

C. elegans Vulval Development:

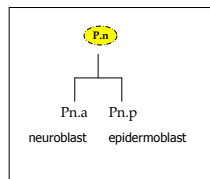
Induction at single cell resolution

The worm is born with 12 ectodermal 'P cells' along the ventral midline of the body.

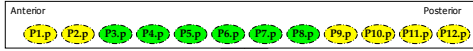


C. elegans L1 larva hatching

Each ectodermal P cell divides once to form a neuroblast and an epidermoblast.



The central Pn.p cells (P3-8.p) can give rise to vulva.

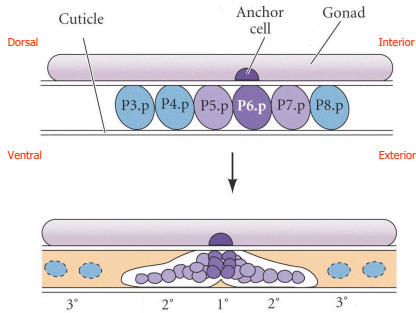


The central Pn.p cells are the vulval precursor cells (VPCs).



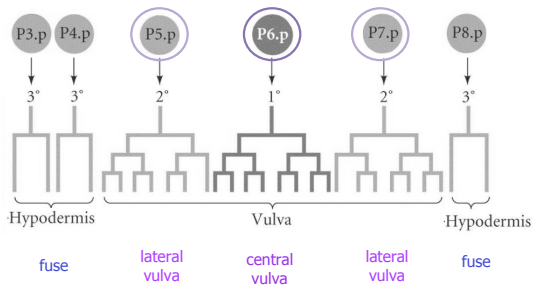
The vulva under construction during L4 larval stage

The central Pn.P cells can give rise to vulva.



Gilbert, Fig 6.19 (turned 90°)

P5 - 7.p normally form the vulva. The other cells fuse with the hypodermis.



Ablation experiments show that these 6 cells are equivalent

	Pn.p cell					
	3	4	5	6	7	8
normal	3°	3°	2°	1°	2°	3°
kill P6.p	3°	2°	1°	X	2°	3°
kill P6.p	3°	3°	2°	X	1°	2°
kill P5.p	3°	2°	X	1°	2°	3°
kill P5,6.p	3°	2°	X	X	1°	2°
kill P5-7.p	2°	1°	X	X	X	2°

The Anchor Cell (AC) is also essential for vulval formation.

	Pn.p cell					
	3	4	5	6	7	8
normal	3°	3°	2°	1°	2°	3°
kill AC	3°	3°	3°	3°	3°	3°
kill all other somatic gonad	3°	3°	2°	1°	2°	3°

The genetic approach:

Isolate mutants that alter vulval development

Mutants lacking a vulva may disrupt signaling from the AC to the VPCs - either sending or receiving the signal

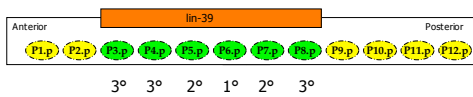
Mutants altering vulval formation were easy to isolate:
 Vul - vulvaless mutants - severe Vuls form a "bag-of-worms"



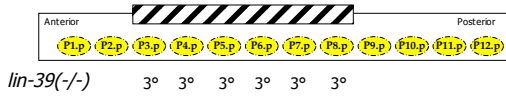
Mutants altering vulval formation were easy to isolate:
 Vul - vulvaless mutants
 Muv - multivulva mutants



The six central Pn.p cells (P3.p - P8.p) are specified as Vulval Precursor Cells (VPCs) by the central body HOX gene *lin-39*.



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In *lin-39(-)* mutants, all six VPCs fuse with the hypodermal syncytium (3° fate), leaving the animal vulvaless.

Examples of Vul mutants:

lin-39 (encodes HOM-C gene)

lin-3

let-23

sem-5

let-60

Example of Muv mutant:

lin-15

Genetic mosaic analysis shows where genes are functioning.

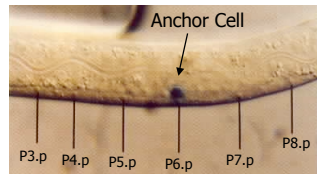
	Genotype of		Result
	AC	VPCs	
<i>lin-3(+)</i>	+	+	wt vulva
<i>lin-3(-)</i>	-	-	no vulva
Mosaics			
<i>lin-3(+/-)</i>	-	+	no vulva
<i>lin-3(+/-)</i>	+	-	wt vulva

Conclusion: *lin-3* function required in AC, but not VPCs

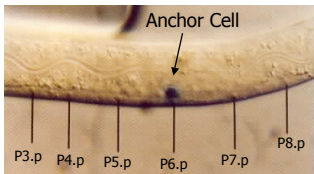
Where is the Lin-3 protein expressed?



lin-3::lacZ reporter gene fusion transgenic worm



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Genetic mosaic analysis shows where genes are functioning.

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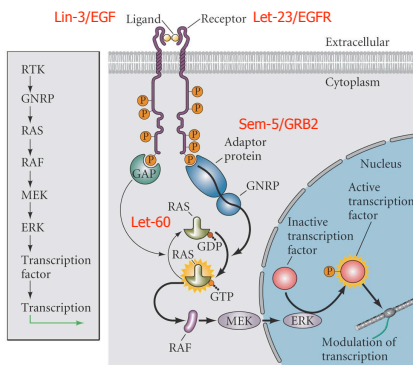
Conclusion: *let-23* function required in VPCs, but not AC

Molecular identities of genes involved in vulval formation:

Worm	Mammal	Type of protein
Lin-3	EGF	Growth factor - secreted signal
Let-23	EGF Receptor	Receptor Tyrosine Kinase
Sem-5	GRB2	SH2-SH3 adaptor
Let-60	Ras	GTP binding ('G protein')

All members of classic RTK signaling pathway

Figure 6.15 The Widely Used RTK Signal Transduction Pathway

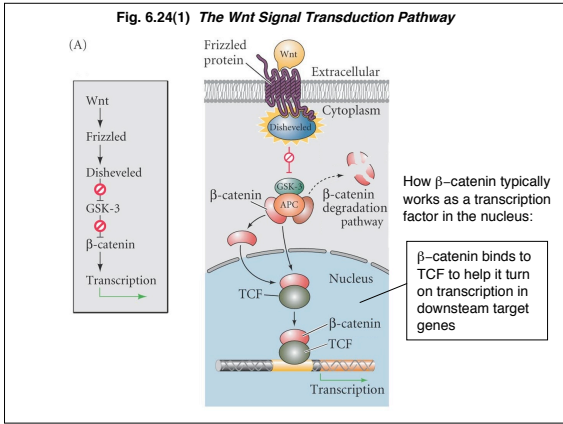


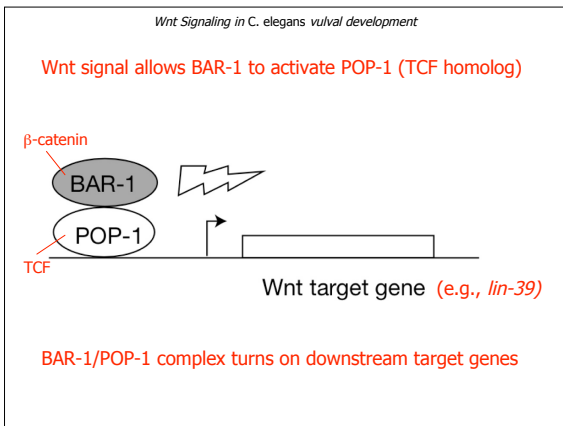
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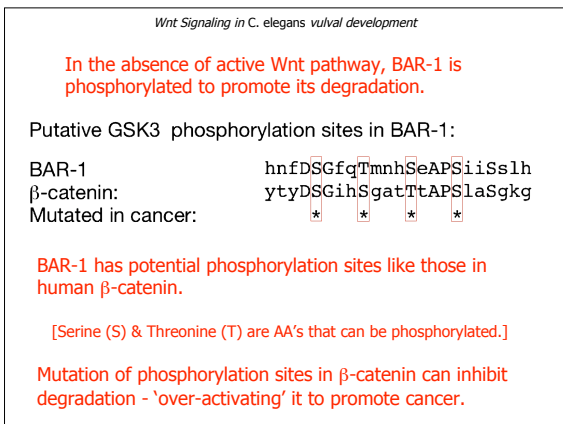
Wnt signaling in *C. elegans* vulva development activates *lin-39*

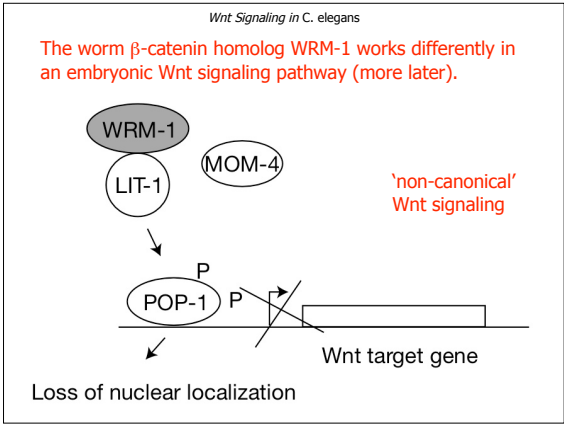
The vulval Wnt signaling pathway is 'canonical'

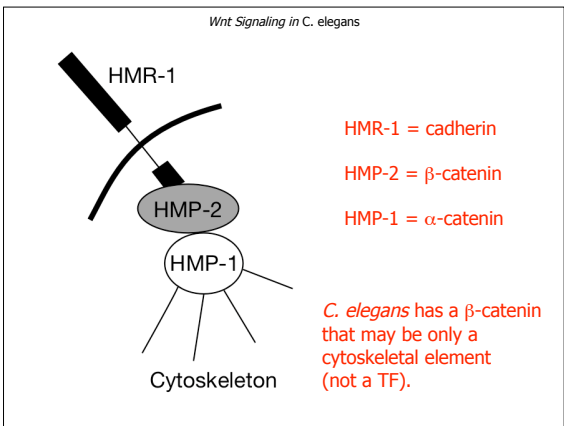
β -catenin homolog in vulva signaling is BAR-1

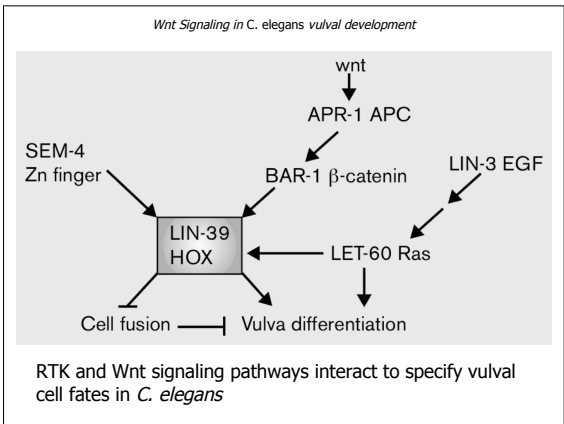


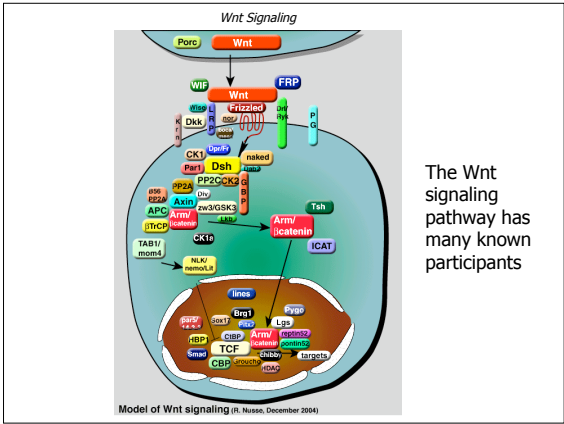












The Wnt signaling pathway has many known participants
