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# Loss of *dpy-2* and *dpy-9* has stage-specific effects on DBL-1 pathway signaling

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## Abstract

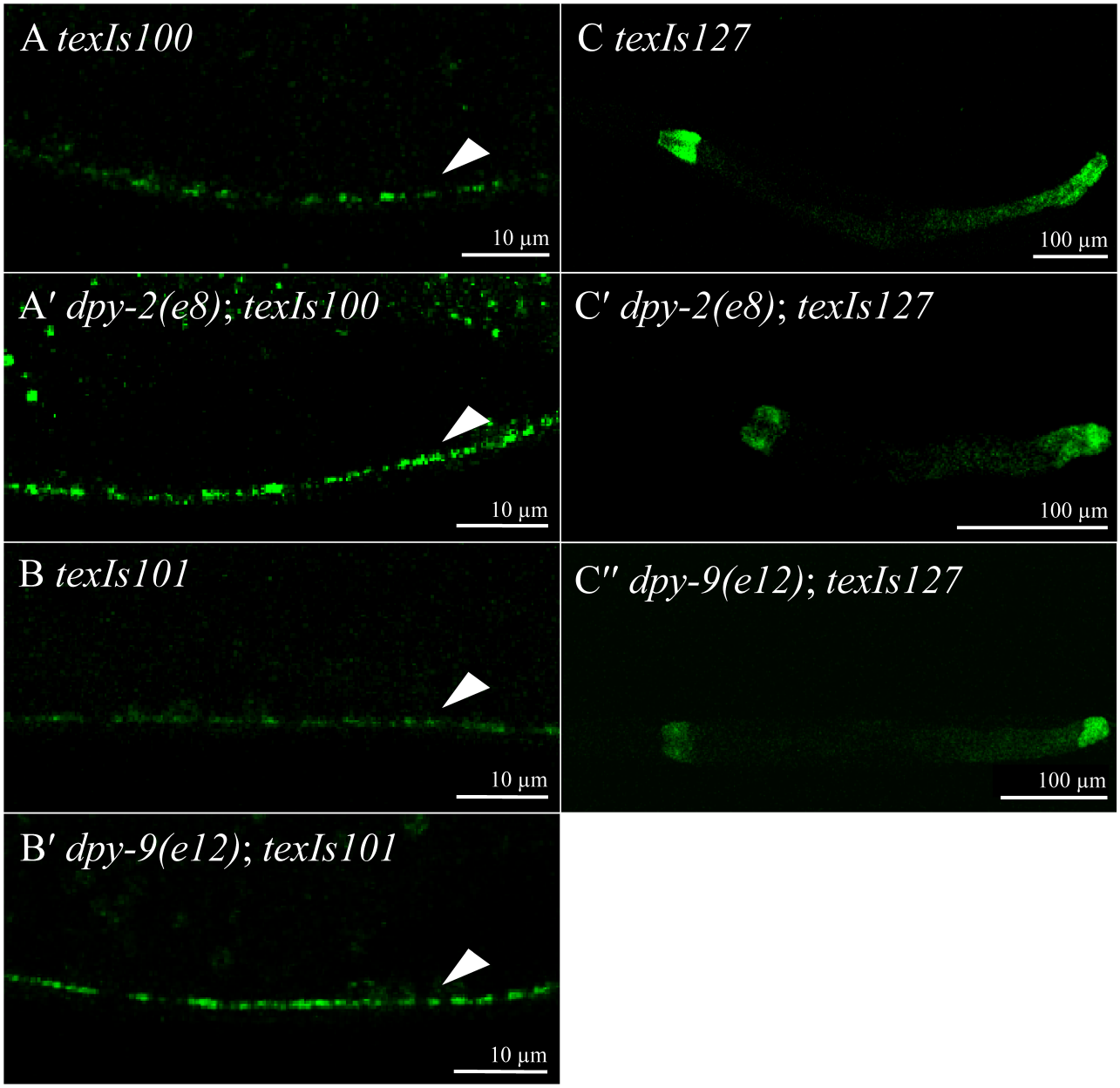


**Figure 1:** *dpy-2* or *dpy-9* loss-of-function mutations affect GFP::DBL-1 and DBL-1 pathway reporter fluorescence in L4 animals. Arrows point to GFP::DBL-1 fluorescent punctae in A and B. Representative images show that loss of *dpy-2* or *dpy-9* gene function is associated with increased GFP::DBL-1 fluorescence from *texIs100* or *texIs101* as shown in (A′) and (B′), respectively. *dpy-2(e8)* and *dpy-9(e12)* mutants also have reduced *spp-9p::gfp* reporter activity compared to control (C), as shown in (C′) and (C″), respectively.

## Description

Loss of some cuticle collagens negatively affects DBL-1 pathway signaling in a stage-dependent manner (Lakdawala *et al.* 2019; Madaan *et al.* 2019). We previously observed that in one-day old adult animals, loss of *dpy-2* or *dpy-9* had no effect on GFP::DBL-1 expressed from the *dbl-1* promoter (Beifuss and Gumienny 2012; Lakdawala *et al.* 2019). We also observed that expression of *spp-9p::gfp*, a reporter that is negatively regulated by the DBL-1 pathway, was not affected in one-day old adult animals (Roberts *et al.* 2010; Lakdawala *et al.* 2019). Post-embryonic expression of *dpy-2* and *dpy-9* is highest in L2 and L3, but low in L4 and even lower in young adults (Gerstein *et al.* 2010). Because cuticle secreted in one stage creates the cuticle in the next stage, this is consistent with the observation that loss of *dpy-2* and *dpy-9* has no effect on DBL-1 signaling in the adult (Hall and Altun 2008; Lakdawala *et al.* 2019). However, the DPY-2 and DPY-9 expression patterns led us to ask if DBL-1 signaling is affected at L4 by loss of *dpy-2* or *dpy-9*. To our surprise, we found that *dpy-2(e8)* or *dpy-9(e12)* resulted in significant increases of GFP::DBL-1 fluorescence within DBL-1-secreting cells in L4 animals compared to control populations (Figure 1, Table 1). We also tested DBL-1 pathway reporter activity in these *dpy-2* and *dpy-9* mutants. Consistent with the increased GFP::DBL-1 fluorescence at L4, we observed significantly decreased fluorescence from the *spp-9p::gfp* reporter at L4 (Figure 1, Table 1). These results are consistent with DPY-2 and DPY-9 affecting DBL-1 signaling at the L4 stage but not at the adult stage. This suggests that these two collagens have a stage-specific effect on DBL-1 signaling, but this effect is normally inhibitory, as loss of *dpy-2* or *dpy-9* increased GFP::DBL-1 fluorescence and decreased *spp-9p*::GFP fluorescence.

Table 1: Effects of *dpy-2* and *dpy-9* gene mutations on GFP::DBL-1 and DBL-1 pathway reporter *spp-9p*::GFP fluorescence

| Gene | Genotype | GFP::DBL-1 fluorescence % control ± 95% CI | P value | Genotype | *spp-9p*::GFP % control ± 95% CI | P value |
|---|---|---|---|---|---|---|
| Animals at L4 stage | | | | | | |
| control | *texIs100* | 100±29.58 | – | *texIs127* | 100±7.94 | – |
| control | *texIs101* | 100±54.28 | – | – | – | – |
| *dpy-2* | *dpy-2; texIs100* | 155.47±55.58 | 0.0263 | *dpy-2; texIs127* | 80.26±10.62 | 0.0009 |
| *dpy-9* | *dpy-9; texIs101* | 212.94±98.06 | 0.0009 | *dpy-9; texIs127* | 84.37±9.56 | 0.0028 |
| Animals at adult stage (data from (Lakdawala *et al.* 2019)) | | | | | | |
| control | *texIs100* | 100±15.57 | – | *texIs127* | 100±11.47 | – |
| control | *texIs101* | 100±25.95 | – | – | – | – |
| *dpy-2* | *dpy-2; texIs100* | 115±52.15 | 0.5080 | *dpy-2; texIs127* | 107.04±12.20 | 0.2344 |
| *dpy-9* | *dpy-9; texIs101* | 95.02±29.01 | 0.7248 | *dpy-9; texIs127* | 100.29±10.24 | 0.9533 |

## Methods

Request a detailed protocol

Nematode maintenance and imaging All the strains were maintained at 20°C on EZ media (Madhu et al. 2019). L4 animals were anesthetized using 1 mM levamisole hydrochloride (Sigma, St. Louis, MO) and imaged on a Nikon A1 confocal system (Nikon Instruments, Melville, NY). GFP::DBL-1 fluorescence was captured using a 60X objective and *spp-9p::gfp* fluorescence was captured using a 10X objective. The imaging conditions were optimized and kept constant between control and experimental samples. Nikon NIS Elements AR-5.02 software was used to quantify fluorescence intensities. Statistical analyses were performed using the unpaired t-test to compare control and experimental sample means. ‘‘% control ± 95% CI” is the ratio of the indicated strain mean to the control strain mean ± 95% confidence interval. n=10 for each strain imaged for the GFP::DBL-1 experiment, and n=15 for each strain imaged for the *spp-9p*::GFP experiment.

## Reagents

### Strains

Strains used in this study are:

TLG182 *texIs100 [dbl-1::dbl-1:gfp; ttx-3p::rfp]* IV

TLG205 *texIs101 [dbl-1::dbl-1:gfp; ttx-3p::rfp]* V

TLG697 *texIs127 [spp-9p::gfp] X*

TLG701 *dpy-2(e8); texIs100*

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TLG702 *dpy-9(e12); texIs101*

TLG725 *dpy-2(e8); texIs127*

TLG724 *dpy-9(e12); texIs127*

Strains are available upon request.

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