HLH-29 Regulates Ovulation in C. Elegans by Targeting Genes in the Inositol Triphosphate Signaling Pathway

Casonya Johnson  
*Georgia State University, cjohnson113@gsu.edu*

Ana White  
*Georgia State University*

Abegail Fearon  
*Georgia State University*

Follow this and additional works at: [https://scholarworks.gsu.edu/biology_facpub](https://scholarworks.gsu.edu/biology_facpub)

Part of the Biology Commons

**Recommended Citation**

Johnson, Casonya; White, Ana; and Fearon, Abegail, "HLH-29 Regulates Ovulation in C. Elegans by Targeting Genes in the Inositol Triphosphate Signaling Pathway" (2012). *Biology Faculty Publications*. 6. [https://scholarworks.gsu.edu/biology_facpub/6](https://scholarworks.gsu.edu/biology_facpub/6)

This Article is brought to you for free and open access by the Department of Biology at ScholarWorks @ Georgia State University. It has been accepted for inclusion in Biology Faculty Publications by an authorized administrator of ScholarWorks @ Georgia State University. For more information, please contact scholarworks@gsu.edu.
HLH-29 regulates ovulation in C. elegans by targeting genes in the inositol triphosphate signaling pathway

Ana White¹, Abegail Fearon¹ and Casonya M. Johnson¹,*
¹Department of Biology, College of Arts and Sciences, Georgia State University, Atlanta, GA 30303, USA
*Author for correspondence (cjohnson113@gsu.edu)

Summary
The reproductive cycle in the nematode Caenorhabditis elegans depends in part on the ability of the mature oocyte to ovulate into the spermatheca, fuse with the sperm during fertilization, and then exit the spermatheca as a fertilized egg. This cycle requires the integration of signals between the germ cells and the somatic gonad and relies heavily on the precise control of inositol 1,4,5 triphosphate (IP₃) levels. The HLH-29 protein, one of five Hairy/Enhancer of Split (HES) homologs in C. elegans, was previously shown to affect development of the somatic gonad. Here we show that HLH-29 expression in the adult spermatheca is strongly localized to the distal spermatheca valve and to the spermatheca-uterine valve, and that loss of hlh-29 activity interferes with oocyte entry into and egg exit from the spermatheca. We show that HLH-29 can regulate the transcriptional activity of the IP₃ signaling pathway genes ppk-1, ipp-5, and plc-1 and provide evidence that hlh-29 acts in a genetic pathway with each of these genes. We propose that the HES-like protein HLH-29 acts in the spermatheca of larval and adult animals to effectively increase IP₃ levels during the reproductive cycle.

Introduction
The basic helix loop helix (bHLH) transcription factors are critical regulators of early development (Maroto et al., 2008; Barnes and Firulli, 2009; Egoz-Matia et al., 2011), with roles in regulating organogenesis and neurosensory development (Pin et al., 2001; Ligon et al., 2006; Zhao et al., 2006; Du and Yip, 2011). Members of the Hairy-Enhancer of Split (HES) subfamily act primarily in response to Notch signaling (Fischer and Gessler, 2006; Kageyama et al., 2007), and are known for roles in neural (Kageyama et al., 2008; Li et al., 2008; Webb et al., 2007; Wiese et al., 2010) development. In humans, mutations in the HES genes are often associated with neuroblastoma (Axelson, 2004; Stockhausen et al., 2005), neuroendocrine tumors of the breast, lung, and prostate (Hartman et al., 2009; Lu et al., 2010; Nasgashio et al., 2011), and early developmental disorders (Sparrow et al., 2008; Sparrow et al., 2010). While members of the bHLH superfamily are also needed post-embryonically for metamorphosis (Lo et al., 2007; Parthasarathy et al., 2008; Bitra et al., 2009), sexual development and gamete formation (Van Wayenbergh et al., 2003; Ballow et al., 2006; Lu et al., 2008), and maintaining homeostasis (Zhou et al., 2009; Li et al., 2010; Long et al., 2010), little is known about the post-embryonic, non-developmental roles of the HES family proteins.

Ovulation in Caenorhabditis elegans is a tightly regulated process that demonstrates how cells and tissues must coordinate major signaling events to function. Critical to the ovulation cycle are a series of communication events between the proximal oocyte and sperm, and between the gametes and the surrounding sheath cells of the somatic gonad (Han et al., 2010; see Fig. 1). After receiving molecular signals from the sperm, the proximal oocyte undergoes maturation and sends its own signal to the gonadal sheath cells to amplify contractions that were initiated by the sperm-derived signals (Greenstein, 2005; Govindan et al., 2006). These oocyte-derived signals, which include the C. elegans epidermal growth factor homolog, LIN-3, also activate the dilation of the distal spermatheca valve. Ovulation occurs when the oocyte is propelled into the spermatheca. Sheath cell contractions, dilation of the distal spermatheca valve, and dilation of the spermatheca-uterine (SP-UT) valve after fertilization all require intracellular calcium release that is induced by LIN-3 dependent, inositol triphosphate (IP₃) signaling in the spermatheca and sheath cells (Clandinin et al., 1998; Bui and Sternberg, 2002; Yin et al., 2004). Here we show that the HES-like protein HLH-29 controls spermatheca entry and exit by altering the expression of genes required for IP₃ signaling, thereby providing one of the first links between a HES protein and the coordination of a rhythmic post-embryonic biological process.

Materials and Methods
Nematode handling and strains
The following strains were used: N2 Bristol wild-type (Brenner, 1974); TM284, hlh-29(tm284); SL232, unc-51(e1189) fog-2(q71); fog-2(q71) rol-6(sc446); PS2286, unc-18(e20) lfe-2(ery326); PS3656, ipp-5(e603). Transgenic lines carrying an integrated hlh-29::GFP transgene were previously described (McMiller et al., 2007). Culture growth and synchronization by alkaline hypochlorite treatment were as previously described (Lewis and Fleming, 1995).

Brood Size and Epistasis Analysis
Animals were raised for at least two generations at 22˚C prior to the start of assays, and were fed bacteria producing dsRNA for either the control gene unc-55 or for...
the test genes (Kamath and Ahringer, 2003). It should be noted that because hli-28 and hli-29 produce identical mRNAs, RNAi against hli-29 mRNA also knocks down hli-28 mRNA. Therefore, the RNAi effects described here may be the result of knockdown of both hli-28 and hli-29 and we will refer to this treatment as hli-29/hli-28 RNAi. For brood size assays and for ovulation assays with fog-2 animals, L4 stage hermaphrodites were serially transferred to fresh plates every 24 hours throughout their egg laying period. For epistasis analysis, L1 stage animals were fed control bacteria for 56 hours at 22.5°C and then moved, individually, to 35 mm NGM plates seeded with bacteria producing the appropriate dsRNA. Eggs and oocytes were counted twice: when the adult hermaphrodite was first removed from the plates, and again 24 hours later. The data are presented as the number of viable progeny (brood size), and oocyte percentage (number oocytes/number oocytes + viable progeny). Inviable and abnormally shaped eggs were eliminated because we could not consistently differentiate between fragmented eggs and fragmented oocytes. Finally, the hli-29(tm284) animals used for these assays were homozygous for the tm284 allele.

**Time-lapse Microscopy**

Animals were anesthetized with 0.1% levamisole (Govindan et al., 2009), mounted on 2.0% agarose pads and imaged using a 60X oil-immersion objective with a Nikon Eclipse 90i microscope equipped with a Nikon Coolsnap CCD camera.

---

**Fig. 1. HLH-29 is expressed in the adult spermatheca during ovulation.** (A) Ovulation in wild-type animals is stimulated when major sperm (MSP) proteins bind to receptors on the proximal oocyte and the proximal gonad to stimulate meiotic maturation and gonad contractions, respectively. The oocyte then secretes LIN-3 protein, which binds to the receptor protein LET-23 and amplifies the gonad contractions. LET-23 activation also triggers the dilation of the distal spermatheca valve. The oocyte is propelled into the spermatheca, where it is fertilized and subsequently expelled through the SP-UT valve into the uterus. (B-G) hli-29::GFP expression in the spermatheca of animals bearing an integrated transcriptional reporter construct. Corresponding DIC images are shown on the left of each epifluorescence micrograph. (B,C) Lateral view, L2/L3 stage. Cells of the somatic primordium are circled. (D,E) Dorsal view, early L4 stage. Anterior spermatheca is circled; arrow indicates uterus and vulval lumen. (F,G) Lateral view, adult stage. Posterior spermatheca is circled; arrow indicates vulva muscles.
Images were captured at 3 second intervals over a 115 minute period using NIS-elements, version 3.2 software (Nikon). Audio video interleave (AVI) files were generated by NIS-elements and compressed using H.264, then exported as moving picture expert group, standard 4 (MPEG-4) movies using Xilisoft Video Converter Ultimate, Version 6.0.7. Because ovulation occurred sporadically in hlh-29(tm284) homozygous animals due in part to variable defects in the gonad morphology, we used hlh-29/hlh-28 RNAi treated hlh-29(tm284) heterozygotes to capture ovulation by time lapse microscopy.

Gene Expression Analysis
Gene expression analysis, including total RNA extraction, cDNA synthesis, and real-time PCR were carried out as previously described (Felton and Johnson, 2011). Total RNA was extracted from late L4 or early adult stage populations, and cDNA synthesis reactions were performed in 50 μL reaction volumes containing 10.0 μg of total RNA. Real time PCR assays were performed with Tagman Gene Expression Assays (Applied Biosystems) specific for each putative target (supplementary material Table S1), using relative quantitation with normalization against the endogenous control gene pmp-3 (Hoogeewijns et al., 2008).

Egg-laying Measurements
Egg-laying in the presence of food was assayed using synchronized cultures that were fed for 72 hours after hatching at 22°C. Two hermaphrodites were placed onto seeded, 35 mm NGM plates and incubated at 22°C. After two hours, the number of eggs and unfertilized oocytes were counted. Animals that did not produce progeny or who ruptured through vulva (exp) during the assay were excluded. For this assay N=30; each experiment was repeated in triplicate.

For egg laying assays in the absence of food, each culture of L1 stage animals was fed bacteria producing the appropriate dsRNA for 72 hours. Animals were then picked onto an unseeded NGM plate and allowed to crawl around for 30 minutes so that all bacteria could be removed from their bodies. Hermaphrodites were then placed individually into each well of a 48-well tissue culture plate containing 25 μL of M9 buffer, and incubated at 22°C for 1 hour. Animals that ruptured through the vulva while in buffer or that displayed anatomical defects were excluded from the assay. For this assay, N=288; each experiment was repeated 5 times.

Results and Discussion
HLH-29 is expressed in the spermatheca and plays a role in egg-laying
Previously, we reported that RNAi knockdown of hlh-29 and the duplicate gene hlh-28 results in the exploded through vulva (exp), protruding vulva (pvl), and accumulated endomitotic oocytes (emo) phenotypes (McMiller et al., 2007). Additionally, a fraction of RNAi animals failed to form a uterus, failed to develop one of the two gonad arms, or accumulated either unfertilized or emo oocytes in egg-laying. The morphology of the gonad arms is affected in animals lacking HLH-29 (McMiller et al, 2007), making it difficult to analyze ovulation in hlh-29(tm284) homozygous animals. HLH-29’s effect on gonad morphology appears to be dose and developmental stage dependent, and we were able to separate this phenotype from the ovulation phenotype by subjecting L3 stage heterozygous hlh-29(tm284) animals, to hlh-29/hlh-28 RNAi. The ovulation defects were the same in N2 and in hlh-29(tm284) heterozygous animals treated with hlh-29/hlh-28 RNAi, and also in hlh-29(tm284) homozygous animals. These defects ranged from complete failure of the oocyte to enter the spermatheca to complete failure of the fertilized egg to exit the spermatheca. We found, however, that defective ovulation occurred more frequently in RNAi treated hlh-29(tm284) heterozygotes than in N2 animals. Therefore, we used hlh-29/hlh-28 RNAi treat hlh-29/hlh-28 heterozygotes, referred to henceforth as hlh-29(tm284)/+ hlh-29/hlh-28(RNAi) animals, to capture the ovulation defects by time lapse microscopy.

Ovulation is defective in hlh-29 animals
In wild-type animals, each gonad arm ovulates one mature oocyte on average of every 23 minutes. In the oocyte, the events leading up to ovulation include distal nuclear migration, breakdown of the nuclear envelope, and cortical rearrangement. Ovulation itself, the propulsion of the mature oocyte into the spermatheca, requires intense rhythmic contractions of the surrounding gonad sheath cells, followed by dilation or extension of the distal spermatheca valve. In wild-type animals, the ovulated oocyte is fertilized almost immediately, and within five minutes of ovulation, the fertilized egg emerges through the SP-UT valve into the uterus (McCarter et al., 1999).

The morphology of the gonad arms is affected in animals lacking HLH-29 (McMiller et al, 2007), making it difficult to analyze ovulation in hlh-29(tm284) homozygous animals. HLH-29’s effect on gonad morphology appears to be dose and developmental stage dependent, and we were able to separate this phenotype from the ovulation phenotype by subjecting L3 stage heterozygous hlh-29(tm284) animals, to hlh-29/hlh-28 RNAi. The ovulation defects were the same in N2 and in hlh-29(tm284) heterozygous animals treated with hlh-29/hlh-28 RNAi, and also in hlh-29(tm284) homozygous animals. These defects ranged from complete failure of the oocyte to enter the spermatheca to complete failure of the fertilized egg to exit the spermatheca. We found, however, that defective ovulation occurred more frequently in RNAi treated hlh-29(tm284) heterozygotes than in N2 animals. Therefore, we used hlh-29/hlh-28 RNAi treat hlh-29/hlh-28 heterozygotes, referred to henceforth as hlh-29(tm284)/+ hlh-29/hlh-28(RNAi) animals, to capture the ovulation defects by time-lapse microscopy.

Distal nuclear migration, breakdown of the nuclear envelope, and cortical rearrangement appeared to occur normally in hlh-29(tm284)/+ hlh-29/hlh-28(RNAi) animals, suggesting that HLH-29 is not required for oocyte maturation. This result was unexpected in light of the fog-2; hlh-29/hlh-28 (RNAi) results described above. Genes previously shown to influence ovulation in fog-2 animals are believed to negatively regulate meiotic maturation. RNAi knockdown of those genes normally increases maturation rates in unmated fog-2 females, though not to the same rates as those seen in hermaphrodites or in mated fog-2...
females (Govindan et al., 2006). It is possible that HLH-29 and HLH-28 function in different tissues to regulate the expression of two separate sets of genes that are required to separate meiotic maturation and the initiation of ovulation from physical movement through the spermatheca. This possibility would explain why ovulation increases in *fog-2*; *hlh-29/hlh-28* (RNAi) animals and also may explain why *hlh-29/hlh-28* RNAi rescues the brood size phenotypes of *hlh-29(tm284)* animals (see below).

Loss of HLH-29 did not appear to affect gonadal sheath contractions; however, the ability of oocytes and fertilized eggs to enter and exit the spermatheca, respectively, was affected (supplementary material Movies 1–3). This ovulation defect was highly variable and occurred randomly in either gonad arm. We compared ovulation events over a 90 minute period in wild-type and in *hlh-29(tm284)/+* *hlh-29/hlh-28* (RNAi) animals (supplementary material Table S2), and defined a successful...
HLH-29 regulates IP₃ signaling in C. elegans

A.

![Graph showing transcriptional activity of genes in the IP₃ signaling transduction pathway affected in hlh-29(tm284) animals.](image)

**Fig. 3. HLH-29 and the IP₃ signaling.** (A) Transcriptional activity of genes in the IP₃ signaling transduction pathway are affected in hlh-29(tm284) animals. Bars represent the relative fold-change in mRNA levels as detected by RT-qPCR when compared to expression in wild-type animals, error bars represent standard error of the mean. Fold expression range considered to be the same as wild-type expression is indicated by light gray shading, centered around the value of 1.0. P values are indicated to the right of each bar. (B) Components of the LIN-3/LET-23 activated IP₃ signaling transduction pathway are transcriptionally regulated by HLH-29.

B.

![Diagram illustrating spermatheca and IP₃ signaling](image)

HLH-29 acts as a positive and a negative regulator of genes expressed in the adult spermatheca

We tested the effect of loss of hlh-29 activity on genes whose expression overlapped with hlh-29 in the spermatheca and that were previously shown to affect ovulation. We found that loss of HLH-29 reduced the activity of ppk-1 and increased the activities of plc-1 and ipp-5 (Fig. 3A), three genes required for IP₃ signaling. Loss of HLH-29 also caused a 1.3 fold increase in lfe-2 activity, though this change was not found to be statistically significant.

We looked for genetic interactions between hlh-29 and ppk-1, ipp-5, and lfe-2 using measurements of brood size and percentage of unfertilized oocytes (see methods and materials). ppk-1 encodes a kinase that converts phosphatidylinositol-4-phosphate (PIP) into phosatidylinositol-4, 5-bisphosphate (PIP₂), which is subsequently hydrolyzed by phospholipase C into the second messengers diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP₃) (Fig. 3B). Loss of PPK-1 causes the accumulation of oocytes in the gonad arms due, in part, to the absence of gonadal sheath contractions during ovulation (Xu et al., 2007). Feeding newly hatched N2 or homozygous hlh-29(tm284) animals bacteria producing ppk-1dsRNA resulted in 100% sterility (not shown). We found that N2 and hlh-29(tm284) animals that received ppk-1 RNAi after L3 stage produced almost as many viable progeny as those that were subjected to control RNAi (Fig. 3B) during the same time period, and so we used these animals for the epistasis assays. Wild-type animals laid a small percentage of unfertilized oocytes at the end of their egg-laying period (Fig. 4A), but this percentage was reduced in ppk-1 (RNAi) animals. hlh-29(tm284) animals laid unfertilized oocytes...
throughout their egg-laying period and laid a significantly higher percentage than wild-type animals. This phenotype was suppressed by \textit{ppk-1} RNAi (Fig. 4A), indicating that \textit{ppk-1} is epistatic to \textit{hlh-29}, and further supporting our molecular finding that \textit{hlh-29} acts upstream of \textit{ppk-1}.

The enzymes IP₃ kinase, encoded by \textit{lfe-2}, and inositol polyphosphate 5-phosphatase, encoded by \textit{ipp-5}, function to reduce the intracellular levels of IP₃ (Clandinin et al., 1998; Bui and Sternberg, 2002). Loss of either \textit{ipp-5} or \textit{lfe-2} effectively increases IP₃ levels, but results in only moderate ovulation defects. \textit{ipp-5} animals produce fewer progeny than wild-type animals and lay unfertilized oocytes because of a hyperactive distal spermatheca valve (Bui and Sternberg, 2002). \textit{lfe-2} animals also have small brood sizes, but no other ovulation defects are evident (Clandinin et al., 1998). Interestingly, animals that carry loss-of-function alleles in both \textit{ipp-5} and \textit{lfe-2}, and animals that over-express \textit{lfe-2} are sterile. In the latter case, sterility is caused by failure of the spermatheca-uterine valve to open (Clandinin et al., 1998), a phenotype very similar to the ovulation defect of \textit{hlh-29(tm284)+/hlh-29/hlh-28} (RNAi) animals and in support of slightly increased \textit{lfe-2} activity in \textit{hlh-29(tm284)} animals. In our genetic interaction studies, \textit{lfe-2(sy36)}; \textit{hlh-29/hlh-28} (RNAi) animals and \textit{ipp-5(sy605)}; \textit{hlh-29/hlh-28} (RNAi) animals laid a significantly higher percentage of unfertilized oocytes than either wild-type or single mutant animals (Fig. 3B,C). Additionally, brood sizes were significantly reduced in \textit{hlh-29(tm284)}, \textit{ipp-5(sy605)}, and \textit{lfe-2(sy36)} animals when compared to wild-type, and were reduced further in \textit{ipp-5(sy605)}; \textit{hlh-29/hlh-28} (RNAi) and \textit{lfe-2(sy36)};\textit{hlh-29/hlh-28} (RNAi) animals. These results show genetic interactions between \textit{hlh-29} and \textit{ipp-5} and between \textit{hlh-29} and \textit{lfe-2}, and we suggest that they are representative of the phenotypes that would be expected in either \textit{lfe-2} (gain-of-function); \textit{ipp-5} (loss-of-function) or \textit{lfe-2} (loss-of-function); \textit{ipp-5} (gain-of-function) animals.

The percentage of unfertilized oocytes in \textit{hlh-29(tm284)} animals does not change significantly upon treatment with \textit{hlh-29/hlh-28} RNAi, suggesting that HLH-29 and HLH-28 do not function redundantly to control ovulation (Fig. 4A). Interestingly, the brood size data contradicts this interpretation, as \textit{hlh-29(tm284)} animals show significantly larger brood sizes after treatment with \textit{hlh-29/hlh-28} RNAi. One possibility for this discrepancy would be that RNAi artificially induces egg-laying via a process that is independent of HLH-29 and HLH-28. We do...
not believe this explanation is plausible, however, because brood size was not increased in hhl-29(tm284) animals subjected to control RNAi when compared to those that were untreated with RNAi (compare Fig. 4A to Fig. 4C). We suggest that this result and the fog-2(q71); hhl-29/hhl-28 (RNAi) results presented above are indicative of a separate function for HLH-28 in controlling ovulation events upstream of spermatheca entry and exit.

HLH-29 and HLH-28 affect the morphology of the gonad arm
As mentioned above, animals that are homozygous for hhl-29(tm284) show variably abnormal morphological defects of the somatic gonad which became more pronounced when animals were cultured at higher temperatures. Because our data showed that hhl-29/hhl-28 RNAi can rescue the brood size phenotype of hhl-29(tm284) animals, we expected that the gonad morphology defects would also be rescued. Surprisingly, gonad defects were more pronounced in hhl-29/hhl-28 RNAi treated hhl-29(tm284) animals than in untreated animals, though the phenotype was still variably expressed and incompletely penetrant.

Supplementary material Fig. S2D,E shows an animal variably expressed and incompletely penetrant. animals than in untreated animals, though the phenotype was still variably expressed and incompletely penetrant. To identify genes that may be targeted by either HLH-29 or HLH-28 during gonad development, and to determine if HLH-28 and HLH-29 may be jointly regulating expression of those genes, we compared the activities of the IP3 signaling genes and the activities of six other genes whose expression overlapped with hhl-29 in at least two tissues and that were previously shown to affect reproduction (supplementary material Table S1A) in hhl-29(tm284) and hhl-29/28 (RNAi) animals. The expression of five of these genes was affected in hhl-29(tm284) animals (supplementary material Table S1B). These genes and the proteins they encode are: nhr-6, a NR4A nuclear receptor protein required for spermatheca development (Heard et al., 2010); emb-9, a Type IV basement membrane collagen required for embryonic morphogenesis (Guo et al., 1991); sca-1, a calcium transporting ATPase that is predicted to interact with the IP3 receptor protein, ITR-1 (Nehrke et al., 2008); and two genes required for gonadal morphogenesis, mig-6, an extracellular matrix protein (Kawano et al., 2009) and pyp-1, nuclease remodeling factor (Ko et al., 2007). We found that the expression of sca-1, nhr-6, emb-9, and most of the IP3 signaling genes was similarly affected in RNAi treated animals; however, the expression of pyp-1, mig-6, and the IP3 signaling pathway gene let-23 was affected differently in RNAi treated hhl-29(tm284) animals (supplementary material Table S1C). Altogether, these results suggest that both HLH-28 and HLH-29 are required for normal development of the somatic gonad.

Conclusions
Our molecular and genetic data indicate that HLH-29 acts in both the distal spermatheca valve and the spermatheca-uterine valve to regulate ovulation by mediating IP3 signaling (Fig. 3B). Previous studies have identified bHLH proteins as regulators of the IP3 receptor genes in mice (Konishi et al., 1999) and in yeast (Shetty and Lopes, 2010); however, this is the first to show coordinated regulation of multiple genes within the IP3 signaling pathway. HLH-29 is a member of the C. elegans REF-1 family, functional homologs of HES proteins (Neves and Priess, 2005). Our results, then, are one of the first to demonstrate the involvement of a HES protein in the modification and regulation of an adult phenotype and a possible link between IP3 and Notch signaling in this organism. These results also underscore the importance of tight regulation of the IP3 signaling cascade, and demonstrate how IP3 signaling can be modulated at multiple inputs in the pathway. Previous results show that perturbing any of the genes in this pathway can have moderate to severe effects on ovulation. HLH-29 seems to act to increase the levels of IP3, either by activating the ppk-1 gene, or by repressing lif-2 and ipp-5, two genes needed to reduce intracellular levels of IP3. Our gene expression data also suggest that HLH-29 represses the gene for phospholipase C e, plc-1. This result contradicts our genetic data, which strongly correlate with ovulation phenotypes seen in plc-1 loss of function mutants. One plausible explanation is that the expression levels reported here are indicative of HLH-29 dependent regulation of plc-1 levels in cells outside of the spermatheca. Finally, our results indicate that HLH-29 regulates genes required for the development of the spermatheca and of the somatic gonad, and are particularly exciting in that they underscore the ability of HES proteins to regulate tissue morphogenesis and organ development in larval and adult animals. This study did not directly address the possible roles of HLH-28 in reproduction; however, treating either fog-2 animals or hhl-29 animals with hhl-29/hhl-28 RNAi increased the total number of ovulation events in both strains. These results suggest that HLH-28 may negatively regulate ovulation separately from HLH-29.

Acknowledgements
We gratefully acknowledge Drs. W.W. Walthall, M. Zellars, and R. Kohn for helpful advice; undergraduate research assistant Stephanie Pritchett for performing brood size assays at 16˚C and 20˚C and the Caenorhabditis Genetics Center for providing strains. While performing these studies A. White and S. Pritchett were Biotech Scholars, supported by Howard Hughes Medical Institute, Division of Undergraduate Education, Grant #52005875. This research and undergraduate student researcher A. Fearon were supported by National Science Foundation grant #MCB0919413 to C. Johnson.

References

Genes Dev. 18, 901-911.


Curr. Biol. 16, 1257-1268.


Development 136, 2211-2221.


WormBook 1-12.


Nature 349, 707-709.


Dev. Curr. 239, 1265-1281.


WormBook.


Mol. Cancer 9, 304.


Dev. Biol. 205, 111-128.


Lung Cancer 74, 405-410.


Dev. Cell 8, 867-879.


Eukaryot. Cell 9, 1845-1855.


Dev. Dyn. 229, 716-725.


BMC Dev. Biol. 11, 27.


Mol. Endocrinol. 20, 2179-2198.


J. Plant Physiol. 166, 1296-1306.