Microtubules regulate intracellular trafficking to mediate apical constriction during tissue invagination

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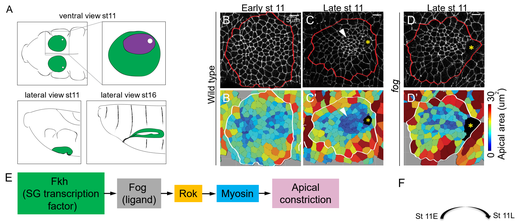
Abstract

The Drosophila embryonic salivary gland (SG) invaginates by budding to form a three-dimensional tube. Coordinated apical constriction during SG invagination is critical for proper tube shape. We previously showed that Folded gastrulation (Fog)-dependent Rho-associated kinase (Rok) accumulation in the apicomedial region of the SG cells is required for apicomedial myosin formation and clustered apical constriction near the invagination pit. Here, we show that microtubule (MT)-dependent intracellular trafficking has a role in regulating apical constriction during SG invagination. Key components involved in protein trafficking, including dynein heavy chain, Rab11 and Nuclear fallout (Nuf), are apically enriched near the invagination pit in a MT-dependent manner during SG invagination. This enrichment is crucial for apical constriction as disruption of the MT networks or intracellular trafficking impairs formation of apicomedial myosin, which leads to apical constriction defects. We show that apical transport of several proteins along MTs, either in a Rab11-dependent or independent manner, mediates clustered apical constriction during SG invagination. Key proteins that are transported include the Fog ligand, the apical determinant protein Crb, the key adherens junction protein E-Cad and the scaffolding protein Bazooka/Par3, and knockdown of these genes in the SG results in apical constriction defects. These results define a role of MT-dependent intracellular trafficking in regulating the actomyosin networks ands cell junctions to coordinate cell behaviors during tubular organ formation.

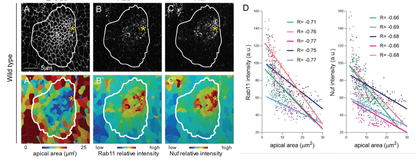
Introduction



(A) A cartoon of the anterior region of stage 11 and stage 16 embryos. SG placodes are labeled in green. The dorsal/posterior region where SG cells undergo clustered apical constriction during invagination is shown in purple. (B-C) SG cells show coordinated apical constriction at stage 11 (white arrowheads). (B'-C') Heat maps of apical area from SGs in (B-C). Dark blue, cells with small apical areas. (D-D') *fog* mutant SG shows uncoordinated apical constriction. (E) The Fog pathway regulates apical constriction via regulating Rok/Myosin activity. (F) Microtubules rearrange themselves during apical constriction at stage 11. Asterisks, invagination pit.

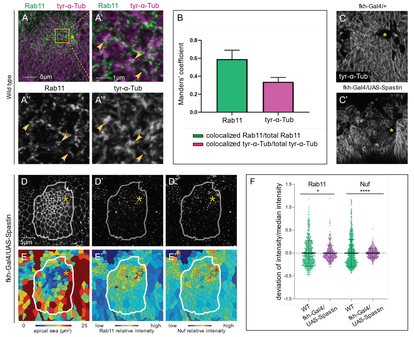
Results

1. Intracellular trafficking components are apically enriched near the invagination pit



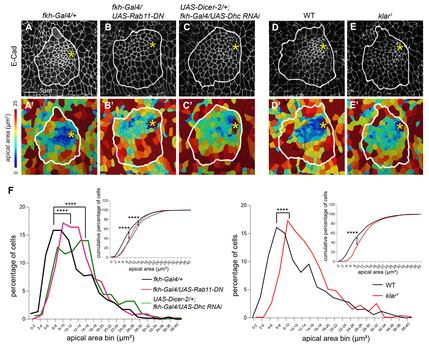
(A-C) A SG immunostained with E-Cad, Rab11 and Nuf. Rab11 and Nuf show upregulation in the apical region near the invagination pit. (A'-C') Heat maps of apical area and intensity of Rab11 and Nuf of images in A-C. Near the invagination pit, intensity of Rab11 and Nuf is high (red cells in B'-C'). (D) Negative correlation between Rab11/Nuf intensities and apical areas of SG cells. n= 5 SGs (690 cells).

2. Disruption of MTs results in reduction of apical vesicle numbers



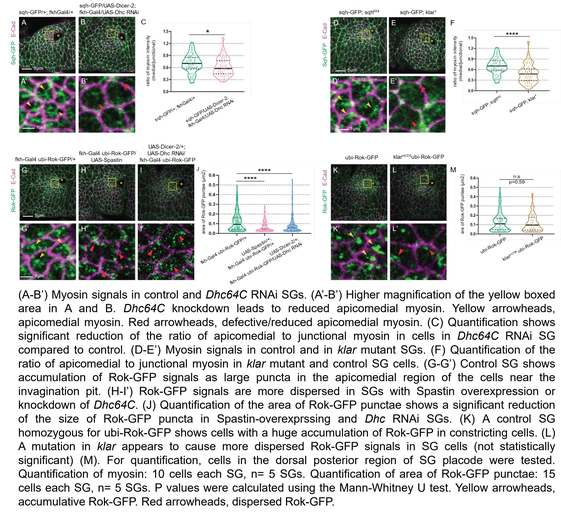
(A-A''') Co-localization of Rab11 and tyrosinated α-Tubulin (tyr-α-Tub) in SG cells during invagination. Yellow arrowheads, co-localized Rab11 and tyr-α-Tub. (B) Thresholded Manders' colocalization coefficients show the degree of colocalization between Rab11 and tyr-α-Tub. Examined area= 100 μm² (dorsal posterior region of SG placode). n= 6 SGs. (C-C') Spastin overexpression in the SG disrupts MT networks. (D-D'') Confocal images of a SG overexpressing Spastin. Rab11 and Nuf show are significantly reduced. (E-E''') Color-coded images corresponding to (D-D''). (G) Quantification of the ratio of deviation of Rab11/Nuf intensity to median intensity of all cells of the SG placode show significant reduction when Spastin is expressed. P values were calculated using the Mann-Whitney U test. n= 5 SGs (690 cells).

3. Endotrafficking is required for apical constriction in the SG



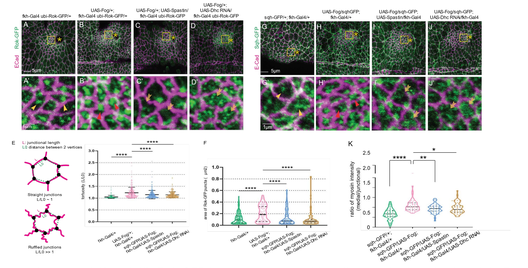
(A-E') SGs with disrupted intracellular trafficking show fewer cells with small apical area (dark blue cells) than control. (A-C) Confocal images of the control (A), Rab11-DN-overexpressing (B) and *Dhc64C* RNAi (C) SGs that are stained with E-Cad. (A'-C') Heat maps corresponding to images in A-C. (D-E') Confocal images and corresponding heat maps of wild type and *klar* mutant SGs. (F) Percentage and cumulative percentage of cells with different apical area in the SG placodes of the genotypes shown in A-E. P values were calculated using the Mann-Whitney U test (for percentage of cells) and the Kolmogorov-Smirnov test (for cumulative percentage of cells). n= 5 SGs (~550 cells). Asterisks: invagination pit. White lines: SG placode region.

4. Compromised intracellular trafficking leads to failure in accumulation of apicomedial Rok and reduction of apicomedial myosin formation in SG cells



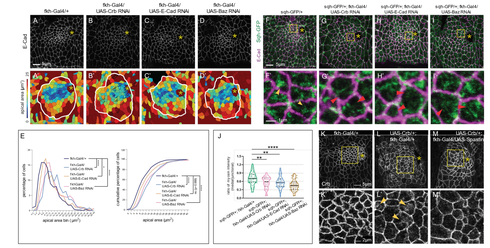
(A-B') Myosin signals in control and *Dhc64C* RNAi SGs. (A'-B') Higher magnification of the yellow boxed area in A and B. *Dhc64C* knockdown leads to reduced apicomedial myosin. Yellow arrowheads, apicomedial myosin. Red arrowheads, defective/reduced apicomedial myosin. (C) Quantification shows significant reduction of the ratio of apicomedial to junctional myosin in cells in *Dhc64C* RNAi SG compared to control. (D-E') Myosin signals in control and in *klar* mutant SGs. (F) Quantification of the ratio of apicomedial to junctional myosin in *klar* mutant and control SG cells. (G-G') Control SG shows accumulation of Rok-GFP signals as large puncta in the apicomedial region of the cells near the invagination pit. (H-I') Rok-GFP signals are more dispersed in SGs with Spastin overexpression or knockdown of *Dhc64C*. (J) Quantification of the area of Rok-GFP punctae shows a significant reduction of the size of Rok-GFP puncta in Spastin-overexprssing and *Dhc* RNAi SGs. (K) A control SG homozygous for ubi-Rok-GFP shows cells with a huge accumulation of Rok-GFP in constricting cells. (L) A mutation in *klar* appears to cause more dispersed Rok-GFP signals in SG cells (not statistically significant) (M). For quantification, cells in the dorsal posterior region of SG placode were tested. Quantification of myosin: 10 cells each SG, n= 5 SGs. Quantification of area of Rok-GFP punctae: 15 cells each SG, n= 5 SGs. P values were calculated using the Mann-Whitney U test. Yellow arrowheads, accumulative Rok-GFP. Red arrowheads, dispersed Rok-GFP.

5. Fog gain-of-function phenotypes are suppressed when intracellular trafficking is compromised



Compared to control (A and A'), Rok-GFP is over-accumulated in cells near the invagination pit in Fog-overexpressing SGs (B and B'). E-Cad signals show wavy cell junctions in Fog-overexpressing SGs. The Fog gain-of-function phenotypes are suppressed when Spastin is overexpressed or *Dhc* is knocked down using RNAi. (A'-D', G'-J') Higher magnification of the yellow boxed area shown in A-D and G-J. For quantification, cells in the dorsal posterior region of SG placode were tested. Quantification of tortuosity: 10 cells each SG, n= 5 SGs (~280 junctions). Quantification of area of Rok-GFP puntae: 15 cells each SG, n= 5 SGs. Quantification of myosin: 10 cells each SG, n= 5 SGs. P values were calculated using the Mann-Whitney U test. Asterisks: invagination pit.

6. Crb, E-Cad and Baz have a role in regulating apical myosin and apical constriction during SG invagination



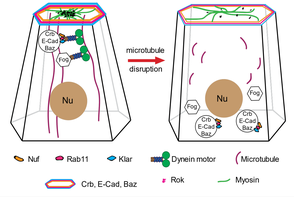
(A-D'') Knockdown of *crb*, *E-Cad* or *bazooka/par3* (*baz/par3*) results in apical constriction defects. (E) Quantification of percentage of cells and cumulative percentage of cells with different areas from SGs of different genotypes shown in A-D. P values were calculated using the Mann-Whitney U test (for percentage of cells) and the Kolmogorov-Smirnov test (for cumulative percentage of cells). n= 5 SGs (~550 cells). (F-I') Knockdown of *crb*, *E-Cad* or *baz* leads to reduction of apicomedial myosin in SG cells near the invagination pit. (F'-I') Higher magnification of yellow boxed areas shown in F-G. Yellow arrowheads, high level of apicomedial myosin. Red arrowheads, defective/reduced apicomedial myosin. (J) Quantification of the ratio of apicomedial to junctional myosin of SG cells from different genotypes shown in A-D. 10 cells in the dorsal posterior region were tested. n= 5 SGs. P values were calculated using the Mann-Whitney U test. (K) Crb is apically upregulated in SG during invagination. (L) Overexpression of Crb results in accumulation of Crb on the cell surface (yellow arrowheads). (M') Co-overexpression of Spastin and Crb suppresses accumulation of Crb, suggesting a role of MTs in apical trafficking of Crb during SG invagination. Asterisks: invagination pit.

7. MTs play a role in apical trafficking of key apical and junctional proteins during SG formation.

(A-B''') Overexpression of Spastin results in mislocalization of Rab11 and Nuf at stage 16 SGs. (A-A''') Rab11 and Nuf localize in the apical region of SG cells (red arrowheads). (B-B''') Rab11 and Nuf are mislocalized to the basal and cytoplasmic region of the cell (yellow arrowheads). (C-C''') Overexpression of Crb results in upregulation of Crb, with Crb highly enriched in the apical domain (red arrowheads). (D-D''') Co-overexpression of Spastin and Crb results in mislocalization of Crb to the basal and cytoplasmic region, which overlaps with mislocalized Rab11 (yellow arrowheads). (E-E''') Overexpression of E-Cad leads to an increase of E-Cad level, with the majority of E-Cad localizes in the apical region (red arrowheads), and some punctate structures of E-Cad observed in the basolateral region (blue arrows). (F-F''') Co-overexpression of E-Cad and Spastin causes mislocalized E-Cad, which is overlapped with Nuf to the basal and cytoplasmic region of the cell (yellow arrowheads). (G-G''') Overexpression of Baz shows an enlarged lumen phenotype. Red arrowheads show apical localization of Baz and Nuf. (H-H''') The enlarged lumen phenotype caused by Baz overexpression is suppressed when Spastin is overexpressed. Baz and Nuf partially overlaps in the basal region of SG cells (blue arrows). Yellow arrowheads, mislocalized Nuf in the basal region of cells.

Summary

1. Key components of the intracellular trafficking pathway, including Rab11 and Nuf, are upregulated in the dorsal posterior region of the SG placode during invagination.
2. Compromised microtubule-dependent trafficking results in the failure of apical Rok accumulation and apicomedial myosin formation, which leads to apical constriction defects.
3. Disruption of MTs suppresses the Fog gain-of-function effect on Rok accumulation and ruffled junctional morphology, suggesting a role of MTs in regulating Fog signaling during SG invagination.
4. MT-dependent trafficking regulates apical constriction during SG invagination via regulating apical trafficking of the apical transmembrane protein Crb and junctional proteins E-Cad and Baz.



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