CELL0023 Cell Biology of Development – LECTURE 1
Development and morphogenesis of the *C. elegans* vulva

Outline (LECTURE 1&2):

General introduction to *C. elegans*
egg-laying apparatus and vulval development

Vulval precursor patterning

Anchor cell invasion

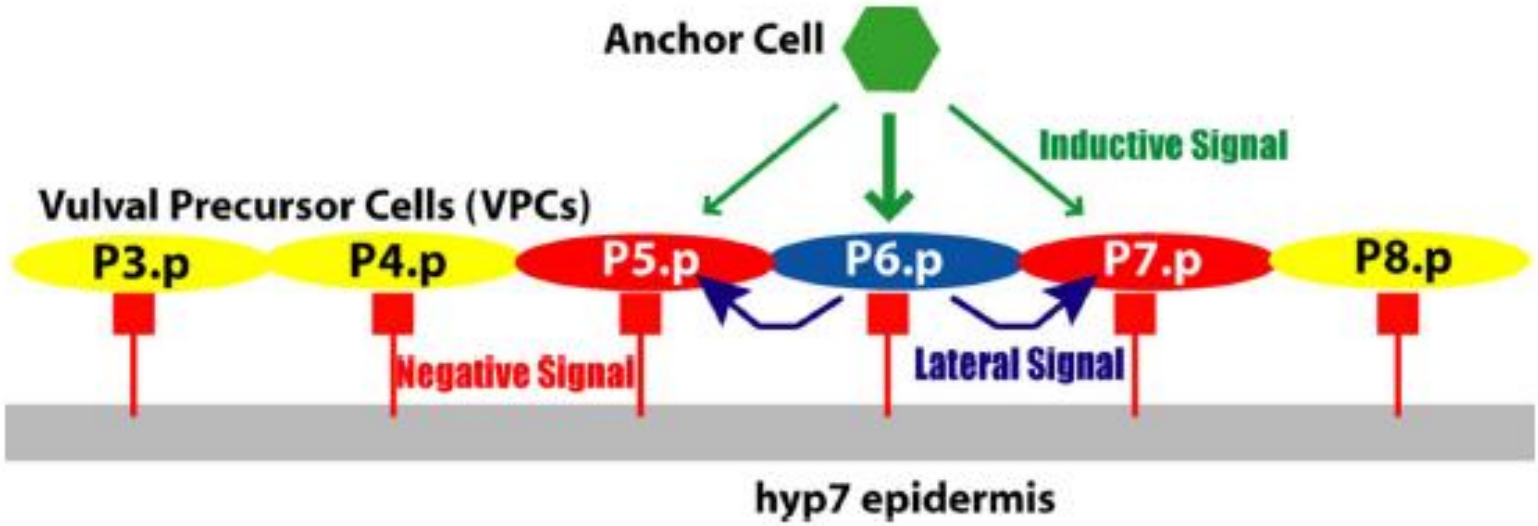
Key terms (LECTURE 2):

subcellular localization of receptors, polarized secretion, cellular polarity, Netrin signaling, whole-genome RNAi screens, robustness, basement membrane invasion, compensatory mechanisms, metabolic networks

Major concepts (LECTURE 2):

Subcellular localization of LET-23 EGF receptors and polarized secretion of LIN-3 EGF contribute to robustness of VPC patterning.
Netrin signaling is required for polarized LIN-3 EGF secretion.
The anchor cell removes the basement membrane that separates the uterus and vulva and invades, initiating a connection between uterus and vulva.
Anchor cell invasion is mediated by matrix metalloproteases (MMP).
An increase in F-actin structures at the invasive membrane can compensate for a loss of MMPs.
F-actin mediated invasion depends on the establishment of a localised metabolic network.

Several signaling events are involved in patterning the VPCs



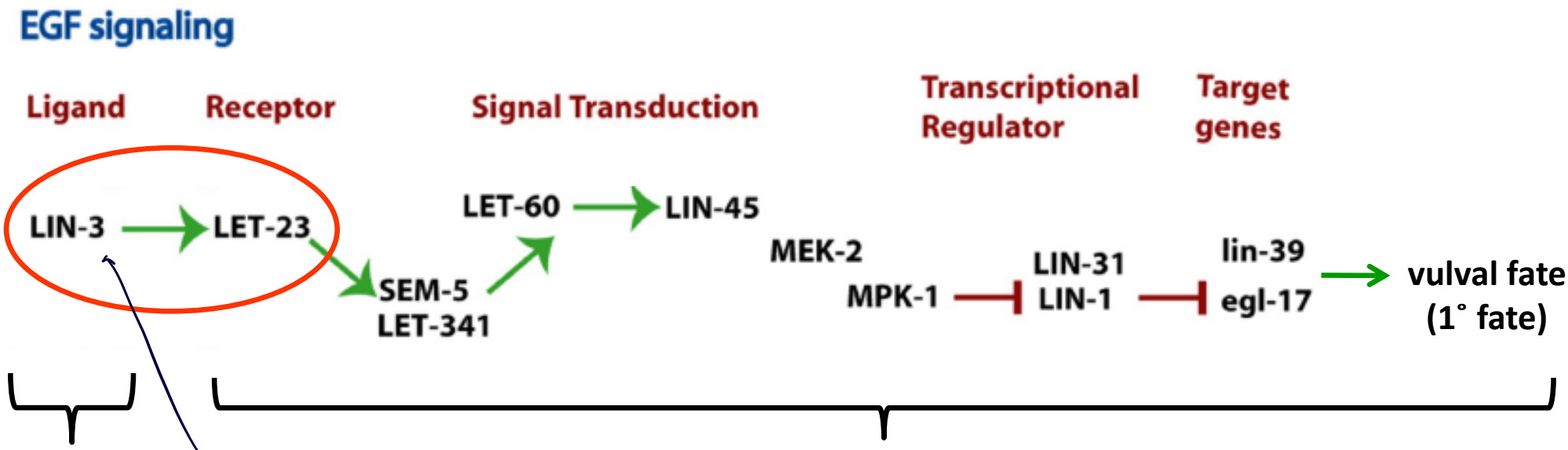
Signal 1 - inductive signal (is graded and acts at a distance)
Signal 2 - lateral signal (is sequential)
Signal 3 - *lin-3* EGF repression in hyp7

Prevent induction of 1° & 2° fate.

hyp7 from hyperdermis prevent P 3.4.8 from 1° & 2°

Sternberg, 2005, WormBook

The inductive signal is mediated by a EGF signaling pathway



Sternberg, 2005, WormBook

LIN	lineage defective
LET	lethal
SEM	sex-muscle defective
MEK	MAP kinase kinase/Erk kinase
EGL	egg-laying defective

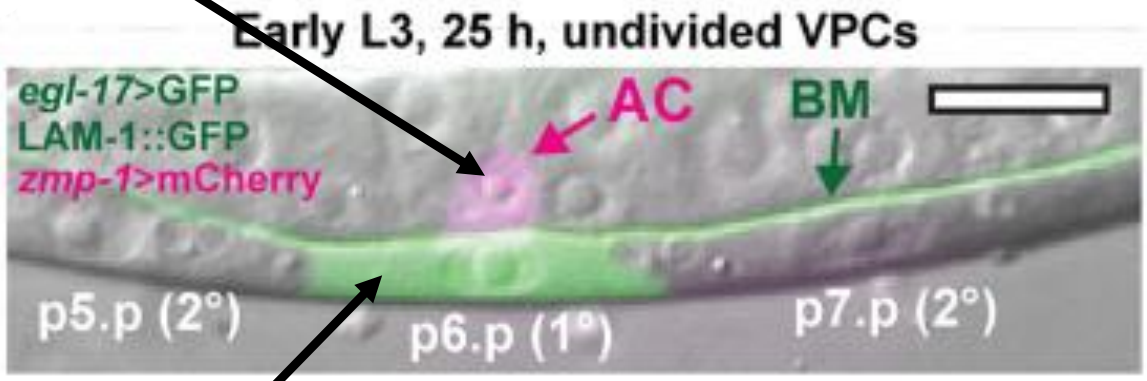
SynMuv genes in hypodermis inhibit Lin expression

Mutation lead to 3→7 cells becoming vulva.

Multivulva phenotype with loss of SynMuv.

Interactions between the AC and P6.p

LIN-3 EGF, ligand



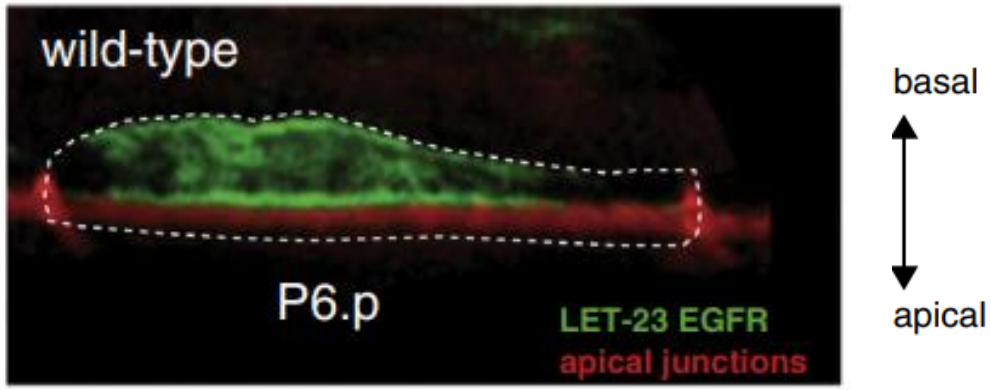
LET-23 EGF receptor

Anchor cell release Lin3 ligand

P6 p cell have LET 23 EGF receptor.

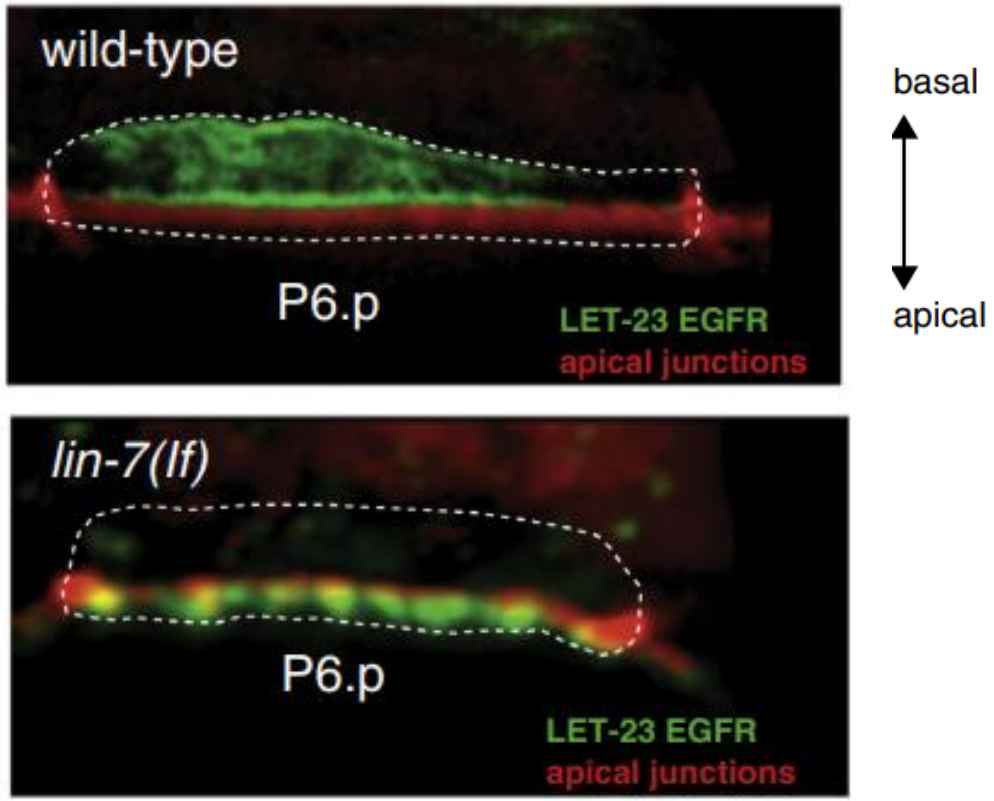
Schindler et al., 2014

The LET-23 EGFR protein localises to the basolateral membrane….



Schmid and Hajnal, 2015

…and that is dependent on the genes *lin-2*, *lin-7* and *lin-10*



Lin7 mutation
LET23 localise apically
rather than basally

Schmid and Hajnal, 2015

The loss of *lin-2*, *lin-7* or *lin-10* causes a 'strong' Vul phenotype…

Table 1. A High Copy Number *let-23(+)* Array Suppresses the *lin-2*, *lin-7*, and *lin-10* Vulvaless Phenotypes

Genotype[a]	Vul (%)[b]	n[c]
Wildtype	0	Many
let-23	95 ± 3	145
let-23; let-23(+++)	14 ± 7	104
lin-7	91 ± 7	67
lin-7; let-23(+++)	3 ± 3	115
lin-2	92 ± 3	239
lin-2; let-23(+++)	3 ± 2	445
lin-10	92 ± 8	37
lin-10; let-23(+++)	3 ± 3	142
lin-45	77 ± 12	44
lin-45; let-23(+++)	76 ± 8	119

[a] *let-23(+++)* refers to the high copy number *let-23(+)* array *gaEx50*, which contains multiple copies of pk7-13.8 (Aroian et al., 1990) and the *rol-6(su1006d)* transformation marker. Alleles used: *let-23(sy1)*, *lin-2(e1309)*, *lin-7(e1413)*, *lin-10(n1508)*, *lin-3(n378)*, and *lin-45(n2018)*.

[b] Percent egg-laying defective scored with a dissecting microscope with 95% confidence interval. Vul, vulvaless phenotype.

[c] Number of animals counted.

Vulvaless

as EGFR not present basally

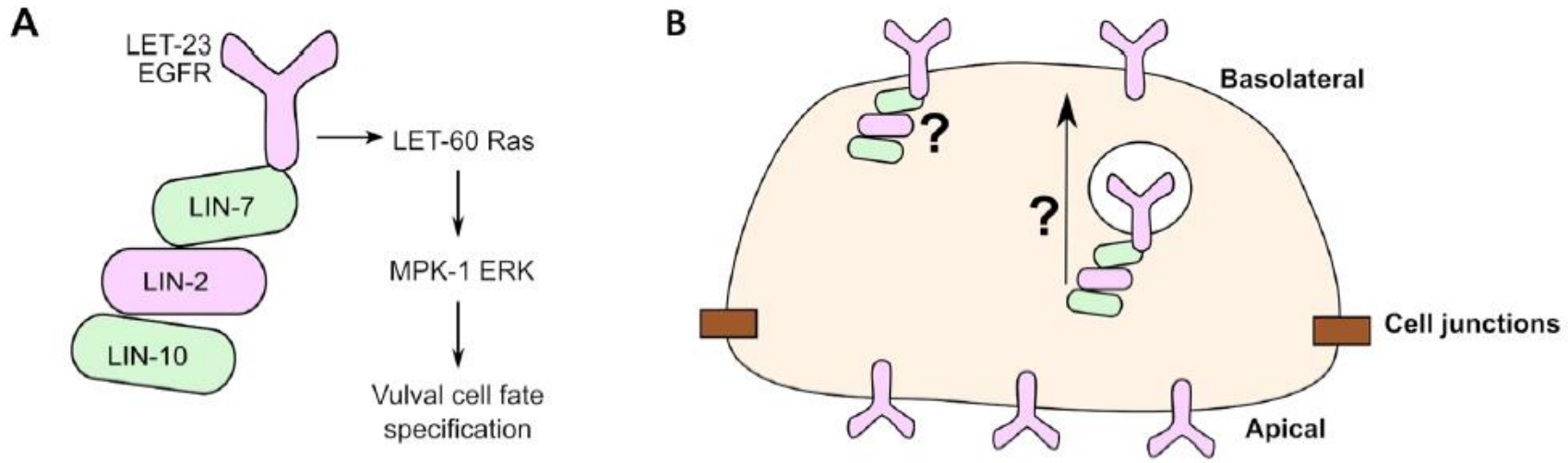
No Lin3 signaling

…which can be suppressed by overexpression of *let-23* EGFR

Simske et al., 1996

The LIN-2, -7 and -10 proteins form a conserved complex that could:

1) tether LET-23 EGFR to the basolateral membrane
OR
2) target LET-23 EGFR trafficking to the basolateral membrane

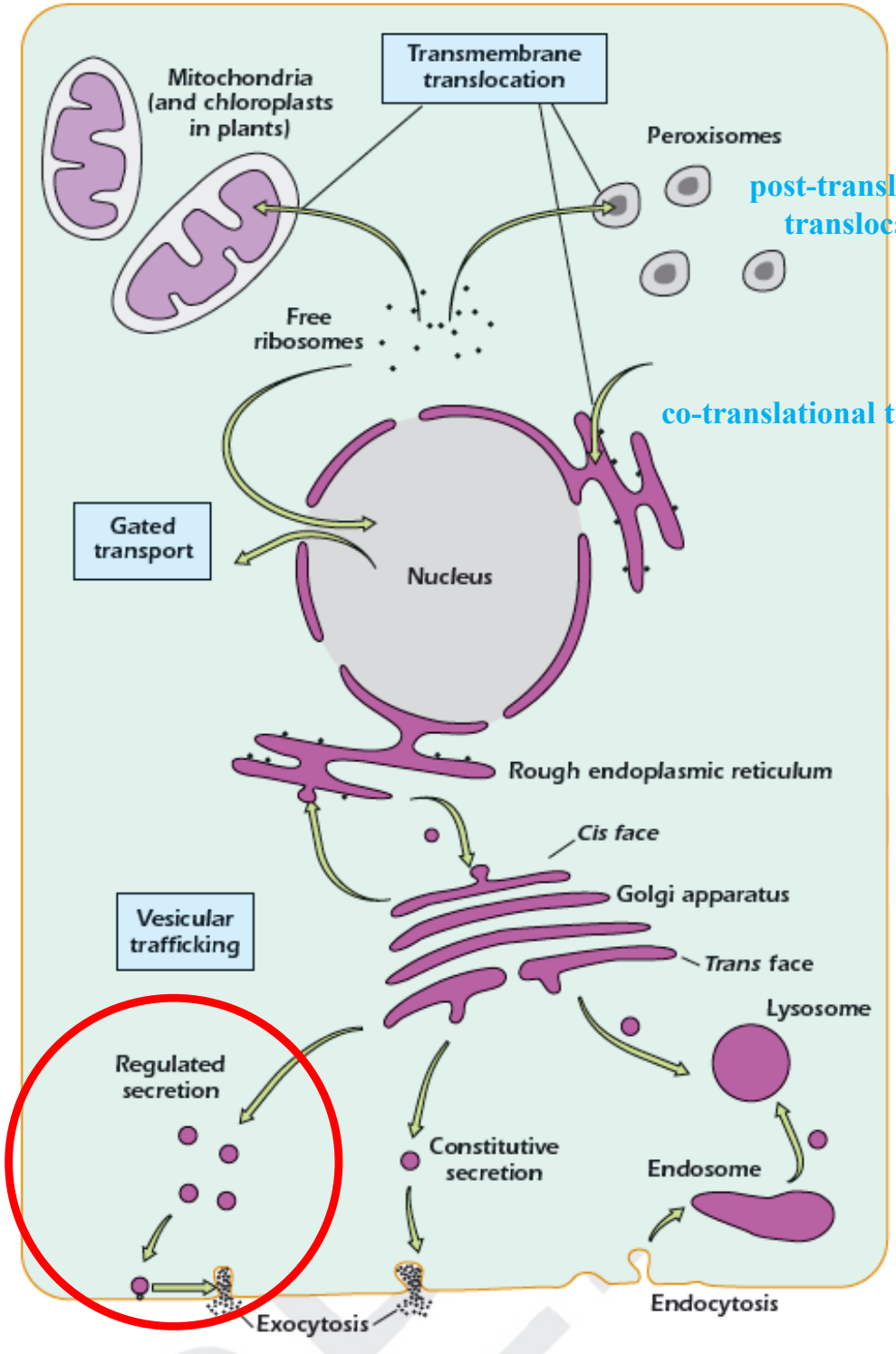


LIN
2. 7. 10
important for transport & integration
of EGF R → PM.

Gauthier and Rocheleau, 2021

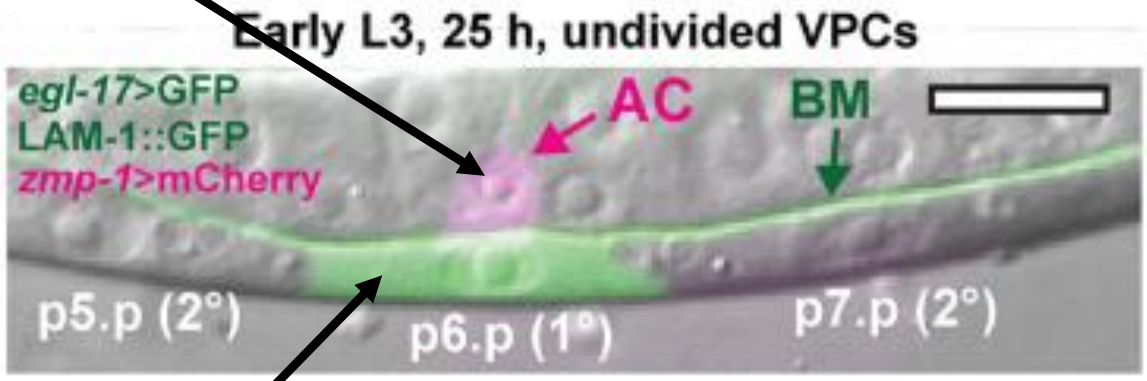
LIN-2	CASK
LIN-7	Lin7, Veli
LIN-10	APBA/Mint1

CELL0007
Intracellular protein trafficking



Interactions between the AC and P6.p

LIN-3 EGF, ligand



LET-23 EGF receptor

Schindler et al., 2014

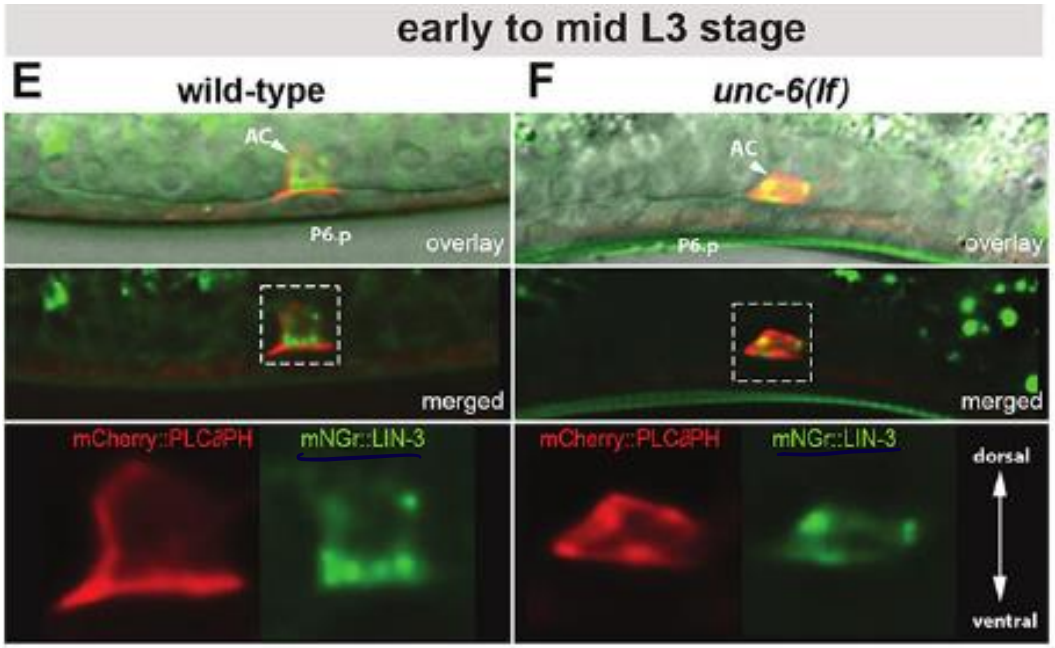
Previous in P6.p

Now in AC...

LIN-3 EGF is secreted from the AC in a polarised manner and this is dependent on *unc-6* Netrin

Mereu et al., 2020

Netrin signaling

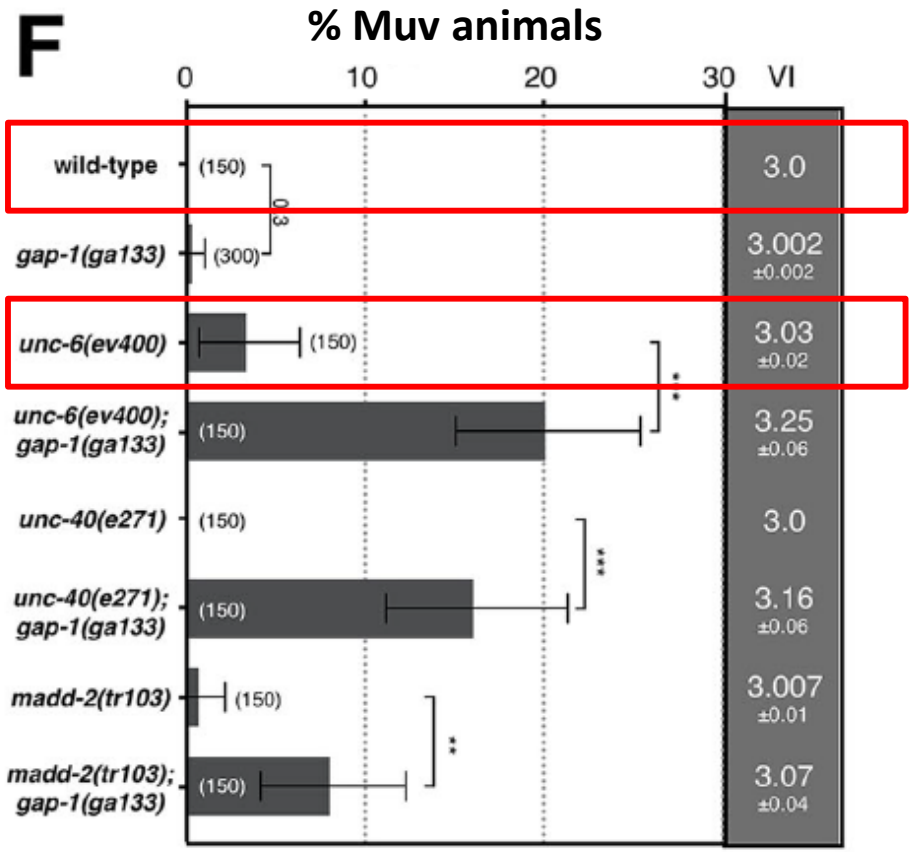


No localisation of Lin 3 to ventral when unc 6 netrin absent.

In *unc-6* Netrin mutants, the AC is not properly polarised

Loss of *unc-6* Netrin causes a 'weak' Muv phenotype….

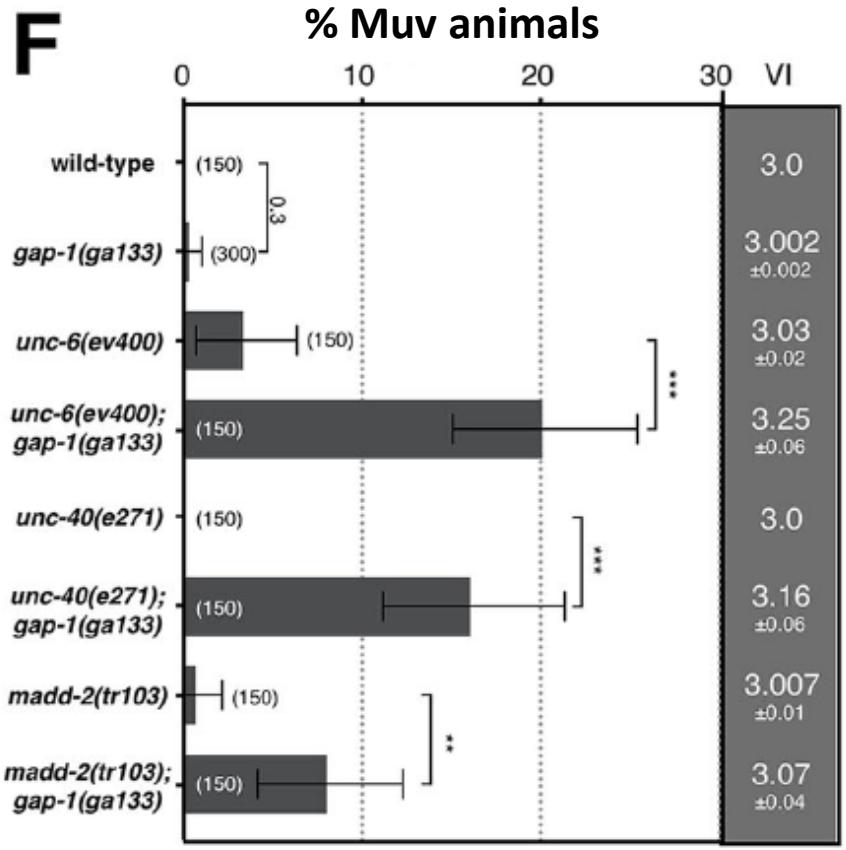
Multi vulva

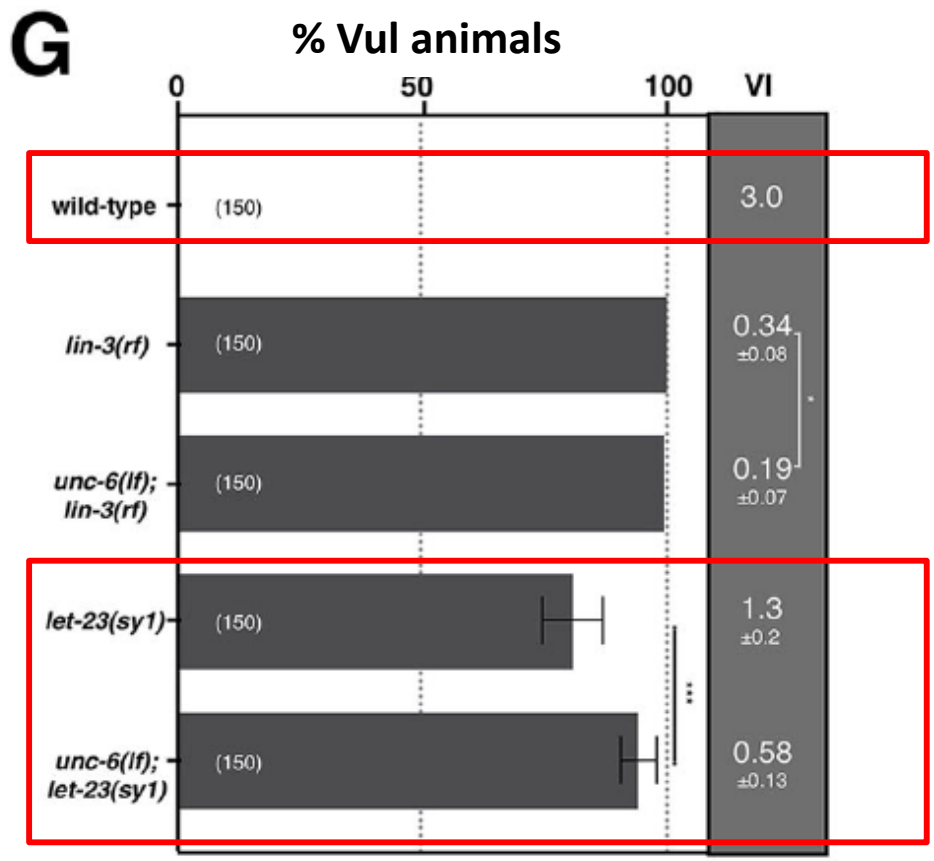


Mereu et al., 2020

….and enhances the Vul phenotype of *let-23*(*sy1*)

Vulvaless. No Lin ligand & receptor



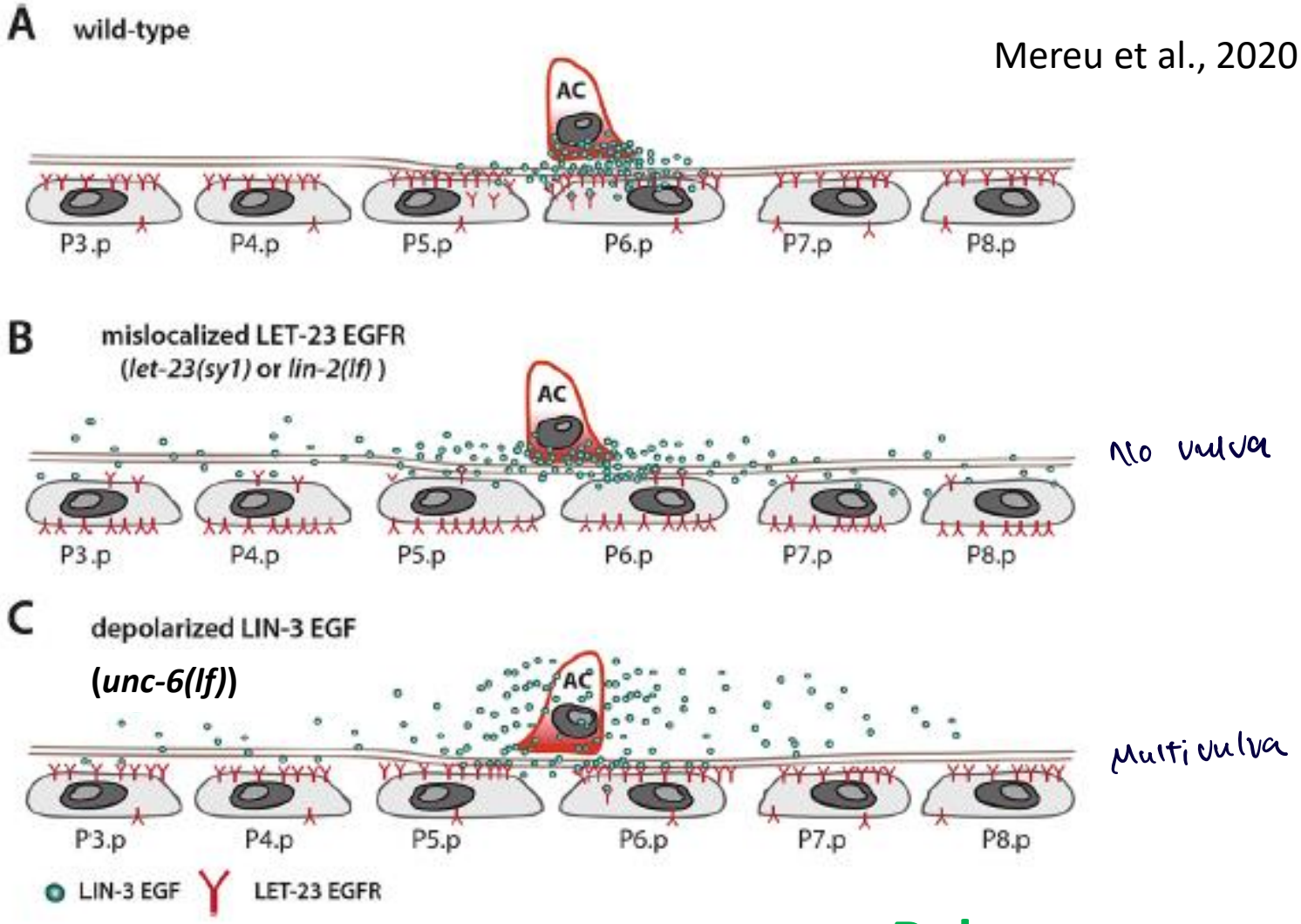


let-23(*sy1*) causes mislocalization of LET-23 receptor

Mereu et al., 2020

Polarised LIN-3 EGF secretion AND basolateral localization of LET-23 EGFR are required for robust vulval development

Mereu et al., 2020



Robustness

Vulval development is a stepwise process

STEP 1 **Generation of the vulval precursor cells (VPCs: P3.p-P8.p)**

STEP 2 **Vulval precursor patterning (1˚, 2 ˚, 3 ˚ fate)**

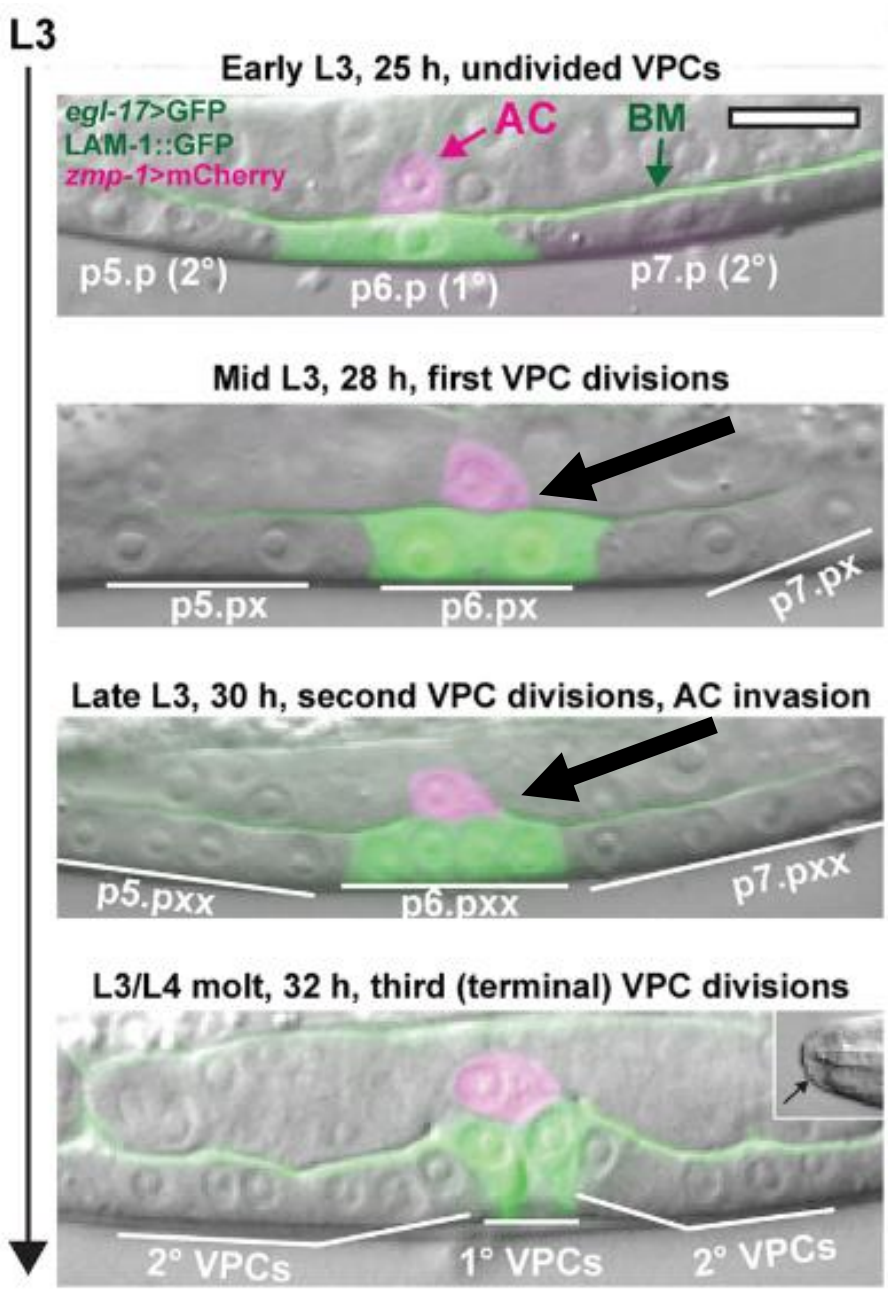
- Inductive signal (EGF signaling), Lateral signal (Notch signaling)
- Subcellular localization of LET-23 EGFR and polarized secretion of LIN-3 EGF
- Polarising signaling (Netrin signaling)

STEP 3 **Generation of adult cells**

STEP 4 **Anchor cell (AC) invasion**

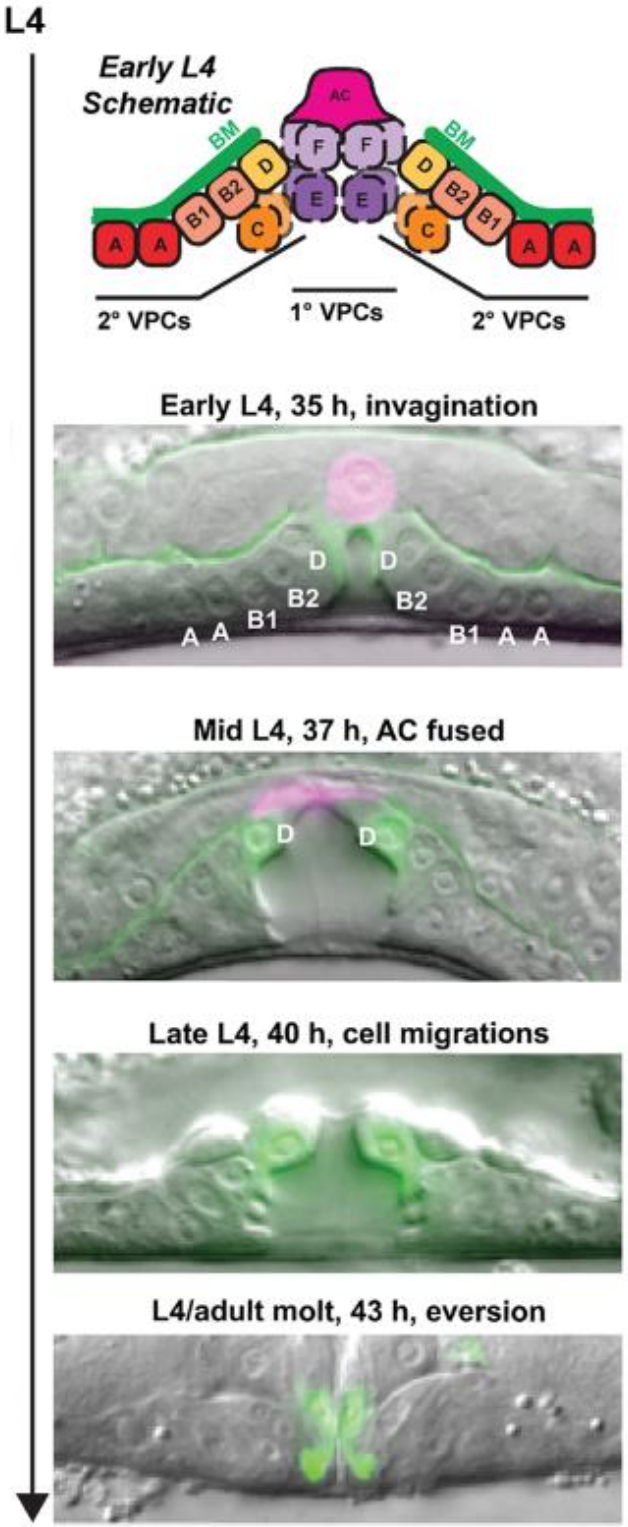
STEP 5 **Morphogenesis of the vulva**

In late L3 larvae,
the AC 'breaks' through basement membranes (BM)



Schindler et al., 2014

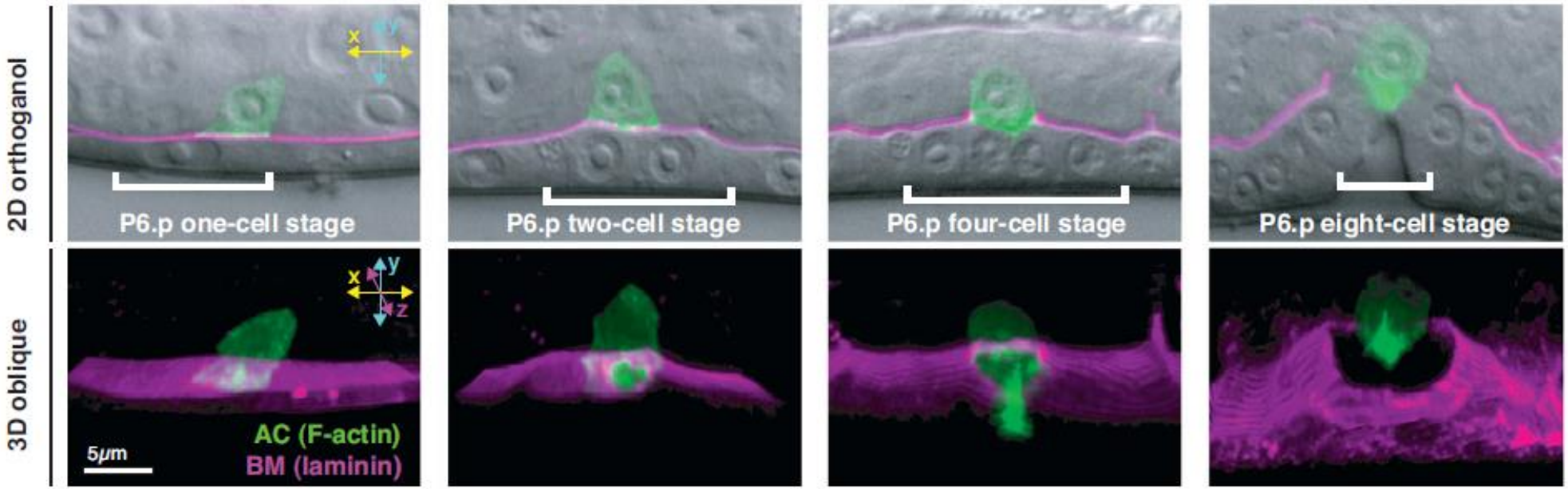
The anchor cell removes the basement membrane that separates the uterus and vulva and invades, initiating the connection between the uterus and the vulva. Finally, the anchor cell fuses with cells of the somatic gonad to form the uterine seam cell.



Schindler et al., 2014

Anchor cell invasion

Hagedorn and Sherwood, 2011



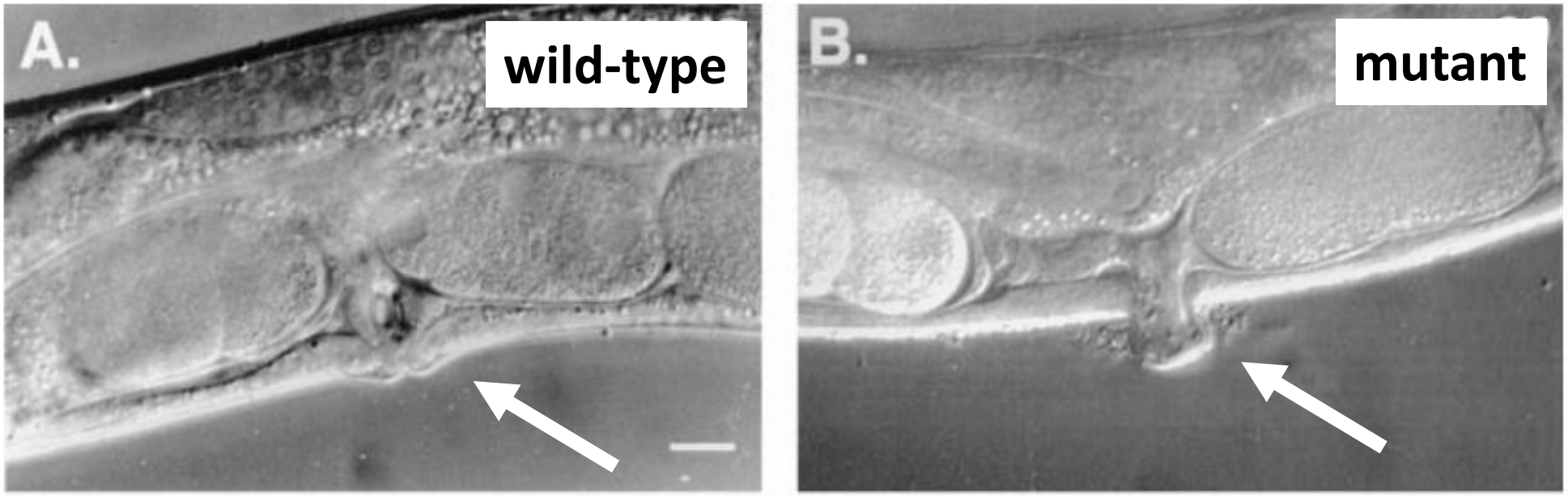
Current Opinion in Cell Biology

Actin polymerisation Rac protrude through 1° VPC
Laminin digestion of BM.

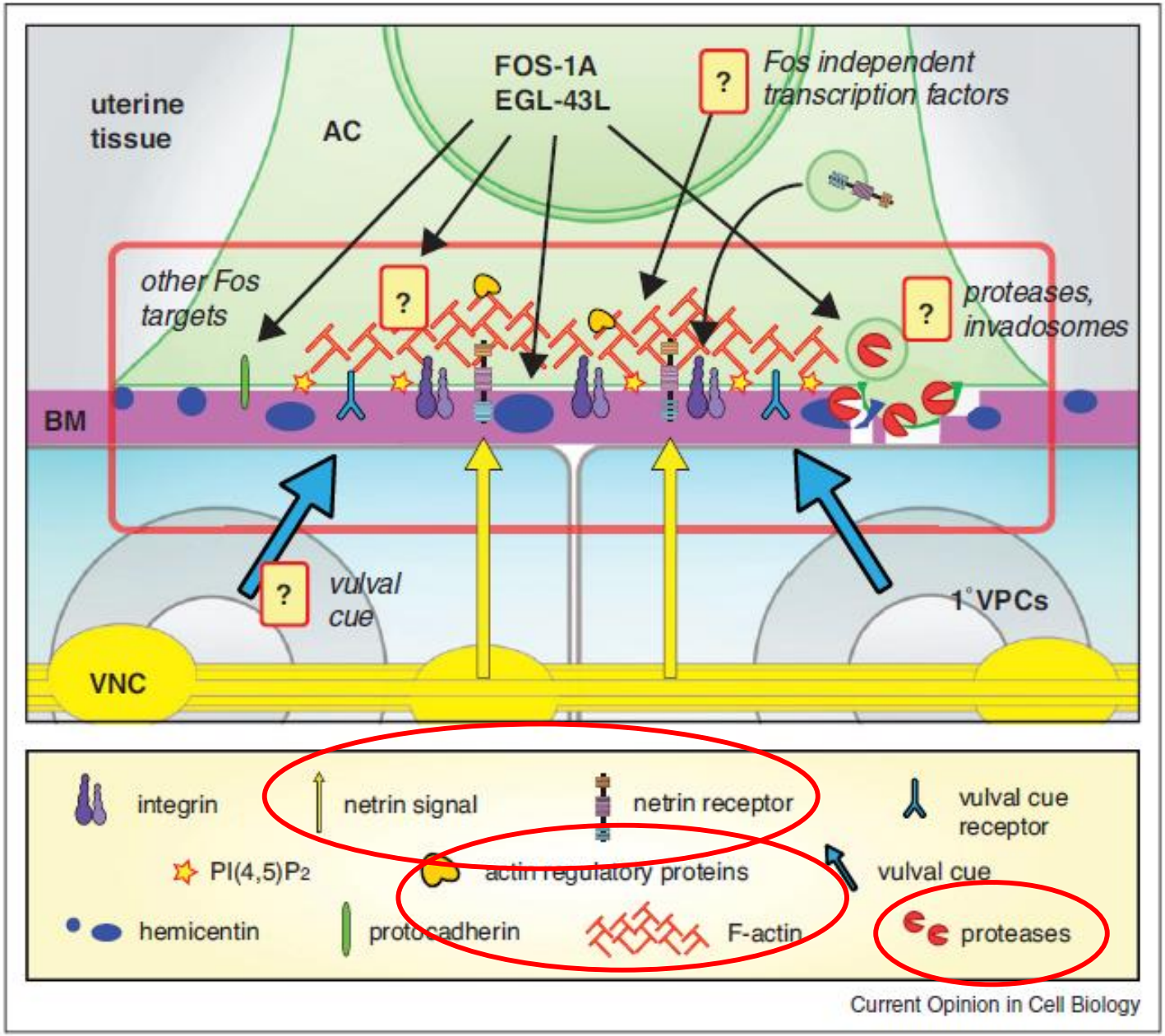
Cytoskeletal rearrangements (actin)
Proteases (MMP, matrix metalloproteases)

Energy!

A block in AC invasion and AC fusion with the somatic gonad causes a ‘Protruding vulva’ or Pvl phenotype



David M Eisenmann
https://www.researchgate.net/figure/Vulval-phenotypes-of-Pvl-mutants-Wild-type-adult-hermaphrodite-A-and-Pvl-adult_fig1_12262668



Hagedorn and Sherwood, 2011

Article

Developmental Cell

Adaptive F-Actin Polymerization and Localized ATP Production Drive Basement Membrane Invasion in the Absence of MMPs

Kelley et al., 2019, Developmental Cell *48*, 313–328
February 11, 2019
https://doi.org/10.1016/j.devcel.2018.12.018

MMPs are not required for AC invasion….

Table 1. Genetic Analysis of the Role of MMPs during AC Invasion

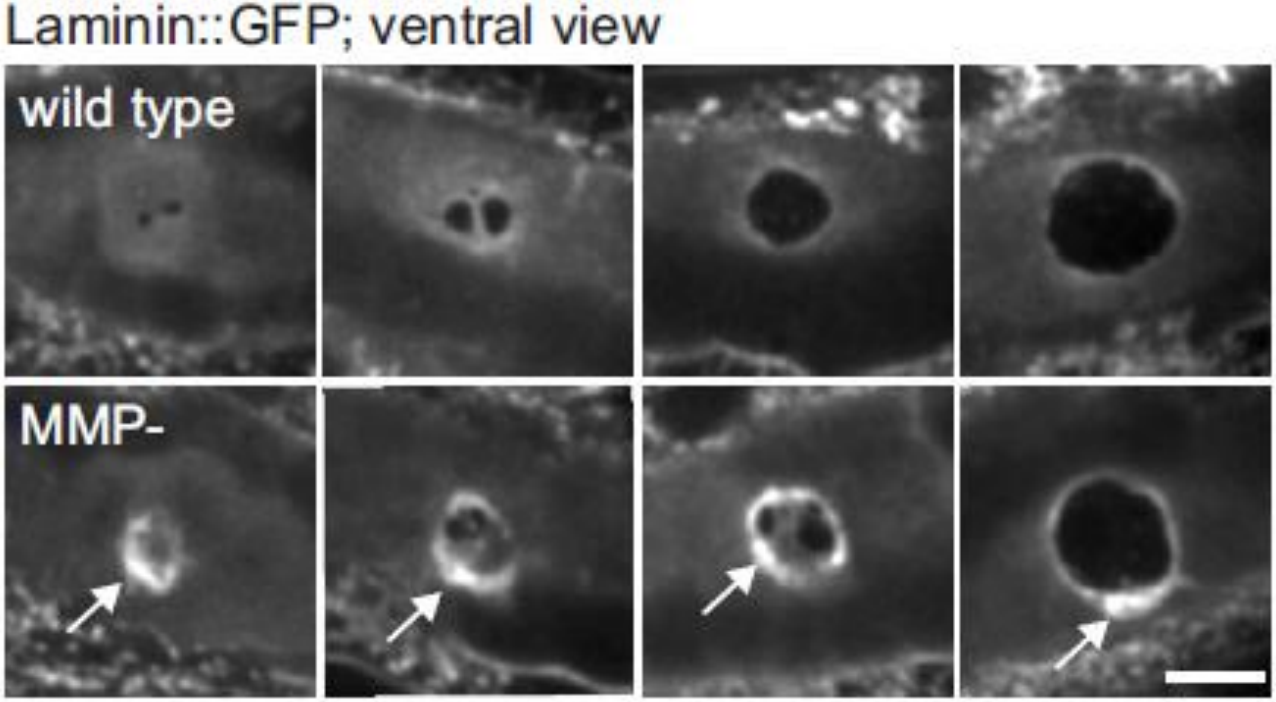
Genotype	RNAi Tx	Developmental P6.p Stage	Invasion Complete	n
Screen of *C. elegans* MMP Genes				
wild-type	n/a	late 4-cell	100%	50
zmp-1 (cg115)	n/a	late 4-cell	100%	29
zmp-2 (tm3529)/+	n/a	late 4-cell	100%	22
zmp-3 (tm3482)	n/a	late 4-cell	100%	50
zmp-4 (tm3078)	n/a	late 4-cell	100%	59
zmp-4 (tm3484)	n/a	late 4-cell	100%	26
zmp-5 (tm3209)	n/a	late 4-cell	100%	15
zmp-6 (tm3073)	n/a	late 4-cell	100%	50
zmp-6 (tm3385)	n/a	late 4-cell	100%	23
zmp-1 (cg115); zmp-3 (tm3482)	n/a	late 4-cell	100%	29
zmp-3 (tm3482); zmp-6 (tm3073)	n/a	late 4-cell	100%	50
zmp-3 (tm3482); zmp-4 (tm3484)	n/a	late 4-cell	100%	50
zmp-1 (cg115); zmp-6 (tm3073)	n/a	late 4-cell	100%	50
zmp-1 (cg115); zmp-3 (tm3482); zmp-6 (tm3073)	n/a	late 4-cell	100%	50
zmp-1 (cg115); zmp-3 (tm3482); zmp-4 (tm3484); zmp-6 (tm3073)	n/a	late 4-cell	100%	50
MMP–	n/a	late 4-cell	100%	50

Matrix Metallo protease

Candidate ‚reverse' genetic screen

Kelley et al, 2019

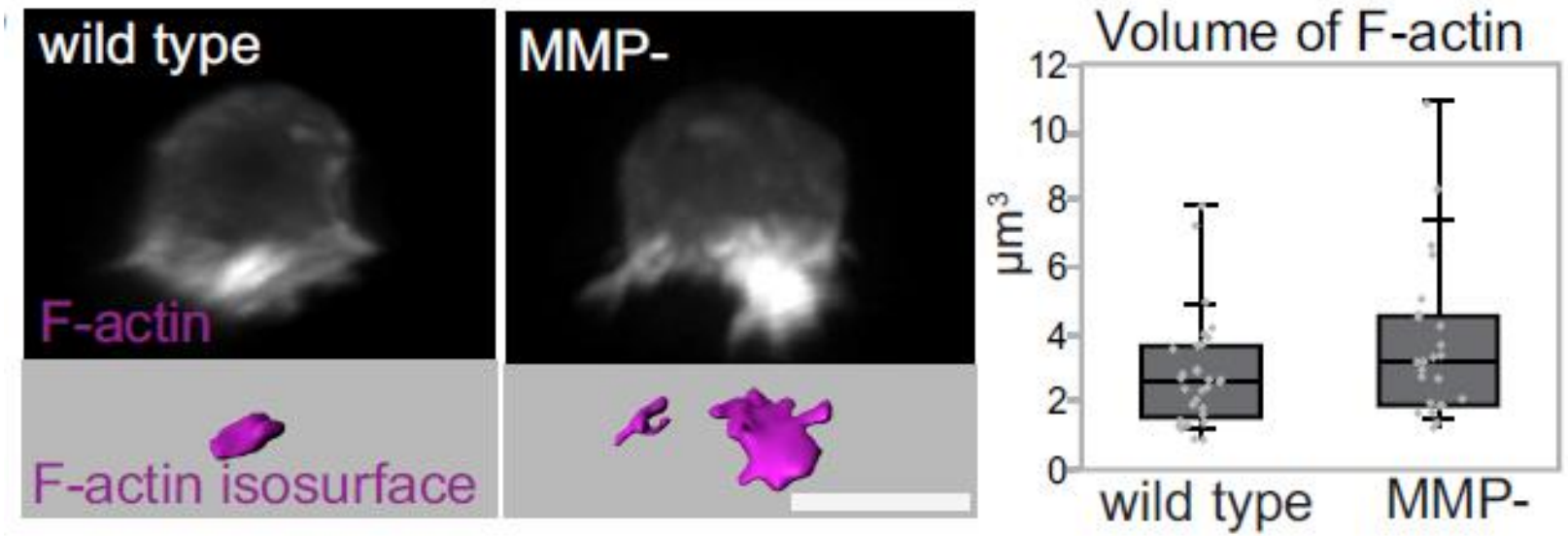
…but their loss delays BM breaching time and decreases ECM removal



Kelley et al, 2019

An increase in F-Actin supports MMP-independent invasion

Greater polymerisation push through.



Arp2/3 complex – promotes nucleation of actin filaments

Compensatory mechanism

Kelley et al, 2019

The loss of Arp2/3 function (*arx-2* RNAi) in an MMP⁻ background causes a strong AC invasion defect

No push no openly

Table 1. Genetic Analysis of the Role of MMPs during AC Invasion

Genotype	RNAi Tx	Developmental P6.p Stage	Invasion Complete	n
RNAi Genetic Interaction Experiments with AC Membrane and BM Markers				
wild type	ctl	early 4-cell	91%	71
		mid 4-cell	100%	
		late 4-cell	100%	
MMP–	ctl	early 4-cell	33%	71
		mid 4-cell	79%	
		late 4-cell	100%	
wild-type	*arx-2*	early 4-cell	31%	97
		mid 4-cell	59%	
MMP–	*arx-2*	early 4-cell	8%	100
		mid 4-cell	15%	

arx-2 ARp2/3 compleX component

Kelley et al, 2019

Whole genome RNAi screen for Pvl animals in an MMP- background

Protruding vulva

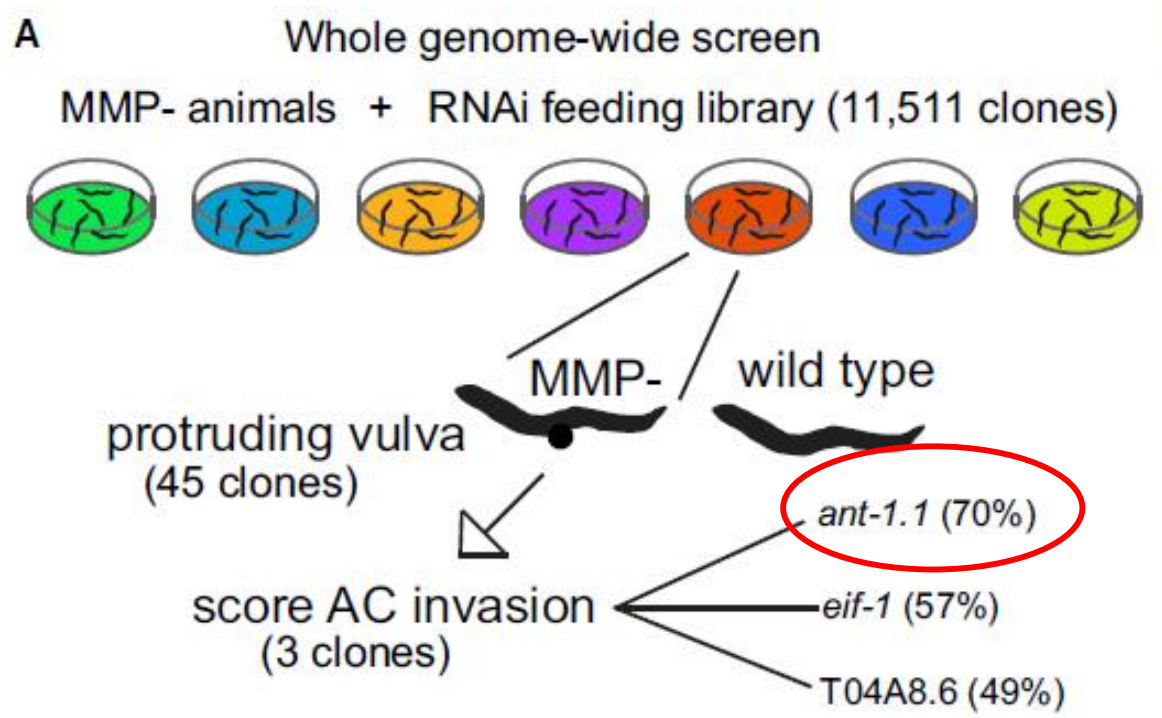
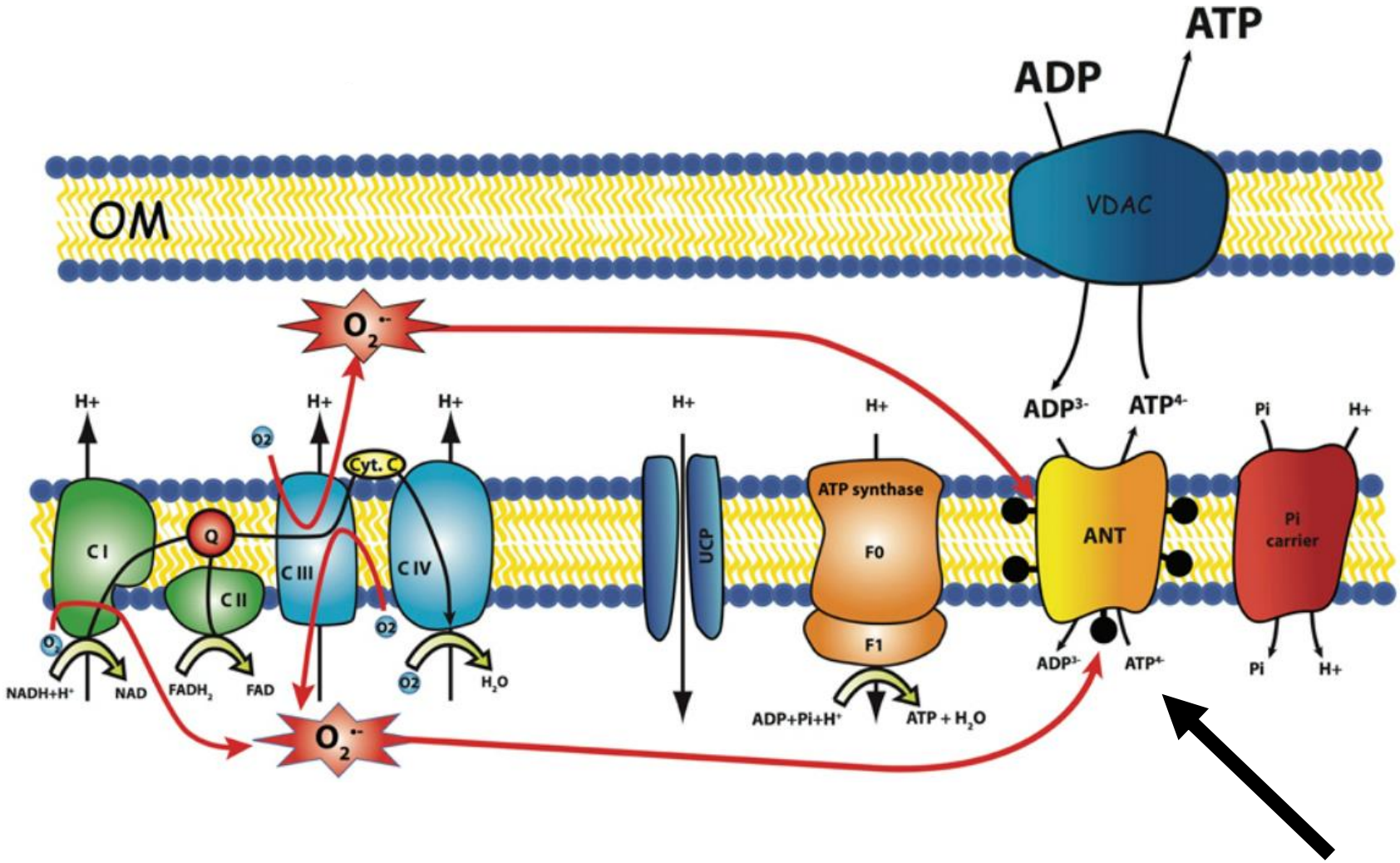


Figure 5. MMP– Synergistic Screen Identifies a Mitochondrial ADP/ATP Translocase

(A) RNAi clones targeting 11,511 genes were fed to newly hatched MMP– L1 animals. Adult worms with a protruding vulval (Pvl) phenotype were scored for AC invasion defects if the gene did not cause Pvls in wild-type worms.

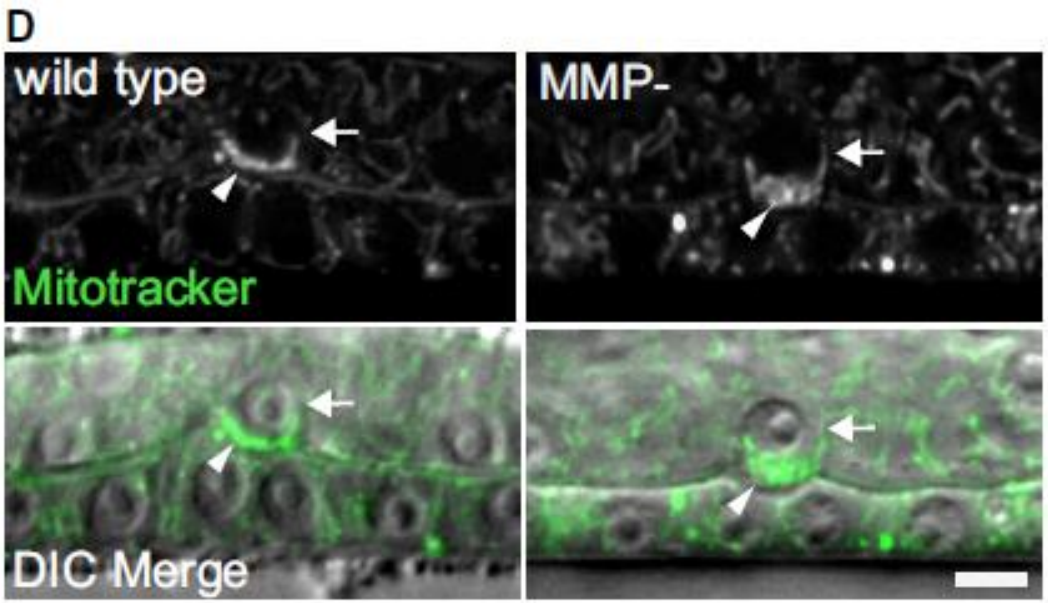
Kelley et al, 2019

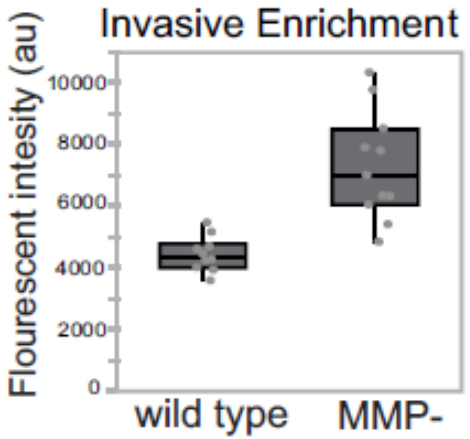
ant-1.1 encodes an adenine nucleotide translocator that is localised in the inner mitochondrial membrane



Diolez et al, 2015

Mitochondria are enriched near the invading membrane…

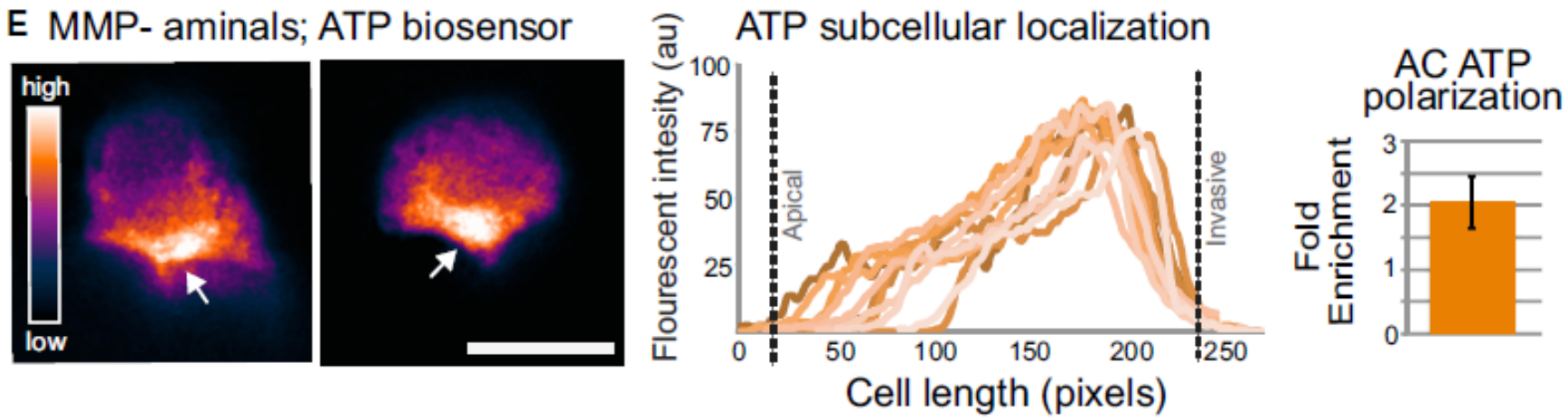




Arf 1.1 used to import asterlin
for ATP generation

Kelley et al, 2019

…and so is ATP



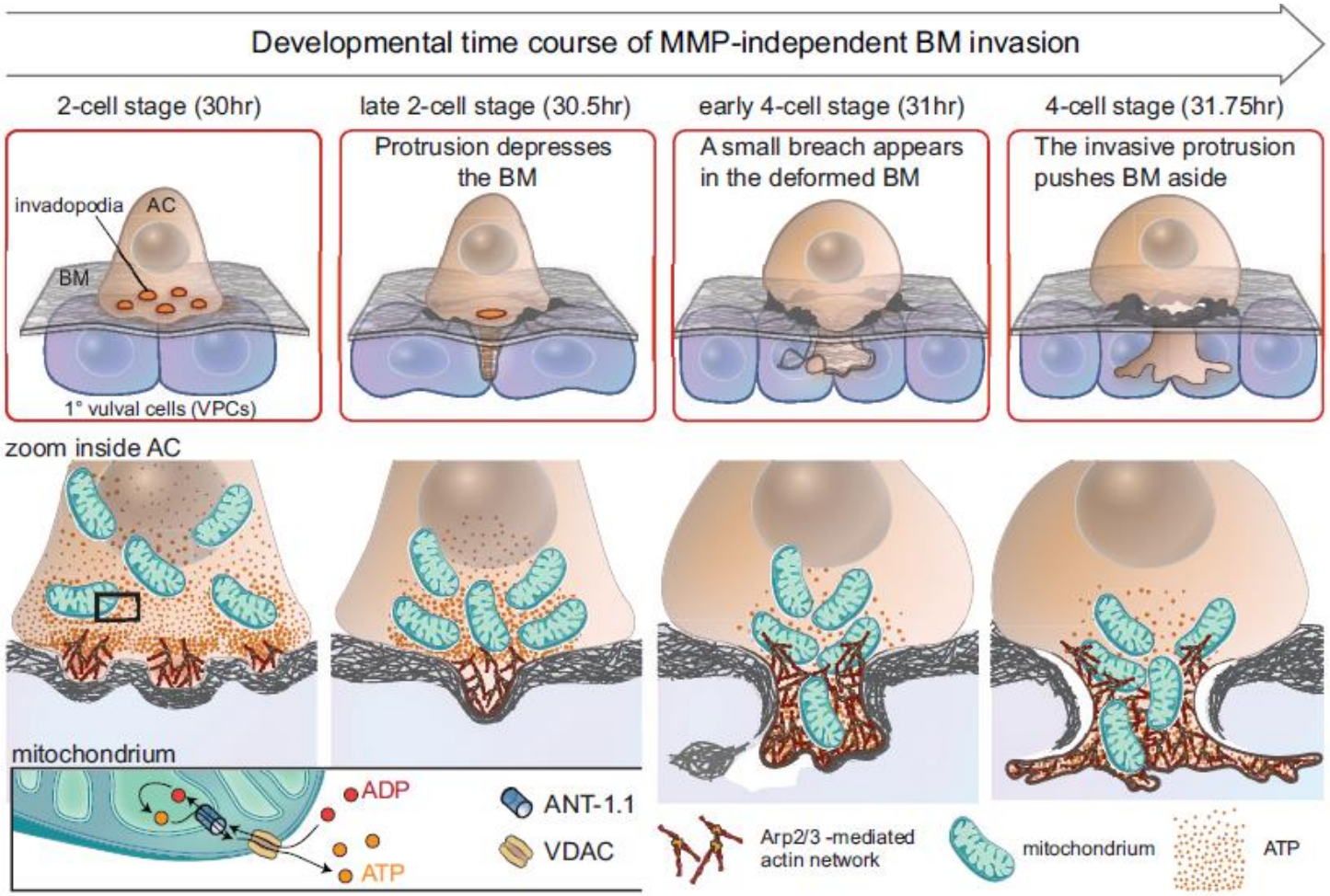
High mito & ATP near invady membrane.

For polymerization & MMP

Kelley et al, 2019

Mitochondria Are Tightly Juxtaposed to the Invasive F-Actin Networks

Accompany



(C) Schematic diagram showing the time course of adaptive MMP– invasion. Invasion is delayed and is propelled by increased Arp2/3-mediated F-actin networks and enrichment of mitochondria/ATP (via ANT-1.1 ADP/ATP translocase), which helps form a large protrusion that breaches and displaces BM through physical forces. VDAC is an outer mitochondrial membrane pore that facilitates diffusion of small hydrophilic molecules such as ATP and ADP. Scale bars, 5 μm.

Kelley et al, 2019

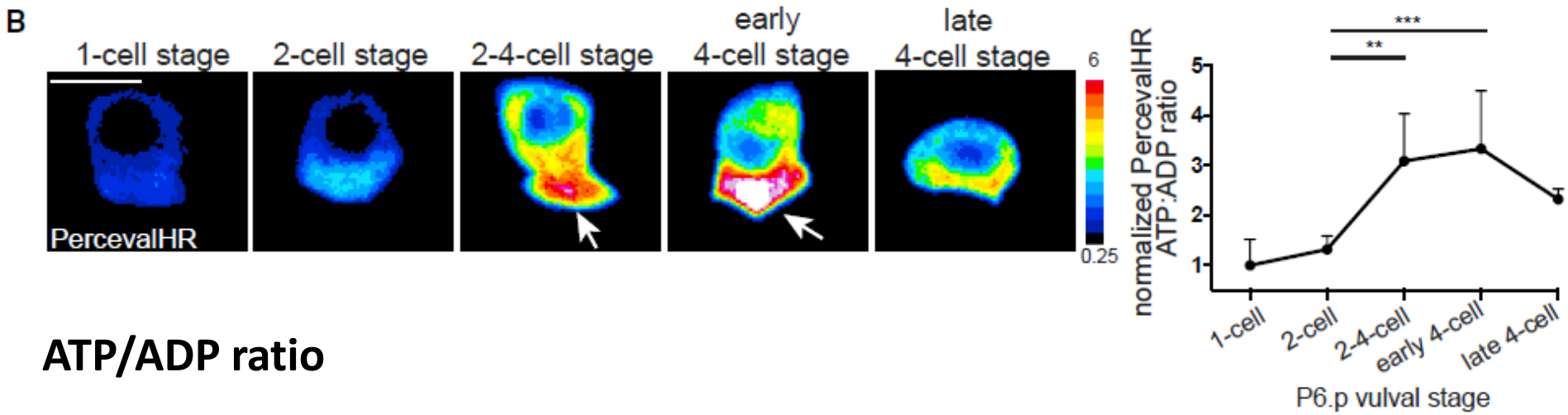
Article

Developmental Cell

Localized glucose import, glycolytic processing, and mitochondria generate a focused ATP burst to power basement-membrane invasion

Garde et al., 2022, Developmental Cell 57, 732–749
March 28, 2022
https://doi.org/10.1016/j.devcel.2022.02.019

During AC invasion, a burst of ATP can be detected



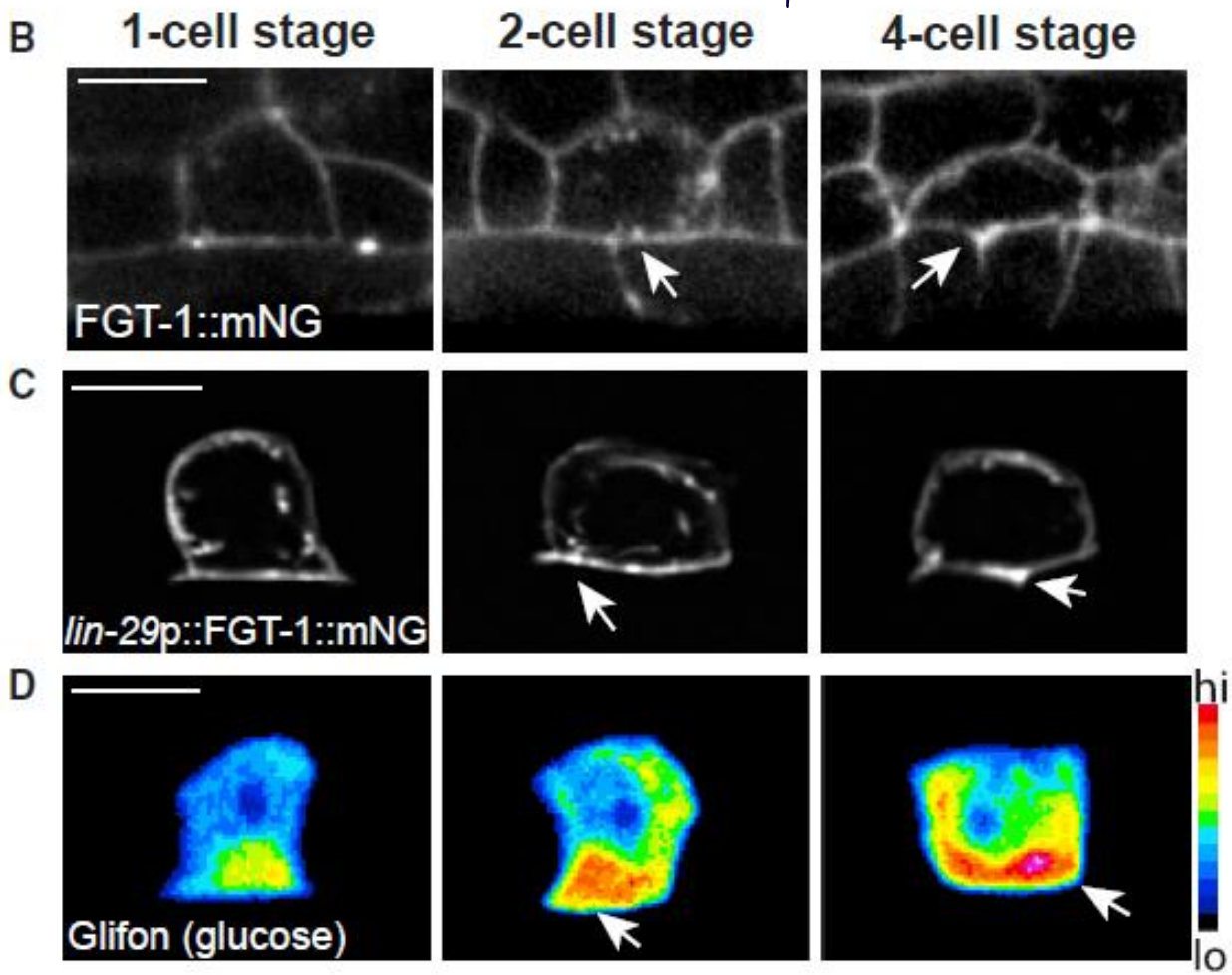
ATP/ADP ratio

Glucose imported for ATP.

Garde et al, 2022

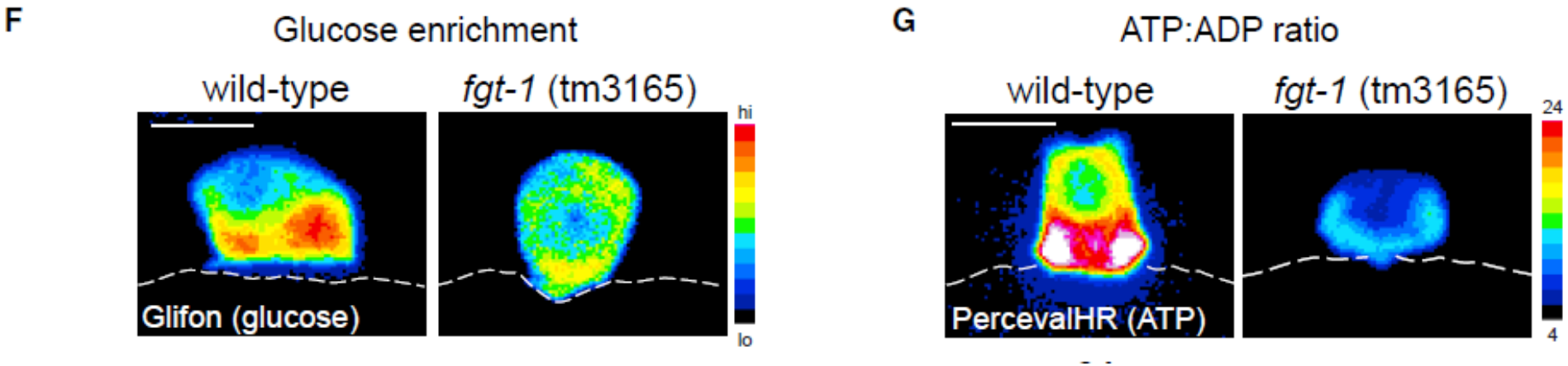
To enable this burst in ATP, the AC imports glucose at the invading membrane, which is mediated through the glucose transporter FGT-1

Facilitated



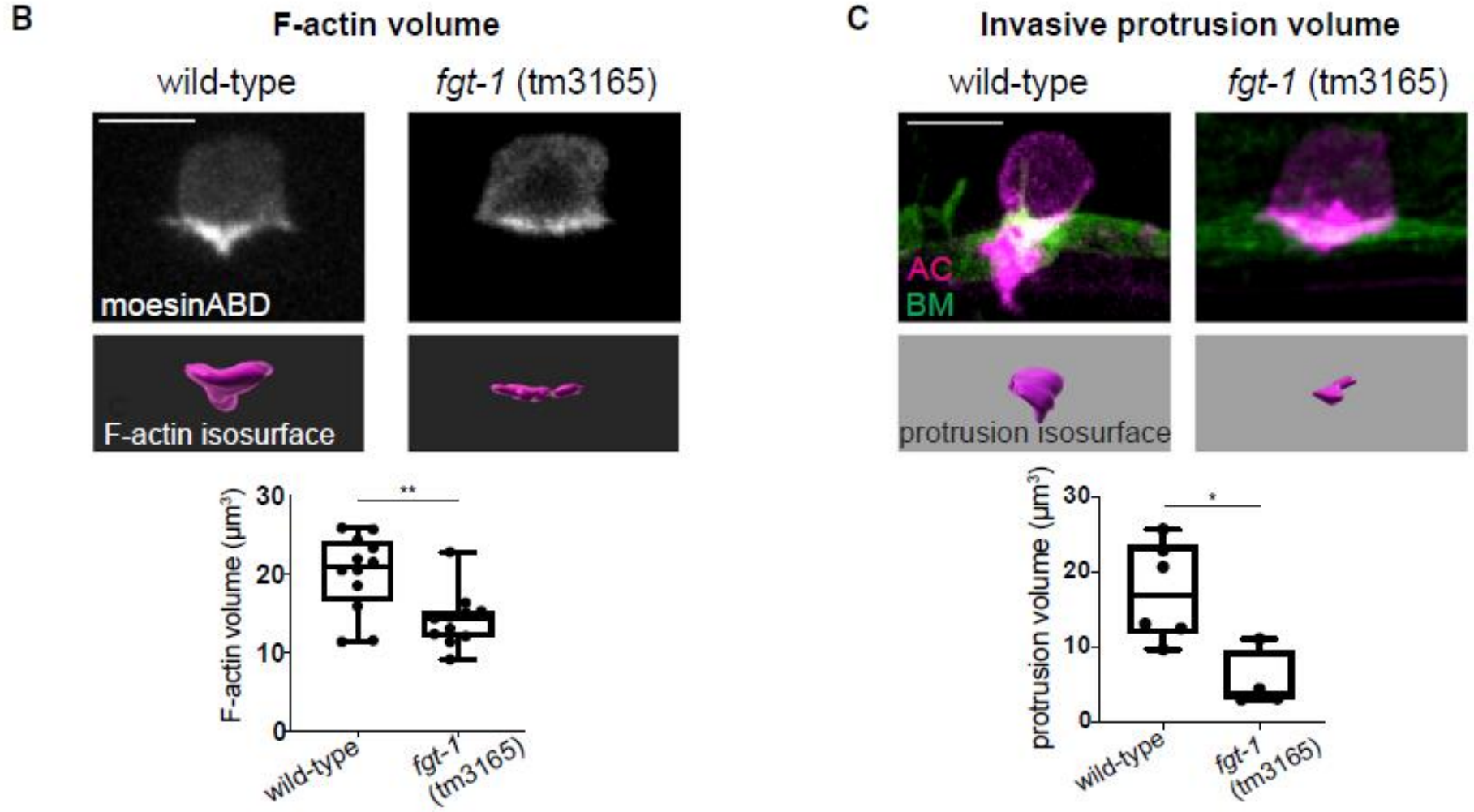
Garde et al, 2022

In *fgt-1* mutants, there is no enrichment of glucose at the invading membrane and also no burst in ATP



Garde et al, 2022

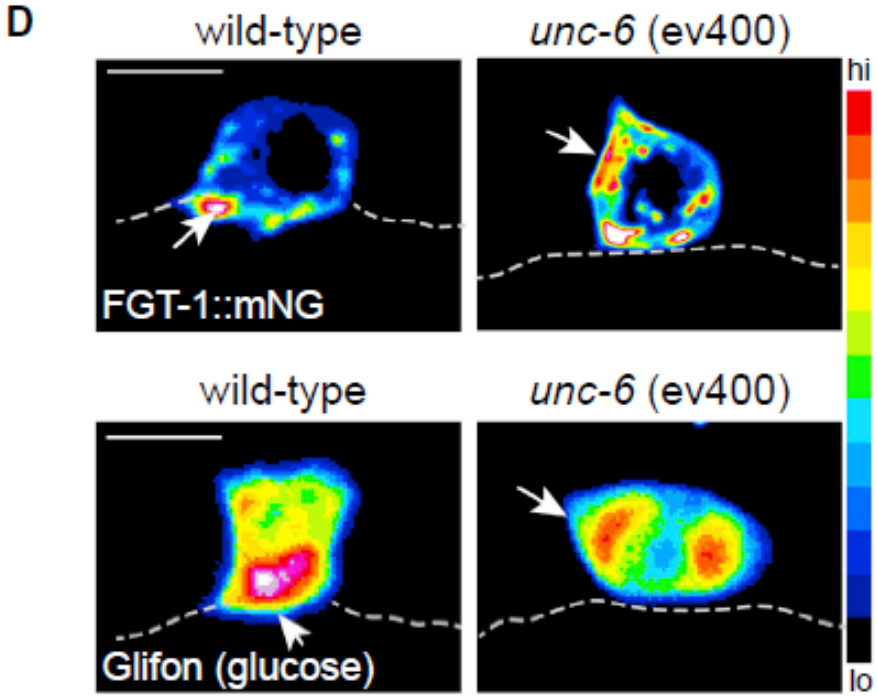
In *fgt-1* mutants, there is no increase in F-actin at the invading membrane and the invasive protrusion volume is reduced

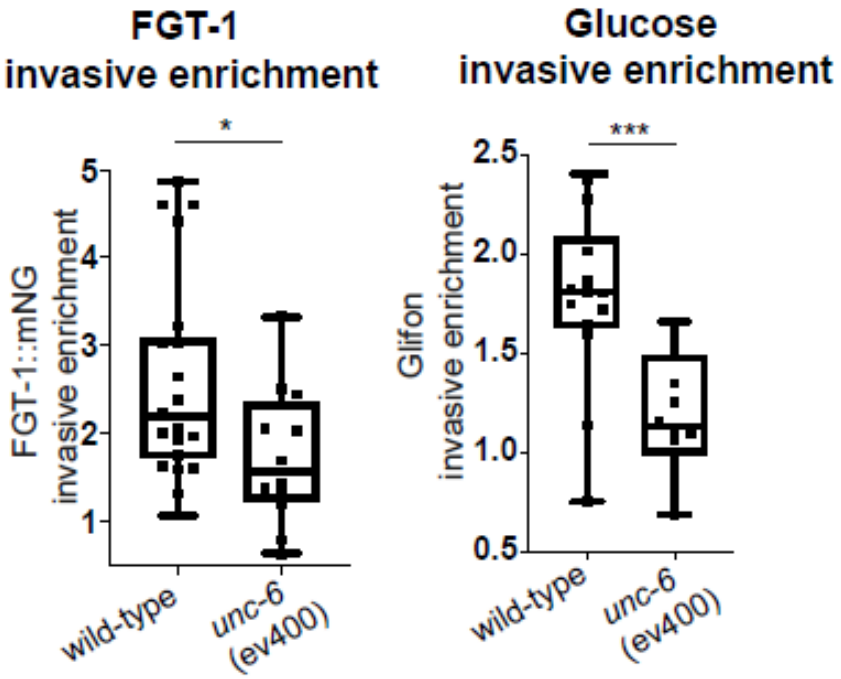


Garde et al, 2022

Less glu → less ATP.

Polar localization of FGT-1 and glucose enrichment is dependent on *unc-6* Netrin signaling

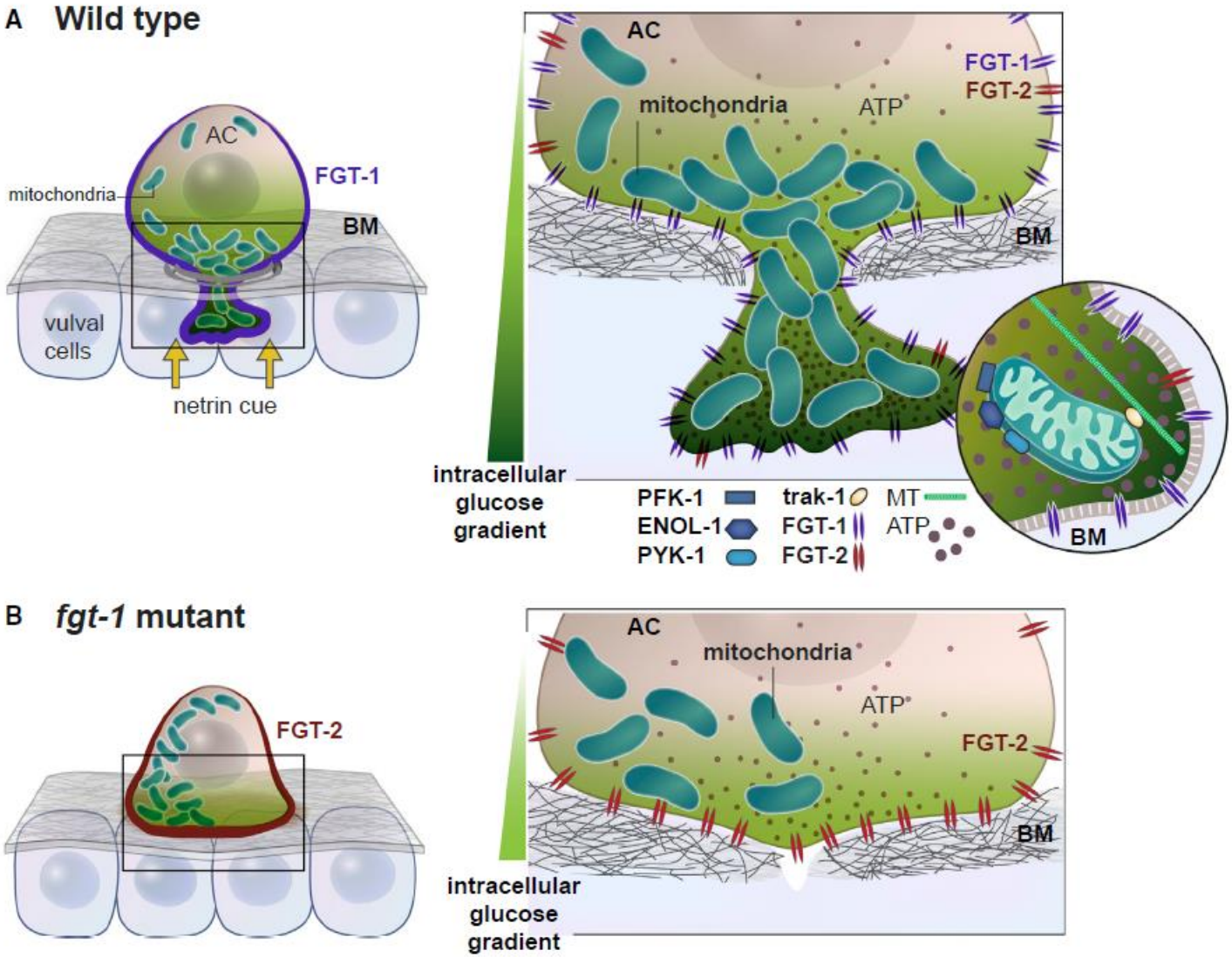




Localised FGT by netrin
Lot of glucose — mitochondria → ATP
Adenine - Ant1.1
Actin & pol

Garde et al, 2022

A localized, integrated, and adaptive metabolic network fuels AC-BM invasion



Garde et al, 2022

Vulval development is a stepwise process

STEP 1 Generation of the vulval precursor cells (VPCs: P3.p-P8.p)

STEP 2 Vulval precursor patterning (1˚, 2 ˚, 3 ˚ fate)

- Inductive signal (EGF signaling), Lateral signal (Notch signaling)
- Subcellular localization of LET-23 EGFR and polarized secretion of LIN-3 EGF
- Polarising signaling (Netrin signaling)

STEP 3 Generation of adult cells

STEP 4 Anchor cell (AC) invasion

- Role of F-actin and matrix metalloproteases
- Subcellular localization of metabolic network
- Polarising signaling (Netrin signaling)

STEP 5 Morphogenesis of the vulva

CELL0023 Cell Biology of Development – LECTURE 1
Development and morphogenesis of the *C. elegans* vulva

Outline (LECTURE 1&2):

General introduction to *C. elegans*
egg-laying apparatus and vulval development

Vulval precursor patterning

Anchor cell invasion

Key terms (LECTURE 2):

subcellular localization of receptors, polarized secretion, cellular polarity, Netrin signaling, whole-genome RNAi screens, robustness, basement membrane invasion, compensatory mechanisms, metabolic networks

Major concepts (LECTURE 2):

Subcellular localization of LET-23 EGF receptors and polarized secretion of LIN-3 EGF contribute to robustness of VPC patterning.
Netrin signaling is required for polarized LIN-3 EGF secretion.
The anchor cell removes the basement membrane that separates the uterus and vulva and invades, initiating a connection between uterus and vulva.
Anchor cell invasion is mediated by matrix metalloproteases (MMP).
An increase in F-actin structures at the invasive membrane can compensate for a loss of MMPs.
F-actin mediated invasion depends on the establishment of a localised metabolic network.

C. elegans vulva development

Coupling of EGF signalling pathway and the lateral inhibitory Notch signalling pathway (Sternberg, 2005)

- Closest proximity of P6.p from anchor cell - highest concentration of LIN-3 ligand
- LIN-3 bind to LET-23 on P6.p cell surface - induce downstream transcription of 1° fate genes (e.g. Egl17)
- 1° fate gene transcription stimulate production of Notch signalling ligand DSL and inhibit Notch receptor LIN-12 expression.
- Lower stimulation of neighbouring P5.p and P7.p - less activation of 1° fate, Notch signalling pathway override the EGF signalling, LIN-12 downstream pathway stimulate expression of 2° cell fate genes, and inhibits EGF pathway.

Components of the EGF signalling pathway:

- LET-23 EGF receptor: important to be localised to the basolateral surface of the VPC to receive ligand
    - Lin2, Lin7 and Lin10 are important for LET-23 EGFR localisation.
    - **Mutants exhibit Vulvaless phenotype**, which can be rescued with EGFR overexpression.
        - Lin2/Lin7/Lin10 complex act to either tether EGFR on the membrane to transduce the LET-23 Ras pathway.
        - Or the complex bind to EGFR, target its trafficking to the membrane

- LIN-3 EGF ligand: important to be localised to the ventral side of the anchor cell, specific release
    - Unc-6 is important for the localised secretion of LIN-3 ventrally, mutant show delocalised LIN-3 in AC.
    - Unc-6 mutant**:** exhibit weak multivulva phenotype, more even secretion of LIN-3 lead to many cells adopting 1° fate.
    - Unc-6 mutant enhances the LET-23 vulvaless phenotype, lack of LET-23 + delocalised LIN-3 = less EGF signalling.

Anchor cell invasion components: Anchor cell would fuse with the vulva cells and form the uterine seam cells.

- Matric Metalloprotease: involved but not essential
    - **(Kelley et al., 2019)** MMP- mutant lead to slower BM breaching time, and more actin involvement (increased arp2/3 activity)
    - When RNAi silencing arp2/3 in MMP- mutants, there is a significant decrease in invasion rate
    - Further genetic RNAi screen shows ant-1.1 RNAi produce synergistic effect with MMP mutation
        - Ant-1.1 code for adenine nucleotide translocase, used in mitochondria, suggest role of mitochobndria

- Involvement of mitochondria
    - In WT and MMP worms, mitochondria probe shows increased localisation at invading site, with greater localisation in MMP- mutants, possibly as a compensatory mechanism.
    - ATP probe also shows higher and bursts of ATP concentration near the invading site
- Involvement of glucose transporter **(Garde et al., 2022)**
    - Fgt1 glucose transporter is localised at the ventral side regulated by Unc-6 Netrin
    - Fgt1 mutant shows less ATP production
    - Unc-6 mutant show defect in Fgt localisation, defect in ATP localisation and lack of actin polymerisation, lack of protrusion volume.