Matrix assembly and function of a *C. elegans* ZP protein

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Abstract

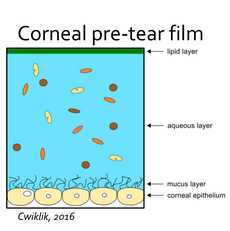
Apical extracellular matrices (aECMs) shape and protect apical surfaces, like tube lumens and the epidermis. aECMs are rich in Zona Pellucida domain (ZP) proteins, whose dysfunction is associated with human diseases. How ZP proteins assemble in the aECM is unclear.

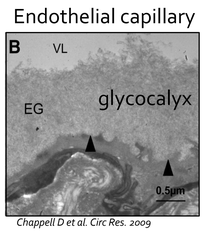
We are using *C. elegans* to study how one ZP protein, LET-653, incorporates in the aECM. LET-653 shapes a narrow, single-celled tube and a much larger, multicellular tube, the vulva. Endogenous, tagged LET-653 is visible alongside other aECM components in distinct aECM layers of the vulva lumen. Surprisingly, LET-653 localizes and functions via the ZPc subdomain of the ZP domain. LET-653(ZPc) aECM incorporation is driven by C-terminal cleavage that relieves ZPn-mediated inhibition. Finally, we show that LET-653(ZPc) incorporated into aggregates *in vitro*. Together, these data offer a novel model for ZP protein aECM assembly.

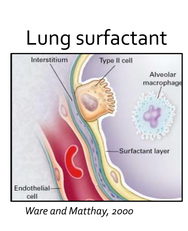
Background

apical extracellular matrices (aECMs) shape and protect apical surfaces

Lumens and external epithelia are lined by a poorly understood, often layered, aECM

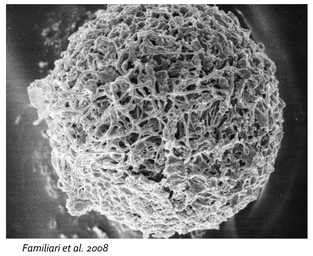






Zona Pellucida domain (ZP) proteins are crucial components of apical ECMs

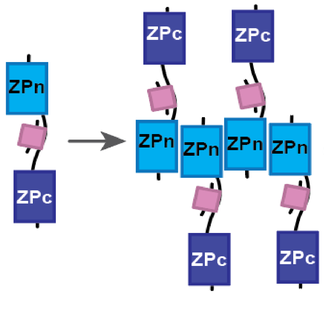
Mammalian Oocyte Zona Pellucida



Familiari et al. 2008

Uromodulin (kidney)
GP2, DNMT1 (GI tract)
Tectorins (ear)
Endoglin,
Betaglycan (vasculature)

Existing model for ZP protein assembly



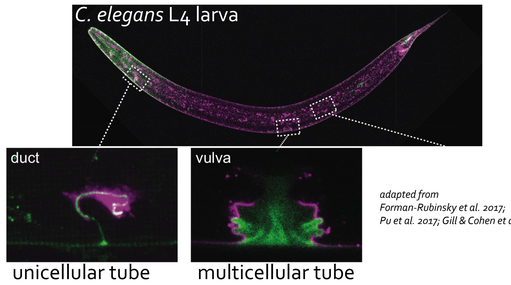
ZP subdomains (ZPn, ZPc) may mediate different functions

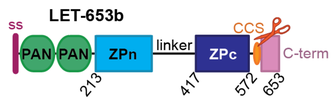
ZPn subdomains may polymerize ZPc subdomain functions are poorly defined

Results

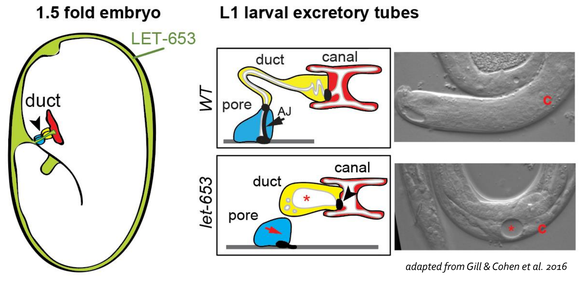
LET-653 shapes narrow and large biological tubes

The ZP protein LET-653 is present in *C. elegans* aECMs



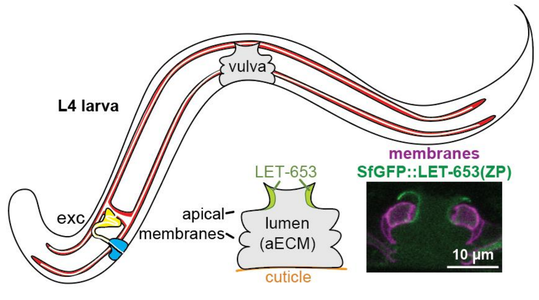


LET-653 is present in and maintains the integrity of the narrow duct tube lumen



The duct offers a functional assay for let-653 function

LET-653 shapes the large vulva tube lumen



The vulva offers a visual assay for LET-653 matrix incorporation

let-653 also shapes the vulva tube

See poster 1408A and preprint

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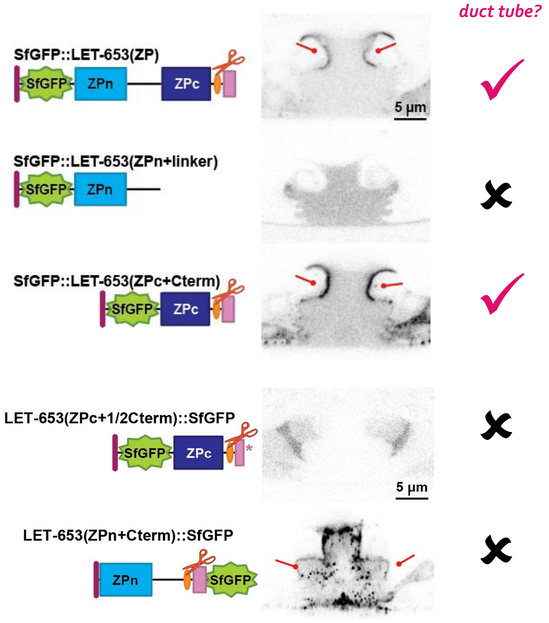
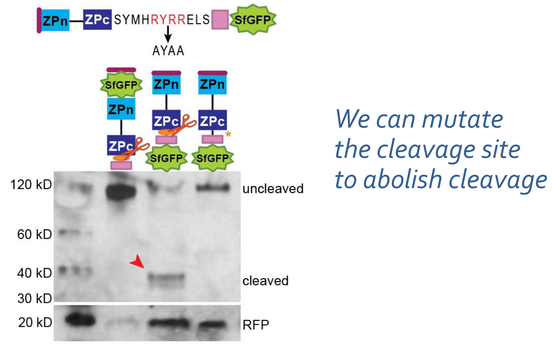
What domains are sufficient for LET-653 localization and function?

LET-653 ZPc subdomain recruits the protein to specific apical membranes



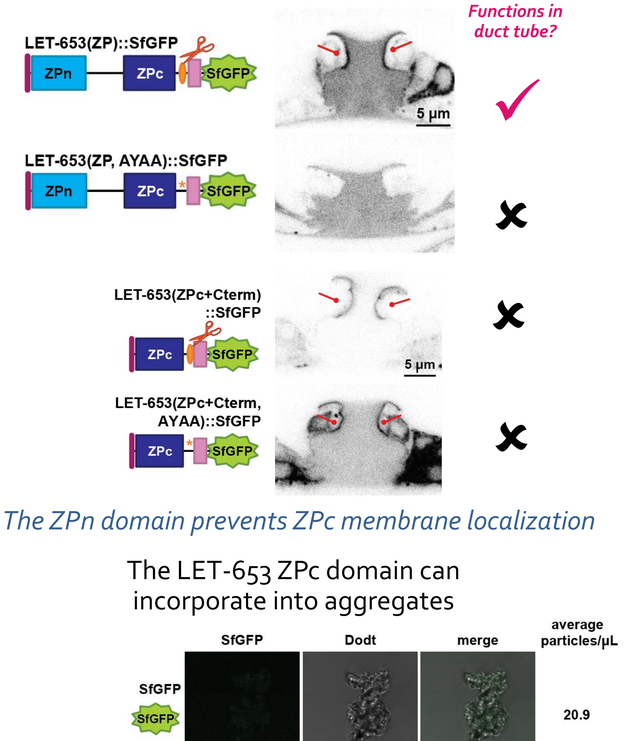
LET-653 is cleaved at its C-terminus



We can mutate the cleavage site to abolish cleavage

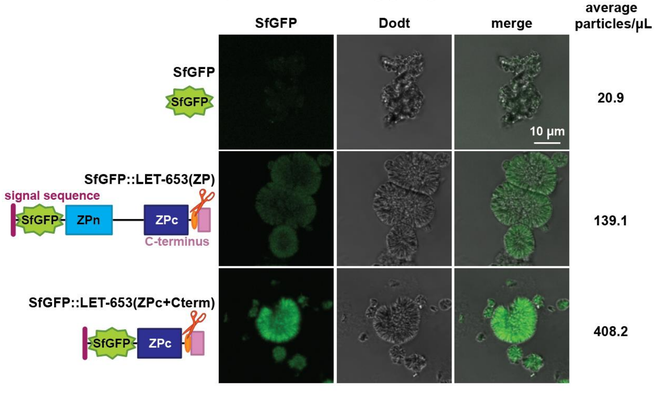
How does the ZPc domain assemble in the matrix?

Cleavage relieves ZPn inhibition of the ZPc domain

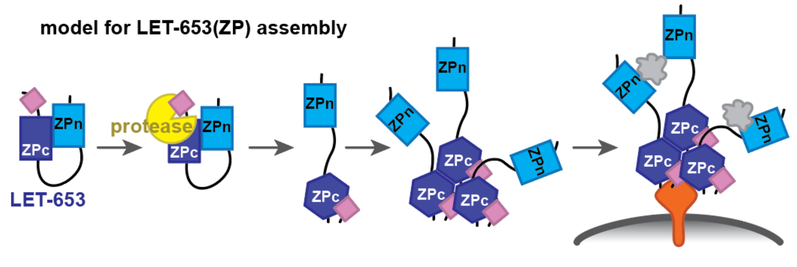


The ZPn domain prevents ZPc membrane localization

The LET-653 ZPc domain can incorporate into aggregates



Conclusions, Future Directions



ZPn inhibits ZPc

ZPc is free of ZPn inhibition; ZPc changes conformation; ZPc aggregates

ZPc binds membrane partner(s) ZPn binds partner(s) that prevent it from inhibiting ZPc aggregation?

Canonical ZP domains polymerize via their ZPn domains.
In contrast, LET-653 assembles in the matrix and functions via its ZPc domain.
Cleavage releases ZPn inhibition of the ZPc domain.
The LET-653 ZPc domain binds an apical membrane partner(s) and may also aggregate.

What is the identity of the ZPc domain membrane partner?

Do other ZP domains assemble this way?

Acknowledgements

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