Composition and Formation of Intercellular Junctions in Epithelial Cells

Elisabeth Knust* and Olaf Bossinger*

The polarized nature of epithelial cells is manifested by the nonrandom partitioning of organelles within the cells, the concentration of intercellular junctions at one pole, and the asymmetric distribution of proteins and lipids within the plasma membrane. These features allow epithelia to fulfill their specific tasks, such as targeted uptake and secretion of molecules and the segregation of different tissue compartments. The accessibility of *Drosophila melanogaster* and *Caenorhabditis elegans* to genetic and cell biological analyses, combined with the study of mammalian cells in culture, provides an ideal basis for understanding the mechanisms that control the establishment and maintenance of epithelial cell polarity and tissue integrity. Here, we focus on some of the best-studied junctions and membrane-associated protein complexes and their relation to cell polarity. Comparisons between fly, worm, and vertebrate epithelia reveal marked similarities with respect to the molecules used, and pronounced differences in the organization of the junctions themselves.

Introduction

Epithelial tissues are present in most metazoa and perform two closely related functions: They delimit compartments within tissues and participate in the controlled exchange of molecules and ions between them, thus regulating homeostasis. In the developing embryo, morphogenetic movements, such as the invagination of cell sheets or the formation of tubes, rods, or placodes, are associated with a variety of shape changes in epithelial cells. Two particular features allow epithelial cells to fulfill their specialized functions during morphogenesis and in the differentiated state, where they may be subjected to mechanical stresses or strong pressures, e.g., in the intestine or the bladder: (i) they have a polarized phenotype and (ii) they form highly elaborate cell-cell junctions. Polarization is reflected in the shape of the cell, the uneven distribution of organelles and molecules, and the oriented alignment of the cytoskeletal networks. But the plasma membrane itself is also polarized, being divided into two distinct regions: the apical domain facing the external environment or a lumen; and the basolateral domain, which is in contact with neighboring cells or a basal substratum. Various cell-cell contacts guarantee close adhesion between the cells and provide the barrier function characteristic of epithelia.

Studies performed on epithelia of different species have uncovered a high degree of similarity between them in structural and molecular terms, but have also revealed remarkable differences, e.g., with regard to the organization and composition of cellular junctions (Figs. 1 and 2). The cells of all epithelia analyzed so far have an adhesive belt that encircles the cell just below the apical surface, which is called the zonula adherens (ZA) and is associated with an electron-dense cytoplasmic plaque of actin. Vertebrate epithelial cells develop a tight junction (TJ), a specialized plasma membrane microdomain apical to the ZA. In *Drosophila* epithelial cells, the septate junction (SJ) lies basal to the ZA and forms a region of close membrane contacts that extends over large parts of the lateral plasma membrane domain. In the nematode *Caenorhabditis elegans*, only a single, tripartite junction, the *C. elegans* apical junction (CeAJ), has been identified, which resembles the ZA of *Drosophila* and vertebrates (*1*–*3*).

A plethora of molecules contribute to the formation of spatially and functionally distinct domains in the plasma membrane and the membrane-associated cytoskeleton. These domains mediate cell-cell adhesion by forming elaborate junctions visible under the electron microscope. In addition, they are sites of intensive intercellular signaling, and defects in their organization may result in apoptosis or uncontrolled cell division. In most cases, these domains exhibit a common molecular organization, in that one or several transmembrane proteins are linked by their intracellular tails to cytoplasmic proteins. Similar structures in different species tend to be made up of similar proteins. For example, the transmembrane protein E-cadherin and its associated cytoplasmic proteins α- and β-catenin are found in the ZA (Fig. 2). Sometimes, however, corresponding ultrastructures are missing in cells of different species, yet homologous molecules are localized at comparable sites. For example, although epithelial cells in *Drosophila* do not develop TJs, a distinct region apical to the ZA, the subapical region (SAR), harbors protein complexes that colocalize with tight junctions in vertebrate cells. These observations are striking and raise the question of how this stereotypic pattern is established during development and what mechanisms are involved in its maintenance. Of course, other cell types, such as neurons, also exhibit a polarized phenotype, but these will not be considered here.

Formation of Intercellular Junctions in the *Drosophila* Embryo

Because the *Drosophila* embryo is readily accessible to genetic analysis, mutations have been identified that affect the establishment and/or maintenance of epithelial cell polarity. Here we will concentrate on epithelia in the developing embryo, but additional data have been obtained from the analysis of the egg follicle and the imaginal discs (*4*).

The first epithelium to form in the *Drosophila* embryo, the blastoderm, develops from a syncytium by multiple invaginations of the plasma membrane, forming the cleavage furrows. This process increases the surface area of the plasma membrane nearly 25-fold and leads to the orderly segregation of the ~5000 nuclei located in the cortex beneath the plasma membrane. The establishment of cell polarity occurs concomitantly with the polarized growth of the plasma membrane (*5*). The furrow canal, a distinct membrane region that is specified first and occupies the basalmost part of the invaginating membrane, will later become the basal membrane domain. During cellularization, a transient, so-called basal adherens junction (BAJ) is formed, just apical to the furrow canal. It is made up of a protein complex containing the known ZA components E-cadherin, α- and β-catenin (Armadillo, Arm), and the protein Discs lost (Dlt), which contains four PDZ domains. Midway through the cellularization process, additional Arm accumulates in spot junctions along the lateral membrane; these later coalesce to form the apical ZA (see below). By the end of cellularization, several distinct domains can be distinguished

Institut für Genetik, Heinrich-Heine Universität Düsseldorf, Universitätsstrasse 1, 40225 Düsseldorf, Germany.

*To whom correspondence should be addressed. E-mail: knust@uni-duesseldorf.de, bossinge@uni-duesseldorf.de

in the plasma membrane by the differential expression of specific proteins (*5*).

Formation of the BAJ requires several additional factors, among them Nullo and Slam, two recently identified proteins with no similarity to any other sequences in the database. Slam has been suggested to specify the membrane domain that will form the furrow canal. In embryos lacking *slam* function, furrow canals fail to appear, and neither BAJs nor the lateral membrane domains are specified. Nullo is required to separate the lateral membrane from the furrow canal. In its absence, BAJ formation is compromised, Arm is not restricted to the BAJ, and cleavage furrow formation is not initiated. Discs Lost is essential for the establishment of polarized epithelial cells. Embryos in which both maternal and zygotic *dlt* mRNAs have been inactivated by injection of double-stranded RNA (RNA interference) fail to establish the typical columnar shape of blastoderm cells (*6*).

Once cellularization is complete, the laterally dispersed spot adherens junctions coalesce apically and eventually form a belt around the cell, the ZA (*4*). In addition to the components of the ZA already mentioned (E-cadherin, α- and β-catenin), the correct positioning and the integrity of the ZA depend on the formation of specialized protein complexes apical and basal to the ZA itself. In the SAR, one complex comprises the transmembrane protein Crumbs, which binds to the MAGUK (membrane-associated guanylate kinase) protein Stardust (Sdt) by way of its four COOH-terminal amino acids, and recruits Dlt into the complex (*7*–*9*). The composition of this complex is conserved in vertebrate epithelial cells (see below). Crumbs also provides a link to the apical spectrin membrane cytoskeleton, which may contribute to the reinforcement of the ZA (*10*). This latter function is mediated by an interaction between the conserved FERM binding site in the cytoplasmic tail of Crumbs and DMoesin, a member of the 4.1 superfamily of cytoplasmic proteins. This protein family includes protein 4.1; the ERM proteins Ezrin, Radixin, and Moesin; the NF2 tumor suppressor Merlin; and others (*11*). Embryos lacking either Crumbs or Stardust fail to establish a continuous adherens belt and die with severe defects in epithelial cell polarity and tissue structure. The second well-characterized protein complex in the SAR, which has also been conserved during evolution (*12*), is formed by the PDZ proteins Bazooka, DmPar-6, an atypical protein kinase (DaPKC), and, at least in vertebrate cells, the monomeric guanosine triphosphatase (GTPase) Cdc42 (see below). *Drosophila* embryos that are completely devoid of Bazooka or DaPKC fail to establish plasma membrane polarity after cellularization and, consequently, the ZA fails to form. Embryos lacking DmPar-6 cellularize normally but do not assemble a functional ZA (*4*).

A further protein complex, which lies basal to the ZA, has been uncovered in epithelial cells of several species, including *Drosophila*. It is composed of the multi-PDZ and leucine rich–repeat protein Scribble (Scrib), a member of a newly defined protein family with high homology to proteins known to interact with small GTPases (*13*); the MAGUK protein Discs large (Dlg); and Lethal giant larvae (Lgl), a protein that contains WD-40 repeats. Localization of each of these proteins to the lateral membrane depends on the presence of each of the other proteins. Failure to establish this complex results in the expansion of proteins of the SAR, e.g., Crumbs, into more lateral positions. Consequently, the ZA is not formed and the epithelium becomes multilayered (*14*). Recent results suggest a single regulatory hierarchy between the Crb-Sdt-Dlt, the Baz-DmPar6-DaPKC, and the Scrib-Lgl-Dlg complexes (*15*). According to this model, the function of the Baz-DmPar6-DaPKC complex (which forms first) is antagonized by the Scrib-Lgl-Dlg complex, which represses apical membrane identity. Finally, apical recruitment of the Crb-Sdt-Dlt complex by the Baz-DmPar6-DaPKC complex counteracts the activity of the Scrib-Lgl-Dlg complex. How these protein complexes interact with components of the ZA remains unclear.

The Scrib-Lgl-Dlg complex is gradually integrated into the SJ. The SJs first become manifest midway through *Drosophila* embryogenesis and are characterized by electron-dense septae between adjacent lateral membranes. A barrier function of the SJs has been demonstrated in another cell type in *Drosophila*, the periglia; these cells surround the central nervous system, thus providing a brain-hemolymph barrier. Lack of the transmembrane protein Neurexin IV (Nrx-IV), a component of the SJ, leads to paralysis of the larvae as a result of leaks in the brain-hemolymph barrier caused by the breakdown of the SJs (*16*). However, mutations in Nrx-IV do not affect epithelial polarity or adhesion in the embryo, although the structure of the SJ is disrupted. Nrx-IV binds by way of its cytoplasmic tail to the con-

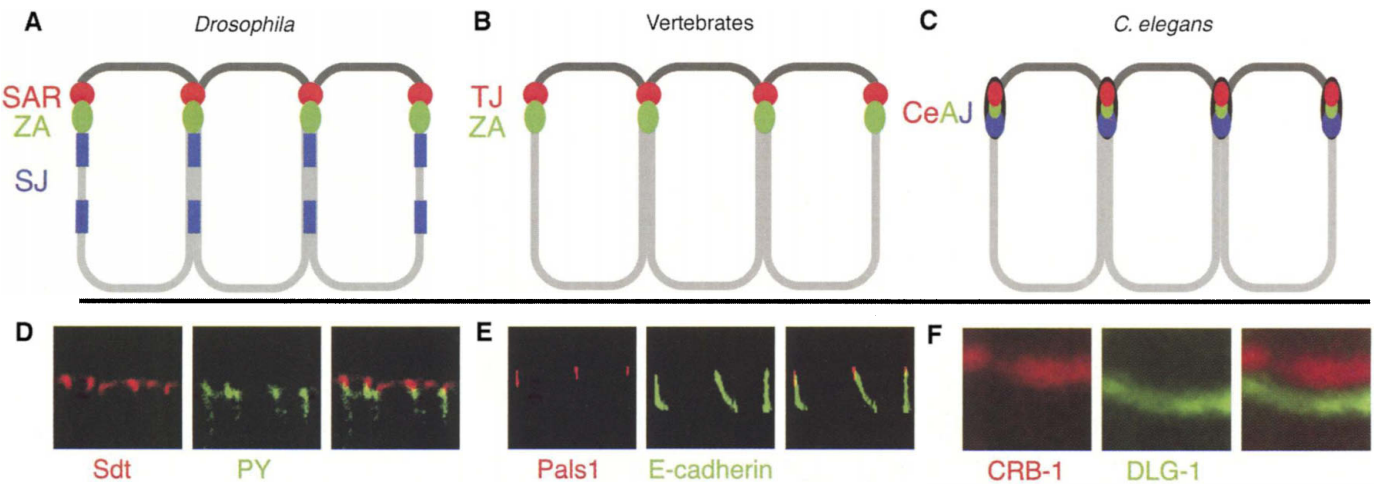

Fig. 1. Comparison of the organization of epithelia cells in *Drosophila*, vertebrates, and *C. elegans*. (**A** to **C**) Schematic representation of epithelial cells and the localization of the junctions. Whereas in *Drosophila* and vertebrate epithelia corresponding junctional regions are clearly separated, the *C. elegans* junction presents a single structure, subdivided into three parts. The colors of the individual regions/junctions (SAR, subapical region; TJ, tight junction; ZA, zonula adherens; SJ, septate junction; CeAJ, *C. elegans* apical junction) correspond with those used in Fig. 2. (**D** to **F**) Immunofluorescent labels demonstrate the different domains in an epidermal cell of a *Drosophila* embryo (D) (red: Stardust, Sdt; green: phosphotyrosine, PY), in MDCK cells (E) (red: Pals1; green: E-cadherin), and in *C. elegans* gut cells (F) (red: CRB-1; green: DLG-1). In (D) to (F), apical is up and basal is down.

served NH_2-terminal domain of Coracle (Cor), a member of the 4.1 superfamily (*17*). It is not clear whether Cor and Nrx-IV are recruited to the SJ by the MAGUK protein Dlg and its partners Lgl and Scrib. Dlg, the first protein shown to localize preferentially to the SJ (*18*), acts as a tumor suppressor, a finding that highlights the importance of intact SJs for proper cell-cell communication.

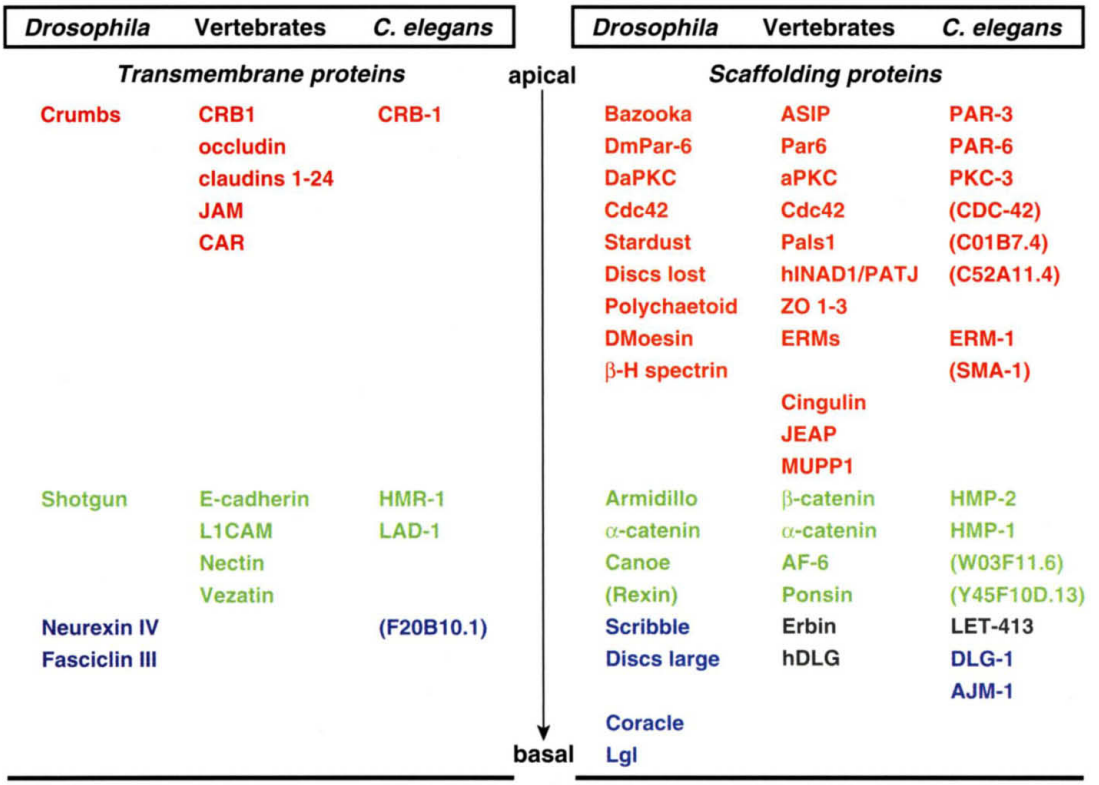

Fig. 2. Comparison of transmembrane and scaffold proteins in epithelial junctions of *Drosophila*, vertebrates, and *C. elegans*. The colors represent individual junctions or membrane regions corresponding to those used in Fig. 1. Homologous proteins appear in the same row. Proteins in parentheses either have only been identified in genome projects or their exact localization in epithelia remains to be determined. Red: subapical region in *Drosophila*; tight junction in vertebrates; *C. elegans* apical junction (CeAJ) and/or apical membrane domain. Green: zonula adherens in *Drosophila* and vertebrates; CeAJ. Blue: septate junction in *Drosophila*. Gray: basolateral membrane domains in vertebrates and *C. elegans*.

Development and Maintenance of the *C. elegans* Apical Junction

The hypodermis and the intestine are the main epithelia in the *C. elegans* embryo (*3*, *19*). Their relative simplicity and tractability make them ideally suited for the investigation of epithelial development. Only one type of intercellular junction, the *C. elegans* apical junction (CeAJ) (Fig. 1C), has been described at the ultrastructural level (*20*–*22*). The intestine is formed by a polarized monolayer of only 20 cells (*23*). Correct establishment of intestinal cell fate and subsequent development rely on an inductive event and the activation of a cascade of transcription factors (*24*). During gut development, cells undergo a mesenchymal-to-epithelial transition, and the establishment of the apico-basal axis occurs in response to unidentified spatial cues (*21*). At the onset of morphogenesis, junctional proteins and components of the future apical membrane domain (e.g. PAR-3, PAR-6, and PKC-3) are intermingled at the apical pole. Later, they are sorted and junctional proteins become distributed more laterally, whereas the other proteins remain apically. During ongoing morphogenesis, the junctional complexes form a continuous adhesion belt around the apex of gut epithelial cells (Fig. 1F). This stereotypic pattern makes it easy to detect even slight perturbances in wild-type morphology induced by the lack of any gene function (*25*). In contrast to the gut primordium, junctional proteins in the differentiating hypodermis are first detected as a discontinuous punctate pattern of cell contacts (*26*). At first, these are concentrated near the base of the lateral membranes, but finally colocalize at the site of the mature CeAJ (*20*, *22*). Hence, the initial distribution of junctional proteins along the lateral membrane of the hypodermis is reminiscent of the situation in the *Drosophila* embryo (see above).

Few structural components of the CeAJ have been identified, but they clearly reveal the tripartite nature of the junction (Fig. 1). The product of *ajm-1*, a previously unknown protein with a coiled-coil domain, colocalizes with DLG-1, the *C. elegans* homolog of the *Drosophila* SJ protein Dlg. Both proteins occupy a domain basal to the catenin-cadherin complex and CRB-1 (Fig. 1C), the *C. elegans* homolog of *Drosophila* Crumbs (*20*, *22*, *25*). The combination of ultrastructural and molecular data suggests that the CeAJ comprises distinct units that have the characteristics of the SAR, the ZA, and the SJ in *Drosophila*. The identification of further junctional proteins and their characterization will help to elucidate the nature of the CeAJ and contribute to an understanding of junction development across phyla.

In *C. elegans*, classical genetic screens and reverse genetic approaches have been used to reveal genes that control the establishment and the integrity of the CeAJ (*22*, *27*). One of the genes identified in genetic screens is *let-413*, which encodes a homolog of the *Drosophila* protein Scrib (*28*). During early embryogenesis, LET-413 is ubiquitously expressed in the cytocortex of all cells, but it becomes restricted to the basolateral membranes of epithelial cells during later stages (*28*). In *let-413* embryos, extended, but generally discontinuous, electron-dense structures form, and the AJM-1–DLG-1 complex is mislocated (*20*, *22*, *25*). In contrast, the establishment of components of the apical part of the CeAJ, including the cadherin-catenin complex, and the apical membrane domain itself (see above) is less severely affected. This points to the existence of independent mechanisms for the establishment of distinct membrane domains in *C. elegans*. Although the PAR-3–PAR-6–PKC-3 complex is involved in setting up polarity in the one-cell *C. elegans* embryo (*29*), its role during epithelial development in the *C. elegans* embryo is still elusive, whereas the polarity protein PAR-1 also plays an essential role in the development of the vulva (*30*).

How do junctional proteins ensure the integrity of the apical junction in the *C. elegans* embryo? DLG-1 physically interacts with AJM-1, and its conserved NH_2-terminus directs its localization to the CeAJ (*20*, *31*). In *dlg-1* embryos, most cell-cell contacts are devoid of any electron-dense structures. In contrast, *ajm-1* embryos exhibit gaps between the ZAs of neighboring cells (*20*). Structural defects in

dlg-1 embryos are more severe than those in *let-413* embryos (*22*), a result that seems contradictory at first glance, because LET-413 is responsible for correct localization of the AJM-1–DLG-1 complex. Nevertheless, in a *let-413* background both proteins are incorporated into the ZA, at least to some extent, resulting in partial restoration of the electron-dense structure. Thus, in the *C. elegans* embryo, correct spatiotemporal localization of the AJM-1–DLG-1 complex by LET-413 appears to be a prerequisite for the establishment and integrity of the CeAJ, whereas the catenin-cadherin complex can form in the absence of LET-413.

Mutations in E-cadherin, α-catenin, and β-catenin (encoded by the genes *hmr-1*, *hmp-1*, and *hmp-2*) cause defects in epithelial sheet sealing but do not affect cell adhesion in general (*19*). These data point to the existence of additional cell adhesion molecules in *C. elegans*. Twenty-six genes encoding predicted transmembrane or glycophosphatidylinositol-anchored proteins with extracellular immunoglobulin (Ig) modules have been identified (*32*) that are candidates for this function. LAD-1 appears to colocalize with AJM-1 in the gut epithelium, after phosphorylation of its COOH-terminus; the modification may create a binding site for known or as yet uncharacterized PDZ proteins (*33*). Among the latter, a protein with strong structural homology to human afadin and *Drosophila* Canoe has been identified in a yeast two-hybrid screen for putative LET-60/Ras effectors (*34*). In vertebrate cells, afadin is required to recruit the Ig-like cell adhesion molecule nectin and E-cadherin to the ZA (*35*). Demonstration of the recruitment of molecules of the Ig superfamily to the CeAJs by way of PDZ proteins would add another piece to the genetic puzzle of junction formation and cell adhesion in *C. elegans*.

Protein Complexes Involved in Tight Junction Formation in Vertebrates

In vertebrate epithelia, the TJs mark the border between the apical and basolateral membrane domains. They act as an intramembrane diffusion barrier and as a paracellular seal (*36*). Transmembrane proteins of the TJs include occludin and members of the claudin family, which span the membrane four times; the single-span transmembrane proteins JAM (junctional adhesion molecule); and CRB1 (*37*). All of them interact directly with cytoplasmic, PDZ domain–containing proteins (e.g. ZO-1, ZO-2, ZO-3, ASIP/Par3, and Pals1), which function as adapters to recruit other cytoskeletal and/or signaling molecules. Although TJs can be formed without occludin, accumulating evidence indicates that claudins represent the backbone of TJs. The generation of a claudin-1–deficient mouse has demonstrated that the claudin-based TJs of keratinocytes are fundamental for the barrier function of the skin (*38*). Furthermore, ectopic expression of claudin in fibroblasts induces the formation of TJ-like cellular junctions (*39*). The analysis of the transmembrane protein JAM (*40*), which bears two Ig-like loops in its extracellular domain, has led to a model for TJ assembly (*41*). According to this model, ZO-1 directly binds to the COOH-termini of claudins and JAM through its PDZ1 and PDZ3 domains, respectively, whereas ASIP/Par3 directly associates with the COOH-terminus of JAM. In the next step, ASIP-binding proteins such as Par6, aPKC, and Cdc42 could be recruited into TJs. Par6 plays a central role in building the protein complex by directly binding to aPKC, to the GTP-bound form of Cdc42, and to ASIP/Par3 (*12*).

When introduced into Madin-Darby canine kidney (MDCK) cells, vertebrate Crb1 forms a complex with Pals1 and PATJ at the TJ (*37*, *42*, *43*). In mammalian epithelia, Crb1 interacts with the PDZ domain of the MAGUK protein Pals1 by way of its cytoplasmic tail. The NH_2-terminal LIN-2/LIN-7 domain of Pals1 binds to the NH_2-terminal MAGUK recruitment domain of the multi-PDZ protein PATJ, which in turn interacts by way of its sixth and eighth PDZ domains with the COOH-termini of ZO-3 and claudin-1, respectively (*37*, *43*). Taken together, these results emphasize claudin's central role in the anchorage of the two scaffolds—the Par–aPKC–Cdc42 and the Crb1–Pals1–PATJ complex—at the TJ of mammalian epithelial cells. It will be exciting to see if any cross talk between the two complexes exists and, if so, whether they regulate TJ formation in a cooperative or competitive way.

Strikingly, the position of the TJ corresponds to that of the SAR in *Drosophila* epithelia, where the homologous Crb–Sdt–Dlt complex resides. This close conservation in localization suggests conservation of function, but our knowledge of the function of this complex in mammalian and *C. elegans* epithelia is still fragmentary (*25*, *37*). Crb1 is unlikely to play a general role in epithelial polarity of vertebrate cells for two reasons. First, in mouse and human, Crb1 expression is restricted to the eye and the central nervous system (*44*). Second, mutations in the human Crb1 gene are associated with retinitis pigmentosa 12 and Leber's congenital amaurosis, two severe forms of retinal dystrophy, but do not affect other organs (*45*). This finding prompted an analysis of the function of *crumbs* in the *Drosophila* eye, where it not only is required for the morphogenesis of photoreceptor cells (*46*, *47*) but also, more surprisingly, is necessary for their survival upon exposure to light (*48*).

Future Perspectives

Studies performed in recent years have enormously increased our knowledge of the composition and formation of intercellular junctions in epithelia of various organisms. Yet, we are still far from fully understanding the mechanisms by which the complex architecture of epithelial cells, or even the relatively simpler polarized organization of nonepithelial cells, is established and preserved. Similarly, we know very little about how the different protein complexes communicate with each other. Although many genes have been implicated in cell polarity, many missing links remain. This is in part due to the abundance and variety of transmembrane proteins and their adapter molecules, which mediate the interaction with the cytoskeleton and may have redundant functions. High-throughput approaches, such as systematic studies of gene function by RNA interference, construction of protein-interaction maps, and genome-wide DNA microarrays, will lead to the identification of novel participants. However, genetic and reverse genetic approaches in model organisms such as *Drosophila* and *C. elegans*, and extensive cell biological studies, will be equally important in dissecting the sequence of events that promote the polarization of a cell. These results, complemented by data obtained from targeted gene inactivation experiments in the mouse, will not only expand our knowledge of epithelial development and function but also contribute to our understanding of human diseases—many of which are caused by defects in epithelial functions. Additional information obtained from the analysis of other polarized cell types will further our knowledge of the process. For example, neurons, which transmit signals in one direction; migrating cells; and egg cells or embryonic blastomeres, which undergo asymmetric divisions, all rely on a polarized phenotype to fulfill their specialized tasks (*29*, *49*). Although polarity in these various cell types serves quite distinct functions, the basic mechanisms used to establish and maintain polarization may be conserved, and the same protein complexes may be used repeatedly to build different sorts of polarized cells.

References and Notes

1. T. P. Fleming, T. Papenbrock, I. Fesenko, P. Hausen, B. Sheth, *Semin. Cell Dev. Biol.* **11**, 291 (2000).
2. H. A. Müller, *Dev. Dyn.* **218**, 52 (2000).
3. G. Michaux, R. Legouis, M. Labouesse, *Gene* **277**, 83 (2001).
4. U. Tepass, G. Tanentzapf, R. Ward, R. Fehon, *Annu. Rev. Genet.* **35**, 747 (2001).
5. T. Lecuit, E. Wieschaus, *Traffic* **3**, 92 (2002).
6. M. A. Bhat *et al.*, *Cell* **96**, 833 (1999).
7. A. Bachmann, M. Schneider, E. Theilenberg, F. Grawe, E. Knust, *Nature* **414**, 638 (2001).
8. Y. Hong, B. Stronach, N. Perrimon, L. Y. Jan, Y. N. Jan, *Nature* **414**, 634 (2001).
9. A. Klebes, E. Knust, *Curr. Biol.* **10**, 76 (2000).
10. E. Medina *et al.*, *J. Cell Biol.* **158**, 941 (2002).
11. A. Bretscher, K. Edwards, R. G. Fehon, *Nature Rev. Mol. Cell. Biol.* **3**, 586 (2002).

12. S. Ohno, *Curr. Opin. Cell Biol.* **13**, 641 (2001).
13. D. Bilder *et al.*, *Nature Cell Biol.* **2**, E114 (2000).
14. D. Bilder, M. Li, N. Perrimon, *Science* **289**, 113 (2000).
15. D. Bilder, M. Schober, N. Perrimon, *Nature Cell Biol.*, in press.
16. S. Baumgartner *et al.*, *Cell* **87**, 1059 (1996).
17. R. E. Ward, R. S. Lamb, R. G. Fehon, *J. Cell Biol.* **140**, 1463 (1998).
18. D. F. Woods, P. J. Bryant, *Cell* **66**, 451 (1991).
19. J. S. Simske, J. Hardin, *BioEssays* **23**, 12 (2001).
20. M. Köppen *et al.*, *Nature Cell Biol.* **3**, 983 (2001).
21. B. Leung, G. J. Hermann, J. R. Priess, *Dev. Biol.* **216**, 114 (1999).
22. L. McMahon, R. Legouis, J. L. Vonesch, M. Labouesse, *J. Cell Sci.* **114**, 2265 (2001).
23. J. E. Sulston, E. Schierenberg, J. G. White, J. N. Thomson, *Dev. Biol.* **100**, 64 (1983).
24. M. F. Maduro, J. H. Rothman, *Dev. Biol.* **246**, 68 (2002).
25. O. Bossinger, A. Klebes, C. Segbert, C. Theres, E. Knust, *Dev. Biol.* **230**, 29 (2001).
26. B. Podbilewicz, J. G. White, *Dev. Biol.* **161**, 408 (1994).
27. M. Labouesse, *Dev. Dyn.* **210**, 19 (1997).
28. R. Legouis *et al.*, *Nature Cell Biol.* **2**, 415 (2000).
29. J. Pellettieri, G. Seydoux, *Science* **298**, 1946 (2002).
30. D. D. Hurd, K. Kemphues, *Dev. Biol.*, in press.
31. B. L. Firestein, C. Rongo, *Mol. Biol. Cell* **12**, 3465 (2001).
32. H. Hutter *et al.*, *Science* **287**, 989 (2000).
33. L. Chen, B. Ong, V. Bennett, *J. Cell Biol.* **154**, 841 (2001).
34. Y. Watari *et al.*, *Gene* **224**, 53 (1998).
35. N. Kioka, K. Ueda, T. Amachi, *Cell Struct. Funct.* **27**, 1 (2002).
36. S. Tsukita, M. Furuse, M. Itoh, *Nature Rev. Mol. Cell. Biol.* **2**, 285 (2001).
37. M. H. Roh *et al.*, *J. Cell Biol.* **157**, 161 (2002).
38. M. Furuse *et al.*, *J. Cell Biol.* **156**, 1099 (2002).
39. M. Furuse, H. Sasaki, K. Fujimoto, S. Tsukita, *J. Cell Biol.* **143**, 391 (1998).
40. K. Ebnet *et al.*, *EMBO J.* **20**, 3738 (2001).
41. M. Itoh *et al.*, *J. Cell Biol.* **154**, 491 (2001).
42. C. Lemmers *et al.*, *J. Biol. Chem.* **277**, 25408 (2002).
43. M. H. Roh, C. J. Liu, S. Laurinec, B. Margolis, *J. Biol. Chem.* **277**, 27501 (2002).
44. A. I. den Hollander *et al.*, *Mech. Dev.* **110**, 203 (2002).
45. A. I. den Hollander *et al.*, *Am. J. Hum. Genet.* **69**, 198 (2001).
46. M. Pellikka *et al.*, *Nature* **416**, 143 (2002).
47. S. Izaddoost, S. C. Nam, M. A. Bhat, H. J. Bellen, K. W. Choi, *Nature* **416**, 178 (2002).
48. K. Johnson, F. Grawe, N. Grzeschik, E. Knust, *Curr. Biol.* **12**, 1675 (2002).
49. A. Wodarz, *Nature Cell Biol.* **4**, E39 (2002).
50. We thank B. Margolis for providing Fig. 1E and P. Hardy for critical reading of the manuscript. We apologize to colleagues whose work is not described or referenced here because of space limitations. Work in the laboratories of E.K. and O.B. is supported by grants from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

REVIEW

Molecular Mechanisms of Axon Guidance

Barry J. Dickson

Axons are guided along specific pathways by attractive and repulsive cues in the extracellular environment. Genetic and biochemical studies have led to the identification of highly conserved families of guidance molecules, including netrins, Slits, semaphorins, and ephrins. Guidance cues steer axons by regulating cytoskeletal dynamics in the growth cone through signaling pathways that are still only poorly understood. Elaborate regulatory mechanisms ensure that a given cue elicits the right response from the right axons at the right time but is otherwise ignored. With such regulatory mechanisms in place, a relatively small number of guidance factors can be used to generate intricate patterns of neuronal wiring.

The correct wiring of the nervous system relies on the uncanny ability of axons and dendrites to locate and recognize their appropriate synaptic partners. To help them find their way in the developing embryo, axons and dendrites are tipped with a highly motile and exquisitely sensitive structure, the growth cone. Extracellular guidance cues can either attract or repel growth cones, and can operate either at close range or over a distance (*1*). By responding to the appropriate set of cues, growth cones are able to select the correct path toward their target.

Ten years ago (*2*), very few of the molecules that guide axons in vivo were known. But the 1970s and '80s had seen the introduction of several powerful in vitro assays to detect guidance activities in the developing vertebrate nervous system, and the growing interest of invertebrate geneticists in the problem of axon guidance. So by the early 1990s, the stage had been set for a burst of activity that led to the discovery of several conserved families of axon guidance molecules. Prominent among these are the netrins, Slits, semaphorins, and ephrins (Fig. 1). These are not the only known guidance molecules, but they are by far the best understood. With these molecules in hand, we can now begin to ask how growth cones sense and respond to guidance cues, and how a relatively small number of cues can be used to assemble complex neuronal networks.

Research Institute of Molecular Pathology, Dr. Bohrgasse 7, A-1030 Vienna, Austria. E-mail: dickson@nt.imp.univie.ac.at

Guidance Cues and Their Receptors

Netrins. The discovery of netrins came as the remarkable convergence of the search for a chemoattractant for vertebrate commissural axons (*3*, *4*), and the analysis of genes required for circumferential axon guidance in *Caenorhabditis elegans* (*5*, *6*). Across more than 600 million years of evolution, netrins have retained the function of attracting axons ventrally toward the midline (*7*). Netrins can also repel some axons, and this function too has been conserved. This was initially inferred from defects in dorsal as well as ventral guidance in *unc-6/netrin* mutant worms (*5*), and subsequently confirmed by the direct demonstration of netrin's repulsive activity in vertebrates (*8*) and in flies (*9*, *10*).

Identification of the netrin receptors followed from the characterization of two other worm mutants with defects in circumferential guidance: *unc-40*, which primarily disrupts ventral guidance; and *unc-5*, which affects only dorsal guidance (*5*). Both *unc-40* and *unc-5* encode conserved transmembrane proteins (*7*), with UNC-40 belonging to the DCC (deleted in colorectal carcinoma) family. Biochemical and genetic studies have confirmed their functions as netrin receptors in several different species (*7*, *10*). DCC receptors mediate attraction to netrins but can also participate in repulsion. UNC-5 receptors appear to function exclusively in repulsion, either alone or in combination with DCC receptors. UNC-5 receptors may require a DCC coreceptor for repulsion farther away from the netrin source, where ligand concentration is likely to be lower (*5*, *10*). This may involve a direct interaction between the cytoplasmic domains of the two receptors (*11*).

Netrins guide many different axons in vivo. In some cases, netrin can exert its effects from distances of up to a few millimeters (*12*), but in others it appears to act only at short range (*9*). Netrins have high affinity for cell membranes (*3*, *4*), and it is unclear how far they can diffuse in vivo and how their diffusion is regulated. Indeed, a netrin gradient has not yet been visualized directly in any system, and formal proof that netrin must diffuse away from its source to exert its long-range effects is lacking.

Slits. Slits are large secreted proteins that signal through Roundabout (Robo) family receptors. Robo was first identified in a genetic screen for midline guidance defects in *Drosophila* (*13*, *14*). Genetic studies suggested that Robo is the receptor for a midline repellent (*14*), subsequently identified as Slit (*15*, *16*). This repulsive action of Slit was found to be conserved in vertebrates (*17*, *18*). However, in a parallel approach, Slit was also purified as a factor that stimulates sensory axon branching