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Epithelial polarity proteins regulate *Drosophila* tracheal tube size independently of the luminal matrix pathway

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Summary

Regulation of epithelial tube size is critical for organ function. However, the mechanisms of tube-size control remain poorly understood. In the *Drosophila* trachea, tube dimensions are regulated by a luminal extracellular matrix (ECM) [1–4]. ECM organization requires apical (luminal) secretion of the protein Vermiform (Verm), which depends on the basolateral septate junction (SJ) [5,6]. Here, we show that apical and basolateral epithelial polarity proteins interact to control tracheal tube-size independently of the Verm pathway. Mutations in *yurt* (*yrt*) and *scribble* (*scrib*), which encode SJ-associated polarity proteins [7,8], cause an expansion of tracheal tubes, but do not disrupt Verm secretion. Reducing activity of the apical polarity protein Crumbs (Crb) suppresses the length defects in *yrt* but not *scrib* mutants, suggesting that Yrt acts by negatively regulating Crb. Conversely, Crb overexpression increases tracheal tube dimensions. Reducing *crb* dosage also rescues tracheal size defects caused by mutations in *coracle* (*cora*), which encodes a SJ-associated polarity protein [8,9]. In addition, *crb* mutations suppress *cora* length defects without restoring Verm secretion. Together, these data indicate that Yrt, Cora, Crb and Scrib operate independently of the Verm pathway. Our data support a model in which Cora and Yrt act through Crb to regulate epithelial tube size.

Keywords

Tracheal morphogenesis; Tubulogenesis; epithelial polarity; Crumbs; Yurt; Coracle Scribble; Septate junction; Vermiform

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Results and Discussion

Yrt is essential for tracheal tube size control, but is not required for Verm and Serpentine (Serp) secretion

The transmembrane protein Crb acts as an apical determinant during establishment of epithelial apical-basal polarity [10,11]. During later stages of epithelial differentiation, Crb promotes apical membrane growth independently of its role in apical-basal polarity [12]. Crb activity is counteracted by different groups of basolateral polarity proteins including the Yrt/Cora group, which is composed of Yrt, Cora, Na^+/K^+-ATPase and Neurexin IV (Nrx-IV) [8]. Loss of Yrt results in Crb-dependent apical membrane growth during late stages of epithelial cell maturation in *Drosophila* [13]. Thus, the equilibrium between the activities of these polarity proteins is important to define the size of the apical domain. Precise control of the apical surface of tracheal cells is crucial to define epithelial tube size in the *Drosophila* respiratory system [14], suggesting a potential role for polarity proteins in tube morphogenesis. However, the contribution of polarity regulators to the regulation of epithelial tube shape and size is poorly understood. Controlling length and diameter of the lumen is important for organ function as illustrated by the deleterious tubule enlargements that occur in polycystic kidney disease [15].

To better understand the role of polarity regulators and apical membrane growth in epithelial tube morphogenesis, we investigated the role of Yrt in the formation of the *Drosophila* respiratory system, a network of interconnected tubules that delivers oxygen throughout the body [16]. Yrt is mainly associated with the lateral membrane in tracheal cells and is enriched at SJs as shown by its co-localization with the SJ marker Nrx-IV (Fig. 1A,B) [17]. We characterized tracheal development in zygotic *yrt* mutants or *yrt* null embryos devoid of both maternal and zygotic *yrt* (*yrt*M/Z). Segmental tracheal placodes invaginated and established a normal branching pattern of tracheal tubes with intersegmental connections in both *yrt* and *yrt*M/Z mutants. *yrt*M/Z embryos display apical-basal polarity defects and irregularities in the tracheal epithelium at mid-embryogenesis. However, apical-basal polarity normalized during terminal differentiation [8,13]. In contrast, zygotic *yrt* mutants have only minor, if any, polarity defects in tracheal cells (Fig. 4C) [13]. The most apparent defect in *yrt* mutant trachea was the excessively long and convoluted dorsal trunks compared to the straight dorsal trunks seen wild type (Fig. 1C–E). The average dorsal trunk length was 417 +/− 12 μm in wild type embryos, whereas it was 476 +/− 22 μm in *yrt* and 470 +/− 23 μm in *yrt*M/Z mutant embryos (Fig. S1A,B). Similar but milder tube-length defects were observed in other tracheal branches. In addition, the diameter of dorsal trunks in *yrt* and *yrt*M/Z mutants was uniform, but wider than in wild type embryos (Fig. 1C–E). The average dorsal trunk diameter was 9.1 +/− 0.6 μm in tracheal segment seven of wild type embryos compared to 12.2 +/− 0.9 μm in *yrt* and 12.7 +/− 0.6 μm in *yrt*M/Z mutant embryos (Fig. S1C,D). In smaller branches, some diameter expansions were also apparent (Fig. 1D). In addition, the smaller tracheal branches in *yrt*M/Z embryos showed frequent interruptions indicating either breaks or a failure in the luminal accumulation of the 2A12 antigen (Fig. 1E). These findings indicate that Yrt regulates the size of tracheal tubes and supports the integrity of segmental tracheal branches. Remarkably, despite the prominent differences in the apical-basal polarity defects between *yrt* and *yrt*M/Z mutant embryos [8,13], both mutants exhibit very similar dorsal trunk elongation and diameter defects. This finding suggests that the transient loss of apical-basal polarity in *yrt*M/Z embryos is not the cause of dorsal trunk size defects, and that Yrt has therefore distinct functions during early and late stages of tracheal morphogenesis.

The enlargement of the tracheal tube lumen observed in *yrt* mutants could be caused by an increase in cell number or an increase in the dimension of the apical surface of tracheal cells that surround the lumen. To address this question, we counted the number of dorsal trunk cells in *yrt* mutants and wild type embryos. No significant differences in cell numbers between wild

type and *yrt* mutant embryos were found (Fig. S1E), indicating that the enlargement of tracheal tubes observed in *yrt* and *yrt*M/Z mutants must be accompanied by an increase in the dimension of the apical surface of tracheal cells.

Several other mutants display enlarged dorsal trunks similar to *yrt*. One group of genes required for limiting tube length encodes components of the SJ, including the Na^+/K^+ ATPase (α and β subunits), Cora, Nrx-IV, Scrib, Lachesin (Lac), Sinuous (Sinu), Megatrachea (Mega), and Varicose (Vari) [18–23]. Among these SJ proteins, Na^+/K^+ ATPase, Cora, Nrx-IV and Scrib also play a role as basolateral polarity proteins [7,8]. In *Drosophila* and other invertebrates, SJs appear as a ladder-like group of septa basal to the cadherin-based adherens junctions. SJs have functions analogous to vertebrate tight junctions as they provide a transepithelial diffusion barrier [24]. Yrt is not required for normal septa formation [8] or localization of SJ components such as Cora (Fig. 1K–N), but is essential for the barrier function of SJs [8]. Zygotic *yrt* mutants show only minor defects in paracellular barrier function, whereas barrier function is fully compromised in *yrt*M/Z embryos [8]. This observation supports the notion that transepithelial barrier function and the regulation of tube dimension are independent functions of Yrt as *yrt* and *yrt*M/Z mutant embryos show similar tube size defects (Fig. 1D,E,S1A,C). This conclusion is consistent with previous findings suggesting that the regulation of tracheal tube elongation and of the paracellular diffusion barrier are distinct roles of SJ proteins [18,25].

A second class of mutants showing abnormally long tracheal tubes are defective in the genes *verm* and *serp*, which encode enzymes that are predicted to modify the chitin-based luminal ECM, and mutants of which show structural defects in the luminal ECM [5,6]. The chitin matrix filling the tracheal lumen is transiently present during lumen morphogenesis and is critical for determining lumen diameter and length [4]. Interestingly, all the mutations affecting SJ components tested so far are associated with a failure to secrete Verm into the tracheal lumen [5,23]. This suggests that SJ proteins control tube size by regulating apical secretion and remodeling of the apical chitin matrix. Although Yrt is required for the barrier function of SJs [8], we found that luminal secretion of Verm (Fig. 1H,I) and Serp (Fig. S2A,B) was normal in *yrt* and *yrt*M/Z mutants. In contrast, low but detectable levels of Verm were observed in *cora* mutants (Fig. 1G). This finding suggests that Yrt regulates tracheal tube length through a pathway that is independent of Verm and Serp.

Antagonistic interactions between Yrt and Crb regulate tracheal tube length independent of Verm secretion

During late stages of epithelia maturation, Yrt is known to restrict apical membrane growth in epidermal and photoreceptor cells by limiting Crb activity. This interplay between Yrt and Crb governs apical membrane size in stage 14 and later embryos when tracheal tube size is defined [13]. This raises the possibility that Crb-dependent apical membrane growth is responsible for dorsal trunk expansion in *yrt* mutant embryos. To test this hypothesis, we reduced *crb* dosage in *yrt* mutant embryos by introducing one copy of a *crb* null allele into a *yrt* mutant background. Loss of one copy of *crb* suppressed the dorsal trunk elongation defects seen in *yrt* mutants, as the dorsal trunks appeared similar to wild type in *yrt crb/yrt* + mutants (Fig. 2B,S1A, S3F). In addition, moderate Crb overexpression increased dorsal trunk length and diameter without interfering with the integrity of the tracheal epithelium or the secretion of Verm or Serp, (Fig. 2A,C and Fig. S1A, S2C, S3C). These results show that Crb is required for promoting the expansion of tracheal tubes at late stages of embryogenesis. It was previously suggested that Crb also acts during early tracheal branch outgrowth [26], in addition to its role in apical-basal polarity. Therefore, Crb plays a critical role at several steps of tracheal development. Together, our findings indicate that the antagonistic interactions between Yrt and Crb determine tracheal tube size.

Verm levels in *yrt crb*/*yrt* + and in *yrt/yrt* mutants were indistinguishable (not shown), indicating that the minor reduction in Verm levels sometime seen in *yrt* mutants were not the cause of the tracheal elongation defects. Accordingly, reduction of *crb* dosage in a *verm* or *serp* mutant background did not suppress tube size defects (Fig. 2D,E, S1A, S3G and not shown). Similarly, loss of one copy of *crb* in *sinu* mutant embryos, which fail to secrete Verm [5], had no impact on the length of dorsal trunks that remained enlarged as in *sinu* single mutants (Fig. 2F,G, S1A, S3G). These findings suggest that the apical secretion of matrix-modifying enzymes such as Verm and the control of Crb activity by Yrt are two independent and non-redundant modes of tracheal tube size regulation. Our data also establish that epithelial tube size control by SJ-associated proteins involves Verm-dependent and Verm-independent mechanisms.

The SJ-associated polarity proteins Yrt and Scrib define distinct Verm-independent mechanisms of tube size-control

To further characterize the function of SJ-associated polarity proteins in the regulation of tracheal tube size, we investigated tube elongation, the integrity of SJs and the secretion of Verm in *scrib*, *lethal giant larvae* (*lgl)* and *discs large (dlg*) mutant embryos [7,21,27–29]. Zygotic loss of *scrib*, *lgl* or *dlg* resulted in excessively long dorsal trunks, indicating that these genes are critical for tube size control (Fig. S4) [21]. Zygotic loss of *lgl* expression caused fully penetrant defects in SJ paracellular barrier function (trachea in 10/10 lgl^4 embryos are dye permeable) (Fig. S4E), whereas zygotic *scrib* or *dlg* mutants did not have compromised transepithelial barriers (0/14 trachea in $scrib^2$ embryos and 0/10 trachea in dlg^{m52} embryos were dye permeable; Fig. S4H,K). Luminal Verm deposition was not detected in *lgl* mutants but appeared near normal in *dlg* and normal in *scrib* mutants (Fig. S4D,G,J). Thus, Scrib and Dlg act like Yrt by controlling tracheal tube size through mechanisms distinct from Verm secretion.

We concentrated our further analysis on Scrib and asked whether this protein, like Yrt, controls tracheal tube size by negatively regulating Crb activity. Scrib together with Lgl and Dlg show antagonistic interactions with Crb to regulate apical-basal epithelial polarity in early *Drosophila* embryos [29,30]. However, the tracheal tube defects were not ameliorated in *scrib crb/scrib* + embryos compared to *scrib* single mutants (Fig. 3C,D). Thus, in contrast to Yrt, Scrib does not seem to limit tube length by restricting Crb activity. As Yrt and Scrib appear to control tracheal tube size through different mechanisms, we tested whether *yrt scrib* double homozygous mutant embryos had a more severe phenotype. The double mutants had Verm levels that were lower compared to *yrt*M/Z and *scrib* mutants (Fig. 1I,3A,B). Moreover, the tracheal defects also appeared more severe in *yrt scrib* mutants than in *yrt* null mutant embryos, particularly in the smaller diameter branches. The defects in small diameter branches of the *yrt scrib* double mutants are not likely caused by the reduction in Verm secretion as the complete loss of Verm has only a mild effect on smaller branches (Fig. 2D) [5,6]. The enhanced severity of the *yrt scrib* double mutant tracheal defects compared to the defects seen in *yrt* null embryos and the differences in the genetic interactions of *scrib* and *yrt* with *crb* suggest that Scrib and Yrt act in separate pathways to regulate the size of tracheal tubes, and that Scrib does not act by modulating Crb activity. It is possible that Scrib acts through other proteins, such as proteins of the Par complex [31], that promote apical domain formation. Therefore, SJ-associated polarity proteins use at least two Verm-independent mechanisms to restrict the dimension of tracheal tubes.

Cora limits Crb activity to restrict tracheal tube length

Cora is a SJ associated protein [9] required for optimal secretion of Verm (Fig. 1G,4I). This suggests that Cora may control tracheal tube length through a Verm-luminal matrix pathway. However, Cora is also a basolateral polarity protein restricting the activity of Crb [8], which

promotes Verm-independent expansion of the dorsal trunk (Fig. 2A,C). This led us to investigate the functional relationship between Cora and Crb in tracheal morphogenesis. In the epidermis, a striking redundancy between *yrt* and *cora* was observed in the regulation of apical-basal polarity [8]. Similarly, we found that tracheal cells in *yrt cora* double mutants show severe apicalization defects characterized by a broad expansion of the surface distribution of Crb (Fig. 4A–D). The antigen recognized by the monoclonal antibody 2A12 was found surrounding tracheal cells and not confined to the luminal cavity (Fig. 4E). In addition, the 2A12 antigen was not only associated with tracheal cells, but also with epidermal cells (Fig. 4E and S5A). These tracheal defects seen in *cora yrt* mutant embryos mimic defects that result from high levels of Crb overexpression (Fig. 4F,S5B). This observation argues that the tracheal defects observed in *cora yrt* double mutant embryos result from strong Crb over-activation, which is associated with a loss of basolateral polarity and an expansion of apical membrane character. As epidermal cells did not acquire expression of the tracheal cell marker Tango (Fig. S5C) [32], it is unlikely that epidermal cells adopt a tracheal cell fate in *cora yrt* mutants. The association of 2A12 with epidermal cells is therefore presumably due to the apicalization of tracheal cells (Fig. 4D), which would consequently secrete the 2A12 antigen not only on the luminal side but all around their cell surface allowing the 2A12 antigen to diffuse and bind to surrounding cells. Accordingly, cuticle deposition, taking place at the apical membrane, was seen at both luminal and abluminal sides of tracheal cells overexpressing Crb (Fig. S5D).

Our data indicate that Yrt and Cora cooperate to control apical-basal polarity of tracheal cells by limiting Crb to the apical cell pole, but they do not reveal whether Cora and Crb interact to control the length of tracheal tubes. To address this question, we examined *cora +/cora crb* embryos for a suppression of the tracheal size defects seen in *cora* single mutants. Reduction of *crb* dosage suppresses tube over-elongation defects resulting from the loss of Cora (Fig. 4G,H, S1A, S3G). This restriction of dorsal trunk elongation does not result from the restoration of Verm secretion as the level of Verm present in the dorsal trunk lumen was as low in *cora +/cora crb* embryos as in single *cora* mutants (Fig. 4I,J). Together, these data suggest that Crb overactivation is the primary cause of epithelial tube length defects observed in the absence of Cora. Thus, Cora and Yrt act independently from each other to counteract Crb activity and maintain the appropriate size of epithelial tubes. As the reduction of *crb* dosage does not rescue the *verm* mutant phenotype (Fig. 2D,E), we conclude that the residual amount of Verm found in *cora* mutants (Fig. 1G, 4I,J) is sufficient to maintain Verm pathway activity.

Conclusions

Our analysis suggests that basolateral proteins that are enriched at SJs have several critical functions in determining the size of epithelial tubes in the *Drosophila* tracheal system. We show that the increase in tube size is not caused by an increase in cell number, and, therefore, must be accompanied by an increase in the apical surface area of individual tracheal cells. Given that Crb is a well-known regulator of apical membrane size [9,10], our findings suggest that the interplay between Yrt, Cora and Crb modulates the dimensions of the apical surface of tracheal cells to control tracheal tube size. Moreover, this mechanism acts independently and in parallel to a previously proposed pathway depending on the apical secretion of the matrix modifying enzymes Verm and Serp, which requires several SJ-associated proteins [5,23]. Yet another mechanism is revealed by our results that *scrib* mutants also have long trachea with normal Verm levels, but that in contrast to *cora* and *yrt*, tracheal defects in *scrib* mutants are not suppressed by loss of one copy of *crb*. Together, our findings suggest that basolateral proteins utilize at least three distinct mechanisms to regulate tube size in the *Drosophila* tracheal system (Fig. 4K). Unexpectedly, these mechanisms involve functional interactions between polarity proteins that appear to be different than those in establishing apical-basal polarity at earlier stages of development. For example, in promoting apical-basal polarity, Yrt and Cora act redundantly so that *cora* mutants show polarity defects only in a *yrt* mutant

background and polarity defects in *yrt* mutants are strongly enhanced by removal of Cora [8]. In contrast, both *cora* and *yrt* single mutants show similar strong tracheal size defects. Furthermore, Scrib and Crb display antagonistic functional interactions during establishment of apical-basal polarity [29,30], but not during tracheal elongation. An important challenge for future investigations will be to uncover the adaptations in the molecular pathways that allow polarity proteins to contribute to different aspects of epithelial development.

Experimental procedures

Drosophila genetics

The alleles used in this study were: yrt^{75a} and yrt^{65a} [13], $cora^{1}$ [34], crb^{11A22} [10], $scrib^{1}$ [7], $scrib^{2}$ [35], $verm^{KG07819}$ [6], $serp^{e02821}$ [6], $sinu^{nwu7}$ [21], lgl^{4} [36] and dlg^{m52} [27]. Recombinant chromosomes generated: *serp* crb^{11A22}, *verm* crb^{11A22}. Overexpression of Crb was accomplished by crossing *UAS-*crb^{wt2e} (strong overexpression) or *UAS-*crb^{wt1d} (moderate overexpression) to the ubiquitous driver *da-GAL4* [11] or the tracheal specific driver *btl-GAL4* [37].

Immunofluorescence

Drosophila embryos were fixed as previously described [10]. Primary antibodies used: guinea pig anti-Yrt GP7 (1/500 dilution) [13], mouse anti-2A12 (1/5) and anti-Tango (1/50) (Developmental Studies Hybridoma Bank, DSHB), rabbit anti-Verm (1/500) [6], rabbit anti-Serp (1/500) [6], anti-Nrx-IV (1/500) [17] and rat anti-Crb (1/500) [12]. Secondary antibodies were conjugated to Cy3, Cy5 (Jackson Immunoresearch Laboratories) or Alexafluor 488 (Molecular Probes).

Measurements and statistical analysis

Dorsal trunks were analyzed in stage 16 embryos. Dorsal trunk length and diameter were determined using the Olympus Fluoview software. Dorsal trunk length was measured from the anterior in tracheal segment 1 to the junction of dorsal trunk and transverse connective in tracheal segment 10. Dorsal trunk diameter measurements were performed on maximal projections of Z-stacks. For each embryo, dorsal trunk diameter was determined by the average value of three independent measures taken in the central region of tracheal segment 7. Dorsal trunk length and diameter are expressed as average values +/− standard deviation. The Student two-sided unpaired t-test with equal variance was used to assess the statistical significance.

Electron microscopy

Electron microscopy was done following a modified version of the protocol described by Tepass and Hartenstein [38]. Embryos were dechorionated in a 50% bleach solution and pre-fixed in a 25% glutaraldehyde solution (diluted in 100 mM cacodylate buffer, pH 7.2) under a heptane phase. After removal of the vitelline membrane with a sharp needle, the embryos were incubated for 1 hour on ice in 2% glutaraldehyde solution (diluted in 100 mM cacodylate buffer pH 7.2). Following three washes in cacodylate buffer, embryos were fixed for 1 hour in a 1% OsO_4, 0.8% w/v potassium ferricyanide solution (prepared in 100 mM cacodylate buffer pH 7.2) on ice (protected from light). The embryos were then washed three times in cacodylate buffer and twice with water and incubated overnight at 4°C in 2% uranylacetate. The embryos were finally dehydrated in an ethanol series (50%, 70%, 80%, 90% and 100%) and embedded in Spurr's resin.

Septate junction permeability assay

Transepithelial permeability assays were carried out as described [18]. Stage 17 embryos were injected with 10 kDa Texan-Red dextran and dye diffusion across epithelia of the main trachea trunk was monitored by microscopy 10 minutes after injection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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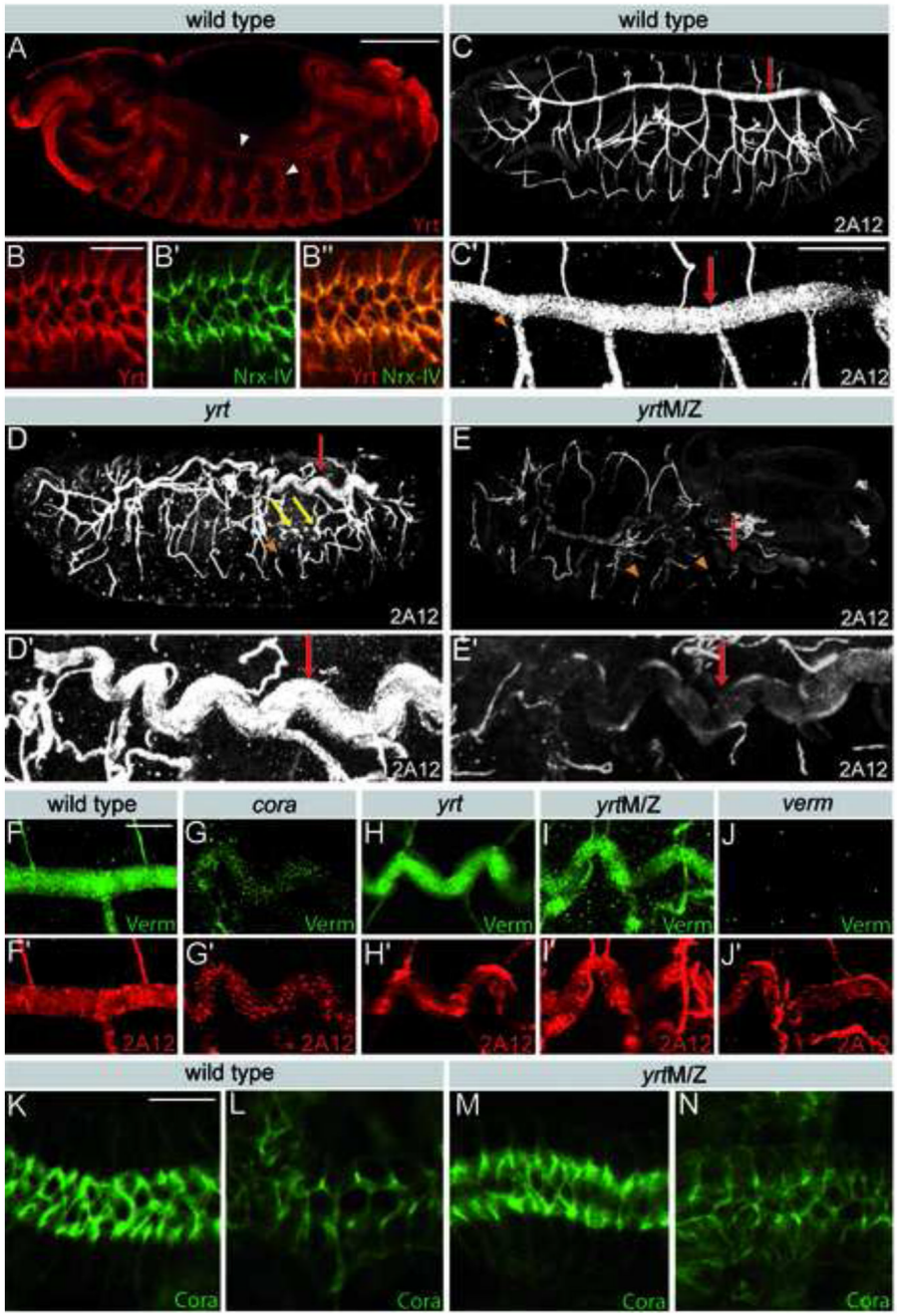

Figure 1. Yrt is required for epithelial tube size regulation, but not luminal secretion

A) Stage 14 embryo stained for Yrt (red), which is expressed in the tracheal system (arrowheads). B) Yrt (red) is enriched in the upper region of the lateral membrane of tracheal cells and co-localizes with the SJ marker Nrx-IV (green). C–E) Stage 16 wild-type (C), *yrt* (D) or *yrt*M/Z (E) embryos stained with the tracheal luminal antigen 2A12 show the tracheal tree (upper panels) or a close up of a dorsal trunk of the same embryo (lower panels). Loss of Yrt causes an elongation of dorsal trunks (red arrows), results in diameter irregularities in small tracheal branches (yellow arrows) and is associated with discontinuous 2A12 staining (arrowheads). F–J) Portion of a dorsal trunk co-stained for Verm (green) and 2A12 (red) in stage 16 wild type embryo (F) or *cora* (G), *yrt* (H), *yrt*M/Z (I) and *verm* (J) mutant embryos.

Note that loss of *yrt* does not interfere with Verm or 2A12 secretion. K,L) Staining of Cora in the salivary gland (K) and dorsal trunk (L) of a stage 16 wild-type embryo. M,N) Staining of Cora in the salivary gland (M) and dorsal trunk (N) of a stage 16 *yrt*M/Z mutant embryo. Cora distribution is normal in the absence of Yrt. Scale bars: A,C,D,E, 100 μm; C′-E′, 30 μm; B, F–N, 10 μm.

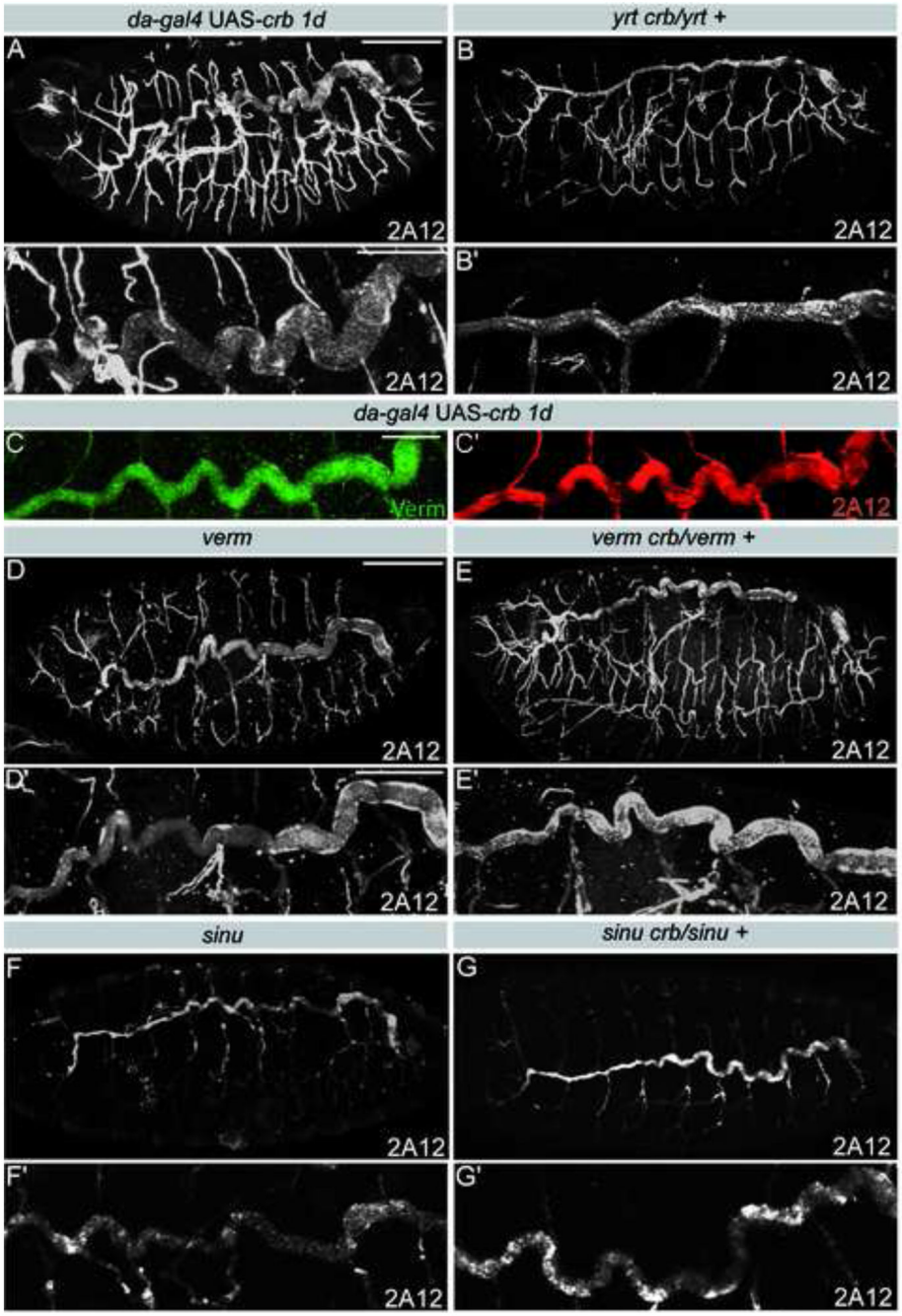

Figure 2. Epithelial tube size defects in *yrt* mutants results from Crb overactivation

A,B, D–G) Stage 16 embryos stained for the 2A12 luminal antigen showing the tracheal tree (upper panels) or a close up of a dorsal trunk of the same embryo (lower panels). Moderate overexpression of Crb lengthens and widens dorsal trunks (A). Reducing *crb* dosage rescues tube size defects in *yrt* mutant embryos (B), but not in *verm* (D,E) or *sinu* (F,G) mutant embryos. C) Staining showing Verm (green) and 2A12 (red) in a dorsal trunk of an embryo overexpressing Crb. Overexpression of Crb does not interfere with Verm secretion. Scale bars: A,B,D–G, 100 μm; C, 30 μm; A′,B′,D′-G′, 30 μm.

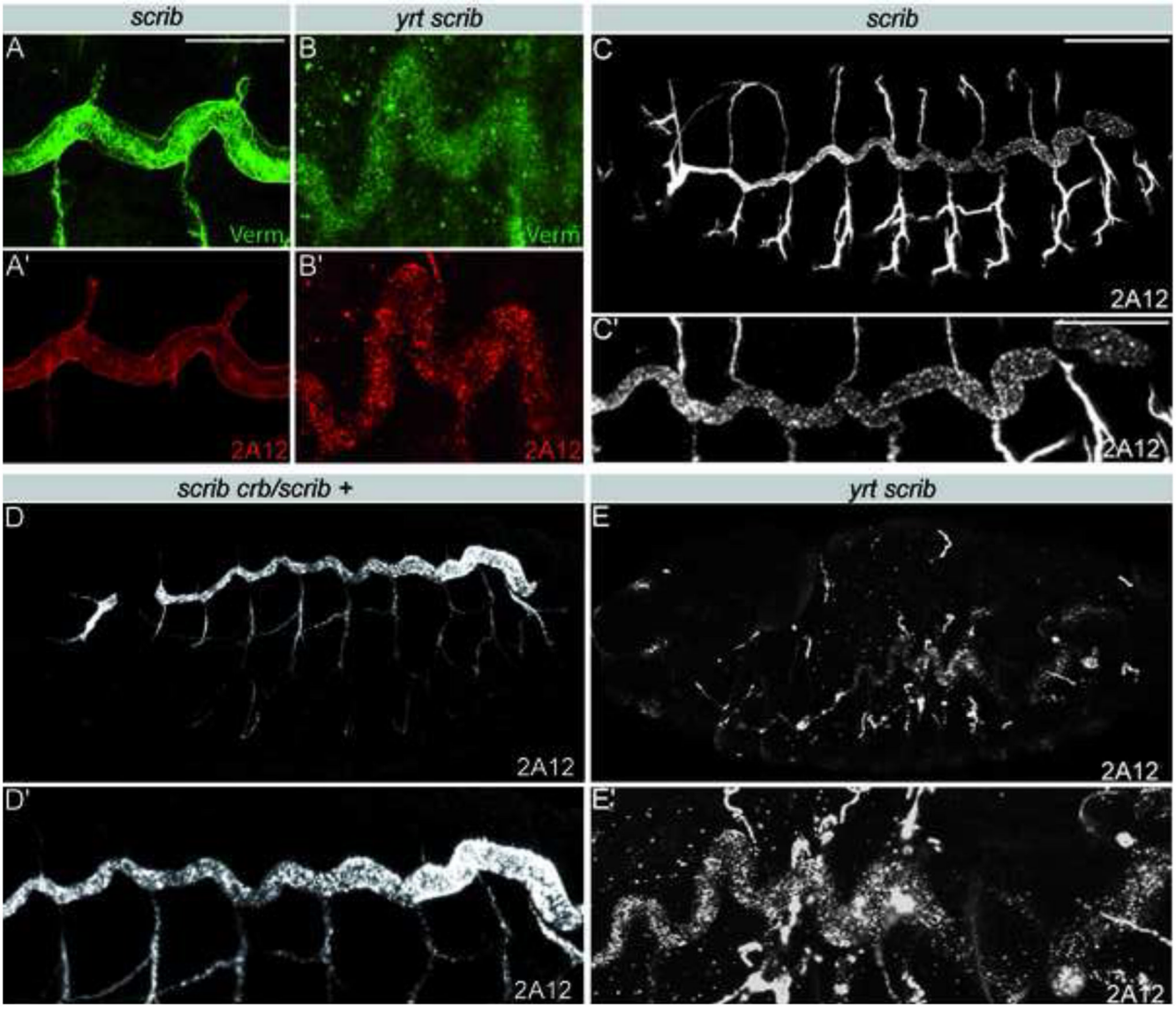

Figure 3. Yrt and Scrib control epithelial tube size through distinct Verm-independent pathways

A,B) Portions of a dorsal trunk co-stained for Verm (green) and 2A12 (red) in *scrib* (A) and *yrt scrib* (B) mutant embryos. Loss of Scrib does not interfere with Verm accumulation in the tracheal lumen, whereas simultaneous loss of zygotic Yrt and Scrib reduces Verm secretion. C–E) 2A12 staining showing the tracheal system (upper panels) or a close up of a dorsal trunk (lower panels) in a *scrib* (C), *scrib crb/scrib* + (D) or *yrt scrib* (E) mutant embryos. Reducing the dosage of *crb* does not ameliorate the *scrib* mutant tracheal defects. *yrt scrib* double mutants show more severe tracheal defects than single mutants (B and E compare to A and C and Figure 1D and H). Scale bars: A,B 20 μm; C–E, 100 μm; C′–E′, 30 μm.

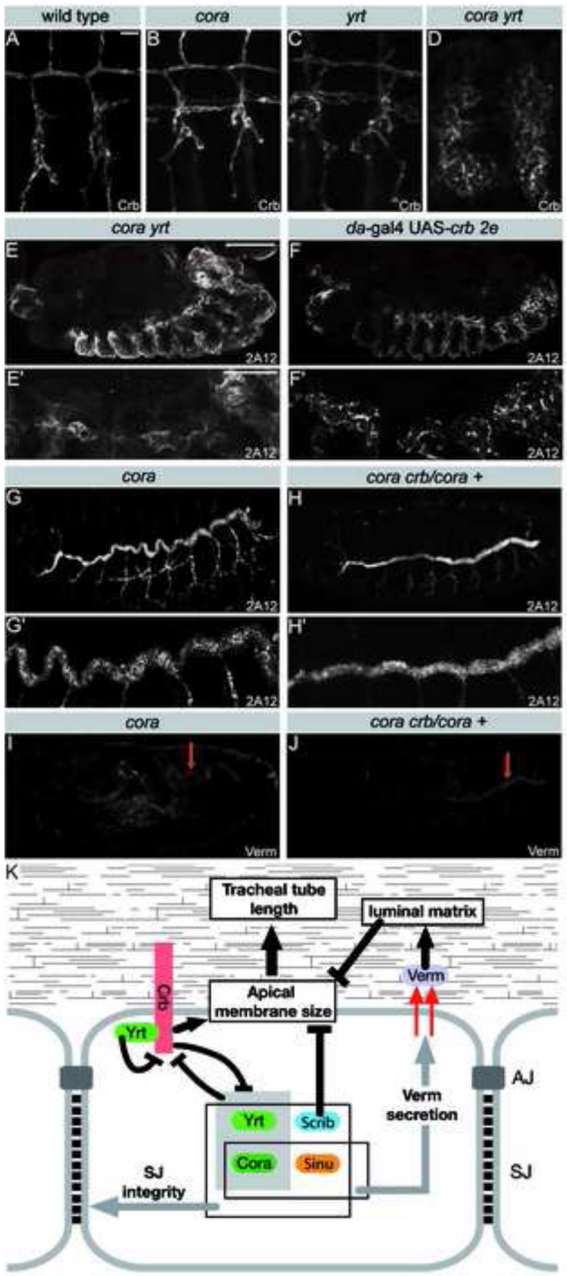

Figure 4. Cora and Yrt inhibit Crb to limit tracheal tube size

A–D) Crb staining in tracheal cells of a stage 16 wild-type (A), *cora* (B), *yrt* (C) or *cora yrt* (D) embryo. E–H) Stage 16 embryos stained with the 2A12 luminal antigen showing the tracheal tree (upper panels) or a close up of a dorsal trunk (lower panels) of a *cora yrt* mutant embryo (E), an embryo overexpressing high levels of Crb (F), a *cora* mutant embryo (G) or a *cora +/cora crb* embryo (H). Simultaneous loss of Cora and Yrt or strong overexpression of Crb cause similar dramatic apicalization defects in the trachea. I,J) Verm staining in a *cora* mutant embryo (I) and a *cora crb/cora +* mutant embryo. K) Model of the pathways involved in the control of tracheal tube length by septate junction proteins. Scale bars: A–D, 10 μm; E–J, 100 μm; E′–J′, 30 μm.