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 \textit{spp-9p::gfp}, a reporter that is negatively regulated by the DBL-1 pathway, was not affected in one-day old adult animals (Roberts \textit{et al.} 2010; Lakdawala \textit{et al.} 2019). Post-embryonic expression of \textit{dpy-2} and \textit{dpy-9} is highest in L2 and L3, but low in L4 and even lower in young adults (Gerstein \textit{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 \textit{dpy-2} and \textit{dpy-9} has no effect on DBL-1 signaling in the adult (Hall and Altun 2008; Lakdawala \textit{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 \textit{dpy-2} or \textit{dpy-9}. To our surprise, we found that \textit{dpy-2(e8)} or \textit{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 \textit{dpy-2} and \textit{dpy-9} mutants. Consistent with the increased GFP::DBL-1 fluorescence at L4, we observed significantly decreased fluorescence from the \textit{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 \textit{dpy-2} or \textit{dpy-9} increased GFP::DBL-1 fluorescence and decreased \textit{spp-9p::GFP} fluorescence.

### Table 1: Effects of \textit{dpy-2} and \textit{dpy-9} gene mutations on GFP::DBL-1 and DBL-1 pathway reporter \textit{spp-9p::GFP} fluorescence

<table>
<thead>
<tr>
<th>Gene at L4 stage</th>
<th>Genotype</th>
<th>GFP::DBL-1 fluorescence</th>
<th>P value</th>
<th>Genotype</th>
<th>\textit{spp-9p::GFP}</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% control ± 95% CI</td>
<td></td>
<td></td>
<td>% control ± 95% CI</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>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>\textit{dpy-2}</td>
<td>\textit{dpy-2}; texIs100</td>
<td>155.47±55.58</td>
<td>0.0263</td>
<td>\textit{dpy-2}; texIs127</td>
<td>80.26±10.62</td>
<td>0.0009</td>
</tr>
<tr>
<td>\textit{dpy-9}</td>
<td>\textit{dpy-9}; texIs101</td>
<td>212.94±98.06</td>
<td>0.0009</td>
<td>\textit{dpy-9}; texIs127</td>
<td>84.37±9.56</td>
<td>0.0028</td>
</tr>
<tr>
<td>Animals at adult stage (data from (Lakdawala \textit{et al.} 2019))</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>control</td>
<td>texIs100</td>
<td>100±15.57</td>
<td>–</td>
<td>texIs127</td>
<td>100±11.47</td>
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<tr>
<td>control</td>
<td>texIs101</td>
<td>100±25.95</td>
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<tr>
<td>\textit{dpy-2}</td>
<td>\textit{dpy-2}; texIs100</td>
<td>115±52.15</td>
<td>0.5080</td>
<td>\textit{dpy-2}; texIs127</td>
<td>107.04±12.20</td>
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<tr>
<td>\textit{dpy-9}</td>
<td>\textit{dpy-9}; texIs101</td>
<td>95.02±29.01</td>
<td>0.7248</td>
<td>\textit{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 \textit{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 \textit{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 \textit{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. \textit{n}=10 for each strain imaged for the GFP::DBL-1 experiment, and \textit{n}=15 for each strain imaged for the \textit{spp-9p::GFP} experiment.

### Reagents

#### Strains

Strains used in this study are:

- TLG182 texIs100 [\textit{dbl-1::dbl-1:gfp}; \textit{txs-3p::rfp}] IV
- TLG205 texIs101 [\textit{dbl-1::dbl-1:gfp}; \textit{txs-3p::rfp}] V
- TLG697 texIs127 [\textit{spp-9p::gfp}] X
- TLG701 \textit{dpy-2(e8); texIs100}
- TLG702 \textit{dpy-9(e12); texIs101}
- TLG725 \textit{dpy-2(e8); texIs127}
- TLG724 \textit{dpy-9(e12); texIs127}
Strains are available upon request.

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**References**


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**Reviewed By:** Anonymous

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