Molecular and functional analysis of apical junction formation in the gut epithelium of Caenorhabditis elegans

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This paper is dedicated to the memory of Christoph Segbert, an outstanding young scientist

Abstract

The Caenorhabditis elegans intestine is a simple and accessible model system to analyze the mechanism of junction assembly. In comparison to Drosophila and vertebrates, the C. elegans apical junction is remarkable because a single electron-dense structure is implicated in complex processes such as epithelial tightness, vectorial transport and cell adhesion. Here we present evidence in support of a heterogeneous molecular assembly of junctional proteins found in Drosophila and vertebrate epithelia associated with different junctions or regions of the plasma membrane. In addition, we show that molecularly diverse complexes participate in different aspects of epithelial maturation in the C. elegans intestine. DLG-1 (Discs large) acts synergistically with the catenin–cadherin complex (HMP-1–HMP-2–HMR-1) and the Ezrin-Radixin-Moesin homolog (ERM-1) to ensure tissue integrity of the intestinal tube. The correct localization of DLG-1 itself depends on AJM-1, a coiled-coil protein. Double depletion of HMP-1 (α-catenin) and LET-413 (C. elegans homolog of Drosophila Scribble) suggests that the catenin–cadherin complex is epistatic to LET-413, while additional depletion of subapically expressed CRB-1 (Crumbs) emphasizes a role of CRB-1 concerning apical junction formation in the C. elegans intestine.

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Introduction

In epithelial cells, the distribution of intercellular junctions and other protein complexes along the lateral plasma membrane manifests their structural polarity. However, the evolutionary solution to the requirements of epithelial tightness, vectorial transport and cell adhesion differs between vertebrates, Drosophila and Caenorhabditis elegans (Knust and Bossinger, 2002; Nelson, 2003; Tepass et al., 2001; Tsukita et al., 2001). In vertebrates, the most apicolateral membrane domain includes the tight junction, followed by the zonula adherens (ZA) and, more basally, by the desmosomes. In many epithelia of Drosophila, the lateral membrane domain is subdivided by the subapical region, followed by the ZA and, more basally, by the septate junction. In C. elegans, only one type of intercellular junction, the C. elegans apical junction (CeAJ, McMahon et al., 2001), has been described at the ultrastructural level (Michaux et al., 2001).

In the Drosophila embryo, the formation of the first identifiable junction (the ZA) is a stepwise process, in which first spot adherence junctions are recruited to two separate structures, the apical and basal junction, and later fuse during gastrulation to form a continuous belt (Knust, 2000; Müller, 2000; Tepass, 2002). Elimination of core components of the ZA (α-, β-catenin and E-cadherin) has dramatic effects on polarization, ZA formation and epithelial structure in Drosophila (Cox et al., 1996; Müller and Wieschaus, 1996; Tepass et al., 1996; Uemura et al., 1996). In C. elegans, however, mutations in the catenin–cadherin complex (HMP-1–HMP-2–HMR-1) only cause defects in epithelial sheet sealing, but do not affect cell polarity and...
adhesion in general (Costa et al., 1998; Raich et al., 1999). These data suggest the existence of additional cell adhesion molecules in C. elegans.

The correct positioning and the integrity of the ZA in Drosophila further depend on the formation of specialized protein complexes apical and basal to the ZA itself (Johnson and Wodarz, 2003; Müller, 2003). In the subapical region, one complex comprises the transmembrane protein Crumbs (Crb), which binds to the MAGUK protein Stardust (Sdt), which in turn recruits the PDZ-containing protein Discs lost (Dlt) into the complex (Bachmann et al., 2001; Hong et al., 2001; Klebes and Knust, 2000). Embryos lacking either Crb or Sdt fail to establish a continuous adherens belt in the epidermis, the cells loose their polarity and undergo apoptosis. Injection of dsRNA (RNAi, Fire et al., 1998) against crb-1 in C. elegans leads to embryos that develop normally and show the typical CeAJ pattern (Bosinger et al., 2001). This suggests that the genetic control of junction formation differs in flies and worms.

The second well-characterized protein complex in the subapical region of Drosophila epithelia is formed by the PDZ proteins Bazooka (PAR-3), DmPar-6 and an atypical protein kinase C (DaPKC). Drosophila embryos that are completely devoid of Bazooka or DaPKC fail to establish plasma membrane polarity after cellularization, and consequently the ZA fails to form (Kuchinke et al., 1998; Wodarz et al., 2000). Embryos deficient of DmPar-6 cellularize normally but do not assemble a functional ZA (Petronczki and Knoblich, 2001). In C. elegans, proteins of the PAR-3–PAR-6–PKC-3 complex localize to the apical membrane domain (Bosinger et al., 2001; Leung et al., 1999; McMahon et al., 2001) but a possible role in epithelial development is still elusive because this complex is involved in setting up the polarity in the one-cell embryo (Pellettier and Seydoux, 2002).

In Drosophila, a third group of genes influences ZA formation from the baso-lateral plasma membrane. The tumor-suppressor genes scrib (scribble), dlg (discs large) and lethal giant larvae (lgl) are mutually dependent on each other for proper lateral localization. They contain multiple protein–protein interaction motifs (Wodarz, 2000): Scrib, a multi-PDZ and leucine-rich repeat protein; Dlg, a MAGUK protein; and Lgl, a protein that contains WD-40 repeats. Failure to establish appropriate interactions results in the expansion of proteins of the subapical region (e.g. Crumbs) into more lateral positions. As a consequence, the ZA is not formed and the epithelium becomes multilayered (Bilder et al., 2000). The tumor suppressor genes also have homologs in C. elegans, suggesting a conserved signaling pathway. For example, let-413 encodes the homolog of the Drosophila Scrib (Legouis et al., 2000). During embryogenesis, LET-413 becomes restricted to the basolateral membranes of epithelial cells. In let-413 embryos, an extended CeAJ forms, suggesting that the function of LET-413 is to correctly position the CeAJ (Legouis et al., 2000; McMahon et al., 2001). After depletion of C. elegans DLG-1, embryos fail to form a continuous belt of junction-associated antigens and the majority of cell–cell contacts are either devoid or faint of electron-dense structures (Bosinger et al., 2001; McMahon et al., 2001). DLG-1 physically interacts with AJM-1, a novel coiled-coil protein, and its conserved N-terminus directs its localization to the CeAJ (Firestein and Rongo, 2001; Köppen et al., 2001).

In this study, we present evidence in support of a heterogeneous assembly of different junctional complexes within the CeAJ of the intestine at the confocal microscopy level. Furthermore, we demonstrate complex interactions of distinct protein activities that are involved in the assembly of the apical junction and the maintenance of tissue integrity.

Materials and methods

Strains and alleles

C. elegans was maintained essentially as described by Brenner (1974). Bristol strain N2 was used as the WT strain. The following mutations were used: LGI, hmr-1(zu389); LGV, hmp-1(zu278), let-413(s128); and LGX, ajm-1(ok160).

Protein depletion using RNA-mediated interference

The following cDNA clones were obtained from Y. Kohara (Gene Network Lab, NIG, Mishima 411, Japan): for ajm-1, yk445g1 (2.6 kB) and yk531e7 (4.3 kB); for dlg-1, yk25e5 (2.6 kB) and yk128b7 (2.4 kB); for hmp-1, yk271a9 (3.3 kB) and yk675e4 (3.3 kB); for hmp-2, yk493f7 (2.4 kB) and yk614f10 (1.3 kB); for hmr-1, yk662b10 (3.6 kB); for let-413, yk126a10 (2.6 kB) and yk524b7 (3.1 kB); for crb-1, yk39b11 (2.9 kB) and yk313a11 (3.9 kB, no 3’ sequence available); and for C01B7.4 (sdt-1, the C. elegans homolog of Drosophila stardust). The 5’- and 3’-sequences are specific to the genes being tested, as determined by blast search and worm base (http://www.wormbase.org/). Phagemid DNA was prepared as described in the Stratagene ExAssist protocol. Sense and antisense strands of RNA were synthesized using the RibomAX Large-scale RNA in vitro transcription kit (Promega, Inc.) and annealed before injection at 0.5 mg/ml (Fire et al., 1998). The quality of RNAs was checked on a formaldehyde-containing (1.8%) agarose gel. Microinjections into the gonads of hermaphrodites were observed with Nomarski optics using a Leica DM IRBE inverted microscope equipped with a 40× PL Fluotar objective (NA 1.3). In general, one syncytial gonad of each of 15–20 young adult hermaphrodites was injected, and each individual was placed on a separate agar plate seeded with Escherichia coli (after 18–20 h). Embryos produced by the injected worms were collected from agar plates (after 24–60 h) and prepared for immunostaining (see below).
Antibody production

A bacterial 6xHis fusion expression construct was made by cloning a 1.17 kb fragment, corresponding amino acids 204–593 (PDZ domain 1–3 of DLG-1), into the BamHI and KpnI sites of the pQE-30 vector (Qiagen). This fragment was generated by PCR of the yk435h12 cDNA (a kind gift of Y. Kohara; EMBL accession number AJ295228) using oligonucleotides containing a KpnI and BamHI site (3’ primer: AGGGATCC GTCTTTGAGAAGGGTCAC) and a KpnI site (3’ primer: ATGGTACCCTCTTGTTGGTCTGACTG). The fusion protein was expressed in E. coli strain M15 (pREP4), purified using Ni-NTA agarose columns (Qiagen) and injected into one rabbit and one rat by Eurogentec. The fusion protein was expressed in E. coli (pREP4), purified using Ni-NTA agarose columns (Qiagen) and injected into one rabbit and one rat by Eurogentec. The specificity of the antiserum was tested by staining and injected into one rabbit and one rat by Eurogentec. The specificity of the antiserum was tested by staining and injected into one rabbit and one rat by Eurogentec. Antibody production by the freeze-crack method (Strome and Wood, 1983) and incubated at 4°C overnight (or for 2 h at room temperature or for 1 h at 37°C) with primary antibodies in TBT (plus 1% BSA and 1% nonfat dry milk powder), washed three times for 10 min each with TBT [Tris-buffered saline (25 mM Tris), plus 0.1% Tween 20], incubated at 4°C overnight (or for 2 h at room temperature or for 1 h at 37°C) with primary antibodies in TBT (plus 1% BSA and 1% nonfat dry milk powder), washed three times for 10 min each with TBT at room temperature, and finally incubated at room temperature for 1–3 h (or for 1 h at 37°C) with secondary, Cy-2 conjugated donkey α-goat, Cy-2 conjugated donkey α-mouse, Cy-3 conjugated donkey α-rabbit antibody and/or Cy-5 conjugated donkey α-guinea pig (all from Jackson ImmunoResearch Laboratories) in TBT (plus 1% BSA and 1% nonfat dry milk powder). Finally, slides were washed three times for 10 min each in TBT and mounted in Mowiol containing DABCO (1,4-diazabicyclo (2.2.2) octane) as antifading reagents. The following primary antibodies were used at the dilutions indicated: mabMH27 (α-AJM-1, mouse, at 1:1500), mabMH33 (α-IFB-2, mouse, at 1:150), α-DLG-1 (rabbit, at 1:400; see above), α-HMP-1 (mouse, at 1:10), α-HMP-2 (goat, at 1:10; Santa Cruz Biotechnologies) and α-IFC-2 (guinea pig, at 1:100). The different stages of morphogenesis become apparent by their shape and elongation of the embryo: lima bean (epiboly), comma (end of epiboly), tadpole (1.5 fold), plum (1.75 fold), loop (twofold) and pretzel (fourfold). Immuno-FL analysis of embryos was performed on a Leica TCS NT confocal microscope (100× PL Fluotar oil-immersion objective, NA 1.3) using as excitation wavelength(s) 488 nm and/or 520 nm. Final image layout with a resolution of 300 dpi was performed on a Macintosh G4 computer, using Photoshop 7 (Adobe) and Canvas 9 (Deneba) software.

Numbers of experiments and recorded embryos

Each described experiment was repeated at least three times. The recorded embryos represent projections of Z-series containing 15–30 sections (approximately 1 section per 0.5 μm). In each experiment, each of the presented morphogenesis stages were at least recorded five times.

Results

At the EM level, the CeAJ in the gut appears as a single electron-dense structure (Figs. 1A,A’) and its invariable pattern (Fig. 1B) ensures correct attachment of epithelial cells to form the intestinal tube (Fig. 2A). At the immuno-EM level, only AJM-1 has been localized to the CeAJ (Köppen et al., 2001). In the first part, we will describe the molecular composition of the CeAJ in more detail at the immuno-FL level using antibodies against several proteins, which localize to different units of the CeAJ or regions of the plasma membrane. We will start our analysis at the basal unit of the CeAJ moving toward the apical cytocortex of the intestine. In the second part, we investigate the function and genetic interactions of different junctional complexes and molecules concerning the maintenance of tissue integrity and formation of the apical junction in the C. elegans gut epithelium.

Staining of DLG-1, the homolog of Drosophila Discs large and a component of the septate junction, completely colocalizes with AJM-1 in the basal unit of the CeAJ. We obtained similar results using an antibody against IFC-2, belonging to the group of C. elegans intermediate filaments (Dodemont et al., 1994; Karabinos et al., 2001, 2002), which normally anchor to desmosomes in vertebrates (Figs. 1B,C,C’; data not shown). The catenin–cadherin complex (α-catenin–β-catenin–E-cadherin; HMP-1–HMP-2–HMR-1) is found in immediate vicinity to DLG-1 in the apical unit of the CeAJ, but immuno-FLs seem to overlap partially (Figs. 1D,D’; data not shown). In contrast, α-CRB-1 (Crumbs) and α-DLG-1 staining is clearly separated, while CRB-1 and HMP-1 are found nearest to each other (Figs. 1E,E’; data not shown). In Drosophila immuno-EM has clearly shown that the catenin–cadherin complex localizes to the ZA, while Crb is a component of the subapical region (Tepass, 1996). This level of resolution is difficult to achieve in C. elegans because most antibodies have failed to recognize their antigens at the immuno-EM level so far (e.g. α-DLG-1 or α-HMP-1; G. Borgonie personal communication). The mabMH33 recognizes another intermediate filament protein called IFB-2 (Karabinos et al., 2001; J. McGhee personal communication, manuscript in preparation) that, in contrast to IFC-2, localizes to the apical
cytocortex of the gut epithelium. α-IFB-2 staining clearly overlaps subapically with CRB-1 and seems not to colocalize with DLG-1 in the basal unit of the CeAJ (Figs. 1F, F′). We observed a similar pattern using FITC-Phalloidin to stain the actin cytoskeleton (data not shown).

Taken together, our results reveal the distinct localization of molecularly diverse complexes to the CeAJ at the immuno-FL level (see also Fig. 5), while at the EM level the CeAJ appears as a single electron dense structure in the gut epithelium and the hypodermis (Köppen et al., 2001; Legouis et al., 2000; Leung et al., 1999; McMahon et al., 2001). A detailed analysis of the pharyngeal epithelium has now identified a specialized cell-to-cell junctional region apical to the CeAJ (Asano et al., 2003), demonstrating that heterogeneity also exists at the EM level, at least in some tissues of C. elegans.

The correct adhesion of gut epithelial cells is a prerequisite to ensure the integrity of the intestinal tube. This is demonstrated by α-IFB-2 staining, which sharply delineates the course of the future gut lumen (Fig. 2A). We employed RNAi (Fire et al., 1998) to investigate the role of junctional complexes concerning cell adhesion and the formation of

Fig. 1. Molecular subdivision of the CeAJ in the embryonic gut epithelium of C. elegans. (A, A′) Electron micrograph (cross section) and a magnification showing the ultrastructure of the apical junction (arrowhead) in the C. elegans gut epithelium (asterisk marks the lumen while arrow points to the basement membrane surrounding the basal surface). (B, C) α-AJM-1 (red) and α-DLG-1 (green) antibodies (ABs) colocalize (yellow) to the basal unit of the CeAJ [single staining against AJM-1 and DLG-1 are shown in left corners (B), respectively]. (D) α-HMP-1 and α-DLG-1 staining show little overlap (orange color), while (E) α-CRB-1 and α-DLG-1 staining are clearly separated. (F) The intermediate filament, IFB-2, localizes to the apical cytocortex of the gut epithelium. Note that AJM-1, DLG-1, HMP-1 and CRB-1 form belts around the apex of intestinal cells, whereas IFB-2 surrounds the whole apical cytocortex of the future gut lumen. (C′–F′) Schematic cross section of two gut epithelial cells, enclosing a common lumen. Colors represent localization of proteins as adapted from antibody staining in (B–F). (B) Orientation: anterior, left; dorsal, top; lateral view; arrowheads point to linkage of the intestine to the pharyngo-intestinal and intestino-rectal valves, respectively. (C) Magnification of the CeAJ, as shown in (B) (white rectangle). (D–F) Magnifications of the CeAJ were taken from similar morphogenesis stages as in (B). Scale bars: (A, A′) 0.1 μm, respectively; (B) 10 μm.
the CeAJ. Analysis of embryos either depleted for DLG-1 or HMP-1 reveals an almost WT-like IFB-2 pattern, respectively (Figs. 2B,C). In contrast, genetic epistasis experiments demonstrate that in \textit{dlg-1/hmp-1\textsc{(RNAi)}} embryos the outline of the future gut lumen is preserved only in fragmentary form, reflecting detachment of intestinal cells (Fig. 2D). In addition, instead of the usually continuous belt of \(\alpha\)-AJM-1 staining around the cellular apex (Fig. 1B), a greatly reduced and distorted pattern arises (Fig. 3A). We noticed the occurrence of a similar CeAJ staining pattern (Fig. 3B) after double depletion of HMP-1 together with \textit{C. elegans} ERM-1 (manuscript submitted), a member of the Band 4.1/Ezrin-Radixin-Moesin protein family, which is proposed throughout the literature as membrane-cytoskeleton linkers (Bretscher et al., 2002; Gautreau et al., 2002). These phenotypes significantly diverge from the single or double RNAi phenotypes in \textit{dlg-1}, \textit{hmp-1}, \textit{erm-1} and \textit{dlg-1/erm-1} embryos, respectively (Figs. 3D,F; data not shown). In contrast, the AJM-1 pattern is more severely perturbed in \textit{dlg-1\textsc{(RNAi)}} embryos (Fig. 3D), indicating a requirement of DLG-1 for AJM-1 but little dependence vice versa (see Discussion).

The correct localization of the AJM-1–DLG-1 complex in \textit{C. elegans} is under the control of basolaterally expressed LET-413 (which is not mislocalized in the absence of DLG-1) but is independent of the catenin–cadherin complex (Bossinger et al., 2001; Köppen et al., 2001; Legouis et al., 2000; McMahon et al., 2001). These observations raise the question of how these systems interact with each other to control the assembly of the basal unit of the CeAJ. While the phenotypes of the double knockdown (\textit{hmp-1/let-413}, Fig. 1E) are identical to the \textit{hmp-1\textsc{(RNAi)}} phenotype, while the phenotypes of the single RNAi knockdowns are distinct (\textit{hmp-1} and \textit{let-413}; Figs. 3F,G). Similar results were obtained using either \textit{hmp-2} (\(\beta\)-catenin) or \textit{hmr-1} (E-cadherin) together with \textit{let-413} in such double knockdowns (data not shown). Thus, the catenin–cadherin complex is epistatic to \textit{let-413}. This surprising result prompted us to reinvestigate the role of \textit{crb-1} concerning the localization of proteins in the basal unit of the CeAJ. While the basal unit of the CeAJ is profoundly affected in a \textit{let-413\textsc{(RNAi)}} single

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\caption{Gut-specific cell adhesion and tubulogenesis defects occur after double knockdown of \textit{dlg-1/hmp-1\textsc{(RNAi)}}. (A) The intermediate filament IFB-2, recognized by mabMH33, is a marker for the apical cytocortex of the embryonic gut epithelium and it sharply delineates the course of the future gut lumen. (B, C) \textit{dlg-1\textsc{(RNAi)}} and \textit{hmp-1\textsc{(RNAi)}} embryos still exhibit an uninterrupted gut lumen, whereas \textit{dlg-1/hmp-1\textsc{(RNAi)}} embryos show a disintegration of the gut lumen. Scale bar: 10 \(\mu\)m. Orientation: anterior, left; dorsal, top; lateral view.}

\end{figure}
knockdown (Fig. 3G), the pattern of the CeAJ is recovered if the catenin–cadherin complex is weakened in addition to LET-413 (Fig. 3E). This recovery seems to depend on CRB-1, since a concerted triple knockdown against hmp-1, let-413 and crb-1 affects the establishment of the basal unit (Fig. 3H) to the same extend as in let-413(RNAi) embryos (Fig. 3G). Replacement of crb-1 by the putative C. elegans homolog of Drosophila stardust (C01B7.4, Bachmann et al., 2001) gave similar results (data not shown). This result suggests a role of subapically expressed CRB-1 and Stardust (data not shown) as a redundant mechanism to ensure proper apical junction formation in the intestine.

The use of RNAi to produce multiply phenocopied embryos has been successfully demonstrated (Maduro et
al., 2001). To demonstrate that a triple knockdown of gene function involved in CeAJ formation is possible in C. elegans, we used α-HMP-1 and α-CRB-1 antibodies to test the reduction of both proteins in our RNAi experiments. In comparison to WT embryos, α-HMP-1 and α-CRB-1 immuno-FL in the gut epithelium is significantly reduced in hmp-1/let-413/crb-1(RNAi) embryos and was not detectable at all under the laser-scanning microscope (Fig. 4).

Discussion

We report here that separate complexes of the C. elegans apical junction (CeAJ; Figs. 1 and 5), the catenin–cadherin system (Costa et al., 1998) and the AJM-1–DLG-1 complex (Köppen et al., 2001), are both responsible for tissue-specific adhesion in the gut epithelium. RNAi depletion of HMP-1 (α-catenin) in combination with DLG-1 (Discs large) results in embryos that fail to maintain cell contact structures resulting in a fragmentized tubular lumen of the intestine (Figs. 2 and 3). We hypothesize the existence of additional transmembrane proteins, which together with HMR-1 (E-cadherin) are stabilized by DLG-1, a scaffolding protein of the MAGUK family (Dimitratos et al., 1999). This view is supported by recent studies of the C. elegans hypodermis (epidermis), suggesting that VAB-9 (a predicted four-pass integral membrane protein and a member of the PMP22/EMP/Claudin family of cell junction proteins) and the AJM-1–DLG-1 complex mediate partially redundant adhesive functions (Simske et al., 2003). In the gut epithelium, possible candidates include transmembrane proteins belonging to the immunoglobulin (Ig) superfamily in C. elegans (Hutter et al., 2000). After phosphorylation of its COOH-terminus, LAD-1, the only L1-related cell adhesion molecule in C. elegans localizes to the basal unit of the CeAJ in the gut epithelium. It contains a PDZ binding motif at the COOH-terminus, which may be accessible to PDZ proteins after phosphorylation (Chen et al., 2001). PDZ domain containing proteins with strong structural homology to human afadin and Drosophila Canoe have been identified in a yeast two-hybrid screen for putative LET-60/Ras effectors (Watari et al., 1998). In vertebrate cells, afadin is required to recruit the Ig-like cell adhesion molecule nectin and E-cadherin to the ZA (Kioka et al., 2002). Demonstration of the recruitment of IgCAMs to the CeAJ via PDZ proteins would add another piece to the genetic puzzle of cell adhesion in the intestine.

The molecular nature of the components involved in cell adhesion implies the existence of redundant mechanisms for apical junction assembly. The depletion of the coiled-coil protein AJM-1 results in spreading of DLG-1 to the lateral membrane domain but does not interfere with correct morphogenesis of the gut epithelium in C. elegans (Fig. 3). How do C. elegans AJM-1 and LET-413 contribute to the correct localization of DLG-1 in the embryonic gut epithelium? In the WT, shortly after cytoplasmic polarization (Leung et al., 1999), proteins of the apical membrane domain and the CeAJ are delivered to the future
epithelium of C. elegans

hmp-1/let-413 basolaterally expressed LET-413 (the ERM-1 (the catenin – cadherin complex (HMP-1– HMP-2– HMR-1), the AJM-1– DLG-1 (Disc large) complex, and the intermediate filament (IFC-2)), other proteins [e.g. CeAJ appears to consist of a basal and an apical unit. While some proteins of these regions form belts around the cellular apex (e.g. CRB-1 (Crumbs), the epsin – cadherin complex. Recent results in Drosophila embryo, a subapical scaffold containing Crb, Sdt and Dlt seems to mediate the localization and stabilization of the ZA component DE-cadherin (Klebes and Knust, 2000). In contrast to Drosophila, however, knockdowns of C. elegans crb-1 and/or a second crb-like gene (eat-20) show no defects in epithelial development or morphology as a whole in a WT background (Bossinger et al., 2001; Shibata et al., 2000). In C. elegans, CRB-1 represents a positional cue for the localization of DLG-1 after deletion of hmp-1 and let-413 (Fig. 3). We propose that during normal development, the downstream activity of CRB-1 is redundant to the positional cue generated by the catenin–cadherin complex. Recent results in Drosophila suggest a regulatory hierarchy along the apico-basal axis between the Baz–DmPar6–DaPKC complex, the Crb–Sdt–Dlt complex and the putative Scrib–Lgl–Dlg complex (Bilder et al., 2003; Tanentzapf and Tepass, 2003). ZA formation directly depends on Crb and Scrib complex activities but only in their absence a function of Baz (PAR-3 homolog) for ZA establishment is revealed (Tanentzapf and Tepass, 2003). In C. elegans, apical junction formation is primarily independent of CRB-1 but its activity can direct junction assembly in the absence of the cadherin–cadherin system and let-413 (Scrib homolog). In C. elegans, the PAR-3–PAR-6–PKC-3 complex and PAR-1 and PAR-2 are involved in setting up the polarity in the one-cell embryo (Kemphues, 2000). Recently, it was shown that PAR-1 also plays an essential role in the development of the vulva (Hurd and Kemphues, 2003). This result begs the question of whether the PAR-3 complex also has a later function during development in C. elegans. The future challenge will be to devise methods to specifically knockdown gene function in the intestine to reveal a

Fig. 5. Molecular composition and genetic interactions between different units of the intestinal C. elegans apical junction. Based on immuno-FL analysis, the CeAJ appears to consist of a basal and an apical unit. While some proteins of these regions form belts around the cellular apex (e.g. CRB-1 (Crumbs), the catenin–cadherin complex (HMP-1– HMP-2– HMR-1), the AJM-1– DLG-1 (Disc large) complex, and the intermediate filament (IFC-2)), other proteins [e.g. ERM-1 (the C. elegans Ezrin-Radixin-Moesin homolog), actin, and the intermediate filament IFB-2] surround the whole apical cytocortex (see also Figs. 1C–F). Correct localization of AJM-1– DLG-1 in the basal unit of the CeAJ is mutually dependent on each other (black double arrow) and under the control of basolaterally expressed LET-413 (the C. elegans Scribble homolog, blue arrow). CRB-1 has the capacity to restore the localization of the DLG-1 pattern in an hmp-1/let-413-deficient background (red arrow). Proteins of the CeAJ and the apical membrane domain are required to maintain cell adhesion in the gut epithelium of C. elegans (see text for further explanations).
participation in cell adhesion and/or the formation of apical junction in C. elegans.

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