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***C. elegans* Metabolic Gene Regulatory Networks Govern the Cellular Economy**

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Abstract

Diet greatly impacts metabolism in health and disease. In response to the presence or absence of specific nutrients, metabolic gene regulatory networks sense the metabolic state of the cell and regulate metabolic flux accordingly, for instance by the transcriptional control of metabolic enzymes. Here we discuss recent insights regarding metazoan metabolic regulatory networks using the nematode *Caenorhabditis elegans* as a model, including the modular organization of metabolic gene regulatory networks, the prominent impact of diet on the transcriptome and metabolome, specialized roles of nuclear hormone receptors in responding to dietary conditions, regulation of metabolic genes and metabolic regulators by microRNAs, and feedback between metabolic genes and their regulators.

Keywords

C. elegans; metabolic network; gene regulatory network; gene expression; nutrient response; life history traits; nuclear hormone receptors; transcription; bacteria

Introduction

Maintaining cellular homeostasis is a complex task that involves monitoring energy states and levels of essential nutrients, regulating metabolic flux to accommodate energy and biomass needs, and preventing buildup of potentially toxic intermediates and byproducts of metabolism. These measures help maintain a healthy cellular economy and inherently depend on the composition of resources available to the organism through its diet and environment. Many diseases are characterized by disruption of metabolic homeostasis, including inborn errors of metabolism (cumulative incidence estimated at 1 in 784 live births [1]), as well as multifactorial diseases such as diabetes (2.8% worldwide incidence in 2000, estimated to double by 2030 [2]). For patients with genetic metabolic disorders, diet can be therapeutic or detrimental, depending on the composition and quantities of dietary nutrients

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and the nature of the metabolic impairment. Diet can also have a primary causal role in diseases such as obesity and type II diabetes [3], however the mechanisms by which diet can lead to metabolic syndrome and insulin resistance are not fully understood.

This review will focus on studies using the bacterivorous nematode *Caenorhabditis elegans* to elucidate mechanisms of metabolic network regulation. *C. elegans* has similar nutritional requirements as humans, including the same essential amino acids and vitamins, and homologous metabolic pathways, as well as canonical metabolic regulatory pathways such as insulin and TOR (target of rapamycin) signaling. *C. elegans* provides clear advantages compared to mammals for system-level studies of metabolism. The nematode is small (~1.5 mm), has a transparent body, a short lifespan (~2-3 weeks), and a well-annotated genome [4, 5]. In addition, a variety of genome-wide technologies are available that enable the genome-scale characterization of metabolic phenotypes, for instance in response to dietary changes. These include two genome-wide RNAi libraries [6, 7] and a growing number of deletion mutants generated and maintained by the *Caenorhabditis* Genetics Center (CGC). In addition, largescale protein-protein and protein-DNA interaction mapping efforts have identified many molecular connections that can be integrated with phenotypic data [8-10]. These tools have in recent years helped researchers gain new insights into metabolic gene regulatory networks.

Several principles have begun to emerge with respect to *C. elegans* metabolic gene regulatory networks – a modular organization of transcription factors (TFs) and targets, an enrichment of nuclear hormone receptors (NHRs) among the transcriptional regulators, microRNAs that regulate metabolic genes directly or indirectly by targeting their regulators, feedback between metabolic pathways and their regulators, and the capacity to sense various metabolite signals, which are dependent on diet and metabolic flux.

Metabolic regulatory networks are enriched for nuclear hormone receptors

Transcriptional regulation provides a major mechanism of metabolic network control, and diet-induced metabolic gene expression changes have been observed in organisms ranging from bacteria to humans. In mammals, many metabolites have regulatory power. For instance, glucose triggers the insulin signaling cascade, which represses the transcription of gluconeogenesis genes [11], and amino acids such as leucine activate the TOR pathway, which controls gene product expression at the translational level [12]. These nutrient-sensing pathways are central to cell survival, growth and proliferation. Other metabolites interact directly with NHRs to modulate their function, such as vitamin A activating the retinoic acid receptor [13], vitamin D activating the vitamin D receptor [14], and free fatty acids and eicosanoids binding to peroxisome proliferator-activated receptor alpha (PPAR α) [15]. Aberrant transcriptional control of metabolic pathways and subsequently altered metabolic flux, especially pertaining to fatty acids, are hallmarks of diabetes. Indeed, PPAR α , a lipidsensing NHR that promotes lipid catabolism is a target of anti-diabetic drugs [16].

Interestingly, the NHR family has greatly expanded in nematodes: whereas humans and mice have 48 and 49 NHRs, respectively, the *C. elegans* genome encodes 274 receptors

[17]. While ligands have been identified for many human NHRs, only one ligand has been identified for a *C. elegans* NHR (dafachronic acid, which binds and activates DAF-12 [18, 19]). Thus, all other *C. elegans* NHRs are currently orphan receptors, and the gene targets of the vast majority of NHRs remain undefined. Yeast-one-hybrid assays have identified the repertoire of TFs that can interact with a set of *C. elegans* metabolic gene promoters [20]. These TFs are enriched for NHRs [20], suggesting that, like their mammalian orthologs, *C. elegans* NHRs function in metabolic network control [16]. Binding of TFs to *C. elegans* metabolic gene promoters is highly modular: TFs tend to separate into densely interconnected groups that share targets [20]. Modularity in biological networks has been proposed to facilitate a rapid and robust response to variable environmental cues [21-24]. In *C. elegans*, the NHR gene family expansion and functional organization into highly interconnected regulatory modules controlling metabolic genes may enable rapid and adaptive metabolic rewiring in response to different conditions, such as diet or environmental toxins. This network organization may provide the animal with a mechanism that ensures robust development and reproductive fitness on diets of highly metabolically diverse bacterial species encountered in its natural habitat (Figure 1). The current state of knowledge regarding *C. elegans* NHRs in metabolic regulatory roles is reviewed in Table 1, and several examples are discussed throughout this review.

The role of diet in regulating the metabolic network

C. elegans can be found in temperate climates around the world and is likely to encounter a wide variety of bacterial species in its natural habitat [25]. Therefore, the worm must adjust to potentially large differences in macro- and micronutrients provided by different bacterial diets. *C. elegans* exhibit a range of differences in life history traits, including development rate, fecundity and lifespan, when fed different bacteria [26-29]. For instance, animals fed the soil bacterium *Comamonas* DA1877 develop faster, have fewer progeny and a shorter lifespan than animals fed the standard *E. coli* OP50 diet. Gene expression profiling of *C. elegans* fed different bacterial diets revealed an enrichment of metabolic genes among differentially expressed genes [28, 30]. This suggests that the transcriptional regulation of metabolic genes in response to diet is an important regulatory mechanism.

Relatively little is known about the mechanisms that govern transcriptional responses of metabolic genes to different bacterial diets, or how these changes lead to physiological responses in the animal. To dissect these mechanisms, it is pertinent to: (i) identify which *C. elegans* genes are involved in mediating a dietary response; (ii) determine which bacterial nutrients or metabolites drive the response; and (iii) characterize diet-specific phenotypes for metabolic and regulatory gene mutants.

Identifying *C. elegans* genes that control the metabolic response to diet

Genetic screens can be used to reveal *C. elegans* factors that control the expression of metabolic genes in response to diet. For instance, genome-scale RNAi and mutagenesis screens can be performed using transgenic animals that express green fluorescent protein (GFP) under control of a diet-responsive gene promoter. One such diet-responsive promoter is that of *acdh-1*, a short/branched-chain acyl-CoA dehydrogenase-encoding gene, which is dramatically repressed when animals are fed a diet of *Comamonas* DA1877 relative to a diet

of *E. coli* OP50 [28]. Complimentary forward and reverse genetic screens with *Pacdh-1::GFP* animals fed *Comamonas* and *E. coli* diets identified activators and repressors of the *C. elegans* transcriptional response to these bacteria [31]. Together these genes form a nutrient-sensing gene regulatory network that transcriptionally controls metabolic gene expression in response to diet [31]. This network includes six NHRs, again highlighting their role in metabolic network control, as well as ACDH-1 itself in a negative feedback loop. Interestingly, two of the NHRs in the network, *nhr-10* and *nhr-68*, are required for the feedback between ACDH-1 and its own promoter.

Similar unbiased, reporter-based screening approaches identified factors responsible for the transcriptional activation of xenobiotic metabolism genes in response to specific xenobiotic compounds [32], the repression of xenobiotic metabolism genes under normal conditions [33], and the induction of triglyceride lipases in response to starvation [34]. NHRs have also emerged as important mediators in the response of drug metabolism to xenobiotic compounds. For instance, *nhr-176* is required for inducing expression of the cytochrome P450 *cyp-35d1* specifically in response to thiabendazole, an antifungal/antiparasitic drug. Interestingly, knockdown of *nhr-176* by RNAi negatively affects fertility when animals are exposed to thiabendazole, but not under normal conditions [32]. In humans, NHRs including the pregnane X receptor (PXR) and constitutive androstane receptor (CAR) are known to play pivotal roles in the induction of detoxifying cytochrome P450's by xenobiotic compounds [35].

Identifying metabolite signals that drive responses to different bacterial diets

Bacterial nutrients that induce a response in *C. elegans* can be identified in different ways. For instance, metabolomics might reveal differences in nutrient levels between bacterial diets. However, the number of metabolites that can be measured is rather limited and usually there are multiple differences in nutrient levels, thus identifying the key nutrients driving physiological responses in *C. elegans* is difficult (see below for more discussion on Metabolomics). Another approach is to identify mutant bacterial strains that, when fed to *C. elegans* elicit a distinct response from the wild-type diet. For instance, a strain of *E. coli* HT115 with a spontaneous mutation in the *aroD* gene was serendipitously found to extend lifespan when fed to *C. elegans*. This mutation reduces bacterial folate synthesis, which extends lifespan in *C. elegans* [36]. Similarly, by feeding a nitric oxide synthase-deficient *Bacillus subtilis* strain and comparing the effects to a wild-type *Bacillus subtilis* diet, it was discovered that bacterially supplemented nitric oxide can impact *C. elegans* longevity [37].

An unbiased bacterial genetics approach followed by a targeted chemical screen was recently employed to identify bacterial metabolites that can activate or repress the diet-responsive *acdH-1* promoter [38]. Previously it was found that the *acdH-1* promoter, which is highly activated on the *E. coli* OP50 diet, could be repressed by mixing in small amounts of *Comamonas* DA1877, indicating that *Comamonas* produces a dilutable compound with potent repressive effects on the *acdH-1* promoter [28]. A transposon-based mutagenesis screen in *Comamonas* revealed mutations in several vitamin B12 biosynthesis genes [38]. Vitamin B12 was also implicated as a repressor of *Pacdh-1* in a screen of the Keio *E. coli* deletion collection; a mutant strain with a deletion in *tonB*, a gene required for vitamin B12

import in *E. coli*, results in an increase in GFP expression when fed to the worm [38]. Mass spectrometry confirmed that *Comamonas* DA1877 synthesizes this essential vitamin, while *E. coli* OP50 does not. Indeed, low nanomolar concentrations of vitamin B12 supplemented to the *E. coli* OP50 diet were sufficient to repress *Pacdh-1* [38].

Vitamin B12 can only be synthesized by prokaryotes, yet is an essential cofactor for two enzymes in many animals including *C. elegans* and mammals: methionine synthase, which functions in the methionine/S-adenosylmethionine (SAM) cycle, and methylmalonyl-CoA mutase, which functions in propionyl-CoA breakdown. Interestingly, the *acdH-1* promoter was strongly induced when animals were fed excess propionic acid, suggesting that the repressive effect of vitamin B12 may lie in its role in propionyl-CoA breakdown [38]. In fact, genetic perturbation of the *C. elegans* propionyl-CoA breakdown pathway rendered vitamin B12 unable to repress the *acdH-1* promoter [38]. Thus, the induction of *acdH-1* expression on low-B12 diets such as *E. coli* can be attributed to reduced flux through the propionyl-CoA breakdown pathway, which causes a buildup of propionyl-CoA and/or propionic acid within the animal.

Characterizing diet-specific phenotypes

Do the transcriptional responses to diet have physiological consequences? The response of *Pacdh-1* to vitamin B12 centered around propionic acid homeostasis, and interestingly *acdH-1* mutants are particularly sensitive to propionic acid-induced toxicity when this short chain fatty acid is supplemented in excess [38]. This suggests that increased ACDH-1 activity on B12-deficient diets serves to alleviate the buildup or the toxic effects of this short chain fatty acid [38]. Currently the exact metabolic role of *acdH-1* is unknown, as it is one of many short-chain acyl-CoA dehydrogenases encoded by the *C. elegans* genome.

Another metabolic gene with a diet-specific phenotype is *alh-6*, which is involved in the breakdown of proline. *alh-6* mutants exhibit normal lifespan when fed *E. coli* HT115, but exhibit reduced lifespan when fed *E. coli* OP50 or *E. coli* HT115 supplemented with excess proline [39]. Knockdown of the enzyme directly upstream of *alh-6*, *proDH*, eliminated the negative effect of proline on *alh-6* mutant longevity [39]. This suggests that the proline breakdown intermediate and *alh-6* substrate 1-pyrroline-5-carboxylate (P5C) builds up in the *alh-6* mutant, and negatively affects longevity in these animals [39].

Diet-specific phenotypes have not only been observed for metabolic genes [30, 38, 39], but also for NHRs that regulate metabolic genes. For instance, *nhr-114*, which targets stress response and detoxification genes (including cytochrome P450's, UDP-glucuronosyltransferases and glutathione S-transferases) and lipid metabolism genes (*lip1-2*, *acs-10*) [40], was identified in an RNAi screen for factors that are necessary for germline development [40]. Surprisingly, the associated sterility phenotype is diet-dependent: *nhr-114* mutants fed *E. coli* OP50 were sterile, while mutants fed *E. coli* HT115 were not [40]. Fertility could be restored in *nhr-114* mutants fed *E. coli* OP50 if the diet was supplemented with tryptophan [40]. The suppression by tryptophan is likely due to its conversion by *E. coli* to another as-yet-undetermined bacterial metabolite, as its effect required a living bacterial diet [40].

nhr-8 was also identified in an RNAi screen, this time for enhancers of *daf-36* null mutants, which have reduced dafachronic acid (DA) production and thus impaired *daf-12* function [41]. *nhr-8* mutants exhibit decreased DA production, enhanced dauer formation and gonad migration defects, and these phenotypes are mitigated by dietary cholesterol supplementation [41]. Gene expression profiling of *nhr-8* mutants revealed differences in lipid and DA metabolism gene expression [41], indicating a regulatory role in maintaining adequate levels of DA synthesis genes that utilize cholesterol as a precursor. Future studies will be required to determine whether *nhr-8* and *nhr-114* are ligand-gated, and whether their ligands are diet-derived.

microRNAs in metabolic gene regulatory networks

Several mammalian microRNAs have been implicated in the control of glucose and lipid metabolism, insulin production, and the pathogenesis of diabetes [42]. microRNAs are emerging as an important class of metabolic regulators in *C. elegans* as well, with examples including the regulation of dietary restriction-induced metabolic pathways by *mir-80* [43], and the regulation of proteoglycan biosynthetic pathway genes by *mir-79* [44].

An interesting example of metabolic regulation by a microRNA is the regulation of *elo-2* by *mir-786*. A null mutation in *mir-786* results in long, arrhythmic defecation cycles, and after knocking down predicted *mir-786* targets, only one gene suppressed the *mir-786* phenotype; the fatty acid elongase *elo-2*, which controls cellular palmitate levels [45]. *mir-786* targets *elo-2* specifically in the two most posterior intestinal cells (int9; defecation “pacemaker” cells), and loss of *mir-786* results in increased *elo-2* expression, and subsequently decreased palmitate levels [45]. Supplementation of *mir-786* mutants with palmitate could rescue the defecation defects in these mutants [45]. Therefore, a microRNA plays a pivotal role in controlling the concentration of fatty acids in int9 cells via control of a fatty acid elongase, and this regulatory role is critical for proper defecation timing in the animal.

There are several examples of microRNAs involved in regulating the regulators of metabolism. For instance, *let-7* microRNA family members regulate (and are regulated by) *daf-12*, a regulator of several metabolic processes activated by the sterol-derived ligand DA [46], and this regulatory loop is essential for developmental progression [47, 48]. Another example is *mir-235*, which functions downstream of insulin signaling to regulate *nhr-91* and, ultimately, the decision of whether to arrest development in response to low nutrients [49]. Normally animals enter a developmentally arrested state called L1 diapause when food is anticipated to be scarce. *mir-235* mutants, however, fail to enter L1 arrest when subjected to starvation in the L1 stage. This failure to arrest is due to an overexpression of the *mir-235* target gene *nhr-91* in the *mir-235* mutant, as loss of *nhr-91* suppresses this defect [49]. Thus, in response to low nutrients, the insulin signaling pathway triggers *mir-235* expression, which negatively regulates *nhr-91*, thus enabling the animal can enter L1 diapause. Although the full spectrum of *nhr-91* targets has yet to be characterized, it likely regulates metabolic processes (directly or indirectly), as it binds the promoters of several well-known metabolic regulators including *nhr-49* and *sbp-1*, and its knockdown alters fat content [20].

Metabolomics, transcriptomics, and proteomics– towards understanding regulatory consequences to metabolic flux

The goal of regulating a metabolic gene is to regulate flux through the pathway in which the enzyme encoded by the gene functions. However, not all enzymes are regulated at the level of transcription; many can be regulated allosterically or by post-translational protein modifications. Thus, more directly measuring protein and metabolite levels may be required to uncover the outputs of transcriptional, allosteric and protein-modification changes.

Metabolomics is the simultaneous quantification of many different metabolites in a biological sample. Current technologies enable several hundred metabolites to be detected from biological samples, which is impressive considering the chemical diversity of these molecules. However, it only represents a small fraction of the thousands of metabolites present in the cell. In *C. elegans*, metabolomics using mass spectrometry and/or nuclear magnetic resonance (NMR) spectroscopy has uncovered metabolic signatures of insulin receptor (*daf-2*) mutants [50-52], a peptide transport (*pept-1*) mutant [51], a histone deacetylase (*sir-2.1*) mutant [52, 53], mitochondrial mutants (*isp-1*, *clk-1*, *mev-1*, *ucr-2.3*, *gas-1*) [52, 54, 55], a TCA cycle (*idh-1*) mutant [52], fatty acid synthesis mutants (*fat-5*, *fat-6*, *fat-7*) [56], as well as wild-type animals exposed to anoxic conditions [57]. For metabolic gene mutants, metabolomic profiling can shed light on the block caused by the lack of enzymatic function and its rippling effects throughout the metabolic network. For instance, aqueous and lipid fraction profiling of $\Delta 9$ desaturase mutants required for monounsaturated fatty acid (MUFA) synthesis revealed that a lack of these enzymes had far-reaching effects beyond just decreases in MUFAs, and the overall trend was a shift towards a catabolic state [56].

Integration of metabolomic data with transcriptomic and proteomic data can provide a more complete picture of how metabolic gene regulation affects metabolic flux. For instance, proteomic analysis of a peptide transport mutant, *pept-1* (*lg601*), revealed 23 proteins with significantly altered abundance compared to wild type, and four of these were methionine/SAM cycle enzymes, which were all downregulated in the mutant. Metabolomics revealed increased homocysteine and decreased methionine levels accompanying the downregulation of methionine/SAM cycle enzyme expression, indicating reduced flux through this metabolic pathway in *pept-1* mutants [51].

One major challenge when interpreting metabolomics data is overcoming variability that naturally arises within a population of animals and across experiments. In the study of various MUFA synthesis mutants (*fat-5*, *fat-6*, *fat-7*) the major principle component explaining the largest portion of sample variance in the metabolomics data was not genotype, but rather batch effects [56]. Two studies found that, surprisingly, the metabolomic differences between cohorts of wild-type *C. elegans* fed two different *E. coli* strains (OP50 and HT115) were equal to, or greater than the metabolomic differences caused by perturbing *daf-2*/insulin signaling [52, 58]. This suggests that *C. elegans* metabolic profiles are exquisitely sensitive to differences in diet and environment, as well as to genotypic differences. These two *E. coli* diets were previously shown to have different carbohydrate content, but similar protein and fat content [59], however differences in

micronutrient content are yet to be determined. Importantly, since wild type animals fed *E. coli* OP50 and those fed *E. coli* HT115 are more-or-less equally fit [28], yet have different metabolomic and transcriptomic profiles [28], we can hypothesize that *C. elegans* may have a range of homeostatic metabolic states that are conducive to healthy development, reproduction and aging.

Concluding remarks and future directions

C. elegans is a powerful model to study metabolic regulatory networks, and how they connect diet to gene expression and physiology. The nematode is extremely well suited to provide valuable insights into mammalian metabolic networks due to its high degree of homology to mammalian metabolism and similar nutrient requirements, as well as its amenability to genetic manipulation and high content “omics” data generation. Researchers have utilized high-throughput screening techniques thanks to the availability of genome-wide RNAi libraries and transgenic tools to reveal key players in the regulation of metabolic genes and dietary responses.

While impressive progress has been made, a full understanding of metabolic regulatory networks will require expansion to additional research areas. One such area is the biochemistry of *C. elegans* enzymes, which is necessary to identify substrates and products and provide thermodynamic parameters associated with enzyme-catalyzed reactions. This information, when integrated with metabolomic and transcriptomic data, will be critical to understand how changes in enzymes expression may translate to changes in metabolic flux both locally and throughout the metabolic network. Metabolic network modeling is a research area that has been largely unexplored for *C. elegans*. In other systems, such network models provide a platform with which biological data can be used to model metabolic flux and thus provide hypotheses about the consequences of metabolic regulation and dysregulation. A high-quality *C. elegans* metabolic network model will greatly facilitate investigation into the mechanisms by which metabolic gene regulatory networks orchestrate metabolic flux to maintain homeostasis.

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GLOSSARY

Essential nutrients	Small molecules that are required for normal cellular and organismal function, but are not synthesized by the organism and must be provided exogenously by the organism’s diet.
Mass spectrometry	An analytical technique that measures mass-to-charge ratios of ionized chemical compounds within a sample. Here we

	specifically discuss small molecule mass spectrometry used in metabolomic studies.
Metabolic gene regulatory network	A network connecting regulatory factors to the metabolic genes that they regulate.
Metabolic flux	The conversion rate of substrates to products through a metabolic pathway, which depends on enzyme expression levels, thermodynamic constraints, and substrate/product concentrations.
Metabolic homeostasis	The maintenance of internal metabolite equilibria by adjusting the metabolic network to compensate for environmental changes, especially dietary changes.
Metabolic network	A bipartite network connecting enzymes to the metabolites involved in the chemical reactions that they catalyze. The metabolic network describes the entire metabolic capacity of an organism.
Metabolomics	The study of small molecule metabolites within a cell, tissue or organism, also called the metabolome. Metabolomics is usually accomplished by techniques such as mass spectrometry and NMR.
microRNA (miRNA)	22 nucleotide RNA molecule that targets mRNAs as part of the RNAi machinery. Here, miRNA refers to endogenously encoded miRNAs.
Network modularity	The tendency for groups of nodes to be more highly interconnected than the average connectedness within the entire network.
Nuclear hormone receptors (NHRs)	A class of TFs characterized by a DNA binding domain and a ligand binding domain, which allows them to sense small molecules such as hormones or metabolites. NHRs for which a ligand has yet to be identified are called orphan receptors.
Nuclear magnetic resonance (NMR) spectroscopy	An analytical technique based on magnetic properties of atomic nuclei, which conveys the chemical properties of atoms and the molecules in which they are incorporated. Here we discuss small molecule NMR spectroscopy used in metabolomic studies.
RNA interference (RNAi)	The posttranscriptional inhibition of gene expression by small RNA molecules.
Transcriptomics	The study of mRNA transcripts within a cell, tissue or organism, also called the transcriptome. Transcriptomics is usually accomplished by techniques such as microarray expression profiling or RNAseq (deep-sequencing of mRNA-derived cDNA libraries).

**Transcription factors
(TFs)**

Proteins that bind DNA and regulate the transcription of genes.

**Yeast one hybrid
(Y1H)**

A yeast-based assay that reports physical interactions between protein and DNA.

Box 1**OUTSTANDING QUESTIONS**

- What are the ligands for *C. elegans* NHRs?
- How does ligand binding to NHRs affect metabolic gene expression?
- Does the metabolic gene regulatory network sense metabolic flux by sampling certain metabolite concentrations?
- Are feedback loops between NHRs and their metabolic targets a method of metabolic flux constraint?
- Is the *C. elegans* metabolic network comprehensively annotated, or are there new/alternate metabolic pathways yet to be discovered?
- What are the tissue-specific differences in metabolic network regulation and how do tissue-specific metabolic roles enhance organismal fitness?

HIGHLIGHTS

C. elegans is an outstanding model to study dietary effects

Metabolic networks are regulated transcriptionally

C. elegans metabolic networks are regulated by nuclear hormone receptors

Figure 1. *C. elegans* metabolic gene regulatory networks

Model illustrating principles of metabolic regulation in the worm. *C. elegans* likely encounters a wide variety of bacterial diets with different nutrient composition in the wild. These nutritional differences trigger transcriptional changes in metabolic genes, mediated by gene regulatory networks enriched for NHRs. Yeast one-hybrid studies have revealed that metabolic gene regulatory networks are highly modular, which may ensure the rapid and coordinated response of metabolic pathways to environmental cues. Metabolic genes can also be regulated posttranscriptionally by microRNAs. Feedback between NHRs and metabolic pathways via metabolite signals may be an important mechanism for regulating metabolic flux and maintaining cellular homeostasis on diverse diets.

Table 1

Nuclear Hormone Receptors that regulate *C. elegans* metabolism

NHR	Metabolic Targets	Physiological Role	Reference
<i>daf-12</i>	TCA cycle enzymes (e.g. <i>cts-1</i> , <i>aco-1</i>)*; Fatty acyl-transferases (e.g. <i>acl-1</i> , <i>acl-2</i> & <i>acl-4</i>)* short-chain dehydrogenases/reductases (e.g. <i>dhs-21</i> & <i>dhs-26</i>)	Required for proper developmental timing; mutants are heterochronic. The DAF-12 ligand is a sterol derivative, daifachronic acid.	[60, 61]
<i>nhr-10</i> & <i>nhr-68</i>	<i>acdh-1</i>	Both NHRs are required for transcriptional activation of <i>acdh-1</i> in response to perturbation of propionyl-CoA breakdown, and for negative feedback from ACDH-1 to its promoter. NHR-10 binds directly to the <i>acdh-1</i> promoter.	[20, 28, 31]
<i>nhr-8</i>	Fatty acid metabolism (e.g. <i>fat-5</i> , <i>fat-7</i> , <i>acdh-5</i> , <i>ech-9</i>) and daifachronic acid metabolism genes (<i>daf-36</i>)	On cholesterol-deficient diets, <i>nhr-8</i> partial loss-of-function mutants display enhanced dauer formation, gonad migration defects, and larval arrest at high temperatures	[41]
<i>nhr-176</i>	<i>cyp-35d1</i>	Knockdown of <i>nhr-176</i> causes reduced fertility when animals are exposed to thiabendazole	[32]
<i>nhr-49</i>	Fatty acid metabolism (e.g. <i>fat-5</i> , <i>fat-6</i> , <i>fat-7</i> , <i>acs-2</i>) and sphingolipid metabolism genes (B0222.4, F27E5.1)	Impairment of <i>nhr-49</i> results in a high fat phenotype and reduced lifespan, as well as decreased oxygen consumption rates and abnormal mitochondrial morphology.	[62, 63]
<i>nhr-80</i>	Fatty acid metabolism genes (<i>fat-5</i> , <i>fat-6</i> , <i>fat-7</i>)	Required for increased lifespan induced by loss of the germline; also required for proper mitochondrial morphology and oxygen consumption. Binds <i>nhr-49</i> and has overlapping targets.	[63-65]
<i>nhr-66</i>	Sphingolipid metabolism (B0222.4, F27E5.1)	Mutants exhibit reduced basal oxygen consumption and beta-oxidation rates. Binds <i>nhr-49</i> and has overlapping targets.	[63]
<i>nhr-62</i>	Fatty acid localization and transport genes (e.g. <i>vit-1/2/3/4/5/6</i> , <i>lbp-8</i>) short chain dehydrogenases (e.g. <i>dhs-25</i> , <i>dhs-14</i> , <i>acdh-2</i>)	Regulates dietary restriction (DR)-induced genes; overexpression leads to DR-like phenotypes such as small body size, reduced fat and increased longevity	[66]
<i>nhr-40</i>	Lipid binding proteins (<i>lbp-6</i> , <i>lbp-9</i>), glycolysis (<i>tpi-1</i>) and TCA cycle genes (<i>aco-1</i>)	Required for larval development	[67, 68]
<i>nhr-114</i>	Stress response (e.g. <i>gst-8</i>), detoxification (e.g. <i>cyp-35B1</i> , <i>cyb-35B2</i>) and lipid metabolism genes (e.g. <i>lip1-2</i> , <i>acs-10</i>)	Mutant is sterile on the <i>E. coli</i> OP50 diet, but fertile on the <i>E. coli</i> HT115 diet. Sterility on <i>E. coli</i> OP50 is rescued by a tryptophan-derived <i>E. coli</i> metabolite	[40]
<i>nhr-64</i>	Lipid binding (e.g. <i>lbp-6</i>) and fatty acid metabolism genes (e.g. <i>acs-1/2/4/5</i> , <i>ech-5</i>)	Knockdown partially suppressed the developmental delay caused by <i>sbp-1</i> mutation. Also partially suppressed developmental delay and reduced fertility of <i>fat-6;fat-7</i> double mutant.	[69]

* Representative targets of DAF-12 from enriched metabolic GO categories based on ChIP data, re-analyzed for this review.