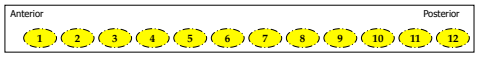
*C. elegans* Vulval Development:

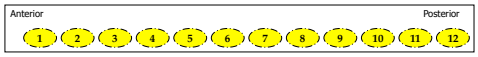
Induction at single cell resolution

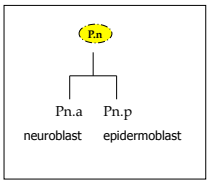
The worm is born with 12 ectodermal 'P cells' along the ventral midline of the body.



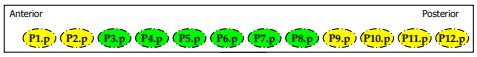

*C. elegans* L1 larva hatchling

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



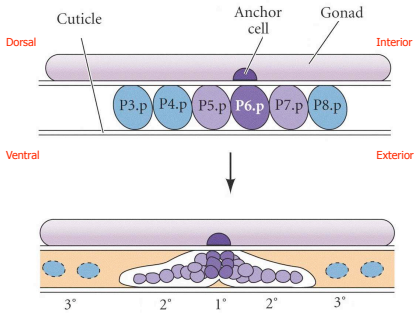
The central Pn.p cells (P3-8.p) can give rise to vulva.




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

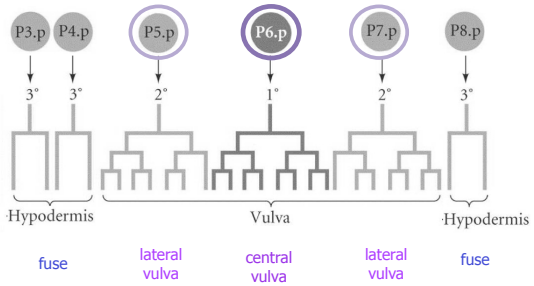
The vulva under construction during larval stage 4 (L4)

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 essential to signal 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° |

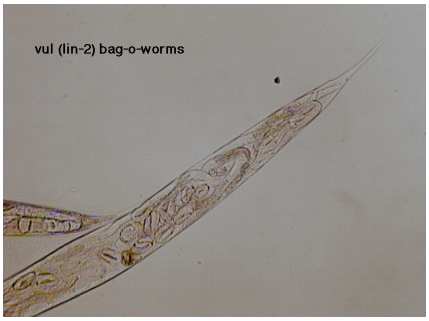
The genetic approach:

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"

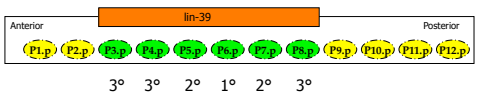



Mutants altering vulval formation were easy to isolate:

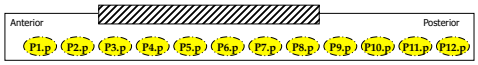
Vul - vulvaless mutants

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*.



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*.




*lin-39(-/-)* 3° 3° 3° 3° 3° 3°

In *lin-39(-)* mutants, all six VPCs fuse with the hypodermal syncitium (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*

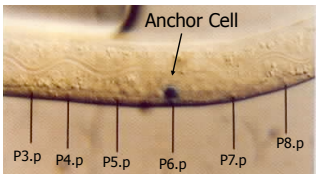
Genetic mosaic analysis shows where genes are functioning.

| | Genotype of | | |
|---|---|---|---|
| | AC | VPCs | Result |
| *lin-3(+/+)* | + | + | wt vulva |
| *lin-3(-/-)* | - | - | no vulva |
| Mosaics | | | |
| *lin-3(+/-)* | - | + | no vulva |
| *lin-3(+/-)* | + | - | wt vulva |

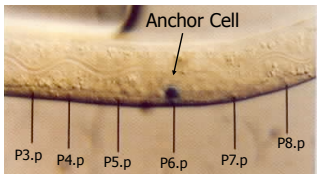
Conclusion: *lin-3* function required in AC, but not VPCs

Where is the Lin-3 protein expressed?

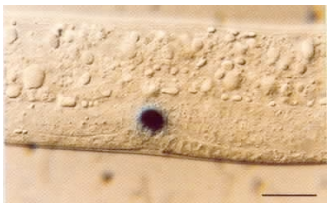
*lin-3::lacZ* reporter gene fusion transgenic worm




Where is the Lin-3 protein expressed?




*lin-3::lacZ* reporter gene fusion transgenic worm



Genetic mosaic analysis shows where genes are functioning.

| | Genotype of | | |
|---|---|---|---|
| | AC | VPCs | Result |
| *let-23(+/+)* | + | + | wt vulva |
| *let-23(-/-)* | - | - | no vulva |
| Mosaics | | | |
| *let-23(+/-)* | - | + | wt vulva |
| *let-23(+/-)* | + | - | no vulva |

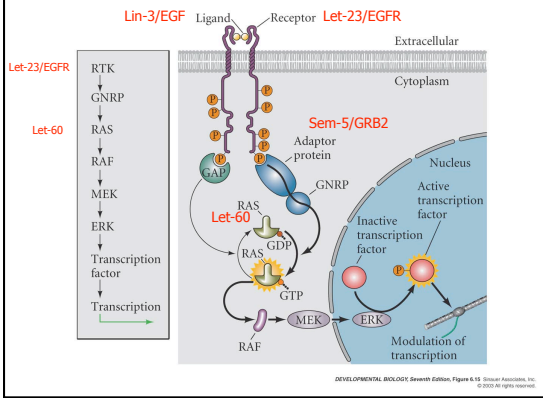
Conclusion: *let-23* function required in VPCs, but not AC

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

**Figure 6.15** ***The Widely Used RTK Signal Transduction Pathway***



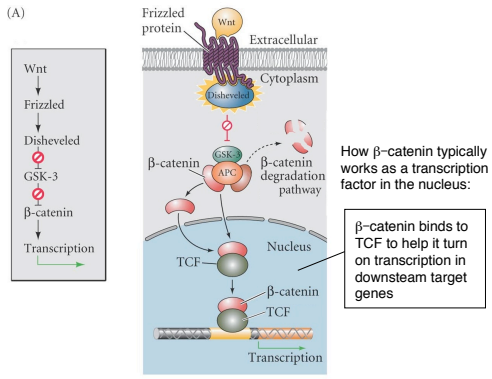

Molecular identities of genes involved in vulval formation:

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

**Fig. 6.24(1)** ***The Wnt Signal Transduction Pathway***

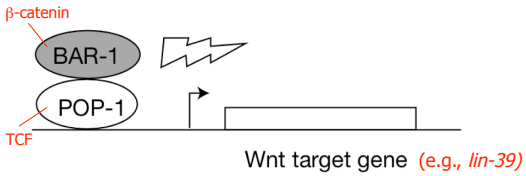



How β-catenin typically works as a transcription factor in the nucleus:

β-catenin binds to TCF to help it turn on transcription in downsteam target genes

*Wnt Signaling in* C. elegans *vulval development*

Wnt signal allows BAR-1 to activate POP-1 (TCF homolog)




BAR-1/POP-1 complex turns on downstream target genes

*Wnt Signaling in* C. elegans *vulval development*

In the absence of active Wnt pathway, BAR-1 is phosphorylated to promote its degradation.

Putative GSK3 phosphorylation sites in BAR-1:

```
BAR-1                hnfDSGfqTmnhSeAPSiiSslh
β-catenin:           ytyDSGihSgatTtAPSlaSgkg
Mutated in cancer:      *   *   *   *
```

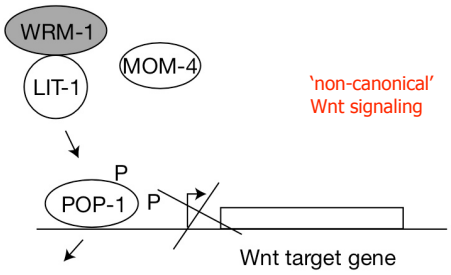
BAR-1 has potential phosphorylation sites like those in human β-catenin.

[Serine (S) & Threonine (T) are AA's that can be phosphorylated.]

Mutation of phosphorylation sites in β-catenin can inhibit degradation - 'over-activating' it to promote cancer.

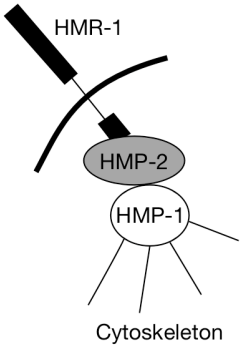
*Wnt Signaling in* C. elegans

The worm β-catenin homolog WRM-1 works differently in an embryonic Wnt signaling pathway (more later).




Loss of nuclear localization

*Wnt Signaling in* C. elegans



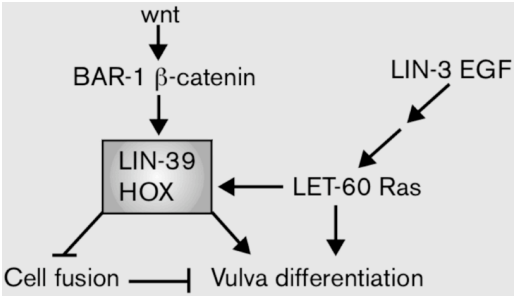

HMR-1 = cadherin

HMP-2 = β-catenin

HMP-1 = α-catenin

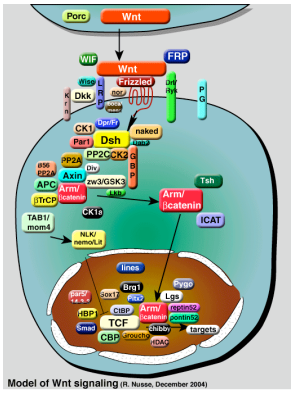
*C. elegans* has a β-catenin that may be only a cytoskeletal element (not a TF).

*Wnt Signaling in* C. elegans *vulval development*




RTK and Wnt signaling pathways interact to specify vulval cell fates in *C. elegans*

*Wnt Signaling*




The Wnt signaling pathway has many known participants