The regulation and function of *C. elegans flavin-containing monooxygenase-2*

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A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2021

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Program Authorized to Offer Degree:

Pathology

University of Washington

ABSTRACT

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In the last one hundred years, aging has become an increasingly tractable problem. The pioneering dietary restriction experiments of the 1920s and '30s, the robust evolutionary and molecular theories of the 1950s -'70s, and the identification of conserved longevity genes in the 1980s – 2000s all paved the way for the aging research field's current rapid expansion and mainstream traction. Along with metformin, senolytics, and numerous other promising avenues, the field is still characterizing the molecular mechanisms through which major interventions like dietary restriction and the inhibition of insulin and mTOR signaling promote longevity. In this dissertation, I use the nematode roundworm model species *Caenorhabditis elegans* to define the regulation and function of the conserved pro-longevity target gene *flavin-containing monooxygenase-2 (fmo-2)*. I find that both the regulation and function of *fmo-2* is

dependent on endogenous sulfur amino acid metabolism, placing *fmo*-2 at a nexus of redox, cellular energetics, and other processes central to aging.

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ACKNOWLEDGEMENTS

First, I would like to thank Matt Kaeberlein. He gave me an opportunity, supported me as a mentor, and consistently provided an excellent example to imitate and learn from. I would also like to thank Scott Leiser. Scott reached out to me to encourage me to not give up on science, he provided me an opportunity, and he supported me as a mentor while also leading by example.

Second, I would like to thank Alex Mendenhall. Alex freely shared some of the most exciting scientific ideas I have ever heard, he always raised our spirits when he visited the lab, and he has been an excellent mentor.

I would like to thank all the members of my committee. Allan and Cathy, thank you for sharing your expertise about FMOs with me. It has been exciting to hear your insiders' view. Dirk, thank you for being my clinical mentor. I learned a lot in the neurodegeneration course I took with you, and I hope to ask you a few clinical questions before I leave. Maitreya, thank you for being my GSR. Your genetics course was awesome, and I've always felt confident with you as my advocate.

I would also like to thank the M3D PhD program, in particular Nancy Maizels, Bill Mahoney, and Megan Barker. From recruitment through the ups and downs of grad school, you have always been there for me, offering support and finding a way to work with me.

Additionally, I would like to thank the GAATG and later BMHA training grants that UW won and awarded to me. Being on these grants gave me invaluable access to guest speakers,

stimulating exposure to ideas at the speaker series and at journal club, and made me feel like part of the aging research community at UW.

I would like to thank several of the aging faculty at UW: George Martin, Peter Rabinovitch, Daniel Promislow, Alan Herr, Scott Kennedy, and Shane Rea.

Thanks to George Martin for attending events and sharing his insights born from decades of involvement. Thanks also to Peter Rabinovitch for helming the GAATG and also attending and sharing his thoughts.

Thanks to Daniel Promislow. My second rotation was with Daniel, and he spent time discussing theories of aging with me in a way that really kindled my enthusiasm and deepened my understanding. In the ensuing years, he also always made time to talk to me.

Thanks to Shane Rea for loaning me reagents, sharing his carefully thought out scientific ideas, and for giving me career advice.

Thanks to Alan Herr for being an inspiring example of perseverance, for always being encouraging and supportive, and for sharing exciting scientific ideas one-on-one.

Special thanks to my undergrads Leo Yeh, Ethan Le, Cara Stacy, Ehmer Taj, Mitch Lopes, and Robert Chavez. Each of them performed excellently across the board, and they made my job easier and more fun.

Finally, deepest thanks to everyone who I worked with in the Kaeberlein lab. Without exception, everyone contributed to the friendly, intellectually stimulating culture of the lab, and there probably isn't a single senior member I didn't both learn something from and share a joke with at some point.

CHAPTER 1: A BRIEF HISTORY OF AGING RESEARCH

1930s: First experimental lifespan extension

In the 1930s, the first successful attempt to increase the lifespan of experimental animals was achieved. These experiments laid the foundation for modern experimental aging research. Scientists at Cornell University subjected rats to either control diet or dietary restriction (DR), and found that the DR animals had greater maximum lifespans[1]. These experiments were incredibly pioneering and seminal. For the next fifty years, the field would primarily develop theories of aging, including attempts to explain how DR lengthened lifespan.

1950s-1970s: Theories of aging

From the 1950s through the 1970s, the primary progress in aging research consisted of the development of theories that offered plausible explanations of aging. In the decades since, none of these theories has been proven completely false, but two of them have accumulated outstanding experimental support. The mechanistic Mitochondrial Free Radical Theory[2], along with its many subsequent variants, has found widespread experimental support[3–6]. The Disposable Soma Theory, an evolutionary theory of aging, has similarly grown in strength[7].

1980s-2004: Genetic breakthroughs

In the 1980s and 1990s, genetic experiments in *C. elegans* began to identify pathways that, when perturbed, lengthen lifespan. Genetic screens continued in yeast, worms, and flies, and by 2005, a handful of major genetic regulators of aging had been established. Two pathways that stand out among those identified include insulin and mTOR signaling.

2005-2013: Expansion of the field

Once major pathways had been identified, the field both followed up on them and pursued new directions. Screens and other experiments were undertaken to identify downstream effectors of the major therapies and signaling pathways. Additionally, diverse approaches began to yield results (Table 1). Senolytics and blood-based therapies are two particularly promising therapies.

2013-present: Mainstream traction

After the rapid expansion of the field, an approach that had proven beneficial to the broad field of cancer research years prior was undertaken. In 2013, "The Hallmarks of Aging" was published in Cell[8]. This paper coalesced the diverse insights and approaches that comprise aging research into a unified framework. It was successful in that it has become a near ubiquitous reference point in the field.

In the mid-2010s, several developments occurred that signified aging research was beginning to gain real mainstream traction. Important developments include the formulation and successful promotion of the "Geroscience" paradigm, the successful engagement of the FDA leading to the first clinical trials with aging as a primary endpoint, and the emergence major aging-specific research journals. The latter two of these developments are self-explanatory, but it is worthwhile to define the paradigm-shifting concept of geroscience. For decades, research into the major killer diseases of countries like the U.S. has been conducted by focusing on each disease separately. The geroscience paradigm points out that aging is the biggest risk factor for all of the major killer diseases. Therefore, funding aging research is likely

to lead to therapies that are effective against multiple major killer diseases, effectively killing multiple birds with one stone[9–11].

fmo-2 in the context of the history of aging research

fmo-2 has a neatly defined place in the history of aging research. The major interventions and genetic manipulations capable of extending lifespan each modulate the expression of hundreds of target genes. FMOs are activated by many, if not most, of these interventions[12–17]. Therefore, fmo-2 is an undercharacterized target of multiple longevity-promoting interventions.

Figure 1. Pubmed citations per year for search term "aging"

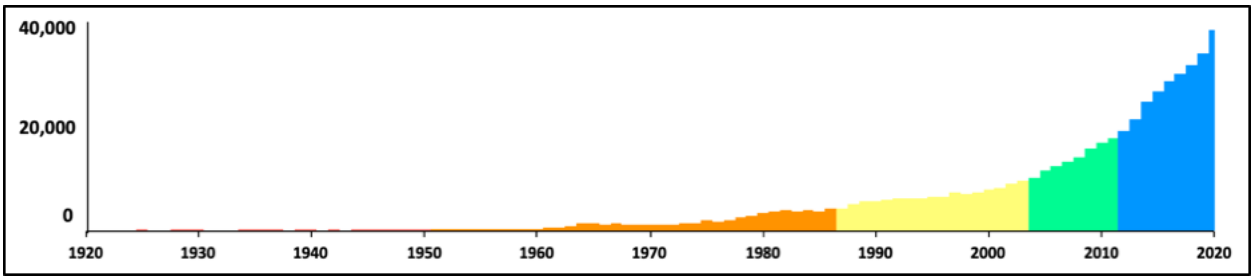


Table 1. Selected major events in the history of aging research

PERIOD	YEAR	EVENT
SEMINAL EXPERIMENTS (1930s)	1928	Fly lifespans experimentally altered[18]
	1935	Dietary restriction increases rat maximum lifespan[1]
THEORIES OF AGING (1950s-1970s)	1952	"Unsolved Problem of Biology"[19]
	1956	Mitochondrial free radical theory of aging[2]
	1961	Hayflick limit[20]
	1979	Disposable soma theory[7]
GENETIC BREAKTHROUGHS (1988-2004)	1988	age-1 worms[21]
	1993	daf-2 worms[22]
	1990s-	Long-lived dwarf mice[23]
	1998	Extant evolved "negligible senescence" postulated[24]
	2001	CHICO (insulin) flies[25]
	2004	mTOR yeast[26]
RAPID EXPANSION (2005-2012)	2005-	High-throughput lifespan screens continue in model species[27]
	2005	Mouse heterochronic parabiosis[28]
	2008	SASP identified[29]
	2009	Nobel Prize for telomerase discovery
MAINSTREAM TRACTION (2013-present)	2013	"Hallmarks of Aging" paper coalesces field[8]
	2014	Google's Calico invests over $700 million into its private sector aging research
	2014	"Geroscience" paradigm shift[9–11]
	2016	TAME trial approved by FDA[30]
	2020	Nature Ageing and The Lancet Healthy Longevity begin publication

CHAPTER 2: INTRODUCTION TO FLAVIN-CONTAINING MONOOXYGENASES

(Text and figures modified from *Rossner et al., 2017*)

INTRODUCTION

Since their discovery in 1972, flavin-containing monooxygenases (FMOs) have been primarily studied as phase I xenobiotic metabolizing enzymes, with FMO-mediated drug metabolism having been of particular interest. In contrast, endogenous functions and substrates of FMO enzymes have been far less studied. A growing body of recent evidence, however, implicates FMOs in aging, several diseases, and various biochemical and metabolic pathways. The evidence suggests a more critical role for this well-conserved protein subgroup in multiple processes and raises new questions about the relevant endogenous FMO substrate(s) and the control of FMO expression and activity in cells and animals. While numerous primary studies and literature reviews discuss the classical xenobiotic metabolizing function of FMOs, few publications focus on the endogenous role of these proteins. Here, we present a brief overview of evidence for FMOs' involvement in aging and disease, discussing the relevant basic biological context, and arguing for increased investigation into the function of these enzymes.

Evolution and classification

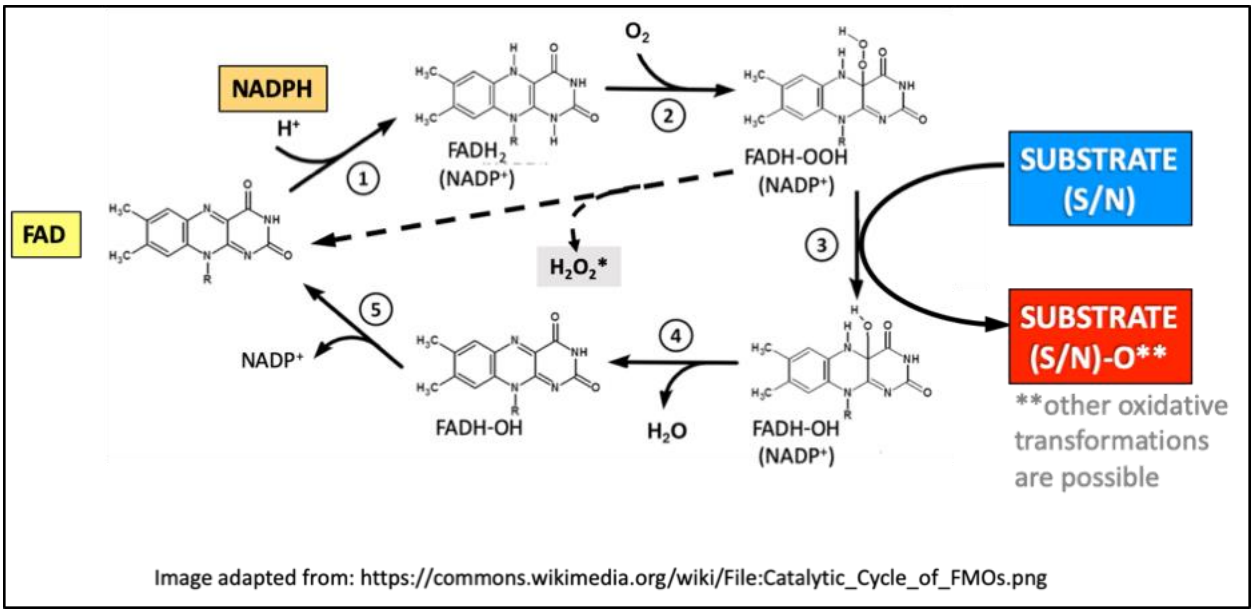
Flavin-containing monooxygenases (FMOs) are ancient and widely conserved, being present in all kingdoms of life [31,32]. FMOs make up a subgroup of the Group B flavin-dependent monooxygenases (EC 1.14.13) along with Baeyer-Villiger monooxygenases (BVMOs) and N-hydroxylating monooxygenases (NMOs) [33]. FMOs, BVMOs, and NMOs are each distinguished by variations in primary structural motifs, substrate preferences, and catalytic mechanisms. FMOs are also notably similar to the Group A enzymes dihydrolipoamide dehydrogenase (DLD),

glutathione reductase (GR), and low-molecular weight thioredoxin reductase (TRXR) [32,34]. These groups are distinguished from other flavin-dependent monooxygenases (Groups C-H) by their combined use of FAD and NAD(P)H. Any functional interplay between FMOs and Group A enzymes, perhaps as an NAD(P)H-thiol redox buffering system, is largely unexplored [35].

Catalytic cycle

The catalytic cycle of FMO enzymes is fairly well understood. FMOs utilize a tightly bound FAD prosthetic group, NAD(P)H, and molecular oxygen to monooxygenate or otherwise oxidize substrates, producing water and NAD(P)+ as byproducts [36] (Fig. 1). Uncoupling has been observed in both the presence and absence of substrate *in vitro*, resulting in "leakage" of hydrogen peroxide, and, less frequently, superoxide [37]. FMOs are notable for their "cocked and loaded" mechanism where they bind NAD(P)H and reduce FAD in the absence of substrate, creating an unusually stable C4a-hydroperoxyflavin ready to oxidize any substrate that accesses the active site [36]. This mechanism is in contrast to that of cytochrome P450s and the Group A enzymes discussed above, which each require the presence of substrate to begin their catalytic cycles [36]. The rate-limiting step of FMOs is thought to be either the release of H_2O or $NADP^+$. The significance of FMOs' reaction kinetics, ROS leakage, and effects on cellular NAD(P)H is not known [37,38].

Figure 2. FMO catalytic cycle



Substrates

FMOs typically monooxygenate the sulfur or nitrogen atoms of small, soft nucleophiles in a charge- and stereo-selective manner, but important exceptions exist. In addition to monooxygenation, FMOs can also catalyze oxidative decarboxylation [39], oxidative demethylation [40], and disulfide bond formation [41]. FMOs act on a wide variety of sulfur- and nitrogen-containing compounds, but carbon, phosphorus, selenium, and other elements are also amenable to FMO-mediated oxidation [36]. Pig FMO1 has a K_m from 0.3-10μM *in vitro* for several organic selenium-containing xenobiotics [36], and both hFMO1 and hFMO3 are capable of catalyzing the formation of pyruvate from selenocysteine [42], consistent with a role in endogenous selenium metabolism. Mammalian FMO5 does not metabolize typical FMO substrates, and may act more like a BVMO [38,43].

The depth and width of the channel leading to the active site are thought to restrict overly large substrates' access [44], but this mechanism is not fully understood because no crystal structure has been solved for a mammalian FMO. The closest orthologs' crystal structures are from bacterial trimethylamine monooxygenase [45–47] and yeast FMO [48], but their sequences are both shorter and significantly different from mammalian FMO sequences [49] (Fig. 2).

FMOs prefer uncharged substrates or substrates with a single positive charge. Neutral zwitterions are likely to be poor substrates [36], but the lone *S. cerevisiae* FMO (yFMO1) can oxygenate free cysteine [50]. FMOs' charge selectivity is the proposed mechanism by which they preferentially act on xenobiotics, as charged compounds cannot easily enter cells through the plasma membrane [36]. Classically studied FMO substrates include xenobiotics such as imipramine, nicotine, clozapine, tamoxifen, and amphetamine [36]. The oxygenation of these compounds increases their solubility and aids in their subsequent excretion, although it can occasionally also activate the compounds and increase their toxicity [51].

FMO metabolism of xenobiotics largely detoxifies compounds, while the role of FMO in endogenous metabolism is less characterized. FMOs can metabolize the sulfur amino acid pathway metabolites methionine, cysteine and cysteamine, and the sulfur-containing cofactor lipoic acid [36,50], but many related endogenous compounds remain untested.

Genomic organization and expression

Humans have five protein-encoding *FMO* genes (*hFMO1-5*) on chromosome 1 [52], each of which displays one-to-one orthology with *FMO1-5* across mammalian species [53]. Humans and other mammals also have several Fmo pseudogenes [54]. Alternative splicing of *hFMO1-5*,

especially *hFMO4*, is possible, but its function is unknown [55]. *hFMO1-4* are tightly clustered, likely the result of a gene duplication event that predates mammals, while the ancestral *hFMO5* is approximately 20Mb away [52,56]. Consistent with this hypothesis, mouse *Fmo5* (*mFmo5*) *is* on chromosome 3, while *mFmo1-4* are clustered together on chromosome 1 [52]. Subfunctionalization of mammalian FMOs probably followed duplication, possibly in response to new xenobiotics encountered in terrestrial environments [56].

hFMO1-5 show distinct developmental and tissue-specific patterns of expression [57,58]. Briefly, *hFMO1* predominates in the fetal liver, whereas *hFMO3* and *hFMO5* are the major isoforms expressed in the postnatal liver [57,58]. In contrast, *mFmo1* and *mFmo5* are the major adult mouse liver *FMOs*, while *mFmo3* is much more highly expressed in female mice [59]. There are conflicting reports regarding conservation in humans of this sex-dependent differential expression, but it is clear that the conserved differences reported in humans are of a smaller magnitude than those in mice [60–62].

Several factors that regulate *FMO* transcription are known [63–67]. Estrogen [59] and insulin [64] activate *FMOs* while testosterone [59] and glucagon [67] are *FMO* repressors, but *FMO5* is again an exception [68]. Several hormone receptors have been placed both upstream and downstream of FMOs [69,70]. Large differences can exist between *FMO* mRNA abundance, FMO protein levels, and FMO functional activity [69,71,72], indicating multiple levels of regulation that require further study.

FMOs AND DISEASE

While FMOs have been causally linked to only one disease, trimethylaminuria, evidence is accumulating that FMOs affect the pathology of multiple major diseases (Table 1). The nature of FMOs' involvement in these diseases, however, remains largely undefined.

Trimethylaminuria (TMAU)

Much of the FMO-related literature is focused on the only disease known to be caused by altered FMO activity, trimethylaminuria (TMAU) [73]. TMAU, or "Fish Odor Syndrome," is a disorder in which the volatile compound trimethylamine (TMA) cannot be converted to the soluble trimethylamine-N-oxide (TMAO), leading to excretion of TMA through the skin. TMA is a small metabolite derived from dietary intake or produced by gut bacteria that has a distinctive “fishy” smell. *hFMO3* mutations are the main cause of TMAU, but other causes exist including variations in *hFMO3* expression and microbiome overproduction of TMA. There is no cure for TMAU, so treatment consists of limiting dietary intake of TMA and its precursors such as choline and carnitine.

Scant epidemiological data exist on whether TMAU alters risk for other diseases. An early study observed TMAU sufferers to have a high incidence of hypertension [73], and there are at least two reported cases of TMAU co-presenting with neurological disorders unlikely to be related to sufferers' social stress [74]. These cases are interesting in relation to the other diseases recently connected to FMOs.

Atherosclerosis and cardiovascular disease (CVD)

Beginning in 2011, several studies concluded, based on both mouse and human data, that FMO3-dependent production of TMAO increases the risk for atherosclerosis and general cardiovascular disease (CVD) [75–77]. These studies shed light on the importance of gut microbiota in determining disease risk and demonstrated a clear association between elevated TMAO and CVD. In the proposed model, gut bacteria produce TMA from dietary precursors, hepatic FMO3 converts TMA to TMAO, and TMAO increases the risk for atherosclerosis and CVD. Proposed mechanisms for TMAO increasing CVD risk include effects on cholesterol [78], a prolongation of the pressor effects of angiotensin II [79], and platelet hyperreactivity and thrombosis potential [80].

These mechanistic explanations are not fully convincing, however, and contrast with other data more consistent with TMAO production being a protective response to CVD. First, seafood rich in TMA and TMAO is widely thought to lower CVD risk [81]. Second, as mentioned, an early study of TMAU sufferers found a high incidence of hypertension in the TMAU-afflicted group [73]. The proposed mechanism was that FMO3 could metabolize tyramine, an endogenous pressor molecule, thereby reducing its pressor effects and lowering blood pressure [73]. A follow-up study found that none of three examined *FMO3* polymorphisms predispose to hypertension in a sample of several hundred Caucasian patients, but also noted that severe, highly-penetrant loss-of-function mutations could "unmask pressor effects of variation in other drug metabolizing enzymes previously buffered by FMO3" [82]. At least two more recent studies are consistent with TMAO production having a reactive, protective function in response to CVD pathology [83,84], with one of these studies asserting that TMAO is, in fact, protective against CVD risk [84]. What is clear

from these publications is that TMAO is a molecule of great interest due to its diverse functions including osmolyte, chemical chaperone, ROS scavenger, and, now, potential risk factor [85].

Diabetes and metabolic disorders

FMOs expression is altered in human diabetic patients and rodent models of diabetes [86,87], and recent reports have revealed that FMOs can alter carbohydrate and lipid metabolism [70,87–91]. Two studies using streptotozocin-induced diabetes in rats show altered *FMO* expression in diabetic states [87]. A third study comparing liver biopsy samples from Type 2 diabetes mellitus patients with samples from non-diabetic patients found *hFMO5* downregulated [86]. A study examining expression of *FMOs* in diabetes finds a trend that *FMO3* is upregulated and *FMO5* is downregulated in the disease state [92]. As in CVD, it is not clear whether these *FMO* transcriptional changes are causative, protective, or have little effect on the disease process.

Recently, mammalian FMOs have been shown to affect intermediary carbon metabolism. Researchers who first linked *FMO3* to atherosclerosis also find that mFMO3 activity correlated with hepatic and/or plasma lipids and glucose levels [70] and that *FMO3* inhibition can divert cholesterol away from bilary excretion [88]. The former paper suggested that mFMO3's effects were PPARα- and KLF15-mediated, while the latter paper concluded that mFMO3 affected cholesterol balance through TMAO production, but that mFMO3's effects on lipids and inflammation were mediated by another substrate. Two other reports examining *mFmo1* and *mFmo5* knockout mice, respectively, found that both were capable of altering metabolism and energy balance sufficiently to cause gross alterations in body size [89,90]. A recent review discusses these mFMO-related effects on carbohydrate and lipid metabolism and energy balance [91].

Neurodegeneration and neurological disease

There is substantial evidence connecting *FMO* expression to neurodegenerative diseases including sporadic amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and schizophrenia. *hFMO1* expression is consistently decreased in the spinal cord of ALS patients [93], and single nucleotide polymorphisms in the *hFMO1* 3' untranslated region occur more frequently in female patients with sporadic ALS [93]. Additionally, *mFmo1* is upregulated in a mouse model of ALS, which, while in the opposite direction of human findings, may be explained by different stages of the disease affecting expression differently [93].

The role of FMOs in other neurodegenerative diseases is also intriguing but largely correlative. The *FMO* gene cluster containing *FMO1-4* is associated with lentiform nucleus volume, a physiological marker associated with PD, schizophrenia, and other neurological disorders [94]. Additionally, *hFMO1* and Parkin are downregulated in a rotenone model of PD carried out in cell culture [95]. The same study showed upregulation of caspase 3, and that *hFMO1* inhibition was sufficient to activate caspase 3, an executor of apoptotic cell death implicated in the loss of dopaminergic neurons in PD [95]. These data suggest a possible role for FMO1 in protecting against multiple neurodegenerative disorders.

Iron dyshomeostasis

Studies by a single research group support FMOs acting in iron homeostasis. Building on a known FMO-calreticulin complex [96], a ferrireductase role for FMO in an iron import complex termed "paraferritin" was described [97]. The complex was further described as including FMO,

calreticulin, DMT1, and other proteins [97]. Its suggested roles include serving as an alternative, non-transferrin mechanism for cellular iron uptake and as a means to deliver iron to mitochondrial ferrochelatase for incorporation into heme. While acknowledging that larger studies would be necessary to determine prevalence, this group also suggested diminished FMO activity as a risk factor for sideroblastic anemia based on four cases [98].

Mouse models of hereditary hemochromatosis, a disease characterized by excessive intestinal iron absorption and subsequent iron overload throughout bodily tissues, alter hepatic *mFmo* transcription. Mutation of the hemochromatosis gene Hfe (High Iron Fe) and high dietary iron both cause liver iron loading, but they do so via secondary and primary iron overload, respectively. Hepatic *mFmo3* transcription was highly upregulated by genetic hereditary hemochromatosis, but, counterintuitively, heavily downregulated in mice fed high dietary iron [99]. By magnitude, *mFmo3* was the most altered transcript across both conditions. In a separate study, *mFmo3* was upregulated in Hfe-deficient D2 mice, and, though the data weren't shown, *mFmo3* was described not to change in WT mice fed a high-iron diet [100]. These studies, in addition to those describing paraferritin, suggest that FMOs act in iron homeostatic pathways in the liver or elsewhere, and that this novel role for FMOs requires further exploration.

Table 2. FMOs in human disease and disease models

FMO GENE	DISEASES	NATURE OF ASSOCIATION
Fmo1	Sporadic ALS	• 3'UTR SNPs associated with increased disease risk [93]
Fmo2	**NO DATA**	**NO DATA**
Fmo3	TMAU	• Fmo3 mutations cause TMAU [73]
	Atherosclerosis	• Increased FMO3 activity increases TMAO, in turn increasing atherosclerosis risk [78]
	Chronic kidney disease	• Minor alleles at hFMO3 residue 158 were associated with increased circulating TMAO and faster eGFR decline [101]
	Diabetes	• Fmo3 downregulated in rodent models of diabetes
	Sideroblastic anemia	• Single study with small sample size found Fmo3 dysfunction in cases of sideroblastic anemia [98]
	Hemochromatosis	• Most differentially expressed hepatic transcript in mouse model of hemochromatosis [99]
Fmo4	**NO DATA**	**NO DATA**
Fmo5	Diabetes	• Hepatic *FMO5* expressed at ratio of 0.41 in diabetic:non-diabetic patients [86]
	Sporadic ALS	• *FMO5* SNP found associated with ALS, especially in female patients [102]
Locus 1q24.3	Neurodegeneration (multiple diseases)	• GWAS: Fmo polymorphisms affect lentiform nucleus volume, itself associated with multiple neurodegenerative diseases [94]
	Parkinson's	• Decreased expression of *FMO1* in rotenone model of Parkinson's in cultured primary midbrain dopaminergic neurons [95]

FMOS AND AGING

Published data from the past decade provide increasing evidence that FMOs play an important role during aging. Specific *Fmo* genes are transcriptionally activated in numerous mouse longevity models including dietary restriction (DR), growth hormone/insulin-like growth factor 1 (GH/IGF1) signaling disruption, and rapamycin treatment [12,14,103,104]. These are among the most robustly conserved longevity-promoting interventions [105]. The correlation between increased *mFmo* expression and longevity suggests that FMOs could play a causal role in promoting longevity. Further supporting this, *C. elegans* (nematode worm) *fmo-2* is upregulated by DR and is necessary for lifespan extension from sDR, a form of DR [106]. Nematode FMO-2 is also sufficient to extend lifespan and improve healthspan and stress resistance when ubiquitously overexpressed [106].

FMOs and mammalian aging

Multiple gene expression analyses from mouse models of delayed aging show that *mFmo* gene expression is often increased in long-lived mice (Table 2). For example, a 2007 meta-analysis of published liver microarray data found that *mFmo3* is consistently upregulated in a variety of long-lived knockout models and in response to longevity-promoting interventions [12]. Similarly, a 2008 study of XME gene expression in liver found *mFmo3* and *mFmo4* to be upregulated in long-lived male mice following DR, GH/IGF1 mutation, or rapamycin treatment [103]. Of particular interest, an independent study showed that growth hormone receptor mutant mice have increased levels of both *mFmo3* and TMAO, further correlating *mFmo3* expression and activity with longevity [107].

Dietary restriction and treatment with the drug rapamycin are the two best-documented and most effective interventions for delaying diseases of aging and increasing lifespan in mice [108], and several studies, including those listed above, have observed increased *FMO* gene expression in animals subjected to both interventions (Table 2). For example, a comparison of mouse DR and gene expression over time found multiple *FMOs* upregulated by DR [14], with *mFmo3* and *mFmo5* among the most significantly upregulated liver transcripts, along with heart *mFmo3*. Overall, *mFmo1* and *mFmo2* were among the most significantly elevated genes when all 17 tested tissues were considered as a group. Interestingly, the same study found that *mFmo1* was significantly downregulated with age in animals fed a normal diet, suggesting that reduced *FMO1* expression could be a biomarker of normative aging or even causally involved in the aging process. The most extensive analysis to date of gene expression changes associated with rapamycin treatment in mice found that hepatic *mFMO* levels are consistently elevated by both DR and rapamycin in both male and female mice [104].

Crowded litter mice represent an alternative model of DR where animals experience nutrient restriction only during the first three weeks of life [109]. Interestingly, even this early life restriction is sufficient to cause persistent induction of *mFmo3* in liver up to 12 months later. This could suggest that epigenetic changes associated with DR, and perhaps other longevity interventions, induce persistent changes in *FMO* expression that contribute to healthy aging even after the intervention is discontinued.

In contrast to data supporting a role for FMOs in promoting longevity, loss of *mFmo5* results in a blood profile of cholesterol, glucose/insulin, and other biomarkers that resembles a more youthful state [90]. The authors suggest that this could mean FMO5 itself promotes metabolic

aging. However, since this study did not test the long-term health effects of the metabolic profile, it is unclear whether these results are representative of aging or metabolic reprogramming [90]. FMO5 also has a unique substrate profile among mammalian FMOs [38], so it may not be representative of the majority of FMOs. Given their wide taxonomic distribution and numerous evolutionary modifications, there are likely to be exceptions to any broad claims about FMOs as a group.

FMOs and aging in non-mammalian species

Extensive data linking FMO function to aging have come from studies performed in the nematode *C. elegans.* Several independent studies show that *fmo-2* is upregulated by both genetic and environmental models of increased lifespan in this organism, including DR, hypoxia, mutation of the Von Hippel Lindau tumor suppressor, stabilization of the hypoxic response transcription factor, and developmental electron transport chain inhibition [110–114]. Recently a direct, causal role for FMO-2 as a longevity-promoting factor was uncovered by work showing that either ubiquitous or intestine-specific overexpression of FMO-2 in otherwise wild type animals is sufficient to extend lifespan in worms [106]. Consistent with this, deletion of *fmo-2* prevented full lifespan extension following activation of the hypoxic response or DR, further supporting a model that activation of *fmo-2* contributes to lifespan extension under these conditions [106].

Additional worm *fmo* genes are upregulated by these same longevity interventions, but they have not yet been studied for their roles in aging. Of note, *fmo-1* is upregulated in a more lasting manner than *fmo-2* by fasting [114]. Also, *fmo-4* is induced similarly to *fmo-2* by hypoxia [110].

Worm *fmo-4* is expressed in the hypodermis, whereas *fmo-2* is expressed in the intestine, pharynx, and excretory cells [115]. Worm *fmo-4* also displays a hypoosmotic sensitivity phenotype unique among worm *fmo* genes [116]. Taken together, this evidence suggests that further valuable details can be learned about worm FMOs' roles in healthy aging, stress resistance, and normal physiological processes.

Evidence from flies and plants further suggests that FMOs promote longevity. RNAi knockdown of *Drosophila melanogaster* (fly) *Fmo2* shortens adult lifespan [117]. As with worm and mammalian *FMOs*, fly *Fmo2* is not directly orthologous to worm *fmo-2* or mammalian *FMO2*. In fact, both fly Fmo genes are more similar to the ancestral yeast Fmo genes [49]. Plants have evolved a distinct group of FMOs termed "YUCCAs." *Arabidopsis thaliana* YUC6 overexpression in potato plants results in increased height, erect stature, and longevity due to YUC6's role in auxin production [118]. These phenotypes may be plant-specific, or there may be overlap with FMO functions conserved in animal species. YUCCAs also promote drought resistance, and some FMOs are involved in osmoregulation in worms [116] and fish [119], in addition to producing the osmolyte TMAO in humans [73]. Interestingly, there is overlap between the DR and osmotic stress pathways [120,121], and it is plausible that FMOs act at this intersection.

Table 3. FMOs and aging in model systems

SPECIES	FMO GENES	LIFESPAN/HEALTHSPAN EFFECTS	EXPRESSION CHANGES IN LONGEVITY INTERVENTIONS
M. musculus (Mouse)	*Fmo1*	**NO DATA**	**NO DATA**
	Fmo2	**NO DATA**	• Caloric restriction ↑[14]
	Fmo3	• Regulates whole-body cholesterol balance in mice [88]	• Caloric restriction ↑[14,103] • ΔIIS ↑[12,103] • Rapamycin ↑[103] • Methionine restriction ↑
	Fmo4	**NO DATA**	• Caloric restriction ↑[103] • ΔIIS (GHRKO, Little, Snell) ↑[103] • Rapamycin ↑[103]
	Fmo5	• Suggested to be a metabolic regulator of aging [90]	• Caloric restriction ↑[14]
D. melanogaster (Fly)	*Fmo1*	**NO DATA**	**NO DATA**
	Fmo2	• RNAi shortens adult lifespan [117]	**NO DATA**
C. elegans (Worm)	*fmo-1*	**NO DATA**	• Dietary restriction ↑[113,114]
	fmo-2	• Overexpression sufficient for LS extension [106,122] and stress resistance [106] • Necessary for sDR longevity [106] • Necessary for hypoxic response longevity [106]	• Dietary restriction ↑[106,113] • Hypoxic response ↑[110,111] • Mitochondrial disruption ↑[112] • Long-telomere worms ↑[122]
	fmo-3	**NO DATA**	**NO DATA**
	fmo-4	**NO DATA**	• Dietary restriction ↑[106,113] • Hypoxic response ↑ [110,111]
	fmo-5	**NO DATA**	**NO DATA**
	C46H11.2	**NO DATA**	• Dietary restriction ↑[114]
	C01H6.4	**NO DATA**	**NO DATA**
S. cerevisiae (Yeast)	*fmo1*	**NO DATA**	• responds to altered sulfur availability (possibly related to methionine restriction) [123,124]
A. thaliana (Plant)	*YUC6*	• OE sufficient for longevity and stress resistance [118]	**NO DATA**

DISCUSSION

FMOs and disease: unifying mechanisms?

Our understanding of FMO-disease relationships is nascent, but the data already suggest two common mechanisms. Altered sulfur amino acid (SAA) metabolism affects CVD [125], metabolic disease [126], and neurodegenerative disease pathology [127]. Again, several SAA pathway metabolites that are credible FMO substrates remain untested as such. Iron metabolism is a second pathway that links FMOs, SAAs, CVD, metabolic disease, and neurological disease.

FMOs and aging: evolutionary considerations

Data from non-mammalian model systems demonstrate that FMOs can play a direct, causal role in promoting longevity. In worms, *fmo-2* is sufficient to extend lifespan and is required for lifespan extension from numerous interventions. *FMO* induction in response to multiple longevity-enhancing interventions in mice supports a conserved role, as do the primary structural similarities shared by worm and mammalian FMOs (Fig. 2).

If FMOs promote longevity in a conserved manner, then the conditions shaping both the evolution of FMOs and aging will merit attention. Induction of *FMOs* in response to both DR and osmotic stress, for example, suggests a "harsh times" survival strategy can underlie longevity. Model systems will be continue to be of outstanding utility in such investigations.

Future directions

FMOs are emerging as enzymes of considerable interest, with clear experimental and theoretical research directions identifiable. Two lines of experimentation will greatly solidify our

foundational knowledge of FMOs. First, solving the crystal structures of hFMOs would conclusively answer structural questions and directly inform functional ones. Second, thorough testing for endogenous substrates aging, disease, and basic cellular function would solidify the conserved endogenous role of these proteins. There are several candidate endogenous substrates of major biological interest that have not been tested *in vitro* or otherwise. Metabolites in the sulfur amino acid metabolism pathway, including S-adenosyl methionine, homocysteine [125], and homocysteine adducts, are potential targets for prioritization.

Evidence points to FMO involvement in multiple major diseases and the aging process. There is an ongoing debate whether aging should be reclassified as a disease, but it is clearly established that aging is the biggest risk factor for the major causes of death including CVD, cancer, and neurodegeneration [11]. The risk for each of these increases exponentially with age, independent of other risk factors. Taking everything into consideration, FMOs are an exciting, undercharacterized subgroup of well-conserved enzymes that may play central roles in basic biological processes affecting human health, and there are clear first steps to characterizing them more fully.

ACKNOWLEDGEMENTS

This work was supported by NIH grant R00AG045200 to SFL, NIH grant R01AG038518 to MK, and NIH training grant T32AG000057 to RR.

Figure 3. FMO sequence and structure analysis

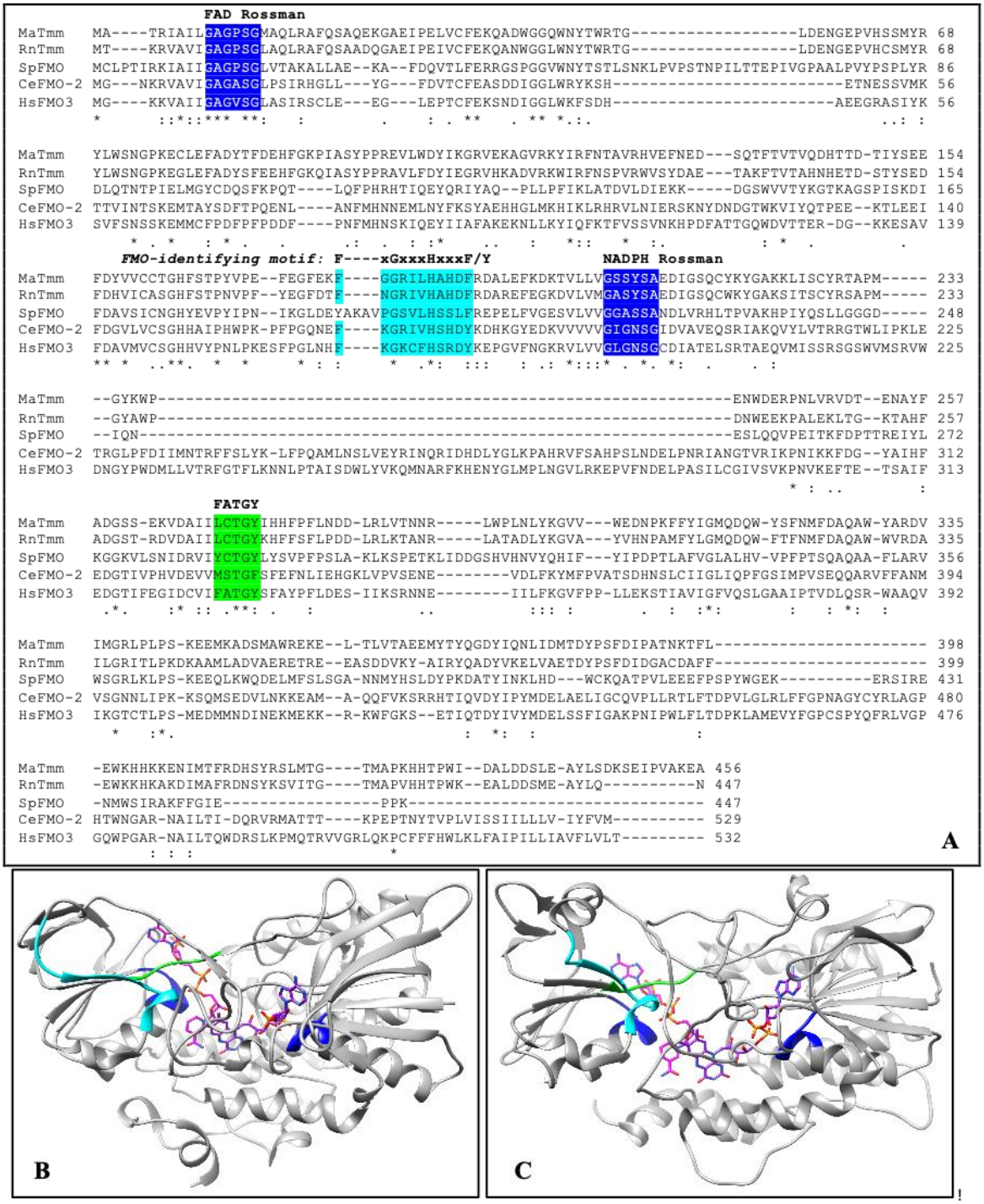


Figure 2. FMO ribbon structures with cofactors and highlighted conserved motifs. A. Alignment of *Methylophaga aminisulfidivorans* trimethylamine monooxygenase (MaTmm), *Roseovarius nubinhibens* Tmm (RnTmm), *Schizosaccharomyces pombe* FMO (SpFMO), *Caenorhabditis elegans* FMO-2 (CeFMO-2), and human FMO3 (HsFMO3). B,C. Ribbon diagrams for (B) RnTmm (PDB: 5ipy) and (C) SpFMO (PDB: 2gv8). FAD is purple, NADPH is magenta, and motif colors correspond to the annotated alignment.

Figure 4. Model of FMO regulation and function

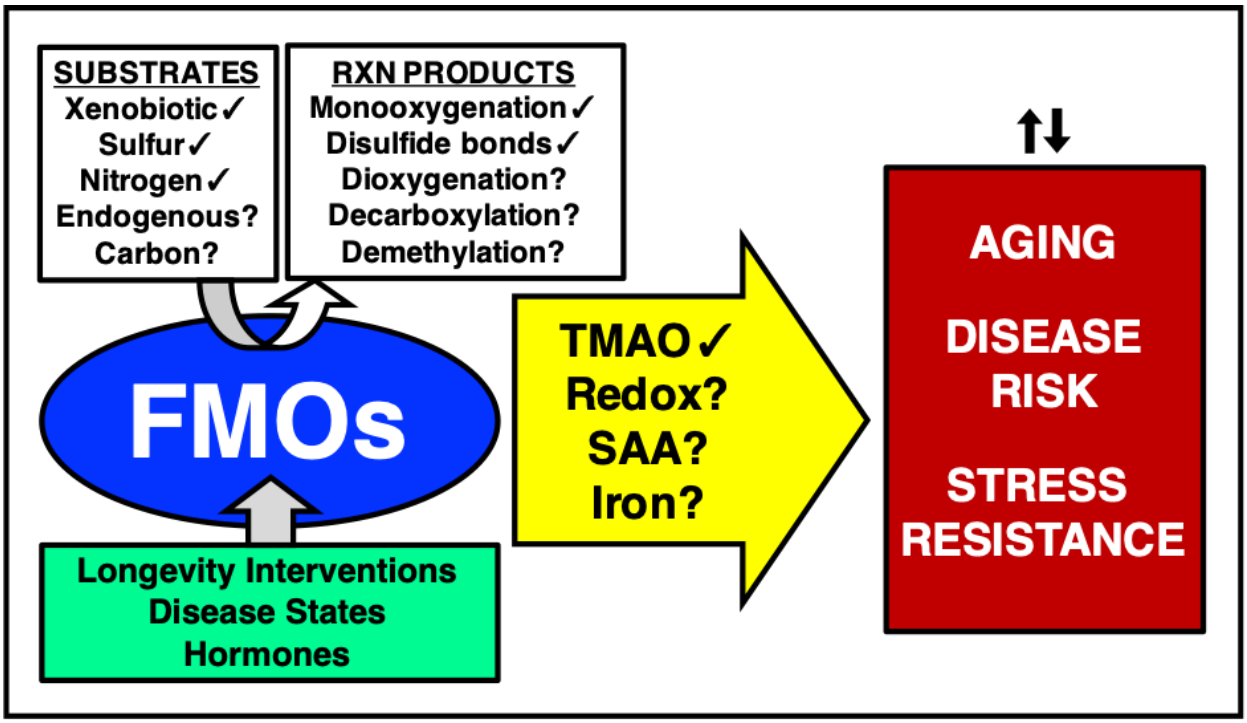


Figure 3. Summary of current knowledge about the regulation and broader role of FMOs. The green box (lower left) depicts known regulators of FMO transcription. The white boxes (upper left) and arrow (center) show both observed **(✓)** and hypothetical (?) reactions and mechanisms of FMOs, leading to the broader effects in the red box (right). FMO = flavin-containing monooxygenase, TMAO = trimethylamine N-oxide, SAA = sulfur amino acids. "Redox" refers to changes in oxidation/reduction balance resulting from changes to the NADPH/NADP+ ratio and/or increased production of hydrogen peroxide.

CHAPTER 3; REGULATION OF FMO-2

INTRODUCTION

Since their discovery five decades ago, flavin-containing monooxygenases (FMOs) have been primarily defined and studied as Phase 1 xenobiotic metabolizing enzymes[36]. FMOs use an FAD prosthetic group, NADPH, and molecular oxygen to, typically, monooxygenate or otherwise oxidize the nitrogen or sulfur atoms of small, minimally charged xenobiotic molecules, rendering them more polar and therefore more ready for aqueous excretion[36].

Also since FMOs' discovery, however, there has been an undercurrent of interest in their ability to metabolize endogenous substrates, particularly sulfur amino acid (SAA) pathway intermediates[35]. Results from early mammalian microsome studies[35], later yeast studies[50], and a recent mouse study[128] are consistent with such a role. SAA metabolism can be thought of as having two branches: the methionine cycle, which enables almost all cellular transmethylation, and transsulfuration, which results in the synthesis of cysteine and subsequently glutathione and taurine (Fig. 1A) Homocysteine is the molecular hub between the two branches (REFS). In C. elegans nematode roundworms (worms), fmo-2 is activated by several stressors and by multiple lifespan-extending interventions, is necessary for lifespan extension from several aforementioned lifespan-extending interventions, and FMO-2 overexpression is sufficient to extend lifespan[16,129,130]. The mechanisms by which fmo-2 promotes longevity are undefined. Some mechanisms of fmo-2 regulation have been defined, mostly transcription factors (Table 1), but the cellular events that link interventions to fmo-2 activation by these transcription factors are undefined.

Based on the connections between FMOs and SAA[35,50,131], FMOs and longevity[103,106,129,130], and SAA and longevity[132–135], we sought to test the hypothesis that fmo-2 responds to changes in SAA metabolism. Our results show that inhibition of transsulfuration, subsequent glutathione synthesis, or S-adenosylhomocysteine (SAH) hydrolysis are all sufficient to activate fmo-2, and that the folate-dependent remethylation of homocysteine to methionine, subsequent S-adenosylmethionine (SAM) synthesis, and its major output, SAM-dependent phosphatidylcholine (PC) synthesis, are all necessary for fmo-2 activation by the stressor ethanol and by fasting, a form of dietary restriction, the most robust conserved lifespan- and healthspan-extending environmental intervention[136]. Thus, SAA metabolism and one of its major outputs, SAM-dependent PC synthesis, regulate fmo-2 in multiple contexts including longevity.

RESULTS

Inhibition of transsulfuration activates fmo-2

To test the hypothesis that fmo-2 responds to changes in SAA metabolism, we first performed a targeted RNAi screen of SAA genes in which we exposed fmo-2p::GFP transcriptional reporter worms to SAA and control RNAi from egg, then photographed all conditions on Day 2 of adulthood to measure activation. RNAi against genes encoding the enzymes of transsulfuration (cbs-1, cth-2), subsequent glutathione synthesis (gcs-1), or reversible SAH hydrolysis increased fmo-2p::GFP fluorescence compared to negative control RNAi (Fig. 1B,C). Thus, inhibition of transsulfuration, subsequent glutathione synthesis, or reversible SAH hydrolysis is each sufficient to activate fmo-2 (Fig. 1D).

SAM synthesis is required for ethanol-mediated activation of fmo-2

To test whether any SAA gene(s) were necessary for fmo-2 activation by a non-SAA intervention, we grew fmo-2p::GFP worms from egg on the same set of SAA RNAi clones, but at LY/YA, we transferred worms to RNAi plates containing 200mM ethanol, a potent activator of fmo-2[137,138]. RNAi against genes encoding the enzymes of folate-dependent remethylation of homocysteine to methionine (mthf-1, metr-1) or SAM synthesis (sams-1) was each sufficient to attenuate ethanol-mediated activation of fmo-2 (Fig. 2A,B). RNAi against sams-1 attenuated ethanol-mediated activation of fmo-2 more strongly than did RNAi against mthf-1 or metr-1. Thus, the folate-dependent remethylation of homocysteine to methionine (mthf-1, metr-1) and subsequent SAM synthesis (sams-1) are necessary for fmo-2 activation by ethanol (Fig. 2C).

PC PC synthesis is required for ethanol-mediated activation of fmo-2

Having established that SAM synthesis was necessary for ethanol-mediated activation of fmo-2, we next wanted to test whether SAM-dependent PC synthesis was also necessary. SAM is used by hundreds of methyltransferases in worms, a highly conserved promiscuity, and SAM also contributes to methylation-independent processes including polyamine synthesis. SAM-dependent PC synthesis, however, accounts for most SAM utilization, such that SAM- and PC-deficient worms are phenotypically similar and can both be rescued with exogenous choline[139].

To test whether SAM-dependent PC synthesis was necessary for ethanol-mediated activation of fmo-2, we repeated our previous screen but replaced SAA RNAi with PC synthesis RNAi. RNAi against pmt-1, which encodes the first of two methyltransferases that convert phosphoethanolamine to phosphocholine in worms, strongly attenuated ethanol-mediated activation of fmo-2 (Fig. 3A,B), similarly to sams-1 RNAi. RNAi against pcyt-1, which encodes the enzyme that performs the subsequent, rate-limiting CDP transfer step of PC synthesis, also attenuated ethanol-mediated activation of fmo-2, but it did not do so as strongly as pmt-1 RNAi (Fig. 3A,B). RNAi against cept-1, which encodes the enzyme that performs the final diacylglycerol transfer step of PC synthesis, did not attenuate ethanol-mediated activation of fmo-2, but previous results from another group have shown that insufficiency of cept-1 RNAi is not inconsistent with a requirement for PC[140]. Thus, SAM-dependent PC synthesis is required for ethanol-mediated activation of fmo-2.

Fasting also requires SAM synthesis and subsequent SAM-dependent phosphocholine synthesis to activate fmo-2

We then sought to determine whether fasting also required SAM synthesis and subsequent SAM-dependent phosphocholine synthesis to activate fmo-2. We grew fmo-2p::GFP worms from egg to adulthood on sams-1, pmt-1, and positive (nhr-49) and negative control RNAi, transferred Day 2 adults to plates with no bacterial food, and photographed them after 24 hours of fasting. Fasting, like ethanol, required both sams-1 and pmt-1 to activate fmo-2 (Fig. 4A,B). Thus, SAM synthesis and subsequent SAM-dependent phosphocholine synthesis are required by multiple interventions, including fasting, a conserved lifespan- and healthspan-extending intervention, to activate fmo-2.

Exogenous phosphocholine is sufficient to activate fmo-2, and it requires nhr-49 and hlh-30, but not hif-1, to do so

Next, we wanted to determine which transcription factor(s) were necessary downstream of methylation-dependent PC synthesis for fmo-2 activation. We first established that exogenous phosphocholine (10mM) was sufficient to activate fmo-2. Then, we screened RNAi clones of transcription factors known to regulate fmo-2 for their ability to block phosphocholine-mediated activation of fmo-2. We found that nhr-49 and hlh-30, but not hif-1, were required for phosphocholine-mediated activation of fmo-2 (Fig. 5A,B).

Table 4. Interventions that activate fmo-2 and associated regulatory genes

INTERVENTION	**GENES**
Dietary Restriction/Fasting	hlh-30[16,129], nhr-49[141]
Hypoxic Response	hif-1[16,110,111], tph-1[16], ser-7[16], elt-2[142]
Pentose Phosphate Pathway Inhibition	pmk-1[129], hlh-30[129]
Electron Transport Chain Inhibition[112,129]	N.D.
Sulfa Drugs (DDS, Sulfadiazine)[130]	N.D.
Ethanol[137,138]	N.D.
Oxidative Stress (AgNP, tBOOH)	pmk-1[143], hif-1[143], nhr-49[141], mdt-15[141]
Infection[144–146]	nhr-49[147], hlh-30[147]

*N.D. = No Data

Figure 5. Model of sulfur amino acid (SAA) metabolism

SAA metabolism has two branches. Cytoprotective products of each branch are brightly colored.

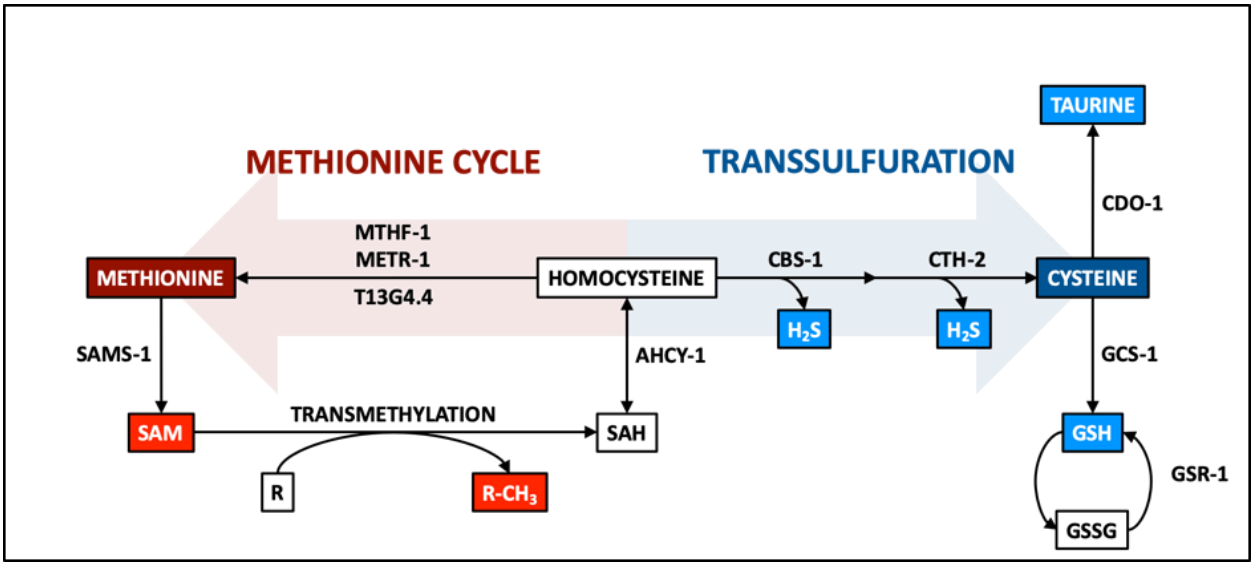
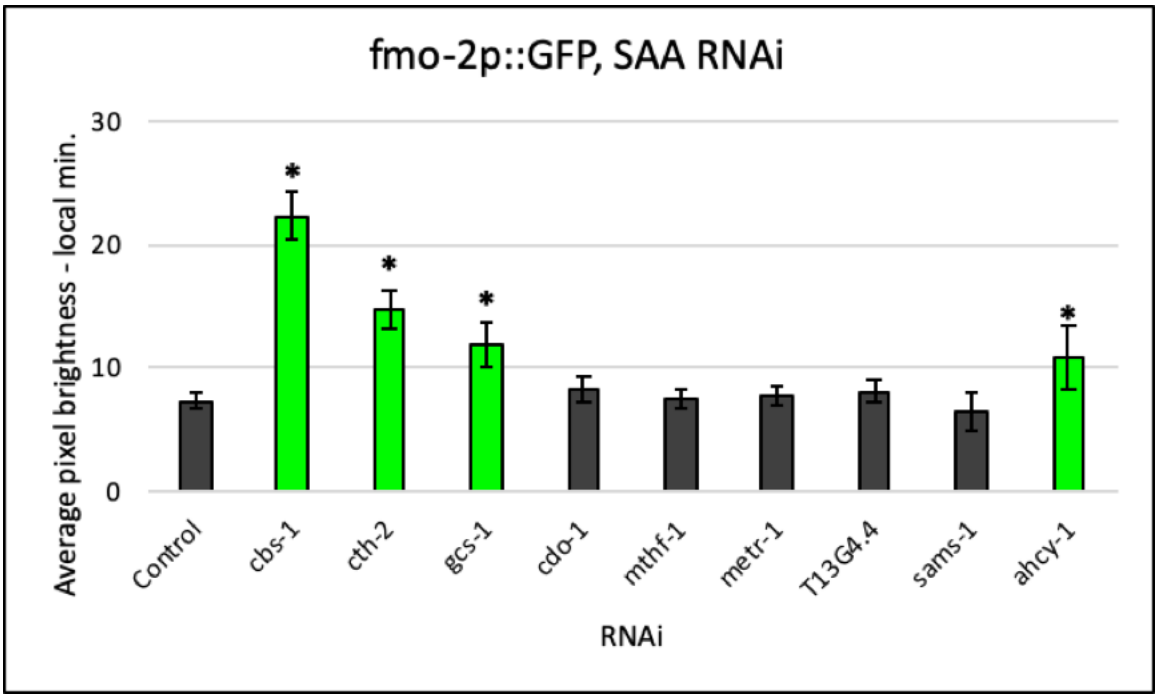


Figure 6. RNAi inhibition of transsulfuration activates fmo-2

A. Quantification of fmo-2p::GFP activation by SAA RNAi.



B. Representative photos of fmo-2p::GFP worms on SAA and negative control RNAi.

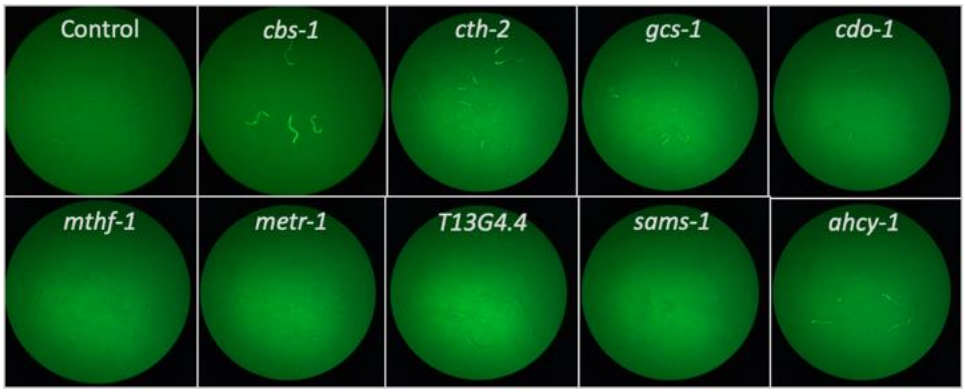
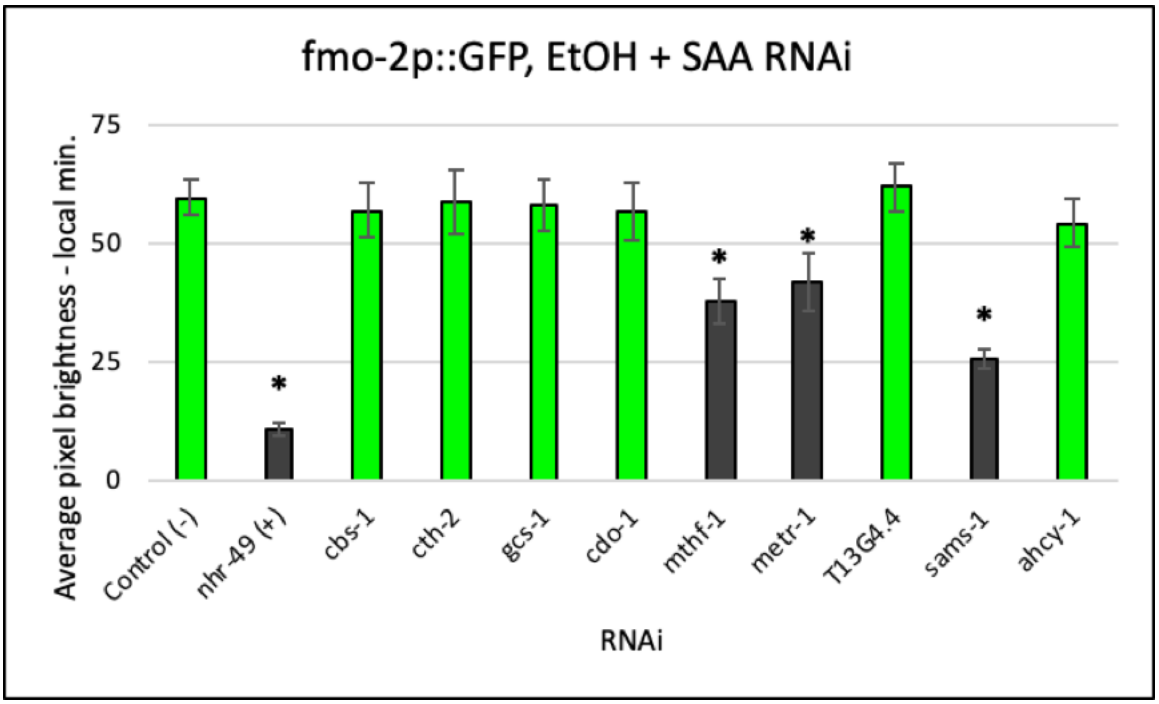


Figure 7. SAM synthesis (sams-1) is necessary for ethanol-mediated activation of fmo-2

A. Quantification of ethanol-mediated activation of fmo-2p::GFP on SAA and control RNAi.



B. Representative photos of ethanol-mediated activation of fmo-2p::GFP on SAA and control RNAi.

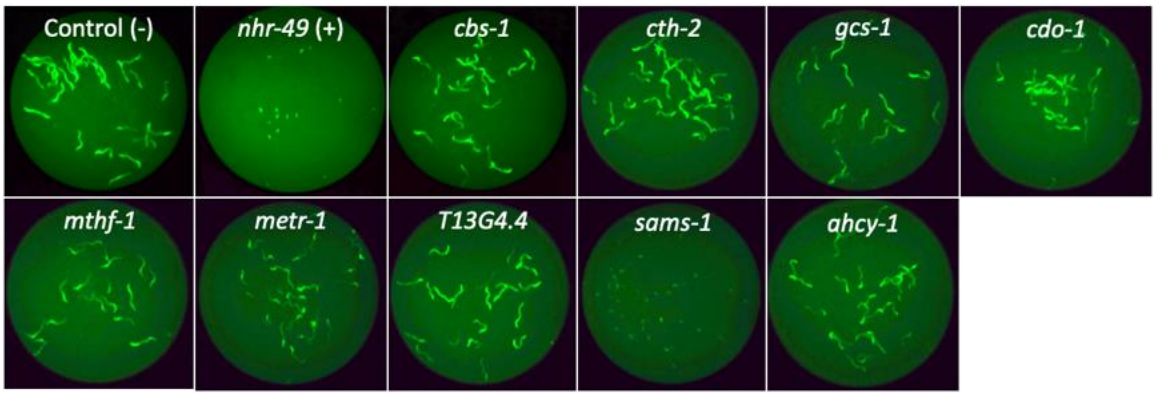
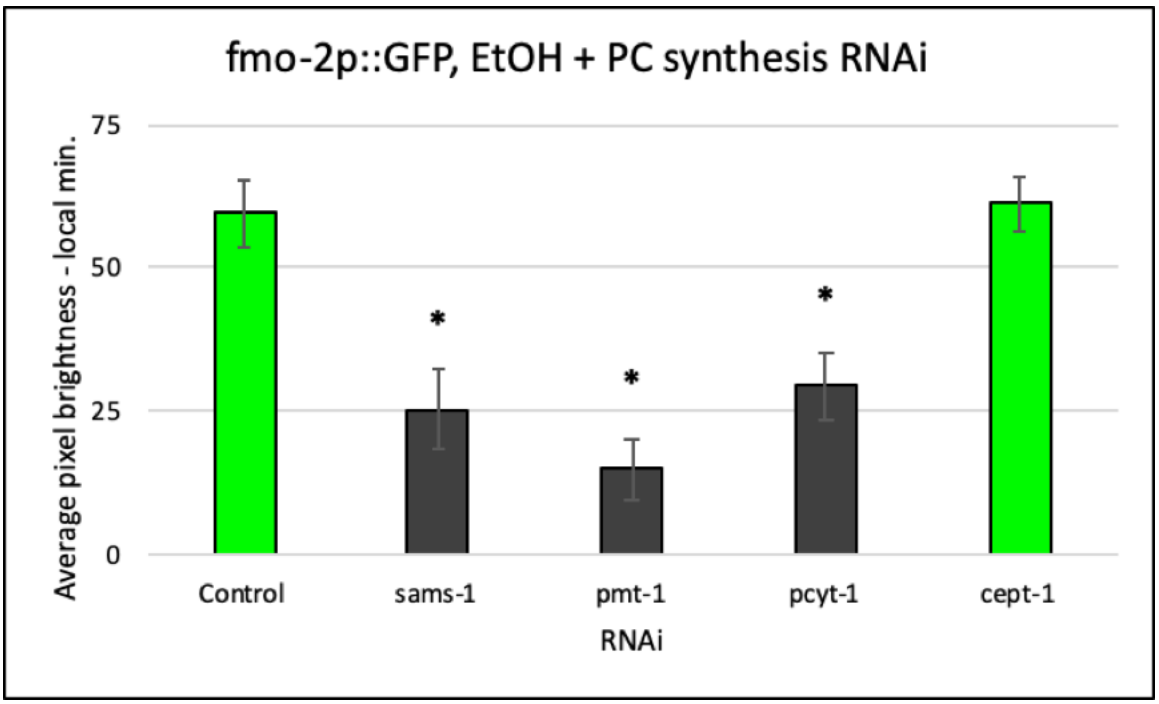


Figure 8. PC synthesis is necessary for ethanol-mediated activation of fmo-2

A. Quantification of ethanol-mediated activation of fmo-2p::GFP on SAM-dependent PC synthesis pathway RNAi.



B. Representative photos of ethanol-mediated activation of fmo-2p::GFP on methylation-dependent phosphatidylcholine synthesis pathway RNAi.

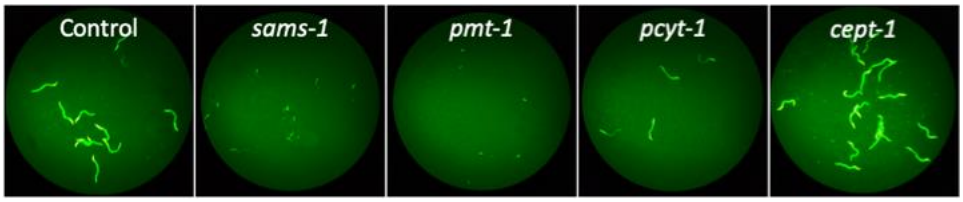
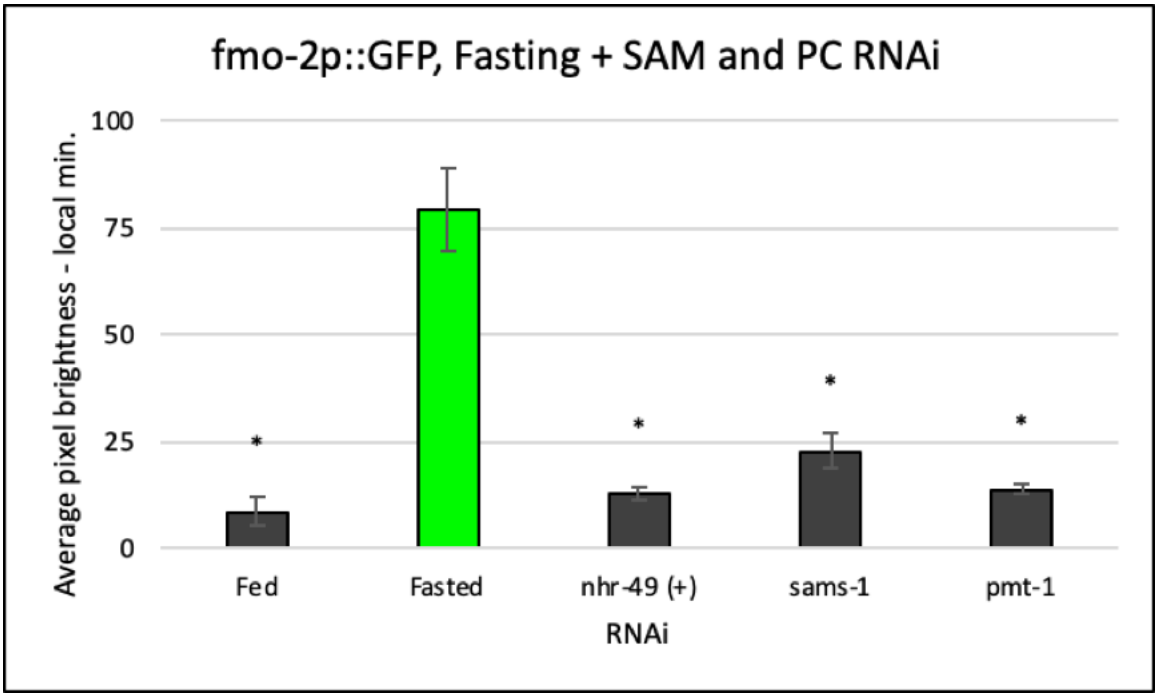


Figure 9. SAM synthesis and subsequent SAM-dependent phosphocholine synthesis are also necessary for fasting-mediated activation of fmo-2

A. Quantification of fasting-mediated activation of fmo-2p::GFP on control, sams-1, and pmt-1 RNAi.



B. Representative photos of fasting-mediated activation of fmo-2p::GFP on control, sams-1, and pmt-1 RNAi.

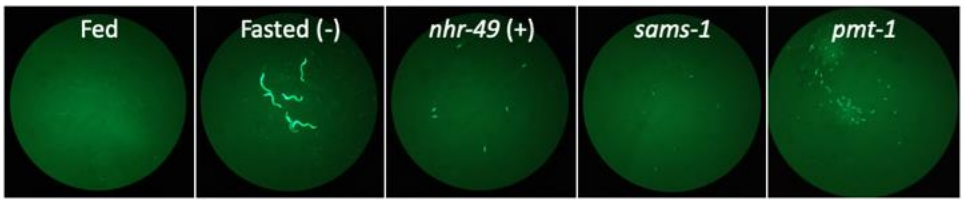
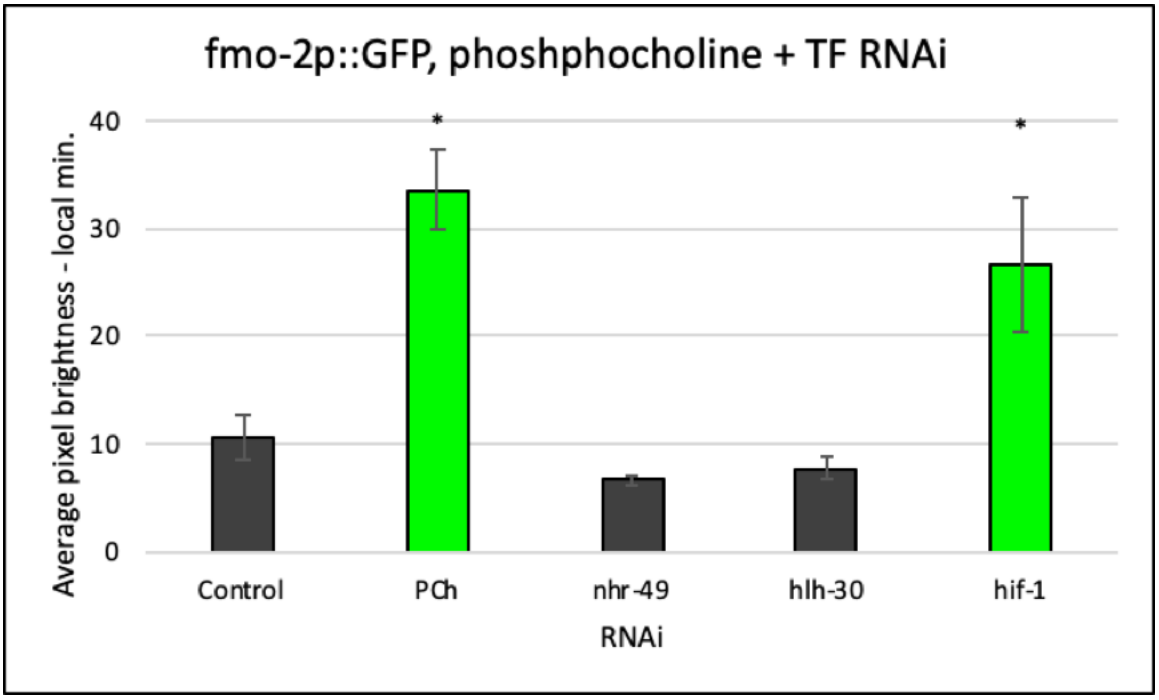


Figure 10. Exogenous phosphocholine activates fmo-2 and requires the transcription factors nhr-49 and hlh-30, but not hif-1, to do so

A. Quantification of fmo-2p::GFP activation by 10mM exogenous phosphocholine on control and transcription factor RNAi.



B. Representative photos of fmo-2p::GFP activation by 10mM exogenous phosphocholine on control and transcription factor RNAi.

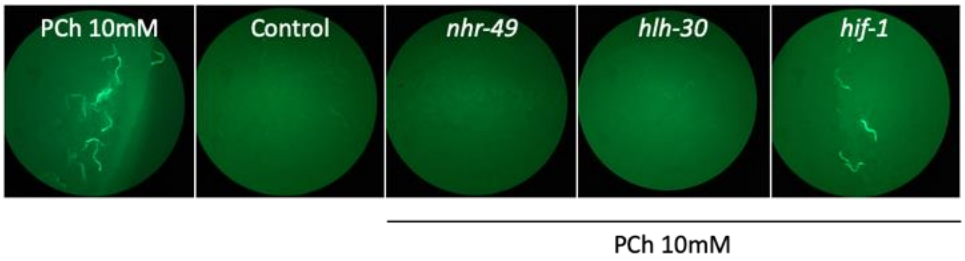
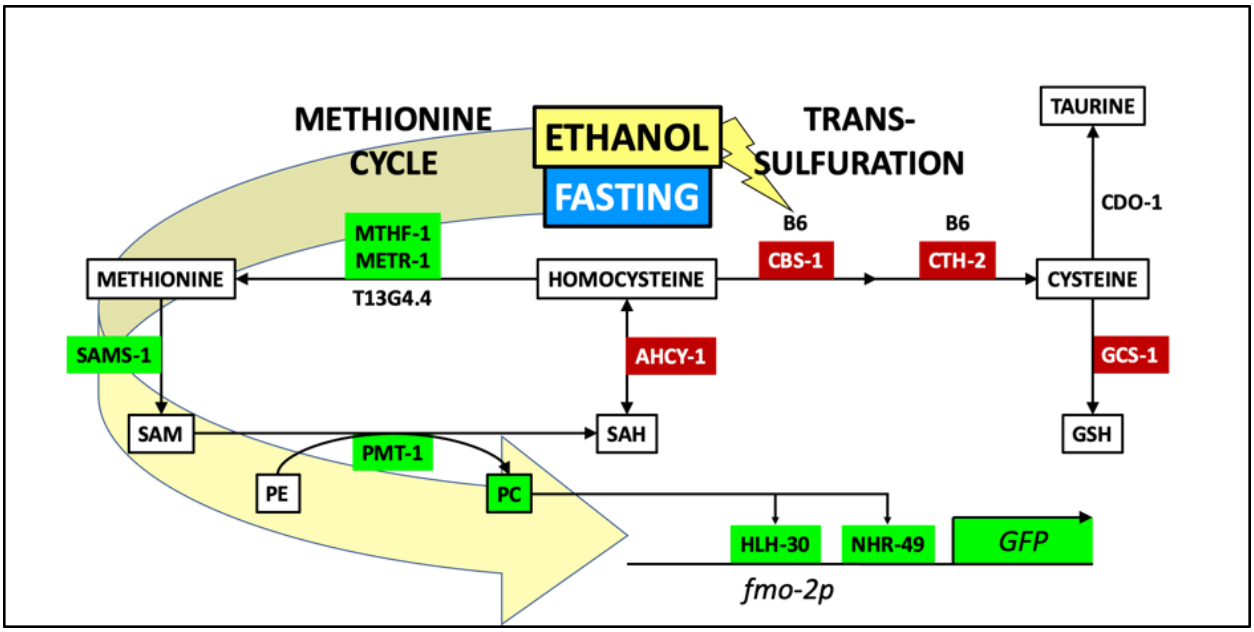


Figure 11. Model of fmo-2 regulation



DISCUSSION

RNAi-directed flux through the methionine cycle activates fmo-2

In considering why only certain SAA RNAi are sufficient to activate fmo-2, it is useful to examine empirical data defining their effects on SAA metabolism in worms where possible, or in other animal models where reasonable, especially because some of the effects are counterintuitive (Table 2).

Inhibition of cbs-1, which encodes the enzyme that catalyzes the first step of transsulfuration, increases homocysteine concentrations approximately 10-fold[148]. In CBS-deficient mice, all intermediates of the methionine cycle are also highly elevated[149]. Inhibition of cth-2, which catalyzes the second step of transsulfuration, may be modeled by mammalian genetic deficiencies of CSE, the mammalian ortholog of cth-2. CSE deficiency increases cystathionine concentrations over 35-fold, but, less expectedly, also modestly increases homocysteine concentrations[150]. Inhibition of gcs-1, which encodes the catalytic subunit of the enzymatic complex responsible for the rate-limiting first step in the two-step synthesis of glutathione from cysteine, may be modeled approximately by GCLM-deficient mice. GCLM is the modifier subunit that increases efficiency of gcs-1 ortholog GCLC. GCLC -/- homozygous mice are embryonic lethal, so metabolomic data are not available. GCLM-deficient mice have low glutathione, but, unexpectedly, they also have low cysteine and high homocysteine[149]. Inhibition of ahcy-1, which encodes the enzyme that catalyzes the reversible hydrolysis of transmethylation waste product SAH into homocysteine and adenosine, may be modeled by

human genetic deficiencies of AHCY. AHCY deficiency only slightly elevates homocysteine, but it elevates methionine over 10-fold, SAM over 20-fold, and SAH over 90-fold.

The mechanism by which these four SAA RNAi might activate fmo-2 is by increasing SAM-dependent PC synthesis downstream of elevated homocysteine. SAA RNAi that increase homocysteine but inhibit folate-dependent remethylation of homocysteine to methionine (mthf-1, metr-1) or subsequent SAM synthesis (sams-1) are not only insufficient to activate fmo-2, but they also attenuate both ethanol- and fasting-mediated activation of fmo-2. The relatively small increase in fmo-2 activation resulting from ahcy-1 RNAi can be explained by its approximately order-of-magnitude greater increase in SAH relative to SAM. SAH is a potent inhibitor of transmethylation, so it could significantly attenuate SAM-dependent fmo-2 activation resulting from ahcy-1 RNAi.

Previous work from our lab showed that the sulfa drugs diaminodiphenyl sulfone (DDS/Dapsone) and sulfadiazine (SD) activate fmo-2 and that DDS extends lifespan in an fmo-2-dependent manner[130]. DDS also requires folate depletion to extend lifespan and lowers methionine cycle intermediates and the SAM/SAH ratio in both worms and their bacterial food source (Table 3). Here, we did not find that RNAi inhibition of the folate cycle, methionine synthesis, or SAM synthesis was sufficient to activate fmo-2, inconsistent with a model in which decreases in related metabolites activate fmo-2.

Several models could reconcile our current and previous results. The trivial explanation is that fmo-2 is activated by DDS and SD because they are sulfur-containing xenobiotics. Another explanation is that DDS and SD limit folate, methionine, and SAM less severely than does RNAi, and that metabolite decreases caused by RNAi are too severe to activate fmo-2.

The gentler inhibition from folate cycle-disrupting drugs could stimulate compensatory upregulation of the BHMT remethylation pathway and cause prioritization of phosphocholine production among transmethylation reactions, resulting in a sufficient net increase in PC to activate fmo-2. Our current result indicating that ahcy-1 RNAi activates fmo-2 is consistent with another model in which ahcy-1 RNAi-mediated lowering of the SAM/SAH ratio more closely reproduces the fmo-2-activating conditions of DDS and SD than does RNAi against mthf-1, metr-1, or sams-1. Consistent with this model, ahcy-1 RNAi modestly activates fmo-2 similarly to DDS and SD. It is also possible that highly increased flux through the methionine cycle and subsequent phosphatidylcholine synthesis activate fmo-2 to a large degree, whereas a decreased SAM/SAH ratio activates fmo-2 more modestly by a different mechanism, perhaps by inhibition of an fmo-2-repressing methyltransferase. Consistent with this possibility, we found that inhibition of H3K4me3 methyltransferase activity was sufficient to modestly activate fmo-2.

SAM synthesis is necessary for ethanol-mediated activation of fmo-2

Ethanol inhibits transsulfuration by depleting the cofactor necessary for both its steps, pyridoxal-5-phosphate. Acetaldehyde, the first product of the oxidative metabolism of ethanol, which is the predominant mode of ethanol metabolism, depletes pyridoxal-5-phosphate[151,152]. Consistent with this, ethanol exposure, like inhibition of cbs-1, is known to cause homocysteine buildup[153]. Our data are consistent with a model in which ethanol inhibits transsulfuration, causes homocysteine buildup, and subsequently increases methionine cycle flux to activate fmo-2.

PC synthesis is required for both ethanol-and fasting-mediated activation of fmo-2

Phosphatidylcholine synthesis is the largest single consumer of SAM in the cell. Inhibition of sams-1 or pmt-1 produces overlapping phenotypes, and either is sufficient to transcriptionally alter the lipid profile of the cell[154]. Therefore, it is not surprising that SAM and PC are similarly necessary for ethanol- and fasting-mediated activation of fmo-2.

It is less obvious why ethanol or fasting would increase flux through SAM-dependent PC synthesis, as is apparently necessary for both conditions to activate fmo-2. There are data, however, that suggest a model consistent with our results. Faced with nutrient scarcity including methionine scarcity as could result from ethanol metabolite acetaldehyde inhibiting methionine synthase[155], the cell can increase methionine cycle flux[156,157] and specifically prioritize phosphatidylcholine transmethylation[158]. This would explain our results, and is consistent with the rapid, transient induction of fmo-2 by fasting[114]. Both acute and chronic

ethanol exposure activate fmo-2[137], but the latter could be due to oxidative stress rather than increased PC production.

Exogenous choline metabolites activate fmo-2

Multiple interventions that activate fmo-2 do so through hlh-30 and nhr-49 (Table 2), so it is not surprising that exogenous phosphocholine also activates fmo-2 via these transcription factors. In silico analysis of the fmo-2 upstream promoter identifies binding motifs for hlh-30 and nhr-49 within 500bp of the fmo-2 start codon (Fig. 12). hif-1 is required for fmo-2 activation by both hypoxia and silver nanoparticle exposure, but fmo-2 activation by genetic inhibition of mitochondrial respiration is, contrastingly, amplified by hif-1 knockdown[112]. In light of this complexity, our finding that hif-1 is not necessary for activation of fmo-2 by exogenous phosphocholine is also not surprising.

METHODS

Strains

The following strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440): N2, VC1668 (fmo-2(ok2147)), KAE9 (seaSi39 [(pCFJ448) (eft-3p::fmo-2 + H2B::GFP) + Cbr-unc-119(+)] I), and KAE28 (seaEx13 [fmo-2p::GFP + unc-119(+)]).

Growth Conditions

Worms were grown under standard conditions[159] at 20°C.

Imaging and Analysis

Worms were visualized using a Zeiss SteREO Lumar.V12 microscope. Photographs were taken using an iPhone 6S and a LabCam Microscope Adapter for iPhone 6S/6S Plus. Imaged worms were then quantified using ImageJ, and the data were compiled into an excel spreadsheet. The minimum pixel brightness per worm, an approximation of background brightness, was subtracted from average pixel brightness per worm to get a brightness value from 0-255 per worm. Results from at least three replicates per experiment were combined to produce charts.

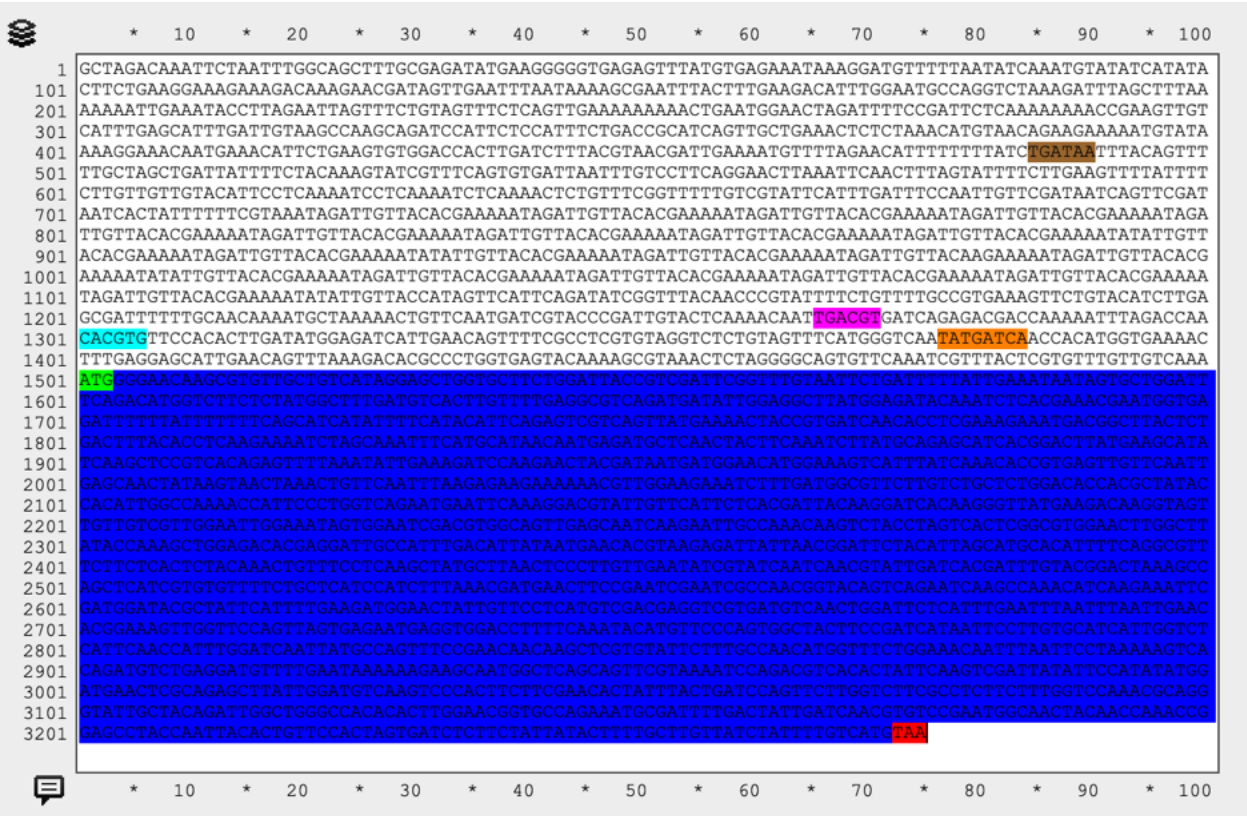
Ethanol and Phosphocholine

Ethanol was added in accordance with an established protocol[160]. For phosphocholine, 100µl of 1M stock solution dissolved in water was added topically to the OP50 lawn and allowed to dry before worm transfer.

SUPPLEMENTARY MATERIALS

Figure 12. fmo-2 promoter and transcription factor binding motifs

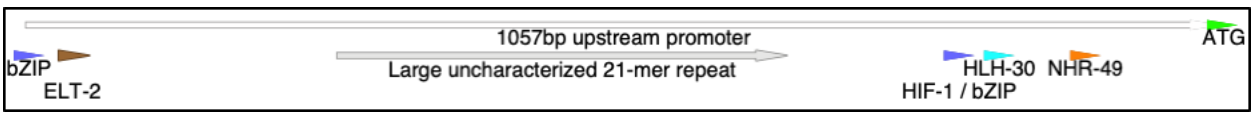
A. Screenshot of ApE file containing fmo-2 gene and 1000bp upstream promoter region.



B. Color coding of features in ApE file.

ELT-2	HIF-1	HLH-30	NHR-49	ATG	fmo-2	STOP

C. Graphical representation of promoter (1057bp) and binding motifs.



CHAPTER 4: THE FUNCTION OF C. ELEGANS FMO-2 IN LONGEVITY

INTRODUCTION

C. elegans flavin-containing monooxygenase-2 (fmo-2) is activated by several longevity-promoting interventions, and several of these interventions require fmo-2 to fully extend lifespan (TABLE 4.1). Additionally, FMO-2 overexpression is sufficient to extend lifespan. Despite this, the mechanism(s) by which fmo-2 promotes longevity remain defined.

Based on data defining the function of FMOs in species other than worms (TABLE 4.2), our own RNA-Seq data on fmo-2-modulated worms (TABLE 4.3), and other pieces of evidence, I devised two approaches to defining the function of fmo-2. First, I would perform a biased RNAi screen, primarily of sulfur amino acid metabolism pathway genes, for their ability to eliminate the longevity of the FMO-2 OE strain. Second, I would attempt to use a biochemical assay to define the precise molecule(s) on which FMO-2 might act.

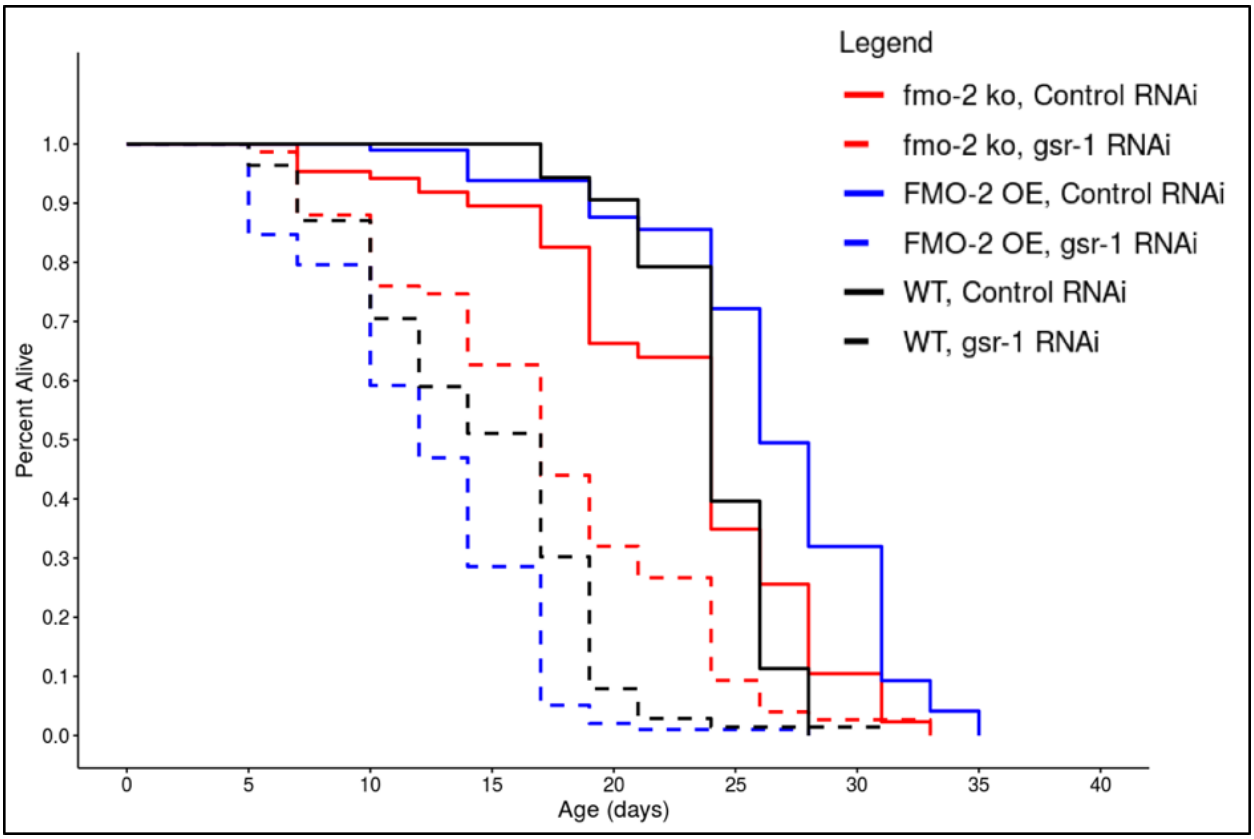
RESULTS

fmo-2 activity exacerbates the lifespan-shortening effects of gsr-1 RNAi

RNAi against glutathione reductase (gsr-1) shortens wildtype worms' lifespans[161]. FMO-2 OE worms are more sensitive than wildtype to gsr-1 RNAi-mediated lifespan shortening, despite being longer-lived on control RNAi (Figure 15). Additionally, Δfmo-2(ok2147) worms are less sensitive to gsr-1 RNAi-mediated lifespan shortening (Figure 15).

Figure 13. fmo-2 activity exacerbates the lifespan-shortening effects of gsr-1 RNAi

A. Survival curves



B. Data table

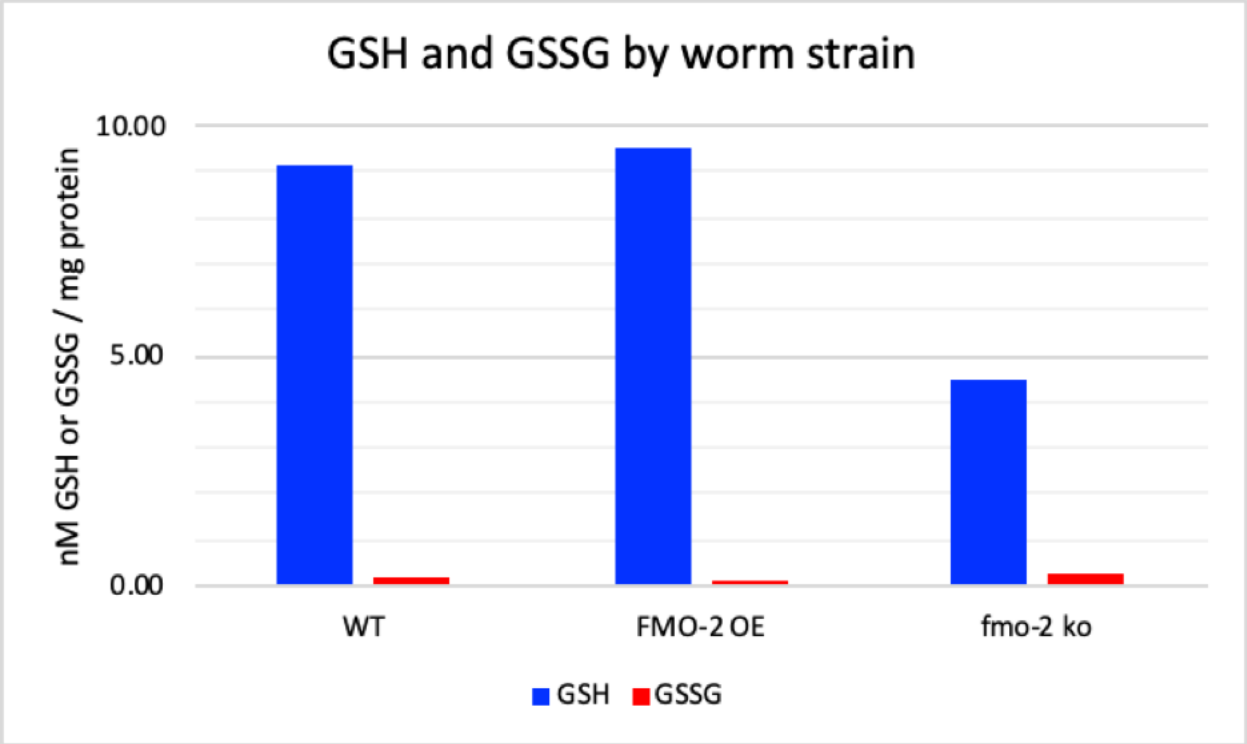
Strain	RNAi	Median	N
fmo-2 ko	Control	24	86
fmo-2 ko	gsr-1	17	75
FMO-2 OE	Control	26	97
FMO-2 OE	gsr-1	12	98
WT	Control	24	53
WT	gsr-1	17	139

fmo-2 is necessary for normal levels of glutathione in adult worms

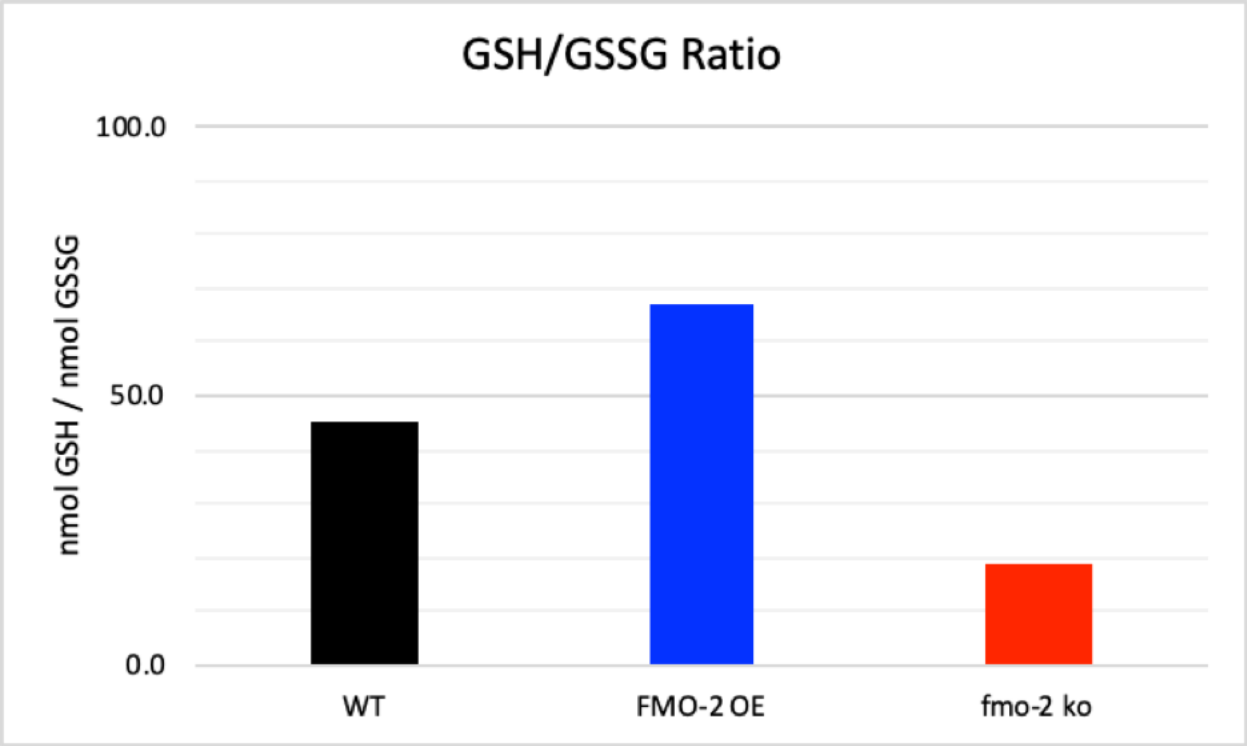
To test whether fmo-2 modulation affects glutathione levels, we analyzed whole-worm lysate using HPLC. The data show that fmo-2 is required for worms to have a normal amount of glutathione. fmo-2(ok2147) worms only show about half the glutathione (normalized to nmol/mg protein) of wildtype and FMO-2 OE worms (Figure 4.7).

Figure 14. fmo-2 is required for normal adult glutathione levels

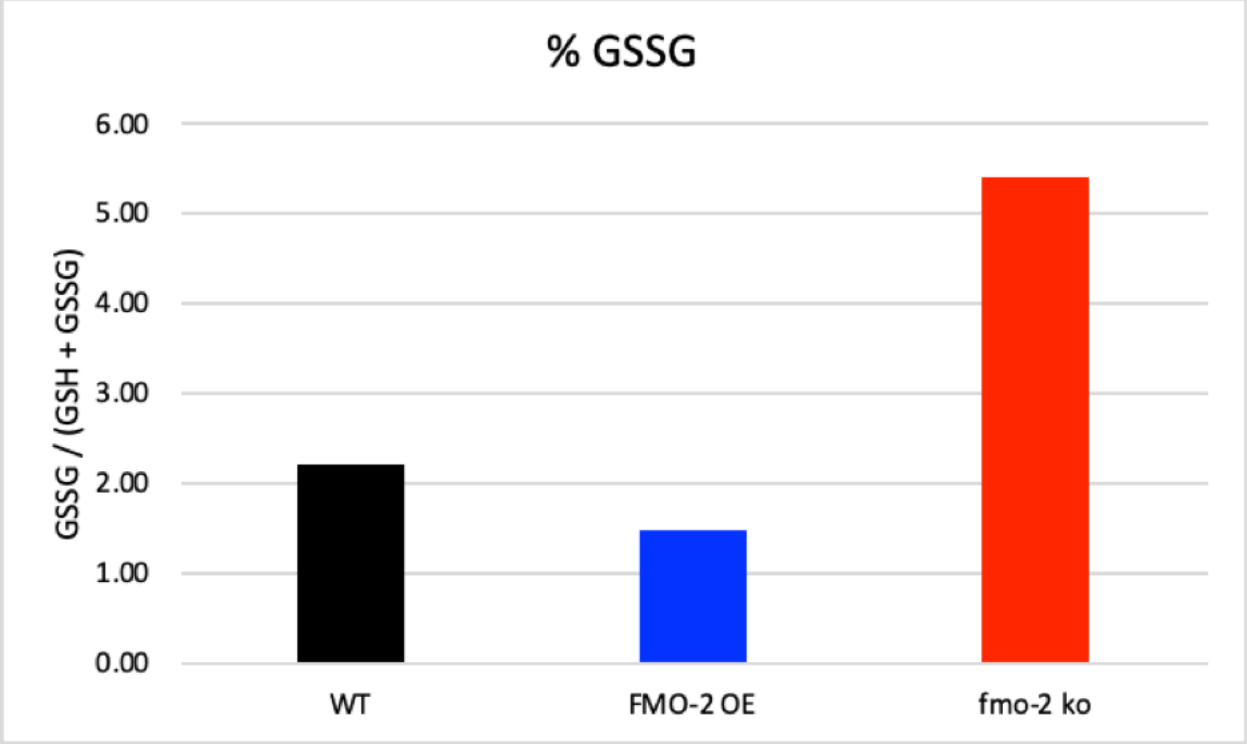
A. Quantification of GSH and GSSG in each worm strain.



B. GSH/GSSG ratio in each worm strain.



C. Percentage of total glutathione pool in oxidized (GSSG) state, by worm strain.

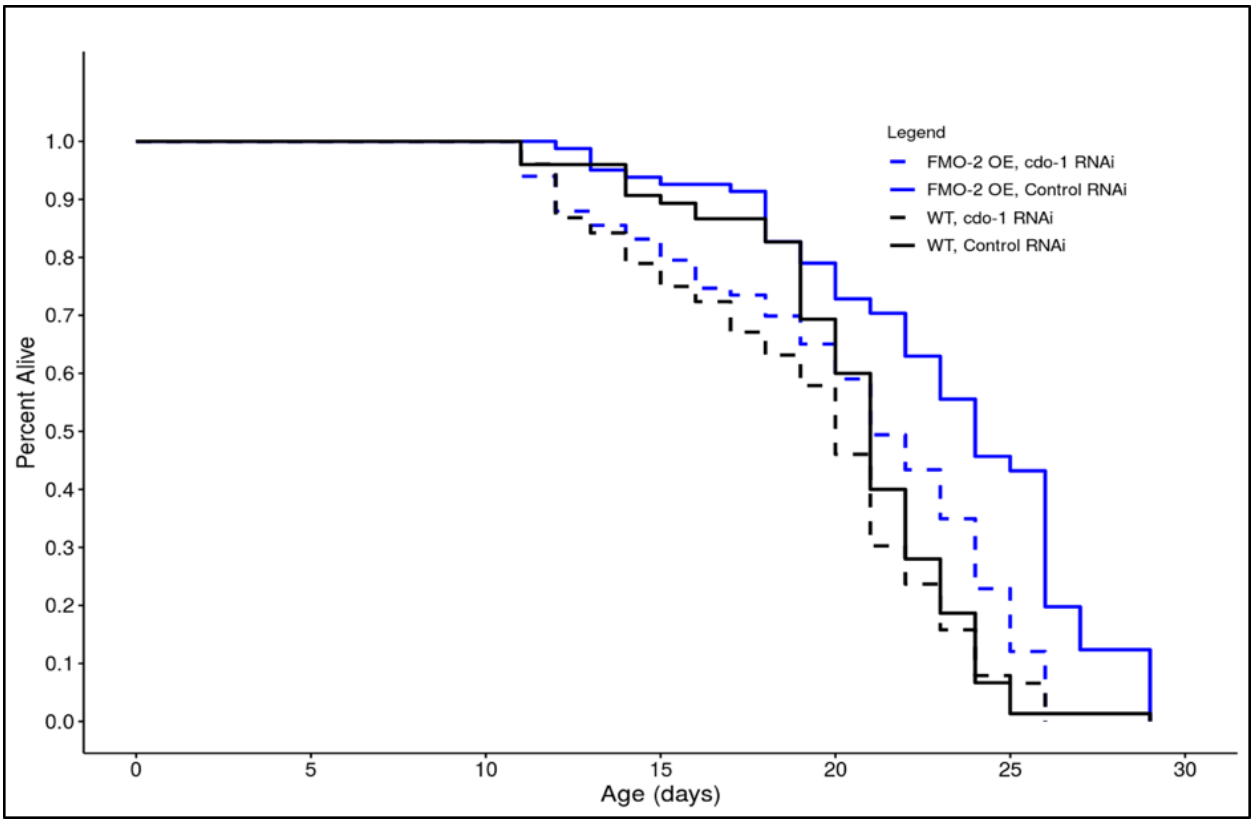


FMO-2 OE longevity requires cysteine dioxygenase (cdo-1)

RNAi against cysteine dioxygenase (cdo-1), a conserved gene that encodes the enzyme that performs the first of three steps in the conversion of cysteine to taurine, was also required for FMO-2 OE longevity (Figure 16).

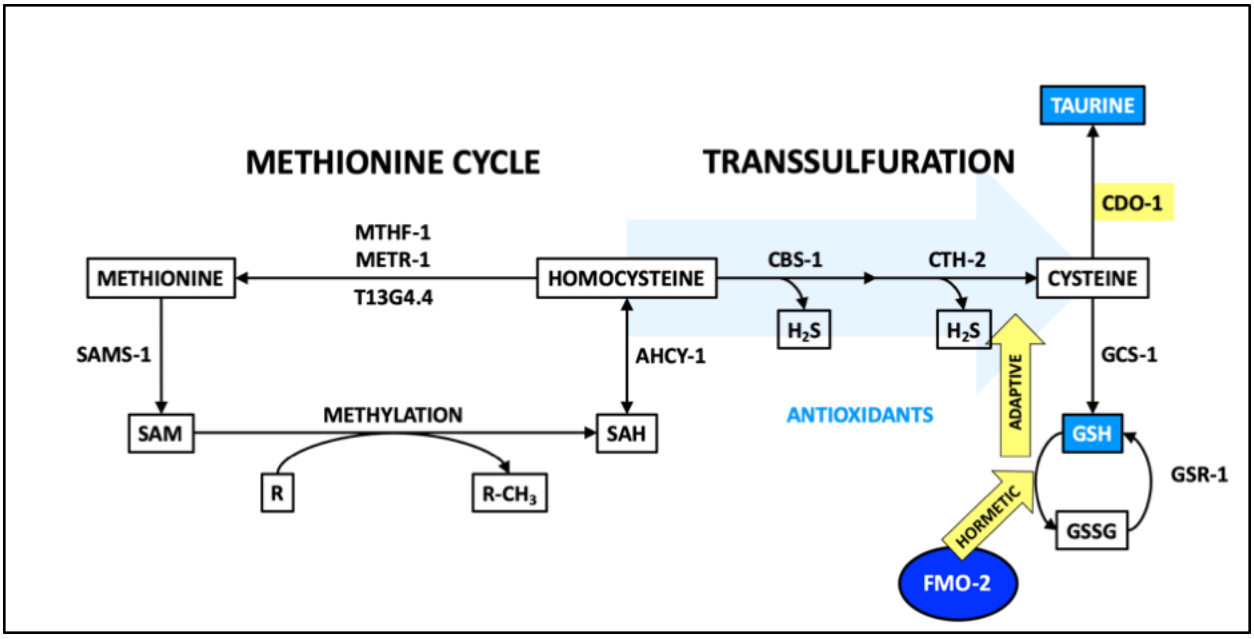
Figure 15. FMO-2 OE longevity requires taurine synthesis (cdo-1)

A. Survival curves. B. Data table.



Strain	RNAi	Median	N
FMO-2 OE	cdo-1	21	83
FMO-2 OE	Control	24	81
WT	cdo-1	20	76
WT	Control	21	75

Figure 16. Model: FMO-2 hormetically oxidizes glutathione to stimulate transsulfuration and subsequent cytoprotective taurine synthesis



FMO-2 and glutathione

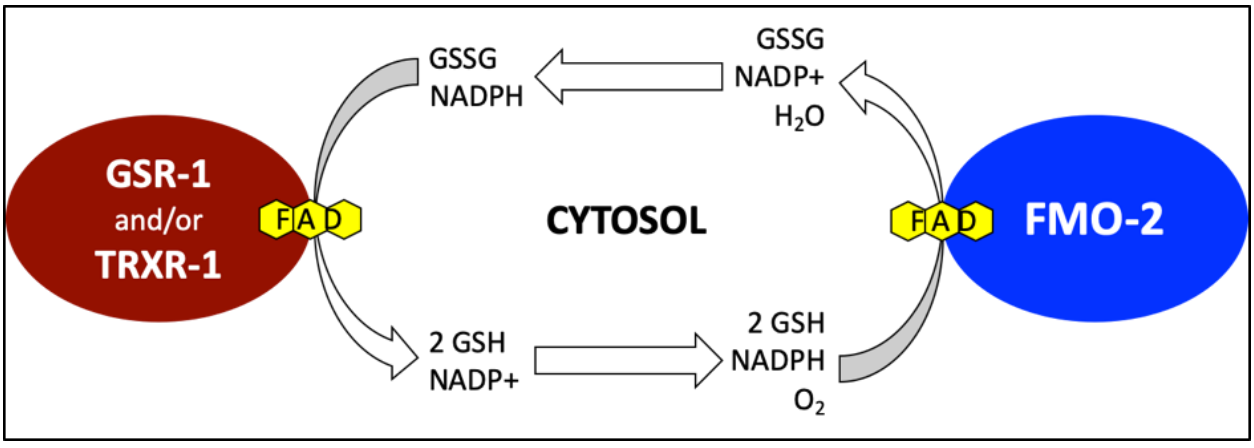
fmo-2 activity sensitizes worms to the lifespan-shortening effects of gsr-1 RNAi. Like FMO-2 overexpression, GSR-1 overexpression extends worm lifespan[161]. In flies, however, glutathione reductase (GR) overexpression extends lifespan in hyperoxia but not in normoxia[162]. Additionally, fmo-2 is necessary for lifespan extension from genetic stabilization of the worm hypoxic response[106]. Taken together, these data are consistent with a model in which FMO-2 and G(S)R use NADPH to exert opposing effects on glutathione and subsequently the redox status of the cell.

It has been known for decades that FMOs can oxidize glutathione and alter at least ER-specific redox balance[50,163], but specifically defining FMO as an oxidizing counterbalance to GR

is novel and important. Recent major reviews covering glutathione homeostasis and even NADPH-dependent glutathione and redox homeostasis in aging focus on GR and thioredoxin reductase (TRXR) without mentioning FMOs[164]. FMOs now need to be included with GR and TRXR as enzymatic components of cells' NADPH-dependent, glutathione-centric redox homeostatic system.

The necessity of fmo-2 for normal glutathione levels makes sense in light of this new paradigm. GSH can feedback inhibit its own synthesis, and GSSG can stimulate GSH synthesis[165]. Given fmo-2 low basal expression and unique enzymatic kinetics including the steady creation of H2O2 even in the absence of substrate, it is reasonable to suggest a model in which FMO-2 acts as a hormetic stimulus during baseline conditions. This hormetic signal can be amplified effectively to a point, thus the sharp but transient spike in fmo-2 transcription seen in various stress conditions (Table 4).

Figure 17. FMO-2 is an oxidizing counterbalance to GSR-1 and/or TRXR-1 in NADPH- and glutathione-dependent regulation of redox homeostasis



FMO-2 and taurine

Our data indicate that FMO-2 OE longevity requires cdo-1, the gene encoding the first step of taurine synthesis. This is interesting for at least two reasons. First, mouse Fmo1 has recently been shown to catalyze the third, final step of taurine synthesis from cysteine *in vivo*[128]. Thus, it is conceivable that FMO-2 is stimulating transsulfuration by hormetically oxidizing glutathione, then contributing directly to the conversion of transsulfuration-generated cysteine to taurine.

The second reason this is interesting is that while taurine is known to be broadly cytoprotective and health-promoting, its role in longevity is underdefined[166–170]. Transsulfuration has been shown to be necessary for DR-mediated longevity in multiple species[134,135], and CBS overexpression has been shown to be sufficient to extend lifespan in multiple species[134,135]. These benefits, however, have been attributed to transsulfuration enzyme-mediated production of hydrogen sulphide (H_2S) through non-transsulfuration reactions[135].

Transsulfuration has also been shown to be upregulated in long-lived Ames dwarf mice, and taurine has been shown to be elevated in long-lived Snell dwarf mice[171,172]. Epistasis experiments have not been performed in worms or other model systems testing the necessity of cdo-1 for DR-mediated lifespan extension, but they should be viewed as a logical next step.

METHODS

Lifespan assays

Lifespan assays were conducted using methods previously described with minor modifications[106]. Briefly, eggs were synchronized via bleach prep, 50 L4/young adults x 2 plates each were transferred to FUDR, and worms were scored alive/dead based on their ability to respond to gentle prodding with a pick. Worms that escaped up the plate walls and desiccated were censored from analysis.

HPLC

HPLC was carried out in conjunction with Collin White of the Kavanagh lab in accordance with his protocols[173,174]. Worms were grown using standard methods then frozen and lysed to produce sufficient adult whole-worm lysate.

CHAPTER 5: CONCLUSION

SUMMARY OF RESULTS

fmo-2 transcription is regulated by the direction of SAA metabolic flux

First, a biased screen of SAA RNAi clones for negative regulators of fmo-2 revealed that inhibition of transsulfuration or subsequent glutathione synthesis is sufficient to activate fmo-2 transcription. Next, a screen of the same RNAi clones for their ability to block ethanol-mediated induction of fmo-2p::GFP demonstrated that SAM synthesis is necessary for such induction. Taken together, these results are consistent with a model in which fmo-2 transcription is activated by flux away from transsulfuration and subsequently through SAM synthesis via the methionine cycle.

fmo-2 activity increases sensitivity to glutathione reductase knockdown

FMO-2 OE worms are long-lived relative to wildtype worms when both are fed control bacteria, but are short-lived relative to wildtype worms when fed glutathione reductase (gsr-1) RNAi bacteria. Additionally, fmo-2(ok2147) loss-of-function mutant worms are long-lived relative to wildtype worms when both are fed gsr-1 RNAi bacteria. These data are consistent with a model in which FMO-2 and GSR-1 each use NADPH to antagonistically oxidize GSH to GSSG or reduce GSSG to GSH respectively.

fmo-2 activity is necessary for normal glutathione production

HPLC analysis of whole adult worm lysate revealed that fmo-2(ok2147) loss-of-function mutant worms only contain only approximately half the total glutathione, normalized to

nmol/mg protein, compared to that seen in age-matched wildtype worms. FMO-2 OE worms, by contrast, did not display altered levels of glutathione compared to those measured in age-matched wildtype worms. These data are consistent with a model in which fmo-2 activity is necessary for production and/or maintenance of normal glutathione levels in adult worms.

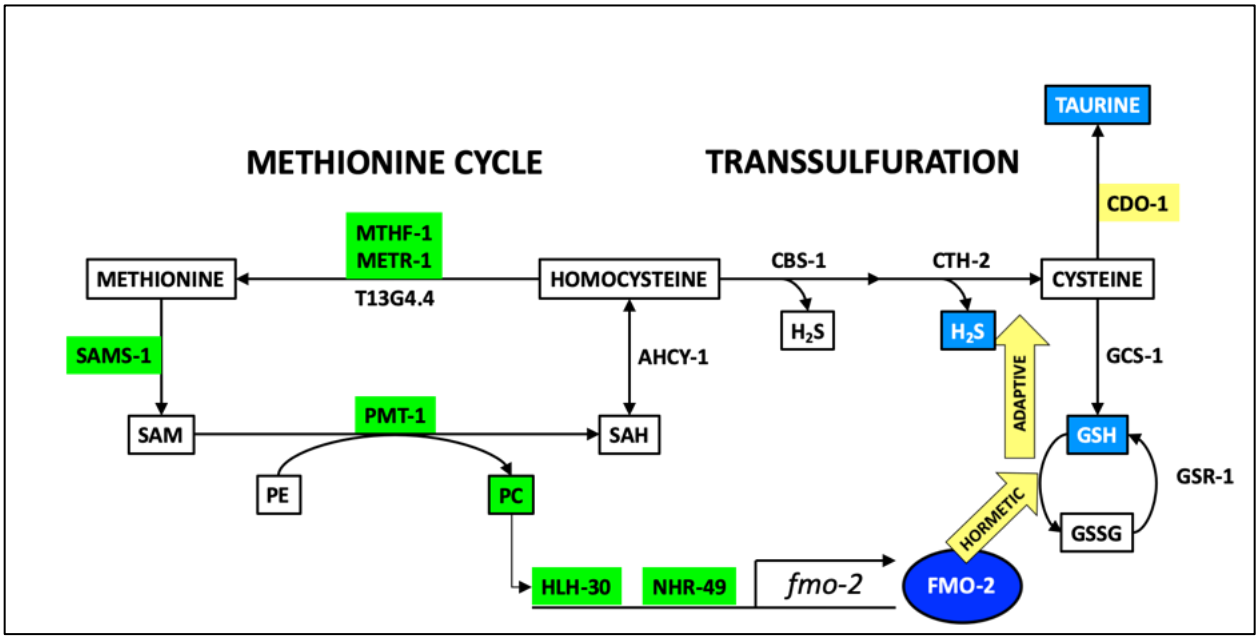
MODEL

Taken together, the aforementioned collected data are consistent with a model in which FMO-2 activity hormetically oxidizes glutathione to stimulate normal glutathione synthesis. Decreased transsulfuration and/or increased methionine cycle flux activate fmo-2 transcription as an effort to rebalance sulfur amino acid metabolism. SAM-derived PC is the signaling molecule responsible for activating fmo-2 transcription through the transcription factors nhr-49 and hlh-30.

RELEVANCE TO AGING

Transsulfuration is necessary for dietary restriction-mediated longevity in a conserved manner[134,135], and both transsulfuration enzymes and enzymes of subsequent glutathione synthesis are sufficient, when overexpressed, to extend lifespan[175,176]. Similarly, fmo-2 is necessary for dietary restriction-mediated longevity and is sufficient, when overexpressed, to extend lifespan[16]. These observations are consistent with a model in which fmo-2 promotes longevity by stimulating transsulfuration and subsequent cytoprotective product synthesis.

Figure 18. Model of fmo-2 regulation and function and SAA metabolism



REFERENCES

1. McCay, C. M., Crowell, M. & Maynard, L. A. The effect of retarded growth upon the length of life span and upon the ultimate body size. *J. Nutr.* (1935).
2. Harman, D. Aging: A Theory Based on Free Radical and Radiation Chemistry. *J. Gerontol.* **11**, 298–300 (1956).
3. Larsen, P. L. Aging and resistance to oxidative damage in Caenorhabditis elegans. *Proc. Natl. Acad. Sci. U. S. A.* **90**, 8905–9 (1993).
4. Vanfleteren, J. R. Oxidative stress and ageing in Caenorhabditis elegans. *Biochem. J.* **292 (Pt 2**, 605–8 (1993).
5. Schriner, S. E. *et al.* Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* **308**, 1909–1911 (2005).
6. Melov, S. *et al.* Extension of Life-Span with Superoxide Dismutase / Catalase Mimetics. **289**, 1567–1570 (2000).
7. Kirkwood, T. B. L. & Holliday, R. The Evolution of Ageing and Longevity. *Proc. R. Soc. B Biol. Sci.* **205**, 531–546 (1979).
8. López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. The Hallmarks of Aging. *Cell* **153**, 1194–217 (2013).
9. Kennedy, B. K. *et al.* Geroscience: Linking aging to chronic disease. *Cell* **159**, 709–713 (2014).
10. Fontana, L., Kennedy, B. K., Longo, V. D., Seals, D. & Melov, S. Medical research: Treat ageing. *Nature* **511**, 405–7 (2014).

11. Kaeberlein, M. Longevity and aging. *F1000Prime Rep.* **5**, 1–8 (2013).

12. Swindell, W. R. Gene expression profiling of long-lived dwarf mice: longevity-associated genes and relationships with diet, gender and aging. *BMC Genomics* **8**, 353 (2007).

13. Swindell, W. R. Comparative analysis of microarray data identifies common responses to caloric restriction among mouse tissues. *Mech. Ageing Dev.* **129**, 138–53 (2008).

14. Swindell, W. R. Genes and gene expression modules associated with caloric restriction and aging in the laboratory mouse. *BMC Genomics* **10**, 585 (2009).

15. Steinbaugh, M. J. Xenobiotic-metabolizing enzymes elevated in multiple mouse models of slow aging by. (2012).

16. Leiser, S. F. *et al.* Cell nonautonomous activation of flavin-containing monooxygenase promotes longevity and health span. *Science (80-.).* **350**, (2015).

17. Rossner, R., Kaeberlein, M. & Leiser, S. F. *Flavin containing monooxygenases in aging and disease: emerging roles for ancient enzymes*. *Journal of Biological Chemistry* (2017).

18. Wright, S. & Pearl, R. The Rate of Living. *J. Am. Stat. Assoc.* **23**, 336 (1928).

19. Medawar, P. B. An Unsolved Problem of Biology. *Evolution in Health and Disease* 24 (1952). doi:10.1016/S0140-6736(00)99799-X

20. Hayflick, L. & Moorhead, P. S. The serial cultivation of human diploid cell strains. *Exp. Cell Res.* 585–621 (1961).

21. Friedman, D. B. & Johnson, T. E. A Mutation in the age-1 Gene in Caenorhabditis elegans Lenghtens Life and Reduces Hermaphrodite Fertility. *Genetics* **118**, 75–86 (1988).

22. Kenyon, C., Chang, J., Gensch, E., Rudner, A. & Tabtlang, R. A C. elegans mutant that lives twice as long as wild type. *Nature* **366**, 461–464 (1993).

23. Bartke, A. Life Extension in the Dwarf Mouse. *Handb. Model. Hum. Aging* **63**, 403–414 (2006).

24. Finch, C. E. Variations in senescence and longevity include the possibility of negligible senescence. *Journals Gerontol. - Ser. A Biol. Sci. Med. Sci.* **53**, 235–239 (1998).

25. Clancy, D. J. *et al.* Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein. *Science (80-.).* **292**, 104–106 (2001).

26. Kaeberlein, M. *et al.* Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. *Science* **310**, 1193–1196 (2005).

27. E. Yanos, M., F. Bennett, C. & Kaeberlein, M. Genome-Wide RNAi Longevity Screens in Caenorhabditis elegans. *Curr. Genomics* **13**, 508–518 (2012).

28. Conboy, I. M. *et al.* Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* **433**, 760–764 (2005).

29. Coppé, J. P. *et al.* Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol.* **6**, (2008).

30. Barzilai, N. *et al.* Metformin as a Tool to Target Aging. *Cell Metab.* **23**, 1060–1065 (2016).

31. Mascotti, M. L., Lapadula, W. J. & Juri Ayub, M. The Origin and Evolution of Baeyer—Villiger Monooxygenases (BVMOs): An Ancestral Family of Flavin Monooxygenases. *PLoS One* **10**, e0132689 (2015).

32. Mascotti, M. L., Ayub, M. J., Furnham, N., Thornton, J. M. & Laskowski, R. A. Chopping and changing: The evolution of the flavin-dependent monooxygenases. *J. Mol. Biol.* **428**, 3131–3146 (2016).

33. Huijbers, M. M. E., Montersino, S., Westphal, A. H., Tischler, D. & Van Berkel, W. J. H. Flavin dependent monooxygenases. *Arch. Biochem. Biophys.* **544**, 2–17 (2014).

34. Ojha, S., Meng, E. C. & Babbitt, P. C. Evolution of function in the 'two dinucleotide binding domains' flavoproteins. *PLoS Comput. Biol.* **3**, 1268–1280 (2007).

35. Ziegler, D. M., Duffel, M. W. & Poulsen, L. L. Studies on the Nature and Regulation of the Cellular Thiol:Disulphide Potential. in *Sulphur in Biology* 191–204 (1980). doi:10.1002/9780470720554.ch12

36. Krueger, S. K. & Williams, D. E. Mammalian flavin-containing monooxygenases: structure/function, genetic polymorphisms and role in drug metabolism. *Pharmacol. Ther.* **106**, 357–87 (2005).

37. Siddens, L. K., Krueger, S. K., Henderson, M. C. & Williams, D. E. Mammalian flavin-containing monooxygenase (FMO) as a source of hydrogen peroxide. *Biochem. Pharmacol.* **89**, 141–7 (2014).

38. Fiorentini, F. *et al.* Biocatalytic Characterization of Human FMO5: Unearthing Baeyer–Villiger Reactions in Humans. *ACS Chem. Biol.* acschembio.5b01016 (2016). doi:10.1021/acschembio.5b01016

39. Mashiguchi, K. *et al.* The main auxin biosynthesis pathway in Arabidopsis. *Proc. Natl. Acad. Sci.* **108**, 18512–18517 (2011).

40. Gut, I. & Conney, A. H. Trimethylamine N-oxygenation and N-demethylation in rat liver microsomes. *Biochem. Pharmacol.* **46**, 239–44. (1993).

41. Suh, J. K., Poulsen, L. L., Ziegler, D. M. & Robertus, J. D. Yeast flavin-containing monooxygenase generates oxidizing equivalents that control protein folding in the

endoplasmic reticulum. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 2687–91 (1999).

42. Rooseboom, M., Commandeur, J. N. M., Floor, G. C., Rettie, A. E. & Vermeiden, N. P. E. Selenoxidation by flavin-containing monooxygenases as a novel pathway for beta-elimination of selenocysteine Se-conjugates. *Chem. Res. Toxicol.* **14**, 127–134 (2001).

43. Neuhoff, C. *et al.* Preliminary study of FMO1, FMO5, CYP21, ESR1, PLIN2 and SULT2A1 as candidate gene for compounds related to boar taint. *Meat Sci.* **108**, 67–73 (2015).

44. Ziegler, D. M. An overview of the mechanism, substrate specificities, and structure of FMOs. *Drug Metab. Rev.* **34**, 503–11 (2002).

45. Alfieri, A., Malito, E., Orru, R., Fraaije, M. W. & Mattevi, A. Revealing the moonlighting role of NADP in the structure of a flavin-containing monooxygenase. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 6572–6577 (2008).

46. Cho, H. J. *et al.* Structural and functional analysis of bacterial flavin-containing monooxygenase reveals its ping-pong-type reaction mechanism. *J. Struct. Biol.* **175**, 39–48 (2011).

47. Li, C. *et al.* Structural mechanism for bacterial oxidation of oceanic trimethylamine into trimethylamine N-oxide. *Mol. Microbiol.* **103**, 992–1003 (2017).

48. Eswaramoorthy, S., Bonanno, J. B., Burley, S. K. & Swaminathan, S. Mechanism of action of a flavin-containing monooxygenase. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 9832–7 (2006).

49. Bateman, A. *et al.* UniProt: A hub for protein information. *Nucleic Acids Res.* **43**, D204–D212 (2015).

50. Suh, J. K., Poulsen, L. L., Ziegler, D. M. & Robertus, J. D. Molecular cloning and kinetic characterization of a flavin-containing monooxygenase from Saccharomyces cerevisiae.

Arch. Biochem. Biophys. **336**, 268–74 (1996).

51. Henderson, M. C., Krueger, S. K., Stevens, J. F. & Williams, D. E. Human flavin-containing monooxygenase form 2 S-oxygenation: sulfenic acid formation from thioureas and oxidation of glutathione. *Chem. Res. Toxicol.* **17**, 633–40 (2004).

52. Hernandez, D., Janmohamed, A., Chandan, P., Phillips, I. R. & Shephard, E. A. Organization and evolution of the flavin-containing monooxygenase genes of human and mouse: identification of novel gene and pseudogene clusters. *Pharmacogenetics* **14**, 117–130 (2004).

53. Phillips, I. R. *et al.* The molecular biology of the flavin-containing monooxygenases of man. *Chem. Biol. Interact.* **96**, 17–32 (1995).

54. Hines, R. N., Hopp, K. a, Franco, J., Saeian, K. & Begun, F. P. Alternative processing of the human FMO6 gene renders transcripts incapable of encoding a functional flavin-containing monooxygenase. *Mol. Pharmacol.* **62**, 320–5 (2002).

55. Lattard, V., Zhang, J. & Cashman, J. R. Alternative processing events in human FMO genes. *Mol. Pharmacol.* **65**, 1517–1525 (2004).

56. Hao, D. C., Chen, S. L., Mu, J. & Xiao, P. G. Molecular phylogeny, long-term evolution, and functional divergence of flavin-containing monooxygenases. *Genetica* **137**, 173–187 (2009).

57. Koukouritaki, S. B., Simpson, P., Yeung, C. K., Rettie, A. E. & Hines, R. N. Human hepatic flavin-containing monooxygenases 1 (FMO1) and 3 (FMO3) developmental expression. *Pediatr. Res.* **51**, 236–243 (2002).

58. Zhang, J. & Cashman, J. R. QUANTITATIVE ANALYSIS OF FMO GENE mRNA LEVELS IN

HUMAN TISSUES ABSTRACT : **34**, 19–26 (2006).

59. Falls, J. G., Blake, B. L., Cao, Y., Levi, P. E. & Hodgson, E. Gender differences in hepatic expression of flavin-containing monooxygenase isoforms (FMO1, FMO3, and FMO5) in mice. *J. Biochem. Toxicol.* **10**, 171–7 (1995).

60. Cashman, J., Yang, Z., Yang, L. & Wrighton, S. Stereo- and regioselective N- and S-oxidation of tertiary amines and sulfides in the presence of adult human liver microsomes. *Drug Metab. Dispos.* (1993).

61. Sadeque, A. J., Thummel, K. E. & Rettie, A. E. Purification of macaque liver flavin-containing monooxygenase: a form of the enzyme related immunochemically to an isozyme expressed selectively in adult human liver. *Biochim. Biophys. Acta* **1162**, 127–34 (1993).

62. Bennett, B. J. *et al.* Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metab.* **17**, 49–60 (2013).

63. Luo, Z. & Hines, R. N. Regulation of flavin-containing monooxygenase 1 expression by ying yang 1 and hepatic nuclear factors 1 and 4. *Mol. Pharmacol.* **60**, 1421–1430 (2001).

64. Borbás, T., Benko, B., Dalmadi, B., Szabó, I. & Tihanyi, K. Insulin in flavin-containing monooxygenase regulation: Flavin-containing monooxygenase and cytochrome P450 activities in experimental diabetes. *Eur. J. Pharm. Sci.* **28**, 51–58 (2006).

65. Klick, D. E., Shadley, J. D. & Hines, R. N. Differential regulation of human hepatic flavin containing monooxygenase 3 (FMO3) by CCAAT/enhancer-binding protein β (C/EBPβ) liver inhibitory and liver activating proteins. *Biochem. Pharmacol.* **76**, 268–278 (2008).

66. Zhang, J. *et al.* Hepatic flavin-containing monooxygenase gene regulation in different mouse inflammation models. *Drug Metab. Dispos.* **37**, 462–8 (2009).

67. Miao, J. *et al.* Flavin-containing monooxygenase 3 as a potential player in diabetes-associated atherosclerosis. *Nat. Commun.* **6**, 6498 (2015).

68. Houseman, L. Flavin-containing monooxygenases : Regulation , endogenous roles and dietary supplements for the degree o f Doctor o f Philosophy. (2008).

69. Celius, T. *et al.* Aryl hydrocarbon receptor-dependent induction of flavin-containing monooxygenase mRNAs in mouse liver. *Drug Metab. Dispos.* **36**, 2499–2505 (2008).

70. Shih, D. M. *et al.* Flavin containing monooxygenase 3 exerts broad effects on glucose and lipid metabolism and atherosclerosis. *J. Lipid Res.* **56**, 22–37 (2015).

71. Celius, T. *et al.* Flavin-containing monooxygenase-3: induction by 3-methylcholanthrene and complex regulation by xenobiotic chemicals in hepatoma cells and mouse liver. *Toxicol. Appl. Pharmacol.* **247**, 60–9 (2010).

72. Novick, R. M., Vezina, C. M. & Elfarra, A. a. Isoform distinct time-, dose-, and castration-dependent alterations in flavin-containing monooxygenase expression in mouse liver after 2,3,7,8-tetrachlorodibenzo-p-dioxin treatment. *Biochem. Pharmacol.* **79**, 1345–51 (2010).

73. Treacy, E. P. *et al.* Mutations of the flavin-containing monooxygenase gene (FMO3) cause trimethylaminuria, a defect in detoxication. *Hum. Mol. Genet.* **7**, 839–845 (1998).

74. McConell, H. W., Mitchell, S. C., Smith, R. L. & Brewster, M. Trimethylaminuria associated with seizures and behavioural disturbance: a case report. *Seizure* (1997). doi:10.1038/2091178c0

75. Wang, Z. *et al.* Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* **472**, 57–63 (2011).

76. Tang, W. H. W. *et al.* Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N. Engl. J. Med.* **368**, 1575–84 (2013).

77. Koeth, R. a *et al.* Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat. Med.* **19**, 576–85 (2013).

78. Wang, Z. *et al.* Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* **472**, 57–63 (2011).

79. Ufnal, M. *et al.* Trimethylamine-N-Oxide : A Carnitine-Derived Metabolite That Prolongs the Hypertensive Effect of Angiotensin II in Rats. *Can. J. Cardiol.* **30**, 1700–1705 (2014).

80. Zhu, W. *et al.* Gut Microbial Metabolite TMAO Enhances Platelet Article Gut Microbial Metabolite TMAO Enhances Platelet Hyperreactivity and Thrombosis Risk. *Cell* **165**, 111–124 (2016).

81. Tomasova, L., Konopelski, P. & Ufnal, M. Gut bacteria and hydrogen sulfide: The new old players in circulatory system homeostasis. *Molecules* **21**, 1–18 (2016).

82. Dolan, C. *et al.* Polymorphisms of the Flavin containing monooxygenase 3 (FMO3) gene do not predispose to essential hypertension in Caucasians. *BMC Med. Genet.* **6**, (2005).

83. Fukami, K., Yamagishi, S. & Sakai, K. Oral L -Carnitine Supplementation Increases Trimethylamine- N -oxide but Reduces Markers of Vascular Injury in Hemodialysis Patients. **65**, 289–295 (2015).

84. Collins, H. L. *et al.* L-Carnitine intake and high trimethylamine N-oxide plasma levels correlate with low aortic lesions in ApoE-/- transgenic mice expressing CETP.

Atherosclerosis **244**, 29–37 (2016).

85. Ufnal, M., Zadlo, A. & Ostaszewski, R. TMAO, a small molecule of great expectations. *Nutrition* **48**, 1–8 (2015).

86. Takamura, T. *et al.* Genes for systemic vascular complications are differentially expressed in the livers of Type 2 diabetic patients. *Diabetologia* **47**, 638–647 (2004).

87. Vahabzadeh, M. & Mohammadpour, A. Effect of Diabetes Mellitus on the Metabolism of Drugs and Toxins. *Clin. Toxicol.* **5**, (2015).

88. Warrier, M. *et al.* The TMAO-Generating Enzyme Flavin Monooxygenase 3 Is a Central Regulator of Cholesterol Balance. *Cell Rep.* **10**, 326–338 (2015).

89. Veeravalli, S. *et al.* The phenotype of a flavin-containing monooyxgenase knockout mouse implicates the drug-metabolizing enzyme FMO1 as a novel regulator of energy balance. *Biochem. Pharmacol.* **90**, 88–95 (2014).

90. Malagon, S. G. G. *et al.* The phenotype of a knockout mouse identifies flavin-containing monooxygenase 5 (FMO5) as a regulator of metabolic ageing. *Biochem. Pharmacol.* **96**, 267–277 (2015).

91. Petriello, M. C., Hoffman, J. B., Morris, A. J. & Hennig, B. Emerging roles of xenobiotic detoxification enzymes in metabolic diseases. *Rev. Environ. Health* **32**, 105–110 (2017).

92. Motika, M. S., Zhang, J. & Cashman, J. R. Flavin-containing monooxygenase 3 and human disease. *Expert Opin. Drug Metab. Toxicol.* **3**, 831–845 (2007).

93. Bozzoni, V. *et al.* Amyotrophic lateral sclerosis and environmental factors. *Funct. Neurol.* **31**, 7–19 (2016).

94. Hibar, D. P. *et al.* Genome-wide association identifies genetic variants associated with

lentiform nucleus volume in N=1345 young and elderly subjects. *Brain Imaging Behav.* **7**, 102–115 (2013).

95. Li, B. *et al.* Flavin-containing monooxygenase, a new clue of pathological proteins in the rotenone model of parkinsonism. *Neurosci. Lett.* **566**, 11–6 (2014).

96. Guan, S. H., Falick, a M., Williams, D. E. & Cashman, J. R. Evidence for complex formation between rabbit lung flavin-containing monooxygenase and calreticulin. *Biochemistry* **30**, 9892–9900 (1991).

97. Umbreit, J. N., Conrad, M. E., Hainsworth, L. N. & Simovich, M. The ferrireductase paraferritin contains divalent metal transporter as well as mobilferrin. *Am. J. Physiol. Gastrointest. Liver Physiol.* **282**, G534–G539 (2002).

98. Barber, M., Conrad, M. E., Umbreit, J. N., Barton, J. C. & Moore, E. G. Abnormalities of flavin monooxygenase as an etiology for sideroblastic anemia. *Am. J. Hematol.* **65**, 149–153 (2000).

99. Muckenthaler, M. *et al.* Regulatory defects in liver and intestine implicate abnormal hepcidin and Cybrd1 expression in mouse hemochromatosis. *Nat. Genet.* **34**, 102–7 (2003).

100. Coppin, H. *et al.* Gene expression profiling of Hfe-/- liver and duodenum in mouse strains with differing susceptibilities to iron loading: identification of transcriptional regulatory targets of Hfe and potential hemochromatosis modifiers. *Genome Biol.* **8**, R221 (2007).

101. Robinson-Cohen, C. *et al.* Association of FMO3 variants and trimethylamine N-oxide concentration, disease progression, and mortality in CKD patients. *PLoS One* **11**, 1–11 (2016).

102. Gagliardi, S. *et al.* Environmental and Genetic Factors in ALS : Positive Correlation of Snps in Flavin-Containing Monooxygenase 5 Gene. *Ann. Neurodegener. Disord.* **1**, 1014 (2016).

103. Steinbaugh, M. J., Sun, L. Y., Bartke, A. & Miller, R. a. Activation of genes involved in xenobiotic metabolism is a shared signature of mouse models with extended lifespan. *Am. J. Physiol. Endocrinol. Metab.* **303**, E488-95 (2012).

104. Miller, R. a *et al.* Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging Cell* **13**, 468–77 (2014).

105. Pitt, J. N. & Kaeberlein, M. Why Is Aging Conserved and What Can We Do about It? *PLOS Biol.* **13**, e1002131 (2015).

106. Leiser, S. F. *et al.* Cell nonautonamous activation of flavin-containing monooxygenase promotes longevity and health span. *Science (80-.).* **350**, 1375–1378 (2015).

107. Schirra, H. J. *et al.* Altered Metabolism of Growth Hormone Receptor Mutant Mice: A Combined NMR Metabonomics and Microarray Study. *PLoS One* **3**, e2764 (2008).

108. Kaeberlein, M., Rabinovitch, P. S. & Martin, G. M. Healthy aging: The ultimate preventative medicine. *Science (80-.).* **350**, 1191–1193 (2015).

109. Sun, L., Sadighi Akha, A. A., Miller, R. A. & Harper, J. M. Life-span extension in mice by preweaning food restriction and by methionine restriction in middle age. *Journals Gerontol. - Ser. A Biol. Sci. Med. Sci.* **64**, 711–722 (2009).

110. Shen, C., Nettleton, D., Jiang, M., Kim, S. K. & Powell-Coffman, J. A. Roles of the HIF-1 hypoxia-inducible factor during hypoxia response in Caenorhabditis elegans. *J. Biol. Chem.* **280**, 20580–8 (2005).

111. Bishop, T. *et al.* Genetic analysis of pathways regulated by the von Hippel-Lindau tumor suppressor in Caenorhabditis elegans. *PLoS Biol.* **2**, e289 (2004).

112. Lee, S.-J., Hwang, A. B. & Kenyon, C. Inhibition of respiration extends C. elegans life span via reactive oxygen species that increase HIF-1 activity. *Curr. Biol.* **20**, 2131–6 (2010).

113. Honjoh, S., Yamamoto, T., Uno, M. & Nishida, E. Signalling through RHEB-1 mediates intermittent fasting-induced longevity in C. elegans. *Nature* **457**, 726–730 (2009).

114. Uno, M. *et al.* A Fasting-Responsive Signaling Pathway that Extends Life Span in C. elegans. *Cell Rep.* **3**, 79–91 (2013).

115. Petalcorin, M. I. R., Joshua, G. W., Agapow, P. M. & Dolphin, C. T. The fmo genes of Caenorhabditis elegans and C. briggsae: Characterisation, gene expression and comparative genomic analysis. *Gene* **346**, 83–96 (2005).

116. Hirani, N., Westenberg, M., Seed, P. T. & Dolphin, C. T. C. elegans flavin-containing monooxygenase-4 is essential for osmoregulation in hypotonic stress. *Biol. Open* 1–13 (2016). doi:10.1242/bio.017400

117. Attrill, H. *et al.* FlyBase: establishing a Gene Group resource for Drosophila melanogaster. *Nucleic Acids Res.* **44**, D786–D792 (2015).

118. Cheol Park, H., Cha, J.-Y. & Yun, D.-J. Roles of YUCCAs in auxin biosynthesis and drought stress responses in plants. *Plant Signal. Behav.* **8**, e24495 (2013).

119. Schlenk, D. A comparison of endogenous and exogenous substrates of the flavin-containing monooxygenases in aquatic organisms. *Aquat. Toxicol.* **26**, 157–162 (1993).

120. Matzkin, L. M. & Markow, T. A. Transcriptional regulation of metabolism associated with the increased desiccation resistance of the cactophilic Drosophila mojavensis. *Genetics*

182, 1279–1288 (2009).

121. Chandler-Brown, D. *et al.* Sorbitol treatment extends lifespan and induces the osmotic stress response in Caenorhabditis elegans. *Front. Genet.* **6**, 1–9 (2015).

122. Park, M. C., Park, D., Lee, E. K., Park, T. & Lee, J. Genomic analysis of the telomeric length effect on organismic lifespan in Caenorhabditis elegans. *Biochem. Biophys. Res. Commun.* **396**, 382–387 (2010).

123. Brauer, M. Coordination of Growth Rate, Cell Cycle, Stress Response, and Metabolic Activity in Yeast. *Mol. Biol. Cell* **19**, 308–317 (2008).

124. Kumar, A. *et al.* Converging evidence of mitochondrial dysfunction in a yeast model of homocysteine metabolism imbalance. *J. Biol. Chem.* **286**, 21779–21795 (2011).

125. McCully, K. S. Homocysteine Metabolism, Atherosclerosis, and Diseases of Aging. *Compr. Physiol.* **6**, 471–505 (2015).

126. Poloni, S., Blom, H. & Schwartz, I. Stearoyl-CoA Desaturase-1: Is It the Link between Sulfur Amino Acids and Lipid Metabolism? *Biology (Basel).* **4**, 383–396 (2015).

127. Gibrat, C. & Cicchetti, F. Potential of cystamine and cysteamine in the treatment of neurodegenerative diseases. *Prog. Neuro-Psychopharmacology Biol. Psychiatry* **35**, 380–389 (2011).

128. Veeravalli, S. *et al.* Flavin-containing monooxygenase 1 catalyzes the production of taurine from hypotaurine. *Drug Metab. Dispos.* **48**, 378–385 (2020).

129. Bennett, C. F. *et al. Transaldolase inhibition impairs mitochondrial respiration and induces a starvation-like longevity response in Caenorhabditis elegans*. *PLOS Genetics* **13**, (2017).

130. Choi, H. *et al.* DDS promotes longevity through a microbiome-mediated starvation signal. *Transl. Med. Aging* **1**, 64–69 (2019).

131. Rossner, R., Kaeberlein, M. & Leiser, S. F. Flavin-containing monooxygenases in aging and disease: Emerging roles for ancient enzymes. *J. Biol. Chem.* **292**, (2017).

132. Hansen, M., Hsu, A. L., Dillin, A. & Kenyon, C. New genes tied to endocrine, metabolic, and dietary regulation of lifespan from a Caenorhabditis elegans genomic RNAi screen. *PLoS Genet.* **1**, 0119–0128 (2005).

133. Cabreiro, F. *et al.* Metformin retards aging in C. elegans by altering microbial folate and methionine metabolism. *Cell* **153**, 228–239 (2013).

134. Kabil, H., Kabil, O., Banerjee, R., Harshman, L. G. & Pletcher, S. D. Increased transsulfuration mediates longevity and dietary restriction in Drosophila. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 16831–6 (2011).

135. Hine, C. M., Mair, W. B. & Mitchell, J. R. Endogenous Hydrogen Sulfide Production Is Essential for Dietary Restriction Benefits. *Cell* (2014). doi:10.1016/j.cell.2014.11.048

136. Kapahi, P., Kaeberlein, M. & Hansen, M. Dietary restriction and lifespan: Lessons from invertebrate models. *Ageing Res. Rev.* **39**, 3–14 (2017).

137. Peltonen, J. & Wong, G. Chronic Ethanol Exposure Increases Cytochrome P-450 and Decreases Activated in Blocked Unfolded Protein Response Gene Family Transcripts in Caenorhabditis elegans. *J. Biochem. Mol. Toxicol.* **27**, (2013).

138. Patananan, A. N., Budenholzer, L. M., Eskin, A., Torres, E. R. & Clarke, S. G. Ethanol-induced differential gene expression and acetyl-CoA metabolism in a longevity model of the nematode Caenorhabditis elegans. *Exp. Gerontol.* **61C**, 20–30 (2014).

139. Walker, A. K., Jacobs, R. L., Watts, J. L., Rottiers, V. & Jiang, K. A conserved SREBP-1 / phosphatidylcholine feedback circuit regulates lipogenesis in metazoans.

140. Crook, M., Upadhyay, A., Ido, L. J. & Hanna-rose, W. Epidermal Growth Factor Receptor Cell Survival Signaling Requires Phosphatidylcholine Biosynthesis. **6**, 3533–3540 (2016).

141. Goh, G. Y. S. *et al.* NHR-49/HNF4 integrates regulation of fatty acid metabolism with a protective transcriptional response to oxidative stress and fasting. *Aging Cell* e12743 (2018). doi:10.1111/acel.12743

142. Schieber, M. & Chandel, N. S. TOR Signaling Couples Oxygen Sensing to Lifespan in C. elegans. *CellReports* **9**, 9–15 (2014).

143. Eom, H.-J., Ahn, J.-M., Kim, Y. & Choi, J. Hypoxia inducible factor-1 (HIF-1)-flavin containing monooxygenase-2 (FMO-2) signaling acts in silver nanoparticles and silver ion toxicity in the nematode, Caenorhabditis elegans. *Toxicol. Appl. Pharmacol.* **270**, 106–13 (2013).

144. Irazoqui, J. E. *et al.* Distinct pathogenesis and host responses during infection of C. elegans by P. aeruginosa and S. aureus. *PLoS Pathog.* **6**, 1–24 (2010).

145. Kirienko, N. V. *et al.* Pseudomonas aeruginosa disrupts Caenorhabditis elegans iron homeostasis, causing a hypoxic response and death. *Cell Host Microbe* **13**, 406–416 (2013).

146. Kirienko, N. V, Ausubel, F. M. & Ruvkun, G. Mitophagy confers resistance to siderophore-mediated killing by Pseudomonas aeruginosa. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 1821–6 (2015).

147. Wani, K., Taubert, S. & Irazoqui, J. NHR-49/PPARα regulates HLH-30/TFEB-mediated

innate immune response via a flavincontaining monooxygenase in C. elegans *J. Immunol.* **202**, 64.6 LP-64.6 (2019).

148. Vozdek, R., Hnízda, A., Krijt, J., Kostrouchová, M. & Kožich, V. Novel structural arrangement of nematode cystathionine β-synthases: characterization of Caenorhabditis elegans CBS-1. *Biochem. J.* **443**, 535–47 (2012).

149. Elshorbagy, A. K. Body composition in gene knockouts of sulfur amino acid-metabolizing enzymes. *Mamm. Genome* **25**, 455–463 (2014).

150. Finkelstein, J. D. Inborn Errors of Sulfur-Containing Amino Acid Metabolism. *Am. Soc. Nutr.* 1751S-1754S (2006).

151. Veitch, R. L., Lumeng, L. & Li, T. Vitamin B6 Metabolism in Chronic Alcohol Abuse THE EFFECT OF ETHANOL OXIDATION ON HEPATIC PYRIDOXAL 5'-PHOSPHATE METABOLISM. **55**, 1026–1032 (1975).

152. Lumeng, L. & Medicine, D. The Role of Acetaldehyde in Mediating the Deleterious Effect of Ethanol on Pyridoxal 5 ' -Phosphate Metabolism. **62**, 286–293 (1978).

153. Kamat, P. K., Mallonee, C. J., George, A. K., Suresh, C. & Tyagi, N. Homocysteine, alcoholism and its potential epigenetic mechanism. *Alcohol Clin Exp Res* **40**, 2474–2481 (2017).

154. Walker, A. K. *et al.* A conserved SREBP-1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. *Cell* **147**, 840–852 (2011).

155. Barak, A. J., Beckenhauer, H. C. & Tuma, D. J. Methionine synthase: A possible prime site of the ethanolic lesion in liver. *Alcohol* **26**, 65–67 (2002).

156. Sakata, S. F. *et al.* Effect of Fasting on Methionine Adenosyltransferase Expression and

the Methionine Cycle in the Mouse Liver. 118–123 (2005).

157. Brown-Borg, H. M. & Rakoczy, S. Metabolic adaptations to short-term every-other-day feeding in long-living Ames dwarf mice. *Exp. Gerontol.* **48**, 905–919 (2013).

158. Robinson, J. L., McBreairty, L. E., Randell, E. W., Brunton, J. A. & Bertolo, R. F. Restriction of dietary methyl donors limits methionine availability and affects the partitioning of dietary methionine for creatine and phosphatidylcholine synthesis in the neonatal piglet. *J. Nutr. Biochem.* **35**, 81–86 (2016).

159. Stiernagle, T. No Title. *Maintenance of C. elegans (February 11, 2006), WormBook, ed. The C. elegans Research Community, WormBook* (2006). doi:doi/10.1895/wormbook.1.101.1

160. Davis, J. R., Li, Y. & Rankin, C. H. Effects of developmental exposure to ethanol on Caenorhabditis elegans. *Alcohol. Clin. Exp. Res.* **32**, 853–867 (2008).

161. Lüersen, K. *et al.* The Glutathione Reductase GSR-1 Determines Stress Tolerance and Longevity in Caenorhabditis elegans. *PLoS One* **8**, 1–16 (2013).

162. Mockett, R. J., Sohal, R. S. & Orr, W. C. Overexpression of glutathione reductase extends survival in transgenic Drosophila melanogaster under hyperoxia but not normoxia. *FASEB J.* **13**, 1733–1742 (1999).

163. Suh, J. K. & Robertus, J. D. Yeast flavin-containing monooxygenase is induced by the unfolded protein response. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 121–6 (2000).

164. Bradshaw, P. C. Cytoplasmic and mitochondrial NADPH-coupled Redox systems in the regulation of aging. *Nutrients* **11**, (2019).

165. Reed, M. C. *et al.* A mathematical model of glutathione metabolism. *Theor. Biol. Med.*

Model. **5**, 1–16 (2008).

166. Ju Jong, C., Azuma, J. & Schaffer, S. Mechanism underlying the antioxidant activity of taurine: prevention of mitochondrial oxidant production. *Amino Acids* **42**, 2223–2232 (2012).

167. Chaturvedi, S. K. *et al.* Biophysical insight into the anti-amyloidogenic behavior of taurine. *Int. J. Biol. Macromol.* **80**, 375–384 (2015).

168. Lambert, I. H., Kristensen, D. M., Holm, J. B. & Mortensen, O. H. Physiological role of taurine--from organism to organelle. *Acta Physiol. (Oxf).* **213**, 191–212 (2015).

169. Kim, H. M., Do, C.-H. & Lee, D. H. Taurine reduces ER stress in C. elegans. *J. Biomed. Sci.* **17 Suppl 1**, S26 (2010).

170. Murakami, S. Role of taurine in the pathogenesis of obesity. *Mol. Nutr. Food Res.* **59**, 1353–1363 (2015).

171. Uthus, E. O. & Brown-Borg, H. M. Methionine flux to transsulfuration is enhanced in the long living Ames dwarf mouse. *Mech. Ageing Dev.* **127**, 444–450 (2006).

172. Vitvitsky, V., Martinov, M., Ataullakhanov, F., Miller, R. A. & Banerjee, R. Sulfur-based redox alterations in long-lived Snell dwarf mice. *Mech. Ageing Dev.* **134**, 321–330 (2013).

173. Giordano, G., White, C. C. & Costa, L. G. Assessment of glutathione homeostasis. *Methods Mol. Biol.* **758**, 205–214 (2011).

174. Botta, D. *et al.* Modulating GSH synthesis using glutamate cysteine ligase transgenic and gene-targeted mice. *Drug Metab. Rev.* **40**, 465–477 (2008).

175. Luchak, J. M., Prabhudesai, L., Sohal, R. S., Radyuk, S. N. & Orr, W. C. Modulating longevity in Drosophila by over- and underexpression of glutamate-cysteine ligase. *Ann.*

N. Y. Acad. Sci. **1119**, 260–273 (2007).

176. Orr, W. C. *et al.* Overexpression of glutamate-cysteine ligase extends life span in Drosophila melanogaster. *J. Biol. Chem.* **280**, 37331–37338 (2005).