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A *Caenorhabditis elegans* model for epithelial–neuronal transdifferentiation

Sophie Jarriault^{*†‡}, Yannick Schwab^{†§}, and Iva Greenwald^{¶||}

^{*}Department of Cell and Developmental Biology and [§]Electron Microscopy Platform, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Institut National de la Santé et de la Recherche Médicale U596, Centre National de la Recherche Scientifique Unité Mixte de Recherche 7104, F-67404 Illkirch, France; [†]Université Louis Pasteur, F-67000 Strasbourg, France; and [¶]Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032

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Understanding transdifferentiation—the conversion of one differentiated cell type into another—is important from both basic science and clinical perspectives. In *Caenorhabditis elegans*, an epithelial cell named Y is initially part of the rectum but later appears to withdraw, migrate, and then become a motor neuron named PDA. Here, we show that this represents a bona fide transdifferentiation event: Y has epithelial hallmarks without detectable neural characteristics, and PDA has no residual epithelial characteristics. Using available mutants and laser microsurgery, we found that transdifferentiation does not depend on fusion with a neighboring cell or require migration of Y away from the rectum, that other rectal epithelial cells are not competent to transdifferentiate, and that transdifferentiation requires the EGL-5 and SEM-4 transcription factors and LIN-12/Notch signaling. Our results establish Y-to-PDA transdifferentiation as a genetically tractable model for deciphering the mechanisms underlying cellular plasticity *in vivo*.

cell plasticity | motor neuron | rectum | hindgut

Although it is commonly believed that commitment and differentiation are stable events, in fact, under some circumstances, committed or differentiated cells have the ability to change their fates (1). Various examples of cell plasticity, from the reprogramming of a nucleus through cloning to the reprogramming of tissue stem cells, have suggested that the final identity of a cell is not locked. Transdifferentiation, the process by which one differentiated cell type changes into another directly (2, 3), is one kind of cell plasticity.

Classic work on the complete cell lineage of *Caenorhabditis elegans* is consistent with the possibility that transdifferentiation occurs naturally during *C. elegans* development: Observation of nuclear division and morphology using Nomarski microscopy suggests that a few cells seem to change identity during larval development (4). However, for any of these apparent identity changes to be true examples of transdifferentiation, it must be established that the cell is fully differentiated into different cell types both before and after the apparent transdifferentiation event (2).

Here, we have focused on a cell called “Y,” which is born in the embryo and forms part of the rectum until the second larval stage, when it rescinds from the rectum, migrates anteriorly, and becomes a motor neuron named PDA (4–6). PDA has a characteristic axonal process and synaptic connections that have been described at the ultrastructural level (5, 6). Here, we demonstrate the epithelial nature of Y by ultrastructural and molecular criteria and show that it does not express neuronal markers, establishing it as a fully differentiated rectal epithelial cell. We also show that PDA lacks expression of epithelial markers and has specific neuronal characteristics. Thus, the Y-to-PDA change appears to be a bona fide example of transdifferentiation. We also perform an initial characterization of this process, using genetics and cell ablation to explore factors pertaining to competence, lineage, and local environment.

Results

The rectum is a vital organ formed during embryogenesis and is made of three pairs of cells, named K and K', U and F, and Y and B. Each pair forms a toroid of the rectal epithelium (Fig. 1A). Based on nuclear morphology and position, Sulston *et al.* (4) reported that during the second larval stage (L2), Y rescinds from the rectum and migrates anterodorsally. Another cell, named P12.pa, born at the end of the L1 stage just anterior to the position of Y, replaces Y in the rectum, completing the toroid with B. Y subsequently differentiates as the PDA motor neuron (Fig. 1B), with a characteristic axonal process that extends ventrally toward the posterior end past the rectum, makes a right-handed commissure and extends along the dorsal cord toward the anterior of the worm (5, 6). By contrast, the rectal cells B, U, F, and K' remain in the rectum at all stages. We note that Y migration appears to involve the whole cell, not just its nucleus as previously observed by using Nomarski optics (4), because marker expression (see below) shows that Y and PDA have a totally different cell shape and position.

The timing of these morphological events is stereotyped; different phases can be correlated with the presumably independent events of somatic gonad development (7) (Fig. 1B), facilitating the analysis of mutants with defects in Y-to-PDA plasticity). Despite the stereotyped timing, the heterochronic genes *lin-4*, *lin-14*, and *lin-28*, which control the timing of many L1 and L2 stage-specific events (8), do not affect the Y-to-PDA change [supporting information (SI) Table 4].

In the next three sections, we use multiple markers (summarized in SI Table 5), several identified expressly for this purpose here, and ultrastructural features to show that Y has only epithelial character while it is part of the rectum and that these features are completely lost and replaced with neuronal characteristics when it becomes the PDA neuron, supporting the view that the Y-to-PDA change is a transdifferentiation event. We then examine environmental and genetic factors that might influence this event.

Y Displays the Hallmark Ultrastructural Characteristics of Rectal Epithelial Cells. We reconstructed the rectal area of newly hatched L1 and L4 hermaphrodites using serial section electron microscopy. We compared L1 Y to the other L1 rectal cells and to L4 P12.pa, the cell that replaced it (Fig. 2 and SI Fig. 3). All of these

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[†]To whom correspondence may be addressed at: Department of Cell and Developmental Biology, Institut de Génétique et de Biologie Moléculaire et Cellulaire, 1 Rue Laurent Fries, BP 10142, Illkirch Cedex 67404, France. E-mail: sophie@igbmc.u-strasbg.fr.

[¶]To whom correspondence may be addressed. E-mail: greenwald@cancercenter.columbia.edu.

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Table 1. Cell ablation experiments

Genotype operated	Ablated cell(s)	No. of Y-to-PDA per total no.
<i>cog-1::gfp</i>	B	13 of 15*
<i>egl-26::gfp</i>	B	28 of 29 [†]
<i>egl-26::gfp</i>	U	16 of 19 [†]
<i>egl-26::gfp</i>	F	13 of 14 [†]
<i>egl-26::gfp</i>	Phasmid sheath [‡]	13 of 13 [†]
<i>cog-1::gfp</i>	P12	0 of 10 [§]
<i>cog-1::gfp</i>	P12.p	2 of 6 [§]
<i>cog-1::gfp</i>	P12.pa	11 of 11 [§]
<i>egl-5(0); cog-1::gfp</i>	Extra P11	0 of 6*
<i>egl-5(0); cog-1::gfp</i>	Extra P11.p	0 of 8*
<i>egl-38(0); egl-26::gfp</i>	Y	9 of 10 [¶]

Ablations were performed in newly hatched L1 larvae or as soon as the relevant cell was born; operated animals were scored when they reached the L3 stage or older.

*Presence of a PDA neuron (i.e., WT phenotype; *cog-1::gfp* scored).

[†]Absence of a persistent Y cell (i.e., WT phenotype; *egl-26::gfp* scored, confirmed by Nomarski scoring).

[‡]The two phasmid sheath cells seem to extend toward B and Y and hence were candidates for a PDA-inducing signal.

[§]WT position of a *cog-1::gfp*-expressing PDA neuron scored; a PDA motor neuron was always found.

[¶]Presence of the *egl-26::gfp*-expressing extra Y-like cell in the rectum scored.

express a marker associated with engulfment (*ced-1::gfp*, data not shown). These results suggest that fusion with prospective neurons is unlikely to initiate Y-to-PDA transdifferentiation.

We also examined the effect of removing cells that are in close contact with Y (Table 1). Laser ablation of the rectal cells B, U, or F or phasmid sheath cells in newly hatched *egl-26::gfp* L1 larvae did not affect Y migration and PDA formation (Table 1). Furthermore, ablation of P12 or P12.p, the precursors to P12.pa, does not affect the formation of PDA (Table 1). These ablation results suggest that no single neighboring cell acts as a source of a putative signal required for Y-to-PDA transdifferentiation. However, the fragility of operated animals prohibited the scoring of individuals that had undergone ablation of multiple cells to assess potential cellular redundancy.

Interestingly, although ablation of P12 did not affect transdifferentiation, it blocked Y migration: In 10 of 10 animals in which P12 and 4 of 6 in which P12.p had been ablated, PDA formed ventrally near the rectum—the position of Y in unoperated L1 animals (Table 1). Wnt or EGF pathway mutants that lack a P12 cell (20) display a similar phenotype (SI Fig. 5 and SI Table 7). Thus, P12 and/or P12.p may provide a signal that promotes or sustains Y migration, but migration *per se* does not appear to provide a microenvironment necessary for transdifferentiation.

***egl-5* and *sem-4* Are Required for an Early Step of the Y-to-PDA Identity Change.** *egl-5* and *sem-4* mutations affect the fates of many adjacent cells in the tail that are related by position but not lineage (21, 22), and, in these mutants, Y has been reported to sustain an epithelial appearance in later larvae, as judged by Nomarski optics. We have confirmed this inference by using epithelial and PDA markers.

In *egl-5(n945)* null mutants, the epithelial marker *ajm-1::gfp* is expressed in Y when the worms hatch (38 of 38 animals) and continues to be so in older animals (Table 2), and the PDA marker *ace-3/4::gfp* is not expressed (Table 2). Thus, in *egl-5* null mutants, Y stays part of the rectum, suggesting that transdifferentiation is not initiated. We note that P12 adopts the fate of P11 so that no P12.pa cell is formed, and two cells that have nuclei

Table 2. Transdifferentiation of Y to PDA does not occur in *egl-5* and *sem-4* mutants

Relevant genotype	2 P11.p, % (n)	Persistent Y, % (n)	No PDA, % (n)
Wild type	0 (34)	0 (34)	5.8 (51)
<i>egl-5(n945)</i>	97.5 (41)	100 (41)	100 (34)
<i>sem-4(n1971)</i>	12.5 (32)	84.8 (79)*	100 (38)

(n), total number of L3 and older hermaphrodites scored; 2 P11.p, percentage of animals with 2 P11.p-like cells; persistent Y, percentage of animals in which Y remained at its initial position with an epithelial appearance and expressed an epithelial or a Y marker. *ajm-1::gfp* was used to score *egl-5* mutants and *egl-26::gfp* was used for *sem-4* mutants; No PDA, percentage of animals that did not express *ace-3/4::gfp*.

*In 15% *sem-4* mutants, P12.pa is not formed or does not differentiate as a rectal epithelial cell, as assessed by using both *egl-26::gfp* and *egl-5::gfp* reporters. This result suggests that hermaphrodites scored here as lacking a persistent Y probably had one but no recognizable P12.pa consistent with the results obtained with a PDA marker.

with epithelial appearance in Nomarski are found in the anterior rectum area (21). Laser ablation of the “extra P11” or “extra P11.p” did not restore a PDA motor neuron in operated *egl-5* hermaphrodites (Table 1), and, because the absence of P12 does not impair Y transdifferentiation (Table 1), the altered cellular neighborhood of Y in *egl-5(n945)* does not appear to be responsible for the block in transdifferentiation.

Approximately 85% of *sem-4(n1971)* null mutants appear to exhibit a persistent Y phenotype by morphological criteria or continued expression of a Y marker (*egl-26::gfp*, Table 2 and SI Fig. 5). We also observed a weakly penetrant P12.pa defect in *sem-4* mutants, accounting for the remaining animals that have two cells in the rectum, appearing as if Y were not affected. In addition, the PDA marker was never observed to be expressed in *sem-4* mutants, consistent with a complete failure of Y-to-PDA transdifferentiation (Table 2). The rectal epithelial character of the persistent Y, found at its original location, was confirmed by *egl-5* and *ajm-1* expression (23 of 26 and 26 of 26 L3 and older animals, respectively), indicating that it was blocked at an early step in transdifferentiation. We note that *sem-4* regulates the expression of various *hox* genes, including *egl-5*, during *C. elegans* development (23, 24); however, an *egl-5::gfp* reporter is expressed normally in the rectal area of *sem-4* null mutants (data not shown), suggesting that such a regulation is not the basis of their similar Y phenotype.

In summary, Y expresses epithelial markers and remains as part of the rectum in *egl-5* and *sem-4* mutants. We conclude that the transdifferentiation of Y-to-PDA is affected at a very early step, which, assuming cell-autonomy, may reflect compromised competence or defective reception/implementation of a hypothetical transdifferentiation signal.

Ectopic Y Cells Generated by Transformation of Other Rectal Cells Are Not Competent to Transdifferentiate. We first tested whether there is a “counting mechanism” in the rectal epithelium by asking whether an extra rectal-bound P12.pa can differentiate as PDA. We examined *lin-15(n765ts)* animals grown at 25°C, under which conditions P11 is sometimes transformed into a supernumerary P12 cell, resulting in two P12.pa cells (20). Although 32% of the time, P11.p has undergone such a transformation (n = 112), we never observed an additional cell expressing a PDA marker (0%, n = 37), suggesting that an extra rectal cell *per se* does not become competent to transdifferentiate as PDA.

We next assessed the fate of “supernumerary Y” cells made at the expense of other rectal epithelial cells. We considered a cell to be a supernumerary Y if it had the appropriate morphology and

Table 3. The ectopic Y cell in *lin-12* mutants, but not in *egl-38* or *mab-9* mutants, undergoes transdifferentiation

Genotype	2 PDA in L4, % (n)	B GFP + in L1, % (n)	B GFP + in L4, % (n)	U GFP + in L1, % (n)	U GFP + in L4, % (n)
<i>egl-26::gfp</i> (Y marker)	—	100 (39)	100 (34)	25 (39)	0 (34)
<i>mab-9(e2410);egl-26::gfp</i>	—	93 (30)*	88 (51)*	—	—
<i>egl-38(sy294);egl-26::gfp</i>	—	—	—	100 (21) [†]	74 (23) [†]
<i>cog-1::gfp</i> (PDA marker)	0 (59)	—	0 (59)	—	—
<i>mab-9(e2410);cog-1::gfp</i>	0 (37)	—	0 (37)*	—	—
<i>ace-3/4::gfp</i> (PDA marker)	0 (131)	—	—	—	0 (51)
<i>egl-38(sy294);ace-3/4::gfp</i>	0 (95)	—	—	—	0 (95) [†]
<i>lin-12(n137);ace-3/4::gfp</i>	76 (54) [‡]	—	—	—	—

The percentage of newly hatched (L1) or L3 to adult (collectively called L4) hermaphrodites were scored for the presence of a GFP-positive cell at the position of the extra Y; (n), total number of animal scored.

*The cell at B position forming the extra Y cell was scored.

[†]The cell at U position forming the extra Y cell was scored.

[‡]In some *lin-12(n137)* mutant hermaphrodites (20/54), the axon of one or both of the neurons expressing the PDA marker went more posteriorly than it does in wild type before joining the dorsal cord, perhaps reflecting an effect of elevated LIN-12/Notch activity on axon guidance.

marker expression in the L1 stage and then assessed its ability to transdifferentiate into PDA in parallel with the “real Y.”

In *egl-38* loss-of-function mutant males, U is transformed into Y, and expression of genes normally expressed in U are lost in hermaphrodites (25, 26). We confirmed that in *egl-38* mutant hermaphrodites, as in males, U is transformed into Y: In an *egl-38* mutant, in contrast to wild type, the cell at U's position ectopically expresses *egl-26::gfp* in L1 (Table 3 and SI Fig. 5) and continues to do so in older larvae (0% WT L4 versus 74% *egl-38* L4 mutants express *egl-26::GFP*, Table 3). Although there is apparently a supernumerary Y, only one PDA cell is observed (Table 3), suggesting that only the “real Y” transdifferentiates into PDA. The supernumerary Y appears instead to be part of the rectum, displays an epithelial appearance and expresses *egl-26::gfp* (Table 3). Furthermore, when Y is ablated in *egl-38* mutant L1 hermaphrodites, the cell at the U position keeps its epithelial morphology and stays at its position in the rectum in older larvae or adult (Table 1). In a *mab-9* null mutant male, B adopts the identity of Y (27). In hermaphrodites, the cell at the B position has normal nuclear morphology in the L1 stage and expresses the epithelial marker *egl-26::gfp* (Table 3 and SI Fig. 5). Although in some older *mab-9* mutants, the B nucleus appears smaller and sometimes lacks a nucleolus (24 of 51 animals), B continues to express *egl-26::gfp* in most L4 hermaphrodites, suggesting that it retains epithelial characteristics, and fails to express the PDA marker (Table 3). These results suggest that the ectopic Y does not undergo transdifferentiation. In sum, in both *egl-38* and *mab-9* mutants, the normal Y transdifferentiates into PDA but the extra Y-like cell does not. Because the extra Y-like looks like the true Y in all other respects, we believe that the results suggest that other rectal cells are lineally or otherwise intrinsically restricted such that they lack the potential to transdifferentiate or to respond to a hypothetical transdifferentiation-inducing signal.

Ectopic Y Cells Caused by Activation of *lin-12/Notch* Transdifferentiation. In the wild-type embryo, ABprpppaaaa is the future Y, and its contralateral lineal homolog ABplpppaaaa is the future neuron DA9 (28). In *lin-12(n137)* mutants, LIN-12 is constitutively active, and, in males, both of these cells adopt the Y fate (29). Both cells appear to adopt the Y fate in hermaphrodites, too, as in 53% of *lin-12(n137)* L1 hermaphrodites (19 of 36), an extra epithelial cell is found in the anterior rectal area (as seen by anatomy or expression of *egl-26::gfp*). Furthermore a PDA marker is expressed in two adjacent neurons in L4 hermaphrodites (SI Fig. 5 and Table 3), suggesting that both the normal and the extra Y cell transdifferentiated into PDA neurons.

To assess when *lin-12* activity is required for transdifferentiation, we used a temperature-sensitive partial loss-of-function allele, *lin-12(n676n930)*. *lin-12* activity is required for a Y cell to be formed: When *lin-12(n676n930)* mutants are grown at 25°C, no L1 hermaphrodites have a Y cell; at 15°C, approximately two-thirds of them do (30). To remove LIN-12 function shortly after, or around the time of Y cell specification, we allowed *lin-12(n676n930); cog-1::gfp* embryos to develop at 15°C until embryonic stages ranging from just before Y cell birth to the 3-fold stage (a time window of ≈250 min at 25°C), at which point, embryos were shifted to 25°C. Half of the newly hatched L1s were then scored for successful Y cell formation (based on anatomy), whereas the other half were scored as L4s or adults for the presence of a PDA motor-neuron (using *cog-1::gfp* expression). We observed that 72% (43 of 60) of newly hatched L1s had a Y cell, and 60% of older animals (33 of 55) had a PDA motor-neuron, statistically indistinguishable numbers ($P = 0.4589$, Fisher's exact test). There appears to be little perdurance of active LIN-12 protein upon temperature shift, because only 3 of 23 embryos that were transferred at 25°C within 100 min of ABprpppaaaa birth had a cell with Y characteristics. We interpret these results as suggesting that *lin-12* acts at the same time that Y is specified to endow it with the competence to transdifferentiate and is not required at the time of transdifferentiation *per se*.

Discussion

In this study, we have provided evidence that the apparent change in fate of the rectal epithelial cell Y into the motor neuron PDA is a bona fide transdifferentiation event. We have also investigated the effect of various cellular and genetic factors on transdifferentiation. The results presented here, together with the many virtues of *C. elegans* for genetic and other experimental manipulations, establish Y-to-PDA transdifferentiation as a compelling model to characterize cellular plasticity *in vivo*.

Observations of wild-type and mutant hermaphrodites suggest that there are at least five phases in the Y-to-PDA transdifferentiation process: establishment of the Y epithelial identity; establishment of competence to undergo transdifferentiation; retraction from the rectum, in a process that resembles epithelial-to-mesenchyme transition; migration of Y away from the rectum; and establishment of neural identity as PDA (4–6). Altering the cellular environment of Y and varying the number, identity, and position of cells in the rectum suggest that transdifferentiation does not appear to require Y to migrate to the PDA position, to fuse with prospective neurons or other cells, or to interact with specific single

neighboring rectal cells. Furthermore, the onset of transdifferentiation does not appear to require the activity of heterochronic genes that control many other L1 or L2-specific events, raising the possibility that the onset of transdifferentiation is controlled by an unidentified developmental timer acting independently of the heterochronic pathway (31).

The *egl-5/Abd-B* or *sem-4/spalt* genes are required for transdifferentiation. In these mutants, Y remains a rectal epithelial cell, raising the possibility that *egl-5* and *sem-4* set or maintain the competence of Y to become PDA or are involved in triggering the transdifferentiation program. If so, neither gene activity is sufficient to promote transdifferentiation, because both genes are expressed in other rectal cells that do not transdifferentiate [*egl-5* in U, F and B (32); *sem-4*, U, F, and B (ref. 23 and this study)]. Alternatively, *egl-5* and *sem-4* activities might be important in Y neighboring cell(s) to establish a necessary “niche” for Y transdifferentiation.

LIN-12/Notch signaling appears to act during hermaphrodite embryogenesis both to specify Y and also to endow Y with the potential to transdifferentiate. In *lin-12(d)* mutants, when LIN-12 is constitutively active, the normal Y is formed, as well as an “extra Y” instead of the prospective neuron DA9 (29); both the normal and the supernumerary Y cells transdifferentiate into PDA neurons. In contrast, in other mutants in which a supernumerary Y cell is created, *egl-38* (U transformed into Y) and *mab-9* (B transformed into Y), only the normal Y transdifferentiates, whereas the supernumerary Y remains an epithelial cell. Y differs from the other rectal cells in that it alone must experience *lin-12* activity at the time it is born to differentiate as a rectal cell: In the absence of *lin-12* activity, no epithelial Y cell is formed (29). Together, these observations suggest that the competence to transdifferentiate is specified in parallel to the distinct Y epithelial fate, because lack of the competence to transdifferentiate does not involve loss of Y rectal epithelial identity. A simple model is that activation of *lin-12* in the future Y results in activation of two distinct sets of genes, one needed to ensure that the right contralateral homolog becomes a Y epithelial cell and one giving it the competence to change its identity.

We note two salient features of Y-to-PDA transdifferentiation in the context of other developmental phenomena or putative or confirmed transdifferentiation events. First, the Y-to-PDA epithelial–neuronal transdifferentiation phenomenon does not involve cell division, unlike, for example, neurogenesis during *Drosophila* development, which involves the generation of neuroblasts from a transient, proliferating epithelium. Cell division is also a feature of other transdifferentiation models, such as regeneration in urodeles (33–35) or the presumptive transdifferentiation of astrocytes into neurons during adult neurogenesis (36, 37). It is not clear, however, whether cell division *per se* is needed for transdifferentiation in these contexts. If so, transdifferentiation of Y may involve a distinct mechanism. In another *C. elegans* model for transdifferentiation, female germ cells can differentiate into various somatic cell types in mutants lacking certain translational regulators. In this model, entry into meiosis is critical for transdifferentiation (38), so the mechanistic relationship of this interesting phenomenon to somatic transdifferentiation is not clear.

Second, transdifferentiation during regeneration in urodeles, in cell culture or adult neurogenesis (33, 34, 37, 39), appears to involve, at least partially, progression through a proliferative dedifferentiated state. It is interesting to note that Y undergoes what superficially resembles an epithelial-to-mesenchymal transition, suggesting a transition through an intermediary state. However, we do not yet know whether Y undergoes a transition to a dedifferentiated state, or whether loss of its epithelial identity happens in parallel to gain of the neural one.

The tractability of *C. elegans* to genetic analysis should allow us to explore in a systematic way the genetic circuitry and consequent molecular cascades underlying transdifferentiation *in vivo*. Indeed, a pilot screen initiated to isolate transdifferentiation mutants has yielded 10 mutants in which a Y cell is initially found but no PDA is made (V. Pavet, N. Vaucamps, and S.J., unpublished data). The understanding of the factors that permit a differentiated cell to change its identity has significant consequences for our understanding of the appearance and progression of various cancers and for our ability to reprogram cells for therapeutic purposes.

Materials and Methods

Genetics. Experiments were conducted at 20°C unless otherwise indicated. The wild-type parent for most strains used in this study is the *C. elegans* var. Bristol strain N2. The relevant mutations used in this study are: LG I: *sem-4(n1971)*, *lin-44(n1792)*, *lin-28(n719)*, *ced-1(n1735)*, *ced-12(k149)*; LG II: *mab-9(e2410)*, *lin-4(e912)*; LG III: *egl-5(n945)*, *lin-12(n676n930ts)*, *lin-12(n137)*; LG IV: *egl-38(sy294)*, *ced-3(n717)*, *ced-3(n1286)*, *ced-10(n3246)*, *let-60(sy93dn)*; and LG X: *lin-15(n765ts)*, *bar-1(ga80)*, *lin-14(n179ts)*.

Information about these alleles can be obtained from Wormbase, www.wormbase.org. See *SI Text* for details about the epithelial markers *mcEx242* [CHE-14::GFP], *jcls1* [AJM-1::GFP], *mcls47* [DLG-1::GFP], *mcEx* [LIN-26::GFP]; the rectal epithelial markers *bxls7* [*egl-5::gfp*], *kuls36* [*egl-26::gfp*]; *kuls34* [*sem-4::gfp*]; the PDA markers *fpls1* [*ace-3/4::gfp*], *syIs63* [*cog-1::gfp*], and *arEx627* [*exp-1::gfp*]; and the other markers used or assessed for expression in Y or PDA.

Anatomy and Laser Ablation. Methods used for electron microscopy are described in *SI Text*. For live-animal analyses, cells were identified based on their characteristic morphology and position by Nomarski optics or GFP expression from transgenes on a Zeiss Z1 Axio imager. A Micropoint laser beam (4) was used to ablate Y, B, U, F, or the phasmid sheath cells in newly hatched L1 hermaphrodites. Ablations of P11 or P12 were performed on L1 hermaphrodites after these cells had entered the ventral cord (6–9 h after hatching). Ablations of P11.p, P12.p, or P12.pa were performed on older L1 hermaphrodites in which the somatic gonadal precursor cells had divided at least twice.

Antibody Staining and Expression in the Y Cell. Synchronized L1 worms expressing CHE-14::GFP (*mcEx242*) or DLG-1::GFP (*mcls47*) were stained as described (40) by using antibodies against GFP protein (Molecular Probes) and Cy3- or FITC-conjugated secondary antibodies, together with DAPI. The *C. elegans* junction marker MH27 antibody against AJM-1 (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) was also used on the fixed animals. Worms were mounted in a drop of antifade (80% glycerol, 20% PBS, 5% propylgalate) and analyzed with a Zeiss Z1 IMAGER 2 microscope or a Leica SP2 AOBs confocal microscope. Alternatively, live worms were anesthetized in 10 mM sodium azide and analyzed with a Zeiss Z1 IMAGER 2 microscope.

Mutant Analysis and Scoring. The Y nucleus has a characteristic morphology and appearance in wild-type hermaphrodites and, in the L1 stage, Y expresses the epithelial markers *ajm-1*, *dlg-1*, *che-14*, *lin-26*, and *egl-26*; it also expresses *egl-5* and *sem-4*. After transdifferentiation into PDA, its nucleus has a different morphology characteristic of neurons and a characteristic axon and expresses the PDA markers *ace-3/4*, *cog-1*, and *exp-1*.

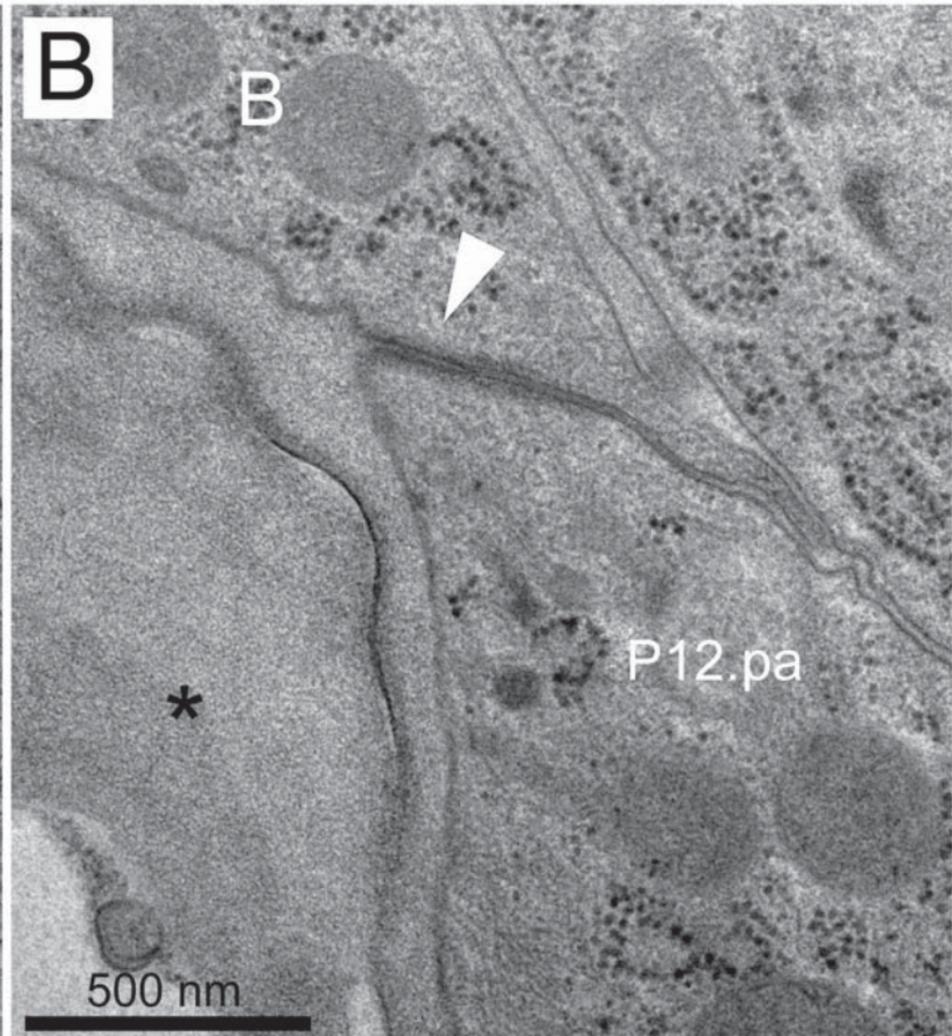
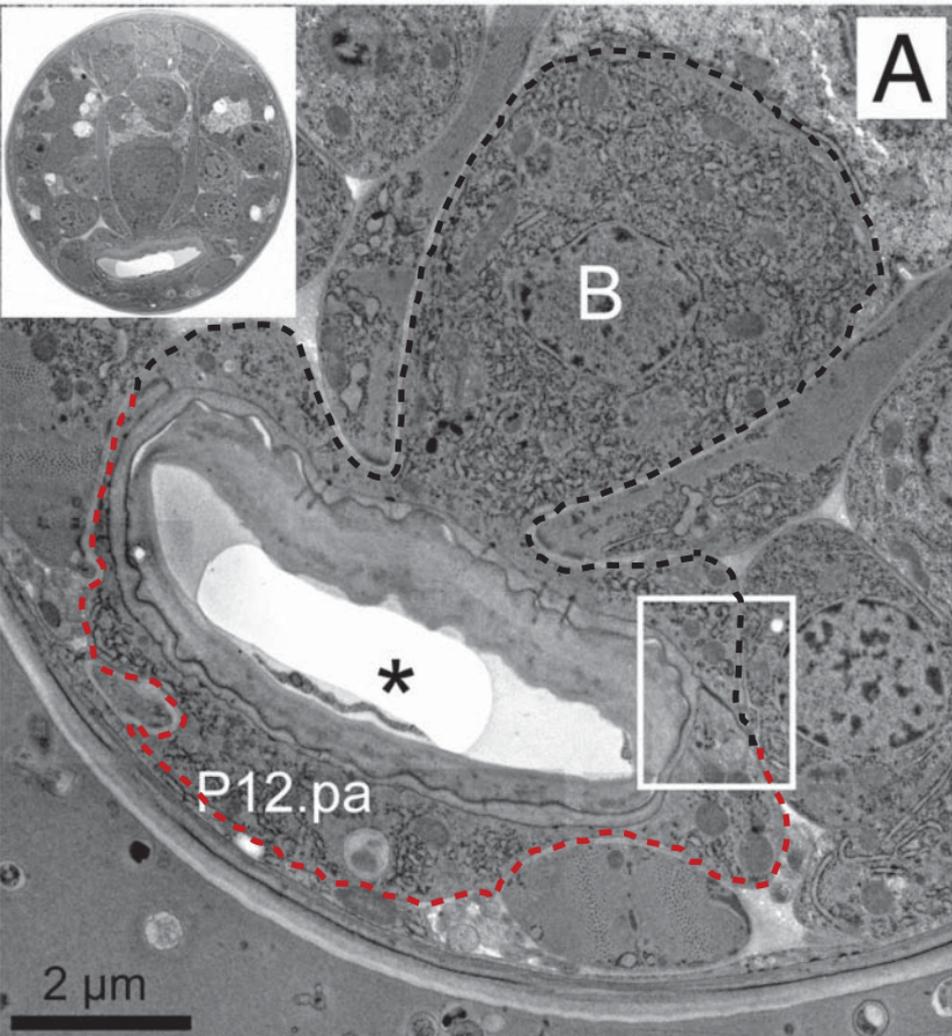
To assess whether Y is present and transdifferentiates normally in mutant backgrounds, all or a subset of the following criteria were used. For Y identity, epithelial appearance, and epithelial and/or Y marker gene expression in the early L1 stage. For PDA identity, neuronal appearance and PDA marker gene expression in the L3 stage and later was used. A mutant phenotype can be inferred from an altered nuclear position and/or morphology and marker gene expression patterns. For Nomarski optics scoring, we relied on the characteristic rectal cell and P11.p morphologies. In L3 and older wild-type animals, there are three cells with epithelial appearance in the anterior rectum area: U, P12.pa, and P11.p. Mutants in which Y does not transdifferentiate have four epithelial cells in the same area: U, Y, P12.pa, and P11.p or, if a P12 cell is not made, U, Y, and 2 P11.p. Note that some of the mutants used in this study are constipated, making scoring by Nomarski microscopy less reliable and GFP scoring the method of choice.

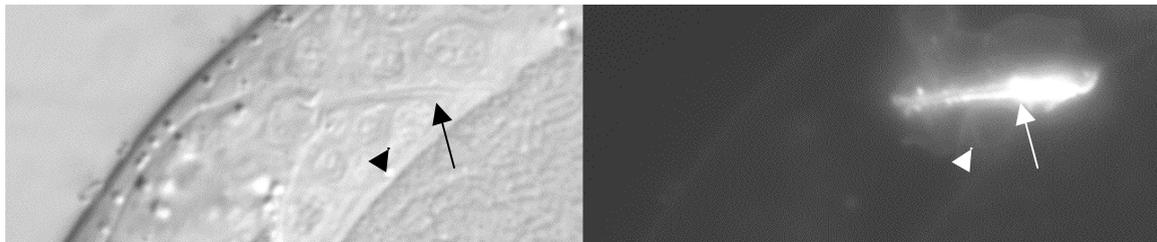
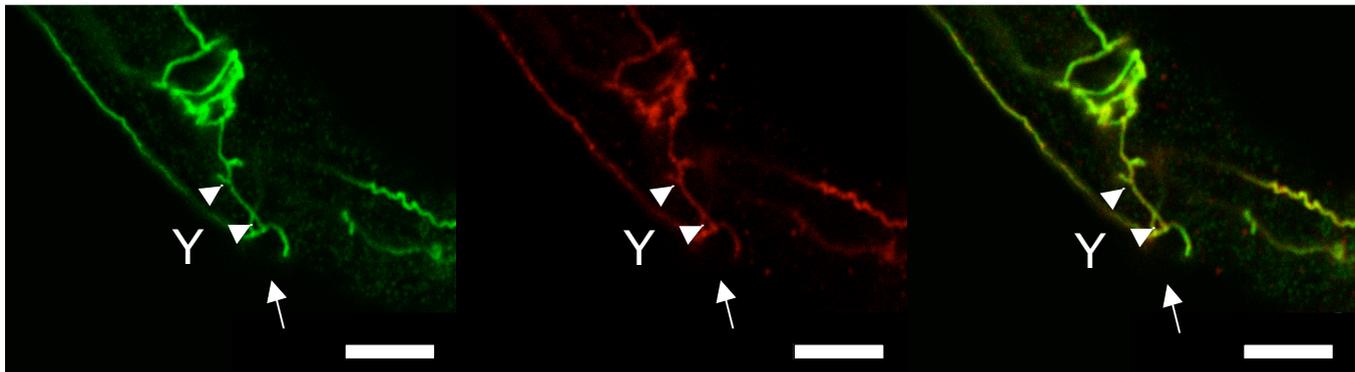
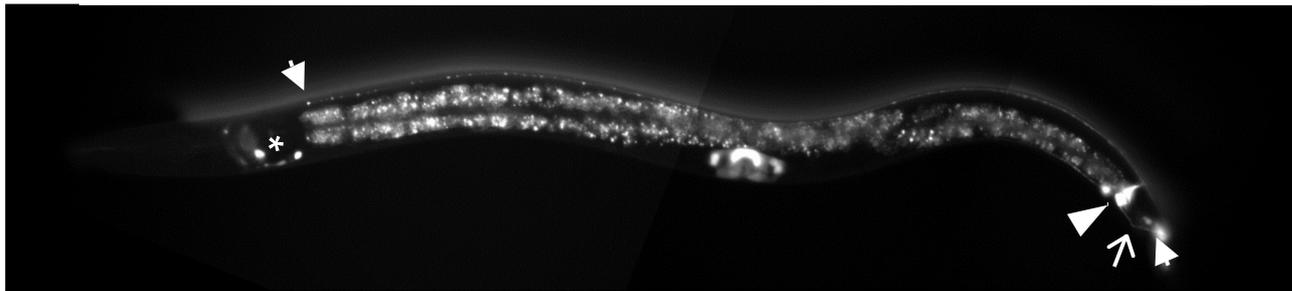
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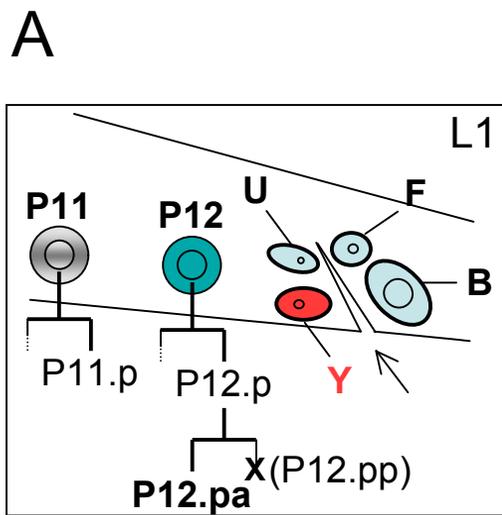
Wendy Hanna-Rose (Pennsylvania State University, University Park, PA), Mike Krause (National Institutes of Health, Bethesda), Michel Labouesse (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France), Zheng Zhou (Baylor College of Medicine, Houston), Theresa Stienagle (University of Minnesota, Minneapolis), and the *Caenorhabditis* Genetics Center, which is funded by the National Institutes of Health National Center for Research Resources, for providing useful plasmids or nematode strains; Scott Clark, Caroline Goutte, Oliver Hobert, Michel Labouesse, Xiajun Li, and Sophie Quintin for a critical reading of the manuscript; Dave Hall, Renaud Legouis, Michel Labouesse, and Erik Jorgensen for helpful discussions; Valeria Pavet for the initial scoring of *cog-1*

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A**B****C**

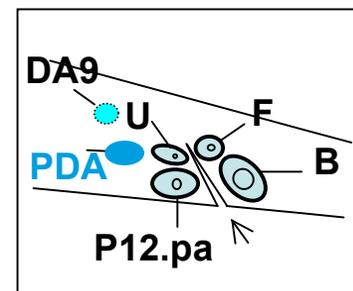
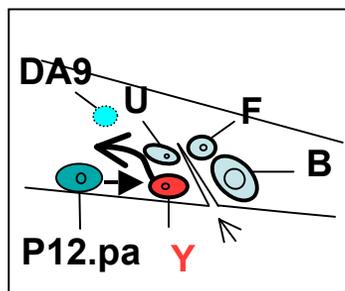


B

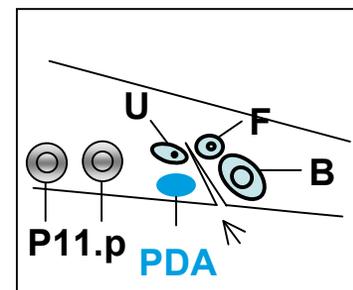
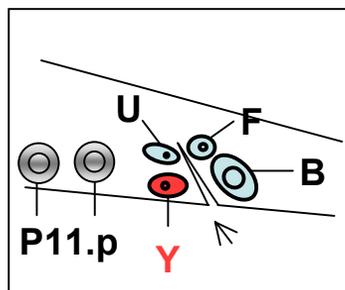
L1

L3

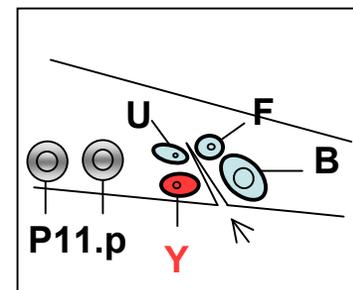
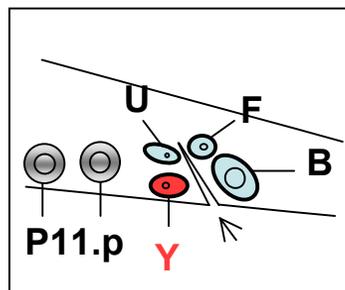
WT



bar-1(0)



egl-5(0)



sem-4(0)

