The biotin-ligating protein BPL-1 is critical for lipid biosynthesis and polarization of the Caenorhabditis elegans embryo

Biotin is an essential cofactor for multiple metabolic reactions catalyzed by carboxylases. Biotin is covalently linked to apoproteins by holocarboxylase synthetase (HCS). Accordingly, some mutations in HCS cause holocarboxylase deficiency, a rare metabolic disorder that can be life-threatening if left untreated. However, the long-term effects of HCS deficiency are poorly understood. Here, we report our investigations of bpl-1, which encodes the Caenorhabditis elegans ortholog of HCS. We found that mutations in the biotin-binding region of bpl-1 are maternal-effect lethal and cause defects in embryonic polarity establishment, meiosis, and the integrity of the eggshell permeability barrier. We confirmed that BPL-1 biotinylates four carboxylase enzymes, and we demonstrate that BPL-1 is required for efficient de novo fatty acid biosynthesis. We also show that the lack of larval growth defects as well as nearly normal fatty acid composition in young adult worms is due to sufficient fatty acid precursors provided by dietary bacteria. However, BPL-1 disruption strongly decreased levels of polyunsaturated fatty acids in embryos produced by bpl-1 mutant hermaphrodites, revealing a critical role for BPL-1 in lipid biosynthesis during embryogenesis and demonstrating that dietary fatty acids and lipid precursors are not adequate to support early embryogenesis in the absence of BPL-1. Our findings highlight that studying BPL-1 function in C. elegans could help dissect the roles of this important metabolic enzyme under different environmental and dietary conditions.

Biotin is an essential B vitamin that acts as a critical cofactor in metabolic reactions. Animals cannot synthesize biotin but rather must obtain biotin from plant or bacterial sources. In humans, biotin deficiency is rare because it is available from numerous dietary sources and synthesized by gut microbes (1). However, biotin deficiency does occur rarely in individuals who consume raw egg whites or who are fed parenteral diets without biotin, leading to hair loss, dermatitis, skin rash, ataxia, and neurologic dysfunction. Additionally, subclinical biotin deficiency was shown to be common during pregnancy, a condition that in mice prohibits normal fetal development (2).

Biotin functions primarily as a cofactor in carboxylation reactions. In mammals, five biotin-dependent carboxylases perform diverse metabolic functions in lipid synthesis, gluconeogenesis, amino acid catabolism, and odd-chain fatty acid oxidation. Biotin is covalently attached to carboxylase enzymes by the action of holocarboxylase synthetase (HCS), a biotin-ligating protein (1, 3). In humans, mutations in the holocarboxylase synthetase gene result in a disease called holocarboxylase synthetase deficiency, or multiple carboxylase deficiency (MCD) (4, 5). The symptoms of MCD are phenotypically similar to biotin deficiency and present during infancy. If left untreated, MCD can cause coma and death. The underlying mutations causing holocarboxylase synthetase deficiency are well-documented, and the disease can be treated with large doses of biotin in most cases (6). However, the long-term effects of perturbing multiple metabolic pathways in these patients are poorly understood.

The nematode Caenorhabditis elegans offers a powerful model for studying complex metabolic systems. The invertebrate is amenable to forward and reverse genetics, has a short life cycle, and produces large numbers of offspring with well-characterized developmental processes that can be easily studied with light microscopy. Additionally, the C. elegans genome contains many orthologs to mammalian metabolic enzymes. These enzymes include orthologs to carboxylase enzymes and holocarboxylase synthetase, which previously were uncharacterized in C. elegans.

In a forward genetic screen, we obtained two mutations in a biotin ligase orthologous to mammalian holocarboxylase synthetase. These mutations cause maternal-effect embryonic lethality, demonstrating a key role for HCS in embryonic development. We show that BPL-1 biotinylates four key carboxylase enzymes, including acetyl-CoA carboxylase (ACC/POD-2), which is critical for maintaining the levels of embryonic polyunsaturated fatty acids and for the formation of the permeability barrier in the newly formed zygote. In the absence of BPL-1,
ACC/POD-2, and fatty acid desaturation, embryos fail to complete meiosis and fail to establish proper polarity during the first zygotic cell divisions. Unlike ACC/POD-2 deficiency, BPL-1 activity is dispensable during larval growth and development, because *C. elegans* are able to utilize fatty acid precursors from their bacterial diet. Our findings suggest that dietary precursors are partitioned away from embryonic lipids, and thus *de novo* lipid machinery is critical for the first steps of embryogenesis.

**Results**

**mel-3 mutations correspond to the holocarboxylase synthetase gene**

Mutations that defined the *mel-3* gene of *C. elegans* were first identified in a screen for maternal-effect lethal mutants. The *mel-3* mutations resulted in 100% lethality in embryos produced by homozygous mutant mothers and mapped to a region of linkage group II uncovered by the *mnDf30* deletion (7). Further mapping using deletion strains combined with complementation analysis and transformation rescue allowed us to identify a 7.5-kb BamH1 fragment containing a single predicted gene, F13H8.10, that was capable of rescuing the embryo lethality of *mel-3(b281)* and *mel-3(it8)*.

The F13H8.10 gene encodes a biotin protein ligase, homologous to the enzyme HCS, which is responsible for attaching biotin to carboxylases. For the remainder of this work, the *mel-3* gene will be referred to as *bpl-1*, biotin protein ligase-1. Sequence analysis of *b281* and *it8* mutants revealed point mutations in the coding sequence of F13H8.10 that result in single amino acid substitutions in the predicted protein product. These mutations affect amino acids within the biotin protein ligase catalytic domain that are highly conserved among all holocarboxylase synthetase genes (8). The *b281* mutation causes an A730E amino acid change, and *it8* results in G912D substitution in *bpl-1* (Fig. 1A). Many human mutations of HCS resulting in multiple carboxylase deficiency disease have also been found in this region (6, 9). The *bpl-1* deletion alleles *tm5867* and *tn6621* remove portions of the conserved biotin protein ligase domain and are predicted to result in frameshift and early termination (Fig. 1A). These deletion mutations also result in 100% maternal-effect lethality (*n* > 5000 for each allele).

**BPL-1 is essential for early embryonic development in *C. elegans***

Our phenotypic studies demonstrate that *bpl-1* plays an essential role in early embryonic development. 100% of embryos produced by homozygous mutant *bpl-1* worms fail to hatch. We used time-lapse video microscopy to compare early embryogenesis in *bpl-1* mutants with wild type. In wild-type *C. elegans*, the one-cell embryo divides asymmetrically to produce two daughter cells, AB and P1, which undergo asynchronous second divisions. First, the AB cell divides with a spindle orientation perpendicular to the anterior–posterior axis, followed by division of the P1 cell along the anterior–posterior axis (10). In contrast, *bpl-1* embryos show abnormal early division patterns, including synchronous and symmetrical divisions and abnormal spindle orientations (Fig. 1B, Table 1 and Movies S1–S3).

We found that 100% of two-cell stage *bpl-1* embryos are permeable to FM4-64 dye, indicating improper formation of the eggshell permeability barrier (Fig. 1C; *n* = 45). Embryos also display aneuploidy due to earlier meiotic defects (Fig. 1C). We observed extra nuclei in one-cell and two-cell embryos, consistent with failure of meiosis in 90% of *bpl-1* embryos (Table 1).

PAR (partitioning-defective) proteins are ancient, conserved proteins that localize asymmetrically and regulate cell polarity (10). We found that *bpl-1* embryos fail to segregate PAR proteins properly (Fig. 1D). Specifically, the posterior PAR-2 domain is severely restricted, whereas the anterior PAR-6/PAR-3/PKC-3 domain extends further into the posterior of the embryo. Like *b281* and *it8*, the deletion mutants *tm5867* and *tn6621* show the same range of embryo phenotypes, with the same penetrance (Table 1). *bpl-1* embryos do not undergo morphogenesis but arrest as a mass of many cells.

**BPL-1 biotinylates carboxylase enzymes**

The predicted amino acid sequence of BPL-1 is orthologous to holocarboxylase synthetase, which in mammals covalently links biotin to multiple carboxylase enzymes, including acetyl-CoA carboxylases 1 and 2, pyruvate carboxylase, 3-methylcrotonyl-CoA carboxylase, and propionyl-CoA carboxylase. These carboxylase enzymes function in a diverse set of metabolic pathways. Both the cytoplasmic acetyl-CoA carboxylase 1 and the mitochondrial acetyl-CoA carboxylase 2 catalyze the rate-limiting step in *de novo* fatty acid synthesis. Pyruvate carboxylase converts pyruvate to oxaloacetate in gluconeogenesis. Both 3-methylcrotonyl-CoA carboxylase and propionyl-CoA carboxylase are involved in catabolism of branched-chain amino acids, and propionyl-CoA carboxylase also functions in odd-chain fatty acid catabolism.

To determine whether BPL-1 biotinylates carboxylase enzymes in *C. elegans*, we probed total protein blots with streptavidin-horseradish peroxidase conjugates. Blots of proteins from wild-type control worms display prominent protein bands at ~240 and 130 kDa and two at ~70 kDa (Fig. 2A). The intensity of each of these bands is reduced in *bpl-1(b281)* homozygous adult worms, indicating a reduction in biotinylating of the proteins. To determine whether the biotinylated protein bands correspond to carboxylase enzymes, we knocked down *pod-2*, *pyc-1*, *pcc-1*, and *mcc-1* with RNAi. The *pod-2* gene is predicted to encode acetyl-CoA carboxylase isoforms of 91.4, 230.6, and 242.6 kDa. When we knocked down *pod-2* with RNAi, the highest molecular mass band was absent from the protein blot (Fig. 2A). The same band is absent in *bpl-1* homozygotes, indicating that BPL-1 biotinylates the 242.6-kDa POD-2 isofrom. We did not detect the lower molecular mass isoforms in our assay, which implies that the 242.6-kDa isoform is the most abundant biotinylated form under laboratory growth conditions.

The *pyc-1* gene encodes pyruvate carboxylase, which catalyzes the carboxylation of pyruvate to oxaloacetate in gluconeogenesis. The predicted molecular mass of PYC-1 is 129.3 and 67.8 kDa. Knockdown of *pyc-1* with RNAi resulted in reduction of intensity of the ~130 kDa protein band, and it is also reduced in *bpl-1* homozygotes.
BPL-1 is required for embryogenesis

Propionyl-CoA carboxylase converts propionyl-CoA to D-methylmalonyl-CoA in the breakdown of the branched-chain amino acids valine and isoleucine and in the metabolism of odd-chain fatty acids. Subsequent steps of D-malonylmalonyl-CoA metabolism provide succinyl-CoA to the tricarboxylic acid cycle. The presence of this pathway has been confirmed in C. elegans (11). The C. elegans genome contains orthologs for propionyl-CoA carboxylase subunits A and B (pcca-1 and pccb-1). The pcca-1 gene is predicted to encode a 79.7-kDa protein. Only PCCA-1 is predicted to contain a biotin-binding domain. Knockdown of pcca-1 with RNAi caused a decrease in intensity of biotinylated protein bands at ~75 kDa (Fig. 2A). This protein

A

BPL domain

b281

it8

BPL-1a 1215 aa

BPL-1b 632 aa

BPL-1c 558 aa

tm5867 Δ 603-711 + frameshift

tm6621 Δ712-842 + frameshift

b281 AQ94E

it8 G1076D

C

Control

bpl-1 (tm6621)

D

Control

fasn-1 RNAi

pod-2 RNAi

bpl-1 (b281)

DIC

PAR-2

PAR-6

PAR-6/PAR-2

FM4-64

FM4-64

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Table 1
Summary of bpl-1(b281) embryo phenotypes

| Phenotype            | Control bpl-1 (b281) | bpl-1 (tm5857) | bpl-1 (tm6621) |
|----------------------|----------------------|----------------|----------------|
| % Abnormal Spindle (N) | 0% (20)              | 79% (17)       | 53% (17)       |
| % Synchronous 2nd Division (N) | 0% (20)              | 47% (17)       | 77% (17)       |
| % Symmetric (N)   | 0% (20)              | 33% (21)       | 26% (23)       |

| Dye Permeability  | Briothin Rescue | Olate Rescue |
|------------------|----------------|--------------|
| Dye Permeability | 100% (50)      | N            |
| Constricted PAR-2 Domain | 80% (15) | N             |
| 2-Cell Multinucleation | 87% (23) | N             |
| Daid Embryo      | 100% (596)     | N            |

Pod-2 sodium salts were added to NGM at 0.1 mM with 0.1% Tergitol. Dye permeability was assayed with FM4-64. N, no rescue.

BPL-1 is required for embryogenesis

Biotin was dissolved in water, and 5 µg was added to feeding plates. Oleic acid sodium salts were added to NGM at 0.1% Tergitol. Dye permeability was assayed with FM4-64. N, no rescue.

Band could contain a modified PCCA-1 protein, and it is reduced in bpl-1 homozygotes.

The 3-methylcrotonyl-CoA carboxylase is also involved in branched-chain amino acid metabolism, converting 3-methylcrotonyl-CoA (derived from leucine) to 3-methylglutaconyl-CoA, which is further metabolized to acetyl-CoA and acetoacetate (1). Knockdown of mccc-1 reduced intensity of the 73-kDa biotinylated protein band (Fig. 2A), indicating that mccc-1 is the major biotinylated protein at 73 kDa.

To determine whether loss of carboxylase function explained the bpl-1 embryonic phenotypes, we assessed viability and permeability of embryos when each of the carboxylases were knocked down by RNAi (Fig. 2, B and C). Mutations in the pod-2 gene were previously shown to cause phenotypes very similar to those of bpl-1 embryos (12, 13). We found that treating worms with RNAi against pyc-1, pcca-1, and mccc-1 for 2 generations resulted in normal growth, impermeable embryos, and very low levels of embryonic lethality, similar to worms treated with an empty RNAi vector. Conversely, RNAi against pod-2 starting at the L3 larval stage caused 100% permeable embryos and 100% embryonic lethality. Thus, these data provide evidence that loss of POD-2 biotinylation is responsible for embryonic defects caused by perturbed BPL-1 function.

Cytosolic, but not mitochondrial, fatty acid synthesis is required for C. elegans embryogenesis

Acetyl-CoA carboxylase, encoded by the pod-2 gene in C. elegans (12), produces malonyl-CoA, which is a required substrate for several reactions, including cytosolic fatty acid synthesis, elongation of C16 and C18 fatty acids in the endoplasmic reticulum, and mitochondrial fatty acid synthesis. Interestingly, embryo phenotypes very similar to those we observe in bpl-1 mutants have also been reported in a pod-2 mutant and for RNAi knockdown of fasn-1, the major cytosolic fatty acid synthase gene of C. elegans. Early embryos of pod-2(−) or fasn-1(RNAi) exhibit egg shell permeability, multiple nuclei, cell cycle synchrony, and PAR protein mislocalization (Figs. 1D and 2C) (12).

In contrast, RNAi knockdown for genes required for mitochondrial fatty acid synthesis failed to show abnormal embryo phenotypes, although the disruption of mitochondrial synthesis of α-lipoic acid, which requires mitochondrial fatty acid synthesis, causes embryonic lethality in mice (14). Because malonyl-CoA is required for both cytosolic and mitochondrial fatty acid synthesis, we examined the embryo phenotypes of RNAi knockdowns for mccc-1, the mitochondrial trans-2-enoyl-CoA reductase (15). This RNAi knockdown did not result in embryo lethality or permeability, indicating that the fatty acid synthesis defects in bpl-1 and pod-2 mutants are probably mediated by the limitations of malonyl-CoA substrates required for cytosolic fatty acid synthesis and fatty acid elongation in the endoplasmic reticulum.

BPL-1 functions in fatty acid biosynthesis

To directly assess BPL-1’s role in fatty acid synthesis, we used a stable isotope-labeling strategy that allows the identification of newly synthesized fatty acids as opposed to those obtained from the diet (16). We measured the relative proportion of newly synthesized fatty acids that animals accumulated over a 12-h period during the L4 larval stage and young adulthood. Knockdown of bpl-1 led to reduced synthesis of several fatty acids, including the long-chain fatty acids 18:0, 18:1(n-7), and 18:2 by 25–50% (Fig. 3A), confirming that BPL-1 plays an important role in de novo fat synthesis. RNAi knockdown of pod-2 also causes a reduction in de novo fat synthesis (17).

Knockdown of BPL-1 and other fat synthesis genes reduces embryonic polyunsaturated fats

We asked whether the reduction in fatty acid synthesis when bpl-1, fasn-1, or pod-2 is knocked down causes changes to the overall proportions of fatty acids in the worm. Wild-type C. elegans contain a mix of saturated fatty acids, cyclopropane fatty acids, branched-chain fatty acids, and long-chain polyunsaturated fatty acids.

Figure 1. Phenotypic analysis of bpl-1 mutant embryos. A, mutations in the C. elegans bpl-1 gene alter conserved biotin protein ligase residues. The C. elegans bpl-1 gene encodes three protein isoforms (black lines) differing in length at the N terminus (WormBase WS251). These isoforms all contain the conserved biotin protein ligase/lipoyl protein ligase catalytic domain (BPL- LPL catalytic domain IPR004143, amino acids 958–1141 in BPL-1a), indicated by a dark blue bar within a larger conserved biotin protein ligase domain (lighter blue bar, PTHR12835, amino acids 526–1215 in BPL-1a). The N-terminal region preceding the BPL domain of C. elegans BPL-1a is not conserved. The positions of the single amino acid changes produced by b281 and i8 mutations are within the conserved biotin-binding pocket of the protein and are indicated with green arrows. tms5867 is a deletion resulting in removal of amino acids 603–711 and subsequent frameshift resulting in premature termination. tm6621 is a deletion that removes codons for amino acids 712–842 and causes a frameshift and premature stop. Thus, neither deletion mutant retains the BPL domain. COBALT alignment between C. elegans BPL-1a and holocarboxylase synthetase proteins of human and zebrafish shows sequence conservation. Amino acid identities are in red. Amino acid changes by C. elegans point mutations are highlighted and indicated with green arrows. b281 changes the codon for alanine 994 (A) to glutamic acid (E); i8 changes the codon for glycine 1056 (G) to aspartic acid (D). Amino acid numbering is with respect to the longest BPL-1 isofrom, BPL-1a. B, time-lapse differential interference contrast microscopy shows symmetric first cleavage in bpl-1(tm6621) and synchrony at the second division, compared with control embryos, which have asymmetric first cleavage and asynchronous second division. Both cells of the bpl-1 two-cell embryo are shown divided transverse to the long axis of the embryo; in control embryos, only the posterior P1 cell divides with this orientation. Time-lapse movies from which these images were taken are included as Movie S1 (control) and Movie S2 (bpl-1(tm6621)). C, differential interference contrast (DIC) imaging of bpl-1(b281) two-cell embryo with multinucleation (top) and FM4-64 dye permeability (bottom). D, imaging of PAR-2:GFP and PAR-6::mCherry in live embryos with compromised fatty acid synthase activity. The PAR-2 domain is restricted, whereas the PAR-6 domain extends further into the posterior in fasn-1 and pod-2 RNAi and bpl-1 mutant embryos than in controls. Bottom, outline of PAR-6 domain (red) and PAR-2 domain (green). The anterior is to the left and posterior is to the right in all images.
saturated fatty acids (PUFAs) (18). These fats are obtained both from the bacterial diet and by de novo synthesis (16). We used GC/MS to measure the fatty acid composition of late L4/young adult worms. Knockdown of \textit{bpl-1} by RNAi slightly, but significantly, reduced the relative amounts of PUFAs and increased saturated fatty acids in worms (Fig. 3B and Table 2). Many of the fatty acids in \textit{C. elegans}, including the 14- and 16-carbon saturated fatty acids, which are the end product of de novo synthesis, are obtained directly from the bacterial diet (19, 20), and these precursors can be elongated and desaturated to form a range of 18- and 20-carbon PUFAs (18). Our data demonstrate that nearly normal fatty acid composition of adult worms can be attained under conditions in which de novo fat synthesis is reduced.

Because \textit{bpl-1} and \textit{pod-2} depletion lead to maternal-effect lethality, we asked whether reduction of de novo fatty acid biosynthesis enzymes cause changes in embryonic lipids. We carried out parallel GC/MS analysis of embryos obtained by hypochlorite treatment of semi-synchronous, RNAi-treated \textit{C. elegans}. Contrary to the subtle fatty acid changes in the whole worm lipid profile, knockdown of \textit{bpl-1}, \textit{pod-2}, or \textit{fasn-1} resulted in drastic changes to the embryonic fatty acid composition: a >2-fold decrease in the total amount of PUFAs and an increased proportion of saturated fatty acids and cyclopropane fatty acids (Fig. 3C and Table 2). The dramatic decrease in embryonic PUFAs, which primarily are components of membrane phospholipids (21), suggests that membrane biophysical properties such as fluidity may be compromised.

\textbf{Polyunsaturated fatty acids are required for early embryonic development}

To test whether any of the embryonic defects observed in \textit{bpl-1} were caused by loss of embryonic PUFAs, we examined mutants in the PUFA biosynthesis pathway. Synthesis of PUFAs requires the sequential addition of two double bonds to a satu-rated fatty acid by Δ9 and Δ12 desaturase enzymes (18). The \textit{C. elegans} genome encodes three redundant Δ9 desaturases, \textit{FAT-5}, \textit{FAT-6}, and \textit{FAT-7} (22, 23), but only a single Δ12 desaturase, \textit{FAT-2}. Mutations in the \textit{fat-2} gene result in the accumulation of monounsaturated fatty acids and a nearly total loss of PUFAs (18), whereas the double mutant strain \textit{fat-6;fat-7} has greatly increased saturated fatty acids and reduced PUFA composition, consisting of unusual PUFAs that are not normally synthesized (24).
We found that similar to bpl-1 mutants, mutations in fat-2 and, to a lesser extent, fat-6/fat-7 resulted in embryonic lethality, disruption of the permeability barrier, and aneuploidy (Fig. 4, A and B). The penetrance of these embryonic abnormalities was incomplete, suggesting that disruption of PUFA synthesis cannot entirely explain the embryonic defects caused by mutations in the genes encoding earlier components of the fat biosynthesis pathway. However, these genetic data provide strong evidence that PUFAS are important for the formation of the embryonic permeability barrier and that the embryonic defects resulting from loss of BPL-1, POD-2, and FASN-1 are at least partially due to reduction of PUFAs.

To further investigate the role of PUFAs in the early embryo, we supplemented the C. elegans diet with either the monounsaturated fatty acid, oleic acid, or with the PUFA, linoleic acid, and assessed lethality and permeability in embryos of fat-supplemented animals compared with controls. Treatment with the 18-carbon PUFA linoleic acid, but not the monounsaturated fatty acid oleic acid, rescued embryonic lethality and permeability barrier defects in fat-2, confirming that PUFAs, but not MUFAs, are critical for the early embryo (Fig. 4, C and D). Interestingly, neither supplemental MUFAs nor PUFAs rescued embryonic lethality or permeability barrier defects in bpl-1 (git8). That bpl-1 is not rescued by supplemental PUFAs probably means that simpler fatty acid precursors, such as malonyl-CoA, are needed for de novo synthesis of complex permeability barrier components. Given the requirement for cytochrome P450s, cytochrome P450 reductase, and the putative sugar-modifying enzyme PERM-1 for the formation of the permeability barrier (25, 26), malonyl-CoA synthesis could be required for de novo synthesis and/or elongation of PUFAs as well as for synthesis of a complex lipid, such as a hydroxylated glycosphingolipid. Because these types of complex lipids are digested by lipases upon ingestion by C. elegans, it is not possible to test these molecules for rescue by dietary supplementation.

**Bacterial sources of malonyl-CoA or other fatty acid precursors compensate for BPL-1 activity during larval growth and development but not during embryogenesis**

Previous studies showed that both ACC/POD-2 and FASN-1 are needed for synthesis of specific sphingolipids during larval development. Knockdown of either gene caused defects in the intestinal lumen and early embryonic arrest due to a defect in the synthesis of glucosylceramides (27, 28). Thus, the ability of homozygous bpl-1 deletion mutant strains to develop normally throughout their larval growth stages was puzzling, because malonyl-CoA is required for fatty acid synthesis reactions catalyzed by ACC/POD-2 and FASN-1.

The finding that bpl-1 depletion only modestly reduced de novo lipid synthesis in young adult nematodes (Fig. 3A) led us to hypothesize that C. elegans obtains precursors for de novo fatty acid synthesis, such as malonyl-CoA, malonate, or short-chain fatty acids, directly from their bacterial diet to support lipid biosynthesis. Malonyl-CoA levels are highly regulated in animal cells; however, malonyl-CoA has been shown to be present in micromolar concentrations in the intracellular pool of Escherichia coli (29). To test whether bacterial fatty acid precursors...
were providing sufficient metabolites for lipid synthesis during larval development, we provided a malonyl-CoA–restricted food source by feeding K12-derived E. coli L8, containing a temperature-sensitive mutation in the accB gene, which encodes the /H9252 subunit of the E. coli acetyl-CoA carboxylase. Growth of the bacteria at restrictive temperature depletes malonyl-CoA stores and impedes new bacterial lipid synthesis compared with L8 grown at non-restrictive temperature. Growth and lipid composition of both wild-type and bpl-1 mutant C. elegans larvae fed L8 bacteria grown at non-restrictive temperature were comparable with those of E. coli strain OP50. In contrast, we found that bpl-1(b281) mutants were much more severely affected than wild type when fed acetyl-CoA carboxylase–restricted L8 bacteria that were grown at restrictive temperature; bpl-1(b281) worms arrested growth at the L3 larval stage, whereas wild-type worms reached adulthood but had a smaller size. A small proportion of wild type arrested similarly to bpl-1 (Fig. 5, A–C). Growth of both bpl-1 and wild type was completely rescued by supplementation with biotin (Fig. 5, A and B), but not oleate, malonate, or malonyl-CoA (not shown). This is in contrast to the embryonic phenotypes described above, where neither biotin nor supplemented fatty acid precursors or fatty acids are able to rescue the permeability barrier and polarity defects of embryos produced by bpl-1 mutant mothers. Finally, we tested the ability of worms to synthesize fatty acids when dietary malonyl-CoA and other fatty acid precursors were restricted. Interestingly, wild-type worms significantly increased de novo fat synthesis in response to the L8 diet (Fig. 5D), suggesting that the worms can detect dietary malonate and other fatty acid precursors and increase de novo synthesis to compensate when these dietary substrates are limited. Together, these data indicate that C. elegans maintains a balance of malonate or other precursors obtained from the diet and malonyl-CoA synthesized de novo from acetyl-CoA to support lipid synthesis for larval growth and development but that dietary lipids and precursors are not available or are not utilized during oogenesis and embryogenesis, and thus de novo synthesized lipids are required at this stage (Fig. 5, E and F).
Discussion

We have demonstrated that the C. elegans gene bpl-1 encodes a biotin-ligating enzyme homologous to mammalian holocarboxylase synthetase. BPL-1 is required for normal biotinylation of at least four conserved carboxylase enzymes. Mutations in the conserved biotin-binding domain of BPL-1 are detrimental to embryonic and larval development. Further, by knocking down each of four carboxylase genes with RNAi, we showed that reduction of the POD-2 acetyl-CoA carboxylase produces embryonic defects similar to bpl-1 mutants, whereas reduction of other carboxylase gene products showed no detrimental developmental effects. Thus, under laboratory conditions when nutrients are abundantly available, de novo fatty acid synthesis and elongation, reactions that required malonyl-CoA produced by acetyl-CoA carboxylase, are the primary biotin-dependent pathways affected by loss of BPL-1.

BPL-1 plays a central role in lipid de novo fatty acid synthesis and elongation and is required for the synthesis of the embryonic permeability barrier and polarity in the embryo. Embryo production is a major consumer of nutrients, and young adult hermaphrodites convert their body mass to embryos every 24 h (30). Bacterial nutrients provide intact fatty acids as well as precursors, such as malonyl-CoA, for growth and development. Our finding that loss of de novo synthesis genes causes a large reduction of PUFA s in embryos, but not in the adult worm, supports a model in which dietary fatty acid precursors and de novo synthesized malonyl-CoA and fatty acids contribute to the lipid requirements for rapid growth and development during larval stages. However, in the sequestered environment of the early embryo, dietary support is unavailable and lipid incorporation is dependent on de novo synthesis and elongation, particularly for PUFA production. Stable isotope labeling studies indicate that PUFA s are preferentially synthesized from de novo derived, as opposed to dietary, precursors (16, 17). The inaccessibility of dietary fats during the oocyte–embryo transition perhaps ensures that fresher, less oxidized fatty acids are present at the start of embryonic life. In addition to providing precursors for membrane and storage lipid synthesis, recent studies revealed that fatty acid levels signal environmental conditions that influence sex determination in nematodes (31), and microbial metabolites, including lipid precursors, affect host metabolism, influencing reproduction and lifespan (32–34).

The embryonic permeability barrier performs the critical function of maintaining osmotic integrity of the embryo. Depletions of the lipid biosynthetic genes pod-2 and fasn-1 both
cause osmotic integrity defects, as does loss of EMB-8, the NADPH reductase, and two lipid-modifying cytochrome P450 enzymes, as well as the putative sugar-modifying enzyme PERM-1 (12, 25, 26), which suggests that the permeability barrier may contain glycosylated and hydroxylated lipids. We have demonstrated that permeability barrier defects caused by depletion of lipid biosynthesis genes result, at least partially, from loss of embryonic PUFAs, which could be incorporated into the barrier as a component of complex hydroxylated glycolipids. In mammals, the PUFAs linoleic and linolenic acid are required for the synthesis of the impermeable corneocyte lipid envelope of the skin, in which PUFAs are esterified to the ω-hydroxyl of the amide-linked very-long-chain fatty acid of a unique class of esterified ceramides (35). A cytochrome P450 enzyme is required for the ω-hydroxylation, and lipoxygenase enzymes are proposed to further oxidize the esterified ceramides to allow binding of the lipid to proteins of the cell envelope, forming the permeability barrier (35, 36).

Similar to other permeability barrier genes, bpl-1 is important for embryonic polarity. We found that loss of bpl-1 caused symmetric first divisions, restricted PAR-2 domains, synchronous second divisions, and transverse spindle orientations during the second division. The relationship between the permeability barrier and polarity is not understood. The permeability
barrier could affect polarity via direct interactions between the barrier and the cell membrane or cell cortex, or polarity defects could result from loss of osmotic regulation. Alternatively, the polarity defects associated with fat synthesis could be independent of permeability barrier function. Previous work suggested that pod-2- and emb-8-depleted embryos displayed weakened interaction between the paternal pronucleus and the cell cortex, which correlated with symmetric first divisions (12). This weakened interaction with the cortex could be explained by either a defective interaction with the permeability barrier or altered embryonic membrane composition. Future work is needed to determine the structure and function of the specific lipids that compose the permeability barrier and how they influence polarity.

The rate-limiting step in de novo fatty acid synthesis is carboxylation of acetyl-CoA by acetyl-CoA carboxylase to generate malonyl-CoA. In humans, holocarboxylase synthetase deficiency is an autosomal recessive disorder that usually manifests early in life, with patients showing defects in fatty acid biosynthesis, gluconeogenesis, and amino acid metabolism that lead to severe conditions of the skin, damage to the nervous system, coma, and early death (37). Fortunately, continual supplementation with biotin is sufficient to treat the syndrome in most cases, suggesting that excess biotin can drive carboxylase reactions in the absence of fully functioning holocarboxylase synthetase (5). Due to rapid turnover, it is not generally believed that appreciable malonyl-CoA pools accumulate during fatty acid synthesis (38, 39). Despite this, our work demonstrates that C. elegans uses both dietary fatty acid precursors and de novo synthesized malonyl-CoA for lipid synthesis. Restricting dietary fatty acid precursors by feeding E. coli deficient in acetyl-CoA carboxylase activity caused severe arrest during larval development in worms without functioning BPL-1 and even showed a measurable effect on growth in wild-type worms. Dietary biotin rescues these growth deficiencies in both wild type and the bpl-1 mutants.

Our studies indicate that C. elegans obtain malonyl-CoA from both dietary precursors and from acetyl-CoA carboxylase reactions. Our observation that supplemental biotin rescued larval development is consistent with the success of treatments for holocarboxylase synthetase deficiency in humans, although it is not known whether holocarboxylase synthetase–deficient humans treated with dietary biotin experience fertility loss or birth defects in their offspring. Furthermore, malonyl-CoA is an important regulator of lipid metabolism. In hepatocytes, malonyl-CoA levels regulate the balance between fat synthesis and fatty acid oxidation (40, 41). We found that in C. elegans, loss of dietary fatty acid precursors caused an increase in de novo fatty acid synthesis, demonstrating that de novo fat synthesis is regulated depending on the availability of dietary precursors.

This study is the first to characterize the C. elegans holocarboxylase synthetase, BPL-1. Holocarboxylase synthetase is a critical regulator of biotin utilization at the epicenter of multiple metabolic pathways. Here, we demonstrated that BPL-1 function is critical for maintaining fatty acid composition during embryonic development, establishment of embryonic polarity, integrity of the embryo permeability barrier, and support of post-embryonic development. In addition to the role of HCS in metabolism, the enzyme also appears to play a role in gene regulation through biotinylation and direct interaction with histones (42–44). Study of HCS function in C. elegans will provide a powerful tool for dissecting the diverse roles of HCS in a multicellular organism under different environmental and dietary conditions.

Experimental procedures

C. elegans strains and RNAi

Nematodes were maintained on nematode growth medium (NGM) at 20 °C seeded with E. coli (OP50) unless otherwise stated (45). The wild-type strain was N2, and the mel-3(b281) and mel-3(i8) mutants were isolated in screens for maternal-effect lethal mutations (7) and maintained as heterozygotes over the mnC1 balancer as mel-3(b281 or i8) unc-4(e120)/mnC1 II. Strains carrying deletions for deletion mapping with mel-3 mutants were provided by the Caenorhabditis Genetics Center. Two deletion alleles, tm5867 and tm6621, were obtained from the Mitani laboratory, Tokyo Women’s Medical University School of Medicine. These were outcrossed, marked with unc-4(e120), and balanced with mnC1. Deletion end points of tm5867 and tm6621 were confirmed by DNA sequence analysis.

Transformation rescue of b281 and i8 mutants with cosmid or plasmid DNA was performed as described (46). Approximately 6.5 kb of the bpl-1 gene, including 600 bp upstream of the first predicted transcription start point, were sequenced from b281 and i8 homozygous worms. Only a single point mutation was found for each mutant.

Feeding RNAi experiments used NGM supplemented with 100 µg/ml ampicillin, 2 mM isopropyl β-D-thiogalactopyranoside and seeded with the appropriate HT115 RNAi bacteria. Feeding RNAi was performed by plating embryos of the RNAi-sensitive strain rrf-3(pk1426) onto seeded RNAi plates, with the exception of RNAi of pod-2 and fasn-1, in which feeding RNAi was initiated at the L3 larval stage. The pod-2, fasn-1, mec-1, and pyc-1 feeding constructs were obtained from the Ahringer RNAi library and verified by sequencing (47) (Source BioSource). For RNAi of bpl-1, pcca-1, and mcc-1, a 250–550-bp portion of each gene was amplified from N2 DNA using primer sequences TTCTTTCGATTCATTTGGGAGTC and GTT-TCCGGGCCATTATCACCTCAT for bpl-1, GTGATGTG-GGGCCAGGAT and TTTCGAGGAGCCAGGAAAGC for mcc-1, and CTTGAAGGCTGGAGCGCAA for pcca-1. Amplified DNA was cloned into L4440, and RNAi constructs were sequence-verified. Empty vector L4440 was used as the control.

RNA extraction and quantitative RT-PCR

RNA was extracted from ~2000 nematodes using 1 ml of TRIzol reagent and multiple freeze–thaw cycles to break up nematodes. After chloroform addition and phase separation, the aqueous phase was precipitated with isopropyl alcohol, and RNA was further purified using a Qiagen RNeasy Plus minikit with genomic DNA elimination column as per the manufacturer’s instructions. cDNA was synthesized from 1 µg of RNA using oligo(dT) primer and superscript IV. Real-time quantita-
BPL-1 is required for embryogenesis

tive PCR was performed in triplicate on each of two biological replicates using PowerUp SYBR Green master mix (Applied Biosystems) using an Applied Biosystems 7300 real-time PCR system. The ΔΔCt method was used to determine the relative expression of the target gene in the RNAi knockdowns compared with empty vector controls. The ΔCt values were calculated using the geometric mean of the Ct values for two reference genes, tbb-2 and Y45F10D.4 (Fig. 2D).

Microscopy

To image live embryos, adult worms were dissected with a syringe needle in 0.8× egg buffer on a glass coverslip and compressed against a pad of 1% agarose dissolved in 0.8× egg buffer and cushioned with petroleum jelly (48). To visualize eggshell defects, 2–6 μM FM4-64 (Thermo Fisher catalog no. T-3166) dye was added to the dissecting buffer (26). Imaging was performed on a Leica DM RA2 microscope with Hamamatsu Orca-ER using OpenLab (Improvision) or BioVision software. Additional imaging was performed on an Olympus BX53 microscope with a QImaging Retiga 2000R CCD camera and analyzed with the QCapture software and ImageJ. Embryo permeability was measured for all bpl-1 alleles using Nile Blue A uptake (49).

PAR-2 and PAR-6 localization in living early embryos was visualized with the transgenes itls272[par-6ːpar-6ːmCherry] (50), and itls297[pie-1ːpar-2ːgfp + unc-119(+)] (provided by Aaron Schetter). The unc-4-marked bpl-1 mutants b281, tm5867, and tm6621 were each crossed into KK1213 itls272[par-6ːpar-6ːmCherry + unc-119(+)]; itls297[pie-1ːpar-2ːgfp + unc-119(+)] to create the strains KK1205, KK1212, and KK1230, respectively. To confirm PAR protein mislocalization phenotypes in b281, it8, tm5867, and tm6621 without transgenes, we fixed unc-4 bpl-1 and control unc-4 worms and embryos on slides in methanol at −20 °C and stained with primary antibodies to PAR-2 (51) and PAR-3 (52). AF488− or Cy-3−labeled secondary antibodies were from Invitrogen and Jackson ImmunoResearch.

Meiosis was examined in bpl-1 oocytes and fertilized zygotes in utero using strain KK1193 bpl-1(b281) unc-4(e120)/mnc1 II; unc-119(ed3) rds13(pie-1ːgfp::H2B + unc-119(+)) III and their heterozygous siblings from the same strain, which have fully viable embryos. rds132 was derived from strain AZ212 (53).

Protein blot detection of biotinylated proteins

To extract worm protein, 300 young adult worms were collected in M9 buffer and allowed to settle on ice before washing one time with M9, resetting on ice, and then removing the supernatant until −20 μl of worm pellet remained. We then added 20 μl of 2× Laemmli buffer and boiled the samples for 5 min. After boiling, each sample was Dounce-homogenized 100× with the melted end of a pipette tip and frozen at −20 °C. Before use, samples were reheated to 90 °C.

Proteins were separated by SDS-PAGE with Bio-Rad 7% Mini-Protein precast gels and transferred with Towbin buffer with no SDS for 20 min using semidynd transfer onto PVDF membranes. Membranes were blocked with 2% BSA in PBST (0.05% Tween 20). We probed for biotinylated proteins by incubating the membranes with 2 μl of streptavidin-horseradish peroxidase conjugate (Abcam ab74403; 1 mg/ml) in 10 ml of PBST (0.1% Tween 20) for 30 min, followed by washing with PBST (six 7-min washes). Bands were visualized using the Pierce ECL Western blotting substrate with a 2-min incubation time. Biotinylated proteins were visualized on X-ray film, and protein standards (Bio-Rad dual color standards) were visualized using a Bio-Rad imager.

Lipid extraction for fatty acid composition

Lipids were extracted as described previously (21). Briefly, worms were washed from feeding plates with water, washed once to remove residual bacteria, and allowed to settle on ice. After residual water was removed, the worm pellet was incubated for 1 h at 70 °C in 2.5% sulfuric acid in methanol. After stopping the reaction with water, the fatty acid methyl esters were extracted with hexane.

Stable isotope labeling assay

As described elsewhere (16, 17), to quantify the amount of de novo fatty acid synthesis, worms were fed a mixed culture of E. coli, where bacteria was grown in either LB broth or 13C-labeled Isogro medium (Sigma-Aldrich). Overnight cultures of each were pelleted and mixed (1:1, v/v) after resuspension to 0.075 mg/ml in M9. The RNAi-hypersensitive rrf-3(pk1426) strain was treated with RNAi or the empty vector control plasmid starting at the L4 larval stage. Their progeny were fed for 12 h starting at the L4 larval stage on HT115 bacteria grown in medium containing a mix of 13C and 12C media (1:1, v/v).

Lipid analysis

Fatty acid methyl esters were analyzed by GC/MS using an Agilent 7890 GC/5975C MS. For de novo synthesis experiments, we operated the MS in scanning mode to monitor ions m/z 260−300 for 16 carbon fatty acids and m/z 290−320 for 18 carbon fatty acids. The percentages of individual fatty acids that derived completely via de novo synthesis from acetyl-CoA were determined using the ratio of ions m/z 273−283 to total isopologs for 16 carbon fatty acids and the ratio of ions m/z 290−314 for 18 carbon fatty acids.

Isolation of embryos for lipid analysis

To isolate early embryos for lipid analysis, worms were synchronized with hypochlorite treatment, and isolated eggs were allowed to hatch and develop on bpl-1 RNAi plates until adult stage. Embryos were collected from the uteri of adult worms by hypochlorite treatment and washed twice with water. The embryo pellet was then flash-frozen in liquid nitrogen and stored at −80 °C before lipid analysis. For pod-2 and fasn-1 RNAi treatment, the semisynchronized offspring were allowed to develop for 48 h on empty vector plates to avoid larval arrest and were then washed with M9 buffer and moved to pod-2 or fasn-1 RNAi plates for 24 h before embryo collection.

Malonyl-CoA restriction plates

To restrict dietary malonyl-CoA or malonic acid, we used the E. coli K12-derived strain L8. The L8 strain contains a temperature-sensitive mutation in the acetyl-CoA carboxylase β-sub-
unit gene, accB22 (54). The L8 strain normally grows at 30 °C but does not grow above 37 °C due to inhibited malonyl-CoA synthesis. For our feeding experiments, we grew overnight cultures of L8 at 30 °C until the A600 of the culture was 2.0. We then collected half of the overnight culture, centrifuged, and concentrated 10× in M9 buffer; plated 300 μl of the bacterial suspension on nutrient-free plates (control); and exposed it to 15 min of UV light in a cell culture hood to prevent further growth. The remaining culture was incubated for an additional 6 h at 40 °C before plating (malonyl-CoA–restricted). Nutrient-free plates contained 1.5% agarose, 50 mM NaCl, 1 mM CaCl2, 1 mM MgSO4, and 25 mM KPO4.

**Biotin, malonyl-CoA, malonic acid, and fatty acid supplementation**

Biotin (Sigma-Aldrich, B4501) was dissolved in water at 22 mg/100 ml and filter-sterilized before top-dressing directly onto bacterial feeding plates at 0.05–50 μg/plate. We found that 0.5 μg/plate was sufficient to rescue growth of worms grown on an ACC-restricted diet. Malonic acid (Sigma-Aldrich, M1296) and malonyl-CoA lithium salt (Sigma-Aldrich, M4263) were dissolved in water at 100 mg/ml and then added to the top of bacterial lawns in concentrations ranging from 1 to 200 μg/plate for malonyl-CoA and up to 1000 μg/plate for malonic acid. The 1000–μg/plate replicates caused toxicity and growth arrest of nematodes, which was alleviated by adding NaOH solution to correct the pH; however, no rescue of embryo lethality of *bpl-1 (b2821)* was observed. Individual fatty acid sodium salts (Nu-Check Prep, Elysian, MN) dissolved in water were added to NGM at 0.1 mM with 0.1% Tergitol before plating as described previously (55).

**Author contributions**—J. S. W. conducted most of the experiments and wrote most of the paper. D. G. M. and K. J. K. isolated the *bpl-1* mutant strains. D. G. M. performed the mapping and complementation tests and carried out the embryonic phenotype characterization shown in Fig. 1, including filming of the movies. J. S. W., D. G. M., K. J. K., and J. L. W. conceived ideas for the experiments. D. G. M., K. J. K., and J. L. W. edited the paper.

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**References**

1. Zempleni, J., Liu, D., Camara, D. T., and Cordonier, E. L. (2014) Novel roles of holocarboxylase synthetase in gene regulation and intermediary metabolism. *Nutr. Rev.* 72, 369–376 CrossRef Medline
2. Mock, D. M. (2009) Marginal biotin deficiency is common in normal human pregnancy and is highly teratogenic in mice. *J. Nutr.* 139, 154–157 Medline
3. Stermick, L. M., Wegener, K. L., Bruning, J. B., Booker, G. W., and Polyaak, S. W. (2017) Mechanisms governing precise protein biotinylation. *Trends Biochem. Sci.* 42, 383–394 CrossRef Medline
4. Rios-Avila, L., Prince, S. A., Wijeratne, S. S., and Zempleni, J. (2011) A 96-well plate assay for high-throughput analysis of holocarboxylase synthetase activity. *Clin. Chim. Acta* 412, 735–739 CrossRef Medline
5. Wolf, B., Hsia, Y. E., Sweetman, L., Feldman, G., Boychuk, R. B., Bart, R. D., Crowell, D. H., Di Mauro, R. M., and Nyhan, W. L. (1981) Multiple carboxylase deficiency: clinical and biochemical improvement following neonatal biotin treatment. *Pediatrics* 68, 113–118 Medline
6. Suzuki, Y., Yang, X., Aoki, Y., Kure, S., and Matsubara, Y. (2005) Mutations in the holocarboxylase synthetase gene HCLS. *Hum. Mutat.* 26, 285–290 CrossRef Medline
7. Kemphues, K. J., Kusch, M., and Wolf, N. (1988) Maternal-effect lethal mutations on linkage group II of *Caenorhabditis elegans*. *Genetics* 120, 977–986 Medline
8. Reche, P. A. (2000) Lipoylating and biotinylating enzymes contain a homologous catalytic module. *Protein Sci.* 9, 1922–1929 CrossRef Medline
9. Yang, X., Aoki, Y., Li, X., Sakamoto, O., Hiratsuka, M., Kure, S., Taheri, S., Christensen, E., Inui, K., Kubota, M., Ohira, M., Okhi, K., Kudoh, J., Kawasaki, K., Shibuya, K., Shintani, A., Asakawa, S., Minoshima, S., Shimizu, N., Narisawa, K., Matsubara, Y., and Suzuki, Y. (2001) Structure of human holocarboxylase synthetase gene and mutation spectrum of holocarboxylase synthetase deficiency. *Hum. Genet.* 109, 526–534 CrossRef Medline
10. Rose, L., and Gonczy, P. (Dec 30, 2014) Polarity establishment, asymmetric division and segregation of fate determinants in early *C. elegans* embryos. *WormBook* 10.1895/wormbook.1.30.2
11. Chandler, R. J., Aswani, V., Tsai, M. S., Falk, M., Wehrli, N., Stable, S., Allen, R., Sedensky, M., Kazazian, H. H., and Venditti, C. P. (2006) Propionyl-CoA and adenosylcobalamin metabolism in *Caenorhabditis elegans*: evidence for a role of methylmalonyl-CoA epimerase in intermediary metabolism. *Mol. Genet. Metab.* 89, 64–73 CrossRef Medline
12. Rappleye, C. A., Tagawa, A., Le Bot, N., Ahringer, J., and Aroian, R. V. (2003) Involvement of fatty acid pathways and cortical interaction of the pronuclear complex in *Caenorhabditis elegans* embryonic polarity. *BMC Dev. Biol.* 3, 8 CrossRef Medline
13. Tagawa, A., Rappleye, C. A., and Aroian, R. V. (2001) Pod-2, along with pod-1, defines a new class of genes required for polarity in the early *Caenorhabditis elegans* embryo. *Dev. Biol.* 233, 412–424 CrossRef Medline
14. Yi, X., and Maeda, N. (2005) Endogenous production of lipoic acid is essential for mouse development. *Mol. Cell. Biol.* 25, 8387–8392 CrossRef Medline
15. Gurtitz, A. (2009) A *C. elegans* model for mitochondrial fatty acid synthase II: the longevity-associated gene W09H1.5/mecr-1 encodes a 2-trans-enoyl-thioester reductase. *PLoS One* 4, e7791 CrossRef Medline
16. Perez, C. L., and Van Gilst, M. R. (2008) A 13C isotope labeling strategy reveals the influence of insulin signaling on lipogenesis in *C. elegans*. *Cell Metab.* 8, 266–277 CrossRef Medline
17. Dancy, B. C., Chen, S. W., Drechsler, R., Gañán, P. R., and Olsen, C. P. (2015) 13C- and 15N-labeling strategies combined with mass spectrometry comprehensively quantify phospholipid dynamics in *C. elegans*. *PLoS One* 10, e011850 CrossRef Medline
18. Watts, J. L., and Browse, J. (2002) Genetic dissection of polynsaturated fatty acid synthesis in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 99, 5854–5859 CrossRef Medline
19. Brooks, K. K., Liang, B., and Watts, J. L. (2009) The influence of bacterial diet on fat storage in *C. elegans*. *PLoS One* 4, e7545 CrossRef Medline
20. Tanaka, T., Ikita, K., Ashida, T., Motoyama, Y., Yamaguchi, Y., and Satouchi, K. (1996) Effects of growth temperature on the fatty acid composition of the free-living nematode *Caenorhabditis elegans*. *Lipids* 31, 1173–1178 CrossRef Medline
21. Shi, X., Li, J., Zou, X., Greggain, J., Redkaier, S. V., Faergeman, N. J., Liang, B., and Watts, J. L. (2013) Regulation of lipid droplet size and phospholipid composition by stearoyl-CoA desaturase. *J. Lipid Res.* 54, 2504–2514 CrossRef Medline
22. Brock, T. J., Browse, J., and Watts, J. L. (2006) Genetic regulation of unsaturated fatty acid composition in *C. elegans*. *PLoS Genet.* 2, e108 CrossRef Medline

BPL-1 is required for embryogenesis

J. Biol. Chem. (2018) 293(2) 610–622 621
BPL-1 is required for embryogenesis

23. Watts, J. L., and Browse, J. (2000) A palmitoyl-CoA-specific Δ9 fatty acid desaturase from Caenorhabditis elegans. Biochem. Biophys. Res. Commun. 272, 263–269 CrossRef Medline

24. Brock, T. J., Browse, J., and Watts, J. L. (2007) Fatty acid desaturation and the regulation of adiposity in Caenorhabditis elegans. Genetics 167, 865–875 Medline

25. Benenati, G., Penkov, S., Müller-Reichert, T., Entchev, E. V., and Kurzchalia, T. V. (2009) Two cytochrome P450s in Caenorhabditis elegans are essential for the organization of eggshell, correct execution of meiosis and the polarization of embryo. Mech. Dev. 126, 382–393 CrossRef Medline

26. Olson, S. K., Greenan, G., Desai, A., Müller-Reichert, T., and Oegema, K. (2012) Hierarchical assembly of the eggshell and permeability barrier in C. elegans. J. Cell Biol. 198, 731–748 CrossRef Medline

27. Zhu, H., Shen, H., Sewell, A. K., Kniazeva, M., and Han, M. (2013) A novel sphingolipid:TORC1 pathway critically promotes postembryonic development in Caenorhabditis elegans. Elife 2, e00429 Medline

28. Zhang, H., Abraham, N., Khan, L. A., Hall, D. H., Fleming, J. T., and Göbel, V. (2011) Apicobasal domain identities of expanding tubular membranes depend on glycosphingolipid biosynthesis. Nat. Cell Biol. 13, 1189–1201 CrossRef Medline

29. Takamura, Y., and Nomura, G. (1988) Changes in the intracellular concentration of acetyl-CoA and malonyl-CoA in relation to the carbon and energy metabolism of Escherichia coli K12. J. Gen. Microbiol. 134, 2249–2253 Medline

30. Hirsh, D., Oppenheim, D., and Klass, M. (1976) Development of the reproductive system of Caenorhabditis elegans. Dev. Biol. 49, 200–219 CrossRef Medline

31. Tang, H., and Han, M. (2017) Fatty acids regulate germline sex determination through ACS-4-dependent myristoylation. Cell 169, 457–469.e13 CrossRef Medline

32. Watson, E., MacNeil, L. T., Ritter, A. D., Yilmaz, L. S., Rosebrock, A. P., Bomar, J. D., and Mathur, A. J. (2014) Interspecies systems biology uncovers metabolites affecting C. elegans gene expression and life history traits. Cell 156, 759–770 CrossRef Medline

33. Watson, E., Olin-Sandoval, V., Hoy, M. J., Li, C. H., Louisse, T., Yao, V., Mori, A., Holdorf, A. D., Troyanskaya, O. G., Ralser, M., and Wallout, A. J. (2016) Metabolic network rewiring of propionate flux compensates vitamin B12 deficiency in C. elegans. Elife 5, e17670 CrossRef Medline

34. Lin, C. J., and Wang, M. C. (2017) Microbial metabolites regulate host lipid metabolism through NRS5A-Hedgehog signalling. Nat. Cell Biol. 19, 550–557 CrossRef Medline

35. Uchida, Y., and Holleran, W. M. (2008) ω-0-acylceramide, a lipid essential for mammalian survival. J. Dermatol. Sci. 51, 77–87 CrossRef Medline

36. Zheng, Y., Yin, H., Boeglin, W. E., Elias, P. M., Crumrine, D., Beier, D. R., and Brash, A. R. (2011) Lipoxigenases mediate the effect of essential fatty acid in skin barrier formation: a proposed role in releasing amide for construction of the corneocyte lipid envelope. J. Biol. Chem. 286, 24046–24056 CrossRef Medline

37. Burri, B. J., Sweetman, L., and Nyhan, W. L. (1981) Mutant holocarboxylase synthetase: evidence for the enzyme defect in early infanteile biontrusive multiple carboxylase deficiency. J. Clin. Invest. 68, 1491–1495 CrossRef Medline

38. Moore, J. H., and Christie, W. W. (1979) Lipid metabolism in the mammary gland of ruminant animals. Prog. Lipid Res. 17, 347–395 CrossRef Medline

39. Post-Bettenmiller, D., Roughan, G., and Ohlrogge, J. B. (1992) Regulation of plant fatty acid biosynthesis: analysis of acyl-coenzyme a and acyl-acyl carrier protein substrate pools in spinach and pea chloroplasts. Plant Physiol. 100, 923–930 CrossRef Medline

40. McGarry, J. D., Leatherman, G. F., and Foster, D. W. (1978) Carnitine palmitoyltransferase I: the site of inhibition of hepatic fatty acid oxidation by malonyl-CoA. J. Biol. Chem. 253, 4128–4136 Medline

41. McGarry, J. D., Mannaeerts, G. P., and Foster, D. W. (1977) A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis. J. Clin. Invest. 60, 265–270 CrossRef Medline

42. Bao, B., Wijeratne, S. S. K., Rodriguez-Melendez, R., and Zemplen, J. (2011) Human holocarboxylase synthetase with a start site at methionine-58 is the predominant nuclear variant of this protein and has catalytic activity. Biochem. Biophys. Res. Commun. 412, 115–120 CrossRef Medline

43. Narang, M. A., Dumas, R., Ayer, L. M., and Gravel, R. A. (2004) Reduced histone butylation in multiple carboxylase deficiency patients: a nuclear role for holocarboxylase synthetase. Hum. Mol. Genet. 13, 15–23 Medline

44. Trujillo-Gonzalez, I., Cervantes-Roldan, R., Gonzalez-Noriega, A., Michalak, C., Reyes-Carmona, S., Barrios-Garcia, T., Meneses-Morales, I., and Leon-Del-Rio, A. (2014) Holocarboxylase synthetase acts as a biotin-independent transcriptional repressor interacting with HDAC1, HDAC2 and HDAC7. Mol. Genet. Metab. 111, 321–330 CrossRef Medline

45. Steriennagle, T. (2006) Maintenance of C. elegans (February 11, 2006) WormBook (The C. elegans Research Community, ed) CrossRef

46. Mello, C. C., Kramer, J. M., Stinchcomb, D., and Ambros, V. (1991) Efficient gene transfer in C. elegans: extrachromosomal maintenance and integration of transforming sequences. EMBO J. 10, 3959–3970 Medline

47. Fraser, A. G., Kanath, R. S., Zipperlen, P., Martinez-Campos, M., Sohrmann, M., and Abringer, J. (2000) Functional genomic analysis of C. elegans chromosome 1 by systematic RNA interference. Nature 408, 325–330 CrossRef Medline

48. Verbrugge, K. J., and Chan, R. C. (2011) Imaging C. elegans embryos using an epifluorescent microscope and open source software. J. Vis. Exp. CrossRef Medline

49. Rappleye, C. A., Tagawa, A., Lyczak, R., Boweman, B., and Aroian, R. V. (2002) The anaphase-promoting complex and separin are required for embryonic anterior-posterior axis formation. Dev. Cell 2, 195–206 CrossRef Medline

50. Brennan, L. D., Roland, T., Morton, D. G., Fellman, S. M., Chung, S., Soltani, M., Kevek, J. W., McEuen, P. M., Kemphues, K. J., and Wang, M. D. (2013) Small molecule injection into single-cell C. elegans embryos via carbon-reinforced nanopipettes. PLoS One 8, e75712 CrossRef Medline

51. Boyd, L., Guo, S., Levitan, D., Stinchcomb, D. T., and Kemphues, K. J. (1996) PAR-2 is asymetrically distributed and promotes association of P granules and PAR-1 with the cortex in C. elegans embryos. Development 122, 3075–3084 Medline

52. Etemad-Moghadam, B., Guo, S., and Kemphues, K. J. (1995) Asymmetrically distributed PAR-3 protein contributes to cell polarity and spindle alignment in early C. elegans embryos. Cell 83, 743–752 CrossRef Medline

53. Robert, V. J., Sijen, T., van Wolfskinkel, J., and Plasterk, R. H. (2005) Chromatin and RNAi factors protect the C. elegans germline against repetitive sequences. Genes Dev. 19, 782–787 CrossRef Medline

54. Harder, M. E., Beacham, I. R., Cronan, J. E., Jr., Beacham, K., Honegger, T. V., McEuen, P. M., Kemphues, K. J., Wang, M. D. (2013) Par-1 and chromosomal localization of par-3 in the AB zygote. Elife 2, 200–219 CrossRef Medline

55. Deline, M. L., Vrablik, T. L., and Watts, J. L. (2013) Dietary supplementa-tion of Caenorhabditis elegans. J. L. and Silbert, D. F. (2012) Temperature-sensitive mutants of Escherichia coli requiring saturated and unsaturated fatty acids for growth: isolation and properties. Proc. Natl. Acad. Sci. U.S.A. 69, 3105–3109 CrossRef Medline

56. Deline, M. L., Vrablik, T. L., and Watts, J. L. (2013) Dietary supplementation of polyunsaturated fatty acids in Caenorhabditis elegans. J. Vis. Exp. CrossRef Medline