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DEPARTMENT OF BIOLOGY

C. elegans surface barrier lipid composition is regulated by the DBL-1/BMP signaling pathway

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Background

Due to parasitic nematode infection, millions of dollars and large yields of crops are lost worldwide. Nematodes are resistant to currently available anthelmintic drugs[1]. Alternative strategies to treat nematode infections are required. The nematodes can be sensitized to the existing anthelmintics by weakening their defense system. Using the related but free-living nematode *C. elegans*, targets for weakening the nematode defense system can be identified. To do so, characterizing the nematode defense system in detail is warranted. One of the nematode defenses is protection by the cuticle, including the lipid-rich epicuticle[2]. Based on previous studies by our lab and others, it is well established that DBL-1 helps protect nematodes from their harmful environment[3,4]. The molecular mechanisms of protection mediated by DBL-1 through epicuticular lipid composition have not been characterized[5]. Our study will result in identification of lipids of the protective epicuticle that can be targeted for developing novel therapeutic strategies against parasitic nematode infection.

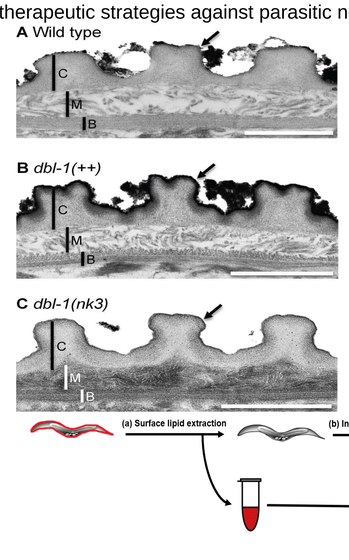

Figure 1. The DBL-1 pathway regulates cuticular organization and composition.
C indicates cortical layer;
M indicates medial layer;
B indicates basal layer;
and the arrow marks the surface coat and epicuticular layer.

Scale bars = 1 mm.

Figure 2. Schematic representation of surface-enriched and inside lipid extraction from *C. elegans* by exposing worms to organic solvent mix.

Results

1. Does DBL-1 affect the lipid classes of surface-enriched and inside lipids?

A: Internal triacylglycerol levels decrease in animals lacking *dbl-1*

Common lipid classes were observed in surface and inside lipids independent of DBL-1 levels

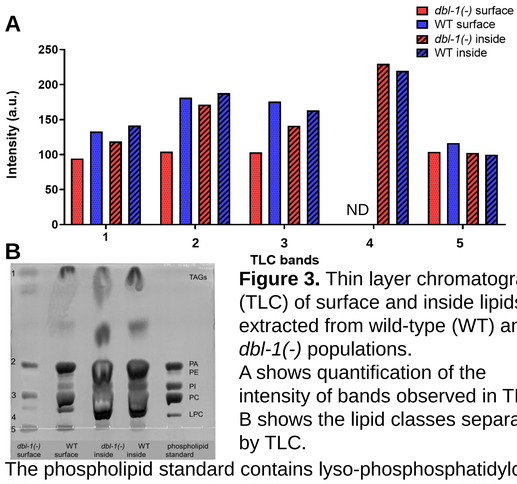

Figure 3. Thin layer chromatography (TLC) of surface and inside lipids extracted from wild-type (WT) and *dbl-1(-)* populations.
A shows quantification of the intensity of bands observed in TLC.
B shows the lipid classes separated by TLC.
The phospholipid standard contains lyso-phosphatidylcholine (LPC), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE), and phosphatidic acid (PA).
ND- not determined

2. Does DBL-1 affect the total lipid composition?

A: Loss of DBL-1 does not affect total lipid composition but reduces unsaturated fatty acid quantity

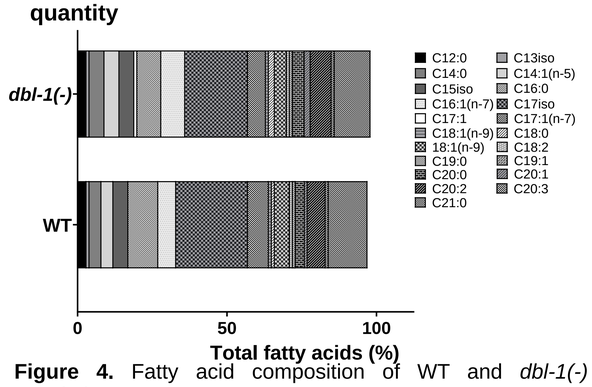

Figure 4. Fatty acid composition of WT and *dbl-1(-)* populations.

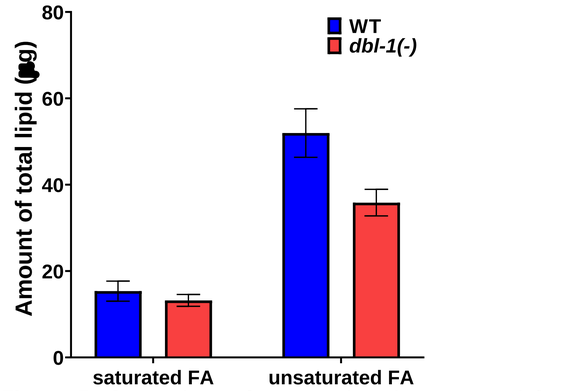

Figure 5. Comparison of saturated and unsaturated fatty acids (FA) between WT and *dbl-1(-)* populations of at least 6 independent trials. Error bars represent standard deviation.

3. Does DBL-1 affect the surface and inside lipid levels?

A: DBL-1 affects both surface-enriched and inside lipid levels, including saturated and unsaturated FA

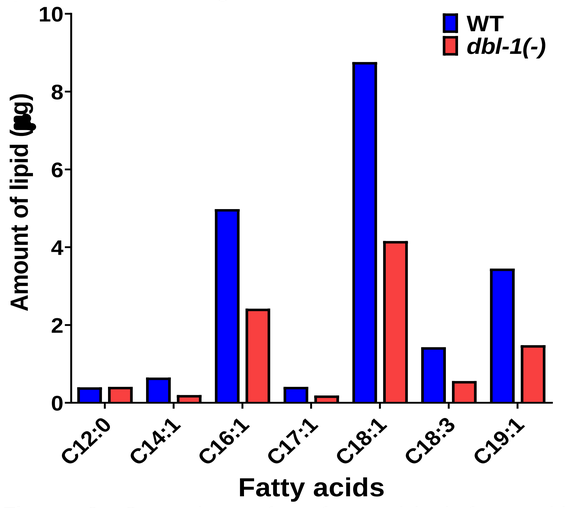

Figure 6. Comparison of surface-enriched fatty acids between WT and *dbl-1(-)* populations.

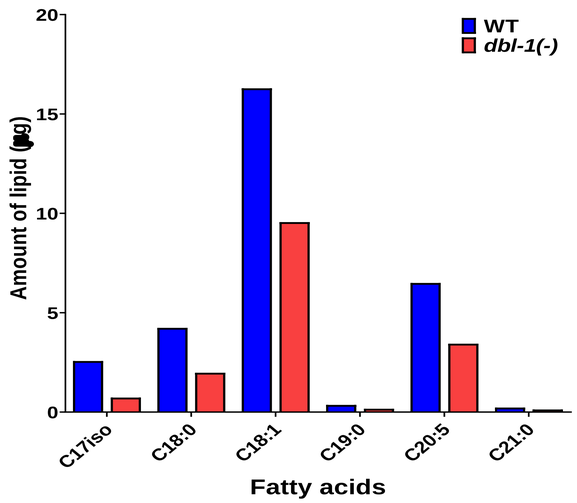

Figure 7. Comparison of inside fatty acids between WT and *dbl-1(-)* populations.

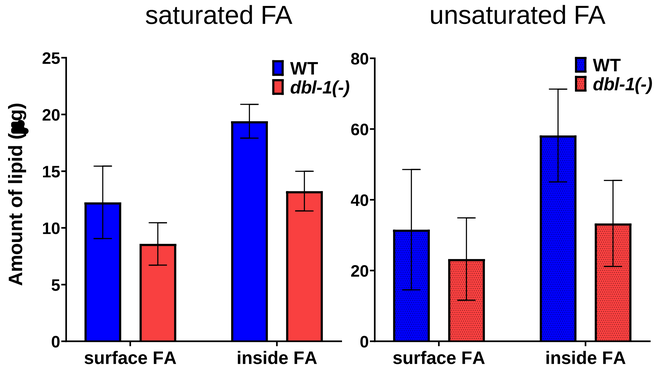

Figure 8. Comparison of saturated and unsaturated fatty acids between WT and *dbl-1(-)* populations from surface-enriched and inside lipid fractions of 3 independent trials. Error bars represent standard deviation.

4. Are lipid metabolism genes affected by DBL-1?

A: RNA-Seq data reveal that some lipid metabolism genes are regulated by DBL-1

Gene name	Function	Read count WT	Read count *dbl-1(-)*	Log fold change *dbl-1(-)*/WT
fat-7	Fatty acid biosynthesis	610.95	77.78	-2.97
fat-5	Fatty acid biosynthesis	934.87	360.00	-1.38
F10D11.6	Ortholog of human phospholipid transfer protein	2126.86	772.81	-1.46
fasn-1	Fatty acid synthesis	12104.75	7319.67	-0.73
oac-5	Acyl transferase activity	1.93	31.33	4.02
far-6	Lipid binding activity	1014.29	2677.87	1.40
acs-2	β-oxidation	1942.28	3773.98	0.96

Table 1. Lipid metabolism genes regulated by DBL-1.

A: qRT-PCR analyses indicate some lipid metabolism genes are regulated positively by DBL-1

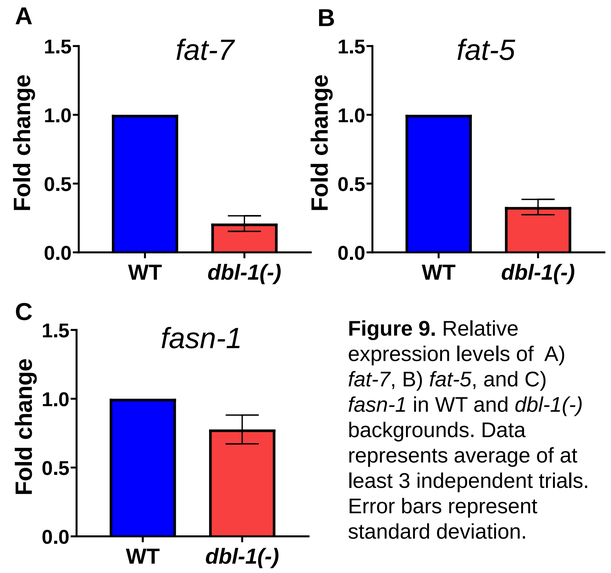

Figure 9. Relative expression levels of A) *fat-7*, B) *fat-5*, and C) *fasn-1* in WT and *dbl-1(-)* backgrounds. Data represents average of at least 3 independent trials. Error bars represent standard deviation.

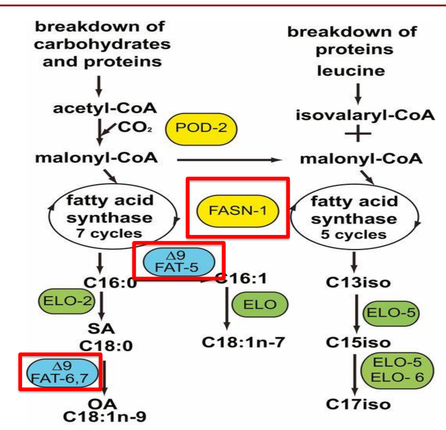

Figure 10. Fatty acid synthesis genes regulated by DBL-1 (Adapted from Watts and Ristow, 2017[6]).

Conclusions

1. Animals have common lipid classes in both surface and inside lipids independent of DBL-1 levels. However, levels of triacylglycerols (TAGs) are reduced with loss of DBL-1. This is consistent with previous reports of TAG-rich lipid droplet reduction in *dbl-1* pathway mutant strains[5].
2. DBL-1 does not alter total lipid composition, but loss of DBL-1 reduces unsaturated fatty acid quantities.
3. DBL-1 alters both surface and inside lipids and loss of DBL-1 reduces quantities of unsaturated fatty acids in both surface and inside lipids
4. DBL-1 regulates expression of lipid metabolism genes. DBL-1 positively regulates expression of *fat-5*, *fat-7*, and *fasn-1*.

Future Directions

1. Identify fatty acid composition of the individual lipid classes in both WT and *dbl-1(-)* populations.
2. Identify lipid transport genes that are highly regulated by DBL-1.

References

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Acknowledgements

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