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Department of Biology

Interaction of the DBL-1/BMP signaling pathway with BLMP-1/BLIMP1 in *Caenorhabditis elegans*

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Abstract

Animals use multiple signaling pathways for cell-to-cell communication, which are essential for proper development. One signaling pathway is defined by its ligand family of bone morphogenetic proteins (BMP). In the roundworm *C. elegans*, BMP member DBL-1 has a well-defined pathway that includes conserved core components and regulators. The DBL-1 signaling pathway is involved in a spectrum of traits, including body size, brood size, male tail morphogenesis, and distal tip cell migration. How does this BMP pathway control target gene expression? We are using the *C. elegans system*, which has a toolbox of genetic and molecular resources available, to specifically address this question. Previous studies in *C. elegans* show that transcriptional regulator BLMP-1 affects a similar array of traits as DBL-1. In mammals, both BMPs and BLIMP1 affect overlapping developmental and homeostatic processes and are implicated in cancers. However, the relationship between BMP and BLIMP1 is not clear. We discovered that DBL-1 and DBL-1 signaling are affected by loss of BLMP-1. Notably, we also found that DBL-1 negatively regulates blmp-1 expression in a stage-specific manner. In addition, ChIP-seq data analyses of SMA-3, a DBL-1 pathway transcription factor, and BLMP-1 suggest that these two transcriptional regulators control expression of some common target genes and may act together. In the future, we will elucidate the molecular mechanism underlying the interaction between this BMP pathway and BLIMP-1 in *C. elegans*, to gain an understanding of how BMP regulates proper growth and development of animals.

Background

- The DBL-1/BMP-like pathway and BLMP-1/BLIMP-1 play significant roles in *C. elegans* developmental processes, like body size, and brood size (1,2).
- In other systems, BLIMP-1 is downstream of the BMP signaling pathway.
- BMP4 is required for BLIMP-1 expression. However, the mechanism of how BMP regulates blimp-1 expression is not known (3,4).

Conclusions

- DBL-1 signaling pathway and BLMP-1 work in parallel to control body size.
- DBL-1 signaling pathway and BLMP-1 work together to control brood size.
- The DBL-1 signaling pathway negatively regulates *blmp-1* expression at L4 and positively regulates *blmp-1* expression in adults.
- SMA-3 and BLMP-1 have some common target genes at L2.

Future directions

- Determine if SMA-3 binds the upstream regulatory region of the *blmp-1* gene.
- Determine if SMA-3 and BLMP-1 physically interact with each other.
- Identify target genes regulated by SMA-3 and BLMP-1 together which play a role in morphogenesis.

References

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Loss *of blmp-1* suppresses long body size caused by DBL-1 overexpression

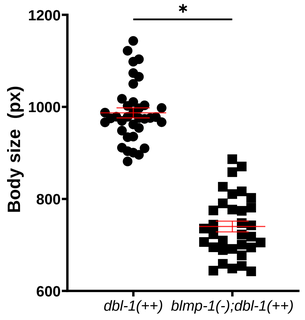
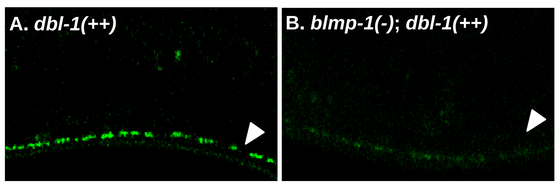


Figure 1. The long phenotype caused by overexpressed DBL-1 (*dbl-1(++)*) is suppressed in a *blmp-1* mutant background. Animals were imaged and measured 24 hr. post-L4 stage. Error bars represents standard error of the mean (SEM), $p<0.0001$ (5).

Loss of *blmp-1* decreases GFP-DBL-1



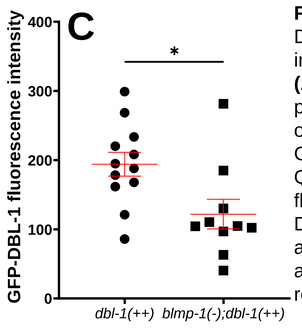
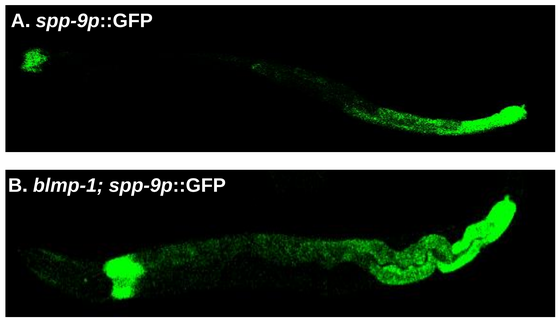


Figure. 2. Fluorescence of GFP-DBL-1 in nerve cord cells is indicated by white arrowheads. **(A)** GFP-DBL-1 (*dbl-1(++)*) is punctate in nerve cord. **(B)** Loss of BLMP-1 function decreases GFP-DBL-1 fluorescence. **(C)** Quantitation of mean fluorescence intensity of GFP-DBL-1 in different wild-type (WT) and *blmp-1(-)* backgrounds in adult animals. Error bars represent SEM, $p<0.05$ (5).

Loss of *blmp-1* decreases DBL-1 signaling



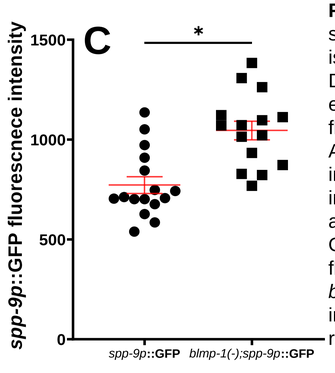


Figure 3. A DBL-1 pathway signaling reporter (*spp-9p::gfp*) is negatively regulated by the DBL-1 pathway (6). **(A)** Animals expressing *spp-9p::gfp* show fluorescence in the intestine. **(B)** Animals expressing *spp-9p::gfp* in a *blmp-1(-)* background show increased fluorescence intensity as compared to control **(A)**. **(C)** Quantitation of mean GFP fluorescence intensity in WT and *blmp-1(-)* genetic backgrounds in adult animals. Error bars represent SEM, $p<0.0001$ (5).

Genetic interaction between *blmp-1* and *dbl-1*

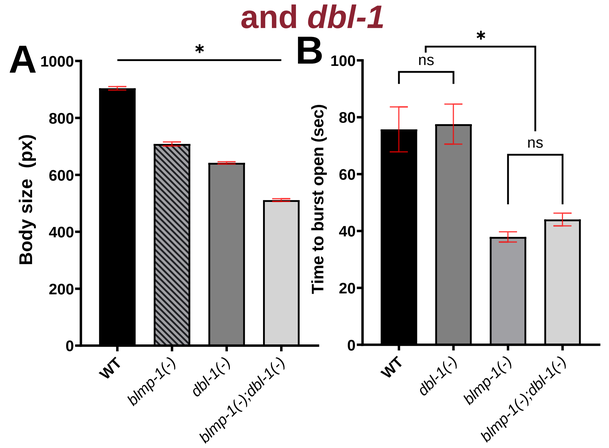


Figure 4. Body size and cuticle integrity of adult animals in different genetic backgrounds. **(A)** Animals lacking *blmp-1* or *dbl-1* have reduced body size as compared to wild type (WT). Animals lacking *blmp-1* and *dbl-1* are significantly smaller than WT and single mutants. (**B)** In 2% bleach solution, the time taken to break open by WT animals and *dbl-1(-)* is similar. Animals lacking *blmp-1* burst open significantly sooner than WT or *dbl-1(-)* animals. Time taken by animals lacking *blmp-1* and *dbl-1* to burst open is similar to that of *blmp-1(-)* animals. Error bars represent SEM, $p<0.0001$.

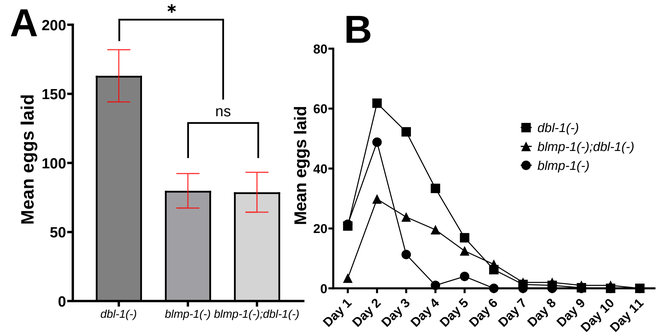


Figure 5. Brood size and egg laying pattern in different genetic backgrounds. **(A)** Animals lacking *blmp-1* have reduced brood size as compared to *dbl-1(-)*. Brood size of animals lacking both *blmp-1* and *dbl-1* is similar to that of *blmp-1(-)* animals. **(B)** Reproductive span of animals lacking both *blmp-1* and *dbl-1* is similar to that of animals lacking *dbl-1*. Error bars represent SEM, $p<0.0001$.

DBL-1 pathway regulates *blmp-1* expression in a stage-specific manner

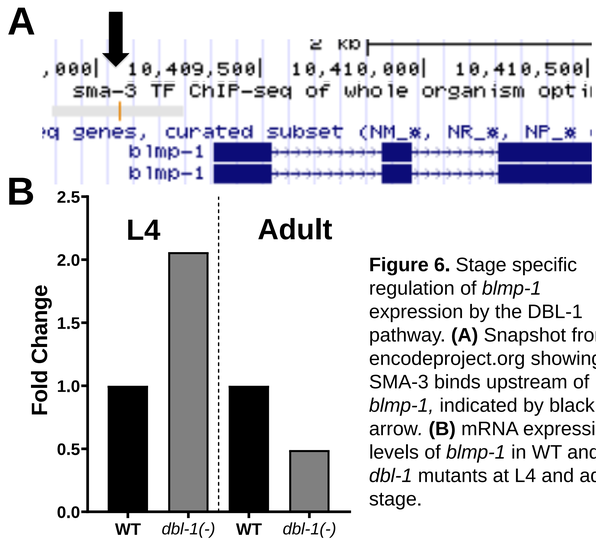


Figure 6. Stage specific regulation of *blmp-1* expression by the DBL-1 pathway. **(A)** Snapshot from encodeproject.org showing SMA-3 binds upstream of *blmp-1,* indicated by black arrow. **(B)** mRNA expression levels of *blmp-1* in WT and *dbl-1* mutants at L4 and adult stage.

DBL-1 signaling pathway and BLMP-1 regulate common target genes

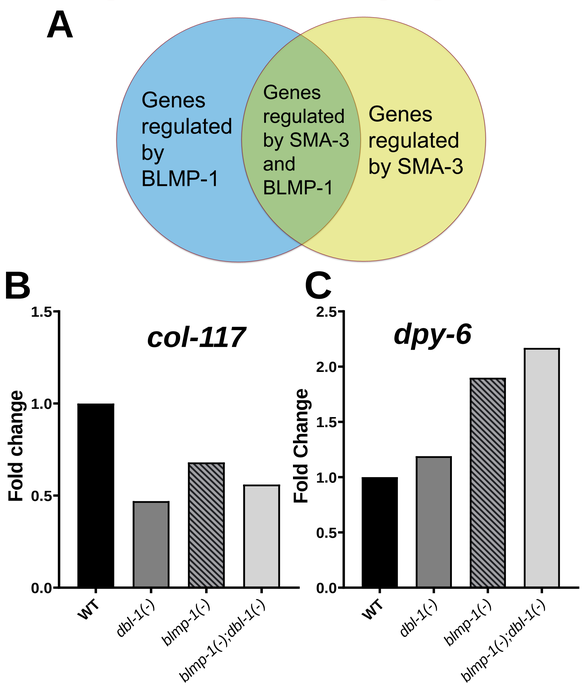


Figure 7. Co-regulation of certain target genes by SMA-3 and BLMP-1. **(A)** ChIP-seq data analysis suggests SMA-3 and BLMP-1 bind upstream regulatory regions of some common target genes at L2 stage. **(B)** mRNA expression of *col-117,* a putative target gene of both BLMP-1 and SMA-3, is reduced in L2 animals lacking *blmp-1, dbl-1,* or both *blmp-1* and *dbl-1.* **(C)** Animals lacking *blmp-1* or both *blmp-1 and dbl-1,* but not *dbl-1,* have increased mRNA expression levels of *dpy-6,* another putative target of BLMP-1 and SMA-3.

Proposed Model

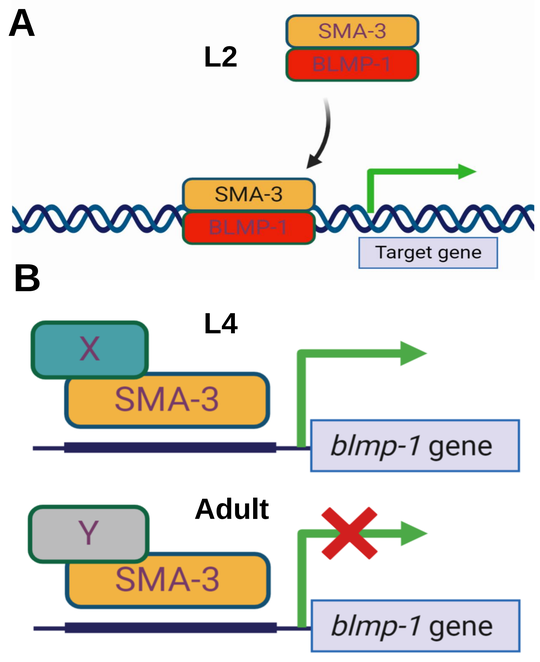


Figure 8. Proposed working model. **(A)** At L2 stage, DBL-1 signaling pathway transcription factor SMA-3 and BLMP-1 interact with each other to regulate common target genes. **(B)** At L4 and adult stages, SMA-3 binds upstream of the *blmp-1* gene to differentially regulate *blmp-1* transcription.