectrin), and lateral markers such as Arm and β-Spectrin are mixed in the apical part of the lateral membrane (97, 149, 161; A. Wodarz, personal communication). The segregation of these molecules into distinct plasma membrane domains leads to the formation of apical and basolateral domains that are separated by a ZA. The apical membrane domain is subdivided into two regions at this point, the free apical surface and the marginal zone, which represents a narrow region of cell-cell contact apical to the ZA (Figure 2) (149).

The formation of SAJs during cellularization presumably depends on the CCC, although direct evidence for such a requirement is still lacking. The CCC consists of DE-cadherin, the predominant epithelial cadherin in *Drosophila* encoded by the *shg* gene, Arm, the homolog of vertebrate α-catenin, Dα-catenin, and Dp120$^{\text{ctn}}$ (103, 104, 107, 109, 152, 167; R. Cavallo & M. Peifer, personal communication). In addition to the pool of Arm molecules that are part of the CCC, Arm is also an effector of Wingless (Wg) signaling. The relation between cytosolic Arm that participates in Wg signaling and junctional Arm remains unresolved. The CCC is essential in the female germline and thus embryos that lack the maternal components of either DE-cadherin or Arm cannot be studied (27, 48, 105, 110, 152, 173) (mutations for Dα-catenin and Dp120$^{\text{ctn}}$ are currently not available). However, if intermediate alleles of *shg* or *arm* are used, a limited number of fertilized eggs is recovered from females with a mutant germline. In such embryos, in which maternal and zygotic expression of *shg* or *arm* is strongly reduced, all epithelia that were examined lose integrity (27, 152). The development of *arm* mutant germline clone (*arm*^{GLC}) embryos was analyzed in detail (27). Here, plasma membrane-associated DE-cadherin is reduced and Dα-catenin is not membrane associated.

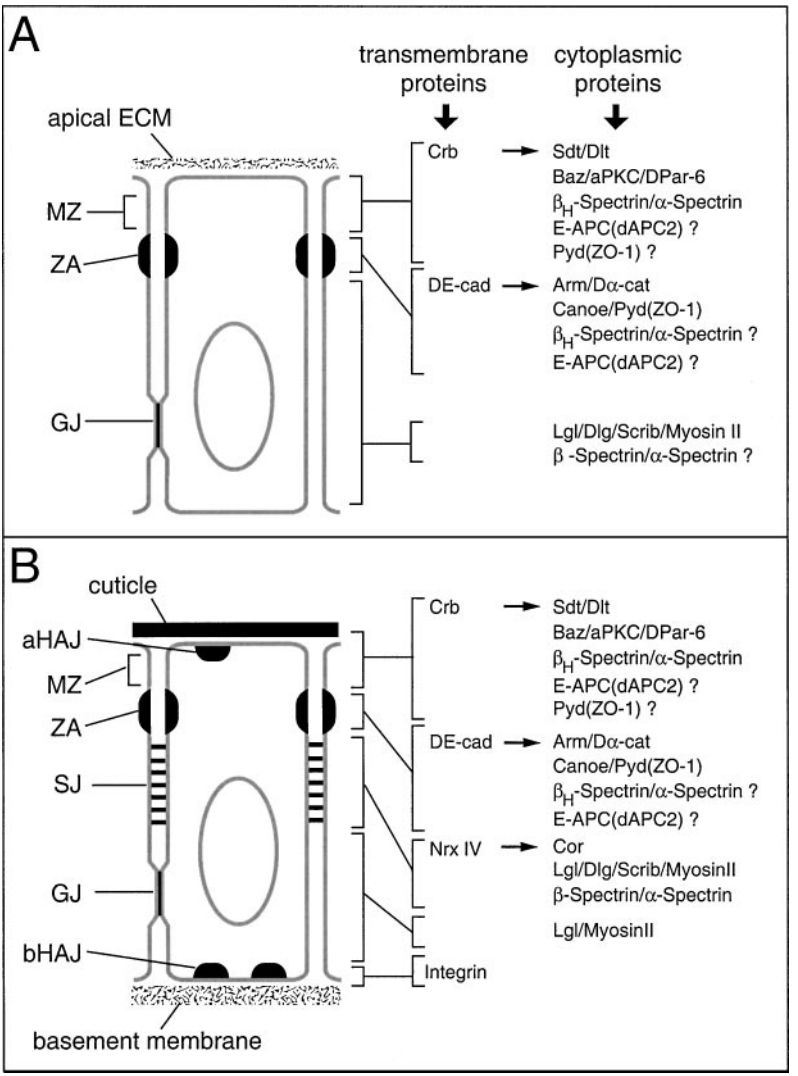


Figure 2 Schematic of epithelial cell structure in an ectodermal epithelial cell during gastrulation (*A*) and a late-embryonic/larval epidermal cell (*B*). Subdomains of the plasma membrane and cellular junctions are indicated to the left and the distribution of proteins discussed in this review are listed to the right. Cytoplasmic proteins that bind directly to a transmembrane protein are indicated by the small arrow. Synonyms of protein names are given in parenthesis. The question marks indicate that the localization of these proteins at the given position require confirmation, e.g., E-APC/dAPC2 might localize to the marginal zone, the zonula adherens or both regions. Abbreviations: aHAJ, apical hemi adherens junction; bHAJ, basal HAJ; DE-cad, DE-cadherin; ECM, extracellular matrix; GJ, gap junction; MZ, marginal zone; SJ, septate junction; ZA, zonula adherens.

Nevertheless, cellularization proceeds normally in these embryos, suggesting that very limited CCC activity is sufficient to promote cellularization. Alternatively, epithelium formation during cellularization might not require the CCC at all. This latter interpretation is supported by the radical shift in cell morphology seen in arm^{GLC} embryos at the onset of gastrulation (27). While the blastoderm forms normally in arm^{GLC} embryos, epithelia rapidly acquire a multilayered mesenchymal morphology at early gastrulation. At later stages of embryonic development, the CCC is needed to maintain integrity of all epithelial tissues that were studied (52, 151–153, 167). Thus, with the possible exception of the blastoderm, the CCC is essential to maintain adhesion and tissue architecture of *Drosophila* primary and secondary epithelia.

In the ovary, recent work indicates that the CCC is not required for the formation of the follicular epithelium but plays a role in its maintenance (147). Adherens junctions in the follicular epithelium contain DE-cadherin and DN-cadherin, which both disappear if follicle cells are rendered null for *arm*. *arm* mutant follicle cells are irregular in shape and sometimes form a multilayered epithelium. In most cases, however, *arm* mutant follicle cells remain within the epithelial layer and acquire a flat shape rather than being cuboidal or columnar, suggesting that the lateral membrane domain is reduced in size when the CCC is disrupted (96, 147). Interestingly, apical markers are lost (Crb and β_H-Spectrin) or mislocalized (Dlt) in *arm* mutant follicle cells although these cells retain a monolayered epithelial arrangement (147). These findings indicate that the loss of the CCC in the follicular epithelium disrupts the architecture of the apical domain without necessitating the breakdown of a monolayered epithelial tissue structure.

Adherens Junctions and Cell Signaling

Recent work in mammalian cell culture has established that the ZA, in addition to the CCC, contains a second complex composed of the immunoglobulin-like adhesion molecule Nectin and the cytoplasmic factors Afadin, a PDZ domain protein, and Ponsin, a SH3 domain protein. This complex interacts with both the CCC and the actin cytoskeleton. The knockout phenotype of Afadin in mice suggests that it has an essential role in maintaining epithelial integrity in the mouse ectoderm (56, 84, 85, 141, 144). *Drosophila* Canoe is the apparent ortholog of Afadin. Canoe was localized at the ZA in photoreceptor cells by immunoelectron microscopy (88), and appears to be a ubiquitous component of the ZA (143). Mutational analysis of *canoe* did not reveal a general requirement for epithelial or ZA integrity.

canoe mutant embryos show defects in dorsal closure, an epithelial migration process regulated by Jun N-terminal kinase (JNK) and Wg signaling (91, 101, 143). In fact, Canoe appears to be an upstream regulator of JNK signaling. In this process, Canoe colocalizes and interacts genetically and physically with *Drosophila* ZO-1, a MAGUK (membrane-associated guanylate kinase) protein encoded by the gene *polychaetoid* (previously also known as *tamou*) (143, 145, 170). Moreover, analysis of the role of Canoe in imaginal development suggests that it can physically interact with Ras1 and modulate Ras and Notch signaling (87, 95, 145). A close spatial

association between the ZA and Ras and Notch signaling components has been described previously (22, 40, 163, 188). Taken together, next to Arm/β-catenin, the work on Canoe represents the best evidence that a bona fide component of the ZA regulates cell signaling. How Canoe modulates signaling remains to be elucidated. It will also be interesting to see whether and how Canoe and Pyd/ZO-1 interact with the CCC and whether Canoe interacts with Nectin and Ponsin-like molecules in *Drosophila*.

In addition to Arm/β-catenin, a second connection between the ZA and Wg signaling has now been made. E-APC/dAPC2, one of two *Drosophila* homologs of the adenomatous polyposis coli (APC) tumor suppressor, is predominantly expressed in epithelial cells and localizes to the apicolateral plasma membrane, a region that includes the ZA and the MZ (89, 187). Whether E-APC/dAPC2 is a component of the ZA or whether its association with the ZA is more peripheral remains to be established. Recent data from mammalian epithelial cells suggest that the majority of APC associates with the apical membrane domain and does not colocalize with the CCC at the lateral domain (120). The localization of E-APC/dAPC2 to the apicolateral region depends on the integrity of the ZA and the actin cytoskeleton (164, 187). The analysis of a hypomorphic E-APC/dAPC2 mutation and RNA interference experiments did not reveal overt effects on epithelial polarity, although junctional Armadillo is reduced in some tissues.

Both human APC and E-APC/dAPC2 act as part of a "destruction complex" that destabilizes Arm/β-catenin and thus negatively regulate Wg/Wnt signaling (111). A temperature-sensitive missense mutation in E-APC/dAPC2, dAPC$^{\Delta S}$, causes E-APC/dAPC2 to accumulate in the cytoplasm and compromises its role in Wg signaling, suggesting that the localization of E-APC/dAPC2 to the apicolateral region of the plasma membrane is essential for its signaling function (89). Interestingly, apical secretion of Wg, which is controlled by the apical localization of its mRNA, is important for effective signaling (128), and also the Wg receptor Frizzled is a component of the apical membrane (138). The concentration of both positive and negative elements of the Wg signaling cascade, in addition to the components of the Notch, Ras, and JNK signaling pathways, found at the marginal zone and/or ZA suggest that this region is at the crossroad of several signaling pathways.

The ZA and E-APC/dAPC2 have now been identified as sources of a cue that controls the orientation and symmetry of cell division in the ectodermal epithelium of *Drosophila* embryos (82). As epithelial cells divide they round off and rise to the apical surface of the epithelium, where they remain connected to adjacent cells via the ZA (154). The spindle is oriented in parallel to the planar axis of the epithelium. Two equally sized daughter cells form that receive a similar share of apical and basolateral membrane and an equal amount of polarity determinants that associate with these membrane domains from the mother cell (82). If the ZA or E-APC/dAPC2 activity is disrupted, spindle orientation is abnormal and cell division is asymmetric (82). Two daughter cells of unequal size form, with the smaller cell receiving only basal membrane. This type of division pattern is reminiscent of mesenchymal neural progenitor cells (neuroblasts) or epithelial cells that express

Inscutable, a key regulator of asymmetric cell division in the neuroectoderm (65). Thus, epithelial cells have the potential to divide asymmetrically. However, this potential is normally overridden by a ZA-associated cue that requires the activity of E-APC/dAPC2. Disruption of dEB1 causes similar defects as blocking of E-APC/dAPC2 activity (82). dEB1 is the *Drosophila* homolog of mammalian EB1 that binds to APC (139). Although dEB1 does not appear to interact physically with E-APC/dAPC2 as the latter lacks a dEB1 binding site, the phenotypic similarities suggest that both proteins interact functionally (82). EB1 is known to preferentially interact with the (+) end of microtubules (10). These findings raise the intriguing possibility that E-APC/dAPC2 and dEB1 may connect the ZA to the (+) end of astral microtubules during division, thereby orienting the spindle along the planar axis of the epithelium as a prerequisite of symmetric division.

Apical Polarization I: The Crumbs/Stardust/Discs Lost Complex

Aside from bona fide components of the adherens junction, the formation of the ZA depends on two protein complexes that associate with the apical membrane. The first complex is composed of Crb, Sdt, and Dlt (the Crb complex). *crb*, which encodes an apical transmembrane protein, was the first *Drosophila* gene characterized as a key regulator of epithelial polarization (155, 157). Currently, no interaction partners are known for the 30 EGF-like and 4 LG domains found in the extracellular part of Crb. The short cytoplasmic domain of Crb contains two functionally important motifs (64). One of these, the C-terminal amino acids ERLI, is a PDZ binding motif that interacts with Dlt and Sdt (7, 11, 54, 64). The physical interactions observed between Crb and Sdt are consistent with a previous genetic analysis suggesting that *sdt* acts downstream of *crb* (156). Dlt contains 4 PDZ domains whereas *sdt* gives rise to several splice forms, encoding either a MAGUK protein with a single PDZ, a SH3, and a GUK domain or a smaller protein containing only the GUK domain (7, 54). Crb, Dlt, and Sdt are conserved in *C. elegans* and mammalian species (7, 17, 29, 54, 112; own unpublished results). The current release of the human genome contains three *crb*-like genes (*CRB1*, *CRB2*, *CRB3*) (29, 112). *CRB1* was shown to correspond to the retinitis pigmentosa 12 (RP12) gene, mutations in which cause a degeneration of the retina (29).

As mentioned above, Dlt is found at the furrow canal during cellularization. In contrast, Crb and Sdt are first seen at the onset of gastrulation in association with the apical membrane, at which time Dlt is also recruited to the apical membrane (7, 11, 54, 149, 157). How the apical localization of the Crb complex is established initially is unclear. Recruitment of Sdt and Dlt to the apical membrane depends on interactions with the cytoplasmic tail of Crb (7, 11, 54, 64), whereas maintenance of apical Crb depends on Sdt and Dlt (11, 156). The distribution of the Crb complex within the apical domain is not uniform. Low concentrations are seen in the central region of the apical membrane, typically the "free" apical surface, whereas high levels of the Crb complex are found at the marginal zone. This accumulation of the

Crb complex may be driven by homophilic interactions between Crb molecules on opposing cell membranes, as the localization to the marginal zone depends on the presence of Crb in both contacting cells (112). Crb expression persists in all epithelia that have a ZA throughout development.

The ZA is not established in *crb* and *sdt* mutant embryos, and adherens junction material retains a spot-like distribution (50, 97, 149). Failure to assemble a ZA is likely to be a major contributor to the tissue breakdown seen in mutants that are affected for a component of the Crb complex. In addition, a number of apical markers disappear from the cell surface in these mutants, suggesting that the apical surface domain is lost (7, 11, 54, 156, 178). Failure to assemble adherens junctions may contribute to the loss of apical markers, as seen in the follicular epithelium (147). The Crb complex is presumably a component of a larger scaffold that controls the molecular composition of the apical membrane similar to the mutual dependencies seen between Crb complex components. In fact, Crb is sufficient to promote apical membrane differentiation as Crb overexpression results in an apicalization of the cell surface at the expense of the basolateral membrane and a complete disruption of the CJC (50, 179). Overexpression of the membrane-tethered cytoplasmic domain of Crb can rescue the *crb* mutant phenotype and cause membrane apicalization to a similar degree as overexpression of full-length Crb (179). The C-terminal PDZ binding motif in the cytoplasmic domain of Crb is essential for this activity (64). In contrast, overexpression of Dlt does not cause an apicalization phenotype (11; G.T. & U.T. unpublished data), whereas overexpression of Sdt still needs to be examined.

A functional overlap between Crb activity and other polarization mechanisms becomes apparent when the effects of *crb* mutations on different epithelial tissues that express Crb at similar levels and with similar subcellular distributions are compared. While the disruption of the ZA is uniform throughout ectodermal and endodermal epithelia during gastrulation (50, 149), tissue-specific responses to the lack of Crb or Sdt become apparent when individual organ primordia are established (155, 156). The epidermis is most strongly affected, and the great majority of cells die through programmed cell death. On the other extreme, there are tissues that appear more or less normal in late mutant embryos such as the Malphigian tubules or parts of the foregut and hindgut, implying that these cells were able to recover and establish normal polarity and a CJC although their ZA did not form during gastrulation (155–157). In fact, if epidermal cell death is prevented, surviving cells form small epithelial vesicles in which individual cells show normal polarity and possess a CJC (G.T. & U.T. unpublished data). Thus, it appears that Crb is required to establish normal polarity at early stages but is not needed to maintain epithelial polarity at later stages of development. Moreover, epithelial development in *crb* null mutants can be rescued to a large extent by increasing the wild-type gene copy number of *sdt* from two to three, suggesting that Sdt has a Crb independent apicalization activity (156). Further, in *crb* null mutant cells that do not die, Dlt is reduced but not lost entirely from the apical membrane, suggesting that a Crb-independent apical targeting mechanism must exist for Dlt that may partially or completely compensate for the loss of Crb (147). Taken together,

these observations hint at complexities in the organization of protein scaffolds that define the apical surface domain of epithelial cells that remain largely elusive.

Apical Polarization II: The Bazooka/aPKC/DPar-6 Complex

A second complex that is important for the formation of the ZA but not a ZA component is composed of Bazooka (Baz), the *Drosophila* homolog of *C. elegans* Par-3 and vertebrate ASIP (66), *Drosophila* Par-6 (DPar-6) (115), and the *Drosophila* homolog of atypical Protein Kinase C (DaPKC) (180). This complex, called here the Baz/Par-3 complex, is conserved in *C. elegans* embryos where it contributes to the asymmetric division of the egg (122). In *Xenopus* oocytes this complex associates with the animal pole during maturation (99), and it localizes to tight junctions in mammalian epithelial cells where it is required for normal cell polarization (57–59, 78, 119, 140). Moreover, recent work in cell culture and *C. elegans* has shown that the small Rho family GTPases Cdc42 or Rac1 interact in their active, GTP-bound state with Par-6 and control localization and activity of the Par-3/Par-6/aPKC complex (49, 58, 59, 63, 78, 119).

Embryos that lack Baz function show defects at late cellularization/early gastrulation, at which time SAJs fail to concentrate at the apex of blastoderm cells and do not form a ZA (97). At early gastrulation in these mutant embryos, epithelial cells lose polarity, acquire mesenchymal characteristics, and gastrulation movements are compromised as a consequence. Later in development most cells die by programmed cell death (97, 174; U.T. unpublished data). This phenotype is very similar to the phenotype seen in arm^{GLC} mutants, suggesting that the failure of ZA assembly may be the major consequence of lack of Baz function. A similar phenotype is seen in embryos that lack DaPKC (180), whereas embryos that lack DPar-6 appear to undergo gastrulation movements normally and then subsequently lose epithelial integrity (115). Although the interactions between Cdc42 and the Baz/Par-3 complex have not been studied so far, embryos with reduced Cdc42 activity show defects in epithelial differentiation related to those in embryos with reduced activity of the Baz/Par-3 complex (46), raising the possibility that Cdc42 interacts with this complex as seen in other systems. The Baz/Par-3 complex associates with the entire apical membrane but is concentrated at the marginal zone similar to the Crb complex (180). It remains unclear as to how the Baz/Par-3 complex is linked to the plasma membrane, how it controls ZA assembly, and whether it has other important roles in epithelial polarity, in addition to ZA formation.

Apical Polarization III: The Lethal Giant Larvae/Discs Large/Scribble Complex

Molecular integrity and size of the apical domain also relies on the function of lateral protein complexes. Disruption of the CCC leads to loss of Crb and other factors from the apical membrane as mentioned above. A second interacting group of proteins that was recently implicated in the control of apical polarization is composed of Lgl, Dlg, and Scribble (Scrib) (14). Lgl is a Myosin II binding protein that contains WD40 repeats (93, 135), whereas Dlg and Scrib are multi-PDZ

domain proteins; Dlg is a MAGUK (183) and Scrib belongs to the LAP subfamily of PDZ domain proteins that also contain leucine-rich repeats (13, 15). *lgl*, *dlg*, and *scrib* mutants display similar defects in the embryo, imaginal discs, and the follicular epithelium. In addition, colocalization and genetic interactions observed between these genes suggest that these proteins may form a biochemical complex, called here the Lgl complex (14). Lgl, Dlg, and Scrib homologs are found in *C. elegans* and vertebrates where they play a role in epithelial polarization as well (16, 17, 75, 91a). Also the Lgl homologs in yeast and humans have been shown to interact with Myosin II (62, 137), suggesting that Lgl may regulate Myosin II function. In fact, suppression of myosin II function by Lgl has recently been demonstarted in *Drosophila* neuroblast where Lgl and Dlg control neuroblast polarity during asymmetric division (106, 113).

lgl and *dlg* were identified as tumor suppressor genes that control proliferation and tissue integrity of imaginal discs (45, 93, 183). In imaginal discs and in late embryos, Dlg and Scrib are specific components of the SJ, whereas Lgl overlaps with the SJ but retains a broader distribution at the basolateral membrane (14, 15, 136, 183, 184). The role of the Lgl complex as a component of the SJ is discussed in more detail below. Defects in epithelial polarity in *lgl*, *dlg*, or *scrib* mutant embryos, which lack both maternal and zygotic expression of these genes, or mutant follicular epithelia appear long before SJ form (14, 15, 114). Cells of the ectodermal epithelium show a mislocalization of apical markers, such as Crb, and ZA markers, such as Arm. Both apical and ZA markers spread basally, suggesting that the marginal zone and the ZA do not form normally and the apical membrane expands basolaterally (14, 15). These defects are most prominent during gastrulation, whereas at later stages of development normal polarity is re-established and a normal CJC forms (G.T. & U.T. unpublished data). These findings suggest that the Lgl complex controls the segregation of apical and basolateral membrane domains at gastrulation and contributes to confinement of apical and apico-lateral markers to their normal position. How the Lgl complex acts to support normal differentiation of epithelial surface domains is unclear at present. As mentioned, the link to Myosin II may suggest that the regulation of Myosin activity is one target of the Lgl complex also in epithelial cells. Alternatively, the recent finding that the yeast homolog of Lgl interacts with a SNARE protein in polarized vesicle targeting (76) raises the possibility that the Lgl complex may regulate vesicle targeting to control epithelial polarity. This notion is also supported by the finding that a human Scrib homolog, ERBIN, restricts the ERBB2/HER2 receptor to the basolateral membrane (16).

THE SEPTATE JUNCTION

Structure and Functions of Septate Junctions

One of the most distinctive ultrastructural features of the CJC in invertebrate epithelial cells is the SJ. During *Drosophila* development, SJs first appear midway

through embryogenesis, well after cellularization is completed, epithelial polarity has been established, and the ZA has formed. The SJ lies just basal to the ZA in epithelial cells, and within the SJ the membranes of adjacent cells maintain a constant distance of approximately 15 nm. In the pleated SJ (found in ectodermally derived epithelia and the glia sheets), regular arrays of electron-dense septae span the intermembranal space. In addition, freeze-fracture analysis reveals the presence of parallel rows of intramembranal particles (100a, 171) that presumably represent transmembrane proteins within the SJ. The septae form circumferential spirals around the cell much like the threads of a screw, and thereby greatly increase the distance that molecules must travel to pass between the apical and basolateral compartments of the epithelial sheet (25). Smooth SJs, which lack these ladder-like structures, are found only in the midgut and its derivatives. Relatively little is known of this variant of the SJ and for this reason we concentrate on the pleated SJ in this review.

Like other intercellular junctions, SJs have been proposed to play a role in formation of a trans-epithelial diffusion barrier, establishing and/or maintaining cell polarity, cell adhesion, and mediating interactions between cells. A variety of observations have led to the suggestion that SJs function in the formation of a trans-epithelial barrier. Morphological analysis, which revealed the existence of septae that fill the space between cells, led to the suggestion that SJs function to block direct paracellular flow between the apical and basolateral surfaces of epithelial sheets. This hypothesis was confirmed using injection of electron-dense dyes, which show restriction of dye diffusion at the SJ (25). More recently, mutational analysis of genes that encode SJ components has shown that disruption of the intercellular septae also results in disruption of the transepithelial seal (8, 68). A similar function has been ascribed to tight junctions in vertebrate epithelia, though SJs are quite different from tight junctions both morphologically and molecularly. Recent studies show that tight junctions regulate paracellular Na^+ and Mg^{++} ion flow by means of a selective "channel" function (129). Whether SJs display a similar ability to selectively regulate paracellular flow is a question that remains to be answered.

In addition to creating the paracellular barrier in ectodermally derived epithelia, pleated SJs have an essential role in the formation of the blood-nerve barrier in insect nervous systems (25). Although insect neurons are not myelinated as they are in vertebrates, neurons are typically surrounded by perineurial and glial sheath cells that form a diffusional barrier between the neurons of the central or peripheral nervous systems and the surrounding hemolymph. In some insects, though not in *Drosophila*, tight junction-like structures have been identified, in addition to SJs, in the surrounding cells (25, 70). Specific evidence regarding tight junctions in insects is discussed in a later section. All available evidence suggests that the SJs in epithelial cells and those in ensheathing perineurial and glia sheets are essentially indistinguishable.

Because SJs appear developmentally well after the time that epithelial polarity is established, they do not seem to be directly involved in this process, though they could be required to maintain that polarity once established. Thus the relationship

between the SJ and apical-basal polarity is currently unclear. Mutations in some known SJ components disrupt the structure of the junction and the localization of other SJ components, but do not seem to affect the ZA, transmembrane proteins such as Notch, or apically localized components of the cytoskeleton (68). These observations suggest that SJs do not function as a fence that blocks diffusion of membrane components between the apical and basolateral surfaces. However, previous experiments have indicated that such a fence does exist in invertebrate epithelia that lack tight junctions, though the SJ was not directly shown to be the source of the fence function (181). In addition, mutations in other known SJ-associated proteins, notably *lgl*, *dlg* and *scrib*, do affect epithelial polarity (see earlier discussion). These seemingly contradictory results may indicate that the SJ has a selective fence function for particular proteins and that a mutation in one component may affect only a subset of apically or basally localized proteins. Alternatively, the early function of the Lgl complex in cell polarity may be distinct from its later role at the SJ.

Molecular Architecture of the Septate Junction

Although no systematic attempt has been made yet to characterize the molecular components of the SJ, molecular genetic analysis of several developmentally interesting genes has led to the discovery of SJ components. Of the SJ-associated proteins thus far identified, two, Coracle (Cor) and Neurexin-IV (Nrx-IV), appear to be most central to the morphologically defined SJ. Cor is a member of the Protein 4.1 superfamily of cytoplasmic proteins (39) that includes Protein 4.1, the Ezrin, Radixin, and Moesin (ERM) proteins, the NF2 tumor suppressor Merlin, Talin, several protein tyrosine phosphatases, unconventional myosins, and *Drosophila* Expanded (146). Cor is most similar to Protein 4.1, showing approximately 60% identity with Protein 4.1 in the amino-terminal 400 amino acids, a region of the molecule that is highly conserved in all members of the superfamily. This domain has been termed the FERM domain (26). In Cor, this domain appears to provide all functions that are required for localization to the SJ (168), as well as for SJ structure and function (169). Cor also shares a region of similarity with Protein 4.1 at the carboxy terminus that is not required for SJ function but is essential for viability. Phenotypic analysis of *cor* mutants revealed a role for *cor* during dorsal closure, salivary gland morphogenesis, and cuticle formation during embryonic development (39, 68, 168).

Examination of a null *cor* allele demonstrated a requirement for Cor in the formation of the SJ (68). *cor* mutant embryos lack the intercellular septae that are characteristic of the pleated SJ. The functional significance of this observed defect was tested by examining permeability of a 10-kD rhodamine-labeled dextran in living embryos. Dextran injected into the hemocoel of *cor* mutant embryos freely crosses the salivary gland epithelium, whereas in wild-type embryos injected in a similar manner dye cannot cross the epithelial barrier for at least one hour. Thus, *cor* function is clearly required for the trans-epithelial barrier function of the SJ,

although, as noted previously, *cor* is not required for overall epithelial polarity or to restrict cell proliferation.

A significant step in understanding the role of Cor in SJ function was made with the discovery of the *Nrx-IV* gene (8). Nrx-IV is a *Drosophila* member of the Caspr (Contactin associated protein) family of neuronal receptors, which have a large extracellular domain with EGF and LG domains, and a single discoidin-like domain. *Drosophila* Nrx-IV possesses a single membrane-spanning region and, of particular interest, a short cytoplasmic domain that displays greater than 60% similarity to the cytoplasmic domain of glycophorin C, a transmembrane binding partner for Protein 4.1 in the erythrocyte (5). Like Cor, Nrx-IV is expressed in all cells that produce SJs, and its expression profile and subcellular localization are almost indistinguishable from that of Cor (8, 168).

Nrx-IV mutants display dorsal closure defects similar to those in *cor* and *dlg* mutant embryos (8, 168). Ultrastructural analysis revealed that the SJ is disrupted in *Nrx-IV* mutant embryos just as it is in *cor* mutants, identifying Nrx-IV as an important structural component of the SJ. *Nrx-IV* mutant embryos also display paralysis due to a breakdown of the blood-nerve barrier. Because this barrier is thought to be maintained by SJs, this result suggests that Nrx-IV, like Cor, is necessary for the barrier function of the SJ. Interestingly, mutations in *Drosophila gliotactin*, which encodes a neuroligin-like protein, also disrupt the blood-nerve barrier (6, 47). Neuroligins were originally identified as ligands for neurexins in neuronal synapses (23). Thus, it is possible that Gliotactin functions as a ligand for Nrx-IV in the SJ.

The similarity between the cytoplasmic tail of Nrx-IV and glycophorin C, the colocalization of Nrx-IV and Cor in the SJ, and the similarity of *cor* and *Nrx-IV* mutant phenotypes all suggest that *cor* and *Nrx-IV* may physically and functionally interact. Consistent with this notion, in *Nrx-IV* mutant embryos Cor fails to localize to the SJ and instead is distributed along the plasma membrane and in the cytoplasm (8, 168). Conversely, loss of *cor* function also affects Nrx-IV subcellular localization. Further studies have also shown that Cor and Nrx-IV can be co-immunoprecipitated from cell extracts, and that these proteins bind directly via the N-terminal conserved domain of Cor and the cytoplasmic tail of Nrx-IV (168). An unresolved question is how Cor and Nrx-IV initially target to the SJ, since they appear to show an interdependence that is incompatible with either one of them having this role. This observation suggests that at least one other protein whose identity is not yet known must be involved. By analogy with Protein 4.1 and glycophorin C, which interact with the PDZ domain containing proteins hDLG and p55 (83, 86), this third protein likely contains PDZ domains. The obvious candidate for this role is Dlg, but attempts to identify interactions between Dlg and either Cor or Nrx-IV have thus far produced negative results (168).

As indicated earlier, Dlg is another SJ-associated protein, and in fact was the first protein shown to localize preferentially to the SJ (183). Dlg is initially uniformly distributed along the lateral membrane and to a lesser extent throughout

the cytoplasm. During mid-embryogenesis, this subcellular localization is refined to the presumptive SJ (183). Although the precise cellular function of Dlg in the SJ is not known, previous work has demonstrated a direct role for *dlg* in the ultrastructure and function of the SJ (184). Imaginal discs in *dlg* mutant larvae lack the septae that characterize the pleated SJ, whereas some ZA material is mislocalized to a more basal location. In addition, the apical-basal polarity of the imaginal tissues is disrupted in *dlg* mutants. Interestingly, these effects are less severe in the nondividing salivary gland epithelial cells, perhaps suggesting that the SJ can be maintained once established, at least in cells that are not mitotically active, and therefore are not disassembling and reassembling the CJC.

dlg mutant imaginal discs display loss of epithelial polarity, cellular apoptosis, and overproliferation that becomes apparent during the extended larval period (more than twice the normal length) that is a consequence of this mutation (1, 182). As described previously, Dlg is part of the Lgl complex that is essential for epithelial polarity in the early embryo (14). The core function of this complex is likely the same in early embryogenesis, before the SJ forms, and later when the complex is associated with the SJ. Less clear is how the function of these proteins relates to the structure of the SJ itself. One possibility is that Dlg, Scrib, and Lgl, cooperatively, establish a unique domain in the apical lateral membrane that serves as a scaffold upon which the later-acting SJ components, such as Cor and Nrx-IV, can assemble during the formation of the SJ.

In addition to their effects on epithelial polarity, mutations in *dlg*, *lgl*, and *scrib* also result in tumor-like overgrowth of imaginal epithelia. However, because null mutations in these genes also affect the formation of other junctions, it is unclear if the observed overproliferation effect results from a direct role in restricting cell proliferation, or instead from disruption of intercellular interactions. In contrast to the overproliferation phenotypes of *lgl*, *dlg*, and *scrib*, mutations in *cor* result in a decreased rate of cell proliferation and eventual loss of cells from the epithelium due to cell competition (68). In addition, *cor* mutations dominantly suppress the hypermorphic *Ellipse* allele of the *EGF receptor* gene (39). Unlike *dlg*, *scrib*, and *lgl*, *cor* mutations do not affect overall epithelial polarity or the ZA. This result could suggest that some aspect of SJ function is required to promote cell proliferation, whereas disruption of apical-basal polarity and CJC formation results in overproliferation, perhaps due to the loss of intercellular interactions that normally function to restrict proliferation.

Relationship between the Insect Epithelial and the Vertebrate Paranodal Septate Junction

Until recently, septate junctions were believed to be unique to invertebrates, unlike the ZA, which appears to be widespread throughout the metazoans. However, it is now apparent that within the vertebrate nervous system a structurally

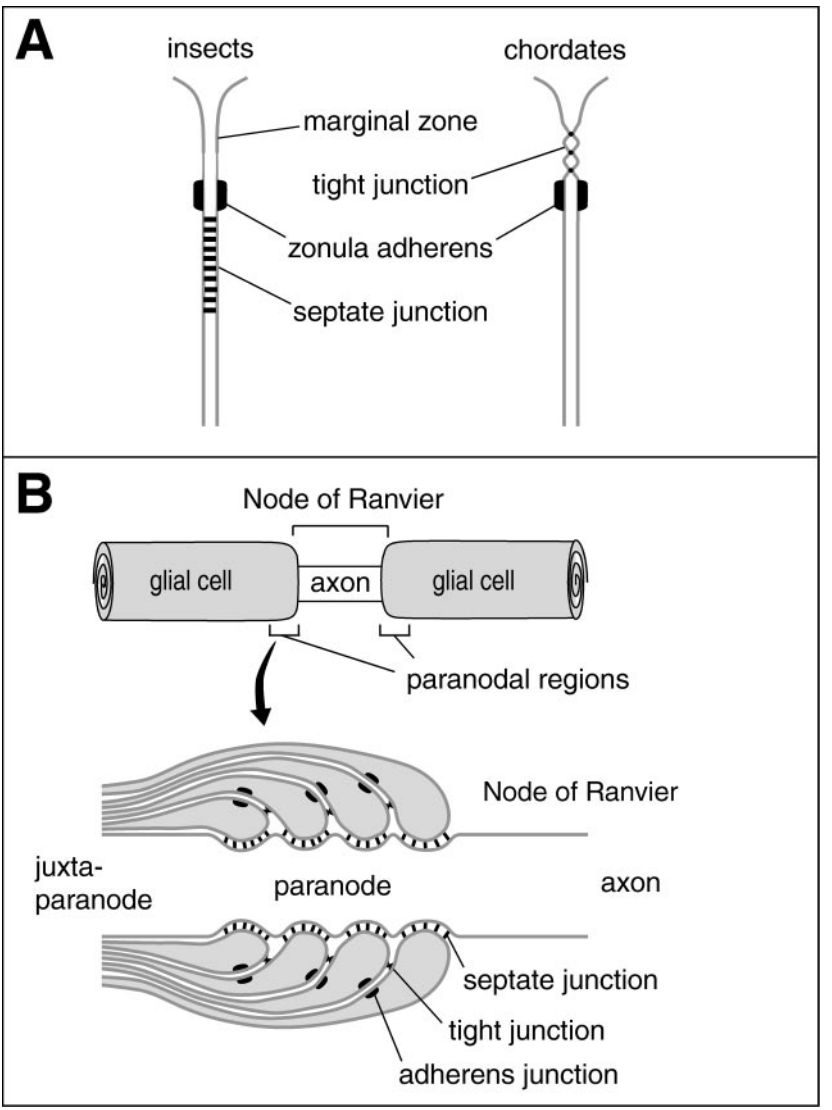
and functionally analogous SJ exists. Paranodal SJs are found in myelinated neurons at either end of each node of Ranvier, the region between adjacent sections of the myelin sheath in which components necessary for the action potential (primarily the voltage gated Na^+ channel) are clustered (Figure 3). These SJs form between the loops of myelinating oligodendrocytes and Schwann cells and the axons they ensheath. Morphologically, paranodal SJs are quite similar to SJs found in insect epithelial cells and glia, displaying a characteristic array of ladder-like cross-bridges (9, 108, 176). Functionally, paranodal SJs are thought to provide insulation between the nodal and internodal regions of the axon, thereby allowing the saltatory conduction that is essential for rapid transmission of electrical signals along myelinated nerve fibers. In addition, as the primary site of contact between axons and glia, they are almost certainly important in mediating signals between these very different but closely interlinked cell types.

Recent studies have made significant progress in understanding the molecular composition and genetic functions of the paranodal SJ. One of the first identified components of this junction, Caspr (Contactin-associated protein; also known as Paranodin), is the mammalian homologue of *Drosophila* Nrx-IV. Caspr is expressed only in neurons, and in mature myelinated neurons it is found exclusively in the paranodal SJs (36, 94). Caspr binds to a neuronal isoform of Protein 4.1 via its cytoplasmic tail (94), just as Nrx-IV binds to Cor (168). Thus two primary components of the invertebrate SJ have homologous counterparts in the vertebrate paranodal SJ. As its name implies, Caspr was isolated via its association with Contactin, a GPI-linked protein that is also expressed by neurons and localizes to the paranodal SJ. In addition to binding Caspr (in *cis* within the neuronal membrane), Contactin colocalizes with Neurofascin-155, an immunoglobulin superfamily adhesion molecule that is expressed on myelinating glia cells, although they do not appear to interact directly (142).

The functions of two components of the paranodal SJ, Caspr and Contactin, have recently been examined using knockout mutations in the mouse (12, 18). Mutation of either gene results in dramatic disruption of paranodal architecture and junctional function. As expected, both mutations alter the electrical properties of myelinated nerve fibers, resulting in reduced conduction velocity along the nerve. More surprising is the effect of these mutations on the organization of other proteins within the node of Ranvier, the paranodal regions, and in the myelinating cells. In *Caspr* knockout mice, the paranodal localizations of Contactin in the neuron and Neurofascin-155 in the myelinating cells are disrupted (12). Furthermore, Na^+ channels that are normally restricted to the node spread laterally along the axon into the paranodal region. Conversely, in *Caspr* mutant mice neuronal K^+ channels that normally are found just outside the paranodal region (in the juxtaparanode) redistribute into the paranodal region and into the node itself. Similar effects, including abnormal localizations of Neurofascin-155 and K^+ channels, are seen in the *contactin* knockout mutant (18). In addition, Caspr protein failed to be transported from the neuronal cell body to the axon in *contactin* mutant neurons, consistent with previous reports

that these proteins form a complex prior to transport to the plasma membrane (37).

Taken together, these results provide strong evidence that the paranodal SJ not only provides a site of contact between the neuron and myelinating glia cell, but also serves as a molecular sieve that organizes the nodal, paranodal, and juxtaparanodal regions of myelinated neurons (108). This sieving effect appears to be analogous to the fence function within the plane of the plasma membrane that has



been shown for tight junctions in mammalian epithelial cells (133) and proposed for the SJ in invertebrate epithelia (181). Given their molecular and morphological similarities, the invertebrate SJ and the mammalian paranodal SJ probably both derive from a common ancestral junction and they are both structurally and functionally homologous. This observation has important implications for both SJs. By analogy with the paranodal SJ, epithelial SJ may have a selective fence function within the plane of the plasma membrane that has not yet been well characterized, and an as yet unidentified Contactin-like molecule may serve as a binding partner for Nrx-IV. Conversely, genetic studies of the epithelial SJ and epithelial polarity in *Drosophila* should provide new insights into the components and functions of the paranodal SJ in vertebrates. For example, we currently know little about the mammalian homologues of *lgl*, *scrib*, and *dlg* in neuronal development; however, the localization of these proteins to the epithelial SJ and their importance in epithelial polarity and SJ function suggest that their mammalian homologues are significant components of paranodal SJs and axonal cell polarity.

TIGHT JUNCTIONS IN DROSOPHILA?

As mentioned earlier, in vertebrate epithelia tight junctions are believed to form the principle paracellular barrier to transepithelial diffusion. Morphologically, tight junctions are characterized by strands of intramembranous particles in freeze fracture analysis. Based on this criterion, previous studies have reported the existence of tight junctions in a variety of invertebrate species, including insects (25, 69–71). However, careful morphological studies have so far failed to identify a tight junction-like structure in *Drosophila* (25, 154). For this reason, and because the SJ seems to provide at least some of the functions ascribed to tight junctions in vertebrate cells, there has not been a clear consensus on the existence of tight

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Figure 3 Comparison of *Drosophila* and chordate apical junctional complexes (*A*) and the structure of the vertebrate paranodal junction (*B*). Insect and chordate epithelia are similar in that the junctional complex in both contains a zonula adherens. In insects the marginal zone is apical to the zonula adherens, whereas in chordates the tight junction is located at this position. Insect epithelial cells have in addition a septate junction that lies basal to the zonula adherens. In (*B*), the structure of the node of Ranvier in myelinated neurons is diagrammed above, with a higher magnification view of the paranodal region presented below. In the node of Ranvier, the myelin sheath is interrupted. At the edge of the sheath (the paranodal region) loops from the myelinating cell are closely apposed to the axon. At the point of contact between the neuron and the myelinating cell, a septate junction forms that is structurally and molecularly similar to the septate junction of insect epithelial and glial cells.

junctions in insects, nor have any functional studies been performed in *Drosophila*.

Despite the lack of evidence for tight junctions in *Drosophila*, molecular genetic analysis of developmentally important genes and the *Drosophila* genome project have identified apparent homologues of known components of the vertebrate tight junction. For example, the previously mentioned *pyd* gene encodes a protein that is similar to the mammalian ZO-1 protein, the first identified tight junction component (134, 145). Although PYD/ZO-1 was originally described as an SJ component, subsequent studies indicate that one isoform is localized apical to the SJ, while another seems more broadly distributed in the apical region of the cell (170). Analysis of vertebrate tight junctions has identified two other types of proteins that seem to be integral to the tight junction, the occludins and the claudins, although recent studies indicate that only the claudins are essential for tight junction function (165). The *Drosophila* genome does not contain any convincing occludin homologues (3). In contrast, there are at least two possible claudin-like genes in the genomic sequence (CG3770 and CG6982) that have four predicted transmembrane domains in a similar arrangement to the claudins (R. Fehon, unpublished observations). So far, neither of these predicted genes nor the proteins they encode have been studied.

Why then have tight junctions not been observed in *Drosophila*? Note that although PYD/ZO-1 is expressed apically in epithelial cells (170), we do not yet know how widely the claudin-like proteins are expressed. Thus, *Drosophila* tight junctions might be restricted to a particular developmental stage or tissue that has not been examined carefully enough to detect tight junctions (24). However, *Drosophila* epithelia might also retain some tight junctional structure, at least at the molecular level, but not have the occluding function of the mammalian tight junction (that is instead provided by the SJ). In mammalian epithelia, the tight junction is found at the apical-most point of contact between cells, just apical to the ZA. In *Drosophila*, the corresponding region is the marginal zone (149) (Figure 3), an area that lacks obvious junctional morphology but does seem to have an accumulation of transmembrane receptors and associated proteins (7, 11, 54, 66, 115, 149, 170, 180). Of particular interest in this regard is the recent demonstration that the Baz/Par-3 complex, which localizes to the marginal zone (66, 115, 180), has mammalian homologues that reside in the tight junction and is essential for tight junction assembly (57, 58; also see earlier discussion of these genes). Taken together, these results indicate that a number of tight junction proteins localize to the marginal zone in *Drosophila*, whereas currently no *Drosophila* homologues of tight junction proteins are known that associate with the SJ. These data suggest that the marginal zone in *Drosophila* epithelia may share some functions, in particular cell-cell signaling and perhaps the fence function within the plane of the plasma membrane, with the mammalian tight junction. In this regard it would be particularly interesting to know the subcellular localizations of the claudin-like proteins in *Drosophila* epithelial cells, if indeed they are expressed in these tissues.

THE SPECTRIN CYTOSKELETON IN EPITHELIAL DIFFERENTIATION

One important aspect of epithelial polarity is the corresponding polarization of the underlying actin-based cytoskeleton that occurs via interactions between polarized transmembrane proteins, membrane-associated cytoplasmic proteins, and cytoskeletal proteins (100, 185). Among the many proteins that appear to be involved in this process, spectrin seems to play a crucial role. The spectrin protein is a tetrameric actin crosslinking protein comprised of two α and two β subunits. Epithelial cells contain a polarized spectrin cytoskeleton, in which distinct isoforms of spectrin associate with the apical or basolateral membrane. Spectrin contributes to polarized membrane organization by binding, and thus trapping membrane proteins at the basolateral surface (92, 100).

Drosophila has three different spectrin subunits, α, β, and β_H-Spectrin, which assemble into two different isoforms, $\alpha_2\beta_2$-Spectrin and $\alpha_2\beta_{H2}$-Spectrin. (32, 159). The two isoforms show non-overlapping polarized distributions in epithelial cells. $\alpha_2\beta_2$-Spectrin is found at the basolateral membrane where it forms a complex with Ankyrin (33, 35, 74). In contrast, the $\alpha_2\beta_{H2}$-Spectrin associates with the apical domain where it is enriched in the marginal zone and, possibly, the ZA (160, 162) (Figure 2). Before the onset of cellularization, $\alpha_2\beta_{H2}$-Spectrin associates with the egg membrane, and during cellularization it remains with the furrow canals, whereas $\alpha_2\beta_2$-Spectrin is added to the lateral membrane as it forms. At late cellularization, β-Spectrin and β_H-Spectrin overlap in the apical-lateral membrane, as mentioned above, before they segregate into their final distinct apical and basolateral positions (161).

Mutational analyses have been carried out for all three *Drosophila* spectrin genes but so far did not reveal a general role of the spectrin cytoskeleton in epithelial polarity. Lack of β_H-Spectrin does not cause defects in cellularization or epithelial polarity in early embryos (J.A. Williams & G.H. Thomas, personal communication), whereas the requirement of β- and α-Spectrin in early embryos remains to be analyzed. However, spectrin mutants exhibit a number of interesting defects, which suggest that spectrin has cell type-specific roles in epithelial differentiation. *α-spectrin* mutants die as larvae and exhibit loss of cell-cell contacts in the midgut whereas other epithelial tissues differentiate normally (74). *α-spectrin* mutations affect the cuprophilic cells of the midgut epithelium that are responsible for the acidification of the midgut content. In *α-spectrin* mutant larvae, β_H-Spectrin is lost from the apical membrane of cuprophilic cells. In addition, the actin cytoskeleton appears disorganized, and acid secretion is impaired (31, 74). In contrast, cuprophilic cells mutant for *β-spectrin* show a disrupted organization of the basolateral membrane that fails to accumulate the Na^+, K^+-ATPase, a defect not seen in *α-spectrin* mutants (34). These findings suggest that $\alpha_2\beta_{H2}$-Spectrin is required for the differentiation of the apical membrane in cuprophilic cells, and that β-Spectrin functions independently of α-Spectrin in the differentiation of the basolateral membrane domain.

β_H-Spectrin in encoded by the *karst* gene. *karst* mutations are semiviable and adult escapers exhibit bent wings, tracheal defects, sterility, and rough eyes (162). However, no obvious polarity defects were observed in *karst* mutant imaginal discs. A role for spectrin in the maintenance of epithelial polarity has been found in the ovarian follicular epithelium (73). Follicle cells that lack α-Spectrin form a normal follicular epithelium initially, but exhibit overproliferation, multilayering, and loss of the apical β_H-Spectrin at later stages. *α-spectrin* mutant follicle cells retain lateral β-Spectrin, suggesting that recruitment of β-Spectrin to the basolateral membrane is independent of α-Spectrin (73). Also, follicle cells that lack β_H-Spectrin form a follicular epithelium but lose apical α-Spectrin. No polarity defects are detected in *karst* mutant follicle cells. However, fragmentation of the ZA is observed in *karst* mutant follicle cells as they migrate posteriorly to cover the oocyte. At this time follicle cells in *karst* mutants fail to constrict apically, suggesting that β_H-Spectrin may stabilize the ZA during apical constriction (159, 190). These results suggest that the defects seen in *α-spectrin* mutants are largely independent of β_H-Spectrin, and that β_H-Spectrin is involved in maintaining the ZA during epithelial morphogenesis.

CONCLUSIONS AND PROSPECTS

The recent progress in our understanding of the mechanisms involved in epithelial polarization has focused our attention on a number of protein complexes that play essential roles in the formation of distinct plasma membrane domains. These protein complexes either congregate around transmembrane proteins (Cadherin, Crb, Nrx-IV) or represent cytocortical protein assemblies for which the mechanism of plasma membrane association remains obscure (Baz/Par-3 complex, Lgl complex). Additional components of these protein complexes and molecular interactions within these complexes remain to be characterized. Further, a remaining major challenge is to uncover how the activity of these complexes is integrated to generate a single polarized cellular architecture. How, for example, does the Crb complex control ZA formation and how, in turn, do adherens junctions control the stability of the Crb complex? Similarly, how does the Lgl complex, which localizes to the lateral membrane, confine the extent of the apical domain? These functional relationships suggest connections between these complexes, either in the form of physical linkages or perhaps via intracellular signaling pathways, which are currently not understood.

We have not discussed in detail a number of additional *Drosophila* genes that act in epithelial differentiation because their function and their relation to the larger themes elaborated in this review are not well understood. Among these genes is *bloated tubules*, which encodes a transmembrane protein related to vertebrate neurotransmitter symporters and controls the extent of the apical domain in Malphigian tubules (60). The *arc* gene encodes a PDZ domain protein that associates with the marginal zone and/or ZA of portions of embryonic and imaginal

epithelia and controls the morphogenesis of imaginal discs (79). *faint sausage* encodes a GPI anchored adhesion molecule of the immunoglobulin superfamily and is required for maintenance of epithelial intergity from mid- to late-embryogenesis (77). Moesin and Merlin, members of the Protein 4.1 superfamily, are found in the apical region of epithelial cells (90). *Merlin* mutations do not appear to affect overall cell polarity (67), and the effects of *Moesin* mutations on polarity and epithelial integrity are currently being examined (O. Nikiforova & R. Fehon, unpublished results). A major challenge for the near future will be to explore the activity and molecular interactions of these proteins, and other yet unidentified genes that play a role in epithelial polarity. Moreover, one issue that plays a central role in the discussion of epithelial polarity in mammalian cell culture models, the contribution of protein and lipid sorting in the biosynthetic pathway, has so far not been vigorously pursued in *Drosophila*.

Intriguing parallels and differences become apparent when polarity in epithelial cells and non-epithelial neuroblasts are compared. Neuroblasts in *Drosophila* (similar to the one-cell *C. elegans* embryo and budding yeast) have two surface domains, an apical and a basal domain (anterior and posterior in *C. elegans* and bud site versus non-bud site in yeast) (30, 81, 122). In contrast, in differentiated epithelial cells we can distinguish at least six membrane domains, the free apical surface, the marginal zone, the ZA, the SJ, the lateral membrane basal to the SJ, and the basal membrane. Only the Baz/Par-3 complex and the Lgl complex act in both neuroblast and epithelial polarity, whereas the complexes that associate with Cadherin, Crb, and Nrx-IV are not needed for neuroblast polarity. This comparison emphasizes the central role of adhesive interactions mediated by transmembrane adhesion receptors in defining epithelial membrane domains. Moreover, it raises the question of how the polarized cortical localization of the Baz/Par-3 complex and the Lgl complex is generated in epithelial cells and neuroblasts in the absence of any known transmembrane components or other localization cues. Two inferences can be drawn from these observations. First, we may view polarity as seen in yeast, the *C. elegans* embryo, or *Drosophila* neuroblasts as a simpler form of cell polarization that is elaborated upon in epithelial cells by the impact of cell adhesion receptors and their associated protein complexes. Second, we are still far from fully understanding the mechanisms by which the intricate cellular architecture of epithelial cells, or even the relatively simpler polarized organization of other cells, is established and maintained in developing organisms.

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