Distribution Agreement

In presenting this thesis a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Daniel Krinichanskiy April 7, 2022

Investigation of the Germline Functions of CFP-1 and MRG-1 in *Caenorhabditis elegans*

By

Daniel Krinichanskiy

William Kelly, Ph.D.

Adviser

Department of Biology

William Kelly, Ph.D.

Adviser

David Lynn, Ph.D.

Committee Member

Roger Deal, Ph.D.

Committee Member

2022

Investigation of the Germline Functions of CFP-1 and MRG-1 in *Caenorhabditis elegans*

By

Daniel Krinichanskiy

William Kelly, Ph.D.

Adviser

An abstract of a thesis submitted to the Faculty of Emory College of Arts and Sciences of Emory University in partial fulfillment of the requirements of the degree of Bachelor of Sciences with Honors

Department of Biology

2022

Abstract

Investigation of the Germline Functions of CFP-1 and MRG-1 in *Caenorhabditis elegans*

By Daniel Krinichanskiy

MRG-1 is the *C. elegans* ortholog of human MRG-15 protein – a factor which recognizes H3K36me3 to guide both histone acetylase and deacetylase complexes to their respective sites in the genome. Though it is known to be an essential epigenetic factor in embryonic and germline development in *C. elegans,* it was recently shown that its chromodomain (the domain with which it recognizes the histone modification) is not required for normal germline development. These findings suggested that its function within the germline could be redundant with another factor. CFP-1 is another highly conserved protein, likewise shown to be involved in guiding histone deacetylase complexes, and is required for normal establishment and maintenance of H3K4me3. Here, we examine the genetic interactions between the two proteins. Our findings demonstrate that the double knockdown of both CFP-1 and MRG-1 causes synergistic sterility, whereas loss of either alone has no such effect in the generation assayed. They exhibit a novel sterility phenotype, wherein germ cells initially proceed through normal development but are seemingly degraded in older adults. The singular effect of *cfp-1* is decreased fertility in both wildtype animals and *mrg-1* deletion heterozygotes, whereas the embryonic mortality rate is not affected. Removal of *mrg-1* and knockdown of *cfp-1* correlates with increases in germline levels of H3K9ac and H3K9me3, which may contribute to the observed phenotype.

Investigation of the Germline Functions of CFP-1 and MRG-1 in *Caenorhabditis elegans*

By

Daniel Krinichanskiy

William Kelly, Ph.D.

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences of Emory University in partial fulfillment of the requirements of the degree of Bachelor of Sciences with Honors

Department of Biology

2022

Table of Contents

Index of Figures

Chapter 1. Introduction

Regulation of gene expression is essential for an organism's survival. Since every cell carries the entire genome in its nucleus, it is vital that each cell only expresses appropriate genes at appropriate times. The high energy costs of expressing "useless" proteins are one concern, however, not the only one. There are tissue-specific proteins that need to be expressed in each specific type of cells and not in the others. Failure to suppress inappropriate gene expression can result in incorrect cell function and ultimately an organism's inability to survive. Furthermore, some proteins are only needed to be expressed during the initial stages of development, and their continued expression in the cells of an adult organism can likewise be detrimental. Thus, correct coordination of cellular function requires correct gene expression.

At one level, gene expression is regulated via accessibility of the gene to the cellular transcription machinery, including RNA polymerases and transcription factors. Since DNA is a very large molecule, cells employ a "packaging" mechanism in order to "fit" it into the nucleus. Eukaryotic DNA is organized into chromosomes by wrapping around proteins called histones. The basic unit of such packaging is a nucleosome, which consists of eight histones, namely, two copies each of H2A, H2B, H3 and H4.

There are two general ways of remodeling chromatin and regulating gene accessibility: either through methylation or demethylation of the DNA itself or through chemically modifying the histones. The former is done by DNA methyltransferase enzymes that add methyl groups to cytosines in a CpG context, i.e., in regions, where a cytosine is followed by guanine. The addition of

methyl groups to cytosines can cause the DNA to be more tightly packaged by other proteins that bind to this modification and hence restrict access of RNA polymerases to the promoters of the genes, lowering gene expression.

Histone modifications are more numerous and diverse as to their effect (for example, a methyl mark can be repressive or permissive depending on the amino acid it is added to) but can serve essentially the same purpose. By tightening or loosening the packaging of the cell's genetic material around histones, through recruitment of specific factors that recognize the histone modifications, they increase or decrease its accessibility to transcription machinery and thus help control gene expression. The modifications are regulated by various protein complexes as well as non-coding RNA. Apart from regulating accessibility, however, histone modifications can, for example, also indicate regions where transcription has occurred to provide a "memory" of gene activity. The heritable regulation of gene activity provided by such "memory", both cell-to-cell and intergenerational, is considered to be the hallmark of the field of epigenetics.

MRG-1 (mortality factor-related gene protein 1) is a *C. elegans* ortholog of the human protein MRG15. These proteins contain two conserved domains, a chromodomain and the MRG domain, located, respectively, at the N and C termini of the molecule. MRG15 is known to be involved in a number of regulatory processes. Specifically, it interacts with H3K36me3 (trimethylation of lysine 36 on histone 3) a well-known mark indicating gene transcription, through binding by chromodomain region. Recent studies (1) indicate that MRG15 is associated with histone acetyltransferase and deacetylase complexes and is

proposed to guide these complexes to genomic sites through its recognition of H3K36me3. Through this involvement in chromatin-remodeling complexes, MRG15 is important in both differentiation of cells and proper embryonic development in general (2,3). MRG15 is also known to recruit proteins like PALB2 to stimulate repair of chromosomal breaks (4), and DNA repair mechanisms in mice (5).

Like its human ortholog, MRG-1 is an important agent in *C. elegans* development. It is maternally loaded, meaning that it must be present within the fertilized egg before any zygotic gene expression occurs. Studies show that loss of maternal MRG-1 results in defective embryonic germline development, showing that the protein is critical in the development and proliferation of primordial germ cells (6). This is consistent with its ortholog, MRG15, which was found to be crucial in spermatogenesis (7). Additionally, absence of MRG-1 germ cells also leads to other abnormalities, including problems with homologous chromosome alignment during meiosis (8).

CFP-1 (CXXC finger binding protein 1) is a highly conserved protein that is present in both *C. elegans* and mammals. Studies have shown that it interacts with histone deacetylase (HDAC1/2) complexes in the nematode's development (9). CFP-1 is known to also form complexes with SET-1A and SET-1B methyltransferases in mammals and has been shown to be required for the normal establishment and maintenance of a histone modification associated with active transcription, H3K4me3 (10) and bind to unmethylated CpG isles, (11). Since MRG-1's orthologs have been found to be involved in both deacetylases and

acetyltransferases, this opens room for speculation about the genetic interaction between *mrg-1* and *cfp-1*.

Doronio et al., 2022 (12) have shown that MRG-1's essential function within the germline, but not the soma, is independent of its chromodomain. In other words, animals homozygous for an *mrg-1* deletion mutation grow up sterile, however, animals expressing only the chromodomain mutation are fertile. However, the same result opens up speculation that there may be some redundancy in factors essential in germline establishment of *C. elegans*. In other words, possible genetic interaction between *mrg-1* and *cfp-1* could be playing into that redundancy.

mrg1(tm1227)/qC1 heterozygous animals (qC1 serves a balancer chromosome) were used to obtain mutants homozygous for *mrg-1* deletion. Since the protein is maternally loaded, the first generation of *mrg-1/mrg-1* homozygotes are rescued by the maternal protein from the single wild-type allele of the mother and the animals grow up fertile. These animals are designated M+Z-; i.e., they have maternal provision but no zygotic function. However, without the maternal rescue, the second-generation offspring of *mrg-1/mrg-1* (M-Z-) are sterile. We examined the effect of loss of CFP-1 in the context of the *mrg-1* mutant. *cfp-1* RNAi was used so as to simulate the knockdown and analyze the fertility of the M+Z- animals in the first generation. The animals were then analyzed under immunofluorescence staining in order to investigate any resulting epigenetic changes. Here, we show that the double knockdown of *mrg-1* and *cfp-1* leads to infertility in the first generation, with maternally loaded *mrg-1* being insufficient to rescue the offspring when CFP-1 is also

depleted. The sterility phenotype is an unusual one, with germ cells progressing relatively far into development never to develop into mature eggs and "disappear" or degrade eventually in older adults. Additionally, the singular effect of *cpf-1* on fertility is adverse, with both wildtype (N2) and *mrg-1* heterozygous animals exhibiting a statistically significant reduction in their brood sizes. There are also subtle increases in H3K9Ac and H3K9me3 in maturing eggs of following *cfp-1*RNAi treatment, suggesting misregulation of these modifications may contribute to the synergistic phenotype.

Chapter 2. Results

Double knockdown of *mrg-1* and *cfp-1* leads to sterility in the F1 generation

In order to establish whether there is any genetic interaction between *mrg-1* and *cfp-1,* the heterozygous *mrg-1(tm2770)/qC1* F0 animals were taken at an L3-L4 stage and grown either in presence or absence of *cfp-1* RNAi, with exposure to the L4440 empty RNAi vector serving as a control. If any interaction was evident, one would expect that the simulated double-knockdown would lead to a novel phenotype in the F1, with maternal MRG-1 being insufficient to rescue the sterility phenotype. 123 animals grown in *cfp-1* RNAi and 80 animals grown in the control were scored for fertility (classified as either fertile or infertile based on inspection under a dissection scope) after they had grown to early adulthood. As expected, heterozygous animals retained their fertility, when exposed to RNAi, with the F1 generation having both the maternal rescue and the endogenous *mrg-1* countinuously expressed: 97% of heterozygotes grown in

the RNAi were fertile (Figure 1A). In contrast, 95% the *mrg-1/mrg-1 M+Z-* (maternal MRG-1 present, zygotic not expressed) F1 homozygotes were sterile.

As expected, control RNAi had no effect on fertility, with 100% of heterozygotes being fertile (Figure 1B). The majority (86%) of M+Z- homozygotes were likewise fertile. The results obtained led to conclusion that there is indeed interaction between *mrg-1* and *cfp-1* in the development of *C. elegans* germline, with the former being a necessary but insufficient condition for establishment of the fertile phenotype. The maternal rescue provided in the F1 does not work in the absence of *cfp-1* and the animals grow up sterile.

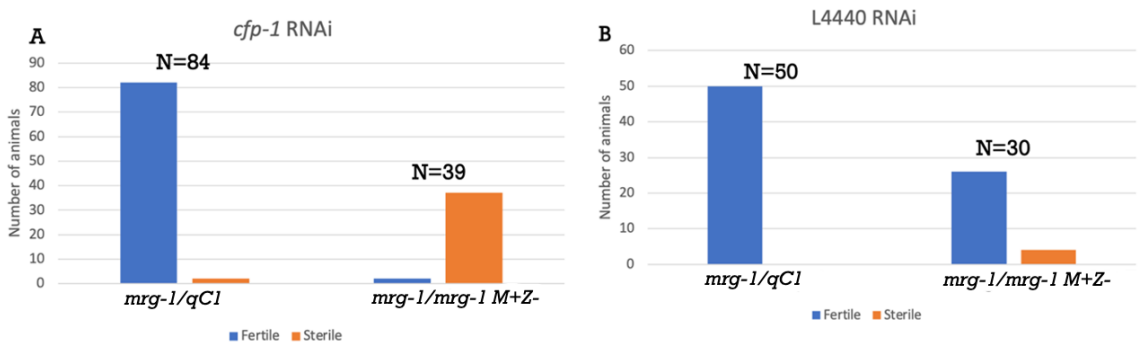

Figure 1. Double knockdown of *cfp-1* and *mrg-1* results in the sterile phenotype. Fertility of heterozygous (*mrg-1/qC1*) and homozygous (*mrg-1/mrg-1 M+Z-*) F1 animals grown either in (A) *cfp-1* RNAi or in (B) control L4440 empty vector. Fertility was assessed by looking for worms exhibiting "black sterile" phenotype.

Cfp-1 and *mrg-1* double knockdown exhibits a strange phenotype, wherein the germline initially proliferates but then degrades

In order to investigate the origins of the sterility phenotype, the gonads of the M+Z- homozygous animals were analyzed under high magnification and compared to those of the heterozygous adult animal grown in the presence of the

same *cfp-1* RNAi. Closer analysis has shown that the germ cells in the homozygotes do go through the initial divisions in the young adult and some maturation into eggs can be observed (Figure 2A, B). However, in older adults the gonad is filled with large empty vacuoles with germ cells seemingly "disappearing" or undergoing degradation following perhaps some programmed cell death or other event currently unaccounted for. In contrast, the heterozygous animals develop a normal germline (Figure 2C), leading to the conclusion that the simulated double knockdown of *mrg-1* and *cfp-1* and not one of them alone is responsible for the observed phenotype.

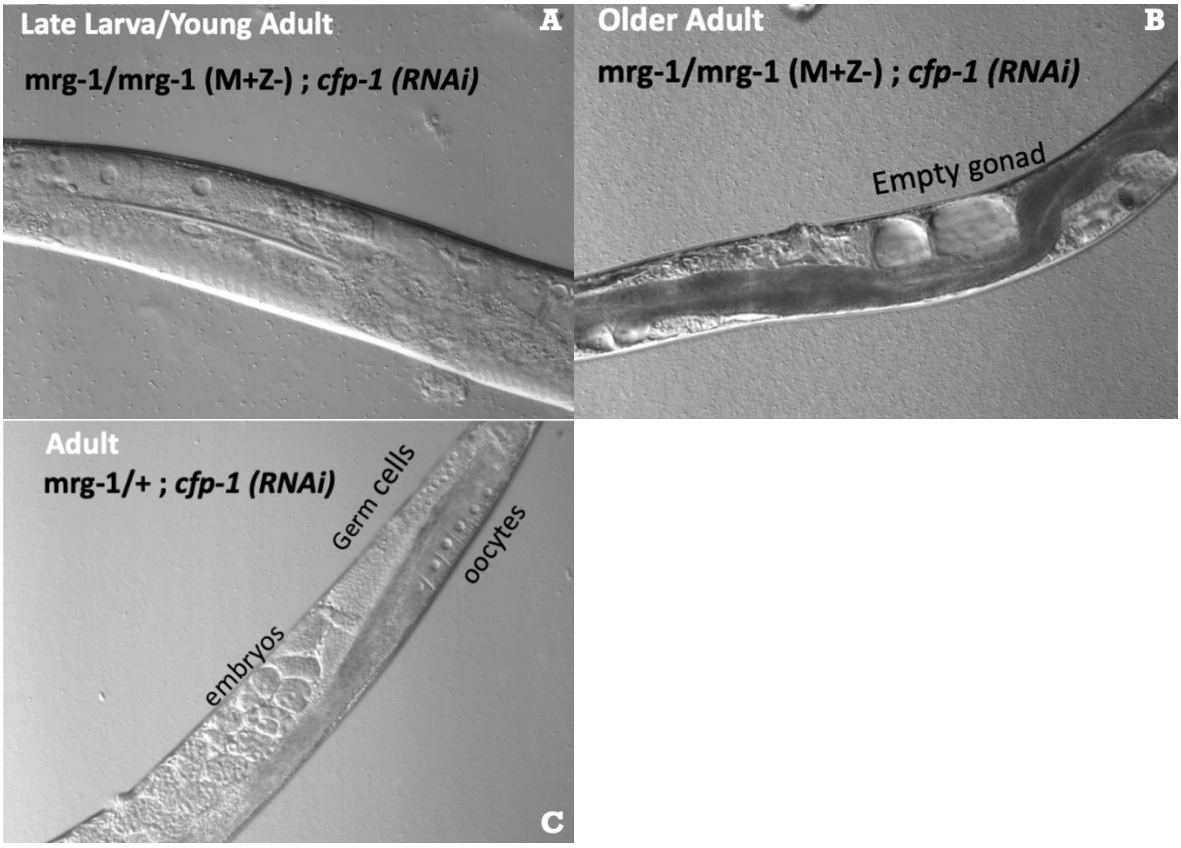

Figure 2. Germ cells of *mrg-1 M+Z-* animals grown in RNAi undergo initial divisions but "disappear" in the gonad by maturation into older adults. High magnification images of the gonads in (A) an M+Z- homozygous animal at L4-young adult stage of development, (B) an older M+Z- homozygous adult and (C) a heterozygous adult grown in *cfp-1* RNAi.

***Cfp-1* significantly decreases fertility of both wildtype and heterozygous animals but is not a necessary factor for oogenesis.**

Next, in order to determine the singular effect of *cfp-1* on F1 fertility, F1 wildtype (N2), *mrg-1/qC1* and *mrg-1/mrg-1 M+Z-* worms were obtained from F0 parents grown in either *cfp-1* or control L4440 RNAi. The total brood size of F1 animals grown in the same RNAi as their parents was then calculated.

In wildtype N2 animals, the effect of *cfp-1* RNAi was almost a two-fold decrease in F1 brood size (i.e., in the F2 population), statistically significant with p value several degrees of ten below the 0.01 significance margin (Figure 3). Such a dramatic decrease highlights the essential role *cfp-1* plays in germline development and is consistent with the findings of previous studies (9). One has to note however, that none of the N2 animals were sterile, indicating that *cfp-1* is not a strictly necessary factor for germline development.

The brood size of *mrg-1/mrg-1* homozygous animals confirmed the results of the previous experiment with all M+Z- specimens grown under *cfp-1* producing zero offspring, while their counterparts grown on L4440-expressing control had been fertile. One note, however, on the fertility of the latter has to be added: their brood sizes are significantly lower than wildtypes or heterozygous animals, with them producing offspring in low 20's, while the brood sizes of N2 animals of the strain the lab uses are normally up to 200+ embryos. This suggests that the maternal rescue is barely enough to develop fertility in and of itself, with zygotic expression required for normal embryonic development.

The brood size of *mrg-1/qC1* homozygotes is likewise significantly decreased following *cfp-1* exposure. However, the effects are more subtle with p-

value from two-tailed t-test being statistically insignificant at the critical value of $p<0.01$ but statistically significant at $p<0.05$. The implications of this result are somewhat harder to interpret, since one would think that between the highly significant almost two-fold fertility decrease in the wildtypes and the complete sterility in the homozygotes, there should exist an intermediate phenotype in the heterozygotes between the two groups.

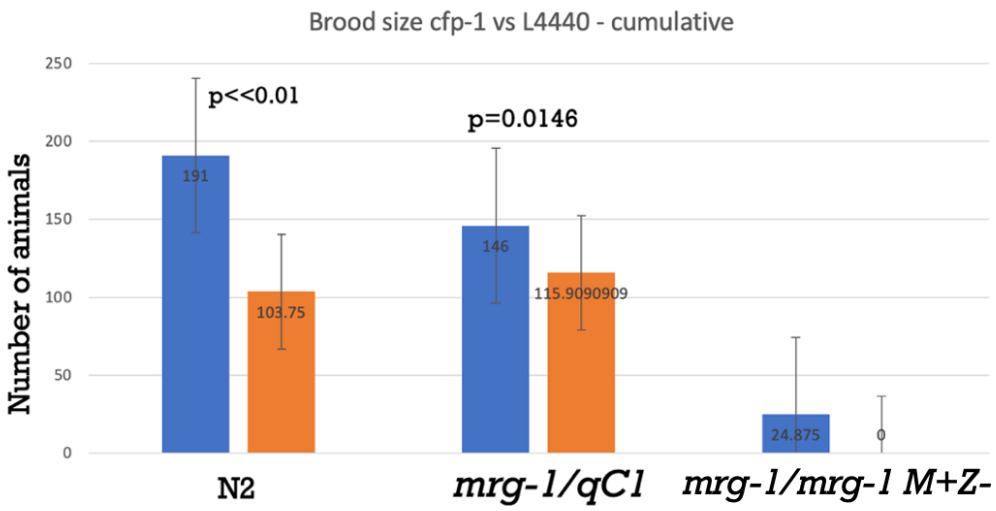

Figure 3. Brood sizes of N2, *mrg-1/qC1* and *mrg-1/mrg-1* animals grown in either cfp-1 (orange) or L4440 (blue) RNAi. Total number of F2 offspring produced by F1 animals grown in the corresponding RNAi. The lower axis indicates the genotype. Error bars represent standard error. Values on the bar plots indicate exact averages within each category. P values are calculated using a standard two-tailed t-test.

Cfp-1 has no effect on embryonic mortality of either wildtype or heterozygous animals

In order to control for possible embryonic mortality stemming from unaccounted for "side-effects" of the gene knockdown, as well as to investigate

whether removal of either protein from the germline leads to such a phenotype, a record of dead embryos was kept, expressed herein as a percentage of the brood size. Overall, there is no statistically significant change in embryonic mortality between either N2 growing in control RNAi vs *cfp-1* or the *mrg-1/qC1* heterozygotes. The slight increase in N2 animals grown in *cfp-1* expressing bacteria is not statistically significant and both values are well within the normal embryonic mortality of below 1% (Figure 4).

mrg-1/mrg-1 animals grown on L4440 expressing bacteria naturally have a high embryonic mortality of 38% which may highlight the effects of losing zygotic MRG-1 on embryonic development. Naturally, since no embryos are obtained from worms grown on *cfp-1*, there is no mortality to be accounted for.

mrg-1/qC1 animals like N2s do not exhibit any statistically significant differences in embryonic mortality with both specimens grown in control vs knockdown RNAi showing embryonic mortality of 15%. The difference between the averages obtained here and the values observed and expected in N2 worms lies in the role of qC1 balancer chromosome, which is homozygous embryonic lethal. The reason the value is below the expected Mendelian 25% is probably due to non-Mendelian segregation of the balancer chromosome, which carries an inversion. Basically, all the embryonic mortality that had been observed most likely stems from the presence of *qC1/qC1* homozygotes and no other factor.

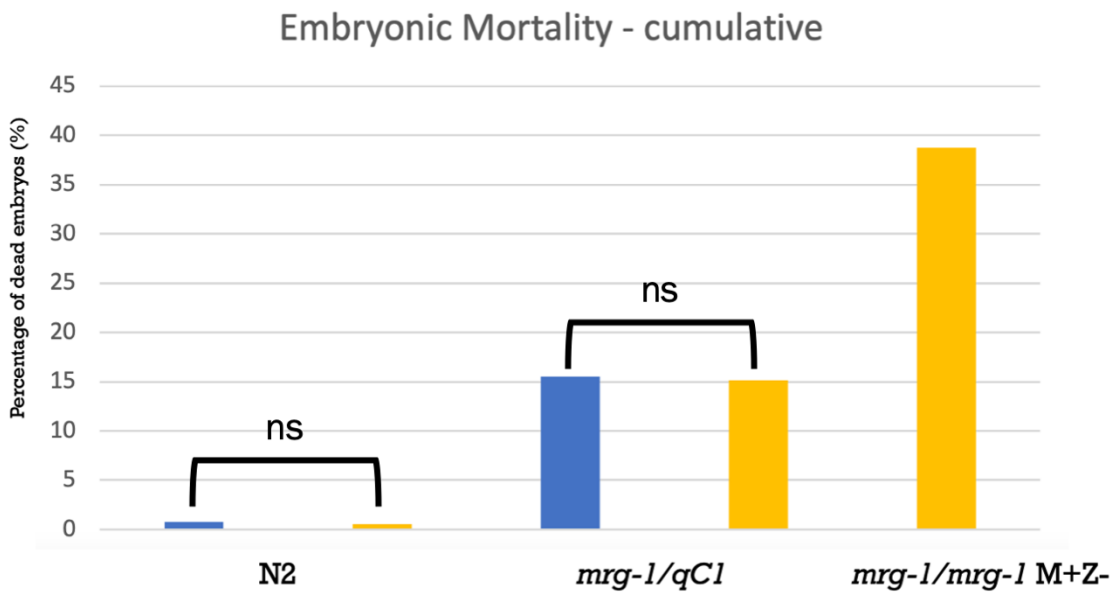

Figure 4. Embryonic mortality for animals grown in either cfp-1 or L4440 RNAi. Percentages were obtained by dividing the number of observed dead embryos by total brood size. In blue, worms growing in *cfp-1* are shown and those in L4440 are in yellow.

Immunofluorescence staining shows changes in H3K9Ac, H3K9Me3 between worms grown on *cfp-1* and L4440 RNAi

In order to determine the possible reasons for the observed phenotypes, the effects of repressing *mrg-1* and *cfp-1* on cells in the germline were investigated. F1 animals were analyzed under immunofluorescence staining for presence of H3K9Ac and H3K9me3 marks in the oocytes progressing into eggs. In N2 worms, cells entering the ovaries lack any noticeable presence of K9 acetylation or trimethylation (Figure 5A,B). In contrast to that, exposure of N2 animals to *cfp-1* RNAi results in subtle but noticeable appearance of the mark in the maturing eggs. Homozygotes and heterozygotes for *mrg-1* experience

virtually the same pattern of increased K9 acetylation upon exposure to the interfering nucleic acid.

Interestingly, in addition to that, RNAi-fed animals simultaneously exhibit patterns of increased H3K9 trimethylation, which is likewise absent from eggs maturing through the ovaries in N2 animals (Figure 5B). This pattern is observed in both N2 animals and in *mrg-1* deletion homozygotes.

Additionally, possible changes in H3K36me3 between homozygous *mrg-1/mrg-1, mrg-1/qC1* and N2 animals were investigated, however no noticeable aberrations could be identified (data not shown). This is expected and not notable since none of the marks affected is known to be directly involved in establishment of K36 trimethylation.

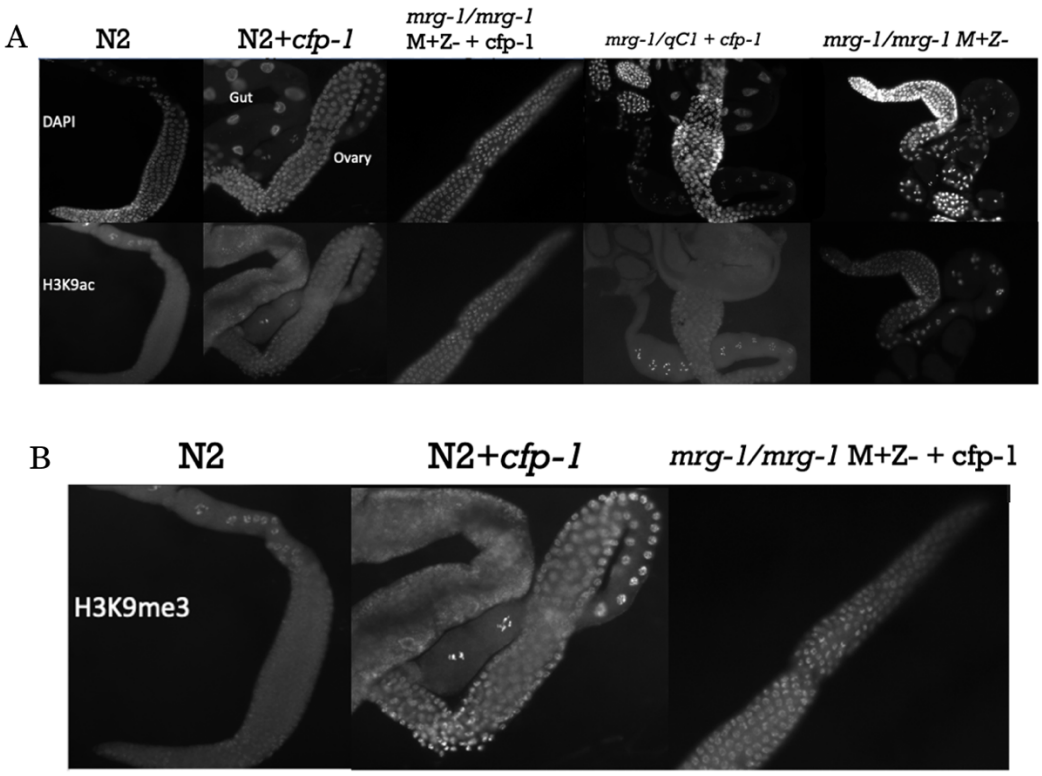

Figure 5. Immunofluorescence staining of gonads from worms grown in either *cfp-1* or L4440 RNAi. Dissected and fixed gonads were stained with (A) anti-H3K9Ac and (B) anti-H3K9me3 antibodies and DAPI.

Argatroban has no effect on F1 mortality in N2 worms

Wei et al. (2020) (13) describe the FDA-approved drug argatroban to be an antagonizing agent to mammalian MRG-15 within the context of attenuating murine liver steatosis. In order to establish whether a sterility phenotype could be produced in *C. elegans,* F1 worms were obtained from parents grown in argatroban and OP50 and scored for sterility. None of the animals scored were sterile or had any changes in germline noticeable under a dissecting scope, so it was concluded that argatroban does not antagonize MRG-1 in context of C. elegans germline development. It has to be noted that the result is not unexpected, since the original researchers specifically describe no changes in overall levels of H3K9 acetylation, instead observing alterations in H4 acetylation patterns.

Chapter 3: Discussion

The genetic interaction shown in this work presents an interesting system, wherein germline development in *C. elegans* is heavily influenced by two interacting epigenetic factors: MRG-1 and CFP-1. The possible reasons of observed phenotypical changes, most probably lie in the increase in epigenetic modifications that were herein identified, namely H3K9Ac and H3K9me3. The origin of the first one most probably lies in the role of both factors in function of histone deacetylases. Lack of any one of them or both leads to necessary overall increase in acetylation throughout the germline. Presence, or rather, noticeable increase in the latter is more difficult for me to explain. Increased K9 methylation in worms exposed to *cfp-1* RNAi can suggest aberrant function of

SET-1/COMPASS methyltransferase complex (14) in which CFP-1 is known to be a crucial targeting component. By losing specificity to CpG isles, the complex may be erroneously led to establish lower levels of H3K4 trimethylation, allowing for increase in the levels of H3K9me3. This, however, offers no explanation as to why similar patterns seen in N2s and homozygous *mrg-1* deletion animals do not lead to same infertility phenotype.

The notion of both acetylation and methylation present at K9 is intriguing in and of itself, since the two marks are normally understood to be in direct opposition to each other, with trimethylation at this amino acid being a well-known repressive mark and acetylation a permissive one. Failure of the worm to develop its own germline in the deletion homozygotes could indeed be attributed to that factor. We do see that on average, *mrg-1/qC1* animals have a notably lower brood size than N2s on their own, and also when exposed to *cfp-1* RNAi. The overall downward trend continues with *mrg-1/mrg-1* homozygotes producing the lowest brood size on control RNAi and being absolutely sterile when exposed to *cfp-1* RNAi. But again, K9 methylation conflicts with K9 acetylation in all groups exposed to *cfp*-1 and it's been established that *cfp-1* is not the singular cause of infertility, so while this may play an essential role in our explanation, we do not have the data to either confirm or rule it out, since the experiments performed do not indicate whether the antagonizing marks are present in the same places. Further experiments, like a ChIP-seq need to be performed to investigate this further.

Another antagonizing relationship that could be responsible for the phenotypes is that between H3K4me3 and H3K9me3. Since CFP-1 is important

for directing the SET methyltransferases to establish K4 trimethylation, loss thereof might adversely impact fertility. This is one of the more immediate future directions of the experiment, which, unfortunately, could not be performed in this work due to time constraints and technical issues with antibodies used for staining.

And all of that does not fully explain the role of MRG-1 maternal rescue in the model being studied. As mentioned before, it seems that in *mrg-1* deletion homozygotes the maternal rescue serves allow fertility, albeit with a high percentage of embryonic mortality, possibly attributed to loss of some essential function of zygotic MRG-1 production. Since MRG-1 (and MRG-15 in mammalian models) is known to play a role in embryonic development, this explanation seems to be very likely. CFP-1 on the other hand, does not seem to affect embryonic development very much, as no changes in embryonic mortality were observed in either N2 worms or the *mrg-1* deletion heterozygotes.

Reiterating on what had been said above, the conflict between the repressive and permissive epigenetic marks may be part of the explanation of the strange phenotype observed in *mrg-1* deletion homozygotes combined with *cfp*-1 knockdown. The fact that the germ cells progress far into development only to suddenly "disappear" in older adults, is a phenotype we failed to find described anywhere else. It seems that there could be a programmed cell death event happening at some point between late larva transition to young adult, since the older adults lack germ cells, whereas younger adults do have their germlines develop quite far, even forming eggs (albeit failing to have any of them fertilized). Determining whether presence of some eggs in younger adults is a

question of variable RNAi penetrance or a characteristic of this novel phenotype is the immediate next step to this work. But returning to the basic interaction between the two epigenetic factors, the epigenetic changes accounted and unaccounted for in this work are probably contributors to the phenotype, which is driven by lack of zygotic expression of *mrg-1,* with sterility demonstrating the basic insufficiency of the maternally loaded factor to in and of itself develop a successful germline. Highlighting this is the fact that M+Z+ animals, subjected to *cfp-1* knockdown grow up fertile. Overall, the seemingly distinct roles maternal and zygotic MRG-1 plays in germline and embryonic development is an interesting topic for potential further investigation.

Returning to the background for this investigation, the role of chromodomain of MRG-1 is another direction that requires further study. As I mentioned, Doronio et al. (12) demonstrated that there are several important aspects of MRG-1's chromodomain that can potentially further explain its role and interaction with other factors like CFP-1. The chromodomain mutation animal does not survive (exhibits an embryonic lethal phenotype) if the deletion occurs in somatic cells. In other words, the maternal rescue once again seems to be insufficient for proper embryonic development. Moreover, it is better to have the entire protein deleted than for it to lack a functional chromodomain and be present.

However, since chromodomain mutation worms are fertile, just like their complete deletion cousins, provided that the said mutation occurs in the germline, the next logical step would be to examine the relationship between CFP-1 and MRG-1 ΔCD in worms, seeing if an infertility phenotype persists

under a *cfp-1* knockdown. Coupled with monitoring of other histone modifications (and the ones included in this work), for example, H3K4me3, this can further elucidate the nature of the genetic interaction herein described.

Overall, any future directions will have to take into account four basic dimensions: maternal vs zygotic and somatic vs germline.

Chapter 4: Experimental Design and Methods

Strains

C. elegans strains used in this work are wildtype N2 specimens and *mrg-1(tm1227)/qC1.* The name of the latter strain is XA6227. The genotype is *mrg-1(tm1227)/qC1 [dpy-19(e1259) glp-1(q339) qls26] III. qls26* insertion contains lag2::GFP and rol-6(su1006). Heterozygous animals exhibit "roller phenotype", making the worms roll in a circular fashion. Integration of *qls26* results in *qC1/qC1* homozygotes to be embryonic lethal. Worms were kept on OP50 *Escherichia coli* in a 16-degree Celsius incubator. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

RNAi

Plasmids used are L4440 empty vector and *cfp-1;* the latter was cloned into L4440 plasmid from library constructed by Kamath et al. (2003) (15). The ID of the *cfp-1* plasmid is F52B11.1, forward primer AACATCCTCGTCAATGTCTCAAT and reverse primer ATCATAATCGACAGAGCATTCGT. L4440 plasmids are engineered to contain genes conferring bacterial resistance to tetracycline and ampicillin (so *cfp-1*

plasmid likewise has that resistance encoded). Plasmids were transformed into HT115 competent cells and grown on regular LB agar plates containing 1:2.5 ampicillin and tetracycline/water solution under 2 degrees Celsius on a monthly basis. Overnight cultures were prepared weekly by taking a bacterial colony from LB plates and transferring it to LB broth containing 0.1% ampicillin solution. Incubation of overnight cultures took place for 12-20 hours under 37 degrees Celsius.

Brood size and embryonic mortality

Brood size LB agar plates were prepared by plating overnight cultures onto plates containing 1:1 ampicillin-IPTG solution in deionized water and incubating overnight under 37 degrees Celsius. F0 worms were taken at L2-L3 or L3-L4 stage and placed onto RNAi plates to be left to themselves until they laid eggs. The F0 adults were then disposed of and the F1 generation worms were left to themselves until reaching L3-L4 stage. After that, 10-12 worms were transferred to either *cfp-1* or L4440 RNAi plates to conduct the brood size and obtain at least 10 biological replicates for each RNAi experiment. All animals were kept under 20 degrees Celsius and transferred unto a new plate for 3-4 days. Embryos were counted the day they had been laid, then one same plate was counted two more times: one to get embryonic mortality numbers from the unhatched embryos on the successive day and one on the next day after that. Where numbers diverged, averages were reported as the brood size number. The total brood size accounts for all embryos, hatched and unhatched and the embryonic mortality number is the ratio of unhatched embryos to all. Unfertilized eggs were excluded from consideration. Statistical significance of the results was assessed using a two-

tailed t-test with highly significant result interpreted below the confidence level of $p<0.01$ and statistically significant result below $p<0.05$.

Immunofluorescence staining

Immunofluorescence staining was performed on positively charged poly-lysine coated SuperFrost slides, using a dissection buffer of 2x sperm salts solution and levamisole. Worms were dissected with a simple scalpel by cutting the head directly behind the pharynx and fixed using 4% PFA solution and 100% ethanol using a freeze-cracking technique. Rat anti-H3K9Ac and mouse anti-H3K9me3 antibodies were obtained from Abcam. For secondary stain donkey anti-rat Alexafluor 594 and goat anti-mouse Alexafluor 488 were used, respectively. Slides were washed in TBS-T solution and stained with DAPI. Dissected gonads were analyzed using compound microscopy.

Argatroban

Argatroban drug was obtained from Sigma-Aldrich. F0 N2 animals were taken at L3-L4 stage and transferred onto OP50 LB plates coated with argatroban solution. The drug was dissolved in DMSO, and worms were exposed to 3 different concentrations thereof (500, 50 and 5 mg/mL). They were left to themselves until the F1 generation had been obtained. The F0 worms were then disposed of and the plates were left to themselves until F1 began laying eggs. Worms were analyzed for presence/abundance of black sterile phenotype.

References

1. Pena, A.N., Tominaga, K., Pereira-Smith, O.M. *MRG15 activates the cdc2 promoter via histone acetylation in human cells.* Exp Cell Res, 2011. **317**(11): p. 1534-40.

2. Chen M., Takano-Maruyama M., Pereira-Smith O.M., Gaufo G.O., Tominaga K. *MRG15, a component of HAT and HDAC complexes, is essential for proliferation and differentiation of neural precursor cells.* J Neurosci Res. 2009; **87**(7): p. 1522-31.

3. Tominaga K., Kirtane B., Jackson J.G., Ikeno Y., Ikeda T., Hawks C., Smith J.R., Matzuk M.M., Pereira-Smith O.M. *MRG15 regulates embryonic development and cell proliferation.* Mol Cell Biol. 2005. **25**(8): p. 2924-37.

4. Hayakawa T., Zhang F., Hayakawa N., Ohtani Y., Shinmyozu K., Nakayama J., Andreassen P.R. *MRG15 binds directly to PALB2 and stimulates homology-directed repair of chromosomal breaks.* J Cell Sci. 2010; **123**(Pt 7): p. 1124-30.

5. Garcia S.N., Kirtane B.M., Podlutsky A.J., Pereira-Smith O.M., Tominaga K. *Mrg15 null and heterozygous mouse embryonic fibroblasts exhibit DNA-repair defects post exposure to gamma ionizing radiation.* FEBS Lett. 2007; **581**(27): p. 5275-81.

6. Fujita M., Takasaki T., Nakajima N., Kawano T., Shimura Y., Sakamoto H. *MRG-1, a mortality factor-related chromodomain protein, is required maternally for primordial germ cells to initiate mitotic proliferation in C. elegans.* Mech Dev. 2002; **114**(1-2): p. 61-9.

7. Iwamori N., Tominaga K., Sato T., Riehle K., Iwamori T., Ohkawa Y., Coarfa C., Ono E., Matzuk M.M. *MRG15 is required for pre-mRNA splicing and spermatogenesis.* Proceedings of the National Academy of Sciences of the USA. 2016;**113**(37): p. E5408-15.

8. Dombecki C.R., Chiang A.C., Kang H.J., Bilgir C., Stefanski N.A., Neva B.J., Klerkx E.P., Nabeshima K. *The chromodomain protein MRG-1 facilitates SC-independent homologous pairing during meiosis in Caenorhabditis elegans.* Dev Cell. 2011; **21**(6): p. 1092-103.

9. Pokhrel, B., Chen, Y. and Biro, J.J. *CFP-1 interacts with HDAC1/2 complexes in C. elegans development.* FEBS J, 2019 **286**: p. 2490-2504

10. Sha Q.Q., Jiang Y., Yu C., Xiang Y., Dai X.X., Jiang J.C., Ou X.H., Fan H.Y. *CFP1-dependent histone H3K4 trimethylation in murine oocytes facilitates ovarian follicle recruitment and ovulation in a cell-nonautonomous manner*. Cell Mol Life Sci., 2020; **77**(15): p. 2997-3012

11. van de Lagemaat, L.N., Flenley, M., Lynch, M.D. *et al. CpG binding protein (CFP1) occupies open chromatin regions of active genes, including enhancers and non-CpG islands.* Epigenetics & Chromatin **11,** 59 (2018).

12. Doronio, C.A., Ling H., Gleason E.J., Kelly W.G. *A Chromodomain Mutation Identifies Separable Roles for C. elegans MRG-1 in germline and somatic development*. bioRxiv 2022.02.19.479917

13. Wei Y., Tian C., Zhao Y., Liu X., Liu F., Li S., Chen Y., Qiu Y., Feng Z., Chen L., Zhou T., Ren X., Feng C., Liu Y., Yu W., Ying H., Ding Q. *MRG15 orchestrates rhythmic epigenomic remodelling and controls hepatic lipid metabolism*. Nat Metab. 2020; **2**(5): p. 447-460.

14. Beurton F., Stempor P., Caron M., et al. *Physical and functional interaction between SET1/COMPASS complex component CFP-1 and a Sin3S HDAC complex in C. elegans.* Nucleic Acids Res. 2019; **47**(21): p. 11164-11180.

15. Kamath, R., Fraser, A., Dong, Y. et al. *Systematic functional analysis of the Caenorhabditis elegans genome using RNAi.* Nature **421,** 231–237 (2003).