Examining the colocalization CLU-1 in the germline of *Caenorhabditis elegans*

A Thesis Presented to:
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Abstract

Embryonic germ cells are pluripotent stem cells derived from primordial germ cells. Germ cells play an important role in reproduction because they give rise to gametes. Much is still to be uncovered about the complex mechanisms that confer and maintain germline pluripotency. Previous work has shown that non-membrane bound cytoplasmic organelles play an important role in determining germline pluripotency. One such organelle is the germ granule. Germ granules are specific to the germline and highly conserved across species. These granules are RNA and protein-rich and are generally found clustering around the nuclear pore complex. Germ granules have been cited as germline determinants and in some cases, transplantation can induce pluripotency. Recently, germ granules have been relevant for cancer diagnostics because many essential germ granule proteins are expressed in late stage cancers. Although germ granules are of great importance in development and hold clinical relevance, little is known about germ granule dynamics and binding partners. Studying germ granule components in a nematode model can provide clarity on their complex role in the germline. In *C. elegans,* germ granules are called P granules and they are necessary for normal nematode germline development. Depletion of P granules in *C. elegans* causes immediate differentiation and loss of pluripotency and fertility. Some studies have shown that many proteins present in P granules can bind RNA, effectively regulating translation. It is hypothesized that P granules accomplish this through the creation of a protective environment that polices mRNA exiting the nucleus. The germline helicases (GLHs) and P granule abnormality (PGLs) are constitutive components of P granules. GLHs and PGLs are essential for proper P granule structure and function and are present in P granules at all stages of *C. elegans* development. GLH-1 is one of four Vasa homologs in *C. elegans.* First discovered in *Drosophila Melanogaster,* Vasa has been extensively studied for its role in translational activities and malfunctions that in some cases, lead to tumorigenesis. However, much is still unknown about the specific localization and activity of Vasa that is detrimental for the cell. Studying Vasa homolog, GLH-1, can inform us on how Vasa functions in the cell. Furthermore,

the use of DDX4, the Vasa ortholog in humans, as a diagnostics tool to establish prognosis in late stage cancers also suggests clinical relevance for the study of GLH-1. In an effort to learn more about the molecular dynamics of P granules, a recent co-immunoprecipitation of GLH-1 followed by mass spectrometry showed significant enrichment of mitochondrial clustering protein, CLU-1. Limited evidence based on homology suggests CLU-1 comprises the A subunit of eukaryotic initiation factor three (eIF3). eIF3 is a highly conserved, multiprotein complex that acts as a gatekeeper of translation. In this study, a V5 and mCherry tag were added to the N-terminus of CLU-1 to explore its localization in the germ cells of a *C. elegans* strain with GLH-1 expressing green fluorescent protein (GFP). Using microscopy, we studied the interactions between GLH-1 and CLU-1. Our results suggest that CLU-1 is present in aggregates in the rachis of germ cells and exhibits occasional colocalization with GLH-1. This study also suggests that CLU-1 is not expressed in the mature oocyte which may shed light on the complex mechanisms behind embryogenesis. This research gives new insight into P granule structure and activity with other cytoplasmic factors that regulate translation and maintain pluripotency.

Introduction

In sexually reproducing metazoans, germ cells are responsible for ensuring genetic information is passed on from one generation to the next. In most animals, germ cells are pluripotent cells that are destined to become sperm or egg. Germ granules are present in germ cells across species and are thought to have a significant role in germ cell identity through the regulation of transcripts involved in fertility and differentiation (Voronina et. al., 2011). Germ granules, which are called P granules in *C. elegans* because they arise from the P lineage, create a microenvironment of RNA and protein around nuclear pore complexes (Voronina et. al., 2011). P granule components are thought to regulate translation through the binding of mRNA that exits the nucleus (Voronina et. al., 2011). Much is still unknown about P granule composition and function and genetic analysis has begun to identify proteins and RNA that make up these nonmembrane bound organelles.

P granule lifecycle in *C. elegans*

Establishment of the germline in *C. elegans* is accomplished through several, highly dynamic cell divisions in which P granules are always present. In the growing oocyte, P

granules can be found dotting the cytoplasmic face of the nucleus, clustering around the nuclear pore complexes. As the oocyte matures, the P granules separate from the nuclear pore complex and disperse throughout the cytoplasm where they remain during ovulation and fertilization (Updike & Strome, 2010). After fertilization the cell becomes a one-cell zygote (P0) and few, if any, P granules remain around the nucleus. In the P0 cell, the maternally contributed P granules segregate to the posterior side of the cell which will eventually give rise to the germline while the anterior portion will become somatic cells (Seydoux, 2018). P granule partitioning is accomplished through strategic disassembly of anterior and assembly of posterior P granules (Seydoux, 2018). This was demonstrated through imaging of PGL-1, a defining constituent of P granules, tagged with GFP (Seydoux, 2018). This process is repeated during four consecutive cell divisions which gives rise to germ line blastomeres known as P cells (P1, P2, P3, P4) which are responsible for the name P granules (Seydoux, 2018). During these four divisions, P granules are segregated and maintained in the P lineage while the other cells lacking P granules give rise to somatic cell lineages. P granules begin to attach themselves once again to nuclear periphery during P2 and this continues in P3 and P4 until almost all the granules have clustered around the nuclear periphery (Updike & Strome, 2010). P4 is the *C. elegans* primordial germ cell as it is responsible for giving rise to all of the germ cells in the animal. P4 divides a single time during embryogenesis to give rise to primordial germ cell daughter cells Z2 and Z3 (Updike & Strome, 2010). Z2 and Z3 mitotically divide at the end of the first larval stage and their daughter continue to divide to produce about 1000 germ cells in the adult gonad (Updike & Strome, 2010).

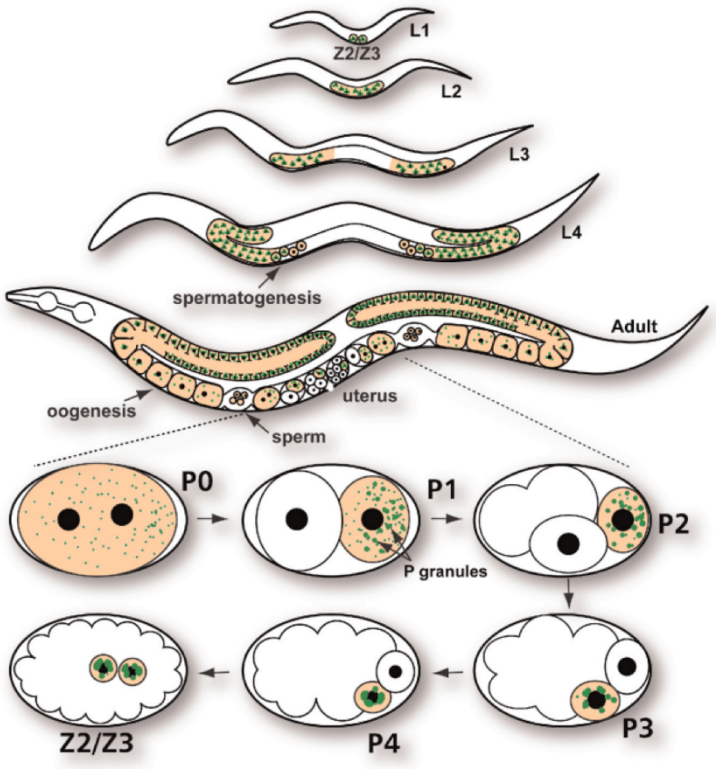

Figure 1. Germ line development in *C. elegans.* Shortly after hatching, the two primordial germ cells (Z2 and Z3) begin to divide. Approximately 40 meiotically produced germ cells are involved in spermatogenesis during the fourth larval stage (L4). In the adult hermaphrodite, spermatogenesis is halted, and oogenesis begins. Leading up to fertilization, as early as the P(-3) oocyte, P granules detach from the nuclear periphery and disperse throughout the cytoplasm. Following fertilization, the P0 zygote begins to divide and P granules separate into germ line blastomeres (P1, P2, P3, P4) through preferential assembly and disassembly. P4 is the primordial germ cell and divides into daughter cells Z2 and Z3, completing the germ line cycle. The germ line is in orange and P granules are in green. (Figure used with permission from Dustin Updike)

<u>P granule function</u>

Nobel Prize winning research performed in 1962 by John Gurdon demonstrated that the nucleus of mature cells can be reprogrammed to become pluripotent. Gurdon transplanted a frog's intestinal nucleus into an enucleated frog zygote. Amazingly, the zygote developed into a new frog. Up until this point, it was thought that pluripotency was strictly nuclear, but this discovery led scientists to look to the cytoplasm for factors that regulate pluripotency (Gurdon, 1962). P granules are thought to be one such factor based on their translational regulation capabilities and indispensable role in the germ line. In one study, *C. elegans* with RNAi a single knockdown of PGL-1, PGL-3, GLH-1, or GLH-2 exhibited the same phenotype: they grew normally through larval stages but did not begin oogenesis when transitioning to adulthood and thus, grew into sterile adults without oocytes (Seydoux, 2018). These sterile adults expressed somatic transcripts in the germ tissue itself and some germ cells even differentiated into neuronal-like cells (Seydoux, 2018). Analysis of the transcriptome showed a number of misregulated transcripts, including a significant number of transcripts normally

expressed in sperm and somatic cells (Seydoux, 2018). These results suggest that P granules protect the pluripotent capabilities of germ cells by preventing ectopic gene expression through the regulation of transcripts as they exit the nuclear pore complex (Seydoux, 2018.

P granule structure

P granules are nonmembrane bound organelles consisting of RNA and protein, many of which are predicted to bind RNA and some of which are involved in translation regulation (Strome, 2005). P granules have a core complex of proteins containing the following three domains: LOTUS/TUDOR, ARGONAUTE, and DEAD-box helicase (Marnik & Updike, 2019). P granules phase separate from the cytoplasm and are found clustered around the nuclear pore complex where they vet transcripts that exit the nucleus (Seydoux, 2018). The phase separation and nuclear pore complex clustering activity of P granules can be largely attributed to proteins with intrinsically disordered regions (IDRs) which have been shown to aggregate *in vivo* (Marnik & Updike, 2019). One common IDR in P-granule proteins consists of FG-repeats – hydrophobic phenylalanine residues interspersed in glycine-rich domains (Marnik & Updike, 2019). Directed P granules assembly to the nuclear pore complex is accomplished by FG-repeat protein, GLH-1, along with FG-containing nucleoporins (Seydoux, 2018). GLH and PGL are constitutive components of P granules and are present throughout P granule lifecycle (Strome, 2005). *In vitro,* multimers of GLH-1 and FG domains can form perinuclear granules when atypically expressed in the intestine; however, wild type GLH-1 must be co-expressed with PGL-1 to form granules (Seydoux, 2018). Recent coimmunoprecipitation and mass spectrometry of P granule scaffolding protein, GLH-1, showed enrichment of members of the 43S preinitiation complex (PIC) (Updike, personal communication, 2019).

eIF3 and eukaryotic translation

Eukaryotic translation initiation is a highly regulated, rate-limiting process that is the culmination of numerous complex events. This process results in the construction of a ribosome containing the methionyl initiator tRNA (Met-tRNA) positioned on the start codon of an mRNA (Jackson et al., 2010). The first step in eukaryotic translation initiation is the formation of the 43S preinitiation complex (PIC) (Jackson et al. 2010). The PIC complex consists of the 40S ribosomal subunit, eukaryotic initiation factors eIF1, eIF1A, eIF3 and eIF5, and the eIF2–GTP–Met-tRNA$_i$ ternary complex (Jackson et al. 2010). eIFs helps mediate the translation initiation

pathway (Cate, 2017). eIF3 is broadly conserved across eukaryotes and has roles in both translational repression and activation. It is a large translational initiation factor with a molar mass approximately 800 kDa. This multiprotein complex consists of five lobes and is evolutionarily similar to the COP9 signalosome and 26S proteasome regulatory lid complex (Cate, 2017). In an experiment using radiolabeled eIF3, it was demonstrated that without the presence of other translation factors, eIF3 binds to the 40S subunit and stabilizes the biding of eIF2/Met-tRNA/GTP to the 40S subunit (Cate, 2017). This is needed for the binding of mRNA to the 40S subunit and inhibition of the 60S subunit joining.

CLU-1

Recent co-immunoprecipitation followed by mass spectrometry of *C. elegans* GLH-1 identified several transcription preinitiation complex related proteins, most significantly of which was CLU-1 (Updike, personal communication, 2019). CLU-1 is largely unstudied in *C. elegans* but limited evidence based on homology in *Saccharomyces cerevisiae* suggests that it may comprise a subunit of eIF3. CLU-1 is thought to be a homolog of *Saccharomyces cerevisiae* eIF3a, a sterically unhindered and easily tagged region of eIF3. It is unknown if CLU-1 can operate outside of eIF3. Most of what is known about CLU-1 comes from studies in *Saccharomyces cerevisiae* and *Drosophila melanogaster*. *Drosophila* protein Clueless (Clu), whose vertebrate homolog is clustered mitochondria homolog (Cluh), exhibits a mitochondrial clumping phenotype (Sheard et al., 2019). Preliminary results may suggest that RNAi knockdown of *clu-1* in *C. elegans* also displays a mitochondrial clumping phenotype (Updike, personal communication). WormBook lists CLU-1 as an eIF3a homolog with predicted mRNA binding activity and structure alignments show conservation with eIF3a.

Using CRISPR/Cas9 to tag CLU-1 with V5 and mCherry

To observe CLU-1's localization in the germline and learn about its associated protein interactions *in vivo*, the CRISPR/Cas9 system was employed to add a fluorescent mCherry tag and V5 epitope tag to the N-terminus of CLU-1.

Clustered regularly interspaced short palindromic repeats (CRISPR) and RNA guided nuclease Cas9 have revolutionized genetic engineering by allowing for streamlined gene editing. CRISPR/Cas9 has proven to be an effective tool for gene editing in *C. elegans.* Cas9 and two small non-coding RNA make up an adaptive immunity in prokaryotes. (Adli, 2018). A fusion of

the CRISPR RNA (crRNA) and tracer RNA (tracrRNA) directs site specific cleavage of target DNA by Cas9. The only sequence requirement for cleavage is the presence of an NGG nucleotide sequence known as the protospacer adjacent motif (PAM) at the 3' end of the target DNA sequence (Adli, 2018). Once the site is cleaved, the break will be repaired by homology directed repair or nonhomologous end joining. By supplying synthetic homologous repair templates such as a gBlock, knock-ins can be created (Dickinson et al., 2013).

Using the Mello Lab overhang method (Fig 2), the small epitope, V5, and fluorescent marker, mCherry, were added to the N-terminus of CLU-1 to study its colocalization with P granules in the *C. elegans* germline. Surprisingly, we observed limited colocalization between GLH-1 and CLU-1, with most of the CLU-1 signal aggregating in the rachis or dispersing throughout the cytoplasm in mature oocytes. Although unexpected, these results may indicate a novel model of P granule structure and function. This study sheds light on previously unspecified P granule structure, function, and interactions in the germline. By discovering more P granules associated proteins, other cytoplasmic factors that regulate translation and pluripotency can be better understood. Furthermore, germ granules' present use as a diagnostics tool to establish prognosis in late stage cancers might suggest relevance to clinical therapies.

Methods

Enhanced recombination using the Mello lab overhang method

A custom gBlock was ordered from IDT consisting of 120 base pairs downstream of the *clu-1* stop codon, V5, mCherry, and the first 120 base pairs upstream of *clu-1* stop codon respectively. The gBlock was diluted to a concentration of 1uM. Two sets of primers were ordered from IDT – an external set of primers, to amplify the entire gBlock, and an internal set of primers, to only amplify V5 and mCherry. The primers were diluted to a concentration of 10uM. Two separate PCR reactions were run – one with the internal primers and one with the external primers. Each PCR reaction consisted of four, 50uL reactions that were aliquoted from a 200uL master mix consisting of 130uL deionized H2O, 40uL 5X Phusion green HF buffer, 4uL dNTP (10mM), 10uL reverse primer (10uM), 10uL forward primer (10uM), 4uL gBlock (1uM), 2uL phusion (2 U/uL). The PCR products were confirmed on a 10% agarose gel which ran at 100V for 50 minutes next to a 1kbp ladder (New England BioLabs, 50 ug/mL). 190uL of the PCR product was purified following the specifications in the

Qiagen MiniElute purification kit. Following purification, the external product and the internal product were combined and hybridized by heating to 98C for 30 seconds and then cooling to room temperature. The concentration of the purified hybridized DNA was measured using a Nanodrop (see Table 1).

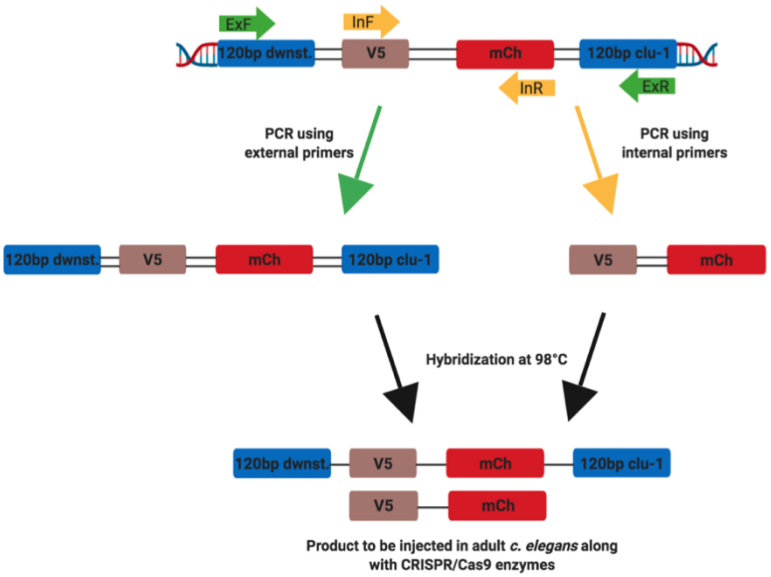

Figure 2. The Mello Lab overhang method. A gBlock of 1077bp contained 120bp downstream of the *clu-1* stop codon, V5, mCherry, and the first 120bp upstream of the *clu-1* stop codon respectively. Two PCR reactions were run using each set of primers and the two products were hybridized. The overhangs of the hybridized product stimulate homologous recombination. This construct, along with CRISPR/Cas9 enzymes were injected into DUP64 to tag *clu-1* with mCherry and V5.

Co-CRISPR Injection

L4 stage DUP64 *(glh-1(sam24[glh-1::gfp::3xFlag]clu-1(sam25[clu-1::mCh::V5) C. elegans* were picked onto a plate 24 hours prior to injection. The 10uL injection mix consisted of 1uL Cas9 (61uM), 1uL trRNA (400uM), .3uL *dpy-10* crRNA (400uM), .5uL *dpy-10* ssODN (10uM), 4.0uL hybridized *clu-1* dsDNA (10uM), .7uL *clu-1* crRNA (400uM), 1uL HEPES (200mM), 1.5uL KCL (1M). After combining the reagents, the injection mix was centrifuged on a mini centrifuge for 5 minutes and kept at -4C until injected directly into the *C. elegans* germline. 57 *C. elegans* were injected and placed in a 20C incubator. All injections were carried out by Catherine Sharp. 30 Dpy and 30 Rol *C. elegans* from the F_1 generation were picked onto individual plates to proliferate. Of those plates, 3 plates contained *C. elegans* with mCherry expression. *C. elegans* exhibiting mCherry were passaged to individual plates in groups of three to reproduce. The subsequent generations of wild type *C. elegans* that also exhibited mCherry were passaged every two days.

Imaging

Adult hermaphrodites were immobilized in a 10% levamisole solution and fixed in a drop of 2% agarose to a glass slide. They were imaged on a Nikon spinning disc confocal microscope at 600X.

Genotyping

To confirm the presence of the correct insert in the fluorescent *C. elegans* a lysate was made from the three strains exhibiting mCherry. The lysis buffer contained 3uL proteinase K (60ug/mL), 10uL tris-Cl (pH 8.3, 10mM), 50uL KCl (50mM), 2.5uL MgCl2 (2.5mM), 4.5uL NP40 (.45%), 4.5uL tween-20 (.45%), 5uL gelatin (.01%), .843mL H2O. 20-30 adult *C. elegans* were picked into the lysis buffer and placed in the –80C freezer for 10 minutes. The *C. elegans* were then placed in the thermocycler at 65C for one hour followed by 95C for 20 minutes. After the lysis, 1uL of lysate, 32.5uL deionized H2O, 10uL 5X Phusion green HF buffer, 1uL dNTP (10mM), 2.5 uL external reverse primer (10uM), 2.5uL external forward primer (10uM), .5uL Phusion (Thermo Fisher, 2 U/uL) was used in a PCR reaction. The product was run on a 10% agarose gel at 100V for 50 minutes with a 100bp purple ladder (New England BioLabs, 50 ug/mL). The PCR was purified following the specifications in the Qiagen MiniElute purification kit, measured for concentration (see Table 1.), and sent for sequencing.

Sequencing

Sequencing was carried out at the MDI Biological Laboratory Sequencing Core. Six DNA samples were used from the worm lysate specified above. Each sample contained 0.8uL of primer (either forward or reverse), 10ng of template for every 100 bases of PCR fragment length, and deionized H2O to bring the volume to 24uL. Sequences were aligned to the gBlock using Geneious software.

Results

Coimmunoprecipitation followed by mass spectrometry of P granule constituent component, GLH-1, identified CLU-1 as a protein that interacts with GLH-1. To study the interaction between CLU-1 and GLH-1 *in vivo*, we used CRISPR/Cas9 to incorporate a V5 and

mCherry tag onto CLU-1. Following confirmation of successful editing, *in vivo* fluorescence microscopy was used to characterize the interaction between GLH-1 and CLU-1.

Generating a V5 and mCherry tag

To learn about the interactions between GLH-1 and CLU-1, the epitope V5 and fluorescent marker mCherry were added to the N-terminus of CLU-1. This was accomplished using the enhanced recombination technique following the Mello Lab overhang method and Co-CRISPR injections (Fig 2).

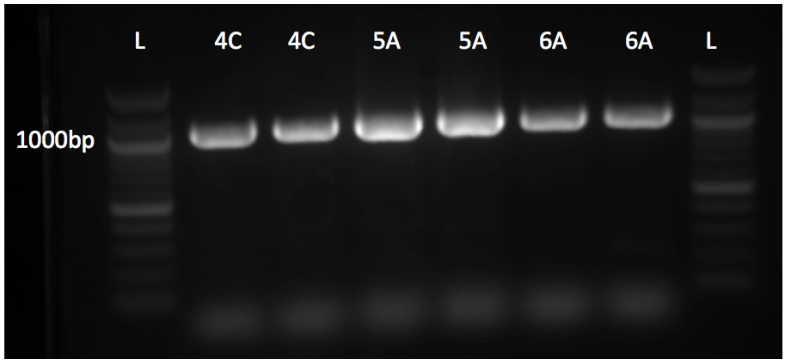

Figure 3. Genotype results of CRISPR/Cas9 edited *C. elegans*. Duplicates of three strains (4C, 5A, 6A) of fluorescent *C. elegans* were genotyped to confirm proper insertion of the gBlock construct based on length. Expected band size was 1077 base pairs. 100 base pair ladder (L) can be seen in the rightmost and leftmost lanes.

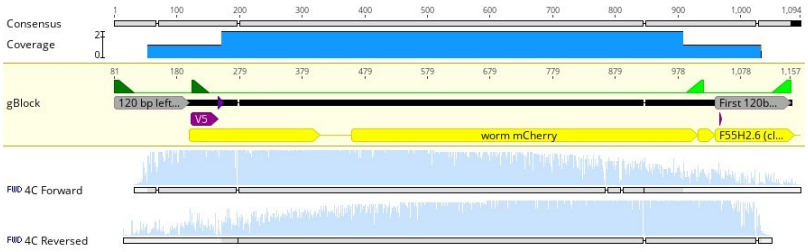

Figure 4. Sanger sequencing results of DUP-201 in Geneious. Following successful genotyping, each sample was sent for sequencing at the MDI Biological Laboratory Bioinformatics Core. Sequences were aligned to the gBlock using Geneious.

Genotyping and sequencing to confirm proper insertion of the construct

Successful addition of V5 and mCherry to *clu-1* was confirmed though preliminary genotyping progeny and finally, Sanger sequencing. Genotyping results confirmed an insertion of the proper 1077bp PCR product (Fig. 3). Once the correct size insertion was confirmed, progeny was sequenced to confirm an insertion of proper length and orientation (Fig.

4). Sequencing confirmed the construct was inserted correctly and the novel strain was named DUP201 *(glh-1(sam24[glh-1::gfp::3xFlag])clu-1(sam25[clu-1::mCh::V5]).*

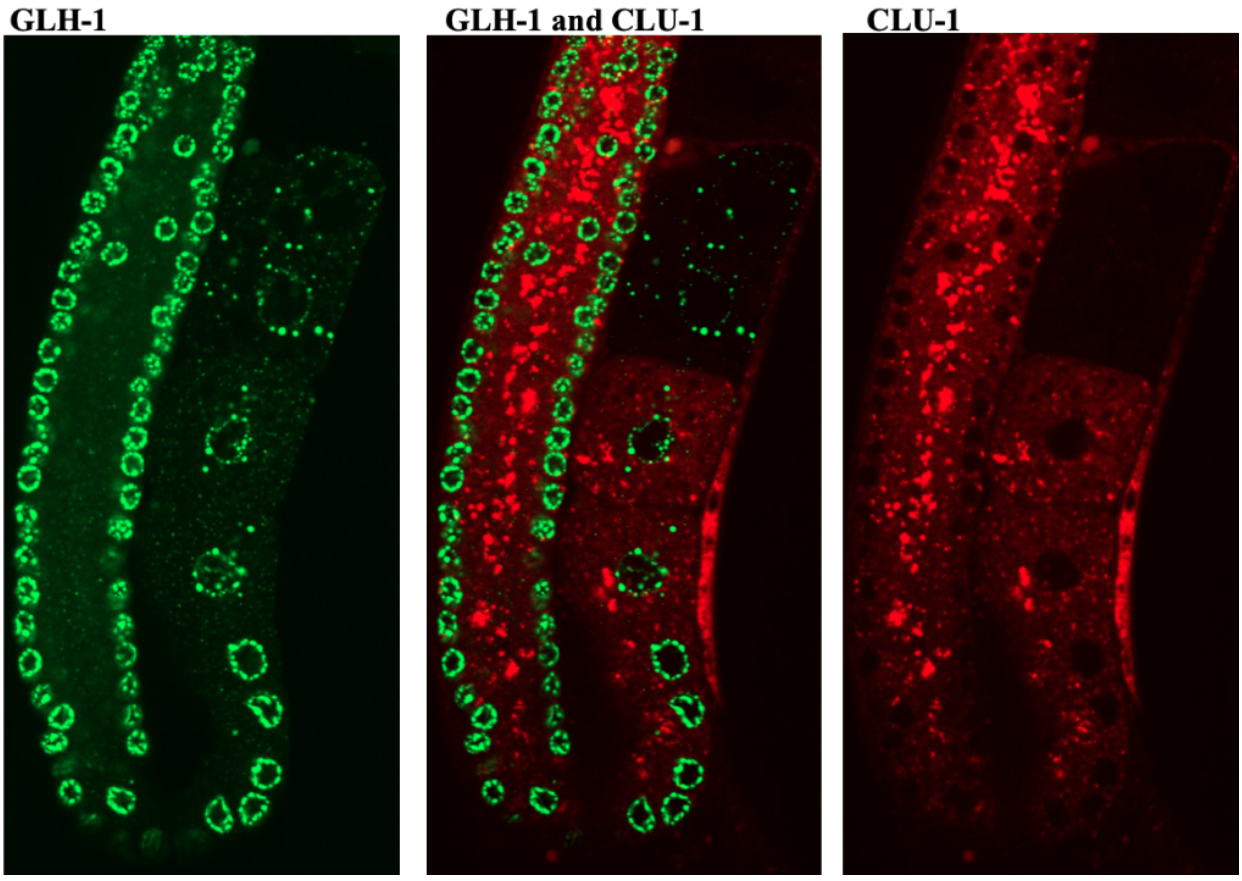

Figure 5. GLH-1 and CLU-1 localization in the germline of *C. elegans* strain DUP-201. Essential P granule scaffolding protein, GLH-1 was tagged with GFP and 3XFLAG, it can be seen in green. CLU-1 was tagged with mCherry and V5, it can be seen in red. GLH-1 can be seen clustering around the nuclear periphery. CLU-1 exhibits some docking behavior with GLH-1 and is mostly present in a granular structure in the rachis. GLH-1 can be seen dispersing as the oocyte matures. CLU-1 is not expressed in the P(-2) oocyte. Images were taken of adult hermaphrodites. The P(-2) oocyte is two oocytes before fertilization.

CLU-1 is dynamic, forming aggregates in the rachis and colocalizing with GLH-1

In order to identify the location of CLU-1 in the germline, we performed fluorescence microscopy. CLU-1 is mainly present in aggregates in the rachis and sequestered on the southwestern side of the nucleus in formed oocytes (Fig. 5). CLU-1 aggregates sparsely decorate some P granules, docking on an individual P granule or docking between two P granules (Fig. 5). This docking activity, along with the previous coimmunoprecipitation and mass spectrometry performed on GLH-1, provides further evidence that GLH-1 and CLU-1 colocalize *in vivo*.

Although mainly found in aggregates, there is also dispersion of CLU-1 in the rachis and formed oocytes (Fig. 5).

P granules disperse and CLU-1 is not expressed in the most proximal oocyte, P(-1), and sometimes P(-2) oocyte

Consistent with previous studies, P granules seem to detach from the nuclear pore complex and disperse in the cytoplasm in the mature oocyte. Imaging reveals that this detachment occurs as early as P(-3) (Fig. 5). CLU-1 is not detectable in the P(-1) and is often cleared from the P(-2) as well. CLU-1 expression changes drastically in the mature oocyte, from full expression to complete absence of expression (Fig. 5).

Discussion

CLU-1 may act as a shuttle protein

It is likely that the CLU-1 aggregates in the rachis are sites of translation. The observed colocalization between CLU-1 and GLH-1 in P granules and the presence of CLU-1 in the rachis could suggest a role as a shuttle protein. If CLU-1 does comprise eIF3a, it could function to transport mRNA from P granules to ribosomes for translation. There is little known about the dynamics of transcript shuttling from P granules to the cytoplasm. eIF3's known role in translation initiation and affinity for both ribosomes and mRNA make it an ideal candidate to shuttle transcripts from P granules to ribosomes.

CLU-1 location in the rachis may be indicative of previously unobserved GLH-1 expression

Although CLU-1 likely has GLH-1 independent roles, surprisingly little colocalization was observed between GLH-1 and CLU-1. Based on the level of enrichment of CLU-1 in the coimmunoprecipitation and mass spectrometry of GLH-1, this lack of colocalization may suggest additional interactions between GLH-1 and CLU-1 other than that observed in P granules with microscopy. Although CLU-1 is typically found in large aggregates in the rachis or interacting with GLH-1 in P granules, small aggregates of CLU-1 are present throughout the cytoplasm (Fig. 5). This phenomenon could be explained by the presence of small amounts of GLH-1 in the cytoplasm or a GLH-1 independent role. It is unknown if GLH-1 has a role outside of P granules but preliminary data suggests GLH-1 may be present and extremely dynamic in the germline cytoplasm (Updike, personal communication).

CLU-1 expression in mature oocytes

CLU-1 is not expressed in the P(-1) and often P(-2) oocytes. The variation in CLU-1 clearing P(-2) is likely due to P(-1) maturity. The closer P(-1) is to fertilization, the more likely P(-2) will lack CLU-1. Absence of CLU-1 in the mature oocyte may indicate the presence of a different initiation factor, effectively replacing CLU-1, or repression of translation in the cell.

Upcoming future studies

Upcoming future studies aim to better characterize CLU-1 and GLH-1 interactions and as discern the function CLU-1 in germ cells through knockout experiments, live imaging, and split sfGFP. Studying the CLU-1 knockout phenotype could shed light on CLU-1 function in the germline. Live imaging of the DUP201 germline will allow for a more complete visual characterization of CLU-1 and GLH-1 activity. Using the split sfGFP model described by Hefel and Smolikove in 2019, GLH-1 and CLU-1 colocalization can be better observed and quantified. The split sfGFP method will test hypothesized activity of GLH-1 in the cytoplasm and give precise docking activity in P granules. This method splits GFP into two parts of not necessarily equal size. Such a flexible approach allows for to strategic CRISPR/Cas9 tagging of proteins with varying sized fragments of GFP at two different loci (Hefel & Smolikove, 2019). When the loci interact, green florescence is produced.

Finally, coimmunoprecipitation followed by mass spectrometry of CLU-1 will be carried out to confirm GLH-1 docking activity and identify other proteins that interact with CLU-1. These proteins can be tagged using the methods described in this paper to determine their role in P granule function.

Broader Impacts

Germ granules are crucial for normal germ line development. They are one, of possibly many, cytoplasmic factors that regulate translation and guard the germline. In some cases, transplantation of germ granules is sufficient to confer multipotent properties, imbuing the host cell with the ability to produce sperm or egg cells. Studying the P granules of *C. elegans* is an effective model to identify components involved in maintaining and conferring germline pluripotency.

Mounting evidence suggests that genes originally thought to be germ line specific may have a more diverse role. Many of these previously germline specific genes have important roles in somatic cells and in some cases, contribute to tumorigenesis (Poon et. al., 2016). GLH homolog VASA, has been shown to have a highly regulated function in somatic cells and an indispensable role in the regulation of germline cells and regenerating tissues (Poon et. al., 2016). In some somatic cases, VASA may influence the efficiency and location of translation and have an impact on cellular dysfunction and tumorigenesis (Poon et. al., 2016). Ectopic expression of VASA is currently used as a diagnostic tool to aid in establishing cancer prognosis. Studying GLH may provide avenues to prevent tumorigenesis. Domains such as the GLHs, that were once thought to be germ line specific, may have a larger role to play and could offer key insights into cancer biology.

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Appendices

Table 1.

Sample	Concentration	Units	260/230	260/280
Clu-1 Hybridized DNA	832.715	ng/uL	2.19	1.88
DUP-201 Lysate DNA	269.961	ng/uL	1.75	1.88