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REVIEW

Hemicentins: What have we learned from worms?

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Hemicentins are conserved extracellular matrix proteins discovered in *Caenorhabditis elegans,* with orthologs in all vertebrate species including human and mouse. Hemicentins share a single, highly conserved amino-terminal von Willebrand A domain, followed by a long (>40) stretch of immunoglobulin repeats, multiple tandem epidermal growth factors and a fibulin-like carboxy-terminal module. *C. elegans* has a single hemicentin gene that has pleiotropic functions in transient cell contacts that are required for cell migration and basement membrane invasion and in stable contacts at hemidesmosome-mediated cell junctions and elastic fiber-like structures. Here, we summarize what is known about the function of hemicentin in *C. elegans* and discuss implications for hemicentin function in other species.

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Introduction

Extracellular matrix (ECM) proteins form networks that contain structural and regulatory information that influence cell adhesion, migration, survival, differentiation and polarity. ECM networks have distinct geometries and vary from tissue to tissue, and they are determined by the availability of ECM components and their assembly mechanism. Hemicentins are a novel family of ECM proteins that assemble into polymers that refine broad regions of cell contact into discrete cell junctions with a line-shaped architecture. Here we discuss what we have learned about the function, distribution, assembly and interactions of hemicentin in *C. elegans* and some of the implications of this work for hemicentins in other species.

Hemicentin structure

Hemicentins are among the most ancient ECM proteins, with highly conserved orthologs in nearly all metazoans that share a number of common structural motifs [1]. The most highly conserved domain is an amino-terminal von Willebrand A (VWA) domain [2]. This domain is followed by a long (>40) stretch of tandem immunoglobulin (Ig) repeats, multiple tandem epidermal growth factors (EGFs) and a fibulin carboxy-terminal (FC) module that is found in members of the fibulin family of ECM proteins [3-5]. The two human and mouse hemicentins have a nidogen G2F motif between the Ig and EGF domains. In addition, vertebrate hemicentin-1 proteins also have a series of six tandem thrombospondin repeats inserted between Ig and nidogen G2F domains (Figure 1, Table 1).

Hemicentin functions

History of hemicentin – discovery as him-4 locus

Perhaps the most surprising phenotype associated with hemicentin mutations is the one that led to its initial discovery. The *C. elegans* karyotype has five pairs of autosomes and one or two X-chromosomes in males and hermaphrodites, respectively. Rare meiotic non-disjunction of the X-chromosome produces a low frequency of nullo-X gametes that results in a low frequency of males (~0.2%) among the self-progeny of wild-type hermaphrodites. A series of 17 *him* (high incidence of male progeny) mutants increase the frequency of male self-progeny 10-150-fold [6]. Among the *him* loci, *him-4* is unique in

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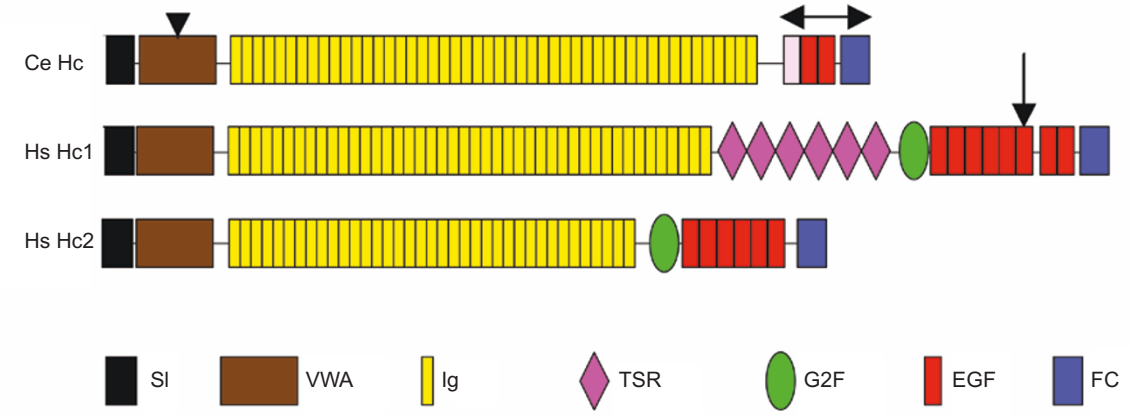

Figure 1 Schematic diagram of *C. elegans* hemicentin and human hemicentin-1 and -2. Represented protein modules include signal sequence (SI); von Willebrand A (VWA); immunoglobulin (Ig); thrombospondin (TSR); nidogen G2F (G2F); epidermal growth factor (EGF); and fibulin type carboxy-terminal modules (FCs). Arrowhead indicates VWA domain in *C. elegans* hemicentin with homing activity and double arrowhead indicates EGF/FC modules in *C. elegans* hemicentin that have assembly activity. An additional homing domain is located within the 48 Ig repeats and an additional assembly domain is located between Ig13 and Ig48. Arrow indicates location of ARMD-1-associated mutation in EGF 6 of human hemicentin-1. The human hemicentin-1 cDNA sequence has been determined experimentally (Accession No. AAK68690), but hemicentin-2 structure is a prediction based on publicly available partial cDNA sequences and analysis of genomic DNA sequence by exon identification algorithms.

Table 1 Hemicentin gene locations

Organism	Gene name/Protein name	Chromosome
C. elegans	*him-4*/Hemicentin	X
Mouse	Hemicentin-1	1
Mouse	Hemicentin-2	2
Human	HMCN1/hemicentin-1	1q25–31
Human	HMCN2/hemicentin-2	9q34

that mutants also have a series of pleiotropic defects in cell adhesion and behavior (see below). Positional cloning of the *him-4* locus revealed that this gene encodes a novel secreted protein that is conserved in vertebrates and named hemicentin because of its approximately 50 (hemi-centi) structural modules and frequent association with hemidesmosomes [3]. The *him* phenotype raises an obvious question: How can a large ECM protein affect chromosome segregation?

The germline of *C. elegans* consists of a cortical layer of cells separated by incomplete cleavage furrows. The cells are peripheral, surrounding a common cytoplasmic core, or rachis. Hemicentin assembles in the extracellular space, between syncytial germ cell plasma membranes (Figure 2). In the absence of hemicentin, these membranes are extremely disorganized, suggesting that hemicentin is required for the structure and/or stability of these membranes [3]. Although direct evidence for the role of hemicentin in chromosome segregation is lacking, a reasonable model is that defects in plasma membrane structure produce corresponding defects in the cortical cytoskeleton that result in mis-positioning of the chromosomes and/or spindle, interfering with their function during mitosis. An alternative model is that in the absence of enveloping cell membranes, chromosomes could be captured or interfered with by spindle microtubules invading from adjacent germ cells. In both cases, chromosome segregation defects would not be X-specific and should affect all chromosomes equally. The observations of oocytes with abnormal karyotypes and inviable, presumably autosomal aneuploid embryos at a high frequency are consistent with these models [3].

Hemidesmosome-mediated anchorages

In *C. elegans*, hemidesmosome-like structures mediate multiple tissue attachments to the epidermis (called hypodermis in *C. elegans*), including those of muscle, mechanosensory neurons and uterus (Figure 2). Line-shaped uterine and mechanosensory neuron anchorages are hemicentin dependent, while punctate muscle anchorages are not, suggesting that hemicentin may be critical only for cell junctions with specific elongated shapes. (It should be noted that several components of vertebrate hemidesmosomes—e.g. the integrin β4-subunit—are not present in the *C. elegans* genome, suggesting that structures that are

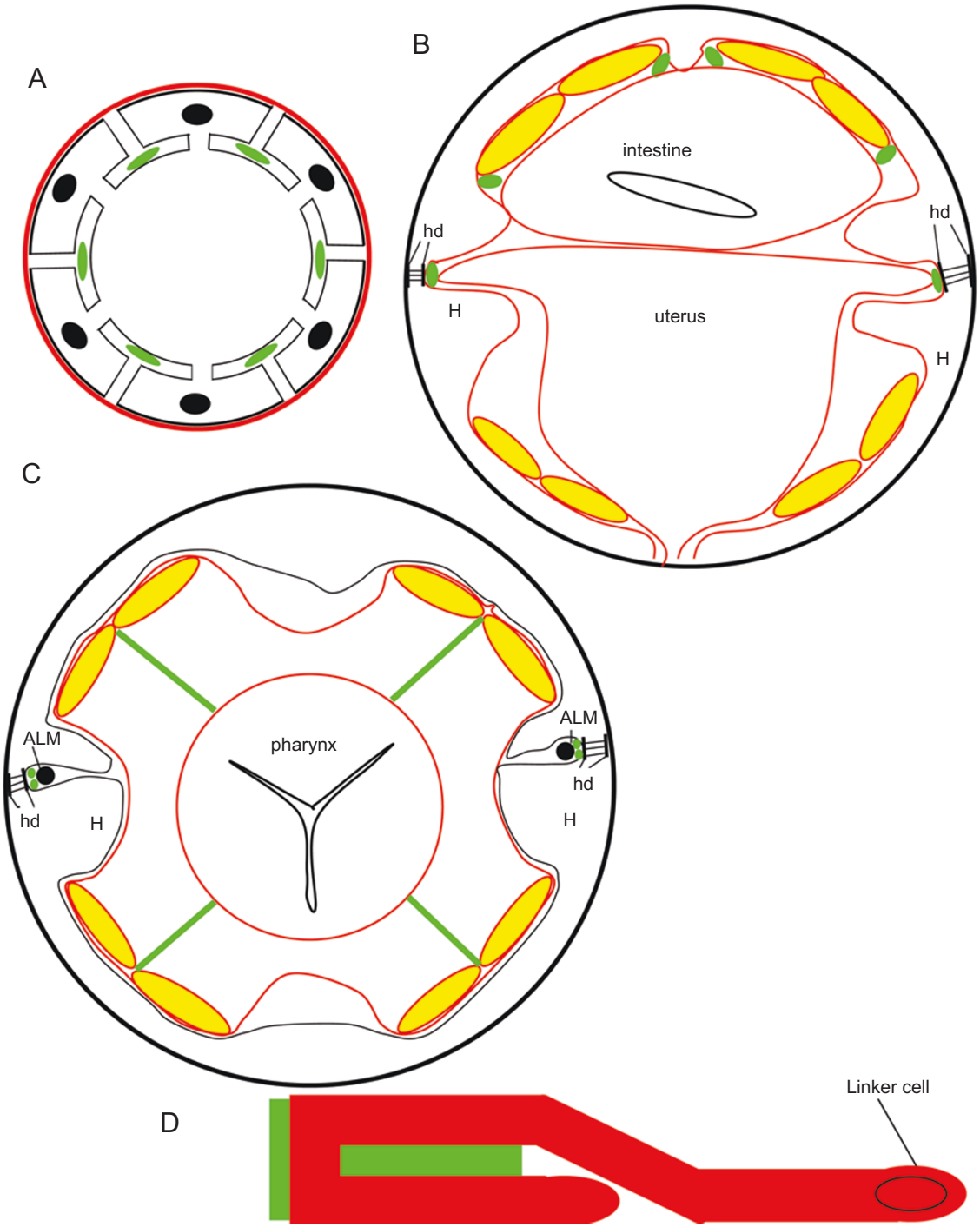

Figure 2 Schematic diagram of hemicentin assembly locations (green) on *C. elegans* germline and somatic cells. Basement membranes are shown in red and muscle cells are shown in yellow. **(A)** Hemicentin assembly on plasma membranes of syncytial germ cells in hermaphrodites and males. Based on the mutant phenotype, hemicentin appears to be essential for the synthesis or stability of these membranes. **(B)** Hemicentin assembly at hemidesmosome (hd)-mediated junctions between the uterus and hypodermis (H) and at junctions where the intestine, hypodermis and muscle converge (n.b. hds and intermediate filaments also mediate anchorage of body wall muscle to hypodermis but are not depicted here). **(C)** Hemicentin assembly in anterior locations where ALM mechanosensory neurons (and PLM mechanosensory neurons in the posterior) are anchored by hds and intermediate filaments to cuticular exoskeleton through a thin sheet of hypodermal cell (H) cytoplasm. Also shown are hemicentin and fibulin-1-containing elastic fiber-like structures that connect basement membrane of pharynx at one end and insert between adjacent lateral and medial body wall muscle cells in all four quadrants. **(D)** Sites of hemicentin deposition on male gonad between proximal and distal gonad arms and between gonad and hypodermis. For primary data, see reference [3].

functionally and ultrastructurally very similar to hemidesmosomes can be assembled from distinct combinations of molecular components [1].)

Late in larval development, a multinucleate uterine cell (utse) contacts lateral epidermal (seam) cells on their basal surface, inducing epidermal squamification and thickening of the basement membranes between them [7]. Within the seam cell cytoplasm, hemidesmosomes and intermediate filaments form an assembly that indirectly links the uterine cell to the collagenous exoskeleton (cuticle) located on the apical surface of the seam cells [7, 8]. In *him-4* mutants, hemidesmosome and intermediate filament assembly is defective and the initial broad attachment of utse to seam cells fails to mature and narrow into a long thin line. As a result, the uterus is not anchored properly to the cuticle. Once egg laying commences, the uterus prolapses through the vulva, resulting in small brood sizes and premature death [3].

Hemidesmosome and intermediate filament anchorages to the cuticle are also induced by four lateral mechanosensory neurons that mediate the nematode escape reflex [9]. Mechanosensory neuron axons, located between epidermal cell surfaces and basement membranes, accrete a thick ECM, known as mantle, between the axon and epidermal cell surface. Hemicentin assembles into parallel tracks that are 0.2 μm thick and over 300 μm long on each side of mechanosensory neuron axons (Figure 2). In the absence of hemicentin, mantle, hemidesmosomes and intermediate filaments are absent and the axons are unanchored, remaining in their juvenile position, adjacent to body wall muscle. Despite the ultrastructural defects, mechanosensation is relatively unaffected in *him-4* mutants, suggesting that mantle- and hemidesmosome-mediated anchorages are not essential components of the mechanotransduction apparatus [3, 10].

Anchor cell invasion

In a recently described *in vivo* model of basement membrane invasion, the *C. elegans* anchor cell, found within uterine tissue, penetrates uterine and vulval basement membranes and invades vulval tissue, forming a connection that allows fertilized eggs to pass from the uterus to the exterior of the animal [11]. Anchor cell invasion of vulval epithelium is dependent on FOS-1, a transcription factor that controls the expression of the *cdh-3*, *zmp-1* and *him-4* genes, which encode a fat-like protocadherin, a membrane-bound matrix metalloproteinase and hemicentin, respectively [11]. Prior to invasion, hemicentin accumulates at the site of invasion and the anchor cell extends processes at the site of hemicentin deposition. Once invasion begins, hemicentin appears to be degraded, forming punctate aggregates. In the absence of hemicentin, invasion is delayed but can still occur [11]. Hemicentin appears to promote anchor cell contact with the basement membrane and may help identify the region of the basement membrane that is targeted for destruction. It will be interesting to determine whether vertebrate hemicentins promote basement membrane invasion during normal development and during tumor cell metastasis.

Linker cell migration

As *C. elegans* males develop through four larval stages (L1–4), gonad morphogenesis is directed by the linker cell as it migrates. This cell pulls the vas deferens behind it as it makes a dorsal turn in the L2 stage followed by a posterior turn. In *him-4* mutants, this migration is defective and males are sterile because the vas deferens never reaches the cloaca. Hemicentin is secreted by the linker cell as it migrates and hemicentin tracks assemble on its surface and on the trailing gonad as it is passively pulled along. It is possible that hemicentin has an adhesive role at these sites. However, in the L3 stage, tracks accumulate between the basement membranes of proximal and distal gonad arms, which are sites of the greatest relative tissue movement (Figure 2). We therefore speculate that hemicentin may act as a molecular lubricant, allowing the basement membranes of adjacent tissues to glide past each other [3].

Intestine

Like the uterus, anterior and posterior intestinal cells make line-shaped attachments to the body wall where intestine meets muscle and hypodermis (Figure 2). These junctions are mediated by hemicentin, although these attachments are not associated with hemidesmosomes. In the uterus and intestine, line-shaped attachments have an advantage over uniform attachments since they allow the lumen of these tube-shaped organs to fill and empty freely while remaining attached to the body wall. In the absence of hemicentin, the intestine is unattached, and floats freely in the body cavity, leading to occasional rectal prolapse [3].

Hemicentin assembly

Several common themes have emerged in the assembly of ECM molecules like collagens, laminins and fibronectins [12-14]. ECM molecules can be assembled into polymers inside the cell and activated before or after secretion as a result of proteolytic processing or interaction with cell surface proteins. Activation changes the structure of specific domains and results in increased homophilic interactions or heterophilic interactions with other ECM proteins as molecules assemble into higher order structures. Hemicentin is secreted from lateral muscle and gonad leader cells into the pseudocolemic cavity and, like type IV collagen, is re-

cruited by an unknown mechanism to diverse cell surfaces where it assembles into polymers [3, 15]. The amino-terminal VWA domain is sufficient to target hemicentin to cell surfaces, and the metal ion-dependent adhesion site within the VWA domain is necessary for targeting function ([16], see Figure 1). A construct with the VWA domain missing can also target to these cell surfaces, suggesting that, like fibronectin and laminin, hemicentin also appears to have at least one other cell binding sequence located within the Ig domain.

The carboxy-terminal EGF and FC modules combine to form a functional unit that is only found in hemicentins and another family of ECM proteins, the fibulins. The hemicentin EGF/FC module can bind to existing hemicentin polymers that are found in wild-type animals, but has no apparent function in the absence of endogenous hemicentin (i.e. *him-4* null mutants [16]). This suggests that the EGF/FC module is involved in mediating hemicentin-hemicentin interactions as it assembles into higher order structures. A hemicentin construct with the EGF and FC modules deleted can still bind to existing hemicentin polymers found in wild-type animals, while a construct consisting of the VWA motif and first 12 Ig modules cannot bind to endogenous hemicentin [16]. Together, this suggests that at least one additional hemicentin binding motif is located between Ig 13 and Ig 48. Since a minimum of two self-assembly domains are required for assembly into a polymer larger than a dimer, it is expected that large ECM molecules will have multiple self-assembly and cell binding domains. Fibronectin, for example, assembles into large, complex fibrillar structures and has at least two cell-binding and four self-assembly domains in addition to multiple binding sites for other ECM components [17].

Interaction with fibulin-1

Fibulins are a family of five glycoproteins that are associated with basement membranes and elastic fibers in vertebrates. Fibulins have been associated with diseases including macular degeneration, cutis laxa and cancer [18-21]. *C. elegans* has a single fibulin gene, orthologous to vertebrate fibulin-1 [22]. Hermaphrodite gonad migration defects caused by mutations in ADAM metalloproteases GON-1 and MIG-17 are suppressed by mutations in fibulin-1 [23, 24]. One model suggests that fibulin and the GON-1 metalloprotease act in opposition to one another in the inhibition or promotion of tissue expansion [23].

Structural conservation of the nematode fibulin-1 gene includes fibulin-1C and fibulin-1D splice variants [22, 25]. Fibulin-1C and 1D splice variants are required at hemidesmosome-mediated uterine and mechanosensory neuron anchorages for hemicentin assembly. Although hemicentin still localizes to these structures in the absence of fibulin-1, it has a discontinuous and frayed appearance [25]. Therefore, it appears that one function of the fibulin-1 splice variants is to refine hemicentin into smooth, continuous tracks. Conversely, hemicentin is required for the localization and assembly of fibulin-1C and 1D since there is no detectable fibulin-1C or fibulin-1D at these structures in the absence of hemicentin [25]. Although we do not know if the interaction between hemicentin and fibulin is direct or indirect, the specificity of the interaction is suggested by the observation that fibulin-1 assembly at mechanosensory neurons appears to be normal in *mec-1* mutants. The *mec-1* gene encodes a secreted ECM protein with multiple isoforms containing two EGFs and up to 15 Kunitz domains [10]. Since the *mec-1* gene product is needed for assembly of hemidesmosomes, intermediate filaments and mantle at mechanosensory neuron anchorages, this indicates that although hemicentin is required for fibulin assembly at these structures, MEC-1, hemidesmosomes, mantle and the large number of other proteins associated with these structures are not required for fibulin-1 assembly [25]. The loss of fibulin, like the loss of hemicentin, at mechanosensory neuron anchorages does not appear to affect mechanosensation [3, 10, 25].

Fibulin-1D, but not fibulin-1C, is required on elastic fiber-like structures that extend from pharynx basement membrane to body wall muscle basement membranes and insert between lateral and medial muscle in all four quadrants (Figure 2). These fibulin-1- and hemicentin-containing structures bend and flex as the animals forage for food and may be an evolutionary precursor to the elastic fibers that are found in vertebrate skin, lung and blood vessels [3, 25].

Fibulins and hemicentins share a region of high homology at the carboxy-terminus of both proteins that contain EGF and FC modules. Based on this homology, human hemicentin-1 has occasionally been included as an unusual member of the fibulin family and referred to as fibulin-6. In *C. elegans*, this region of hemicentin is thought to mediate homophilic interactions (see above; [16]). We speculate that this region of fibulin-1 mediates direct interaction between hemicentin and fibulin-1. It is tempting to speculate further that fibulin-1 and possibly other fibulin family members co-assemble with hemicentins in other species as well.

Summary

Although little is known about vertebrate hemicentins, based on their high sequence conservation with the nematode proteins, it is likely that the vertebrate hemicentin genes have a similar role in the architecture of dynamic cell junctions formed during a variety of developmental

and pathological processes, including migration and invasion, and more static cell junctions that are subjected to significant amounts of mechanical stress in select vertebrate epithelia (summarized in Table 2).

Interestingly, Schultz *et al*. [26] identified a mutation in the carboxy-terminal EGF repeats of human hemicentin-1 that segregates with the disease phenotype in a multi-generation family with age-related macular degeneration (ARMD) (Figure 1). Based on our studies in *C. elegans*, we suggest that a mutation in this region of hemicentin-1 is likely to interfere with the hemicentin assembly process.

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Table 2 Summary of *C. elegans him-4* phenotypes

Cell(s) affected	Structures affected	Phenotypic defect
Pharynx/muscle	Elastic fiber-like structures between pharynx basement membrane and body wall muscles	None observed
ALM and PLM mechanosensory neurons	Hemidesmosome-mediated attachments of neuron to epidermis (hypodermis)	Axons mis-positioned
Anterior and posterior intestinal cells	Basement membrane contacts between intestine and epidermis	Ends of intestine are unanchored
Utse cell	Hemidesmosome-mediated attachments of uterus to seam cells	Uterine prolapse
Anchor cell	Anchor cell contacts with underlying basement membrane	Delayed invasion
Linker cell	Basement membrane contacts between gonad and epidermis and between proximal and distal gonad arms	Male gonad migration/male sterility
Mitotic germ cells	Syncytial plasma membranes	*him, zyg* (i.e. chromosome segregation)

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