Protein synthesis regulation in the germ line affects gamete differentiation

Mentor: Brett D. Keiper, Ph.D

Department of Biochemistry at the Brody School of Medicine

Jacob Subash

Jacob Subash

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102 Churchside Drive Greenville, NC

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During germ cell differentiation, embryogenesis and oncogenesis, eIF4E genes are highly expressed. This translation initiation factor regulates the selection of mRNAs for translation encoding new proteins. The hermaphroditic nematode worm, Caenorhabditis elegans, has five genes encoding eIF4E (IFE) isoforms (Keiper 2000). The *ife* genes identify the 5'-cap on mRNAs and recruit them to the 40S subunit of the ribosome for translatio (Keiper 2000). Therefore, IFE proteins play a critical role in translation control by moderating initiation, the most regulated part of protein synthesis (Dinkova 2005). Identifying the roles of specific IFE isoforms in differentiation is part of understanding mRNA translational control during animal development. Essentially all eukaryotic organisms express several eIF4E proteins (Song 2010).

My two objectives in this project were to derive *ife-3*, a deletion strain, males to determine if they were fertile, and to examine the gonads of *ife-3* worms via microscopy. IFE-1 and IFE-3 are two isoforms found in the worm that play crucial roles in the development of meiotic germ cells (oocytes and sperm). Previous research has determined that loss of *ife-1* cripples spermatogenesis and, to a lesser extent, oogenesis (Henderson 2009). Worms lacking IFE-1 have lower fertility compared to wildtype worms (Amiri 2001). The ife-1 mutants did not translate pos-1, pal-1, mex-1 and oma-1 mRNAs efficiently relative to wildtype worms (Henderson 2009). The mutants had an 80% decrease in fertility at 20°C, and were infertile at 25°C. IFE-3 is another essential isoform that is expressed in both germ cells and embryos

(Keiper 2000). Initially, *ife-3* was ascertained to be completely embryonically lethal, but worms lacking the gene generally arrest at the embryonic stage; approximately 10% "escape" that restriction and develop into infertile, adult hermaphrodites (Keiper 2000). These IFE-3-deficient worms are infertile because due to a lack of oocytes. Due to the infertility of the *ife-3* worms at all temperatures a balanced strain was derived. The wildtype *ife-3* gene was fused with a gfp (green fluorescent protein) marker and a homozygous lethal gene. This resulted in a homozygous modified "wildtype" that was embryonically lethal due to the lethality gene, and the homozygous null mutant was lethal due to the characteristics of deletion strain, except for forementioned sterile escapers. The heterozygous worms that had a single copy of the modified wildtype gene and the deletion gene were also viable. Therefore, the offspring of the heterozygously balanced strain were also heterozygous or were homozygous deletion escapers.

Deriving males of the *ife-3/gfp* balanced strain will be useful for crossing the *ife-3* gene into other strains. C. elegans are usually hermaphroditic (XX), but one in thousand worms are male (XO) due to spontaneous non-disjunction of the sex chromosome (Lints 2009). To increase the chance of getting a male, the strain can be "heat shocked" at 32°C for six hours in larval stages 3-4 (Lints 2009). This often times boosts the chances of generating a male due to the spermatogenic abnormities that occur at higher than normal temperatures in all sexually reproducing organisms. However, heat shocking the *ife-3* strain did not generate any observable males. Thus, another approach was needed. So, I crossed 2 *ife-3/gfp* hermaphrodites with 6 wildtype (N2) males. The offspring follow normal Punnett square genetics. The gfp minus male F1 offspring had the *ife-3 (+/-)* genotype. 10 of the resulting males were then crossed with 4 *ife-3/gfp* hermaphrodites. The F2 offspring that expressed gfp were either *ife-3 (g/+)* or *ife-3 (g/-)*; gfp males were then clonally picked and crossed with *ife-3/gfp* hermaphrodites. The genotype

was determined via polymerase chain reaction (PCR) and gel electrophoresis. This process acquired the *ife-3/gfp* males. We then determined the fertility of ife-3 males to assess sperm viability. Male ife-3 worms were crossed into the fem-2 strain, which doesn't produce sperm. Since the *fem-2* strain only produces oocytes any F1 offspring from the cross result from *ife-3* fertilization. The experiment was conducted at 20°C. The crosses were set up at 5 males to 2 hermaphrodites. N2s were used as control males, and *ife-1* hermaphrodites were used as controls. Our data, in Table 1, demonstrated that the *ife-3* males were fertile and able to produce fecund sperm. In some of the crosses the mothers were not able to be identified because they either died, or they were indistinguishable from their daughters.

The second part of this project was viewing the gonads of the *ife-3* mutants after immunostaining under a microscope. The gonads were excised and fixed in 3% paraformaldehyde and phosphate buffer saline. The gonads were then stained with DAPI, to visualize DNA, or antibody stained for the sperm marker 56 (that is only expressed in sperm cells). All pictures were taken in the 40x air objective. The DAPI images for the N2, *ife-3/gfp* heterozygote, and *ife-3* hermaphrodite gonads are shown in figures 1 and 2. The wildtype and the heterozygote have normal adult gonads with mitotic stem cells, in the distal end, that differentiate into oocytes with bivalent chromosomes in the proximal end. Sperm only exists at the end of the gonad in the spermatheca. However, the *ife-3* mutant has cells with nuclei that are condensed tightly akin to sperm cells. The gonads were then antibody stained with the sperm marker sp-56 in figure 3. The color coded images illustrate that the *ife-3* mutant gonad does have sperm in the proximal end like a male gonad instead of maturing oocytes found in hermaphrodites. This indicates that the *ife-3* mutants express a phenotype that masculinizes the gonad. However, only the gonad is masculinized, the rest of the body resembles a hermaphrodite.

Thus, the IFE-3 protein is critical for oocyte production. Worms that do not express this protein revert back to sperm production as adults. My survey of gonad morphology and immunostaining indicate that all *ife-3* mutant germ cells are converted to sperm that is viable. This leads me to hypothesize that IFE-3 is working to determine germ cell differentiation.

A few future experiments with the *ife-3* worms would be to test the expression of the phenotype at different temperatures ranging from 15°C to 25°C and a 32°C heat shock. Sperm production is sensitive to temperature, and the cell fate might change as it does with *ife-1* deficient worms. Furthermore, creating a double mutant that lacks *ife-3* and *ife-1* would illuminate how gamete differentiation is affected by their translation initiation factors. If the double mutant is not able to be created, then RNA interference can be utilized to illustrate the same results.

Data

Table 1: Fertility crosses

Name (male x herm.)	#mothers alive	Male F1s	Herm. F1s	Note
ife-3 x fem-2 A	1	22	26	1 mother dead
ife-3 x fem-2 B	2	25	30	
ife-3 x fem-2 C	?	46	54	
N2 x fem-2 A	1	41	32	
N2 x fem-2 B	?	71	78	starved 1 day

N2 x fem-2 C	?	68	71	starved 1 day
ife-3 x ife-1 A	2	17	10	
ife-3 x ife-1 B	?	68	69	1 mother dead
ife-3 x ife-1 C	?	99	101	starved 1 day
N2 x ife-1 A	1	54	70	starved 1 day
N2 x ife-1 C	?	66	66	starved 1 day
N2 x ife-1 C	?	93	84	starved 1 day
NON-CROSS CONTROL				
2 ife-1 herm. A	2	0	0	
2 ife-1 herm. B	?	0	35	
2 ife-1 herm. C	2	0	1	
2 fem-2 herm. A	1	0	0	
2 fem-2 herm. B	2	0	0	
2 fem-2 herm. C	2	0	0	
2 ife-3 herm. A	0	0	0	both mothers died
2 ife-3 herm. B	2	0	0	
2 ife-3 herm. C	2	0	0	

Figure 1: Wild type adult gonad stained with DAPI.

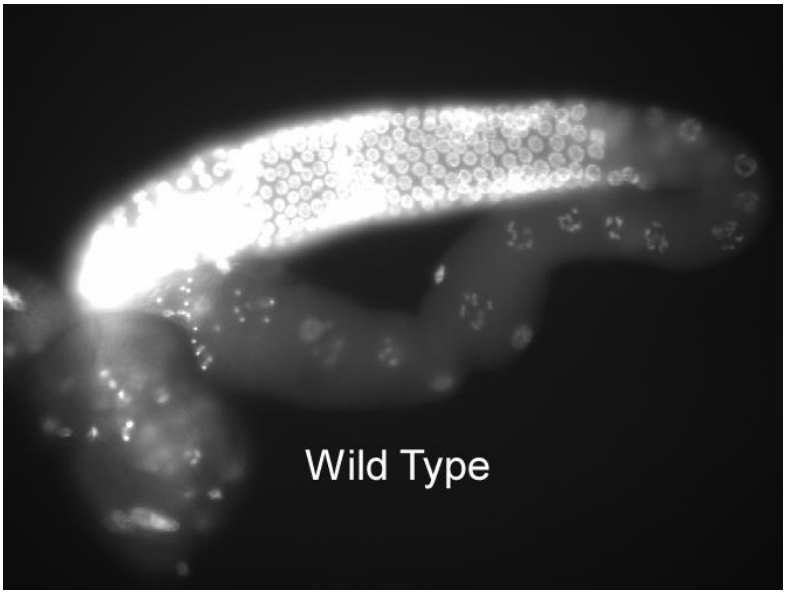

Wild Type

Figure 2: *ife-3*, and *ife-3/gfp* adult gonads stained with DAPI

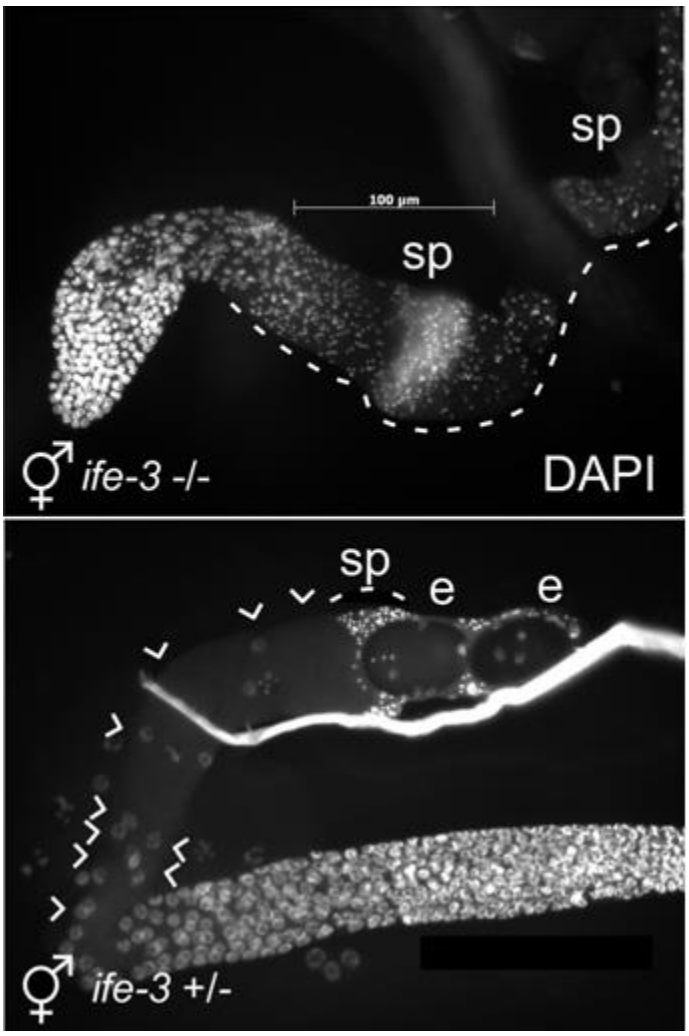
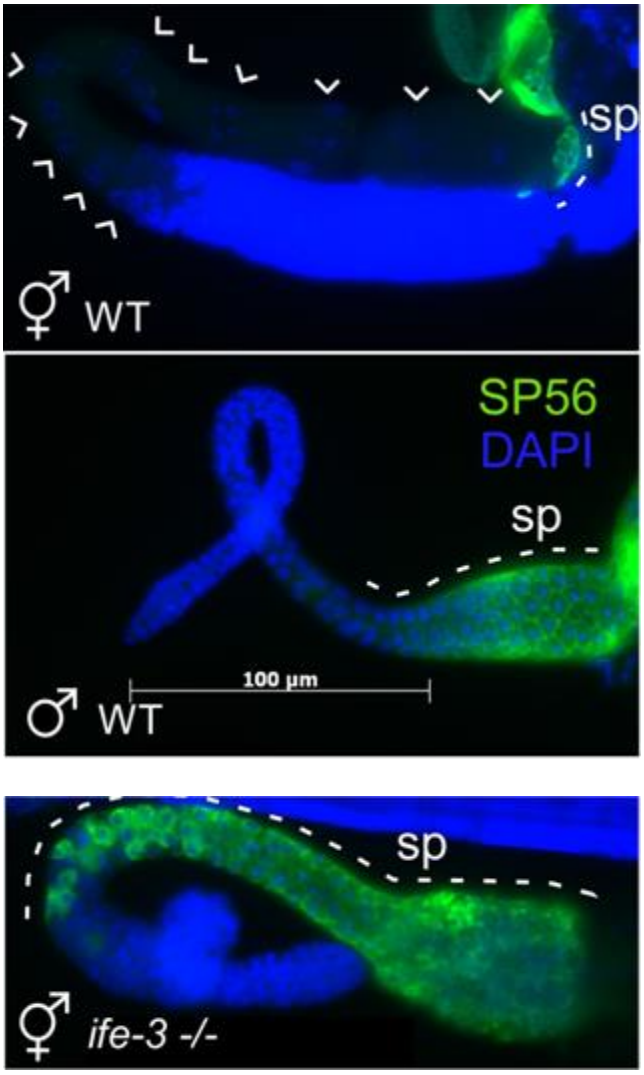


Figure 3: Wildtype hermaphrodite, wildtype male and *ife-3* hermphrodite adult gonads stained with DAPI and sp-56. Staining is color coded.



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