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Epithelium—The Primary Building Block for Metazoan Complexity[1]

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SYNOPSIS. In simplest terms, the complexity of the metazoan body arises through various combinations of but two tissue types: epithelium and mesenchyme. Through mutual inductions and interactions, these tissues produce all of the organs of the body. Of the two, epithelium must be considered the default type in the Eumetazoa because it arises first in embryonic development and because mesenchyme arises from it by a switching off of the mechanisms that underly differentiation and maintenance of epithelial cells. In the few model metazoans whose epithelia have been studied by molecular techniques (largely *Drosophila, Caenorhabditis,* mouse), the molecular mechanisms underlying differentiation of epithelia show remarkable similarity. Extrapolating from these studies and from comparisons of the morphology of epithelia in lower metazoans, I propose how epithelia arose in the stem metazoan. Steps in epithelial differentiation include 1) establishment of cell polarity by molecular markers confined to either apical or basolateral domains in the plasma membrane; 2) aggregation of cells into sheets by localization of cell-adhesion molecules like cadherin to the lateral membrane; 3) formation of a zonula adherens junction from the cadherins by their localization to a discrete belt; 4) cell-to-cell linking of certain transmembrane proteins (primitively in the septate junction) to produce gates that physiologically isolate compartments delimited by the cells; and 5) synthesis of a basal lamina and adaptation of receptors (integrins) to its components. Despite morphological differences in the variety of cell junctions evident in various epithelia, the underlying molecular markers of these junctions are probably universally present in all eumetazoan epithelia.

INTRODUCTION

At the root of complexity in the Metazoa is the capacity to develop organ systems. Organs—for example, limbs, organs of the gut, and various feeding structures—are the modules that are variously constructed and combined in different ways to build the variety of body plans we recognize among metazoan phyla. The capacity to develop organs can, itself, be traced to the differentiation of epithelia and mesenchyme, because all organs arise through the interaction of an epithelium with an associated mesenchyme (Gilbert, 2000). While the term ''organ'' has been loosely applied to other structures, sometimes even anything composed of more than one cell type or of a single tissue complicatedly folded, this stricter definition, specifying mesenchymal-epithelial interactions, is the only one that conserves the highest information content—anything short of this robs the term of both evolutionary and developmental meaning. In the embryonic development of an organ, an epithelium and a mesenchyme induce each other to differentiate and then maintain that differentiated state in the fully formed organ by continued interactions. To understand the origins of complexity in metazoan body plans, then, we must find where these tissues came from; that is, how epithelia and mesenchyme arose.

The dichotomy between epithelia and mesenchyme is well defined, with specific criteria marking the distinction between the two (Rieger, 1986): Epithelia are tissues in which the component cells share an aligned polarity (*i.e.,* have apical and basal surfaces distinguished and aligned in parallel), are joined by belt-form junctions (zonulae adherentes and tight junctions, for instance), and associate with extracellular matrix only on their basal and apical sides (that is, always to basal lamina and sometimes apically also to cuticle); mesenchyme, on the other hand, is constructed of cells with polarities of any orientation (not necessarily in parallel), with only spot-form junctions, and in contact with extracellular matrix on any or all sides (Fig. 1).

The basal phyla of metazoans provide models of the steps leading to organ formation seen in higher phyla, particularly the steps in the differentiation of epithelia (Fig. 2). The selective advantage driving the development of epithelium would have been the ability to separate compartments. Sponges form small sealed compartments only transiently, such as between cells secreting spicules (see Green and Bergquist, 1982), but their epithelia-like monolayers—choanoderm and pinacoderm—lack the typical belt-form junctions of epithelia and so are not likely to be isolating any compartments; they are usually considered not to be true epithelia, and, in fact these layers are probably quite permeable, almost mesenchymal in nature. The non-epithelial nature of these layers is also evident in the lack of a basal lamina, but, curiously, sponges do have a gene sequence coding for collagen type IV, a diagnostic feature of the basal lamina (Boute *et al.*, 1996). By contrast with sponges, Cnidarians are virtually wholly epithelial: their epidermis and gastrodermis fulfill all the criteria of epithelia, and the only mesenchyme-like cells are isolated amoeboid cells sunk into the extracellular matrix (the mesoglea). Isolating a digestive cavity, where enzymes are incubated, from the external environment obviously gives cnidarians the advantage in feeding on larger prey.

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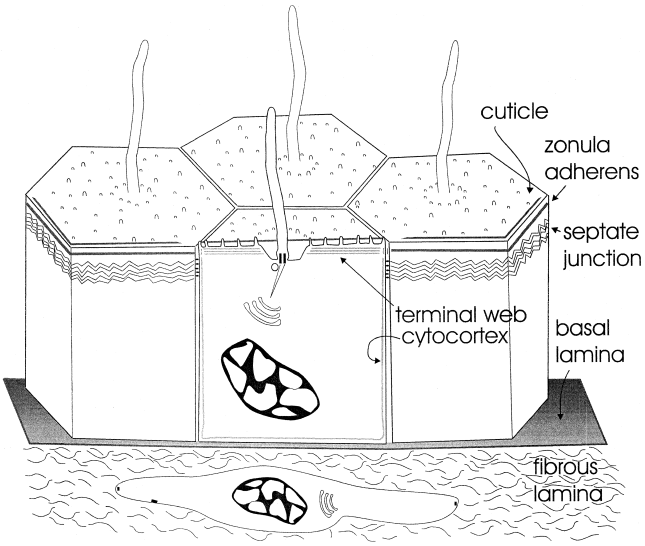

FIG. 1. Schematic of a representative epithelium. The epithelial cells are polarized, with their axes aligned in parallel with each other; they are joined by belt-form junctions (the zonula adherens and septate junction); and only their basal and apical surfaces associate with extracellular matrix (basal lamina of a basement membrane basally and cuticle apically). By contrast, the mesenchymal cell below has no particular aligment with other mesenchymal cells, bears only spot-form junctions, and is essentially surrounded by the extracellular matrix.

Compartmentalization is also the underlying advantage epithelia provide in triploblastic descendants of a diploblastic ancestor. In the triploblasts, a second compartment, the coelom, is isolated by the newly developed epithelium of the mesoderm. The coelom provides for a hydrostatic skeleton as well as for an internal layer from which dedicated musculature of the body wall and gut can be produced. The mesoderm also provides the mesenchyme (by sinking of cells from its epithelium into the extracellular matrix) from which organ development becomes possible.

The primary building block providing for complex differentiation, then, is epithelium. It is the epithelial tissue type that sets the Eumetazoa apart from the sponges, and it is epithelia in the Bilateria that provides the mesenchyme, the second building block in organ formation. Inasmuch as the mesenchyme comes from epithelium, epithelium can be viewed as the primary building block of the metazoan body plan. In fact, epithelium is the default state of cells in the Eumetazoa. It is the tissue that arises first in embryonic development, and it is only from epithelium that mesenchyme arises (by the so-called epithelial-mesenchymal transition, or EMT, which is largely a suppression of the factors that cause epithelial differentiation).

Despite the variety of forms of epithelia and their morphological differences among phyla (see, *e.g.,* reviews in Bareiter-Hahn *et al.,* 1984), the underlying determinants of epithelial cell structure appear to be the same in all animals. Studies of epithelial cell differentiation in model organisms such as the insect *Drosophila melanogaster*, the nematode *Caenorhabditis elegans*, the cnidarian *Hydra*, and rat and mouse, show that the proteins determining epithelial differentiation are remarkably similar among them (see reviews, *e.g.,* by Krämer [2000] and Knust and Bossinger [2002]). These are rather derived species, not particularly well suited for models of evolution, but the consistencies among them in structure and function of the molecules controlling epithelial differentiation show these molecules are evolutionarily conserved even here.

As a comparative morphologist, I want to extrapolate from these models and from similarities in the structure of epithelial cells in these and in lower invertebrates to construct a hypothesis of how epithelia arose in an ancestral metazoan. The overarching principles that emerge show that the mechanisms of epithelial determination are not only common to all eumetazons but probably are largely already developed in even the most basal of the metazoan phyla, the Porifera.

THE NATURE OF EPITHELIA

Ultrastructure of epithelia

Epithelia are easily recognized as sheet-like arrangements of cells that have an apical surface facing some space, that are joined along their adjacent surfaces by belt-like junctions, and that attach on their basal side to a basal lamina (Fig. 3). Among the vertebrates, whose epithelia are typically taken as models, the belt-form junctions are the zonula occludens (ZO; also called tight junction), which is apical-most, and the zonula adherens (ZA) arrayed just proximal to it (Fig. 3A). Together with occassional maculae adherentes (desmosomes), which are spot-like, these two junctions form what is evident even at the light-microscope level as a ''junctional complex.'' Invertebrate chordates, specifically the tunicates, also have what appears to be a ZO (Fig. 3B). Among the other invertebrates, on the other hand, the ZA is apical-most and there is no ZO, its role as a sealing junction being filled instead by the septate junction which lies just basal to the ZA (Fig. 3C).

The ZA adjoins the cytoskeleton of the cell by links to actin microfilaments, and some of these may form a distinct apical sheet-like array, the terminal web. Depending on the degree of development of the terminal web, either the ZA (where there is well-developed cell web) or the SJ (where it is not) may appear to dominate, even to the extent that the other junction is so inconspicuous as to be overlooked. It is highly probable that both ZA and SJ are present in all invertebrate epithelia (Tyler, 1984), especially given what we now know of molecular markers in the plasma membrane of epithelial cells (see below). The ZA dominates in the model organism *Caenorhabditis elegans*, for example, making it appear that its epithelia have but a single junctional type, the *C. elegans* Apical Junction or CeAJ (*e.g.,* Leung *et al.,* 1999), but molecular

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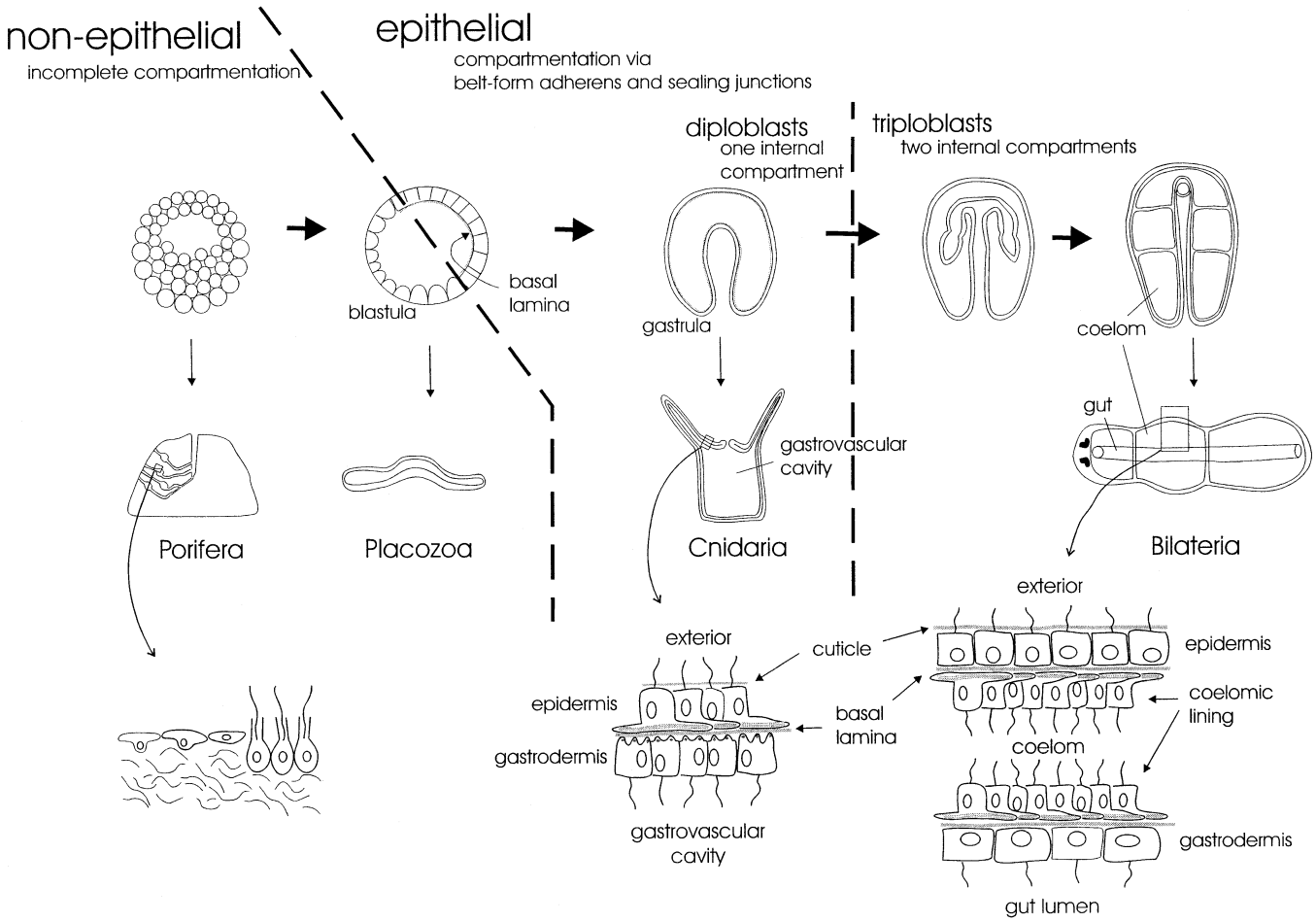

FIG. 2. Embryonic development of epithelia in lower metazoans (center pathway) and grades of organization reflected at the various embryonic stages. The morula and early blastula stages are non-epithelial, lacking a basal lamina, and can be taken as reflecting cell organization in the lower two phyla, Porifera and Placozoa, because of the lack of a basal lamina. The cells of the late blastula (lower side of dividing line) become epithelial by forming a basal lamina and sealing junctions. Gastrulation produces two epithelial layers—epidermis and gastrodermis—and from such a stage the diploblasts, with a single internal compartment, the gastrovascular cavity, are derived. Triploblasts develop by setting off a third epithelial germ layer that delimits another internal compartment, the coelom. Mesenchymal cells derive from this epithelium in particular. Epithelia and mesenchyme are the building blocks from which organs form, so true organs arise only in the triploblasts.

markers for both ZA and SJ are still present (Knust and Bossinger, 2002).

Links to actin, sometimes in prominent bundles, also characterize the hemi-adherens junction, otherwise known as the focal contact, that joins the cell to extracellular matrix with spot-like welds at the basal lamina or apical cuticle. On the other hand, the tension-bearing cytoplasmic filaments in the desmosome (macula adherens) and hemidesmosome (which spot-weld epithelial cells of vertebrates to each other or to the basal lamina, respectively) are intermediate filaments. A macula adherens is not part of the junctional complex in invertebrates, and although intercellular spot-like adherens junctions appear in specialized epithelial cells in many invertebrate groups (for example, ctenophores; Hernandez-Nicole, 1991), they probably do not satisfy the definition of desmosomes because their stress-bearing cytoplasmic filaments appear to be actin bundles rather than intermediate filaments.

Mammalian vertebrates may also have the morphological equivalent of the septate junction where Schwann cells link to nerve axons at the node of Ranvier (Wiley and Ellisman, 1980; Tepass *et al.*, 2001). Even though its position is topographically reversed from that of the invertebrate SJ (that is, it sits apical to the ZA rather than basal to it), this paranodal junction does have molecular markers homologous with SJ's of invertebrates (see below).

Molecular markers of the epithelial cell

The polarity of an epithelial cell is evident microscopically by the polarized distribution of cell organelles, but it is reflected at the molecular level, as well, by division of its plasma membrane into distinct apical and basolateral domains (Yeaman *et al.*, 1999; Krämer, 2000; Tepass *et al.*, 2001). Transmembrane proteins with cytoplasmic tails that link to elements of the cytoskeleton establish these domains. These protein complexes not only establish the polarity of the plasma membrane, they provide for cell-to-cell adhesion at in-

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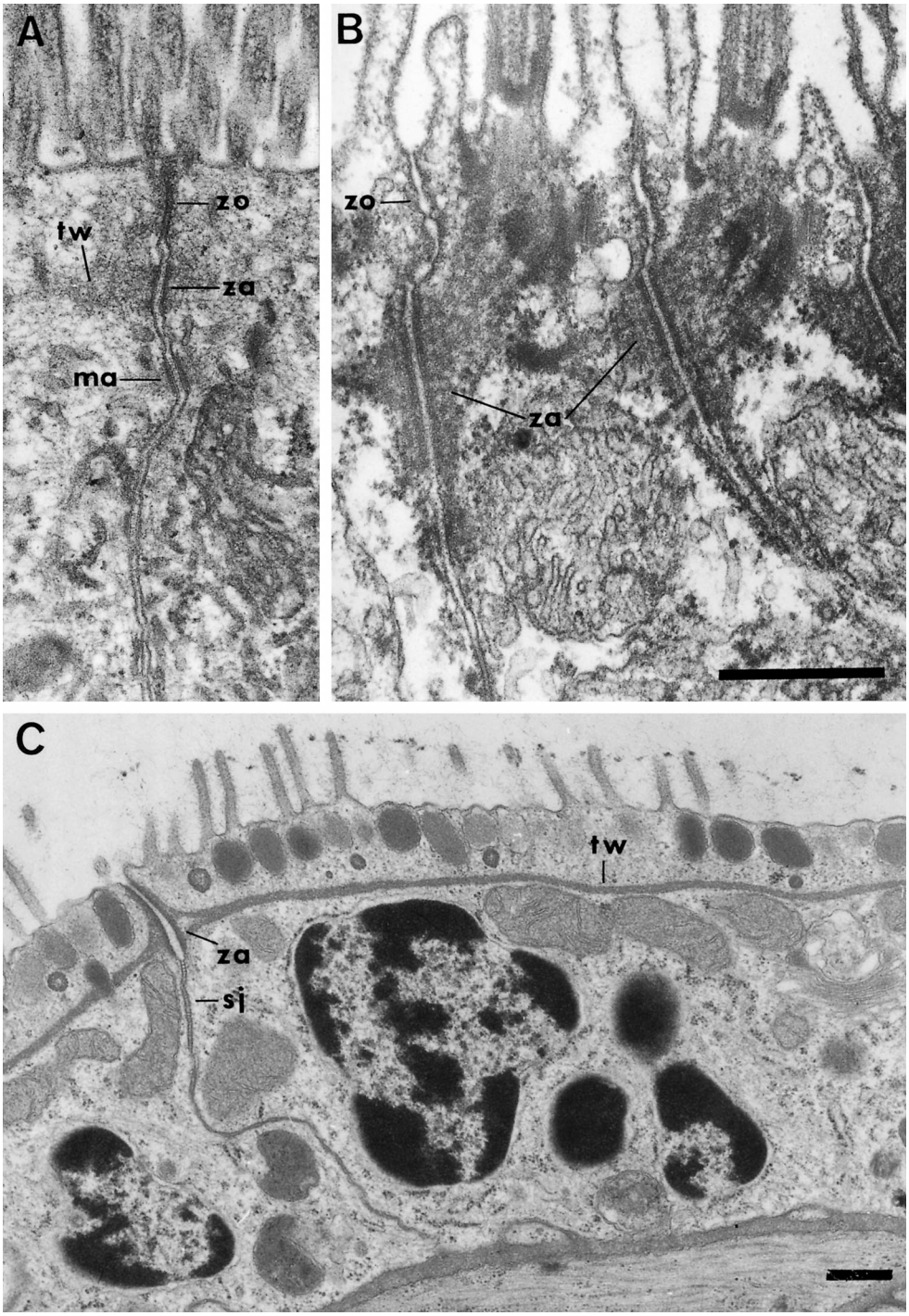

FIG. 3. Typical cell junctions in epithelia. A) The junctional complex in intestinal epithelia of a vertebrate (the mouse *Peromyscus leucopus*) consists of a tight junction (zonula occludens, zo) and a zonula adherens (za) basal to it, both belt-form junctions, plus, at selected spots, a desmosome (macula adherens: ma). B) Cell junctions in a tunicate (*Aplidium pellucidum*) are similar, with zonula occludens and zonula adherens. C) Epidermis of the platyhelminth *Halopharynx quadristimulus* showing junctions typical of most invertebrates, with zonula adherens apical to a septate junction (sj). Scale bar for A and B = 0.5 μm; scale bar for C = 0.5 μm.

tercellular junctions as well as links to intracellular signaling molecules controlling the differentiated state of the epithelial cell (Fig. 4). The zonula adherens junction, for example, comprises the transmembrane protein E-cadherin and its associated cytoplasmic proteins α- and β-catenin. The apical plasma membrane is marked by the transmembrane protein Crumbs (Crb) in *Drosophila* and homologues CRB1 and CRB-1 in vertebrates and *C. elegans*, respectively, and this transmembrane protein links to scaffolding proteins in the

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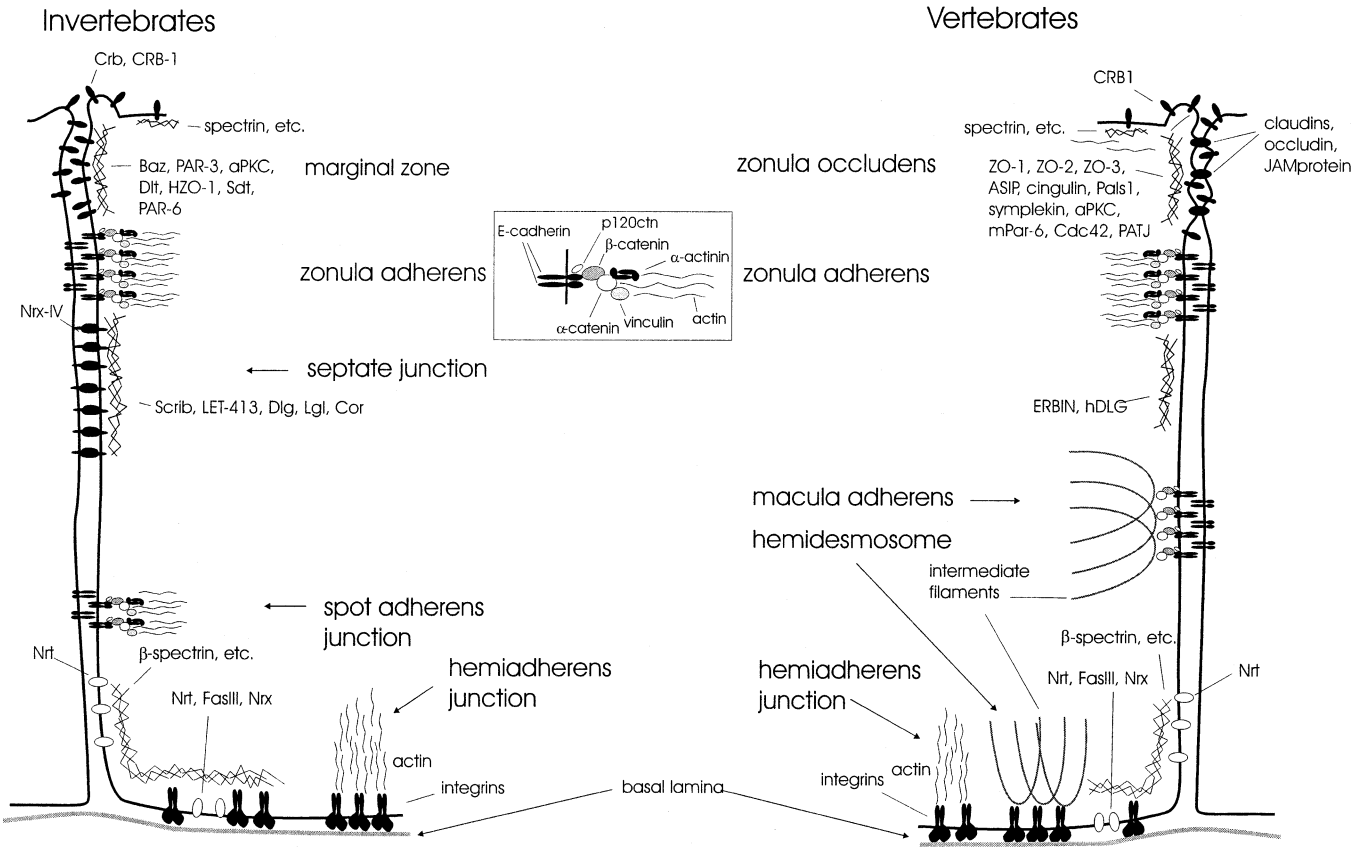

FIG. 4. Highly schematic representation of plasma-membrane and cytocortical proteins in epithelial cells of invertebrates (left) and vertebrates (right) depicted as at the juncture of two cells. Presumed homologous parts are drawn in parallel at the same levels; probably unique to vertebrates are just some transmembrane proteins at the ZO (claudins, etc.) and the intermediate-filament linked parts of the macula adherens and hemidesmosome; other transmembrane and scaffolding proteins have homologues in invertebrates. Inset: arrangement of components of the zonula adherens, with transmembrane protein E-cadherin linked via other proteins, including β-catenin, to actin filaments of the cell web.

cell cortex, particularly Stardust and Discs Lost in the case of *Drosophila*, or Pals1 and hIND/PATJ in vertebrates; these localize to a subapical region at the edge of the cell just apical to the ZA where the zonula occludens occurs in the vertebrate epithelial cell (Tepass *et al.*, 2001; Knust and Bossinger, 2002). Determination of the basal plasma membrane is mediated by integrins, trans-membrane glycoproteins that link to components of extracellular matrix (Tepass, 1997; Burke, 1999; Lussier *et al.*, 2000; van de Flier and Sonnenberg, 2001).

In development of epithelia, it is only after these protein complexes have been established in the plasma membrane that the cytoskeleton reorganizes and, as a result, that the cell organelles show their polarized distribution (Tanentzapf *et al.*, 2000).

The zonula occludens (ZO, tight junction) has long been considered unique to the vertebrates, but several of the proteins that characterize it can be seen to have homologues in the invertebrates. The ZO is unique in having the transmembrane proteins occludin, members of the claudin family, and JAM or Junctional Adhesion Molecule (Roh *et al.*, 2002), but their positioning is dependent on interaction with scaffolding proteins in the cytoplasm like ZO-1, ZO-2, ZO-3, ASIP/Par3, and Pals1 that have homologues among scaffolding proteins identified in *Drosophila* and *Caenorhabditis* (Polychaetoid for ZO-1-3, Bazooka for ASIP, Stardust for Pals1; see Knust and Bossinger, 2002); and a homologue of ZO-1 has even been found in the cnidarian *Hydra* (Fei *et al.*, 2000). The ZO appears comparable, therefore, to a specific subapical region of the plasma membrane of *Drosophila* where the Crib-Stardust-Discs-lost complex is positioned and this conservation of position suggests conservation of function (Knust and Bossinger, 2002). Curiously, however, the mammalian homologue of Crib, Crb1, is expressed only in epithelia of the eye and central nervous system (den Hollander *et al.*, 2002).

Septate junctions in epithelia of *Drosophila* are marked by the proteins Scribble (Scrib), Lethal giant larvae (Lgl), and Discs large (Dlg). These are scaffolding proteins, linked to the plasmamembrane by the transmembrane protein Neurexin IV (Nrx-IV). Homologous to Scrib and Dlg in *C. elegans* are LET-413 and DLG-1, and in vertebrates the homologues are Erbin and hDLG (Knust and Bossinger, 2002). Caspr in the septate-junction-like paranodal junction of mammals is the homologue of Nrx-IV (Tepass *et al.*, 2001).

Desmosomes (maculae adherentes) and hemides-

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mosomes involve intermediate filaments (Kowalczyk *et al.*, 1999) and because of the apparent lack of cytoplasmic intermediate filaments in invertebrate epithelia, such junctions may be properly considered specific to the chordates. While desmosomes link to the intermediate filaments by cadherins in a manner like that of the ZA, these are distinct from the classic ZA cadherins and, by sequence analysis, appear to have evolved from them within the chordate lineage (Tepass *et al.*, 2000). Reports of desmosomes and hemidesmosomes in invertebrates (*e.g.*, in Bareiter-Hahn *et al.*, 1984) are probably confused with spot adherens and hemiadherens junctions and have actin bundles as the cytoplasmic stress-bearing elements.

The basal domain of the epithelial cell is established by integrins, which are αβ heterodimeric glycoproteins (reviewed by van der Flier and Sonnenberg, 2001). These transmembrane molecules link the cell to laminins and collagen in the basal lamina. Like the proteins composing the apical junctions, the integrins appear to be highly conserved in evolution, being similar in all animals from sponges to humans (Burke, 1999; van der Flier and Sonnenberg, 2001).

DEVELOPMENT OF CELL JUNCTIONS

Clues to the origin of the epithelial cell type may be gleaned from studies of the embryonic development of epithelia. Particularly well studied are epithelial differentiation in *Drosophila* (Tepass, 1997; Tanentzapf *et al.*, 2000; Tepass *et al.*, 2001; Lecuit and Wieschaus, 2002) and *Caenorhabditis* (Podbilewicz and White, 1994; Köppen *et al.*, 2001; Knust and Bossinger, 2002). In *Drosophila*, the first indication of the epithelial nature of the blastomeres following their cellularization is their lateral adhesion through cadherins (that is, E-cadherin, Armadillo/β-catenin, and α-catenin) to produce a two-dimensional sheet. Three protein complexes that assemble at the subapical region of the developing epithelial cell—Baz-DmPar6-DaPKC, Scrib-Lgl-Dlg, and Crb-Sdt-Dlt—appear to interact sequentially and in a hierarchical fashion to set up membrane domains and delimit the cell junctions (Tepass *et al.*, 2001; Knust and Bossinger, 2002). The ZA coalesces at its final site from initially spottily distributed ZA components, and the Scrib-Lgl-Dlg complex is gradually integrated into the SJ. Similarly, junctional proteins in *Caenorhabditis* appear first in a punctate pattern in the basolateral membrane and then coalesce at the site of the final ZA. In addition to cadherin, other as-yet unidentified cell-adhesion molecules appear to play a role in the lateral adhesion of the epithelial cells in *Caenorhabditis* and to partly compensate in mutant phenotypes (Knust and Bossinger, 2002).

A similar two-step process is evident in the vertebrate embryo (reviewed by Gilbert, 2000). Cells become epithelial by 1) synthesizing E-cadherin and another adhesive protein, syndecan, both of which cause clumping of the cells into aggregates, and then, 2) secreting the basal lamina, to which newly developed receptors for basal-lamina components like laminin promote the final differentiation to epithelium.

EVOLUTION OF EPITHELIUM

Epithelium as the default state of the eumetazoan cell

Differentiation into an epithelial state is essentially the first coordinated activity the blastomeres of the animal embryo undertake. Cells of the 8-cell embryo in mammals, for instance, are epithelial, complete with ZO's as well as E-cadherin and epithelium-specific intermediate filaments, cytokeratins (Frisch, 1997); and the blastula stage of animals in general is composed entirely of epithelial cells.

By extension, then, mesenchymal cells must arise from epithelial precursors. In the sea-urchin embryo, for instance, the primary mesenchyme originates from epithelial cells of the blastula by their losing cadherins and producing enzymes that allow them to invade the basal lamina. Where the epithelial-mesenchymal transition (EMT) has been studied most extensively is in its role in generation of cancer. The first step in the transition to the cancer cell is down-regulation of cell-adhesion molecules; then phosphorylation of β-catenin leads to loss of cell-adhesion and further degradation of β-catenin; and finally transcriptional activation occurs as excess unphosphorylated β-catenin translocates to nucleus and interacts with the LEF transcription factor family there (Savagner, 2001). The transcription factors that promote epithelial cells are repressed in order to produce mesenchymal cells. From his studies of the EMT in cancer, Frisch (1997) has proposed that epithelium is the default state in the healthy body: ''Malignancy of carcinoma cells is inversely related to the robustness of their epithelial phenotype'' (Frisch, 1997, p. 70).

In fact, the default state of cells in all eumetazoans must be epithelial. All animals, including the essentially epithelial cnidarians, nevertheless have the capacity to produce mesenchymal cells. Amoeboid cells in the mesoglea of cnidarians probably serve to increase production of the mesoglea and hence the bulk of the animal, and they must arise by the common mechanism of EMT. In the Bilateria, the mesenchyme produced has further capacity to interact with epithelia in ways unrealized in the diploblasts, specifically to allow inductive events that generate organs, the ultimate use of the tissue building blocks for producing metazoan complexity.

Evolution of the epithelial cell type

Common threads in the differentiation of epithelial cells of eumetazoans show that the epithelial cell type arose early in the metazoan stem—definitively in the stem to the Cnidaria—and that the same mechanisms producing epithelium here are conserved throughout the Metazoa. In terms of the signature proteins governing epithelial differentiation, we see, for example, that the MAGuK family of proteins—including ZO-1 which has homologues in the cnidarians and mammals

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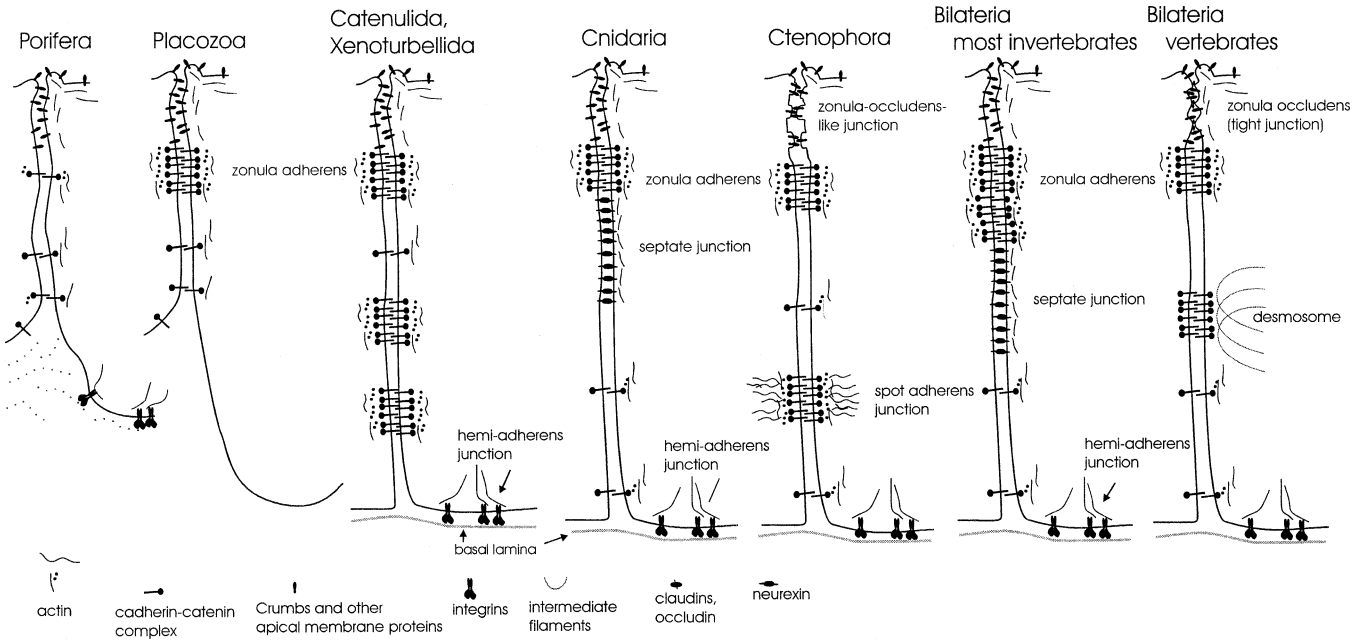

FIG. 5. Presumed (hypothetical) distribution of plasma-membrane proteins in epithelial cells of various metazoan phyla based on comparative morphology of junctions in these phyla and comparison with known arrangements in *Drosophila* (representing bilaterian invertebrates) and mammals (representing vertebrates).

and polychaetoid in *Drosophila*—likely arose early in evolution (Fei *et al.*, 2000). The mechanism for establishing epithelial cell polarity by localization of aPKC is also evolutionarily conserved, appearing in mammals and nematodes (Suzuki *et al.*, 2001) and *Drosophila* (Wodarz *et al.*, 2000). Integrins, too, are conserved, appearing throughout the metazoan phyla (Burke, 1999; van de Flier and Sonnenberg, 2001), although those specific to epithelia (*i.e.*, linkage to basal lamina) are likely restricted to phyla other than the Porifera. Other components are likely to be shared among epithelial cell junctions inasmuch as the morphology of cell junctions is conserved across the diversity of metazoan phyla.

By comparing the form of epithelial cell junctions among the lower metazoans, in particular, we can hypothesize how the molecular determinants in these junctions may be distributed and how they may have become specialized in the form of junctions most widely seen among higher metazoans (Fig. 5).

Inherent in the origin of the epithelial cell type must first be the ability to establish cell polarity. Even the pinacocytes and choanocytes of sponges display such polarity, and it is probable that some apical-domain determinant exists in these cells, useful, for example, in directing phagocytosis or differentiation of microvilli at one pole of the cell. A second step would likely involve the development of integrins to link the basal plasma membrane to ECM; Burke (1999) proposes that such ability actually led to multicellularity. Aggregation of cells into sheet-like arrays would be dependent on the origin of E-cadherin (as happens in blastomeres in the blastula) or a comparable cell-cell adhesion molecule (Tepass *et al.*, 2000); sponges may have cadherins, as evidenced by cell-dissociation experiments which show calcium-dependent cell adhesion, though other complex aggregation factors may be involved in cell aggregation instead (Müller *et al.*, 1985).

Development of a ZA junction would be the next step, a step requiring the fence function of apical plasma-membrane markers to localize the cadherins to a specific apical belt; among lower metazoans, such junctions may be in their most basic form in the Placozoa, which have a single belt-form ZA (Grell and Ruthmann, 1991), and the worm groups Xenoturbellida and Catenulida, which have multiple belts (Tyler, 2001). The belt form of the junction is critical and may first involve the signal-transduction pathway via aPKC; establishment of the ZA junction reorganizes the cytoskeleton and β-catenin for cell-cell communication and control of cell differentiation.

Beyond simple mechanical linking of cells, as is the responsibility of the ZA, the ability to physiologically isolate compartments with a belt-form sealing junction would be a critical next step. Even sponges show this capacity in the transient development of septate junctions between certain cells (Green and Bergquist, 1982). Complete development of septate junctions in an epithelial sheet, however, is first seen in Cnidaria. The other diploblastic phylum, the Ctenophora, surprisingly, has not been reported to have septate junctions. Instead, it appears that such a sealing function may be assumed by a special junction that more resembles the ZO of vertebrates, composed of a series of punctate contacts (cf. Hernandez-Nicole, 1991, her Fig. 8).

Completing the compartment-isolating capacity—

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and, thus, full differentiation of an epithelium—would be the synthesis of a basal lamina comprising collagen type IV and laminin, as well as production of the receptors capable of linking the cell to such basal-lamina components, namely the epithelium-specific integrins.

These steps are represented in early development of the embryo. In the mammalian embryo, for instance, the first compaction of the blastomeres is under the influence of E-cadherin to produce the trophectoderm (Fleming and Johnson, 1988). Then the ZA forms and establishes polarity of the cell, sequentially followed by formation of the ZO and desmosomes.

The vertebrate epithelium may mark a certain specialization beyond that found in most animal phyla in that the ZO appears to have replaced the septate junction in the role of physiologically sealing the epithelium. The distinction may not be as marked a dichotomy as morphology might suggest, however. Homologues of certain proteins of the ZO are evident in invertebrates, and, similarly, homologues of certain marker proteins of the septate junction can be found in the same position (or in other sealing positions in the case of paranodal junctions of Schwann cells) in vertebrate epithelia. Perhaps the vertebrates (and tunicates) have secondarily lost the septate junction because of the development of the integral plasma-membrane claudins. Invention of the claudins and occludin may have made superfluous the transmembrane proteins of the septate junction. Moreover, the equivalent of the ZO may be evident in even as basal a group as the Ctenophora, as explained above.

CONCLUSION

The structure of epithelia, specifically the arrangement of the proteins and other molecules that determine epithelial function, is remarkably similar in all eumetazoans and reflects a highly complex and conserved mode of cell differentiation. Comparison with sponge cell layers suggests that many of the same determinants are present even in this non-eumetazoan phylum, though these layers do not qualify by established criteria as true epithelia. Sponges still have the capacity to form certain limited isolating compartments, as they do, for example in secreting spicules, and likely have developed many, if not all, of the mechanisms deemed specific to true epithelium. Mechanisms establishing the epithelial cell type appear to be recapitulated in embryonic development of eumetazoans, and so we have a model for the steps involved in the evolutionary origins of epithelia in the stem to the Eumetazoa. From epithelia develop mesenchymal cells by a specific process of epithelial-mesenchymal transition, largely through disruption of the determinants that cause differentiation and maintenance of the epithelial cell type. There are no ''master genes'' to switch on the epithelial cell type as there are for other cell types, such as Myo-D for muscles (Davies and Garrod, 1997); instead, epithelium appears to be the default eumetazoan cell type. It is a surprisingly complex cell type nonetheless, with the capacity, through interactions with mesenchyme, to produce the variety of organ systems that underly the higher-level complexity of metazoans.

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