TBC-2 Is Required for Embryonic Yolk Protein Storage and Larval Survival during L1 Diapause in Caenorhabditis elegans

Laëtitia Chotard, Olga Skorobogata, Marc-André Sylvain, Sanhita Shrivastava, Christian E. Rocheleau*

Division of Endocrinology and Metabolism, Department of Medicine, Royal Victoria Hospital, McGill University Health Centre Research Institute, McGill University, Montreal, Quebec, Canada

Abstract

C. elegans first stage (L1) larvae hatched in the absence of food, arrest development and enter an L1 diapause, whereby they can survive starvation for several weeks. The physiological and metabolic requirements for survival during L1 diapause are poorly understood. However, yolk, a cholesterol binding/transport protein, has been suggested to serve as an energy source. Here, we demonstrate that C. elegans TBC-2, a RAB-5 GTPase Activating Protein (GAP) involved in early-to-late endosome transition, is important for yolk protein storage during embryogenesis and for L1 survival during starvation. We found during embryogenesis, that a yolk green fluorescent protein fusion (YP170::GFP), disappeared much more quickly in tbc-2 mutant embryos as compared with wild-type control embryos. The premature disappearance of YP170::GFP in tbc-2 mutants is likely due to premature degradation in the lysosomes as we found that YP170::GFP showed increased colocalization with Lysotracker Red, a marker for acidic compartments. Furthermore, YP170::GFP disappearance in tbc-2 mutants required RAB-7, a regulator of endosome to lysosome trafficking. Although tbc-2 is not essential in fed animals, we discovered that tbc-2 mutant L1 larvae have strongly reduced survival when hatched in the absence of food. We show that tbc-2 mutant larvae are not defective in maintaining L1 diapause and that mutants defective in yolk uptake, rme-1 and rme-6, also had strongly reduced L1 survival when hatched in the absence of food. Our findings demonstrate that TBC-2 is required for yolk protein storage during embryonic development and provide strong correlative data indicating that yolk constitutes an important energy source for larval survival during L1 diapause.

Introduction

In many oviparous species, yolk proteins or vitellogenins, and their associated lipids, are an important food source for developing embryos [1]. Caenorhabditis elegans vitellogenins have homology with human ApoB-100, the primary component of low-density lipoprotein (LDL) [2-3], and like ApoB-100, they mediate cholesterol transport [4]. C. elegans YP170, YP115 and YP88 yolk proteins are synthesized in hermaphrodite intestine and secreted into the body cavity [5]. Yolk protein is then internalized into maturing oocytes via receptor-mediated endocytosis of the RME-2/LDL receptor where it resides in puncta or vesicles [6]. It has been shown in other organisms that internalized yolk is stored in yolk granules or yolk platelets, lysosomal-like compartments with a neutral or mildly acidic pH, and that regulated acidification can activate latent proteases such as cathepsin L to control yolk degradation [1]. During C. elegans embryogenesis, yolk granules are present in the blastomeres of the dividing embryo, and during morphogenesis yolk is transferred into the intestinal cells [7]. Yolk protein trafficking can be followed using a yolk protein::green fluorescent protein fusion (YP170::GFP) which has been used to identify genes required for yolk uptake and trafficking such as rme-2 [6]. Mutations in rme-2, the yolk receptor/LDLR homolog, block YP170::GFP internalization, display slow growth and partial embryonic lethal phenotypes. Mutations in other rme genes identify endocytic regulators such as rme-1, a conserved EH-domain protein required for endosome recycling, and rme-6, a RAB-5 Guanine nucleotide Exchange Factor (GEF) [8;9]. Despite having strongly reduced YP170::GFP uptake, rme-1(b1045) and rme-6(b1014) embryos are viable. The absolute requirements for yolk proteins during C. elegans development remains unknown, due in part to the fact that there are six genes coding for vitellogenins.

When L1 larvae hatch in absence of food, they enter an “L1 diapause” or developmental arrest [10]. These arrested animals can survive for a few weeks under starvation conditions without changing morphology and can resume larval development when reintroduced to food without affecting lifespan. Since yolk has been detected in the intestine of hatching larvae, it has been

Copyright: © 2010 Chotard et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was supported by a Canadian Institutes for Health Research (CIHR) operating grant (MOP-86719) to C.E.R. C.E.R. was supported by a Canada Graduate Scholarship and a FRSQ Master’s training award. The Research Institute at the McGill University Health Centre is supported in part by the FRSQ. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: christian.rocheleau@mcgill.ca

Citation: Chotard L, Skorobogata O, Sylvain M-A, Shrivastava S, Rocheleau CE (2010) TBC-2 Is Required for Embryonic Yolk Protein Storage and Larval Survival during L1 Diapause in Caenorhabditis elegans. PLoS ONE 5(12): e15662. doi:10.1371/journal.pone.0015662

Received August 31, 2010; Accepted November 18, 2010; Published December 28, 2010
suggested that yolk, in addition to serving as an energy source during embryogenesis, could serve as an energy source for larvae that hatch in the absence of food [7].

TBC-2 is a RAB-5 GAP that regulates endosome and phagosome maturation [11;12]. In tbc-2 mutant oocytes, YP170::GFP accumulates in enlarged granules/vesicles [11]. Here, we follow the fate of YP170::GFP during embryonic development in tbc-2(tm2241) mutants. We found that YP170::GFP colocalized with Lysotracker Red, a marker for lysosomes, and was rapidly degraded. The localization and degradation of YP170::GFP in tbc-2(tm2241) embryos was RAB-7 dependent, consistent with TBC-2 antagonizing Rab GTPase activity. Despite the rapid depletion of yolk protein, tbc-2(tm2241) embryos are viable. However, we found that tbc-2(tm2241) L1 larvae had reduced survival under starvation conditions. This reduced survival is not due to a defect in entering or maintaining L1 diapause, but may be due to reduced yolk stores, as other mutants with reduced YP170::GFP uptake, rme-1 and rme-6, were similarly compromised for L1 survival during starvation conditions.

**Results**

**Yolk protein YP170 is prematurely lost in tbc-2(tm2241) embryos**

YP170::GFP is internalized into maturing oocytes in both wild-type and tbc-2(-) animals, however YP170::GFP granules are larger in tbc-2(-) oocytes and early embryos (Fig. 1A-1D; [11]). We quantified the levels of YP170::GFP fluorescence in oocytes of wild-type and tbc-2(tm2241) mutants and found no significant difference (Fig. 1X), consistent with tbc-2(tm2241) animals not having significant defects in yolk synthesis, secretion, or uptake. To determine if tbc-2(-) embryos are defective in the trafficking or storage of yolk, we followed the localization of YP170::GFP during later stages of embryonic development and found that the YP170::GFP fluorescence was strongly reduced in tbc-2(tm2241) embryos as compared to similarly staged wild-type embryos (Fig. 1E-1P). We start to see a decrease in YP170::GFP fluorescence as early as the 4-cell stage of embryogenesis (Fig. 1E-1H), and by the blast stage, prior to elongation, YP170::GFP fluorescence is reduced by 50% in tbc-2(tm2241) embryos as compared to wild-type (Fig. 1I-1L, 1Y). By the 1.5 fold stage, very little fluorescence is evident in tbc-2(tm2241) embryos (Fig. 1M-1P). We see the same loss of YP170::GFP by immunostaining with an anti-GFP antibody (Fig. 1Q-1T), suggesting that YP170::GFP is prematurely degraded in tbc-2(tm2241) embryos.

YP170 is targeted to lysosomes in a RAB-7-dependent manner in tbc-2(-) embryos

Consistent with yolk being stored in distinct lysosomal-related compartments, YP170::GFP does not significantly colocalize with Lysotracker Red, a marker for acidic lysosomes in wild-type embryos (Fig. 2A-2C). TBC-2 regulates endosomal trafficking, therefore we hypothesized that yolk granules are prematurely acidified or yolk may be mistargeted to lysosomes in tbc-2(-) embryos. Consistent with this hypothesis, there was a higher incidence of colocalization between YP170::GFP and Lysotracker Red in tbc-2(tm2241) embryos (Fig. 2D-2F, 2J), suggesting that YP170::GFP is being prematurely degraded in the lysosomes of tbc-2(tm2241) embryos.

Rab7 mediates endosome to lysosome trafficking [13-16], therefore, we tested whether RAB-7 was required for YP170::GFP degradation in tbc-2(-) embryos. We found that YP170::GFP persisted in embryos of the-2(tm2241) rab-7(ok511) double mutants (Fig. 1U and 1V), and almost no colocalization of YP170::GFP with Lysotracker Red was seen in the-2(tm2241) rab-7(ok511) double mutants (Fig. 2G-2J), indicating that RAB-7 is required for the lysosomal trafficking and degradation of YP170::GFP in tbc-2(tm2241) embryos.

**tbc-2(tm2241) animals display reduced survival during starvation-induced L1 diapause**

Under normal laboratory culture conditions, tbc-2(-) animals do not show overt defects in development or fertility, suggesting that the reduced yolk protein in tbc-2(-) animals does not have any deleterious effects when there is an abundance of food. Animals that hatch in the absence of food enter into an L1 diapause or developmental arrest and can survive for several weeks. Yolk stored in the gut of hatching L1s has been proposed to serve as an energy source during L1 diapause as it has been observed that yolk disappears in L1 larvae after starvation [7]. Therefore we tested whether tbc-2(tm2241) larvae display reduced survival when hatched in the absence of food. We found that tbc-2(tm2241) larvae hatched in the absence of food have a significantly reduced survival with a mean survival of 13 days, as compared to wild-type controls with mean survival of 19 days (P<0.0001) (Fig. 3). Thus, the survival of the-2(tm2241) larvae hatched in the absence of food is compromised.

**Starved tbc-2(tm2241) larvae are not defective in maintaining L1 diapause**

A possible explanation for the reduced survival of tbc-2(tm2241) animals during starvation could be a failure to initiate or maintain L1 diapause in the absence of food. To assess whether tbc-2(tm2241) larvae were defective in developmental arrest, we followed the lineage of the M blast cell in day 1 and day 10 starved L1 larvae. The M blast cell gives rise to mesodermal tissues and normally divides several hours into the L1 stage in fed larvae [17] and can be easily followed with an hh::GFP marker [18;19]. We found that the M blast cell never divided in starved wild-type (n=115 day 1, n=175 day 10) or tbc-2(tm2241) (n=116 day 1, n=172 day 10) larvae at either time point (Fig. 4A-4D).

The expression of the cell cycle inhibitor, cki-1, is upregulated in the seam cells of starved L1 larvae and low in fed larvae as assessed with the transcriptional reporter, cki-1::GFP [20;21]. Similarly, we found that cki-1::GFP expression was low in fed wild-type and tbc-2(tm2241) larvae and upregulated in the seam cells of day 10 starved wild-type and tbc-2(tm2241) larvae. (Fig. 4E-4H). Together, these data indicated that the-2(tm2241) larvae arrest development and maintain L1 diapause during starvation.

**rme-1 and rme-6 mutants have reduced survival during starvation-induced L1 diapause**

If the reduced L1 survival of starved tbc-2(tm2241) larvae is due to having reduced levels of yolk, then we would predict that other viable mutants with reduced yolk should also have reduced L1 survival during starvation. Therefore we measured the survival of rme-1(b1045) and rme-6(b1014) mutants, which like tbc-2(tm2241) are viable and have strongly reduced embryonic YP170::GFP expression. We found that rme-1(b1045) and rme-6(b1014) L1 larvae hatched in the absence of food also have significantly reduced mean survival of 13 days (P<0.0001) and 10 days (P<0.0001) respectively, as compared to wild-type (19 days), and similar to that of tbc-2(tm2241) (13 days). These findings are consistent with yolk having an important role in L1 survival during starvation conditions.
Discussion

C. elegans yolk protein trafficking in oocytes requires the activities of RAB-5 and RME-6 (the RAB-5 GEF, an activator of RAB-5) for endocytosis/internalization and RAB-7 for movement away from the cell periphery [6,9]. We have recently demonstrated a role for TBC-2 as a RAB-5 GAP and a negative regulator of Rab GTPase-mediated endosome trafficking [11]. In addition to having enlarged yolk vesicles in oocytes, tbc-2(-) animals have enlarged endosomes in coelomocytes and intestinal cells. The large late endosome phenotype in the intestine requires the activities of both RAB-5 and RAB-7, consistent with the phenotypes being due to increased Rab GTPase activity. As with the intestinal phenotype, the premature disappearance of yolk protein in tbc-2(tm2241) embryos is RAB-7-dependent. While it is not known how internalized yolk comes to be in specialized yolk granules/platelets, our data indicate that TBC-2 is required either for the sorting of yolk into yolk granules or for the stability/maintenance of yolk granules. In the later case, TBC-2 could be required for the sorting of a critical component of yolk granules, preventing premature acidification or fusion with lysosomes. Since Rab7 mediates fusion of late endosomes with lysosomes in mammalian cells [22,16] and the yeast Rab7, Ypt7p mediates homotypic fusion of vacuoles [23], we would predict that too much RAB-7 activity drives fusion between yolk granules and lysosomes in tbc-2(tm2241) mutants, but further analysis will be required to determine the molecular mechanisms involved.

While yolk serves as a food source for the embryo in egg laying species, the full requirements for yolk in C. elegans is not known.
Since mutations in the yolk receptor, rme-2, are partially lethal, it would suggest that yolk is important for embryonic development, but it is also possible that RME-2 has additional requirements during embryogenesis [6]. Other genes that regulate yolk trafficking, such as rab-5, rab-7, and cup-5 (a late endocytic regulator), are embryonic lethal, however this lethality is probably not specific to defects in yolk trafficking [24;6]. cpl-1 (a cathepsin L cysteine protease) mutants are embryonic lethal and defective in yolk processing, but lethality may be due to yolk platelet aggregation in the cytoplasm rather than an energetic requirement for yolk [25]. Meanwhile, mutations in rme-1 and rme-6 have strongly reduced yolk uptake, yet are viable [8;9]. Bossinger and Schierenberg (1996) hypothesize that yolk present in the intestine of hatching larvae might be important for animal survival. Our

Figure 2. YP170::GFP localizes to lysosomes in tbc-2(tm2241) embryos. Confocal images of wild-type (A–C), tbc-2(tm2241) (D–F), and tbc-2(tm2241) rab-7(ok511) (G–I) embryos carrying YP170::GFP (green; A, D, and G) and stained with Lysotracker Red (B, E, and H) with the colocalization shown in the Merge images (C, F, and I). Arrows mark colocalization between YP170::GFP vesicles and Lysotracker Red. Quantification of the percentage of YP170::GFP fluorescence overlapping with Lysotracker Red compared to the total YP170::GFP fluorescence (J). n = 25, wild-type; 17, tbc-2(tm2241); 10, tbc-2(tm2241) rab-7(ok511). Statistical significance was determined using a two-tailed unpaired Student t-test. n.s., not significant. *** p < 0.0001. Error bars represent standard deviations. Bar, 5 μm.

doi:10.1371/journal.pone.0015662.g002
data showing that tbc-2(-), rme-1(-) and rme-6(-) larvae have reduced survival during starvation-induced L1 diapause support this hypothesis, but do not preclude a role for yolk during embryogenesis or that these genes could regulate survival by other means. Assuming that the reduced survival is due to depletion of energetic resources, these endocytic regulators could affect other processes required for survival such as autophagy or insulin/IGF signaling. Our results show that tbc-2, rme-1, and rme-6, while not essential under standard laboratory conditions, are likely important for survival in the wild.

Materials and Methods

C. elegans alleles and general methods

General methods for the handling and culturing C. elegans were as previously described [26]. C. elegans var Bristol strain N2 is the wild-type parent for all strains used in this work. E. coli strain HB101 was used as a food source. Specific genes and alleles are described on Wormbase (www.wormbase.org) and are available from the Caenorhabditis Genetics Center. LGII: tbc-2(tm2241), rab-7(ak511); LGV: rme-1(b1045); LGX: rme-6(b1014), bli1[vat-2::GFP + rol-6]; Linkage unknown: maIs113[cki-1::GFP + dpy-20(+)]; ayIs7[hlh-8::GFP + dpy-20(+)].

Microscopy and Phenotype Analysis

General methods for Nomarski differential interference contrast (DIC) microscopy of live animals were as previously described [17]. Animals were analyzed on an Axio Zeiss A1 Imager compound microscope (Zeiss, Oberkochen, Germany) and images were captured using an AxioCam MRm camera and AxioVision software (Zeiss, Oberkochen, Germany). Comparison of YP170::GFP expression in wild-type and tbc-2(tm2241) embryos was performed using identical exposure times and image modifications for each embryonic stage. Oocyte images were taken with reduced exposure.

Confocal analysis was performed using a Zeiss LSM-510 Meta laser scanning microscope with 63X oil immersion lens in a multi-track mode using a single or dual excitation (488 nm for GFP and/or 543 nm for mCherry). Images were captured using LSM Image software (Zeiss, Oberkochen, Germany). Immunostaining of YP170::GFP in C. elegans embryos was carried out using the freeze-crack method and methanol/acetoacet fixation [27]. A Goat anti-GFP antibody (Rockland Inc., Gilbertsville, PA) was used at a 1:100 dilution and a secondary rabbit anti-goat antibody conjugated to AlexaFluor 488 (Invitrogen, Carlsbad, CA) was used at a 1:200 dilution. Lysotracker Red staining was performed as previously described [24]. NGM plates were complemented with Lysotracker Red (Invitrogen, Carlsbad, CA) at a concentration of 50 nM, and then plates were seeded with HB101 bacteria containing 2 μM Lysotracker Red. Quantification of fluorescence and colocalization images was performed using Metamorph (Universal Imaging Corp.). Statistical analysis and graphing was done using Prism 5 (GraphPad Software, Inc., La Jolla, CA).

L1 Survival Assays

Starvation survival assays were performed as previously described [28]. Synchronized L1 larvae were incubated in 3 mL of sterilized M9 buffer and animals were kept at 20°C. In triplicate, 20 μL aliquots from each sample were placed on individual seeded plates every 3 days. The number of survivors was determined after 3 days at 20°C. Day 1 is considered to be the

Figure 3. tbc-2(tm2241), rme-1(b1045) and rme-6(b1014) L1 larvae have reduced survival during starvation. Survival curve of wild-type (blue), tbc-2(tm2241) (green), rme-1(b1045) (purple), and rme-6(b1014) (red) L1 larvae hatched in the absence of food. Graph represents the average of three independent experiments. doi:10.1371/journal.pone.0015662.g003

Figure 4. tbc-2 is not required for maintaining L1 starvation-induced diapause. (A–D) Merged DIC and epifluorescence images of hlh-8::GFP expression in the M cell of wild-type (A and C) and tbc-2(tm2241) (B and D) L1 larvae at day 1 (A and B) and day 10 (C and D) of starvation. (E–H) Epifluorescence images of cki-1::GFP expression in wild-type (E and G) and tbc-2(tm2241) (F and H) in fed L1 larvae (E and F) and L1 larvae at day 10 of starvation (G and H). Bar, 10 μm. doi:10.1371/journal.pone.0015662.g004
first day of starvation and was used as the 100% survival point to calculate the percentage of survivors for the proceeding time points. Results are derived from at least three independent experiments. The average percent survival for each time point of the three independent experiments was determined to derive the survival curve in Fig. 3. Statistical analysis and graphing was done using Prism 5 (GraphPad Software, Inc., La Jolla, CA). Log-rank (Mantel-Cox) test was used to compare the percentage of survivors and create survival curves for each strain.

Acknowledgments

We thank Tim Schell and Siegfried Hekimi for helpful discussions, Eugénie Goupil for assistance with statistical analysis, Stephane Laporte for use of the McGill University Health Centre Research Institute Confocal Microscopy Facility, and Claire M. Brown of the McGill University Life Sciences Complex Imaging Facility for assistance with image analysis. Most of the nematode strains used in this work were provided by the Caenorhabditis Genetics Center.

Author Contributions

Conceived and designed the experiments: LC. Performed the experiments: LC OS MAS SS. Analyzed the data: LC CER. Wrote the paper: LC CER.

References

1. Pagotto F (1995) Regulation of yolk degradation, or how to make sleepy lysosomes. J Cell Sci 108: 3645–3647.
2. Sperth J, Nettleton M, Zarker-Aprenon E, Lea K, Blumenthal T (1991) Vitellogenin mRNAs conserved in nematodes and vertebrates. J Mol Evol 32: 429–438.
3. Baker ME (1988) Is vitellogenin an ancestor of apolipoprotein B-100 of human low-density lipoprotein and human lipoprotein lipase? Biochem J 255: 1057–1060.
4. Matyas V, Geier C, Henrke A, Mukherjee S, Hirsh D, et al. (2001) Distribution and transport of cholesterol in Caenorhabditis elegans. Mol Biol Cell 12: 1725–1736.
5. Kimble J, Sharrock WJ (1983) Tissue-specific synthesis of yolk proteins in Caenorhabditis elegans. Dev Biol 96: 189–196.
6. Grant B, Hirsh D (1999) Receptor-mediated endocytosis in the Caenorhabditis elegans oocyte. Mol Biol Cell 10: 4311–4326.
7. Bossinger O, Schierenberg E (1996) The use of fluorescent marker dyes for studying intercellular communication in nematode embryos. Int J Dev Biol 40: 431–439.
8. Grant B, Zhang Y, Paupard MC, Lin SX, Hall DH, et al. (2001) Evidence that RME-1, a conserved C. elegans EH-domain protein, functions in endocytic recycling. Nat Cell Biol 5: 573–579.
9. Sato M, Sato K, Fonarev P, Huang CJ, Liu W, et al. (2005) Caenorhabditis elegans RME-6 is a novel regulator of RAB-5 at the clathrin-coated pit. Nat Cell Biol 7: 559–569.
10. Johnson TE, Mitchell DH, Kline S, Kemal R, Foy J (1984) Arresting development arrests aging in the nematode Caenorhabditis elegans. Mech Ageing Dev 28: 23–40.
11. Chotard L, Mishra AK, Sylvain MA, Tuck S, Lambrieft DG, et al. (2010) TBC-2 regulates RAB-5/RAB-7-mediated endosomal trafficking in Caenorhabditis elegans. Mol Biol Cell 21: 2285–2296.
12. Li W, Zou W, Zhao D, Han J, Zhu Z, et al. (2009) C. elegans Rab GTPase activating protein TBC-2 promotes cell corpse degradation by regulating the small GTPase RAB-5. Development 136: 2443–2453.
13. Vincelli R, Santillo M, Lattiero D, Chiariello M, Bininda E, et al. (1997) Role of the small GTPase Rab7 in the late endocytic pathway. J Biol Chem 272: 4391–4397.
14. Feng Y, Pres B, Waddington-Neas A (1995) Rab 7: an important regulator of late endocytic membrane traffic. J Cell Biol 131: 1435–1452.
15. Meresse S, Gorvel JP, Chavrier P (1995) The rab7 GTPase resides on a vesicular compartment connected to lysosomes. J Cell Sci 108: 3349–3355.
16. Bucci C, Thomsen P, Nicoziani P, McCarthy J, van Deurs B (2008) Rab7: a key to lysosome biogenesis. Mol Biol Cell 11: 467–480.
17. Suhiton JE, Horvitz HR (1977) Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. Dev Biol 56: 110–156.
18. Harfe BD, Vaz Gomes A, Kenyon C, Liu J, Krause M, et al. (1998) Analysis of a Caenorhabditis elegans Twist homolog identifies conserved and divergent aspects of mesodermal patterning. Genes Dev 12: 2623–2635.
19. Corsi AK, Kostas SA, Fire A, Krause M (2000) Caenorhabditis elegans twist plays an essential role in non-striated muscle development. Development 127: 2041–2051.
20. Horv T, Roy R, Ambros V (1998) Developmental regulation of a cyclin-dependent kinase inhibitor controls postembryonic cell cycle progression in Caenorhabditis elegans. Development 125: 3585–3597.
21. Baugh LR, Sternberg PW (2006) DAF-16/FOXO regulates transcription of dcl-1/Cip/Kip and repression of law-1 during Caenorhabditis elegans L1 arrest. Curr Biol 16: 780–785.
22. Papini E, Satin B, Bucci C, de Bernard M, Telford JL, et al. (1997) The small GTP binding protein rab7 is essential for cellular vacuolation induced by Helicobacter pylori cytotoxin. EMBO J 16: 15–24.
23. Haas A, Scheidegger D, Lazar T, Gallwitz D, Wicken W (1995) The GTPase Ypt7p of Saccharomyces cerevisiae is required on both partner vacuoles for the homotypic fusion step of vacuole inheritance. EMBO J 14: 5258–5270.
24. Schabes L, Dang H, Fares H (2006) Basis of lethality in Caenorhabditis elegans lacking CUP-5, the Mucoepithelial Type IV orthologue. Dev Biol 293: 382–391.
25. Britton C, Murray L (2002) A cathepsin L protease essential for Caenorhabditis elegans embryogenesis is functionally conserved in parasitic nematodes. Mol Biochem Parasitol 122: 21–33.
26. Brenner S (1974) The genetics of Caenorhabditis elegans. Genetics 77: 71–94.
27. Miller DM, Shakes DC (1995) Immunofluorescence microscopy. Methods Cell Biol 48: 365–394.
28. You YJ, Kim J, Gobb M, Avery L (2006) Starvation activates MAP kinase through the mucinase acrylylcholine pathway in Caenorhabditis elegans pharynx. Cell Metab 3: 237–245.