Cadherin-mediated cell adhesion and cell motility in *Drosophila* trachea regulated by the transcription factor Escargot

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SUMMARY

Coordination of cell motility and adhesion is essential for concerted movement of tissues during animal morphogenesis. The *Drosophila* tracheal network is formed by branching, migration and fusion of tubular ectodermal epithelia. Tracheal tip cells, located at the end of each branch that is going to fuse, extend filopodia to search for targets and later change their cell shape to a seamless ring to allow passage of lumen. The cell adhesion molecule DE-cadherin accumulates at the site of contact to form a ring that marks the site of lumen entry and is essential for the fusion. DE-cadherin expression in tip cells of a subset of branches is dependent on escargot, a zinc finger gene expressed in all tip cells. Such escargot mutant tip cells failed to adhere to each other and continued to search for alternative targets by extending long filopodia. We present evidence indicating escargot positively regulates transcription of the DE-cadherin gene, shotgun. Overexpression of DE-cadherin rescued the defect in one of the fusion points in escargot mutants, demonstrating an essential role of DE-cadherin in target recognition and identifying escargot as a key regulator of cell adhesion and motility in tracheal morphogenesis.

Key words: epithelium fusion, cell shape change, filopodium, adherens junction, *Drosophila*, escargot, shotgun, cadherin

INTRODUCTION

During embryogenesis, multiple cell types are specified and are assembled to form functional organs. This process involves cell sorting, migration of individual cells and movement of cells as a group (Hynes and Lander, 1992). Cell-to-cell adhesion is essential (Takeichi, 1995) and is required to be efficiently coupled to cell motility during morphogenesis (Lauffenburger and Horwitz, 1996). Cell motility has mostly been studied by use of in vitro culture systems but more detailed assessment in the developmental context awaits genetic analyses at the single cell resolution.

The insect trachea is a network of tubular epithelium that serves as a respiratory system (Manning and Krasnow, 1993; Samakovlis et al., 1996a). In *Drosophila*, the tracheal system is derived from ten pairs of ectodermal invaginations that each extends six tubular branches without cell division. These branch further to generate terminal branches and fusion branches. Fusion branches migrate to find branches from an adjacent segment and fuse to form a network (Fig. 1A). There are eleven fusion points on the dorsal midline (one of cerebral branch and ten of dorsal branch [DB], Fig. 4B), eighteen on each side (nine each of dorsal trunk [DT] and lateral trunk [LT], Fig. 4A), and three on the ventral midline in the thorax (ventral branch [VB]), all of which are joined with remarkable precision. Genes essential for tracheal formation have been identified. These include genes required for invagination of the tracheal placode (Isaac and Andrew, 1996; Wilk et al., 1996), branch migration (Klämbt et al., 1992) and cell specification (Guillemin et al., 1996). However, no gene that is specifically involved in the fusion has been identified.

At the tip of migrating branches, there are specialized tip cells that express lacZ in several enhancer trap lines (Hartenstein and Jan, 1992; Samakovlis et al., 1996a). Since tip cells lead the migration of fusion branches and are the first cells to make contact during fusion, they are thought to play important roles in branch migration and in epithelial reorganization during fusion (Nardi, 1990; Samakovlis et al., 1996a). The escargot (esg) gene is expressed in tip cells (Uemura et al., 1996). Esg expression in the trachea is first detectable soon after the start of tracheal branch migration. Expression of Esg protein and mRNA becomes weaker after fusion but continues throughout the rest of embryogenesis. Esg is a zinc-finger type DNA-binding protein that regulates transcription of specific target genes (Whiteley et al., 1992; Fuse et al., 1994). esg has been shown to be essential for the maintenance of diplomy of imaginal cells (Hayashi et al., 1993; Fuse et al., 1994; Hayashi, 1996) and for specification of the wing primordium during embryogenesis (Fuse et al., 1996). Function of Esg in tracheal formation is not known.

Inside the trachea there is a lumen that is connected to the outside of the embryo. The tracheal cells are in tight contact with each other by the junctional complex in the lateral side of the cells that restricts the movement of ions and small
molecules between the apical (facing luminal side) and the basal (facing hemolymph) side. The junctional complex is composed of two parts. The basally located septate junction is the invertebrate equivalent of the tight junction and includes Fasciclin III (FasIII; Patel et al., 1987), a member of the immunoglobulin superfamily. The apically localized adherens junction includes DE-cadherin, which, like its vertebrate counterparts, mediates cell-to-cell adhesion by homophilic association (Oda et al., 1994). Its cytoplasmic domain is associated with Armadillo (Arm; a Drosophila homolog of β-catenin, reviewed in Peifer, 1995) and Dac-catenin (Oda et al., 1993) that are linked to the actin network. When tracheal branches fuse, they first make contact at their basal side, which is normally devoid of the junctional complex. This situation poses a problem. Tracheal branches have to either break existing junctions and rejoin with their fusion partner, or reverse their apical-basal polarity prior to fusion to create new junctions. In either case, a smooth transition must occur since leakage of luminal contents is not observed. Epithelia encounter a similar situation many times during vertebrate development (for example, kidney development; Gilbert, 1994). How epithelial cells manage this problem is not understood.

The gene encoding DE-cadherin, shotgun (shg), has been identified (Nüsslein-Volhard et al., 1984; Tepass et al., 1996; Uemura et al., 1996). In the case of an intermediate loss of shg from the function, the fusion branches migrate to contact their targets, but fusion does not occur. The effect of the loss of zygotic DE-cadherin appears to be specific to cell rearrangement and the formation of a new cell-cell junction.

In this study, we analyzed the migration and fusion of tracheal branches by following the morphology of and molecular marker expression in the tip cells. Expression of DE-cadherin was closely correlated with a dynamic cell shape change associated with epithelial reorganization during fusion. In esg mutants, tip cells of a subset of the tracheal branch failed to accumulate DE-cadherin and such branches did not fuse but became hyperactive in motility. This phenotype was occasionally rescued by expression of DE-cadherin from the heat-shock promoter, leading to complete restoration of fusion. These results suggest that Esg stimulates DE-cadherin accumulation in the tracheal tip cell and that this accumulation is sufficient to induce a series of events leading to tracheal branch fusion.

**RESULTS**

In this study, we classified tracheal branches into two types, thin and thick. Ventral and dorsal branches were thin, as were those seen in the lateral trunk (Figs 4A,B, 5A). The diameter of these branches was small, and a single cell surrounded their circumference except for the tenth dorsal branch (DB10), the circumference of which consists of two cells. Tip cells in thin branches use filopodia to make initial contact as described in detail below. The dorsal trunk contained thick tracheal branches that consisted of multiple cells in their circumference. No filopodia were apparent on the tip cells in their branches. These two types of branches are described separately below.

**MATERIALS AND METHODS**

**Drosophila strains**

The null mutation esg/Y (Whiteley et al., 1992) and the strong hypomorphic mutation esg/Y (Fuse et al., 1994) caused essentially the same tracheal phenotype in homozygous condition and in hemizygous condition over Df(2L)A48 (Ashburner et al., 1982). esg/Y is an enhancer trap line that express β-galactosidase in the cytoplasm in a pattern nearly identical to that of esg. Development of heterozygous esg/Y embryos was indistinguishable from that of wild-type Oregon R embryos. shg/Y and hsp-cadE were described by Uemura et al. (1996). hsp/Y (shg-YZ) expresses β-galactosidase in a pattern similar to that of DE-cadherin. shg/Y was described by Nüsslein-Volhard et al. (1984), and HS-esg and HS-esg ΔZ lines were described by Fuse et al. (1994). 6-81a is a homozygous viable enhancer trap line of brtl (Klämbt et al., 1992) that frequently shows failure of tracheal fusion.

**Immunological procedures**

Embryos and larval tissue were immunostained as described by Hayashi et al. (1993). The following primary antibodies were used for immunostaining and western blotting: rat anti-DE-cadherin (DCAD1 and 2; Oda et al, 1994), rat anti-FasIII (Brower et al., 1980), mouse anti-Crums (Tepass et al., 1990), mouse anti-β-galactosidase (Boehringer Mannheim), and rabbit anti-β-galactosidase (Cappel). To stain the tracheal lumen, we employed lumen-specific antibody #55 (Klambt et al., 1992) and rabbit anti-actin (Biomedical Technologies Inc. cat#BT-560), which fortuitously recognize a luminal antigen. The following secondary antibodies were used: FITC-conjugated antibodies against rat, mouse and guinea pig IgG (Jackson Laboratory), Cy3-conjugated anti-rabbit IgG (Chemicon), HRP-conjugated goat anti-mouse IgG (Jackson) and HRP-conjugated sheep anti-rat IgG (Amersham). Sometimes the mouse and the rat primary antibodies were detected with biotinylated secondary antibodies and FITC-streptavidin (Vector Lab). Photomicrographs were taken with a confocal microscope (Zeiss LSM410).

**Heat-shock experiment**

Stage 12-13 embryos collected on apple juice-agar plates were transferred to a plastic dish together with a slice of agar (1 mm thick) and covered with paraffin. Heat shock was applied by floating the dishes in a 37°C water bath for 15 minutes and then incubating them at 25°C for 30 minutes for recovery. After repeating this procedure, embryos were allowed to develop to appropriate stage and were fixed for immunostaining or mounted in 70% glycerol for direct observation under bright-field optics. For Western blotting, embryos were homogenized in SDS sample buffer (3% SDS, 10% glycerol, 65 mM Tris-HCl [pH 6.8], 5.25% 2-mercaptoethanol, 0.2 mM PMSF, 1 μg/ml aprotonin, 10 μg/ml chymostatin, 2 μg/ml leupeptin, 1 μg/ml pepstatin). Protein concentration was determined by the Bradford assay (Bio-Rad Laboratories). Protein samples (120 μg) were separated in 7.5% SDS-PAGE, blotted as described by Sambrook et al. (1989) and detected with a chemiluminescence kit (NEN). Filters were reprobed according to the manufacturer’s instruction and quantitated by use of densitometric analysis software (Quantity One, PDI).

**Dynamic changes in cell shape and cadherin expression during tracheal branch fusion**

According to the shapes and position of tip cells, and to the temporal sequence of DE-cadherin expression, the fusion of branches could be separated into five stages (Fig. 1). Since the tracheal development proceeds in the anterior-to-posterior direction along the body axis, it was possible to unambiguously determine the temporal order of the fusion. In stage 0, while tracheal branches were migrating to their targets, DE-cadherin accumulation was restricted to the apical side of the tip cell at...
**Fig. 1.** Change in cell shape and the surface marker expression in tip cells during tracheal fusion. (A) Overall view of the tracheal system stained with the lumen-specific antibody. Arrows and arrowheads indicate dorsal branch (DB), dorsal trunk (DT) and lateral trunk (LT) and their fusion points, respectively. (B–F) escG66B heterozygotes were stained for β-galactosidase to reveal the shape of tip cells (red) and for cell-surface markers (green). (B) DE-cadherin expression during lateral trunk fusion. In stage I (B1), tip cells extended filopodia that contact each other and DE-cadherin accumulates in a line. Tip cells retracted their filopodia in stage II (B2) and the line of DE-cadherin assumed a dot shape. Subsequently, the dot of DE-cadherin changed to a ring in stage III (B3) as the lumen forms (Fig. 2C). In stage IV (B4), the major part of cell body was displaced laterally and three rings of DE-cadherin moved close to each other. The rings expanded to their final size in this stage. (C) DE-cadherin expression during dorsal trunk fusion. No filopodia were detected in this branch. DE-cadherin was first detected within one of the tip cells as a line (C1). The line of DE-cadherin subsequently extended to encompass both of the tip cells in stage II (C2). In stage III (C3), the DE-cadherin was expressed in a ring and underwent the same series of events as observed in the lateral trunk. DE-cadherin accumulated in the two tip cells in a seamless ring. (D) Dα-catenin expression in the dorsal trunk. The pattern was indistinguishable from that of DE-cadherin. (E) FasIII expression in the dorsal trunk. Fas III between contact interface was first detected in late stage III (E3). (F) Crb expression in the dorsal trunk. Crb expression followed expansion of the lumen (F2 to F4). Its accumulation between tip cells was only detected in stage IV during expansion of the lumen. In this and other figures, arrows indicate the boundary between a tip cell and the adjacent tracheal cell. Arrowheads indicate contact sites between tip cells. Numbers corresponds to stage of fusion. Bar, 5 μm. Anterior, left; dorsal, up.

**Fig. 2.** A ring of DE-cadherin protects the site of lumen fusion. (A–C) Dorsal branch 10 stained for tracheal lumen (red) and DE-cadherin (green). Tracheal lumen was not detected within tip cell while DB10 was migrating (A; stage 0) or when the tip cells have just contacted each other (B; stage II). When DE-cadherin forms a ring in stage III (C), the lumen rapidly forms in the ring. (A′–C′) Schematic diagrams of tip cells during fusion. Note that rings of DE-cadherin (green) separate lumen (red) from hemolymph (dark background). Bar, 10 μm; anterior, top.
the interface between the tip cell and the tracheal cell next to it. When the thin tracheal branches had migrated to within 5 μm of their targets (stage I, Fig. 1B1), the tip cells started to extend filopodia to make contact with each other. This was the first time that a line of DE-cadherin accumulation was observed at the contact interface. Tip cells then moved closer in tandem as the filopodia shortened (stage II, Fig. 1B2) and the DE-cadherin-positive area became a dot in shape at the site of contact. In stage III, the dot became a ring (Fig. 1B3) and, shortly afterward, a lumen appeared in it (Fig. 2C). During stage IV, the ring of DE-cadherin expanded and the cell bodies were displaced laterally, leaving thin discs of cytoplasm between branches flanked by three rings of DE-cadherin (Fig. 1B4). Fusion of the thick tracheal branch was more straightforward. The tip cells of migrating branches contacted one another directly without extending filopodia and accumulated DE-cadherin in a dot shape (stage I, Fig. 1C1). In stage II, the pattern of DE-cadherin became a line parallel to the orientation of the branch (Fig. 1C2). It subsequently changed to a ring and the tip cells underwent a series of events similar to those that occurred in thin branches in stages III and IV. The final shape of the tip cell was a seamless ring as judged by the pattern of DE-cadherin localization. We next followed fusion of lumen in the tenth dorsal branch (DB10). Staining of luminal antigen was not detectable within the tip cell until stage II (Fig. 2B). In stage III (Fig. 2C) when a ring of DE-cadherin was first detectable, a thin lumen penetrated through each tip cell and both lumens became connected at the ring. The expression pattern of Dα-catenin was very similar to that of DE-cadherin in the DT (Fig. 1D) and in thin branches (data not shown). Thus the ring of DE-cadherin and Dα-catenin marking the site of the lumen connection is a common feature of tracheal branch fusion, but distinct steps are taken by thin and thick branches to reach this stage. In contrast to DE-cadherin and Dα-catenin, which were detectable in the contact interface in fusion stage I, FasIII accumulated in the interface in late stage III when the tracheal lumens had already connected (Fig. 1E3). The transmembrane protein Crumbs (Crb) is normally localized to the apical cell membrane of epithelia (Tepaß et al., 1990). Crb accumulation appeared to follow expansion of the apical membrane (Fig. 1F).

Multiple roles of DE-cadherin during fusion

To understand the role of DE-cadherin in mediating fusion, we studied the effect of reducing DE-cadherin activity. Uemura et al. (1996) described tracheal defects of shg mutants that could be ordered in a phenotypic series according to severity. They ranged from complete failure of fusion in the null mutants to failure in expansion of the lumen in mild mutants. DE-cadherin expression was examined in two classes of mutants. In the ‘mild’ shgE17B embryo, overall intensity of DE-cadherin staining was comparable to that of the control; however, the signal was not localized on the apical side but was more broadly distributed over all of the plasma membrane. Thus the amount of DE-cadherin available for adhesion at the adherens junction was reduced. In such embryos, the majority of tracheal branches fused, but did not expand to normal width and exhibited a ‘bottleneck’ appearance (Uemura et al., 1996; Fig. 3A). Such fusion points were arrested in stage III as a small ring of DE-cadherin with a thin lumen inside. A minor fraction of fusion points was arrested in stage 0; tip cells contacted one another but did not accumulate DE-cadherin at the contact interface. In no case was fusion arrested at any other stage. The ‘intermediate’ shgE17B embryos produced unstable DE-cadherin molecules as observed in Western blots (Uemura et al., 1996) and showed an increased cytoplasmic DE-cadherin signal in immunostained preparations. Tracheal cells in shgE17B embryo appeared rounded, indicating decreased cell-to-cell adhesive- ness (Fig. 3B). Fusion points of such embryos showed one of the two phenotypes as seen in shgE17B embryo; in this case, the majority of fusion points were arrested in stage 0 (Fig. 3B). It thus appears that tip cells in stage 0 and III with insufficient amount of DE-cadherin cannot continue to the next stage. These results indicate that DE-cadherin is not only essential for initial adhesion, but also for expansion of lumen.

Loss of DE-cadherin accumulation and failure of tracheal branch fusion in esg mutants

Expression of Esg in tip cells during fusion makes it a good candidate for a regulator of tip cell function. In the null esgG66B embryo, none of the fusion points in LT, DB and VB were connected (Figs 4, 5B, and data not shown), a phenotype reminiscent of that of intermediate shg mutants (Uemura et al., 1996, Fig. 3B). Tip cells of DB10 in the esg mutant migrated and contacted one another normally but never accumulated DE-cadherin and subsequently detached (Fig. 5B). They developed a blind-ended lumen inside, as revealed by deposition of a cuticle in the mature embryo and staining of a luminal antigen (Fig. 5B, data not shown). Branches of DT expressed DE-cadherin and were in most cases successfully connected. However, the shape of the dorsal trunk appeared unusual after cuticle deposition. It was occasionally constricted at the tracheal node, suggesting that expansion of lumen was incom-
complete (Fig. 5B). HS-esg transgene was used to rescue the esg mutant defects. In the embryos heat shocked seven times, tip cells of DB10 often stably contacted one another and accumulated 26.7% (n=105) of DB10 were found to be connected (Fig. 5C). A regime of less than five heat shocks was not effective. Since the heat-shocked period had to cover the entire stage of dorsal branch migration and fusion, the continuous presence of Esg appears to be essential for fusion.

Hyperactive cell motility in esg mutant tip cells

Tip cells on thin branches showed an additional interesting phenotype. They migrated and extended filopodia in esgG66B embryos, but did not accumulate DE-cadherin or stably adhere to each other. The mutant filopodia detached and continued to extend to various directions (Fig. 6A). They contained a lumen inside and deposited a cuticle in their blind-ended structure (Fig. 4B’), that sometimes reached more than 25 μm in length and displayed a coiled appearance with its terminus floating freely in the body. These features are distinct from the morphology of a terminal branch, which migrates in a relatively straight path and becomes fixed to the tissue. Furthermore, the esg mutant tip cells appeared larger than normal and occasionally extended additional lamellodium-like cytoplasmic protrusions (Fig. 6A). This phenotype could be due to a general property of tip cells that fail to adhere to targets, or could be due to a specific effect of the loss of esg activity. To distinguish between these possibilities, we examined phenotypes of branches that did not fuse due to reasons other than esg mutations. In the wild-type embryo, fusion of tracheal branches sometimes fails and the frequency can be increased by heat shocks. Fusion of tracheal branches also failed in breathless (btl)6-81a and shg-embryos. In such embryos, no extension of filopodia was observed (Fig. 6B, data not shown), suggesting that the elongated filopodia phenotype is specific to the esg mutations. In the dorsal trunk of esg mutants, the shape of the tip cells after fusion was unusual. They appeared larger and sometimes extended cytoplasmic protrusions. These phenotypes suggest that, in esg mutants, cell motility is hyperactivated in tip cells of both thin and thick tracheal branches.

Transcriptional regulation of DE-cadherin by Esg

The promoter activity of DE-cadherin was studied by use of the lacZ reporter under the control of the shg enhancer in shgP2370 (shg-lacZ). shg-lacZ was expressed uniformly within migrating tracheal precursors (data not shown). When tracheal branches started to fuse, apposed tip cells accumulated a higher amount of β-galactosidase in both thick and thin branches (Fig. 7A,B), suggesting that shg transcription is stimulated upon fusion. This stimulation in thin branches requires Esg; upregulation of shg-lacZ did not occur in any of thin branches of hemizygous esgV38 embryos (Fig. 7C, data not shown). These results suggest that Esg is required for transcriptional upregulation of shg in thin branches to provide a sufficient amount of DE-cadherin to support fusion. DE-cadherin and shg-lacZ expression were unaffected in DT of esgV38 hemizygotes (data not shown), indicating that a distinct mechanism regulates DE-cadherin transcription in thick branches.

The above observation suggests that Esg normally stimulates transcription of DE-cadherin in tip cells of thin branches. Western blotting experiments were performed to quantitate the effect HS-esg on shg-lacZ and DE-cadherin expression. We observed a consistent increase in β-galactosidase and DE-cadherin levels upon induction of HS-esg (Fig. 8A,B,D). The amounts of Dacatenin and protein phosphatase 2A did not vary significantly between different strains and were not affected by heat shock (Fig. 8C,D). Time-course experiments showed that the amount of DE-cadherin reached maximum 30 minutes after the end of a heat shock and then started to decline (Fig. 8E). DE-cadherin induced by HS-esg was shown to be localized in the cytoplasm (data not shown), suggesting that most of the induced DE-cadherin did not participate in cell adhesion and was rapidly degraded. These results indicate that Esg can stimulate transcription of DE-cadherin in non-tracheal cells when expressed in a large amount.

Rescue of the tracheal defect in esg mutant by DE-cadherin

To test whether the fusion defect in the esg mutant is due to the loss of DE-cadherin expression, we examined an effect of
Increased and completely fused DB10 were frequently observed in the cytoplasm, the frequency of successful adhesion was high. E-cadherin accumulated in the contact interface (Fig. 5G). DB10 in some of the treated embryo adhered to each other and to contact to the target (Fig. 5F). 30 minutes later, tip cells of DB10 in the treated embryo heat shocked seven times. DE-cadherin expression and adhesion were restored, and the larvae formed completely fused DB10. (D) hsp-cadE; esgG66B embryo strongly heat shocked two times. Completely fused DB10 is indicated by the solid arrowhead. This photo is less clear than A', B', C' because the embryo is still covered by the vitellin membrane. (E) Dorsal trunk of hsp-cadE; esgG66B embryo that was mildly heat shocked (32°C for 15 minutes) and immediately fixed. Note punctuated DE-cadherin stainings not normally seen in wild-type or esgG66B embryos (yellow arrows). (F) The fusion point of DB10 of an embryo similar to that shown in E. Punctuated DE-cadherin staining is observed at the end of filopodium. (G) Similar to E, fixed 30 minutes after the end of a mild heat shock. Tip cells adhered to each other with high concentration of DE-cadherin (arrowhead). DE-cadherin staining on surface is also found in the other part of the tip cells (yellow arrows). Bars, 5 μm.

DISCUSSION

Cell motility and cell adhesion play essential roles in the construction of organs during development of multicellular organisms. We studied the function of Esg in coordinating these two activities in the formation of the tracheal network in Drosophila. The tracheal tip cells were shown to dynamically change cell shape and apical-basal polarity, and activate transcription of the essential cell adhesion molecule DE-cadherin. In the thin tracheal branch of esg mutant, motility of filopodia was hyperactivated and DE-cadherin was not properly expressed. The rescue of fusion in DB10 by hsp-cadE suggests that DE-cadherin is a major target for Esg in regulating the fusion of thin branches.

The effects of HS-esg and hsp-cadE to rescue the fusion defect was limited to DB10, which has a circumference comprising two cells instead of one as usually seen in other thin branches, and it migrates the shortest distance among the dorsal branches. In addition, movement of DB is coupled to dorsal closure, which may make path finding less problematic. We think these properties hold tip cells on DB10 close together in the esg mutant condition and make the rescue more effective. However, we think it highly likely that the genetic interaction seen in DB10 is relevant to other thin branches. Samakovlis et al. (1996b) recently reported cellular analyses of tracheal fusion and its regulation by esg. Their results are basically in good agreement with the results in this paper, although they presented a somewhat different view on the role of esg, which remains to be clarified.

Dynamic changes in adhesion and polarity of tip cells during tracheal fusion

This study revealed the dynamic behavior of the tracheal tip cell. Once thin branches have migrated to within 5 μm of one another, the tip cells start to extend filopodia to contact each other. Their behavior is similar to mammalian epithelial cells.
in culture, which extend filopodia to make multiple transient contacts with each other prior to stable adhesion via E-cadherin (Mcneill et al., 1993; Yonemura et al., 1995). The behavior of tip cells suggests that they search and recognize their target by direct contact with filopodia. Once in contact, the tip cells accumulate $\text{DE-cadherin}$ at the contact interface. This accumulation probably occurs very rapidly after contact, since we rarely found any examples of contacted tip cells without accumulation of $\text{DE-cadherin}$. The polarity of the tip cell in this stage is unusual in that $\text{DE-cadherin}$, normally localized on the apical side, is expressed on both apical and basal sides of the cell. As this ‘bipolar’ tip cell retracts its filopodia, the pattern of $\text{DE-cadherin}$ accumulation changes from a dot to a ring where lumen penetrated. This $\text{DE-cadherin}$ accumulation is associated with $\text{a}-\text{catenin}$ and with F-actin (Fig. 1D, our unpublished observation). Inside the ring of accumulated $\text{DE-cadherin}$, the gap between the two adjacent tip cells is presumably separated from hemolymph, thus creating a space where the cell membrane can be opened, without leakage of luminal contents. We speculate that the ring of $\text{DE-cadherin}$ and its associated proteins form a barrier to separate the lumen and hemolymph (see Fig. 2C'), minimizing the risk of mixing of the luminal content and hemolymph, since no breakage of existing junction occurs. FasIII and Crb are first detectable at the contact interface when the ring of $\text{DE-cadherin}$ accumulation is expanding. These observations suggest that the tracheal lumen is already continuous prior to the full accumulation of FasIII and Crb, and raise the possibility that these two molecules are dispensable for the initial formation of the barrier. The function of FasIII may be to consolidate adhesion in later stage of fusion. The coincident pattern of Crb expression with lumen formation suggests that the function of Crb is to restore apical-basal polarity (Wodarz et al., 1995).

The observed temporal order of the cell-surface marker...
Esg regulates cell motility and adhesion of the tip cells in the thin tracheal branches

The use of filopodia to make initial contact is a unique property of the thin tracheal branches. Cells in tissue culture migrate on a substratum by use of filopodia that repeat a cycle of protrusion, adhesion and traction (Lauffenburger and Horwitz, 1996).

In the case of the tip cell, cell-cell adhesion replaces cell-substratum adhesion in the adhesion step. In esg mutants, tip cells of the thin branches extend filopodia normally but fail to adhere to their target and stay in a prolonged protrusion step. Furthermore, additional short protrusions appear in esg mutant tip cells. Hyperactivated tip cell motility is also evident in the fused dorsal trunk which showed numerous protrusions. We have shown that activated cell motility is unique to the esg mutations among other conditions that affect fusion, suggesting that esg regulates cell motility independent of its function to regulate cell adhesion. Cells injected with activated forms of the small GTP-binding proteins Rac and Cdc42 showed a phenotype similar to that of esg mutant tip cells (Nobes and Hall, 1995) suggesting that esg is involved in actin-based motility. However, it is not known how the transcriptional regulatory activity of esg is related to motility of a filopodium.

Differential requirement for Esg in the fusion branches

In contrast to the striking phenotype in the thin tracheal branches, the esg mutations did not block the fusion of the thick tracheal branch, which is different from the thin tracheal branch in several aspects. Thick branches contain multiple cells in their circumference and migrate in a straight path to their targets. No filopodia are apparent during fusion. In the absence of Esg, the tip cells of thick tracheal branches contact, accumulate DE-cadherin at the interface, upregulate shg-lacZ and fuse as in the wild-type embryo. This suggests that an esg-independent pathway activates DE-cadherin transcription upon fusion of DT.

Regulation of DE-cadherin by Esg

There was no apparent correlation between the temporal change of esg expression and the onset of the tracheal defect due to the esg mutations. esg RNA and protein were detectable in tip cells before fusion and their expression was maintained, albeit at a lower level, in later stages of embryogenesis. This continuous expression of esg is probably essential for tracheal fusion, since only repeated induction of esg starting prior to the contact between tip cells supported the fusion. We speculate that Esg is essential, but is not sufficient, for expression of target genes such as shg and genes regulating cell motility. Contact between tip cells may invoke a signal that activates a rate-limiting factor regulating motility and adhesion. Perhaps such a signal is also important for the switch from the adhesion to the traction step. In the case of thick tracheal branches, in which tip cells contact with large interface from the beginning, the contact-initiated signaling may be sufficient to stimulate shg transcription. In the thin tracheal branches, in which initial contact is made in a small region between filopodia, an additional boost from Esg may be essential to activate shg. The ability of HS-esg to stimulate shg transcription in many cell types suggests that Esg is one of many regulators that activate the shg promoter and that the amount of Esg is rate limiting in many cells. We have shown...
that widely expressed DE-cadherin is under dynamic transcriptional control in a cell-type-specific manner. Given the enormous variety of cell types and morphogenetic processes requiring DE-cadherin, its regulatory mechanism must be very complex. Focusing on a single cell type in a defined developmental context should be a fruitful way to understand the function of cell adhesion molecules during morphogenesis.

Yasuhiro Shiga contributed to the initial phase of this work. We thank Mark Krasnow and Christos Samakovlis for communicating results prior to publication. We also thank Judith Kassis, Michael Ashburner and the Bloomington Stock Center for fly stocks, Elizabeth Knust, Danny Brewer and Ben-Zion Shilo for antibodies. We are grateful to Jun-ichi Tanizawa for critically reading the manuscript. This work was supported by Research Grant from the Ministry of Science and Culture to SH, TU and MT. SH acknowledges the support from Naito Foundation and Ciba-Geigy Japan.

REFERENCES


Fuse, N., Hirose, S. and Hayashi, S. (1994). Identification of a monoclonal antibody recognizing E-cadherin, its regulatory mechanism must be very complex. Focusing on a single cell type in a defined developmental context should be a fruitful way to understand the function of cell adhesion molecules during morphogenesis.


(Accepted 16 September 1996)