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

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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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>GFP::DBL-1 fluorescence</th>
<th>P value</th>
<th>Genotype</th>
<th><em>spp-9p::GFP</em></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% control ± 95% CI</td>
<td></td>
<td></td>
<td>% control ± 95% CI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals at L4 stage</td>
<td></td>
<td></td>
<td></td>
<td>Animals at adult stage (data from (Lakdawala et al. 2019))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>texIs100</td>
<td>100±29.58</td>
<td>–</td>
<td>texIs127</td>
<td>100±7.94</td>
<td>–</td>
</tr>
<tr>
<td>control</td>
<td>texIs101</td>
<td>100±54.28</td>
<td>–</td>
<td>texIs127</td>
<td>80.26±10.62</td>
<td>0.0009</td>
</tr>
<tr>
<td>dpy-2</td>
<td>dpy-2; texIs100</td>
<td>155.47±55.58</td>
<td>0.0263</td>
<td>dpy-2; texIs127</td>
<td>84.37±9.56</td>
<td>0.0028</td>
</tr>
<tr>
<td>dpy-9</td>
<td>dpy-9; texIs101</td>
<td>212.94±98.06</td>
<td>0.0009</td>
<td>dpy-9; texIs127</td>
<td>100.29±10.24</td>
<td>0.9533</td>
</tr>
</tbody>
</table>

**Methods**

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
- TLG702 dpy-9(e12); texIs101
- TLG725 dpy-2(e8); texIs127
- TLG724 dpy-9(e12); texIs127
Strains are available upon request.

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References


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