Sticky worms: adhesion complexes in *C. elegans*

Elisabeth A. Cox* and Jeff Hardin

Department of Zoology, University of Wisconsin, 1117 W. Johnson Street, Madison, WI 53706, USA
*Author for correspondence (e-mail: eacox@wisc.edu)

Journal of Cell Science 117, 1885-1897 Published by The Company of Biologists 2004
doi:10.1242/jcs.01176

Summary

***Caenorhabditis elegans* is a powerful model system for investigating the establishment, regulation and function of adhesive structures in vivo. *C. elegans* has several adhesion complexes related to those in vertebrates. These include: (1) epithelial apical junctions, which have features of both adherens and tight junctions; (2) dense bodies, which are muscle-attachment structures similar to focal adhesions; (3) fibrous organelles, which resemble hemidesmosomes and mediate mechanical coupling between tissues; and (4) a putative dystrophin-glycoprotein complex that has potential roles in muscle function and embryogenesis. Recent work has increased our understanding of these structures and has given new insights into the functions of their vertebrate counterparts.**

Movies available online

Key words: Cell adhesion, Adherens junctions, Integrins, Hemidesmosomes, Dystrophin-glycoprotein complex

Introduction

Adhesion complexes play key roles in many events, including cell migration, differentiation, proliferation, apoptosis and growth cone guidance. The underlying strategies used to accomplish cell adhesion are remarkably similar in *Caenorhabditis elegans*, *Drosophila* and humans (Hynes and Zhao, 2000). Therefore, studies in relatively simple organisms such as *C. elegans* have the potential to reveal much about the basic, conserved molecular mechanisms that mediate and regulate cell adhesion.

Several aspects of *C. elegans* make it highly amenable to in vivo studies of cell adhesion. Its small size, simple body plan, optical transparency, invariant cell lineage, fast generation time and genetic manipulability facilitate the analysis of adhesion complexes. Forward genetic screening has identified numerous proteins that are important for cell adhesion in *C. elegans*, including integrins (Williams and Waterston, 1994), a classical cadherin (Costa et al., 1998) and many others. Furthermore, analysis of the *C. elegans* genome has identified numerous putative adhesion receptors, many of which have vertebrate homologs [see Cell Science at a Glance article in this issue, pp. 1867-1870 (Cox et al., 2004)] (Hutter et al., 2000). Several adhesion complexes in *C. elegans* are similar to those in vertebrates, including: epithelial apical junctions, which have traits of adherens and tight junctions; integrin-mediated dense bodies, which orchestrate sarcomere assembly and are similar to focal adhesions; hemidesmosome-like fibrous organelles, which anchor intermediate filaments and mechanically couple tissues; and a putative dystrophin-glycoprotein complex, which may link the extracellular matrix (ECM) and actin cytoskeleton in epithelial cells, neurons and, potentially, muscle. These complexes have been studied predominantly during epithelial and muscle morphogenesis. Cell adhesion also plays an important role in the nervous system of *C. elegans*, particularly in synapse formation (reviewed by Broadie and Richmond, 2002; Jin, 2002) and growth cone guidance (reviewed by Branda and Stern, 1999; Cooper, 2002); however, these topics are beyond the scope of this review.

C. elegans apical junctions

Epithelial junctions in *C. elegans* and higher eukaryotes are strikingly similar in composition and function, but have some overall differences in organization (Fig. 1A) (reviewed by Knust and Bossinger, 2002; Müller and Bossinger, 2003). In the epithelia of *C. elegans* (which include the epidermis, pharynx, intestine and vulva), a single, electron-dense region is typically restricted to the apical contact zone between cells (Fig. 1B) (Priess and Hirsh, 1986; Leung et al., 1999). This *C. elegans* apical junction (CeAJ) has several domains with distinct multiprotein complexes (Fig. 1A).

The apical region of CeAJs contains a cadherin-catenin complex that mediates adhesion and anchorage to the actin cytoskeleton. Because of its similarity to adherens junctions of other organisms, we refer to this as the adherens junctional domain. More basal is a domain containing the Discs large (Dlg) homolog, DLG-1, and its binding partner, AJM-1. The DLG-AJM-containing domain regulates adhesion and might also regulate paracellular permeability.

In cells of the intestine and pharynx, a region apical to the adherens junctional domain has a composition that is similar to that of the vertebrate tight junction and the subapical region (SAR) in *Drosophila*. This SAR-like region contains a PAR-3–PAR-6–PKC-3 complex and at least one homolog of Crumbs, a transmembrane protein that regulates cell polarity in *Drosophila* and vertebrates (reviewed by Müller and Bossinger, 2003). Although the PAR-atypical PKC (aPKC) complex regulates cell polarization in the one-cell *C. elegans*

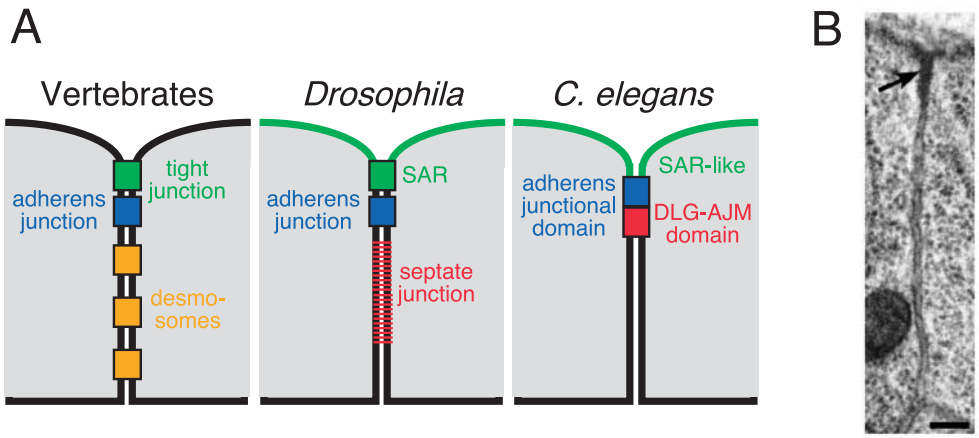


Fig. 1. The *C. elegans* apical junction. (A) Schematic diagram showing epithelial junctional organization in vertebrates, *Drosophila* and *C. elegans*. In vertebrates and *Drosophila*, different junctions are distinguishable by electron microscopy (EM), whereas in *C. elegans* there is one electron-dense region called the *C. elegans* apical junction (CeAJ). CeAJs consist of at least three distinct domains, although how these domains correlate to the electron-dense region is currently unclear. Note that the SAR of *Drosophila* is concentrated at the marginal zone but extends to the apical surface (reviewed by Tepass et al., 2001). Components of the SAR-like region of *C. elegans* (observed in pharynx and intestine) also localize to both the marginal zone and apical surface (Bossinger et al., 2001). Despite some differences in organization, there is compositional similarity between the regions shown in green (vertebrate tight junction, *Drosophila* SAR, and *C. elegans* SAR-like domain), blue (vertebrate, *Drosophila*, and *C. elegans* adherens junctions) and red (*Drosophila* septate junction and the *C. elegans* DLG-AJM domain) (reviewed by Knust and Bossinger, 2002). (B) TEM image of a CeAJ (arrow indicates the electron-dense region). Bar, 100 nm.

embryo (reviewed by Ohno, 2001; Etienne-Manneville and Hall, 2003), its functional role in *C. elegans* epithelia is not known. *C. elegans* has one *Crumbs* homolog (*crb-1*) and a second Crumbs-like gene (*crl-1* or *eat-20*). Loss-of-function studies indicate that neither is essential for epithelial development (Bossinger et al., 2001), although EAT-20 is necessary for proper muscle pumping in the pharynx (Shibata et al., 2000). Thus, the function of the SAR-like region remains unclear. Accordingly, we focus here on describing recent progress in understanding the adherens junctional and DLG-AJM domains.

Adherens junctional domain

Classic cadherins are calcium-dependent homophilic cell-cell adhesion receptors linked to the actin cytoskeleton through β-catenin, which binds to α-catenin. Cadherins also bind to p120 catenin (p120ctn), which has a complex role in regulating cadherin function (reviewed by Anastasiadis and Reynolds, 2000). *C. elegans* has a cadherin-catenin complex consisting of the classical cadherin, HMR-1A, HMP-2 (β-catenin), HMP-1 (α-catenin) and JAC-1 (p120ctn) (Fig. 2A) (Costa et al., 1998; Pettitt et al., 2003). These proteins have low overall sequence similarity to their vertebrate homologs, but have conserved functional domains and protein-protein interactions (reviewed by Simske and Hardin, 2001; Pettitt et al., 2003).

The *C. elegans* HMR-HMP complex was identified through its essential role in morphogenesis of the hypodermis (Costa et al., 1998) (reviewed by Simske and Hardin, 2001), a specialized epidermis that forms the outer layer of the worm. Epidermal cells are born on the dorsal side of the embryo after gastrulation and adhere to form a cell sheet. The free edges migrate to the ventral midline, where they seal through the formation of adhesive junctions in a process called ventral enclosure (Fig. 2B) (Williams-Masson et al., 1997). The formation of junctions is rapid (taking fewer than 15 minutes) and is mediated by contact between actin-rich protrusions (see Movie 1, http://jcs.biologists.org/supplemental/) (Raich et al., 1999). Junction formation in cultured cells occurs more slowly, but is similarly initiated by cell protrusions (reviewed by Vasioukhin and Fuchs, 2001). Circumferential actin filament bundles (CFBs) anchor to CeAJs and their contraction aids the four-fold elongation of the embryo into a worm-like shape (Fig. 2B).

hmr-1-null mutants exhibit a ‘hammerhead’ (Hmr) phenotype (Movie 2, http://jcs.biologists.org/supplemental/), in which epidermal cells fail to enclose the anterior, and cells spill out of this region (Costa et al., 1998). *hmp-1*-null and *hmp-2*-null embryos exhibit a more mild ‘humpback’ (Hmp) phenotype (Movie 3, http://jcs.biologists.org/supplemental/), in which embryos enclose but form abnormal bulges when they attempt to elongate (Costa et al., 1998). However, *hmp-1*-null or *hmp-2*-null embryos that also lack maternal *hmp-1* or *hmp-2* exhibit the more severe Hmr phenotype. JAC-1, like the *Drosophila* p120ctn homolog (Myster et al., 2003), positively modulates cadherin-catenin function in *C. elegans* but is not essential for development (Pettitt et al., 2003).

The putative four-pass transmembrane protein VAB-9 also localizes to the adherens junctional domain (Simske et al., 2003). VAB-9 has *Drosophila* and vertebrate homologs (BCMP1) and is distantly related to claudins, which are tetraspan cell adhesion receptors that localize to vertebrate tight junctions (Tsukita et al., 2001; Colegio et al., 2002). *vab-9*-null mutants are viable but have a ‘variably abnormal’ (Vab) phenotype, characterized by elongation and body-shape defects. VAB-9 requires HMR-1 for its localization and regulates CFB attachment to CeAJs (Simske et al., 2003), although the mechanisms involved are unclear.

Several proteins have emerged as potential regulators of the adherens junctional domain including APR-1, Rho family GTPases and semaphorins. APR-1, a homolog of the APC tumor suppressor, localizes to epidermal cell borders, and mutants exhibit Hmr-like phenotypes in addition to defects in cell fate specification, among others (Hoier et al., 2000). The potential role of APR-1 in regulating the HMR-HMP complex has not been explored. Rho-family GTPases are key regulators of adherens junctions in other systems (reviewed by Van Aelst and Symons, 2002). *C. elegans* homologs of Cdc42 (CeCDC-

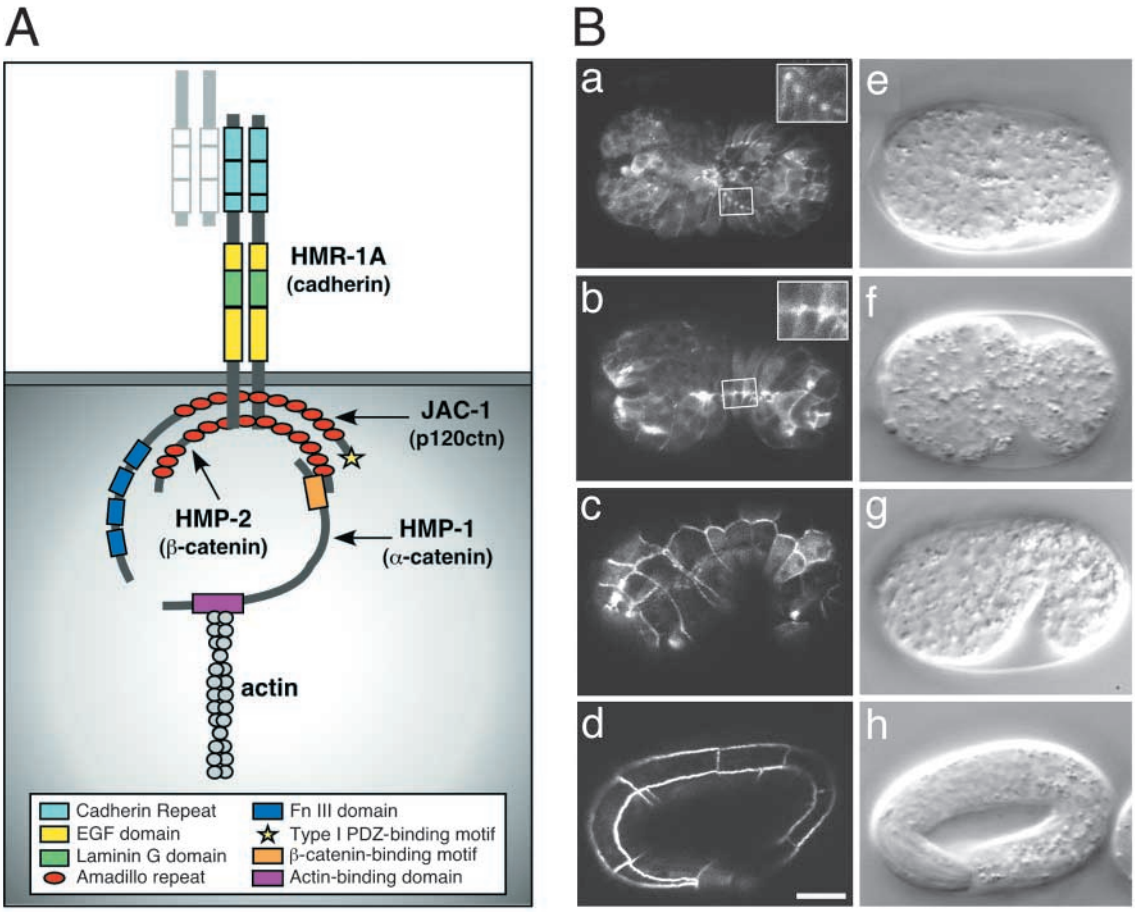


Fig. 2. The cadherin-catenin complex in *C. elegans*. (A) Diagram of the cadherin-catenin complex in *C. elegans*. This complex consists of HMR-1A (cadherin), HMP-2 (β-catenin), HMP-1 (α-catenin) and JAC-1 (p120ctn). (B) A time course showing localization of the cadherin-catenin complex during epidermal morphogenesis in *C. elegans*. Confocal images of a living embryo expressing a JAC-1–GFP fusion protein. During ventral enclosure (a-b), edges of the epidermal cell sheet extend around the embryo, meet at the ventral midline, and seal through the formation of cell junctions. Note that JAC-1–GFP is not present at the leading edge of migrating epidermal cells, but is rapidly recruited to the contact region between cells at the ventral midline. Other adherens junctional components show a similar localization during enclosure. After ventral enclosure, contraction of the epidermis helps to drive the four-fold elongation of the embryo (c,d). Nomarski images of similarly aged embryos are shown for comparison (e-h). A Nomarski movie (Movie 4, http://jcs.biologists.org/supplemental/) showing development of a wild-type embryo is available online. In all images, anterior is left. Bar, 10 μm.

42) and Rac-1 (CED-10) localize to epidermal cell borders (Chen et al., 1996), and Rho-family GTPases appear to regulate actomyosin contractility during elongation (Wissmann et al., 1997; Wissmann et al., 1999). Beyond this, the role of Rho-family GTPases in CeAJs is not understood. Interestingly, depleting the semaphorin MAB-20/Sema2A causes formation of ectopic epidermal cell contacts during morphogenesis (Roy et al., 2000). Abnormal contacts might be an indirect consequence of increased protrusive activity, although a more direct role for MAB-20 in regulating cell contacts cannot be ruled out. Homologs of Sema1A (SMP-1) and Sema1B (SMP-2) also appear to modulate epidermal cell contacts, but the mechanisms involved are unknown (Ginzburg et al., 2002).

C. elegans also has homologs of many proteins that localize to adherens junctions in other organisms, including ATN-1 (α-actinin), DEB-1 (vinculin), PES-7 (IQGAP) and ZO-1 (Y105E8A.26), but their roles at CeAJs are unclear (McMahon et al., 2001) (T. Tuskey, M. Köppen and J.H., unpublished). Several other proteins regulate epidermal cell migration during enclosure, including components of a conserved Arp2/3 complex (Sawa et al., 2003), an N-Wasp homolog (Sawa et al., 2003), GEX-2 and GEX-3 (Soto et al., 2002), and EVL-20/Arl2 (Antoshechkin and Han, 2002); however, a role for these proteins in cell junction formation has not been demonstrated.

Cadherin-independent mechanisms for mediating cell-cell adhesion

Since inactivation of cadherins and catenins in mice (Larue et al., 1994; Riethmacher et al., 1995), *Xenopus* (Heasman et al., 1994) and *Drosophila* (Tepass et al., 1996; Uemura et al., 1996) disrupts early embryonic cell adhesion, it is surprising that this is not the case in *C. elegans*. Other putative cadherins in *C. elegans* (Cox et al., 2004; Hill et al., 2001) could act redundantly with HMR-1. However, the only other cadherin characterized to date is the Fat-like cadherin, CDH-3, which regulates morphogenesis of the epidermal cell (hyp10) at the

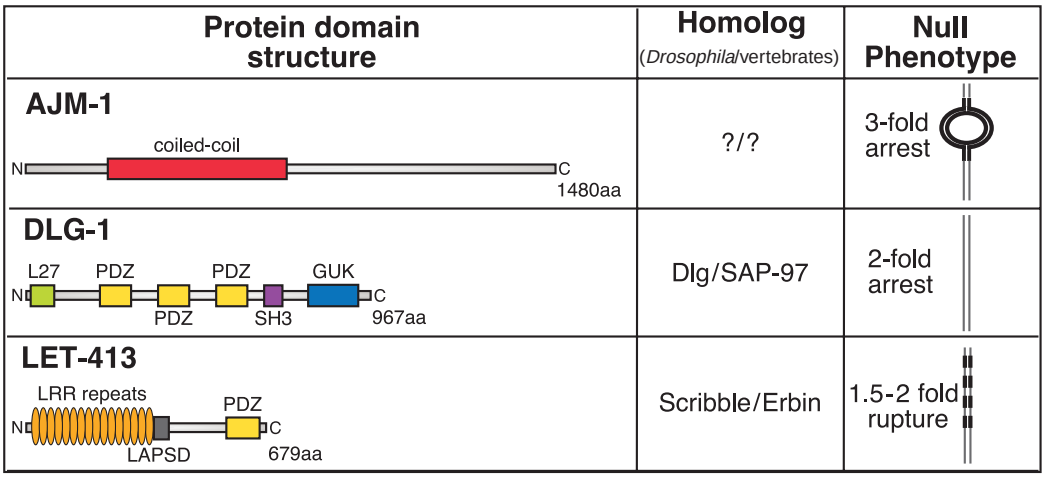


Fig. 3. The structure, homologs and mutant phenotypes of AJM-1, DLG-1 and LET-413. The strongest *Drosophila* and vertebrate homologs are listed. For the null phenotype, the stage of arrest is indicated and the drawing represents abnormalities in the electron-dense region, as observed by TEM (see text for details).

tip of the tail (Pettitt et al., 1996). Another possibility is the L1-CAM homolog, LAD-1 (Chen et al., 2001). LAD-1 localizes to cell-cell contacts in the early embryo and epithelia. Expression of dominant-negative LAD-1 causes body-shape defects, which could reflect perturbed epithelial adhesion. Another candidate is an analog of the ponsin-nectin-afadin complex, which provides adhesive function redundantly with the cadherin-catenin system in vertebrates (reviewed by Takai and Nakanishi, 2003). *C. elegans* lacks a clear nectin homolog, but has homologs of afadin (W03F11.6 and C43E11.6) and ponsin (Y45F10D.13) (McMahon et al., 2001). RNA interference (RNAi) directed against these genes does not produce lethality (McMahon et al., 2001). However, RNAi directed against W03F11.6 enhances the lethality of embryos that have a weak hypomorphic mutation in *hmp-1* (M. Köppen, J.H. and J. Pettitt, unpublished), indicating that it might either act in the same pathway or one parallel to that involving the HMR-HMP complex.

DLG-AJM domain

The DLG-AJM domain lies basal to the adherens junctional domain (Fig. 1) and plays a role in adhesion and, potentially, in paracellular permeability. DLG-1 is a membrane-associated guanylate kinase (MAGUK; Fig. 3) that has homologs in *Drosophila* (Dlg) and vertebrates (hDlg/SAP97). *Drosophila* Dlg regulates formation of adherens and septate junctions (reviewed by Müller and Bossinger, 2003), and is also important for synapse formation (Budnik, 1996) and preventing follicular cell overgrowth during oogenesis (Goode and Perrimon, 1997). Like *Drosophila* Dlg, vertebrate SAP97 localizes to epithelial cell contacts, acts as a tumor suppressor, and aids assembly of synaptic junctions (reviewed by Fujita and Kurachi, 2000). Mice with a partial deletion of SAP97 exhibit cleft palates (Caruana and Bernstein, 2001), highlighting its importance in morphogenesis.

AJM-1 is a novel coiled-coil protein (Fig. 3) (Köppen et al., 2001). The coiled-coil region is similar to that of the keratin-binding protein trichohyalin (E.A.C. and J.H., unpublished), and its topology is predicted to be similar to that of the vertebrate tight junction proteins cingulin and JEAP (S. Citi, personal communication). DLG-1 is necessary for the proper localization of AJM-1 and physically interacts with AJM-1 in vitro (Bossinger et al., 2001; Firestein and Rongo, 2001; Köppen et al., 2001; McMahon et al., 2001).

Loss of DLG-1 or AJM-1 causes embryonic arrest at the two- to three-fold stage of elongation, which appears to be accompanied by necrotic cell death (C. Lockwood and J.H., unpublished) (Bossinger et al., 2001; Firestein and Rongo, 2001; Köppen et al., 2001; McMahon et al., 2001). Additionally, loss of DLG-1 results in leakage of cytoplasm from the tail and ventral midline during elongation (McMahon et al., 2001) and abnormalities in actin bundles of the pharynx and epidermis (Firestein and Rongo, 2001).

The precise function of the DLG-AJM domain is not known, but transmission electron microscopy (TEM) analysis has provided some insights. In *ajm-1* null mutants, junctions occasionally separate, leaving paracellular gaps of 50-200 nm (Köppen et al., 2001). This is speculated to cause loss of solute gating, leading to osmotic shock (Köppen et al., 2001). By contrast, embryos subject to RNAi directed against *dlg-1* exhibit loss of electron-dense material from CeAJs (Firestein and Rongo, 2001; McMahon et al., 2001), indicating that DLG-1 might act as a scaffold for the formation of this material. Intriguingly, epithelial cell membranes nonetheless remain closely apposed. They may be held together by the HMR-HMP complex, which remains intact in *dlg-1(RNAi)* embryos (Bossinger et al., 2001; McMahon et al., 2001). In light of this, it is interesting that the severity of the adhesion phenotypes induced by loss of DLG-1 or AJM-1 is enhanced by RNAi directed against the adherens junctional component *vab-9* (Simske et al., 2003). This suggests that the adherens junctional and DLG-AJM domains act together to promote epithelial integrity.

Recent work shows that the claudin-like protein CLC-1 colocalizes with AJM-1 in pharyngeal cell junctions, where it might regulate paracellular permeability (Asano et al., 2003). Determining whether CLC-1 or other claudin-like proteins (CLC-2, CLC-3 and CLC-4) function within the DLG-AJM domain will be an important topic for future study. Although they have not been extensively characterized, *C. elegans* also has several homologs of tight and septate junctional proteins (McMahon et al., 2001; Knust and Bossinger, 2002), including Lethal giant larvae (M01A10.2), Band 4.1/Coracle (ZK270.2, C48D5.2A, T04C9.6, H05G16.2), Symplekin (F25G6.2) and Neurexin IV (F20B10.1). Identifying more components of the

DLG-AJM domain, and determining whether it interacts with the cytoskeleton, will help clarify its function.

Regulation of CeAJ organization and polarity

Currently, little is known about how CeAJ polarity is established and maintained. Components of the adherens junctional domain (the HMR-HMP complex and VAB-9) are not necessary for localization of the DLG-AJM complex and vice versa (Costa et al., 1998; Bossinger et al., 2001; Firestein and Rongo, 2001; Köppen et al., 2001; McMahon et al., 2001; Simske et al., 2003). Recent insights into junctional polarity in *C. elegans* are derived from studies of the LAP ('leucine-rich repeat and PDZ') protein LET-413 (Fig. 3). LET-413 localizes through its leucine-rich repeat region to the basolateral membrane of epithelial cells and partially overlaps the DLG-AJM domain (Legouis et al., 2000; Legouis et al., 2003). Like its homologs in *Drosophila* (Scribble) (Bilder et al., 2000; Bilder and Perrimon, 2000) and vertebrates (Erbin) (Borg et al., 2000), LET-413 has an important role in regulating junctional organization.

In *C. elegans* embryos lacking LET-413, the electron-dense material at CeAJs is discontinuous, extended basally, or absent entirely (Legouis et al., 2000; McMahon et al., 2001). What accounts for this phenotype is not entirely clear, but several recent studies offer clues. First, LET-413 prevents basal diffusion of some apical CeAJ proteins, including PAR-3 and PAR-6 (Bossinger et al., 2001). Second, although LET-413 is not necessary for apical targeting of adherens junctional components (Legouis et al., 2000; McMahon et al., 2001; Simske et al., 2003), it does act with DLG-1 to exclude them from the basolateral membrane (Simske et al., 2003). Third, LET-413 is important for promoting proper distribution of DLG-1 and AJM-1 and preventing them from spreading basally (Köppen et al., 2001; McMahon et al., 2001). The molecular mechanisms through which LET-413 accomplishes these functions are unknown. LET-413 could interact with a motor complex (McMahon et al., 2001) or the Rho-family GTPases (Legouis et al., 2000), although direct evidence for either possibility is lacking. Further studies on LET-413 and the identification of other regulators of CeAJ polarity are likely to clarify how epithelial cell polarity is established in vivo.

In summary, despite organizational differences between epithelial junctions in *C. elegans*, *Drosophila* and vertebrates, there are several conserved proteins that share some analogous functions (see also Müller and Bossinger, 2003). These include the HMR-HMP (cadherin-catenin) complex, DLG-1 (Dlg/SAP-97) and LET-413 (Scribble/Erbin). *C. elegans* also has a PAR-aPKC complex, and it will be important to determine its functional role in epithelial cell adhesion and polarity. The conservation of these proteins indicates their importance as core elements of epithelial cell junctions and indicates that *C. elegans* is an excellent model system for studying their functions.

Dense bodies and M-lines

C. elegans has four longitudinal muscle quadrants that underlie the epidermis (reviewed by Francis and Waterston, 1985). Muscle quadrants are first detectable just after ventral enclosure is complete and sarcomeres are evident at the two-fold stage of elongation (reviewed by Moerman and Fire, 1997). Force transduction in muscle is accomplished through anchorage of actin and myosin filaments to the muscle cell membrane. In *C. elegans*, mutations that perturb this process cause arrest at the two-fold stage of elongation owing to muscle paralysis (Hresko et al., 1994; Williams and Waterston, 1994; Gettner et al., 1995). Characterization of 'paralyzed at two-fold' (Pat) mutants has shown that integrin attachment structures are essential for sarcomere assembly in *C. elegans*. Similarly, integrins are also required for the formation of functional muscles in *Drosophila* and mammals (reviewed by Bokel and Brown, 2002).

C. elegans is a powerful model system for exploring integrin function, in part because it has just two α-integrins (PAT-2 and INA-1) and one β-integrin (PAT-3). In *C. elegans*, roles for integrins in cell migration (Baum and Garriga, 1997; Lee et al., 2001), neuron fasciculation (Baum and Garriga, 1997), gonadal morphogenesis (Lee et al., 2001) and muscle tissue integrity (see below) have been documented. However, the adhesive structures organized by integrins have been most extensively examined in muscle.

During sarcomere assembly in *C. elegans*, parallel bundles of actin (thin filaments) and myosin (thick filaments) link to the cell membrane through dense bodies (Z-disc analogs) and M-lines, respectively (Fig. 4A,B). Dense bodies are similar to vertebrate focal adhesions, which are integrin-based complexes that mediate linkage between the ECM and the actin cytoskeleton (Hynes, 1992). In addition to their structural role, focal adhesions also mediate cell signaling (reviewed by Zamir and Geiger, 2001; Hynes, 2002). M-lines are also integrin based and are compositionally similar to dense bodies; however, they are inherently different from focal adhesions since they anchor to myosin rather than actin.

Dense bodies and M-lines are organized by αPAT-2–βPAT-3 integrin heterodimers, which recruit numerous cytoplasmic proteins also present in focal adhesions (Fig. 5A). Like focal adhesions, dense bodies contain DEB-1/vinculin (Barstead and Waterston, 1991), ATN-1/α-actinin (Francis and Waterston, 1985; Barstead et al., 1991), CeTalin (Moulder et al., 1996), UNC-97/PINCH (Hobert et al., 1999), UNC-112/Mig-2 (Rogalski et al., 2000), PAT-4/ILK (Mackinnon et al., 2002) and PAT-6/actopaxin (Lin et al., 2003). M-lines lack actin-binding proteins (DEB-1 and ATN-1), and instead contain a novel immunoglobulin-fold protein, UNC-89 (Benian et al., 1996), that binds to myosin.

Assembly of dense bodies and M-lines

The first step in dense body and M-line assembly is recruitment of integrins to the plasma membrane, where they cluster into bands, one along the longitudinal axis of each body-wall muscle quadrant. Subsequently, these initial attachment sites segregate into a highly ordered pattern of dense bodies and M-lines (Fig. 4B).

UNC-52/perlecan, a secreted heparan sulfate proteoglycan, is the only protein known to be necessary for integrin localization to the basal muscle cell membrane (Rogalski et al., 1993; Hresko et al., 1994; Williams and Waterston, 1994; Mullen et al., 1999). Whether UNC-52 is a ligand for αPAT-2–βPAT-3, or has an indirect role in integrin recruitment, is currently unknown. Recent studies indicate that the laminin αB subunit (one of two laminin α subunits), like UNC-52,

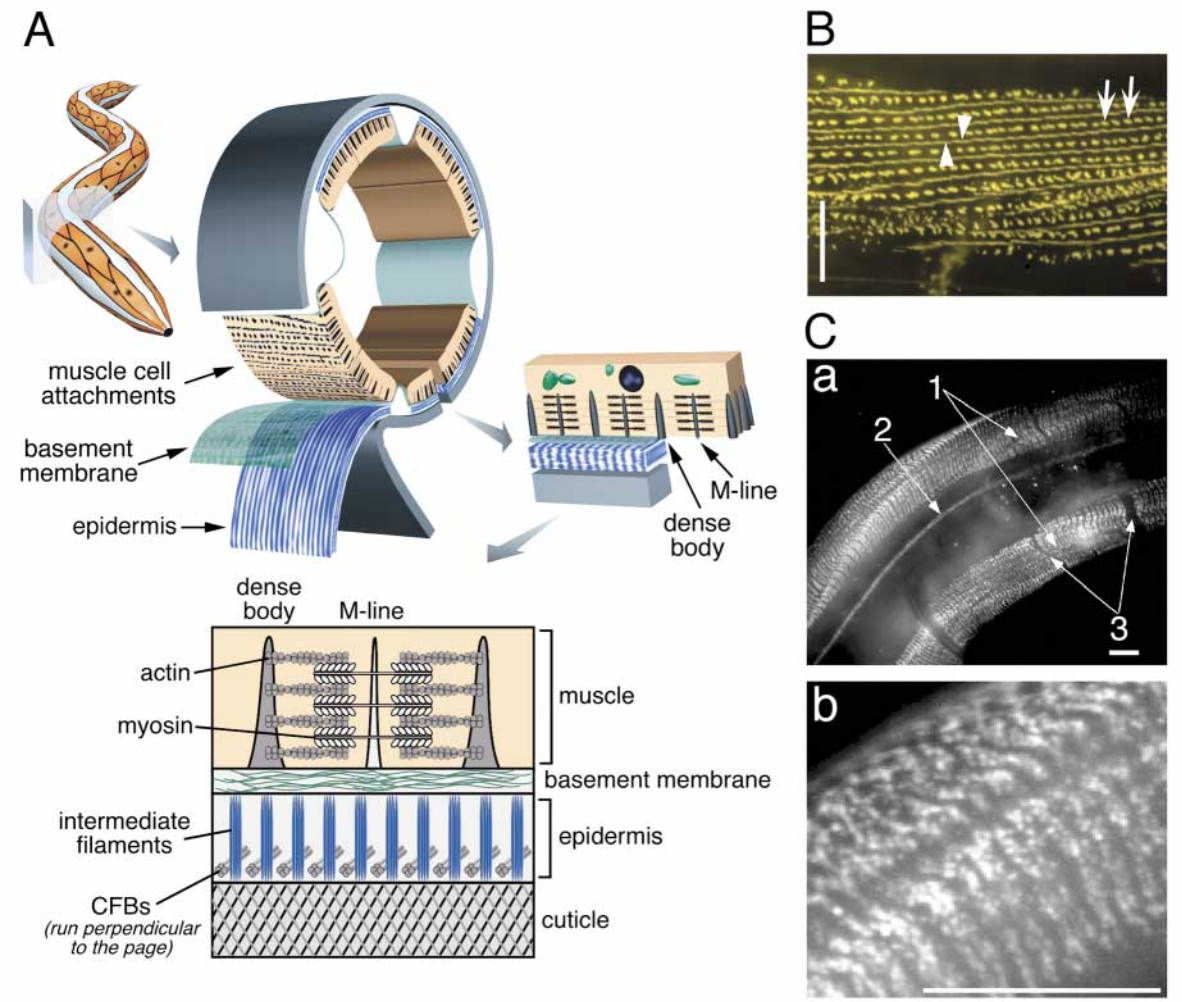


Fig. 4. Dense bodies, M-lines and fibrous organelles. (A) Schematic showing the localization and arrangement of dense bodies and M-lines in *C. elegans* body wall muscle and fibrous organelles in the epidermis. (Adapted from Mackinnon et al., 2002.) (B) Dense bodies (arrows) and M-lines (arrowheads), visualized with PAT-4–YFP. (Adapted from Mackinnon et al., 2002.) Bar, 5 μm. (C) Fibrous organelles, visualized by MUA-3 staining. Fibrous organelles are shown in regions of epidermal cell contact with body wall muscle (1) and the ALM touch neuron (2). Gaps in fibrous organelles are seen where nerves pass between the epidermis and muscle (3). The image in (b) is an enlargement of (a). (Adapted from Bercher et al., 2001.) Bars, 10 μm.

localizes to the extracellular face of dense bodies and M-lines (Huang et al., 2003). However, rather than mediating assembly, laminin αB is necessary for restricting dense body formation to the basal membrane (Huang et al., 2003).

There are at least two distinct protein complexes that are recruited separately to nascent adhesion sites. One complex contains DEB-1/vinculin, which mediates actin filament attachment to dense bodies (Barstead and Waterston, 1991; Hresko et al., 1994). Another complex, containing UNC-112, PAT-4 and PAT-6, acts as a scaffold for the development of nascent attachment sites into dense bodies and M-lines (Rogalski et al., 2000; Mackinnon et al., 2002; Lin et al., 2003). In embryos with null mutations in these genes, UNC-52 localizes normally in the basement membrane, and integrin foci form and become associated with DEB-1, but dense bodies and M-lines do not develop.

UNC-112 has a conserved sequence present in FERM-domain proteins (Rogalski et al., 2000) that might mediate attachment to the plasma membrane (Chishti et al., 1998). Recently, Mig-2, a human homolog of UNC-112, has been shown to localize to integrin-mediated adhesions in tissue culture, where it recruits the filamin-binding protein migfilin (Tu et al., 2003). Loss of Mig-2 or migfilin impairs cell spreading and shape modulation, although cell-substratum contacts still form (Tu et al., 2003). Currently, it is unknown whether Mig-2, like UNC-112, forms a complex with ILK and actopaxin. Conversely, whether or not UNC-112, like Mig-2, provides a link to filamin is not known.

PAT-4 is the only integrin-linked kinase (ILK) homolog in *C. elegans* (Mackinnon et al., 2002) and participates in recruiting proteins to nascent integrin attachment sites. Surprisingly, transgenically expressed kinase-dead PAT-4 can rescue *pat-4*-null mutants (Mackinnon et al., 2002) and a similar result has been obtained with the *Drosophila* ILK homolog (Zervas et al., 2001). This suggests that ILK primarily functions as an adaptor protein rather than a kinase (Zervas and Brown, 2002). Consistent with its role as an adaptor protein in *C. elegans* is the observation that PAT-4/ILK binds to UNC-

112/Mig-2, UNC-97/PINCH and PAT-6/actopaxin in yeast-two-hybrid assays (Mackinnon et al., 2002; Lin et al., 2003).

PAT-6 is an actopaxin homolog that has an important role in promoting the linkage of actin and myosin filaments to dense bodies and M-lines, respectively (Lin et al., 2003). PAT-6 might directly recruit actin to dense bodies through its two tandem, unconventional calponin-homology domains and appears to recruit myosin to M-lines indirectly through binding UNC-89 (Lin et al., 2003).

Maintaining integrity of dense bodies and M-lines

Although not necessary for the formation of dense bodies and M-lines, UNC-97 is essential for their integrity (Hobert et al., 1999). UNC-97 belongs to the PINCH family of adaptor proteins, which have important roles in integrin-dependent processes in *Drosophila* and mammalian cells (reviewed by Wu et al., 1999). Notably, mammalian PINCH1 binds ILK, and this interaction is important for cell spreading and motility in tissue culture (Zhang et al., 2002). Similarly, UNC-97 binds PAT-4 in yeast two-hybrid assays and is recruited to dense bodies and M-lines by PAT-4 (Hobert et al., 1999). UNC-97 also interacts with UNC-98, a C2H2 Zn-finger protein that localizes to M-lines (and possibly dense bodies) and to the nucleus (Mercer et al., 2003). UNC-98 might be involved in maintaining muscle structure, although the underlying mechanisms are not understood.

Other regulators of dense bodies and M-lines

Several other less well-characterized proteins regulate dense bodies and M-lines. The single β-G spectrin in *C. elegans*, UNC-70, localizes to several sites on the sarcolemmal membrane, including dense bodies and M-lines (Moorthy et al., 2000). Loss of UNC-70 results in progressive muscular dystrophy, which is associated with discontinuous dense bodies and disruption of the myofilament lattice (Moorthy et al., 2000; Hammarlund et al., 2000). Spectrin similarly localizes to Z- and M-lines in vertebrate skeletal muscle (Porter et al., 1992; Zhou et al., 1998). DIM-1 (‘disorganized muscle 1’) is a novel immunoglobulin-domain protein that localizes between and around dense bodies and stabilizes actin filament attachment (Rogalski et al., 2003).

Other integrin-mediated adhesions in *C. elegans*

Integrin-based adhesions can differ in morphology, composition and function (reviewed by Zamir and Geiger, 2001) (Wehrle-Haller and Imhof, 2002). Some, such as focal adhesions and dense bodies, are relatively large structures that mediate stable attachment to the ECM. By contrast, the smaller focal complexes at the leading edge of migrating cells and growth cones are rapidly turned over, permitting dynamic changes in adhesion. In addition to their role in muscle, *C. elegans* integrins also play a role in the formation of dynamic contacts in motile cells. For instance, migration of the distal tip cells, which directs morphogenesis of the gonad arms, is integrin dependent (Lee et al., 2001) and has recently been shown to involve CeTalin (Cram et al., 2003). Additionally, αINA-1/βPAT-3 regulates neuronal cell migration and fasciculation (Baum and Garriga, 1997) and acts at least in part through the Nck-interacting kinase (NIK) homolog MIG-15 (Poinat et al., 2002).

There is evidence that phosphorylation of βPAT-3 is important for its function in motile cells. Integrin β tails have one or two NPXY motifs that can be tyrosine phosphorylated, which thereby modulates integrin function (reviewed by Calderwood, 2004). Interestingly, the muscle defects of *pat-3*-null mutants can be rescued by a *pat-3* transgene that has tyrosine-to-phenylalanine mutations in its two NPXY motifs (Lee et al., 2001). However, these embryos have defects in distal tip cell migration. Further studies on the structure, regulation and signaling of integrin-mediated adhesions during developmental events in *C. elegans* are likely to increase our understanding of dynamic requirements for integrins in vivo.

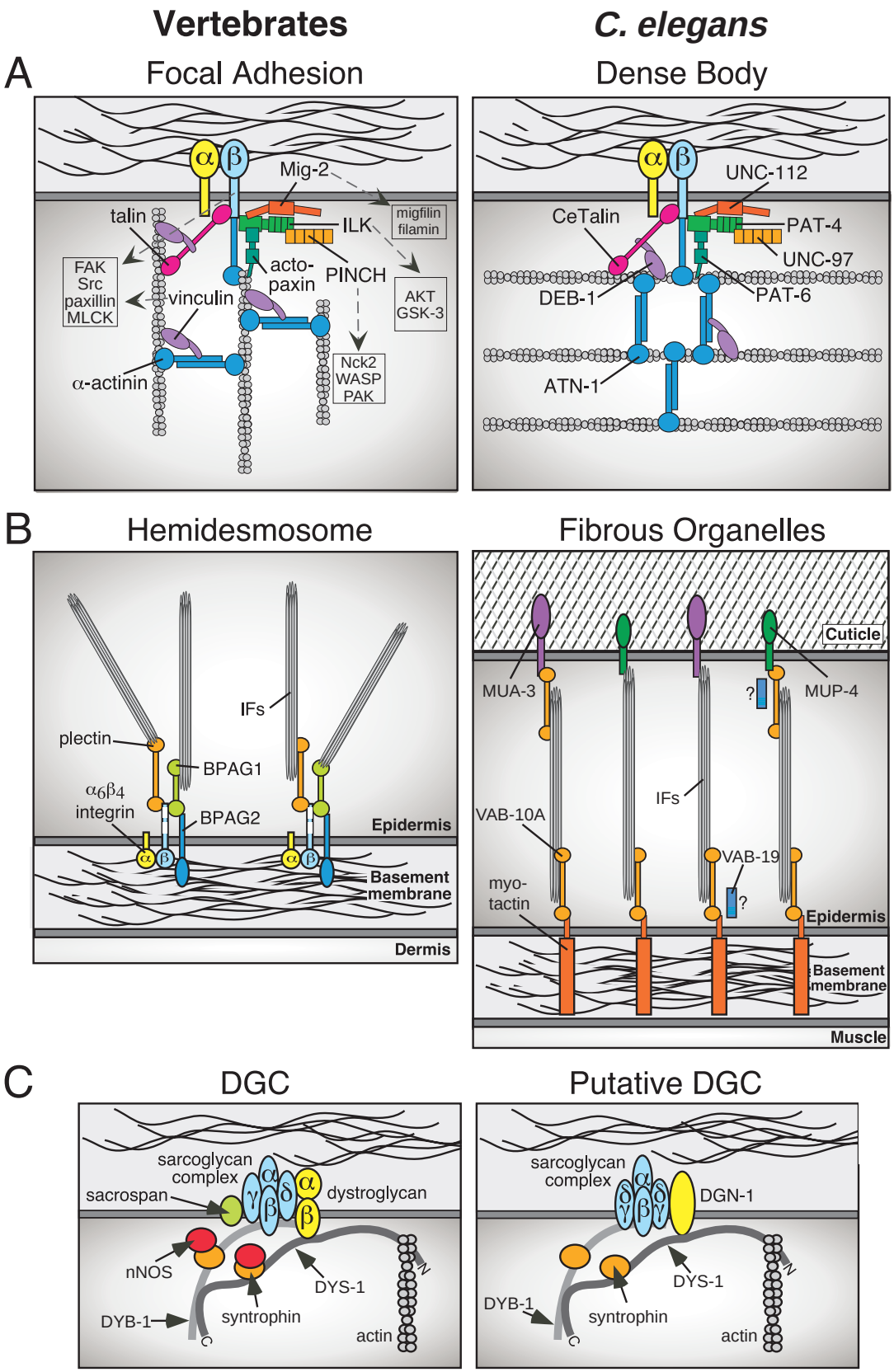
Fibrous organelles

Muscle cells in *C. elegans* are mechanically linked to the cuticle through trans-epidermal attachment structures called fibrous organelles (FOs). FOs comprise an epidermal intermediate filament (IF) array that is anchored basally to the underlying basement membrane and associated muscle and apically to the cuticle (Fig. 4A). FOs are restricted to epidermal regions that overlie body wall muscles, and their formation occurs concurrently with development of muscle sarcomeres (Francis and Waterston, 1991; Hresko et al., 1994; Hresko et al., 1999). In adults, FOs form circumferential bands (of 0.4 μm periodicity) composed of individual attachment spots (Bercher et al., 2001) that are in register with the spacing of cuticular ridges called annuli (Fig. 4C). By contrast, there is not a strict correlation between the location of FOs and dense bodies or M-lines (Francis and Waterston, 1991). FOs also form in other areas where force is transduced to or through the epidermis, including regions of epidermal contact with intestinal, anal and vulval muscles, and mechanosensory neurons (White, 1988; Francis and Waterston, 1991).

FOs are structurally similar to vertebrate type I hemidesmosomes (Fig. 5B) (reviewed by Hahn and Labouesse, 2001), in which transmembrane receptors form a link between cytoplasmic IFs and the basement membrane (reviewed by Nievers et al., 1999). Disruption of hemidesmosomal components causes separation of the epidermis from the basal lamina, resulting in various skin-blistering diseases (Jones et al., 1998; Nievers et al., 1999). Similarly, FOs confer structural integrity to the epidermis through anchoring IFs. In vertebrate hemidesmosomes, $\alpha_6\beta_4$ integrin and BPAG2 provide anchorage to the basement membrane, and plectin and BPAG1 bind IFs (reviewed by Jones et al., 1998; Nievers et al., 1999). The only conserved protein present in FOs is the plectin homolog VAB-10A (Bosher et al., 2003). FOs do not contain integrins or a BPAG2 homolog, but have other transmembrane proteins (myotactin, MUA-3 and MUP-4) that might function analogously.

Composition and assembly of FOs

The apical and basal attachment plaques of FOs have some similarities and differences in composition (Fig. 5B). VAB-10A/plectin and VAB-19/Kank appear to be present in both apical and basal attachment plaques. Apical attachments



contain the transmembrane receptors MUA-3 and MUP-4, whereas basal attachments contain myotactin. This probably reflects the need to have apical receptors specific for the collagen-rich cuticle and basal receptors specific for the basement membrane and/or muscle cell surface.

In regions of muscle contact with the epidermis, underlying

Fig. 5. Comparison of adhesion complexes in vertebrates and *C. elegans*. (A) A vertebrate focal adhesion and *C. elegans* dense body. Only conserved proteins are shown. Focal adhesions contain numerous adaptor and signaling proteins not shown here (refer to Zamir and Geiger, 2001). A few downstream effectors are indicated by the dashed arrows. (B) A vertebrate hemidesmosome and *C. elegans* fibrous organelles. (For more on hemidesmosome composition, see Nievers et al., 1999; Roper et al., 2002.) Like hemidesmosomes, fibrous organelles anchor IFs through a plectin-family member (VAB-10A). The transmembrane proteins MUA-3 and MUP-4 are located apically and myotactin is located basally. VAB-19 may be located both basally and apically. The protein-protein interactions shown for fibrous organelles are speculative, since it is currently unclear whether myotactin, MUA-3 or MUP-4 interacts directly with VAB-10A or IFs. (C) The dystrophin-glycoprotein complex (DGC) in vertebrates and *C. elegans*. Other proteins are known to associate with the mammalian DGC (reviewed by Ehmsen et al., 2002), but have not been shown for simplicity. *C. elegans* does not have any clear sarcospan or nNOS homologs (Segalat, 2002). Currently, it is unclear whether the putative *C. elegans* DGC is present in epidermal, muscle, and/or neuronal tissues (see text for details).

muscle initiates FO formation (Hresko et al., 1999); however, the nature of this cue is not well understood. Candidates include collagen IV (comprising EMB-9/α1 and LET-2/α2 subunits) and laminin αB. All are produced by myoblasts, and embryos lacking these proteins exhibit phenotypes consistent with perturbed FOs, including muscle separation from the epidermis (Gupta et al., 1997; Graham et al., 1997; Huang et al., 2003). Note that, in regions of contact with non-muscle tissues, the novel ECM protein hemicentin (HIM-4) is required for FO assembly (Vogel and Hedgecock, 2001), although the mechanisms involved are not understood.

Maintaining integrity of FOs

Several proteins are important for FO maintenance, including VAB-10A, VAB-10B, VAB-19 and the transmembrane proteins MUA-3, MUP-4 and myotactin. *vab-10* produces two isoforms with distinct functions (Bosher et al., 2003). As mentioned above, VAB-10A is similar to plectins, whereas VAB-10B is similar to plakins, which are actin-microtubule crosslinking proteins. VAB-10A localizes to FOs and VAB-10B localizes to bands between FOs (Bosher et al., 2003). Loss of either VAB-10 isoform results in epidermal detachment from the cuticle and muscle and perturbation of IFs and CFBs. VAB-10A mutants exhibit a Pat phenotype, whereas VAB-10B mutants typically die in the first larval stage and have elongation and body-shape defects. In VAB-10A mutants, the number of FOs is greatly reduced, indicating a potential role in FO assembly. By analogy to vertebrate plectins, VAB-10A might mediate the link between transmembrane receptors and IFs. VAB-10B appears to regulate the thickness of the epidermis and could provide resistance against tension, although the mechanisms involved remain unclear (Bosher et al., 2003).

MUA-3 and MUP-4 are single-span transmembrane proteins that maintain adhesion between the epidermis and cuticle (Plenefisch et al., 2000; Bercher et al., 2001; Hong et al., 2001). *mua-3* was identified in a screen for fragile muscle-attachment (Mua) mutants (Plenefisch et al., 2000), and *mup-4* was identified on the basis of a muscle-position-defective (Mup) phenotype (Gatewood and Bucher, 1997). In *mua-3* mutants, FOs form normally but the epidermis detaches from the cuticle post-embryonically (Bercher et al., 2001). *mup-4*-null animals exhibit a similar phenotype, but detachment occurs earlier, typically at the three-fold stage of elongation (Hong et al., 2001). MUA-3 and MUP-4 share structural similarities but do not have any clear non-nematode homologs (reviewed by Cox et al., 2004; Hahn and Labouesse, 2001). Their extracellular domains contain a von Willebrand factor type A domain, which mediates collagen binding in other proteins (Colombatti and Bonaldo, 1991), whereas their cytoplasmic domains have some sequence similarity to filaggrins, which are IF-binding proteins (reviewed by Hahn and Labouesse, 2001).

Myotactin (LET-805) is a novel transmembrane protein required for adhesion between the epidermis and muscle (Hresko et al., 1999). The longest splice form encodes a single-span transmembrane protein with at least 32 fibronectin type III (FNIII) repeats in the extracellular domain and a novel cytoplasmic domain. VAB-19, an ankrin repeat protein with sequence similarity to the human tumor suppressor Kank, is also required for attachment between muscle and the epidermis (Ding et al., 2003). Recruitment of VAB-19 to FOs requires VAB-10A; however, myotactin and VAB-19 require each other for their proper final localization. In myotactin and VAB-19 mutants, IFs maintain their annular arrangement but are not restricted to the area mirroring muscle/epidermal cell contact (Hresko et al., 1999; Ding et al., 2003). Apparently as a result, shortly after muscle contraction begins, muscle detaches from the epidermis.

Interestingly, the mutant phenotype of animals lacking VAB-19, but not that of other FO components, can be suppressed by mutations in the actin-binding protein *sma-1*/β-H spectrin (Ding et al., 2003). This suggests, along with previous studies on VAB-10 (see above) (Bosher et al., 2003), that cross-talk between FOs and actin is essential for proper cytoskeletal patterning in the epidermis. Continued studies on FOs are likely to yield important insights into how IFs and their attachment structures regulate cytoskeletal architecture and provide mechanical stability to tissues.

Putative dystrophin-glycoprotein complex

In vertebrates, the dystrophin-glycoprotein complex (DGC) confers stability to the muscle cell membrane during cycles of muscle contraction (Campbell, 1995). It is centered around dystroglycan (Fig. 5C) (reviewed by Ehmsen et al., 2002), a transmembrane receptor that binds various ligands, including the ECM components laminin, perlecan and agrin. Dystroglycan associates with a membrane-embedded sarcoglycan complex, and forms a link to the actin cytoskeleton through binding dystrophin. In addition to actin, dystrophin binds dystrobrevin and syntrophins, which interact with nitric oxide synthase (nNOS). Mutations in DGC components and in proteins that affect the ligand-binding activity of dystoglycan are found in various forms of muscular dystrophy (reviewed by Cohn and Campbell, 2000) (Michele and Campbell, 2003).

C. elegans has many conserved DGC components (Fig. 5C), including homologs of dystroglycan (*dgn-1* and F56C3.6), dystrophin (*dys-1*), dystrobrevin (*dyb-1*), α-sacroglycan (H22K11.4), β-sarcoglycan (K01A2.1), δ/γ-sarcoglycan

(F07H5.2) and syntrophin (F27D9.8, F30A10.8) (Bessou et al., 1998; Grisoni et al., 2002). The dystroglycan-binding region of DYS-1 is well conserved (Bessou et al., 1998), and DYS-1 and DYB-1 physically interact through their C-terminal coiled-coil domains (Gieseler et al., 1999). In addition, both DYS-1 and DYB-1 bind the syntrophin homolog F30A10.8 in vitro (Gieseler et al., 1999). The conservation of these protein-protein interactions suggests that the structure and function of the DGC is highly conserved between *C. elegans* and vertebrates.

The expression and subcellular localization of DGC components in *C. elegans* have not been extensively studied. Some DGC components are expressed in *C. elegans* muscle, including DYS-1 and DYB-1 (Segalat, 2002). However, DGN-1 and a second dystroglycan-like protein (F56C3.6) do not appear to be expressed in muscle, but are expressed in epithelial and neuronal tissues (R. Johnson and J. Kramer, personal communication). Consistent with this localization is the observation that embryos lacking either gene exhibit various epithelial and neural defects (R. Johnson and J. Kramer, personal communication). Similarly, dystroglycan is present in many non-muscle tissues in mammals (reviewed by Durbeej et al., 1998; Hemler, 1999). Whether or not *C. elegans* assembles a DGC analogously to vertebrates and, if so, where it is present, remain unclear.

In *C. elegans*, loss of DYS-1/dystrophin does not cause significant muscle degeneration. However, in ten-day-old *dys-1*-null animals, a small percentage (<1%) of body-wall muscle cells show an abnormal striation pattern, which may indicate the beginning of degeneration (Bessou et al., 1998). Loss of DYS-1 also results in movement defects consistent with increased muscle excitability (Bessou et al., 1998), and RNAi directed against *dgn-1*, δ/γ-sarcoglycan (F07H5.2) and syntrophin (F30A10.8) in adult worms causes a similar phenotype (Grisoni et al., 2002). Discerning the composition of dystroglycan-mediated adhesion complexes in the muscle and non-muscle tissues of *C. elegans*, and learning more about their precise functions, should yield important insights into the role of the DGC in development and disease.

Conclusion

Despite its small size and relative simplicity, *C. elegans* employs many of the same strategies for accomplishing cell adhesion as higher organisms. Most work to date has centered on defining the composition and basic roles of these structures, particularly during development. Despite this progress, there is still much to learn about how adhesion complexes in *C. elegans* accomplish their functions. In particular, their roles in mediating cell signaling have been largely unexamined. Since adherens junctions, focal adhesions, hemidesmosomes and the DGC all regulate cell signaling in tissue culture (reviewed by Nievers et al., 1999; Zamir and Geiger, 2001; Rando, 2001; Braga et al., 2002), it will be important to determine whether and how the analogs of these structures in *C. elegans* mediate signaling and how this affects in vivo events.

In the future, *C. elegans* might be a useful system for analyzing cross-talk between adhesion complexes. Adhesion receptor cross-talk occurs during convergent extension in *Xenopus* (Marsden and DeSimone, 2003) and is likely also to be important in many other in vivo events. Elongation of the *C. elegans* embryo, which involves the coordinated activity of adherens junctions, FOs and dense bodies, might be a useful context for studying how biochemical and physical cross-talk between adhesion receptors contributes to complex cellular events.

Additionally, *C. elegans* might be useful in the study of diseases involving aberrant cell adhesion, including tumor metastasis, chronic inflammatory disorders, tissue fragility disorders, muscular dystrophy, and many others. In this regard, *C. elegans* is beginning to be used as a model for dystrophin-mediated myopathy (reviewed by Chamberlain and Benian, 2000; Culetto and Sattelle, 2000). Thus, future studies on 'sticky worms' have the potential to advance our understanding of the basic mechanisms underlying cell adhesion and might also give insights as to how their dysregulation contributes to disease.

We thank R. Johnson and J. Kramer for sharing data prior to publication; B. Williams and J. Plenefisch for giving us permission to reproduce images of their work; C. Thomas-Virnig and P. Sims for supplying materials for use in the figures and movies; and T. Tuskey and R. King for critical reading of the manuscript. We also extend our thanks to the reviewers for their thoughtful and helpful comments. This work was funded by an NIH postdoctoral fellowship (E.A.C.) and NIH grant GM58038 (J.H.).

References

Anastasiadis, P. Z. and Reynolds, A. B. (2000). The p120 catenin family: complex roles in adhesion, signaling and cancer. *J. Cell Sci.* **113**, 1319-1334.

Antoshechkin, I. and Han, M. (2002). The *C. elegans evl-20* gene is a homolog of the small GTPase ARL2 and regulates cytoskeleton dynamics during cytokinesis and morphogenesis. *Dev. Cell* **2**, 579-591.

Asano, A., Asano, K., Sasaki, H., Furuse, M. and Tsukita, S. (2003). Claudins in *Caenorhabditis elegans*: their distribution and barrier function in the epithelium. *Curr. Biol.* **13**, 1042-1046.

Barstead, R. J. and Waterston, R. H. (1991). Vinculin is essential for muscle function in the nematode. *J. Cell Biol.* **114**, 715-724.

Barstead, R. J., Kleiman, L. and Waterston, R. H. (1991). Cloning, sequencing, and mapping of an α-actinin gene from the nematode *Caenorhabditis elegans*. *Cell Motil. Cytoskeleton* **20**, 69-78.

Baum, P. D. and Garriga, G. (1997). Neuronal migrations and axon fasciculation are disrupted in *ina-1* integrin mutants. *Neuron* **19**, 51-62.

Benian, G. M., Tinley, T. L., Tang, X. and Borodovsky, M. (1996). The *Caenorhabditis elegans* gene *unc-89*, required for muscle M-line assembly, encodes a giant modular protein composed of Ig and signal transduction domains. *J. Cell Biol.* **132**, 835-848.

Bercher, M., Wahl, J., Vogel, B. E., Lu, C., Hedgecock, E. M., Hall, D. H. and Plenefisch, J. D. (2001). *mua-3*, a gene required for mechanical tissue integrity in *Caenorhabditis elegans*, encodes a novel transmembrane protein of epithelial attachment complexes. *J. Cell Biol.* **154**, 415-426.

Bessou, C., Giugia, J. B., Franks, C. J., Holden-Dye, L. and Segalat, L. (1998). Mutations in the *Caenorhabditis elegans* dystrophin-like gene *dys-1* lead to hyperactivity and suggest a link with cholinergic transmission. *Neurogenetics* **2**, 61-72.

Bilder, D. and Perrimon, N. (2000). Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. *Nature* **403**, 676-680.

Bilder, D., Li, M. and Perrimon, N. (2000). Cooperative regulation of cell polarity and growth by *Drosophila* tumor suppressors. *Science* **289**, 113-116.

Bokel, C. and Brown, N. H. (2002). Integrins in development: moving on, responding to, and sticking to the extracellular matrix. *Dev. Cell* **3**, 311-321.

Borg, J. P., Marchetto, S., le Bivic, A., Ollendorff, V., Jaulin-Bastard, F., Saito, H., Fournier, E., Adelaide, J., Margolis, B. and Birnbaum, D. (2000). ERBIN: a basolateral PDZ protein that interacts with the mammalian ERBB2/HER2 receptor. *Nat. Cell Biol.* **2**, 407-414.

Bosher, J. M., Hahn, B. S., Legouis, R., Sookhareea, S., Weimer, R. M., Gansmuller, A., Chisholm, A. D., Rose, A. M., Bessereau, J. L. and Labouesse, M. (2003). The *Caenorhabditis elegans vab-10* spectraplakin

isoforms protect the epidermis against internal and external forces. *J. Cell Biol.* **161**, 757-768.

Bossinger, O., Klebes, A., Segbert, C., Theres, C. and Knust, E. (2001). Zonula adherens formation in *Caenorhabditis elegans* requires *dlg-1*, the homologue of the *Drosophila* gene Discs large. *Dev. Biol.* **230**, 29-42.

Braga, V. M. (2002). Cell-cell adhesion and signalling. *Curr. Opin. Cell Biol.* **14**, 546-556.

Branda, C. S. and Stern, M. J. (1999). Cell migration and axon growth cone guidance in *Caenorhabditis elegans*. *Curr. Opin. Genet. Dev.* **9**, 479-484.

Broadie, K. S. and Richmond, J. E. (2002). Establishing and sculpting the synapse in *Drosophila* and *C. elegans*. *Curr. Opin. Neurobiol.* **12**, 491-498.

Budnik, V., Koh, Y. H., Guan, B., Hartmann, B., Hough, C., Woods, D. and Gorczyca, M. (1996). Regulation of synapse structure and function by the *Drosophila* tumor suppressor gene *dlg*. *Neuron* **17**, 627-640.

Calderwood D, A. Integrin activation. (2004). *J. Cell Sci.* **15**, 657-666.

Campbell, K. P. (1995). Three muscular dystrophies: loss of cytoskeleton-extracellular matrix linkage. *Cell* **80**, 675-679.

Caruana, G. and Bernstein, A. (2001). Craniofacial dysmorphogenesis including cleft palate in mice with an insertional mutation in the discs large gene. *Mol. Cell. Biol.* **21**, 1475-1483.

Chamberlain, J. S. and Benian, G. M. (2000). Muscular dystrophy: the worm turns to genetic disease. *Curr. Biol.* **10**, R795-R797.

Chen, L., Ong, B. and Bennett, V. (2001). LAD-1, the *Caenorhabditis elegans* L1CAM homologue, participates in embryonic and gonadal morphogenesis and is a substrate for fibroblast growth factor receptor pathway-dependent phosphotyrosine-based signaling. *J. Cell Biol.* **154**, 841-855.

Chen, W., Chen, S., Yap, S. F. and Lim, L. (1996). The *Caenorhabditis elegans* p21-activated kinase (CePAK) colocalizes with CeRac1 and CDC42Ce at hypodermal cell boundaries during embryo elongation. *J. Biol. Chem.* **271**, 26362-26368.

Chishti, A. H., Kim, A. C., Marfatia, S. M., Lutchman, M., Hanspal, M., Jindal, H., Liu, S. C., Low, P. S., Rouleau, G. A., Mohandas, N. et al. (1998). The FERM domain: a unique module involved in the linkage of cytoplasmic proteins to the membrane. *Trends Biochem. Sci.* **23**, 281-282.

Cohn, R. D. and Campbell, K. P. (2000). Molecular basis of muscular dystrophies. *Muscle Nerve* **23**, 1456-1471.

Colegio, O. R., van Itallie, C. M., McCrea, H. J., Rahner, C. and Anderson, J. M. (2002). Claudins create charge-selective channels in the paracellular pathway between epithelial cells. *Am. J. Physiol. Cell Physiol.* **283**, C142-C147.

Colombatti, A. and Bonaldo, P. (1991). The superfamily of proteins with von Willebrand factor type A-like domains: one theme common to components of extracellular matrix, hemostasis, cellular adhesion, and defense mechanisms. *Blood* **77**, 2305-2315.

Cooper, H. M. (2002). Axon guidance receptors direct growth cone pathfinding: rivalry at the leading edge. *Int. J. Dev. Biol.* **46**, 621-631.

Costa, M., Raich, W., Agbunag, C., Leung, B., Hardin, J. and Priess, J. R. (1998). A putative catenin-cadherin system mediates morphogenesis of the *Caenorhabditis elegans* embryo. *J. Cell Biol.* **141**, 297-308.

Cox, E. A., Tuskey, C. and Hardin, J. (2004). Cell adhesion receptors in C. elegans. *J. Cell Sci.* **117**, 1867-1870.

Cram, E. J., Clark, S. G. and Schwarzbauer, J. E. (2003). Talin loss-of-function uncovers roles in cell contractility and migration in *C. elegans*. *J. Cell Sci.* **116**, 3871-3888.

Culetto, E. and Sattelle, D. B. (2000). A role for *Caenorhabditis elegans* in understanding the function and interactions of human disease genes. *Hum. Mol. Genet.* **9**, 869-877.

Ding, M., Goncharov, A., Jin, Y. and Chisholm, A. D. (2003). *C. elegans* ankyrin repeat protein VAB-19 is a component of epidermal attachment structures and is essential for epidermal morphogenesis. *Development* **130**, 5791-5801.

Durbeej, M., Henry, M. D. and Campbell, K. P. (1998). Dystroglycan in development and disease. *Curr. Opin. Cell Biol.* **10**, 594-601.

Ehmsen, J., Poon, E. and Davies, K. (2002). The dystrophin-associated protein complex. *J. Cell Sci.* **115**, 2801-2803.

Etienne-Manneville, S. and Hall, A. (2003). Cell polarity: Par6, aPKC and cytoskeletal crosstalk. *Curr. Opin. Cell Biol.* **15**, 67-72.

Firestein, B. L. and Rongo, C. (2001). DLG-1 is a MAGUK similar to SAP97 and is required for adherens junction formation. *Mol. Biol. Cell* **12**, 3465-3475.

Francis, G. R. and Waterston, R. H. (1985). Muscle organization in *Caenorhabditis elegans*: localization of proteins implicated in thin filament attachment and I-band organization. *J. Cell Biol.* **101**, 1532-1549.

Francis, R. and Waterston, R. H. (1991). Muscle cell attachment in *Caenorhabditis elegans*. *J. Cell Biol.* **114**, 465-479.

Fujita, A. and Kurachi, Y. (2000). SAP family proteins. *Biochem. Biophys. Res. Commun.* **269**, 1-6.

Gatewood, B. K. and Bucher, E. A. (1997). The *mup-4* locus in *Caenorhabditis elegans* is essential for hypodermal integrity, organismal morphogenesis and embryonic body wall muscle position. *Genetics* **146**, 165-183.

Gettner, S. N., Kenyon, C. and Reichardt, L. F. (1995). Characterization of β *pat-3* heterodimers, a family of essential integrin receptors in *C. elegans*. *J. Cell Biol.* **129**, 1127-1141.

Gieseler, K., Abdel-Dayem, M. and Segalat, L. (1999). In vitro interactions of *Caenorhabditis elegans* dystrophin with dystrobrevin and syntrophin. *FEBS Lett.* **461**, 59-62.

Ginzburg, V. E., Roy, P. J. and Culotti, J. G. (2002). Semaphorin 1a and semaphorin 1b are required for correct epidermal cell positioning and adhesion during morphogenesis in *C. elegans*. *Development* **129**, 2065-2078.

Goode, S. and Perrimon, N. (1997). Inhibition of patterned cell shape change and cell invasion by Discs large during *Drosophila* oogenesis. *Genes Dev.* **11**, 2532-2544.

Graham, P. L., Johnson, J. J., Wang, S., Sibley, M. H., Gupta, M. C. and Kramer, J. M. (1997). Type IV collagen is detectable in most, but not all, basement membranes of *Caenorhabditis elegans* and assembles on tissues that do not express it. *J. Cell Biol.* **137**, 1171-1183.

Grisoni, K., Martin, E., Gieseler, K., Mariol, M. C. and Segalat, L. (2002). Genetic evidence for a dystrophin-glycoprotein complex (DGC) in *Caenorhabditis elegans*. *Gene* **294**, 77-86.

Gupta, M. C., Graham, P. L. and Kramer, J. M. (1997). Characterization of alpha1(IV) collagen mutations in *Caenorhabditis elegans* and the effects of alpha1 and alpha2(IV) mutations on type IV collagen distribution. *J. Cell Biol.* **137**, 1185-1196.

Hahn, B. S. and Labouesse, M. (2001). Tissue integrity: hemidesmosomes and resistance to stress. *Curr. Biol.* **11**, R858-R861.

Hammarlund, M., Davis, W. S. and Jorgensen, E. M. (2000). Mutations in β-spectrin disrupt axon outgrowth and sarcomere structure. *J. Cell Biol.* **149**, 931-942.

Heasman, J., Ginsberg, D., Geiger, B., Goldstone, K., Pratt, T., Yoshida-Noro, C. and Wylie, C. (1994). A functional test for maternally inherited cadherin in *Xenopus* shows its importance in cell adhesion at the blastula stage. *Development* **120**, 49-57.

Hemler, M. E. (1999). Dystroglycan versatility. *Cell* **97**, 543-546.

Hill, E., Broadbent, I. D., Chothia, C. and Pettitt, J. (2001). Cadherin superfamily proteins in *Caenorhabditis elegans* and *Drosophila melanogaster*. *J. Mol. Biol.* **305**, 1011-1024.

Hobert, O., Moerman, D. G., Clark, K. A., Beckerle, M. C. and Ruvkun, G. (1999). A conserved LIM protein that affects muscular adherens junction integrity and mechanosensory function in *Caenorhabditis elegans*. *J. Cell Biol.* **144**, 45-57.

Hoier, E. F., Mohler, W. A., Kim, S. K. and Hajnal, A. (2000). The *Caenorhabditis elegans* APC-related gene *apr-1* is required for epithelial cell migration and Hox gene expression. *Genes Dev.* **14**, 874-886.

Hong, L., Elbl, T., Ward, J., Franzini-Armstrong, C., Rybicka, K. K., Gatewood, B. K., Baillie, D. L. and Bucher, E. A. (2001). MUP-4 is a novel transmembrane protein with functions in epithelial cell adhesion in *Caenorhabditis elegans*. *J. Cell Biol.* **154**, 403-414.

Hresko, M. C., Williams, B. D. and Waterston, R. H. (1994). Assembly of body wall muscle and muscle cell attachment structures in *Caenorhabditis elegans*. *J. Cell Biol.* **124**, 491-506.

Hresko, M. C., Schriefer, L. A., Shrimankar, P. and Waterston, R. H. (1999). Myotactin, a novel hypodermal protein involved in muscle-cell adhesion in *Caenorhabditis elegans*. *J. Cell Biol.* **146**, 659-672.

Huang, C. C., Hall, D. H., Hedgecock, E. M., Kao, G., Karantza, V., Vogel, B. E., Hutter, H., Chisholm, A. D., Yurchenco, P. D. and Wadsworth, W. G. (2003). Laminin α subunits and their role in *C. elegans* development. *Development* **130**, 3343-3358.

Hutter, H., Vogel, B. E., Plenefisch, J. D., Norris, C. R., Proenca, R. B., Spieth, J., Guo, C., Mastwal, S., Zhu, X., Scheel, J. et al. (2000). Conservation and novelty in the evolution of cell adhesion and extracellular matrix genes. *Science* **287**, 989-994.

Hynes, R. O. (1992). Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* **69**, 11-25.

Hynes, R. O. (2002). Integrins: bidirectional, allosteric signaling machines. *Cell* **110**, 673-687.

Hynes, R. O. and Zhao, Q. (2000). The evolution of cell adhesion. *J. Cell Biol.* **150**, F89-F96.

Jin, Y. (2002). Synaptogenesis: insights from worm and fly. *Curr. Opin. Neurobiol.* **12**, 71-79.

Jones, J. C., Hopkinson, S. B. and Goldfinger, L. E. (1998). Structure and assembly of hemidesmosomes. *Bioessays* **20**, 488-494.

Knust, E. and Bossinger, O. (2002). Composition and formation of intercellular junctions in epithelial cells. *Science* **298**, 1955-1959.

Köppen, M., Simske, J. S., Sims, P. A., Firestein, B. L., Hall, D. H., Radice, A. D., Rongo, C. and Hardin, J. D. (2001). Cooperative regulation of AJM-1 controls junctional integrity in *Caenorhabditis elegans* epithelia. *Nat. Cell Biol.* **3**, 983-991.

Larue, L., Ohsugi, M., Hirchenhain, J. and Kemler, R. (1994). E-cadherin null mutant embryos fail to form a trophectoderm epithelium. *Proc. Natl. Acad. Sci. USA* **91**, 8263-8267.

Lee, M., Cram, E. J., Shen, B. and Schwarzbauer, J. E. (2001). Roles for β*pat-3* integrins in development and function of *Caenorhabditis elegans* muscles and gonads. *J. Biol. Chem.* **276**, 36404-36410.

Legouis, R., Gansmuller, A., Sookhareea, S., Bosher, J. M., Baillie, D. L. and Labouesse, M. (2000). LET-413 is a basolateral protein required for the assembly of adherens junctions in *Caenorhabditis elegans*. *Nat. Cell Biol.* **2**, 415-422.

Legouis, R., Jaulin-Bastard, F., Schott, S., Navarro, C., Borg, J. P. and Labouesse, M. (2003). Basolateral targeting by leucine-rich repeat domains in epithelial cells. *EMBO Rep.* **4**, 1096-1102.

Leung, B., Hermann, G. J. and Priess, J. R. (1999). Organogenesis of the *Caenorhabditis elegans* intestine. *Dev. Biol.* **216**, 114-134.

Lin, X., Qadota, H., Moerman, D. G. and Williams, B. D. (2003). *C. elegans* PAT-6/actopaxin plays a critical role in the assembly of integrin adhesion complexes in vivo. *Curr. Biol.* **13**, 922-932.

Mackinnon, A. C., Qadota, H., Norman, K. R., Moerman, D. G. and Williams, B. D. (2002). *C. elegans* PAT-4/ILK functions as an adaptor protein within integrin adhesion complexes. *Curr. Biol.* **12**, 787-797.

Marsden, M. and DeSimone, D. W. (2003). Integrin-ECM interactions regulate cadherin-dependent cell adhesion and are required for convergent extension in *Xenopus*. *Curr. Biol.* **13**, 1182-1191.

McMahon, L., Legouis, R., Vonesch, J. L. and Labouesse, M. (2001). Assembly of *C. elegans* apical junctions involves positioning and compaction by LET-413 and protein aggregation by the MAGUK protein DLG-1. *J. Cell Sci.* **114**, 2265-2277.

Mercer, K. B., Flaherty, D. B., Miller, R. K., Qadota, H., Tinley, T. L., Moerman, D. G. and Benian, G. M. (2003). *Caenorhabditis elegans* UNC-98, a C2H2 Zn finger protein, is a novel partner of UNC-97/PINCH in muscle adhesion complexes. *Mol. Biol. Cell* **14**, 2492-2507.

Michele, D. E. and Campbell, K. P. (2003). Dystrophin-glycoprotein complex: post-translational processing and dystroglycan function. *J. Biol. Chem.* **278**, 15457-15460.

Moerman, D. G. and Fire, A. (1997). Muscle: Structure, Function, and Development. In *Caenorhabditis elegans* II (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess) pp. 417-470. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Moorthy, S., Chen, L. and Bennett, V. (2000). *Caenorhabditis elegans* β-G spectrin is dispensable for establishment of epithelial polarity, but essential for muscular and neuronal function. *J. Cell Biol.* **149**, 915-930.

Moulder, G. L., Huang, M. M., Waterston, R. H. and Barstead, R. J. (1996). Talin requires β-integrin, but not vinculin, for its assembly into focal adhesion-like structures in the nematode *Caenorhabditis elegans*. *Mol. Biol. Cell* **7**, 1181-1193.

Mullen, G. P., Rogalski, T. M., Bush, J. A., Gorji, P. R. and Moerman, D. G. (1999). Complex patterns of alternative splicing mediate the spatial and temporal distribution of perlecan/UNC-52 in *Caenorhabditis elegans*. *Mol. Biol. Cell* **10**, 3205-3221.

Müller, H. A. and Bossinger, O. (2003). Molecular networks controlling epithelial cell polarity in development. *Mech. Dev.* **120**, 1231-1256.

Myster, S. H., Cavallo, R., Anderson, C. T., Fox, D. T. and Peifer, M. (2003). *Drosophila* p120catenin plays a supporting role in cell adhesion but is not an essential adherens junction component. *J. Cell Biol.* **160**, 433-449.

Nievers, M. G., Schaapveld, R. Q. and Sonnenberg, A. (1999). Biology and function of hemidesmosomes. *Matrix Biol.* **18**, 5-17.

Ohno, S. (2001). Intercellular junctions and cellular polarity: the PAR-aPKC complex, a conserved core cassette playing fundamental roles in cell polarity. *Curr. Opin. Cell Biol.* **13**, 641-648.

Pettitt, J., Wood, W. B. and Plasterk, R. H. (1996). *cdh-3*, a gene encoding a member of the cadherin superfamily, functions in epithelial cell morphogenesis in *Caenorhabditis elegans*. *Development* **122**, 4149-4157.

Pettitt, J., Cox, E. A., Broadbent, I. D., Flett, A. and Hardin, J. (2003). The *Caenorhabditis elegans* p120 catenin homologue, JAC-1, modulates cadherin-catenin function during epidermal morphogenesis. *J. Cell Biol.* **162**, 15-22.

Plenefisch, J. D., Zhu, X. and Hedgecock, E. M. (2000). Fragile skeletal muscle attachments in dystrophic mutants of *Caenorhabditis elegans*: isolation and characterization of the *mua* genes. *Development* **127**, 1197-1207.

Poinat, P., de Arcangelis, A., Sookhareea, S., Zhu, X., Hedgecock, E. M., Labouesse, M. and Georges-Labouesse, E. (2002). A conserved interaction between β1 integrin/PAT-3 and Nck-interacting kinase/MIG-15 that mediates commissural axon navigation in *C. elegans*. *Curr. Biol.* **12**, 622-631.

Porter, G. A., Dmytrenko, G. M., Winkelmann, J. C. and Bloch, R. J. (1992). Dystrophin colocalizes with β-spectrin in distinct subsarcolemmal domains in mammalian skeletal muscle. *J. Cell Biol.* **117**, 997-1005.

Priess, J. R. and Hirsh, D. I. (1986). *Caenorhabditis elegans* morphogenesis: the role of the cytoskeleton in elongation of the embryo. *Dev. Biol.* **117**, 156-173.

Raich, W. B., Agbunag, C. and Hardin, J. (1999). Rapid epithelial-sheet sealing in the *Caenorhabditis elegans* embryo requires cadherin-dependent filopodial priming. *Curr. Biol.* **9**, 1139-1146.

Rando, T. A. (2001). The dystrophin-glycoprotein complex, cellular signaling, and the regulation of cell survival in the muscular dystrophies. *Muscle Nerve* **24**, 1575-1594.

Riethmacher, D., Brinkmann, V. and Birchmeier, C. (1995). A targeted mutation in the mouse E-cadherin gene results in defective preimplantation development. *Proc. Natl Acad. Sci. USA* **92**, 855-859.

Rogalski, T. M., Williams, B. D., Mullen, G. P. and Moerman, D. G. (1993). Products of the *unc-52* gene in *Caenorhabditis elegans* are homologous to the core protein of the mammalian basement membrane heparan sulfate proteoglycan. *Genes Dev.* **7**, 1471-1484.

Rogalski, T. M., Mullen, G. P., Gilbert, M. M., Williams, B. D. and Moerman, D. G. (2000). The UNC-112 gene in *Caenorhabditis elegans* encodes a novel component of cell-matrix adhesion structures required for integrin localization in the muscle cell membrane. *J. Cell Biol.* **150**, 253-264.

Rogalski, T. M., Gilbert, M. M., Devenport, D., Norman, K. R. and Moerman, D. G. (2003). DIM-1, a novel immunoglobulin superfamily protein in *Caenorhabditis elegans*, is necessary for maintaining bodywall muscle integrity. *Genetics* **163**, 905-915.

Roper, K., Gregory, S. L. and Brown, N. H. (2002). The 'spectraplakins': cytoskeletal giants with characteristics of both spectrin and plakin families. *J. Cell Sci.* **115**, 4215-4225.

Sakai, T., Zhang, Q., Fassler, R. and Mosher, D. F. (1998). Modulation of β1A integrin functions by tyrosine residues in the β1 cytoplasmic domain. *J. Cell Biol.* **141**, 527-538.

Sawa, M., Suetsugu, S., Sugimoto, A., Miki, H., Yamamoto, M. and Takenawa, T. (2003). Essential role of the *C. elegans* Arp2/3 complex in cell migration during ventral enclosure. *J. Cell Sci.* **116**, 1505-1518.

Segalat, L. (2002). Dystrophin and functionally related proteins in the nematode *Caenorhabditis elegans*. *Neuromuscular Disord.* **12**, S105-S109.

Shibata, Y., Fujii, T., Dent, J. A., Fujisawa, H. and Takagi, S. (2000). EAT-20, a novel transmembrane protein with EGF motifs, is required for efficient feeding in *Caenorhabditis elegans*. *Genetics* **154**, 635-646.

Simske, J. S. and Hardin, J. (2001). Getting into shape: epidermal morphogenesis in *Caenorhabditis elegans* embryos. *Bioessays* **23**, 12-23.

Simske, J. S., Köppen, M., Sims, P., Hodgkin, J., Yonkof, A. and Hardin, J. (2003). The cell junction protein VAB-9 regulates adhesion and epidermal morphology in *C. elegans*. *Nat. Cell Biol.* **5**, 619-625.

Soto, M. C., Qadota, H., Kasuya, K., Inoue, M., Tsuboi, D., Mello, C. C. and Kaibuchi, K. (2002). The GEX-2 and GEX-3 proteins are required for tissue morphogenesis and cell migrations in *C. elegans*. *Genes Dev.* **16**, 620-632.

Takai, Y. and Nakanishi, H. (2003). Nectin and afadin: novel organizers of intercellular junctions. *J. Cell Sci.* **116**, 17-27.

Tepass, U., Gruszynski-DeFeo, E., Haag, T. A., Omatyar, L., Torok, T. and Hartenstein, V. (1996). *shotgun* encodes *Drosophila* E-cadherin and is preferentially required during cell rearrangement in the neurectoderm and other morphogenetically active epithelia. *Genes Dev.* **10**, 672-685.

Tepass, U., Tanentzapf, G., Ward, R., and Fehon, R. (2001). Epithelial cell polarity and cell junctions in *Drosophila*. *Annu. Rev. Genet.* **35**, 747-784.

Tsukita, S., Furuse, M. and Itoh, M. (2001). Multifunctional strands in tight junctions. *Nat. Rev. Mol. Cell Biol.* **2**, 285-293.

Tu, Y., Wu, S., Shi, X., Chen, K. and Wu, C. (2003). Migfilin and Mig-2 link focal adhesions to filamin and the actin cytoskeleton and function in cell shape modulation. *Cell* **113**, 37-47.

Uemura, T., Oda, H., Kraut, R., Hayashi, S., Kotaoka, Y. and Takeichi, M. (1996). Zygotic *Drosophila* E-cadherin expression is required for processes of dynamic epithelial cell rearrangement in the *Drosophila* embryo. *Genes Dev.* **10**, 659-671.

Van Aelst, L. and Symons, M. (2002). Role of Rho family GTPases in epithelial morphogenesis. *Genes Dev.* **16**, 1032-1054.

Vasioukhin, V. and Fuchs, E. (2001). Actin dynamics and cell-cell adhesion in epithelia. *Curr. Opin. Cell Biol.* **13**, 76-84.

Vogel, B. E. and Hedgecock, E. M. (2001). Hemicentin, a conserved extracellular member of the immunoglobulin superfamily, organizes epithelial and other cell attachments into oriented line-shaped junctions. *Development* **128**, 883-894.

Wehrle-Haller, B. and Imhof, B. (2002). The inner lives of focal adhesions. *Trends Cell Biol.* **12**, 382-389.

White, J. (1988). The Anatomy. In *The Nematode* Caenorhabditis elegans (ed. W. W. Wood and the community of *C. elegans* researchers), pp. 81-122. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Williams, B. D. and Waterston, R. H. (1994). Genes critical for muscle development and function in *Caenorhabditis elegans* identified through lethal mutations. *J. Cell Biol.* **124**, 475-490.

Williams-Masson, E. M., Malik, A. N. and Hardin, J. (1997). An actin-mediated two-step mechanism is required for ventral enclosure of the *C. elegans* hypodermis. *Development* **124**, 2889-2901.

Wissmann, A., Ingles, J., McGhee, J. D. and Mains, P. E. (1997). *Caenorhabditis elegans* LET-502 is related to Rho-binding kinases and human myotonic dystrophy kinase and interacts genetically with a homolog of the regulatory subunit of smooth muscle myosin phosphatase to affect cell shape. *Genes Dev.* **11**, 409-422.

Wissmann, A., Ingles, J. and Mains, P. E. (1999). The *Caenorhabditis elegans mel-11* myosin phosphatase regulatory subunit affects tissue contraction in the somatic gonad and the embryonic epidermis and genetically interacts with the Rac signaling pathway. *Dev. Biol.* **209**, 111-127.

Wu, C. (1999). Integrin-linked kinase and PINCH: partners in regulation of cell-extracellular matrix interaction and signal transduction. *J. Cell Sci.* **112**, 4485-4489.

Zamir, E. and Geiger, B. (2001). Molecular complexity and dynamics of cell-matrix adhesions. *J. Cell Sci.* **114**, 3583-3590.

Zervas, C. G. and Brown, N. H. (2002). Integrin adhesion: when is a kinase a kinase? *Curr. Biol.* **12**, R350-R351.

Zervas, C. G., Gregory, S. L. and Brown, N. H. (2001). *Drosophila* integrin-linked kinase is required at sites of integrin adhesion to link the cytoskeleton to the plasma membrane. *J. Cell Biol.* **152**, 1007-1018.

Zhang, Y., Guo, L., Chen, K. and Wu, C. (2002). A critical role of the PINCH-integrin-linked kinase interaction in the regulation of cell shape change and migration. *J. Biol. Chem.* **277**, 318-326.

Zhou, D., Ursitti, J. A. and Bloch, R. J. (1998). Developmental expression of spectrins in rat skeletal muscle. *Mol. Biol. Cell* **9**, 47-61.