

SUPPLEMENTARY MATERIAL & METHODS

Markers for Y:

mcIs47 [*dlg-1::gfp*; *rol-6(d)*] a gift of Michel Labouesse; expresses a GFP-tagged adherens junction marker in epithelial cells;

mcEx242 [*che-14::gfp*; *rol-6(d)* (1)] a gift of Michel Labouesse; expresses a GFP-tagged apical membrane marker in epithelial cells;

jcIs1 [*ajm-1::gfp*; *rol-6(d)* (2)]; expresses a GFP-tagged adherens junction marker in epithelial cells;

mcEx [*lin-26::gfp*; *rol-6(d)*] a gift of Michel Labouesse; expresses *gfp* in all rectal cells and P12.pa;

bxIs7 [*egl-5::gfp*; (3)] a gift of Scott Emmons; we found that *gfp* is expressed in all rectal cells in transgenic L1 larvae, including Y, and then in all rectal cells except for P12.pa in transgenic L4 and older animals;

kuIs36 [*pegl-26::gfp*; *unc-119(+)* (4)] a transcriptional fusion gift of Wendy Hanna-Rose; *egl-26* is expressed in cells forming tubes, including Y, B and P12.pa;

kuIs34 [*psem-4::gfp*; *unc-119(+)* (5)] a gift of Wendy Hanna-Rose; *sem-4* has been reported to be expressed among other cells in the rectal B and F cells as well as in cells in the preanal ganglion (5). We extended these observations by scoring a *sem-4::gfp* reporter in the rectal area and found that *sem-4* is expressed in all rectal cells in L1s hermaphrodites (ie Y, U, B, F, K and K') and in all rectal cells, including P12.pa, in L3 or older worms.

Markers for PDA:

fpIs1 [*pace-3/4::gfp*; *pha-1(+)*(this study)]; a transcriptional fusion;

syIs63 [*cog-1::cog-1::gfp*; *unc-119(+)* (6)]; a translational fusion; we identified *cog-1*, which had been described to be expressed in unidentified cells in the preanal ganglion of late larvae (6), as a useful PDA marker.

arEx627 [*pexp-1::GFP*; *dpy-20(+)*(this study)]; a transcriptional fusion.

Other markers:

ccIs4251 [*pmyo-3::NgfplacZ*; *pmyo-3::Mtgfp*; *dpy-20(+)*, (7)] a gift of Mike Krause. *myo-3* is expressed in muscles and we confirmed it is not expressed in Y (0/35 L1 animals);

edIs6 [*unc-119::gfp*; *rol-6(d)* (8)], a pan-neuronal marker;

evIs111 [*F25B3.3::gfp*; *dpy-20(+)* (9)], a pan-neuronal marker;

enIs7 [*pced-1::ced-1::gfp*; *unc-76(+)* (10)] a gift of Zheng Zhou. *ced-1* is expressed in engulfing cells.

Identification of molecular markers for PDA:

PDA is easily confused with two other neurons in the preanal ganglion (PAG), named DA8 and DA9s (11, 12). However, they may be distinguished using two criteria: time of appearance (DA8 and DA9 are present in early L1 larvae; the transdifferentiation of Y into PDA occurs later) and axon trajectory (DA8 sends its process through a left hand commissure, PDA and DA9 through a right hand commissure, (11, 12). A transgene was scored positive for expression in PDA if all of the following conditions were met: the GFP-positive cell in the PAG is NOT seen in L1s; the axonal process of the GFP positive neuron is as expected (commisure and trajectory); the GFP-positive neuron sends its axon towards the dorsal cord through a right-handed commissure; the position of the cell body in the PAG is just anterior to the U cell in young L3 hermaphrodites. In addition, when possible, the transgenic worms were lineaged to follow Y's transformation into PDA in those strains and/or Y was killed using a laser beam in a newly hatched L1 bearing a transgene of interest and the absence of the PDA neuron was analysed in L4 animals. Using these criteria, only three genes were found or confirmed to be expressed in PDA: *ace-3/4*, *exp-1* and *cog-1*. A number of markers previously thought to be expressed in PDA were reclassified [*wrk-1*, *dop-2*, *ksv-1*, *dbl-1*].

Electron microscopy: Two newly hatched N2 L1 hermaphrodites (0 to 1h after hatching) and one L4 hermaphrodite were cryo-fixed by high pressure freezing as described previously (13). Freeze substitution was processed in the Leica AFS for 60 hours at -90°C and then for 6 hours at -30°C in 1% osmium tetroxide, 0.5% uranyl acetate, 0.5% glutaraldehyde and 2% H₂O in pure acetone. Samples were then rinsed and infiltrated with graded concentrations of an epoxy resin (Epon812, Fluka). The final steps of the infiltration were done at room temperature, when the epon concentration was greater than 70%. Ten microns of serial ultra-thin sections (50 to 70 nm) of the rectal area for 2 L1 and 1 L4 hermaphrodites were collected and contrasted in lead citrate and uranyl acetate before imaging with a SiS Megaview 3 ccd camera mounted on a FEI Morgagni TEM operated at 70kV.

T shift experiments: To assess whether *lin-12* activity was needed after Y's birth and specification, we used the temperature sensitive allele *lin-12(n676n930ts)* (14). L1 larvae were scored for the presence of a Y cell in the anterior rectum by Nomarski optics and L3 and older larvae were scored for the presence of a PDA motor-neuron by checking expression of the *cog-1::gfp* marker. When grown at 15°C, 15/24 *lin-12(n676n930)* L1s have a Y cell in the anterior rectum and 33/50 L3s or older have a PDA neuron made. By comparison, no Y cell is found in L1 grown at 25°C (15) and 0/35 *lin-12(n676n930)* L3 or older grown at 25°C have a PDA neuron made. In a first set of experiments, *lin-12(n676n930)* embryos laid at 15°C were transferred to 25°C. The embryos used corresponded to stages ranging from just before Y's birth to past the 3 fold stage [around 253 min to 578 min of development after the first cleavage at 20°C; the Y cell is born around 290 min after the first cleavage at 20°C in wild type embryo (16)]. 2h50 later, larvae that had already hatched were removed. After an additional 4h10, half of the L1 found on the plate were scored for the presence of a Y cell,

while the other half was transferred to a new plate at 25°C and scored when the worms reached the L3 stage or older for the presence of the PDA neuron. In a second set of experiment, we sought to restrict the developmental time window of the embryos analysed and transferred to 25°C embryos grown at 15°C that had reached anywhere between the lima bean stage to the 1 ½ fold stage [around 320 min to 417 min after the first cleavage at 20°C].

SI table 4: Heterochronic genes affecting early larval stages do not affect PDA formation

Genotype	% no PDA (n)
<i>lin-4(e912)</i>	0% (46)
<i>lin-14(n179)*</i>	1% (98)
<i>lin-28(n719)</i>	0% (39)

The *lin-4*, *lin-14*, and *lin-28* heterochronic genes control the timing of many L1 and L2 stage-specific events (17). L4 hermaphrodites were scored for the presence of a PDA motor-neuron using *syIs63* [*cog-1::gfp*] or *fpIs1* [*ace-3/4::gfp*]. Both the appearance and the position of PDA were wild type. (n), total number of animal scored. In addition, the timing of the Y-to-PDA transdifferentiation appears to be independent of these genes, as precocious PDA formation was not evident in the *lin-14(n179)* precocious mutant: 0/28 L1 animals and 1/17 late L2 had differentiated PDA neurons. * grown and scored at 25°C.

SI table 5: Summary of markers expression

A - Expression of markers in Y and PDA			
transgene	marks	Y	PDA
CHE-14::GFP	apical	40/40	0/40
AJM-1::GFP	CeJ	41/41	0/30
DLG-1::GFP	CeJ	27/27	0/48
<i>egl-5::gfp*</i>	rectal	56/56	0/56
<i>egl-26::gfp*</i>	Y, B	36/39	0/34
<i>unc-119::gfp</i>	neurons	0/26	[all]
<i>F25B3.3::gfp</i>	neurons	0/32	[all]
<i>cog-1::gfp</i>	PDA	0/30	59/59
<i>ace-3/4::gfp</i>	PDA	0/45	48/51

Transgenes are fully described in Supplemental Material. CeJ, *C. elegans* junctions (homologous to adherens junctions, 18). Expression in Y was scored in the L1 stage; expression in PDA was scored in the L3 and L4 stages. * complete profile of expression in rectal cells is shown in SI Table 5B.

B - expression of *egl-5::gfp* and *egl-26::gfp* in the rectal cells

reporter	stage	n	Y	P12.pa	B	U	F	K	K'
<i>egl-5</i>	L1	56	100%	-	100%	100%	92%	100%	100%
	L4*	40	0%	0%	100%	100%	100%	100%	100%
<i>egl-26</i>	L1	39	92%	-	100%	25%	56%	nd	nd
	L4*	34	0%	97%	100%	0%	0%	nd	nd

n, number of newly hatched L1s and L3 or older hermaphrodites scored. *, L3 and older animals, designated here as L4, all show the same pattern. The % of animals with GFP in a given cell is shown. *egl-5::gfp* is expressed in Y in L1 larvae and B, U, F, K and K' throughout development, but not in P12.pa. *egl-26::gfp* is expressed in Y and B in L1 larvae, and in P12.pa and B in older larvae.

SI table 6: Engulfment of prospective neurons does not underlie the Y-to-PDA transformation

Genotype	% persistent Y (n)	% normal PDA (n)
<i>ced-3(n717)^</i>	0% (65)	100% (61)
<i>ced-3(n1286)</i>	nd	100% (29)
<i>ced-10(n3246)</i>	0% (16)	100% (31)
<i>ced-1(n1735)</i>	0% (27)	nd
<i>ced-12(k149)</i>	0% (34)	nd

In cell death mutants, the "undead" cells often take on the differentiated characteristics of their sisters or lineal homologs (19). In the tail, just anterior to the rectum of

hermaphrodites, there are several dying prospective neurons: in the L1 stage, the sisters of the P12.aaa and P11.aaa neuroblasts – as well as the sister of P12.pa - and in the L2 stage, the sisters of the VA11 and VA12 neurons. L4 hermaphrodites were scored for the persistence of Y as an epithelial cell in the anterior rectum by Nomarski optics or for the presence of a PDA motor-neuron using *syIs63 [cog-1::gfp]* or *fpIs1 [ace-3/4::gfp]*. Both the appearance and the position of PDA were wild type. (n), total number of animal scored. ^, P12.pp death was 50% penetrant (33/65).

SI table 7: Y-to-PDA transdifferentiation occurs in mutants with no P12 cell

genotype	% 2 P11.p (n)	% mutant Y (n)	% 2 cells in rectum (n')	% 1 PDA (n)
<i>bar-1(ga80)</i>	94% (87)	77% (87)	81% (82)	98% (50)
<i>let-60(sy93)</i>	27% (92)	10% (92)	36% (25)	nd
<i>lin-44(n1792)</i>	24% (50)	10% (50)	41% (12)	nd

Reduced activity of either the Wnt or EGF pathways causes P12 to adopt the fate of P11, resulting in a characteristic "2 P11.p" phenotype (20). L4 hermaphrodites were scored for the presence of an additional cell in the anterior rectum ; (n), total number of animal scored ; % mutant Y, percentage of worms where Y did not migrate ; % 2 cells in rectum, percentage of animals with 2 cells in the rectum (i.e. Y did not migrate) among those mutants that displayed a 2 P11.p mutant phenotype (n'); % 1 PDA, percentage of the animals that had 1 wild type looking PDA neuron expressing *cog-1 ::gfp*, irrespective of its location.

Ablation of the "extra P11" cell in newly hatched *bar-1(0)* L1 larvae did not rescue the Y migration (0/8 wild-type); two cells were always found but the ventral one at Y's original place had a neuronal morphology suggestive of differentiation into PDA.

SI Fig. 3: Marker expression in Y and PDA

(A) The epithelial marker CHE-14::GFP is expressed in Y. Right, fluorescent photomicrograph of Y expressing CHE-14::GFP in a newly hatched L1 animal (40/40 early L1s expressed *che-14* in Y). Left, the corresponding Nomarski photomicrograph. CHE-14 can be detected at the apical side of the Y cell, bordering the rectal slit (white arrow), and some faint fluorescence can be seen in Y cytoplasm; note that both sides of the rectal slit are marked with CHE-14, although only one is shown in focus; arrowhead, Y nucleus.

(B) The epithelial markers DLG-1 and AJM-1 are expressed in Y. Fluorescent photomicrographs of the subapical part of the Y cell (white arrowheads) expressing both DLG-1 (green, left panel) and AJM-1 (red, middle panel) in the same L1 animal (overlay in yellow, right panel; 41/41 and 27/27 early L1s expressed *dlg-1* or *ajm-1* in Y respectively); thin arrow, rectal slit.

(C) PDA is a neuron in the L3 stage. Photomicrograph of the PDA motor-neuron cell body (arrowhead) and axonal process (thick arrow) in a *cog-1::gfp* L3 larva. PDA's axon is first directed towards the posterior of the animal on its ventral side, then it runs round to the dorsal side through a right-handed commissure past the rectum (thick arrow), and goes towards the anterior part of the worm in the dorsal cord. In *cog-1::gfp* or *ace-3/4::gfp* animals, PDA's process (thick arrow) stops just posterior to the pharynx (asterisk) and synaptic varicosities are observed all along the dorsal cord, consistent with its described synapses onto dorsal muscles (11, 12). The anus is indicated by a thin arrow.

SI Fig. 4: Ultrastructural characteristics of P12.pa

(A) Electron-micrograph of the rectal area of an L4 hermaphrodite showing the B and the P12.pa cells wrapped around the rectum, both displaying a train-rail shape. The cell contour of the P12.pa cell is in a red dotted line whereas the cell contour of the B cell is in a black dotted line. Boxed is the area

shown at a higher magnification in B. Note the similarity between the pictures here and in Fig. 2A.

(B) Blow-up of the boxed area in fig 5A, illustrating an adherens junction (arrowhead) between the apical membranes of P12.pa and B.

An asterisk indicates the rectal slit.

that's because P12 and descendants, which send the migration signal, are not there - not because Wnt directly controls migration

SI Fig. 5: Schematic representation of phenotypes

(A) Partial lineage of P11 and P12 during the L1 stage in a wild-type animal. P11.p does not divide further and displays a characteristic epithelial morphology. P12.p divides at the end of the L1 stage to give rise to P12.pa, which will replace Y in the rectum and to P12.pp, which dies.

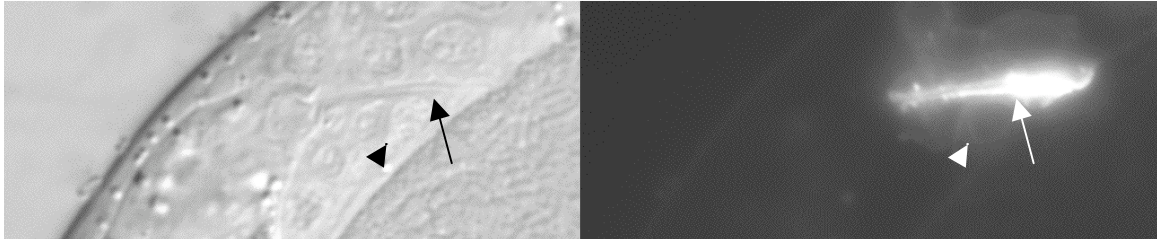
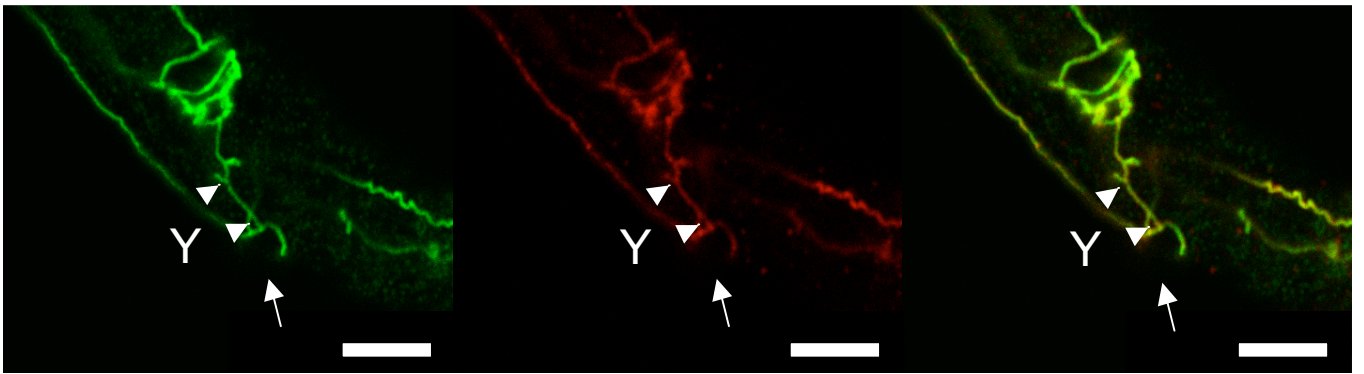
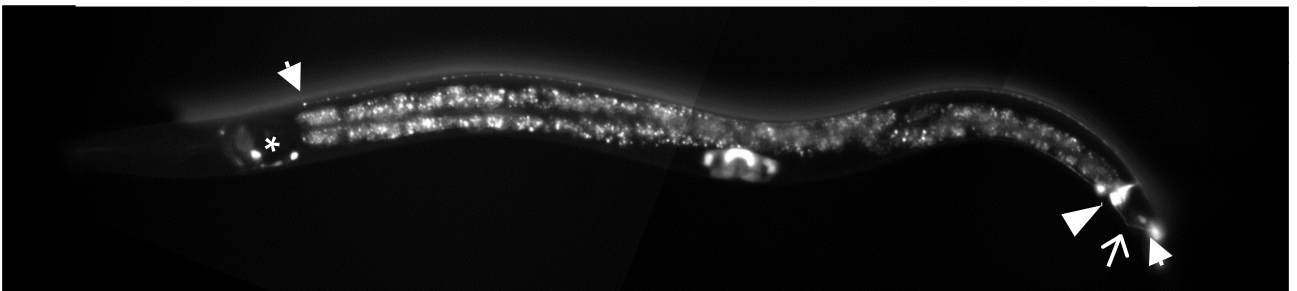
(B) The rectal area at the L1 and L3 stages in wild type animals or in mutant backgrounds as indicated. A thin arrow indicates the anus. In L3 and older wild type animals, 3 epithelial cells can be found in the rectal and preanal area: P11.p, P12.pa and U cells. In *bar-1*, Y migration is blocked but transdifferentiation occurs; in *lin-15*, the extra P12.pa cell fails to transdifferentiate; *egl-5* and *sem-4*, Y transdifferentiation is blocked; *egl-38* and *mab-9*, "extra Y" cells remain rectal and fail to transdifferentiate; *lin-12(d)*, the "extra Y" transdifferentiates as PDA. See text and Tables 2 and 3 for data. The supernumerary Y-like cell is indicated as Y*. For simplicity, K, K' and the axonal projections of PDA and DA9 are not drawn.

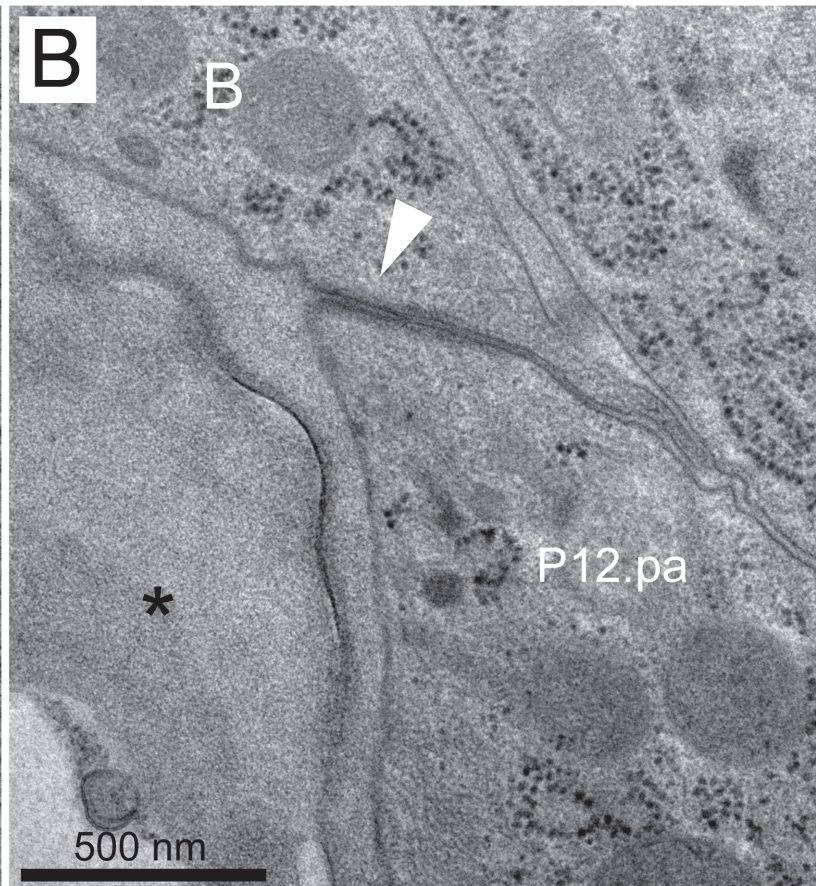
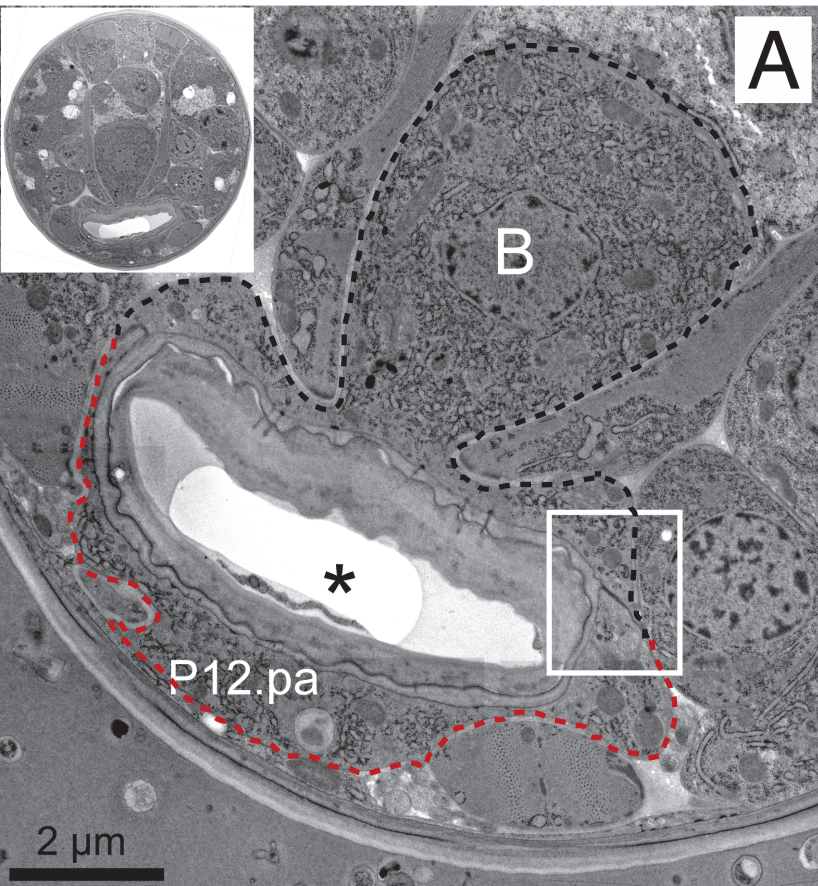
Supplemental references

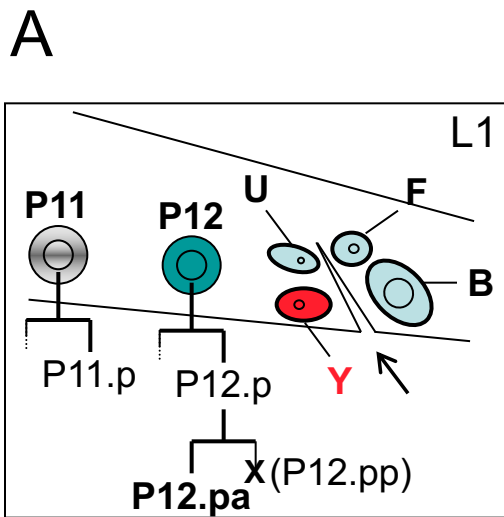
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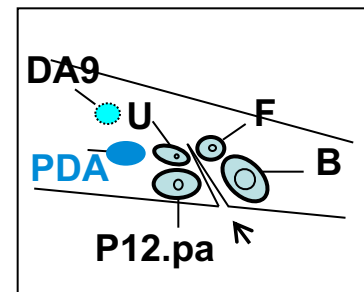
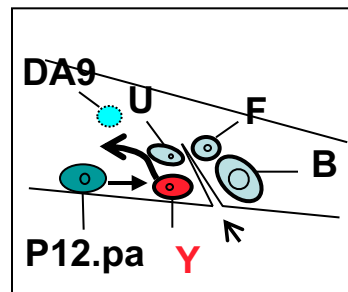


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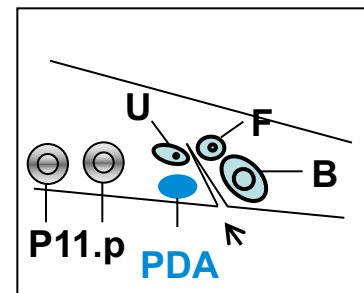
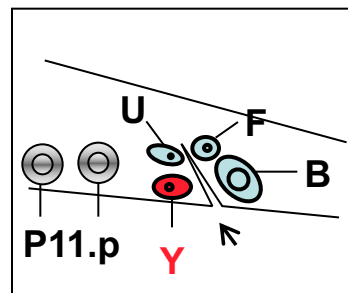
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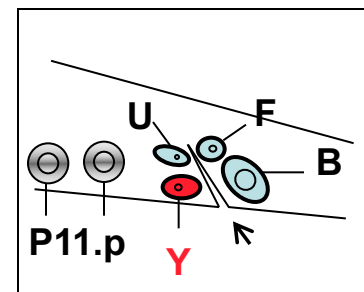
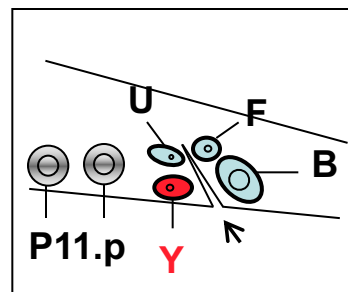
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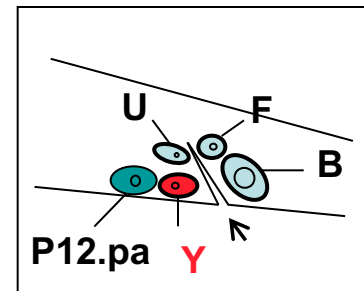
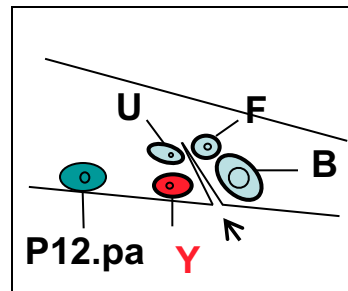
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egl-5(0)



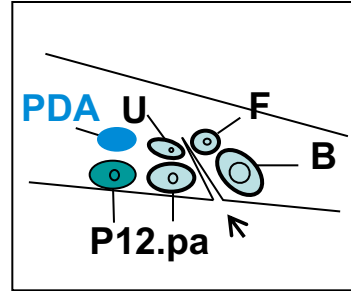
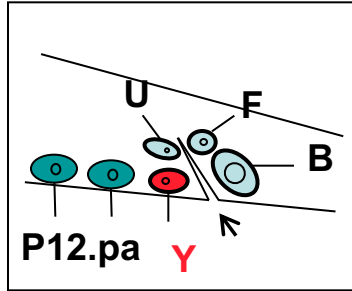
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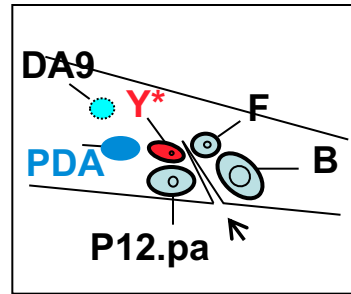
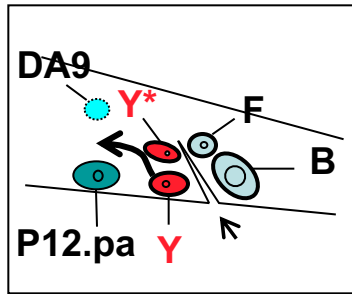
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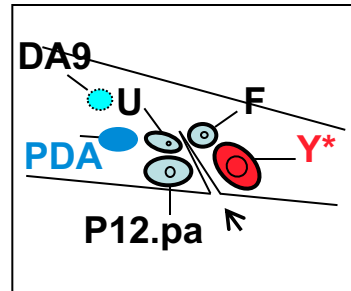
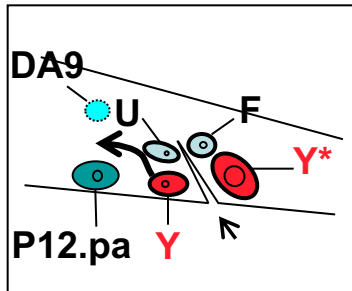
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mab-9(0)



lin-12(d)

