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Live Animal PiMPing and Faster Antibody Staining Methods for *C. elegans* TGF-β Localization Studies

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Transforming growth factor-β (TGF-β) is a family of cell-cell signaling ligands that plays important roles in development and diseases [1]. To understand how this pathway is regulated, others have used antibody staining and fluorescent tags to determine TGF-β ligand localization [2]. However, no study to date has identified post-transcriptional regulation of membrane localized TGF-β. DBL-1 is a secreted, dose-dependent ligand that belongs to the TGF-β superfamily in *Caenorhabditis elegans* (*C. elegans*). While DBL-1 is secreted from nervous tissue [3, 4], it must be trafficked to the epidermis where it binds to receptors, activating a signaling pathway to regulate body size development [5]. While studies have mapped the tissues that secrete DBL-1, no studies have described the subcellular location of DBL-1 protein. Therefore using *C. elegans* TGF-β DBL-1, we developed and optimized labeling techniques to determine the specific localization of DBL-1.

To determine localization of the TGF-β, we used fluorescent and whole mount immunofluorescent approaches in this transparent organism. For direct visualization studies, we expressed a functional, GFP-tagged DBL-1 in otherwise wild-type animals. We imaged the GFP-tagged DBL-1 in immobilized, live animals using confocal microscopy and then enhanced the images using photobleaching microscopy with non-linear processing (PiMP) [6] to obtain super-resolution images. We imaged an area over time, taking 50 images in a single plane using a standard confocal microscope. Next, we processed these images using PiMP software to generate a final image at sub-diffraction resolution. We found that functional, GFP-tagged DBL-1 is expressed in a punctate pattern along the ventral and dorsal nerve cords when visualized using super-resolution microscopy (Figure 1).

For immunocytochemical studies, we stained a transgenically expressed mammalian DBL-1 homolog along with nerve cord markers. We created a microwave-assisted immunocytochemistry protocol to allow better preservation, increased infiltration of antibodies, and decreased fixation and staining time. Fixation, steps to reduce autofluorescence or increase permeability, and antibody staining were all accomplished in the PELCO BioWave® Pro microwave. To preserve the tissues, we fixed animals in paraformaldehyde. To permeabilize the animal's tough exterior cuticular layer, we treated samples with Tris-Triton β-mercaptoethanol, hydrogen peroxide, and dithiothreitol (DTT) solutions. To reduce aldehyde-induced autofluorescence, we treated animals with sodium borohydride. Finally, we performed whole mount antibody staining, where we used a normal serum blocker and then incubated animals three times with primary and once with secondary antibodies. To optimize immunofluorescence, we used specific primary antibodies against the DBL-1 homolog and antibodies targeting specific subcellularly localized neuronal proteins. Using confocal microscopy, we examined the localization of a functional DBL-1 homolog with other proteins expressed within the nerve cord. We found that the DBL-1 homolog localizes to the nerve cord (red in Figures 2 and 3), in a punctate pattern similar to GFP-tagged DBL-1 (Figure 1). Further probing this localization, we found that the DBL-1 homolog colocalizes with two other nerve cord markers, SAX-7, an adhesion protein expressed at plasma membranes at cell-cell

contact sites in the nerve cord (green in Figure 2) and CAV-1, a caveolin that localizes to lipid raft subdomains in caveolar structures (green in Figure 3).

We found that DBL-1 and its mammalian homolog are expressed in punctate pattern (Figures 1-3) in the nervous system, colocalizing with cell-cell contact sites (Figure 2) and caveolin bodies in the ventral nerve cord (Figure 3). These studies are the first to decipher the subcellular localization of DBL-1 TGF-β, providing a basis for understanding TGF-β trafficking in *C. elegans*. Studies in genetic mutant backgrounds using these approaches will yield new insights into regulation of TGF-β signaling at the molecular and cellular level.

References

[1] MY Wu, CS Hill, Developmental Cell **16** (2009) 329.
[2] MC Ramel, CS Hill, FEBS Letters **586** (2012) 1929.
[3] K Morita *et al*, Development **126** (1999), 1337.
[4] Y Suzuki *et al*, Development **126** (1999), 241.
[5] S Krishna *et al*, Development **126** (1999), 251.
[6] S Munck *et al*, Journal of Cell Science **125** (2012), 2257.

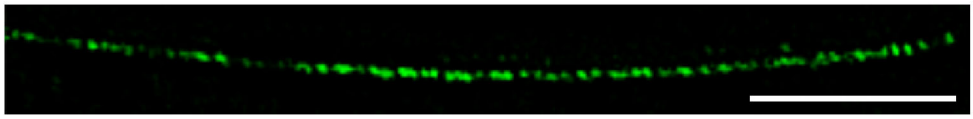

Figure 1. PiMP super-resolution image of punctate GFP-tagged DBL-1 TGF-β in live *C. elegans* neurons. Scale bar = 5 μm.

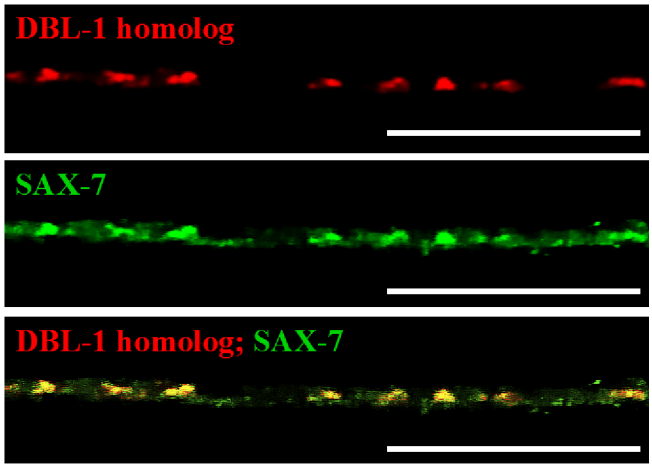

Figure 3. SAX-7 (green) at cell-cell adhesion sites colocalizes with a DBL-1 homolog (red) in nervous tissue upon w hole mount immunofluorescent staining. Scale bars = 5 μm.

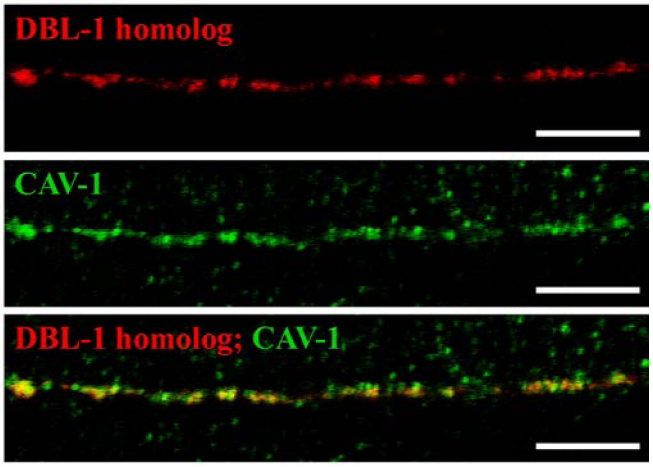

Figure 2. CAV-1 (green) bodies colocalize with a DBL-1 homolog (red) in nervous tissue upon whole mount immunofluorescent staining. Scale bars = 5 μm.

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