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Mahmoud ElJalby April 15, 2015

Characterization of H3.3 Histone Variant HIS-74 in the Germline of *Caenorhabditis elegans*

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Abstract

Characterization of H3.3 Histone Variant HIS-74 in the Germline of *Caenorhabditis elegans*
By Mahmoud ElJalby

Histone proteins wrap DNA to form nucleosomes and compact eukaryotic chromosomes. Histones exist as variants that play significant roles in gene regulation and expression. Histone variant H3.3, which is structurally similar to histone H3, is associated with transcription. Three H3.3 histone variants (HIS-71, HIS-72 and HIS-74) have been identified in *C. elegans* but of these only HIS-71 and HIS-72 have been partially characterized. This study seeks to characterize the role and function of HIS-74, an H3.3 variant that differs from the other H3.3. variants. A GFP-tagged HIS-74 transgene demonstrates germline-specific expression with differences in its stability within the genome between sperm and oocytes. A strain carrying a deletion allele that removes both *his-74* and an adjacent gene, *ccch-3*, exhibits embryonic lethality. RNA interference (RNAi) of the *ccch-3* gene alone exhibits no significant lethality, indicating that deletion of the *ccch-3* gene is not responsible for the lethal phenotype in the strain. Mapping and outcrossing experiments could not separate the lethality from the deletion, further implicating the loss of *his-74* in the lethality. However, a mutation with a premature stop codon in *his-74* has no phenotype and we have not yet been able to rescue the mutant strain with a transgene covering the deletion. A *his-74*$^{-/-}$ knockout line is currently being generated using CRISPR/Cas9 technology. Further studies will seek to better understand the HIS-74 function and interplay with the other two H3.3 histone variants HIS-71 and HIS-72.

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Honors Thesis

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Introduction

Histones are small, highly conserved eukaryotic proteins which the DNA wraps around to form nucleosomes to compact the chromatin while still allowing cellular processes such as replication, DNA repair and transcription (Szenker, Ray-Gallet, & Almouzni, 2011). The nucleosome is formed by packaging about 147 base pairs (bp) of DNA with a tetramer of $(H3\text{-}H4)_2$ as well as two dimers of H2A-H2B histone proteins (Luger, Mäder, Richmond, Sargent, & Richmond, 1997; Ramaswamy & Ioshikhes, 2013). Histone proteins are generally categorized into "canonical" and "replacement" proteins. Canonical histones are defined as histone proteins with an expression level that peaks during the S phase and are hence implicated in DNA replication (Szenker et al., 2011). Replacement proteins are defined as histone proteins with an expression level that does not peak during the S phase or are expressed throughout the cell cycle (Szenker et al., 2011). These histone proteins are incorporated into the nucleosomes in a replication-independent manner (Szenker et al., 2011).

Five H3 variants have been previously identified: two are canonical variants—H3.1 and H3.2—and three replacement variants—H3.3, the centromere-specific CenH3 (or CENP-A in mammals) (Allshire & Karpen, 2008) and the testis-specific H3t (Witt, Albig, & Doenecke, 1996). The H3.3 histone variant, found in all eukaryotes, is a replication and cell-cycle phase independent variant and is incorporated at sites of active transcription (Ahmad & Henikoff, 2002; Chow et al., 2005; Gabrielli et al., 1984; Mito, Henikoff, & Henikoff, 2005). Recent findings suggest an increasingly important role for the H3.3 histone variant in regulating transcription and epigenetic memory (Szenker et al., 2011). Indeed, H3.3 is enriched in actively

transcribed genes and its depletion has been associated with silent chromatin (Hake et al., 2006; McKittrick, Gafken, Ahmad, & Henikoff, 2004; Stroud et al., 2012; Waterborg, 1990). Recently, pediatric glioblastoma has been associated with H3.3 mutations leading to defects of chromatin architecture (Schwartzentruber et al., 2012).

While only one H3.3 gene—H3F3A—has been identified in humans, with the other genes shown to be pseudogenes (Wells, Hoffman, & Kedes, 1987), three H3.3 histone variants—HIS-71, HIS-72 and HIS-74—have been previously identified in *Caenorhabditis elegans* (Ooi, Priess, & Henikoff, 2006). While all three H3.3 variants have the H3.3-specific AIG residues at positions 87, 89 and 90, HIS-74 is uniquely lacking the conserved serine residue at position 31 (Ooi et al., 2006). Little is known about the role of HIS-74, however. Previous data have shown that HIS-74 expression is restricted to the germline and varies across development (Figure 1; Tengguo Li, personal communication). In the present study, we sought to characterize HIS-74 expression and possible functions.

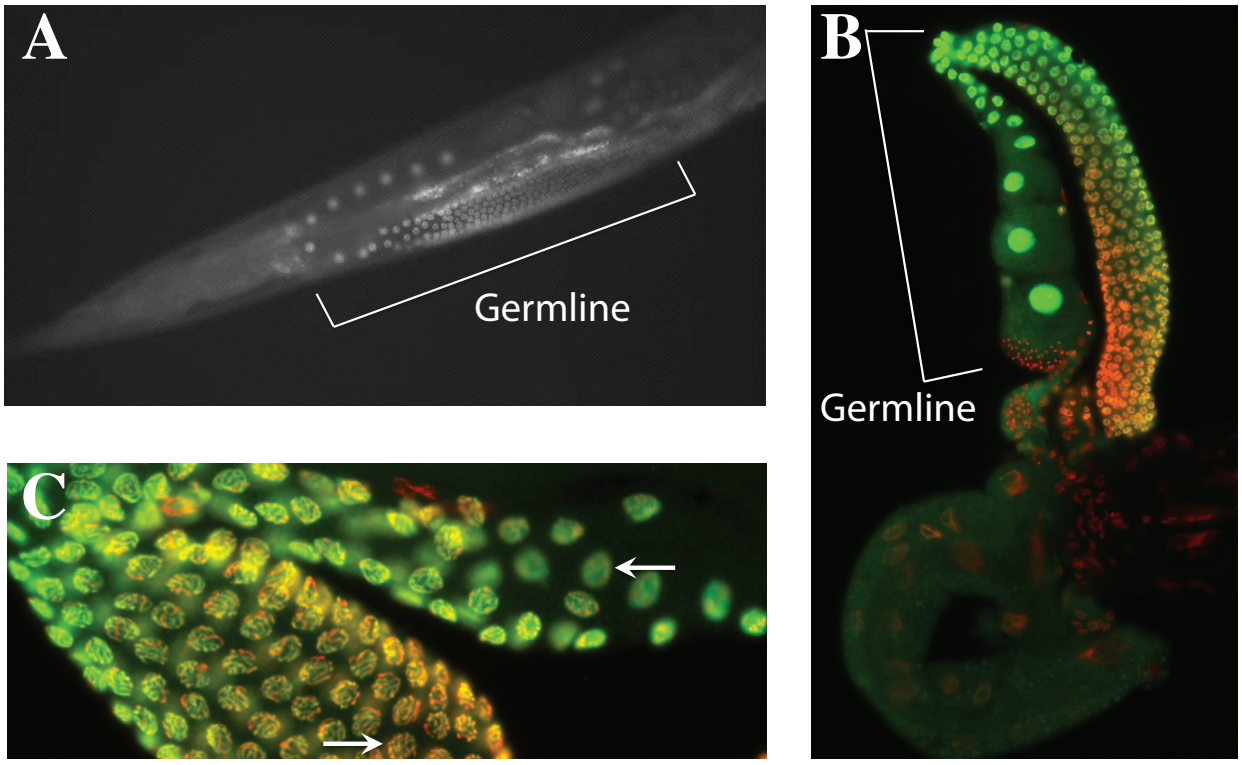

Figure 1. HIS-74 expression is restricted to the germline. A *C. elegans* line expressing a *his-74*::GFP tagged transgene was used. (A) Image of GFP in adult worms. Immunostaining of adult *C. elegans* with anti-GFP (green) and DAPI (red) (B) HIS-74 expression seems germline specific. (C) HIS-74 is tightly associated with DNA at early stages (left arrow pointing to the right) before coming off of it at later stages in germline development (right arrow pointing to the left).

Results

The *his-74* Deletion May be Responsible for Embryonic Lethality

C. elegans contain an operon of the *his-74* and *ccch-3* genes (Figure 2). A previous study in our lab found that a deletion of the *ccch-3* and *his-74* DNA fragment in *C. elegans* (Figure 2) results in a complete embryonic lethality (T.M. Edwards, personal communication).

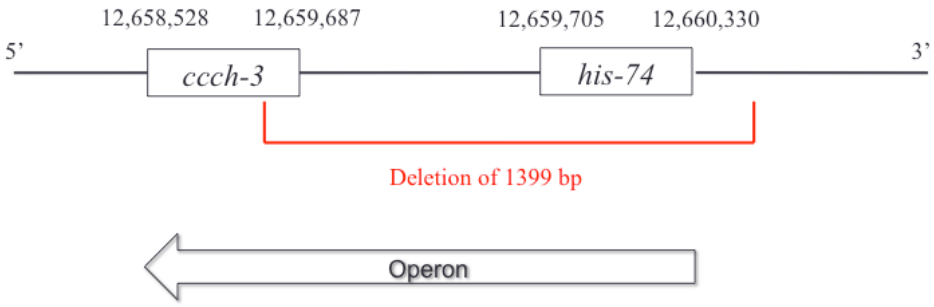

Figure 2. Map of the 1.4 kb DNA deletion that removes the *his-74* gene and a portion of the *ccch-3* gene in the *ok1219* allele on chromosome V.

A *ccch-3* RNAi on wild-type (N2) *C. elegans* from larval stage 1 (L1) was hence conducted to determine whether the *ccch-3* deletion causes, by itself, the observed lethal phenotype. RNAi of the RNA Polymerase II *ama-1* subunit or control RNAi (only the L4440 plasmid backbone) on N2 *C. elegans* were used as positive and negative controls, respectively. The number of hatched embryos from the total number of embryos was then counted. Results showed that *ccch-3* gene silencing produced 0.8% lethality, similar to the negative control (0.64% lethality) and in contrast to the positive control (100% lethality, Table 1). These results suggest that loss of *ccch-3* is not responsible for the *C. elegans* embryonic lethality.

Table 1. Embryonic lethality of *ccch-3*, *ama-1* and L4440 RNAi.

	ccch-3	***ama-1***	**L4440**
Embryonic lethality (%)	0.88	100	0.64

The Lethality of the *his-74* Deletion is Inconclusive

While the RNAi results suggest that the *ccch-3* gene may not affect *C. elegans* embryonic development, it remains important to test the effect of *his-74* on this phenotype. To this end, we attempted to rescue the *his-74* deletion from the *ok1219* strain by a transgene containing the

entire operon and expressing a C-terminal GFP-tagged version of HIS-74. The *ckIs32* transgenic line was prepared by injecting a *C. elegans* MOS-SCI line with the *ccc-h3* and *his-74:GFP* constructs in pBABH74d plasmid (Elizabeth Bowman, personal communication). After the transgene was homozygosed, the line was crossed to *his-74* (*ok1219*) deletion mutant and a homozygous deletion of the endogenous *his-74* was sought. However, such a line could not be obtained: only worms heterozygous for the mutation or wild-type for the endogenous *his-74* were viable, independent of the presence of the transgene.

To further verify whether the *his-74* deletion is responsible for the lethal phenotype, two strains, *his-74* (*gk603106*)—containing a nonsense mutation that is predicted to result in a short, nonfunctional histone at position 56—and *his-74* (*gk619256*)—containing a missense G91E mutation that may detrimentally affect the protein function—were obtained from the Caenorhabditis Genetics Center (CGC) at the University of Minnesota (Figure 3). These strains were generated through the Million Mutation Project (Thompson et al., 2013). Locus sequencing and embryonic lethality tests were conducted to verify the presence of a *his-74* mutation and assess its effect on embryonic lethality. While both single nucleotide polymorphisms (SNPs) were present, a 0% embryonic lethality was observed for both alleles as well as an N2 control (data not shown).

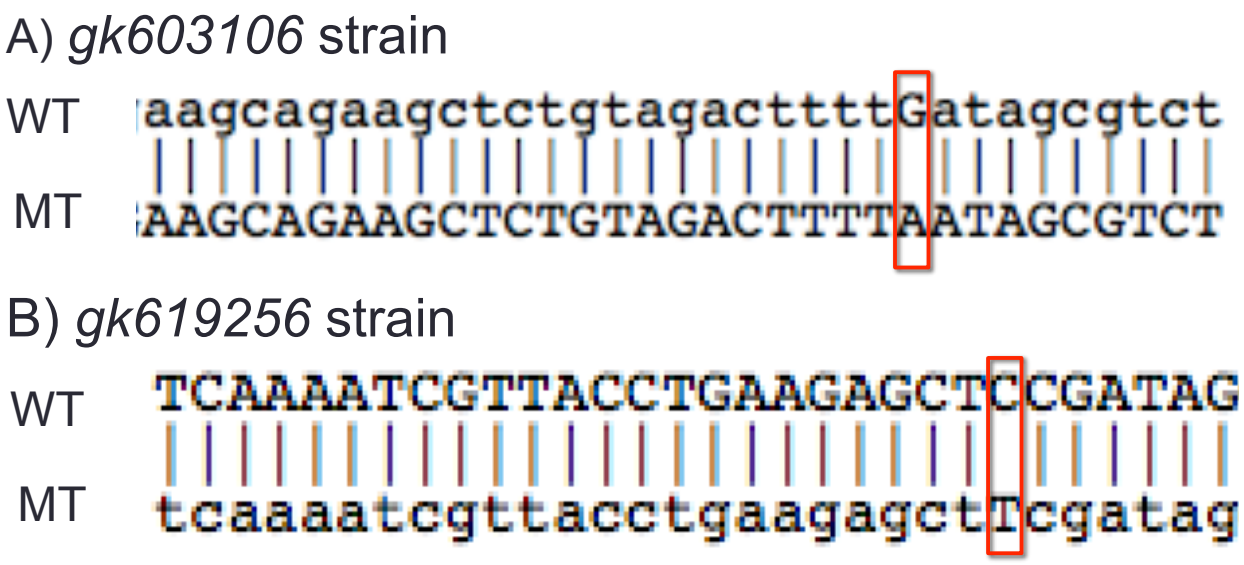

Figure 3. SNP mutations of the *his-74* gene on the A) *gk603106* strain with the Q56 to stop codon and B) the *gk619256* strain with the G91E mutation. The top sequence in each figure corresponds to the wild-type sequence whereas the bottom sequence corresponds to the mutated sequence. WT: Wild-type; MT: mutant.

These results suggested that the *his-74* deletion may not be responsible itself for the observed embryonic lethality in the *ok1219* strain. We hence hypothesized that the lethal phenotype is not due to the *his-74* gene but rather to a lethal mutation that is linked to the *his-74* gene. To test this hypothesis, and to separate the presumed second site mutation from the *his-74* mutation, the *his-74* (*ok1219*) *V* strain was crossed with a *dpy-11(e224) unc-76(e911) V* strain (Figure 4). Recombinants (*dpy/non-unc* or *unc/non-dpy*) were then screened by PCR and a viable homozygous strain was sought. A viable homozygous *his-74 deletion*/*dpy-11* or *his-74 deletion*/*unc-76* line would indicate that *his-74* is not responsible for the embryonically lethal phenotype. Conversely, the presence of a viable recombinant strain only in the presence of *his-74* gene would suggest that either the *his-74* gene or a mutation that is tightly linked to it—or both—is responsible for the lethal phenotype. No viable recombinant *C. elegans*—either

dpy/non-*unc* or *unc*/non-*dpy*—could be obtained with a homozygous deletion of the *his-74* (*ok1219*) *V* gene; only recombinants with either a homozygous or heterozygous wild-type *his-74* were viable.

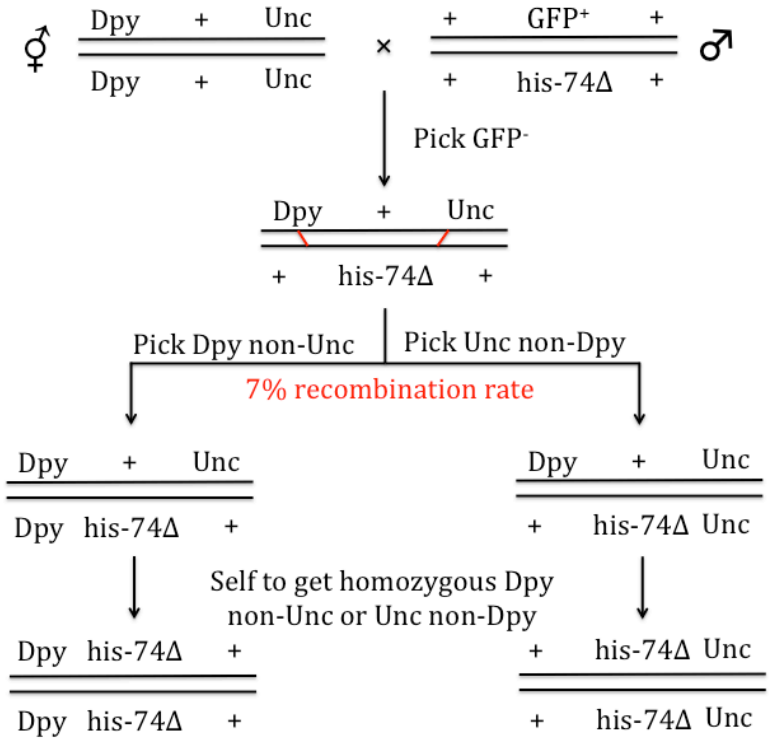

Figure 4. Outcrossing Strategy. The *his-74* (*ok1219*)/GFP balancer *V* strain was crossed with a *dpy-11(e224) unc-76(e911) V* strain. Following the cross, GFP^{-} *C. elegans* (*his-74/+)* were selected before picking recombinant *dpy*/non-*unc* or *unc*/non-*dpy* strains. Recombination rate was found to be about 7%, which matched the recombination rate from the genetic map. The recombinant progeny were then isolated in an attempt to obtain a homozygous *dpy*/non-*unc* or *unc*/non-*dpy* with a homozygous *his-74* deletion—and hence separating the hypothesized lethal mutation from the *his-74* deletion.

HIS-74::GFP Is Strictly Maternally Loaded in the *C. elegans* Embryo

To test whether HIS-74 is zygotically expressed or maternally loaded, a cross between *ckIs32* transgenic line males—which have the *his-74*::*gfp* gene—were crossed with *fem-1 (hc17) IV*, which becomes feminized at temperatures higher than 20^{o}C (Figure 5). The cross was

conducted at 25°C. Twenty-four hours later, the *fem-1 (hc17) IV* females were cloned on unseeded nematode growth media (NGM) plates before obtaining their embryos 24 hours later. Embryos at various stages of embryonic development were then screened for GFP expression. F1 embryos from the cross between the GFP-tagged HIS-74 transgenic line and the *fem-1 (hc17) IV* females did not reveal any GFP expression at all stages of embryonic development (Figure 6), suggesting that the HIS-74::GFP is strictly maternally loaded.

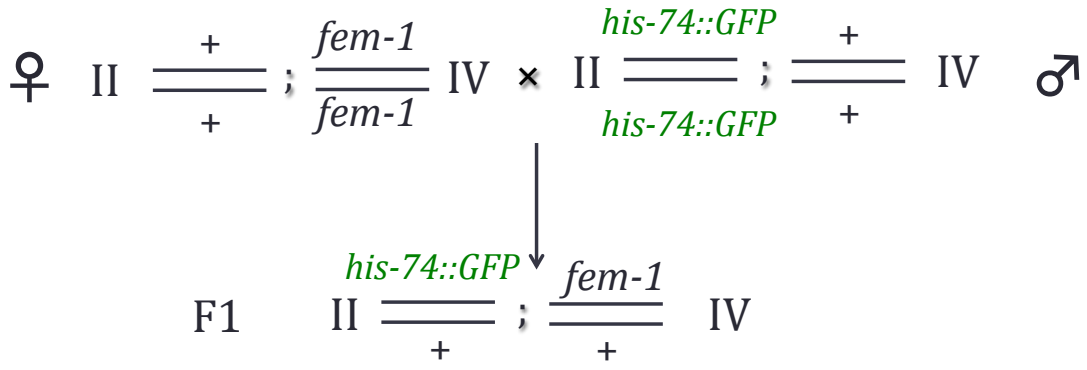

Figure 5. Map of the transgenic *ckIs32* line males and feminized *fem-1 (hc17) IV*.

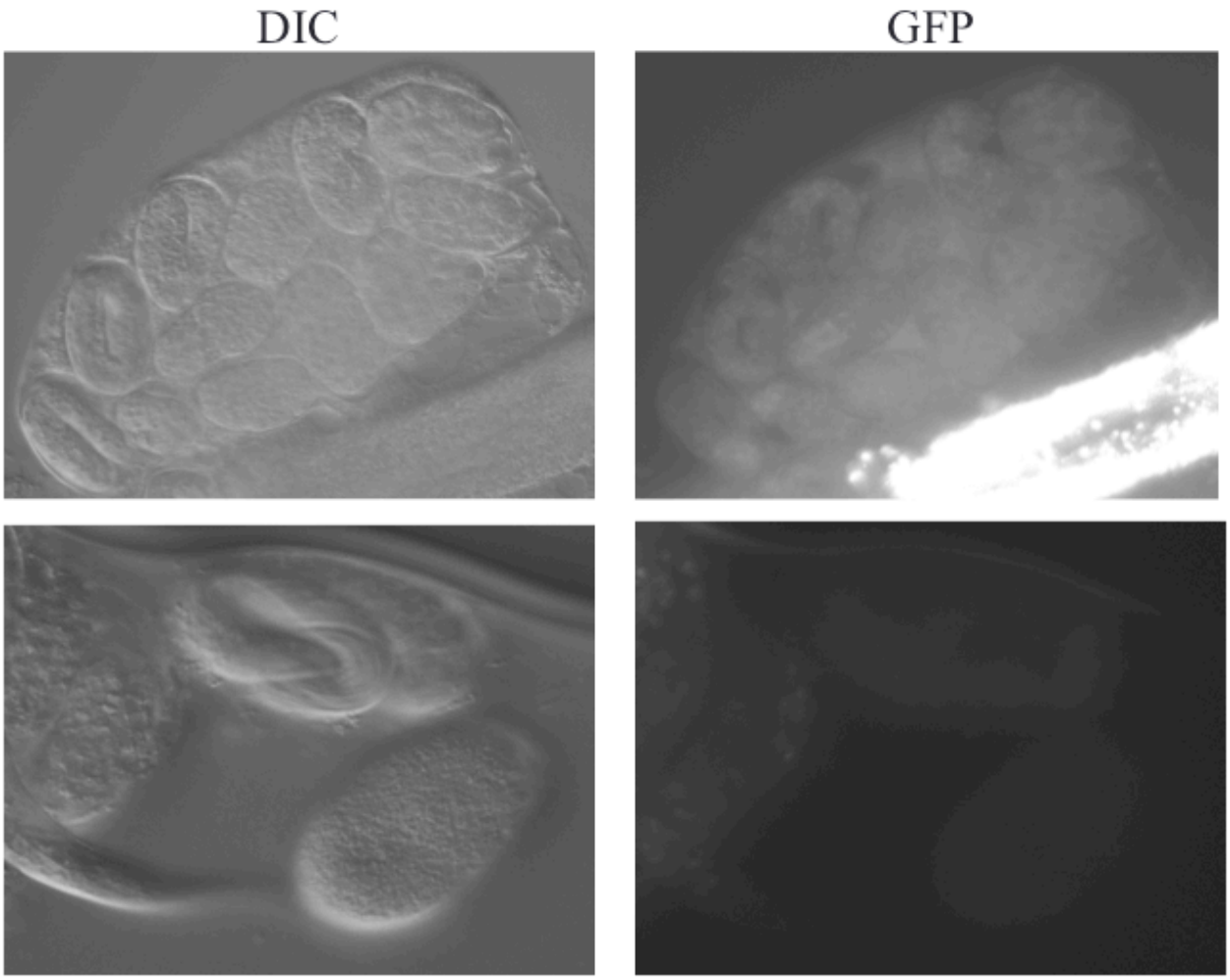

Figure 6. Fluorescence and differential interference microscopy images of the *his-74:GFP* transgenic line and feminized *fem-1 (hc17) IV* cross-progeny embryos. The images on the left are taken under differential interference contrast (DIC) conditions and correspond to the images on the right (under GFP conditions). The images on the top and the bottom are representative images of F11 embryos at different embryonic development stages. The GFP expression observed in the top image is auto-fluorescence from the *C. elegans* gut.

Conclusion

Histone proteins are constantly replaced during the dynamic remodeling of germ cell chromatin throughout gametogenesis and upon fertilization (Ooi et al., 2006). Likewise, several histone variants have been found to play important roles in embryonic development. Our results

are inconclusive with regard to the influence of *his-74* on embryonic development in *C. elegans*. Our finding that the deletion of the *ccch-3* and *his-74* DNA fragment causes complete embryonic lethality and that the silencing of the *ccch-3* gene did not affect embryonic lethality (Table 1) suggested that the *his-74* gene may play a role in this lethal phenotype. However, our inability to rescue the homozygous deletion of the *his-74* gene and the viable *his-74* (*gk603106*) strain containing an ochre stop codon at position 56 suggest that the *his-74* gene may not be involved in embryonic development. Nonetheless, it remains important to verify whether the early stop codon truly results in a truncated HIS-74. To this end, a transgenic line containing the mutated *his-74* gene tagged with GFP can be generated. In this line, GFP expression would suggest that the *his-74* gene is not silenced by the SNP whereas the absence of GFP expression in comparison to a control line with a wild-type *his-74* would suggest that the SNP does indeed silence the *his-74* gene.

It is also unclear whether a mutation separate from the *his-74* gene is responsible for the lethal phenotype. While the separation of a hypothetical lethal mutation outside of the *his-74* gene has not been successful, it still may be possible that the lethal mutation is tightly linked to the *his-74* gene. Consequently, knocking-out (KO) the *his-74* gene in N2 *C. elegans* using CRISPR/Cas9 will be important in order to later obtain a precise deletion of the *his-74* gene without any background mutations that may potentially interfere with the function of the histone variant.

Finally, our data suggest that HIS-74 is expressed in the germline and persists in sperm chromatin of *C. elegans* but is removed from oocyte chromatin (Figure 1). The inability to detect GFP expression in sperm by immunostaining (Figure 1B)—unlike the GFP expression seen by

microscopy (Figure 1A)—may be due to the compact nature of the DNA in mature sperm (Wu & Chu, 2008), suggesting that it is only a limit to the experimental approach.

Similarly, previous data have identified several histone variants with localized expression patterns, with some histone variants only expressed in the germline (Churikov, Zalenskaya, & Zalensky, 2004; Kimmins & Sassone-Corsi, 2005). Our results showing an absence of GFP expression in the developing embryos (Figure 6) following a cross leading to embryos with GFP-tagged HIS-74 (Figure 5) suggests that the *his-74* gene is strictly maternally loaded in *C. elegans* embryos. Previous studies have shown that maternal gene products drive the development of the early embryo (Schier, 2007). Taken together, our data on the complete embryonic lethality of *C. elegans* following the *his-74* deletion and our finding that HIS-74 strictly maternally loaded suggest that *his-74* is essential for early embryonic development. Recently, a study conducted in mice showed that the dynamic changes in the deposition of different H3 variants influence the functional organization of chromatin and are critical for chromatin reorganization during epigenetic reprogramming (Akiyama, Suzuki, Matsuda, & Aoki, 2011).

Future experiments should aim at studying the interplay between *his-74* and *his-71* and *his-72*. A triple KO of the three genes as well as a double KO of *his-74* and either *his-71* or *his-72* may be significant. To determine whether the expression of either *his-71*:*gfp*, *his-72*:*gfp* or *H3.1*:*gfp* changes in the *his-74* deletion mutant, *his-71*:*gfp*, *his-72*:*gfp* and *H3.1*:*gfp* transgenes will each be crossed into the *his-74* CRISPR-Cas9 deletion background. Changes in expression compared to N2 worms will then be monitored. A triple *his-71*, *his-72* and *his-74* mutant will also be obtained to determine whether the deletion of all three histone variants (instead of only one or two) would result in embryonic lethality.

To eliminate the putative background mutations carried in the *his-74* (*ok1219*) *V*; *ckIs32* (*unc-119, his-74:GFP*) *II* line as a result of the *his-74* (*ok1219*) *V* strain, the *his-74* gene will be deleted by CRISPR/cas9. N2 worms will be injected with the Peft-3::Cas9-*his-74*-sgRNA and Peft-3::Cas9-*unc-22*-sgRNA constructs in addition to pRF4 roller plasmid. Peft-3::Cas9-*his-74*-sgRNA guides the Cas9 enzyme to target the *his-74* gene and cause the deletion defined by the sgRNA. Peft-3::Cas9-*unc-22*-sgRNA targets the *unc-22* gene causing animals to become twitchers and serves as a positive control that the Cas-9 enzyme is functional. Finally, pRF4 plasmid causes *C. elegans* to become rollers when injected. It hence serves as a positive control for worms that have been successfully injected with the CRISPR/Cas9 plasmids. The F1 progeny will then be monitored: F1 twitchers will be genotyped by PCR for the *his-74* deletion while F1 rollers will be cloned out and twitchers in the progeny (F2) will be genotyped by PCR for *his-74* deletion.

Materials and Methods

Nematode strains and maintenance

Nematodes were cultured and manipulated as previously described (Brenner, 1974). All strains were grown at 20^{o}C, unless otherwise stated. The following strains were used: N2, *his-74 W05B10.1(ok1219) V/nT1 [qIs51] (IV;V)*, *his-74* (*gk603106*) *V*, *his-74* (*gk619256*) *V*, *fem-1(hc17) IV*, *dpy-11(e224) unc-76(e911) V*, *ttTi5605 II; unc-119(ed3) III; oxEx1578, his-74* (*ok1219*) *V*; *ckIs32* (*unc-119, his-74:GFP*) *II*,. The first nine strains were obtained from the Caenorhabditis Genetics Center (CGC) at the University of Minnesota (Table 2). The last, *ckIs32* transgenic line was made by injecting a *C. elegans* MOS-SCI line with *his-74:GFP* construct in pBABH74d plasmid into the *ttTi5605 II; unc-119(ed3) III; oxEx1578* strain. The transgenic line

was then crossed to *his-74* (*ok1219*) deletion mutant and a transgene rescue with a homozygous *his-74* deletion was sought.

The *his-74* (*gk603106*) strain has a nonsense mutation that is predicted to result in a short, nonfunctional histone whereas the *his-74* (*gk619256*) has a missense G91E mutation that may be significant. Locus sequencing and embryonic lethality tests were conducted to verify the presence of a *his-74* mutation and assess its effect on embryonic lethality.

Table 2. Strains obtained from the CGC.

Strain Name in CGC	Genotype
N2	*C. elegans* wild isolate
RB1221	*W05B10.1(ok1219) V/nT1 [qIs51] (IV;V)*
VC40373	*his-74 (gk603106) V*
VC40398	*his-74 (gk619256) V*
BA17	*fem-1(hc17) IV*
CB2065	*dpy-11(e224) unc-76(e911) V*
EG6699	*ttTi5605 II; unc-119(ed3) III; oxEx1578*

RNAi of the *ccch-3* gene

RNAi of the *ccch-3* was conducted as previously described (Timmons & Fire, 1998). Briefly, liquid cultures were prepared using lysogeny broth (LB) media supplemented with ampicillin (50 μg/mL) and tetracycline (12.5 μg/μL). *Ama-1* and L4440 liquid cultures were prepared as positive and negative controls, respectively. All cultures were incubated in 37^{o}C for 24 hours, shaking at 250 rpm. Each plasmid reaction was then seeded on 12 NGM, which were

supplemented with ampicillin (10 μg/ mL) and Isopropyl β-D-1-thiogalactopyranoside (IPTG, 1 mM).

Worms were prepared as previously described (Mukhopadhyay, Deplancke, Walhout, & Tissenbaum, 2008). Briefly, N2, *his-74* (*gk603106*) and *his-74* (*gk619256*) worms were washed twice in M9 and centrifuged at 600*g* for 1.5 minutes. The pellet was then incubated in a bleach solution (1 mL of commercial Clorox, 0.1 mL of 10N NaOH and 3.9 mL of water), vigorously vortexing for 30 seconds every minute before centrifuging the solution at 600*g* for 1.5 minutes and washing the pellet twice as previously described. The pellet was resuspended and plated on unseeded NGM plates incubated at 20°C. The *his-74* (*gk603106*) and *his-74* (*gk619256*) embryos were plated on NGM plates seeded with OP50 *E. coli* and incubated—along with N2 controls—at 20°C and another set incubated at 25°C. Embryos were counted at the time of plating and two days after.

Obtaining L1 *C. elegans*

L1 *C. elegans* of the N2 strain growing on the unseeded plates from the worm culture were obtained using the method previously described (Mukhopadhyay et al., 2008). N2 *C. elegans* were washed twice with M9 solution and centrifuged at 600*g* for 1.5 minutes before filtering through a 40 μm Nylon cell strainer (BD Falcon). The solution was then centrifuged at 600*g* for 1.5 minutes and the pellet was plated on the NGM plate seeded with the appropriate RNAi culture (*ccch-3*, *ama-1* or L4440) and incubated at 20°C.

Two days later, 12 L4 *C. elegans* of each RNAi plate were cloned on 12 NGM plates with the corresponding RNAi culture (1 worm / plate). After 24 hours, the adult worm was removed and had its embryos counted and compared to unhatched embryos on the following day.

Additionally, adult *C. elegans* from each of the three original RNAi plates were bleached and their embryos were plated on the corresponding RNAi plate as previously described. Embryos were counted at the time of plating and two days later. This method yields a much higher number of embryos per plate, providing greater statistical power.

Identifying whether the lethal phenotype is due to *his-74* gene deletion or a linked mutation

To further identify whether the lethal phenotype observed is due to the his-74 gene deletion instead of a lethal mutation linked to the gene, the *his-74* (*ok1219*) *V* strain was crossed with a *dpy-11(e224) unc-76(e911) V* strain in an attempt to identify whether a hypothesized lethal mutation may be separated from the *his-74* gene.

DNA Extraction and PCR Amplification

DNA from *C. elegans* was extracted using a worm lysis buffer (50 mM KCl, 10 mM Tris at pH 8.3, 2.5 mM of $MgCl_2$, 0.45% NP-40, 0.45% Tween-20 and 0.01% gelatin) with proteinase K (100 ug / mL). *C. elegans* were placed in this solution before freezing at –80^{o}C for a minimum of 30 minutes. The worms were then heated at 60^{o}C for 60 min before inactivating the proteinase K at 95^{o}C for 15 min.

Polymerase chain reaction (PCR) amplification of the DNA template was conducted using Taq polymerase. Primers MME1 (5'-TTCGGGCAGTAATAAAACAGG-3') and MME2 (5'-AAAGGATCCTCTTCCGTTGAA-3'), yielding a band of 588 base pairs (bp), were used to identify the wild-type *his-74* band while primers MME 16 (5'-AGCTTTAATTTGGCCGTGTC-3') and MME 19 (5'-GTGCTTCTGGCGTTTCAGTT-3'), yielding a 1242 bp band, were used to identify the deletion band of *his-74*. Primers ME31 (5'-TTCGGGCAGTAATAAAACAGG-3') and ME32 (5'-GTGAGCTCGTAATATCCGGC-3'), yielding a 2158 bp band, were used to identify the wild-type *his-74* band on the transgenic line that was produced in an attempt to

rescue the *his-74* homozygous deletion. The PCR cycle for primers MME1 and MME2 was run at 98°C for 30 s, then 40 cycles of 98°C for 10 s, 56°C for 30 s and 72°C for 30 s before a final extension step at 72°C for 10 minutes. The cycle for primers MME16 and MME19 was the same but with an annealing temperature of 58°C for 30 seconds and an extension time of 1 min. The cycle for primers ME31 and ME32 had an annealing temperature of 65°C for 1 min and 72°C for 1 min.

The PCR product was then run in a 1% agarose gel (made with 1X TAE buffer and ethidium bromide at 1 μg / mL) and compared to a 1 Kb DNA ladder (Fermantas). Visualization of the PCR products was conducted using the Transilluminator TR-302 (Spectroline®). The number of bands and their respective sizes were determined by side-to-side comparisons with the DNA ladder.

References

Ahmad, K., & Henikoff, S. (2002). The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. *Molecular Cell*, *9*(6), 1191–1200.

Akiyama, T., Suzuki, O., Matsuda, J., & Aoki, F. (2011). Dynamic Replacement of Histone H3 Variants Reprograms Epigenetic Marks in Early Mouse Embryos. *PLoS Genet*, *7*(10), e1002279. http://doi.org/10.1371/journal.pgen.1002279

Allshire, R. C., & Karpen, G. H. (2008). Epigenetic regulation of centromeric chromatin: old dogs, new tricks? *Nature Reviews. Genetics*, *9*(12), 923–937. http://doi.org/10.1038/nrg2466

Brenner, S. (1974). The genetics of Caenorhabditis elegans. *Genetics*, *77*(1), 71–94.

Chow, C.-M., Georgiou, A., Szutorisz, H., Maia e Silva, A., Pombo, A., Barahona, I., … Dillon, N. (2005). Variant histone H3.3 marks promoters of transcriptionally active genes during mammalian cell division. *EMBO Reports*, *6*(4), 354–360. http://doi.org/10.1038/sj.embor.7400366

Churikov, D., Zalenskaya, I. A., & Zalensky, A. O. (2004). Male germline-specific histones in mouse and man. *Cytogenetic and Genome Research*, *105*(2-4), 203–214. http://doi.org/10.1159/000078190

Gabrielli, F., Aden, D. P., Carrel, S. C., von Bahr, C., Rane, A., Angeletti, C. A., & Hancock, R. (1984). Histone complements of human tissues, carcinomas, and carcinoma-derived cell lines. *Molecular and Cellular Biochemistry*, *65*(1), 57–66.

Hake, S. B., Garcia, B. A., Duncan, E. M., Kauer, M., Dellaire, G., Shabanowitz, J., … Hunt, D. F. (2006). Expression patterns and post-translational modifications associated with

mammalian histone H3 variants. *The Journal of Biological Chemistry*, *281*(1), 559–568. http://doi.org/10.1074/jbc.M509266200

Kimmins, S., & Sassone-Corsi, P. (2005). Chromatin remodelling and epigenetic features of germ cells. *Nature*, *434*(7033), 583–589. http://doi.org/10.1038/nature03368

Luger, K., Mäder, A. W., Richmond, R. K., Sargent, D. F., & Richmond, T. J. (1997). Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature*, *389*(6648), 251–260. http://doi.org/10.1038/38444

McKittrick, E., Gafken, P. R., Ahmad, K., & Henikoff, S. (2004). Histone H3.3 is enriched in covalent modifications associated with active chromatin. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(6), 1525–1530. http://doi.org/10.1073/pnas.0308092100

Mito, Y., Henikoff, J. G., & Henikoff, S. (2005). Genome-scale profiling of histone H3.3 replacement patterns. *Nature Genetics*, *37*(10), 1090–1097. http://doi.org/10.1038/ng1637

Mukhopadhyay, A., Deplancke, B., Walhout, A. J. M., & Tissenbaum, H. A. (2008). Chromatin immunoprecipitation (ChIP) coupled to detection by quantitative real-time PCR to study transcription factor binding to DNA in Caenorhabditis elegans. *Nature Protocols*, *3*(4), 698–709. http://doi.org/10.1038/nprot.2008.38

Ooi, S. L., Priess, J. R., & Henikoff, S. (2006). Histone H3.3 variant dynamics in the germline of Caenorhabditis elegans. *PLoS Genetics*, *2*(6), e97. http://doi.org/10.1371/journal.pgen.0020097

Ramaswamy, A., & Ioshikhes, I. (2013). Dynamics of modeled oligonucleosomes and the role of histone variant proteins in nucleosome organization. *Advances in Protein Chemistry and Structural Biology*, *90*, 119–149. http://doi.org/10.1016/B978-0-12-410523-2.00004-3

Schier, A. F. (2007). The maternal-zygotic transition: death and birth of RNAs. *Science (New York, N.Y.)*, *316*(5823), 406–407. http://doi.org/10.1126/science.1140693

Schwartzentruber, J., Korshunov, A., Liu, X.-Y., Jones, D. T. W., Pfaff, E., Jacob, K., … Jabado, N. (2012). Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature*, *482*(7384), 226–231. http://doi.org/10.1038/nature10833

Stroud, H., Otero, S., Desvoyes, B., Ramírez-Parra, E., Jacobsen, S. E., & Gutierrez, C. (2012). Genome-wide analysis of histone H3.1 and H3.3 variants in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(14), 5370–5375. http://doi.org/10.1073/pnas.1203145109

Szenker, E., Ray-Gallet, D., & Almouzni, G. (2011). The double face of the histone variant H3.3. *Cell Research*, *21*(3), 421–434. http://doi.org/10.1038/cr.2011.14

Thompson, O., Edgley, M., Strasbourger, P., Flibotte, S., Ewing, B., Adair, R., … Waterston, R. H. (2013). The million mutation project: a new approach to genetics in Caenorhabditis elegans. *Genome Research*, *23*(10), 1749–1762. http://doi.org/10.1101/gr.157651.113

Timmons, L., & Fire, A. (1998). Specific interference by ingested dsRNA. *Nature*, *395*(6705), 854. http://doi.org/10.1038/27579

Waterborg, J. H. (1990). Sequence analysis of acetylation and methylation in two histone H3 variants of alfalfa. *The Journal of Biological Chemistry*, *265*(28), 17157–17161.

Wells, D., Hoffman, D., & Kedes, L. (1987). Unusual structure, evolutionary conservation of non-coding sequences and numerous pseudogenes characterize the human H3.3 histone multigene family. *Nucleic Acids Research*, *15*(7), 2871–2889.

Witt, O., Albig, W., & Doenecke, D. (1996). Testis-specific expression of a novel human H3 histone gene. *Experimental Cell Research*, *229*(2), 301–306. http://doi.org/10.1006/excr.1996.0375

Wu, T. F., & Chu, D. S. (2008). Sperm Chromatin. *Molecular & Cellular Proteomics : MCP*, *7*(10), 1876–1886. http://doi.org/10.1074/mcp.R800005-MCP200