

# Basement Membranes in the Worm: A Dynamic Scaffolding that Instructs Cellular Behaviors and Shapes Tissues

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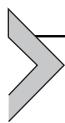
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## Abstract

The nematode worm *Caenorhabditis elegans* has all the major basement membrane proteins found in vertebrates, usually with a smaller gene family encoding each component. With its powerful forward genetics, optical clarity, simple tissue organization, and the capability to functionally tag most basement membrane components with fluorescent proteins, *C. elegans* has facilitated novel insights into the assembly and function of basement membranes. Although basement membranes are generally thought of as static structures, studies in *C. elegans* have revealed their active properties and essential functions in tissue formation and maintenance. Here, we review discoveries from *C. elegans* development that highlight dynamic aspects of basement membrane assembly, function, and regulation during organ growth, tissue polarity, cell migration, cell invasion, and tissue attachment. These studies have helped transform our view of basement membranes from static support structures to dynamic scaffoldings that play broad roles in regulating tissue organization and cellular behavior that are essential for development and have important implications in human diseases.



## 1. INTRODUCTION

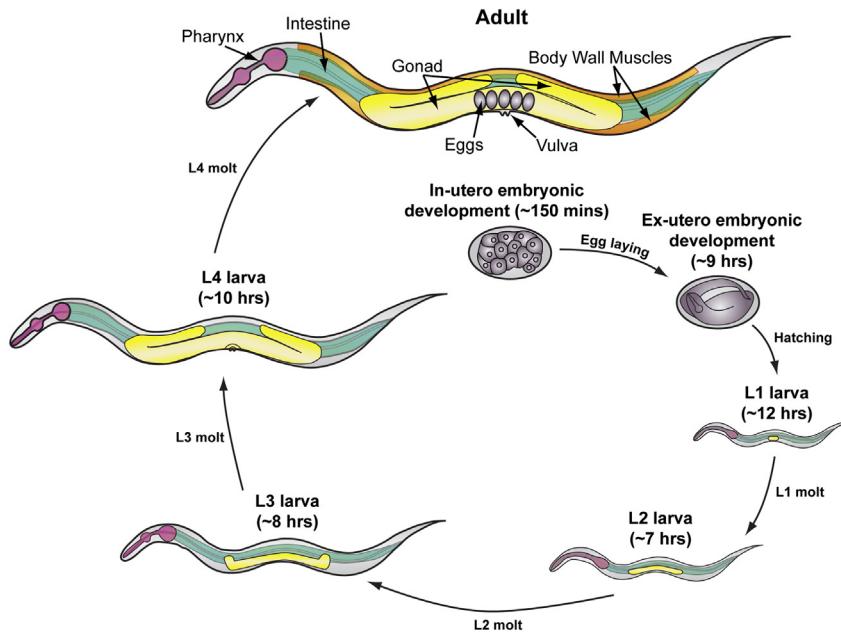
Basement membranes are specialized, cell-associated networks of extracellular matrix that underlie epithelia, endothelia, and enwrap muscles, fat, and Schwann cells (Yurchenco, 2011). The emergence of basement membranes coincided with the origin of metazoan life, suggesting that basement membranes were essential for the construction of complex, multicellular animals (Hynes, 2012; Ozbek, Balasubramanian, Chiquet-Ehrismann, Tucker, & Adams, 2010; Whittaker et al., 2006). Consistent with this notion, loss of key basement membrane components results in early embryonic lethality, and disruption of basement membranes is associated with human diseases involving most organ systems (reviews by Van Agtmael & Bruckner-Tuderman, 2010; Yurchenco & Patton, 2009). Traditionally, basement membranes have been thought of as static assemblies of proteins, glycoproteins, and proteoglycans that function mainly to support tissues. However, recent *in vivo* studies in developmental contexts have revealed that basement membrane components are dynamically secreted, assembled, and rearranged to affect cell shape, tissue polarization, and morphogenesis (reviews by Daley & Yamada, 2013; Morrissey & Sherwood, 2015). These findings have begun to transform our view of the basement membrane into an active participant in many important biological processes.

Studies in cell lines, mice, flies, and worms have supported the idea that a cell-associated network of laminin is the building block of all basement membranes (Urbano et al., 2009; Yurchenco, 2011). Laminin is a secreted heterotrimeric protein composed of an  $\alpha$ ,  $\beta$ , and  $\gamma$  subunit that binds to

receptors and lipids at the cell surface and self-assembles into a sheetlike polymeric network. Following laminin assembly, an independent network of cross-linked type IV collagen, assembled from heterotrimers containing two  $\alpha 1$ -like and one  $\alpha 2$ -like chains, is overlaid onto basement membranes (reviews by Hohenester & Yurchenco, 2013; Yurchenco, 2011). Type IV collagen is often the most abundant protein in basement membranes, and its cross-linking provides basement membranes the ability to withstand mechanical load (Fidler et al., 2014; Hohenester & Yurchenco, 2013; LeBleu, MacDonald, & Kalluri, 2007; Vanacore et al., 2009). The supramolecular grid of laminin and collagen is thought to provide basement membranes with their thin, dense structure, and several proteins, including perlecan and nidogen, have been implicated in connecting the independent laminin and collagen networks (Yurchenco, 2011). Elaborating their shared, core architecture, basement membranes can contain different forms of laminin that associate with collagen, nidogen, and perlecan at different densities (Breitkreutz, Koxholt, Thiemann, & Nischt, 2013; Rohrbach & Murrah, 1993). Various combinations of other proteins, including fibulins, collagen XVIII, agrin, hemicentin, and SPARC can be present in basement membranes, creating diverse structures with unique biochemical and biophysical properties (Candiello, Cole, & Halfter, 2010; LeBleu et al., 2007; Tzu & Marinkovich, 2008). The mechanisms that direct incorporation of specific proteins into basement membranes at particular sites, however, have remained largely unclear.

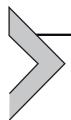
Several factors have limited the study of how vertebrate basement membranes are assembled, and how they ultimately function, in specific tissues. First, laminin and type IV collagen are essential for embryonic survival, making mechanistic loss of function studies difficult (Miner, Cunningham, & Sanes, 1998; Rozario & DeSimone, 2010; Smyth et al., 1999). Second, the expanded families of basement membrane components found in vertebrates further complicate loss of function studies. For example, vertebrates have at least 16 laminin and 3 type IV collagen trimers (Aumailley et al., 2005; Boutaud et al., 2000; Kobayashi & Uchiyama, 2003). Mutation of an individual laminin or collagen subunit frequently affects more than one trimer, thus disrupting basement membranes of multiple tissues (reviewed by Rozario & DeSimone, 2010). Third, the visual inaccessibility of most vertebrate tissues to live-cell microscopy has made it difficult to image basement membranes and basement membrane dynamics during development. The *Caenorhabditis elegans* model system avoids many of these complications. The *C. elegans* genome encodes orthologs of the structural basement membrane components laminin, type IV collagen, type XVIII collagen, nidogen,

perlecan, and agrin; the basement membrane-associated proteins fibulin-1, hemicentin, SPARC, F-spondin, and papilin (Hrus et al., 2007; Kramer, 2005; Woo et al., 2008); and the major basement membrane receptors integrin and dystroglycan (Hutter et al., 2000). Importantly, these gene families have not undergone large expansions in the *C. elegans* lineage, thus simplifying experimental analysis. Further, most basement membrane proteins have been fluorescently tagged to create functional fusion proteins, allowing them to be directly visualized in the optically transparent, rapidly developing, simple body plan of the worm (Figure 1). These features combined with forward genetics (including viable mutant alleles of most basement membrane components) and reverse genetics (including postembryonic RNAi to bypass embryonic lethal effects) make *C. elegans* uniquely suited for studying basement



**Figure 1 Overview of *Caenorhabditis elegans* development.** A schematic diagram depicting *C. elegans* development, life cycle, and several adult tissues. The times indicate the duration spent in each developmental stage when grown at 22 °C. Embryogenesis involves two stages: (1) a proliferative stage that includes gastrulation and (2) organogenesis and morphogenesis. Larval development consists of four stages (L1–L4), each separated by a molt of the stage-specific cuticle. During larval development, several tissues continue morphogenesis and growth. Two notable examples are the expansion and shaping of the gonad (yellow (light gray in print versions)), and establishment of the egg-laying apparatus, which includes morphogenesis of the vulva and formation of the uterine–vulval attachment.

membranes *in vivo*. This chapter highlights recent work from *C. elegans* development that has advanced our understanding of the assembly, dynamic nature, and diverse functions of basement membranes.



## 2. BASEMENT MEMBRANE ASSEMBLY IN THE WORM: DIVERSE COMPOSITIONS DETERMINED BY LOCAL INTERACTIONS AND RECRUITMENT OF MATRIX COMPONENTS

The two primary structural components of basement membranes, laminin and type IV collagen, are assembled into almost all basement membranes. *Caenorhabditis elegans* has two laminin  $\alpha$  subunits (*lam-3* and *epi-1*), one  $\beta$  subunit (*lam-1*), and one  $\gamma$  subunit (*lam-2*) that form two distinct laminin heterotrimers with unique  $\alpha$  subunits (Kramer, 2005). Gene expression and immunolocalization analyses revealed that laminin trimers containing the different  $\alpha$  subunits are secreted between the primary tissue layers at the onset of gastrulation and become differentially enriched along organs as they develop (Huang et al., 2003). Many—but not all—*C. elegans* tissues express the laminin  $\alpha$  subunit that is found in the tissue's basement membrane. For example, during larval development, the *C. elegans* body wall muscles express the  $\alpha$  subunit *epi-1* whereas the epidermis expresses the  $\alpha$  subunit *lam-3*, and these basement membranes contain only the laminin- $\alpha$  subunit expressed locally (Huang et al., 2003). This is similar to the mouse embryonic kidney, where the laminin  $\alpha 5$  subunit shows a restricted localization pattern that is distinct from  $\alpha 1$ , and each correlates strongly with their respective mRNA expression patterns (Ekblom et al., 1990; Sorokin, Pausch, Durbeej, & Ekblom, 1997). Together, these data suggest that tissue-specific expression of laminins may be an important factor in determining basement membrane composition.

In addition to local production, laminin secreted from distant sites can be incorporated into basement membranes from the extracellular fluid. For example, mutants of the laminin  $\beta$  subunit (*lam-1*) have pharyngeal defects that can be rescued by expressing *lam-1* only in the intestine (Rasmussen, Reddy, & Priess, 2012). Furthermore, many tissues do not express either laminin  $\alpha$  subunit, but are nevertheless surrounded by laminin-containing basement membranes. For example, the sublateral nerves do not express laminin, but they are encased by a basement membrane specifically containing the  $\alpha$  subunit LAM-3 (Huang et al., 2003). Similar situations have been described in vertebrate tissues, such as the mouse neural tube basement

membrane, which contains the laminin  $\alpha 5$  subunit that is not expressed by any cells within the neural tube (Copp et al., 2011). Together, these results show that laminin secreted into the extracellular space can be incorporated into basement membranes in a selective manner.

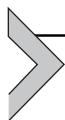
Similar to laminin, type IV collagen is also found in basement membranes of *C. elegans* tissues that do not express it. The *C. elegans* genome encodes one  $\alpha 1$ -like collagen chain (*emb-9/αA*), and one  $\alpha 2$ -like chain (*let-2/αB*), which are thought to make a single heterotrimeric type IV collagen molecule, composed of two EMB-9 chains and one LET-2 chain. Nearly all *C. elegans* basement membranes contain type IV collagen, but it is mainly expressed in the body wall muscle and distal tip cells (DTCs) (specialized cells in the somatic gonad, see below) (Graham et al., 1997). This suggests that collagen secreted into the extracellular space by one tissue can be incorporated into the basement membranes of other tissues. Supporting this notion, expression of *emb-9* in only the body wall muscle of *emb-9* mutants leads to accumulation of type IV collagen in most tissues and rescues the embryonic lethality associated with loss of *emb-9* (Graham et al., 1997). During *Drosophila* larval development, the collagen in most basement membranes is also secreted from a distant source, implying this might be a conserved aspect of type IV collagen addition to and incorporation into basement membranes (Pastor-Pareja & Xu, 2011).

The incorporation of laminin and collagen produced from distant sites into basement membranes suggests that tissues are exposed to multiple isoforms of these proteins circulating in the extracellular space. Thus, cells must have mechanisms that target the deposition of specific basement membrane components. Indeed, data from many experimental models suggest that basement membrane assembly is initiated by cell-anchored components such as receptors. Specifically, it is thought that cell surface binding of laminin induces self-assembly, which builds an initial scaffolding for basement membrane formation (reviews by Hohenester & Yurchenco, 2013; Yurchenco, 2011). Integrins are transmembrane heterodimer complexes composed of an  $\alpha$  and a  $\beta$  subunit and are thought to induce basement membrane assembly by binding laminins (Yurchenco, 2011). In *C. elegans*, loss of integrin disrupts basement membrane integrity, similar to weak mutant alleles of the laminin  $\alpha$  subunit *epi-1* (Baum & Garriga, 1997; Huang et al., 2003). Vertebrate integrins display some differential laminin-binding characteristics in vitro, but most nonetheless bind multiple laminin trimers; therefore, differential binding of integrins to laminin is unlikely to fully account for how laminin is targeted to specific tissues (Hohenester & Yurchenco, 2013; Nishiuchi et al., 2006). Different combinations of integrins and other cell surface receptors

may mediate tissue-specific basement membrane assembly. Dystroglycan is another cell surface receptor that binds laminin (Yurchenco, 2011). Supporting a role for dystroglycan as a laminin receptor, in *C. elegans* loss of dystroglycan disrupts the gonadal basement membrane, similar to loss of laminin (Johnson, 2006). However, dystroglycan is not necessary for the initial localization of laminin to the gonadal basement membrane, suggesting it might be primarily required to maintain the basement membrane as the gonad expands during development (Johnson, 2006). A role for the transmembrane glycoprotein teneurin-1 (encoded by *C. elegans ten-1*; ortholog of *Drosophila ten-m* family and vertebrate ten-m-1–m-4) in regulating basement membrane integrity was also discovered in *C. elegans* (Trzebiatowska, Topf, Sauder, Drabikowski, & Chiquet-Ehrismann, 2008). Loss of teneurin-1 disrupts the basement membranes of tissues where it is expressed such as the pharynx, gonad, and epidermis (Drabikowski, Trzebiatowska, & Chiquet-Ehrismann, 2005; Topf & Chiquet-Ehrismann, 2011; Trzebiatowska et al., 2008). Teneurin-1 interacts genetically with both integrin and dystroglycan, and loss of teneurin-1 enhances the embryonic lethality of hypomorphic type IV collagen mutants, indicating teneurin-1 could act as a receptor for type IV collagen (Topf & Chiquet-Ehrismann, 2011; Trzebiatowska et al., 2008). However, it has not yet been shown whether teneurin-1 binds or directly recruits collagen or any other basement membrane components. Integrin, dystroglycan, and teneurin-1 clearly have functions in mediating basement membrane integrity in *C. elegans*; however, their roles in the recruitment of specific laminin isoforms as well as type IV collagen to basement membrane remain unclear.

In *C. elegans*, the basement membrane components nidogen, fibulin-1, hemicentin, SPARC, and F-spondin have cell-specific expression patterns, but are also secreted into the extracellular fluid and undergo localized assembly on distant tissues (Fitzgerald & Schwarzbauer, 1998; Kawano et al., 2009; Kim & Wadsworth, 2000; Muriel, Dong, Hutter, & Vogel, 2005; Vogel & Hedgecock, 2001; Woo et al., 2008). Although the mechanisms that regulate the tissue-specific distributions of these proteins are largely unknown, several studies have revealed that local interactions between basement membrane components are important for their tissue-specific distribution. For example, at sites where mechanosensory neurons and uterine cells attach to basement membranes, fibulin-1 localization depends on hemicentin and a unique splice variant of perlecan (Muriel et al., 2005; Muriel, Xu, Kramer, & Vogel, 2006). Fibulin-1 in turn regulates hemicentin by refining and concentrating hemicentin localization in these regions (Muriel et al., 2005). In another case,

the extracellular protease MIG-17 is secreted from body wall muscles and localizes to the gonadal basement membrane where it recruits fibulin-1, which in turn recruits nidogen (see below) (Kubota, Kuroki, & Nishiwaki, 2004; Kubota, Ohkura, Tamai, Nagata, & Nishiwaki, 2008). Interestingly, fibulin-1 is also required to retain type IV collagen within the gonadal basement membrane during late larval stages (Kubota, Nagata, Sugimoto, & Nishiwaki, 2012). Together, these observations suggest that dynamic and complex interactions between basement membrane proteins and extracellular proteases might be a common mechanisms to help generate the unique composition of basement membranes surrounding tissues.



### 3. BASEMENT MEMBRANES INSTRUCT DIVERSE CELLULAR BEHAVIORS AND SHAPE TISSUES

The role of basement membranes in establishing and maintaining tissue architecture has long been recognized. Recent studies have also begun to reveal active roles for basement membranes in controlling morphogenesis in many organisms (reviews by Daley & Yamada, 2013; Morrissey & Sherwood, 2015). In particular, pioneering studies in *C. elegans* have shown that basement membranes play dynamic roles in regulating numerous cellular behaviors as well as the shaping of tissues.

#### 3.1 Nidogen regulates axon guidance and synapse formation

Nidogen is a ubiquitous basement membrane glycoprotein that binds both laminin and type IV collagen in vitro (Aumailley, Wiedemann, Mann, & Timpl, 1989; Fox et al., 1991; Paulsson et al., 1987). These characteristics made it an intriguing candidate to mediate the initial connection between laminin and collagen networks. In *C. elegans*, however, nidogen (*nid-1*) mutants undergo embryogenesis normally, are viable, and form basement membranes containing both laminin and type IV collagen (Kang & Kramer, 2000; Kim & Wadsworth, 2000). Although not required for basement membrane formation or collagen recruitment, nidogen is enriched within the sublateral nerves and near the nerve cords at the lateral edges of the body wall muscle where it helps guide longitudinal axons (Hutter, 2003; Kang & Kramer, 2000; Kim & Wadsworth, 2000; Unsoeld, Park, & Hutter, 2013). Genetic studies showed that nidogen in the SDQR sublateral neuron influences signaling through the netrin receptor UNC-40 (Deleted in Colon Cancer, DCC ortholog) to regulate axon guidance (Kim & Wadsworth,

2000; Kim, Ren, Fox, & Wadsworth, 1999). Additionally, nidogen functions in the same axon guidance pathway as the discoidin domain receptors, *ddr-1* and *ddr-2*, which are expressed in the PVPR neurons of the ventral nerve cord (Unsoeld et al., 2013). Thus, nidogen can act in multiple pathways to direct axon guidance of specific longitudinal nerves. Discoidin domain receptors are established collagen receptors in mammals (Leitinger & Hohenester, 2007), so it is possible that nidogen regulates DDR-1 and DDR-2 indirectly through alterations in type IV collagen in the basement membrane. Nidogen is also enriched near neuromuscular junction synapses where it acts with the intracellular adapter protein  $\alpha$  liprin (encoded by *C. elegans syd-2*) to maintain proper synapse size and function (Ackley et al., 2005, 2003). Vertebrates have two nidogens, and mice lacking nidogen-2 have defects in neuromuscular junctions (Fox, Ho, Smyth, & Sanes, 2008). Thus, much like in *C. elegans*, vertebrate nidogens may have instructive roles in regulating cellular behaviors.

### 3.2 Perlecan instructs muscle cell attachment

Perlecan (encoded by *C. elegans unc-52*), a basement membrane heparan sulfate proteoglycan, regulates a number of cellular behaviors including axon guidance (Tang & Wadsworth, 2014; Yang, Lee, Tang, & Wadsworth, 2014), sensory dendrite branching (Liang, Dong, Moerman, Shen, & Wang, 2015), and DTC migration (see below) (Merz, Alves, Kawano, Zheng, & Culotti, 2003). Perlecan is also essential for embryonic viability through its role in organizing muscle cell attachments to the epidermis. Movement in *C. elegans* is generated through contraction of the body wall muscles. To allow efficient motility, the force of muscle contraction must be mechanically transmitted to the epidermis. This force transmission is accomplished through specialized attachments, known as dense bodies and M-lines, two related integrin-mediated adhesions that anchor the muscle sarcomeres to the basement membrane between the muscle and epidermis that are enriched for and require perlecan (Francis & Waterston, 1991; Moerman & Williams, 2006; Mullen, Rogalski, Bush, Gorji, & Moerman, 1999; Rogalski, Williams, Mullen, & Moerman, 1993). In animals lacking perlecan, there is a complete failure to form muscle attachments, resulting in a paralyzed, lethal embryonic arrest (Hresko, Williams, & Waterston, 1994; Rogalski, Gilchrist, Mullen, & Moerman, 1995; Williams & Waterston, 1994). The muscle cells express perlecan, but how it becomes enriched at specific sites in the basement membrane to organize attachment between the muscles and epidermis is unknown.

### 3.3 Laminin mediates coordinated cell polarity

Work from cell culture and organotypic models has shown that the basement membrane component laminin induces cell polarity (Ekblom, Vestweber, & Kemler, 1986; Klein, Langecker, Timpl, & Ekblom, 1988; O'Brien et al., 2001), but due to embryonic lethality of mutants, it has remained unclear if and how laminin regulates tissue polarization in vivo. Recent studies in *C. elegans*, have revealed a specific and highly regulated role for laminin in coordinating tissue polarity. The *C. elegans* digestive tract is composed of three connected, specialized epithelial tubes: the pharynx, which is responsible for capturing and grinding food; the intestine, which digests the food; and specialized valve cells, which link the pharynx and intestine (Mango, 2007). The polarization of the pharyngeal primordium, which also contains the valve cell precursors, depends on signals from laminin. The pharyngeal primordium aligns in two bilaterally symmetric rows of cells then undergoes epithelialization in response to laminin to form a polarized cyst (Rasmussen et al., 2012). The intestinal primordium polarizes independently of laminin at a different time, thus exhibiting a distinct axis of polarity (Leung, Hermann, & Priess, 1999; Rasmussen et al., 2012; Rasmussen, Feldman, Reddy, & Priess, 2013). As the tissue segments connect, the valve cells reorganize to align with the polarity of the intestinal cells (Rasmussen et al., 2013). Throughout attachment, the majority of the pharyngeal and intestinal primordia are covered with laminin; however, laminin is specifically absent at the interface where the intestinal cells contact the posterior valve cells (Rasmussen et al., 2013). In animals where intestinal cells are ablated, or in mutants where intestinal cells do not send out the protrusions that normally contact the valve cells, laminin is ectopically deposited on the posterior valve cells, and they do not develop the normal, radial axis of polarity (Rasmussen et al., 2013). These results suggest that the intestine prevents the accumulation of laminin on the posterior valve cells and that in contrast to the pharyngeal primordium, the absence of laminin is necessary to properly polarize these cells. Therefore, laminin deposition is precisely controlled to regulate specific polarity events within tissues.

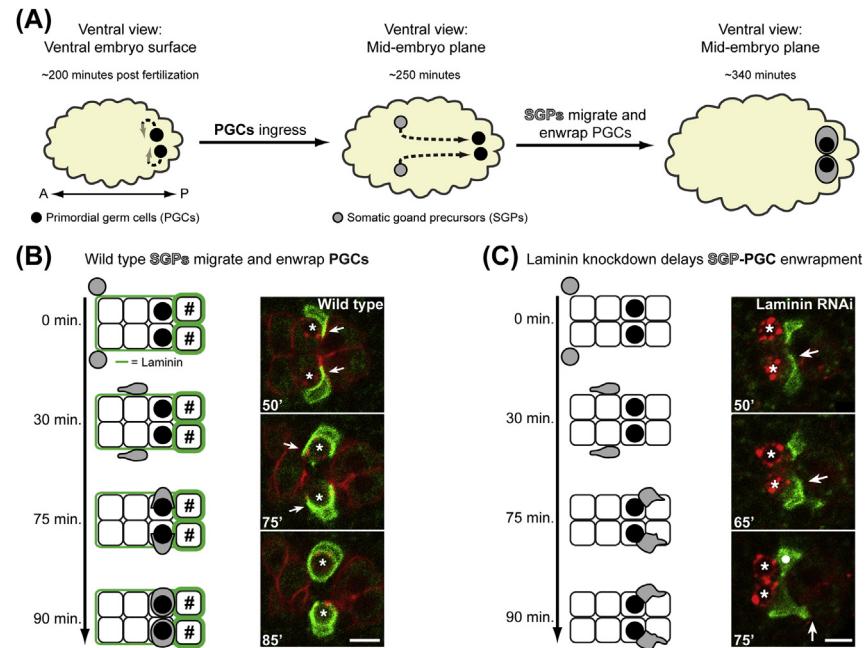
### 3.4 Laminin functions to regulate germ stem cell niche establishment

Many complex tissues arise from and are maintained by stem cells. Adult stem cells are typically found in a niche that supports stem cell survival and pluripotency. Extracellular matrices, including basement membranes,

provide an adhesive substrate that retains the stem cell in the niche and regulates signaling that promotes self-renewal (reviews by Brizzi, Tarone, & Defilippi, 2012; Marthiens, Kazanis, Moss, Long, & Ffrench-Constant, 2010). Work in *C. elegans* has revealed a role for laminin in the establishment of the germ stem cell niche. The germ stem cell niche is formed when the two somatic gonad precursors, which arise in anterior regions of the embryo, migrate posteriorly to enwrap the two primordial germ cells (Figure 2(A) and (B)) (Harrell & Goldstein, 2011; Kimble & White, 1981; Nance & Priess, 2002; Rohrschneider & Nance, 2013; Sulston, Schierenberg, White, & Thomson, 1983). Somatic gonad precursors extend protrusions as they migrate along the surface of the endoderm, which is surrounded by laminin. Interestingly, the endodermal cells just posterior to the primordial germ cells have additional laminin around their cell surfaces, and when the migrating somatic gonad precursors contact these endodermal cells, they stop migrating and enwrap the germ cells (Rohrschneider & Nance, 2013). RNAi-mediated reduction of laminin does not affect initial somatic gonad precursor migration. However, the somatic gonad precursor cells migrate just beyond the primordial germ cells in the absence of laminin, suggesting that laminin provides an important cue for somatic gonad precursors to stop migrating (Figure 2(C)) (Rohrschneider & Nance, 2013). Since somatic gonad precursors eventually retract and enwrap the germ cells despite laminin knockdown, additional signals likely promote enrapment and may regulate where the somatic gonad precursors stop. Laminin is also increased in areas where germ cells stop migrating in mice, and *Drosophila* laminin mutants have primordial germ cells and somatic gonad precursors in ectopic locations (García-Castro, Anderson, Heasman, & Wylie, 1997; Weyers, Milutinovich, Takeda, Jemc, & Van Doren, 2011), suggesting that laminin may be a conserved instructive cue in positioning these cells. Laminin may carry out this function by localizing another cue that halts cell migration, or laminin itself could provide a direct signal to stop migrating cells by binding a cell surface receptor.

### 3.5 Integrins, extracellular signals, and basement membrane components regulate DTC migration

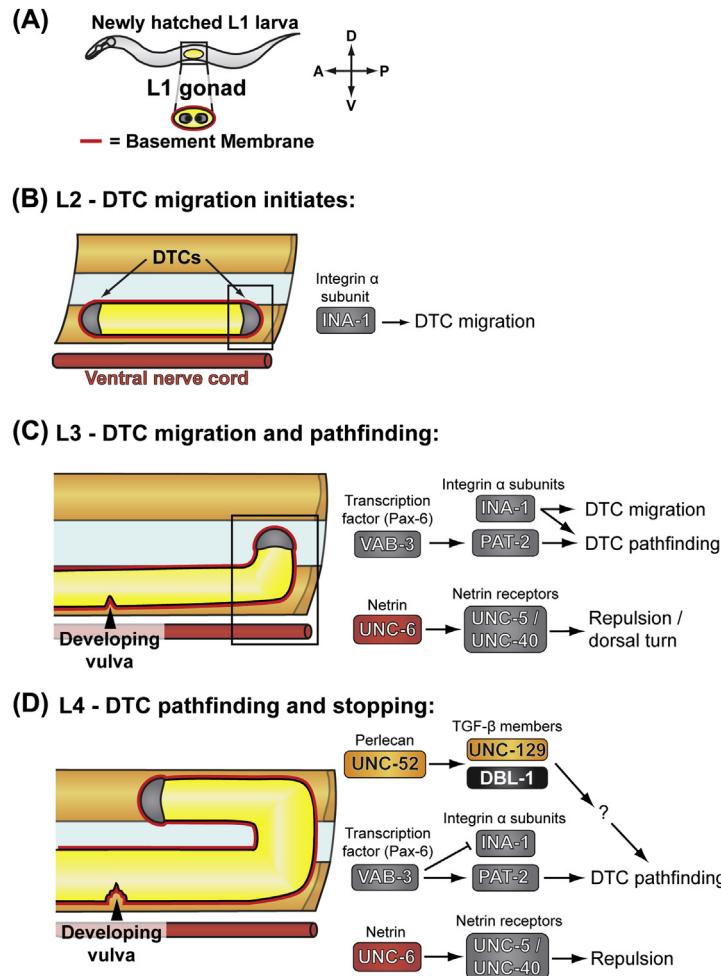
The establishment and shaping of tissues in many organisms often relies on cell migration. For example, in *C. elegans* the migration of the DTCs helps to shape the gonad during larval development (see Figure 1 for staging) (reviewed by Wong & Schwarzbauer, 2012). The entire gonad, including the DTCs and germ cells, is surrounded by a basement membrane



**Figure 2** A laminin-dependent signal halts somatic gonad precursor cell migration. (A) A schematic diagram of germ stem cell niche establishment. During embryogenesis the primordial germ cells (PGCs; black circles) are specified in the posterior region of the ventral embryo surface before ingressing during gastrulation. Somatic gonad precursors (SGPs; gray circles) are born in anterior regions of the embryo and migrate along the endoderm to meet the germ cells. Upon reaching the germ cells, somatic gonad precursors halt their migration and establish the niche by enwrapping the primordial germ cells. (B) Cell behaviors during somatic gonad precursor migration and primordial germ cell enwrapping. The schematic diagrams (left; normal distribution of laminin is shown in green (gray in print versions)) and confocal time-lapse images (right) depict somatic gonad precursors (gray circles (left), green (light gray in print versions) cells (right)) migrating along the endoderm (white boxes (left), red (dark gray in print versions) cells (right)). Somatic gonad precursors stop migrating when they contact endoderm cells that have increased laminin around their cell surfaces (#) that lie just posterior to the primordial germ cells (black circles (left), marked with asterisk (right)). After stopping, somatic gonad precursors extend cellular processes (arrows) that enwrap primordial germ cells to establish the niche. (C) When laminin is decreased or absent, somatic gonad precursors migrate further and extend projections past the primordial germ cells, resulting in delayed enwrapping. PGCs, primordial germ cells; SGPs, somatic gonad precursors. Confocal images in (B) and (C) originally appeared in Rohrschneider and Nance (2013) and were adapted with permission.

(Hall et al., 1999); thus when the DTCs migrate, they are encased by the gonadal basement membrane. During migration, the DTCs secrete many basement membrane components, including laminin, collagen, nidogen, agrin, papilin, and hemicentin (Graham et al., 1997; Hrus et al., 2007; Huang et al., 2003; Kawano et al., 2009; Kim & Wadsworth, 2000; Vogel & Hedgecock, 2001). The DTC has the complex task of laying down new basement membrane, remodeling deposited basement membrane to allow gonad expansion, and using the basement membrane as a substrate to promote or orient migration. In addition to the gonadal basement membrane, DTC migration is influenced by the neighboring muscle and epidermal basement membranes during different stages of migration (Figure 3). Migration is initiated in the L2 larval stage when the two DTCs move in anterior and posterior directions adjacent to the ventral body wall muscle basement membrane (Figure 3(B)). In the mid-to-late L3 stage, DTCs turn toward the dorsal side of the animal and migrate lateral to the epidermal basement membrane (Figure 3(C)). When DTCs reach the dorsal body wall muscle basement membrane, they turn and move back toward the midbody (Figure 3(D)), where they continue to migrate until early adulthood. A complex interplay of integrin receptor activity, extracellular signaling, and basement membrane components directs various aspects of DTC migration.

The *C. elegans* genome encodes two  $\alpha$  integrin subunits (*ina-1* and *pat-2*) and one  $\beta$  integrin subunit (*pat-3*) and form two heterodimeric integrin receptors (INA-1/PAT-3 and PAT-2/PAT-3), which are thought to bind basement membrane components (Kramer, 2005). Both integrin complexes are expressed in the DTCs and regulate distinct aspects of DTC migration. The *ina-1* gene (ortholog of laminin-binding integrin  $\alpha$  subunits) promotes DTC migration, is expressed throughout migration, and is downregulated as migration ceases (Figure 3(B)–(D)) (Baum & Garriga, 1997; Kikuchi et al., 2015; Meighan & Schwarzbauer, 2007). In mutants of the *vab-3* transcription factor (vertebrate paired box Pax6 ortholog), *ina-1* expression is maintained in DTCs and they continue migrating into adulthood, resulting in long, looping gonad arms (Meighan & Schwarzbauer, 2007). INA-1 also regulates DTC pathfinding along the dorsal side of the animal. In animals with reduced *ina-1* function, DTCs undergo multiple, ectopic turns when they reach the dorsal muscle basement membrane. The *pat-2* gene (ortholog of RGD binding  $\alpha$  subunits) is expressed beginning at the L3 stage when DTCs turn to migrate dorsally. In contrast to



**Figure 3** Larval morphogenesis of the gonad and molecular pathways controlling distal tip cell (DTC) migration. (A) A schematic diagram of a newly hatched L1 larva and gonad. The gonadal basement membrane is shown in red (dark gray in print versions) in all illustrations. (B) DTC migration initiates at the L2 stage, and DTCs migrate adjacent to the ventral body wall muscle basement membrane (orange (gray in print versions)). The integrin  $\alpha$  subunit *ina-1* is expressed in DTCs beginning at L2 and promotes DTC migration. (C) In L3, the DTCs turn and migrate toward the dorsal body wall muscle along the epidermal basement membrane (light blue). During L3, the transcription factor VAB-3 (Pax6 ortholog) induces expression of the integrin  $\alpha$  subunit *pat-2*. Both *INA-1* and *PAT-2* regulate DTC turning and proper pathfinding. In addition, the activation of netrin (*UNC-6*) secreted from the ventral nerve cord binds the netrin receptors *UNC-5* and *UNC-40* (DCC) in the DTCs to mediate repulsion from the ventral body wall. (D) Once the DTCs reach the dorsal body wall muscle in the L4 stage, the DTCs turn back toward the animal midbody. This turn is regulated by the integrins *INA-1* and *PAT-2* in the DTCs, the basement membrane protein perlecan (*UNC-52*), and the TGF- $\beta$  family members *UNC-129* and *DBL-1*. Perlecan and *unc-129* are expressed in the body wall muscle, and perlecan might sequester TGF- $\beta$  members within the dorsal body wall muscle basement membrane. During L4, VAB-3 downregulates *ina-1* integrin expression, which is necessary to stop DTC migration in early adulthood.

*ina-1*, which is turned off by *vab-3*, *pat-2* expression is initiated by *vab-3* (Figure 3(C) and (D)) (Meighan & Schwarzbauer, 2007). Knocking down *pat-2* results in DTCs that execute multiple turns, or DTCs that do not turn toward the dorsal muscle and instead migrate back toward the midbody along the ventral side of the animal (Meighan & Schwarzbauer, 2007). Thus, integrins are crucial players in DTC migration, with INA-1 promoting DTC migration and both INA-1 and PAT-2 cooperating to regulate DTC pathfinding.

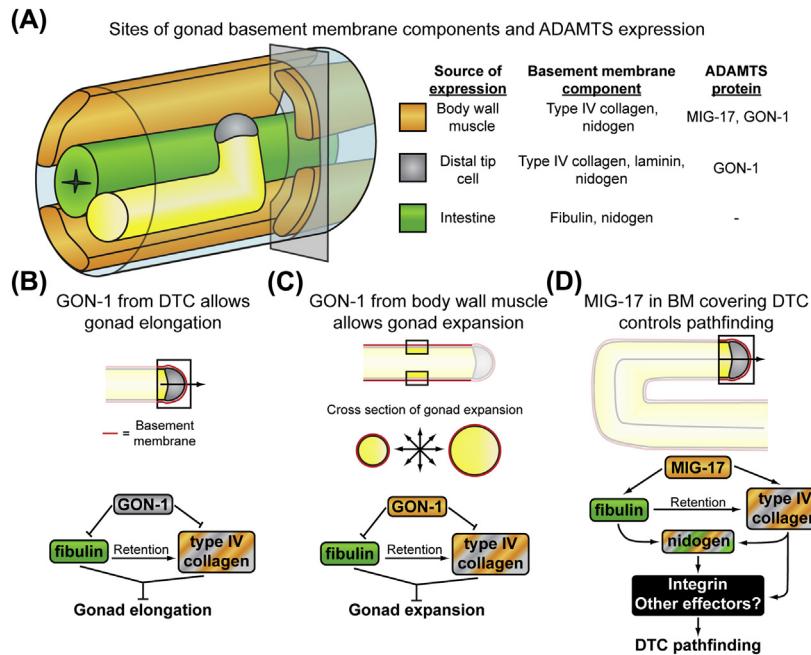
In addition to the coordinated activity of integrins, DTC migration depends on extracellular signaling that can be influenced by the basement membrane component perlecan. Perlecan is expressed in the body wall muscle and appears to influence DTC migration by sequestering or localizing the TGF- $\beta$  family members UNC-129 and DBL-1 (Figure 3(D)) (Merz et al., 2003). This interaction between perlecan and TGF- $\beta$  might affect netrin signaling, since the loss of perlecan, as well as *dbl-1* or *unc-129*, enhances the DTC migration defects seen in weak mutants for the netrin receptor *unc-5* (Merz et al., 2003). The netrin receptors UNC-5 and UNC-40 (DCC) act in the DTC to initiate the turn toward the dorsal muscle by mediating repulsion from the ventrally secreted cue netrin (UNC-6) (Figure 3(C) and (D)).

The functions of integrins, extracellular signals, and basement membrane components are spatiotemporally regulated to control DTC migration, and these pathways may exhibit extensive cross talk. In addition to the signaling interactions between netrin and TGF- $\beta$  mentioned above, interactions between integrins and netrin or TGF- $\beta$  signaling have been characterized in other contexts. For example, during anchor cell (AC) invasion in *C. elegans*, integrin regulates localization of the netrin receptor UNC-40 (DCC) (see below) (Hagedorn et al., 2009; Ziel, Hagedorn, Audhya, & Sherwood, 2009), and in vertebrates integrins play important roles in controlling the expression and activation of TGF- $\beta$  (reviewed by Ivaska & Heino, 2011; Margadant & Sonnenberg, 2010). In the DTC, the loss of the netrin receptor *unc-5* results in DTCs that fail to migrate to the dorsal muscle (Hedgecock, Culotti, & Hall, 1990; Su et al., 2000), which is a phenotype similar to loss of the integrin  $\alpha$  subunit *pat-2* (Meighan & Schwarzbauer, 2007) and it will be interesting to determine if netrin and integrin interact during DTC migration. Further, it will be important to determine if other basement membrane components influence DTCs similar to perlecan, and to explore how other molecules, such as secreted proteases (see below), regulate these pathways.

### 3.6 Extracellular metalloproteases within the gonadal basement membrane regulate DTC migration and gonad shape

Another family of proteins associated with the basement membrane that regulate DTC migration and gonad shape are the ADAMTS (A Disintegrin And Metalloprotease with ThromboSpondin repeats) proteins—conserved, secreted proteases that cleave the extracellular matrix and have roles in development, physiological function, and disease (reviewed by [Dubail & Apté, 2015](#); [Tang, 2001](#)). GON-1 is a *C. elegans* ADAMTS protein that controls gonad elongation and shape ([Blelloch & Kimble, 1999](#); [Hesselson, Newman, Kim, & Kimble, 2004](#); [Kubota et al., 2012](#)). Strong expression of *gon-1* is observed in the DTCs throughout their migration, and *gon-1* is also expressed by the body wall muscles ([Figure 4\(A\)](#)) ([Blelloch & Kimble, 1999](#)). Site of action experiments indicated that GON-1 secreted from the DTCs allows gonad arm elongation via DTC migration, whereas GON-1 secreted from the body wall muscles allows the gonad to expand radially during DTC outgrowth ([Figure 4\(B\) and \(C\)](#)) ([Blelloch & Kimble, 1999](#)). Genetic and localization studies showed that GON-1 opposes the function of the basement membrane-associated protein fibulin-1 and type IV collagen in the gonad, and that fibulin-1 maintains collagen within the basement membrane at late larval stages ([Hesselson et al., 2004](#); [Kubota et al., 2012](#)). Whereas gonads in *gon-1* mutants fail to elongate and expand, mutants for fibulin-1 or type IV collagen have an opposite phenotype of widened gonad arms ([Hesselson et al., 2004](#); [Kubota et al., 2004, 2012](#); [Muriel et al., 2005](#)). The catalytic activity of GON-1 is required for its function, suggesting that it may cleave fibulin-1 or type IV collagen ([Blelloch & Kimble, 1999](#)), however, no such cleavage has been detected ([Hesselson et al., 2004](#)). Given the function of collagen in providing structural stability to basement membranes, it is possible that GON-1 might lower type IV collagen levels or reduce collagen function to allow for a more pliable basement membrane during DTC migration and gonad expansion.

MIG-17 is an ADAMTS that regulates the directionality of DTC migration. Loss of MIG-17 leads to a “wandering” DTC phenotype where DTCs make multiple, incorrect turns throughout their migration ([Nishiwaki, Hisamoto, & Matsumoto, 2000](#)). MIG-17, along with MIG-18, a novel cofactor required for MIG-17 activation, is secreted from the body wall muscle ([Figure 4\(A\)](#)) ([Kim et al., 2014](#); [Nishiwaki et al., 2000](#)). MIG-17 is secreted in a pro-form that is incorporated into the gonadal basement

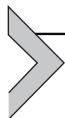


**Figure 4 A Disintegrin And Metalloprotease with ThromboSpndin (ADAMTS) proteins act in the gonadal basement membrane to control gonad elongation, expansion, and distal tip cell (DTC) migration.** (A) A schematic diagram depicting a portion of a *Caenorhabditis elegans* larva showing the tissues that express gonadal basement membrane components and ADAMTS proteins. The body wall muscles (orange (gray in print versions)) express and secrete MIG-17, GON-1, and type IV collagen; the DTCs (gray) secrete GON-1, type IV collagen, laminin, and nidogen; and the intestine (green (dark gray in print versions)) secretes fibulin-1 and nidogen. (B–D) Mechanisms utilized by ADAMTS proteins to control gonad elongation (B), gonad expansion (C), and DTC pathfinding (C). Colors represent the sites of protein secretion and correspond to the key in (A). (B) The action of GON-1 secreted from the DTC antagonizes fibulin and type IV collagen and allows gonad arm elongation. (C) GON-1 secreted from the body wall muscle acts within the gonadal basement membrane to allow gonad expansion. (D) MIG-17 secreted from the body wall muscle acts in the basement membrane covering the DTC to regulate a functional network of fibulin-1, type IV collagen, and nidogen that regulates integrin or other effectors to control DTC pathfinding.

membrane where the pro-domain is removed to activate MIG-17 at the L3 stage (Ihara & Nishiwaki, 2007, 2008). MIG-17 functions in the basement membrane covering the DTC where it recruits fibulin-1 (Kubota et al., 2004; Nishiwaki et al., 2000). Genetic and overexpression studies suggest that MIG-17 “activates” fibulin-1 in the basement membrane—possibly

through direct proteolytic cleavage or through indirect interactions with another substrate—such that fibulin-1 recruits nidogen, which has a subtle role in DTC pathfinding (Figure 4(D)) (Kubota et al., 2008). In addition to regulating fibulin-1, genetic studies suggest that MIG-17 also activates type IV collagen within the gonadal basement membrane to promote DTC pathfinding (Kubota et al., 2012). Notably, type IV collagen determines the level of the INA-1/PAT-3 integrin complex in the DTC (Kubota et al., 2012). As integrins are also known regulators of DTC pathfinding (see above), they may be key effectors of the MIG-17 pathway (Figure 4(D)) (Baum & Garriga, 1997; Kubota et al., 2012; Lee, Cram, Shen, & Schwarzbauer, 2001; Meighan & Schwarzbauer, 2007).

Together these studies have indicated that ADAMTS proteins within the basement membrane control cell migration and organ shape in gonad formation. These proteins appear to be important in shaping other organs as well. For example, MIG-17 and several basement membrane components are important for pharyngeal morphogenesis (Jafari et al., 2010), and GON-1 and type IV collagen play a role in the formation and growth of motor neuron presynaptic boutons (Qin, Liang, & Ding, 2014). The functions of ADAMTS proteins may be context dependent, however, as GON-1 restrains growth of presynaptic boutons, whereas it promotes growth in the gonad.



#### 4. CREATING GAPS IN—OR LINKS BETWEEN— BASEMENT MEMBRANES HELPS SHAPE TISSUES

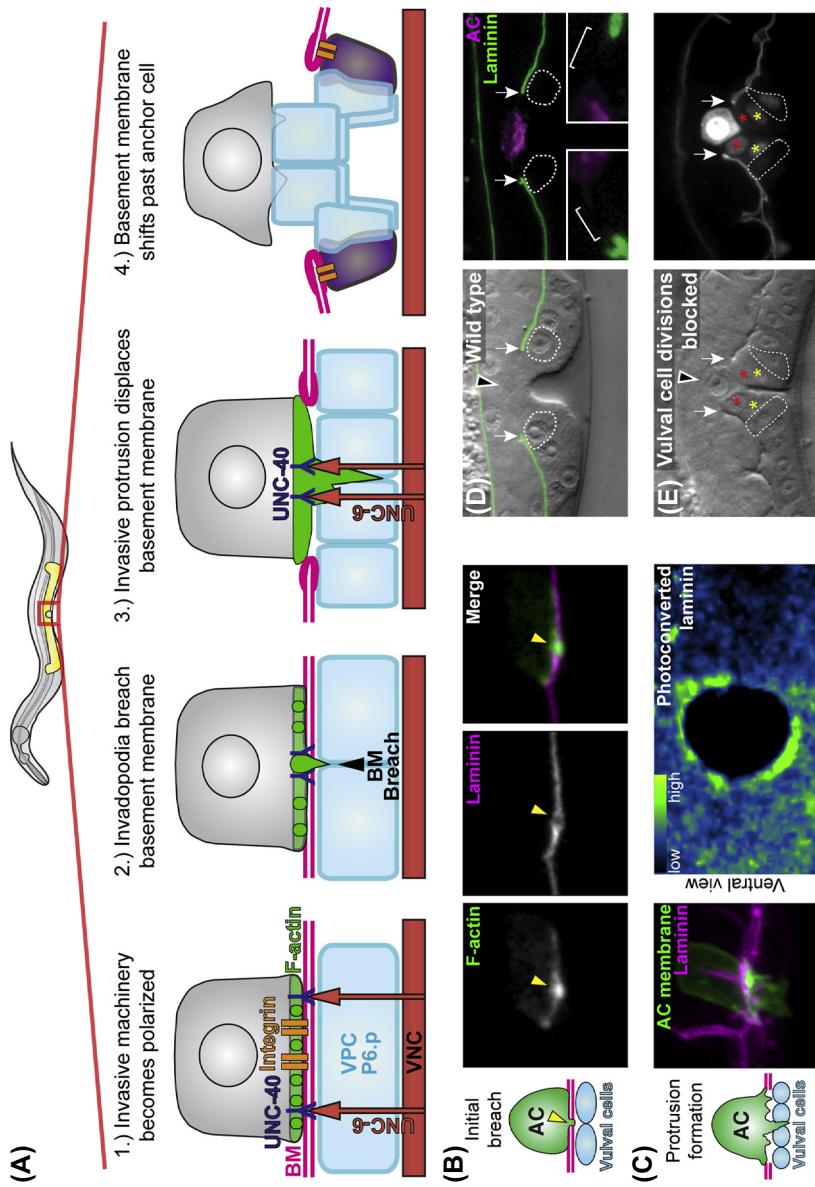
Basement membranes act as formidable barriers that contain and separate tissues. During morphogenesis, distinct tissues often join together, and dramatic structural changes in basement membranes must occur to facilitate the migration or exchange of cells from one tissue to another. Recent studies in *C. elegans* have identified important molecular mechanisms that control the creation of large openings in basement membranes that allow the direct connection of cells between tissues. In addition, work in the worm has identified a new adhesion system that links neighboring tissues through adjoining basement membranes.

##### 4.1 Creating de novo gaps in the basement membrane

Formation of de novo gaps in basement membranes occurs frequently during development and also underlies the progression of diseases such as cancer (reviewed by Kelley, Lohmer, Hagedorn, & Sherwood, 2014). In spite of this widespread importance, how openings in the basement membrane are

created is not well understood. During *C. elegans* larval development, the uterine and vulval tissues are initially separated by their respective basement membranes (Sherwood & Sternberg, 2003). To initiate uterine–vulval attachment, the AC, a specialized uterine cell, invades across the uterine and vulval basement membranes. AC invasion creates an initial breach in the basement membrane that is widened as the vulval cells invaginate to connect the tissues (Figure 5) (reviewed by Hagedorn & Sherwood, 2011; see Hagedorn et al., 2013; Sherwood & Sternberg, 2003; Ziel et al., 2009). Studies of uterine–vulval attachment have begun to reveal the cellular and molecular mechanisms that create gaps in basement membranes.

AC invasion is highly stereotyped and occurs in synchrony with the divisions of the underlying vulval precursor cell (P6.p; Figure 5(A)) (Sherwood & Sternberg, 2003). The initial breach in the basement membrane is generated by invadopodia—small, membrane-associated, actin-rich structures (Figure 5(A) and (B)) that are also present in metastatic cancer cells (Hagedorn et al., 2014, 2013; Murphy & Courtneidge, 2011). Similar to invadopodia in cancer cells, the AC’s invadopodia are dependent on integrin–basement membrane interactions (Figure 5(A)) (Hagedorn et al., 2014, 2009, 2013; Lohmer, Kelley, Hagedorn, & Sherwood, 2014). Therefore, the cellular machinery responsible for breaching the basement membrane is organized, in part, by the basement membrane itself. After breach, the basement membrane is removed underneath the entire AC footprint. A variety of mechanisms for opening holes in basement membrane exist (reviewed by Kelley et al., 2014), but in many cases the activity of matrix metalloproteinases (MMPs) are thought to dissolve basement membrane (Overall & Kleifeld, 2006; Shay, Lynch, & Fingleton, 2015; Valastyan & Weinberg, 2011). Consistent with this notion, AC invasion depends on the action of *fos-1*—the single *C. elegans* ortholog of the *fos* family of bZIP transcription factors—which induces expression of the MMP *zmp-1* (Sherwood, Butler, Kramer, & Sternberg, 2005). However, animals lacking *zmp-1* still have efficient AC invasion (Sherwood et al., 2005), suggesting that removal of basement membrane may depend on redundant MMPs or might involve proteolysis-independent mechanisms (see below). Prior to AC invasion the netrin receptor UNC-40 (DCC) is polarized to the ACs invasive surface through the action of integrin and the UNC-40 ligand UNC-6 (netrin) (Hagedorn et al., 2009; Wang et al., 2014; Ziel et al., 2009). UNC-40 (DCC) becomes enriched at the site of initial breach where it directs formation of an invasive protrusion that crosses the basement membrane (Figure 5(A)) (Hagedorn et al., 2013). Photoconversion of laminin under the AC revealed that the



**Figure 5 Creating a de novo gap in basement membrane during uterine–vulval attachment.** (A) A schematic diagram of basement membrane breach and hole widening during uterine–vulval attachment. 1.) The anchor cell (AC) invasive cellular machinery is polarized by integrin–basement membrane interactions and netrin (UNC-6) localized within the basement membrane that binds to the netrin receptor UNC-40 (DCC) in the AC. Netrin is secreted from the ventral nerve cord (VNC). 2.) UNC-40 (DCC) traffics to the site where invadopodia (marked by F-actin) breach the basement membrane. 3.) UNC-6 (netrin) binding UNC-40 (DCC) stimulates the formation of an invasive protrusion that displaces basement membrane. 4.) Vulval cell invagination shifts the basement membrane sheet until integrin adhesion in the vulval vulD cell (purple (dark gray in print versions)), which is triggered by increased laminin at the edges of the hole, stops basement membrane sliding. (B) Confocal images showing invadopodia (F-actin) breaching the basement membrane (yellow (light gray in print versions) arrowheads), which has been labeled with fluorescent (gray in print versions) laminin. (C) Confocal images showing the invasive protrusion. Ventral view shows accumulation of laminin that was photoconverted under the AC before being displaced by the invasive protrusion. (D) The basement membrane slides past the AC (black arrowhead) and stops (arrows mark position) over the vulval vulD cell (circled). Brackets mark space between the AC and the basement membrane. (E) Basement membrane does not slide when cell divisions of vulval cells (marked by asterisks) are blocked. AC, anchor cell; BM, basement membrane; VPC, vulval precursor cell; VNC, ventral nerve cord. All confocal images were adapted with permission. Images in (B) and (C) originally appeared in Hagedorn et al. (2013); images in (D) appeared in Hara et al. (2011); images in (E) appeared in Matus et al. (2014).

invasive protrusion displaces the basement membrane to expand the existing hole (Figure 5(C)) (Hagedorn et al., 2013). Approximately 30% of the laminin is displaced, with the remainder removed presumably by proteolysis. Thus, the combined action of proteases and the physical actions of the AC create and widen a gap in the basement membrane.

Once AC invasion is complete, the gap in the basement membrane continues to widen past the footprint of the AC to allow direct contact between the uterine and vulval cells that form the mature uterine–vulval attachment. Live imaging of animals with ablated ACs or vulval cells showed that this further expansion requires only the vulval cells (Ihara et al., 2011). Optical highlighting of laminin and type IV collagen revealed that as the gap widens the basement membrane slides as a sheet over the dividing and invaginating vulval cells. These observations suggest that forces created by vulval cell invagination physically shift the basement membrane. Strikingly, the basement membrane always stops over vulD—the only vulval cell that does not divide during invagination (Figure 5(A) and (D)) (Ihara et al., 2011). Manipulations of vulval cell divisions revealed that dividing vulval cells lose attachment with the basement membrane and allow it to slide (Figure 5(E)) (Matus et al., 2014). At the edge of the gap, laminin is enriched from the original displacement of basement membrane by the AC (Hagedorn et al., 2013; Matus et al., 2014). This higher level of laminin plays a key role in stabilizing the position of the sliding basement membrane by increasing the INA-1/PAT-3 integrin complex within the cell membrane of vulD, which binds laminin and halts basement membrane sliding (Ihara et al., 2011; Matus et al., 2014).

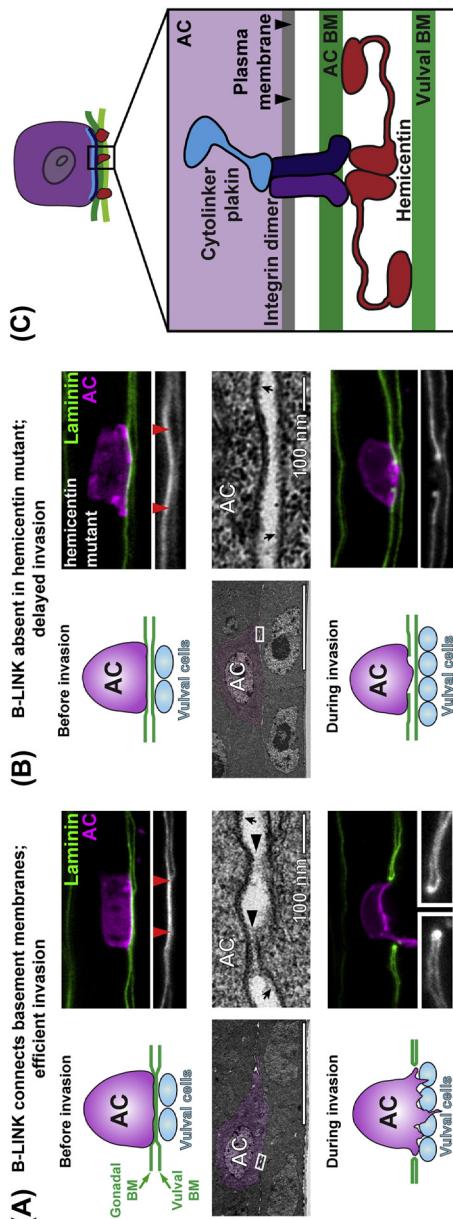
These studies in *C. elegans* revealed that forming and widening the gap in basement membrane during uterine–vulval attachment requires the coordinated actions of a number of cellular behaviors, including cell invasion, cell division, and tissue invagination. Creating the basement membrane gap also depends on instructive cues from the basement membrane itself that promote basement membrane breaching by invadopodia and that stabilize the position of the widening gap by signaling for increased adhesion.

#### 4.2 Adhesion between basement membranes

There are several biological processes where basement membranes of adjacent tissues come into contact and appear connected. For example, the basement membranes of kidney glomerular podocytes and lung alveolar epithelial cells appear to firmly connect or possibly fuse with the basement membrane of the neighboring vasculature; basement membranes of the

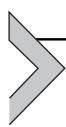
invaginating optic cup meet and link before being removed to form a continuous epithelium; and the basement membranes of tissues at the blood–brain barrier appear to stably attach (Abrahamson, 1985; Morrissey & Sherwood, 2015; Obermeier, Daneman, & Ransohoff, 2013; Tsuji, Kita, Ozaki, Narama, & Matsuura, 2012; Vaccaro & Brody, 1981). It has remained unclear if specific adhesions between basement membranes occur to regulate these basement membrane–basement membrane interactions. Recent work in *C. elegans* has identified the molecular components of a new adhesion system between basement membranes of adjacent tissues and provided insight into several roles for this adhesion.

The uterine and vulval basement membranes under the AC are in close apposition to one another before invasion (Figure 6(A)) (Morrissey et al., 2014; Sherwood & Sternberg, 2003). Studies combining photo-bleaching of the basement membrane and forced tissue shifting revealed that the uterine and vulval basement membranes move independently of one another several hours before invasion (Morrissey et al., 2014). However, just prior to AC invasion, the basement membranes under the AC no longer move independently, suggesting they become linked. Consistent with this notion, electron microscopy revealed punctate structures between the uterine and vulval basement membranes, indicative of adhesions between the basement membranes (Figure 6(A)) (Morrissey et al., 2014). Hemicentin (encoded by *C. elegans him-4*) is a conserved, large, extracellular protein with 48 tandem immunoglobulin repeats that is associated with basement membranes (Vogel & Hedgecock, 2001; Xu, Dong, & Vogel, 2007). Hemicentin is secreted from the AC and assembles into stable puncta in basement membranes underneath the AC (Morrissey et al., 2014; Sherwood et al., 2005; Sherwood & Sternberg, 2003), suggesting it may mediate linkage between uterine and vulval basement membranes. Indeed, in hemicentin mutants the basement membranes are not linked, and the AC breaches the uterine and vulval basement membranes sequentially rather than simultaneously, resulting in a delay in invasion (Figure 6(B)) (Morrissey et al., 2014). Additionally, the cytolinker plakin (*vab-10a*) and integrin (*ina-1/pat-3*) are expressed in the AC and are essential for hemicentin puncta organization and basement membrane connection. These newly identified, cell-associated linkages between juxtaposed basement membranes are termed B-LINKs for Basement membrane LINKage (Figure 6(C)) (Morrissey et al., 2014). In the AC, B-LINKs are transient adhesions, but stable B-LINKs also form between the basement membranes of the uterus and epidermis to maintain uterine structure during egg laying



**Figure 6 Basement membrane LINKages (B-LINKs) connect adjacent basement membranes.** (A) During anchor cell (AC) invasion, the gonadal and vulval basement membranes (BMs; labeled with fluorescent (gray in print versions) laminin) under the anchor cell are connected together (red (dark gray in print versions) arrowheads). Tight adhesion between the basement membranes is mediated by the B-LINK, seen as punctate structures (black arrowheads) between the gonadal and vulval basement membranes (black arrows) in transmission electron micrographs. The B-LINK allows efficient invasion. (B) In hemicentin mutants the B-LINK is absent and the gonadal and vulval basement membranes are no longer linked, evidenced by space between the basement membranes seen by fluorescent (gray in print versions) microscopy (red (dark gray in print versions) arrowheads) and lack of punctate structures between the basement membranes (black arrows) in electron micrographs. Disrupting the B-LINK leads to stepwise breaching of the gonadal and vulval basement membranes and delayed invasion. (C) A schematic diagram showing molecular components of the B-LINK. Hemicentin is secreted from the AC and forms punctate structures in the BM. The cytolinker plakin (VAB-10A, blue (dark gray in print versions)) and the transmembrane integrin complex (INA-1/PAT-3; purple (gray in print versions)) are necessary for B-LINK formation. AC, anchor cell; BM, basement membrane. Confocal images, electron microscopy images, and illustration of molecular mechanism originally appeared in Morrissey et al. (2014) and were adapted with permission.

(Morrissey et al., 2014; Newman, White, & Sternberg, 1996; Vogel & Hedgecock, 2001). Further, B-LINKs may be conserved across taxa, as zebra fish hemicentins regulate the interaction between neighboring epithelia in the fin epidermis, as well as the connection between somites and the epidermis (Carney et al., 2010; Feitosa et al., 2012). While work from *C. elegans* definitively identified B-LINKs, these studies suggest that B-LINKs likely have broad roles in connecting various tissues during development and in forming stable structures that mediate long-term tissue connections in adults.



## 5. FUTURE DIRECTIONS AND PERSPECTIVES

Throughout this chapter, we have highlighted studies from *C. elegans* that have advanced our understanding of basement membranes. In *C. elegans*, as in vertebrates, early loss of laminin, the key building block of all basement membranes, results in gross disruptions in all tissues and early embryonic lethality (Kao, Huang, Hedgecock, Hall, & Wadsworth, 2006). These observations clearly highlight the widespread and essential importance of basement membranes to organogenesis and development. By using the worm system's strengths including RNAi, null and hypomorphic genetic mutations, and live imaging of basement membrane components, work in *C. elegans* has extended our understanding of basement membranes beyond their essential role in tissue support. In particular, studies in *C. elegans* have provided new insights into how basement membranes are built *in vivo*, how individual components within the basement membrane actively regulate numerous cellular behaviors, and how basement membranes are remodeled to facilitate tissue connections. Many aspects of basement membrane assembly, function, and mechanics, however, remain unclear.

### 5.1 Identifying key determinants of basement membrane composition and selective deposition

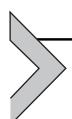
One important area of future study is to further understand how extracellular matrix components are localized to specific basement membranes *in vivo*, as the specific localization or concentration of basement membrane proteins is crucial in regulating many cellular processes. It is clear that localized secretion of basement membrane components is often important. For example, the AC secretes hemicentin to link basement membranes (Morrissey et al., 2014). However, in many cases matrix components are recruited from the extracellular fluid to specific basement membranes or

concentrated within specific cellular regions, and in most of these instances we do not have a clear understanding of how this occurs. One glaring example is type IV collagen. Studies in cell culture have suggested that basement membrane proteins nidogen, perlecan, and agrin act redundantly to recruit collagen to basement membranes (reviews by Hohenester & Yurchenco, 2013; Yurchenco, 2011). Due to the difficulties associated with removing all of these basement membrane components in vertebrates, this has not yet been verified *in vivo*. With single genes encoding nidogen, perlecan, and agrin in *C. elegans*, it should be possible to definitively determine if these proteins act redundantly, or in various combinations, to incorporate collagen into basement membranes. Furthermore, it is conceivable that known extracellular matrix receptors play a role in recruiting collagen to basement membranes *in vivo*. With small gene families encoding basement membrane receptors in *C. elegans* (Kramer, 2005) it is feasible to knock down known receptors individually, in different combinations, and in a tissue-specific manner to determine whether they affect collagen distribution. It is also possible that unidentified or uncharacterized collagen-specific receptors, interactions between collagen and other basement membrane components, or the action of enzymes such as proteases are important for directing incorporation of basement membrane collagen. Unbiased screens in *C. elegans* could identify such genes, and the genetic, cellular, and molecular tools of *C. elegans* would allow in-depth analysis of how these molecules function in a cell-specific manner. Similar approaches could be taken to identify genes involved in generating the specific localization patterns of other basement membrane proteins we have discussed: for example, the presence of laminin along the sublateral nerves, the enrichment nidogen at muscle edges, the concentrated perlecan at muscle cell basement membrane attachments, the increased laminin around the endoderm cells posterior to the primordial germ cells, and hemicentin specifically at the attachments of mechanosensory and uterine cells (Huang et al., 2003; Kang & Kramer, 2000; Kim & Wadsworth, 2000; Muriel et al., 2005; Rogalski et al., 1993; Rohrschneider & Nance, 2013).

## 5.2 Physical forces on basement membranes

We have also highlighted diverse aspects of organ growth and morphogenesis for which the mechanical properties of basement membranes are likely important in maintaining tissue shape and integrity. The biomechanical properties of the basement membrane change over time, and basement membranes are thought to sense and respond to external physical forces

(Candiello et al., 2007; Halfter et al., 2013; Ingber, 2003; Moore et al., 2005). How basement membranes detect and mediate responses to force is largely unknown. During morphogenesis, basement membranes likely experience changes in force as a result of expanding or contracting tissues, changes in basement membrane composition, or the actions of enzymes such as proteases. For example, the *C. elegans* gonad undergoes a dramatic expansion during larval development (Hirsh, Oppenheim, & Klass, 1976; Kimble & White, 1981; Keeley and Sherwood, unpublished data), and the gonadal basement membrane likely experiences increased tension during this process. This tension could be relieved by addition of new basement membrane from sources such as the DTCs. Alternatively, the protease GON-1, which is important for gonad expansion (see above), may relieve basement membrane tension through reducing type IV collagen levels or function, thus allowing flexibility in the basement membrane for the gonad to expand and take on complex shapes. The forces borne by the basement membrane have not been precisely measured, and how basement membranes bear load is poorly understood. FRET-based sensors have been used to visualize tension across various molecules (Borghi et al., 2012; Grashoff et al., 2010; Meng, Suchyna, Lazakowitch, Gronostajski, & Sachs, 2011). These sensors could be adapted to *C. elegans* basement membrane components to determine the load that basement membranes bear, and if these loads are altered during morphogenetic processes, by proteases, or by alterations in basement membrane composition. These tools could also be important for determining whether the physical properties of the basement membrane are altered before it is breached and displaced by the AC during invasion, or as it is shifted by invaginating cells during uterine–vulval attachment.



## 6. CONCLUSION

Relative to the millions of years of evolution that have shaped basement membrane structure and function, our study and understanding of basement membrane biology is in its infancy. Our knowledge of basement membranes has transformed greatly since they were first described in 1840 (Bowman, 1840), and there is an emerging appreciation for basement membranes as dynamic structures that interact with cells and influence various aspects of their biology. In recent decades, the study of basement membranes in *C. elegans* has led to significant advancements in our understanding of

these important biological structures. We expect that further studies harnessing the strengths of the *C. elegans* system will continue to provide a more complete view of the diverse, conserved, and crucial functions of basement membranes during development, which will also provide insight into the many human diseases where basement membranes are misregulated.

## ACKNOWLEDGMENTS

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