A Caenorhabditis elegans model for epithelial–neuronal transdifferentiation

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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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Expression of both ceh-6 and peb-1, which are required for proper differentiation or function of the rectal cells, are expressed in Y in the L1 stage but disappear from Y as it withdraws from the rectum (15, 16).

Neuronal Markers Are Not Expressed in Y. Two previous studies establish PDA as being a motor neuron based on its ultrastructural features (5, 6). To test whether Y has any neuronal characteristics while functioning and appearing epithelial in the L1 stage, we first looked at the pan-neuronal markers unc-119:gfp (17) and F25B3.3::gfp (18). The panneuronal markers are absent in Y in the L1 stage (0 of 26 and 0 of 32 animals expressed unc-119:gfp and F25B3.3::gfp, respectively) and, as expected, appear present in PDA neurons (data not shown). To assay specifically for PDA identity, we examined a number of transgenes (see SI Text) and found that cog-1::gfp and ace-3::gfp are useful PDA markers. In particular, cog-1::gfp and ace-3::gfp are strongly and reliably expressed in PDA (59 of 59 cog-1::gfp and 48 of 51 ace-3::gfp L3 or older hermaphrodites) but are never expressed in Y (0 of 30 cog-1::gfp and 0 of 45 ace-3::gfp L1 hermaphrodites).

Our results strongly support the view that the Y-to-PDA identity change is a bona fide example of transdifferentiation: Y has epithelial hallmarks and no evident neuronal character, whereas PDA has neuronal hallmarks and no residual epithelial character.

Assessment of the Role of the Cellular Environment on Y Transdifferentiation. We investigated whether the microenvironment, sometimes referred to as “niche,” could influence Y-to-PDA transdifferentiation.

Grafted head nerve cells change their fates after fusion with differentiated cells of the host (19). Thus, we asked whether the Y-to-PDA identity change is triggered by the fusion of Y with a neuron or a cell with neuronal potential in the local environment. Although all neurons initially present in the neighborhood of Y remain intact throughout development (4–6), there are transient prospective neurons that normally undergo apoptosis and are engulfed by nearby hypodermal cells (4). We found that Y-to-PDA transdifferentiation occurs normally when apoptosis or engulfment is defective (SI Table 6); furthermore, Y does not

Fig. 2. Ultrastructural characteristics of Y. (A) Electron micrograph of the rectal area of a newly hatched L1 hermaphrodite showing the B and the Y cells (outlined in black and red, respectively) wrapped around the rectum, both displaying a train-rail-like shape. (Inset) The section of the whole worm from which the rectal area is magnified in A. (B) Blow-up of the boxed area in A, illustrating a C. elegans junction (arrowhead) between the apical membranes of Y and B. (C) Another section of the same L1 animal illustrating a junction between the apical side of the Y cell, or the B cell, and the cuticle of the rectum (arrows). This structure is called a fibrous organelle in C. elegans. An asterisk indicates the rectal slit.
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; cog-1::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.
¶Presence 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.

Table 1. Cell ablation experiments

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

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).

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

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

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.

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 ABprppppaaa 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-tomesenchyme 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 (39); 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 neurons 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 models, such as blastemal cells from a transient, proliferating epithelium. Cell division is also 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 isolates, in which a Y cell is initially specified but no PDA is made (V. Pavot, 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-64(n1792), lin-28(n17), ced-1(n1735), ced-12(k149); LG II: mab-9(e2410), lin-4(e912); LG III: egl-5(n454), lin-12(n6769ts); LG IV: egl-38(y294), ced-3(n17), ced-3(n1286), ced-10(n3246), let-60(y93); 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], jcs1 [AJM-1::GFP], mtc47 [DLG-1::GFP], mcex [LIN-26::GFP]; the rectal epithelial markers bxs7 [egl-5::gfp], kuls36 [egl-26::gfp], kuls34 [sem-4::gfp]; the PDA markers fak1 [ace-34::GFP], syb63 [cog-1::gfp], and arEx267 [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 transgenes on a Zeiss 21 Axio imager. A Microport 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.p 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 (mcex47) 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 AOB5 confocal microscope. Alternatively, live worms were anesthetized for at least twice.

Synchronized L1 worms expressing CHE-14::GFP (mcEx242) or DLG-1::GFP (mcex47) were ablated in the L1 stage, Y expresses the epithelial markers ajm-1, 1(dl-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-24, 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 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.p, and P11.p. Mutants in which Y does not transdifferentiate have four epithelial cells in the same area: U, Y, P12.p, and P11.p or, if a P12 cell is not made, U, Y, and P21.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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