Review

Epithelial biology: lessons from *Caenorhabditis elegans*

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Received 4 May 2001; received in revised form 17 August 2001; accepted 4 September 2001

Received by A.J. van Wijnen

Abstract

Epithelial cells are essential and abundant in all multicellular animals where their dynamic cell shape changes orchestrate morphogenesis of the embryo and individual organs. Genetic analysis in the simple nematode *Caenorhabditis elegans* provides some clues to the mechanisms that are involved in specifying epithelial cell fates and in controlling specific epithelial processes such as junction assembly, trafficking or cell fusion and cell adhesion. Here we review recent findings concerning *C. elegans* epithelial cells, focusing in particular on epithelial polarity, and transcriptional control. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: *Caenorhabditis elegans*; Epithelial cell; Differentiation; Morphogenesis; Adherens junction; Hemidesmosome; Extracellular matrix; Trafficking; Cell fusion; Transcription

1. Introduction

Epithelial cells play an essential role during development and adult life by shaping organs, and by acting as a selective barrier to regulate the exchange of ions, growth factors or nutrients coming from the outside environment (Yeaman et al., 1999). These functions rely on proper cell fate specification and on the acquisition of a polarised phenotype. Epithelial cells can also easily become cancerous and a direct link between tumorigenesis, loss of cell polarity and adhesion has long been noted (Thiery et al., 1988; Behrens et al., 1989).

Despite their importance and widespread occurrence, many aspects concerning the biology of epithelial cells are not fully understood. For instance, we do not know if specific transcription factors control the onset of ‘epithelialisation’ by switching on the expression of proteins that establish cell polarity. Similarly, we do not know, except in a few cases such as branching of the tracheal system (Metzger and Krason, 1999) and dorsal closure in *Drosophila* (Noselli and Agnes, 1999), which genes induce major morphogenetic processes. In addition, the detailed genetic analysis of the mechanisms underlying morphogenesis is a relatively new area, and what we know comes mainly from the fly.

The nematode *Caenorhabditis elegans* provides another powerful system to address the questions raised above using a genetic approach in the integrated context of a live animal. Here we highlight recent progress in the analysis of different aspects of *C. elegans* epithelial biology. We first discuss specification of epithelial cell fates in the embryo (Section 3). Then we review how apico-basal polarity is established and maintained (Sections 4 and 7), how the epidermis becomes connected to the underlying muscles (Section 5), and how the basal lamina influences morphogenesis of internal epithelia (gonad, intestine and pharynx) (Sections 6, 8 and 9). Section 10 is devoted to pattern formation and morphogenesis of the egg-laying system (uterus and vulva).

2. Background anatomy

There are three broad categories of epithelial cells in *C. elegans*: those that contribute to a classical epithelium by
Intestine Specific

Vulva/Uterus Connection

Vulva Specific

C), 300 nm (D, E).

epidermis, dense bodies and sarcomeres can be recognised (dense bodies and sarcomeres are still poorly developed compared to larvae). Scale bar 10

m (B,

m (D, E).

substructures. The micrograph on the right shows an area of muscle-epidermis contact in a late embryo, where the cuticle,

fi

n (Francis and Waterston, 1991). The apical substructure, which attaches the epidermis and muscle to the cuticle, contains the proteins M UP-4

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ing EDR (so far only large chromosomal deficiencies removing both end-1 and end-3 lead to absence of the intestine; the intestinal phenotype of those deficiencies can be rescued by introduction of an end-1 or end-3 transgene); MAPK; mitogen associated protein kinase. Lethality associated with deficiencies removing the EDR are probably linked to the size of the deficiency. * Mutants in the lin-12/glp-1 pathway affect many other processes which contribute to embryonic lethality: 1 (Page et al., 1997); 2 (Koh and Rothman, 2001); 3 (Labouesse et al., 1994); (Labouesse et al., 1996); 5 (Quintin et al., 1995); 20 (Sternberg and Han, 1998); 21 (Herman and Horvitz, 1999); 22 (Herman et al., 1999); 23 (Wissmann et al., 1997); 13 (Bercher et al., 2001); 14 (Gatewood and Bucher, 1997); 15 (Hong et al., 2001); 16 (Hresko et al., 1999); 17 (Plene

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forming either a sheet (i.e. the epidermis and vulva) or a tube (i.e. the intestine and pharynx); those that are isolated (i.e. the excretory cell); those that form a contractile myoe-

stitutional determinants (so far only large chromosomal deficiencies removing both end-1 and end-3 lead to absence of the intestine; the intestinal phenotype of those deficiencies can be rescued by introduction of an end-1 or end-3 transgene); MAPK; mitogen associated protein kinase. Lethality associated with deficiencies removing the EDR are probably linked to the size of the deficiency. * Mutants in the lin-12/glp-1 pathway affect many other processes which contribute to embryonic lethality: 1 (Page et al., 1997); 2 (Koh and Rothman, 2001); 3 (Labouesse et al., 1994); (Labouesse et al., 1996); 5 (Quintin et al., 1995); 20 (Sternberg and Han, 1998); 21 (Herman and Horvitz, 1999); 22 (Herman et al., 1999); 23 (Wissmann et al., 1997); 13 (Bercher et al., 2001); 14 (Gatewood and Bucher, 1997); 15 (Hong et al., 2001); 16 (Hresko et al., 1999); 17 (Plene

epidermal cells can be distinguished: cells which cover

epidermal organ (i.e. the spermatheca) (White, 1988). Mutations that affect the differentiation, structure, morphogenesis or function of epithelial cells generally result in lethality (see Table 1). This review will focus

particular on the first category, particularly on epidermal cells which have been well-characterised in nematodes (Fig. 1A). Epidermal cells are often called hypodermal because they secrete a collageneous cuticle at their apical surface, which acts as an exoskeleton to maintain the shape of the animal and to anchor muscles. Three classes of epidermal cells can be distinguished: cells which cover

Table 1
Phenotypes associated with some genes acting in epithelial cells

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Process</th>
<th>Gene</th>
<th>Phenotype</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major epidermis</td>
<td>Differentiation</td>
<td>elt-1</td>
<td>Emb lethal</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Junction formation</td>
<td>lin-26, elt-5/6</td>
<td>Emb/larval lethal</td>
<td>Ab. cells/defective elongation</td>
</tr>
<tr>
<td>Vulva/Uterus</td>
<td>Connection with muscles</td>
<td>hmr-1/hmp-1/hmp-2, let-502</td>
<td>Emb lethal</td>
<td>No enclosure and/or no elongation</td>
</tr>
<tr>
<td>Vulva Specific</td>
<td>Trafficking</td>
<td>che-14, lrp-1</td>
<td>Part larval lethal</td>
<td>Cuticle defects</td>
</tr>
<tr>
<td>Vulva/Utterus</td>
<td>Connection</td>
<td>cog-2, lin-11</td>
<td>Egl</td>
<td>Vul or Muv</td>
</tr>
<tr>
<td>Intestine</td>
<td>Specification</td>
<td>Ras/MAPK pathway</td>
<td>Egl or Ste</td>
<td>Squashed vulva</td>
</tr>
<tr>
<td></td>
<td>Differentiation</td>
<td>sqw-1, sqw-7, sqw-8</td>
<td>Egl</td>
<td>No connection</td>
</tr>
<tr>
<td></td>
<td>Morphogenesis</td>
<td>line-12/glp-1 pathway</td>
<td>Larval lethal</td>
<td>Ab. cells/no food intake</td>
</tr>
</tbody>
</table>

* This table provides a few examples of the terminal phenotypes associated with mutations affecting epithelial cells but is not an exhaustive list of all genes acting in epithelia. Emb: embryonic; Egl: egg-laying defective; Ste: sterile; Vul: vulvaless; Muv: multivulva; Ab: abnormal; Part: partial; EDR: endoderm determining region (so far only large chromosomal deficiencies removing both end-1 and end-3 lead to absence of the intestine; the intestinal phenotype of those deficiencies can be rescued by introduction of an end-1 or end-3 transgene); MAPK: mitogen associated protein kinase. Lethality associated with deficiencies removing the EDR are probably linked to the size of the deficiency. * Mutants in the lin-12/glp-1 pathway affect many other processes which contribute to embryonic lethality: 1 (Page et al., 1997); 2 (Koh and Rothman, 2001); 3 (Labouesse et al., 1994); (Labouesse et al., 1996); 5 (Quintin et al., 2001); 6 (Bosssinger et al., 2001); 7 (Koeppen et al., 2001); 8 (Legouis et al., 2000); 9 (McMahon et al., 2001); 10 (Costa et al., 1998); 11 (Raich et al., 1999); 12 (Wissmann et al., 1997); 13 (Bercher et al., 2001); 14 (Gatewood and Bucher, 1997); 15 (Hong et al., 2001); 16 (Hresko et al., 1999); 17 (Plenisch et al., 2000); 18 (Michaux et al., 2000); 19 (Yochem et al., 1999); 20 (Sternberg and Han, 1998); 21 (Herman and Horvitz, 1999); 22 (Herman et al., 1999); 23 (Hanna-Rose and Han, 1999); 24 (Newman et al., 1999); 25 (Zhu et al., 1997); 26 (Zhu et al., 1998); 27 (Maduro et al., 2001); 28 (Fukushige et al., 1998); 29 (Hermmann et al., 2000).
the body and form the so-called major epidermis; small specialised epidermal syncytia which cover the head and the tail; and interfacial epidermal cells which connect internal organs to the epidermis generally by adopting a toroidal structure as in the rectum and the vulva (White, 1988). The morphogenetic events that allow a functional vulval-uterine connection to be made have recently been thoroughly investigated and will be discussed in detail. Intestinal cells represent also extensively studied epithelial cells; we will describe the morphogenetic events that lead to an epithelial tube. Due to space constraints, we will not deal with the development of isolated epithelial cells. Similarly, we will only briefly mention morphogenesis of the embryo and of the somatic gonad, since these subjects have been the topic of excellent recent reviews (Chin-Sang and Chisholm, 2000; Hubbard and Greenstein, 2000; Simske and Hardin, 2001).

3. Specification of epithelial cell fates in the embryo

The pathways involved in specifying epithelial cell fates differ in detail between various cell types, but are quite similar in a more global sense, at least in the embryo (for an earlier review, see Labouesse and Mango, 1999). We will concentrate mostly on late aspects of epidermal differentiation.

A model for the specification of the intestine has recently been suggested and can be taken as a paradigm
for other tissues (Fig. 2) (Newman-Smith and Rothman, 1998; Maduro et al., 2001). According to this model, two almost identical GATA factors, MED-1 and MED-2, which are direct targets of the maternal gene \textit{skn-1}, confer mesendoderm identity to a single blastomere (EMS) at the 4-cell stage (Maduro et al., 2001). In response to a polarising Wnt pathway acting together with a MAP kinase pathway, \textit{med} genes promote endoderm formation by turning on the GATA factors \textit{end-1} and \textit{end-3}, which redundantly specify intestine identity (Zhu et al., 1997; Maduro et al., 2001). \textit{END-1} in turn activates the expression of several transcription factors present in the gut, including \textit{elt-2}, which is another intestine-specific GATA factor (Hawkins and McGhee, 1995). \textit{elt-2} and \textit{end-1} probably act redundantly to activate intestine-specific genes in the embryo, including \textit{elt-2} itself, but \textit{elt-2} is essential to maintain intestinal differentiation (Fukushige et al., 1998; Zhu et al., 1998; Fukushige et al., 1999). In other words, \textit{end-1} and/or \textit{end-3} may compensate for the loss of \textit{elt-2} in the early embryo, perhaps by up-regulating their own expression. This pathway has probably been evolutionarily conserved, as GATA factors play an important role in endoderm specification in other species (Reuter, 1994; Reiter et al., 1999), and injection of \textit{end-1} mRNA in \textit{Xenopus} embryos can trigger the expression of endodermal specific markers (Shoichet et al., 2000).

The pathway specifying epidermal differentiation reveals a similar level of redundancy (Fig. 2). As in the intestine, a GATA factor with two zinc-fingers, ELT-1, acts at the top of the hierarchy to specify epidermal identity (Page et al., 1997) and turn on several regulatory genes, including genes encoding the GATA factors ELT-3, ELT-5 and ELT-6, which redundantly specify seam cell identity (seam cells correspond to lateral epidermal cells, see Fig. 3A). Other potential ELT-1 targets are genes encoding the nuclear hormone receptors NHR-23 and NHR-25, which are essential for embryonic development and control moulting in larvae. The bottom arrows indicate possible targets of these proven or putative transcription factors (see Section 3).

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\begin{figure}
\centering
\includegraphics[width=\textwidth]{cell_fate_specification.png}
\caption{Cell fate specification in the epidermis and intestine. Four distinct levels of regulation are involved in specifying intestinal cell fates. (Left) In response to a Wnt and a MAP kinase pathway, the GATA factors MED-1 and MED-2, perhaps with the bZIP transcription factor SKN-1, induce expression of the GATA factors END-1 and END-3, which redundantly act to specify and maintain intestine identity. END-1/3 proteins then activate expression of ELT-2, another GATA factor that turns on expression of different terminal differentiation factors such as the junction component AJM-1, the gut esterase GES-1, or the epitope recognised by the monoclonal antibody MH33. PHA-4, which specifies pharynx identity, is also expressed in the intestine where its specific function is not known. (Right) In lineages generating the major epidermal cells, the GATA factor ELT-1 specifies epidermal identity. Its most likely targets are genes encoding the atypical C$_2$H$_2$ zinc-finger protein LIN-26 which is required for epithelial differentiation, the GATA factor ELT-3 which can induce AJM-1 and collagen gene expression in non-seam cells, the GATA factors ELT-5 and ELT-6 which redundantly specify seam cell identity (seam cells correspond to lateral epidermal cells, see Fig. 3A). Other potential ELT-1 targets are genes encoding the nuclear hormone receptors NHR-23 and NHR-25, which are essential for embryonic development and control moulting in larvae. The bottom arrows indicate possible targets of these proven or putative transcription factors (see Section 3).}
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\footnote{In accordance with the general \textit{C. elegans} nomenclature for genes and proteins, we write gene names in italics with small case letters (i.e. \textit{skn-1}) and the corresponding proteins with non-italicised capital letters (i.e. \textit{SKN-1}).}
coupled with ectopic expression experiments, suggests the following simplified model for the generation of the ‘major epidermal’ cells (Gilleard and McGhee, 2001; Koh and Rothman, 2001; Quintin et al., 2001). In the dorsal and ventral epidermis, elt-3, acting redundantly with other genes, turns on and maintains epidermal-specific genes, such as the cuticle collagen gene dpy-7, and the junction marker gene ajm-1 (see Section 4) (Page et al., 1997; Gilleard et al., 1999; Gilleard and McGhee, 2001). In cells of the lateral epidermis (seam cells, see Fig. 3A), elt-5 and elt-6 redundantly activate and maintain the expression of several seam-specific genes, the synthesis of cuticular ridges known as alae and probably junction formation. These genes also repress the expression of non-seam genes (elt-3), in particular genes bestowing the potential to fuse with other cells (Koh and Rothman, 2001). In parallel, lin-26 acts to turn on genes that are important for epithelial integrity (‘epithelial-specific’), but is not required to turn on genes specifically expressed in the epidermis and not in other epithelia (i.e. cuticle collagens) (Labouesse et al., 1994, 1996; Quintin et al., 2001).

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**Fig. 3. Morphogenesis of epithelial tissues in the embryo.** The major epithelial cells are schematised along with their major morphogenetic changes. Anterior is left, dorsal is up. The different organs are not drawn to scale. (A) Epidermal cells are subdivided into ‘major epidermal’ cells, which cover most of the body, and ‘minor epidermal’ cells, which cover other areas and are not represented. Among cells of the ‘major epidermis’, dorsal (top), lateral (middle, dark grey) and ventral (bottom) have different functions and express different regulatory genes. In particular, all major epidermal cells initially express the GATA factor ELT-1 and the atypical zinc-finger protein LIN-26; subsequently, seam cells express the GATA factors ELT-1, ELT-5 and ELT-6, but not ELT-3 whose expression is restricted to ventral and dorsal cells. The main morphogenetic processes that take place in the embryo are indicated, for further details see recent reviews (Chin-Sang and Chisholm, 2000; Simske and Hardin, 2001). (B) The pharynx has a tube-cyst-tube-cyst structure (see Fig. 1A) (Albertson and Thomson, 1976; Leung et al., 1999). It is surrounded by a basement membrane and is composed of five different cell types. The adult pharynx displays a typical triradiate symmetry in cross-section with a lumen adopting a triangular shape in the buccal cavity, and a Y-shape elsewhere (B-right). Epithelial marginal cells play an important role in giving the lumen its Y-shape: their nuclei migrate towards the basement membrane following cytoplasmic polarisation and their cell bodies retract. (C) The intestine primordium initially displays a radial symmetry except in the first row (C-left). However, soon after intestinal cells become polarised, left ventral (or right ventral) cells intercalate with left dorsal (respectively, right dorsal) cells, resulting in a bilaterally symmetrical tube (C-middle) (Leung et al., 1999). Soon after these cell movements, three pairs of anterior cells move counter-clockwise to the left to initiate a left-handed twist in the anterior part of the intestine; by the time the larva hatches this twist is about 180° (C-right).
Some of the key genetic data supporting this model are mentioned below. Firstly, forced expression of ELT-3 in early blastomeres can turn on the expression of AJM-1 and of the collagen DPY-7, in the same way as forced expression of ELT-1 does (Gilleard and McGhee, 2001). However, elt-3 inactivation does not affect embryonic viability, or epidermal development. This suggests that other genes act redundantly with elt-3 to maintain epidermal differentiation, although elt-1 is not a candidate since it is down-regulated in dorsal and ventral epidermal cells where elt-3 is expressed at mid-embryogenesis (Gilleard and McGhee, 2001). Secondly, simultaneous inactivation of elt-5 and elt-6, which are both expressed in seam cells and have related DNA binding domains (80% identical), results in embryonic lethality with seam cell defects, or in larval lethality with molting defects. Finally, forced expression of LIN-26 in early embryonic blastomeres can turn on expression of AJM-1, DLG-1 and CHE-14 (these proteins are involved in junction formation or trafficking), but not the collagen DPY-7 (Quintin et al., 2001). Although these proteins are normally expressed in lin-26 null mutant embryos their sub-cellular distribution is affected, suggesting that other genes can compensate for loss of lin-26 function to initiate their expression (Quintin et al., 2001). Further evidence that lin-26 is required for epithelial differentiation comes from an analysis of animals engineered to specifically lack lin-26 expression in their somatic gonad. In such animals, AJM-1 is strongly down-regulated in the uterus and the uterine lumen fails to form (den Boer et al., 1998).

Two important features of this model should be outlined: the involvement of GATA factors to redundantly control epidermal differentiation and specify differences among dorsal/lateral/ventral epidermal cells; and the requirement for a specific factor (LIN-26) to turn on genes that are important for epithelial integrity. Redundancy in tissue specification is a rather general feature in many species, for instance during intestine formation in C. elegans (see above), or myogenesis in vertebrates (Arnold and Braun, 2000). The necessity for a differential control among dorsal/lateral/ventral epidermal cells is not surprising, since these cells perform different morphogenetic functions. As outlined in Fig. 3A, dorsal cells intercalate and subsequently fuse together, ventral cells mediate the process known as ventral enclosure of the embryo and seam cells undergo concerted elongation during embryonic elongation. Potential targets of the GATA factors could be genes specifically expressed in a subset of epidermal cells that control morphogenesis of the embryo. For instance, the C2H2 zinc-finger transcription factor DIE-1, which is required for the process of dorsal intercalation, is specifically expressed in dorsal epidermal cells (Heid et al., 2001). Similarly, the LET-502 Rho-binding kinase is specifically expressed in seam cells and is essential for the concerted elongation process, whereas its antagonist, the MEL-11 myosin phosphatase, is specifically expressed in non-seam cells (Wissmann et al., 1997, 1999). The requirement for a gene such as lin-26 was unexpected. It raises the possibility that specific transcription factors control the epithelial cell fate, irrespective of the nature of the epithelium being made.

The analysis of genes required for epithelial differentiation is only starting and remains poorly understood in many cell types. The biochemistry of the above-mentioned proteins is limited, and several conclusions have not yet been confirmed by DNA-binding assays. An intriguing question is what confers binding specificity to the different ELT/GATA factors involved in specifying intestinal and epidermal cell fates. Many other partially characterised genes are expressed in the epidermis, in particular several nuclear hormone receptor homologues. Although their specific targets have not been identified, it is known that their silencing by RNA interference results in lethality (Kostrouchova et al., 1998; Miyabayashi et al., 1999; Gissendanner and Sluder, 2000).

4. Epithelial junction structure and assembly

The first major cellular processes that occur in response to genes (such as those mentioned above) that induce differentiation of epithelial tissues are the establishment of cell polarity and the assembly of sub-apical junctional belts. Two major types of junctions can be distinguished by electron microscopy in epithelial cells (for general reviews, see Drubin and Nelson, 1996; Yeoman et al., 1999). Adherens junctions (AJs), which are present in vertebrates and invertebrates, are essential for cell adhesion. Tight junctions (TJs) in vertebrates and septate junctions (SJs) in Drosophila are involved in the control of paracellular solute transport. These two belts, whose molecular composition is quite well known in vertebrates and in Drosophila demarcate the apical from the basolateral domains (Yap et al., 1997; Tsukita et al., 1999; Muller, 2000).

Electron microscopic examination of C. elegans embryonic epithelial cells has revealed only one type of junctional belt in the embryo (Fig. 1D), which has an adherens junction-like structure (Priess and Hirsh, 1986; Leung et al., 1999; Legouis et al., 2000; Koeppen et al., 2001). We will subsequently refer to it as the CeAJ (C. elegans apical junction). A septate junction has not been observed, except occasionally (D. Hall, pers. comm.; R. Legouis, unpublished data). Below, we discuss junction formation in the embryo, and particularly in the epidermis. It is not yet clear whether all junctions share the same components and are assembled in the same way in all C. elegans epithelial cells. In particular it is unknown if there are important differences between the formation of junctions in sheet-like epithelia (i.e. the epidermis) and tube-like epithelia (i.e. the intestine).

Components of the CeAJ have been identified either in a screen for monoclonal antibodies recognising membrane-
rich or insoluble extracts from embryos (AJM-1), in genetic screens for mutations which affect embryonic morphogenesis (hmr-1, hmp-1 and hmp-2), or in RNA interference screens for C. elegans homologues of Drosophila and vertebrate junction components (dlg-1). AJM-1, the first identified CeAJ component, corresponds to the epitope recognised by the monoclonal antibody MH27 (Francis and Waterston, 1991). Immunostaining with MH27 reveals a belt-like pattern around epithelial cells in whole-mount embryos (red in Fig. 1B) (Priess and Hirsh, 1986), and immunogold experiments show that AJM-1 localises to the electron dense part of the CeAJ at the ultrastructural level (Koeppen et al., 2001). The ajm-1 gene has recently been identified by expression cloning using MH27 as a probe, showing that it encodes a large hydrophilic coiled-coil protein with no obvious homology in other species (Koeppen et al., 2001). The genes hmr-1, hmp-1 and hmp-2 encode E-cadherin, α-catenin and β-catenin homologues, respectively (Costa et al., 1998). HMP-2 is one of three C. elegans β-catenin homologues. It is the only β-catenin homologue to interact with HMR-1 (the closest E-cadherin homologue in the C. elegans genome), but it has lost a function associated with classical β-catenins, the potential to signal in a Wnt pathway. Instead, Wnt signalling involves BAR-1, another β-catenin homologue, or WRM-1 which acts in a non-conventional manner (Rocheleau et al., 1997; Eisenmann et al., 1998; Korswagen et al., 2000). Staining with antibodies against HMP-1 and HMR-1 reveal in first approximation a belt similar in position and structure to that formed by AJM-1 (Costa et al., 1998). The most recently identified CeAJ component, named DLG-1, is the C. elegans orthologue of Drosophila Lethal Discs Large (Dlg) and human hDlg proteins and colocalises with AJM-1 in epithelial cells (Bossinger et al., 2001; Koeppen et al., 2001; McMahon et al., 2001).

A combination of genetic, immunochemical and electron microscopic studies suggest that the apical junction of C. elegans epithelial cells contain two independent units with at least partially distinct functions (Fig. 1D). The first and most apical unit would comprise HMR-1, HMP-1, HMP-2 and possibly other proteins, while the other unit would include at least AJM-1 and DLG-1 (Koeppen et al., 2001; McMahon et al., 2001). Indeed, as observed by immunostaining, AJM-1 and DLG-1 do not appear to colocalise with HMP-1 in transverse sections through the apico-basal axis of epidermal cells (McMahon et al., 2001) or in the pharynx (Koeppen et al., 2001). Mutations in hmr-1, hmp-1 and hmp-2 do not affect the distribution of AJM-1, but cause defects in actin cytoskeleton organisation and anchoring (Costa et al., 1998; Raich et al., 1999). Conversely, in the absence of DLG-1, AJM-1 distribution becomes very irregular, whereas the HMR-1/HMP-1/HMP-2 complex and actin anchoring remain largely unaffected (Bossinger et al., 2001; Koeppen et al., 2001; McMahon et al., 2001). Similarly, in ajm-1 mutants the HMR-1/HMP-1/HMP-2 complex and actin anchoring are unaffected (Koeppen et al., 2001). At the ultrastructural level, inactivation of dlg-1 causes an almost complete disappearance of the electron dense structure of CeAJs where AJM-1 is located, suggesting that DLG-1 functions to aggregate proteins that form this structure (McMahon et al., 2001). Interestingly, AJM-1 and DLG-1 physically interact as shown by yeast two-hybrid and GST-pull down experiments (Koeppen et al., 2001). In ajm-1 mutants, local paracellular gaps of 50–200 nm between the normally closely apposed sides of the junctional complex can be observed, showing that AJM-1 is required to maintain the tightness of the CeAJ electron dense domain (Koeppen et al., 2001). The CeAJs of hmr-1, hmp-1 and hmp-2 mutants have not yet been examined by electron microscopy; it is not clear whether one should expect them to be normal since HMP-1 is more apical than AJM-1, or defective. Surprisingly, neither a loss of the HMR-1/HMP-1/HMP-2 complex, nor of the DLG-1/AJM-1 complex, strongly affects cell adhesion and polarity (Costa et al., 1998; Raich et al., 1999; Bossinger et al., 2001; Koeppen et al., 2001; McMahon et al., 2001). A reasonable hypothesis is that both complexes act redundantly to maintain adhesion and cell polarity. In addition, DLG-1 might be required to maintain some unique aspects of cell polarity, since the apical protein CRB-1 (see below) is no longer detected in the intestine of embryos lacking DLG-1 (Bossinger et al., 2001).

The results summarised above suggest that the CeAJ combines the functions of both adherens junctions and septate/tight junctions in other species. Thus, although C. elegans epithelial cells do not contain a distinguishable septate junction, the CeAJ appears to be more similar to epithelial junctions in other species than initially anticipated. Interestingly, in vertebrates, adherens and tight junctions might also be two aspects of a single unit, since the tight junction protein ZO-2 can also associate with adherens junctions (Itoh et al., 1999). In addition, recent results suggest that a second complex, the afadin/nectin complex, contributes to cell adhesion in addition to the cadherin/catenin complex (Tachibana et al., 2000). Some unique properties of the CeAJ, in particular the likely redundancy in the maintenance of adhesion and polarity, make C. elegans a particularly attractive model to dissect the functions of junctional proteins, such as actin anchoring by HMP-1 or protein aggregation by DLG-1. Indeed, in Drosophila and mammals the severe polarity defects associated with loss of cadherin/catenin or Dlg function make it more difficult to study these functions in a live embryo.

How junctions become positioned sub-apically has been revealed by characterisation of the gene let-413 (Legouis et al., 2000). This gene was identified through a screen in which embryos homozygous for chromosomal deficiencies were examined for their AJM-1 pattern (Labouesse, 1997).
In *let-413* mutants, the AJM-1 distribution within epithelial cells is severely disrupted. *LET-413* is a basolateral plasma membrane protein, which possesses one PDZ domain and sixteen leucine-rich repeats (LRR domain). The LRR domain is quite similar to the Ras-binding protein SUR-8, raising the possibility that *LET-413* could bind a GTPase. Electron microscopic and confocal analysis revealed that in *let-413* mutants CeAJs and their components are spread along the lateral membrane (Legouis et al., 2000; Koeppen et al., 2001; McMahon et al., 2001). Interestingly, the distribution of junction components in *let-413* deficient embryos resembles that observed in immature wild-type epidermal cells, where markers are initially located along the lateral membrane, instead of sub-apically (McMahon et al., 2001). These observations suggest that in epidermal cells *let-413* acts in a process leading to the coalescence of junction components at a sub-apical position, or possibly to prevent junction assembly laterally. The function played by *LET-413* is likely to be general since the *Drosophila* homologue Scribble has a directly related role and subcellular localisation (Bilder et al., 2000a,b). In vertebrates, ERBIN, one of the human *LET-413* homologues, is a basolateral protein required to localise the EGF receptor ERBB2/HER2 at the basolateral membrane (Borg et al., 2000).

In *Drosophila*, two additional complexes, localised apical to the AJ, are essential to establish cell polarity and assemble AJs. One complex involves aPKC, an atypical protein kinase C, the PDZ proteins Bazooka and Par-6 (Kuchinke et al., 1998; Schober et al., 1999; Wodarz et al., 2000; Petronczki and Knoblich, 2001), and the other includes the transmembrane protein Crumbs with the multi-PDZ protein Disc Lost (Bhat et al., 1999; Klebes and Knust, 2000). Bazooka, Par-6 and aPKC are the respective homologues of the worm proteins PAR-3, PAR-6 and PKC-3, which colocalise at the anterior cortex of the early *C. elegans* embryo where they are essential to establish embryonic polarity (for reviews, see Rose and Kemphues, 1998; Doe and Bowerman, 2001). Their vertebrate homologues ASIP, mPAR6 and PKCζ are apical in mammalian epithelial cells, and also form a complex together with the GTPase CDC42 (for a review, see Kim et al., 2000). In *C. elegans*, PAR-3, PAR-6 and PKC-3 are also present at the apical surface of intestinal and pharyngeal cells (green in Fig. 1B) but their role in these cells has not yet been examined (Leung et al., 1999). There is also a Crumbs homologue in *C. elegans*, CRB-1, which is localised at the apical surface of intestinal cells. However, RNA interference against *crb-1* or against a *disc lost* homologue does not affect cell polarity or junction assembly (Bosinger et al., 2001).

In summary, there are some differences in the specific functions that homologous proteins play in epithelial cells from *C. elegans* and other systems (*Drosophila* and vertebrates). Nonetheless there are also common features, one of which is the presence of homologous proteins at the same positions. Future studies and comparisons will certainly reveal the relevance of these similarities and differences.

5. Hemidesmosomes, intermediate filaments and the connection with other tissues

Epithelial cells are also tightly linked to non-epithelial cells through the extracellular matrix. These interactions, which must be integrated throughout development, are essential to ensure mechanical coupling between adjacent tissues, to maintain tissue architecture and to facilitate cell-cell communication (for review, see Hynes, 1999).

Several studies have recently highlighted the role of hemidesmosomes and intermediate filaments (IFs) in the epidermis to link the underlying muscle on its basal surface and the external cuticle on its apical surface (Fig. 1E). In *C. elegans* as in many invertebrates, the cuticle acts as an exoskeleton onto which muscle cells attach to allow locomotion. Sarcomeres are mechanically coupled to the basal lamina that separates muscles from the epidermis through integrins (Hresko et al., 1994; Williams and Waterston, 1994). In regions of muscle contacts, epidermal cells become flattened and contain IF arrays connecting electron dense structures similar to hemidesmosomes, which together form what is known as fibrous organelles (Francis and Waterston, 1991). IFs represent a major cytoskeletal system found in animal cells that transmit mechanical stress (Fuchs and Cleveland, 1998). The diversity of this family (more than 50 genes in mouse or human) explains the poor understanding of its functions. In *C. elegans*, there are only 11 genes coding for IFs, which can be divided into five groups A1–A4, B1–B2, C1–C2, D1–D2, E1. Inactivation by RNA interference has revealed that four of them (A1–A3, B1) are essential for embryonic or larval development (Karabinos et al., 2001). In embryos and larvae lacking the A2 and A3 IFs, muscle cells become detached from the body wall although it is not yet known at which level this detachment takes place. Consistent with a role in epidermal cells, GFP fusion experiments indicate that A3, and possibly A2, are specifically expressed in the epidermis (Karabinos et al., 2001). This confirms the essential role of IFs in epithelial cells to establish and maintain connections between several tissues during organogenesis.

Two screens for mutants presenting detachment of muscle cells have led to the identification of the genes *miaa-3* (fragile muscle attachment) and *mup-4* (muscle positioning defective) (Gatewood and Bucher, 1997; Pleneuf et al., 2000), which have recently been cloned (Bercher et al., 2001; Hong et al., 2001). In these mutants, muscle cells are detached from the body wall and animals die during embryonic or larval stages. Electron microscopy analysis has shown that the epidermis detaches from the cuticle and is less strongly flattened in these mutants, but that muscles remain attached to the epidermis (Bercher et al., 2001; Hong et al., 2001). *MUA-3* and *MUP-4* are single pass transmembrane proteins with a large extracellular domain mainly formed of EGF repeats and a cytoplasmic domain with weak homology to filaggrins, which are known to bind intermediate filaments (Steinert et al., 1981). Amino acid identity between MUA-3 and MUP-4
reaches 50% in the extracellular region. Both proteins are expressed in the epidermis and localised at sites of muscle contacts, in a pattern similar to that of intermediate filaments. The authors suggest that MUA-3 and MUP-4 act at the epidermal apical surface, binding on the one hand to the cuticle via their extracellular domain, and on the other to intermediate filaments presumably through their filaggrin-like cytoplasmic tail (Bercher et al., 2001; Hong et al., 2001).

Another transmembrane epithelial protein required for muscle attachment is called myotactin. The gene encoding this protein, let-805, was isolated by screening an expression library with the monoclonal antibody MH46 (Hresko et al., 1999), which was identified in the same screen as MH27 (see Section 4) (Francis and Waterston, 1991). The extracellular region of myotactin contains 32 fibronectin type III (FNIII) repeats, but its cytoplasmic part does not present any homology. Immunohistochemical analysis suggests that myotactin is localised at the basal surface of epidermal cells. Based on its number of FNIII repeats, it could expand through the basal lamina to allow anchorage of muscle and maintain the association with the fibrous organelles. In let-805 mutants, muscles detach when contraction begins at the two-fold stage, and fibrous organelles tend to be localised throughout the epidermis instead of just in areas of muscle contact. However fibrous organelles remain surprisingly well organised and maintain a regular pattern, suggesting that myotactin may only be required to maintain the association between muscle and fibrous organelles.

The analysis of the muscle-epidermis-cuticle connection is providing exciting results at a rapid pace, identifying new proteins with known modules. Genetic analysis in the worm embryo should complement biochemical studies in vertebrates to better understand the complex and still poorly understood process of mechanical coupling between tissues.

6. Basement membrane and organogenesis

Epithelial cells are characterised by the presence of a basal lamina, which provides a link with the underlying tissue and an important polarity clue (for a review, see Yeaman et al., 1999). For instance, experiments with tissue culture cells have shown that the presence of a basal lamina can induce polarisation of MDCK cells (Wang et al., 1990a,b). Because of its anatomical and genetic simplicity C. elegans, which has many classical components of the extra-cellular matrix such as laminins, nidogen, perlecan, type IV collagen (Kramer, 1997), is a good model to identify new components of the basement membrane or to clarify the roles of proteins already known in vertebrates. For instance, C. elegans has only two α-laminin chains (LAM-3 and EPI-1) instead of five in vertebrates, only one β-integrin chain (PAT-3) compared to eight in vertebrates and only two α-integrin chains (PAT-2 and INA-1) instead of 17 in vertebrates (Hutter et al., 2000). Below, we will focus mainly on components that affect epithelial cells.

Genetic analysis of somatic gonad morphogenesis has identified a new basal lamina component, GON-1, which is a secreted protein with a metalloprotease domain related to the ADAM family and thrombospondin repeats. GON-1 is required for two aspects of gonad morphogenesis (Blelloch et al., 1999; Blelloch and Kimble, 1999). In gon-1 mutants, the somatic gonad primordium and uterus fail to acquire a clear epithelial character, in particular the uterine lumen is essentially absent, suggesting that GON-1 promotes their epithelialisation. It might do so by providing a basal clue important to polarise somatic gonadal cells. Interestingly, mutations eliminating the α-laminin chain LAM-3 affect organogenesis of the pharynx, and in particular the position at which some CeAJs are assembled (W. Wadsworth, pers. comm.). These observations bring genetic support to the current models regarding the influence of basal cues on the establishment of cell polarity, although its influence may be subtler than anticipated. Independently of its function in setting polarity, GON-1 is also essential for the initial migration of the leader cells (distal tip cells, or DTC, in hermaphrodites; linker cell, or LC, in males) which guide growth and extension of the somatic gonad. MIG-17, another metalloprotease related to GON-1 but lacking the thrombospondin repeats, is required for a distinct phase of DTC/LC migration. It is likely that the function of GON-1 and MIG-17 in cell migration is to locally degrade the basal lamina to allow further migration or to reorient the direction of migration (Blelloch et al., 1999; Blelloch and Kimble, 1999; Nishiwaki et al., 2000).

Hemicentin, which is secreted by muscle and gonadal leader cells, is another novel extracellular matrix protein identified by genetic analysis (Vogel and Hedgecock, 2001). Hemicentin, which is encoded by the gene him-4, contains 48 immunoglobulin repeats flanked by new terminal domains. The HIM-4 protein forms fine tracks at specific sites, particularly in regions of oriented linear junctions, which can always be correlated with phenotypes observed in him-4 mutant larvae. These mutants present pleiotropic defects in different organs, such as defective gonadal movement, loss of adhesion of the intestine to body wall muscles, detachment of the uterus from the lateral epidermis once egg-laying begins (see Section 10 and Fig. 4). It has been suggested that hemicentin organises hemidesmosomes in the overlying epidermis where the uterus contacts the lateral epidermis, and along mechanosensory axons.

7. Differential trafficking to apical and basolateral membrane domains

An important aspect of epithelial cell polarity concerns the differential trafficking of proteins to the apical and basolateral domains. Proteins destined to the apical and basolateral membranes are often sorted out in the Golgi based on specific determinants (Mellman and Warren, 2000). Di-leucine and tyrosine residues can mediate basolateral target-
ing, whereas O-glycosylation, N-glycosylation or a GPI adduct (glycophosphoinositol) can mediate apical targeting. The mechanisms that control protein trafficking in *C. elegans* are just beginning to be analyzed.

In mammalian MDCK cells, Simons and Ikonen have proposed that lipid microdomains enriched in cholesterol (also called lipid rafts) direct trafficking towards the apical membrane (Ikonen and Simons, 1998). Biochemical analysis has led to the identification of several raft components, including caveolin. RNA interference studies indicate that caveolin may not be essential for apical trafficking in *C. elegans* (Scheel et al., 1999). Genetic analysis has led to identification of *che-14*, which encodes a probable component of the apical trafficking machinery in ectodermal
epithelial cells. Ultrastructural studies showed that che-14 mutants accumulate vesicles close to the apical membrane and have a marked reduction of the cuticle thickness (i.e. an apically secreted structure), causing larval lethality or abnormal behaviour (Michaux et al., 2000). Since the CHE-14 protein itself is predominantly located at the apical surface of ectodermal epithelial cells, it has been suggested that it is necessary for normal trafficking of a subset of proteins to the apical domain (Michaux et al., 2000). Interestingly, CHE-14 is a member of the NPC1/Patched/Dispatched family of multi-pass transmembrane proteins, which contain a putative sterol-sensing domain (Lange and Steck, 1998). Consistent with CHE-14 trafficking function, the vertebrate NPC1 protein is thought to control cholesterol trafficking between late endosomes and lysosomes (Neufeld et al., 1999; Cruz et al., 2000), while the phenotype of flies mutated in dispatched may result from defective secretion of the morphogen Hedgehog (Burke et al., 1999). However, the targets of CHE-14 and the precise step in exocytosis to the apical membrane at which CHE-14 acts remain to be identified. It should be pointed out that loss of CHE-14 activity affects epidermal cells in a different way than mutations in LRP-1, a gp330/megalin-related protein necessary for cholesterol endocytosis, which result in an inability to shed and degrade the old cuticle at each larval moult (Yochem et al., 1999).

The mechanism involved in targeting one particular basolateral protein, the EGF receptor homologue LET-23, has been particularly well characterised. LET-23 is involved in several signalling events, particularly during vulva formation (see Figs. 4A and 5). Normally, LET-23 receives a signal made by the anchor cell (AC), which is located dorsal to the basolateral surface of vulval precursor cells (VPCs). Thus, to receive the AC signal, LET-23 should be present at the basolateral membrane of VPCs. Its basolateral restriction is achieved by a complex of three PDZ-containing proteins encoded by the genes lin-2, lin-7 and lin-10 (Hoskins et al., 1996; Sim ske et al., 1996; Kaech et al., 1998; Whitfield et al., 1999). Mutations in these genes result in a vulvaless phenotype due to apical mislocalisation of LET-23. Elegant experiments have shown that the type I PDZ domain of LIN-7 interacts with the final residues of LET-23, which correspond to a canonical type I PDZ target sequence. LIN-7 can also directly interact with LIN-2, which can bind LIN-10 (Kaech et al., 1998). The LIN-7 protein appears to colocalise with junctions, while LIN-10 is a presumptive Golgi protein (Simske et al., 1996; Whitfield et al., 1999), indicating that LIN-2/LIN-7/LIN-10 may not act at the same step in the trafficking pathway that prevents LET-23 from going to the apical membrane. This pathway has been conserved in many species and also operates in non-epithelial cell types, particularly in the nervous system (Bredt, 1998). Analysis of LET-23 localisation in C. elegans vulval precursors allows a powerful genetic dissection of this pathway.

8. Morphogenesis of the intestine

The C. elegans intestine is an extraordinarily simple bilaterally symmetrical organ containing only 20 cells that derive from a unique blastomere, the E blastomere. Most of the intestine is a tube composed of rings of only two cells, such that the lumen of the intestine is a canal between two opposed cells (Figs. 1C and 3C). Elegant work from the Priess laboratory recently described some of the cellular and genetic steps involved in intestinal morphogenesis (Leung et al., 1999; Hermann et al., 2000). It involves cyto...

Fig. 4. Morphogenesis of the egg-laying system. (A) The 12 cells that constitute the ventral epidermis in the embryo (see Fig. 3A) divide in the first larval stage to each generate an epidermal cell (Pn.p) and a neuroblast (Pn.a). The fates of Pn.p cells are symbolised in the top right drawing. At the first larval stage, six Pn.ps fuse with the major epidermal syncytium hyp7 (F fate), while the others become vulval precursor cells (VPCs). At the third larval stage, the three central cells (P(5-7).p) are induced by the anchor cell (AC in blue) to generate the vulva and adopt either a 1” or 2” fate, while the three other Pn.ps adopt the 3” fate (one division and fusion of both daughters to hyp7). The 1” and 2” fates are characterised by three cell divisions along specific cleavage planes to generate eight and seven cells, respectively, with distinct properties. The 22 vulval cells generated by P(5-7).p can adopt seven different fates following a bilateral symmetry, from the outermost vulA to the innermost vulF rings (top left drawing). Each fate is executed either by two or four cells, which undergo short-range migrations and extension of their apical domains to wrap around the more proximal cells and to fuse with other cells of the same fate. Apical extension and fusion push internal rings dorsally and thereby drive the autonomous invagination process. Each ring has a specialised function: vulA connects the vulva to the surrounding epidermis syncytium hyp7; vulE anchors the vulva to the lateral seam cells; vulF connects the vulva to the vul1 uterine cells; vulD and vulF anchor the sex muscles that open the vulva. As explained further in Section 10, the AC by fusing with the utse plays a critical role in making the connection between the vulva and uterine lumens. (B) The uterus is composed of 61 cells that derive from three ventral (VU) and two dorsal (DU) uterine precursors (top left drawing). Each VU descendant generates four intermediate precursors, which adopt a so-called π or r fate depending on whether they are close to the AC or not. After cell fusion, these 61 cells generate on each side of the vulva (from distal to proximal) one spermatheca-uterine valve (sujin), four uterine toroids (ut) and three uterine-vulval cells (uv1-3), and centrally a dorsal uterine cell (du) and an H-shaped uterine-seam cell (utse) (top left and top right drawings). Formation of the uterine lumen may be related to formation of the intestinal lumen. The four ut and the sujn cells on each side of the vulva are formed by the fusion of four to six cells generated by DU and r descendants. In the central part of the uterus, the ventral uv1-3 cells are mononucleate, the dorsal du cell results from the fusion of four cells, and the H-shaped utse results from heterotypic fusion of 8 π descendants with the AC. Each uterine cell has a specialised function: uv2 sends cytoplasmic processes contacting the dorsal uterus; uv1 physically connects the uterus to the vulva by forming apical junctions with the utse and the vulF cells of the vulva; the utse anchors the uterus to the lateral epidermis. The top right drawing shows a longitudinal-section through the uterus, the bottom right drawing shows a transverse-section through the uterus and the vulva. Note the specialised projections made by vulE, uv1, uv2, utse cells, and the positions of vulva opening muscles. Insects correspond to the vulval primordium (A) or the entire egg-laying system (B; sujn cell, arrow; ut cells, diamonds) of transgenic animals expressing a dlg-1::gfp translational fusion, highlighting junctions at the same stage as the drawing above (A) or to the right (B). Drawings in this figure are adapted from Newman and Sternberg (1996) and Sharma-Kishore et al. (1999). Scale bar 10 μm (A) or 40 μm (B).
Plasmic polarisation of each cell, microtubule-dependent nuclear migration to the midline, intercalation of ventral cells with dorsal cells in a stereotyped pattern that does not cross the left/right boundary, formation of the lumen, counter-clockwise rotation of three anterior pairs of cells and appearance of a basement membrane (Leung et al., 1999). The separation of apical membranes at the midline, which initiates lumen formation, coincides with the appearance of vesicles at the apical surface (Leung et al., 1999). These vesicles resemble some endocytic vesicles, raising the possibility that endocytosis might contribute to cell separation. Cytoplasmic polarisation and formation of a lumen are cell-autonomous processes that occur in embryos containing only descendants of the E blastomere. However, intercalation does not occur in these partial embryos, such that the intestinal cyst made retains a radial symmetry instead of acquiring a bilateral symmetry (Leung et al., 1999). It suggests that external clues play a role in the acquisition of a bilateral symmetry.

The genetic control of these events is not understood, except for the events that induce an anterior-posterior and a left-right asymmetry within the intestinal tube and subsequently allow the anterior pairs of cells II–IV to rotate. Anterior-posterior and left-right asymmetries within the intestine are specified by a MAP kinase-like and a Notch-like pathway that are repeatedly used in the early embryo (Hermann et al., 2000). These pathways might modify the adhesive properties of left versus right cells in the anterior part of the intestine and create favourable conditions to allow a rotation. The anterior-posterior asymmetry is generated within the intestinal primordium at or after the third division of the E blastomere, which occurs along the anterior-posterior axis. It involves the LIT-1 kinase, the final effector of a non-conventional MAP kinase pathway that results in excluding the Tcf/Lef homologue POP-1 from posterior daughters after anterior-posterior divisions (Kaletta et al., 1997; Meneghini et al., 1999; Rocheleau et al., 1999). In parallel, a Delta-related ligand encoded by the gene lag-2 down-regulates expression of the Notch-related receptor LIN-12 in the left part of the intestinal primordium (for a review of the LIN-12/Notch pathway, see Greenwald, 1998). All intestinal cells initially express LIN-12, while only cells located on the right side of the intestine express LIN-12, which suggests that external clues play a role in the acquisition of a bilateral symmetry.
tine express LAG-2. The initial LIN-12 asymmetry serves to pattern a second, subsequent, LIN-12 mediated interaction. In lin-12, lag-2, or lag-1 (which encodes a Su(H) homologue) mutants, anterior cells fail to rotate (Hermann et al., 2000). It is likely that the basic mechanisms C. elegans uses to establish polarity in its 20-cell intestine will be relevant to studies on organogenesis in more complex systems.

9. Morphogenesis of the pharynx

The pharynx, which corresponds to the C. elegans foregut, is more complex than other embryonic organs as the mature organ includes glands, neurons, epithelial and myoepithelial cells. Most cells are polarised and their apical surfaces border a Y-shaped, cuticle-lined lumen. They are generally arranged in sets of nine cells with three-fold rotational symmetry around the lumen (Albertson and Thomson, 1976). Two recent studies have investigated some of the events that occur during formation of the pharyngeal lumen and the buccal cavity (Leung et al., 1999; Portereiko and Mango, 2001). Cytoplasmic polarisation appears later in the pharyngeal primordium than in the intestinal primordium, when cells become wedge-shaped, with their narrow apical surface extending to the midline and their nuclei moving to the basal surface (Leung et al., 1999). As in the intestine, formation of the lumen starts by a separation of adjacent membranes at the apical midline. Subsequently, and unlike what happens in the intestine, three epithelial cells called the marginal cells retract their apical tips, thereby considerably expanding the apical surface and giving rise to the Y-shaped lumen characteristic of the pharynx (Fig. 3B) (Leung et al., 1999). The mechanisms involved in marginal cell retraction are unknown.

Portereiko and Mango (2001) examined the cellular events involved in connecting the pharynx primordium to the buccal cavity. They have shown that it involves three main steps. First, the apical/basal axis (as defined by the position of CeAJs) of the anterior-most epithelial cells within the primordium, which is initially oriented along the anterior-posterior axis towards the centre of the primordium, rotates approximately 90° to orient along the dorsal ventral axis. Meanwhile, the basement membrane opposed to the anterior surface of these cells disappears, which transforms the cyst-like pharyngeal primordium into an open tube. Second, the so-called arcade cells, which are anterior to the pharynx primordium and will form the buccal cavity, become epithelial and produce a continuous epithelium linked to the epidermis anteriorly and to the developing pharynx posteriorly. Third, the apical surfaces of arcade cells constrict, thereby pulling the entire pharynx anteriorly. Surprisingly, none of the genes that have been mentioned so far affect pharynx organogenesis, except let-413 (Legois et al., 2000), epi-1 and lam-3 (W. Wadsworth, pers. comm.). Genetic analysis of pharynx formation should identify genes playing an important role in forming tubes or connecting cysts.

10. Patterning and morphogenesis of the egg-laying system

The egg-laying system of C. elegans comprises an external epithelial tube, the vulva, an internal epithelial tube, the uterus, sex muscles and neurons. Sex muscles act to open the vulva or squeeze the uterus. Historically, formation of the egg-laying system was the first to be studied by genetic analysis, and as a consequence is the best known (Horvitz and Sulston, 1980). Although its development is comparatively more complex than any other organ discussed so far, it remains much simpler than most vertebrate organs, and offers a unique system in which to dissect the signalling pathways involved in patterning and morphogenesis of epithelia. Formation of the egg-laying system involves the specification of different specialised epithelial cells, fusion of adjacent specialised cells, and finally connection of the uterus and vulva. The main cellular consequences of signalling events that pattern vulval and uterine cell fates are to induce specific short-range cell migrations and fusions. A detailed discussion of all these pathways is beyond the scope of this article (for earlier reviews, see Newman and Sternberg, 1996; Sternberg and Han, 1998; Delattre and Felix, 1999; Moghal and Sternberg, 1999; Fay and Han, 2000; Shemer and Podbilewicz, 2000). Instead, we provide an overview of vulval and uterine development and focus on some key events related to the connection of both organs.

10.1. The vulva, a stack of concentric rings

The vulva is built from 22 epithelial cells which, after their fusion by groups of two or four cells sharing the same fates (homotypic fusion), form a stack of seven concentric rings. These 22 cells originate from three ventral epidermal precursors, which are progressively selected in two steps among 12 cells called the Pn.p cells (Fig. 4A). In the first step, during the first larval stage, the Deformed-like Hox gene lin-39 confers the potential to become vulvar precursor cells (VPCs) to the central epidermal cells (P(3-8).p) (Clark et al., 1993; Salser et al., 1993; Ch’ng and Kenyon, 1999). In the second step, during the third larval stage, the anchor cell (AC) located in the overlying somatic gonad induces P(5-7).p to form the vulva (Kimble, 1981). The AC signal, an EGF-like signal which is transduced by the Ras/MAP kinase pathway also initiates vulval patterning. Normally P6.p adopts a fate called 1°, which is to generate the two innermost vulval rings that are closest to the uterus, while P5.p and P7.p adopt a fate called 2°, which results in the generation of the outermost vulval rings (Sternberg and Horvitz, 1986). Genetic analysis and cell ablation experiments have shown that the graded nature of the AC signal, in conjunction with lateral signalling between P(5-7).p cells mediated by the LIN-12/Notch receptor, ensures that only the cell closest to the AC adopt the 1° fate (Figs. 4A and 5) (Sternberg and Han, 1998; Moghal and Sternberg, 1999). Both pathways interact through a MAP kinase phosphatase,
called LIP-1, which in response to LIN-12 signalling down-regulates Ras/MAP kinase signalling in cells adopting the 2° fate (Berset et al., 2001). Each 1° or 2° precursor divides three times in a specific manner to generate vulval cells that fuse to form the seven rings with specific functions, called vulA to vulF (Fig. 4A). The AC again plays an important role, together with the Ras and a Wnt pathways, to pattern the 1° lineage (Wang and Sternberg, 2000). The process of cell fusion and ring formation transforms the linear array of cells present in the vulva primordium into a sequence of toroidal syncytia (Sharma-Kishore et al., 1999; Shemer et al., 2000).

10.2. The uterus, a set of toroids linked to the vulva by specialised cells

The uterus is initially composed of 61 cells, which fuse to form seven different cell types (Fig. 4B): distal to the vulva and on each side of it, it consists of a series of four toroids; centrally, it consists of three pairs of cells that link the vulva to the toroids, and of a fourth large H-shaped cell that allows the connection to be made. Uterine cells derive from three ventral (VU) and two dorsal (DU) uterine precursors. The AC plays three important roles in patterning the uterus. During the third larval stage, it induces six of the VU granddaughters that are closest to it to adopt the uterine π fate, while the six more distal granddaughters adopt the default ρ fate (Figs. 4B and 5). Normally, ρ descendants contribute to form the distal toroids and the uterine-vulval cells uv2-3, while π descendants contribute to form the centrally located cells (i.e. uv1 cells and the uterine-seam cell or utse). Specification of the uv1 fate involves a reciprocal EGF signal from vulF to induce uv1 differentiation among a subset of π descendants (Chang et al., 1999). The utse also plays an important function in connecting the uterine and vulval lumens. At the fourth larval stage, the AC partially penetrates the basal lamina that separates the growing uterus from the innermost vulval ring (vulF) and contacts both tissues (the mechanisms of this invasion are not known). Subsequently, the AC fuses with the utse, and its nucleus undergoes a migration towards the lateral extension of the utse. This leaves only a thin lamellar process above the vulva which can be broken by the first egg laid (Fig. 4A,B). The final connection between the vulva and the uterus requires that CeAJs physically link the innermost ring vulF to the central-most ventral uterine cells (uv1).

Patterning of the vulva and the uterus thus involves three general principles: cell fate potentials are restricted in a stepwise manner; one cell, the AC, has many properties of an ‘organiser’; and reciprocal signalling from the vulva back to the uterus ensures a linkage of both organs. The AC can be considered as an organiser, because it induces different fates within the vulva and the uterus, makes the connection between both tubes possible by invading the basal lamina, and finally fuses with a larger cell to clear the way for eggs.

10.3. Making the connection and co-ordinating vulva and uterus patterning

The LIN-12 signalling pathway and its effectors (Greenwald, 1998), play a key role in making the vulva-uterus connection. It specifies the π fate upon AC induction and thus allows the generation of the centrally located uterine cells that make the connection (utse and uv1 cells) (Newman et al., 1995). One of the final LIN-12 targets in π cells is the LIM-homeodomain protein LIN-11 (Newman et al., 1999). In lin-11 mutants, the π descendants that contribute to the utse are mis-specified and fail to fuse with the AC, resulting in the absence of a connection (Newman et al., 1999). Formation of the utse requires the activity of a second transcription factor, COG-2, a member of the SOX family which is expressed in π descendants. In cog-2 mutants, the π fate is normally specified but the AC fails to fuse with the utse (Hanna-Rose and Han, 1999), suggesting that COG-2 acts at a late step, possibly by directly controlling the fusion process.

Making the connection between the vulva and the uterus, and anchoring both organs to the lateral or surrounding epidermis, requires that vulval, uterine and other epidermal cells reach the appropriate developmental stage at the same time. Therefore, there must be genes that act to keep vulval, uterine and surrounding epidermis development in register. One such gene is lin-29, which encodes a zinc-finger protein and is a member of the heterochronic gene family (Bettinger et al., 1997; Newman et al., 2000). Mutations in heterochronic genes cause reiterated or precocious programs of development. In lin-29 mutants, there is no terminal differentiation of the lateral epidermal cells, abnormal specification of π cells which all fuse to form the utse, no vulval-uterine connection and abnormal eversion of the vulva. LIN-29 is expressed in the AC where it acts upstream of LAG-2, the LIN-12 ligand (Greenwald, 1998), in the VPCs and their descendants, in π cells where it acts upstream of LIN-12, and in lateral epidermal cells. Therefore lin-29 acts to coordinate development of all three tissues.

Continuous analysis of vulval and uterine development should reveal how signalling can induce cell shape changes and identify possible targets of the numerous transcription factors involved. Some key issues that should be addressed in the future include the determination of how vulval rings or uterine toroids arise. What are the mechanisms involved in short-range migrations that precede cell fusion and are they related to the process of ventral enclosure in the embryo? What drives homotypic or heterotypic cell fusion and are specific homophilic adhesion molecules involved? One strategy will come from a genetic analysis of mutants specifically affecting late aspects of vulva morphogenesis and invagination, resulting in a protruding (Eisenmann and Kim, 2000) or a collapsed vulva (Herman et al., 1999). For instance, three of the genes defined by mutations resulting in a collapsed squashed vulva (squ-1-8 genes) have been cloned and belong to the glycosylation pathway; they may...
modify cell-cell or cell-basal lamina interactions (Herman and Horvitz, 1999). In parallel, ongoing studies of vulval development in the nematodes *Pristionchus pacificus* and *Oscheius* (Eizinger and Sommer, 1997; Dichtel et al., 2001) may prompt a reinvestigation of old problems and lead to the discovery of non-suspected functions. A recent example comes from ablation studies performed a few years ago in the nematode *Oscheius*, which had shown that induction of the vulva requires two nested inductions from the gonad (Felix and Sternberg, 1997). More recently, some careful analysis of the 1° fate with new probes, showed that in *C. elegans* too the AC is necessary not only to induce vulva formation but also to pattern the 1° lineage (see Section 10) (Wang and Sternberg, 2000).

11. Conclusions and perspectives

For a long time, the paucity of useful markers and the lack of well-defined phenotypes have hampered genetic analysis of morphogenesis in *C. elegans*. This gap is now being filled as the field is turning some of its attention to issues of cell biology in development. In addition to the great collection of monoclonal antibodies against membrane markers developed by Francis and Waterston (1991), which has been the only source of markers for some time, a new set of GFP fusions to several proteins localised in different subcellular compartments of epithelial cells is now available. In addition, numerous mutations have been described that define phenotypes to score the different steps involved in a given cellular process. Systematic genomic approaches, such as those made available by the RNA interference technology, global expression profiling and automated protein interaction-mapping (Fraser et al., 2000; Gonczy et al., 2000; Reinke et al., 2000; Walhout et al., 2000), will further increase the number of useful probes. With this in hand, *C. elegans* should keep proving a powerful system to provide further insights into the mechanisms involved in connecting cells and tissues together. As outlined above, one of the real strengths of *C. elegans* lies in its genetic simplicity, which facilitates (sometimes eliminates) issues of genetic redundancy, as it is the case for laminin, integrin or intermediate filament genes. In other cases, and rather unexpectedly as for apical junctions (CeAJs), *C. elegans* offers the possibility to independently study functions which are intermingled in other species. One disadvantage of *C. elegans* to keep in mind is the small size of certain cells (particularly epidermal cells). In the near future, the field will certainly start integrating data from different areas, for instance examine how transcription control influences the expression of genes that orchestrate major morphogenetic changes. It will also begin exploring differential targeting in relation to lipid biology, the complexity of the cytoskeleton, and how higher order structures such as junctional complexes influence signalling. *Caenorhabditis elegans* occupies an ideal place to do so, between unicellular organisms amenable to genetic analysis (yeast) and more complex multicellular organisms where genetics is difficult and/or costly (vertebrates) but biochemical analysis is easy. A comparison between related processes in vertebrates, *Drosophila*, yeast as well as other nematodes should give clues about conserved functions.

Acknowledgements

We are grateful to Marie-Anne Félix and Laura McMahan for critical reading of the manuscript and useful discussions. We thank Jeff Hardin, John Gillette, Susan Mango, Joel Rothman and Bill Wadsworth for sharing unpublished data or communicating preprints. Our work is supported by grants from the EEC-TMR program, the Fondation pour la Recherche Médicale to GM, the Association pour la Recherche contre le Cancer and the Ministère de la Recherche-ACI program to ML and by funds from the CNRS, INSERM and Hôpital Universitaire de Strasbourg.

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