Rapid epithelial-sheet sealing in the *Caenorhabditis elegans* embryo requires cadherin-dependent filopodial priming

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Background: During embryonic development, epithelia with free edges must join together to create continuous tissues that seal the interior of the organism from the outside environment; failure of epithelial sealing underlies several common human birth defects. Sealing of epithelial sheets in embryos can be extremely rapid, dramatically exceeding the rate of adherens junction formation by epithelial cells in culture or during healing of epithelial wounds. Little is known about the dynamic redistribution of cellular junctional components during such events in living embryos.

Results: We have used time-lapse, multiphoton laser-scanning microscopy and green fluorescent protein fusion proteins to analyze the sealing of the *Caenorhabditis elegans* epidermis in living embryos. Rapid recruitment of α-catenin to sites of filopodial contact between contralateral migrating epithelial cells, concomitant with clearing of cytoplasmic α-catenin, resulted in formation of nascent junctions; this preceded the formation of mature junctions. Surprisingly, upon inactivation of the entire cadherin–catenin complex, only adhesive strengthening between filopodia was reproducibly affected. Other ventral epidermal cells, which did not extend filopodia and appeared to seal along the ventral midline by coordinated changes in cell shape, successfully adhered in the absence of these proteins.

Conclusions: We propose that 'filopodial priming' – prealignment of bundled actin in filopodia combined with the rapid recruitment of α-catenin from cytoplasmic reserves at sites of filopodial contact – accounts for the rapid rate of sealing of the embryonic epidermis of *C. elegans*. Filopodial priming may provide a general mechanism for rapid creation of adherens junctions during epithelial-sheet sealing in embryos.

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Background

The ability of epithelial sheets with free edges to join together to create continuous tissues is a crucial event following the injury of epithelia [1] and during embryonic development [2]. During both wound healing and embryogenesis, cells at the free edges of the epithelium must engage in directed motility and must establish new adherens and occluding junctional connections as they make contact with one another to close the sheet. Despite the apparent similarities between wound closure and sealing of embryonic sheets, it is not known whether they use similar molecular mechanisms. Sealing of epithelial sheets in normal embryos can be extremely rapid; for example, the margins of the embryonic epidermis in *Caenorhabditis elegans* can close over an area many cell diameters wide in as little as 15 minutes [3]. Such rapid sealing contrasts sharply with the sealing of wounded epithelial cells, either in culture [4] or in the chick embryo [1,5], in which cases wound sealing requires 2.5–24 hours, depending on the size of the wound.

In all cases of epithelial-sheet sealing, cellular junction components and the cytoskeleton must be redistributed. Epithelial cells are connected by adherens junctions, which provide adhesive stabilization to cells within the sheet. Adherens junctions typically contain high concentrations of the cell-adhesion molecule cadherin and the associated cytoplasmic proteins β-catenin (which binds to the cytoplasmic tail of cadherin) and α-catenin (which binds to β-catenin and also directly and/or indirectly to the actin cytoskeleton). The catenins provide a physical link between the cadherins at the cell surface and the cytoskeleton (reviewed in [6–9]).

The kinetics of adherens junction assembly has been examined in some detail in cultured cells. In individual isolated Madin–Darby canine kidney (MDCK) cells, actin filaments form a cable under the plasma membrane around the entire cell [10,11]. MDCK cell contacts are initially characterized by cadherin/catenin spots associated with thin actin filaments that emerge from the circumferential actin cable but that are oriented perpendicular to

the contact surface. As the junction matures, cadherin and catenins accumulate into plaques at the two ends of the contact; actin between these plaques reorganizes parallel to the contact surface. MDCK cells in culture form stable junctional associations over a period of 4–10 hours [11]. In contrast to junction formation in MDCK cells, little is known about the dynamic redistribution of components of adherens junctions and the cytoskeleton during the sealing of embryonic epithelia. The marked difference in the rate of assembly of contacts between epithelial cells in culture and epithelial sheets in embryos suggests that embryonic epithelial-sheet sealing may use different subcellular mechanisms from those used by cultured cells.

C. elegans hmr-1 (encoding cadherin), *hmp-2* (encoding β-catenin), and *hmp-1* (encoding α-catenin) provide a valuable entry point for assessing cadherin/catenin function during rapid epithelial-sheet sealing, as mutations exist in all three genes [12] and the function of the proteins they encode can be studied in simple, optically transparent, living embryos. HMR-1 is the only cadherin in *C. elegans* that contains a recognizable β-catenin-binding site [12]. Of the three β-catenin family genes in *C. elegans* (*wrm-1*, *hmp-2*, and *bar-1*), only *hmp-2* has been shown to be required for embryonic morphogenesis. The *wrm-1* gene is required maternally for patterning events in the early embryo [13]; *bar-1* is not required in the embryo, but is needed later for patterning events in the vulva [14]. Likewise, of the two α-catenins in *C. elegans* (HMP-1 and a second family member encoded by the clone CELK02251), only loss of HMP-1 function results in morphogenetic defects in the embryo [12], and there is no enhancement of morphogenetic defects when both *hmp-1* and CELK02551 are simultaneously inactivated (our unpublished observations). All reported mutations in *hmr-1*, *hmp-2*, and *hmp-1* are recessive and zygotic-lethal; homozygotes fail to elongate from their initial bean shape into worms and die as embryos [12].

Here, we report the systematic inactivation of both zygotic and maternal *hmr-1/cadherin*, *hmp-2/β-catenin*, and *hmp-1/α-catenin*, either singly or in double and triple combinations using loss-of-function mutations, RNA interference (RNAi; [15,16]), and germ-line mosaics. Cell position and junction formation were monitored in living embryos by multiphoton four-dimensional laser scanning microscopy (MPLSM; [17]), using junctionally localized green fluorescent protein (GFP) translational fusion proteins. We show, for the first time in a living embryo, that the cadherin–catenin complex is required for the stabilization of adhesive contacts between cells that adhere with filopodia. This requirement contrasts sharply with that of other epithelial cells in the early embryo, which do not have a stringent requirement for the cadherin–catenin complex. We propose that filopodial adhesive contacts occur by 'filopodial priming'. In this model, actin bundles within filopodia, which are prealigned perpendicular to sites of nascent junction formation, rapidly bind α-catenin recruited from pre-existing cytoplasmic reserves at sites of filopodial contact. Such a role for cadherin–catenin complexes provides a new mechanism for rapid creation of adherens junctions during epithelial-sheet sealing in embryos.

Results

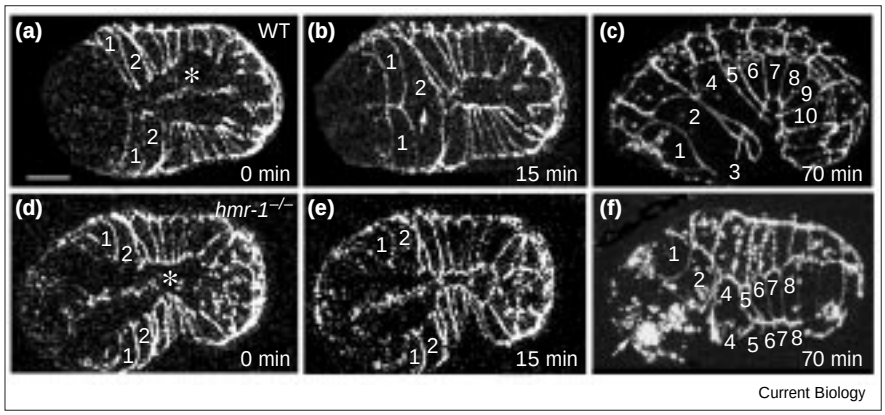
The cadherin–catenin complex is required only in epithelial cells that interact via filopodia

During the process known as ventral enclosure, the embryonic epidermis spreads from the dorsal surface of the *C. elegans* embryo, ultimately enclosing the embryo in an epithelial monolayer [3]. Four cells ('leading cells') within the epidermis initiate enclosure by extending filopodial protrusions towards the ventral midline of the embryo. Leading cells adhere at the midline through these filopodia, which is followed by the coordinated contraction of more posterior ventral epidermal cells ('pocket cells') to complete enclosure [3]. To analyze ventral enclosure at the cellular level, we used MPLSM to image a translational fusion between JAM-1 (for 'junction-associated molecule') and GFP [18]). This JAM-1–GFP fusion protein was used to track epidermal cell position, junction formation, and the formation of syncytia by cell–cell fusions. Immunostaining and immunogold labeling show that JAM-1 localizes to the apical zonula adherens, which form a belt-like structure encircling all *C. elegans* epithelial cells ([18–21]; D. Hall, personal communication). Embryos expressing an integrated JAM-1–GFP construct were phenotypically normal; JAM-1–GFP recapitulated the localization and temporal expression of endogenous JAM-1 antigen, as detected by immunofluorescence with the monoclonal antibody MH27 (data not shown).

In wild-type embryos, ventral enclosure is highly reproducible from embryo to embryo [3]; a representative example is shown in Figure 1a–c. Enclosure is bilaterally symmetric; the leading cells are the first to meet at the ventral midline, as shown by the formation of ventral midline junctions between each pair of cells (Figure 1b). In the second step of ventral enclosure, eight posterior pairs of pocket cells (labeled 3–10 in Figure 1c) migrate to the ventral midline. The leading cells always reach the midline well before the first pocket cells; the most anterior pocket cells make contact 17.1 ± 1.5 minutes after the leading cells, and the pocket cells complete their ventral migration 35.7 ± 3.5 minutes after the leading cells (mean ± SEM, n = 7 embryos).

To elucidate the mechanism of cadherin–catenin function in enclosure, JAM-1–GFP was observed in *hmr-1/cadherin*, *hmp-2/β-catenin*, or *hmp-1/α-catenin* mutant embryos or following gene inactivation using RNAi; the results are summarized in Table 1. Three classes of defective

Figure 1



HMR-1/cadherin is essential for adhesion of leading cells during ventral enclosure. MPLSM was used to image the junctional marker JAM-1–GFP in living (a–c) wild-type (WT) and (d–f) *hmr-1* mutant embryos. Embryos are viewed from the ventral side, anterior to the left. Unless otherwise specified, this convention is used in all figures. (a) In the wild-type embryo, the leading cells (pairs 1 and 2) approach the ventral midline in advance of the cells lining the ventral pocket (asterisk). (b) The leading cells establish adherens junctions at the ventral midline, completing the first stage of enclosure. Cell pair 2 fuses to produce a binucleate cell; this can be observed by the loss of junctional JAM-1–GFP (arrow). The pocket cells have approached the ventral midline. (c) Enclosure is complete. The embryo has turned on its side because of the geometry of the mount. The pocket cells (cell pairs 3–10) have met at the ventral midline. (d) A *hmr-1* mutant embryo at a stage comparable to that in (a). The leading cells (cell pairs 1 and 2) are further from the ventral midline than the cells lining the ventral pocket (asterisk). (e) The pocket cells have moved toward the midline, but the leading cells have retracted further laterally. (f) The retracting leading cells (1 and 2) express JAM-1–GFP at their ventral-most edge. The posterior of the embryo encloses, as cell pairs 4–8 establish ventral midline contacts. The pharynx is extruded through the unenclosed anterior end of the embryo (arrow). The scale bar represents 10 μm.

hmr-1/cadherin embryos were identified in the analysis of RNAi experiments and zygotic mutants: embryos whose epidermis failed to make any permanent attachments at the ventral midline and subsequently retracted dorsally (data not shown; for movie footage, see Supplementary material); embryos in which some or all pocket cells formed stable attachments at the ventral midline; and a minority of embryos in which enclosure appeared successful but subsequent elongation was not. The second, most common class of embryos displayed defects during the later stages of enclosure. As the ventral epidermal cells approached the ventral midline, the pocket cells migrated in advance of the leading cells (Figure 1d,e; compare to Figure 1a,b). The leading cells failed to form junctions at the ventral midline and retracted towards the dorsal surface of the embryo. In contrast, some or all of the pocket cells formed junctions with their contralateral neighbors. Following the completion of epidermal migration, the internal contents of the embryo were extruded through the unenclosed anterior region (Figure 1f).

It was possible that the lack of severity of the adhesion defects exhibited in *hmr-1/cadherin* and *hmp-1/α-catenin* mutants was because of maternal mRNA. Similarly, the embryos that successfully enclosed in RNAi experiments could have arisen due to incomplete depletion of mRNA. To test for these possibilities, germ-line-mosaic hermaphrodites were identified in which functional *hmr-1/cadherin*

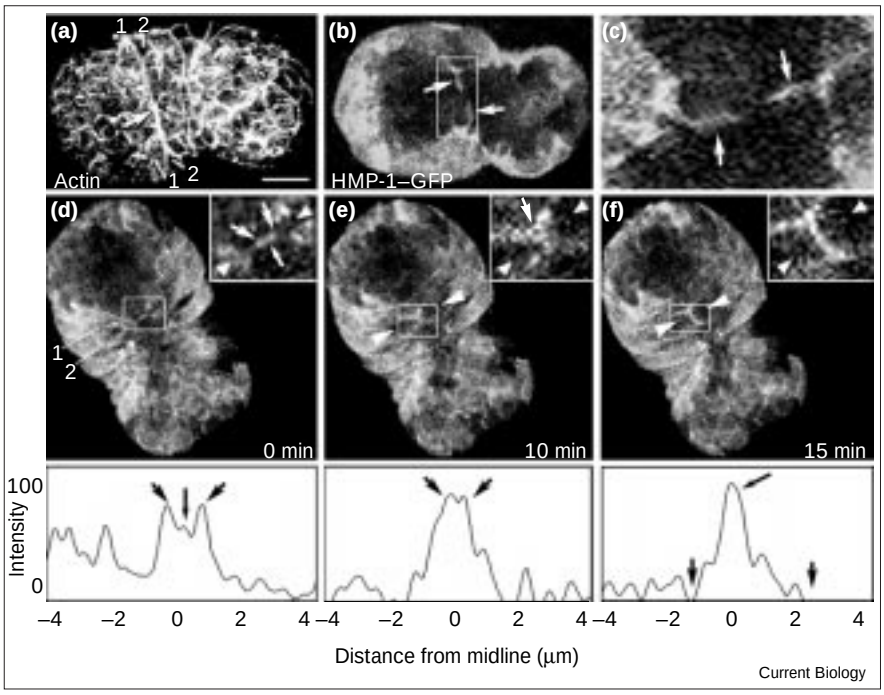
Table 1

Effects of perturbing the cadherin–catenin complex on ventral enclosure.

Inactivated molecules	Number of embryos	No ventral enclosure	Incomplete enclosure	Full ventral enclosure
hmr-1 (RNAi)	186	44 (24%)	120 (64%)	22 (12%)
hmp-2 (RNAi)	67	0 (0%)	54 (81%)	13 (19%)
hmp-1 (RNAi)	44	0 (0%)	36 (82%)	8 (18%)
hmp-2; *hmp-1* (RNAi)	26	0 (0%)	19 (73%)	7 (26%)
hmr-1; *hmp-2*; *hmp-1* (RNAi)	81	6 (20%)	61 (75%)	4 (5%)
hmr-1(zu389)	97	8 (19%)	74 (76%)	5 (5%)
hmp-2(zu364)	13	0 (0%)	2 (15%)	11 (85%)
hmr-1(zu389) (germline mosaic)*	7	2 (29%)	5 (71%)	0 (0%)
hmp-1(zu278) (germline mosaic)*	10	0 (0%)	9 (90%)	1 (10%)

Zygotic and maternal HMR-1/cadherin, HMP-2/β-catenin, and HMP-1/α-catenin were inactivated in single, double, and triple combinations using RNAi, loss-of-function mutations, and germ-line mosaics. Terminal phenotypes were determined by examination of JAM-1–GFP or using Nomarski microscopy (where asterisked). Defective embryos were divided into three categories on the basis of the number of stable, adhesive contacts formed between opposing ventral hypodermal cells. 'No ventral enclosure', fully retracted epidermis; 'incomplete enclosure', some or all pocket cells reached the ventral midline but leading cells retracted (see Figure 1c,d); 'full ventral enclosure', embryos completed ventral enclosure but failed to elongate.

Figure 2



Leading cells extend HMP-1/α-catenin-rich filopodia that initiate adherens junction formation at the ventral midline. (a) Actin microfilaments in a fixed, enclosing embryo. The leading cells extend actin-rich filopodia that meet at the ventral midline (arrow). (b) HMP-1–GFP in an enclosing embryo. At this point, the leading cells are extending filopodia towards the midline (arrows). (c) Twofold magnification of the boxed region in (b), rotated 90° clockwise. Filopodia are clearly visible (arrows). In (d–f), the embryo is viewed from the ventral side; anterior is to the upper-left. Boxed areas in (d–f) are enlarged 3× in the insets. Arrowheads in the insets indicate the endpoints of lines along which fluorescence intensity was measured and plotted in the graphs below each image. HMP-1–GFP fluorescence is plotted as a function of fluorescence intensity (y-axis) versus distance (in μm) from the ventral midline (x-axis). The black arrows in the graph in (d) correspond to the arrows shown in the inset. (d) HMP-1–GFP is upregulated at the point of leading-cell filopodial contact (inset, arrows). Upon contact, two densities of HMP-1–GFP are apparent (graph, arrows), separated by a smaller aggregation of HMP-1/α-catenin between them (graph, small arrow). (e) Junction formation between the posterior pair of leading cells. HMP-1–GFP appears to decrease in concentration in the cytoplasm (arrowheads, inset) concomitant with its localization at the ventral midline (arrow, inset). The aggregates of fluorescence appear to coalesce (graph, arrows). (f) Accumulation of HMP-1–GFP at the junction. Junction formation occurs ~15 min after filopodial contact. In a mature junction, HMP-1–GFP fluorescence appears as a single, intense peak (graph, small arrow), flanked by a region devoid of HMP-1/α-catenin (graph, arrows). The scale bar represents 10 μm.

or *hmp-1/α-catenin* mRNA was present in somatic cells but absent in the germ line. Such animals are expected to produce embryos lacking both maternal and zygotic mRNA for the gene of interest. Offspring from *hmr-1/cadherin* germ-line-mosaic mothers never successfully enclosed (none out of seven embryos analyzed); similarly, all but one of the 10 embryos derived from *hmp-1/α-catenin* mosaic mothers failed to enclose (Table 1). Remarkably, embryos derived from *hmr-1/cadherin* and *hmp-1/α-catenin* germ-line-mosaic hermaphrodites maintain general cell adhesion, JAM-1–GFP localization in all epithelial tissues, and epidermal integrity. Significantly, the pocket cells could make successful contacts at the ventral midline in such embryos (5 of 7 in *hmr-1/cadherin* experiments and 9 of 9 enclosure defective embryos in *hmp-1/α-catenin* experiments; Table 1). As *hmr-1* is the only classical cadherin in the *C. elegans* genome, this result indicates that only the leading cells have a stringent requirement for cadherin-mediated adhesion.

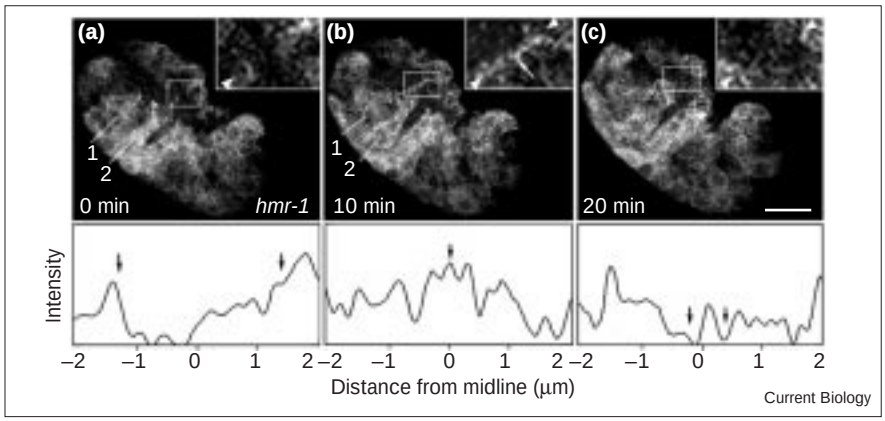
Accumulation of α-catenin at sites of filopodial contact is required for leading-cell adhesion

Because cadherin-dependent adhesion between pairs of leading cells is a crucial event during ventral enclosure, we used a translational fusion between HMP-1/α-catenin and GFP to analyze cadherin–catenin complex formation during ventral enclosure by MPLSM. HMP-1 localizes to adherens junctions in all *C. elegans* epithelia. In addition, a significant proportion of HMP-1 protein remains in the cytoplasm [12]. HMP-1–GFP recapitulated the expression and junctional localization of HMP-1 antigen, and it was functional, as it rescued the *hmp-1(zu278)* null mutant, which produces no HMP-1 protein [12], to viability.

During ventral enclosure, HMP-1–GFP was expressed in every cell in the embryo. The migrating rows of ventral epidermis expressed the reporter at high levels, however, including the filopodia extended by leading cells. These protrusions could be visualized in living embryos using MPLSM, and were very similar to those seen in fixed specimens stained with phalloidin (Figure 2a; also see [3]). Filopodia extended by the leading cells grew to lengths exceeding 5 μm and persisted for periods of more than 20 minutes (Figure 2b); they exhibited dynamic lengthening and retraction, but the result was always directed movement towards the ventral midline.

Leading-cell filopodial contact initiated a dramatic redistribution of HMP-1–GFP (Figure 2c–e), which was confirmed by quantitative analysis (graphs in Figure 2c–e; of

Figure 3



HMR-1/cadherin is required to stabilize leading-cell filopodial contacts. HMP-1–GFP was imaged by MPLSM in embryos in which *hmr-1* has been inactivated by RNAi. In all panels, the embryo is viewed ventro-laterally; anterior is to the upper left. The boxed region in (a–c) is magnified 3× in the insets. HMP-1–GFP fluorescence is plotted as a function of fluorescence intensity (y-axis) versus distance (in μm) from the ventral midline (x-axis). The black arrow in the graph in (b) corresponds to the arrow shown in the inset. Arrowheads in the insets indicate the endpoints of lines along which fluorescence intensity was measured. (a) Leading cells (labeled 1 and 2) are visible during migration, and have elevated levels of HMP-1–GFP (graph, arrows). (b) Leading-cell filopodial contact occurs at the ventral midline (inset, arrow). HMP-1–GFP is elevated in filopodia (graph, arrow), but fails to accumulate at the embryo's midline. (c) The leading cells retract towards the dorsal surface of the embryo, resulting in a region devoid of HMP-1–GFP (graph, arrows). The pocket cells establish adhesive contacts (asterisk) without an observable increase in junctionally localized HMP-1–GFP. The scale bar represents 10 μm.

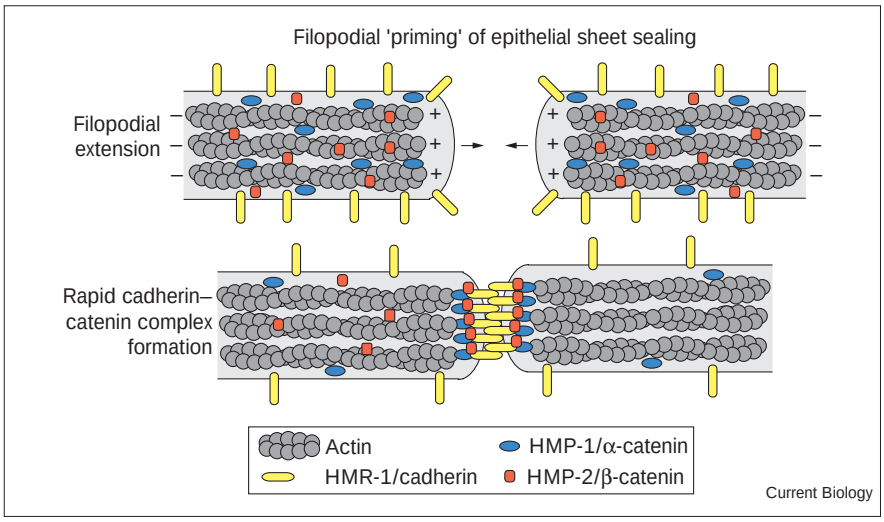
the nine embryos that were examined, all showed a similar quantitative profile). Within 5 minutes of contact, HMP-1–GFP strongly localized to points of contact between the filopodia. Upon initial contact, several densities of α-catenin were present (Figure 2c,d). These then apparently coalesced into a single intense junctional contact and fluorescence intensity in the cytoplasm dropped markedly (Figure 2e). Given the spatial resolution of MPLSM, it is not clear what the multiple aggregates of HMP-1/α-catenin seen in Figure 2d represent. It is possible that HMP-1/α-catenin is recruited to sites near the tips of filopodia before insertion at the midline septum. If this is the case, then the aggregates of HMP-1–GFP directly on the midline may reflect the formation of the actual nascent junction. Alternatively, these foci could represent regions of overlap between filopodial tips. In this case, the aggregates may reflect several discrete regions of nascent junction formation between the dorsal surface of one filopodium and the ventral surface of the other, which are subsequently consolidated into the mature septum. We favor the second of these two alternatives, because phalloidin staining indicates that filopodia often interact obliquely or overlap significantly [3].

Because α-catenin is normally recruited to sites of cell–cell contact by its linkage to cadherin through β catenin, HMP-1–GFP was monitored in the absence of HMR-1/cadherin in RNAi experiments (Figure 3). As expected, following *hmr-1* inactivation HMP-1–GFP no longer localized to adherens junctions; this was confirmed by quantifying HMP-1–GFP fluorescence intensity (graphs in Figure 3a–c; of the five embryos that were examined, all showed a similar profile). In all cases, filopodia were nevertheless extended by both pairs of leading cells, and displayed directed extension towards the ventral midline at a rate and in a manner indistinguishable from wild-type embryos (Figure 3a). As the leading cells approached the ventral midline, physical contact was established between apposing leading-cell filopodia (Figure 3b). In no case, however, was clearing of cytoplasmic α-catenin observed (Figure 3b), and no accumulation of α-catenin occurred at the ventral midline. Following the failure to form an adhesive septum, the leading cells ceased to exhibit protrusive activity. Within 10–30 minutes, the leading cells began to retract towards the embryo's dorsal surface. The filopodial activity characteristic of actively migrating leading cells was noticeably absent during leading cell retraction (Figure 3c).

Discussion

During *C. elegans* ventral enclosure, initial contacts between migrating epithelial cells occur through cadherin–catenin-mediated interactions of leading-cell filopodia. Although conclusive evidence regarding the polarity of actin filaments at epithelial junctional plaques does not exist, the plus ends of actin filaments are thought to insert at junctional complexes between epithelial cells [22]. We propose that cadherin–catenin attachments function to physically link the barbed ends of pre-existing, organized actin bundles in the filopodia, and thus provide stability to the contact. Furthermore, we propose that the high concentration of cytoplasmic HMP-1/α-catenin that was observed before epithelial sealing serves as a premobilized pool of α-catenin that becomes associated with localized transmembrane cadherins at the cell surface. In the absence of cadherin, this pre-mobilized pool of α-catenin does not localize to junctions. The presence of actin filaments pre-aligned perpendicular to the septum with their

Figure 4



A filopodial priming model for rapid adherens-junction formation in the *C. elegans* embryo. The leading cells extend filopodial protrusions, which contain organized bundles of actin filaments and high levels of cytoplasmic HMP-1/α-catenin. Filopodial contact occurs at the embryo's ventral midline and is stabilized through the interactions of apposing complexes of cadherin and catenins, which localize to focal attachments at the site of contact. Cadherin in the plasma membrane and premobilized catenins in the cytoplasm are recruited to the forming septum, causing the contact between cells to increase in breadth (anterior–posterior extent). As junctional complexes form, actin microfilaments, which are already perpendicular to the junction and prealigned with their plus ends towards the nascent junction, are inserted into the junction. The direction of alignment of the actin filaments also primes them for participation in the contractile events that are thought to underlie embryo elongation [19].

plus ends facing the midline, combined with a high concentration of cytoplasmic HMP-1/α-catenin, predisposes filopodia for rapid adherens junction formation. We refer to this mechanism as 'filopodial priming' (Figure 4).

Several pieces of data support the filopodial-priming model. First, HMP-1–GFP accumulated at the tips of adhering filopodia. Clustering of the cadherin–catenin complex is thought to cooperatively increase adhesion strength [23,24], suggesting that HMP-1/α-catenin accumulation is indicative of HMR-1/cadherin–actin linkages. Second, embryos lacking all maternal and zygotic *hmp-1/α-catenin* had consistent defects in leading-cell adhesion (Table 1). This result indicates that cadherin-mediated extracellular contacts between apposing filopodia are not, of themselves, sufficient for leading-cell adhesion. The fact that embryos lacking *hmr-1/cadherin* mRNA displayed an even more severe retraction phenotype may reflect the ability of HMR-1/cadherin to mediate a limited amount of adhesion even when cadherins are not linked to the actin cytoskeleton; such linkage-independent adhesion has been observed in some cases in other systems (reviewed in [25]). Finally, although the polarity of bundled actin filaments in filopodia has not been conclusively demonstrated because of the density of bundled filaments and the limitations of currently available decoration techniques [26], it is likely that the bias in actin-filament polarity in filopodia is equal to or exceeds that in the less-well-ordered lamellipodium. The vast majority of filaments at the leading edges of lamellipodia are oriented with their barbed ends forward [27], and all the short bundles in lamellipodial microspikes appear to have this orientation [28]. When more definitive methods for determining the polarity of large populations of actin filaments in filopodia become available, this aspect of the filopodial priming model can be directly tested in the leading cells of the *C. elegans* epidermis.

In contrast to leading cells, pocket cells did not have a stringent requirement for cadherin-mediated adhesion. One possible reason for this difference is that leading cells may experience more mechanical stress than pocket cells. In order to withstand such stress, these cells may require an additional adhesion system that is dispensable in pocket cells. Pocket cells have been proposed to use a 'purse-string' closure mechanism similar to that envisioned for dorsal closure in *Drosophila* [29] and the healing of small wounds in single cells [30] or epithelia [1,4]. We presume that cadherin-independent cell adhesion is sufficient for this process, as it can still occur in embryos derived from *hmp-1/α-catenin* or *hmr-1/cadherin* germ-line-mosaic mothers. If the pocket does in fact close with a purse string, this result contrasts with wound closure in at least one vertebrate cell type, in which E-cadherin may be required for correctly coordinated wound closure [31]. Curiously, in the absence of a normally enclosed anterior, the pocket could still close, albeit incompletely. This may indicate that the density of actin around its edges does not function as a purse string. Alternatively, the cadherin-independent attachment of leading-cell filopodia to underlying cells may provide sufficient anterior anchorage to the remainder of the purse string for it to close.

The dynamics of ventral midline junction formation in *C. elegans* contrasts markedly with that of MDCK cells in

culture. Cadherin accumulation in MDCK cells initiates with the formation of dispersed E-cadherin spots, and the development of mature junctions between MDCK cells requires several hours [10,11]. In contrast, α-catenin accumulation during *C. elegans* leading-cell formation expands laterally from the point of filopodial contact, and leading-cell junction formation takes less than 15 minutes. We propose that the differences in the dynamics of junction formation seen in MDCK cells and in *C. elegans* epithelial cells result from the architecture of the cytoskeleton before contact. Junction formation between isolated MDCK cells requires substantial actin reorganization [11]. In contrast, the actin filaments within the filopodia of migrating *C. elegans* epidermal cells are pre-aligned perpendicular to sites of junction formation and are thus primed to stabilize extracellular contacts between cadherins and to maximize the areas of contact between the leading cells.

Conclusions

We have shown for the first time in a living embryo that a key step in the adhesion between protrusively active epithelial cells is the cadherin-dependent recruitment of α-catenin to sites of filopodial contact. Only protrusively active cells had a stringent requirement for cadherin-based cell–cell adhesion. In contrast, the pocket cells, which did not utilize long filopodia to initiate adhesive contact, adhered successfully in the absence of the cadherin–catenin complex. Such filopodial priming may be a general mechanism that facilitates the rapid sealing of epithelial sheets in embryos.

Materials and methods

Genetics

The Bristol strain N2 was used as wild type [32]. Nematodes were grown at 20°C in all experiments and were cultured as described in [32]. The following genes and alleles were used in this study [33,34]: LG I: *hmr-1(zu389)*; *hmr-1(zu248)*; *hmp-2(zu364)*; *lin-11(n566)*; *unc-75(e950)*; *hIn1{unc-54(h1040)}*; LG III: *unc-119(e2498::Tc1)*; LG IV: *jcIs1{jam-1::gfp}*; LG V: *hmp-1(zu278)*; *unc-42(e270)*; *him-5(e1467)*. LG, linkage group.

Live fluorescence microscopy

Gravid hermaphrodites were cut transversely through the vulva. The extruded embryos were mounted on a 5% agar pad in M9 solution [33] and filmed using MPLSM. Embryos were imaged using a Spectra Physics Ti:Sapphire laser tuned to 900 nm mounted on a custom built optical workstation (D. Wokosin and J. White, personal communication). Data acquisition and stereo-4D processing were performed as described in [35]; movie sequences were analyzed using a modified version of NIH Image written in our laboratory. All macros, the standard version of NIH Image, and 4D Turnaround/Viewer are available at http://www.loci.wisc.edu/4d/native/4d.html. The modified version of NIH Image is available from the authors. To quantify fluorescence intensity along the mediolateral axis of leading-cell filopodia, a one-pixel-wide selection from the base to the tip of the right and left posterior pair of leading cells was selected, and the eight-bit pixel-intensity values were then measured along this line.

Reporter constructs

JAM-1–GFP has been described previously as MH27–GFP [18]. The stable integrant *jcIs1* was moved into the genetic background of *zu389*, *zu364*, and *zu278* using standard genetic techniques, and no synthetic phenotypes were observed. HMP-1–GFP was constructed by ligating bright variant S65T GFP into the unique *Nae*I site in the final exon of *hmp-1*. The extrachromosomal array *zuEx24* was used for MPLSM, which provides bright, stable expression of HMP-1–GFP, but produces ~20% lethality. Lethality was typically observed in brightly fluorescing embryos, suggesting that overexpression of HMP-1 is lethal. To ensure that we were not observing synthetic phenotypes, embryos that failed to hatch were excluded from this analysis.

Phalloidin staining

Embryos were stained as described in [3], except that Alexa 488 phalloidin (Molecular Probes) was used at a concentration of 10 units/ml.

Mosaic analysis and RNA interference

Germ-line mosaics were identified as described in [36] from a homozygous *hmr-1(zu278)* strain carrying the rescuing cosmid W02B9 on an extrachromosomal array. The *hmp-1(zu278)* germ-line mosaics were produced as described in [12].

RNA was transcribed as described in [15] except that T3 and T7 RNA polymerases were used to synthesize both sense and antisense RNA (MEGAscript kit, Ambion). RNA was recovered by an ethanol ammonium acetate precipitation, and product concentration was assessed by measuring optical density at 260 nm and by electrophoresis on standard agarose gels. Sense–antisense annealing was carried out at 37°C for 15 min as described in [16], and RNA was injected into the gonad of young N2 hermaphrodite adults at a concentration of ~1 μg/ml. The following genes and plasmids were used for RNAi: *hmr-1*, pBR981; *hmp-1*, pBR915; *hmp-2*, pBR982; CELK02251, pBR983; *jam-1*, pMK692 (M. Köppen, personal communication). In all cases, the cDNA used as a template for RNAi spanned the majority of the predicted coding sequence. RNAi of *hmr-1*, *hmp-2*, *hmp-1*, and *jam-1* produced 100% lethality and eliminated epidermal expression of the respective antigens, as assayed by immunostaining ([12]; M. Köppen, personal communication; our unpublished observations).

Supplementary material

Movies showing ventral enclosure in wild-type, *hmr-1/cadherin* and *hmp-1/α-catenin* embryos are available at http://current-biology.com/supmat/supmatin.htm.

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Supplementary material

Rapid epithelial-sheet sealing in the *Caenorhabditis elegans* embryo requires cadherin-dependent filopodial priming

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Movie legends

Movie 1: Ventral enclosure in wild-type embryos (WT-video.mov)

In this sequence, a JAM-1–GFP fusion protein was used to image migration of epithelial cells during ventral enclosure in living embryos using multiphoton laser scanning microscopy (MPLSM). Enclosure is bilaterally symmetric, with the left and right rows of ventral epidermal cells migrating at the same rate and covering the same distance. As a result, the apposing rows of epithelial cells meet in register at the ventral midline. The leading cells are the first to meet at the ventral midline, as evidenced by the formation of ventral midline junctions between each pair of cells. In the second step of ventral enclosure, eight posterior pairs of ventral epidermal cells (pocket cells) migrate to the ventral midline. The pocket cells complete their ventral migration ~30 min after the leading cells.

Movie 2: Loss of cadherin function specifically disrupts leading-cell adhesion (HMR-1-video.mov)

Mutations in the *C. elegans* cadherin–catenin complex prevent embryo elongation. To elucidate the mechanism of cadherin/catenin function in morphogenesis, JAM-1–GFP was observed in embryos derived from mothers in which *hmr-1*/*cadherin* was inactivated by RNA interference. Inactivation of *hmr-1*/*cadherin* disrupts the adhesion of the leading cells, which are known to interact through actin-rich filopodial protrusions at the ventral midline. In contrast, the more posterior pocket cells can seal successfully at the ventral midline. The ability of the pocket cells to establish contact further indicates that only the leading cells have a stringent requirement for cadherin-mediated adhesion.

Movie 3: HMP-1/α-catenin dynamics in wild-type embryos (a-cat.mov)

A fully functional HMP-1/α-catenin–GFP translational fusion was used to image HMP-1/α-catenin accumulation at the midline in living embryos using MPLSM. HMP-1/α-catenin is rapidly recruited (within 5 min) to sites of filopodial contact between contralateral migrating epithelial cells. At the same time, clearing of cytoplasmic HMP-1/α-catenin results in formation of nascent junctions, and precedes the formation of more mature junctions, characterized by the accumulation of JAM-1.