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Department of Biology

Identification of genetic interactions between the DBL-1/BMP-like pathway and other body size-associated genes in *Caenorhabditis elegans*

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Background

Bone morphogenetic protein (BMP) signaling pathways are conserved in animals and are used to control many developmental and homeostatic processes, including cell size and extracellular matrix remodeling.

DBL-1, a BMP in the roundworm *C. elegans*, has a well-defined pathway that includes the core signaling pathway and some regulators of this pathway. There are clear, dose-dependent phenotypes associated with this pathway. For instance, loss of signaling leads to small body size, while increased signaling results in long animals. Genetic screens and powerful molecular techniques available in this system have been used to isolate, identify, and characterize *dbl-1* and associated pathway gene mutations and products.[1-3]

To identify novel regulators of BMP signaling, we performed a forward genetic screen in *C. elegans* for genes involved in body size regulation, a trait under the control of BMP member DBL-1. We isolated mutations that suppress the long phenotype of *lon-2*, a gene that encodes a negative regulator that sequesters DBL-1.[4,5] We screened ~9,000 genomes and identified alleles of:

- genes encoding several core components of the DBL-1 pathway, demonstrating the efficacy of the screen
- novel genes (not shown)
- genes encoding extracellular matrix proteins
- other genes that affect body morphology.

We discovered that loss of some of these genes affects DBL-1 pathway signaling. Two prominent cuticular collagens, DPY-2 and DPY-9, have stage-specific effects on DBL-1 signaling.

We propose a model in which DBL-1 controls multiple body size factors, and the DBL-1 pathway is itself affected by novel regulators.

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Mutations in known DBL-1 pathway genes were isolated in a *lon-2* suppressor screen

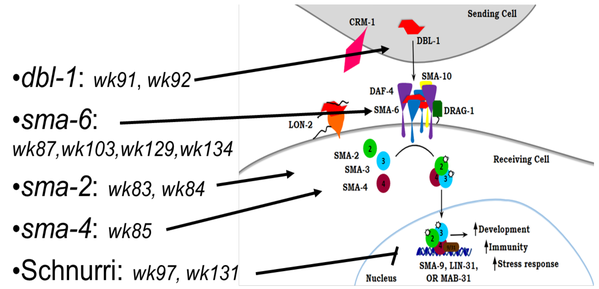


Fig. 1. DBL-1 pathway alleles were isolated that suppressed *lon-2(-)* long body size. Adapted from Gumienny and Savage-Dunn, 2005.

dpy alleles suppress *lon-2(-)* long body size

Gene name	NCBI KOGs or homology to:	*lon-2(lf)* sup cloned
dpy-1	Cuticlin	*wk111, wk142, wk143*
dpy-2	Collagens (type IV and type XIII)	*wk122, wk104*
dpy-3	Collagens (type IV and type XIII)	*wk105*
dpy-4	Collagens (type IV and type XIII)	*wk101, wk102*
dpy-5	Collagens (type IV and type XIII)	*wk137*
dpy-6	Mucin 2 precursor	*wk113, wk118, wk127, wk132*
dpy-7	Collagens (type IV and type XIII)	*wk106*
dpy-8	Collagens (type IV and type XIII)	*wk120*
dpy-9	Collagens (type IV and type XIII)	*wk124*
dpy-10	Collagens (type IV and type XIII)	-
dpy-11	disulfide oxidoreductase	-
dpy-13/16	Collagens (type IV and type XIII)	*wk95, wk145, wk135*
dpy-14	collagen type III(alpha1)	*wk116,wk128*
dpy-15/sqt-3	Collagens (type IV and type XIII)	-
dpy-17	Collagens (type IV and type XIII)	*wk140*
dpy-18	Prolyl 4-hydroxylase alpha subunit	-
dpy-19	C-mannosyltransferase	-
dpy-20	BED zinc finger protein	*wk141*
dpy-21	Deoxygenase	-
dpy-23	Adaptor-related protein complex 2 subunit mu 1	-
dpy-24/ blmp-1	zinc finger transcription repressor	-

Table 1. *dpy* genes used in this work and alleles isolated.

Some *dpy* genes affect GFP-DBL-1

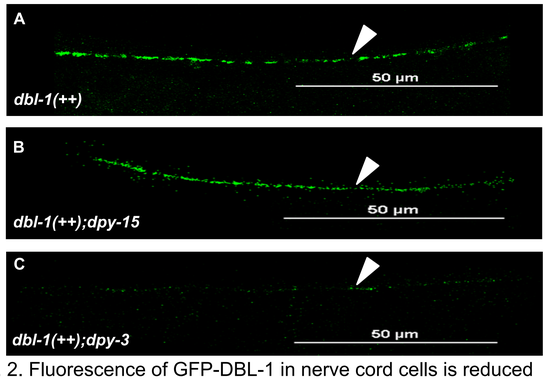


Fig. 2. Fluorescence of GFP-DBL-1 in nerve cord cells is reduced in some *dpy* animals.

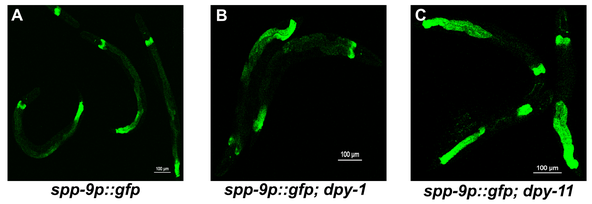
(A) Animals expressing GFP-DBL-1 show a punctate localization of fluorescence in nerve cord.

(B) No change in GFP-DBL-1 fluorescence was observed upon loss of *dpy-15* (shown), *dpy-1*, *dpy-4*, *dpy-10*, *dpy-11*, *dpy-13*, *dpy-17*, *dpy-18*, *dpy-19*, or *dpy-20*.

(C) GFP-DBL-1 fluorescence is significantly reduced upon loss of *dpy-3* (shown), *dpy-5*, *dpy-6*, *dpy-7*, *dpy-8*, *dpy-14*, *dpy-23*, or *dpy-24*.

Animals were imaged 24 hr. post-L4 at 60X using a Nikon A1 confocal. White arrowheads point to GFP-DBL-1 fluorescence.

Some *dpy* mutants affect a DBL-1 pathway reporter



spp-9p::gfp | *spp-9p::gfp; dpy-1* | *spp-9p::gfp; dpy-11*

Fig. 3. Some dpy mutants upregulate expression of GFP from a DBL-1 reporter.

(A) Animals expressing the *spp-9p::gfp* integrated transgene *texIs127* show expression in the intestine.

(B) No change in reporter expression was observed upon loss of *dpy-1* (shown), *dpy-2* (Table 2), *dpy-7*, *dpy-8*, *dpy-9* (Table 2), *dpy-15*, *dpy-18*, or *dpy-20*.

(C) Significantly increased reporter expression was observed upon loss of *dpy-11* (shown), *dpy-3*, *dpy-4*, *dpy-5*, *dpy-6*, *dpy-13*, *dpy-14*, *dpy-17*, *dpy-19*, *dpy-21*, *dpy-23*, or *dpy-24*.

Animals were imaged 24 hr. post-L4 using a Nikon A1 confocal with a 10X objective.

DBL-1 regulates collagen and transcription regulator *blmp-1* gene expression

Genes	Fold change in *dbl-1(-)* vs WT
dbl-1	-13.65
dpy-5	-4.5
dpy-8	-2.94
dpy-9	-2.88
rol-6	-4.22
rol-8	-3.52
sqt-2	-3.81
col-17	-4.26
col-90	-4.66
col-125	-2.28
col-135	2.05
dpy-24/blmp-1	-1.7

Table 2. RNA-seq comparison of *dbl-1(-)* mutant and wild-type mRNA levels of genes encoding extracellular matrix and two transcription factors. Total RNA was extracted 48 hr. post-L4, reverse transcribed, and sequenced using an Illumina MiSeq system. Differential gene analysis was performed using Illumina's Cufflinks Assembly software.

dpy-2 and *dpy-9* have stage-specific effects on DBL-1 signaling

Table 2: 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 %cont±95% CI	*p* value	Genotype	reporter %cont ±95% CI	*p* value
Animals at L4 stage (data from Lakdawala and Gumienny, 2019)[6]						
control	*texIs100*	100±340	-	*texIs127*	100±8	-
control	*texIs101*	100±32	-	-	-	-
dpy-2	*dpy-2; texIs100*	155±56↑	0.026	*dpy-2; texIs127*	80±11↓	0.001
dpy-9	*dpy-9; texIs101*	213±98↑	0.001	*dpy-9; texIs127*	84±10↓	0.003
Animals at adult stage (data from Lakdawala et al., 2019)[7]						
control	*texIs100*	100±30	-	*texIs127*	100±12	-
control	*texIs101*	100±54	-	-	-	-
dpy-2	*dpy-2; texIs100*	115±52	0.508	*dpy-2; texIs127*	107±12	0.234
dpy-9	*dpy-9; texIs101*	95±29	0.725	*dpy-9; texIs127*	100±10	0.953

Table 3. Loss of *dpy-2* or *dpy-9* has no effect on DBL-1 signaling in adults, but increases signaling in L4 animals, suggesting DPY-2 and DPY-9 inhibit DBL-1 signaling in L4 animals.

Proposed model of body size regulation by the DBL-1 pathway and *dpy* body size-associated genes.

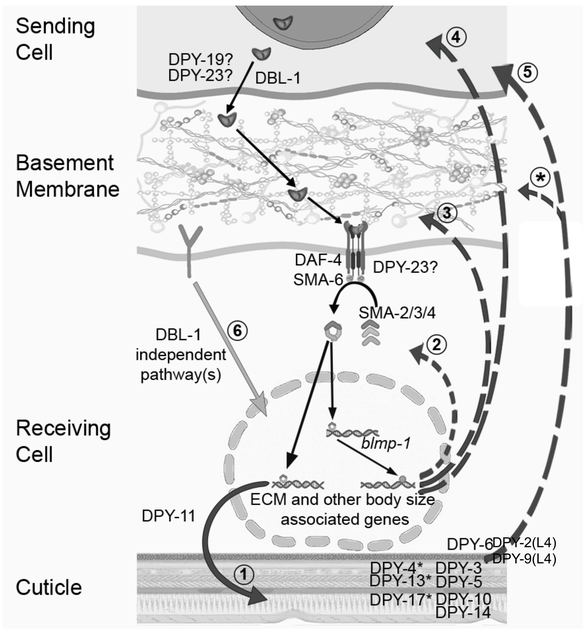


Fig. 4. Model adapted from from Lakdawala et al., 2019.

- DBL-1 signaling directly controls the expression of cuticle components, which affects body size (**arrow 1**).
- DPY-11/disulfide oxidoreductase may process DBL-1-regulated cuticle components before secretion.
- DBL-1-regulated gene expression, in part through BLMP-1, may modulate signaling within the receiving cell (**arrow 2**),
- or between the sending and receiving cells (**arrow 3**),
- or may feed back on the sending cell (**arrow 4**).
- Cues from the receiving cell or cuticle may be received (indirectly) by the sending cell and affect DBL-1 expression or secretion (**arrow 5**)
- or affect signaling downstream of DBL-1 secretion (**starred** arrowhead in arrow 5 and starred DPY collagens).
- Other cell-signaling pathways act independent of DBL-1 to control body size (**arrow 6**). Dashed lines represent potential indirect regulation.

Conclusions

Not only do all tested *dpy* genes suppress the long phenotype of *lon-2(-)*, some affect DBL-1 signaling, as evidenced by reduced fluorescence of a GFP-tagged DBL-1 ligand and increased expression of a reporter that shows an inverse correlation with DBL-1 activity.

DBL-1 may affect body size in part by regulating *dpy* gene expression and the expression of *blmp-1*, a gene encoding a transcription regulator that also affects body size.[8-9]

Future directions

Determine the interaction between the DBL-1 signaling pathway and BLMP-1 (see poster 1422C).

Characterize the roles of DBL-1-interacting proteins in DBL-1 pathway signaling.