Microtubule-dependent balanced cell contraction and luminal-matrix modification accelerate epithelial tube fusion

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Connection of tubules into larger networks is the key process for the development of circulatory systems. In Drosophila development, tip cells of the tracheal system lead the migration of each branch and connect tubules by adhering to each other and simultaneously changing into a torus-shape. We show that as adhesion sites form between fusion cells, myosin and microtubules form polarized bundles that connect the new adhesion site to the cells’ microtubule-organizing centres, and that E-cadherin and retrograde recycling endosomes are preferentially deposited at the new adhesion site. We demonstrate that microtubules help balancing tip cell contraction, which is driven by myosin, and is required for adhesion and tube fusion. We also show that retrograde recycling and directed secretion of a specific matrix protein into the fusion-cell interface promote fusion. We propose that microtubule bundles connecting these cell–cell interfaces coordinate cell contractility and apical secretion to facilitate tube fusion.
Organ formation and modifications in tissue architecture occurring during development involve deconstructing both cell adhesion and the extracellular matrix\(^1\), thereby converting cells into a migratory state with a new tissue identity; this is known as epithelial–mesenchymal transition\(^2\). Although epithelial cells and mesenchymal cells have contrasting properties, the molecular mechanisms behind these properties are not entirely mutually exclusive. Some epithelial cells acquire the ability to migrate while retaining cell–cell adhesion, allowing these cells to migrate as a group\(^3\). This is possible due to the polarized nature of the epithelia; the apical side retains the cell–cell interface, while migratory protrusions form on the basal cell surface. On reaching their destination, these migratory epithelia are integrated into the target tissues. However, how migratory epithelia find their target tissues, establish new cell–cell interfaces and integrate with the target tissue are poorly understood.

Studies of the formation of adhesive interfaces in cultured cells showed that contact with actin-enriched filopodia initiates the assembly of the adherens junction\(^10,11\) and suggested that microtubules are involved in this process\(^12\)–\(^15\). As the formation of cell junctions involves coordinating the assembly of the cell adhesion complex and the generation of tension in each side, the cells forming the junction must be observed simultaneously to understand how mechanical coupling is controlled at the tissue level.

Here, to examine how cell adhesion and cell contractility are coupled to establish a new cell interface, we examined the anastomosis formation in the Drosophila tracheal system. In this process, a pair of tip cell (hereafter called fusion cell, FC) forms an adherens junction \(de novo\) (Fig. 1a,b) and deposit extracellular matrix materials into newly forming lumen. Each FC is converted into a torus shape by plasma membrane fusion via the inner matrix materials into newly forming lumen. Each FC is converted into a torus shape by plasma membrane fusion via the inner matrix materials into newly forming lumen. Each FC is converted into a torus shape by plasma membrane fusion via the inner matrix materials into newly forming lumen. Each FC is converted into a torus shape by plasma membrane fusion via the inner matrix materials into newly forming lumen.

An FC pair is highly coordinated. However, how cell adhesion, cell–shape change and membrane trafficking in each FC pair is highly coordinated. How, however, cell adhesion, cell–shape change and membrane trafficking are regulated coordinately in pairs of FGs is poorly understood.

We show that FCs form a \(de novo\) adherens junction at the contact site through stabilization by a mechanism requiring actomyosin and microtubules; this mechanism applies a balanced pulling force to flatten the FCs. In addition to this cell-intrinsic contractile force, the preferential deposition of molecules via the apical secretion pathway promotes maturation of the matrix in the lumen that forms in the FC contact interface and helps to fuse the plasma membranes.

**Results**

F-actin and microtubule dynamics in migrating FCs. FCs expressing the F-actin markers GFP-moesin (Fig. 1c; see also Supplementary Movie 1) or lifeact-GFP (Supplementary Fig. 1a), each driven by an FC-specific enhancer (\(esg\_FC\) enhancer; see Methods), were closely associated with the basal surface of the dorsal epidermis and showed numerous F-actin-enriched filopodia extending towards the dorsal midline\(^16\)–\(^18\). We also observed invasive filopodia that extended vertically from these FCs into the epidermal layer (Fig. 1c open triangles, Fig. 1c’ and see also Supplementary Fig. 1a); their location did not correspond to epidermal cell junctions, suggesting that the filopodia penetrated the epidermal cells. While migrating, the FCs appeared flat with numerous forward-extending filopodia (Fig. 1c, time 0:00 h), but on reaching their target, their shape became more compact, with the lumen extending from the side of the stalk (Fig. 1c, time 1:54 h). Microtubule labelling with GFP-tau revealed extensive microtubule arrays emanating from the microtubule–organizing centre (MTOC) at the proximal side of the cell (Fig. 1d, time 0:00 h; see also Supplementary Fig. 1b,c and Supplementary Movie 2) and F-actin labelling revealed many, F-actin-rich protrusions extending forward to reach the target cell (Fig. 1e and see also Supplementary Movie 3). On FC contact with its partner FC, the MTOC moved to the contact site (Fig. 1d, time 1:25 h). The vertical protrusions revealed by F-actin marking were not observable with microtubule labelling (compare Fig. 1c,d and Supplementary Fig. 1b for another microtubule marker GFP-Jupiter).

A polarized flux of E-Cadherin to the new cell junction. When two FCs came within a proximity of 15.2\(\mu m\) (on average), fluorescent markers for E-cadherin (Fig. 2a)\(^22\) and its associated protein p120ctn (Supplementary Fig. 3a)\(^23\), accumulated at the contact site. Immunostaining revealed endogenous E-cadherin localized to the extending edge of forward-reaching filopodia on the tip cells (Fig. 2b top). This punctate E-cadherin localization was not observed on the filopodia of terminal cells or stalk cells (Fig. 2b bottom). To monitor the dynamics of E-cadherin transport, we imaged E-cadherin-GFP fluorescence during the fusion process. We found that the E-cadherin-GFP level remained fairly constant at the junction between the FC and stalk cell (Fig. 2a, position and plot marked with ‘L’ and ‘R’), but increased steadily at the FC interface, surpassing the intensity at the FC–stalk cell interface within 20 min after contact (Fig. 2a). We next monitored the E-cadherin-GFP turnover rate at each cell interface by fluorescence recovery after photobleaching (FRAP) analysis. All three E-cadherin-GFP foci (L, R and C) were photobleached simultaneously and their rate of fluorescence recovery was measured (Fig. 2c arrowheads). The fluorescence recovery rate and the mobile E-cadherin-GFP fraction were significantly higher at the FC interface than at the L and R foci (Fig. 2d,e). Imaging with high spatiotemporal resolution revealed that E-cadherin-GFP appeared on the free surface of the plasma membrane, with occasional streams from the cytoplasm to the membrane surface, and then accumulated at the FC contact site (Fig. 2e and Supplementary Movie 4). In addition, Golgi apparatus in FCs became progressively enriched near the contact site (Fig. 2g).

The low E-cadherin-GFP turnover at the FC–stalk cell interface suggests that the exchange rate of the E-cadherin complex at this junction, which was already present at the time of dorsal branch (DB) migration, is low. To analyse E-cadherin’s contribution, we reduced its synthesis by double-stranded RNA-mediated gene knockdown (Supplementary Fig. 3b). Expressing E-cadherin double-stranded RNA in tracheal cells with the trachea-specific \(bit\_Gal4\) driver reduced the level of E-cadherin in DB stalk cell junction by ~27% (Methods), but did not compromise the overall tracheal morphology or the migration speed of the DB before the FCs came into contact with each other (Fig. 2h,i), suggesting that the amount of E-cadherin gene product in this experimental condition was sufficient to sustain the tracheal tissue architecture. However, the FC contraction was significantly delayed after contact and the interface between the FCs failed to mature (Fig. 2h,i). These results suggest that newly synthesized E-cadherin is preferentially partitioned to the distal side, to form the new cell interface.

**FC contraction by a myosin-dependent pulling force.** We next examined the role of myosin II in the contractile force within the FC. When expressed in FCs, a green fluorescent protein (GFP) fusion protein of the myosin heavy chain Zipper formed...
longitudinal bundles at the time of FC–FC contact, to connect the adherens junctions on each FC (Fig. 3a and Supplementary Movie 5). These myosin bundles became shorter as the FCs contracted and became more compact (Fig. 3a). Similar myosin bundles were observed in the dorsal-trunk FCs (Supplementary Fig. 4a).

To determine whether FC contraction is driven by a cell-autonomous force, we assessed the contractile state of laser-perturbed FCs. A microwave-length infrared laser (1,440 nm) effectively penetrated the epidermal layer and produced sufficient heat at the focal point to induce a heat-shock response 24,25. Infrared laser illumination targeted to one of the paired FCs caused a cell-specific relaxation of cell contractility (Fig. 3b), with a simultaneous excessive contraction in the intact FC. The progression of the fusion process, assessed by shortening of the L–R length, was also arrested (Fig. 3c and Supplementary Movie 6, N = 3). This result supports the idea that FC pairs normally exert nearly equal pull on each other to maintain their symmetric appearance, and that this balanced pulling force is required to pull FCs closer together. Furthermore, expressing a dominant-negative form of the myosin heavy chain interfered with FC contraction (Fig. 3d and Supplementary Movie 7, 100% N = 9, 3 embryos) and fusion (Supplementary Fig. 4e,f).

**Polarized microtubule organization in the FC.** We next studied the detailed organization of microtubules. The microtubule minus-end marker Nod-LacZ (ref. 26) and the centriole marker GFP-PACT (ref. 27) were concentrated near the FC–stalk cell interface (Fig. 4a,b). From this proximal MOTC, microtubules extended in a fan-shaped pattern to the migrating FC’s leading edge (Fig. 1d and Supplementary Fig. 1c). We also imaged the microtubule plus-end marker EB1-GFP in FCs (Fig. 4c and Supplementary Movie 8); particle image velocimetry analysis revealed rapid, comet-like EB1-GFP signals with a mode velocity.

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**Figure 1 | Dorsal-branch migration and fusion of the Drosophila trachea.** (a) A stack of confocal GFP images shows the tracheal system of a stage-15 embryo carrying btl > GFP-moe. (b) Spatial relationship of the migrating tracheal branch and the epidermis; FCs, terminal cells and stalk cells of the dorsal branch and peripheral amnioserosa (pAs) are indicated. (c) Time course of dorsal-branch migration and fusion, shown by F-actin labelling with esg_FC-Gal4 and UAS-GFP-moe (FC and pAs), and with sqh-GFP-moe (epidermis). Each panel shows dorsal (top half) and transverse views (bottom half) of images at the indicated time points (top left of the image). Filled triangles indicate F-actin-rich denticles of the apical surface of the epidermis; open triangles indicate FC filopodia penetrating vertically into the epidermis and nearly reaching the apical surface. Red dotted line indicates the basal epidermal surface. (c’) Enlarged view of the vertical filopodia (1:30). (d) Microtubule distribution during tracheal-branch fusion, revealed by expressing the tau-GFP marker in FCs and pAs by the esg_FC-Gal4 driver. Magenta triangles indicate MTOCs. (d’) Enlargement of d, 0:30 time point. (e) Double labelling of microtubules (GFP-tau) and F-actin (GFP-moe). Scale bar, 10 μm.
of 5 μm min⁻¹ moving towards the FC’s leading edge (Fig. 4c) and an EB1-GFP population moving at even higher velocities (>10 μm min⁻¹) in the periphery (Fig. 4c). Dual-colour imaging of EB1-GFP and the cell membrane revealed that the microtubule plus ends projected to the cortical region of the leading edge and extended repeatedly into the filopodial protrusions (Fig. 4d and Supplementary Movie 9). The microtubule-filled protrusions were found most frequently in the leading edge, which made contact with the partner FC at the midline and accumulated E-cadherin-GFP (Fig. 4e and Supplementary Movie 10).

Requirement of microtubules for tracheal fusion. To reveal the role of polarized microtubule arrays in tracheal-tube fusion, we perturbed the FC microtubule organization by expressing Katanin p60 (Kat60), a catalytic subunit of the microtubule-severing factor Kat60/p80 (refs 28–30). Microtubules in control FCs, visualized by the marker GFP-tau, formed dense central bundles directed towards the leading edge, with diffuse fractions filling the rest of the cell (Fig. 4f top and Supplementary Movie 11). Analysis of the signal variance in a 200-s interval (20 frames) revealed stable central microtubule bundles and an unstable diffuse fraction (Fig. 4f bottom). In FCs expressing Kat60, the microtubule bundle was misdirected and became unstable (high signal variance, Fig. 4f), indicating that the ectopic Kat60 could destabilize the microtubules. In this condition, endogenous Zip cables connecting the adherence junctions of FCs was reduced (Supplementary Fig. 2). DBs expressing Kat60 migrated towards the dorsal midline and maintained normal-shaped stalk cells and the FCs contacted each other using filopodia. However, the fusion process was arrested, because the filopodia contact site failed to accumulate E-cadherin or mature into a tight cell contact18 (Fig. 4g,h).
To further elucidate the role of microtubules in forming the adherens junctions, we expressed Spastin (Spas), another microtubule-severing factor, in FCs. Spas was previously demonstrated to inhibit tracheal branch migration and fusion. We found Spas to be less disruptive to the fusion process than Kat60 (Methods), making it suitable for quantifying the E-cadherin-GFP accumulation at the contact site over time. The rate of E-cadherin-GFP accumulation was estimated by the slope of a fitted line (Fig. 4j,k) and the coefficient of determination ($R^2$-value) was used as a measure of fluctuation (Fig. 4l). Spas reduced the E-cadherin-GFP accumulation rate and increased the fluctuation ($R^2$-value; Fig. 4l). Despite these defects, FCs expressing Spas eventually accumulated E-cadherin-GFP and completed branch fusion. Taken together, these results indicate that proper microtubule organization is required for stable, persistent E-cadherin accumulation to the new cell-adhesion sites in tracheal-branch fusion.

Microtubules coordinate deformation of paired FCs. We next analysed the dynamics of cell-shape change during the fusion process by monitoring the distance between the FC contact site (C) and the FC–stalk cell boundaries at the left (L) and right (R) side branch. In control embryos, FC pairs maintained their symmetry, with nearly equal C–L and C–R lengths (Fig. 5a–c and Supplementary Movie 13). However, FCs that expressed Spas deformed more slowly, as measured by the speed at which the L–R length decreased (Fig. 5d), and failed to maintain a balanced bilateral cell contraction (Fig. 5a) as measured by the L/R balance; the L/R balance was calculated as $(a - b)/(a + b)$, where $a = C - L$ and $b = C - R$ (Fig. 5b). The degree of fluctuation, based on the s.d. of the L/R balance, remained low in control cells. However, imbalances in cell length began appearing in $esg_{FC}$ $\text{Spas}$ cells 5 min after the first contact between FC cells (Fig. 5c). These findings indicated that proper microtubule organization is required for balanced cell contraction.

We further analysed the mechanism of the balanced cell contraction. We hypothesized that asymmetry in the length of paired FCs is caused by an imbalance in contractile forces between the cells, creating a back-and-forth tug-of-war (ToW) situation. To demonstrate this concept, we calculated the contraction speed of each FC at every time interval after contact. We used the contraction speed of viscoelastic cell materials, which is proportional to the contractile force, as a surrogate of cellular force. Negative and positive values indicate contractile forces pushing the cell towards the left or right, respectively. (Fig. 3 | FC contraction. (a) GFP-myosin heavy chain formed a longitudinal track connecting two adherens junctions in an FC. (b) Laser perturbation of FC contraction: the left-side cell (asterisk) in a new FC pair was illuminated by infrared laser at the time of contact. (c) The location of each cell-adhesion site (L, C and R) was tracked and the distance between sites was plotted. Laser perturbation caused significant deviations in L/R balance values ($N = 3$, a representative case is shown). (d) Fusion was inhibited by a dominant-negative form of myosin. White arrow indicates the FC contact site. Scale bar, 10 μm.)
and relaxed state of each FC, respectively. If both FCs contracted or relaxed simultaneously at a given time point after contact, the contractile force was considered balanced. If one cell contracted while the other relaxed, the event was considered imbalanced and the ToW ratio deviated greatly from 1.0 for control and esg_FC

Direct apical Serp secretion to the FC interface. Based on our observation that the Golgi apparatus moved towards the site of contact between FCs (Fig. 2g), we reasoned that the localization of secretory machineries is probably influenced by microtubule polarity. Rab9 is required for the endosome-to-Golgi trafficking of a key luminal component, the chitin deacetylase Serpentine (Serp)\(^{33-35}\). RFP-Rab9’s localization in FCs was similar to that of the Golgi apparatus and of Arl3, another small GTPase that is essential for tracheal-branch fusion (Fig. 6a,b)\(^{19,20}\). We therefore examined the secretion of luminal components into the new lumen formed between FCs. Fusion points of dorsal trunk were observed for this study, because detailed analysis of lumen formation is possible with their large tube diameter. In arl3 mutants, isolated chitin-filled lumen was found at the contact site of dorsal-trunk FCs (Fig. 6c) and the lumen contained secreted luminal proteins: the chitin deacetylase Verm\(^{33,34}\), the ZP-domain protein Pio\(^{36}\) and the chitin-binding protein Gasp\(^{37}\) (Fig. 6d-f). However, Serp was specifically missing in the isolated lumen (Fig. 6c). To determine whether FCs can secrete Serp, we expressed Serp-GFP by the FC driver and found that Serp-GFP was absent at the contact site but filled the rest of the tracheal lumen (Fig. 6g). In contrast, in an arl3-mutant background, Serp-GFP was absent at the FC contact site but filled the rest of the tracheal lumen (Fig. 6h). These results indicate that Arl3 is specifically required for the directed secretion of Serp into the lumen in the interface between paired FCs, but not for its secretion into FC–stalk cell interfaces.
we attempted to test this model by imaging vesicular trafficking of endosomes containing E-cadherin towards the contact site and of preferential deposition, in which microtubules transport btl-Gal4 arl3 idea, we examined whether Rab9 overexpression could rescue the aforementioned results suggested that Rab9-dependent Serp Rab9 overexpression overrides the requirement for Arl3. The aforementioned results suggested that Rab9-dependent Serp trafficking might be an important function of Arl3. To test this idea, we examined whether Rab9 overexpression could rescue the arl3-mutant phenotype. We found that GFP-Rab9 expressed by btl-Gal4 or esg_FC-Gal4 rescued the fusion defect of arl3 mutants (Fig. 7a–d,g). However, GFP-fusion proteins of other small GTPases, Rab11 and Rab6, or of Serp failed to rescue the arl3 phenotype (Figs. 7e–g and 6h). These results indicate that Rab9-dependent recycling and apical secretion is a crucial component of the Arl3-dependent conversion of paired FCs into a torus shape.

Discussion
Previous studies showed that F-actin-enriched cell protrusions form in the tip of migrating tracheal branches16–18,38. Here we showed that tracheal FCs form polarized microtubule bundles oriented towards the leading edge of the migrating cells. The function of these microtubules is twofold: to concentrate E-cadherin to the newly contacted cell interface and to initiate the formation of new adherens junctions. We found that the E-cadherin that accumulated at the new cell interface is not recycled from the cell surface, but is instead drawn from a newly synthesized pool and recruited preferentially to the FC contact site, and not to existing adherens junctions between FCs and stalk cells. We speculate that the forward reorientation of the MTOC and the polarization of the microtubule plus ends towards the leading edge underlie the preferential deposition of E-cadherin at the FC contact site59. We considered one possible mechanism of preferential deposition, in which microtubules transport endosomes containing E-cadherin towards the contact site and we attempted to test this model by imaging vesicular trafficking of the complex containing E-cadherin-GFP and other adherens junction components. We did not find definitive evidence for this model. However, we observed that the Golgi apparatus shifted forward, towards the FC contact site, and that an E-cadherin-GFP signal increased in the plasma membrane before becoming concentrated at the contact site. Based on these observations, we favour a model in which the relocalization of the Golgi apparatus near the leading edge of the FC provides a source for E-cadherin that is deposited locally in the plasma membrane and the trans association of E-cadherin between the FCs nucleates a further concentration of E-cadherin via cis clustering40. Brodu and colleagues15,41 have shown that MTOC components are located apically in stalk cells and the microtubule function is required for the apical assembly of adherens junction proteins Par-3 and E-cadherin through regulation of recycling endosomes. This mechanism appears different from FCs, as assembly of new adherens junction occurs in the cell interface enriched with microtubule plus ends opposite to the centriole located in the proximal side.

A second microtubule function was discovered in this study, which was to equalize the contraction in FC pairs after contact. The coordinated contraction in FC pairs pulls two FC–stalk cell junctions simultaneously towards the FC contact site. The contractile force comes from a myosin-driven process; the microtubules may serve as a ‘ratchet’ to fix the length of the FCs after each round of contraction. When microtubules were inhibited, the FCs relaxed to their original length after contracting, which delayed the overall fusion process. When microtubules were destabilized, branch fusion proceeded, albeit with delays and imbalances, and fusion was eventually completed. Even in this condition, the conversion of the FC cells into a torus shape occurred simultaneously, suggesting that there is a mechanism to coordinate the fusion event in FC pairs. The...
Figure 6 | RFP-Rab9 vesicle trafficking in dorsal-branch FCs. (a) In dorsal-branch FCs (indicated by arrowheads and dotted outlines), RFP-Rab9 vesicle clusters (asterisks) were localized near the FC contact site. Anterior, left. (a’) Clustering of RFP-Rab9 vesicles was inhibited when microtubule was disrupted by Kat60. (b) Arl3 was also concentrated adjacent to the apical cell membrane (labelled with Uif) and co-localized with GFP-Rab9 at the FC contact site of the dorsal trunk (yellow arrowheads). (c–f) A specific secretion defect in arl3-mutant embryos: fusion points of stage-16 dorsal trunk were stained for CBP and Serp (c), Verm (d), Pio (e) or Gasp (f). Chitin, Verm, Pio and 2A12 were present in the lumen isolated from the FC contact site (blue arrowheads), whereas Serp (yellow arrowheads) was missing. (g, h) Test of directed Serp trafficking: Serp-GFP was expressed in FCs (blue arrowheads) using the esg_FC-Gal4 driver in control (arl3+/+, g) and arl3 mutant (h) embryos, respectively. Secreted Serp-GFP was present throughout the lumen in control embryos (yellow arrowheads in g) but absent from the FC contact site in arl3 embryos (blue arrowheads in h). (g’, h’) Enlargement of the boxed regions in g and h, respectively. Scale bar, 10 μm.
The equal number of cadherin-catenin complex in each paired sister chromosomes applies pulling force to each spindle microtubule plus ends attached to the kinetochore of each of is similar in configuration to the mitotic spindle, where the E-cadherin conjugated cell interface via microtubule plus ends mechanisms observed in several contraction-dependent and was shown to be required for tracheal branch fusion 43. Here we showed that Arl3 is required for microtubules and intracellular vesicles concentrated at the FC this process requires Arl3 GTPase, which associates with the microtubules and intracellular vesicles and cell pairs are converted simultaneously into a torus shape.

When FCs are fully contracted, plasma membrane of the two adherens junctions in each FC are connected in a single burst and cell pairs are converted simultaneously into a torus shape. This process requires Arl3 GTPase, which associates with the microtubules and intracellular vesicles concentrated at the FC contact site19,20. Here we showed that Arl3 is required for directed Serp trafficking, and that GFP-Rab9 overexpression overrides the requirement for Arl3. We propose that the microtubule-dependent transport of the Golgi apparatus and endosomes facilitates the concentration of Rab9 and Arl3 at the FC contact site, where they act together to increase the concentration of Serp in the lumen. The deacetylation of chitin converts it to the more hydrophilic form chitosan. The increase in water absorption by chitosan would cause the luminal matrix gel to swell, simultaneously pushing the plasma membranes of the FC interface closer to the plasma membrane of the FC-stalk cell interface so that the membrane-fusion machinery triggers the conversion of the paired FCs into a torus shape.

A number of issues remain to be explained. Lumen formation in FCs was clearly detected arl3 mutants, but not in the normal context. This is probably because very small lumen is sufficient to trigger fusion of wild-type FCs. Although Arl3 is absolutely required for fusion, Rab9 and Serp are not, suggesting that the proposed luminal-matrix swelling due to chitin deacetylation is not the sole mechanism of plasma membrane fusion, and additional Arl3-regulated process of fusion control must exist. Moreover, additional Rab9 cargo that acts together with Serp to rescue the arl3 mutants is predicted. To uncover the entire fusion process, it will be necessary to search for additional Arl3 and Rab9 targets, and to analyse FC-specific membrane trafficking and secretion.

**Methods**

**Drosophila strains.** The esg FC-Gal4 strains used here were constructed by injecting a plasmid containing a genomic fragment upstream of the esg gene into the pGatB vector48 and by isolating insertions in the second and third chromosomes. The fusion-cell enhancer was identified during a study of esg
regulatory elements. The Fc-FC-Gal4 strain were constructed by inserting the 7,259 bp of genomic DNA (position 15317399-15320998 of the C. elegans genome) upstream of the Fc-FC-Gal4 (FB:100384), whereas VY-EGFP and SHI were associated with the Genetic Resource Center, National Institute of Genetics in Mishima, Japan. This construct was described as pks-Gal4 expressed in the peripheral amnioserosa. UAS-p120ctx-targFP was constructed with a p120ctx sequence amplified from the complementary DNA library.p10 Fagen and TagRFP (Ovrogen) was constructed as described elsewhere. UAS-TagRFP was constructed by cloning three copies of tagRFP as a tandem fusion. UAS-tagRFP-tau was constructed by fusing tau's microtubule-binding domain (amino acids 165–327) to the carboxy terminus of tagRFP. UAS-TagRFP-Jupiter was constructed by fusing Jupiter then framing sequence ccd 2 to terminator sequence to the C-terminal end of Tag-RFP. UAS-EBI-GFP was constructed by Masako Kaido and Leo Tsuda. All of the fluorescent-protein clones were cloned into pUAST vector and transformed into germ line.33,34

Katz60's microtubule-severing activity was originally discovered in a screen of Gene Search strains for effectors interfering with tracheal morphogenesis (Kenzi Oshima, personal communication; P{w[+]/}). Measurements were processed with Ruby (http://www.ruby-lang.org/) and Perl.40–43. Embryos were devitelinized by addition of 2 ml methanol and vigorous mixing. Devitellinized embryos were washed two times with methanol and rehydrated with PBS. Embryos were stained as described previously.23 Embryos were dechorionated with 1/2 diluted commercial bleach and were fixed by mixing liquid scintillation vial for 30 min at room temperature. After removal of heptane, were dechorionated with 1/2 diluted commercial bleach and were fixed in mixed solutions of 1/4 M PBS, 0.1 M sodium ascorbate, 0.1 M β-mercaptoethanol, 0.1 M glucose, 10% methanol, and 4% paraformaldehyde at 4°C for 15 min and were washed three times with PBS before being placed on pads with PBS. Embryos were stained as described previously. Blocking, antibody incubation and washing were performed with 1% BSA and 0.2% Triton X-100 in PBS. The anti-Katanin 60 antibody (100×) was generously donated by Naoyuki Fuse and Fumio Matsuzaki.

Microscopy. Time-lapse imaging of living embryos was performed with Olympus FV1000 with GaAsP detectors.19,30 FRAP analyses were performed on tracheal cells expressing DE-cad-GFP with a confocal microscope equipped with a second scanner (a × 20 water immersion lens (FV-1000, Olympus)). The scanning rate was every 10 s and specific locations were bleached with a 50-mW, 450-nm diode laser. EBI-GFP was imaged using a TCS SP5 II (Leica) equipped with a × 63 oil-immersion lens (numerical aperture = 1.4). Optical sections (0.46 μm × 13) were scanned at 200 frames every 0.098 s. Infrared laser illumination was applied as a 1-s pulse at 60 mW and the embryos were imaged in 30 s intervals.23

Measurement and data processing. All imaging data were processed and measured with applications developed in-house; these data were written in C and Objective-C and compiled as figures with ImageMagick (Rasband, http://image.ij.nih.gov/ij/). Measurements were processed with Ruby (http://www.ruby-lang.org/) and R (R Development Core Team, http://www.R-project.org/). The spatial coordinates (x-y-z) of areas with high signal intensity (E-cadherin-GFP, p120ctn-RFP or F-actin) were fitted with a 3D Gaussian function that has been previously proposed.26,49,50

Numerical calculation of tug-of-war frequency. To compare the frequency of tug-of-war occurrence between wild-type and MT perturbed conditions, we measured the number of actin filament bundles as a measure of the frequency of ToWs. We calculated the number of actin bundles that displayed significant increases in intensity ratio (r) between the two ends of the bundle. We then compared the number of actin bundles that displayed significant increases in intensity ratio (r) between the two ends of the bundle.


t = \frac{E \cdot \text{intensity ratio}}{\text{bundle length}}

where E is the pulling force and inten\tsity ratio is the ratio of the signal intensity at the two ends of the bundle. The number of actin bundles that displayed significant increases in intensity ratio (r) between the two ends of the bundle was calculated for each condition.

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Author contributions

K.K. performed the microtubule and cadherin experiments and analysed data with help from H.W. B.D. analysed the functions of myosin, Ar and Serp. Y.Y. identified the FC enhancer and M.T.-M. constructed the driver strain. K.K., B.D. and S.H. designed experiments, analysed the data and wrote the manuscript.

Additional information

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