Spring 2016

Coffee may have a protective role against cadmium-induced stress in Caenorhabditis elegans

Patrick J. McMullen
pamc0080@colorado.edu

Coffee may have a protective role against cadmium-induced stress in *Caenorhabditis elegans*

By
Patrick J. McMullen
Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado at Boulder

Defended March 29, 2016

Thesis Advisor:
Dr. Min Han, Dept. of Molecular, Cellular, and Developmental Biology

Defense Committee
Dr. Min Han, Dept. of Molecular, Cellular, and Developmental Biology
Dr. Jennifer Martin, Dept. of Molecular, Cellular, and Developmental Biology
Dr. Joseph Falke, Dept. of Chemistry and Biochemistry

Abstract

Coffee is consumed by many, but the health benefits of coffee are largely unknown. Epidemiological studies have revealed that coffee may play a preventative role against various diseases. I utilize the nematode *Caenorhabditis elegans* to further investigate the preventative or protective role of coffee against stress conditions. More specifically, I explore the possibility that coffee may protect against cadmium-induced stress. My data indicates that coffee treatment suppressed cadmium-induced stress in worms with the stress response ability compromised. Since no obvious negative impact of coffee treatment on development and reproductivity was observed, my data suggests that coffee likely plays a protective role against cadmium-induced stress. Analysis of the functional relationship between coffee and stress response kinases did not lead to a firm conclusion. However, I observed coffee enhances the cadmium-induced ROS response. A small screen was conducted to determine other stress response elements that could be involved in the protective effect. The results of this study may make an important contribution to our understanding of coffee's impact on human health.

Introduction

Coffee is a bean product of the plant *Coffea arabica*, which grows in hot humid climates around the equator. The coffee bean is traditionally roasted, ground, and brewed with hot water to extract the flavor and contents including dietary minerals, hyrdroxycinnamic acids (polyphenols), and many bioactive compounds such as caffeine (Hecimovic et al 2011). The presence of antioxidant polyphenols and other bioactive compounds suggests that coffee may have a significant impact on physiological functions of the organisms.

Correlative studies have shown an inverse relation between coffee consumption and incidence of Parkinson's Disease, Alzheimer's Disease, and cancer suggesting that coffee may play a protective role in aging-related diseases (Barranco Quintana et al 2007, Hu et al 2007, Yu et al 2011). Experimental studies have shown coffee may protect in some diseases by impacting cell signaling networks. Dostal et al showed coffee protects against beta amyloid toxicity, implicated in Alzheimer's Disease, in a SKN-1/NRF2 dependent manner using the *C. elegans* disease model (Dostal et al 2010). SKN-1/NRF2 is a basic leucine zipper transcription factor responsible for response to detrimental (stress) conditions (An & Blackwell 2003). SKN-1 is also known to have an important role in development, but this study will focus on the stress response role of SKN-1.

I have used the nematode *Caenorhabditis elegans* to characterize the impact of coffee under stress conditions. *C. elegans* grow to about 1 mm in length and contain 959 cells at the adult stage. The entire life cycle takes about 3-4 days under optimal conditions (Wood 1988). Under stress conditions the organism may develop more slowly or arrest during development (Koga et al 2000). Development initiates upon the fertilization of an egg. The growth process from hatching to adulthood proceeds via four larval molts characterized by the larval stages

termed L1-L4 (Wood 1988). Stress conditions may arrest larval growth at any larval stage (Koga et al 2000).

Cadmium is a heavy metal present in low quantities on the earth's crust. When present at high concentrations, cadmium is toxic to organisms. The range of pathologies from cadmium toxicity is broad including cancer, mental retardation, Parkinson-like symptoms, kidney damage, lung damage, etc (Wang et al 2013). At the cellular level, cadmium is known to impact the cellular redox state, DNA damage repair machinery, cell cycle checkpoints, apoptosis, and epigenetics (Thevenod et al 2013, Bishak & Payahoo et al 2015). The mechanism by which cadmium inflicts damage on the cell has been studied extensively in the past but remain to be investigated for better understanding. Cadmium is believed to enter the cell via iron-proton co-transporters and calcium transporters (Thevenod 2010). Iron and copper undergo the Fenton reaction forming hydroxyl radicals via the oxidation. The hydroxyl radical will contribute to the formation of reactive oxygen species (ROS), which is known to occur with cadmium toxicity leading to oxidative stress. However, cadmium does not undergo the Fenton reaction. The leading hypothesis explaining the formation of ROS under cadmium toxicity suggests cadmium displaces other iron and copper, which are necessary for biological processes, leading to a liberation of iron and copper that are capable of undergoing the Fenton reaction and forming ROS (Filipic 2012). ROS is deleterious for the cell causing damage to major molecular components including lipids, proteins, and nucleic acids. The resulting changes in signaling cause proteomic state changes throughout the organism to mitigate the detrimental impacts that cadmium has on the cell and to eliminate the presence of cadmium via export and excretion.

The worms respond to the presence of cadmium by increasing glutathione production that function to capture ROS via oxidation and by capturing and eliminating cadmium. GCS-1 is a

glutamate cysteine synthetase. Glutamate cysteine synthetase catalyzes the rate-limiting step in glutathione synthesis (Liao & Yu 2005). Glutathiones function to capture ROS via oxidation. GST-4 is a glutathione s-transferase. Glutathione s-transferase functions to catalyze the oxidation of glutathiones in ROS capture (Leiers et al 2003). PCS-1 encodes a protein that chelates cadmium, which is later exported by HMT-1, a heavy metal transporter (Schwartz et al 2010).

Cadmium is known to impact signaling by activating MAPKinase pathways. MAPKinase activity is believed to cause an increase in cadmium specific detoxification genes. MEK-1 plays a key role in modulating the activity of the MAPKinase pathways under cadmium toxicity, which is supported by experimental data showing *mek-1* mutants have increased sensitivity to cadmium (Koga et al 2000).

MEK-1 has been shown to activate PMK-1, KGB-1, and JNK-1 under cadmium toxicity, which further activate unique sets of transcription factors. PMK-1 is homologous to mammalian p38 kinase that is known to be activated by MKK3, MKK6, and MKK7 in mammals. In *C. elegans*, PMK-1 is only activated by two upstream kinases, SEK-1 and MEK-1. SEK-1 is most homologous to mammalian MKK6 and MKK3, and MEK-1 is homologous to MKK7. SEK-1 is required for PMK-1 activity. MEK-1 contributes to PMK-1 activity, but is not required for PMK-1 activity. *pmk-1(-)* mutants are reported to be sensitive to heavy metals (Kim et al 2004). PMK-1 primarily activates SKN-1, which is known to be a key modulator of antioxidant dependent genes essential in ROS detoxification (Inoue et al 2005).

KGB-1 is known to play a role in cadmium toxicity signaling. KGB-1 shows the most sequence homology to JNK in mammals; however, activation of KGB-1 requires Ser-Asp-Tyr residues, while all other MAPK including mammalian JNK require Thr-X-Tyr residues making

KGB-1 phosphorylation motif unique to C. elegans. *kgb-1(-)* mutants has been shown to be sensitive to heavy metals and downstream of MEK-1 (Mizuno et al 2008, Kim et al 2004).

JNK-1 is known to target FOXO protein DAF-16, which is a general stress response transcription factor important in various stress responses. JNK-1 is partially homologous to JNK in mammals and show more homology to MAPK10 in mammals. *jnk-1(-)* mutant worms shows heavy metal sensitivity like *mek-1(-)* mutants suggesting that JNK-1 may function downstream of MEK-1 (Villanueva et al 2001).

PMK-1, KGB-1, and JNK-1 have all been shown to play a role in cadmium toxicity response; however, it is not known if these genes play a role in dietary dependent protection against stress, in particular coffee protection against cadmium toxicity.

In this study, we will focus on the effects of coffee on cadmium toxicity in an attempt to understand the genetic and molecular impacts of coffee.

Materials and Methods:

Strains: N2, FK171 *mek-1(ks54),* KU21 *kgb-1(km21),* KU25 *pmk-1(km25),* VC8 *jnk-1(gk7),* CB950 *unc-7(e950),* TK22 *mev-1(kn1),* RB1749 *numr-1(ok2239),* RB2496 *cdr-1(ok3456),* RB959 *pgp-5(ok856),* VF2 *pcs-1(tm1748),* VF3 *hmt-1(gk161),* ZG31 *hif-1(ia4),* VC128 *mtl-2(gk125).*

Coffee Preparation: Coffee was obtained from Starbucks (Verona Blend) and stored at 4 degrees Celsius. Coffee grounds were prepared with grinding for 30 seconds with standard grinder prior to each time coffee was brewed. 9.1 g of coffee grounds were added to 500 mL of boiling water and allowed to boil for 30 minutes. Resulting solution was filtered using standard paper coffee

filter and filtered a second time using a Nalgene PES filter unit (Dostal et al 2010). The resulting solution was stored at 4 degrees Celsius. Coffee was only stored for 2 weeks at 4 degrees Celsius before it was deemed unusable.

Cadmium Stock Solution Preparation: Cadmium stock solutions were prepared in 40 mL batches at 24 mM with 4 drops of 12 M HCl and 40 mL of water to dissolve. Control conditions contained equivalent amounts of 12 M HCl.

Plate Preparation: Plates were prepared in 200 mL batches using NGM recipe. 70% of water required for standard NGM recipe was added prior to autoclaving. Proceeding autoclaving NGM recipe was followed for addition of CaCl2, MgSO4, Cholesterol, and PPB. The rest of the water volume for NGM recipe was replaced with coffee solution or water (control). At this time cadmium stock solution or control HCl solution was added. The resulting solution was poured into 5.7 cm petridishes and allowed to solidify. Plates were then seeded the next day with liquid *Escherichia coli* OP50 and allowed to incubate for 2 days after seeding at room temperature.

Larval Growth Scoring: Larval stage of each animal was determined by characteristics described by Wood et al (1988) and binned into Egg, L1, L2 and L3 and L4, Adult.

Cadmium Sensitivity Assay: L4 mutant animals were picked to agar plates and allowed to lay eggs for approximately 20 hours. These animals were then removed from the agar plates making approximately synchronized populations of eggs. These eggs were allowed to develop for 4 days and scored for larval staging. This assay was performed for all strains and conditions used.

Cadmium Survival Assay: 15 L3 *mek-1* animals were transferred to plates containing 100 uM CdCl2. Survival was scored each subsequent day for 3 days. Survival was indicated by movement and pharyngeal pumping. If either movement or pharyngeal pumping was observed, the animal was scored as alive.

GCS-1::GFP Expression Assay: 3 L4 GCS-1::GFP containing animals were picked to agar plates and allowed to lay eggs for approximately 20 hours. These animals were then removed from agar plates. These eggs were allowed to develop for 4 days and scored for GCS-1::GFP expression in the posterior intestine. This assay was performed without coffee or cadmium treatment, coffee without cadmium treatment, 50 μM cadmium treatment, and 50 μM cadmium + coffee treatment.

Results:

Coffee does not obviously impact larval growth timing or brood size in *C. elegans*

To determine the potential impact of coffee supplementation on larval growth, we assayed larval growth timing in worms treated with coffee. Larval growth scoring was performed on day 1-4 with day 0 being time when L4 animals were placed on plates. Plates were scored for the number of animals that belonged to each of three stage groups (1. Egg, L1 and L2, 2. L3, 3. L4 and Adult). We found that coffee did not impact the larval growth rate in wild type and *mek-1* mutants because no difference was observed in the proportion of animals in each stage group over time. Coffee also did not speed up growth in *mek-1* mutants, which have a slower growth rate than wild-type (Figure 1).

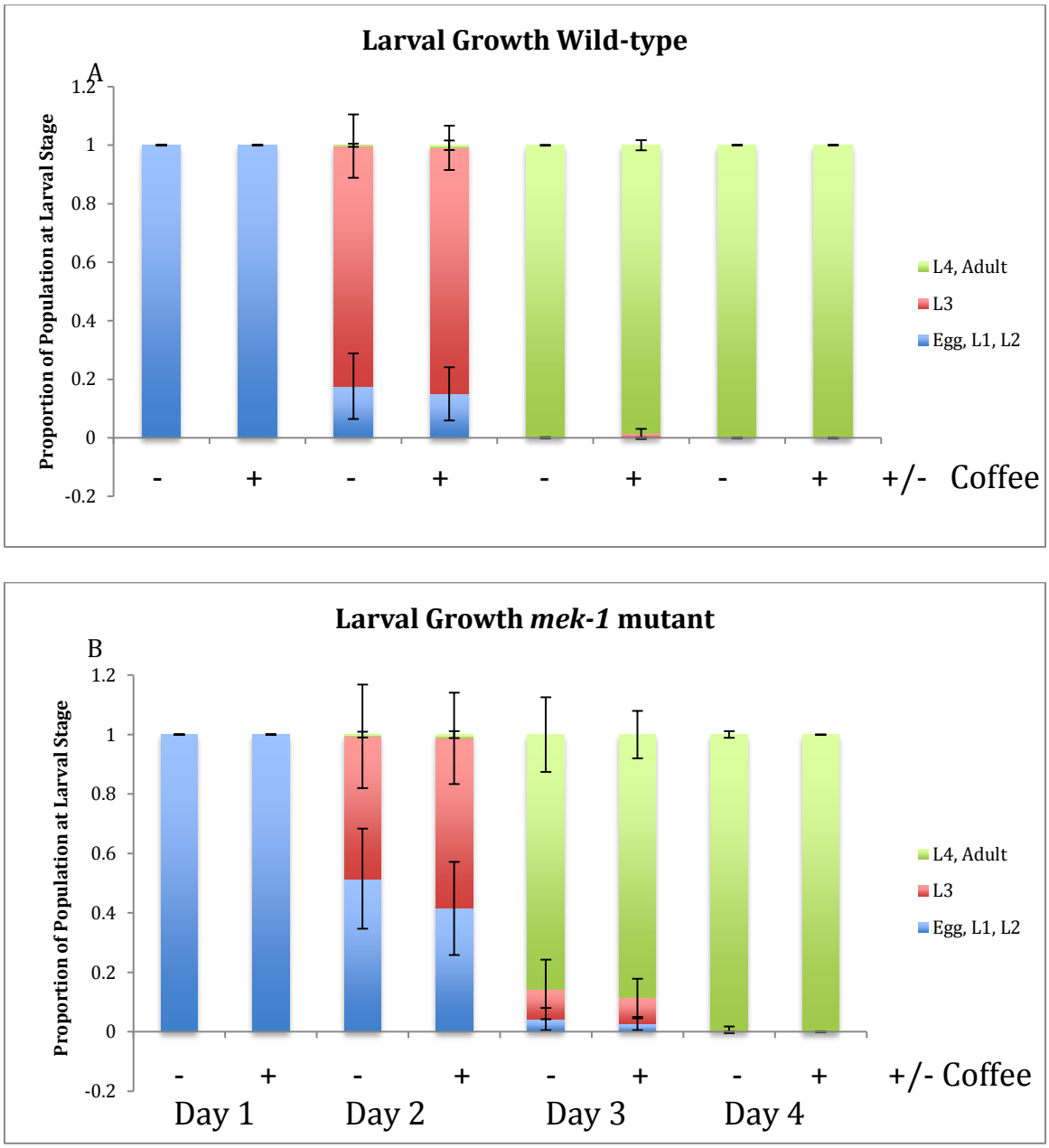


Figure 1: Coffee supplementation does not affect larval growth timing. Larval stages scored day 1-day 4. A) Larval growth of wild-type worms with and without coffee. B) Larval growth of *mek-1* mutants with and without coffee.

During our experiments, we observed that the presence of coffee in the agar plates produced a significant reduction in the thickness of the bacterial lawn. In addition to larval

growth timing (above), we tested worm brood size to determine if coffee or potential dietary restriction from reduced bacterial lawn thickness causes reduction in brood size associated with increased life span and stress response present in insulin signaling mutants (Gems et al 1998). We did not observe any significant change in brood size with coffee treatment (Figure 2). This is congruent with the observation by Dostal et al that coffee does impact pharyngeal pumping. We did not test *mek-1* mutant brood size because brood size with *mek-1* mutants is highly variable. We conclude that coffee does not cause any defects in larval growth rate or brood size.

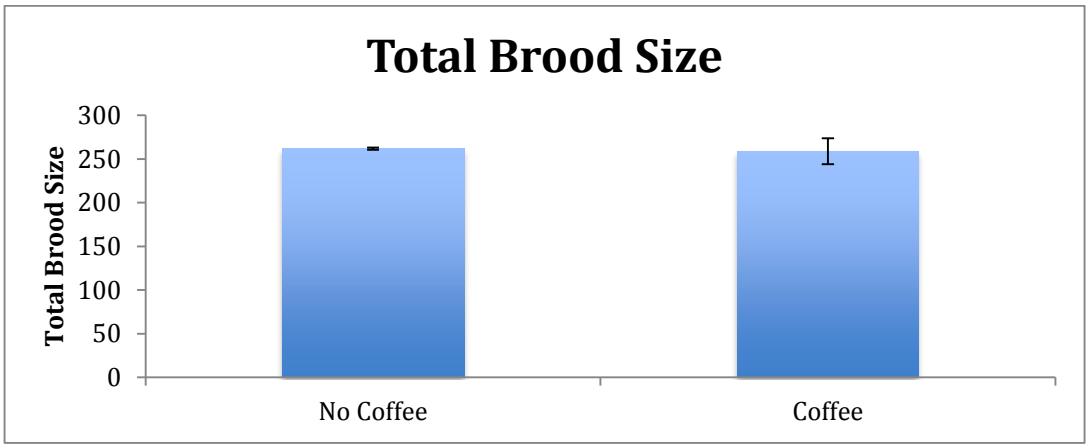
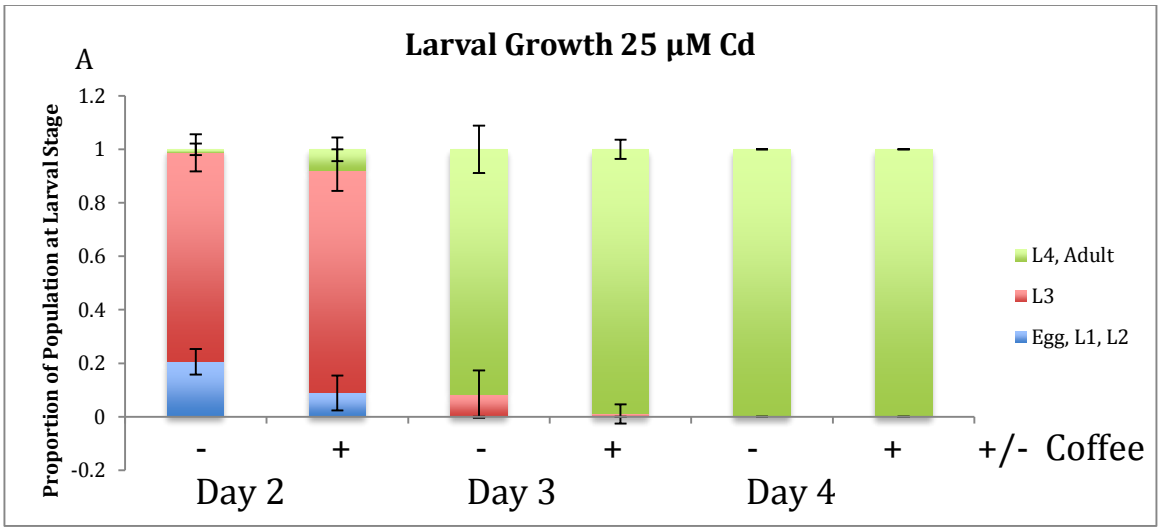


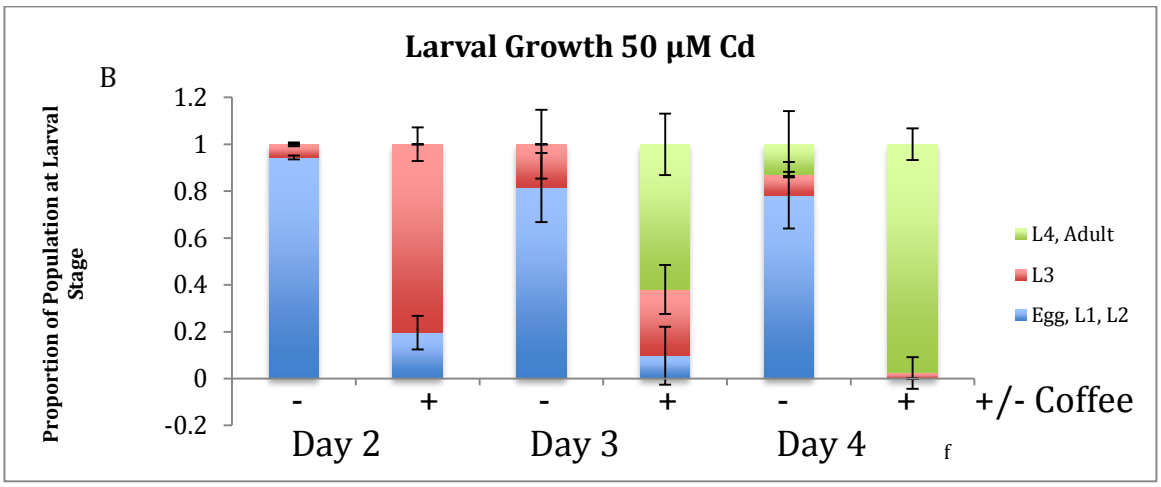
Figure 2: Brood size of wild-type worms scored with and without coffee treatment. Data represents three technical replicates and three biological replicates.

Coffee suppresses cadmium-induced defects in *mek-1* mutants

To test if coffee can play a protective role under heavy metal stress, we chose to study cadmium exposure using a cadmium-sensitive mutant. Wild-type worms did not show a strong sensitivity to cadmium, so we wanted to determine coffee protection on a sensitized background. We tested *mek-1* mutants because they were previously reported to be sensitive to cadmium and to play a role in heavy metal stress response (Koga et al 2000). While *mek-1* mutants show no

larval arrest (Figure 1B), treatment with cadmium at multiple concentrations induced a strong L2 larval stage growth arrest in *mek-1* mutants (Figure 3).


A
Larval Growth 25 µM Cd
Proportion of Population at Larval Stage
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
L4, Adult
L3
Egg, L1, L2
- + - + - +
+/- Coffee
Day 2
Day 3
Day 4


B
Larval Growth 50 µM Cd
Proportion of Population at Larval Stage
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
L4, Adult
L3
Egg, L1, L2
- + - + - +
+/- Coffee
Day 2
Day 3
Day 4

f

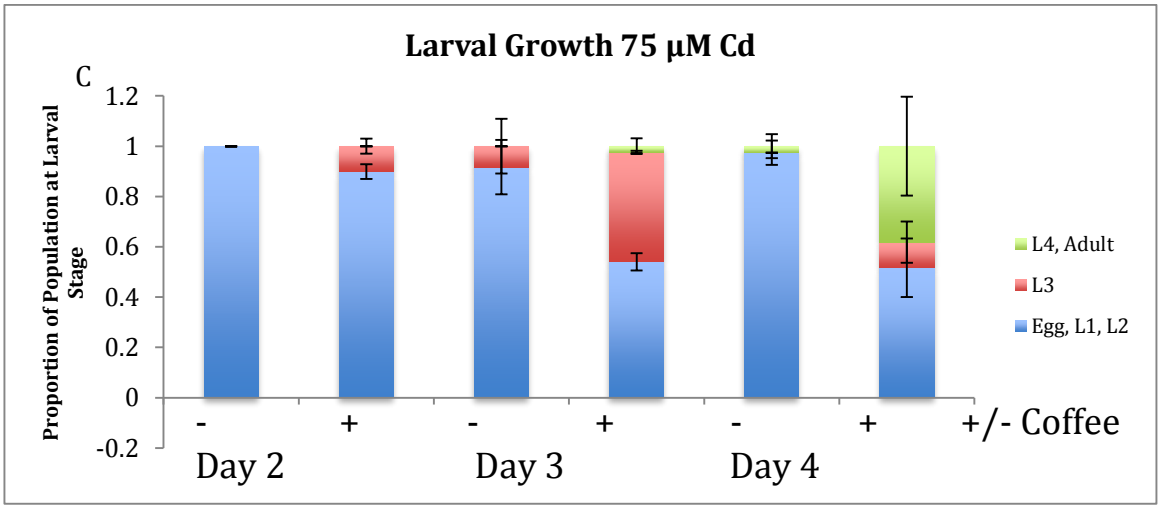

C
Larval Growth 75 µM Cd
Proportion of Population at Larval Stage
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
L4, Adult
L3
Egg, L1, L2
- + - + - +
+/- Coffee
Day 2
Day 3
Day 4

Figure 3: Larval stages scored day 2-4 for *mek-1* mutants exposed to A) 25µM Cd B) 50 µM Cd C) 75 µM Cd on plates with and without coffee. The data from three technical replicates and one biological replicate are shown.

We found that coffee supplementation partially suppresses cadmium-induced arrest allowing *mek-1* mutants to reach the L4 larval stage. The largest observable difference between *mek-1* mutant +/- coffee occurred on day 4 with 50 µM cadmium (Figure 3B). This time point and cadmium concentration was more rigorously tested to confirm the observed difference (Figure 4).

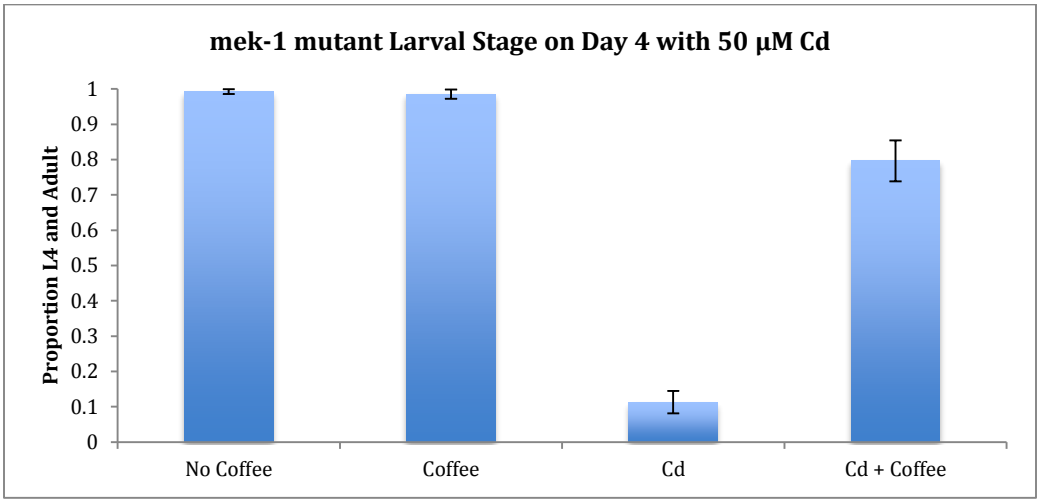


Figure 4: *mek-1* mutants treated with 50 µM Cd +/- coffee scored for larval stage on day 4. Coffee increases the percent of cadmium-treated *mek-1* mutants that reach L4 and adult stages by ~70%.

After observing protective effects with larval growth, we were interested in assaying changes in other cadmium-induced phenotypes. Cadmium is reported to cause death at high concentrations (Koga et al 2000). We determined the optimal concentration to observe cadmium-

induced death in *mek-1* mutants was 100 µM Cd. When supplemented with coffee, we observed a decrease in death in 3 out of 4 trials. On average it took 2.85 days for cadmium treatment to reach 50% survival and 3.40 days for cadmium + coffee treatment to reach 50 % survival.

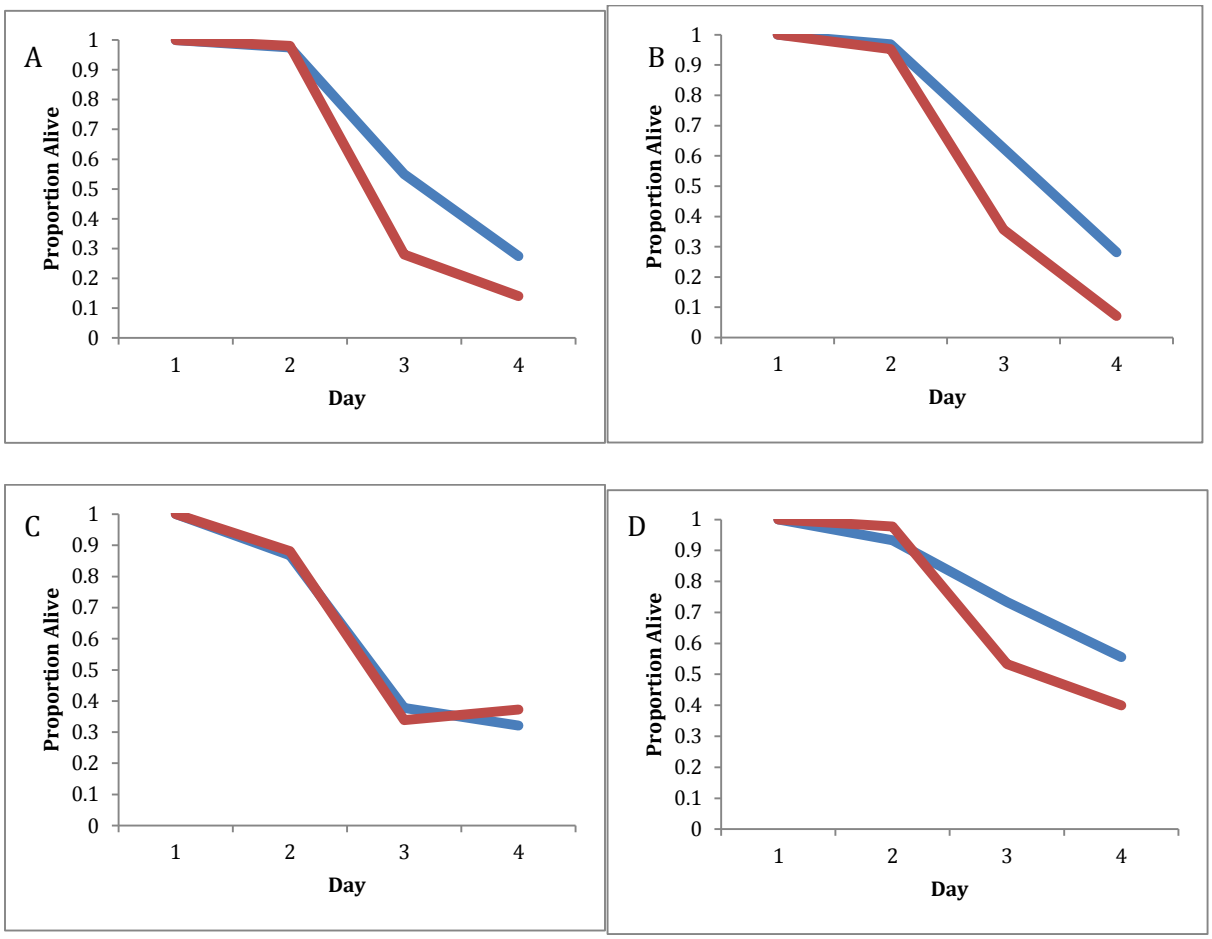


Figure 5: Survival scored for 4 days on 100 µM Cd. Each trial had 3 technical replicates. Four trials summed together p = .0074 between coffee (blue) and no coffee (red) using log rank from survival analysis using JMP software.

Taken together, these data support a beneficial role for coffee supplementation coupled with cadmium exposure in *mek-1* mutants.

Mutations in downstream target genes of MEK-1 do not individually show sensitivity to cadmium

Next, we were interested in testing downstream targets of MEK-1 to see if mutations in those genes would also show cadmium sensitivity and coffee protection. MEK-1 has been shown to increase activity of KGB-1, JNK-1, and PMK-1, which are believed to be intermediate stress signaling kinases that are active in response to cadmium toxicity (Kim et al 2004, Mizuno et al 2008, Villanueva 2001). These genes may play a role in signaling for cadmium detoxification. We reasoned that, in a compromised system, like the *mek-1(-)* mutant condition, where a protective effect is observed with coffee, it is possible that the protective effect is due to a change in activity of genes immediately downstream, such as the stress kinases KGB-1, JNK-1, and PMK-1.

50 μM cadmium treatment did not cause arrest in *pmk-1(-)* mutants; instead more than 90% of worms reached L4 or adult stage after four days (Figure 7). This is similar to wild type assayed with 50 μM cadmium treatment; therefore, we did not further consider the role of PMK-1 for coffee protection against cadmium toxicity.

50 μM cadmium treatment caused some arrest in *jnk-1(-)* mutants. However, 50 μM cadmium did not induce a consistent arrest phenotype in *jnk-1(-)* mutants making the role of JNK-1 in coffee protection unclear (Figure 7).

50 μM cadmium treatment caused some arrest in *kgb-1(-)* mutants as well. 50 μM cadmium induced a more consistent arrest in *kgb-1(-)* mutants than *jnk-1(-)* mutants. However, the arrest phenotype is not as severe as *mek-1(-)* mutants making the role of KGB-1 in coffee

protection unclear. Interestingly, a small suppression of the arrest phenotype is observed in *kgb-1(-)* mutants (Figure 7).

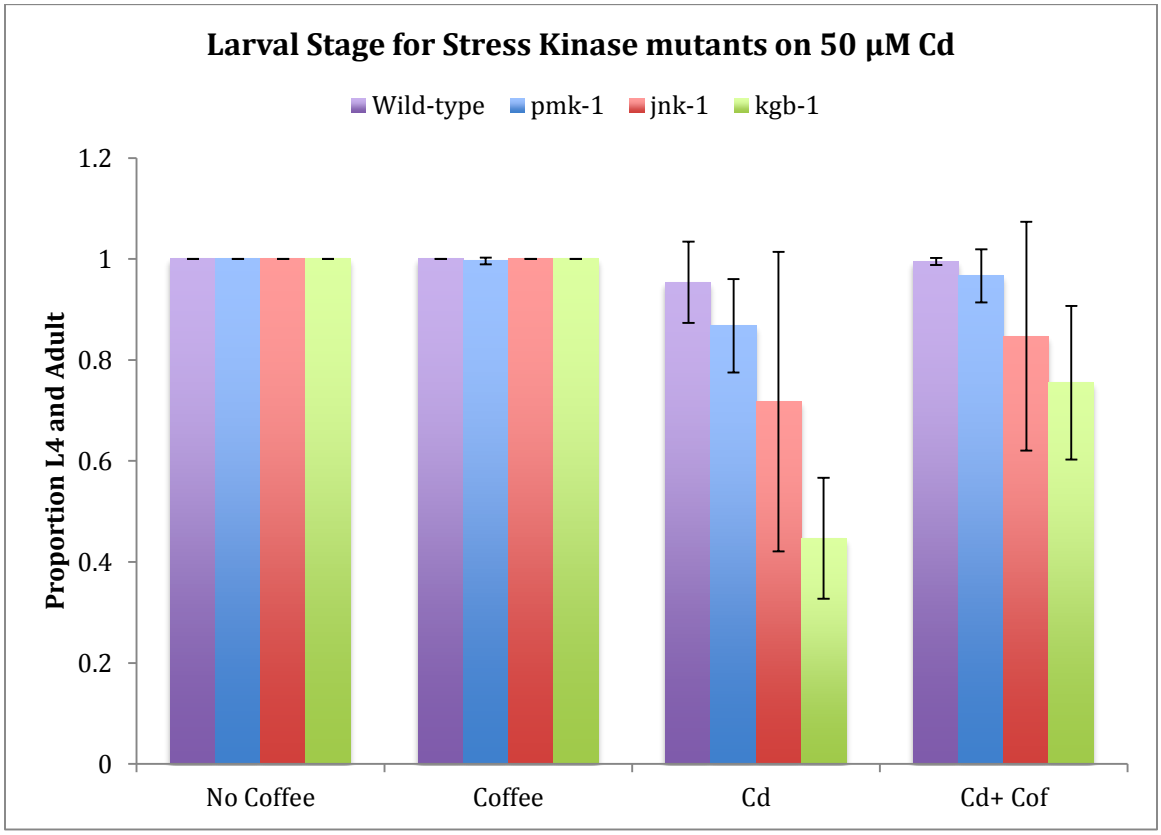


Figure 7: Stress kinase mutants scored on day 4 of 50 µM cadmium treatment +/- coffee. The bar graph represents data acquired from three technical replicates and three biological replicates.

The role of SKN-1 in coffee protection against cadmium toxicity is unclear

After finding that stress kinase mutants (*pmk-1, jnk-1, kgb-1*) did not individually show strong sensitivity to cadmium, we turned our attention to SKN-1, which is known to play a role in stress response. Previous work showed that coffee protects against β amyloid toxicity in a SKN-1 dependent manner (Dostal et al 2010). We hypothesized that coffee may protect against cadmium toxicity in a SKN-1 dependent manner as well.

50 μM cadmium treatment caused some arrest in two out of three trials (Table 1). In each of these trials the variability between technical replicates was high because mutants lay a small number of progeny. Because 50 μM cadmium did not induce consistent arrest in *skn-1* mutants, the role SKN-1 plays in coffee protection against cadmium toxicity remains unclear. Interestingly, we did not observe a large difference between +/- coffee treatments with cadmium when arrest was observed. However, the variability in these experiments prevent us from drawing a conclusion.

	Trial 1	Trial 2	Trial 3
No Coffee	26/26= 100%	38/39= 97%	19/21= 90%
Coffee	23/26= 88%	43/43= 100%	18/19= 95%
Cadmium	16/31= 52%	14/53= 27%	6/7= 86%
Cadmium + Coffee	12/22= 55%	6/40= 15%	11/14= 79%

Table 1: Percent animals reaching L4 or adult with *skn-1* mutant in the 2 trials where arrest was observed.

We attempted to see a difference in SKN-1::GFP nuclear localization with cadmium +/- coffee treatment because Dostal et al showed an increase in SKN-1::GFP nuclear localization with coffee treatment. However, no detectable difference was observed in with cadmium +/- coffee treatment or no cadmium +/- coffee treatment.

Coffee enhances cadmium-induced GCS-1::GFP expression

Because mutant models of genes immediately down stream of MEK-1 did not enhance our mechanistic understanding of coffee protection against cadmium toxicity, we turned our attention to examining the coffee impact on stress-induced fluorescent reporters. We examined the effects of cadmium +/- coffee treatment on two known oxidative stress induced GFP fluorescent strains GCS-1::GFP and GST-4::GFP. GCS-1 and GST-4 are two genes that play an important role in ROS capture and elimination (Liao et al 2005, Leiers et al 2003). We did not qualitatively observe any difference between fluorescent intensity in GST-4::GFP between our two conditions. However, we did observe an increase in the number of animals with GCS-1::GFP expression in the posterior intestine with cadmium + coffee treatment as compared to the cadmium treatment alone. This suggests that coffee enhances cadmium-induced GCS-1::GFP expression.

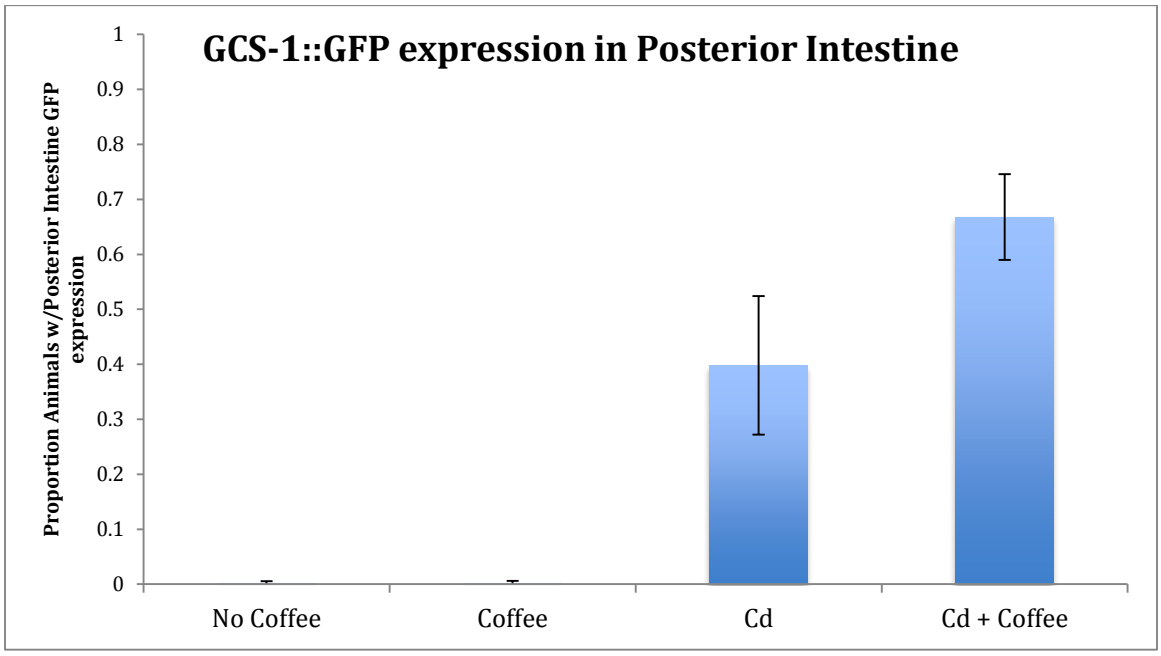


Figure 8: Proportion of worms showing GCS-1::GFP fluorescence in posterior intestine four days after egg stage. Worms scored are L4 and Adult stages. Data represents three technical replicates and three biological replicates.

HMT-1 and PCS-1 may play a cadmium toxicity defense mechanism

To determine if other genes may play a role in coffee's protection against cadmium toxicity, we focused on genes that are reported to be sensitive to cadmium. We tested mutations in eight genes that are reported to result in cadmium sensitivity (WormBase W251) in order to determine the response of each to coffee supplementation. We excluded mutations that are reported to have significant developmental defects, such as a constitutive dauer phenotype, or observed to have significantly abnormal larval growth rate, since these phenotypes would be incompatible with our assays. To test the sensitivity of these mutants to cadmium, we let synchronous populations of mutant worms grow for 4 days on 100 μM Cd and scored the larval staging. We chose to use 100 μM Cd to determine sensitivity because the threshold of sensitivity is low. Worms with significant cadmium sensitivity did not lay eggs on 100 μM Cd plates, so we picked eggs over to 100 μM plates for these strains to score staging. We determined that mutations in PCS-1, HMT-1, and CDR-1 all showed sensitivity to 100 μM Cd(Figure 9).

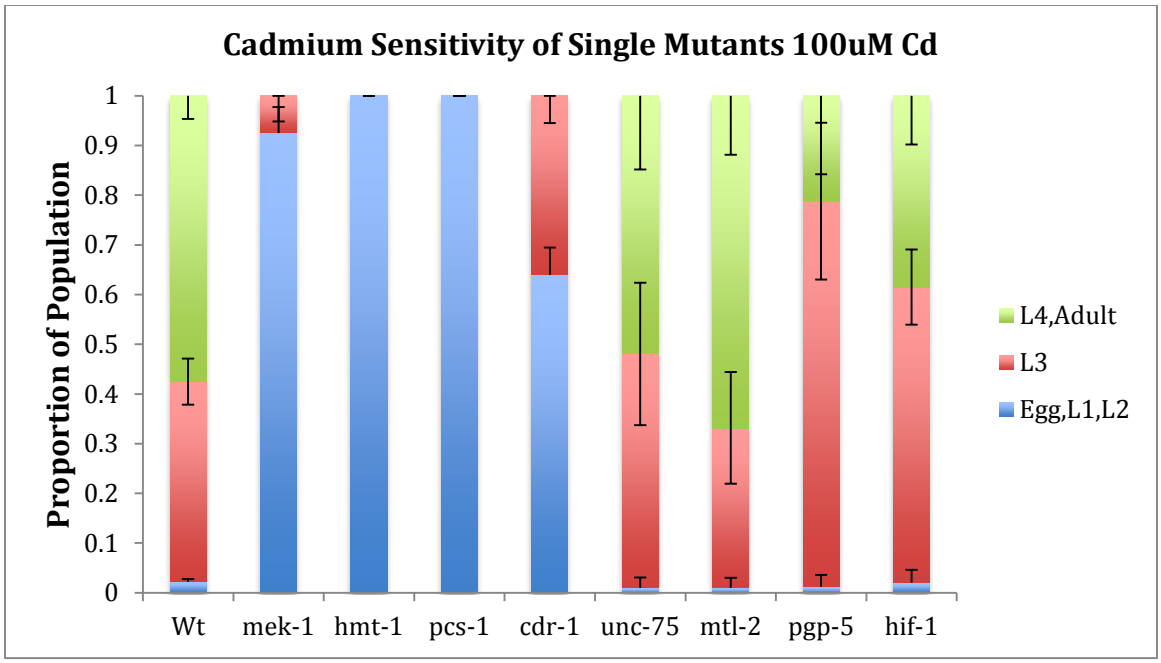


Figure 9: Larval staging for Cd sensitive mutants with 100 µM Cd treatment. Data represents three technical replicates and one biological replicate.

Among *hmt-1*, *pcs-1*, and *cdr-1*, we tested the cadmium sensitivity range for each mutant and found that 5 µM cadmium was sufficient to cause a weak L3 arrest in *pcs-1* and *hmt-1* mutants without additional defects (reduced brood size) that would preclude them from further analysis. *cdr-1(-)* mutants no longer showed cadmium sensitivity with 50 µM cadmium treatment. We are in the process of testing the potential protective effects of coffee in *pcs-1(-)* and *hmt-1(-)* mutants.

Discussion:

Our examination of coffee's impact on health has revealed that coffee protects against cadmium toxicity in *mek-1(-)* mutants by suppressing the cadmium induced larval growth arrest and cadmium induced death phenotypes (Figures 4 & 5). This is very interesting finding and is

consistent with coffee's protection in the Alzheimer's model (Dostal et al 2010). This result raises the intriguing question of how coffee confers protection against cadmium toxicity.

We do not know what components of coffee cause the protective effect. Dostal et al demonstrated that decaffeinated coffee maintained the protective effect. It likely that multiple components play an additive role in the protective effect.

The observed suppression of cadmium toxicity by coffee in *mek-1(-)* mutants suggests that coffee may act in a MEK-1 independent mechanism to protect animals from Cd-induced stress. We also investigated known targets of MEK-1 (PMK-1, KGB-1, and JNK-1), but did not find that individual mutants showed strong sensitivity to cadmium (Figure 7). *kgb-1(-)* mutants and *jnk-1* mutants did show some sensitivity to cadmium; however, these mutants did not phenocopy the cadmium sensitivity of *mek-1* mutants making it unclear if protection was observed. This could be due to the redundancy of cadmium stress response genes that both downstream of KGB-1 and JNK-1. We hypothesize that *kgb-1(-);jnk-1(-)* double mutant may phenocopy *mek-1(-)* mutant cadmium sensitivity, making it possible to evaluate functional relationship of coffee with KGB-1 and JNK-1 in coffee protection of cadmium toxicity.

Based on previous work by Dostal et al, it seemed reasonable to test if SKN-1 played a role in coffee protection against cadmium toxicity. However, *skn-1* mutants did not show consistent sensitivity to cadmium making it unclear whether SKN-1 plays a role (Table 1).

To determine candidate genes that we can evaluate for a role in coffee protection against cadmium toxicity, we performed a screen of reported cadmium sensitive mutants to determine which genes when mutated induced cadmium sensitivity as evidenced by larval growth arrest. We observed that *pcs-1* and *hmt-1* were the most sensitive to cadmium suggesting these genes are important for cadmium detoxification (Figure 9). Because our data suggests that HMT-1 and

PCS-1 are important for cadmium detoxification, we hypothesize that coffee may be altering HMT-1 and PCS-1 expression to confer protection against cadmium toxicity. Interestingly, HMT-1 transcription is reported to be altered by SKN-1 (Wang et al 2014). Because *hmt-1(-)* mutants and *pcs-1(-)* mutants have strong sensitivity to cadmium, we can evaluate the role of HMT-1 and PCS-1 in coffee protection against cadmium toxicity.

It is possible that coffee may protect against cadmium toxicity by inducing a mild stress that acts to promote a greater stress response (Gem et al 1998). However, our data does not suggest that this is the case because we do not observe any reduction in brood size or in larval growth rate from coffee treatment, two indicators of a mild stress induction. This is also supported by our observation that GCS-1::GFP fluorescence is not induced by coffee treatment alone (Figure 8).

We observed that coffee enhances cadmium-induced GCS-1::GFP expression. GCS-1 is known to play a role in protecting against oxidative damage by producing glutathione (Liao et al 2005). Interestingly, SKN-1 is known to increase GCS-1 expression; however, GCS-1 expression is reported to be altered by SKN-1 independent mechanisms as well (Wang et al 2010). The data suggests that coffee increases protein quantities of GCS-1 (Figure 8), which is often correlated with increased glutathione production as GCS-1 is the rate-limiting step of glutathione synthesis. With increased glutathione levels, the capacity of the ROS capture ought to be higher leading to reduced cellular damage from ROS. Interestingly, ROS levels in the cell are tightly regulated suggesting that there are proteins that sense the presence of ROS. GCS-1 has been shown to have a negative feedback mechanism (Crook-McMahon et al 2010, Wang et al 2014). We speculate that glutathione degradation may increase with coffee treatment leading to higher GCS-1 expression under stress conditions. Under non-stress conditions, GCS-1::GFP expression may

not increase even though glutathione degradation is higher because the oxidative state of the cell is stable. In the future, we plan to measure total oxidized and reduced forms of glutathione using a glutathione assay kit (Cell Biolabs, Inc) to determine the rate of degradation and formation of glutathione with coffee.

To understand how coffee may be altering GCS-1::GFP expression, we are interested in screening for genes that will suppress the coffee's enhancement of cadmium induced GCS-1::GFP expression. We have obtained a list of genes that are known to suppress GCS-1::GFP expression under arsenite exposure when the candidate gene expression is knocked down with RNAi (Crook-McMahon et al 2010, Table 2). Like cadmium, arsenite induces heavy metal stress. We hypothesize that RNAi knockdown of these candidate genes may also suppress coffee's enhancement of cadmium induced GCS-1::GFP expression and may negate coffee suppression of cadmium induced defects in *mek-1* mutants.

RNAi Target Gene	Proposed Function
C17G10.1	Regulates Translation
sdc-2	Sex determination/dosage compensation
dpy-22	Mediator Subunit
thoc-2	Transcriptional elongation
cand-1	Cullin-associated NEDD8-dissociated
csn-2	Cop9 signalosome
ufd-2	E4 ubiquitin enzyme
tir-1	Pathogen response
srm-1	Serpentine receptor
inx-9	Innexin
mthf-1	Methylenetetrahydrofolate reductase
C34F11.3	AMP deaminase
hpo-26	Sensitive to pore-forming toxins
apb-3	Adaptin
K04G7.11	Pre-mRNA splicing
F22F7.4	Unknown

Table 2: These RNAi candidate genes are reported to suppress arsenite induced GCS-1::GFP expression (Crook-McMahon et al 2010).

References:

An JH, Blackwell TK. SKN-1 links *C. elegans* mesendodermal specification to a conserved oxidative stress response. Genes Dev. 2003;17:1882–1893.

Barranco Quintana, J. L., M. F. Allam, A. Serrano Del Castillo and R. Fernandez-Crehuet Navajas. Alzheimer's disease and coffee: a quantitative review. Neurol. Res. 2007;29:91–95.

Bishak YK, Payahoo L, Osatdrahimi A, Nourazarian A. Mechanisms of cadmium carcinogenicity in the gastrointestinal tract. Asian Pac J Cancer Prev. 2015;16:9–21.

Crook-McMahon H.M., Oláhová M., Button E.L., Winter J.J., Veal E.A. Genome-wide screening identifies new genes required for stress-induced phase 2 detoxification gene expression in animals. BMC Biol. 2014;12:64.

Filipič M . Mechanisms of cadmium induced genomic instability. Mutat. Res. 2012;733:69–77

Gems D, Sutton AJ, Sundermeyer ML, Albert PS, King KV, Edgley ML, et al. Two pleiotropic classes of daf-2 mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. Genetics. 1998;150:129–155

Hečimović I, Belščak-Cvitanović A, Horžić D and Komes D, Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting. Food Chemistry 2011;129:991–1000.

Hu, G., S. Bidel, P. Jousilahti, R. Antikainen and J. Tuomilehto. Coffee and tea consumption and the risk of Parkinson's disease. Mov. Disord. 2007;22:2242–2248.

Inoue H, Hisamoto N, An JH, Oliveira RP, Nishida E, Blackwell TK, et al. The C. elegans p38 MAPK pathway regulates nuclear localization of the transcription factor SKN-1 in oxidative stress response. Genes Dev. 2005;19:2278–83

Kim DH, Liberati NT, Mizuno T, et al. Integration of *Caenorhabditis elegans* MAPK pathways mediating immunity and stress resistance by MEK-1 MAPK kinase and VHP-1 MAPK phosphatase. *P Natl Acad Sci USA* 2004;101:10990-4.

Koga M, Zwaal R, Guan KL, Avery L, Ohshima Y A *Caenorhabditis elegans* MAP kinase kinase, MEK-1, is involved in stress responses. EMBO J. 2000;19: 5148–5156

Leiers B, Kampkotter A, Grevelding CG, Link CD, Johnson TE, et al. A stress-responsive glutathione S-transferase confers resistance to oxidative stress in Caenorhabditis elegans. Free radical biology & medicine. 2003;34:1405–1415.

Liao VH, Yu CW. *Caenorhabditis elegans* gcs-1 confers resistance to arsenic-induced oxidative stress. Biometals. 2005;18:519–528.

Mizuno T, Fujiki K, Sasakawa A, Hisamoto N, Matsumoto K Role of the Caenorhabditis elegans Shc adaptor protein in the c-Jun N-terminal kinase signaling pathway. Mol Cell Biol. 2008;28:7041–7049

Schwartz MS, Benci JL, Selote DS, Sharma AK, Chen AGY, Dang H, et al. Detoxification of Multiple Heavy Metals by a Half-Molecule ABC Transporter, HMT-1, and Coelomocytes of *Caenorhabditis elegans*. PLoS ONE. 2010;5:e9564.

Thévenod F. Catch me if you can! Novel aspects of cadmium transport in mammalian cells. *BioMetals*. 2010;23:857–875.

Thévenod F., Lee W.K. Cadmium and cellular signaling cascades: Interactions between cell death and survival pathways. Arch. Toxicol. 2013;87:1743–1786.

Villanueva A., Lozano J., Morales A., Lin X., Deng X., Hengartner M. O., et al. . jkk-1 and mek-1 regulate body movement coordination and response to heavy metals through jnk-1 in *Caenorhabditis elegans*. EMBO J. 2001;20:5114–5128

Wang B, Du Y. Cadmium and its neurotoxic effects. Oxid Med Cell Longev. 2013:898034.

Wood WB. The Nematode *Caenorhabditis elegans*. Cold Spring Harbor Press. 1988.

Yu X, Bao Z, Zou J, Dong J Coffee consumption and risk of cancers: a meta-analysis of cohort studies. BMC Cancer 2011;11:96.