

Cadherin complexity: recent insights into cadherin superfamily function in *C. elegans*

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Cadherin superfamily proteins mediate cell–cell adhesion during development. The *C. elegans* embryo is a powerful system for analyzing how cadherins function in highly stereotyped morphogenetic events. In the embryo, the classical cadherin HMR-1 acts along with the Rac pathway and SAX-7/L1CAM during gastrulation. As adherens junctions mature, PAR complex proteins differentially regulate cadherin complex localization, and SRGP-1/Slit/Robo GAP aids adhesion by promoting membrane bending. Once adherens junctions form, actin is linked to the cell surface via HMP-1/ α -catenin, whose actin binding activity is regulated in novel ways. FMI-1/Flamingo and CDH-4/Fat-like regulate axonal morphology of both pioneer and follower neurons. *C. elegans* thus continues to be useful for uncovering precise functions for cadherin superfamily proteins and their associates in a simple metazoan.

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Introduction: *C. elegans*: a simple system for studying cadherins

The classical cadherin system connects cadherins to the actin cytoskeleton via β -catenin and α -catenin to maintain tissue integrity in metazoans [1]. How the cadherin–catenin complex (CCC) is regulated and how it functions together with other adhesion systems in complicated, 3d tissue environments has important implications for understanding morphogenesis and how CCC dysfunction promotes cancer. The nematode *C. elegans* is a useful model for analyzing CCC function in such complex environments. It possesses a single classical cadherin, HMR-1; the short isoform, HMR-1A, forms a complex with HMP-1/ α -catenin and HMP-2/ β -catenin [2] in epithelia, where the HMR/HMP complex functions like

the vertebrate adherens junction (AJ) to mediate cell–cell adhesion and junctional actin organization. An alternative HMR-1 isoform, HMR-1B, is expressed in neurons [3]. *C. elegans* also possesses conserved members of the atypical cadherin superfamily [4] (Table 1). Here we review recent work that sheds light on the CCC during gastrulation and epidermal morphogenesis in *C. elegans*. We also discuss recent studies implicating FMI-1/Flamingo and the Fat-like atypical cadherin, CDH-4, in axon fasciculation in the ventral nerve cord.

Not alone: the cadherin complex cooperates during gastrulation

C. elegans morphogenesis begins with gastrulation, initiated by ingression of the endodermal precursor cells, Ea and Ep (Figure 1A). Ingression of Ea and Ep is partly driven by actomyosin-mediated apical constriction, which helps to pull adjacent cells over the apical surface of Ea/Ep and displaces the pair internally [5[•]]. Cadherin-based adhesion appears to be involved in this process: knockdown of maternal and zygotic *hmr-1* message by RNAi reduces the rate of Ea/Ep apical constriction by approximately 50% [6^{••}]. Nevertheless, the eventual ingression of endodermal precursors in *hmr-1* loss of function embryos indicates that one or more redundant systems contribute to Ea/Ep ingression. SAX-7/L1CAM has recently been identified as a component of such a system. Knockdown of *hmr-1*, *hmp-1*, or *hmp-2* in *sax-7* mutants results in failure of apical constriction and ingression, which may be partly due to reduced adhesion between blastomeres in these backgrounds, based on blastomere isolation experiments [7^{••}].

Additionally, HMR-1 and SAX-7 contribute to endodermal precursor ingression by promoting apical enrichment of NMY-2/nonmuscle myosin II in Ea and Ep [7^{••}], and HMR-1 may help transmit actomyosin tension to the membrane (Figure 1A'). In *hmr-1(RNAi)* endodermal precursors, membrane movements concomitant with myosin translocation are diminished [6^{••}], similar to other systems in which actomyosin contractions are uncoupled from AJs [8,9]. Given the role of the CCC in stabilizing F-actin linkages to the AJ in other tension-driven processes [10,11], it is possible that HMR-1 contributes to stabilizing the cytoskeletal machinery required for apical constriction here as well. Consistent with this possibility, the CCC interacts genetically with the Rac pathway in endodermal precursors. Knockdown of CCC components along with Rac pathway components, including CED-5/Dock180, CED-12/ELMO, and CED-10/Rac, abrogates Ea/Ep

Table 1

Cadherin superfamily proteins in <i>C. elegans</i>			
Class	<i>C. elegans</i> cadherin	Localization	Observed functions
Classical E-cadherin	HMR-1A	Blastomeres	ABar spindle orientation. Blastomere compaction. Ingression of Ea, Ep, and PGCs. Cleft closure.
		Epithelia	Epidermal sheet sealing. Actomyosin force transmission. Maintenance of epidermal and intestinal tissue integrity.
Classical N-cadherin	HMR-1B	Neurons	AS, DD, and VS axon guidance. AS fasciculation.
Flamingo	FMI-1	Neurons	PVP, PVQ, and HSN axon guidance and fasciculation. DD and VD axon guidance and synapse formation.
Fat-like	CDH-3	Excretory cell hyp10, hyp11	Excretory cell outgrowth Tail morphology
		Anchor, arcade, F, seam, U, and uterine seam cells, VC neurons, and vulval precursors	–
Calsyntenin	CDH-4	Neurons, vulva, spermatheca	–
	CASY-1	Intestine, gonadal sheath Neurons	– Associative learning
Daschous?	CDH-1	Pharynx	Larval viability, growth, locomotion.
Dcad96Cb	CDH-9		
Nematode-specific	CDH-5, 7, 8, 12	mc1, mc2, K, K', and seam cells	
	CDH-10		

apical constriction without loss of apical NMY-2 [5,6^{••}]. Thus 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, which neighboring mesodermal precursors extend over Ea/Ep [12,13].

While the specific cells in which HMR-1-mediated adhesion is required remains to be experimentally investigated during Ea/Ep ingression, such specificity has been determined for another HMR-1-dependent internalization event, ingression of the primordial germ cells (PGCs). Following Ea/Ep ingression, PGCs maintain contact with endodermal daughters and are eventually drawn into the interior by the dorsal movement of the endoderm [14^{••}]. PGC internalization requires surface enrichment of HMR-1 in the PGCs, driven post-transcriptionally via a specific element in the 3'-UTR of the *hmr-1* mRNA [14^{••}]. As HMR-1 expression in PGCs alone is both necessary and sufficient for their internalization [14^{••}], it is possible that HMR-1 participates in heterotypic interactions in this context, but this remains to be demonstrated.

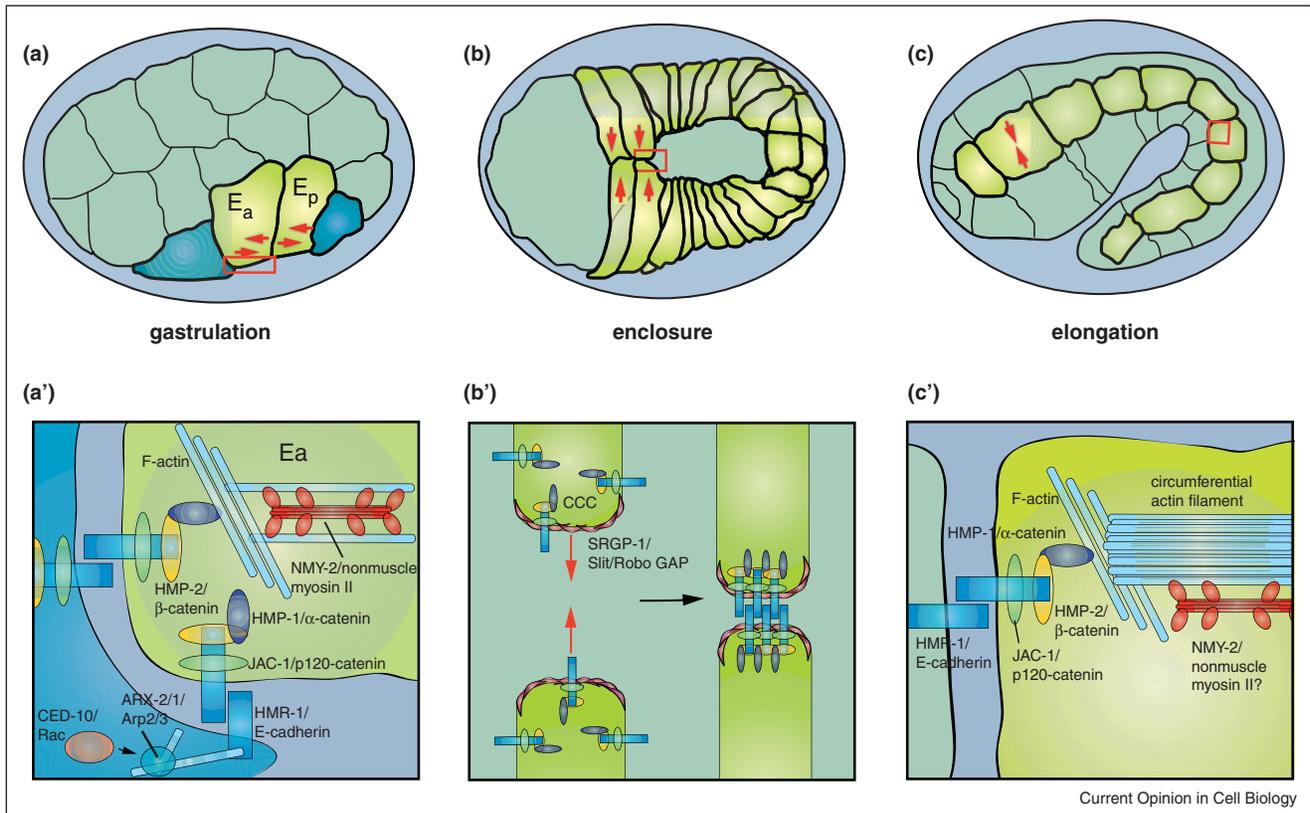
Dropping the HMR: PAR proteins and cadherin complex localization

Cadherin localization in metazoan epithelia depends on the establishment of apicobasal polarity [15], which

crucially depends on PAR proteins [16,17]. While PAR-3/Bazooka and PAR-6 do not appear to affect HMR-1 localization at basolateral cell–cell contacts in the early *C. elegans* embryo [18^{••}], they are critical for establishing apicobasal polarity and CCC localization in epithelia. At the earliest stages of *C. elegans* intestinal differentiation, CCC and polarity complex proteins colocalize to foci, which then migrate and aggregate at the future apical surface [19,20]. As apicobasal polarity is established, CCC puncta coalesce into persistent junctional belts that maintain intestinal tissue integrity. Knockdown of CCC components results in loss of apical F-actin in the embryonic intestine and dilation of the intestinal lumen, consistent with this role [21[•]]. In the absence of PAR-3, intestinal HMR-1 is initially dispersed and mislocalized, whereas HMP-1 is still recruited into foci [19]. HMP-1 localization is aberrant, however; it colocalizes with DLG-1/Discs Large [18^{••}], normally found basal to the CCC [22]. Basolateral foci of HMP-1 and DLG-1 accumulate despite unperturbed localization of LET-413/Scribble [19], which normally excludes AJ components from basolateral surfaces [23,24]. Similar localization defects are seen in the mesendodermal epithelium of the pharynx in *par-3* mutants [19].

While PAR-3 is critical for CCC establishment in endodermal and mesodermal epithelia, it is surprisingly dispensable in the epidermis, although localization of HMR-1 and DLG-1 is progressively lost and the epidermis tears

Figure 1



The classical cadherin–catenin complex (CCC) regulates multiple morphogenetic events during *C. elegans* embryogenesis. 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, E_a and E_p , via apical constriction (A, red arrows), which internalizes E_a and E_p and aids covering of the space they vacate by pulling on neighboring cells (blue). 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 towards the ventral midline (B, red arrows). The first cells to meet at the midline ('leading cells') rapidly make cadherin-dependent contacts, which are aided by the SRGP-1/Slit-Robo GAP. (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, red 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.

[19]. This difference may be due to an inherent difference in tissue organization: the epidermis is in contact with the developing cuticle. PAR-3-independent apical targeting of cadherin is also seen during cellularization of the first epithelium in *Drosophila* [25]. In both the *C. elegans* intestine and the epidermis, PAR-6 is required for junctional maturation, but not targeting of CCC to initial spot junctions [20]. Curiously, apical enrichment of PAR-6 does not occur in *par-3* mutants [19]. How PAR-6 can aid junctional maturation in such mutant backgrounds but not be at its normal location is unclear.

Bending adhesions into shape: membrane curvature and cadherin-based adhesion

Morphogenesis of the *C. elegans* epidermis can be divided into three major events: dorsal intercalation, ventral enclosure, and elongation [26]. To date, no function for the CCC has been identified during intercalation.

However, AJs must be remodeled during intercalation [27] and cadherins and catenins are key proteins in convergent extension events in other systems [28,29], so the possibility remains that functions for HMR-1 will be uncovered during intercalation when examined in an appropriately sensitized backgrounds. By contrast, the CCC is crucial for enclosure and elongation. During ventral enclosure, ventral epidermal cells extend protrusions as they migrate and initiate sealing at the ventral midline via these protrusions (Figure 1B) [30]. Maternal and zygotic CCC knockdown results in loss of adhesion between opposing cells, failure of epidermal enclosure, and consequently the Hammerhead (Hmr) phenotype [31]. Once enclosed, the embryo elongates via actomyosin-mediated tension, which is transmitted via circumferential F-actin filament bundles (CFBs) in the epidermis [32,33] (Figure 1C). In zygotic null mutants for *hmp-1* and *hmp-2*, maternal mRNA carries the embryo through

ventral enclosure, but mutants fail during elongation, as the embryos retract dorsally and adopt a characteristic Humpback (Hmp) phenotype [2].

Owing to the mechanical stress exerted on the epidermis during elongation, its morphogenesis requires robust cell–cell adhesion. However, adhesion must also be dynamically regulated. The hypomorphic catenin alleles *hmp-1(fe4)* and *hmp-2(qm39)* display elongation defects and serve as sensitized backgrounds for identifying genetic modulators of CCC function. Such modulators include UNC-34/Ena, ZOO-1/ZO-1, and JAC-1/p120ctn [34–36]. Another is the Slit-Robo GAP homologue, SRGP-1 [37**]. 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. *srgp-1* knockdown enhances catenin hypomorphic phenotypes, and interestingly, expression of only the F-BAR domain and 200 downstream amino acids of SRGP-1 is sufficient to target the transgene to AJs and rescue *hmp-2(qm39)*; *srgp-1(RNAi)* synthetic phenotypes [37**], independent of its GAP activity. By contrast, in its role during engulfment of cell corpses, SRGP-1's GAP activity is required [38].

The F-BAR domains of vertebrate srGAP2 and srGAP3 produce outward membrane protrusions [39,40]. Similarly, overexpression of SRGP-1 induces numerous sizeable excursions of the junctional membrane, which appear to be outward bends, based on mosaic expression. These excursions contain HMR-1 and HMP-1, but not DLG-1 [37**], suggesting that SRGP-1 may contribute to adhesion by increasing the surface area of contact between adjacent cells specifically at the level of the CCC (Figure 1B'). Consistent with this model, *srgp-1(RNAi)* embryos display defects in gastrulation cleft closure and a decrease in membrane dynamics at nascent contacts during ventral enclosure. BAR proteins also modulate actin dynamics [41,42*]; it remains to be determined whether SRGP-1 possesses this function as well.

Hidden talent: new insights into α -catenin regulation through HMP-1

How the cadherin–catenin complex mediates dynamic connections to the actin cytoskeleton has been a subject of considerable debate. α -catenin can bind and bundle actin at sites of E-cadherin-mediated adhesion, and β -catenin can act as a molecular bridge between cadherin and α -catenin [1,11]. Bringing α -catenin to the junction via direct fusion with cadherin rescues some adhesive functions of adhesion-defective cells, both *in vitro* [11] and in *Drosophila* [43] suggesting that the CCC can function as a quaternary complex. Such a model may not fully capture how vertebrate α E-catenin normally

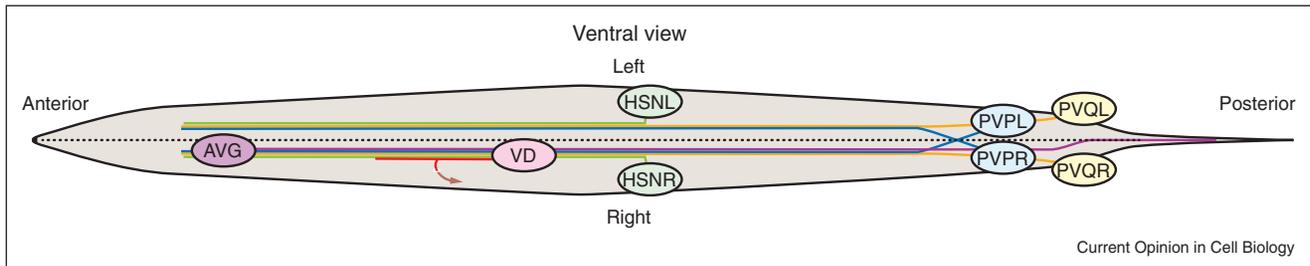
functions, however. α E-catenin can associate with the CCC, but does not bind actin simultaneously *in vitro*; to do so it forms homodimers with high affinity for F-actin [44]. Recent experiments also indicate that the vertebrate CCC can undergo mechanosensitive adhesive strengthening, which involves the actin-binding protein vinculin [45]; α E-catenin may undergo conformational changes as part of this process that allow it to bind and recruit vinculin [46].

HMP-1 provides some advantages that simplify analysis of α -catenin function *in vivo*. Vinculin is not present in epithelia in *C. elegans*, and deletion of the region homologous to the α E-catenin sequence that binds vinculin seems to have little effect on HMP-1 function (S. Maiden and J. Hardin, unpublished). Moreover, the tension-bearing mechanical requirements on HMP-1 owing to the stresses of elongation are stringent (Figure 1C, C'). 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. Deletion of the N-terminal β -catenin binding domain similarly results in morphogenetic failure, with loss of recruitment of HMP-1 to junctions [47**]. Interestingly, HMP-1 appears conformationally autoinhibited *in vitro*: full-length HMP-1 only binds F-actin weakly *in vitro*, whereas C-terminal fragments bind actin avidly [47**]. Whether HMP-1 is regulated *in vivo* by application of tension to relieve this autoinhibition remains to be determined, but a common theme seems to be emerging that α -catenin functions as a switch through which CCC dynamics can be modulated. This pivotal function of α -catenin may reflect its evolutionary origins. Recent work in *Dictyostelium* suggests that an α -catenin-like molecule predates cadherin as a polarized epithelial regulator of morphogenesis [48].

Follow the leader: FMI-1/Flamingo and CDH-4/Fat-like during neuronal morphogenesis

The N-cadherin homolog HMR-1B and the Fat-like atypical cadherin CDH-4 have previously been demonstrated to promote axon guidance and fasciculation of neurons in the ventral nerve cord (VNC) [3,49]. Recent experiments show that FMI-1/Flamingo is also required for these processes, perhaps through a *trans* interaction with CDH-4. During embryogenesis, the left VNC tract is pioneered by the right PVP neuron (PVPR), which extends an axon across the ventral midline and proceeds anteriorly to the nerve ring (Figure 2). Subsequently, neurons such as PVQL and HSN extend axons that follow the established tract by fasciculating with the pioneer. FMI-1 contributes to both of these processes, being required for left VNC pioneering and for pathfinding and fasciculation of the PVQ and HSN axons that follow [50**].

Figure 2



The atypical cadherins FMI-1/Flamingo and CDH-4/Fat-like are required for axon guidance and synapse development. PVP neurons reside in the pre-anal ganglion, where, in the embryo, they extend axons that cross the ventral midline and proceed anteriorly to the nerve ring. Pioneering of the left ventral nerve cord (VNC) by PVPR requires FMI-1 and CDH-4. The right VNC is pioneered by posterior axon extension of AVG, which is unaffected by loss of either cadherin. PVQ axons originating in the lumbar ganglia extend along the ipsilateral nerve cord immediately following PVP processes. PVQL navigation requires both FMI-1 and CDH-4, while PVQR displays only a moderate requirement for FMI-1. After the L1 stage, 13 VD and 6 DD GABAergic motor neurons lie in the right VNC (one pictured), where they extend axons anteriorly and through commissures to the dorsal cord. VD and DD synapse with ventral and dorsal body wall muscles, respectively, and their axon guidance and synapse formation are dependent on both FMI-1 and CDH-4. During L2 and L3 larval stages, HSN neurons extend axons ventrally, innervating the vulva and fasciculating with the ipsilateral VNC to extend anteriorly. Both HSN neurons require FMI-1 for correct axon morphology.

The cadherin domains target FMI-1 to axons and contribute to PVQ and HSN guidance [50^{••}], implicating FMI-1-mediated adhesion in fasciculation of followers in the VNC. Evidence from VD motor neurons in the VNC suggests that FMI-1 may interact with CDH-4 [51[•]], which participates in heterophilic binding in other systems [52]. Mutations in *cdh-4* and *fmi-1* also produce similar synaptic defects that are not enhanced in double mutants [51[•]], suggesting that heterophilic binding between Flamingo and Fat-like cadherins may also promote proper synapse formation at neuromuscular junctions.

Structure–function analysis also sheds light on the intracellular events downstream of FMI-1. The intracellular domain of FMI-1 is necessary for PVP pathfinding but dispensable for PVQ and HSN fasciculation [50^{••}], suggesting that FMI-1 may have signaling activity necessary for pioneer axon guidance. *Drosophila* Flamingo can mediate neuronal morphogenesis through both Planar Cell Polarity (PCP) pathway-dependent and PCP-independent mechanisms [53–55]. However, there is currently no clear evidence for a full PCP pathway in *C. elegans*, and FMI-1 does not appear to function in a PCP-like pathway [50^{••},56]. This raises the possibility that further study of Flamingo cadherins in the VNC of *C. elegans* may shed additional light on PCP-independent mechanisms of atypical cadherin function in neuronal morphogenesis.

Conclusions

C. elegans is a powerful system for studying both classical and atypical cadherins. The ability to identify highly specific cellular behaviors that are regulated by cadherin family members and their associates should continue to make *C. elegans* useful for clarifying how these important

cell adhesion proteins function during morphogenesis in multicellular organisms.

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