Contribution of the Peroxisomal acox Gene to the Dynamic Balance of Daumone Production in Caenorhabditis elegans

Received for publication, March 11, 2010, and in revised form, July 3, 2010. Published, JBC Papers in Press, July 7, 2010, DOI 10.1074/jbc.M110.122663

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Dauer pheromones or daumones, which are signaling molecules that interrupt development and reproduction (dauer larvae) during unfavorable growth conditions, are essential for cellular homeostasis in Caenorhabditis elegans. According to earlier studies, dauer larva formation in strain N2 is enhanced by a temperature increase, suggesting the involvement of a temperature-dependent component in dauer pheromone biosynthesis or sensing. Several naturally occurring daumone analogs (e.g. daumones 1–3) have been identified, and these molecules are predicted to be synthesized in different physiological settings in this nematode. To elucidate the molecular regulatory system that may influence the dynamic balance of specific daumone production in response to sudden temperature changes, we characterized the peroxisomal acox gene encoding acyl-CoA oxidase, which is predicted to catalyze the first reaction during biosynthesis of the fatty acid component of daumones. Using acox-1(ok2257) mutants and a new, robust analytical method, we quantified the three most abundant daumones in worm bodies and showed that acox likely contributes to the dynamic production of various quantities of three different daumones in response to temperature increase, changes that are critical in C. elegans for coping with the natural environmental changes it faces.

The free-living soil nematode Caenorhabditis elegans develops through four larval stages to the adult stage in ~3 days under favorable conditions; however, when C. elegans encounters unfavorable environmental conditions such as increased temperature (e.g. 25 °C) or high population density, dauer pheromones or daumones signal the L2 larvae to enter the nonaging dauer stage (1–3). Because the structure of daumone 1 was reported (4), at least nine naturally occurring daumone analogs with presumably diverse molecular functions have been discovered (5–9). Therefore, these daumones appear to be important signaling molecules for aging, development, and reproduction in C. elegans (4, 7, 10). The three most abundant daumones (daumones 1, 2, and 3) (4, 5) may be produced under different physiological conditions or in response to different stress signals (e.g. higher temperature or different nutritional conditions). Despite the critical roles of these daumones in C. elegans, the molecular regulatory system that controls the biosynthesis of these diverse molecules is unknown.

These daumones are composed of dideoxyhexose ascryllose and various short chain fatty acid moieties. The methyl-branched fatty acid moieties of the three most abundant daumones are likely biosynthesized during peroxisomal β-oxidation, which, in C. elegans, consists of four consecutive reactions catalyzed by three peroxisomal enzymes: acyl-CoA oxidase (ACOX), δHDS-28, and DAF-22 (11, 12) (see Fig. 1A). However, among these enzymes, ACOX has not been identified and characterized in C. elegans. In mammals, various fatty acid substrates for peroxisomal β-oxidation are utilized by two or three different ACOX enzymes with different specificities (13, 14). We addressed one important question as to the possible role of acox with respect to the balance of the concentration of different daumones synthesized in response to sudden environmental shifts. Here we report that C. elegans acox-1 likely contributes to the cellular distribution of daumones and influences dauer entry in response to temperature increases in C. elegans.

EXPERIMENTAL PROCEDURES

Strains and General Procedures—C. elegans strains were maintained on nematode growth medium agar plates at 20 °C. The N2 Bristol strain was used for all experiments unless otherwise noted. The acox-1(ok2257) and daf-22(ok693) mutant strains were obtained from the Caenorhabditis Genetics Center (Minneapolis, MN). We outcrossed each mutant with N2 worms four times to remove any possible unrelated genes before the strains were used in our experiments.

Construction and Expression of ACOX-1::GFP—For construction of acox-1p::GFP::acoX-1, the 1.37-kb upstream promoter of acox-1 was ligated into the BamHI/NotI restriction site upstream of the GFP-coding region in the pPD114.108 GFP vector. The entire coding region of acox-1 (3.559 kb) was ligated into the Nhel/StuI restriction site downstream of the GFP-coding region. Approximately 50 μg/ml of plasmid DNA containing the full acox-1p::GFP::acoX-1 gene construct was injected...
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into N2 worms, which also received 50 μg/ml of the transfection marker pRF4 containing rol-6(su1006). Transgenic rolling animals were selected from the progeny.

Measurement of Growth Defects—To measure the growth defects, five synchronized adult worms were transferred to a fresh nematode growth medium plate to lay eggs for 2 h. The worms were removed, and their progeny were grown at 20 °C for 72 h. The number of worms at each developmental stage was counted.

Yeast Strains and Spotting Assays—The Saccharomyces cerevisiae strain BY4741 was used as the wild-type strain, and the ∆pox1 mutant strain (YGL205W) was purchased from Open Biosystems (Huntsville, AL). Acox-1 cDNA of C. elegans was ligated into the BamHI restriction site of the pRSCupGFP expression vector containing the CUP1 promoter. The cloned construct was transformed into the ∆pox1 mutant strain with standard protocols (15). To investigate the growth rate, strains were grown for 24 h at 30 °C in YPDA-rich medium, and then cultures of each strain were equally diluted to A600 of 1 in YEP medium (1% yeast extract, 2% bacto-peptone) without a carbon source. Three microliters of a 5-fold series of dilutions of each strain was spotted on YEP agar plates containing 0.2% oleic acid and 50 μM copper ion. The spots were observed after 48 h of incubation at 30 °C.

Liquid Culture Bioassays for Quantification of Dauer Induction—Liquid culture bioassays to measure dauer induction were performed as previously described (11). C. elegans were cultured in 100 ml of S basal liquid medium containing Escherichia coli (OP50) (16) at 20 °C for 10 days. Additional E. coli was added to the medium on the fourth and fifth days. The dauer ratio in the liquid was measured after 10 days, with dauers confirmed by treatment with SDS as described previously (11).

 Extraction and Quantification of Specific Daumones—After worms were grown in 100 ml of S basal liquid medium for 10 days on a rotary shaker, the culture broth and worm bodies were separated by centrifugation (4629 × g for 15 min), and then the culture broth was filtered with Whatman paper to remove any remaining worms. Filtered culture broth was evaporated to obtain dried culture broth, which was then subjected to repeated extraction with ethanol for 3 days (11). The ethanol was evaporated, and the residue was dissolved in 1 ml of methanol. Then 5-μl aliquots of this mixture were analyzed by LC-MS/MS in a LTQ mass spectrometer (Thermo Scientific, San Jose, CA) equipped with an electrospray ionization source. Quantification of daumones was performed as described previously (11).

New Microquantitative Method for Measurement of Specific Daumones in Individual Worms—A small number of worms (1–80 worms) was transferred into a 1.5-ml tube containing 20 μl of water, and the tube was stored at −70 °C. For daumone quantification, samples containing frozen worms were thawed at ambient temperature, and then 10 μl of the aqueous layer was evaporated to dryness under vacuum. Then 0.1% formic acid in acetonitrile was added to the residue. The samples were then vortexed for 1.5 min and centrifuged at 18,514 × g for 5 min. Next, 10 μl of supernatant was transferred into the autosampler for injection into the LC-MS/MS system. Stock standard solutions of daumone 1, daumone 2, and daumone 3 were dissolved in methanol, and working standards were diluted serially with methanol to obtain final concentrations of 1, 2, 5, 10, 20, 50, and 100 fmol/μl. Daumones 1, 2, and 3 were quantified with an API 4000 MS/MS system (Applied Biosystems, MDS SCIEX, Toronto, Canada) using a multiple reaction monitoring method. The data were analyzed with Analyst 1.5 software (Applied Biosystems/MDS SCIEX). Compounds were separated on a C18 column (Agilent Eclipse XDB-C18 150 × 4.6 mm, 5 μm) with an HP 1200 series pump and autosampler (Agilent). The mobile phase was 10 mM ammonium acetate buffer containing 0.1% formic acid/acetonitrile (40:60, v/v). The column temperature was 40 °C, and the flow rate was 0.5 ml/min. The total chromatographic run time was 5 min.

Quantitative RT-PCR—Quantitative RT-PCR was performed according to the method described previously (10). The relative expression rate (%) was determined using the ΔCt method, and average expression of the reference gene act-1 was used to control for template levels.

RESULTS

Acox-1 of C. elegans Is an Essential Component of Daumone Biosynthesis—Previously, we characterized two key genes, dhs-28 and daf-22, that participate in three of the four peroxisomal β-oxidation reactions for the production of the fatty acid component of daumones (11) (Fig. 1A). Analogous to the mammalian peroxisomal β-oxidation pathway, C. elegans acox, which encodes ACOX, has remained the only uncharacterized gene in the biosynthesis of the fatty acid component of daumones. To investigate the contribution of ACOX to daumone biosynthesis, we searched for C. elegans acox in the NCBI BLAST database and identified F09A8.1a (acox-I), which exhibits 62% homology in amino acid sequence (43% identity) to that of the human ACOX (supplemental Fig. S1). This C. elegans ACOX-I contains a peroxisomal targeting signal at the C terminus (PSORT II database; Fig. 1A). As previously seen for two other peroxisomal enzymes, DHS-28 and DAF-22 (11), expression of an ACOX-1::GFP construct revealed that ACOX-I is localized in the peroxisomal matrix of the intestine and hypodermis (Fig. 1A; as observed by a punctate pattern in both intestinal and hypodermal cells versus a dispersed appearance in prx-5 RNAi (17) worms). Examination of acox-1(ok2257) mutants with deleted acox-I (Fig. 1A) revealed phenotypes quite similar to dhs-28 and daf-22 mutant worms. Indeed, these worms exhibited large fat granules mainly in the intestinal region of adult tails (supplemental Fig. S2A), serious growth defects (percentages of adult stage worms 72 h after the synchronized eggs: 7.7 ± 2.4% for acox-1 mutant versus 93.8 ± 2.2% for N2; Fig. 1C), reduced body length (12–17% smaller), and reduced brood size (~42%; supplemental Fig. S2, B and C), suggesting an important function in C. elegans peroxisomal β-oxidation. In S. cerevisiae, the peroxisome is the only site where fatty acid β-oxidation occurs, and this process is essential for growth in the presence of fatty acids. S. cerevisiae POX1 is a peroxisomal acyl-CoA oxidase that is very similar to C. elegans ACOX-1 (18). Its mutant strain, ∆pox1, grows very slowly in YEP medium in the presence of oleic acid as the only
To confirm that C. elegans acox-1 encodes a functional ACOX enzyme, we transformed the S. cerevisiae/H9004 pox1 mutant with C. elegans acox-1 cDNA. As shown in Fig. 1D, the growth rate of the acox-1-transformed S. cerevisiae/H9004 pox1 mutant was recovered in the presence of oleic acid, indicating that ACOX-1 is an authentic functional enzyme involved in the peroxisomal β-oxidation pathway. Thus, identification of acox-1 completes the components of the peroxisomal β-oxidation biosynthetic pathway (ACOX-1/ΔDHS-28/ΔDAF-22) that produces the fatty acid moieties of C. elegans daumones.

Acox-1(ok2257) Mutants Have Unexpected Dauer Inducing Activity—To further characterize the acox-1 mutants with respect to dauer formation capacity, we performed dauer assays in liquid medium by growing N2, acox-1(ok2257), and daf-22(ok693) worms for 10 days at 20 °C. N2 worms formed 20.7% dauers, but acox-1(ok2257) mutants yielded only 2.6% dauer formation (Fig. 2A), indicating defects in either daumone biosynthesis or the daumone sensing mechanism, although we predict that it is more likely the former. To determine whether the observed differences were associated with secreted daumones or with a daumone-sensing defect, plate dauer assays (4, 11) were performed in nematode growth medium supplemented with liquid culture supernatant from each strain (N2, acox-1(ok2257), or daf-22(ok693) mutants; Fig. 2A). N2 worms grown in the presence of their own pheromone extracts entered the dauer stage abundantly (57.3 ± 8%), whereas N2 worms grown in the presence of pheromone extracts of daf-22(ok693) mutants, however, did not form dauers in agreement with previous results (11). These results indicate that despite serious defects in daumone biosynthesis, acox-1(ok2257) mutants produced significant dauer inducing activity, suggesting the possibility of another ACOX isozyme(s).

Acox-1 Is Responsible for Production of Daumones 2 and 3 but Not Daumone 1—To determine the specific daumones that are biosynthetically dependent on ACOX-1 at 20 °C, we quantitatively examined the daumone production phenotype of the

![Diagram](image_url)
mutant \textit{acox-1(ok2257)} using a robust LC-MS/MS analysis of supernatant from 10-day cultures at 20 °C of N2, \textit{acox-1(ok2257)}, and \textit{daf-22(ok693)} worms as previously described (11). At 20 °C, the culture broth of \textit{acox-1(ok2257)} mutants showed significantly reduced quantities of daumones 2 and 3 (reduced by 98.2 and 80%, respectively) compared with that of N2 (142.6 ± 4.6 to 2.6 ± 1.1 nmol/ml for daumone 2 and 83.8 ± 2.1 to 16.6 ± 1.6 nmol/ml for daumone 3; Fig. 2B and supplemental Table S1). Daumones were not detected with the LC-MS/MS analysis of culture broth from \textit{daf-22(ok693)} mutants. Interestingly, daumone 1 was sharply induced by as much as 280% (28 ± 2 to 88.3 ± 12.8 nmol/ml) in \textit{acox-1(ok2257)} worms, suggesting that ACOX-1 catalyzes the first reaction in the biosynthesis of daumones 2 and 3 but not daumone 1.

\textbf{Daumones Are Biosynthesized in a Temperature-dependent Manner—}
In earlier studies by the Riddle group (1, 2), a rise in temperature (25 °C) increased the dauer formation rate, suggesting that daumone biosynthesis or a daumone-sensing mechanism may also be influenced by a temperature increase (from 20 to 25 °C). In addition, daumone 1 was more abundant in the supernatants from cultures grown at 20 °C than from cultures grown at 25 °C (6), no similar quantitative analysis for the three major daumones has been performed in the context of temperature change. Furthermore, even though daumone 2 was most abundant in the liquid culture broth (Fig. 2B), the relative concentrations of specific daumones within the worm body and the total amount of these molecules inside a single worm are completely unknown. Therefore, we developed a robust LC-MS/MS-based method for quantitative measurement of daumones present in a single worm body (see “Experimental Procedures”). Plotting the peak areas associated with specific daumones isolated from lysed samples of 1–80 synchronized 1-day N2 adult worms \textit{versus} standard curves (Fig. 3A and supplemental Table S2) yielded an estimate of the total amount of daumones and the relative concentrations of each daumone in a single worm body. For N2 worm bodies (1-day adults) grown on plates at 20 °C, daumone

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure2.png}
\caption{Dauer formation and daumone quantification in liquid culture broth of \textit{C. elegans} N2 wild-type worms, \textit{acox-1(ok2257)} and \textit{daf-22(ok693)} mutants. A, dauer formation in liquid culture for 10 days at 20 °C, and dauer formation of N2 worms on plates containing extracts from the liquid culture supernatants of each worm strain. The results represent one of two independent experiments in which triplicate samples were analyzed (means ± S.D.). **, \( p < 0.001 \) compared with N2. B, quantification of daumones 1, 2, and 3 isolated from supernatants from liquid cultures after 10 days at 20 °C. Quantification was performed using LC-MS/MS by interpolating or extrapolating from standard curves prepared with synthetic daumones (supplemental Fig. S5A). The values and statistical analysis are given in supplemental Table S1, and chromatograms for each daumone are shown in supplemental Fig. S5 (B–D). N.D., not detected.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3.png}
\caption{Daumone quantification in single worm bodies of N2, \textit{acox-1(ok2257)}, and \textit{daf-22(ok693)} mutants. A, quantification of daumones in 1–80 1-day adult N2 worms. Linearity was established by plotting the peak area of synthetic calibration curve samples at seven concentrations from 10 to 1000 fmol (supplemental Table S2). B, total number of daumones in single N2 worm bodies at 20 and 25 °C. The values were calculated from total daumone quantities obtained from 40 1-day adult N2 worms. The results represent one of two independent experiments in which triplicate samples were analyzed (means ± S.D.). **, \( p < 0.001 \) compared with worms grown at 20 °C. C, total daumone number and relative distribution of daumones 1, 2, and 3 in single 1-day adult N2 worms at each temperature. The values below the cylinders indicate the total daumone amount. D, daumone quantities present in one \textit{acox-1} mutant worm at 20 and 25 °C. The results represent one of two independent experiments in which triplicate samples were analyzed (means ± S.D.). **, \( p < 0.001 \) compared with worms grown at 20 °C. N.D., not detected.}
\end{figure}
3 (total 31.8 ± 1.3 fmol/worm body) was the major daumone followed by daumone 1 (total 20.1 ± 3.4 fmol/worm body) and daumone 2 (total 1.8 ± 0.3 fmol/worm body; Fig. 3B). Daumone 2 was, therefore, the least abundant daumone detected in the N2 worm body, although this daumone was the most abundant one in the culture broth (Figs. 2B and 3B) (11). Thus, daumone 2 is the most rapidly secreted daumone in the culture broth. Interestingly, when N2 worms were grown at 25 °C, we observed a 60–120% increase in daumone 1 (20.1 ± 3.4 to 45.4 ± 10.1 fmol/worm body) and daumone 3 (31.8 ± 1.3 to 50.9 ± 3.3 fmol/worm body) but almost no change in daumone 2. These results are in stark contrast to the results of Clardy’s group (6). Our results clearly demonstrate that growth temperature significantly affects the rates of daumone biosynthesis and that daumone 2 appears to be readily secreted into the medium after its synthesis, whereas daumones 1 and 3 remain in the worm body in addition to being secreted into the liquid medium (Figs. 2B and 3B).
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**Temperature**

| Peroxisomal β-oxidation pathway |
|--------------------------------|
| ACOX-1                        |
| DHS-28                        |
| DAF-22                        |
| Daumone 2, 3                  |
| Daumones or SCFA-CoA          |
| Various long chain ascaroside or VLCFA-CoA |

**FIGURE 5. Hypothetical model for the mode of action of ACOX and other enzymes involved in daumone biosynthesis.** Under the various growth temperatures, very long chain fatty acyl-CoA is processed through an intestinal peroxisomal β-oxidation pathway consisting of at least one ACOX enzyme, DHS-28, and DAF-22. This reaction generates different types of short chain fatty acyl-CoA, which are then exported to the cytosol for esterification by an endogenous CDP-ascarlose. This process results in daumone formation (11). Alternatively, daumones may also be made directly through shortening of the fatty acid chains in long chain ascarosides (11).

Daumones were not detected in **daf-22(ok693)** worms at either 20 or 25 °C (data not shown).

To search for the cause of increased daumone quantity in response to temperature change, we examined **acox-1** and **daf-22** expression at both temperatures. Expression of both genes was increased by 60% (**ACOX-1::GFP**) and 47% (**DAF-22::GFP**), respectively, at 25 °C compared with 20 °C (Fig. 4A). This observation was further verified by quantitative RT-PCR (Fig. 4B), which is consistent with our results showing a significant induction of daumone **1** (225%) and daumone **3** (159%) in N2 worm bodies grown at 25 °C (Fig. 3, B and C). Therefore, these results suggest that daumone biosynthesis can be regulated by controlling genes involved in the peroxisomal β-oxidation pathway in response to temperature increase.

**Daumone Biosynthesis May Indirectly Influence the Daumone Sensing Mechanism**—In our previous study (11), we observed a significant decrease in dauer formation rate in daumone synthesis mutants (**e.g. daf-22(ok693)**), indicating linkage between daumone sensing and production. To determine whether daumone sensing is also affected in **acox-1(ok2257)** mutants, we first tested the relative dauer inducing activity of daumones **1**, **2**, and **3** in N2 wild-type worms. As shown in Fig. 4C, all three daumones showed essentially the same dauer inducing activity (**i.e.** the **EC**50 values of daumones **1**, **2**, and **3** were 69.9, 84.6, and 66.1 μM, respectively), which is a stark contradiction to the report of Butcher et al. (5), who showed that daumone **2** (**asc#2**) has >150-fold higher activity over daumone **1**. This difference may be due to the unavailability of an accurate measurement method for daumone quantification when this group determined the relative dauer inducing activity of these daumone analogs. We then examined the relative dauer formation rate of N2 and **acox-1** and **daf-22** mutants at two different temperatures (20 versus 25 °C) by employing a suboptimal daumone **1** plate assay (20). Interestingly, there was an approximately 2–2.7-fold increase in the dauer formation rate in N2 and **daf-22(ok693)** worms when the temperature was shifted from 20 to 25 °C, whereas there was almost no such change in **acox-1(ok2257)** mutants (Fig. 4D). A similar result was also obtained with the standard daumone assay using a higher concentration of daumone (380 μM synthetic daumone **1**) (supplemental Fig. S3). This result suggests that daumone synthesis genes in general (**e.g.** **acox-1** and **daf-22**) likely contain a temperature-dependant regulation system that may be advantageous for **C. elegans** to adapt to sudden temperature increases, leading to entry into the dauer stage.

Other Acox Genes Involved in Daumone Biosynthesis—BLAST searches revealed five homologous **acox** genes (**acox-1**, **F08A8.3**, **F08A8.4**, **F59F4.1**, and **F58F9.7**) in **C. elegans**. Therefore, we hypothesized that these associated **acox** genes may play different roles in the production of different daumones, as reflected in the observations of differential biosynthesis described above. To find other **acox** genes in addition to daumone **2-** and **3-specific** **acox**, we performed RNAi in N2 worms for the four above **acox** homologs and for **acox-1** and **daf-22** and then compared daumone quantities in the synchronized 1-day adult RNAi worms with those of controls. In the F08A8.3 and F08A8.4 RNAi worms, daumone **2** was not detected, and daumone **3** was significantly decreased (supplemental Fig. S4, A–C). In contrast, daumone **1** was increased in these worms as in **acox-1** RNAi worms, suggesting that proteins produced by F08A8.3 and F08A8.4 act similarly to the protein encoded by **acox-1**. Changes in concentrations of daumones **1**, **2**, and **3** compared with controls were not detected in F59F4.1 and F58F9.7 RNAi worms, suggesting that these genes are not involved in daumone biosynthesis. In **daf-22** RNAi worms, all daumones were greatly diminished, as expected (supplemental Fig. S4).

**DISCUSSION**

Although we initially sought to identify and characterize the role of ACOX in the biosynthesis of dauer pheromones, we serendipitously found that daumone biosynthesis genes responsible for the peroxisomal β-oxidation of fatty acid components, which include **acox**,** daf-22** (Fig. 4D), and **dhs-28** (data not shown), may be candidates for the elusive temperature-dependent enzymes for dauer formation. More than 25 years ago, the Riddle group (1, 2) observed that a temperature increase results in an enhanced dauer formation rate in worms of the N2 strain. Interestingly, temperature-sensitive ACOX is also implicated in a peroxisome biogenesis disorder in cultured mammalian cells (21). Our data reveal that daumones are synthesized by
different ACOX enzymes in C. elegans depending on the growth conditions (Figs. 2B and 3, B–D). ACOX-1 appears to govern the selective production of daumones 2 and 3 but not daumone 1 and to induce the production of daumones in response to a temperature increase.

From a structural point of view, various daumones are distinguishable because of the presence of different fatty acid chain lengths (daumone 1, C8; daumone 2, C6; and daumone 3, C9) and different functional groups that bind to the end of the fatty acid chains (daumones 1 and 3, carboxylic acid; daumone 2, ketone group; Fig. 2B) (4, 5). In this context, we asked whether these structural differences affect their biological function, i.e. dauer inducing activity. Our data show that they contain essentially the same dauer formation activity, indicating some critical errors or technical difficulties in assessment of either daumone quantities or dauer inducing activity in a previous report (5) in which daumone 2 was claimed to be greater than 150-fold more potent than daumone 1. Thus, given the availability of robust accurate quantification methods and standard pure daumones as described in this paper, this contradicting result should be resolved by an independent investigation.

Cellular daumones are predicted to be synthesized via complex metabolic pathways, which consist of ascaroylase biosynthesis, binding between ascaroylase and short chain fatty acids, and peroxisomal β-oxidation (containing acox-1, dhs-28, and daf-22) (11, 12). The new robust daumone quantification method with a sensitivity level suitable for a single worm body now enables routine detection of the intermediates or the final forms of specific daumones in individual worms with high accuracy and reproducibility, and it can even be utilized to analyze RNAi worms (supplemental Fig. S4). This method allows us not only to compare relative daumone production in synchronized worms exposed to various conditions but also to more thoroughly decipher the complex daumone metabolic pathway.

The reason why this nematode requires so many acox genes and/or daumones, however, remains unclear. Based on our work, we postulate that C. elegans may need such options to ensure that a sufficient daumone supply is available when the worms encounter sudden environmental changes or stress signals, such as poor nutrition or high population density to prepare for the dauer stage. Perhaps acox-1 functions in concert with other yet-to-be-identified acox genes to maintain the overall balance of daumones (Fig. 5). For example, daumone 1 was elevated in acox-1 mutants lacking synthesis of daumones 2 and 3 compared with that in N2 liquid culture supernatants (Fig. 2B and supplemental Table S1). We propose that if acox-1, which is responsible for production of daumones 2 and 3, is not active (i.e. mutation), a reduced total daumone pool may stimulate yet-to-be-discovered acox-n genes, which are responsible for synthesizing daumone 1 at the transcriptional level as seen with HMG-CoA reductase in cholesterol biosynthesis homeostasis (22). It is also plausible to predict the presence of daumone sensor proteins that signal activation of daumone synthesis upon depletion of daumone levels caused by some biological or physiological intervention.

This homeostasis may necessitate the presence of a rich diversity of daumone molecules to cope with a variety of adverse environmental insults. In conclusion, our work on acox-1 provides new insight into the daumone biosynthesis mechanism as well as the environmental adaptation strategy by C. elegans.

Acknowledgments—We thank the Caenorhabditis Genetics Center for kind provision of mutants used for this study. We also thank Dr. David Chitwood for critical review of this manuscript and Dr. Junho Lee (Seoul National University, Korea) for the kind gift of the RNAi clones. We also thank Dr. Kiwon Song (Yonsei University) for the kind gift of the yeast strains and materials and Namil Kim for excellent technical assistance.

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