Molecules and Cells

Journal Club

"Cutting and Burning Guts" Nourish the Young

Caenorhabditis elegans lyse their guts to produce nutritious yolk milk to feed larvae.

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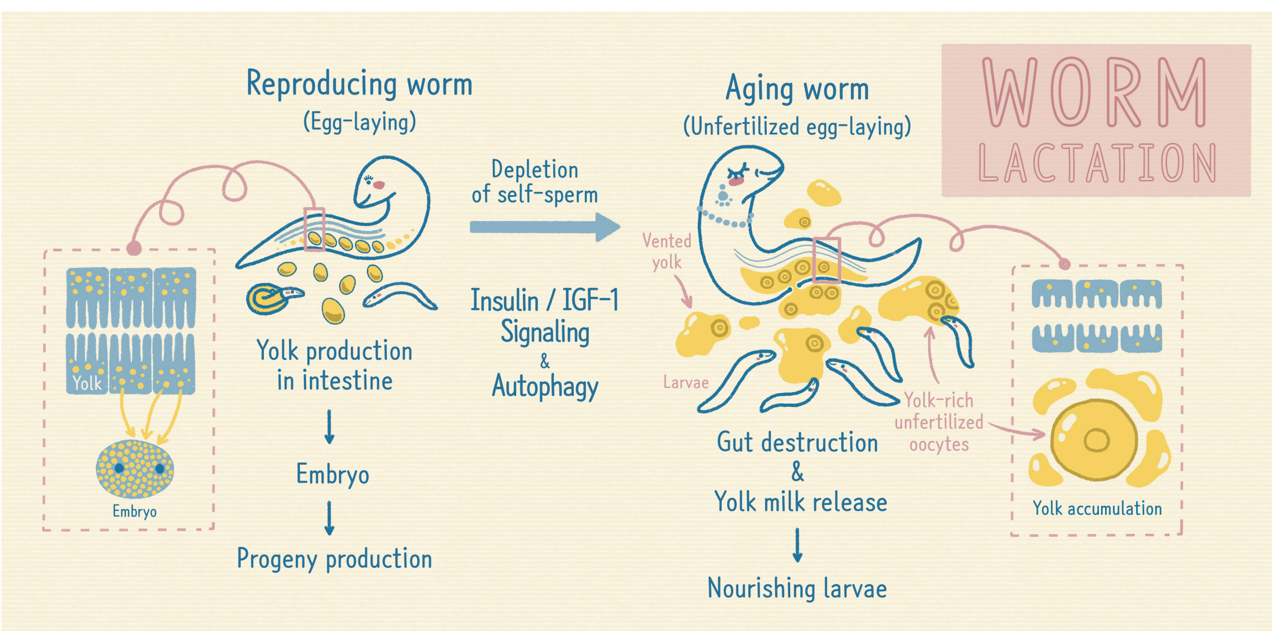

Worm mothers produce yolk milk to maximize the survival chances of larvae at the risk of death. Self-fertilizing *C. elegans* hermaphrodites synthesize yolk proteins in the intestine, which are transported to embryos. After self-sperm depletion and cessation of reproduction, worms repurpose intestines by autophagy to produce yolk, accumulating in the body cavity. These worms release free yolk through the vulva and continue laying unfertilized eggs filled with yolk. Larvae feed on the vented yolk fluid, yolk milk, both free and bottled-in-egg, to grow. Insulin/IGF-1 signaling promotes the entire process, a trade-off between reproduction and aging.

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Parents sacrifice for their children and never retire; they dedicate their time and resources to fulfill their child's needs. While providing the best care for the kids, they often curb their choices of basic human needs, as well as career and social relationships. Thus, parenting takes a significant toll on the emotional health of parents, often affecting physical health. In Korea, this behavior is described by the expression "cutting and burning guts." This expression was first described in old mythology, where a devastated mother monkey who lost her cub had her guts fractured inside. Numerous stories have explained that parents put their lives at stake to care for and save their children in need. Selfless and sacrificial parenting are embedded in the human genome along with evolution. A recent study by Kern et al. (2021) demonstrated never-imagined selfless parenting by worms. *Caenorhabditis elegans*, a nematode, secretes milk-like yolk fluid made by self-destructive intestinal atrophy to repurpose biomass, for the growth young larvae. This self-destructive primitive form of lactation, reminiscent of reproductive death, explains many puzzling aging features of the old, versatile nematode model, which is widely studied for understanding why and how organisms age.

C. elegans are mainly hermaphrodites, i.e., having male and female reproductive organs and reproduce by self-fertilization (Corsi et al., 2015). Although self-sperm runs out first, worms lay many unfertilized eggs, even greater than their body volume (Ward and Carrel, 1979). The intestines of these worms are self-destructive via autophagy and convert intestinal biomass into yolk, which accumulates in the body cavities, causing aging-associated pathology (Ezcurra et al., 2018). Paradoxically, insulin/IGF-1 (insulin-like growth factor 1) signaling (IIS) accelerates the continuous production of oocytes and seemingly wasteful yolks in the mid-aged worms after reproduction, thereby shortening the lifespan. These events in aging worms were considered futile, and were thus assumed to be an old age-associated diseased state.

Kern et al. (2021) reported that mother worms expressing green fluorescent protein (GFP) fusion of vitellogenin VIT-2, a yolk protein, leave patches of lipid-containing green fluorescent substance on culture plates immediately after they finished their egg-laying for reproduction. The yolk is vented through the vulva, occasionally with several unfertilized eggs filled with green fluorescent yolk. The total amount of yolk protein in unfertilized eggs is twice as much as freely vented yolk. Surprisingly, GFP-labeled yolk is also found in larval intestines, indicating that larvae feed on the nutritious fluid, yolk milk, secreted by mother worms. Interestingly, baby worms can suck on the yolk milk in unfertilized eggs, equivalent to baby bottles. The larvae that drink the yolk milk grow significantly larger than those that do not. Additionally, the beneficial growth effects are suppressed when yolk accumulation decreases in mothers but are enhanced when accumulation increases. Therefore, yolk milk feeding promotes larval fitness.

IIS promotes both aging and reproductive processes. Insulin/IGF-1 receptor *daf-2 loss-of-function* (*ls*) mutants live a long life, synthesize and accumulate less yolk, and produce less unfertilized eggs (DePina et al., 2011; Gems et al., 1998; Kenyon et al., 1993; Kimura et al., 1997). As a result, long-living *daf-2 ls* mutants vent less yolk milk and cannot support larval growth. In contrast, *daf-2 gain-of-function* (*gf*) mutants that produce and vent more yolk milk, further promote larval growth. These effects depend on *the daf-16* FOXO transcription factor required for *daf-2* function in the aging process (Ezcurra et al., 2018; Kenyon et al., 1993). Moreover, a null mutation of *daf-18* PTEN phosphatase, which also increases IIS activity similar to *daf-2 gf* mutation, enhances yolk milk venting and larval growth (Mihaylova et al., 1999). Therefore, IIS promotes worm lactation by increasing yolk synthesis and stimulating gut-to-yolk biomass conversion, contributing to the aging of worms. Decreased IIS, as observed in *daf-2 lf* mutants, reduces its contribution to reproductive fitness and associated costs in the later stage of life, when reproduction is inessential, promoting longevity. Furthermore, mothers produce yolk milk to maximize larvae survival chances at the risk of survival.

So, is the worm milk really a kind of milk we know? Kern et al. (2021) analyzed excretory-secretory (ES) proteins of the substance released by mother worms. These proteins include vulval-vented ones, those excreted through the pore and anus, and those shed from the worm bodies. Additionally, proteomic analysis showed 125 mother worm-specific proteins in the ES proteins, of which 21 were IIS-upregulated, 30 upregulated in old age, and 28 contained signal peptides, similar to vitellogenins, major released proteins. Furthermore, 17 proteins showed all three features above, which is 82-fold greater than expected by chance alone and six-fold more when considering the overlap between the sets of proteins defined by each of the three features. Among the most abundant proteins, there were several transthyretin-related proteins, which function as carrier proteins for lipophilic compounds and other lipid-binding proteins, including vulva-expressed factor arrest protein 3 (Goodman, 1985). Lipid carrier proteins are also abundant in mammalian milk; as expected, there is a modest but significant overlap between mother worms' ES proteome and human milk proteome. This suggests functional similarities despite relatively distinct evolutionary origins (Andreas et al., 2015; Brawand et al., 2008).

C. elegans' primitive lactation may provide a fitness benefit, coupling pathological changes associated with aging to reproduction-supporting physiology, akin to reproductive death as seen in semelparous organisms (Gems et al., 2021). For example, Pacific salmons die in hormone-driven pleiotropic atrophy shortly after the one-time spawning of their lives. Self-destructive repurposing of biomass into yolk is observed in mother worms, whereas males or unmated females do not show this behavior (Kern et al., 2020; Kim et al., 2019; Turek et al., 2021). Worm lactation is surprising, and other invertebrates, including tsetse flies and the Pacific beetle cockroach, also secrete nutrient-containing fluid to feed their offsprings (Benoit et al., 2015; Marchal et al., 2013). Additionally, yolk milk feeding is similar to trophallaxis, which is transferring food or nutritious fluids between individuals in a community, for example, in social insects such as ants and bees (LeBoeuf, 2017). In the wild, worms mainly reproduce in highly dense and colony-like clonal populations (Schulenburg and Félix, 2017). Venting yolk into this collection should increase inclusive fitness through kin selection rather than individual fitness. Yolk milk packaged in unfertilized eggs may serve as emergency sustenance for all individuals, including adults in populations, especially if microbial food supplies are scarce. Whether yolk milk specifically improves particular traits, such as neurobehavior and immune responses has not been explored; neither is whether adult worms consume the vented yolk.

Reproduction costs life. In many species, including humans, limiting reproduction can extend lifespan, and overactive reproduction accelerates aging. Castrated servants of the royal court, lived up to 40% longer than uncastrated men, and women who experienced more pregnancies look older in their cells (Kang, 2019; Min et al., 2012; Ryan et al., 2018). Lactation greatly taxes a mothers' resources; nursing mothers lose up to 5% of bone mass. The energetically and emotionally expensive food has been shared in human society in various ways, seemingly supporting human fitness: for example, using wet nurses and a human milk bank for nonbreastfeeding mothers, and a medicinal and nutritional alternative for adults. "Cutting and burning guts" is based in reality. The relentless emotional grip might be a piece of an ancient artifact in evolution that results in adaptation, encouraging parents who receive undeniable and incomparable joy. Pay tribute to all parents feeling "cutting and burning guts," and may there be a lasting peace.

CONFLICT OF INTEREST
The author has no potential conflicts of interest to disclose.

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REFERENCES

Andreas, N.J., Kampmann, B., and Mehring Le-Doare, K. (2015). Human breast milk: a review on its composition and bioactivity. Early Hum. Dev. *91*, 629-635.

Benoit, J.B., Attardo, G.M., Baumann, A.A., Michalkova, V., and Aksoy, S. (2015). Adenotrophic viviparity in tsetse flies: potential for population control and as an insect model for lactation. Annu. Rev. Entomol. *60*, 351-371.

Brawand, D., Wahli, W., and Kaessmann, H. (2008). Loss of egg yolk genes in mammals and the origin of lactation and placentation. PLoS Biol. *6*, e63.

Corsi, A.K., Wightman, B., and Chalfie, M. (2015). A transparent window into biology: a primer on Caenorhabditis elegans. In WormBook, The C. elegans Research Community, ed. (Pasadena, CA: WormBook), https://doi.org/10.1895/wormbook.1.177.1

DePina, A.S., Iser, W.B., Park, S.S., Maudsley, S., Wilson, M.A., and Wolkow, C.A. (2011). Regulation of Caenorhabditis elegans vitellogenesis by DAF-2/IIS through separable transcriptional and posttranscriptional mechanisms. BMC Physiol. *11*, 11.

Ezcurra, M., Benedetto, A., Sornda, T., Gilliat, A.F., Au, C., Zhang, Q., van Schelt, S., Petrache, A.L., Wang, H., de la Guardia, Y., et al. (2018). C. elegans eats its own intestine to make yolk leading to multiple senescent pathologies. Curr. Biol. *28*, 2544-2556.e5.

Gems, D., Kern, C.C., Nour, J., and Ezcurra, M. (2021). Reproductive suicide: similar mechanisms of aging in C. elegans and pacific salmon. Front. Cell Dev. Biol. *9*, 688788.

Gems, D., Sutton, A.J., Sundermeyer, M.L., Albert, P.S., King, K.V., Edgley, M.L., Larsen, P.L., and Riddle, D.L. (1998). Two pleiotropic classes of daf-2 mutation affect larval arrest, adult behavior, reproduction and longevity in Caenorhabditis elegans. Genetics *150*, 129-155.

Goodman, D. (1985). Retinoids and retinoid-binding proteins. Harvey Lect. *81*, 111-132.

Kang, C. (2019). Senolytics and senostatics: a two-pronged approach to target cellular senescence for delaying aging and age-related diseases. Mol. Cells *42*, 821-827.

Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A C. elegans mutant that lives twice as long as wild type. Nature *366*, 461-464.

Kern, C.C., Townsend, S., Salzmann, A., Rendell, N.B., Taylor, G.W., Comisel, R.M., Foukas, L.C., Bähler, J., and Gems, D. (2020). C. elegans provide milk for their young. BioRxiv, https://doi.org/10.1101/2020.11.15.380253

Kern, C.C., Townsend, S., Salzmann, A., Rendell, N.B., Taylor, G.W., Comisel, R.M., Foukas, L.C., Bähler, J., and Gems, D. (2021). C. elegans feed yolk to their young in a form of primitive lactation. Nat. Commun. *12*, 5801.

Kim, S.H., Jung, H., Ahnn, J., and Lee, S.K. (2019). Calcineurin tax-6 regulates male ray development and counteracts with kin-29 kinase in Caenorhabditis elegans. Anim. Cells Syst. (Seoul) *23*, 399-406.

Kimura, K.D., Tissenbaum, H.A., Liu, Y., and Ruvkun, G. (1997). daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. Science *277*, 942-946.

LeBoeuf, A.C. (2017). Trophallaxis. Curr. Biol. *27*, R1299-R1300.

Marchal, E., Hult, E.F., Huang, J., Stay, B., and Tobe, S.S. (2013). Diploptera punctata as a model for studying the endocrinology of arthropod reproduction and development. Gen. Comp. Endocrinol. *188*, 85-93.

Mihaylova, V.T., Borland, C.Z., Manjarrez, L., Stern, M.J., and Sun, H. (1999). The PTEN tumor suppressor homolog in Caenorhabditis elegans regulates longevity and dauer formation in an insulin receptor-like signaling pathway. Proc. Natl. Acad. Sci. U. S. A. *96*, 7427-7432.

Min, K.J., Lee, C.K., and Park, H.N. (2012). The lifespan of Korean eunuchs. Curr. Biol. *22*, R792-R793.

Ryan, C.P., Hayes, M.G., Lee, N.R., McDade, T.W., Jones, M.J., Kobor, M.S., Kuzawa, C.W., and Eisenberg, D.T.A. (2018). Reproduction predicts shorter telomeres and epigenetic age acceleration among young adult women. Sci. Rep. *8*, 11100.

Schulenburg, H. and Félix, M.A. (2017). The natural biotic environment of Caenorhabditis elegans. Genetics *206*, 55-86.

Turek, M., Banasiak, K., Piechota, M., Shanmugam, N., Macias, M., Śliwińska, M.A., Niklewicz, M., Kowalski, K., Nowak, N., Chacinska, A., et al. (2021). Muscle-derived exophers promote reproductive fitness. EMBO Rep. *22*, e52071.

Ward, S. and Carrel, J.S. (1979). Fertilization and sperm competition in the nematode Caenorhabditis elegans. Dev. Biol. *73*, 304-321.