

Cadherins and Their Partners in the Nematode Worm *Caenorhabditis elegans*

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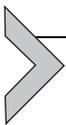
Contents

1. <i>Caenorhabditis elegans</i> : A Model for Studying Cadherin Function <i>In Vivo</i>	240
2. The Classical Cadherin/Catenin Complex in <i>C. elegans</i>	242
2.1 HMR-1A: A classical cadherin required for cell–cell adhesion	243
2.2 HMR-1A regulates cell ingression during gastrulation	243
2.3 HMR-1A and the CCC are crucial during ventral enclosure and embryonic elongation	245
2.4 HMP-2: A β -catenin specialized for adhesion	246
2.5 HMP-1/ α -catenin: Connecting to the actin cytoskeleton	248
2.6 JAC-1: A modulator of the other components of the core CCC	249
2.7 HMR-1B: A classical neuronal cadherin involved in axon guidance	249
3. Regulating the Classical CCC in <i>C. elegans</i>	249
3.1 The PAR/aPKC complex and LET-413/Scribble: Regulators of apical junctional organization	249
3.2 FRK-1/Fer kinase: A regulator of HMP-2/ β -catenin	250
4. New Functional Partners with the Classical CCC in <i>C. elegans</i>	251
4.1 SAX-7/L1CAM: A redundant partner of HMR-1 in blastomere adhesion	251
4.2 VAB-9: A divergent claudin family member regulated by the cadherin complex	251
4.3 ZOO-1/ZO-1: An associate of VAB-9 and the CCC that promotes strong adhesion	252
4.4 UNC-34/Enabled: A potential integrator of the cadherin and DLG-1/AJM-1 complexes	252
4.5 MAGI-1: A SAX-7/L1CAM-dependent regulator of AFD-1/afadin that stabilizes junctions	253
4.6 SRGP-1/srGAP: Bending adhesions into shape	254
4.7 UNC-94: A regulator of HMP-1-dependent actin networks	254
5. Beyond the Classical CCC: Other Cadherin Family Members in <i>C. elegans</i>	255

5.1	CDH-3 and CDH-4: Fat-like cadherins that do not modulate planar cell polarity	255
5.2	FMI-1: A Flamingo/Stan protein that modulates fasciculation in follower axons	256
5.3	CASY-1: A calyntenin involved in associative learning	257
6.	Conclusions	257
	Acknowledgments	257
	References	257

Abstract

The extreme simplicity of *Caenorhabditis elegans* makes it an ideal system to study the basic principles of cadherin function at the level of single cells within the physiologically relevant context of a developing animal. The genetic tractability of *C. elegans* also means that components of cadherin complexes can be identified through genetic modifier screens, allowing a comprehensive *in vivo* characterization of the macromolecular assemblies involved in cadherin function during tissue formation and maintenance in *C. elegans*. This work shows that a single cadherin system, the classical cadherin–catenin complex, is essential for diverse morphogenetic events during embryogenesis through its interactions with a range of mostly conserved proteins that act to modulate its function. The role of other members of the cadherin family in *C. elegans*, including members of the Fat-like, Flamingo/CELSR and calyntenin families is less well characterized, but they have clear roles in neuronal development and function.



1. CAENORHABDITIS ELEGANS: A MODEL FOR STUDYING CADHERIN FUNCTION *IN VIVO*

The relatively small number of cadherin superfamily proteins encoded in the *C. elegans* genome, combined with its well-known transparency, simplicity of organization, and robust forward and reverse genetics makes *C. elegans* an appealing model organism for understanding how cadherins and their binding partners function in defined cellular contexts. *C. elegans* has 12 genes encoding 13 cadherins.^{1–3} *C. elegans* has representatives of most of the main cadherin families that are conserved throughout the metazoa, including members of the classical cadherin, Fat-like cadherin, Dachshous, Flamingo/CELSR, and Calyntenin families (Fig. 11.1). *C. elegans* lacks protocadherins,⁴ a feature that it shares with *Drosophila*; however, the presence of this family in other major invertebrate groups suggests that they may have been lost in the Ecdysozoa.⁴ As is the case for other invertebrates and nonvertebrate chordates, *C. elegans* lacks desmosomal cadherins.⁵ While overall, single loss-of-function experiments suggest that most cadherins have surprisingly limited functions (J. Pettitt, unpublished⁶), the classical cadherin/catenin complex of *C. elegans* is crucial for morphogenesis. We discuss it and other cadherins with known functions in the following sections.

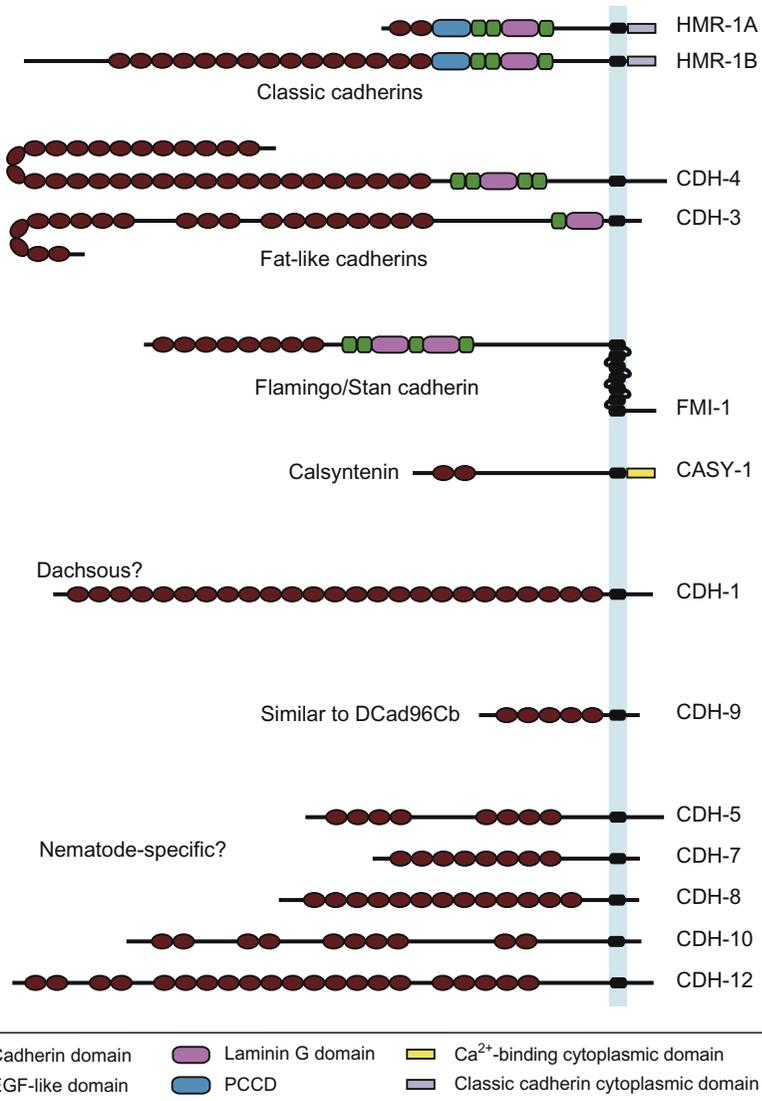


Figure 11.1 The cadherin superfamily in *C. elegans*. The 13 *C. elegans* cadherins are grouped according to their structural similarity with cadherins from other organisms. Each cadherin is positioned with its N-terminus to the left. PCCD, primitive classic cadherin domain. Adapted from Ref. 3 with permission.

2. THE CLASSICAL CADHERIN/CATENIN COMPLEX IN *C. ELEGANS*

Like all nonchordates characterized to date, *C. elegans* has single representatives of the α - and p120-catenin families, and these are encoded by the *hmp-1* and *jac-1* genes, respectively.^{7,8} Unusually, it possesses four β -catenin homologs, but only one, HMP-2, is known to participate in CCC junction formation (Fig. 11.2A). Its single, type III classical cadherin gene, *hmr-1*, encodes two proteins, HMR-1A and HMR-1B, which differ in their ectodomains (Fig. 11.1), and are expressed by alternative splicing and alternative promoter use.⁹ HMR-1A is expressed in all epithelia (Fig. 11.2B) plus some neurons, while HMR-1B appears to be confined to neurons. Thus, *C. elegans* has both epithelial and neuronal classical cadherins, but their origin from a single locus appears to be unique to Caenorhabditids. Based on colocalization and epistasis,⁷ as well as molecular interactions,^{8,10,11}

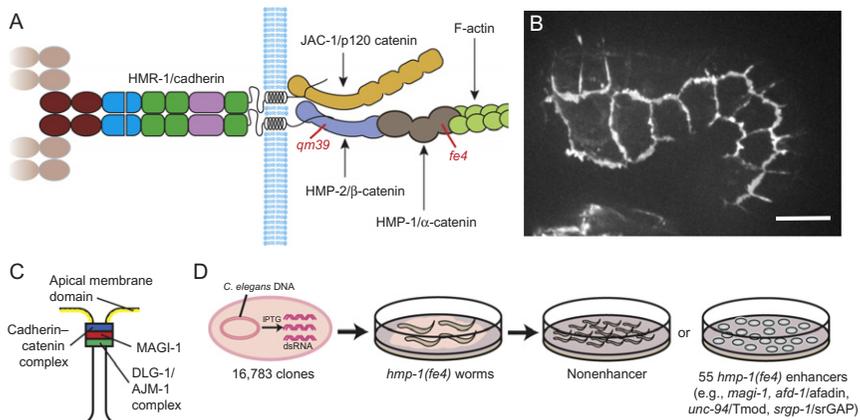


Figure 11.2 The core components of the *C. elegans* classical cadherin/catenin complex (CCC). (A) HMR-1A is the epithelial cadherin, HMP-2 the junctional β -catenin, HMP-1 the *C. elegans* α -catenin, and JAC-1 the p120-catenin homolog. (B) A comma stage embryo expressing *hmr-1::gfp* (image courtesy of B. Lucas). Bar = 10 μ m. (C) Schematic of the *C. elegans* apical junction. MAGI-1 localizes to a domain between the CCC and the DLG-1/AJM-1 complex. (D) Genome-wide screening in *hmp-1(fe4)* mutants identifies CCC partners during morphogenesis. A library of 16,783 RNAi feeding clones was screened to identify enhancers of a hypomorphic allele of α -catenin, *hmp-1(fe4)*. Each bacterial clone was fed to wild-type and *hmp-1(fe4)* worms and phenotypes were assessed in the next generation. Enhancers increased lethality in *hmp-1(fe4)* embryos to >83% and caused <10% lethality in wild-type embryos. Fifty-five enhancers were identified, including *magi-1*, *afd-1/afadin/Canoe*, *srgp-1/srGAP*, and *unc-94/Tropomodulin*. Panel A: Adapted from Ref. 3; C-D adapted from Ref. 71, with permission.

HMR-1A, HMP-1, HMP-2, and JAC-1 form a CCC that is a component of junctions in all *C. elegans* epithelia.

While *C. elegans* has a core CCC, many proteins associated with the vertebrate CCC are missing, including vinculin and α -actinin.^{12,13} Other effectors, such as *C. elegans* Enabled, have surprisingly minor roles (see below). Such surprises suggest that *C. elegans* may be extremely useful as a “minimalist” system for examining the core features of the CCC. Morphologically, epithelial junctions in *C. elegans* are minimalist as well. Unlike in *Drosophila* and vertebrates, they do not possess structural specializations that lead to different electron-dense structures. Instead, the *C. elegans* apical junction possesses a single electron-dense structure, with a stratified set of molecular complexes, including the CCC (Fig. 11.2C).¹⁴

2.1. HMR-1A: A classical cadherin required for cell–cell adhesion

HMR-1 proteins possess all of the canonical domains of other invertebrate classical cadherins. HMR-1A has two extracellular cadherin (EC) repeats as well as a primitive classical cadherin domain (PCCD; see Fig. 11.1), which, together with EGF-like and laminin G repeats, lies between the EC repeats and the transmembrane helix. The PCCD is proteolytically cleaved during the maturation of *Drosophila* E-cadherin¹⁵; this has not been shown for HMR-1.

HMR-1 behaves like a *bona fide* classical cadherin. Based on yeast two-hybrid^{8,10} and biochemical assays,¹¹ the HMR-1 C-terminus can bind HMP-2. Significantly, this association is only efficient when HMR-1 is prephosphorylated by casein kinase II.¹¹ Mammalian E-cadherin’s association with β -catenin increases in a similar, dramatic fashion when it is phosphorylated at a key serine (S840 in human E-cadherin¹⁶; see also Ref. 17). A candidate serine in the same location in HMR-1 (S1212) could potentially serve the same function in *C. elegans*.

HMR-1 also contains a well-conserved juxtamembrane domain that binds JAC-1/p120-catenin.⁸ p120-catenin is an important regulator of trafficking of the CCC in vertebrates.¹⁸ Current evidence does not favor a major role for JAC-1 in similar events in *C. elegans* (see below); it remains to be determined whether HMR-1 undergoes regulation via endocytic trafficking in ways that are similar to mammalian classical cadherins.¹⁹

2.2. HMR-1A regulates cell ingression during gastrulation

C. elegans gastrulation begins with the ingression of the endodermal precursors, Ea and Ep (Fig. 11.3A). This process is partly driven by actomyosin-mediated

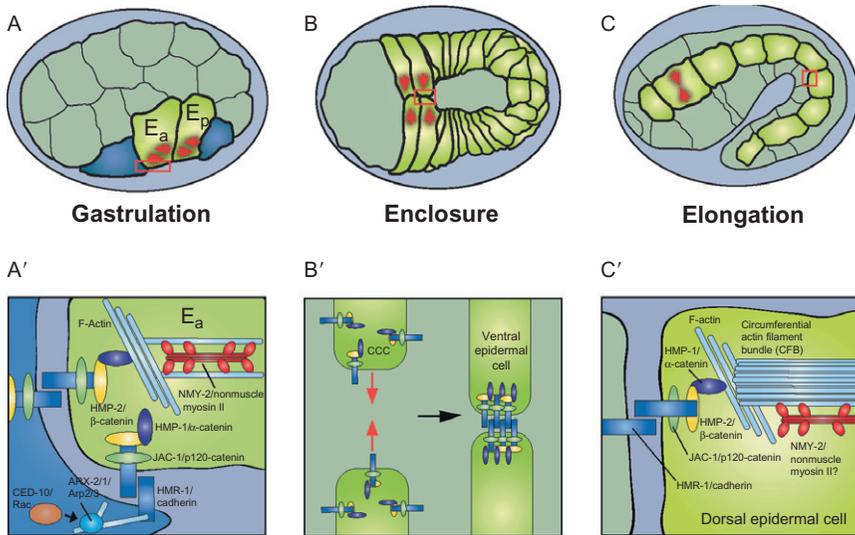


Figure 11.3 Roles of the classical CCC in epidermal morphogenesis. Developmental stages are depicted in A–C, and postulated subcellular events in the boxed regions are depicted in A'–C'. A, A'. Gastrulation involves internalization of the endodermal precursors, Ea and Ep, via apical constriction (A, arrows), which internalizes Ea and Ep and aids covering of the space they vacate by pulling on neighboring cells. Normal apical constriction requires the CCC, as well as SAX-7/L1CAM, and components of Rac signaling. (B, B') Ventral enclosure involves migration of epithelial cells toward the ventral midline (B, arrows). The first cells to meet at the midline (leading cells) rapidly make cadherin-dependent contacts. (C, C') Elongation of the embryo from a bean to a worm shape requires coordinated, actomyosin-mediated changes in the shape of epidermal cells (C, arrows). The CCC is required for attachment of actin, including circumferential filament bundles (CFBs) to the cell surface, and hence for transmission of forces to the cell surface. HMP-1/ α -catenin is particularly crucial in this process. Anterior is to the left in each panel. Adapted from Ref. 20 with permission.

apical constriction, which helps to pull adjacent cells over the apical surface of Ea/Ep and displaces them internally.²¹ Cadherin-based adhesion appears to be involved in this process because knockdown of maternal and zygotic *hmr-1* mRNA by RNAi reduces the rate of Ea/Ep apical constriction by approximately 50%.²² In such embryos with reduced HMR-1 function, endodermal precursors show diminished membrane movements concomitant with myosin translocation.²² Given the role of the CCC in stabilizing F-actin linkages to adherens junctions in other tension-driven processes,^{23,24} HMR-1 may stabilize the cytoskeletal machinery required for apical constriction (Fig. 11.3A'). Consistent with this possibility, the CCC interacts genetically with the Rac

pathway in endodermal precursors. Knockdown of CCC components along with Rac pathway components abrogates Ea/Ep apical constriction without loss of apical nonmuscle myosin.^{6,22} Rac may function as part of a molecular “clutch,” linking actomyosin contractions to the CCC within Ea/Ep. Alternatively, the CCC may synergize with Rac by providing adhesive traction for Arp2/3-dependent lamellipodia extended by neighboring mesodermal precursors.^{25,26}

HMR-1 is also involved in ingression of primordial germ cells (PGCs). Following Ea/Ep ingression, PGCs maintain contact with endodermal daughters, as they are drawn into the interior by the dorsal movement of the endoderm.²⁷ PGC internalization requires surface enrichment of HMR-1 in the PGCs; such enrichment appears to be mediated post-transcriptionally via a specific element in the 3'-UTR of the *hmr-1* mRNA. Surprisingly, however, HMR-1 expression in PGCs alone is both necessary and sufficient for their internalization.²⁷ This suggests that HMR-1 participates in heterotypic interactions in this context. Confirming this interesting possibility and identifying the heterotypic binding partner involved await additional experiments.

2.3. HMR-1A and the CCC are crucial during ventral enclosure and embryonic elongation

Postgastrula morphogenesis in *C. elegans* involves three major events in the epidermis: dorsal intercalation, ventral enclosure, and elongation.²⁸ To date, no function for the CCC during dorsal intercalation has been identified. In contrast, the CCC is crucial for ventral enclosure and elongation.⁷ During enclosure, the free, ventral edges of the epidermis migrate around the embryo toward the ventral midline, where they meet and form nascent junctions^{29–31} (Fig. 11.3B and B'). These events require the CCC: zygotic loss of HMR-1 or maternal + zygotic removal of HMP-2 and HMP-1 leads to failure of ventral enclosure and the Hammerhead (Hmr) phenotype, as anterior cells spill out of the embryo^{7,29} (Fig. 11.4A and B). Zygotic *hmp-1* and *hmp-2* mutants enclose, but as the embryo elongates, it develops characteristic dorsal folds, known as the Humpback (Hmp) phenotype⁷ (Fig. 11.4C), which results from the detachment of circumferential filament bundles (CFBs; Fig. 11.3C and C') from the junction-associated actin network (Fig. 11.4D–F). CFBs presumably transmit forces generated by actomyosin-mediated contraction in the epidermis (reviewed in Ref. 28).

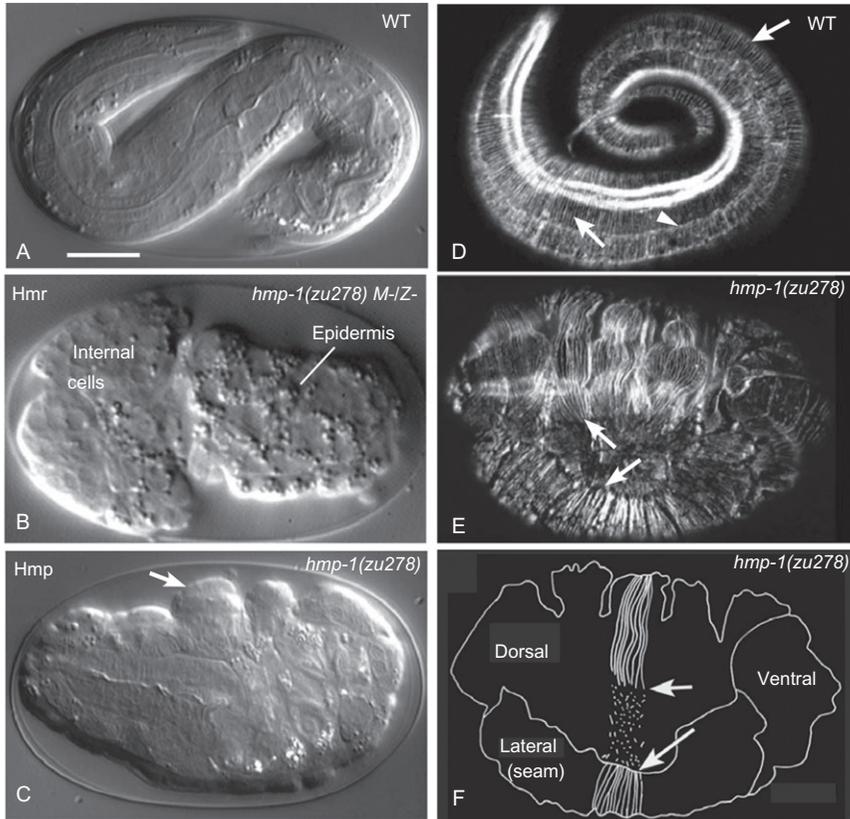


Figure 11.4 Phenotypes associated with loss of CCC proteins. (A–C) Nomarski optics; (D–E) phalloidin staining of F-actin imaged using confocal microscopy. (A) Wild-type embryo at approximately the threefold stage. (B) An embryo from a *hmp-1(zu278)* M-/Z- germline mosaic mother, which therefore lacks both maternal and zygotic HMP-1, displaying the Hmr (*Hammerhead*) phenotype. Enclosure failure in the anterior results in rupture of the embryo and ejection of internal cells through the opening in the epidermis. (C) A *hmp-1(zu278)* homozygote displaying the Hmp (*Humpback*) phenotype. Prominent dorsal bulges in the epidermis are visible (arrow). (D) Phalloidin staining of a wild-type embryo at approximately the threefold stage of elongation. Circumferential filament bundles (CFBs) (arrows) and junctional proximal actin (arrowhead) are clearly visible; the bright staining is one of the four muscle quadrants. (E) A *hmp-1(zu278)* homozygote stained with phalloidin. CFBs have detached from cell–cell junctions (arrows). (F) A schematic of (E). Scale bar = 5 μ m. Images adapted from Ref. 7 with permission.

2.4. HMP-2: A β -catenin specialized for adhesion

Whereas β -catenins in most animals have dual functions, in the CCC and in Wnt signaling³² (see also [Chapter 17](#)), in *C. elegans* these activities are distributed into separate proteins.¹⁰ HMP-2 has clear functions in cell–cell adhesion as part of the classical CCC. However, it is one of four β -catenin

homologs in *C. elegans*, which have undergone subfunctionalization during their evolution. HMP-2 interacts strongly with HMR-1, but not with the single Tcf/Lef homolog in *C. elegans*, POP-1.^{10,33} Instead, β -catenin's role in canonical Wnt signaling is fulfilled by BAR-1.^{34–36} The remaining two β -catenin homologs, WRM-1 and SYS-1, participate in what appears to be a highly diverted Wnt signaling pathway, the “Wnt/ β -catenin asymmetry” pathway,³⁶ which is involved in cell differentiation during anterior–posterior cell divisions. The subfunctionalization of β -catenins extends to other members of the nematode phylum, but importantly does not appear to be the case for the most distant relatives of *C. elegans*: these nematodes possess dual function β -catenins similar to those found in other animal groups, indicating subfunctionalization events occurred after the diversification of the nematode phylum (D. Sarkar and J. Pettitt, unpublished).

Structural comparison of HMP-2 with “dual function” β -catenins from vertebrates and *Drosophila* confirms that HMP-2 retains key features required for interactions with α -catenin and cadherin. The interaction sites with cadherin and α -catenin have been mapped for β -catenin of both vertebrates and *Drosophila* (reviewed in Ref. 32). β -catenin's binding to the CCC can be regulated by phosphorylation of residues in these regions. When Y654 of human β -catenin is phosphorylated, β -catenin's affinity for cadherin is significantly reduced. Phosphorylation of Y142 likewise decreases β -catenin's affinity for α -catenin.^{37–41} HMP-2 possesses similar subdomains (H.-J. Choi and W. Weis, personal communication; T. Loveless and J. Hardin, unpublished); so, it will be interesting to see whether mutating these sites has important functional consequences *in vivo*.

Vertebrate and *Drosophila* β -catenins are well-known transcriptional coactivators acting downstream of canonical Wnt signaling. Residues in the C-terminus of β -catenin just terminal to the 12th armadillo repeat, termed “Helix C”,⁴² are conserved in all β -catenins known to be involved in transcriptional coactivation.³² This domain is conspicuously lacking in HMP-2,^{32,43} suggesting that it should have reduced transcriptional coactivator activities. Indeed, HMP-2 binds POP-1/Tcf weakly, if at all in yeast two-hybrid assays.¹⁰ HMP-2 nevertheless has non-adhesive roles under some circumstances. When FRK-1/Fer kinase is knocked down, HMP-2 can function in the Wnt/ β -catenin asymmetry pathway.⁴⁴ Immunostaining also indicates that HMP-2 is present in the nucleus in the early embryo (Ref. 45; T. Loveless and J. Hardin, unpublished). Moreover, when overexpressed, HMP-2 can rescue *bar-1* mutants.³³ Taken together, the data suggest that HMP-2 normally acts as an “adhesion-only” β -catenin, but that it can function weakly in the nucleus. The physiological significance of such weak activity is unknown.

2.5. HMP-1/ α -catenin: Connecting to the actin cytoskeleton

The traditional view of the cadherin/catenin complex (CCC) assumes that its core components directly tether F-actin to the CCC via a quaternary complex of cadherin/ β -catenin/ α -catenin/F-actin. In favor of this classical model are the findings that direct molecular fusion of α -catenin to cadherin rescues some adhesive functions of adhesion-defective cells, both in vertebrate tissue culture cells²⁴ and *in vivo* in *Drosophila*.⁴⁶ However, the traditional view of the situation in vertebrates has been questioned (see also Chapter 1), leading to a model in which E-cadherin and β -catenin recruit α -catenin to junctions via transient interactions.^{24,47} The subsequent dissociation of α -catenin from the complex would then result in the formation of α -catenin homodimers in the vicinity of junctional actin, where they can influence filament dynamics, possibly by antagonizing the Arp2/3 complex.²⁴ Beyond these questions about whether the core CCC functions as a stoichiometric complex, recent experiments indicate that the vertebrate CCC can undergo mechanosensitive adhesive strengthening involving the actin-binding protein vinculin.⁴⁸ α E-catenin may undergo conformational changes as part of this process that allows it to bind and recruit vinculin.⁴⁹

Given these complexities, *C. elegans* may be an outstanding system in which to address how α -catenin functions. Based on sequence comparisons, several domains of vertebrate α -catenin, including its three vinculin homology domains, an N-terminal β -catenin binding domain, and an F-actin binding site at the C-terminus, are highly conserved.¹¹ Moreover, the tension-bearing mechanical requirements on HMP-1 due to the stresses of elongation are stringent, making it a good system for studying essential features of α -catenin.

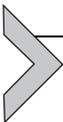
Recent experiments provide unambiguous evidence that both linkage to the CCC via HMP-2 and F-actin binding are essential for HMP-1 function. Mutations that truncate HMP-1 before the C-terminal F-actin binding domain result in complete failure of elongation.¹¹ Deletion of the N-terminal β -catenin binding domain also results in complete morphogenetic failure, with loss of recruitment of HMP-1 to junctions.¹¹ Interestingly, HMP-1 appears conformationally autoinhibited *in vitro*: full-length HMP-1 is very compact and only binds F-actin weakly *in vitro*, whereas C-terminal fragments bind actin avidly.¹¹ Although the details remain to be worked out, HMP-1 shows that a common theme among α -catenins is that their conformational state regulates the CCC.

2.6. JAC-1: A modulator of the other components of the core CCC

Like other p120-catenins, JAC-1 (*Juxtamembrane domain-associated catenin*) has 10 armadillo repeats in its central region, but unlike the N-termini of mammalian and *Drosophila* p120-catenins, the N-terminus of JAC-1 contains four fibronectin type III domains.⁸ The functional significance of these domains is unclear, but they are found in all characterized nematode p120-catenins. JAC-1 is not essential for cadherin-mediated events in the *C. elegans* epidermis.⁸ However, it does positively contribute to CCC function, since reducing its function enhances the phenotype of a weak *hmp-1* loss-of-function mutant.⁸ Indeed, loss of *jac-1* function in this genetic background leads to exacerbated clumping of CFBs and phenocopies the strong *hmp-1* loss-of-function phenotype. Interestingly, *Drosophila* p120-catenin plays a similar modulatory role.⁵⁰ In contrast, vertebrate p120-catenins appear to play a more essential function⁵¹ (see also Chapter 18).

2.7. HMR-1B: A classical neuronal cadherin involved in axon guidance

Functional analysis of the HMR-1B isoform indicates that a classical cadherin also acts during neuronal development in *C. elegans*. Animals with reduced or absent HMR-1B function are viable, but they display incompletely penetrant defects in axon guidance of a subset of motor neurons.⁹ Strong *hmp-1* mutants that express HMP-1 only in the epithelia typically have mild uncoordinated (Unc) phenotypes.¹¹ However, since the penetrance of axonal guidance defects caused by loss of HMR-1B function is relatively low, it is likely that HMR-1B-based adhesion only augments other more important guidance cues.



3. REGULATING THE CLASSICAL CCC IN *C. ELEGANS*

3.1. The PAR/aPKC complex and LET-413/Scribble: Regulators of apical junctional organization

As in other metazoans, PAR-3, PAR-6, and PKC-3/atypical Protein Kinase C3 constitute an apical polarity complex in *C. elegans* epithelia.⁵² PAR protein constructs possessing a C-terminal sequence that allows PAR proteins to persist in early embryos, but leads to their destruction before the birth of epithelial cells, have been used to study PAR proteins in embryonic epithelia.⁵³ Loss of PAR-3 leads to ectopic separations between lateral surfaces of

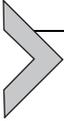
cells and to abnormal HMP-1 localization; it colocalizes with DLG-1/Discs Large, rather than apical to it. In the absence of PAR-3, intestinal HMR-1 is initially dispersed and mislocalized, whereas HMP-1 is still recruited into foci. Basolateral (as opposed to apical) foci of HMP-1 and DLG-1 accumulate, despite unperturbed localization of LET-413/Scribble,⁵⁴ which normally excludes AJ components from basolateral surfaces.^{55,56} Similar localization defects are seen in the pharynx in *par-3* mutants.⁵⁴ Loss of PAR-6 prior to gastrulation results in elongation failure despite successful ventral enclosure.⁵⁷ The distribution of DLG-1, AJM-1, HMP-1, and HMR-1 is fragmentary, with the CCC being affected less severely.

While PAR-3 is critical for CCC establishment in endodermal and mesodermal epithelia, it is relatively less important in the epidermis; *par-3* embryos are still able to correctly localize CCC proteins and form mature epidermal junctions.⁵⁴ This difference may be due to an inherent difference in tissue organization: the epidermis is in contact with the developing cuticle. Eventually, however, localization of HMR-1 and DLG-1 is lost in *par-3* embryos, resulting in ripping of the epidermis.⁵⁷ This may be due to loss of apical PAR-6 in the *par-3* mutant epidermis,⁵⁷ suggesting a role in junctional maintenance rather than formation in this tissue.

The LAP (leucine-rich repeat and PDZ) protein LET-413/Scribble is also involved in apicobasal positioning of junctional proteins. LET-413 localizes to basolateral membranes of *C. elegans* epithelia.⁵⁸ *let-413* loss of function leads to a lack of apical-basal “focusing” of junctional proteins in the epidermis and gut (reviewed in Ref. 14). *let-413* mutants display occasional ventral enclosure defects,⁵⁶ suggesting effects on the CCC as well.

3.2. FRK-1/Fer kinase: A regulator of HMP-2/ β -catenin

FRK-1 is the ortholog of the mammalian nonreceptor tyrosine kinase, Fer. In the early embryo, abrogation of *frk-1* function results in relocalization of HMP-2 to the nucleus in endodermal precursors, and allows it to functionally substitute for WRM-1 during endoderm development.⁴⁴ Later, during morphogenesis, FRK-1 localizes to epithelial junctions and is reported to be necessary for embryonic enclosure and morphogenesis.⁵⁹ FRK-1 mislocalizes in *hmp-2* mutants and *jac-1(RNAi)* embryos, and coimmunoprecipitates with HMP-2.⁵⁹ Surprisingly, some functions of FRK-1 appear to be independent of its function as a *bona fide* kinase,⁵⁹ and it is not known if FRK-1 kinase activity plays a role in regulating the phosphorylation of HMP-2 or other β -catenins.



4. NEW FUNCTIONAL PARTNERS WITH THE CLASSICAL CCC IN *C. ELEGANS*

4.1. SAX-7/L1CAM: A redundant partner of HMR-1 in blastomere adhesion

Because the *C. elegans* CCC is not absolutely essential for general cell–cell adhesion in embryos, it is a useful system for identifying other proteins that act redundantly with the CCC during development. One promising class of adhesion molecules that may function alongside the cadherin complex is the L1CAM family of proteins, which are members of the immunoglobulin superfamily of cell surface proteins.⁶⁰ In *Drosophila*, hypomorphic mutations in the L1CAM homolog neuroglian result in disrupted septate junctions, suggesting a role in cell adhesion.⁶¹ In *C. elegans*, SAX-7/L1CAM localizes to cell–cell contacts in the early embryo and in epithelia at all stages,⁶² and transgenic embryos expressing a dominant-negative SAX-7 display cells with altered shapes and locations.⁶²

Recent work implicates HMR-1 as a functionally redundant partner with SAX-7/L1CAM in the early embryo. Knockdown of *hmr-1*, *hmp-1*, or *hmp-2* in *sax-7* mutants results in failure of apical constriction and defective ingression of Ea and Ep.⁶³ Moreover, removal of HMR-1 and SAX-7 together leads to loss of apical NMY-2/nonmuscle myosin II in endodermal precursors. Double loss-of-function embryos show reduced adhesion between isolated blastomeres.⁶³ How the combined effects of loss of HMR-1 and SAX-7 leads to widespread failure of NMY-2 recruitment is unclear, but these results highlight the utility of *C. elegans* for uncovering functionally interacting networks that affect cell–cell adhesion during embryogenesis.

4.2. VAB-9: A divergent claudin family member regulated by the cadherin complex

Positional cloning of a mutant with variable abnormal (Vab) defects identified a divergent member of the claudin superfamily, *vab-9*.⁶⁴ *vab-9* mutants show a variety of body shape defects, including mild disruption of CFBs similar to hypomorphic *hmp-1* mutants, suggesting *vab-9* functions within the same pathway. Consistent with this possibility, VAB-9 is expressed in all *C. elegans* epithelia and colocalizes with the CCC. HMR-1 is required for VAB-9 membrane localization and HMP-1 is required to maintain uniform VAB-9 distribution.⁶⁴ Mutations in *vab-9* enhance morphological

defects in weak *hmp-1* mutants and enhance cell adhesion defects following loss of AJM-1 or DLG-1. Thus, *vab-9* participates in the organization of F-actin at epithelial junctions and, alongside the AJM-1/DLG-1 complex, maintains proper epithelial adhesion.

4.3. ZOO-1/ZO-1: An associate of VAB-9 and the CCC that promotes strong adhesion

Further insight into the function of *vab-9* came from an analysis of the ZO-1 ortholog, ZOO-1.⁶⁵ Similar to *vab-9* mutants, loss of *zoo-1* function enhances the morphogenetic phenotypes of a weak *hmp-1* loss-of-function allele, *hmp-1(fe4)*. Several data indicate that *zoo-1* closely interacts with *vab-9*. ZOO-1 localizes to cell junctions in the epidermis and, like VAB-9, requires HMR-1, but not HMP-1 or HMP-2, for junctional localization. Moreover, ZOO-1 localization requires VAB-9, and loss of either affects the organization of F-actin in the epidermis of elongating embryos. However, loss of *zoo-1* function does not enhance the phenotypes of *vab-9* mutants, suggesting that they act in a common pathway. Both *vab-9* and *zoo-1* interact with mutations affecting the actomyosin contractile machinery. Loss of *zoo-1* activity enhances *mel-11* (myosin light chain phosphatase) mutants and suppresses *let-502* (ROCK) mutants,⁶⁵ whereas *vab-9* mutations suppress *mel-11* alleles (J. Simske, personal communication). Together these results suggest a regulatory pathway in which HMR-1 regulates VAB-9, and VAB-9, in turn, regulates epidermal morphogenetic processes, some of which are mediated by *zoo-1* and *mel-11*.

4.4. UNC-34/Enabled: A potential integrator of the cadherin and DLG-1/AJM-1 complexes

UNC-34 is the *C. elegans* homolog of *Drosophila* Enabled (Ena), a member of the Ena/VASP family of proteins.⁶⁶ In tissue culture, Ena/VASP proteins have been shown to play important roles in epithelial sealing events in culture,⁶⁷ and during neurulation.⁶⁸ Surprisingly, UNC-34 plays only a minor role in modulating motility in epithelial cells in *C. elegans*, apparently because *unc-34* is genetically redundant with the N-WASP homolog, *wsp-1*, during ventral enclosure.^{30,66} During enclosure, UNC-34::GFP localizes to the leading edges of migrating epidermal cells, but it is also redistributed to junctions when contralateral cells meet.³⁰ In primary mouse keratinocytes, junctional association of VASP depends on CCC function.⁶⁷ However, this does not appear to be the case in *C. elegans* because disrupting CCC function has little effect on UNC-34 localization.³⁰ Surprisingly,

UNC-34 junctional localization requires the activity of the DLG-1/Discs large binding partner AJM-1, which contains a putative consensus Ena/VASP binding motif.³⁰

Interestingly, *unc-34* synergizes with *hmp-1* during morphogenesis. Depleting UNC-34 in *hmp-1(fe4)* embryos results in frequent failure of ventral enclosure, suggesting that UNC-34 might partially compensate for reduced HMP-1 activity.³⁰ The reason for this is unclear; however, if α -catenin acts to regulate actin dynamics partly by antagonizing actin filament branching and promoting filament bundling,^{69,70} it is possible that UNC-34 could compensate for compromised α -catenin function by promoting actin bundles via a parallel pathway. Further experiments will be necessary to test this or alternative models.

4.5. MAGI-1: A SAX-7/L1CAM-dependent regulator of AFD-1/afadin that stabilizes junctions

The functional redundancy between HMR-1 and SAX-7 suggests that other proteins act synergistically or in parallel with the CCC during morphogenesis. A genome-wide RNAi screen identified 55 such enhancers⁷¹ (Fig. 11.2D). Among them was *magi-1*, a highly conserved inverted MAGUK protein.⁷² Loss of *magi-1* function in wild-type embryos results in disorganized epithelial migration and occasional morphogenetic failure during ventral enclosure, with loss of leading edge organization and excessive protrusive activity at the ventral midline. Knocking down *magi-1* in *hmp-1(fe4)* mutants leads to complete morphogenetic failure during elongation and actin disorganization in the epidermis.⁷¹

MAGI-1 localizes to cell junctions in all epithelial tissues in *C. elegans*^{71–73} at a position basal to the CCC and apical to the DLG-1/AJM-1 complex (see Fig. 11.2C). Although MAGI-1 is capable of binding HMP-2 in cellular extracts,⁷⁴ this interaction is apparently unimportant for localization of MAGI-1 in epithelia, since MAGI-1::GFP is initially targeted to junctions following depletion of HMR-1 or DLG-1.^{71,73} However, as embryos age, MAGI-1::GFP begins to lose its tight association with junctions under these conditions,⁷¹ indicating that the CCC and DLG-1/AJM-1 complexes are not crucial for MAGI-1's initial recruitment, but contribute to its maintenance at junctions.

MAGI-1 physically interacts with AFD-1/afadin/Canoe, which was also identified in the same screen, and which colocalizes with MAGI-1.⁷¹ Normal accumulation of MAGI-1 at junctions requires SAX-7, which can bind MAGI-1 via its C-terminus.⁷¹ This MAGI-1/AFD-1/SAX-7 middle layer

may help to spatially organize the other components of the apical junction. Depletion of MAGI-1 leads to loss of spatial segregation and expansion of the CCC and DLG-1/AJM-1 domains and greater mobility of junctional proteins in fluorescence recovery after photobleaching assays.⁷¹ These newly discovered molecular interactions between MAGI-1, AFD-1/afadin, and SAX-7/L1CAM appear to be part of a larger functional interactome that acts in concert with the core components of the CCC. Two other members of this interactome, SRGP-1/srGAP and UNC-94/tropomodulin, are described in the next two sections.

4.6. SRGP-1/srGAP: Bending adhesions into shape

SRGP-1, the *C. elegans* Slit/Robo GAP homolog, is another lethal enhancer of *hmp-1(fe4)*, and also enhances lethality in *hmp-2(qm39)* mutants.⁷⁵ Like its vertebrate counterparts, SRGP-1 contains an N-terminal F-BAR (Bin1, Amphiphysin, RVS167) domain, and a central GTPase activating (GAP) domain, which are thought to allow such proteins to regulate membrane curvature and modulate Rho family GTPase activity, respectively.⁷⁵ SRGP-1 colocalizes with the CCC at junctions; interestingly, expression of only the SRGP-1 F-BAR domain plus 200 downstream amino acids is sufficient to target SRGP-1 to junctions. This downstream sequence may be responsible for SRGP-1 interaction with the CCC as the F-BAR domain alone is not targeted to junctions.⁷⁵ SRGP-1 transgenes lacking GAP activity can rescue synergistic defects in *hmp-2(qm39)* mutants, suggesting that some of its functions are independent of its GAP activity. Whether SRGP-1's GAP activity is required in the more stringent *hmp-1(fe4)* background is currently unknown.

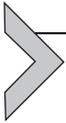
Overexpression of SRGP-1 leads to tubulations in the junctional membrane, which correlate with the level of SRGP-1 overexpression. Significantly, these tubulations contain HMR-1 and HMP-1, but not DLG-1,⁷⁵ suggesting that SRGP-1 aids adhesion by increasing the surface area of contact between adjacent cells at the level of the CCC. F-BAR proteins can also modulate actin dynamics more directly through their C-terminal domains⁷⁶; it remains unclear whether SRGP-1 has this capacity.

4.7. UNC-94: A regulator of HMP-1-dependent actin networks

UNC-94 is a tropomodulin (Tmod) ortholog⁷⁷ that is structurally very similar to vertebrate Tmods,⁷⁸ which cap the minus ends of actin filaments in a tropomyosin-dependent manner.⁷⁹ In body wall muscle sarcomeres,

UNC-94 is found at the minus (pointed) ends of thin filaments.⁷⁷ Biochemically, UNC-94 behaves like other Tmods, protecting pointed ends of actin filaments from depolymerization by UNC-60B/ADF/cofilin *in vitro*⁸⁰ and against minus-end subunit loss when actin monomer concentration is limiting.⁸¹

Like several other *hmp-1(fe4)* enhancers, *unc-94(RNAi);hmp-1(fe4)* embryos fail to elongate.⁸¹ Remarkably, UNC-94 localizes to a subset of cell–cell junctions in the epidermis in a *hmp-1*-dependent manner, at sites where lateral (seam) cells connect to dorsal and ventral cells; these junctions are under considerable mechanical stress.⁸¹ High-speed filming of GFP-tagged proteins at cell–cell junctions confirms the instability of junctions in double loss-of-function embryos: prior to complete mechanical failure, junctions in *unc-94(RNAi);hmp-1(fe4)* embryos exhibit “excursions” of JAC-1::GFP that are pulled away from the junction. Phalloidin staining of *unc-94(RNAi);hmp-1(fe4)* embryos reveals separation of the CCC and its associated junctional actin, and CFB detachment. *In vitro*, UNC-94 promotes formation of F-actin bundles that are longer than in the presence of HMP-1 alone. Taken together, these results suggest that UNC-94 stabilizes actin filaments against minus-end subunit loss at junctions, leading to a more mechanically rigid junction that is better able to withstand stress during elongation.



5. BEYOND THE CLASSICAL CCC: OTHER CADHERIN FAMILY MEMBERS IN *C. ELEGANS*

The nonclassical cadherins in *C. elegans* are much less well understood, in part because single loss-of-function mutations do not result in strong, easily interpretable phenotypes, and in some cases, have no observable phenotype at all. In addition, many of the *C. elegans* cadherins are highly divergent. Coupled with the fact that many of the pathways that their putative homologs impinge upon in other animals are significantly altered in *C. elegans*, this has made functional comparisons difficult. We have thus restricted our discussion to those cadherins that have clear mutant phenotypes, and/or have clear homologs in other organisms.

5.1. CDH-3 and CDH-4: Fat-like cadherins that do not modulate planar cell polarity

Two *C. elegans* cadherins, CDH-3 and CDH-4, structurally resemble the large *Drosophila* cadherin, Fat, with regard to the organization of their ectodomains.⁸² However, their cytoplasmic domains are highly divergent

from Fat or Fat-like cadherins found in other animals and lack sequence motifs conserved across many metazoan phyla⁸³ (see also [Chapters 4 and 10](#)). Thus, unambiguous assignment of orthology is not possible. In addition, while *Drosophila* Fat is a key component of the planar cell polarity (PCP) and Hippo signaling pathways,⁸⁴ there is no functional evidence implicating either CDH-3 or CDH-4 in the regulation of cell polarity through these pathways. Moreover, several members of the Hippo pathway are missing in *C. elegans*,⁸⁵ and despite concerted efforts, there is currently no clear evidence for a true PCP pathway in *C. elegans*.^{34,35,86}

Loss of *cdh-3* function causes mild, variably penetrant defects in the morphogenesis of a single epidermal cell, together with weakly penetrant defects in the morphology of the excretory system³ (L. Hodgson and J. Pettitt, unpublished). In addition, overexpression of CDH-3 enhances the penetrance of defects in the morphogenesis of the buccal epithelium in animals lacking the miR-51 family of microRNAs.⁸⁷ *cdh-4* loss-of-function mutants show defects in axon fasciculation, synapse formation, and the migration of a pair of neuroblasts, as well as incompletely penetrant defects involving epithelial cells.⁸⁸ The precise cellular bases of the *cdh-3/-4* loss-of-function phenotypes are not known, but defects in cell polarity cannot be ruled out.

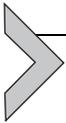
5.2. FMI-1: A Flamingo/Stan protein that modulates fasciculation in follower axons

fmi-1 encodes the sole *C. elegans* homolog of Flamingo/Starry night, a seven-pass transmembrane protein that is a core PCP pathway component in *Drosophila* and mice.⁸⁹ In addition, *Drosophila* Flamingo can mediate neuronal morphogenesis through both PCP-dependent and -independent mechanisms.^{90,91} One of the Flamingo orthologs in mice is likewise involved in fore-brain axon guidance⁹² (see also [Chapter 9](#)). In *C. elegans*, it seems unlikely that FMI-1 functions in a conventional PCP pathway (see above), but recent experiments show that it is involved in axon guidance.

FMI-1 contributes to navigation of both pioneer and follower axons during embryogenesis.⁹³ Guidance of PVQ and HSN axons is dependent on FMI-1's extracellular domain.⁹³ Intriguingly, genetic evidence from VD motor neurons suggests that FMI-1 may interact with CDH-4.⁹⁴ The possibility of such heterophilic interactions has some precedent in other systems.⁹⁵ The intracellular domain of FMI-1 is cell-autonomously necessary for PVP pathfinding but dispensable for PVQ and HSN fasciculation,⁹³ suggesting that FMI-1 may be involved in signaling required for pioneer axon guidance.

5.3. CASY-1: A calsynenin involved in associative learning

Comparison of CASY-1 to its mammalian and *Drosophila* homologs shows that these proteins share significant sequence similarity along their entire lengths.² The mammalian homologs are termed calsynenins because their cytoplasmic domains can bind synaptic calcium.⁹⁶ Calsynenins are associated with the postsynaptic membranes of excitatory CNS synapses. The extracellular domains of calsynenins are proteolytically cleaved close to the membrane, and the transmembrane-intracellular portion is then internalized. This has led to a model whereby calsynenins modulate postsynaptic calcium levels.⁹⁶ CASY-1 may play a similar role in *C. elegans*. Based on transgene assays, CASY-1 is widely expressed in the nervous system,⁹⁷ and loss-of-function studies show that it plays a role during associative learning in *C. elegans*.^{97,98} Increasing the gene dosage of the glutamate receptor subunit GLR-1 can compensate for behavioral defects of *casy-1* mutants, suggesting that CASY-1 positively regulates GLR-1 signaling during olfactory associative learning.⁹⁸ Like its vertebrate homologs, CASY-1 is cleaved and released from neurons; indeed, the ectodomain of CASY-1 is sufficient to rescue defects in salt chemotaxis learning.⁹⁷



6. CONCLUSIONS

C. elegans has emerged as an outstanding model system for the *in vivo* functional analysis of the cadherin/catenin complex during morphogenesis. By analyzing this basal metazoan, it should be possible to identify core functionalities of the CCC that allow cells to specifically adhere to each other, to modulate those adhesions, and to withstand the inherent stresses during embryonic morphogenesis. Recent genome-wide functional screens have been especially fruitful for identifying new molecular complexes that partner with the core components of the CCC during morphogenesis. Understanding in detail how these complexes function in *C. elegans* and in vertebrates is an exciting challenge for the future.

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