Smog as a putative receptor for Fog to regulate apical constriction during *Drosophila* SG invagination

vvishw2@lsu.edu
@vishakha_v96

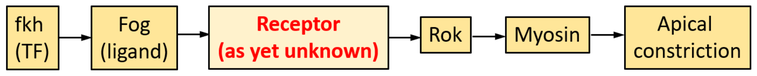
Vishakha Vishwakarma and SeYeon Chung
Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803

LSU
LOUISIANA STATE UNIVERSITY

Summary

A major cell shape change during epithelial tube formation is apical constriction, driven by pulsed contraction of the actomyosin cytoskeleton. During invagination of the *Drosophila* embryonic salivary gland (SG), apical constriction is clustered in the dorsal/posterior region of the SG placode. Coordinated apical constriction is critical for the proper shape of the SG. We previously showed that the Folded gastrulation (Fog) signaling pathway is upregulated in the dorsal/posterior region of SG during invagination. However, the receptor that senses and transduces the Fog signal in the SG has not yet been identified. Here, we show the Smog G protein-coupled receptor (GPCR) as a potential candidate to act as a receptor for Fog in the *Drosophila* SG. Identifying the receptor(s) for Fog will help us to improve our understanding of how the Fog signal is recognized and translated to change cytoskeletal organization.

Introduction



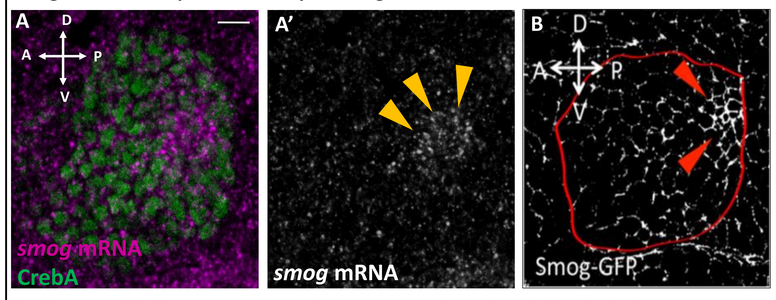
Fog signaling pathway regulates apical constriction during *Drosophila* SG invagination.[1] Fog is regulated by the Fork head (Fkh) transcription factor. Rho-associated kinase (Rok), in response to Fog, accumulates in the apicomedial region of SG cells and further results in apicomedial myosin formation and apical constriction near the invagination pit. There are two known receptors for Fog during *Drosophila* gastrulation- Mist and Smog.[2-4] The receptor acting downstream of Fog in SG has not been identified yet.

My objective is to identify the receptor which transduces Fog signaling in SG.

The candidate that I am testing is **Smog GPCR.**

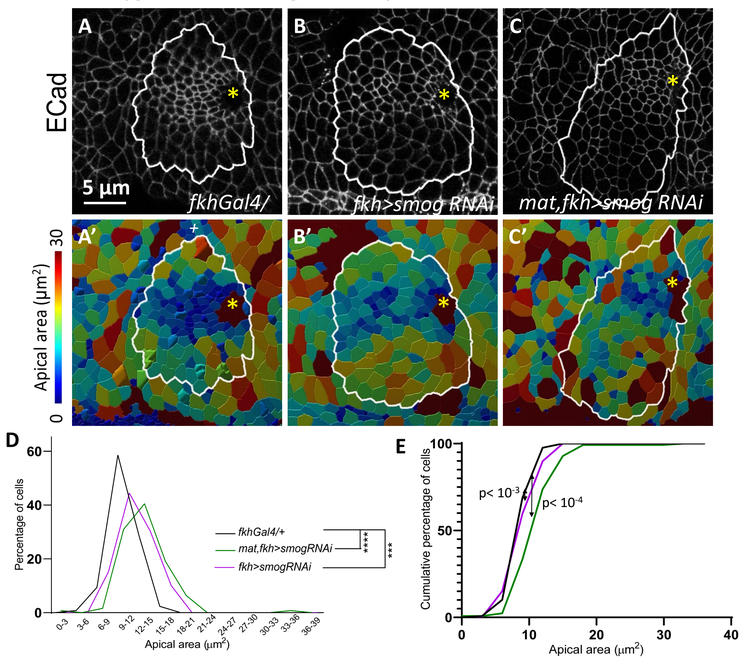
Results

1. Smog is enriched in the dorsal posterior region of the SG placode where SG invagination takes place at embryonic stage 11



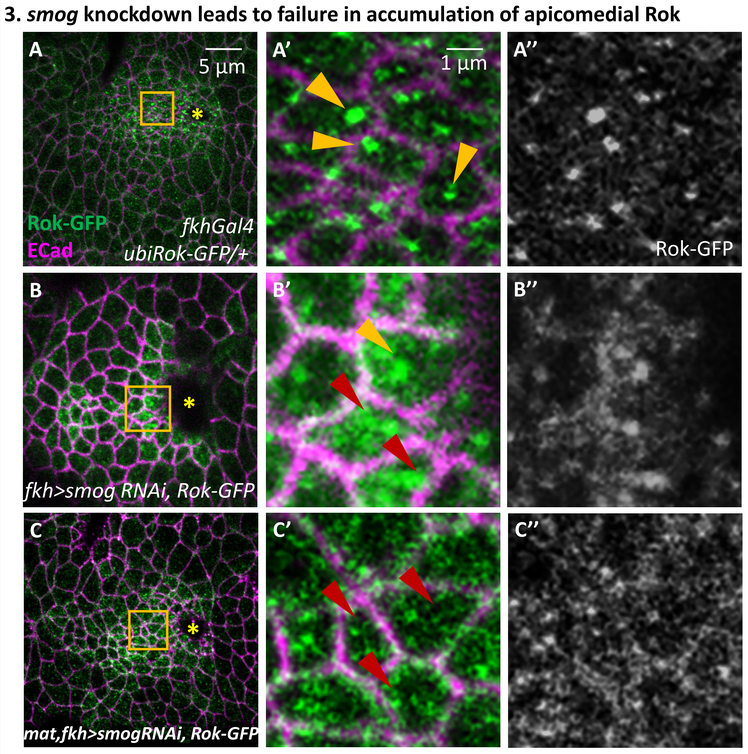
(A,A') *smog* mRNA is highly expressed in dorsal-posterior region of SG cells (yellow arrowheads). **(B)** Upregulated Smog-GFP in SG cells where apical constriction occurs (red arrowheads) Red lines, SG boundary.

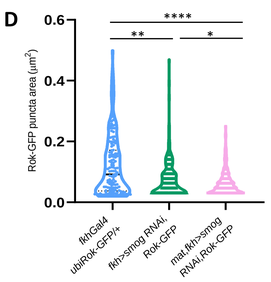
2. Maternal zygotic loss of *smog* causes apical constriction defects in the SG



(A,B,C) Confocal images of the indicated genotypes. Confocal images are used for segmentation using the Imaris program to mark the cells based on apical area. **(A',B',C')** Segmented images of A,B, and C, respectively. **(D)** percentage and **(E)** cumulative percentage of SG cells in indicated apical area bins. White lines, SG boundary. Mann-Whitney U test (D) and Kolmogorov-Smirnov test (E) were performed. ****p<0.0001, ***p<0.001 (A) n= 6 SGs, 659 cells. (B) n = 6 SGs, 688 cells. (C) n= 6 SGs, 672 cells.

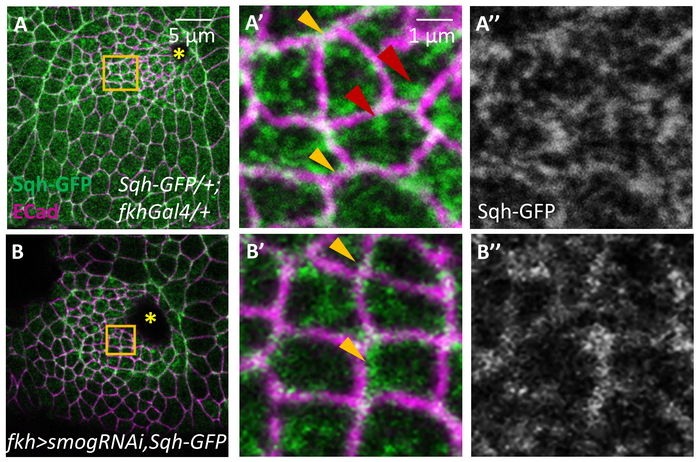
3. *smog* knockdown leads to failure in accumulation of apicomedial Rok

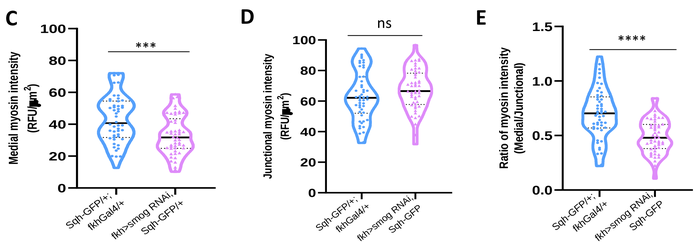




(A) Control SG shows accumulation of Rok-GFP signals in the apicomedial region of the cells near the invagination pit. **(B,C)** Rok-GFP signals are more dispersed in SGs with zygotic knockdown **(B)** and maternal, zygotic knockdown of *smog* **(C)**. **(A'-C')** Higher magnification of yellow boxed area shown in (A-C). **(A''-C'')** Rok-GFP signals only. **(D)** Quantification of area of Rok-GFP puncta in SG cells. Yellow arrowheads, accumulative Rok-GFP structures; Red arrowheads, dispersed Rok-GFP. Mann-Whitney U test was performed. ****p<0.0001, **p<0.01, *p<0.05. (A) n= 5 SGs, 75 cells (B) n = 5 SGs, 75 cells (C) n= 5 SGs, 75 cells

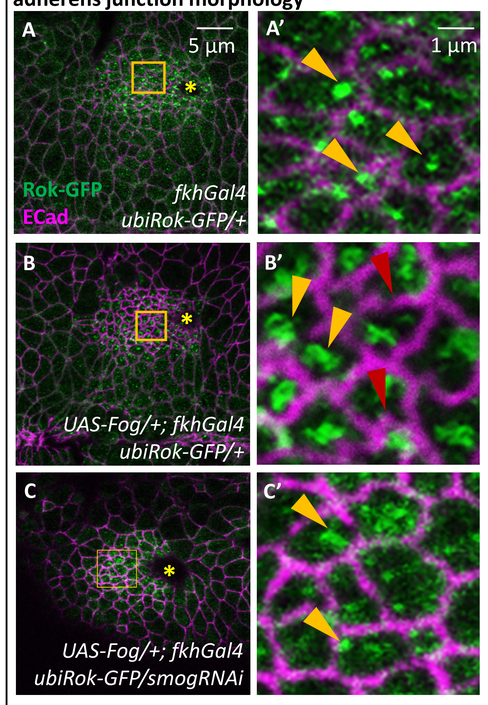
4. Smog is required for proper myosin distribution during SG invagination

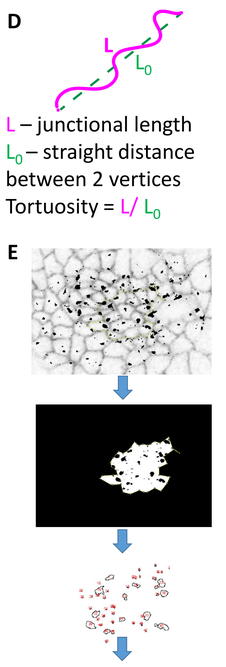


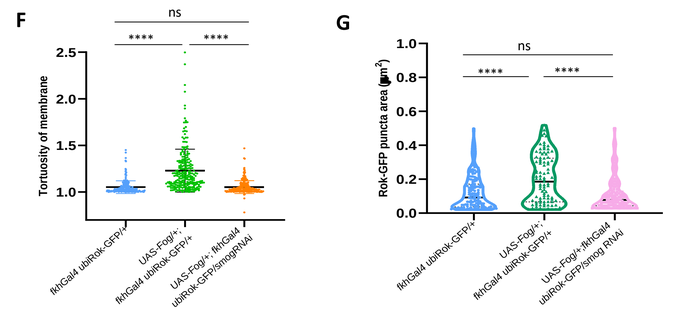


(A) Control SG shows accumulation of Sqh-GFP signals in the apicomedial region and junctions of the SG cells. **(B)** Sqh-GFP signals are more dispersed in SGs with *smog* knockdown. **(A', B')** Higher magnification of yellow boxed area shown in (A,B). **(A'', B'')** Sqh-GFP signals only. Quantification of **(C)** medial myosin **(D)** junctional myosin and **(E)** ratio of medial vs junctional myosin. Yellow arrowheads, junctional myosin.Red arrowheads, apicomedial myosin. Mann-Whitney U test was performed. ****p<0.0001, ***p<0.001, ns=not significant. (A) n= 5 SGs, 50 cells. (B) n = 5 SGs, 50 cells

5. *smog* knockdown suppresses the Fog gain-of-function effect on Rok and adherens junction morphology







(A) Control SG shows accumulation of Rok-GFP in the apicomedial region of SG cells. **(B)** Overexpression of Fog shows over accumulation of Rok-GFP in apicomedial region and wiggliness of the junctions in SG placode cells. **(C)** *smog* knockdown can rescue the Fog overexpression phenotype. **(A'-C')** Higher magnification of yellow boxed area shown in (A-C). **(D)** Cartoon showing how tortuosity of membrane was measured using ImageJ. **(E)** Diagram representing the method of quantification of Rok-GFP particles. 10-15 cells near invagination pit were selected and area of Rok-GFP in those cells were measured and quantified using ImageJ. **(F)** Quantification of wiggliness of junctions (tortuosity) and **(G)** area of Rok-GFP puncta in the indicated genotypes. Yellow arrowheads, Rok-GFP particles. Red arrowheads, wiggliness of junctions. Mann-Whitney U test was performed. ****p<0.0001, ns=not significant. (A) n= 5 SGs, 75 cells (B) n = 5 SGs, 75 cells (C) n= 5 SGs, 75 cells

Future Directions

- Test other candidate genes that might act as a receptor for Fog during *Drosophila* SG invagination. Those genes will be chosen based on FlyBase data (http://flybase.org)
- In vitro assays to test for a role of all the candidate genes in Fog-induced cell contraction.

References

1. Chung et al., (2017) Uncoupling apical constriction from tissue invagination. *eLife*.
2. Manning et al., (2013) Regulation of Epithelial Morphogenesis by the G-Protein Coupled Receptor Mist and its Ligand Fog. *Sci Signal*.
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Acknowledgements

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