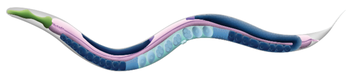
Identifying new functions of the EGF/ EGFR pathway through tissue-specific recombination

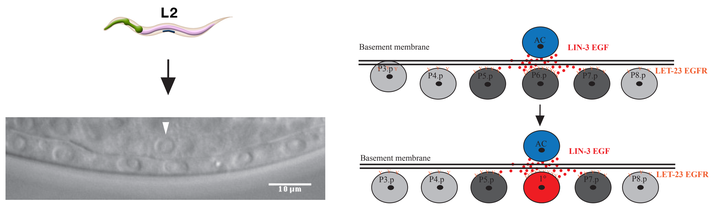


Silvan Spiri, Louisa Mereu, Matthias Morf, Alex Hajnal
Institute of Molecular Life Sciences, University of Zurich

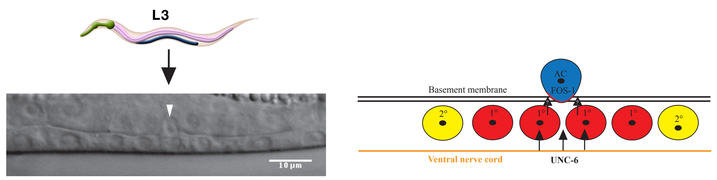
uzh eth
Zurich Ph.D Program in Molecular Life Sciences

Introduction

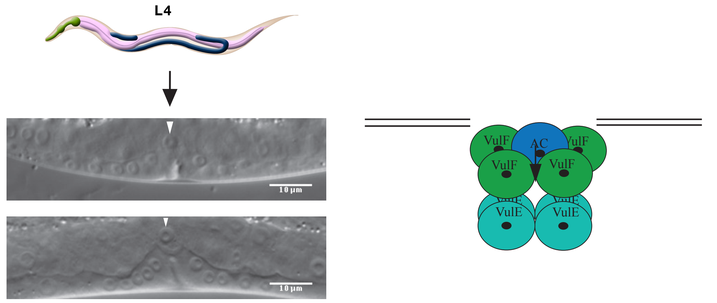
Vulval development and morphogenesis:



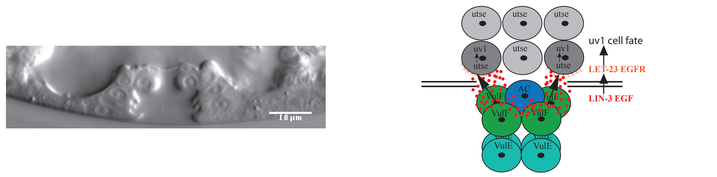
A) EGF/ LIN-3 dependent induction of the vulval precursor cells.



B) The anchor cell (AC), guided by Netrin/ UNC-6 and unkown VPC guidance cue`s, breaches the basement membrane after the VPCs divided the second time.

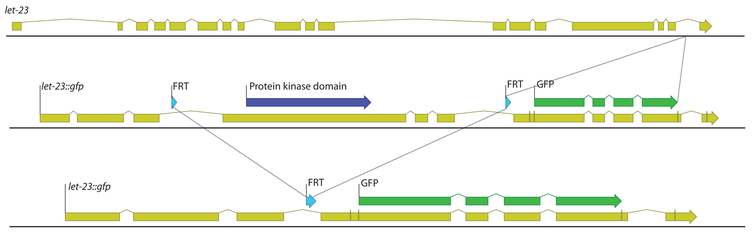


C) The AC directly promotes dorsal lumen formation during vulva morphogenesis.



D) EGF/ LIN-3 is expressed in the 1° vulval cell lineage after vulva induction and is necessary to specify the uterin *uv1* cell fate.

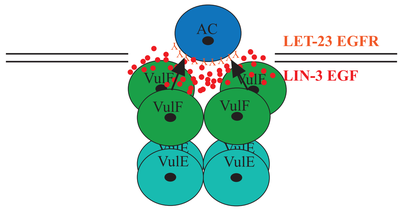
Tool to induce a temporal or spatial specific knock-out of *egfr/ let-23*:



E) Two FRT sites, flanking the sole kinase domain of *egfr/ let-23,* along with a GFP sequence are inserted into the endogenous *egfr/ let-23* locus. Upon Flp expression the kinase domain is excised resulting in inactive receptor and loss of GFP signal.

Model

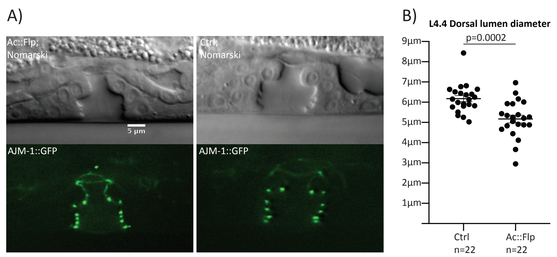
EGFR is expressed in the AC from the onset of vulval morphogenesis, securing dorsal lumen formation by stabilizing cytoskeleton components during lumen opening



Summary

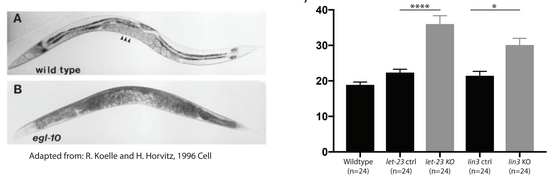
1) EGFR is expressed in the AC during vulval morphogenesis
2) AC mispositioned in AC EGFR-KO animals during L4.1-2
3) F-actin in AC is disorganized during L4.2-3 in AC EGFR-KOs
4) In a sensitized background BM breaching during morphogenesis is affected in AC EGFR-KO animals
5) Narrow dorsal lumen upon AC EGFR-KO during L4.4
6) KO of *lin-3* in the VPCs or *let-23* in the AC leads to egg laying defective adults

6) KO of *egfr/ let-23* in the AC leads to a decreased dorsal lumen diameter during L4.4



AC specific KO of *egfr/ let-23* leads to a smaller dorsal lumen diameter during L4.4: (A) Example images of AC specific KO animals during L4.4 on the left and control animals on the right. Upper row Nomarski images, bottom row AJM-1::GFP to mark the apical junctions. (B) the dorsal lumen diameter was determed by measuring the inner diameter of the vulF toroid using the vulF/ vulE apical junctions.

7) KO of *lin-3* in the VPCs or *let-23* in the AC leads to egg-laying defectie animals

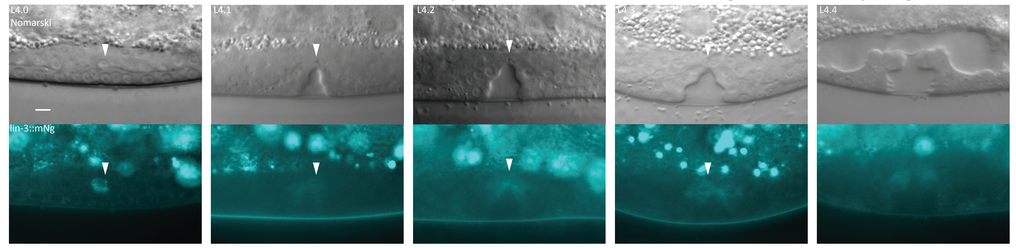


Adapted from: R. Koelle and H. Horvitz, 1996 Cell

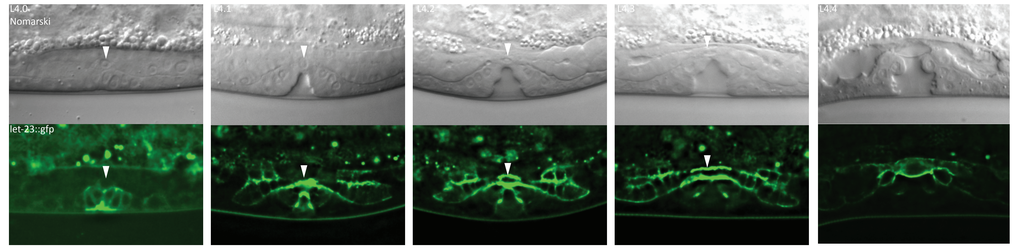
KO of *lin-3* in the VPCs or *let-23* in the AC leads to egg laying defective animals: (A) Wildtype adult. (B) Egg laying defective mutant (*egl-10)* showing increased number of eggs in utero. (C) Graph shows average number of eggs in utero in Wildtype, and KO conditions.

Results

1) After vulva induction, EGF/ LIN-3 is expressed in the VulF during vulval morphogenesis

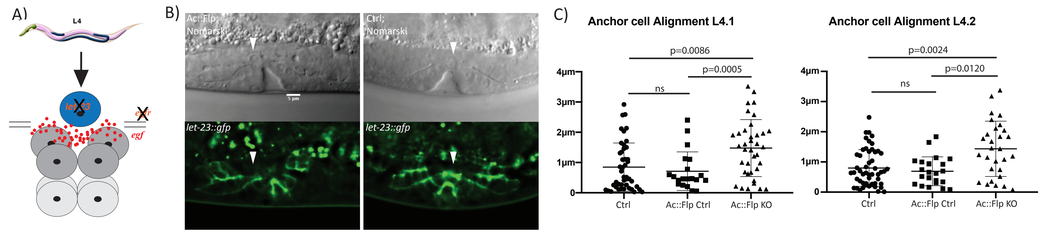


2) EGFR/ LET-23 is expressed in the AC from the start of invagination



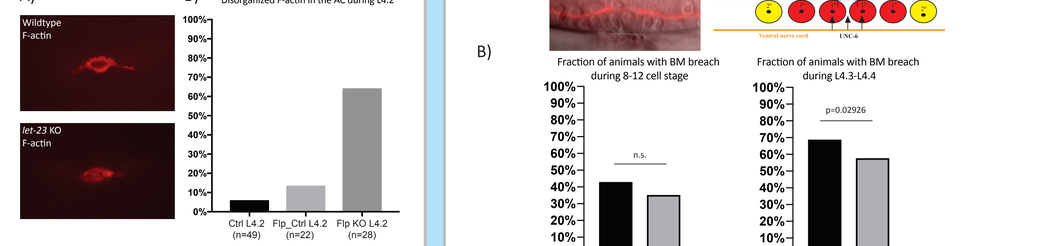
Enodgenous expression pattern of EGF/ LIN-3 and EGFR/ LET-23 was analyzed during vulval development and morphogenesis: (A) Enodgenous LIN-3::mNg reporter during different vulval morphogenesis stages (L4.0-L4.4). Upper row Nomarski images, bottom row Z-projection of LIN-3::mNg. (B) Enodgenous LET-23::GFP reporter during different vulval morphogenesis stages (L4.0-L4.4). Upper row Nomarski images, bottom row Z-projection of LET-23::GFP. Scale bar represents 5 µm, arrowhead indicates the AC.

3) KO of *egfr/ let-23* in the AC leads to more variability in AC alignment during L4.1 and L4.2



LET-23::GFP signal is lost upon AC specific KO of *egfr/ let-23* resulting in a more variable position of the AC during L4.1 and L4.2: (A) schematic of AC specific KO of *egfr/ let-23.* (B) Example images of AC specific KO animals during L4.1 on the left and control animals on the right. Upper row Nomarski images, bottom row LET-23::GFP. Scale bar represents 5 µm, arrowhead indicates the AC. (C) Quantifiaction of AC alignment relative to the vulval midline during L4.1 and L4.2.

4) KO of *egfr/ let-23* in the AC leads to disorganized F-actin network in L4.2



***egfr/ let-23* KO leads to disorganized F-atin in the AC during L4.2:** (A) F-actin reporter in the AC during L4.2, upper image shows wildtype F-actin behaviour and lower image shows disorganized F-actin in *let-23* KO condition. (B) Graph shows fraction of animals with a disorganized F-actin network during L4.2.

5) KO of *egfr/ let-23* in the AC leads to decreased basement membrane breaching during morphogenesis in a sensitized background

***egfr/ let-23* KO increases failure to breach the basement membrane during morphogenesis in a sensitized background:** (A) Left schematic of AC invasion into the vulval tissue, right overlay of Nomarski image with basement membrane (BM). Big arrowhead indicates AC, small arrowhead indicate BM disruption. (B) Percentage of animals with breached BM during 8-12 cell stage (left) and after morphogenesis (rigth). Experiment was performed in an Netrin (*unc-6)* mutant background.