Molded by Matrix:
A multi-layered pre-cuticular apical extracellular matrix shapes the *C. elegans'* vulva lumen

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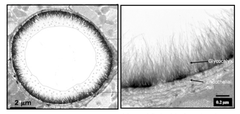
Abstract

Biological tubes must develop and maintain their proper diameter in order to transport materials efficiently. These tubes are molded and protected in part by apical extracellular matrices (aECMs) that line their lumens. Despite their importance, aECMs are difficult to image in most systems and therefore poorly understood. The *C. elegans* vulva has been a paradigm for understanding many aspects of organogenesis. Here we describe the vulva luminal matrix, which contains chondroitin proteoglycans (CPGs), Zona Pellucida (ZP) domain proteins, and other glycoproteins and lipid transporters related to those in mammals. Confocal and transmission electron microscopy revealed, with unprecedented detail, a complex and dynamic aECM. Different matrix factors assemble on the apical surfaces of each vulva cell type, with clear distinctions seen between Ras-dependent (1˚) and Notch-dependent (2˚) cell types. Genetic perturbations suggest that chondroitin and other aECM factors together generate a structured scaffold that both expands and constricts lumen shape.

Background & Questions

Tube lumens are lined by apical extracellular matrix (aECM)

Endothelial capillary



Hans Vink, U. Amsterdam

glycocalyx

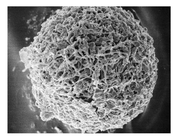
What are aECM components? lipids, glycoproteins, proteoglycans

What are aECM functions? Tissue shaping, infection barrier, etc.

How does aECM traffic to lumen and assemble/disassemble?
How does aECM shape tubes?

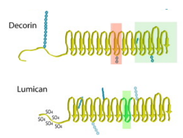
Similar types of proteins are found in/near worm vs. mammalian ECMs

Zona Pellucida (ZP) proteins (fibril-forming proteins in mammalian egg coat and many other aECMs)



Familiari et al. 2008

leucine-rich repeat proteins (e.g. SLRPS = small LRR proteoglycans – bind collagens in various ECMs)



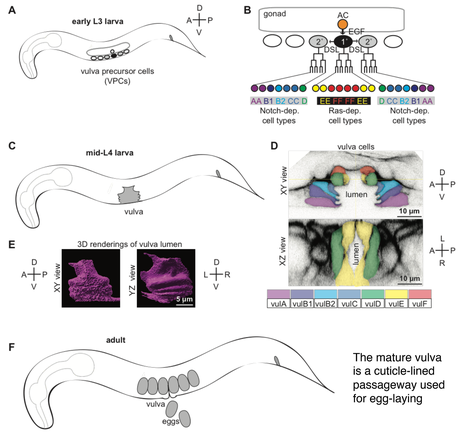
Kalamajski & Oldberg, 2010

Lipocalins (lipid transporters in circulatory systems)

Flower, 1996

What is the role of each protein in aECM organization and function?

The *C. elegans* Vulva contains 22 cells of 7 different cell types

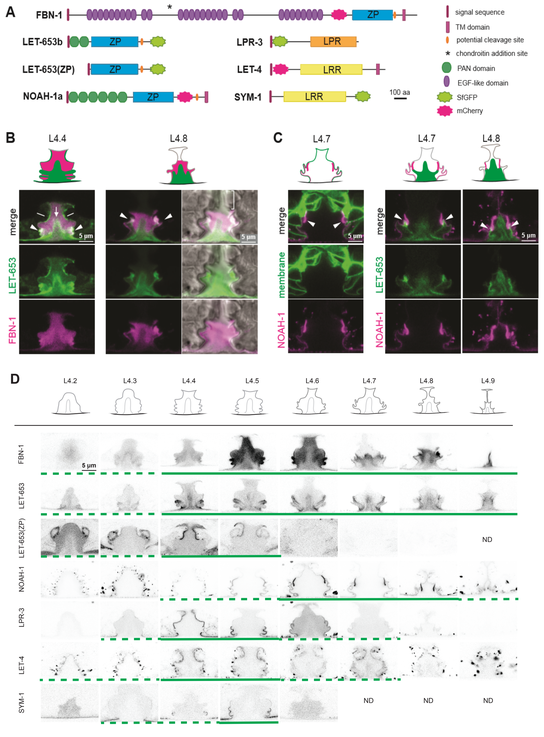


The mature vulva is a cuticle-lined passageway used for egg-laying

What are the contents and roles of the vulva luminal matrix?

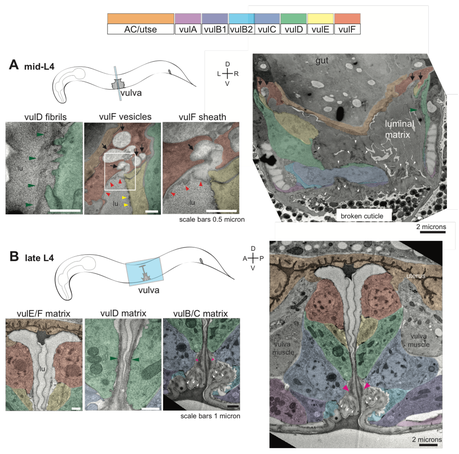
Results

1. A spatially and temporally dynamic aECM fills the vulva lumen during morphogenesis



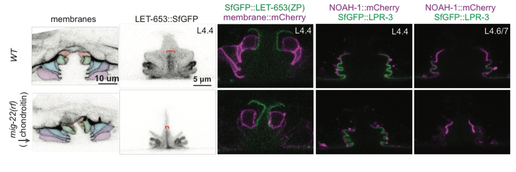
- The vulva lumen changes shape over the course of the L4 larval stage
- ZP proteins LET-653 and FBN-1 have ~complementary luminal patterns
- LET-653 marks core fibril "stalk" and ventrolateral fibrils, and some membranes
- FBN-1 fills much of the lumen outside of the stalk region
- Other aECM factors mark membrane-anchored sheath-like matrices
- Different aECM factors assemble over different cell types
- All the aECM factors are transient and disappear by adulthood

2. Transmission Electron Microscopy reveals changing aECM ultrastructure

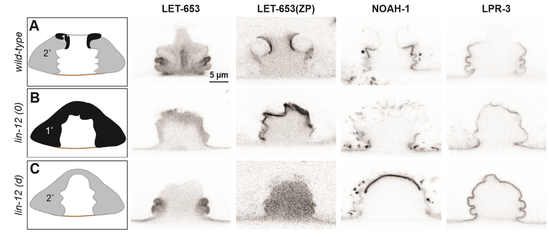


- High Pressure Freezing (HPF) preserved aECM structure
- A granular matrix fills the entire lumen at mid-L4 – this matrix likely includes CPGs.
- A stalk-like fibril structure rises through the central lumen
- Different cell types are covered in different types of aECM layers or fibrils
- vulF contains large secretory vesicles whose contents populate its aECM
- Many structures seen here correlate well with structures seen by confocal imaging

3. Reducing chondroitin has different effects on the shape of different cell types, but does not prevent aECM assembly

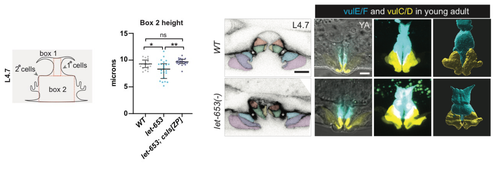


4. Different aECM factors assemble over Ras (1˚) vs Notch (2˚)-dependent cell types

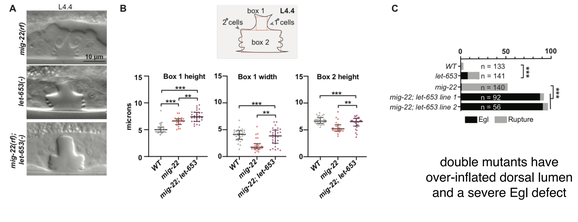


- Both 1˚ and 2˚ cells are needed to form the core fibril structure
- LET-653(ZP) marks 1˚ descendants vulE/F
- NOAH-1 marks 2˚ descendants, especially vulC/D

5. *let-653* mutants have subtle defects in vulva eversion



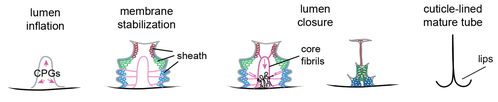
6. Chondroitin and LET-653 have both lumen-expanding and lumen-constraining roles



double mutants have over-inflated dorsal lumen and a severe Egl defect

Conclusions & Model

- Like the cytoskeleton, the aECM is a dynamic, multi-component structure. Now that we can see it, we can study it!
- The vulva aECM is a structured scaffold that likely exerts, resists and distributes multiple types of forces to shape the cells and lumen.
- We propose that after initial inflation of the lumen by chondroitin proteoglycans (CPGs), the sheath matrix and luminal fibrils assemble to stabilize apical domain shape. Changes in the matrix then help generate pulling forces that drive vulva eversion and lumen closure.



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