Review

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Caenorhabditis elegans metabolic gene regulatory networks govern the cellular economy

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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 (NHRs) in responding to dietary conditions, regulation of metabolic genes and metabolic regulators by miRNAs, and feedback between metabolic genes and their regulators.

Introduction

Maintaining cellular homeostasis is a complex task that involves monitoring energy states and levels of essential nutrients (see Glossary), 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, predicted 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 and the nature of the metabolic impairment. Diet can also have a primary causal role in diseases such as obesity and type 2 diabetes mellitus [3]; however, the mechanisms by which diet can lead to metabolic syndrome and insulin resistance are not fully understood.

In this review, we focus on studies using the bacterivorous nematode *Caenorhabditis elegans* to elucidate mechanisms of metabolic network regulation. *Caenorhabditis 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 target of rapamycin (TOR) signaling. It provides clear advantages compared with mammals for system-level studies of metabolism. The nematode is small (approximately 1.5 mm), has a transparent body, a short lifespan (approximately 2–3 weeks), and a well-annotated genome [4,5]. In addition, a variety of genome-wide technologies are available that

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 its diet.
Mass spectrometry (MS): an analytical technique that measures mass-to-charge ratios of ionized chemical compounds within a sample. Here, we specifically discuss small-molecule MS 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 and/or product concentrations.
Metabolic homeostasis: the maintenance of internal metabolite equilibriums by adjusting the metabolic network to compensate for environmental changes, especially dietary changes.
Metabolic network: a 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 MS and NMR.
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.
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.

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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, large-scale 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 NHRs among the transcriptional regulators, miRNAs 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 NHRs

Transcriptional regulation provides a major mechanism of metabolic network control, and nutrient-induced metabolic gene expression changes have been observed in organisms from bacteria to humans. In mammals, many metabolites have regulatory capacity. 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 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 (PPARα) [15]. Aberrant transcriptional control of metabolic pathways and subsequently altered metabolic flux, especially pertaining to fatty acids, are hallmarks of diabetes. Indeed, PPARα, a lipid-sensing NHR that promotes lipid catabolism, is a target of antidiabetic 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 [17]. Although ligands have been identified for many human NHRs, only one ligand has been identified for a *C. elegans* NHR (dafachronic acid, which binds to and activates DAF-12 [18,19]). Thus, all other *C. elegans* NHRs are currently orphan receptors, and, furthermore, the gene targets of most 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, similar to their mammalian orthologs, *C. elegans* NHRs function in metabolic network control [16]. Binding of TFs to *C. elegans* metabolic gene promoters is highly modular in that TFs tend to separate into densely interconnected groups with shared 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

Caenorhabditis elegans can be found in temperate climates around the world and is likely to encounter a variety of bacterial species in its natural habitat [25]. Therefore, the nematode must adjust to potentially large differences in macro- and micronutrients provided by different bacterial diets. It exhibits 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 aquatica* DA1877 develop faster, have fewer progeny and a shorter lifespan than animals fed the standard *Escherichia 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 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 repressed when animals are fed a diet of *Comamonas aq.* 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