The influence of shear forces on *Drosophila* ventral furrow formation

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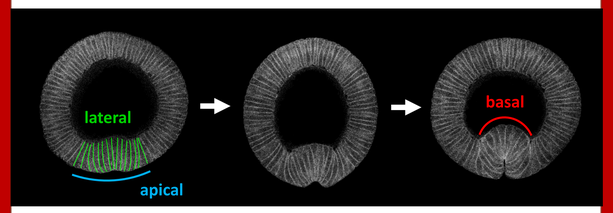
ABSTRACT

In *Drosophila* embryos, gastrulation begins with ventral furrow (VF) formation: cells along the ventral midline constrict apically, lengthen laterally, and finally shorten back to their original length as the monolayered tissue invaginates to form the ventral furrow. Solving the mechanism of these cell shape changes requires knowledge of the forces driving tissue dynamics. Current understanding pinpoints apically-localized forces as the key player in VF formation; however, simple theoretical considerations strongly suggest that non-apical forces also make a significant contribution to folding. We constructed a computational model of VF formation based on *in vivo* measurements of tissue material properties done in our laboratory. Our model shows that furrow formation succeeds in the absence of basal membranes. However, if the level of cytoplasmic viscosity is decreased, lowering the amount of shear force exerted on membranes, the furrow fails to form. We hypothesize that viscous shear forces contribute to tissue invagination by pulling the ventral embryo surface inward. We tested this *in vivo* by observing VF formation in anillin RNAi embryos, which completely lack basal membranes throughout the course of VF formation. Anillin RNAi embryos are fully capable of VF formation despite their lack of basal membranes. Neurotactin staining additionally reveals that ventral cell lateral membranes disintegrate during late stages of VF formation in anillin RNAi embryos. We are further analyzing this phenotype using TEM and live imaging under 2-photon microscopy. Our data suggest viscous shear forces compose a major contribution to tissue folding.

INTRODUCTION

In the initial stage of gastrulation, embryonic tissue forms a fold known as the ventral furrow. Ventral furrow (VF) formation can be further subdivided into two stages:

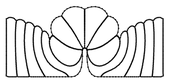
1) Cells constrict apically and elongate laterally
2) Cells shrink laterally and seal basally as tissue invaginates



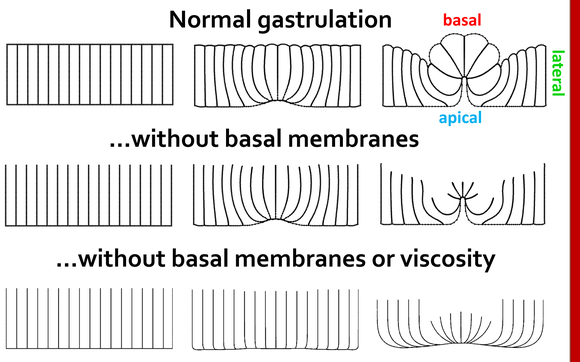
Each part of the cell goes through its own series of shape changes during ventral furrow formation. We want to know if forces present in nonapical membranes or cytosol flow contribute to tissue folding. We will use a modelling approach combined with *in vivo* RNA interference (RNAi) experiments to target these cellular compartments, and observe the effect on furrow phenotype.

MODELS

These simulations represent cross sections of 16 ventral epithelial cells undergoing VF formation. Cells are elastic along all membranes and filled with a viscous fluid.



MODELS (cont.)

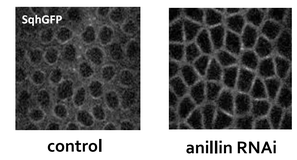


Contractile forces are generated along apical and lateral membranes to form the ventral furrow. If cytoplasmic viscosity is sufficiently high, furrow formation succeeds even in the absence of basal membranes.

METHODS

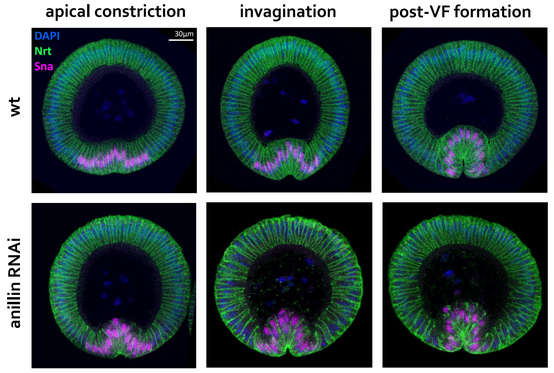
Slowing basal closure: cellularization

Cellularization immediately precedes VF formation and ends with the sealing of basal membranes as the VF folds inwards. Embryos with anillin loss-of-function mutations exhibit slowed actin ring closure during basal membrane formation (Field *et al.* 2005). Driving RNAi against anillin produces a similar phenotype: actomyosin rings seal more slowly.

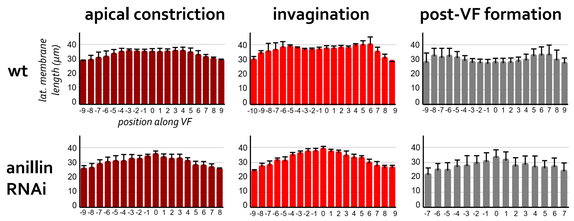


METHODS (cont.)

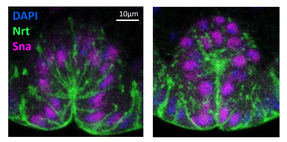
Slowing basal closure: gastrulation



In anillin RNAi embryos, basal membranes remain unformed until well after ventral furrow formation.



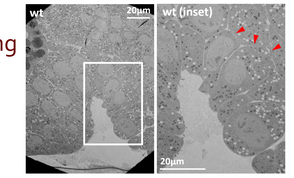
We recorded the distribution of lateral membrane lengths across the VF at different stages of folding (central membrane = 0). Peripheral lateral membranes are shorter in anillin RNAi embryos.



Immunostaining shows a lateral membrane vesiculation phenotype in anillin RNAi embryos that intensifies over time.

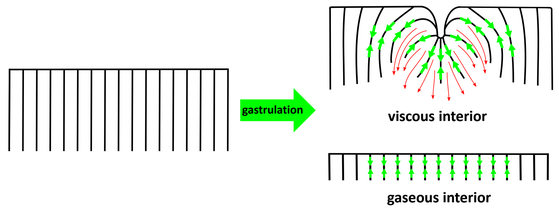
METHODS (cont.)

We are in the process of using high pressure freezing and freeze substitution TEM to capture high resolution images of basal membranes (red arrows) during VF formation.



We are also refining a live imaging protocol using 2-photon confocal microscopy to capture membrane dynamics during VF formation *in vivo*.

As predicted by our model, the ventral furrow succeeds in forming even in the absence of basal membranes. We hypothesize that furrow formation in these embryos relies on not only apical forces, but also lateral membrane tension coupled with viscous shear forces from cytosol that drag the ventral surface inwards by pulling on the lateral membranes.



FUTURE DIRECTIONS

We will use our collection of TEM sections to better quantify the differences in basal closure between WT and anillin RNAi embryos, and use these findings to further develop our mathematical model of VF formation.

REFERENCES

Field C, Coughlin M, Doberstein S, Marty T, Sullivan W. **Development** (2005)

ACKNOWLEDGEMENTS

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