

C. elegans Vulval Development:

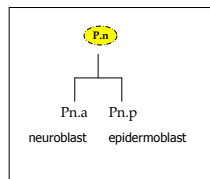
Induction at single cell resolution

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

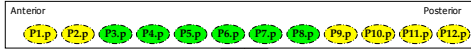


C. elegans L1 larva hatching

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.

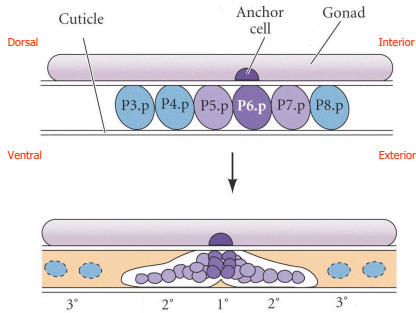


The central Pn.p cells are the vulval precursor cells (VPCs).



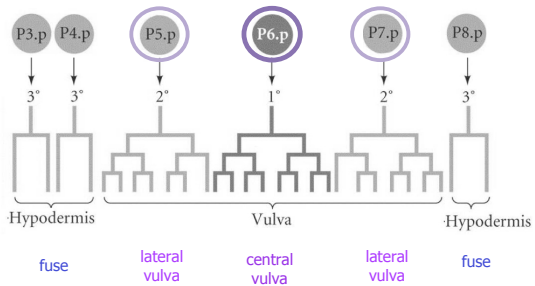
The vulva under construction during larval stage 4 (L4)

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

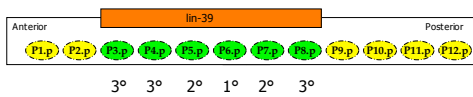
Mutants altering vulval formation were easy to isolate:
 Vul - vulvaless mutants - severe Vuls form a "bag-of-worms"



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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lin-39(-/-) 3° 3° 3° 3° 3° 3°

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.

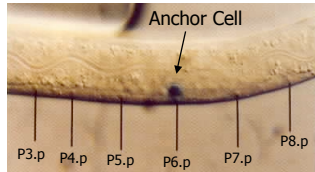
	Genotype of		Result
	AC	VPCs	
<i>lin-3</i> (+)	+	+	wt vulva
<i>lin-3</i> (-)	-	-	no vulva
Mosaics			
<i>lin-3</i> (+/-)	-	+	no vulva
<i>lin-3</i> (+/-)	+	-	wt vulva

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

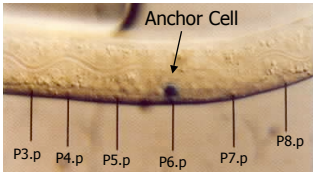
Where is the Lin-3 protein expressed?



lin-3::lacZ reporter gene fusion transgenic worm



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Genetic mosaic analysis shows where genes are functioning.

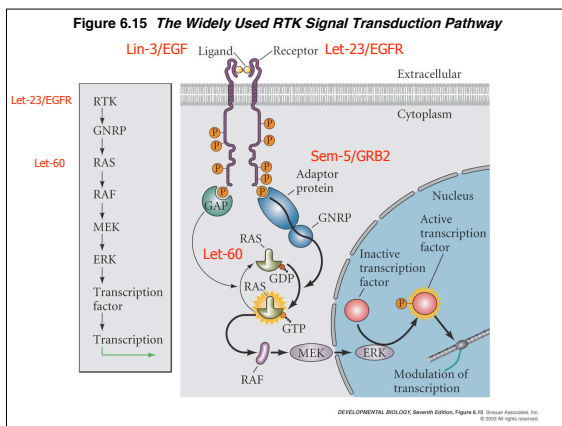
	Genotype of		Result
	AC	VPCs	
<i>let-23(+)</i>	+	+	wt vulva
<i>let-23(-)</i>	-	-	no vulva
Mosaics			
<i>let-23(+/-)</i>	-	+	wt vulva
<i>let-23(+/-)</i>	+	-	no vulva

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

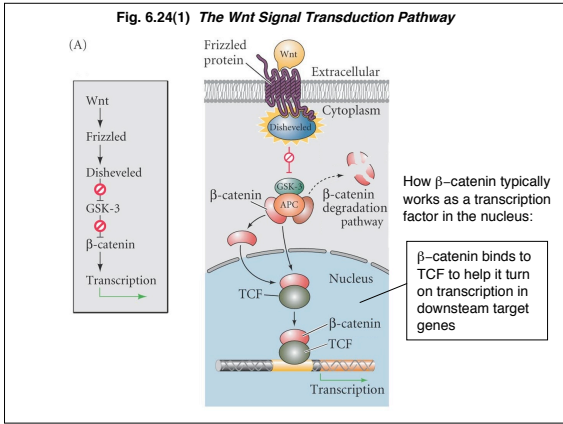


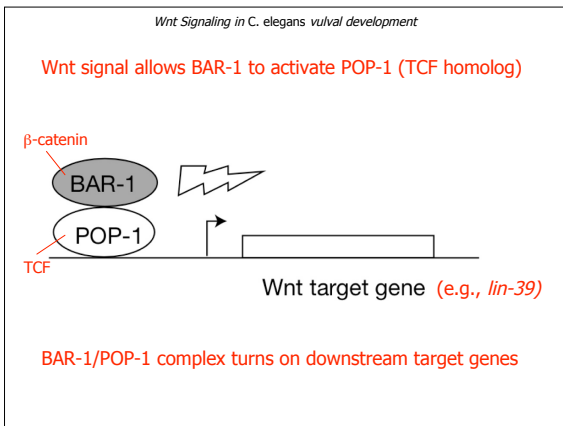
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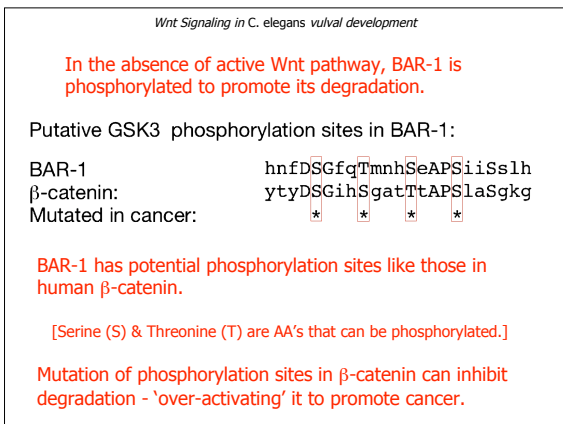
Wnt signaling in *C. elegans* vulva development activates *lin-39*

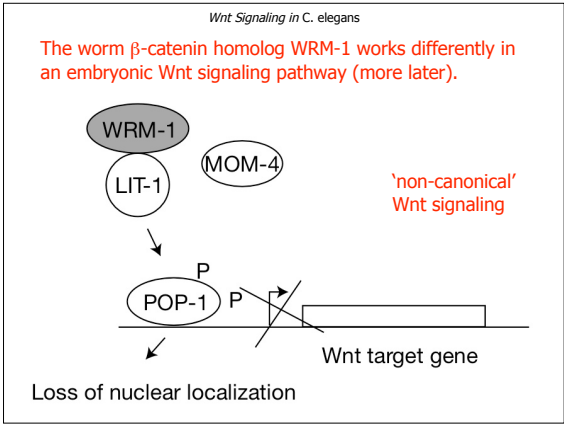
The vulval Wnt signaling pathway is 'canonical'

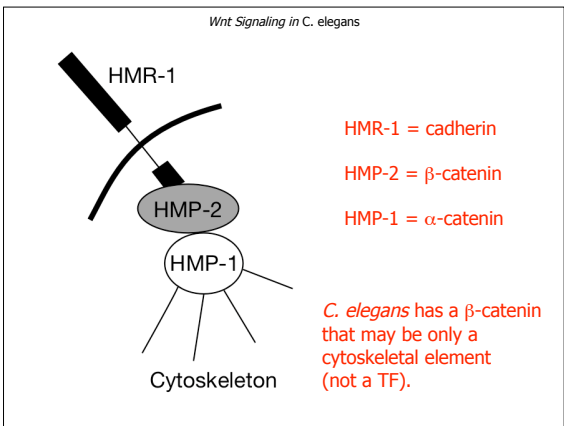
β -catenin homolog in vulva signaling is BAR-1

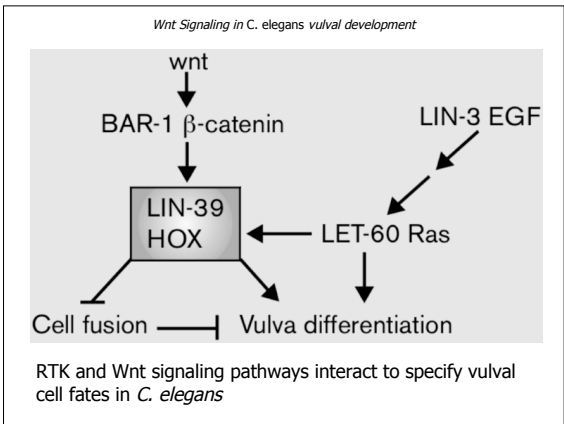


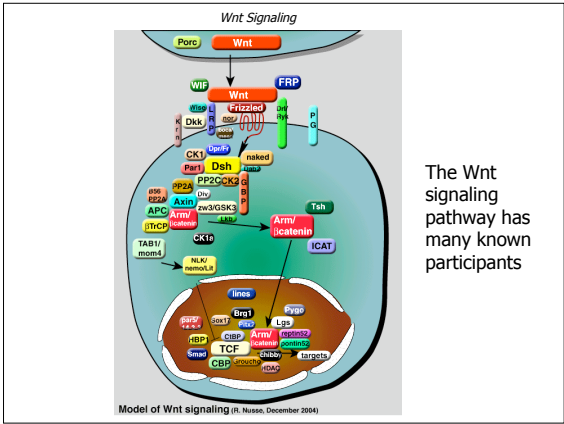












The Wnt signaling pathway has many known participants
