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 1):

cell fates, laser ablations, multipotential, equivalence group, pattern formation, inductive signal, graded signal, action at a distance, EGF signaling, lateral inhibition, lateral signal, Notch signaling, sequential signaling

Major concepts (LECTURE 1):

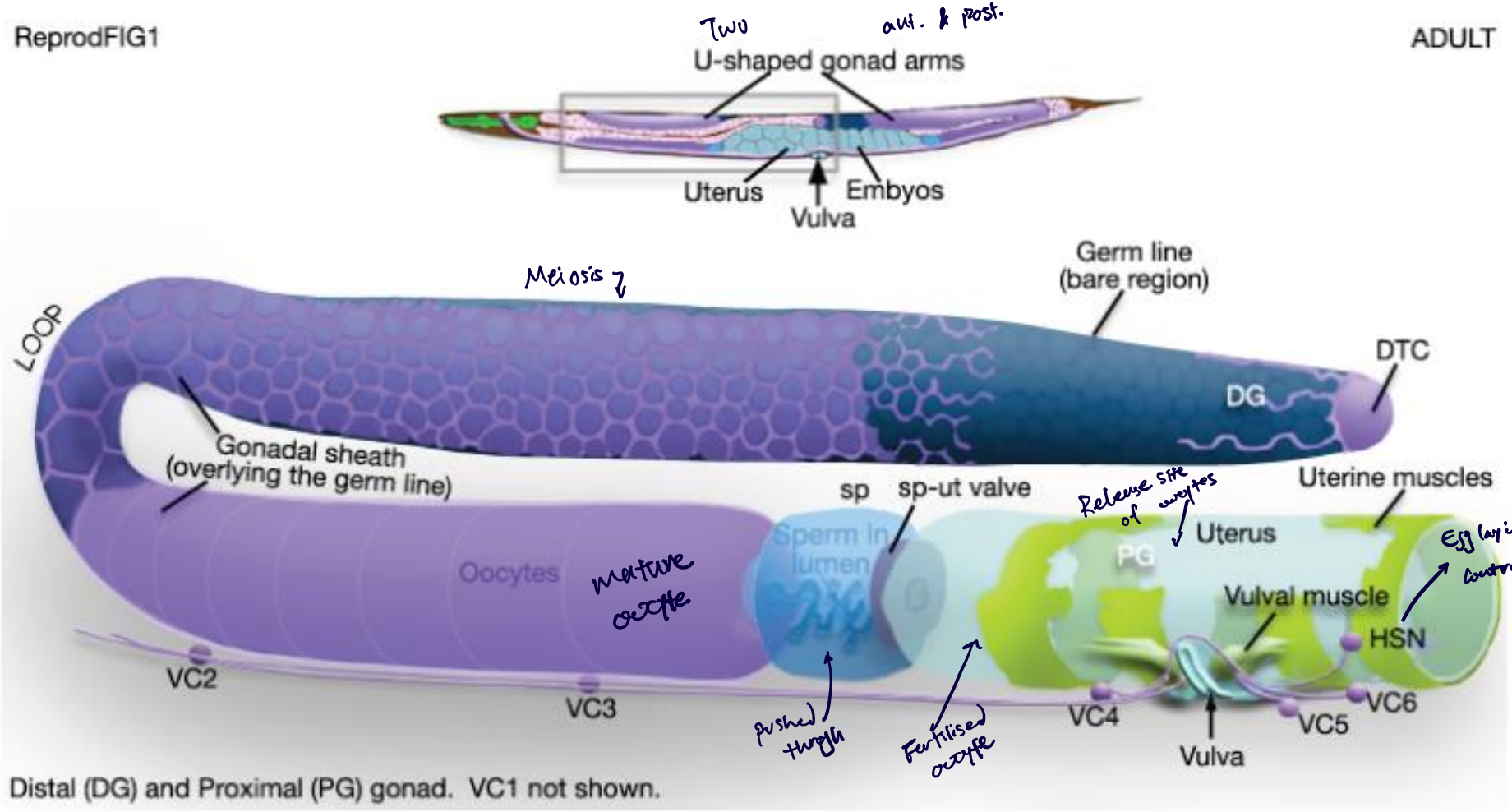
Vulval development is a multistep process.
The vulval equivalence group is a group of multipotential cells.
The commitment of vulval precursor cells to a specific fate is dependent on several signaling events.
EGF and Notch signaling are coupled during VPC patterning.

References

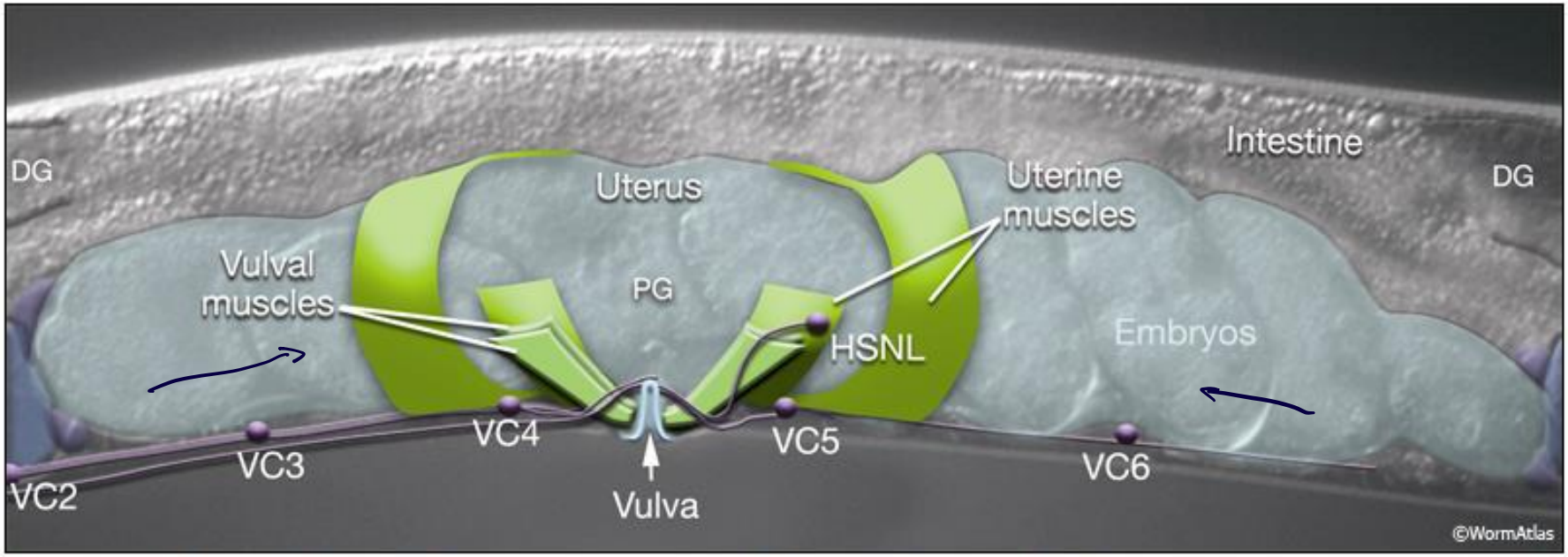
Sternberg, P., 2005, WormBook	**http://www.wormbook.org/**
Gupta et al, 2012, WormBook	**http://www.wormbook.org/**
WormAtlas	**http://www.wormatlas.org/**

C. elegans anatomy

ANATOMY OF THE HERMAPHRODITE REPRODUCTIVE SYSTEM



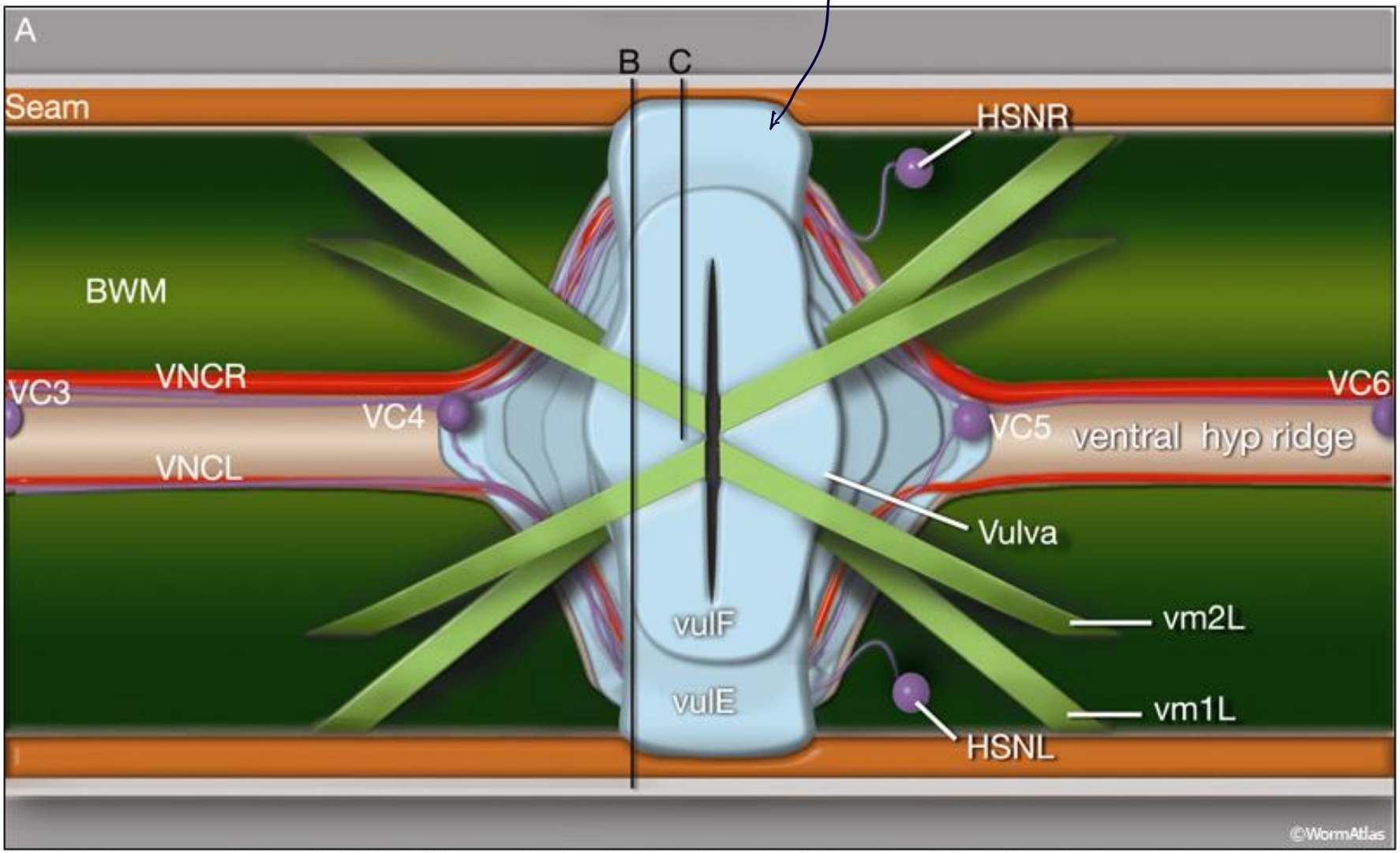
The egg-laying apparatus of an adult hermaphrodite (side view)



EggFIG 1: DIC image of adult hermaphrodite midbody region. Lateral view, left side. Tissues that contribute to the egg-laying apparatus are highlighted by the color overlay. (DG) Distal gonad; (PG) proximal gonad; VC1-6 and HSNL are motor neurons that control egg-laying. VC1 (not shown) is located more anteriorly, outside the field of view. Magnification, 400x.

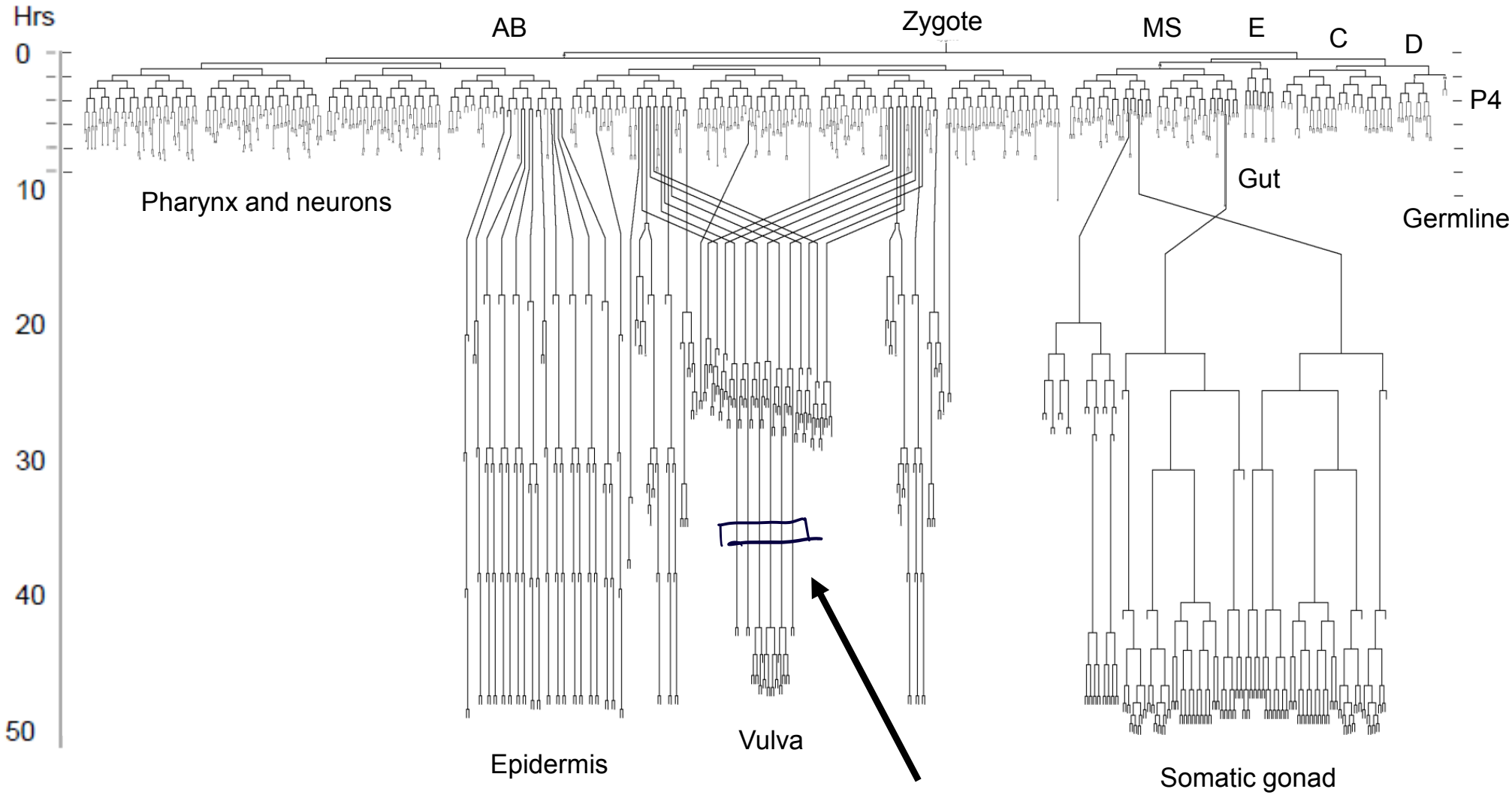
Muscles
Neurons
Uterus (somatic gonad)
Vulva (epidermal structure)

The egg-laying apparatus of an adult hermaphrodite (inside-out view)



EggFIG 16A: Egg-laying neurons. Schematic of the adult hermaphrodite midbody region, dorsal view, showing the vulva, vulval muscles (vm), and egg-laying neurons (VC1 and VC2 cell bodies not shown). (BWM) Body wall muscle; (VNCL, VNCR) ventral nerve cord, left and right fascicle, respectively.

The essentially invariant *C. elegans* cell lineage



6 vulval precursor cells (VPCs)

Sulston and Horvitz, 1977
Sulston et al., 1983

Vulval development is a stepwise process

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

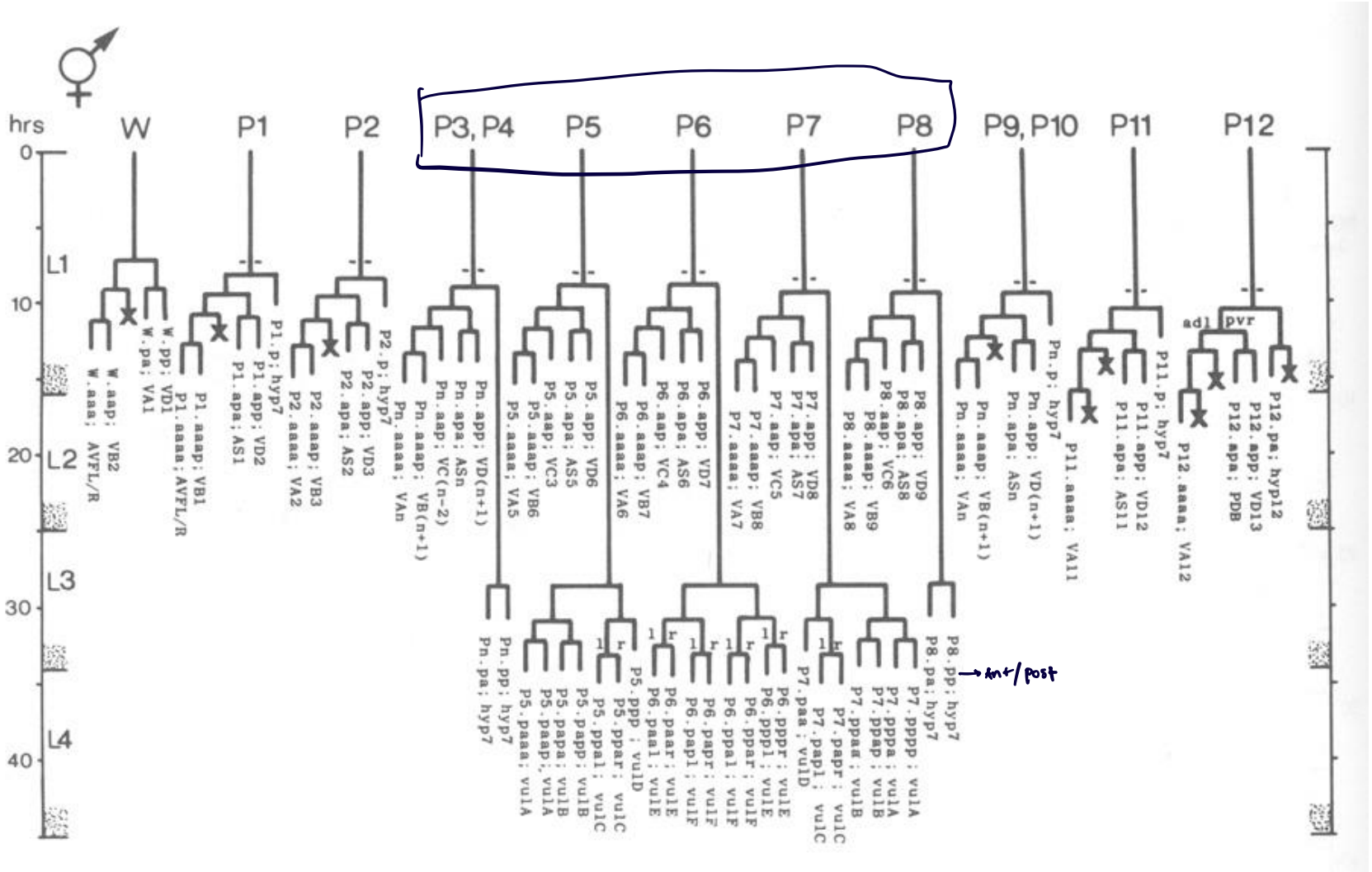
STEP 2 Vulval precursor patterning (1˚, 2 ˚, 3 ˚ fate)

STEP 3 Generation of adult cells

STEP 4 Anchor cell (AC) invasion

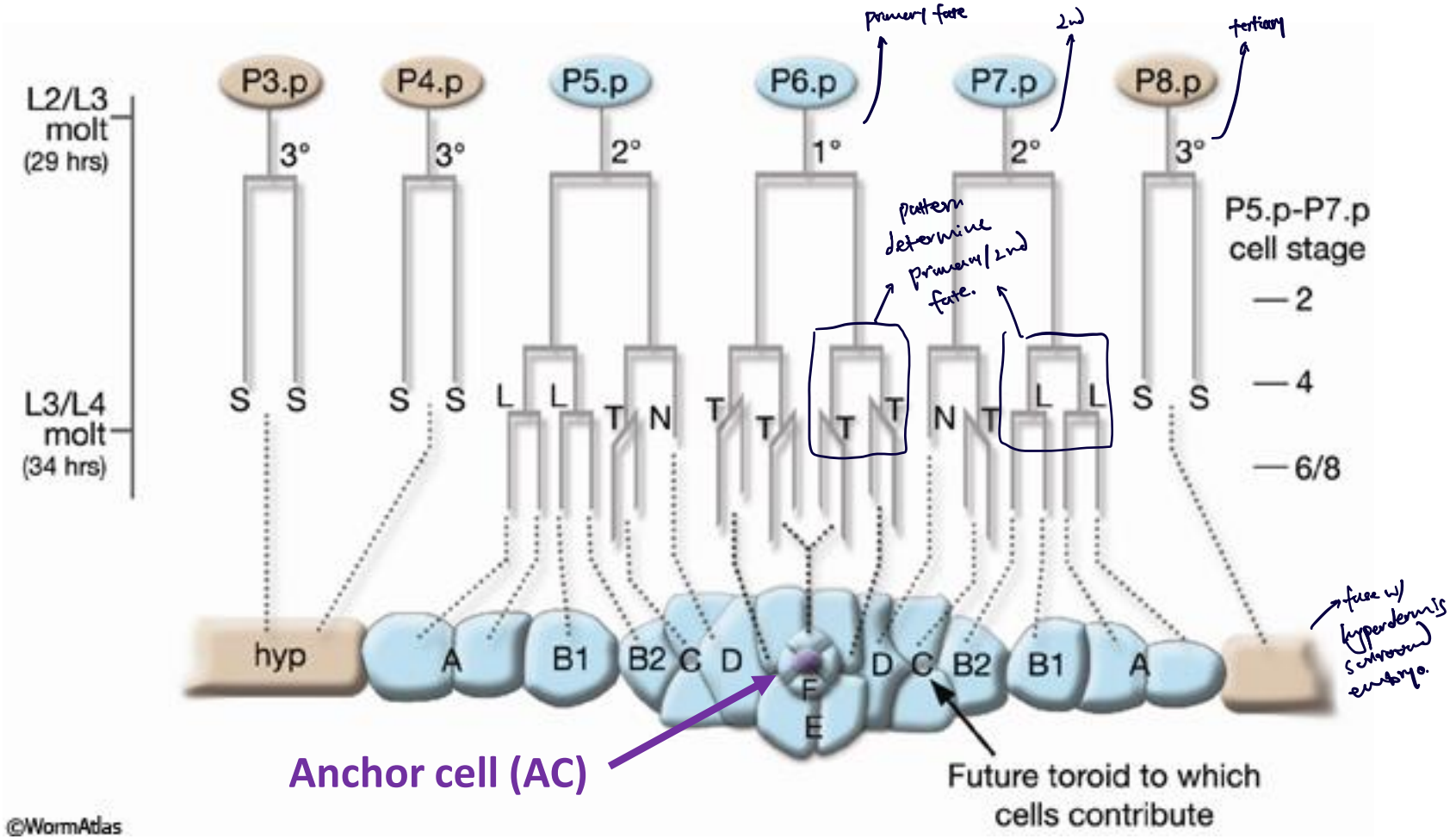
STEP 5 Morphogenesis of the vulva

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



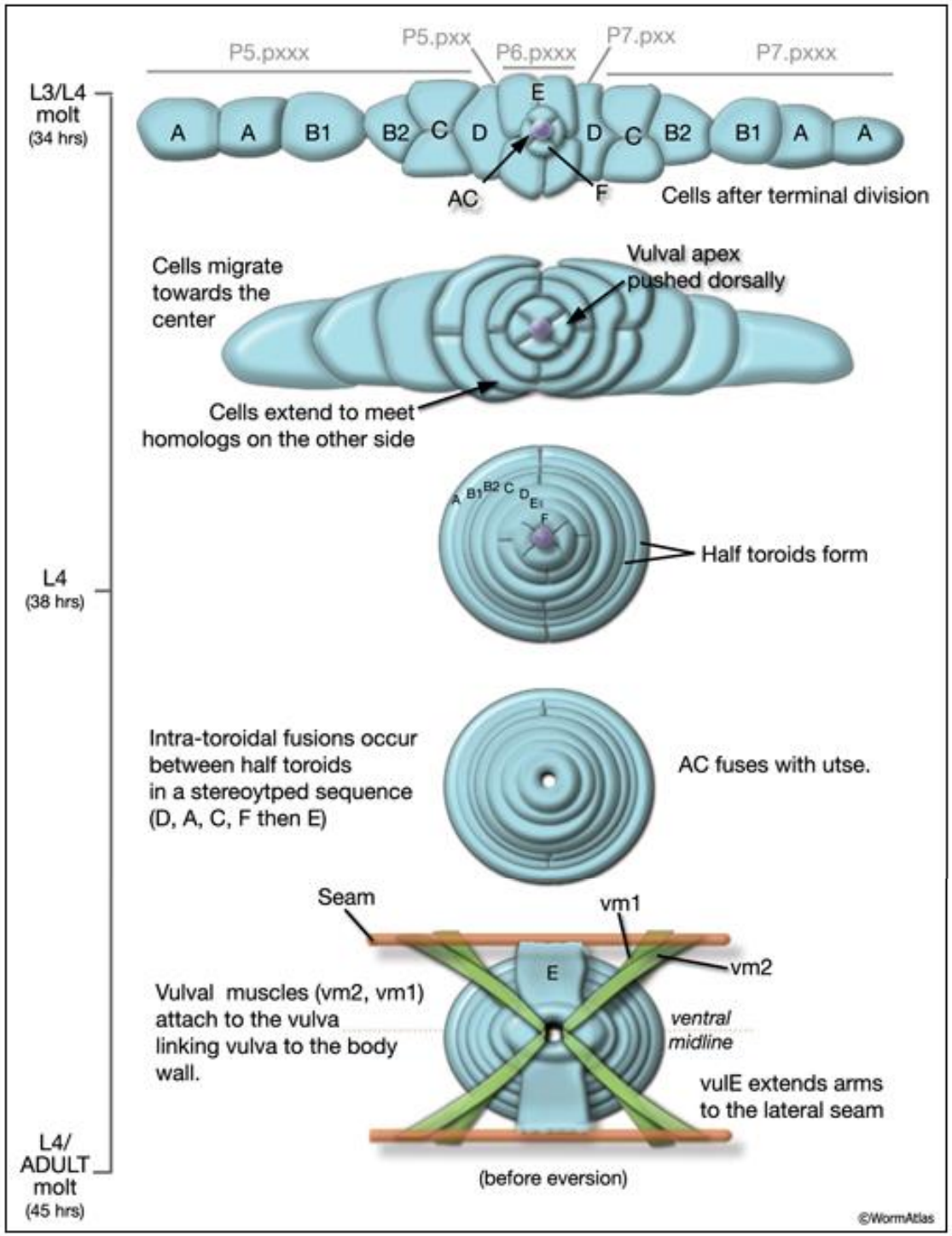
Sulston and Horvitz, 1977
Sulston et al., 1983

STEP 2, 3 and 4:
Patterning of VPCs, generation of adult cells, invasion of AC



- P5.p, P6.p, and P7.p adopt **vulval cell fates** (**1º or 2º**); their descendants form the vulva

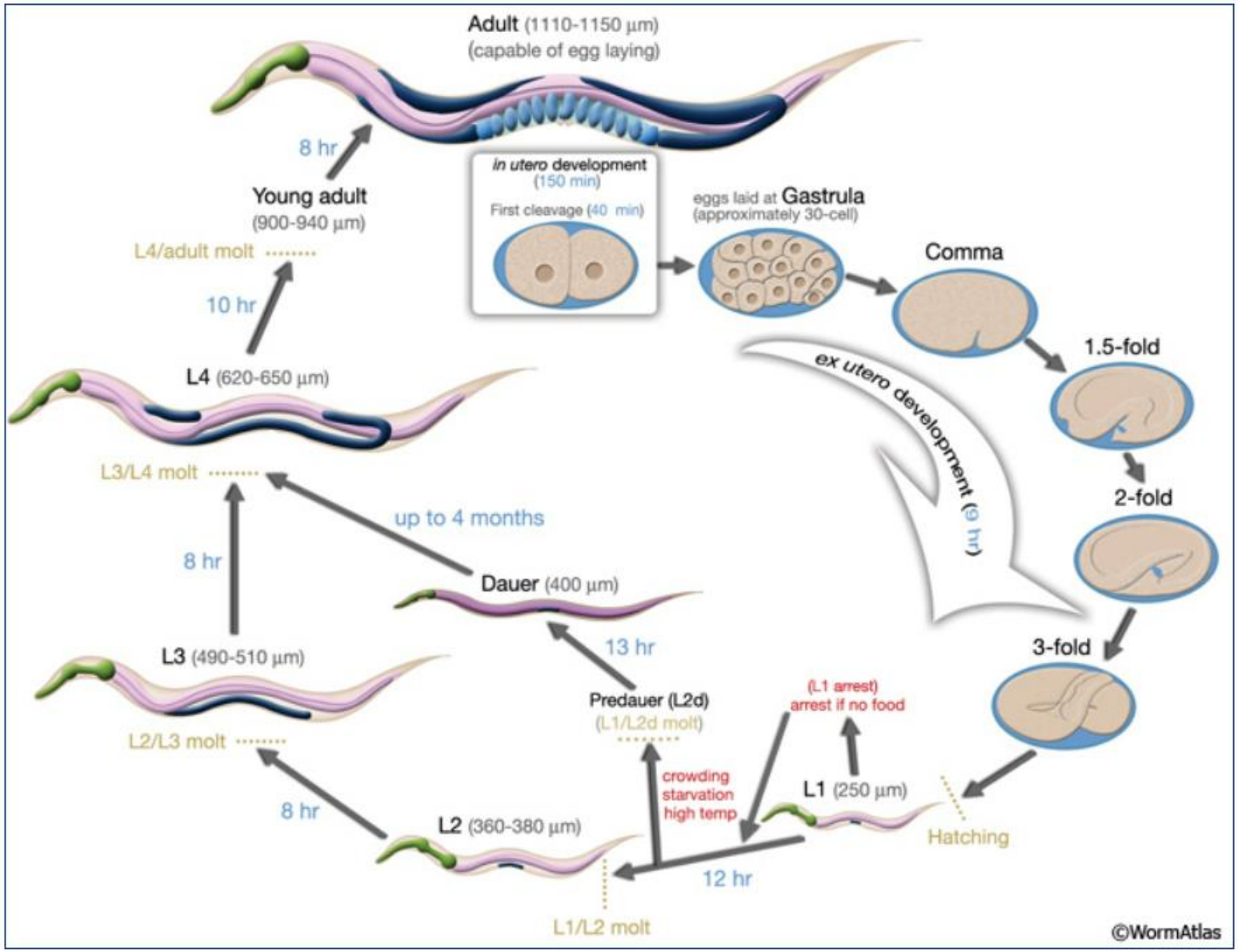
- P3.p, P4.p, and P8.p adopt a **non-vulval cell fate** (**3º**); their descendants fuse with the hypodermis



STEP 5:

Morphogenesis

Life cycle of *C. elegans* at 22°C



Vulval development is a stepwise process

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

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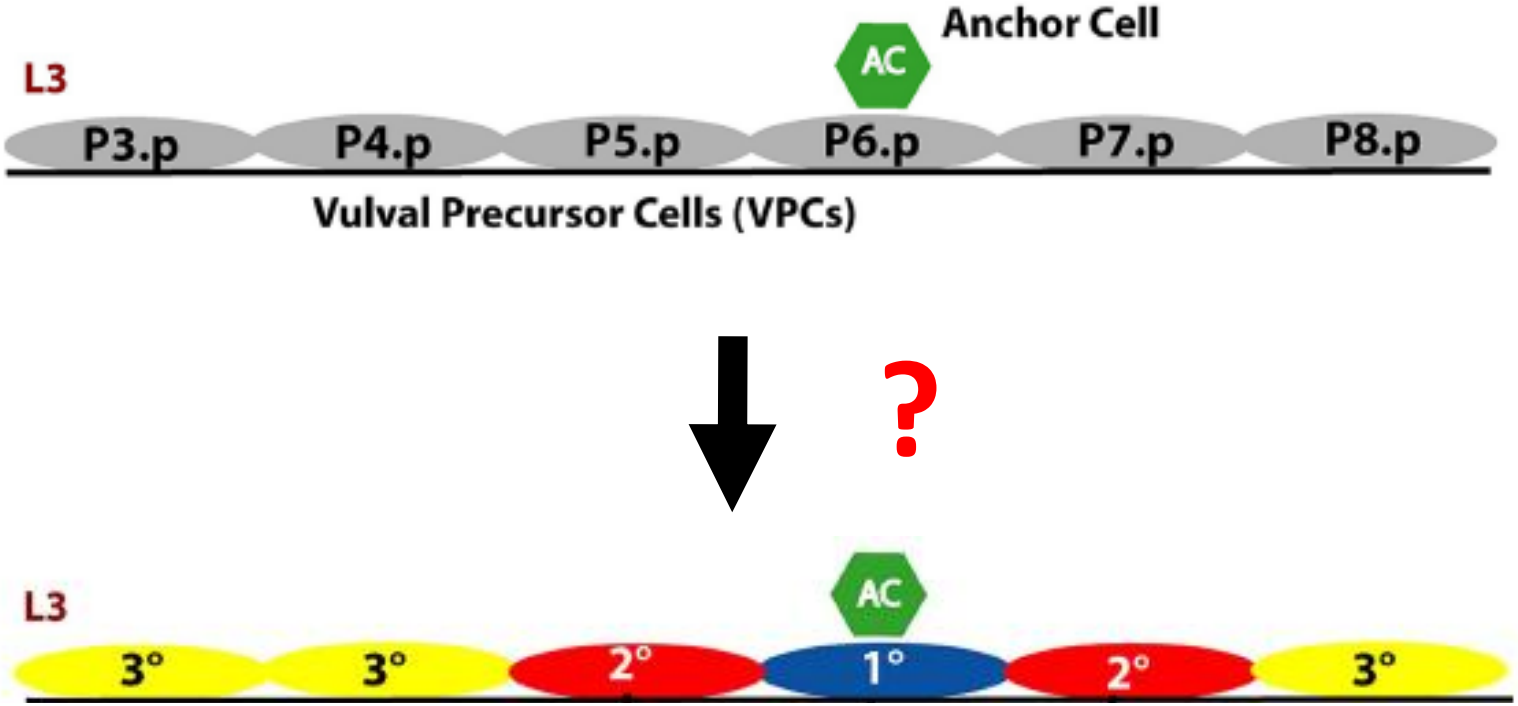
STEP 4 Anchor cell (AC) invasion

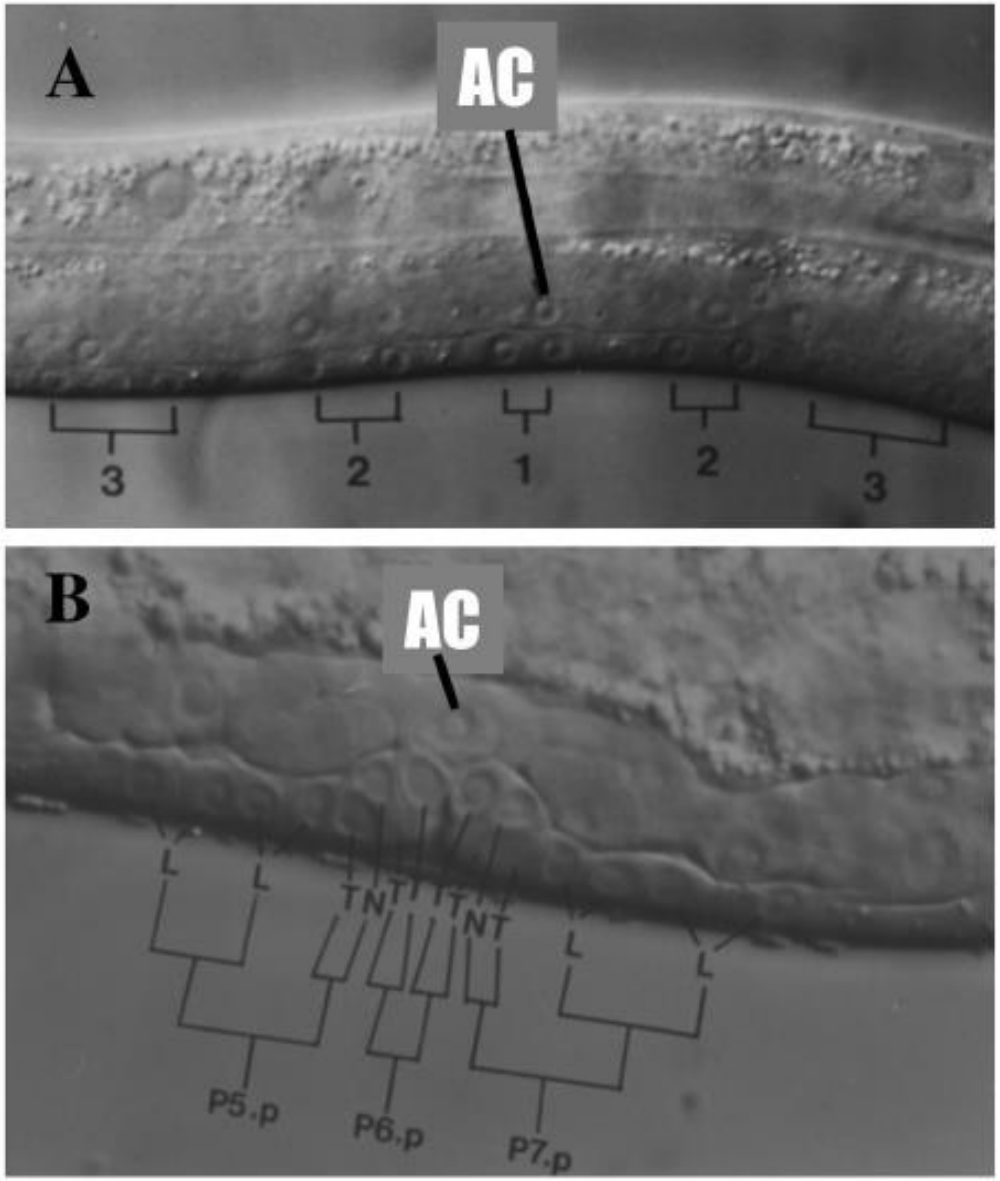
STEP 5 Morphogenesis of the vulva

Step 2 - Vulval precursor patterning (1˚, 2 ˚, 3 ˚ fate)



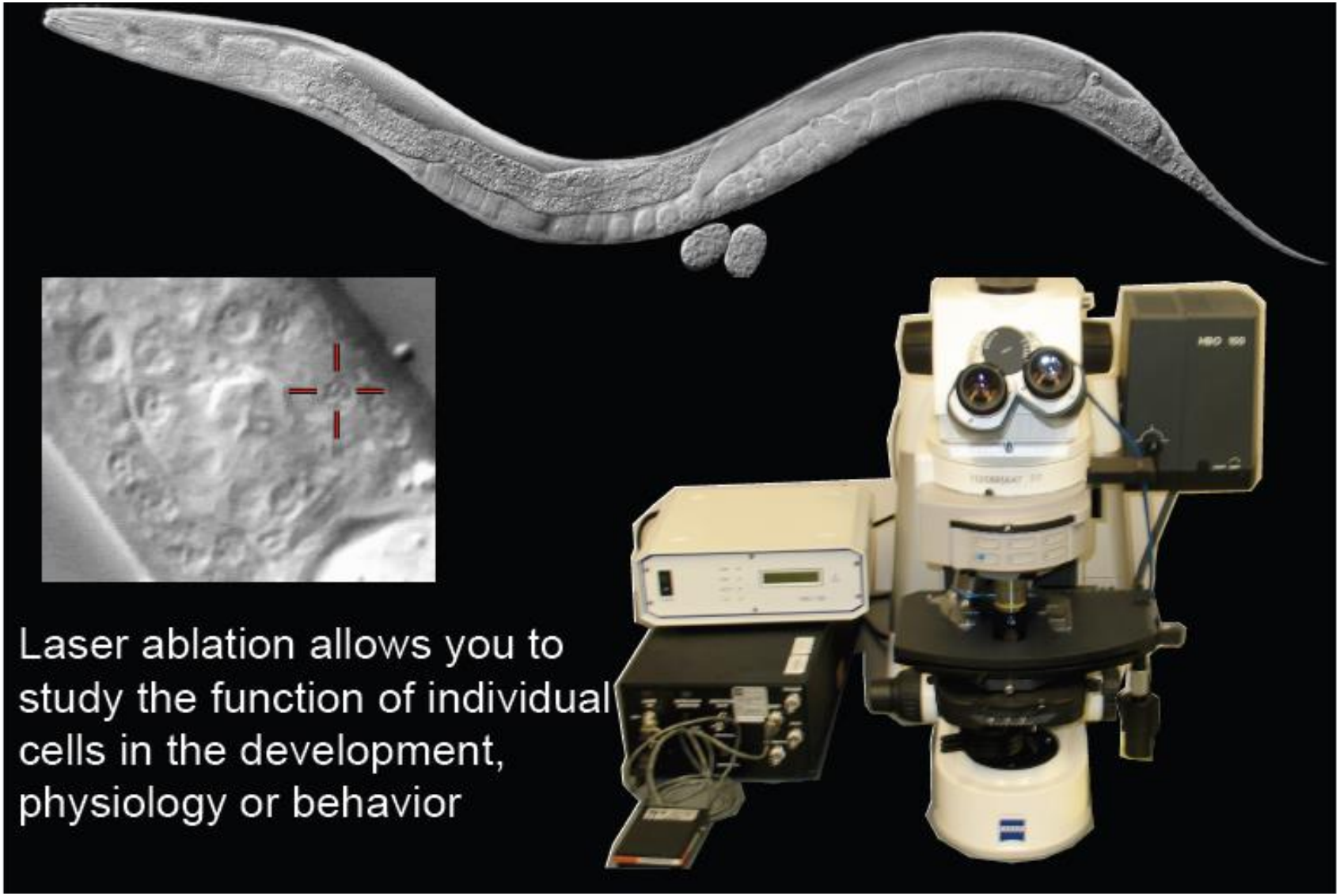
Sternberg, 2005, WormBook

Cell lineage analysis

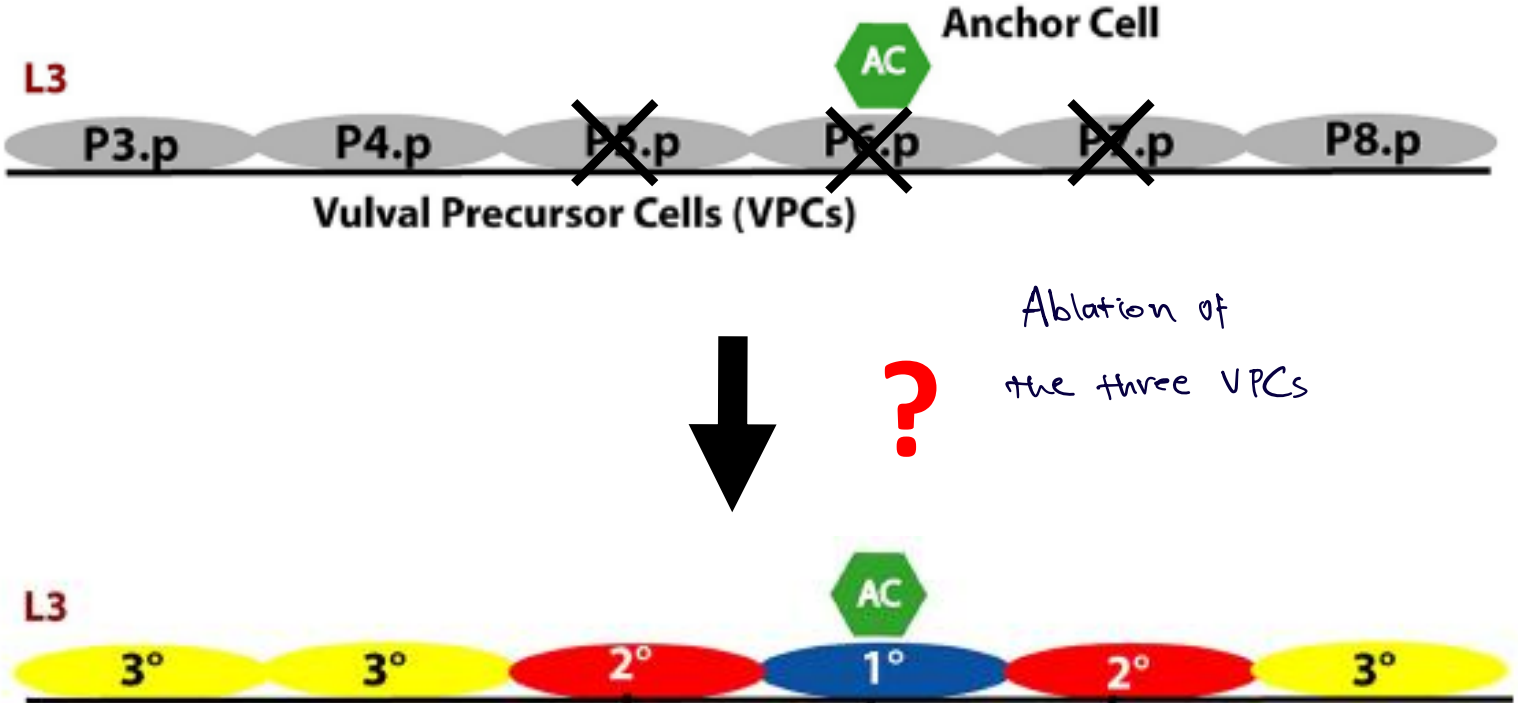


Sternberg, 2005, WormBook

Microsurgery – Laser ablations



Step 2 - Vulval precursor patterning (1˚, 2 ˚, 3 ˚ fate)



Sternberg, 2005, WormBook

DEVELOPMENTAL BIOLOGY 78, 577–597 (1980)

Regulation and Cell Autonomy during Postembryonic Development of *Caenorhabditis elegans*

J. E. SULSTON AND J. G. WHITE

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TABLE 1

REGULATION IN THE VENTRAL HYPODERMIS OF THE HERMAPHRODITE[a]

Ablated	No. of animals	No. of descendants from						Egg Laying
		P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	
0	Many	1–2	2	7	8	7	2	+
P6.p	2	1–2	7	8	—	7	2	+
P(4, 5).p	1	2	—	—	8	7	2	+
P(5, 6).p	1	1	1 + 4	—	—	8	7	+
P(5, 7).p	1	2	8	—	8	—	8	+
P(7, 8).p	1	2	2	7	8	—	—	+
P(5–7).p	1	8	8	—	—	—	8	Slow
P(5–7).p	1	1	8	—	—	—	8	Slow
P(3, 4, 5, 7, 8).p	1	—	—	—	8	—	—	Slow
P(3, 4, 6, 7, 8).p	1	—	—	8	—	—	—	–
P(3–8)	3	—	—	—	—	—	—	–
P(1, 2, 9–12)	2	1–2	2	7	8	7	2	+

originally Tertiary cells can generate Primary fate, lay eggs.

[a] Ablations performed 10–12 hr after hatching, except for P(1–12) which were ablated in the first hour after hatching. Heavy type: incorporated into vulva; italic type: form pseudovulvas; normal type: fuse with large hypodermal syncytium.

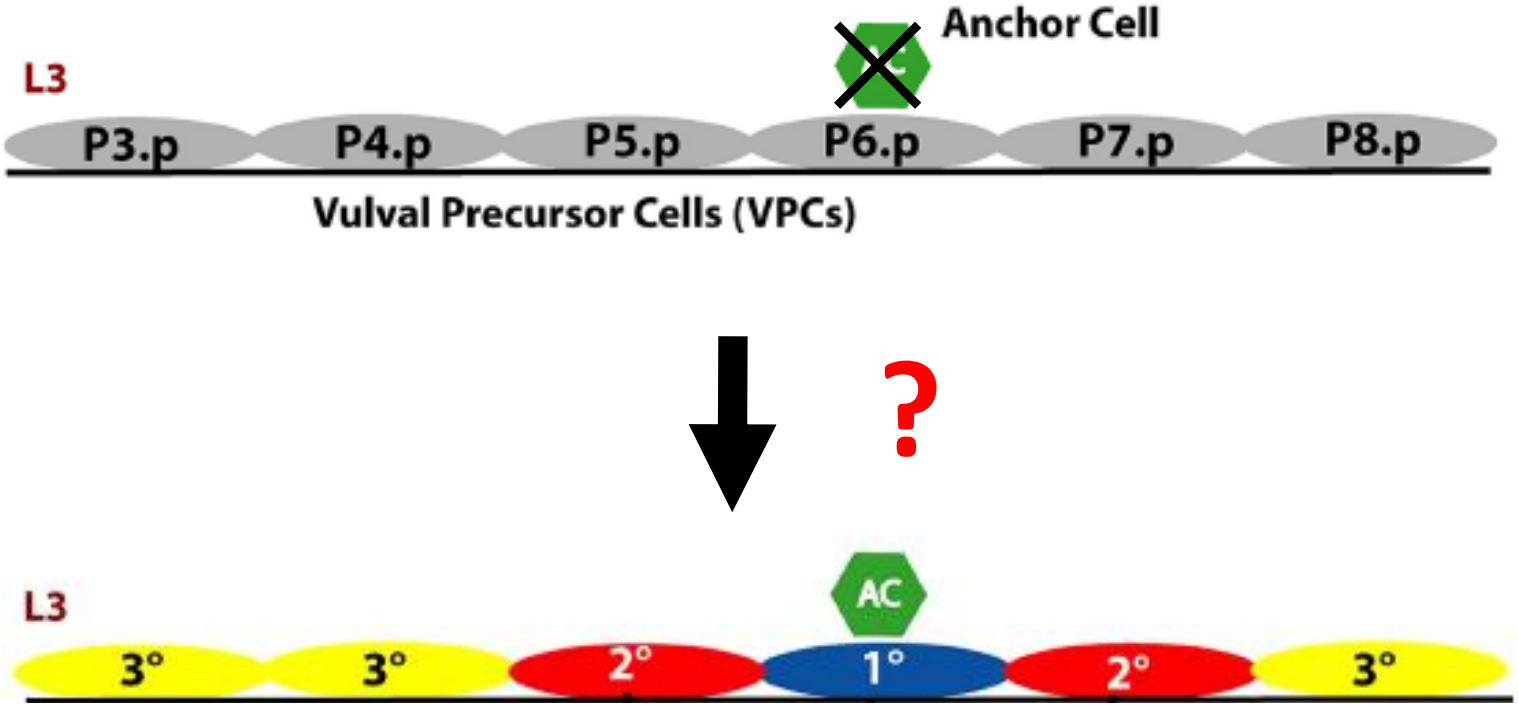
Result:

- If P5.p-P7.p are ablated, P3.p, P4.p and P8.p can adopt vulval cell fates (1˚ or 2˚).

Conclusion:

- P3.p-P8.p (but not their descendents) are ‘multipotential’ and form the ‘vulval equivalence group’.

Step 2 - Vulval precursor patterning (1˚, 2 ˚, 3 ˚ fate)



Sternberg, 2005, WormBook

DEVELOPMENTAL BIOLOGY 87, 286-300 (1981)

Alterations in Cell Lineage following Laser Ablation of Cells in the Somatic Gonad of *Caenorhabditis elegans*

JUDITH KIMBLE

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TABLE 3

TIMING OF VULVA INDUCTION

Cell ablated	Stage of development[a]	Number of animals	Vulva induction[b]			
			−	+	++	+++
Anchor cell	24–26	5	4	1	0	0
	27	8	4	4	0	0
	28	13	0	3	9	1
	29–31	13	0	0	4	8

[a] The time of the operation was scored in hours after hatching (20°C) by standards obtained by Sulston and Horvitz (1977) and by

Results:

- If the anchor cell is ablated, no vulva is induced (P3.p-P8.p all adopt 3˚ fate).
- Timing of ablation is important.

sors, Pn.p, is occurring or has just occurred.

[b] The development of the vulva was scored in early L4 as follows:

Conclusion:

- The anchor cell is required for VPC patterning and hence vulval development.

Cell, Vol. 44, 761–772, March 14, 1986, Copyright © 1986 by Cell Press

Pattern Formation during Vulval Development in C. elegans

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Table 4. Fates of Isolated Pn.p Cells

Experiment	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p
normal	S S	S S	LLTN	TTTT	NTLL	S S
1	TTTO	- - - -	- - - -	- - - -	- - - -	- - - -
2	LLTO	- - - -	- - - -	- - - -	- - - -	- - - -
3	(LLTT)		- - - -	- - - -	- - - -	- - - -
4	- - - -	LTTT	- - - -	- - - -	- - - -	- - - -
5	- - - -	LLTN	- - - -	- - - -	- - - -	- - - -
6	- - - -	- - - -	TTTT	- - - -	- - - -	- - - -
7	- - - -	- - - -	TTTT	- - - -	- - - -	- - - -
8	- - - -	- - - -	- - - -	- - - -	TTTT	- - - -
13						OTTO

Isolated Pn.p cells were obtained as described in Experimental Procedures using *unc-84* hermaphrodites. Dashes indicate ablated cells. ac indicates the relative position of the anchor cell with respect to existing Pn.p cells. The Pn.p cells in most cases moved toward the anchor cell (see Figure 4). Parentheses indicate that an isolated cell was either P3 or P4, or P7 or P8, due to uncertainty in following L1 migrations of P3/4L and P3/4R or P7/8L and P7/8R, respectively (see Experimental Procedures).

← Division Plates.

'Isolated VPCs'

unc-84 mutant background

Anchor cell position variable.

when anchor cell far away
P8 do not adopt 1°/2° fate.

Result:

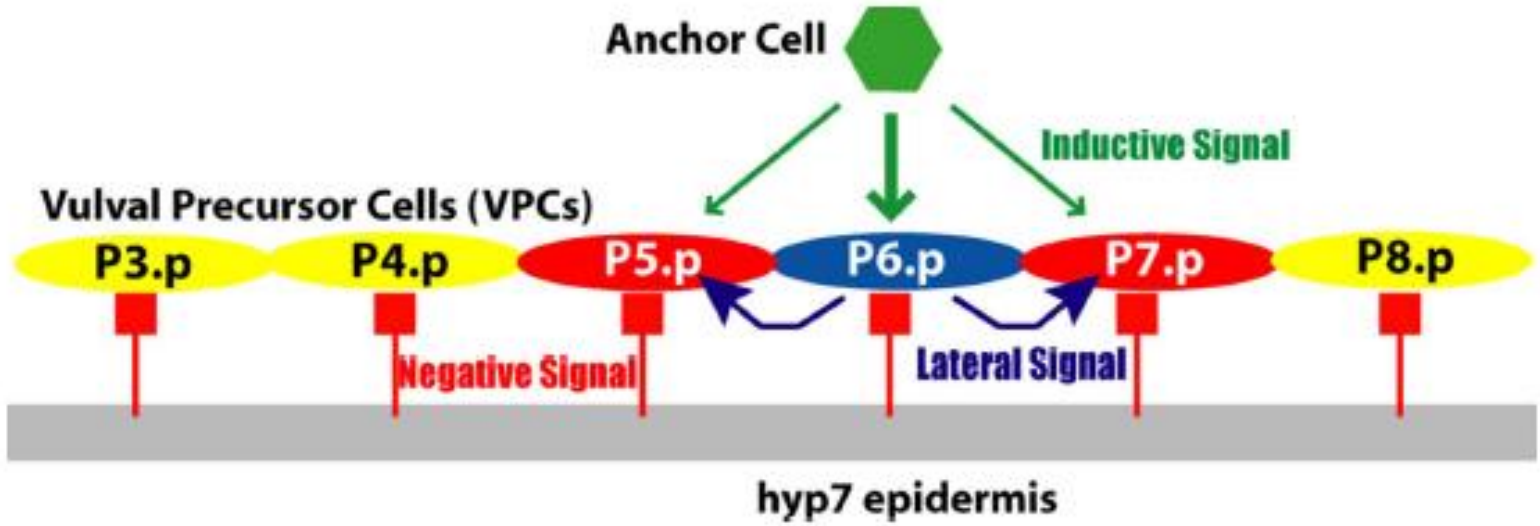
- The likelyhood of an isolated VPC to adopt the 1° or 2° fate depends on its position with respect to the AC. closer → more likely to receive 1°/2° fate

Conclusion:

- The inductive signal from the AC is graded and can act at a distance. ← Induction signal

Sternberg and Horvitz, 1986

Several signaling events are involved in patterning the VPCs



Signal 1 - inductive signal (is graded and acts at a distance)

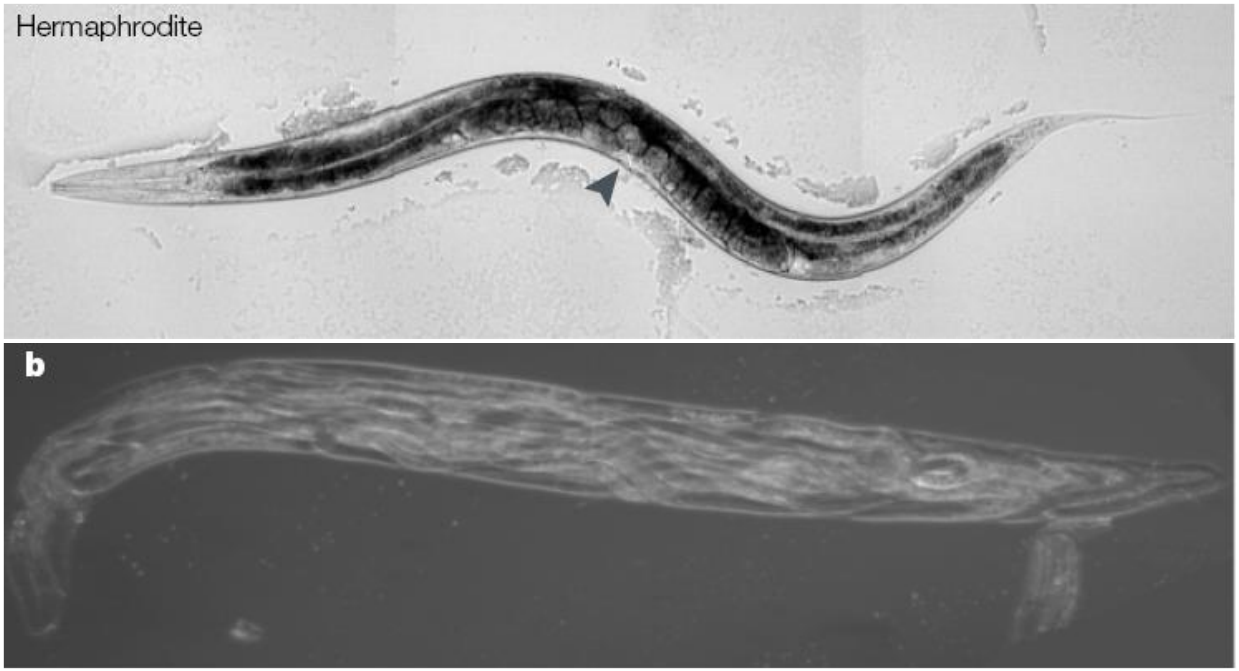
Signal 2 - lateral signal (is sequential)

Signal 3 - 'negative signal'

Sternberg, 2005, WormBook

SIGNAL 1
What is the molecular nature of the inductive signal?

Animals with defects in vulval development have obvious phenotypes



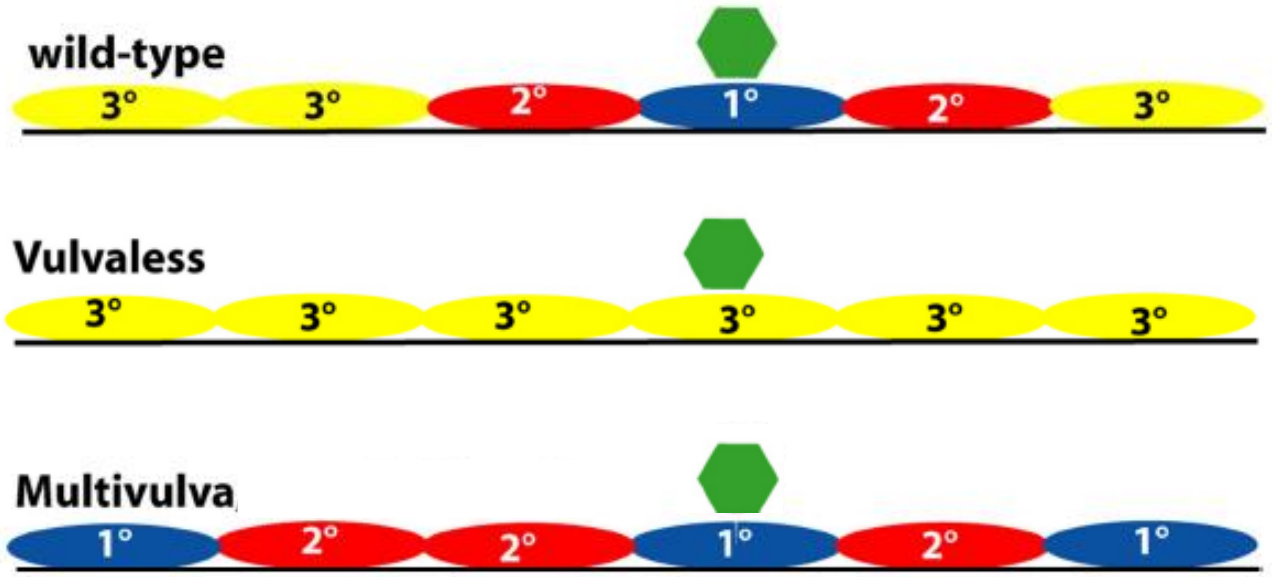
wild-type

Vul - vulvaless
'bag of worms'

Muv - multivulval

Gain of function vural induction.

Jorgensen and Mango, 2002

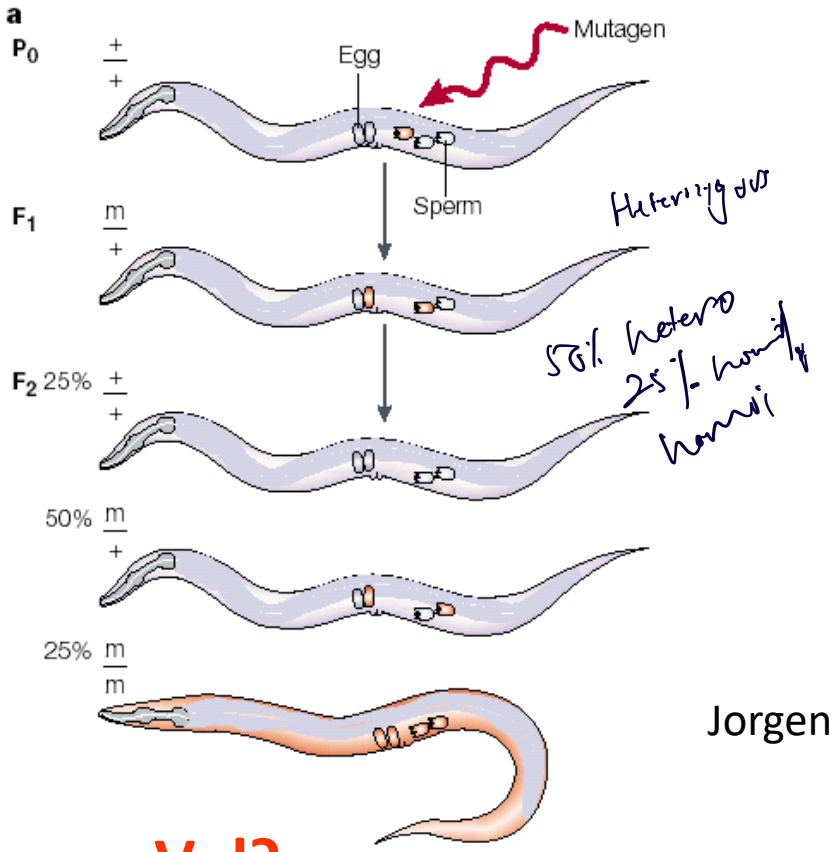

wild-type
3°
3°
2°
1°
2°
3°
Vulvaless
3°
3°
3°
3°
3°
3°
Multivulva
1°
2°
2°
1°
2°
1°

SIGNAL 1

What is the molecular nature of the inductive signal?

Unbiased ‚forward' genetic screens

F2 Screen

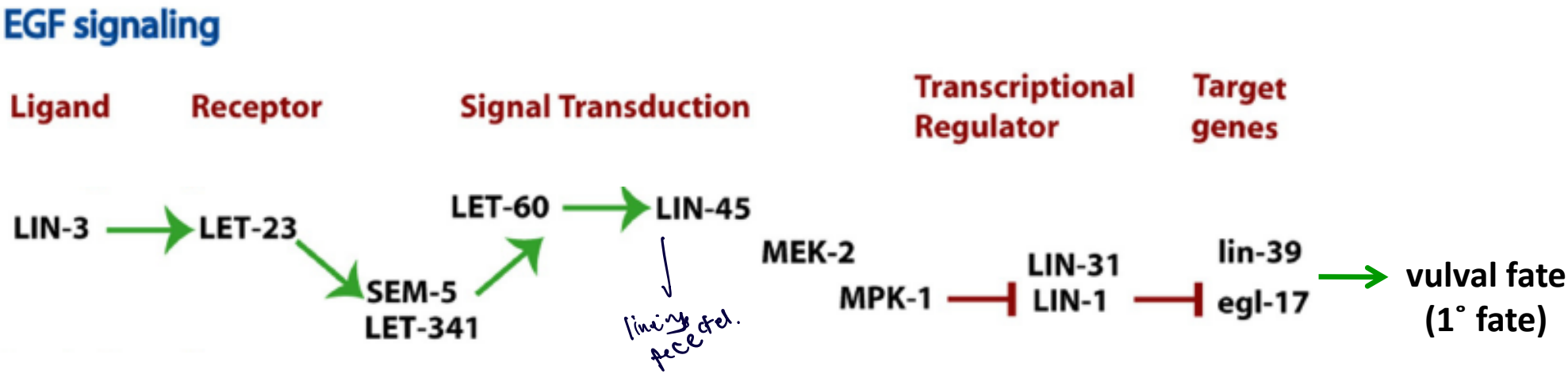


Jorgensen and Mango, 2002

Vul?

Muv?

The inductive signal is mediated by a EGF signaling pathway



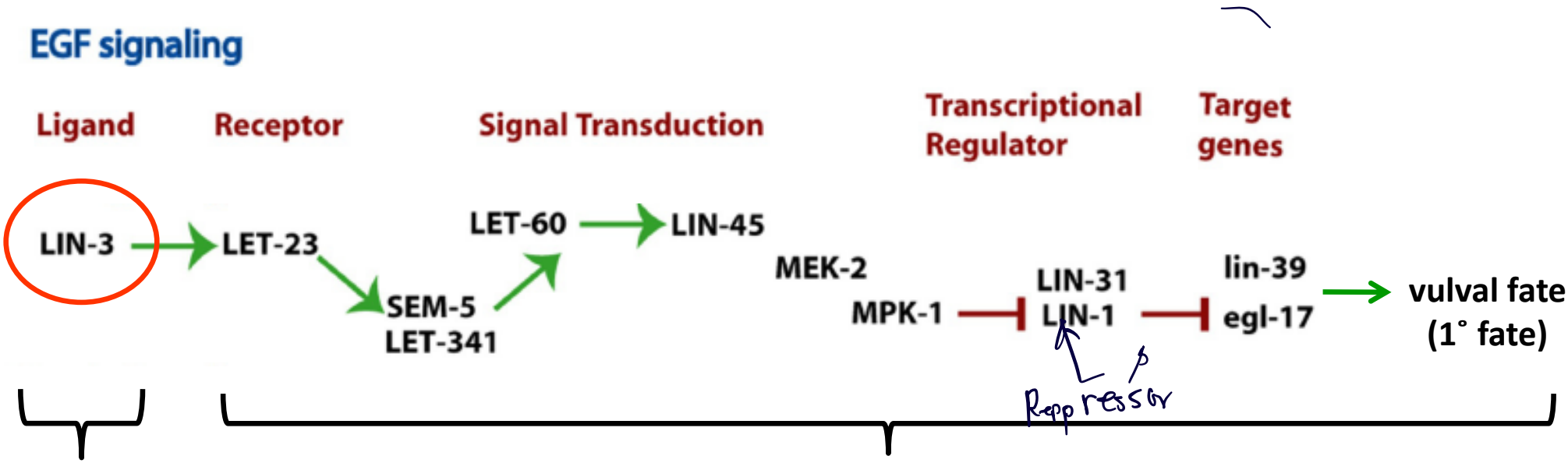
ON	ON	ON	ON	ON	ON	ON	OFF	ON
OFF	OFF	OFF	OFF	OFF	OFF	OFF	ON	OFF

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

EGF & Ras signaling pathway.

Sternberg, 2005, WormBook

The inductive signal is mediated by a EGF signaling pathway



AC Trans-regulator **Vulval precursor cells**

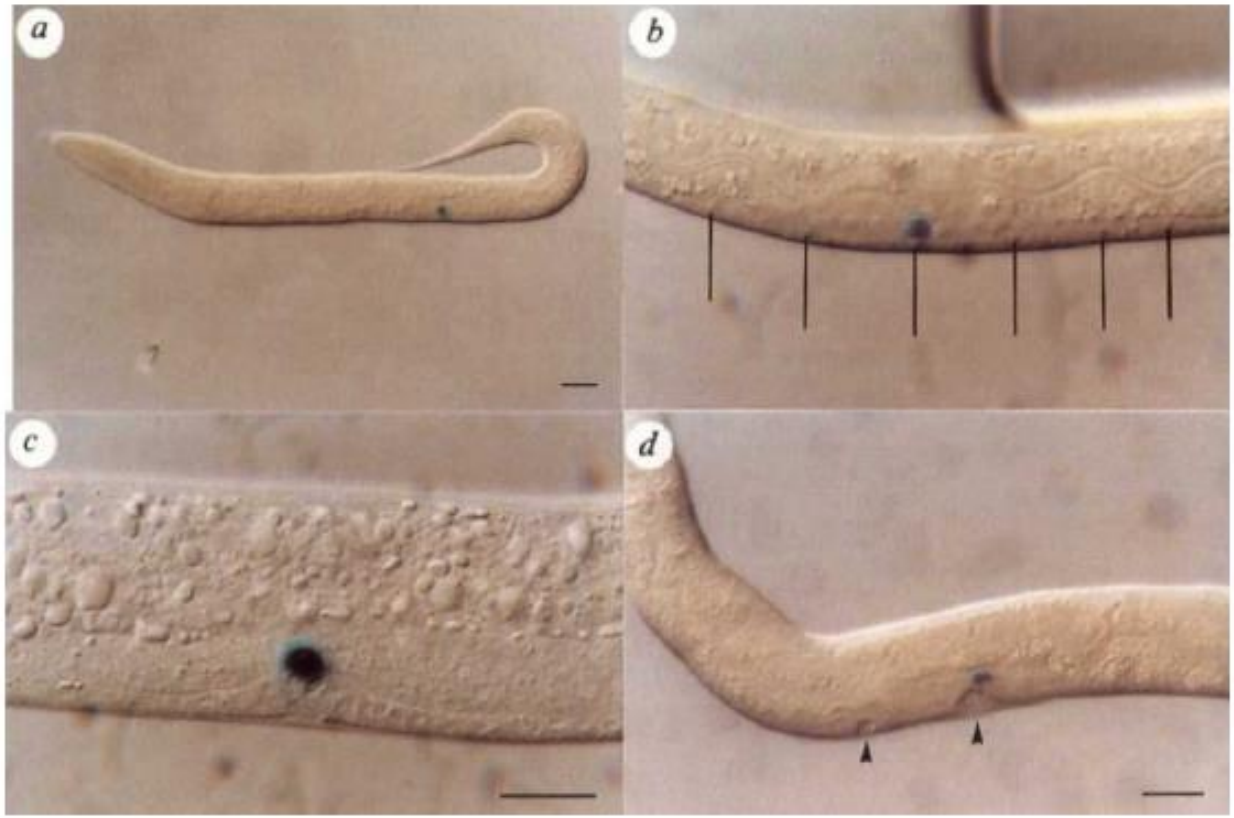
If lin ko. → no vul fate

Sternberg, 2005, WormBook

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

The *lin-3* gene is expressed in the AC but not the VPCs or the hypodermis

Transgenic animals

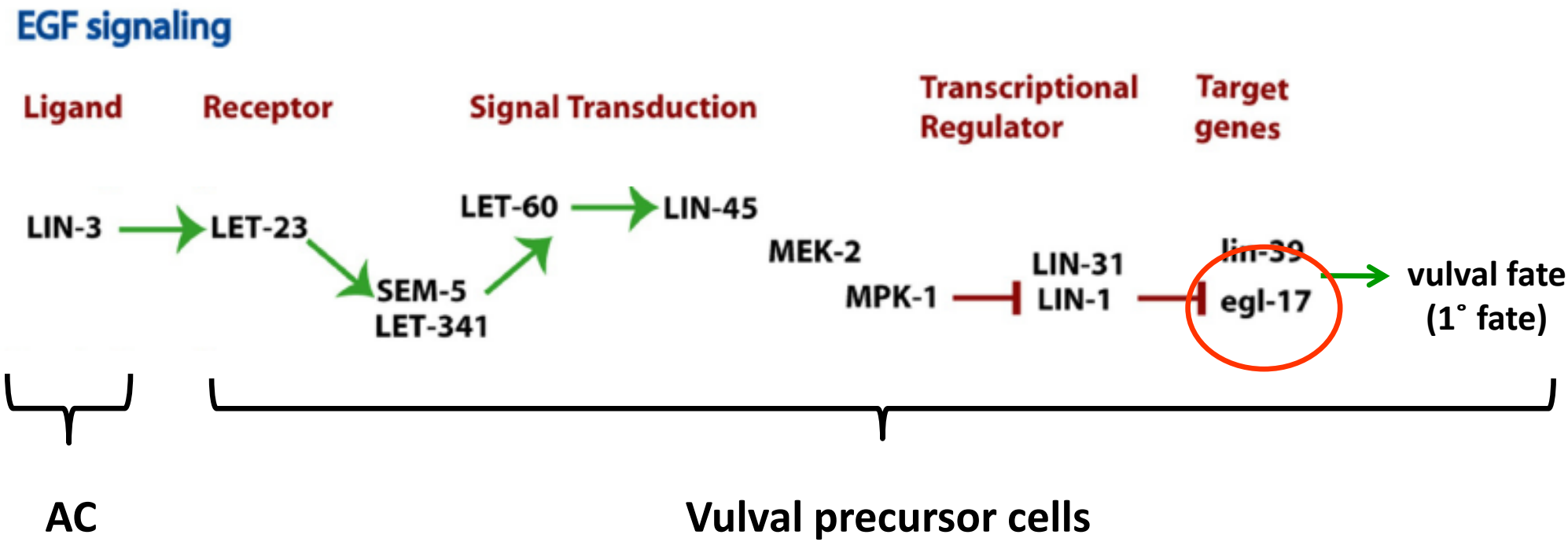


***lin-3*::lacZ reporter**

only expressed by AC.

Hill & Sternberg, 1992

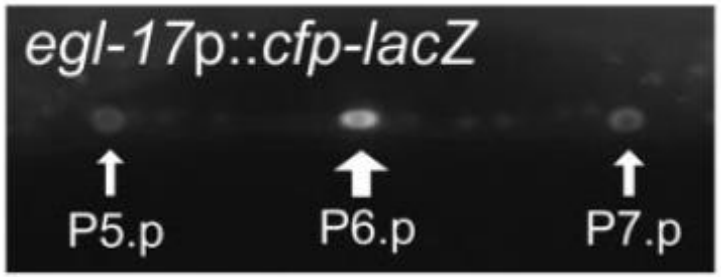
The inductive signal is mediated by a EGF signaling pathway

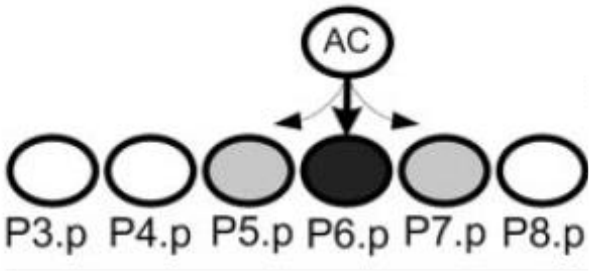


Sternberg, 2005, WormBook

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

The *egl-17* gene is initially expressed in P5.p, P6.p and P7.p but later expression becomes restricted to P6.p and its descendants



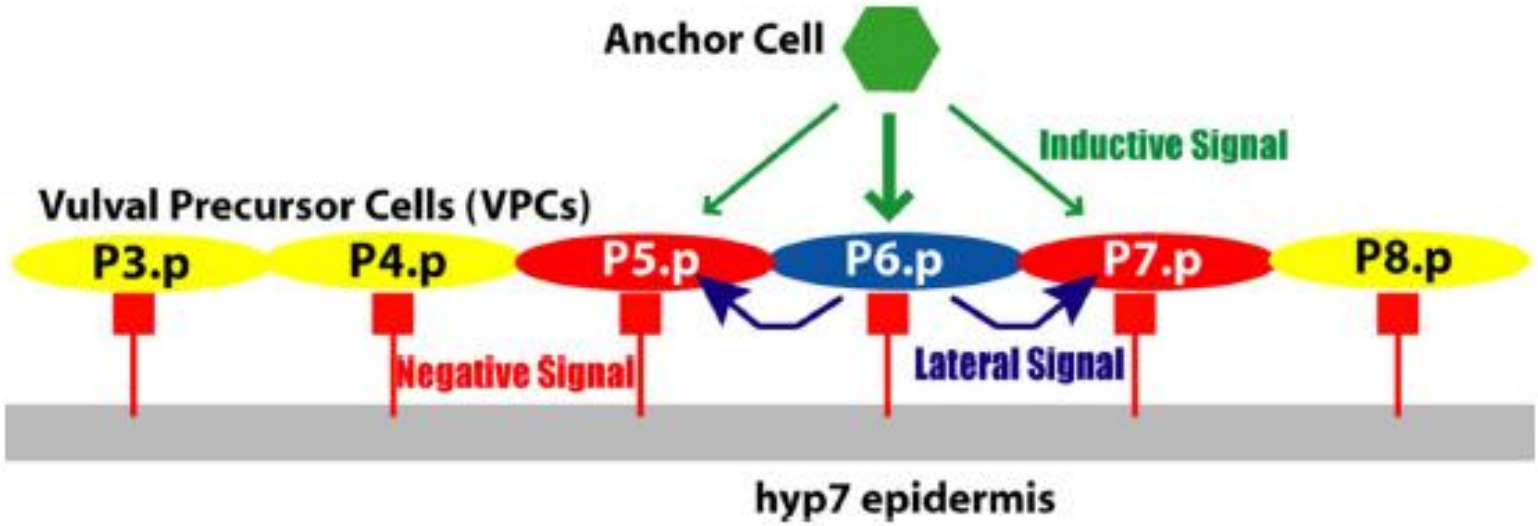


Yoo et al., 2004

A second signaling event occurs that results in the down-regulation of EGF signaling pathway activity in P5.p and P7.p

→ Signal 2 – lateral signal

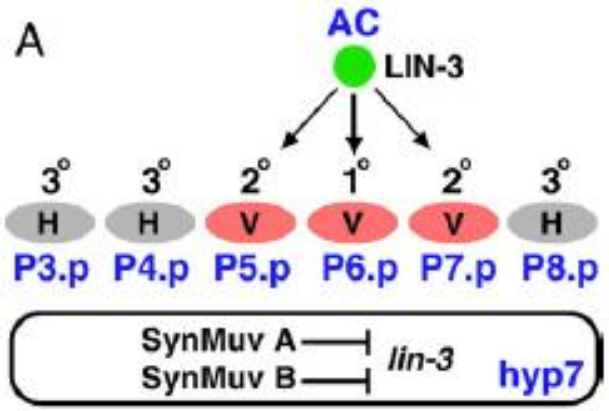
Several signaling events are involved in patterning the VPCs



Signal 1 - inductive signal (is graded and acts at a distance)
Signal 2 - lateral signal (is sequential)
Signal 3 - 'negative signal'

Sternberg, 2005, WormBook

The 'negative signal' from the hypodermis reflects the repression of *lin-3* EGF expression in this tissue through the SynMuv genes

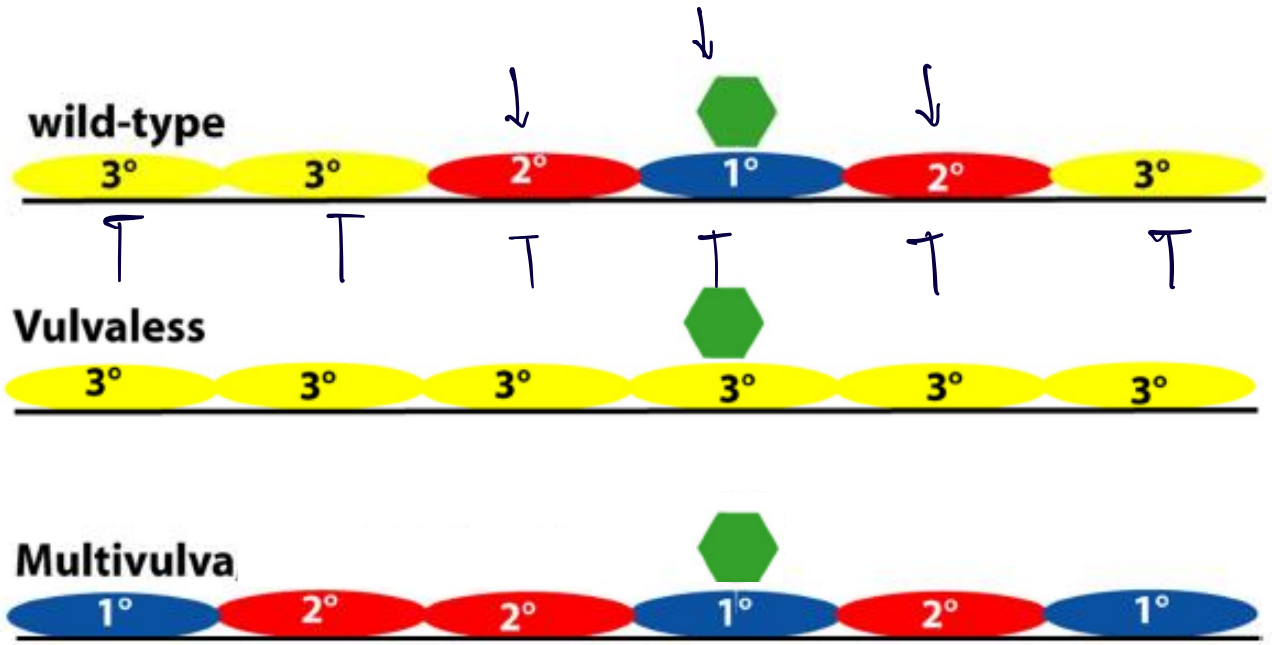


Massive express of PCMs.

Fig. 3. A model of the SynMuv mutant phenotype (Cui et al., 2006a). Vulval induction in wild-type (A) and SynMuv-mutant (B) animals. In wild type, the anchor cell is the only source of the LIN-3 inducing signal. This leads to P5.p, P6.p, and P7.p acquiring the vulval cell fate (V), whereas P3.p, P4.p, and P8.p adopt a default hypodermal fate (H). In SynMuv mutants, the hypodermal hyp7 syncytium secretes ectopic LIN-3 leading to additional VPCs acquiring the vulval cell fate.

Fay and Yochem, 2007

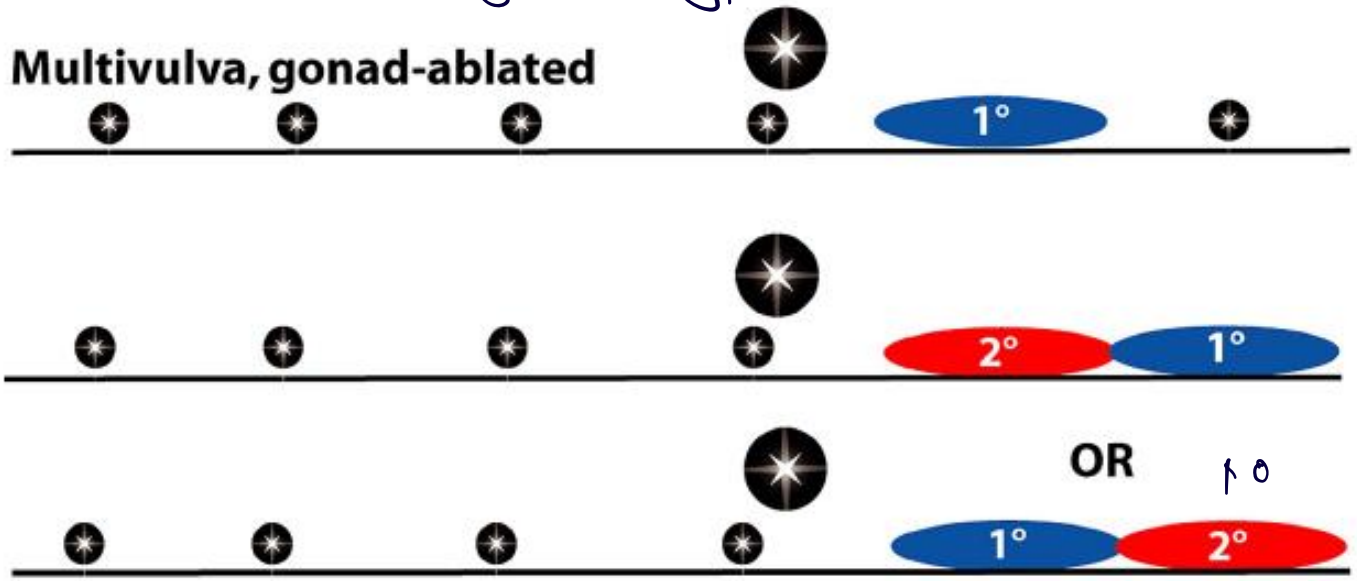
The loss of SynMuv gene activity results in a Multivulva phenotype



SynMuv ⊣ Lin3

SIGNAL 2 – Lateral signal
Ablation experiments in a Muv (*lin-15a, b*[-]) background

Coming from hypodermis.



Results:
- Single VPCs in a Muv background adopt the 1˚ fate.
- Adjacent VPCs in a Muv background will become 1˚- 2˚ or 2˚- 1˚.

Conclusion:
- The 1˚ VPC inhibits adjacent cells from also becoming 1˚ VPCs.

→ **lateral inhibition through a lateral signal**

Sternberg, 2005, WormBook

Inhibit neighbour cells from 1°.

Cell, Vol. 34, 435–444, September 1983, Copyright © 1983 by MIT

The *lin-12* Locus Specifies Cell Fates in Caenorhabditis elegans

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overexpress lz all express 2° fate.

LoF : P5~7 all have 1° fate.

Table 5. Cell Fates in the Ventral Hypodermis

	Hermaphrodite						Male			
Genotype	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	P9.p	P10.p	P11.p	Transformation
lin-12(d)	2°	2°	2°	2°	2°	2°	2°	2°	2°	non-2° → 2°
lin-12(+)	3°	3°	2°	1°	2°	3°	3°	2°	1°	
lin-12(0)	3°	hybrid	1°	1°	1°	3°	3°	3°	1°	2° → non-2°

The fates of P(3–8).p in the hermaphrodite are shown for two *lin-12(d)* alleles that confer a multivulva phenotype, *lin-12(n137)* and *lin-12(n177)*, and for the *lin-12(0)* allele *lin-12(n137 n720)*. 1°, 2°, and 3° fates are as defined in Figure 3; "hybrid" indicates a hybrid lineage that has characteristics of both the 1° and 3° fates: one P(3–8).p daughter either joined the hypodermal syncytium or generated two progeny that joined the hypodermal syncytium, and the other daughter generated three or four progeny that participated in vulva formation.

Complete P(3–8).p lineages were determined in 11 *lin-12(n137)* hermaphrodites. Most *lin-12(d)* P(3–8).p cells adopted the 2° fate: only four P3.p lineages and one P7.p lineage were possibly non-2°. Complete P(3–8).p lineages were determined in 11 *lin-12(0)* hermaphrodites (two of these animals had three anchor cells): no 2° lineages were observed. However, there was some variability in cell fate. P4.p: 7/11 expressed a "hybrid" lineage, 3/11 expressed the 3°

Results:

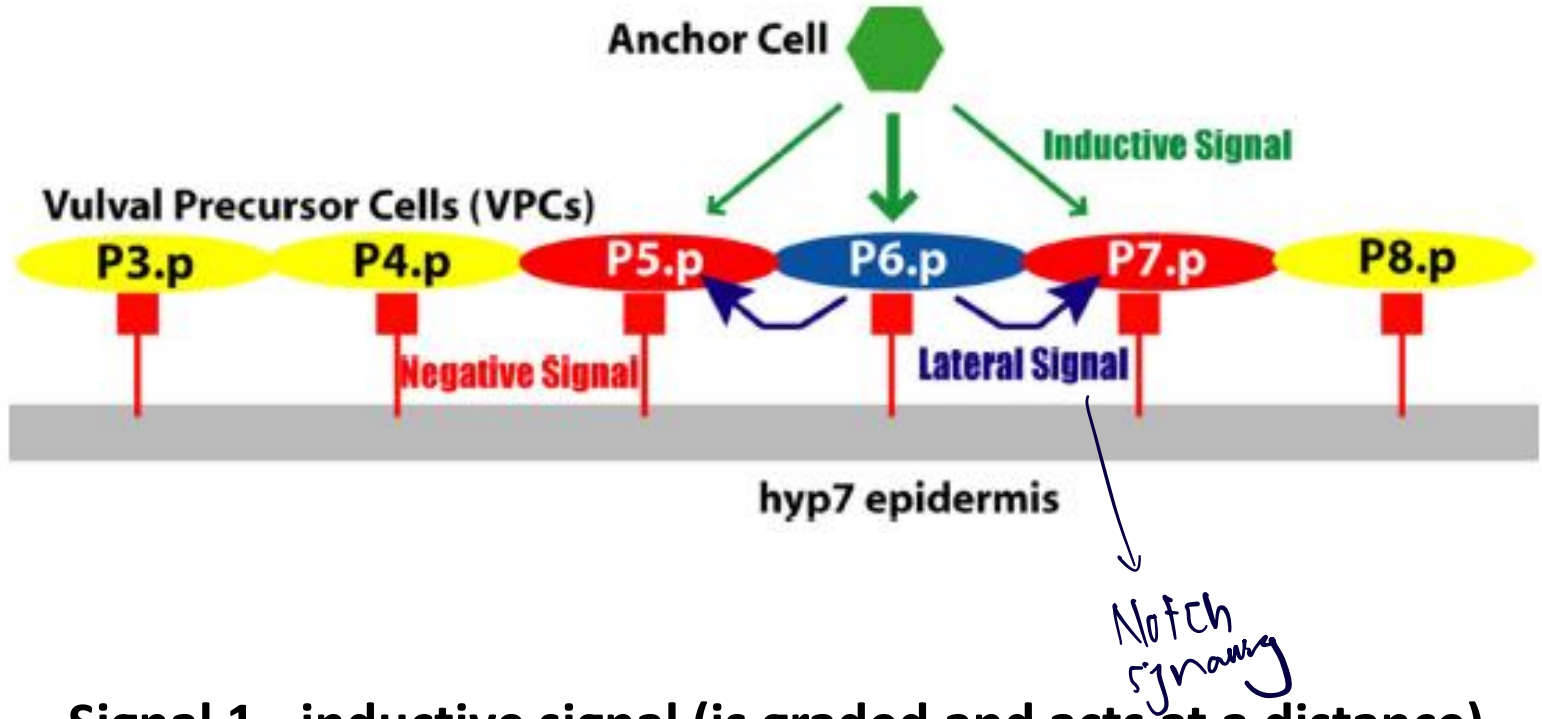
- The loss of *lin-12* causes P5.p, P6.p and P7.p to all adopt the 1˚ fate.
- The gain of *lin-12* function causes all VPCs to adopt the 2˚ fate.

Wild-type background

Conclusion:

- *lin-12* is necessary and sufficient for the specification of the 2˚ fate.
- *lin-12* is also necessary for **lateral inhibition**.

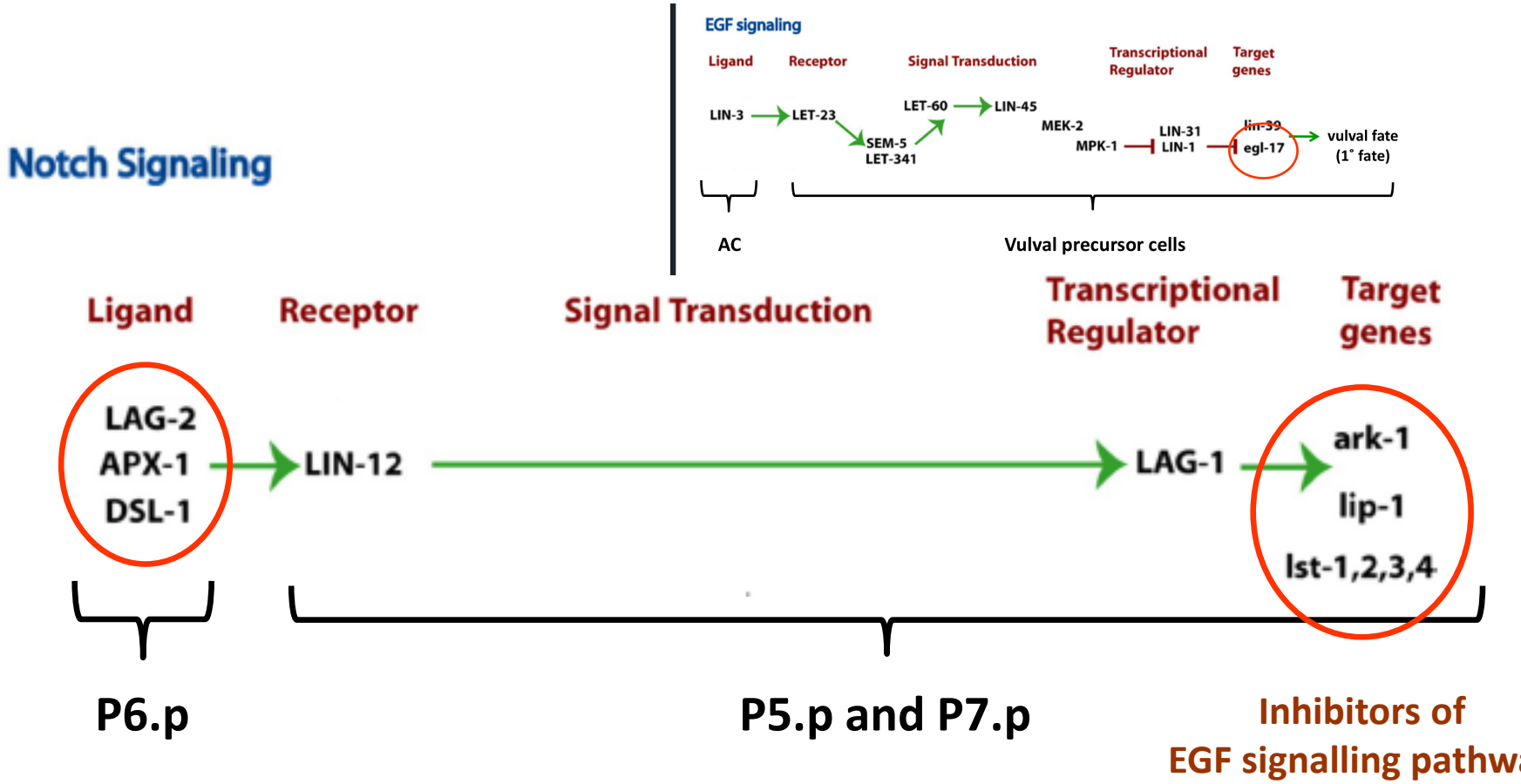
Several signaling events are involved in patterning the VPCs



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

Sternberg, 2005, WormBook

The lateral signal is mediated by a Notch signaling pathway



Notch inhibit EGF signal.
eg. LIN3-LET23 signaling

Sternberg, 2005, WormBook

EGF signaling and Notch signaling occur sequentially and are coupled

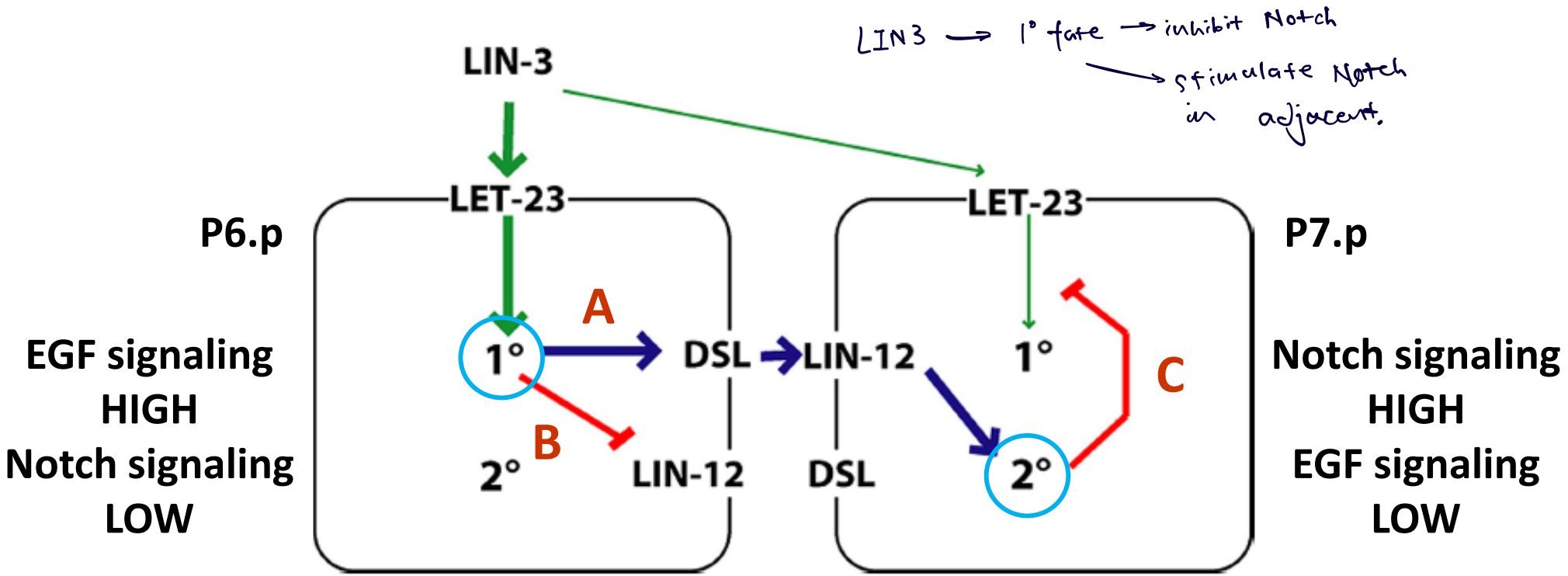
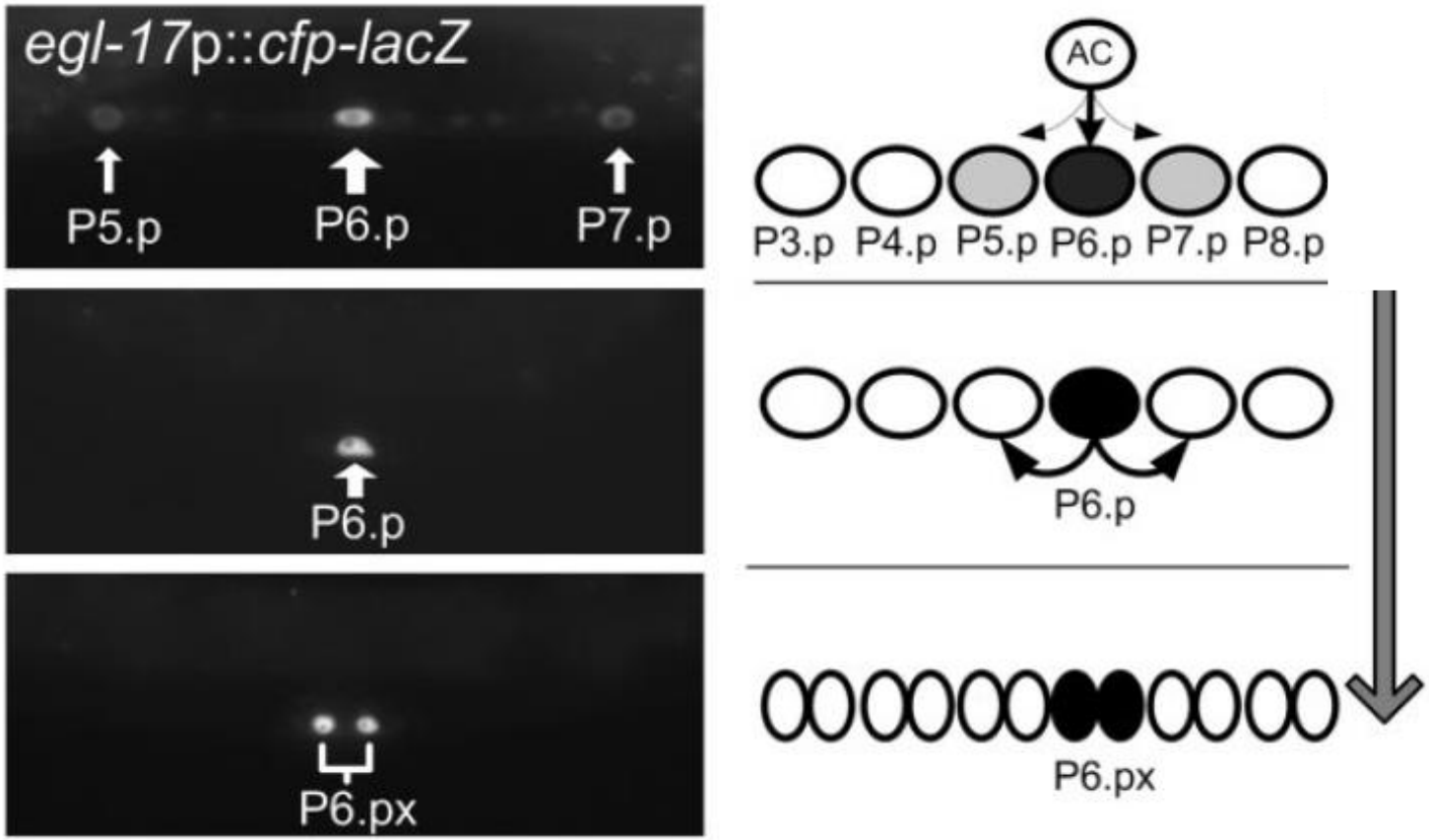


Figure 13. Simple model of antagonism between 1° and 2° VPCs. Signals for 1° specification (green) compete with signals for 2° specification (blue). In this example, the left cell is winning the competition to become 1° because it is receives more LIN-3. LIN-3 signaling via LET-23 promotes the 1° fate, which includes the production of DSL ligands and downregulation of the receptor LIN-12. DSL ligands activate LIN-12 on neighboring cells, thereby promoting the 2° fate, which inhibits LET-23 signaling. Red bars, inhibition. Model based on Sternberg and Horvitz (1989); Berset et al. (2001); Shaye and Greenwald (2002); and Yoo et al. (2004).

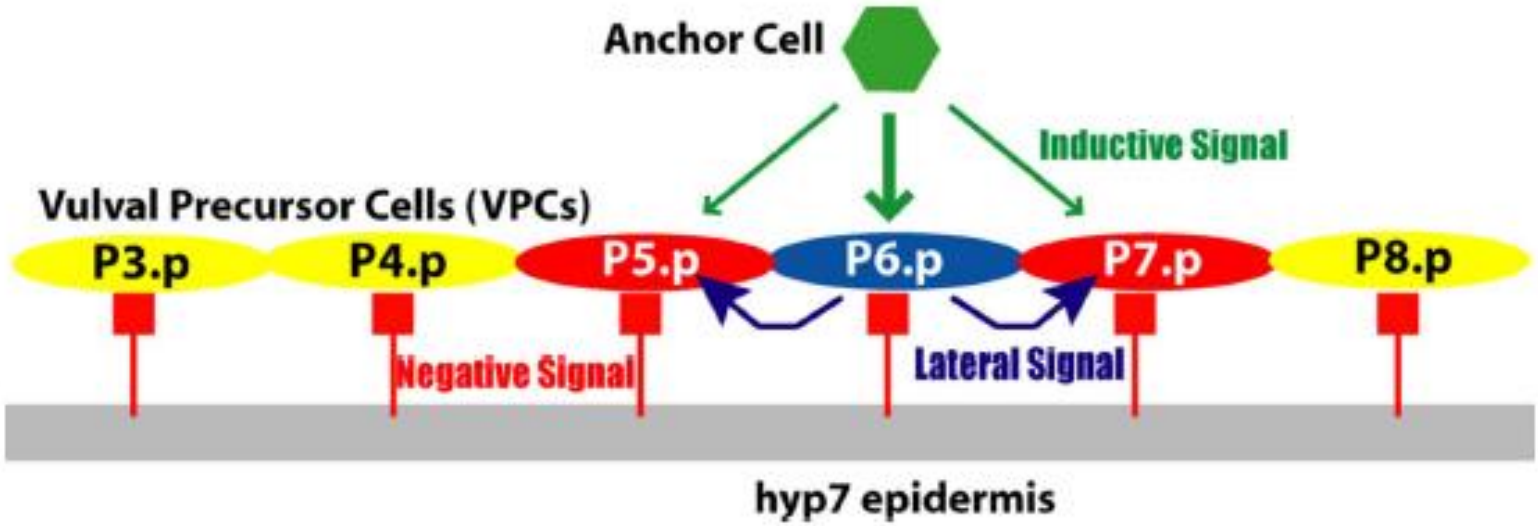
Sternberg, 2005, WormBook

Interaction between EGF and Notch signaling pathway is visualised by dynamics of *egl-17* expression



Yoo et al., 2004

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

Sternberg, 2005, WormBook

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)

STEP 3 **Generation of adult cells**

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

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 1):

cell fates, laser ablations, multipotential, equivalence group, pattern formation, inductive signal, graded signal, action at a distance, EGF signaling, lateral inhibition, lateral signal, Notch signaling, sequential signaling

Major concepts (LECTURE 1):

Vulval development is a multistep process.
The vulval equivalence group is a group of multipotential cells.
The commitment of vulval precursor cells to a specific fate is dependent on several signaling events.
EGF and Notch signaling are coupled during VPC patterning.

C. elegans vulva development.

5 steps: Vulval precursor cell generation

VPC patterning

VPC division

Anchor cell invasion.

Ablation of previous primary fate cells, neighbour cells adopt vulva cell fate.

Ablation of anchor cell at diff. developmental stage shows involved in 1° & 2° fate.

Early ablation → all adopt tertiary fate.

Late ablation → Most adopt previous normal fate.

Ablation of all but one VPC + variable anchor cell position shows VPC position relative to anchor cell determines cell fate. Graded signalling.

C. elegans vulva formation

C. elegans contain gonadal arms, oocytes mature as they move through the arm, pass through the spermatheca (contains sperm made during development), into the uterus, out via ventral vulva.
The vulva of the C. elegans is enclosed by voval muscle, controlls egg-laying.

- 6 vulval precursor cells are generated during development (P3.p - P8.p), anchor cell sits on P6.p
- Adoption of primary/secondary/tertiary fates
    - P5.p, P6.p and P7.p adopt primary and secondary fate, forms the vulva
    - P3.p, P4.p, P8.p adopt tertiary fates, fuses with the hypodermis
- Maturation into adult cells: Different cell fates have distinctive division patterns
- Anchor cell integration: fusion forms the opening of the vulva.

- 1°, 2° and 3° cell fate is not determined at birth, all 6 VPCs are multipotential.
    - **Laser ablation experiment** shows removal of P5.p~P7.p cells induce other cells to adopt 1° cell fate.
- Anchor cell signalling is responsible for fate determination
    - **Ablation of anchor cells at different developmental stages** (Kimble et al., 1981)
        - Early stage ablation lead to lack of vulva induction
        - Late stage ablation lead to less effect
    - **Ablation of VPCs and variable positioning of AC show position-dependent induction**
        - Paper concludes induction occur via a graded potential from anchor cells

Established that the lineage specification is mediated by the EGF signalling pathway
LIN-3 (ligand) - LET-23 (receptor) - signal transduction (MEK/MPK) - inhibited TF LIN-1 - activate 1° cell fate

- **Fluorescence probe** shows LIN-3 is expressed in early stage anchor cells, support inductive effect.
- **Yoo et al., 2004 showed** Egl-17 reporter shows early graded expression of 1° fate genes in P5.p, P6.p, P7.p, but later restricted to P6.p due to lateral inhibition of the EGF pathway

Lateral inhibition signalling: ligand of LIN12 secreted by 1° fate VPC, inhibit lateral 1° adoption, LIN12 expressed by cells adjacent to the primary fate cell

- **Sternberg ablation experiment** shows alternative adoption of 1° fate, but no adjacent 1° fate cells.
- Relating experiment also shows LoF LIN12 - all adopt 1°; LoF LIN12 - all adopt 2°
- Lateral inhibition occur via Notch signalling pathway: ligand bind to LIN12 receptors

Negative signal from the hypodermis regulates 1° fate adoption:

- Hypodermis contains SynMuvA and SynMuvB, inhibit LIN-3 expression in the hypodermis
    - **Experiment:** SynMuv mutant C.elegans shows 1° fate adoption in all 6 VPCs.