Skin tight: cell adhesion in the epidermis of Caenorhabditis elegans
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The powerful genetics, genomics and microscopy tools available for C. elegans make it well suited to studying how epithelial cells adhere to one another and the extracellular matrix, and how the integrated, simultaneous activities of multiple cell adhesion complexes function to shape an organism. Recent studies using forward and reverse genetics have shed light on how phylogenetically conserved cell adhesion complexes, such as the cadherin/catenin complex, claudins, the Discs large complex and hemidesmosome-like attachment structures, regulate epithelial cell adhesion, providing new insights into conserved cell adhesion mechanisms in higher eukaryotes.

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Abbreviations
aPKC atypical protein kinase C
CCC cadherin/catenin complex
CeAJ C. elegans apical junction
ECM extracellular matrix
FO fibrous organelle
GFP green fluorescent protein
Hmp humpback
Hmr hammerhead
IF intermediate filament
MAGUK membrane-associated guanylate kinase
Mua muscle attachment
RNAi RNA interference
ZP zona pellucida

Introduction: C. elegans as a model system for studying epithelial adhesion
Epithelia play a key role in orchestrating the overall architecture of animals. The dynamic adhesive properties of epithelia are especially important in embryos, where adhesion complexes are regulated at an exquisitely refined level to achieve the changes in form associated with morphogenesis. Because of its simplicity, optical transparency, stereotypical cell lineage and suitability for both forward and reverse genetics, the C. elegans embryo is a convenient system for analyzing dynamic changes in epithelial cell adhesion in vivo. Analysis of the C. elegans genome indicates that it has many adhesion proteins in common with other organisms [1*,2,3,4*]. Moreover, unlike Drosophila, which has no intermediate filaments (IFs), C. elegans expresses a number of IFs ([5,6*,7*], allowing powerful genetic approaches to the study of IF-containing adhesion complexes similar to those in vertebrates. Recent reviews have summarized studies of all of the major adhesion complexes in C. elegans [1*,3,8*], including muscle dense bodies, which attach muscle cells to the basal lamina. Dense bodies contain many of the proteins found at focal contacts in vertebrates, including integrins and their associated proteins, such as integrin-linked kinase (ILK; see the article by Grashoff et al. in this issue). Here, we focus primarily on the embryonic epidermis, or hypodermis, of C. elegans as a model for understanding how multiple adhesion complexes are integrated in epithelial cells during embryonic development.

Unlike in Drosophila and vertebrates, there is a single, apically situated electron-dense region connecting epithelial cells in C. elegans, the C. elegans apical junction (CeAJ; see Figure 1a). Although it is currently unclear exactly where specific molecular complexes localize relative to this single electron density, C. elegans epithelia possess several multiprotein complexes similar to those in other organisms (Figure 1b; see [8**,9] for other reviews). These include the following: an adherens junctional subdomain, which contains the cadherin/catenin complex (CCC); a more basal junctional domain containing the C. elegans homologue of Discs large and its binding partner, AJM-1 (the DLG-1/AJM-1 subdomain); and an apical region, which, in some tissues, contains transmembrane proteins of the Crumbs family, the C. elegans PAR-3/PAR-6/atypical protein kinase C (aPKC) complex, and proteins that stabilize cytoskeletal attachments to the apical membrane (Figure 1b). In addition, epidermal cells in C. elegans also possess hemidesmosome-like attachment structures or ‘fibrous organelles’ (FOs), which couple IFs to the apical and basal surfaces of epidermal cells (Figure 1b).

The cadherin/catenin complex: epithelial sealing and actin anchorage
In C. elegans, the only classical cadherin is encoded by the hmr-1 locus [10], which encodes two proteins: a longer, neuronal-specific isoform, HMR-1B, and a shorter
isoform, HMR-1A, which is expressed in epithelia [11].

The other components of the C. elegans CCC are HMP-2/β-catenin, HMP-1/α-catenin, and JAC-1/p120ctn (Figure 1b), whose conserved functional domains and protein–protein interactions indicate that they function in a manner very similar to their vertebrate and Drosophila counterparts [12,13**].

The C. elegans CCC was first identified on the basis of its essential role during morphogenesis [12,14]. During the process of ventral enclosure, the free edges of the epidermis migrate ventrally and rapidly seal as filopodia make contact at the ventral midline [14], a process that bears striking similarities to both dorsal closure in Drosophila and junction formation in vertebrate cells [15–17]. hmr-1/cadherin null mutants and embryos depleted of both maternal and zygotic hmp-2/β-catenin and hmp-1/α-catenin function often exhibit a ‘hammerhead’ (Hmr) phenotype, as anterior ventral epidermal cells fail to make such midline contacts [12,14].

The CCC is also required for the next step in morphogenesis, elongation. As ventral enclosure completes, circumferentially oriented actin bundles in the epidermis become anchored into the CCC, and are thought to help distribute the forces that elongate the embryo, a process
that involves actomyosin-mediated cell shape changes [18]. In embryos lacking zygotic hmp-1/α-catenin or hmp-2/β-catenin activity, circumferential actin filaments consistently detach from the CCC in epidermal cells, as dorsal epidermal cells are thrown into folds (the ‘humpback’, or Hmp, phenotype) [12].

Given the clear role that the core components of the CCC play in regulating morphogenesis in C. elegans, it is surprising that RNA interference (RNAi) directed against the other major component of the CCC, JAC-1/p120ctn, does not cause obvious defects [13**]. However, like its vertebrate counterpart [19], JAC-1/p120ctn does modulate attachment of actin filament bundles to the CCC, as deduced from its ability to enhance elongation defects in weak hmp-1/α-catenin mutants [13**]. Similar results have been reported in Drosophila: loss of p120ctn has little or no phenotypic effect on its own but enhances weak mutations in CCC components [20] and a shotgun/DE-cadherin deletion construct lacking the juxtamembrane p120ctn binding motif can fully rescue shotgun mutants [21]. Since vertebrate p120ctn regulates cadherin turnover and recruitment [22], it is possible that in rapidly developing invertebrate embryos such functions are dispensable. Indeed, C. elegans expresses many other proteins that localize to adherens junctions in vertebrates, yet these proteins are of minor functional importance [8**].

A novel associate of the CCC is a founding member of a new class of claudin superfamily proteins, VAB-9. bona fide claudins are transmembrane proteins that engage in hetero- or homophilic interactions; most claudins are components of occluding junctions in both vertebrates [23,24] and Drosophila [25,26], and are not associated with the CCC (see [27] for a comparison of organizational differences in occluding junctions in invertebrates and vertebrates). In contrast, VAB-9 colocalizes with the CCC and requires HMR-1/cadherin function for correct localization [28**]. vab-9 nulls do not exhibit embryonic lethality, but display elongation and body shape defects and abnormal circumferential actin filament depositions in invertebrate embryos such functions are dispensable. Indeed, C. elegans expresses many other proteins that localize to adherens junctions in vertebrates, yet these proteins are of minor functional importance [8**].

Although the CCC is clearly important for morphogenesis in C. elegans, it is puzzling that complete removal of its components does not abrogate cell–cell adhesion in the early embryo. C. elegans must presumably have other, functionally redundant adhesive complexes that act in the early embryo. Although C. elegans does not have a clear nectin homologue [1*], additional possibilities include other cadherin-like proteins [10] and the L1CAM homologue, LAD-1 [29]. LAD-1 localizes to cell–cell contacts in the early embryo and basolaterally in epithelia, [29], so it could act in parallel with the CCC in the early embryo.

The DLG-1/AJM-1 complex: effectors in search of a partner

The basal domain of the CeAJ is characterized by the presence of DLG-1/Discs large and AJM-1, which colocalize to the electron-dense junctional region [30–33]. Like its orthologues, the Drosophila tumor suppressor Discs-large and vertebrate SAP97, DLG-1 is a member of the membrane-associated guanylate kinase (MAGUK) family. AJM-1 is a novel protein whose major predicted structural feature is a large coiled-coil domain [32]. Loss of either DLG-1 or AJM-1 leads to similar phenotypes: embryos arrest at about the two-fold stage of elongation, and contain swollen cells that may be necrotic [30,32]. TEM reveals that the integrity of the electron-dense region of the CeAJ is affected in both mutants: in ajm-1 mutants, local, bubble-like separations form in this region [32], whereas removal of dlg-1 function leads to a complete loss of electron-dense material [30,33]. DLG-1 is essential for the proper localization of AJM-1 — loss of DLG-1 function results in lateral redistribution of AJM-1 into puncta [30–33]. DLG-1 is likely to be a direct regulator of AJM-1, since the proteins interact in a yeast two-hybrid assay [32]. Recent work suggests that AJM-1 may in turn play a reciprocal role in maintaining the localization of DLG-1 in the intestine [34*]. In the epidermis of ajm-1 mutants, DLG-1 localization has been reported to be normal [30,32,33], although finer-resolution imaging may reveal that similar interactions occur there.

Several studies have characterized the regulation of the DLG-1/AJM-1 complex. The LAP (leucine-rich repeat and PDZ) protein LET-413, a homologue of Drosophila Scribble, localizes to the basolateral membranes of epithelia through its leucine-rich repeats [35*]. Loss of let-413 function leads to basal spreading of DLG-1/AJM-1 along the lateral membrane, resulting in discontinuous stretches of junctional material [30,32,36]. Like its Drosophila counterpart, LET-413 probably regulates apicobasal polarity more generally, at least in the intestine, since its removal also perturbs more apically situated proteins [37*]. The mechanism by which LET-413 acts to maintain apicobasal polarity is currently unknown.

In addition to the role of the DLG/AJM-1 complex in stabilizing the electron-dense region of the CeAJ, double mutant analysis indicates that it may also play a role in regulating adhesion. For example, vab-9/dlg-1 and vab-9/ajm-1 mutants frequently arrest with a ruptured epidermis, a phenotype exhibited in neither of the single mutants [28**]. Similarly, simultaneous removal of DLG-1 function along with those of the apical protein
ERM-1 or CCC components results in synergistic defects in the intestine [34**,38**]. These results suggest that the DLG-1/AJM-1 complex associates with an adhesive function that is at least partially functionally redundant with the CCC. What transmembrane protein might be responsible? One possibility is that DLG-1 interacts with C. elegans claudins. One C. elegans claudin homologue, CLC-1, co-localizes with AJM-1 in the pharynx, and its removal via RNAi leads to compromised epithelial barrier function in adults [39**], consistent with this possibility. Other candidate DLG-1 interactors include LAD-1, which contains a PDZ binding motif at its C terminus [29] that could potentially interact with the PDZ motifs of DLG-1. Further studies will be necessary to determine whether CLC proteins, LAD-1 or other transmembrane proteins interact with the DLG-1/AJM-1 to regulate junctional integrity.

**The apical domain: not all epithelia are created equal**

The tight junctions of vertebrates and the subapical region (SAR) of Drosophila contain two apical protein complexes that are key regulators of apical–basal polarity: first, the PAR-3/PAR-6/aPKC complex, and second, the Crumbs/Stardust/Discs-lost complex, which associates with β1H-spectrin (reviewed in [9,40–43]). Some epithelial cells in C. elegans, including those of the alimentary canal [31] and spermatheca [44**], exhibit a similar apical region, which contains PAR-3, PAR-6 and PKC-3/aPKC, as well as the Crumbs homologue CRB-1 (Figure 1b). Although there is currently no evidence that the PAR/aPKC complex is crucial in most tissues where it is expressed, a functional role for the PAR/aPKC complex in epithelia was recently reported for the spermatheca, where it regulates polarity, including the positioning of the DLG-1/AJM-1 complex [44**]. Similarly, neither the C. elegans Crumbs homologue (crb-1) nor a second Crumbs-like gene (crf-1/earl-20) is essential for epithelial development in C. elegans [31]; however, EAT-20 is required in the pharynx for proper pumping [45], and crb-1 may interact genetically with let-413 and hmp-1/α-catenin in a complex manner in the intestine [34*]. Taken together, such studies do not make it clear how these apical protein complexes function in most C. elegans epithelia. In addition to the PAR/aPKC and Crumbs complexes, the intestine and pharynx express proteins that probably stabilize the apical membrane by linking it to the cytoskeleton. These include the FERM domain protein ERM-1 and SMA-1/β1H-spectrin; removal of the function of either results in tubulogenesis defects [34**,38**,46**].

Surprisingly, in cuticular epithelia, such as the epidermis and the regions of the pharynx and anus that have cuticle associated with them, the PAR/aPKC and Crumbs complexes are absent ([31]; Hardin and Lockwood, unpublished). The one distinguishing feature of epithelia in C. elegans that lack these proteins is their attachment to the cuticle, which could provide a functional substitute. Cuticular epithelia in Drosophila require several proteins containing domains originally identified in proteins of the mammalian zona pellucida (ZP). These ZP-domain-containing proteins are required for proper organization, including Dumpy and Piopio (reviewed in [47]); since the C. elegans genome encodes several ZP domain proteins (Hardin and Lockwood, unpublished), it is possible that these proteins are also important in C. elegans. Two apical components retained in the epidermis are SMA-1/β1H-spectrin and SPC-1/α-spectrin; loss of SMA-1 or SPC-1 function perturbs elongation of the embryo and results in disruption of the apical actin cytoskeleton [48,49]; V Praitis, personal communication).

**Fibrous organelles: mechanical coupling between tissues**

In addition to its role in morphogenesis and as an epithelial barrier, the C. elegans epidermis is crucial for the efficient mechanical transduction of muscle contraction to the cuticle, which occurs through epithelial FOs. FOs, which are structurally similar to the type I hemidesmosomes of vertebrate epidermal cells (reviewed in [8**,50]), attach IFs basally to the underlying basement membrane and associated muscle, and apically to the cuticle (Figure 2). C. elegans has eleven genes that encode cytoplasmic IFs; at least three (A2, A3, B1) are essential components of FOs [6*,7*].

FOs do not contain integrins or a BPAG2 homologue, but instead have other transmembrane proteins that probably form analogous linkages. Apically, MUA-3 and MUP-4, which both contain numerous EGF repeats, a von Willebrand factor type A domain, and two sea-urchin-enterokinase–agrin modules ([51,52]) aid attachment to the cuticle. Basally, myotactin/LET-805, a novel transmembrane protein with at least 32 extracellular fibronectin type III repeats [53], aids attachment of FOs to the basal lamina. The importance of attachment of FOs to the basal lamina is further suggested by the phenotype of embryos lacking laminin αB function, in which the epidermis separates from muscle [54*]. The plectin homologue, VAB-10A, is an important component of both apical and basal FOs [55**]. By analogy to vertebrate plectins, VAB-10A probably links transmembrane receptors and IFs. The vab-10 locus also encodes a second isoform, VAB-10B, which is similar to vertebrate plakins, known actin/microtubule cross-linking proteins; VAB-10B/plakin probably makes an important contribution to the mechanical integrity of the epidermis by regulating the distance between its apical and basal surfaces [55**]. Finally, the basally localized ankyrin-repeat protein, VAB-19/Kank, which requires myotactin function for its localization, appears to regulate attachment structures [56**]. vab-19 mutants have elongation defects, and, interestingly, loss of the function of SMA-1/β1H-spectrin...
studying the integration of epithelial adhesion complexes, which various epithelial adhesion complexes depend on mechanistically, in an intact organism. Despite recent progress in exploring how the functions of multiple epithelial adhesion complexes are integrated, both functionally and mechanically, in an intact organism. Despite recent progress, many questions remain. The first is the extent to which various epithelial adhesion complexes depend on one another for their formation. In some cases, there is clear independence; for example, the CCC and DLG-1/AJM-1 domains appear to form independently of one another [28**,30,32], even though they act synergistically in epithelial adhesion [28**,34,38**]. On the other hand, intact FOs are needed for the proper patterning and function of actin filaments in the epidermis during embryonic elongation [55**,56**], suggesting a potentially important role for IF attachments in regulating the actin cytoskeleton. A second, related question is how epithelial adhesion complexes engage in mechanical cross-talk. For example, epidermal elongation requires SMA-1/B1-spectrin apical to the adherens junctional domain [49], actin anchorage at the CCC [12], junctional integrity mediated by the DLG-1/AJM-1 complex [30,32,33], and linkages between FOs and the IF cytoskeleton [55**,56**]. Using the genetic and molecular tools available in C. elegans it should be possible to explore how each of these multiprotein complexes contributes to the completion of complex tasks, including shaping an organism.

**Conclusions: C. elegans as a system for studying the integration of epithelial adhesion**

Epithelial cell adhesion in C. elegans is strikingly similar to that in higher organisms, making C. elegans a useful system for exploring how the functions of multiple epithelial adhesion complexes are integrated, both functionally and mechanically, in an intact organism. Despite recent progress, many questions remain. The first is the extent to which various epithelial adhesion complexes depend on one another for their formation. In some cases, there is clear independence; for example, the CCC and DLG-1/AJM-1 domains appear to form independently of one another [28**,30,32], even though they act synergistically in epithelial adhesion [28**,34,38**]. On the other hand, intact FOs are needed for the proper patterning and function of actin filaments in the epidermis during embryonic elongation [55**,56**], suggesting a potentially important role for IF attachments in regulating the actin cytoskeleton. A second, related question is how epithelial adhesion complexes engage in mechanical cross-talk. For example, epidermal elongation requires SMA-1/B1-spectrin apical to the adherens junctional domain [49], actin anchorage at the CCC [12], junctional integrity mediated by the DLG-1/AJM-1 complex [30,32,33], and linkages between FOs and the IF cytoskeleton [55**,56**]. Using the genetic and molecular tools available in C. elegans it should be possible to explore how each of these multiprotein complexes contributes to the completion of complex tasks, including shaping an organism.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


This short note clarifies some recent confusion in the literature regarding this class of receptors in C. elegans.


The authors show that the muscle attachment (Mua) mutant, mua-6, corresponds to the ifa-2 gene, that MUA-6/IFA-2 localizes to attachment structures/FOs, and that it is required post-embryonically for muscle attachment.


This paper shows that both isoforms of the intermediate filament protein IFB-1 localize to attachment structures in the epidermis. The authors demonstrate that they play isoform-specific roles in morphogenesis and...
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muscle attachment. Loss of the intermediate filament protein IFA-3 results in a similar phenotype, suggesting that the two proteins are the major components of attachment structures.


A major review of cell adhesion complexes in C. elegans, encompassing both cell–cell and cell–ECM adhesion in most major tissues in C. elegans.


The authors report that the p120 catenin, JAC-1, localizes to the adherens junctional domain using a GFP construct, and that JAC-1 interacts physically with the juxtamembrane domain of HMR-1 in a yeast two-hybrid assay. A role for JAC-1 in cadherin function is indicated on the basis of the enhancement of a weak HMP-1 allele by jac-1(RNAi).


A recent review of morphogenetic movements in the C. elegans embryo, focusing on events in the epidermis.


This paper reports the cloning and characterization of a new claudin-like protein, VAB-9, which localizes to the adherens junctional domain and modulates actin dynamics at junctions. Analysis of vab-9;dlg-1/ajm-1 double mutants suggests the existence of redundant adhesive functions between the adherens junctional and DLG-1/AJM domains.


33. Firestein BL, Rongo C: DLG-1 is a MAGUK similar to SAP97 and is required for adherens junction function. Mol Biol Cell 2001, 12:3465-3475.


The authors use double and triple loss-of-function experiments to determine interactions between molecular complexes in the intestine. They specifically find synergy between dlg-1/hmp-1 and erm-1/hmp-1 loss-of-function during gut formation. The authors also implicate AJM-1 and HMP-1/CRB-1 in DLG-1 localization.


Structure/function experiments with LET-413/Scribble demonstrate that the LRR domain is sufficient for basolateral localization. The authors also report that the PDZ domain of LET-413 is not required for its function.


Using the MH53 antibody, which specifically recognizes IFB-2, a component of the intestinal web, the authors find that LET-413/Scribble is essential for maintaining the apical distribution of the intestinal web.


The authors show that the ezrin/radixin/moesin family protein ERM-1 localizes with F-actin at the apical cell cortex in the alimentary canal. ERM-1 depletion results in collapse of the intestinal lumen and loss of apical actin without wholesale disruption of epithelial polarization; simultaneous removal of CCC components results in synergistic intestinal defects.


The authors demonstrate that CLC-1, a claudin, localizes with the DLG-1/AJM-1 complex in the pharynx, where it functions in paracellular...
permeability. They also show that a CLC-1::GFP construct localizes to the lateral membranes of the epidermal seam cells.


This paper reports the localization of VAB-19, an ankyrin repeat protein, to attachment structures in the epidermis. Loss of vab-19 function leads to mislocalization of the attachment structures and disorganization of circumferential actin bundles. The actin disorganization phenotype can be partially suppressed by reduction of function of the βH-spectrin sma-1.