BMB
reports

A potential role for fatty acid biosynthesis genes during molting and cuticle formation in *Caenorhabditis elegans*

Yingxiu Li[1] *& Young-Ki Paik*[2,*]

[1]Department of Biochemistry, College of Life Science and Biotechnology, [2]Department of Biomedical Science, World Class University Graduate School Program, and Yonsei Proteome Research Center, Yonsei University, Seoul 120-749, Korea

Caenorhabditis elegans **undergoes a developmental molting process that involves a coordinated interplay among diverse intracellular pathways. Here, we investigated the functions of two fatty acid biosynthesis genes; *pod-2*, encoding acetyl-CoA carboxylase, and *fasn-1*, encoding fatty acid synthase, in the *C. elegans* molting process. Although both the *pod-2* and *fasn-1* genes were expressed at constant levels throughout *C. elegans* development, knockdown of the proteins encoded by these genes using RNA interference produced severe defects in triglyceride production, molting, and reproduction that were coupled to suppression of NAS-37, a metalloprotease. An assessment of the structure and integrity of the cuticle using a COL-19::GFP marker and Hoechst 33258 staining showed that downregulation of either *pod-2* or *fasn-1* impaired cuticle formation and disrupted the integrity of the cuticle and the hypodermal membrane. [BMB reports 2011; 44(4): 285-290]**

INTRODUCTION

During development in *Caenorhabditis elegans*, the end of each larval stage is coupled to molting, in which the old cuticles are shed (1). The molting process has four basic steps: (i) lethargus, (ii) apolysis, (iii) cuticle synthesis, and (iv) ecdysis (2-4). The *C. elegans* molting process involves a coordinated interplay between proteases [e.g., NAS-37 (3, 4)] and other proteins. These include cholesterol transport proteins (5), transcription factors [e.g., NHR-23 (6-8)], extracellular transport proteins, transmembrane proteins, and extracellular matrix (ECM) enzymes (9-14). Because this process involves extensive tissue remodeling entailing degradation of old cuticle proteins, inactivation of these proteases during the ecdysis step can cause molting defects in *C. elegans* (4). Cuticles are an exoskeleton of *C. elegans* and function to maintain body shape and shield the worm from external influences. Cuticles cover the entire body, are secreted by the hypodermis, and consist primarily of extracellular matrix, insoluble proteins, glycoproteins, and lipids (15, 16). The lipid components of cuticles are mainly free fatty acids, triglycerides, sterol, cholesterol esters, phosphatidylinositol, and phosphatidylcholine (16).

Because fatty acids are among the key components of the newly formed cuticle, and because molting appears to be directly associated with membrane organization (e.g., cuticle formation and hypodermal membrane), we sought to determine how the *C. elegans* fatty acid biosynthesis genes, *pod-2* and *fasn-1* (17), might contribute to the molting process. Here, we demonstrate that downregulation of the proteins encoded by these two genes causes impairment of the molting process and cuticle formation in *C. elegans*.

*Corresponding author. Tel: 82-2-2123-4242; Fax: 82-2-393-6589; E-mail: paikyk@gmail.com
DOI 10.5483/BMBRep.2011.44.4.285

Received 11 October 2010, Accepted 10 March 2011

Keywords: Acetyl-CoA carboxylase, *C. elegans*, Cuticle, Development, Fatty acid synthase, Molting

RESULTS

pod-2 and *fasn-1* are essential for *C. elegans* development and reproduction

We treated worms with interfering RNA (RNAi) against *pod-2* or *fasn-1* to determine the function of POD-2 and FASN-1 during development. When treatment started at the synchronized N2 L1 stage, all worms became arrested in larval stages and exhibited slow movement (Supplementary Fig. S1B and C). Worms treated with *pod-2* RNAi at the L2 stage or with *fasn-1* RNAi at the L3 stage all reached adulthood, although they were smaller in size than control adults. They also had fewer eggs within their bodies (Supplementary Fig. S1). Unlike L2 stage worms treated with *pod-2* RNAi, worms treated with *fasn-1* RNAi at the L2 stage were not able to reach adulthood. No differences were observed in fat storage of worms receiving *pod-2* RNAi or *fasn-1* RNAi at the early L4 stage, as indicated by Sudan black and Nile red staining (data not shown). However, a significant reduction in triglyceride (TG) levels in these RNAi-treated worms was found compared to control worms (Supplementary Fig. S1G). Apparently, the timing of the knockdown of these two genes makes a difference in terms of the resulting developmental abnormality. The spatial and temporal expression patterns of *pod-2* and *fasn-1* were studied in transgenic *pod-2::gfp* and *fasn1::gfp* worms. *pod-2* was expressed in the hypodermis and intestine (Supplementary Fig.

S1H and I), whereas *fasn-1* was expressed only in the hypodermis throughout all larval stages (Supplementary Fig. S1J).

pod-2 and *fasn-1* are involved in the molting process

When synchronized L1 stage N2 worms were fed *pod-2* RNAi, we observed a molting defect phenotype, as previously reported (12). Late L1 stage N2 worms fed *pod-2* or *fasn-1* RNAi exhibited similar molting defect phenotypes (Fig. 1B and C). This is summarized in Supplementary Table S1, which shows that 10.1 ± 1.2% (n = 3) of *pod-2 (RNAi)* worms and 23 ± 4.5% (n = 3) of *fasn-1 (RNAi)* worms exhibited molting defects when fed RNAi from the late L1 stage compared with 0.45 ± 0.3% (n = 3) of control worms. Worms treated with *pod-2* RNAi and *fasn-1* RNAi from the L2 and L3 stages, respectively, reached adulthood but showed some molting-defect phenotypes. These defects were evident in a scanning electron microscopy (SEM) analysis of adult *pod-2 (RNAi)* (Fig. 1E) and *fasn-1 (RNAi)* worms (Fig. 1F) compared with worms in the control group. In these images, unshed old cuticle can be clearly seen in the bodies of RNAi-treated worms, suggesting that POD-2 and FASN-1 play an important role in the molting process. Furthermore, severe defects in vulva formation were observed in *pod-2 (RNAi)* and *fasn-1 (RNAi)* worms (Fig. 1H and I). These results indicate that POD-2 and FASN-1 may contribute to vulva morphogenesis in *C. elegans*. To validate the relationship between molting defects and other fat metabolism-related genes, we inactivated two genes, *let-767,* which encodes short chain dehydrogenases/reductases, and *fat-7,* which encodes an essential delta-9 fatty acid desaturase, and observed their phenotypes. N2 worms treated with *let-767* RNAi from the L1 stage reached adulthood without showing a molting-defect phenotype. However, the intestines of *let-767 (RNAi)* worms were paler than those of control worms (Supplementary Fig. S2). Furthermore, *let-767 (RNAi)* worms showed an egg-laying defect in which only a few F1 progeny hatched inside the mother's body, consistent with a previous report (18). N2 worms treated with *fat-7* RNAi from the L1 stage also reached adulthood without showing a molting-defect phenotype, but unlike control worms, their intestines were colorless (Supplementary Fig. S2). These results suggest that not all fat metabolism-related genes have identical roles during the molting process.

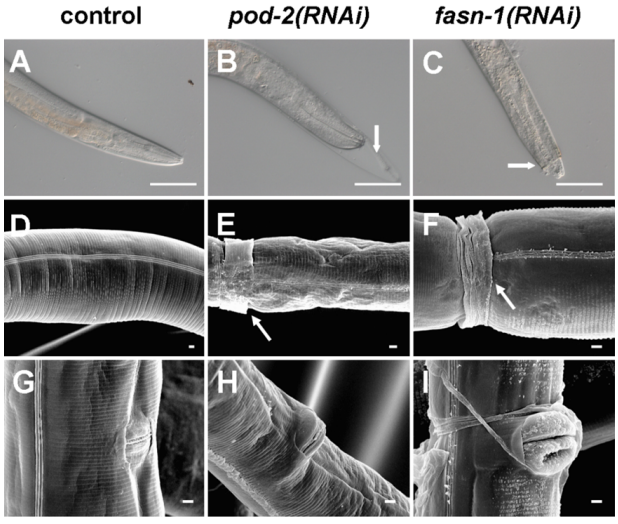

Fig. 1. Molting defects caused by *pod-2* or *fasn-1* RNAi. (A-C) Late L1 N2 larvae were fed bacteria expressing RNAi specific for the indicated genes. Shown here are control (A), *pod-2 (RNAi)* (B), and *fasn-1 (RNAi)* (C) worms. Scale bar, 50 μm. Scanning electron microscopic (SEM) analysis of control (D), *pod-2 (RNAi)* (E), and *fasn-1 (RNAi)* (F) worms. Unshed old cuticles can be seen in *pod-2 (RNAi)* and *fasn-1 (RNAi)* worms. Scale bar, 3 μm. SEM analysis of control (G), *pod-2 (RNAi)* (H), and *fasn-1 (RNAi)* (I) worms. Protruding vulvas are evident (H and I). Scale bar, 3 μm. White arrowheads indicate unshed cuticles.

Downregulation of *pod-2* or *fasn-1* causes inactivation of molting-related protease genes

To identify genes that might be linked to the observed molting defects caused by silencing *pod-2* and *fasn-1*, we examined the expression level of the representative protease gene, *nas-37*, which is involved in molting defects (4). Using *nas-37p::gfp-pest* (4), we assessed a potential link between deficiency in the fatty acid biosynthesis genes and reduced protease activity during the molting process. The positive control used for this experiment was *nhr-23 (RNAi)* worms. NHR-23 is a transcription factor that stimulates genes involved in larval molting, new cuticle formation, and ecdysis (12). To this end, *nas-37p::gfp-pest* worms (at the late L1 stage) were transferred to plates containing *pod-2* RNAi, *fasn-1* RNAi, *nhr-23* RNAi, or control RNAi, and green fluorescent protein (GFP) fluorescence levels indicative of *nas-37* expression were observed every 2 hours. At 16 hours, *nas-37p::gfp-pest* activity was approximately two-fold lower in the *pod-2 (RNAi)* group (40.9 ± 5.7%) than in the control group (81.8 ± 8.9%), whereas the fluorescence intensities of *fasn-1 (RNAi)* and *nhr23 (RNAi)* groups were 5.3-fold and 11.7-fold lower, respectively, than controls (Fig. 2A). At 18 hours, all RNAi-treated groups showed significant reductions in fluorescent intensities: −9.6-fold for *pod-2 (RNAi)*, −58-fold for *fasn-1 (RNAi)*, and −49.7-fold for *nhr-23 (RNAi)* (Fig. 2). The *pod-2 (RNAi)* group also showed patterns of expression in the L3/L4 and L4/adult molt stages that were similar to those of the *nhr23 (RNAi)* group. However, the *fasn-1* RNAi-treated group arrested at the L3 stage (Fig. 2A). These results support the notion that downregulation of fatty acid biosynthesis genes accompanies inactivation of the protease gene and may be involved in the molting process.

Downregulation of *pod-2* or *fasn-1* causes disruption of the cuticle collagen pattern and cuticle integrity

We next assessed the effects of downregulating these fatty acid genes on cuticle structure by observing RNAi worms by SEM (Fig. 3A-C). The adult cuticles of the *pod-2 (RNAi)* and *fasn1*

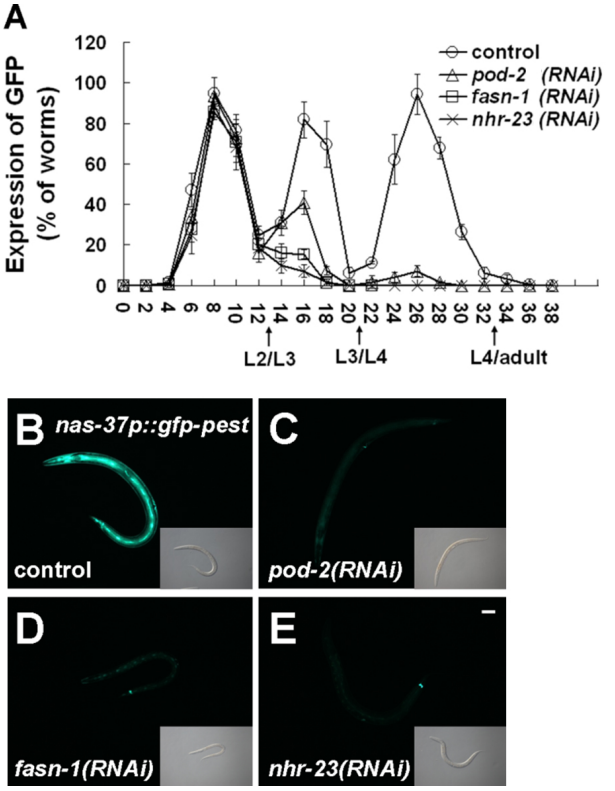

Fig. 2. Downregulation of *nas-37* in *pod-2(RNAi)* and *fasn-1 (RNAi)* worms. (A) GPF fluorescence from *nas-37p::gfp-pest*. Each sample contained 100 or more animals at 0 hours (late L1), 14 hours (L2/L3 molt), 22 hours (L3/L4 molt), or 34 hours (L4/adult molt). (B-E) *nas37p::gfp-pest* were fed bacteria expressing RNAi against the indicated genes from the late L1 stage. GFP fluorescence from *nas-37p::gfp-pest* in larvae. Scale bar, 50 μm.

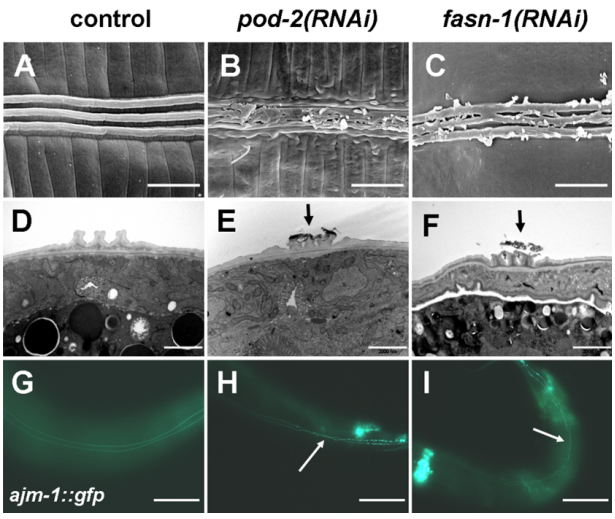

Fig. 3. Effects of *pod-2* and *fasn-1* on cuticle formation and seam cell function. (A-C) Scanning electron micrographs of adult control (A), *pod-2 (RNAi)* (B), and *fasn-1 (RNAi)* (C) worms. Serious defects in alaes are evident in *pod-2 (RNAi)* and *fasn-1 (RNAi)* worms. Scale bar, 3 μm. (D-F) Transmission electron micrographs of adult control (D), *pod-2 (RNAi)* (E) and *fasn-1 (RNAi)* (F) worms. The alaes are not distinctly divided into ternate ridges in *pod-2 (RNAi)* or *fasn-1 (RNAi)* worms (arrowheads). Scale bar, 2 μm. (G, H) *ajm-1::gfp* expression in adult control (G), *pod-2 (RNAi)* (H), and *fasn-1 (RNAi)* (I) worms. Incomplete fusion at the junction of seam cells is evident in part of H and I (arrowheads). Scale bar, 50 μm.

(RNAi) groups showed severe distortion of the alae (Fig. 3B and C) compared to the control group (Fig. 3A). A transmission electron microscopy (TEM) analysis showed that the alae were not distinctly divided and were covered with an undefined substance (Fig. 3E and F). The adult alae are parts of the cuticle structure secreted by seam cells, which are lateral hypodermal cells (14, 19). The seam cells go through several divisions during each larval stage. When larvae enter the adult stage, the seam cells fuse to form a hypodermal syncytium. We attempted to detect any change in the function of seam cells using GFP constructs of the seam cell marker SCM (*scm::gfp*), which is expressed in the nucleus, and the hypodermal cell integrity marker AJM-1 (*ajm-1::gfp*), which is expressed in apical junctions between epithelial cells. Observations of seam cells from RNAi worms using the SCM::GFP marker revealed no apparent defects in seam cell nuclei (data not shown). In contrast, seam cells detected using the AJM-1::GFP marker exhibited an abnormal phenotype in which the seam cells did not retain their linear aspect in small-sized adult *pod-2 (RNAi)* and *fasn-1 (RNAi)* worms (Fig. 3H and I). Furthermore, an examination of the expression of *ajm-1::gfp* in adult *let-767 (RNAi)* and *fat-7 (RNAi)* worms revealed no evident defects (Supplementary Fig. S2E and F). Collectively, these results indicate that POD-2 and FASN-1 play an important role in the function of seam cells in *C. elegans*.

To evaluate the effects of downregulating these fatty acid genes on cuticle structure, we used *col-19::gfp* to examine the expression of COL-19 (adult cuticle collagen marker) in the RNAi worms (Fig. 4A-D). The expression of COL-19::GFP was clearly detected in the form of an annular furrow in control worms, appearing as regularly spaced circumferential rings (Fig. 4A). However, COL-19::GFP expression decreased significantly in *pod-2 (RNAi)* and *fasn-1 (RNAi)* worms (Fig. 4B-D). These results indicate that POD-2 and FASN-1 contribute to cuticle structure formation. Moreover, adult *let-767 (RNAi)* and *fat-7 (RNAi)* worms also showed significantly decreased expression of COL-19::GFP (Supplementary Fig. S3B and C), confirming that fat metabolism plays a crucial role in cuticle formation in *C. elegans*.

The integrity of the hypodermal membrane and cuticle was also examined using Hoechst 33258, which is a membrane-

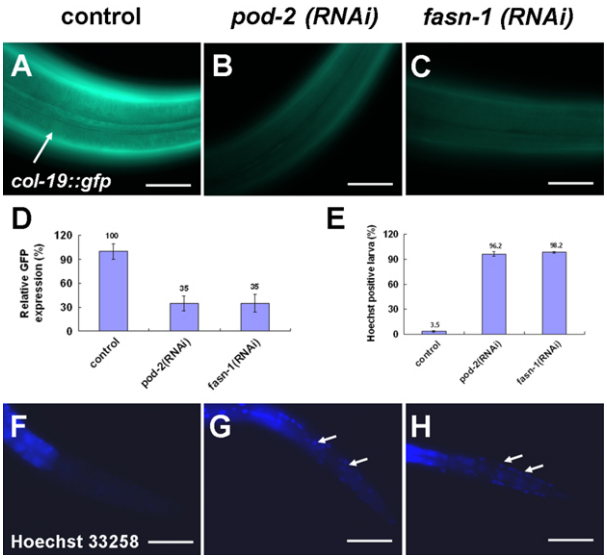

Fig. 4. *pod-2 (RNAi)* and *fasn-1 (RNAi)* worms exhibit disruption of cuticle collagen and cuticle integrity. (A-C) Cuticle collagen COL-19 expression in RNAi worms. Regular annular furrows are expressed strongly in control worms (A, arrowhead); annular furrows are blurry and COL-19 expression level decreased in *pod-2 (RNAi)* (B) and *fasn-1 (RNAi)* (C) worms. Scale bar, 50 μm. (D) The relative level of *col-19::gfp* expression in RNAi worms (n = 20). (E-H) Permeability of the cuticle was examined following an incubation of live worms with the fluorescent nuclear dye Hoechst 33258. The graph (E) shows the ratio of Hoechst-positive nuclei (n > 100) in RNAi worms. Nuclear fluorescence was not observed within living control worms (F) but was frequently seen in *pod-2 (RNAi)* (G) and *fasn-1 (RNAi)* (H) worms (arrowheads). Scale bar, 50 μm.

and cuticle-impermeable dye that stains nuclei upon disruption of the membrane. Hoechst 33258 failed to stain the nuclei of living cells in control worms (Fig. 4F), whereas *pod-2 (RNAi)* and *fasn-1 (RNAi)* worms showed nuclear staining under the same conditions (Fig. 4E, G, and H). However, *let-767 (RNAi)* and *fat-7 (RNAi)* adults showed no serious disruption of cuticle integrity (Supplementary Fig. S3E-H). From these results, we conclude that a reduction in either POD-2 or FASN-1 results in disruption of the hypodermal membrane and diminished cuticle integrity.

DISCUSSION

This study defined a new functional role for *pod-2* and *fasn-1* genes in *C. elegans* development and molting, which was accompanied by significant changes in the integrity of cuticles and the hypodermal membrane. We showed that silencing either *pod-2* or *fasn-1* using RNAi caused molting defects, decreased TG content, and reduced adult body size and egg numbers (Supplementary Fig. S1). *pod-2* is expressed in all types of hypodermis and the intestine (Supplementary Fig. S1), whereas *fasn-1* is expressed only in the main body syncytium (hyp-7) and in head and tail hypodermal cells but not in seam cells (Supplementary Fig. S1). Given that both *pod-2 (RNAi)* and *fasn-1 (RNAi)* worms showed essentially the same phenotypes with respect to the expression of AJM-1::GFP, a hypodermal integrity marker, it is unclear how the difference in tissue specificity of these genes, namely the expression of *pod-2* but not *fasn-1* in seam cells, can be accounted for in the molting process.

Our results on *let-767 (RNAi)* worms, which showed no molting defects, are somewhat different than an earlier report (19), which showed that a *let-767* mutation resulted in hypersensitivity to cholesterol limitation. This difference may be due in part to the fact that RNAi treatment is not strictly equivalent to the mutation tested, a possibility that will require further investigation.

The involvement of both POD-2 and FASN-1 in the molting process appears to be coupled to protease function, which is essential for proteolytic digestion of the old cuticle during the third molting step (ecdysis) (4, 20). These proteins act by influencing both shedding of the old cuticle and maintenance of cuticle structure.

The disruption in cuticle integrity in *pod-2 (RNAi)* and *fasn-1 (RNAi)* worms may be explained, in part, by the fact that these RNAi-treated worms exhibit osmotic sensitivity, which is related to long chain fatty acid biosynthesis (17, 21). The reasoning behind this notion lies in the observation that the plasma membrane of *C. elegans* hypodermal cells is composed of basolateral and apical surfaces (22). Because of this arrangement, the cuticle is secreted above the apical membrane of the hypodermis. If the balance between plasma membrane sphingolipid/cholesterol levels is changed, membrane fluidity would also be changed and consequently affect endocytosis and exocytosis (23, 24). Our results showed that LET-767 did not affect cuticle integrity, whereas FAT-7, which is involved in the biosynthesis of monounsaturated fatty acid (MUFA), influenced cuticle integrity (Supplementary Fig. S3). Furthermore, Kage-Nakadai (25) reported that very long chain fatty acyl-CoA synthetase plays an important role in the cuticle surface barrier. As both POD-2 and FASN-1 contribute to the biosynthesis of MUFA and polyunsaturated fatty acids in *C. elegans* (26), it would be reasonable to predict that RNAi against *pod-2* and *fasn-1* would affect cuticle integrity.

MATERIALS AND METHODS

Strains and general handling

Bristol N2, a standard wild-type *C. elegans* strain, and the *kaIs12 (col-19::gfp)*, *wIs51 (scm::gfp)*, and *jcIs1 (ajm-1::gfp)* strains were obtained from the Caenorhabditis Genetics Center (Minneapolis, MN, USA). The transgenes BC13916 [*W09B6.1::gfp (pod-2::gfp);* (27) and EG3198 (*nas-37p::gfp- pest*) (4)] were also used. All strains were cultured according to standard labo-

ratory procedures.

RNAi analysis

RNAi feeding was outperformed as described previously (28). The feeding constructs consisted of 439-bp *pod-2* (W09B6.1), 524-bp *fasn-1* (F32H2.5), 516-bp *nhr-23* (C01H6.5a), 524-bp *let-767* (C56G2.6.1), and 387-bp *fat-7* (F10D2.9) cDNA fragments inserted into the pPD129.36 vector (L4440), containing two T7 RNA polymerase sites. The construct was transformed into HT115 *E. coli*. The fragment sequence was confirmed by DNA sequencing. Expression in HT115 *E. coli* was induced by isopropyl-β-D-thiogalactopyranoside.

Electron microscopic analyses

After applying a thermal stress for 12 hours, each sample was prepared for TEM by fixation with 2% glutaraldehyde-paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4) for 2 hours, followed by washing three times for 30 minutes in 0.1 M PBS. Samples were post-fixed for 2 hours with 1% OsO_4, dissolved in 0.1 M PBS, dehydrated in an ascending graded series (50-100%) of ethanol, and infiltrated with propylene oxide. Specimens were embedded using a Poly/Bed 812 kit (Polysciences, Warrington, PA, USA). The samples were placed in an electron microscope oven (TD-700, Dosaka, Kyoto, Japan) for 24 hours after embedding in pure fresh resin and polymerizing at 60°C. A 350-nm-thick section was initially cut and stained with toluidine blue for light microscopic observation. A thin (70-nm) section was double-stained for 20 minutes with 7% uranyl acetate and lead citrate for contrast staining. These sections were cut using a LEICA Ultracut UCT ultra-microtome (Leica Microsystems, Vienna, Austria). All thin sections were observed by TEM (JEM-1011, JEOL, Tokyo, Japan) at an acceleration voltage of 80 kV. Samples were prepared and post-fixed for SEM as described for TEM, after which they were treated with isoamyl acetate and dried using a critical point dryer (HCP-2, Hitachi, Tokyo, Japan). Samples were then coated with gold by ion sputter (IB-3 Eiko, Tokyo, Japan) at 6 mA for 6 minutes and photographed with an S-800 FE SEM instrument (Hitachi, Tokyo, Japan) at an acceleration voltage of 20 kV.

Hoechst dye staining

RNAi-treated worms were washed from plates with M9 buffer and then incubated in M9 buffer containing 1.0 μg/ml Hoechst 33258 (Sigma-Aldrich, St. Louis, MO, USA) at room temperature for 15 minutes with gentle agitation. After washing three times with M9 buffer, worms were inspected by fluorescence microscopy (29).

Acknowledgements

This work was supported by a grant from the Korean Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (A030003 to YKP) and WCU (World Class University) program through the National Research Foundation of Korea funded by the Ministry of Education, Science, and Technology (R31-2008-000-10086-0). We thank the Caenorhabditis Genetics Center for providing the *pod-2(ye60)* mutants. We are also grateful to the following scientists for their gifts of various clones: Dr. D. Baillie (Simon Fraser University, Burnaby, Canada) for BC13916 *(W09B6.1::gfp)*, Dr. E. Jorgensen (University of Utah, Salt Lake City, UT) for EG3198 *(nas-37p::gfp-pest)*, Dr. E. Isaac (University of Leeds, Leeds, UK) for *acn-1::gfp* transgenic strain, Dr. M. Kostrouchova (Institute of Inherited Metabolic Disorders, Prague, Czech Republic) for strain 4666 *(nhr-23::gfp)*, Dr. Iain Johnstone (Glasgow) for DPY-7 antibody, Nemapharm Pharmaceuticals for the *nhr-49(nr2041)* strain, and Dr. E. Mekada (Osaka University, Japan) for strain OB1 *(tsp-15::gfp)*.

REFERENCES

1. Page, A. P. and Johnstone, I. L. (2007) The cuticle. *WormBook* **19**, 1-15.
2. Brooks, D. R., Appleford, P. J., Murray, L. and Isaac, R. E. (2003) An essential role in molting and morphogenesis of *Caenorhabditis elegans* for ACN-1, a novel member of the angiotensin-converting enzyme family that lacks a metallopeptidase active site. *J. Biol. Chem.* **278**, 52340-52346.
3. Hashmi, S., Zhang, J., Oksov, Y. and Lustigman, S. (2004) The *Caenorhabditis elegans* cathepsin Z-like cysteine protease, Ce-CPZ-1, has a multifunctional role during the worms' development. *J. Biol. Chem.* **279**, 6035-6045.
4. Davis, M. W., Birnie, A. J., Chan, A. C., Page, A. P. and Jorgensen, E. M. (2004) A conserved metalloprotease mediates ecdysis in *Caenorhabditis elegans*. *Development* **131**, 6001-6008.
5. Yochem, J., Tuck, S., Greenwald, I. and Han, M. (1999) A gp330/megalin-related protein is required in the major epidermis of *Caenorhabditis elegans* for completion of molting. *Development* **126**, 597-606.
6. Kostrouchova, M., Krause, M., Kostrouch, Z. and Rall, J. E. (1998) CHR3: a *Caenorhabditis elegans* orphan nuclear hormone receptor required for proper epidermal development and molting. *Development* **125**, 1617-1626.
7. Kostrouchova, M., Krause, M., Kostrouch, Z. and Rall, J. E. (2001) Nuclear hormone receptor CHR3 is a critical regulator of all four larval molts of the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **98**, 7360-7365.
8. Magner, D. B. and Antebi, A. (2008) *Caenorhabditis elegans* nuclear receptors: insights into life traits. *Trends. Endocrinol. Metab.* **19**, 153-160.
9. Roberts, B., Clucas, C. and Johnstone, I. L. (2003) Loss of SEC-23 in *Caenorhabditis elegans* causes defects in oogenesis, morphogenesis, and extracellular matrix secretion. *Mol. Biol. Cell* **14**, 4414-4426.
10. Winter, A. D., McCormack, G. and Page, A. P. (2007) Protein disulfide isomerase activity is essential for viability and extracellular matrix formation in the nematode *Caenorhabditis elegans*. *Dev. Biol.* **15**, 449-461.
11. Roudier, N., Lefebvre, C. and Legouis, R. (2005) CeVPS-27 is an endosomal protein required for the molting and the endocytic trafficking of the low-density lipoprotein receptor-related protein 1 in *Caenorhabditis elegans*. *Traffic*

6, 695-705.
12. Frand, A. R., Russel, S. and Ruvkun, G. (2005) Functional genomic analysis of *C. elegans* molting. *PLoS Biol.* **3**, e312.
13. Thein, M. C., Winter, A. D., Stepek, G., McCormack, G., Stapleton, G., Johnstone, I. L. and Page, A. P. (2009) The combined extracellular matrix cross-linking activity of the peroxidase MLT-7 and the duox BLI-3 are critical for post-embryonic viability in *Caenorhabditis elegans*. *J. Biol. Chem.* **284**, 17549-17563.
14. Fritz, J. A. and Behm, C. A. (2009) CUTI-1: A novel tetraspan protein involved in *C. elegans* cuticle formation and epithelial integrity. *PLoS One* **4**, e5117.
15. Johnstone, I. L. (2000) Cuticle collagen genes expression in *Caenorhabditis elegans*. *Trends. Genet.* **16**, 21-27.
16. Blaxter, M. L. (1993) Cuticle surface proteins of wild type and mutant *Caenorhabditis\elegans*. *J. Biol. Chem.* **268**, 6600-6609.
17. 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.
18. Entchev, E. V., Schwudke, D., Zagoriy, V., Matyash, V., Bogdanova, A., Habermann, B., Zhu, L., Shevchenko, A. and Kurzchalia, T. V. (2008) LET-767 is required for the production of branched chain and long chain fatty acids in *Caenorhabditis elegans*. *J. Biol. Chem.* **283**, 17550-17560.
19. Kuervers, L. M., Jones, C. L., O'Neil, N. J. and Baillie, D. L. (2003) The sterol modifying enzyme LET-767 is essential for growth, reproduction and development in Caenorhabditis elegans. *Mol. Genet. Genomics* **270**, 121-131.
20. Craig, H., Isaac, R. E. and Brooks, D. R. (2007) Unravelling the moulting degradome: new opportunities for chemotherapy? *Trends Parasitol.* **23**, 248-253.
21. 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.
22. Wood, W. B. (1988) *The Nematode Caenorhabditis elegans*. pp. 82-89, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, U.S.A.
23. Simons, K. and Ikonen, E. (1997) Functional rafts in cell membranes. *Nature* **387**, 569-572.
24. Kurzchalia, T. V. and Ward, S. (2003) Why do worms need cholesterol? *Nat. Cell Biol.* **5**, 684-688.
25. Kage-Nakadai, E., Kobuna, H., Kimura, M., Gengyo-Ando, K., Inoue, T., Arai, H. and Mitani, S. (2010) Two very long chain fatty acid acyl-CoA synthetase genes, acs-20 and acs-22, have roles in the cuticle surface barrier in *Caenorhabditis elegans*. *PLoS. One* **5**, e8857.
26. Watts, J. L. (2009) Fat synthesis and adiposity regulation in *Caenorhabditis elegans*. *Trends Endocrinol. Metab.* **20**, 58-65.
27. McKay, S. J., Johnsen, R., Khattra, J., Asano, J., Baillie, D. L., Chan, S., Dube, N., Fang, L., Goszczynski, B., Ha, E., Halfnight, E., Hollebakken, R., Huang, P., Hung, K., Jensen, V., Jones, S. J., Kai, H., Li, D., Mah, A., Marra, M., McGhee, J., Newbury, R., Pouzyrev, A., Riddle, D. L., Sonnhammer, E., Tian, H., Tu, D., Tyson, J. R., Vatcher, G., Warner, A., Wong, K., Zhao, Z. and Moerman, D. G. (2003) Gene expression profiling of cells, tissues, and developmental stages of the nematode *C. elegans*. *Cold Spring Harb. Symp. Quant. Biol.* **68**, 159-169.
28. Timmons, L. and Fire, A. (1998) Specific Interference by ingested dsRNA. *Nature* **395**, 854.
29. Moribe, H., Yochem, J., Yamada, H., Tabuse, Y., Fujimoto, T. and Mekada, E. (2004) Tetraspanin protein (TSP-15) is required for epidermal integrity in *Caenorhabditis elegans*. *J. Cell Sci.* **117**, 5209-5220.