The Caenorhabditis elegans K10C2.4 Gene Encodes a Member of the Fumarylacetoacetate Hydrolase Family

**A CAENORHABDITIS ELEGANS MODEL OF TYPE I TYROSINEMIA**

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In eukaryotes and many bacteria, tyrosine is degraded to produce energy via a five-step tyrosine degradation pathway. Mutations affecting the tyrosine degradation pathway are also of medical importance as mutations affecting enzymes in the pathway are responsible for type I tyrosinemia, type II tyrosinemia, and type III tyrosinemia. The most severe of these is type I tyrosinemia, which is caused by mutations in the fumarylacetoacetate hydrolase (FAH) gene. So far, tyrosinemia has been studied in the nematode Caenorhabditis elegans. Type I tyrosinemia has been identified in several microarray, proteomic, and RNA interference (RNAi) screens as perhaps being involved in aging and the control of protein folding. We sought to identify and characterize the genes in the worm tyrosine degradation pathway as an initial step in understanding these findings. Here we describe the characterization of the K10C2.4, which encodes a homolog of FAH. RNAi directed against K10C2.4 produces a lethal phenotype consisting of death in young adulthood, extensive damage to the intestine, impaired fertility, and activation of oxidative stress and endoplasmic stress response pathways. This phenotype is due to alterations in tyrosine metabolism as increases in dietary tyrosine enhance it, and inhibition of upstream enzymes in tyrosine degradation with RNAi or genetic mutations reduces the phenotype. We also use our model to identify genes that suppress the damage produced by K10C2.4 RNAi in a pilot genetic screen. Our results establish worms as a model for the study of type I tyrosinemia.

Tyrosine catabolism in all eukaryotes studied so far proceeds by the same five-step biochemical pathway (Fig. 1) (1, 2). This pathway is of medical interest because of several inborn errors of metabolism that affect the pathway (3). Alkaptonuria is caused by mutations affecting homogentisate 1,2-dioxygenase and results in accumulation of HGA (4). Type II and type III tyrosinemia are attributed to mutations affecting tyrosine aminotransferase and 4-hydroxyphenylpyruvate dioxygenase, respectively (5–7). The most severe inborn error of metabolism affecting the pathway is type I tyrosinemia, which is attributed to mutations in the fumarylacetoacetate hydrolase (FAH) gene (8–10). Type I tyrosinemia manifests in two clinical forms: an acute form that often results in death during the first year of life, and a chronic form characterized by the slow development of liver and renal disease and a high risk for the development of liver cancer (3, 11). Organ damage is likely due to the accumulation of MAA, FAA, and their conversion to succinylacetoylacetate (SAA) and succinylacetone (SA) (12). These compounds are highly reactive electrophiles, which can damage proteins and DNA leading to the activation of several stress response pathways as well as apoptotic pathways (13–20).

In Caenorhabditis elegans, genes predicted to be part of the tyrosine degradation pathway have been identified in microarray, proteomic, and RNAi screens looking for genes involved in aging and polyglutamine-repeat protein aggregation (21–25). At this time, it is not clear how these findings relate to the predicted metabolic activity of the proteins. As a first step in understanding how the C. elegans tyrosine degradation pathway relates to these findings, we identified and characterized the genes in this pathway. We find that tyrosine degradation occurs via the same biochemical pathway in C. elegans as in other eukaryotes. Importantly, inhibition of the FAH homolog K10C2.4 via RNAi has dramatic effects on worms and results in destruction of the intestine, death in young adulthood, impaired fertility, and activation of oxidative stress and endoplasmic stress response pathways. These effects are specifically caused by changes in tyrosine metabolism as the produced phenotype is sensitive to levels of tyrosine in the worm media and require an intact tyrosine degradation pathway to cause tissue damage. The intestinal damage may occur because of the accumulation of tyrosine metabolites because treatment of worms with succinylacetone (SA) can mimic the phenotype produced by K10C2.4 RNAi. Our finding that SA is toxic to cells is novel. We further show that damage to the intestine is associated with activation of oxidative stress and ER stress pathways because reporter genes responsive to these cellular stresses are activated in transgenic animals following treatment with K10C2.4 RNAi. Finally, we exploit the genetic power of C. elegans to conduct a pilot genetic.

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* The abbreviations used are: FAH, fumarylacetoacetate hydrolase; MAA, maleylacetoacetate; FAA, fumarylacetoacetate; HGA, homogentisic acid; NGA, nematode growth agar; rde, RNAi defective; SAA, succinylacetoylacetate; SA, succinylacetone; GFP, green fluorescent protein; ER, endoplasmic reticulum; IPTG, isopropyl-1-thio-β-D-galactopyranoside; RNAi, RNA interference.

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screen for resistance to the effects of K10C2.4 RNAi, and we identify the first alleles of homogentisate dioxygenase and tyrosine aminotransferase in this animal. Together our results suggest that *C. elegans* may serve as a new model system for the study of type I tyrosinemia.

**EXPERIMENTAL PROCEDURES**

**Strains**—Wild-type N2 worms and the strains BC11065 (Exl1065) (26), BC13802 (Exl3802) (26), BC14489 (sls13451) (26), NL2099 (rfy-3(pk1426)) (27), CL2070 (dvlts70) (28), DP38 (unc-119(ed3)) (29), CL2166 (dvlts19) (30), TJ356 (zls356) (31), and SJ4005 (zcls4[hsp-4::GFP]) (32) were obtained from the Caenorhabditis Genetics Center, which is supported by a grant from the National Institutes of Health.

**Inhibition of FAH by RNAi**—A K10C2.4 RNAi clone was purchased from Open Biosystems (Huntsville, AL) (33). The identity of this clone was determined by sequencing (data not shown). Bacteria from an overnight culture was spotted on NGA plates containing 25 μg/ml carbenicillin and 6 mM IPTG to induce dsRNA expression (33). NL2099 worms were synchronized by hypochlorite treatment, and the resulting eggs were placed on spotted plates and incubated at 20 °C (34). Effects were assessed in adult worms after 3 days. Effectiveness of RNAi was assayed by Western blotting. ALF100 (baf1s100) worms were grown on control or K10C2.4 RNAi as described above. Adult worms were washed from the plates and resuspended in 1 ml of 10X Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA with protease inhibitors (Complete Mini-EDTA free, Roche Applied Science). Worms were lysed with four passes in a French press at 8000 psi. Debris was pelleted by spinning in a microfuge, and the supernatant was stored frozen. 

**GFP Transgenes**—Cells expressing F42D1.2, T21C12.2, and W06D4.1 were identified by photographing the BC11065, BC13802, and BC14489 strains, respectively. Cells expressing K10C2.4 were identified by photographing the ALF100 (baf1s100), ALF101 (baf1s101), and ALF102 (baf1s102) strains. These carry the WRM0615c09 fosmid containing 34,131 bp of genomic DNA centered on the K10C2.4 gene (Geneservice Ltd., Oxford, UK). This fosmid was modified via recombineering to express an N-terminal GFP fusion protein and fitted with an unc-119 rescue fragment via cre-lox recombination. The modified fosmid was bombarded into DP38 (unc-119(ed3)) worms via microparticle bombardment using a Bio-Rad PDS-1000 (Bio-Rad Laboratories) (35).

**GFP Reporter Assay**—L4 larval worms carrying the GFP reporters hsp-16.2:GFP (strain CL2070), gst-4:GFP (CL2166), hsp-4:GFP (SJ4005), or daf-16:GFP (TJ356) were placed on spotted RNAi plates and allowed to lay eggs. The adult worms were removed, and the effects on GFP expression scored in the F1 progeny at the indicated time.

**RNAi Epistasis**—RNAi clones for F42D1.2 and W06D4.1 were obtained from the Ahringer RNAi library (MRC Geneservice) (36). We found that the Ahringer RNAi library clone for T21C12.2 is incorrect based on sequence data (data not shown). As a result, T21C12.2 genomic DNA was amplified using the GenePair primers listed on Wormbase and subcloned into L1440 (37). The GFP RNAi clone was obtained from the Fire Lab vector kit (pPD128.110, a gift of A. Fire). The identities of these clones were confirmed by sequencing (data not shown). Overnight cultures were mixed 1:1 in 1.5-ml tubes and used to spot NGA plates containing 25 μg/ml carbenicillin and 6 mM IPTG. Eggs were added to plates as described above and scored for phenotypes 3 days later.

**Treatment of Worms with Tyrosine, Homogentisic Acid, and Succinylacetone**—Tyrosine, homogentisic acid, and SA were purchased from Sigma-Aldrich. These were added to NGA containing carbenicillin and IPTG to a final concentration of 1 mg/ml. RNAi clones were spotted on plates as described above. Overnight cultures were mixed 1:1 in 1.5-ml tubes and used to spot NGA plates containing 25 μg/ml carbenicillin and 6 mM IPTG. Eggs were added to plates as described above.

**Pilot Genetic Screen**—We mutagenized 100 worms with 50 mM EMS as previously described and transferred the mutagenized worms to NGA plates (38). F1 progeny were treated with hypochlorite, and the resulting F2 eggs were placed on plates spotted with K10C2.4 RNAi. After 3 days, plates were screened for rare normal-appearing adults. Control experiments demonstrated that even a single normal worm could be reliably identified in a population of affected worms (data not shown). These mutants were used in secondary screens to verify resistance to K10C2.4 RNAi and to demonstrate sensitivity to RNAi via responses to unc-22 RNAi. unc-22 RNAi produces an easily visible “twitcher” phenotype and served to exclude RNAi unresponsive mutants (39). Six mutants passed this secondary screen and were backcrossed to verify that a single gene was responsible for the identified resistance phenotype.

Mutations were mapped backcrossing with CB4586 males, and the F1 progeny were allowed to self-fertilize on K10C2.4 RNAi. Affected and unaffected F2 progeny were collected and used for SNP mapping as previously described (40). Genomic DNA from mutant worms carrying genes localizing to chromosome I were used for PCR using W06D4.1 specific primers, to chromosome III with T21C12.2 primers, and to chromosome X with F42D1.2 primers (sequences available on request). The resulting PCR products were cloned and sequenced. At least four independent clones were sequenced for each gene. This sequencing led to the identification of the strains ALF103 (F42D1.2[baf1]) and ALF104 (W06D4.1[bafl2]).

**RESULTS**

**K10C2.4 Encodes a Homolog of Fumarylacetoacetate Hydrolase**—We wanted to determine whether tyrosine degradation in worms occurs via the same biochemical pathway as in...

\[A. L. Fisher, submitted manuscript.\]
other eukaryotes and identify the genes involved in the pathway. We used the Kyoto Encyclopedia of Genes and Genomes (KEGG) data base to predict homologs for each step in the five enzyme pathway (Fig. 1) (41). We focused our analysis on the gene K10C2.4, which is predicted to encode the C. elegans homolog of FAH given the severe effects of mutations affecting this enzyme in people, rodents, and fungi (8, 42–44). Alignment of K10C2.4 with FAH genes from other species with ClustalW revealed 34.7% identity between K10C2.4 and the other genes (supplemental Fig. S1) (45). Comparison of K10C2.4 with mouse and human FAH in terms of residues identified as critical for enzymatic function based upon functional studies and the enzyme crystal structure demonstrates strong conservation of these residues (supplemental Fig. S1) (9, 46, 47). All nine of the amino acids in the active site of the mouse FAH crystal structure (Tyr-128, His-133, Arg-142, Glu-199, Arg-237, Gln-240, Tyr-244, Lys-253, and Glu-364), all four of the amino acids involved in calcium ion binding (Asp-126, Glu-199, Glu-201, and Asp-233), and all but three of the amino acids mutated in human type I tyrosinemia (Asn-16, Phe-62, Gln-64, Ala-134, Gly-158, Val-166, Cys-193, Gly-207, Asn-232, Asp-233, Trp-234, Pro-249, Pro-261, Thr-294, Gly-337, Arg-341, Pro-342, Gly-369, and Arg-381) are conserved. Together, this suggests that K10C2.4 may represent the C. elegans homolog of FAH.

Given the profound effects of FAH mutations in mammals, we asked whether RNAi directed against K10C2.4 produced a phenotype in worms. We found that worms treated with K10C2.4 RNAi from hatching developed a lethal phenotype characterized by stunted growth, poor fertility, and progressive destruction of the intestine by adulthood (Fig. 2, C and D). The damage to the intestine is striking and easily visible by Nomarski imaging as evidenced by thinning of the intestinal wall and

FIGURE 1. The tyrosine degradation pathway. Shown are the chemical structure of each compound and the names of the predicted worm genes.

FIGURE 2. Inhibition of C. elegans fah produces a lethal phenotype and results in destruction of the intestine. Low power view of worms treated with control (A) or K10C2.4 RNAi (C). High power view of control (B) or K10C2.4 (D) RNAi-treated worms. Arrows, intestine. E, Western blot showing change in K10C2.4 protein levels following RNAi treatment. Thin arrow, K10C2.4. Thick arrow, K10C2.4-GFP fusion. Anti-actin served as a loading control. F, expression of a K10C2.4-GFP transgene in the intestine and hypodermis of worms.
loss of intracellular lipid droplets (Fig. 2D). Additionally, essentially all worms died during the first few days of adulthood (data not shown).

Western blots using an anti-mouse FAH antibody demonstrate the presence of a 46-kDa band consistent with the predicted molecular weight of the K10C2.4 gene product (Fig. 2E). This likely represents the K10C2.4 protein as a K10C2.4::GFP transgene, which fuses GFP to the N terminus of K10C2.4 produces a 77-kDa protein that is recognized both by the anti-FAH antibody and anti-FLAG antibody, which recognizes a FLAG epitope introduced into the GFP sequence (Fig. 2E and data not shown). Following RNAi treatment, both the K10C2.4::GFP fusion protein and K10C2.4 protein are strongly depleted compared with control (Fig. 2E).

In adult worms, a K10C2.4::GFP fusion protein is expressed strongly in the intestine and the hypodermis, which is the connective tissue layer that secretes the cuticle (Fig. 2F). Expression of K10C2.4 in the intestine may account for the damage caused by K10C2.4 RNAi to this tissue. Analysis of GFP reporter genes controlled by the F42D1.2 (predicted to encode tyrosine aminotransferase), T21C12.2 (predicted to encode 4-hydroxyphe-nylpyruvate dioxygenase), and W06D4.1 (predicted to encode maleylacetoacetate isomerase) promoters reveal the intestine to be a common site of expression of each enzyme (supplemental Fig. S2). F42D1.2 shows the broadest expression pattern with expression in muscle, hypodermis, and intestine. In contrast, both T21C12.2 and W06D4.1 are expressed only in the hypodermis and intestine.

The K10C2.4 Phenotype Is Dependent upon Tyrosine and the Tyrosine Degradation Pathway—Mice and fungi with FAH mutations are rescued by mutations in the 4-hydroxyphe-nylpyruvate dioxygenase and homogenisate dioxygenase genes (14, 44, 49, 50). We find that the phenotype is critically dependent on an intact tyrosine degradation pathway, because combining K10C2.4 RNAi with RNAi directed against F42D1.2, T21C12.2, or W06D4.1 is able to completely rescue treated worms from the effects of K10C2.4 RNAi (Fig. 4). Both mice and fungi with FAH mutations are rescued by mutations in the 4-hydroxyphe-nylpyruvate dioxygenase and homogenisate dioxygenase genes (14, 44, 49, 50). We find that the phenotype is critically dependent on an intact tyrosine degradation pathway, because combining K10C2.4 RNAi with RNAi directed against F42D1.2, T21C12.2, or W06D4.1 is able to completely rescue treated worms from the effects of K10C2.4 RNAi (Fig. 4). This effect is specific to these genes as combining K10C2.4 RNAi with either GFP RNAi or RNAi directed against D1053.1 fails to rescue the worms (Fig. 4, D–F, and H). The failure of RNAi directed against D1053.1 is not unexpected as D1053.1 is predicted to encode the enzyme maleylacetoacetate isomerase, which converts MAA to FAA. This isomerization reaction is able to occur even in the absence of this enzyme (48). Hence, RNAi directed against D1053.1 is likely not to rescue worms treated with K10C2.4 RNAi as FAA can still be formed in the absence of D1053.1, MAA may have toxicity on its own, and the tyrosine metabolites SAA and SA can still be formed from MAA. Quantitative data for these treatments are shown in supplemental Fig. S3.

We also tested whether inhibition of K08F8.4, which encodes the worm homolog of phenylalanine 4-hydroxylase, modifies the phenotype produced by K10C2.4 RNAi. The K08F8.4 gene converts phenylalanine to tyrosine, which is then metabolized via K10C2.4 along with dietary tyrosine. We find that K08F8.4 RNAi is able to partially rescue worms treated with K10C2.4...
RNAi suggesting that conversion of phenylalanine to tyrosine contributes significantly to flux through the tyrosine degradation pathway (Fig. 5). Quantitative data for these treatments are shown in supplemental Fig. S4.

Treatment of Worms with Succinylacetone Mimics the K10C2.4 Phenotype—Together these results indicate that the K10C2.4 phenotype is specifically due to the accumulation of tyrosine-derived metabolites downstream of W06D4.1 (Fig. 1), namely MAA and FAA. Additionally, both MAA and FAA are able to undergo spontaneous reduction to succinylacetoacetate (SAA) followed by spontaneous non-enzymatic decarboxylation to SA (12). In both humans with type I tyrosinemia and mice with FAH mutations, MAA, FAA, SAA, and SA have been shown to accumulate and be toxic to cells and tissues (12, 14–18, 20, 42, 48, 50, 51). Because our results suggested that the accumulation of MAA, FAA, SAA, or SA may produce the observed K10C2.4 RNAi phenotype, we tested whether exposure to worms to these chemicals could produce the same effects. We treated worms either with control RNAi or the combination of control RNAi and SA (Fig. 6, A–F). SA was used as it is commercially available, whereas MAA and FAA can only be synthesized in small amounts via enzymatic synthesis. We find that treatment of worms with SA mimics the K10C2.4 phenotype as evidenced by stunted growth, destruction of the intestine, and poor fertility (Fig. 6, B, D, and F). Furthermore, treatment of worms with high doses of SA appears to produce complete sterility with no progeny observed even after several days (data not shown). We then performed titration experiments to test the effects of lower doses of SA. We find that as little as 300 μg/ml SA is able to have significant effects on adult worms (Fig. 6G). Together these results demonstrate that accumulation of at least SA directly may produce the K10C2.4 phenotype in worms.
Tyrosine Degradation in Worms

We have tried to detect MAA, FAA, SAA, and SA in worms treated with K10C2.4 RNAi and grown in mass culture without success. It is not clear whether this result represents low levels of accumulation of these compounds in worms treated with K10C2.4 RNAi or technical issues related to the extraction, derivatization, and detection of these compounds from entire worms instead of biologic fluids as done in fungal and mammalian samples.

Inhibition of K10C2.4 Activates Cell Stress Pathways—To better understand the mechanisms by which tyrosine metabolites produce the K10C2.4 phenotype, we used worms carrying integrated reporter genes that respond to specific cellular stress response pathways. Specifically, we tested strains carrying integrated reporters for hsp16-2::GFP, gst-4::GFP, daf-16::GFP, and hsp-4::GFP (Fig. 7). The hsp16::GFP reporter responds to the heat shock response mediated via hsf-1, which is the C. elegans homolog of heat shock factor (28). The gst-4::GFP reporter responds specifically to oxidative stress, and its activation may be mediated by the skn-1 transcription factor (30, 52). The daf-16::GFP transgene produces functional DAF-16::GFP protein. DAF-16::GFP is normally localized to the cytoplasm but translocates to the nucleus in response to starvation, cell stress, or changes in daf-2 insulin/IGF-1 signaling (31). Finally, the hsp-4::GFP reporter responds to endoplasmic reticulum (ER) stress mediated by activation of the unfolded protein response (32).

We find that treatment of worms with K10C2.4 RNAi fails to activate the heat shock response or trigger the nuclear localization of DAF-16 (Fig. 7, A and B). However, we find that treatment of worms with K10C2.4 RNAi activates both oxidative stress response pathways (gst-4::GFP) and ER stress response pathways specifically in the worm intestine (Fig. 7, C and D). The induction of both reporters specifically in the affected tissue argues that biochemical events due to the accumulation of tyrosine metabolites in the intestine are producing the observed effects.

Interestingly, the induction of oxidative stress responses and ER stress responses differ in timing. The oxidative stress response is activated first with maximal GFP intensity seen prior to visible damage to the worm intestine (Fig. 7C and not shown). In contrast, the maximal activation of the ER stress response occurs later in the young adult after the onset of visible damage to the intestine (Fig. 7D and E).

To test the utility of our model, we conducted a pilot screen to identify mutants resistant to the effects of K10C2.4 RNAi—C. elegans is a powerful genetic system, which provides the opportunity to conduct genetic screens to identify modifiers of the toxicity of tyrosine metabolites. This type of screen could potentially uncover pathways involved in the control of the toxicity of these metabolites, the repair of damage caused by these compounds, and perhaps critical targets of damage. Understanding these pathways would complement studies in mice and cell culture and provide new insights into the pathogenesis of type I tyrosinemia. The small size of C. elegans also offers the potential of conducting drug screens using intact worms to identify new drugs, which may attenuate the toxicity of these metabolites and could provide new therapeutic options in the treatment of type I tyrosinemia.

To test the utility of our model, we conducted a pilot screen to identify mutants resistant to the effects of K10C2.4 RNAi. We mutagenized ~10,000 haploid genomes and placed F2 eggs on K10C2.4 RNAi. After 3 days, plates were screened for normal-appearing adults. As these mutants could either be resistant to either RNAi in general (rde mutants) or specifically K10C2.4 RNAi, we conducted secondary screens to verify resistance to K10C2.4 RNAi and to demonstrate sensitivity to

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5 T. Burlingame and A. Fisher, unpublished results.
unc-22 RNAi. unc-22 RNAi produces an easily visible “twitcher” phenotype and served to exclude rde mutants (39). Six mutants passed this secondary screen and were analyzed further.

Based on the RNAi phenotypes observed in Fig. 4, we figured that some of the genes identified in our screen would represent alleles of upstream enzymes in the tyrosine degradation pathway. Consequently, we used a PCR-based SNP-based mapping technique to localize the mutant genes and then sequenced genomic DNA of mutants that localize near F42D1.2, T21C12.2, or W06D4.1 (data not shown) (40). Among our mutants we identified the strains ALF103 (F42D1.2(baf1)) and ALF104 (W06D4.1(baf2)) through sequencing. ALF103 carries a P224S mutation in the F42D1.2 gene. Proline 224 is conserved in tyrosine aminotransferase genes from worms, humans, mice, and Drosophila (Fig. 8A). Evaluation of this amino acid change using the cSNP analysis tool within the Panther data base suggests a >99% probability that this substitution will be detrimental to protein function (Fig. 8A) (53). No crystal structure of tyrosine aminotransferase is available, but modeling using the Phyre program based on the structure of alanine aminotransferase from Pyrococcus furiosus suggested that Pro-224 lies in the center of the folded protein and is responsible for a bend in the protein backbone (data not shown) (54). ALF104 carries a G173R mutation in the W06D4.1 gene. Glycine 173 is conserved in homogentisate dioxygenase genes from worms, humans, mice, and Drosophila (Fig. 8B). Evaluation of this amino acid change using the cSNP analysis tool within the Panther data base suggests a >88% probability that this substitution will be detrimental to protein function (Fig. 8B) (53). Modeling of W06D4.1 using the Phyre program and the human homogentisate dioxygenase crystal structure suggests that Gly-173 lies on the surface of the protein in a region of β-sheet structure, which is involved in assembly of the hexameric protein structure (data not shown) (54, 55). Together these results suggest that our screen is able to successfully identify suppressors of K10C2.4 RNAi-mediated toxicity in worms.

**DISCUSSION**

Conservation of the Tyrosine Degradation Pathway in Worms—To begin to understand the tyrosine degradation pathway in *C. elegans*, we used bioinformatics to identify homologs of the enzymes in the tyrosine degradation pathway in *C. elegans* and studied the functions of these genes through the use of RNAi. Among the genes identified is a homolog of FAH, which is the enzyme affected by mutations in type I tyrosinemia. Detailed sequence analysis of K10C2.4 supported this prediction and demonstrated strong conservation of the critical amino acids. RNAi directed against this gene potently reduced levels of the K10C2.4 protein and produced a lethal phenotype consisting of destruction of the intestine, stunted growth, and decreased fertility. We were able to use this phenotype to explore the *C. elegans* tyrosine degradation pathway, the toxicity of tyrosine metabolites that accumulate once K10C2.4 is inhibited, and the response of worms to these metabolites.

Importantly, we find that tyrosine degradation in worms proceeds via the same pathway as in other eukaryotes. Through the use of combined RNAi directed against two genes at a time, we are able to demonstrate that the inhibition of the enzymes upstream of K10C2.4 in worms is able to prevent the toxicity of K10C2.4 inhibition. These findings are particularly important as they demonstrate the functional identity of the predicted homologs and order the genes in the degradation pathway at a genetic level. These results are also consistent with findings from fungal and mouse models where mutations in upstream genes are able to rescue the lethality associated with FAH mutations (14, 44, 49, 50).

Study of people with type I tyrosinemia and animal models with FAH mutations has shown that the accumulation of the tyrosine metabolites MAA, FAA, SAA, and SA leads to direct tissue damage (12, 14–18, 20, 42, 48, 50, 51). For example, treatment of cells in culture with purified FAA is able to cause a number of toxic effects including DNA mutagenesis, ER stress, oxidative stress, mitochondrial dysfunction, and activation of apoptosis (15–18, 20). Additionally, animals mutant for both HPD and FAH that are given homogentisate demonstrate the functional identity of the predicted homologs and order the genes in the degradation pathway at a genetic level. These results are also consistent with findings from fungal and mouse models where mutations in upstream genes are able to rescue the lethality associated with FAH mutations (14, 44, 49, 50).

A *C. elegans* Model of Type I Tyrosinemia—The toxicity to worms caused by inhibition of K10C2.4 produces a useful genetic model of type I tyrosinemia. This illness has been modeled both in mice and the fungus *Aspergillus nidans* (44, 51). *C. elegans* is especially useful as a model system as it is a multicellular animal, in contrast to *A. nidans*. Additionally, *C. elegans* possesses most of the cell signaling, stress response, and detoxification pathways present in mammals that allows for study of the responses to the effects of tyrosine metabolites at the level of both tissues and the whole animal. These studies are greatly facilitated by the *C. elegans* transparency, which allows visualization of tissue responses and activation of GFP reporter genes to occur in real-time in a non-invasive manner. Addition-
ally, the ability to control disease onset via the use of RNAi also provides an additional level of flexibility in experimental design. We demonstrate the usefulness of our system by conducting a pilot genetic screen, which identified mutations in expected genes, specifically tyrosine aminotransferase and homogentisate dioxygenase. We additionally found several likely novel but currently unknown genes involved in the pathogenesis of tissue damage in type 1 tyrosinemia.

**Does the Tyrosine Degradation Pathway Have a Role in Protein Aggregation and Aging?**—Recent proteomic and microarray studies have identified three genes in the tyrosine degradation pathway as genes perhaps important in aging. Specifically, T21C12.2 has been shown to be down-regulated in long-liveddaf-2 anddaf-12 mutants via microarray and Northern blot, W06D4.1 has been shown to be up-regulated in long-liveddaf-2 mutants via microarray, and K10C2.4 has been shown to be up-regulated in long-liveddaf-2 mutants via quantitative mass spectrometry (21–25, 56). Interestingly, RNAi studies withT21C12.2 have demonstrated that RNAi directed against this gene can increase the lifespan of treated worms (21). Additionally, K10C2.4 was identified in a genome-wide RNAi screen as a suppressor of the aggregation of a synthetic polyglutamine repeat protein (57). At this point, it is unclear how the biochemical actions of these genes relate to these putative roles in aging and protein misfolding suggested from these functional and genomic studies. Normal physiologic levels of tyrosine metabolites may contribute to protein misfolding and aging through the damage of cellular proteins, and thus reduced levels might lower the cumulative burden and provide an increase in repair capacity. Alternatively, the tyrosine degradation enzymes might have new cellular roles independent of their enzymatic activity. This is the case for phenylalanine metabolism where 4α-carboxylamine dehydratase, which is involved in the synthesis of the biopterin cofactor for phenylalanine hydroxylase, has independent biochemical and transcriptional regulation roles in the cell (58). Our work provides a set of tools to begin to study these findings.

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