Establishing *C. elegans* as a model to study the function of vitamin A metabolism

Pamela A. Joseph[1]
Helen B. Everts[2]
Tina L. Gumienny[3]

Abstract: Vitamin A is critical for cell development, maintaining a healthy immune system, regulating energy metabolism, and eyesight in mammals. In addition, abnormal levels contribute to obesity and cancer. While vitamin A plays these many roles, what is not well known is the impact of individual vitamin A metabolism genes at the cellular level. We asked if the roundworm *Caenorhabditis elegans*, an established animal model system with a sequenced genome and established methods for genetic and cellular analyses, is appropriate for the study of vitamin A metabolism. Our objective was to determine if the *C. elegans* genome contains genes encoding potential vitamin A metabolism genes. We performed literature and database searches and identified potential retinoid metabolism genes in the *C. elegans* genome. Furthermore, some of these genes share phenotypes with their mammalian homologs. These genes include cellular retinol-binding proteins, retinol dehydrogenases, retinal dehydrogenase, cellular retinoic acid-binding proteins, and retinoic acid receptors. However, many of these genes in *C. elegans* and mammals have no known mutant traits. We conclude that the roundworm *C. elegans* may be an excellent model organism for this investigation because all expected genes are conserved. Future research in *C. elegans* will define the functional conservation of the vitamin A metabolism pathway in *C. elegans* and will characterize the physiological relevance of altered and normal vitamin A metabolism at the cellular level.

1. Introduction and literature review

Vitamin A is the general term for several functional compounds that are important nutrients (Ross & Harrison, 2007; Tanumihardjo et al., 2016). These compounds include the precursor vitamin A carotenoids, predominantly β-carotene, α-carotene, and β-cryptoxanthin, which can be obtained from consuming certain fruits along with orange, yellow, or green vegetables such as squash, carrots, collards, spinach, pumpkin, kale, and sweet potatoes. Other compounds stem from active vitamin A. These include retinyl esters and retinol which are found in liver and liver oil from meats and fish, eggs, cheese, and butter. Retinyl esters are the main type of vitamin A in human systems. All-trans retinoic acid (atRA) is the most

[1] Corresponding author. Department of Biology. pjoseph1@twu.edu
[2] Department of Nutrition and Food Sciences. heverts@twu.edu
[3] Department of Nutrition and Food Sciences. heverts@twu.edu

biologically active form of vitamin A. This form alone can improve cell differentiation, development, and prevent fatality in animals with vitamin A deficiency.

Vitamin A is crucial for many animals. In mammals, it is essential for stable eyesight, embryonic development, reproduction, immune activity, cell and tissue differentiation, and the hair cycle (Everts, 2012; Tanumihardjo et al., 2016). Vitamin A deficiency can cause xerophthalmia in the form of night blindness and corneal irregularities, such as keratomalacia (maceration of the cornea) and ulceration, which can lead to permanent blindness. Vitamin A deficiency can also elevate the danger of progressing diarrheal and respiratory infections. It can slow bone growth, growth rate, and decrease recovery from critical illnesses. This deficiency can manifest in the skin as follicular hyperkeratosis linked with sebaceous glands, modification in the thickness of skin, and proteins such as keratin. Birth defects, embryonic death, and hair loss (alopecia) are associated with this deficiency as well as excess storage. Mutations in genes encoding vitamin A metabolism proteins result in traits like those observed with vitamin A deficiencies (Napoli, 2012). Hypervitaminosis A (excess levels of stored vitamin A in the body) can cause lethal symptoms such as dizziness, headache, nausea, vomiting, poor muscular coordination, blurred vision, pain in joints, and irregular liver activity. There is no treatment for hypervitaminosis A, other than reducing the intake. In invertebrates, RA also affects development, consistent with an ancient requirement for vitamin metabolism in animals (Albalat, 2009). The roundworm *Caenorhabditis elegans* stores reservoirs of retinal and RA upon vitamin A supplementation. These stores play a role in fat storage and mobilization and contribute to survival in starvation and high-glucose conditions (Chen et al., 2018).

Identification of mammalian vitamin A metabolism genes

The vitamin A metabolic pathway converts retinol to forms that can be stored or used by cells (Figure 1) (Belyaeva et al., 2020; Napoli, 2012). The liver, which stores the greatest quantity of vitamin A, discharges retinol combined with retinol-binding protein (RBP) into the blood. The transmission of retinol into cells is accompanied by the interaction of RBP and the extra-hepatic plasma membrane receptor, STRA6, which carries it across the cell membrane. Cellular retinol-binding proteins (CRBPs) then bind the retinol and direct the flow of retinol in and out of storage. Lecithin: retinol acyltransferase (LRAT) converts retinol into retinyl esters (RE) for storage. Retinyl ester hydrolase (REH), converts RE into retinol by hydrolysis RE activated by CRBP. LRAT obtains retinol for esterification as it enters storage. The first and second retinol dehydrogenations (RDH) in the pathway convert retinol into retinal. Three retinal dehydrogenases (RALDH) irreversibly dehydrogenate retinal into atRA. Cellular retinoic acid-binding proteins (CRABPs) deliver atRA to retinoic acid receptors (RARs) for transcription or they deliver it to three members of the cytochrome P450 gene family for catabolism.

Evolutionary conservation

The RA system is common to all metazoans (Albalat, 2009). Specifically, retinoid-binding proteins, RA metabolic enzymes, and RA-binding nuclear receptors have been identified in deuterostomes and protostomes. However, the RA system is more streamlined in some species than others. RAR, for example, appears to have vanished in numerous invertebrate species. Similarly, the bilaterian stem retained CYP26, while invertebrate lineages lost this enzyme or preserved it differently. Yet, every species (except the sea urchin *S. purpuratus*)

that retained RAR also retained the CYP26 and RALDH1 enzymes. *C. elegans*, the organism of our study, has retained the RAR subfamily, CYP26, and RALDH1 (Kostrouch et al., 1995). This suggests that retinoid-mediated transcription occurred in ancient organisms (Baker, 1998). STRA6, a critical gene involved in mammalian vision, underwent purifying selection in many vertebrates in regard to habitat surroundings (Wu et al., 2014). This gene may have evolved in vertebrates to maintain retinoid homeostasis and buffer retinoid inconsistencies in natural habitats (Albalat, 2009). However, invertebrate homologs of STRA6 have not been identified (Albalat, 2009).

Problems and questions in the vitamin A field and opportunities with studying vitamin A metabolism in *C. elegans*

Previous research has revealed that *C. elegans* can metabolize vitamin A into retinal and RA (Chen et al., 2018). The *C. elegans* predicted RBP homologs FAR 1-6 have been shown to bind to retinol (Garofalo et al., 2003). A partial *C. elegans* vitamin A metabolism pathway has been predicted (Yilmaz & Walhout, 2016). This evidence suggests that there is a conserved vitamin A pathway in these nematodes, however, most of the enzymes in the pathway have not been identified yet (Chen et al., 2018). Conducting this study on mammalian models poses limitations of cost and time. Also, looking at the cellular and subcellular localization of tagged enzymes would be difficult because mammals are opaque. This limits us from determining which enzyme family members are most important for vitamin A metabolism. However, *C. elegans* is an excellent model for this study because it is a transparent organism, whose cellular details can be seen with ease. Its short and reproducible life span provides great timing to see significant results at its different developmental stages. Also, conducting studies on these nematodes are less costly.

2. Methods

Mammalian vitamin A metabolism genes and phenotypes were identified through previous research from the Napoli lab (Napoli, 2012). Genecards.org, uniprot.org, and genenames.org databases were used to find names of specific human protein sequences. The National Center for Biotechnology Information BLASTP protein database search engine was used to identify *C. elegans* homologs of the known human vitamin A metabolism proteins (https://www.ncbi.nlm.nih.gov/). The top hits are presented in the findings section. Published phenotypes were extracted using wormbase.org.

3. Findings

To identify potential vitamin A metabolism genes in the *C. elegans* genome, we collected the protein sequences of human vitamin A metabolism proteins and identified candidate *C. elegans* homologs by sequence homology using BLASTP, an online search tool that identifies similar protein sequences in an extensive protein database to a submitted protein sequence (Figure 1). While no clear traits are associated with aberrant levels of vitamin A in *C. elegans*, we identified the known traits associated with these potential vitamin A metabolism genes

by interrogating WormBase, a comprehensive database of *C. elegans* genes and traits (Table 1). Many potential *C. elegans* homologs have developmental roles consistent with those seen for mammalian vitamin A metabolism genes (Table 1A, B). Lastly, some *C. elegans* genes we identified have no confirmed function or traits associated with them (Table 1C).

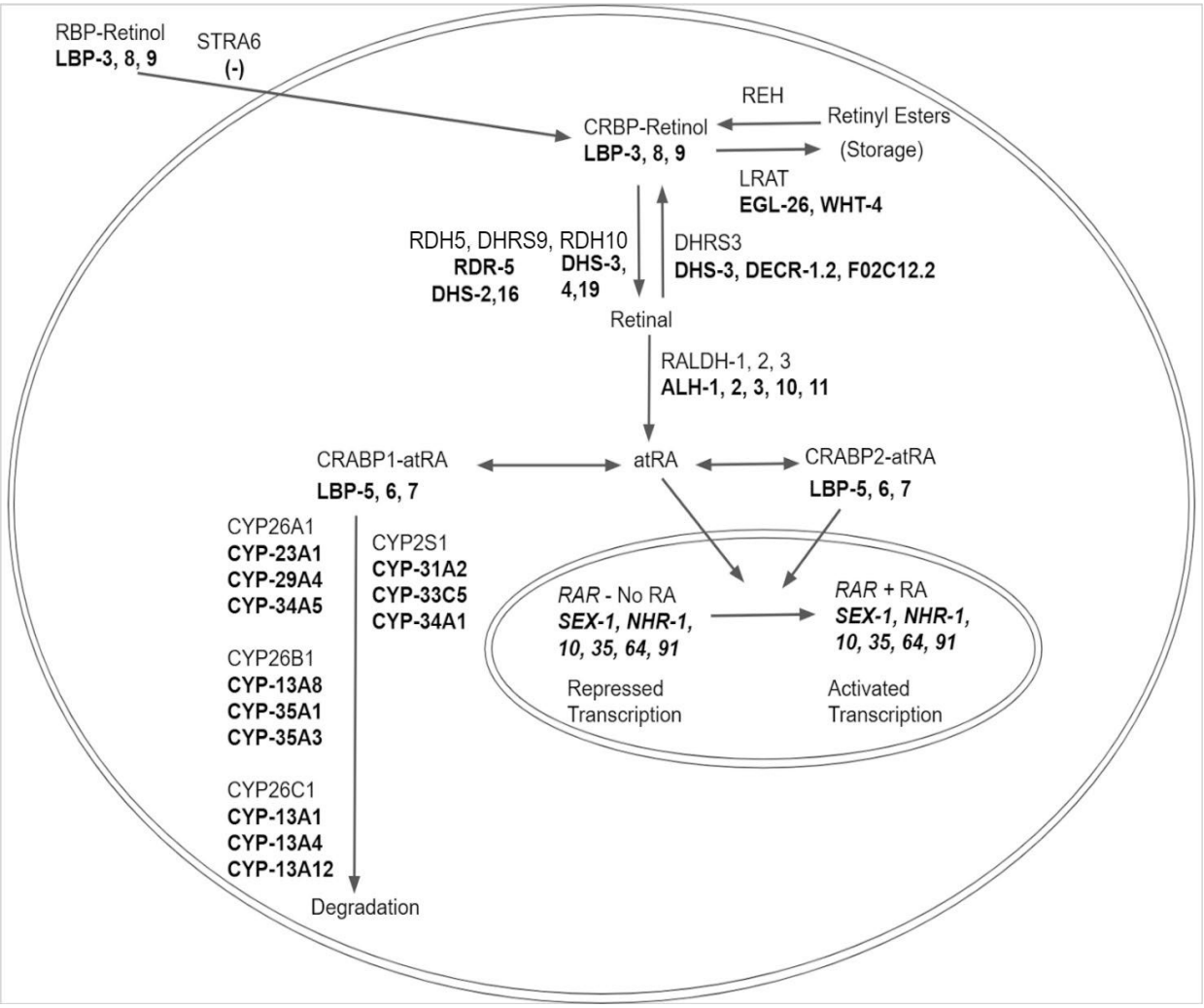

Figure 1. Model of the vitamin A metabolism pathway in humans with *C. elegans* candidates.

Identification of *C. elegans* CRBP/ RBP-like retinol-binding protein candidates

The Lipid Binding Protein family contains potential homologs of human CRBP/RBP. This protein family binds extracellular retinol and brings it into the cell but also help ferry retinols through the cell. LBP-3 (NP_001360012.1), LBP-8 (NP_001033512.1), and LBP-9 (NP_001033511.1) have high homology to human CRBP/RBP (CAA30318.1). LBP-8 was identified as a potential homolog of human CRBP/RBP by querying BLASTP with CRBP, accession number NP_001033512.1. LBP-9 was identified as a potential homolog of human CRBP/RBP by querying BLASTP with CRBP, accession number NP_001033511.1. LBP-3 was identified as a potential homolog of human CRBP/RBP by querying BLASTP with CRBP, accession number NP_001360012.1.

No STRA6-like chaperone was identified in the *C. elegans* genome

STRA6 is a chaperone that helps bring retinol into the cell, but is found only in vertebrates. No *C. elegans* homolog for STRA6 (AAQ89447.1) was identified.

Identification of *C. elegans* LRAT candidates

EGL-26 was identified as a potential homolog of human LRAT by querying BLASTP with LRAT, accession number PDM82548. WHT-4, ABC transporter domain-containing protein, was identified as a potential homolog of human LRAT by querying BLASTP with LRAT, accession number NP_494495.3.

Identification of *C. elegans* dehydrogenase candidates

DeHydrogenases, Short chain family, were identified as potential homologs of human RDH5 and DHRS9. DRD-5 (NP_509415.2), DHS-2 (NP_871815.1), and DHS-16 (NP_504554.1) have high homology to human RDH1 (NP_002896.2) and DHRS9 (NP_001363853.1). DRD-5 was identified as a potential homolog of human RDH5 and DHRS9 by querying BLASTP with RDH1 and DHRS9, accession number NP_509415.2. DHS-16 was identified as a potential homolog of human RDH1 and DHRS9 by querying BLASTP with RDH1 and DHRS9, accession number NP_504554.1. DHS-2 was identified as a potential homolog of human RDH1 and DHRS9 by querying BLASTP with RDH5 and DHRS9, accession number NP_871815.1.

DeHydrogenases, Short chain family, were identified as potential homologs of human RDH10. DHS-3 (NP_001122508.1), DHS-4 (NP_492563.1), and DHS-19 (NP_505915.1) have high homology to human RDH10 (NP_742034.1). DHS-3 was identified as a potential homolog of human RDH10 by querying BLASTP with RDH10, accession number NP_001122508.1. DHS-4 was identified as a potential homolog of human RDH10 by querying BLASTP with RDH10, accession number NP_492563.1. DHS-19 was identified as a potential homolog of human RDH10 by querying BLASTP with RDH10, accession number NP_505915.1.

DeHydrogenases, Short chain family, were identified as potential homologs of human DHRS3. DHS-3 was identified as a potential homolog of human DHRS-3 by querying BLASTP with DHRS-3, accession number NP_001122508.1. DECR-1.2 was identified as a potential homolog of human DHRS-3 by querying BLASTP with DHRS-3, accession number NP_495805.1. F02C12.2 was identified as a potential homolog of human DHRS-3 by querying BLASTP with DHRS-3, accession number NP_510229.1.

Identification of *C. elegans* RALDH-1, 2, and 3 candidates

The Aldedh domain-containing protein family contains potential homologs of human RALDH-1, 2, and 3. ALH-1 (NP_498081.2) and ALH-2 (NP_503467.2) have high homology to human RALDH-1 (NP_000680.2), 2 (NP_003879.2), and 3 (NP_000684.2). ALH-1 was identified as a potential homolog of human RALDH-1, 2, and 3 by querying BLASTP with RALDH-1, 2, and 3, accession number NP_498081.2. ALH-3, 10-formyltetrahydrofolate dehydrogenase, was identified as a potential homolog of human RALDH-1, 2, and 3 by querying BLASTP with RALDH-1, 2, and 3, accession number NP_502054.2. ALH-11 was identified as a potential homolog of human RALDH-1by querying BLASTP with RALDH-1, accession number NP_001367351.1. ALH-10 was identified as a potential homolog of human RALDH-2 by querying BLASTP with RALDH-2, accession number NP_509203.1. ALH-2 was

identified as a potential homolog of human RALDH-3 by querying BLASTP with RALDH-3, accession number NP_503467.2.

Identification of *C. elegans* CRABP 1 and 2 candidates

The Lipid Binding Protein family contains potential homologs of human CRABP1 and 2. LBP7 (NP_506440.1), LBP-6 (NP_491926.1), and LBP-5 (NP_491928.1) have high homology with human CRABP 1 (NP_004369.1) and 2 (NP_001869.1). LBP-7 was identified as a potential homolog of human CRABP-1 and 2 by querying BLASTP with CRABP-1, accession number NP_506440.1. LBP-6 was identified as a potential homolog of human CRABP-1 and 2 by querying BLASTP with CRABP-1, accession number NP_491926.1. LBP-5 was identified as a potential homolog of human CRABP-1 and 2 by querying BLASTP with CRABP-1, accession number NP_491928.1.

Identification of *C. elegans* RARα, β, and γ candidates

The Nuclear Hormone Receptor family contains potential homologs of human RARα, β, and γ. NHR-91 (AAG15183.2), NHR-35 (NP_001024365.1), and NHR-64 (NP_001343721.1) have high homology to human RARA (NP_000955.1). NHR-91 (AAG15183.2), NHR-64 (NP_001343721.1), SEX-1 (NP_001024662.1), and NHR-1 (AAO39170.1) have high homology to human RARB (NP_000956.2). NHR-91 (AAG15183.2), NHR-1 (AAO39170.1), NHR-10 (AAO39172.1) have high homology to human RARG (NP_000957.1). NHR-91 was identified as a potential homolog of human RARα, β, and γ by querying BLASTP with RARα and β, accession number AAG15183.2. NHR-35 was identified as a potential homolog of human RAR α by querying BLASTP with RARα, accession number NP_001024365.1. NHR-64 was identified as a potential homolog of human RAR α and β by querying BLASTP with RARα and β, accession number NP_001343721.1. SEX-1 was identified as a potential homolog of human RAR β by querying BLASTP with RAR β, accession number NP_001024662.1. NHR-1 was identified as a potential homolog of human RARβ and γ by querying BLASTP with RARβ and γ, accession number AAO39170.1. NHR-10 was identified as a potential homolog of human RARγ by querying BLASTP with RARγ, accession number AAO39172.1.

Identification of *C. elegans* cytochrome candidates

The cytochrome P450 superfamily contains potential homologs of human CYP26A, B, and C. CYP-23A1 (NP_494797.1), CYP-34A5 (NP_504099.1) and CYP-29A4 (NP_505490.2) have high homology to human CYP26A1 (NP_000774.2). CYP-23A1 was identified as a potential homolog of human CYP26A1 by querying BLASTP with CYP26A1, accession number NP_494797.1. CYP-34A5 was identified as a potential homolog of human CYP26A1 by querying BLASTP with CYP26A1, accession number NP_504099.1. CYP-29A4 was identified as a potential homolog of human CYP26A1 by querying BLASTP with CYP26A1, accession number NP_505490.2. CYP-13A8 (NP_496115.1), CYP-35A3 (NP_504121.1), CYP-35A1 (NP_001343612.1) have high homology to human CYP26B1 (NP_063938.1). CYP-13A8 was identified as a potential homolog of human CYP26B1 by querying BLASTP with CYP26B1, accession number NP_496115.1. CYP-35A3 was identified as a potential homolog of human CYP26B1 by querying BLASTP with CYP26B1, accession number NP_504121.1. CYP-35A1 was identified as a potential homolog of human CYP26B1 by querying BLASTP with CYP26B1, accession number NP_001343612.1. CYP13A4 (NP_496111.1), CYP-13A1 (NP_496108.1), CYP-13A12 (NP_499705.1) have high homology to human CYP26C1 (NP_899230.2). CYP-13A4 was identified as a potential homolog of human CYP26C1 by

querying BLASTP with CYP26C1, accession number NP_496111.1. CYP-13A1 was identified as a potential homolog of human CYP26C1 by querying BLASTP with CYP26C1, accession number NP_496108.1. CYP-13A12 was identified as a potential homolog of human CYP26C1 by querying BLASTP with CYP26C1, accession number NP_499705.1.

The cytochrome P450 superfamily contains potential homologs of human CYP2S1. CYP-33C5 (NP_503616.1), CYP-34A1 (NP_506787.1), CYP-31A2 (NP_502152.3) have high homology to human CYP2S1 (NP_085125.1). CYP-33C5 was identified as a potential homolog of human CYP2S1 by querying BLASTP with CYP2S1, accession number NP_503616.1. CYP-34A1 was identified as a potential homolog of human CYP2S1 by querying BLASTP with CYP2S1, accession number NP_506787.1. CYP-31A2 was identified as a potential homolog of human CYP2S1 by querying BLASTP with CYP2S1, accession number NP_502152.3.

Enrichment of putative vitamin A metabolism genes in intestine and hypodermis

Although the tissues that metabolize vitamin A in *C. elegans* are not known, the tissues that do process vitamin A should also express the necessary proteins. If the candidate genes we identified are co-expressed, that would increase our confidence that these genes may act in the same pathway. Therefore, we compared the tissue localization of candidate genes with published expression patterns. 22 of 53 candidate vitamin A metabolism genes have known expression in the intestine. This enrichment of candidate genes in the intestine is consistent with the roles of the intestine in processing bacteria, the source of vitamin A for *C. elegans*, and storing lipids, which retinols regulate (Chen et al., 2018).

Mammalian protein	***C. elegans* candidate gene**	**References**
A. *C. elegans* genes with clear non-lethal, non-sterile phenotypes		
CRBP (RBP)	*lbp-3*	(O'Rourke et al., 2013)
CRBP (RBP)	*lbp-8*	(Folick et al., 2015)
CRBP (RBP)	*lbp-9*	(Arda et al., 2010; Ha et al., 2006)
LRAT	*egl-26*	(Hodgkin, 1986; Piano et al., 2002; Shephard et al., 2011; Trent et al., 1983)
DHRS3	*decr-1.2*	(Ashrafi et al., 2003)
RALDH-1, 2, and 3	*alh-3*	(Minogue et al., 2018)
RALDH-1, 2, and 3	*alh-10*	(O'Rourke et al., 2006)

CRABP 1 and 2	*lbp-7*	(Green et al., 2009)
CRABP 1 and 2	*lbp-6*	(Ha et al., 2006)
CRABP 1 and 2	*lbp-5*	(Fraser et al., 2000; Xu et al., 2011)
RARA	*nhr-35*	J-C. Martinou, unpublished
RARA	*nhr-64*	(Liang et al., 2010)
RARG	*nhr-10*	(Arda et al., 2010; MacNeil et al., 2013)
CYP26A1	*cyp-23a1*	(Kraemer et al., 2006)
CYP26B1	*cyp-35a3*	(Ashrafi et al., 2003)
CYP26B1	*cyp-35a1*	(Menzel et al., 2005)
CYP26C1	*cyp-13a4*	(Kamath et al., 2003; Simmer et al., 2003)
CYP26C1	*cyp-13a12*	(Ma et al., 2013)
CYP2S1	*cyp-33c5*	(Cui et al., 2007; Liu et al., 2012)
B. *C. elegans* genes with lethal or sterile phenotypes		
RDH1 and DHRS9	*dhs-16*	(Zhang et al., 2013)
RDH10	*dhs-3*	(Maeda et al., 2001)
RALDH-1, 2, and 3	*alh-1*	(Consortium, 2012)
RARA	*nhr-91*	(Zhao et al., 2004)
RARB	*sex-1*	(Carmi et al., 1998; Kamath et al., 2003; Rual et al., 2004; Simmer et al., 2003; Sonnichsen et al., 2005)
CYP26B1	*cyp-13a8*	(Kamath et al., 2003)
CYP2S1	*cyp-31a2*	(Benenati et al., 2009; Kamath et al., 2003; Piano et al., 2002; Rual et al., 2004; Simmer et al., 2003; Sonnichsen et al., 2005)
C. *C. elegans* genes with no published phenotype		

LRAT	*wht-4*	
RDH1 and DHRS9	*drd-5*	
RDH1 and DHRS9	*dhs-2*	
RDH10	*dhs-4*	
RDH10	*dhs-19*	
DHRS3	F02C12.2 / DHRS3-like	
RALDH-1, 2, and 3	*alh-2*	
RALDH-1, 2, and 3	*alh-11*	
RARB	*nhr-1*	
CYP26A1	*cyp-34a5*	
CYP26A1	*cyp-29a4*	
CYP26C1	*cyp-13a1*	
CYP26A1	*cyp-34a1*	

Table 1. Table of identified vitamin A metabolism gene candidates in *C. elegans* by phenotype.

4. Conclusions

Here, we identified possible vitamin A metabolism genes and gene families based on protein homology with known mammalian genes (Table 1, Figure 1). Most of the nematode homologs were identified through databases, while a few homologs were identified through previous research. Phenotypes and available strains for each homolog were identified for most of the homologs through databases. We believe many of these genes are crucial for these nematodes based on their phenotypes.

Many of these gene families may have functions other than vitamin A metabolism, such as lipid metabolism and reduction of high-glucose toxicity (Chen et al., 2018). Many of the identified genes are required for survival, development, immune responses, and reproduction (Table 1). They are required but their molecular function is not known. Our work will focus on confirming the specific enzymes and genes whose products are required

for vitamin A metabolism in *C. elegans*. Further research could determine the possible interaction between vitamin A and nematode immunity. In addition, previous research discovered a novel *C. elegans* fatty-acid and retinol-binding protein family (FAR) (Garofalo et al., 2003). Future directions will focus on identifying whether these proteins compensate for lack of STRA6 to bring retinol into the cell.

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