Understanding Metabolic Regulation at a Systems Level: Metabolite Sensing, Mathematical Predictions, and Model Organisms

Emma Watson, L. Safak Yilmaz, and Albertha J.M. Walhout*

Program in Systems Biology, Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts 01605; email: emma.watson@umassmed.edu, lutfussafak.yilmaz@umassmed.edu, marian.walhout@umassmed.edu

Annu. Rev. Genet. 2015. 49:553–75

The *Annual Review of Genetics* is online at genet.annualreviews.org

This article's doi: 10.1146/annurev-genet-112414-055257

*Corresponding author

Keywords

metabolic network, feedback loop, homeostasis, gene regulation, flux balance analysis, *Caenorhabditis elegans*

Abstract

Metabolic networks are extensively regulated to facilitate tissue-specific metabolic programs and robustly maintain homeostasis in response to dietary changes. Homeostatic metabolic regulation is achieved through metabolite sensing coupled to feedback regulation of metabolic enzyme activity or expression. With a wealth of transcriptomic, proteomic, and metabolomic data available for different cell types across various conditions, we are challenged with understanding global metabolic network regulation and the resulting metabolic outputs. Stoichiometric metabolic network modeling integrated with "omics" data has addressed this challenge by generating nonintuitive, testable hypotheses about metabolic flux rewiring. Model organism studies have also yielded novel insight into metabolic networks. This review covers three topics: the feedback loops inherent in metabolic regulatory networks, metabolic network modeling, and interspecies studies utilizing *Caenorhabditis elegans* and various bacterial diets that have revealed novel metabolic paradigms.

INTRODUCTION

A metabolic network is a collection of biochemical reactions that maintains chemical homeostasis. This network forms the foundation for the cellular economy, as it controls amino acid levels for protein and neurotransmitter biosynthesis, nucleic acids for DNA and RNA synthesis and repair, methyl and acetyl donors for building the epigenome and synthesizing lipids, and energy for every anabolic, signaling, and general maintenance process.

Most of what is known about the human metabolic network comes from a century of enzymology research, in which enzyme activities were detected, functionally purified, and eventually genetically characterized. The current age of genomics has enabled the study of metazoan metabolism at a genome-wide, or systems, level. Genome sequencing has revealed a predicted parts list of the human metabolic network. Transcriptomic and proteomic studies have revealed that metabolic networks exhibit great diversity between tissues, during proliferation versus senescence, and in health versus disease. This differential use of metabolic subnetworks or pathways is referred to as metabolic network rewiring. Today we are challenged with understanding which metabolic pathways are employed in which tissues and under which conditions, and with dissecting the control systems that drive metabolic rewiring.

To explore metabolic network rewiring we must first consider the mechanisms and outputs of metabolic network regulation. Metabolic genes are extensively regulated at the levels of transcription (77), posttranscription (36, 120, 123), posttranslation (91, 109) including allostery through direct interactions with metabolites (3, 86, 155), and subcellular localization (104, 105). Further, the regulators are themselves regulated at multiple levels (11) and are connected to master endocrine signaling pathways that coordinate metabolism across tissues (82).

Understanding how metabolic network regulation affects global outputs (phenotypes) is another major challenge. This can be addressed by stoichiometric metabolic network models that are built by comprehensively annotating the collection of enzymes encoded by the genome and the reactions they are likely to catalyze. The power of this approach is that such models can provide nonintuitive metabolic and physiological hypotheses. An advantage of these models is that "omics" data can be integrated into the network model to investigate tissue- and condition-specific metabolic programs, such as metabolite and enzyme dependencies of cancer cells (46).

A complementary approach to exploring metabolic network function is to utilize forward and reverse genetics to identify the genes responsible for metabolic phenotypes or metabolic rewiring events in vivo. Metazoan model organisms such as *Caenorhabditis elegans* offer a platform for high-throughput genetic screening to uncover novel functions and biological roles of metabolic enzymes, as well as the regulatory networks involved in metabolic network rewiring. *C. elegans* is particularly powerful because in addition to being genetically tractable itself, its bacterial diet can also be subjected to systematic mutagenesis. This can be used to identify, in an unbiased fashion, the most important players in the orchestration of various metabolic states, such as those induced by caloric restriction or different bacterially supplied nutrients.

This review summarizes what is known about the mechanisms of metabolic regulation from a metabolite-centered perspective, given that metabolites are both the currency of communication between the metabolic network and its regulators, and the commodities that are ultimately regulated. We also discuss the methods by which mathematical models of metabolism are built and integrated with gene expression data to generate hypotheses about global metabolic network function. Finally, we explore genetics studies of *C. elegans* that have shed light on metabolic network regulation and function, with emphasis on dissecting the metabolic responses to diet and commensal relationships with bacteria such as those occurring in the mammalian intestine.

MECHANISMS OF METAZOAN NUTRIENT SENSING AND METABOLIC REGULATION

To maintain metabolic homeostasis, cells must monitor their energy states, the quantities of central chemical building blocks, and essential micronutrients. This information enables the cell to take the most appropriate action given its metabolic circumstances, whether that means a subtle shift in oxidative fuel choice or a dramatic rewiring of the metabolic network in response to starvation or hypoxia. How do cells sense the diverse repertoire of metabolites, and how do they use this information to make regulatory decisions? Organisms have evolved complex networks of proteins with metabolite-sensing capabilities that relay information about the concentrations of key metabolites to regulators or that are themselves direct regulators of metabolic genes. Metabolite sensing is achieved in large part through physical interactions between metabolites and regulators that can affect the function of a regulator by altering its biophysical properties. Many nutrient-sensing regulators are transcription factors and members of signal transduction pathways that regulate metabolic enzyme expression and function at various levels.

Metabolic regulation utilizes negative feedback loops (nFBLs), which are known to confer the properties of robustness (80) and adaptability (93, 96) to biological networks. nFBLs in metabolism center on metabolites, which need to be maintained within proper cellular concentrations. Metabolic nFBLs include additional components such as enzymes to modify metabolite concentrations, regulators to regulate enzyme expression or activity, and sensors to monitor metabolite levels (81). For example, the cellular ATP control system operates as an nFBL to maintain appropriate energy levels (**Figure 1**). In this system, AMP:ATP ratios are sensed by AMPK, which serves both sensor and regulator functions. AMPK is activated by increased AMP:ATP levels and phosphorylates glycolytic enzymes among other metabolic targets to enhance glycolytic flux, ultimately increasing ATP production. As ATP levels increase, AMPK is inhibited, which completes the feedback loop. This section describes how specific metabolic control systems are encoded by the genome, detailing the gene networks responsible for nutrient sensing and metabolic gene regulation as well as the mechanisms they employ.

Glucose Sensing

The insulin-signaling pathway is the major regulator of circulating glucose concentrations and glucose metabolism. It forms a multiorgan nFBL, which is initiated by glucose uptake in pancreatic β-cells. These cells express GLUT2, the highest affinity glucose transporter, and thus are the first cells to be affected by blood glucose spikes after a meal (41). Glucose is metabolized via glycolysis, the tricarboxylic acid (TCA) cycle, and ultimately the electron transport chain, resulting in increased intracellular ATP:ADP ratios (63). This leads to depolarization of the cell through inhibited potassium export from ATP-sensitive potassium channels, which causes an influx of calcium and the release of insulin to the bloodstream through exocytosis of insulin-storage granules (63). Thus, pancreatic β-cells sense an increase in glucose by monitoring their own internal glucose oxidation flux, reflected by ATP:ADP ratios. The release of insulin from the β-cells signifies to other tissues that glucose is abundant and should be imported, metabolized, and/or stored (as glycogen).

In the target tissue, the insulin-signaling pathway initiates with binding of insulin to the insulin receptor, followed by phosphorylation of PI3K and AKT kinase, which are involved in transducing the signal (23). AKT activates GLUT4 translocation to the cell surface to promote glucose import (76), inhibits a negative regulator of glycogen synthase (GSK3) to increase glycogen production and thus glucose storage (34), and inhibits FOXO to halt transcriptional activation of gluconeogenesis enzymes (27, 115). Glucose, therefore, through its proxy signal

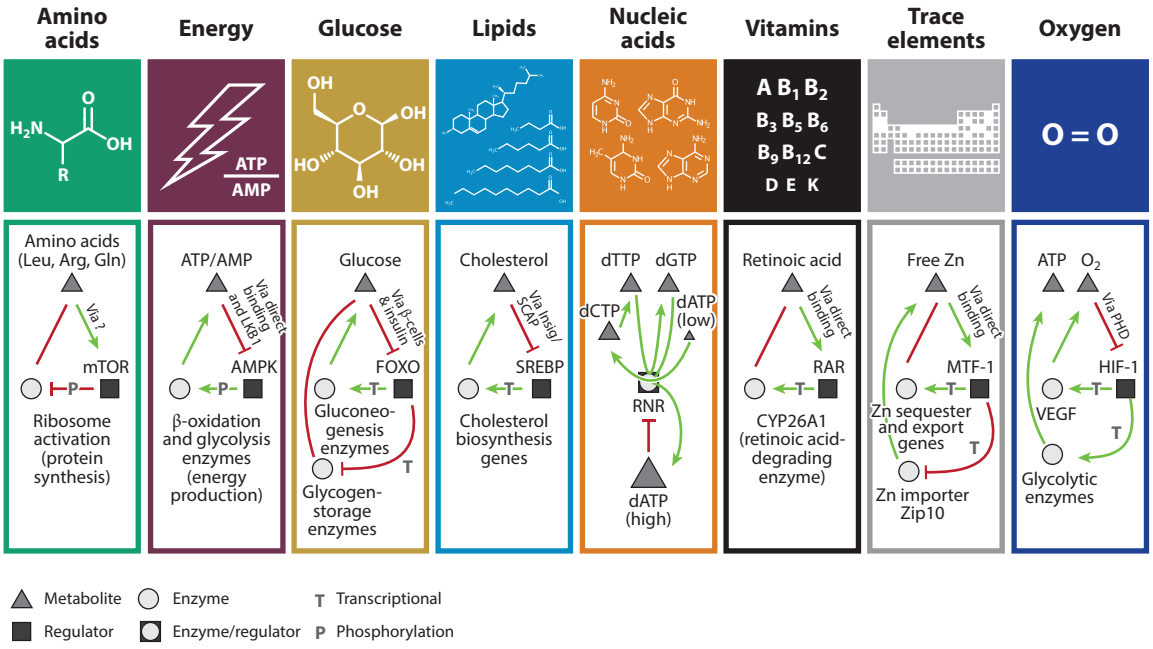


Figure 1

Feedback loops in metabolic network regulation. Feedback loops can consist of small molecules (*triangles*), regulators (*squares*), and enzymes or other metabolic gene targets (*circles*). Edges from metabolites to regulators indicate a repressive (*red*) or activating (*green*) effect of a metabolite on regulator function, with the sensing mechanism noted along the edge. Edges from regulators to enzymes or metabolic genes indicate a repressive (*red*) or activating (*green*) effect on target function. Edges are marked P to indicate phosphorylation and T to indicate transcription. Edges from enzymes/metabolic genes to metabolites indicate a consuming (*red*) or producing (*green*) relationship.

insulin, activates its own uptake and storage as glycogen, and inhibits its own biosynthesis by inhibiting gluconeogenic enzyme expression via FOXO inhibition (**Figure 1**). The glucose control system maintains blood glucose within a certain range; however, this set point is adjustable (to accommodate periods of feast and famine), and this adjustable set point is particularly prone to dysregulation, leading to obesity, metabolic syndrome, and diabetes (81).

Amino Acid Sensing

The mammalian target of rapamycin (mTOR) kinase is a major regulator of protein synthesis, autophagy, cell proliferation, and organ size (99). mTOR provides a link between amino acid availability and translation rates (79), as mTOR activation by amino acids leads to inhibition of a repressor of translation, eukaryotic initiation factor 4E-binding protein (eIF4E-BP1), and activation of the translational activator S6 kinase (S6K1) (61, 67). The mechanisms by which amino acids are sensed to regulate mTOR activity are still being elucidated (5). mTORC1, one of two major mTOR complexes, is regulated by the lysosome-bound GTPase Rheb. Under amino acid depletion, inactive mTORC1 is located diffusely throughout the cytoplasm, whereas when amino acids are abundantly present, active mTORC1 is found at the lysosomal surface where it can access the activator Rheb (129). The most potent amino acid activators of mTORC1 are leucine, arginine, and glutamine, which activate mTORC1 by different mechanisms. In addition to the

lysosomal amino acid–sensing mechanism, leucine sensing may be achieved through interactions between leucyl-tRNA synthase and the mTOR complex (56), whereas glutamine sensing involves the adenosine diphosphate ribosylation factor-1 (Arf1) (73), and arginine sensing requires the lysosomal SLC38A9 transporter (150).

Another mechanism of amino acid sensing is the monitoring of uncharged tRNAs, which represent amino acid depletion. This is accomplished through the highly conserved Gcn2-eIF2α-Gcn4/ATF system. As amino acid levels decline during amino acid starvation, uncharged tRNAs accumulate and bind Gcn2 through a domain with homology to histidyl tRNA synthetase. This domain has higher affinity for uncharged tRNA than charged tRNA, and binding causes a conformational change that activates the kinase domain of Gcn2. Gcn2 phosphorylates eIF2α, resulting in a global decrease in translation rates, with the exception of certain mRNAs, such as those encoding the transcription factors Gcn4 and ATF. Transcriptional targets of Gcn4 include amino acid biosynthetic enzymes and transporters (65). ATF drives the autophagy gene expression program in order to salvage amino acids from existing proteins (8).

Lipid Sensing

We focus on the mechanisms for sensing two types of lipid: cholesterol and free fatty acids. Cholesterol is an important component of mammalian cell membranes and also serves as a precursor in the synthesis of bile acids and steroid hormones. Cholesterol sensing takes place in the endoplasmic reticulum membrane, where SREBP, a transcription factor, resides with its binding partners SCAP and Insig1 (39). When cholesterol levels in the membrane fall below a threshold, Insig1 dissociates, and the SCAP-SREBP complex translocates to the Golgi body, where SCAP-activated S1P and S2P proceed to cleave SREBP, freeing SREBP to migrate to the nucleus (125, 151). Once in the nucleus, SREBP activates the transcription of several genes in the de novo cholesterol biosynthesis pathway, including HMG-CoA reductase (68), the target of widely used cholesterol-lowering statin drugs. Thus, via SREBP, cholesterol negatively regulates its own biosynthesis.

Fatty acids consist of saturated and unsaturated carbon chains with a carboxylic acid head group. Fatty acids serve both structural (as components of cellular membranes) and energy-storage purposes (in their acyl-CoA form). The precise sensing mechanisms for different fatty acids are not entirely clear, but it has been suggested that the peroxisome proliferator-activated receptor (PPAR) subgroup of nuclear hormone receptors sense the fatty acid milieu of the cell through promiscuous, weak binding of diverse fatty acid ligands (20, 147). The binding affinities observed in vitro [2–50 μM range for PPARα binding to free fatty acids (146)] may indicate that PPARs are activated only when ligands reach high concentrations in vivo (128). PPARs regulate the expression of enzymes involved in fatty acid oxidation (78), and increased intracellular levels of fatty acids result in increased PPAR-mediated expression of fatty acid oxidation genes (108). Another nuclear receptor, HNF4-α, also binds lipids and regulates an overlapping set of lipid metabolism genes. Therefore, free fatty acids activate their own oxidation through PPARs and HNF4-α.

Nucleotide Sensing

Nucleotide pools are carefully managed, as nucleotide deficiencies lead to increased mutagenesis rates and genomic instability (12). During S phase, the demand for nucleotides spikes to support DNA replication, and enzymes involved in de novo nucleotide biosynthesis are transcriptionally upregulated, including ribonucleotide reductase (RNR) (13), which converts nucleoside diphosphates (NDPs) to deoxynucleoside diphosphates (dNDPs); these go on to form deoxynucleoside triphosphates (dNTPs). RNR and other nucleotide metabolism genes are direct

targets of c-MYC, a master transcriptional regulator of the cell cycle (90). RNR is subject to further regulation through subcellular localization [for instance, it assembles at sites of DNA damage (141)], proteolytic degradation (19), and an elegant system of allosteric feedback with various end product dNTPs to ensure proper nucleotide ratios (66). This autoregulation occurs via differential binding of ATP, dATP, dTTP, and dGTP to the specificity site, or S site. The identity of the S site–bound nucleotide dictates the substrate preference of RNR: ATP and dATP binding stimulates conversion of CDP to dCDP, dTTP (which is formed from dCTP through the de novo nucleotide synthesis pathway) activates conversion of GDP to dGDP, and finally dGTP activates conversion of ADP to dADP. When dATP levels are high, dATP binds a different site on RNR, the A site, to shut down all RNR activity and halt dNTP synthesis. Thus, RNR plays multiple vital roles in maintaining proper nucleotide pools; it is the enzyme that creates dNTPs, the sensor of dNTP ratios, and the regulator of those ratios.

Trace Element Sensing: Zinc

Trace metals, including cobalt, iron, and zinc, are micronutrients that function extensively throughout the metabolic network, where they bind enzymes to form metalloenzymes, which utilize bioinorganic chemistry to catalyze metabolic reactions that are difficult to achieve with organic chemistry alone. Trace metals are important resources that must be extracted from the diet and carefully incorporated into the cellular economy to avoid the toxicity associated with the overabundance of highly reactive free ions. However, deficiencies in trace elements can be catastrophic for an organism, as they can impair the function of hundreds if not thousands of different proteins.

For instance, zinc is predicted to bind 10% of the human proteome (4), serving both catalytic and structural roles. As a structural component, it generates protein-folding landscapes, which increases the complexity of protein-protein and protein-DNA interactions (101). In human metabolism, the catalytic activity of zinc is used by >100 zinc metalloenzymes (103). A classic example is carbonic anhydrase, which accommodates a zinc atom in its reaction center and reversibly converts carbon dioxide to bicarbonate (139).

An intricate network of zinc importers and exporters, intracellular zinc binding/transporting metallothioneins, and zinc-sensing transcription factors such as MTF-1 (25) regulate the cellular reservoir of zinc ions. As with the other nutrient-sensing mechanisms, zinc sensing involves nFBLs to maintain zinc pools within an optimal cellular threshold. MTF-1, itself a zinc-finger protein, transports to the nucleus upon binding free zinc ions in the cytoplasm. In the nucleus, it binds to metal response elements (MREs) and thereby activates the expression of zinc-sequestering metallothioneins and zinc exporters. MTF-1 can also repress some targets, including the zinc importer Zip10, which has an MRE downstream of its transcription start site, and binding of MTF-1 to this MRE stalls Pol II progression (54). Whereas MTF-1 is conserved from insects to mammals, there is no clear ortholog in the nematode *C. elegans*. *C. elegans* does exhibit transcriptional responses to zinc and a *C. elegans* zinc-responsive DNA element was recently identified (122). However, the transcription factor responsible for sensing zinc and binding this element is still unknown.

Vitamin Sensing

Vitamins are chemical cofactors required for the proper functioning of a species (the vitamin auxotroph) but provided by another species (the vitamin synthesizer). Vitamin auxotrophy is a cornerstone of many symbiotic relationships (33, 62), including the relationship between humans and the vitamin-producing bacteria that make up our microbiota (84). Humans require a suite of vitamins that function in diverse metabolic pathways, and flux through these pathways inherently

depends on the quantity of vitamins derived from diet and provided by our microbiota. Vitamin deficiencies were once a major cause of disease and death in human populations, and continue to cause health problems in underdeveloped countries. In mammals, direct sensing of vitamin A and vitamin D occurs through binding to nuclear hormone receptors. Vitamin D, which is technically a hormone rather than a vitamin given that it can be synthesized from cholesterol in the skin upon UV light exposure, regulates the uptake of calcium, iron, magnesium, and zinc (59). This regulation occurs through vitamin D receptor (VDR)-mediated transcriptional activation of the transporters of these micronutrients (18). Vitamin A, a true vitamin, has a plethora of functions in diverse processes, including development, vision, and immunity (38). Vitamin A does not function in anabolic or catabolic processes, as most other vitamins do, but rather serves as a light-sensing cofactor for rhodopsin and a signaling morphogen to regulate *Hox* genes during development via retinoic acid receptor (RAR) binding (38). There are enzymes that modify dietary Vitamin A (retinol) to generate retinal (the form utilized by rhodopsin) and retinoic acid (the form that directly binds and activates RAR), as well as enzymes that degrade retinoic acid. Vitamin A metabolism is tightly regulated by positive and negative FBLs to maintain proper concentrations of the various forms of Vitamin A during development, given that dysregulation can lead to teratogenesis (35, 134). For instance, the retinoic acid–degrading enzyme CYP26A1 is directly activated by RAR through binding of highly conserved retinoic acid response elements (RAREs) in its promoter (35). Thus, retinoic acid activates its own degradation, preventing deleteriously high levels of retinoic acid from building up. Retinoic acid also represses the expression of the enzymes involved in its synthesis from retinol, although the mechanisms of this repression are unknown (35).

Less is known about the mechanisms employed by mammalian cells to sense other vitamins, if they do exist. However, gene expression studies have revealed regulatory responses to vitamins B1 (thiamine) (47, 88, 142), B2 (riboflavin) (111), B3 (nicotinamide/niacin) (26, 29, 50), B6 [pyridoxal 5′ phosphate (PLP)] (145, 158), B9 (folic acid) (7, 21, 87), C (ascorbic acid) (17, 74, 140), and E (tocopherol/tocotrienols) (83, 100, 110). Indirect vitamin-sensing mechanisms, in which flux through the vitamin-dependent pathway is sensed as a proxy for vitamin abundance, may also exist. For instance, the nematode *C. elegans* exhibits a vitamin B12 response in which the expression of several metabolic genes is tuned according to dietary vitamin B12 intake. However, genetic perturbation of either of the two vitamin B12–dependent pathways disrupts the vitamin B12 responsiveness of several of these genes (154). This demonstrates that vitamin B12 itself is not the mediator of transcriptional changes but rather suggests that some genes are regulated according to flux through vitamin B12–dependent pathways rather than the absolute quantities of the vitamin. Several *C. elegans* nuclear hormone receptors are implicated in this response (153), although the identities of the metabolites that are sensed remain to be identified.

Energy Sensing

As the energy currency of the cell, ATP is a vital molecule required by many diverse cellular processes. A central regulator of energy homeostasis is AMPK, a heterotrimeric kinase that senses AMP/ATP ratios and regulates ATP production accordingly. AMPK is activated by high AMP levels and inhibited by high ATP levels (58). As energy is harvested from the hydrolysis of ATP to ADP, increasing ADP levels push the adenylate kinase reaction ($2ADP \leftrightarrow ATP + AMP$) toward AMP and ATP production to buffer the falling ATP levels. AMPK activity increases several-fold in vitro when AMP is present (24), and increases more than 100-fold when phosphorylated by its upstream regulator, the LKB1-STRAD-MO25 complex (60). Although this complex does not sense AMP itself, it phosphorylates AMPK preferentially when AMP/ATP ratios are high because of conformational changes in AMPK induced by AMP or ADP binding, which enhances

phosphorylation site availability (156). There are many downstream targets of AMPK, including the central carbon metabolism enzymes 6-phosphofructo-2-kinase (glycolysis enzyme activated by AMPK phosphorylation) and acetyl-CoA carboxylase (fatty acid synthesis enzyme inhibited by AMPK). The end result of AMPK activation is enhanced oxidative catabolism of glucose and fatty acids to fuel the electron transport chain to produce more ATP, and the inhibition of anabolic pathways such as gluconeogenesis and fatty acid synthesis (106).

Oxygen Sensing

Cellular oxygen levels must be closely monitored, as major metabolic network rewiring must take place to survive periods of hypoxia. Primarily, a shift from oxidative phosphorylation (OXPHOS) to anaerobic glycolysis is needed for compensatory ATP production. The chief oxygen sensor, conserved in all metazoans, is the prolyl-hydroxylase and hypoxia-inducible factor 1 (HIF-1) system. HIF-1 is a heterodimeric transcription factor, consisting of a basic helix-loop-helix DNA binding domain, a heterodimerization domain, and a transcriptional coactivator-binding domain. Under normoxic conditions, HIF-1 is hydroxylated by prolyl-hydroxylases (42), leading to ubiquitination by a von Hippel–Lindau-guided E3 ubiquitin ligase (116) and proteasomal degradation of HIF-1 (127). Under hypoxic conditions, the prolyl-hydroxylases fail to hydroxylate HIF-1 because this reaction requires molecular oxygen, and the HIF-1 protein is stabilized (130). Stable, active HIF-1 accumulates in the nucleus, where, in addition to upregulating the expression of proangiogenic factors like vascular endothelial growth factor (VEGF) to increase oxygen flow (89), it activates expression of glycolytic enzymes (133), resulting in increased flux through glycolysis to compensate for the lack of mitochondrial ATP production.

The HIF-regulating prolyl-hydroxylases are Fe, O_2, and α-ketoglutarate (α-KG, a TCA cycle intermediate) dependent, suggesting that the HIF-1 oxygen-sensing system also senses and responds to changes in iron and α-KG levels through altered prolyl-hydroxylase substrate availability (95). A lack of α-KG may signify reduced flux through the TCA cycle, and therefore an impending energy crisis, given that the TCA cycle produces reducing equivalents to fuel OXPHOS. Low TCA cycle flux and a lack of O_2 would therefore lead to a decrease in ATP production via OXPHOS and, in either situation, the HIF-1-mediated increase in glycolytic flux provides necessary compensation. Aberrant activation of HIF-1 under normoxic conditions is a common occurrence in cancer cells and helps establish the aerobic glycolysis metabolic program conducive to rapid proliferation (102, 121, 132).

Intercommunication Between Metabolic Regulatory Pathways

Regulatory cross talk between the various metabolite-specific sensing pathways is a useful way for the organism to coordinate metabolism with growth or to cope with nutrient stress. For instance, the insulin-signaling cascade activates mTOR to promote protein synthesis and cell growth. However, under nutrient stress, mTOR is inhibited by AMPK to slow translation rates and halt cell growth and proliferation. Below are several examples of cross talk between pathways.

- The insulin-signaling pathway activates mTOR through AKT-mediated inhibition of TSC1/TSC2, the major mTOR repressor (69).
- mTOR is inhibited by AMPK, both directly through phosphorylation of Raptor (55) and indirectly through activation of mTOR repressors TSC1 and TSC2 (70).
- AMPK activates FOXO (53), which upregulates genes involved in gluconeogenesis, lipid metabolism, and autophagy.

- mTOR can activate HIF-1a under normoxic conditions, leading to aerobic glycolysis (the Warburg effect). This occurs in proliferating macrophages (126) and cancer cells (57).
- mTOR can activate SREBP-1 (119) through Lipin-1 (118) to activate lipid synthesis for cell growth and proliferation.
- AMPK directly phosphorylates SREBP-1 to inhibit its cleavage and translocation into the nucleus, thus repressing SREBP-1-mediated transcriptional activation of lipid synthesis in mammalian liver (85).

BUILDING METABOLIC NETWORK MODELS AND INCORPORATING "OMICS" DATA

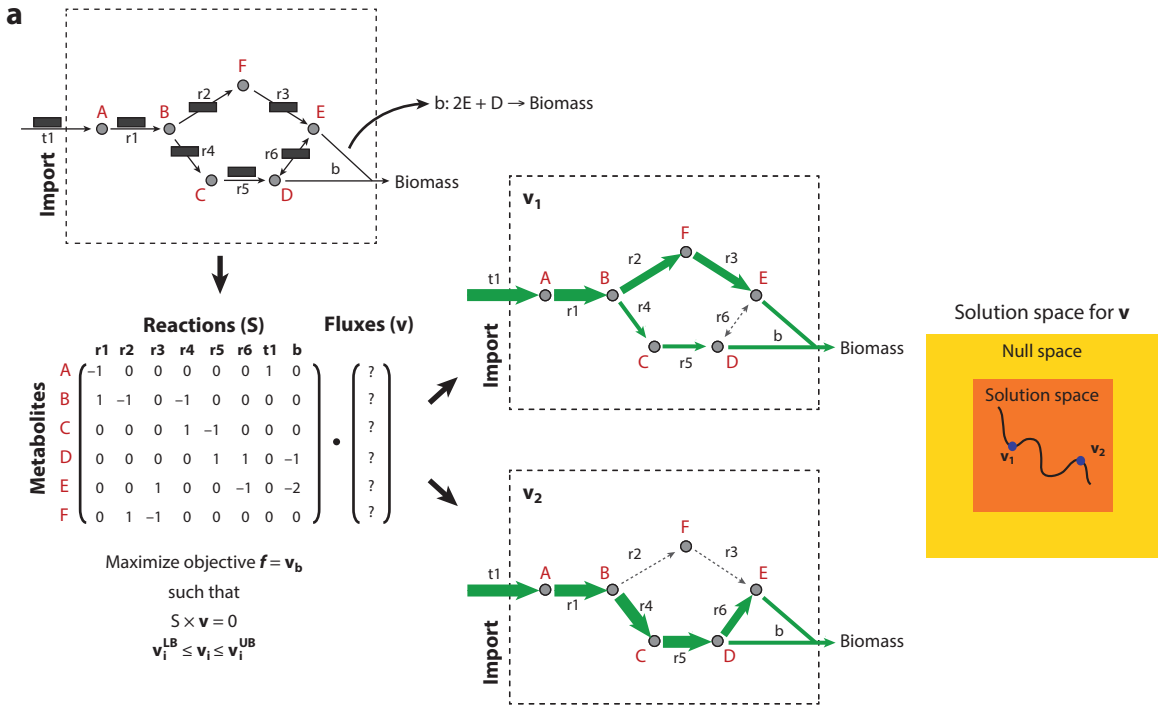
Metabolic regulation targets genes that encode metabolic enzymes or transporters. The output of this regulation is altered metabolic flux in regulated reactions and pathways, known as metabolic network rewiring. A major challenge is to interpret local metabolic network rewiring in the context of the entire metabolic network and to decipher how these rewiring events affect overall phenotypic outputs. To address this challenge, it has become prudent to reconstruct and mathematically model global metabolic networks for major model organisms and humans. In the following section, we first summarize the general principles of metabolic network reconstruction and modeling, and then discuss the application of human metabolic models to the generation of tissue- and condition-specific metabolic networks and to the resolution of metabolic changes associated with disease.

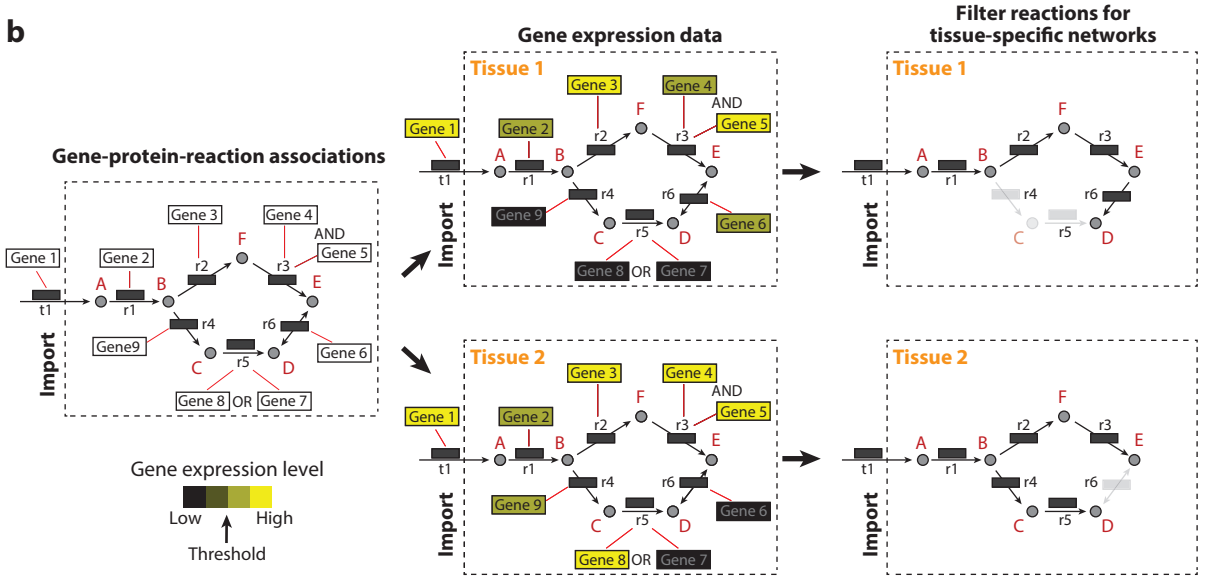
Building Mathematical Models of Genome-Scale Metabolic Networks

Reconstruction of a metabolic model begins with annotation of gene-protein-reaction (GPR) associations from the literature and from bioinformatic analysis of gene sequences (131). Reaction sets for annotated enzymes are derived from several databases, including Brenda (131) and KEGG (75). Boolean language (AND, OR) is used to describe GPRs. For instance, if two isozymes can catalyze the same reaction, they are associated with OR logic to that reaction, and enzyme complex subunits that work in concert but are encoded by different genes are associated to their reaction with AND logic (143). Although most metabolic network modeling efforts to date have targeted microorganisms (44), genome-scale reconstructions have also been built for several metazoans, including humans (40, 92, 144), mouse (138), *Arabidopsis* (37), and zebrafish (10). The most recent reconstruction of the human metabolic network is based on 1,789 enzyme-encoding genes, with a total of 7,440 reactions annotated, including those that transport metabolites between eight compartments and involving 2,626 unique metabolites (144).

An ideal metabolic model can take the composition of nutrients from diet as input and generates as output a range of flux distributions that satisfy experimentally determined measurements of biomass composition and energy requirements of the organism. This is mathematically achieved by representing the network as a stoichiometry (S) matrix that consists of the coefficients of metabolites (rows) in corresponding reactions (columns), such that these coefficients are negative for the reactants and positive for the products of a given reaction (see the toy network in **Figure 2*a***). This matrix is multiplied by a vector of fluxes (v), and the equation is set to equal zero, representing mass balance under steady state conditions (i.e., at every compound node, the total flux of consumption matches that of production). The solution of this equation alone gives the null space of the S matrix. To narrow down the solution to a biologically meaningful space of feasible fluxes, a set of reaction constraints are applied based on available information of the biochemistry. In particular, irreversible reactions are limited by a lower boundary of zero, i.e., flux in the reverse direction is disallowed. In addition, an objective function is set to obtain a

particular flux distribution within this biological solution space to achieve a defined goal, such as the maximization of growth (i.e., the flux of a biomass assembly reaction) or maximization of energy generation. This method is referred to as constraint-based flux balance analysis (FBA) (136). Importantly, after typical constraints are imposed, the same objective may still be achieved by multiple flux distributions, which implicates the existence of alternate pathways (**Figure 2*a***).





Then, additional constraints based on experimental data, such as gene expression levels informing whether a subset of reactions carry flux or not, can be utilized to narrow down the solution space further by eliminating or favoring some of the alternative pathways (**Figure 2*b***).

Using "Omics" Data to Extract Tissue- and Condition-Specific Metabolic Network Models

One way to address the regulation of metabolic networks is to extract from the generic metabolic network of an organism the metabolically rewired states that are tightly regulated, for instance, in different tissues or under specific conditions. Current methods of extraction constrain the global network models with tissue- or condition-specific experimental evidence, including gene expression data (**Figure 2*b***). For instance, mixed integer linear programming was used to penalize FBA for introducing flux in reactions associated with lowly expressed genes and for lack of flux in reactions associated with highly expressed genes, and this way, flux distributions in 10 human tissues were predicted based on transcriptomic and proteomic data sets (136). A more advanced and systematic optimization procedure called the model-building algorithm (MBA) was later developed to take into account not only expression data but also other tissue-specific information, such as phenotypic data and the presence of tissue-specific metabolites (71). This method uses sets of reactions grouped according to their probability of being functional in the targeted tissue based on experimental information, and optimizes the metabolic network to maximize the number of reactions in higher probability categories while minimizing those that are unlikely to be present. MBA was successfully used to derive a liver metabolic network that outperforms the generic human model in predicting hepatic fluxes in different conditions and changes in biomarkers of hepatic disorders related to metabolism (71).

Several other methods for deriving tissue-specific metabolic networks have been developed and validated since the development of MBA, including GIMME, which uses gene expression data combined with objective functions such as energy generation (9), mCADRE, which is similar to MBA (152), and INIT, which is also similar to MBA with a key exception that it allows flux imbalance to address metabolomics data (2). More about tissue-specific applications of metabolic network modeling can be found in a recent review (124).

Constraint-Based Metabolic Models of Cancer

Cancer cells exhibit major metabolic network rewiring in central carbon pathways that allow uncontrolled proliferation. Although the switch from OXPHOS to aerobic glycolysis in cancer cells

Figure 2

General principles of metabolic network modeling. (*a*) Toy network comprising metabolites (*red letters*, *gray circles*) converted by reactions (*black letters*, *black rectangles*). The metabolic network is designated by the stoichiometry (S) matrix with rows representing metabolites and columns representing reactions. The values depict the stoichiometric ratios of metabolites that are consumed (*negative values*) and produced (*positive values*) by reactions. Flux distribution (v) is determined by flux balance analysis (FBA), which satisfies mass balance at every node (compound) by the steady state equation ($S \times \mathbf{v} = 0$), while imposing the boundary conditions on v and maximizing the objective function (rate of reaction $2E + D \rightarrow$ Biomass). Shown on the right is the solution space without any constraints (*yellow*), biologically meaningful subspace with constraints (*orange*), and the landscape of alternative solutions that satisfy the maximum objective function (*black curve*). Two alternative solutions are shown. Arrow thickness and direction depict flux magnitude and direction, respectively. (*b*) Gene-protein-reaction associations for the toy network from panel *a*, represented using Boolean language. Gene expression data (*colored in a scale from low to high*) can be used to constrain fluxes within the global metabolic network and to generate tissue- or condition-specific networks. Two different conditions are shown with corresponding flux distributions that best fit the expression data.

was first described by Otto Warburg more than 90 years ago, we are still determining its causes and consequences. It seems counterintuitive that rapidly proliferating cancer cells would prefer a less-efficient glucose catabolism with respect to ATP production (i.e., 1 mole of glucose yields 2 moles ATP from glycolysis versus 32 moles ATP from OXPHOS) because proliferating cells still require copious amounts of ATP. Recently, metabolic modeling of human cancer has been applied to explore Warburg-like metabolic rewiring. Cancer metabolic models have accurately predicted a preference for flux through the glycolysis-branching pentose phosphate pathway to supply the cell with reducing equivalents (NADPH) for fatty acid synthesis and nucleotide biosynthesis precursors. Other studies utilizing either small kinetic (148) or global stoichiometric (135) metabolic models of cancer have arrived at the same conclusion, which is that in the context of rapid proliferation, glycolysis alone is actually more energetically efficient than OXPHOS if the costs of enzyme synthesis and molecular crowding are taken into account. By constraining a global stoichiometric model with gene expression data, cancer metabolic models have been built for individual tumors to define metabolic phenotypes of premalignant versus malignant tumors (premalignant models actually supported higher growth rates) and estrogen receptor (ER)+ versus ER− breast cancers (72).

Metabolic modeling of an engineered fumarase-deficient cell line was used to generate hypotheses that can explain how fumarase-deficient tumors manage to fuel the electron transport chain without a functional TCA cycle (48). This modeling discovered that these cells compensate for lack of NADH production by synthesizing and degrading heme. Fumarase deficiency leads to a buildup of fumarate, which is believed to act as an oncometabolite by inhibiting prolyl-hydroxylases from activating HIF-1 under normoxic conditions, leading to Warburg-like glucose metabolism and enhanced vascularization via HIF-1-mediated VEGF activation (48). Whether Warburg-like metabolism is a driver or an enabler of tumorigenesis is a subject of debate. Certainly, many proliferating cells exhibit aerobic glycolysis and do not become tumorigenic. Simultaneously, there is compelling evidence supporting the notion that metabolic disruptions such as fumarase deficiency, succinate dehydrogenase deficiency, and neomorphic mutations in isocitrate dehydrogenase (114) can be oncogenic. Interestingly, these metabolic disturbances are each associated with specific types of cancer, suggesting context dependence for the transformative properties of the putative oncometabolites fumarate, succinate, and 2-hydroxyglutarate. Recently, cancer type–specific metabolic models were built and queried with commonly detected cancer type–specific enzyme mutations to explore the context dependence of known oncometabolites and predict novel oncometabolites, resulting in 15 newly predicted oncometabolites (112). Another study utilized constraint-based models of tumor cell metabolism to predict novel drug targets by determining cancer-specific synthetic lethal relationships within the metabolic network (46).

NOVEL PARADIGMS IN METABOLIC NETWORK FUNCTION AND REGULATION FROM *CAENORHABDITIS ELEGANS*

Although metabolic modeling compiles known metabolic data into a mathematical framework, it has limited ability to predict novel metabolic functions and pathways, and cannot critically assess the assignment of genes to reactions. This was illustrated in a recent study of metabolic models, which found that metabolic network reconstruction similarity between organisms did not correlate well with genome similarity or phylogenetic distance, and often metabolically diverse organisms had highly similar metabolic network models (107). This is largely due to the fact that metabolic models are often built upon the framework of an existing model (107), and reactions and pathways outside of common central metabolic pathways are largely unexplored in most species, biochemically or genetically. Advancement in metabolic modeling will require complementary

efforts in biochemistry and genetics to expand our knowledge about metabolism. Here, we focus on recent studies that have taken advantage of the genetic tractability of the nematode *C. elegans* to generate novel insights into metabolic gene function and regulation.

Caenorhabditis elegans Genetics Identify Novel Enzyme Activities and Biological Significance

C. elegans is a bacterivorous soil-dwelling nematode, consisting of 959 somatic cells, whose short life cycle (3 days) and genetic tractability make it amenable to high-throughput genetic screening. Much of the human metabolic network is conserved in *C. elegans*, which also has similar essential nutrient requirements, devoted metabolic tissues (primarily the intestine, comprising 20 cells), and conserved metabolic regulatory pathways such as the insulin and TOR signaling pathways (14). In fact, the regulation of FOXO by insulin signaling and its relationship to aging were identified in *C. elegans* (115), as was the regulation of HIF-1 by prolyl-hydroxylases (42).

C. elegans can be used to genetically dissect metabolic phenotypes and explore metabolic enzymes of unknown function. For instance, ACER-1, a conserved protein of unknown function, was identified as a novel acetyl-CoA hydrolase (converting acetyl-CoA into acetate and CoA) in a search for binding partners of CRA-1, a conserved regulator of global histone acetylation, which functions by an unknown mechanism (49). ACER-1 was found to bind and affect CRA-1-regulated histone acetylation, and metabolomics revealed elevated acetyl-CoA levels in an *acer-1* mutant. Thus, a mechanism was proposed whereby CRA-1 regulates the levels of acetyl-CoA in the nucleus and thus histone acetylation by antagonizing ACER-1 acetyl-CoA hydrolase activity (49). Another protein with novel metabolic activity identified in *C. elegans* is the GDP-D-glucose phosphorylase C10F3.4, which converts GDP-D-glucose to GDP and D-glucose-1-phosphate (1). *C. elegans* mutants lacking C10F3.4 lost all GDP-D-glucose phosphorylase activity and built up the substrate GDP-D-glucose. The mouse ortholog of C10F3.4 was shown to have the same catalytic activity and to exhibit similar tissue expression patterns as *C. elegans*, with highest expression in neuronal and reproductive tissues (1). The exact function of GDP-D-glucose phosphorylation is unknown but was postulated to be a metabolite repair reaction to deplete GDP-D-glucose produced nonspecifically by the enzyme GDP-d-Man pyrophosphorylase, thus preventing the misincorporation of GDP-D-glucose into glycoproteins (1).

Other studies have identified new biological roles for known enzymes and have revealed novel relationships between metabolic pathways and cellular processes. For instance, mutation of tyrosine aminotransferase (*tatn-1*; breakdown of tyrosine to acetoacetate and fumarate) resulted in gene expression changes and enhanced the lifespan extension of insulin-signaling mutants (45). These effects were largely due to elevated tyrosine levels, which activated AMPK to repress FOXO (*daf-16*) (45). Another study identified a diet-specific phenotype for the proline catabolic enzyme *alh-6*, which is the ortholog of human 1-pyrroline-5-carboxylate (P5C) dehydrogenase (P5CDH). *alh-6* mutants exhibited reduced lifespan on the standard laboratory diet of *Escherichia coli* OP50 but on a diet of *E. coli* HT115 *alh-6* mutants and wild-type animals exhibited identical lifespans (117). The specific metabolic differences between these two *E. coli* strains as they relate to the lifespan of the *alh-6* mutant are unknown, although metabolomic profiling has revealed myriad differences in metabolite levels. The buildup of the *alh-6* substrate P5C was proposed to be the cause of premature aging in the mutant because knockdown of the upstream enzyme proline dehydrogenase, which produces P5C from proline, rescued some of the effects of the *alh-6* mutant on aging (117). Thus, a novel role for proline catabolism in metabolic flexibility, healthy aging, and adaptation to dietary changes was established, although the mechanisms underlying these roles are unknown.

Caenorhabditis elegans Genetics Reveal Factors Involved in Metabolic Response to Diet and the Microbiota

Diet and microbiota composition have profound impacts on host metabolic network regulation and function; however, these impacts are difficult to investigate because of the complexity and diversity of dietary inputs, as well as the genetic complexity and diversity within the human population and microbiota. A major advantage of using *C. elegans* is the ability to genetically dissect the regulatory mechanisms that enable metabolic network rewiring in response to changes in the bacterial diet and the resulting physiological outputs. For instance, *C. elegans* has been used extensively to study the effects of caloric restriction on metabolism and lifespan, and more recently to delineate regulatory networks controlling metabolic responses to dietary composition. The genetic tractability of both *C. elegans* and its bacterial diets renders this natural predator-prey system an excellent model to study the principles of metabolic network regulation in response to diet, as well as to model the effects of commensal bacteria on host metabolism and physiology (16, 97, 157). Recently, it was discovered that the lifespan-extending effect of the antidiabetic drug metformin on *C. elegans* is due to alteration of *E. coli* folate metabolism, which affects flux through the folate-dependent one-carbon cycle in *C. elegans*, as demonstrated through genetic inactivation of *C. elegans* one-carbon-cycle genes (15). Another study serendipitously isolated a spontaneous mutation in *E. coli* aroD, a shikimic acid pathway gene required for folate precursor biosynthesis, when screening an RNAi feeding library for *C. elegans* lifespan extension (149), further demonstrating the link between microbial folate production and *C. elegans* longevity.

C. elegans has been fed a variety of metabolically diverse bacterial species in the lab, and the physiological and gene expression responses in *C. elegans* have been measured (28, 98). In one study, many of the genes that exhibit diet-induced expression changes were found to confer diet-specific phenotypes when mutated (28). Genetic inactivation of the regulators of metabolic gene expression can also confer diet-specific phenotypes. For instance, mutations in the nuclear hormone receptor *nhr-114* lead to complete sterility caused by a defective germline on a diet of *E. coli* OP50; however, *nhr-114* mutants develop intact germlines and are fertile when fed *E. coli* HT115 (52). As with the *alh-6* example, the differences between *E. coli* OP50 and HT115 that determine *nhr-114* sterility are unknown, although supplementation of *E. coli* OP50 with tryptophan could rescue the *nhr-114* sterility phenotype on this diet (52). A challenge for the future will be to determine the causal factors among a sea of differences in nutrient compositions between different bacterial diets that drive effects in the animal.

Recently, interspecies genetics has been used to address this very challenge. Genetic screening in both *C. elegans* and its bacterial diets, *Comamonas aquatica* and *E. coli*, revealed bacterially produced vitamin B12 as the driver of several *C. elegans* gene expression and physiological changes (154). These screens employed a transgenic *C. elegans* reporter strain, in which green fluorescent protein expression is driven by the promoter of the *C. elegans* gene *acdh-1*, which is strongly repressed by the *Comamonas* diet but activated by the *E. coli* diet (98). Mutations in *Comamonas* vitamin B12 biosynthetic enzymes were identified in strains that failed to repress *acdh-1* promoter activity. Exogenous supplementation of B12 was sufficient to repress *acdh-1* expression on the low-B12 diet of *E. coli* OP50. Networks of *C. elegans* metabolic and regulatory genes, including nuclear hormone receptors, were required for the repression of the *acdh-1* promoter by the vitamin B12–synthesizing *Comamonas* diet (153). Interestingly, the vitamin B12–mediated repression of *acdh-1* was dependent on flux through the vitamin B12–requiring propionate breakdown pathway, as mutants in this pathway resulted in constitutively activated *acdh-1* expression (154). These genes could also be activated by exogenously supplied propionate, and deletion of *acdh-1* increases sensitivity to propionate-induced toxicity (154), suggesting that this gene may be involved in novel

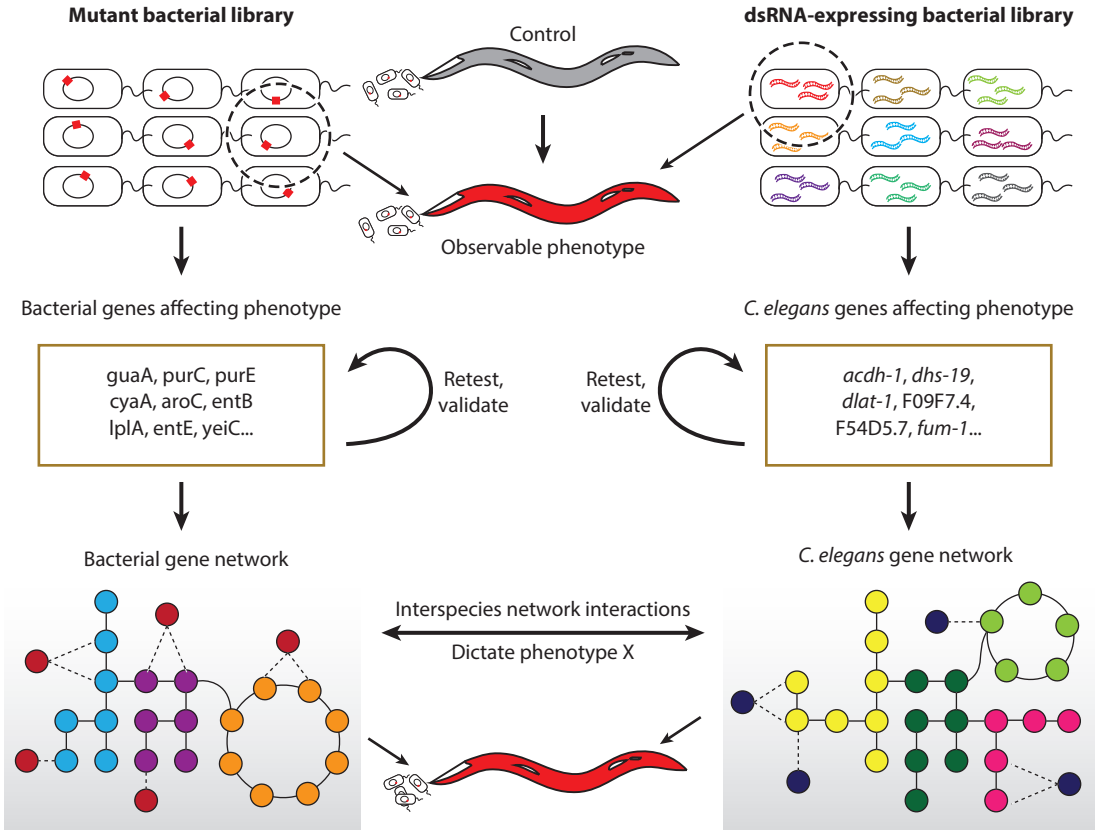


Figure 3

Dissecting diet-related phenotypes in *Caenorhabditis elegans* with interspecies genetics. To determine gene networks involved in diet-related phenotypes in *C. elegans*, genetic screens can be performed in both the bacterial diet and in the animal. *C. elegans* can be fed a library of mutant bacteria to determine bacterial genes that, when mutated, lead to a particular phenotype. To identify *C. elegans* genes that are involved in the diet-associated phenotype, animals can be fed a library of dsRNA-expressing bacteria targeting individual *C. elegans* genes. Hits from either screen should be retested and validated, and can be built into gene networks based on coexpression, cocomplex formation, and/or genetic interaction data, or metabolic pathway annotations. Hypotheses about interspecies gene network interactions and their consequences with respect to the metabolic phenotype can be generated and further tested.

alternative propionate breakdown or detoxification mechanisms on low-B12 diets. This approach utilizing interspecies systems biology serves as an example of how the effects of nutrient composition within complex biotic diets on animal gene expression and physiology can be elucidated (**Figure 3**).

CONCLUSIONS AND FUTURE DIRECTIONS

Metabolic network modeling can benefit from improvements both in the biological knowledge that is integrated into the model and in the computational methods of such integration (107). Although utilization of gene expression data to predict condition-specific fluxes has been advanced by numerous methods (94, 112), techniques to integrate many other types of data sets of potential importance are in the early stages. In particular, methods that constrain FBA with metabolomics data

are emerging (148). Other challenges include the integration of kinetic parameters and metabolite concentrations. Kinetic parameters of central carbon pathways have been incorporated into the *E. coli* model (32). Certain human pathways, such as the one-carbon cycle (113), have also been studied using kinetic models equipped with experimentally determined parameters. Thus, it is reasonable to expect the integration of kinetic data into genome-scale models of metazoan metabolism.

Perhaps the greatest future challenge for genome-scale metabolic network modeling with the greatest potential benefit is the direct incorporation of regulatory networks (such as the regulatory nFBLs discussed in this review) into the model. An existing method named regulatory FBA converts gene regulatory pathways to a Boolean formalism that dictates "on" or "off" states of reactions within stoichiometric metabolic network models (31). This technique has been used to incorporate regulatory networks of *E. coli* (30) and yeast (64) into the respective genome-scale network models. Other Boolean formalism–based methods (6, 22, 32, 43, 51, 137), as well as a novel stochastic approach (22), have also been developed, but applications have been limited to microorganisms to date. Direct integration of metazoan regulatory networks into genome-scale metabolic models will likely await better characterization of the paths from regulators to enzymatic reactions, which is dependent on protein-metabolite, protein-protein, and protein-DNA interactions.

Recently, our lab has reconstructed a global metabolic network model for *C. elegans* (L.S. Yilmaz & A.J.M. Walhout, in preparation). *C. elegans* provides a unique opportunity to rapidly test hypotheses generated by in silico FBA of the metabolic model, such as pathway usage on different diets. Genetic screening in *C. elegans* can be used to improve GPR annotations and, by extrapolation, guide improvement of annotations within the human model.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Adler LN, Gomez TA, Clarke SG, Linster CL. 2011. A novel GDP-D-glucose phosphorylase involved in quality control of the nucleoside diphosphate sugar pool in *Caenorhabditis elegans* and mammals. *J. Biol. Chem.* 286:21511–23
2. Agren R, Bordel S, Mardinoglu A, Pornputtapong N, Nookaew I, Nielsen J. 2012. Reconstruction of genome-scale active metabolic networks for 69 human cell types and 16 cancer types using INIT. *PLoS Comput. Biol.* 8:e1002518
3. Ahmad MF, Dealwis CG. 2013. The structural basis for the allosteric regulation of ribonucleotide reductase. *Prog. Mol. Biol. Transl. Sci.* 117:389–410
4. Andreini C, Banci L, Bertini I, Rosato A. 2006. Counting the zinc-proteins encoded in the human genome. *J. Proteome Res.* 5:196–201
5. Bar-Peled L, Sabatini DM. 2014. Regulation of mTORC1 by amino acids. *Trends Cell Biol.* 24:400–6
6. Barua D, Kim J, Reed JL. 2010. An automated phenotype-driven approach (GeneForce) for refining metabolic and regulatory models. *PLoS Comput. Biol.* 6:e1000970
7. Barua S, Chadman KK, Kuizon S, Buenaventura D, Stapley NW, et al. 2014. Increasing maternal or post-weaning folic acid alters gene expression and moderately changes behavior in the offspring. *PLoS ONE* 9:e101674
8. B'Chir W, Maurin AC, Carraro V, Averous J, Jousse C, et al. 2013. The eIF2α/ATF4 pathway is essential for stress-induced autophagy gene expression. *Nucleic Acids Res.* 41:7683–99
9. Becker SA, Palsson BO. 2008. Context-specific metabolic networks are consistent with experiments. *PLoS Comput. Biol.* 4:e1000082

10. Bekaert M. 2012. Reconstruction of *Danio rerio* metabolic model accounting for subcellular compartmentalisation. *PLoS ONE* 7:e49903
11. Berrabah W, Aumercier P, Lefebvre P, Staels B. 2011. Control of nuclear receptor activities in metabolism by post-translational modifications. *FEBS Lett.* 585:1640–50
12. Bester AC, Roniger M, Oren YS, Im MM, Sarni D, et al. 2011. Nucleotide deficiency promotes genomic instability in early stages of cancer development. *Cell* 145:435–46
13. Bjorklund S, Skog S, Tribukait B, Thelander L. 1990. S-phase-specific expression of mammalian ribonucleotide reductase R1 and R2 subunit mRNAs. *Biochemistry* 29:5452–58
14. Braeckman BP, Houthoofd K, Vanfleteren JR. 2009. Intermediary metabolism. *WormBook*. doi: 10.1895/wormbook.1.146.1
15. Cabreiro F, Au C, Leung KY, Vergara-Irigaray N, Cocheme HM, et al. 2013. Metformin retards aging in *C. elegans* by altering microbial folate and methionine metabolism. *Cell* 153:228–39
16. Cabreiro F, Gems D. 2013. Worms need microbes too: microbiota, health and aging in *Caenorhabditis elegans*. *EMBO Mol. Med.* 5:1300–10
17. Canali R, Natarelli L, Leoni G, Azzini E, Comitato R, et al. 2014. Vitamin C supplementation modulates gene expression in peripheral blood mononuclear cells specifically upon an inflammatory stimulus: a pilot study in healthy subjects. *Genes Nutr.* 9:390
18. Carlberg C, Seuter S. 2009. A genomic perspective on vitamin D signaling. *Anticancer Res.* 29:3485–93
19. Chabes AL, Pfleger CM, Kirschner MW, Thelander L. 2003. Mouse ribonucleotide reductase R2 protein: a new target for anaphase-promoting complex-Cdh1-mediated proteolysis. *PNAS* 100:3925–29
20. Chakravarthy MV, Lodhi IJ, Yin L, Malapaka RR, Xu HE, et al. 2009. Identification of a physiologically relevant endogenous ligand for PPARα in liver. *Cell* 138:476–88
21. Champier J, Claustrat F, Nazaret N, Fevre Montange M, Claustrat B. 2012. Folate depletion changes gene expression of fatty acid metabolism, DNA synthesis, and circadian cycle in male mice. *Nutr. Res.* 32:124–32
22. Chandrasekaran S, Price ND. 2010. Probabilistic integrative modeling of genome-scale metabolic and regulatory networks in *Escherichia coli* and *Mycobacterium tuberculosis*. *PNAS* 107:17845–50
23. Cheng Z, Tseng Y, White MF. 2010. Insulin signaling meets mitochondria in metabolism. *Trends Endocrinol. Metab.* 21:589–98
24. Cheung PC, Salt IP, Davies SP, Hardie DG, Carling D. 2000. Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. *Biochem. J.* 346(Pt. 3):659–69
25. Choi S, Bird AJ. 2014. Zinc'ing sensibly: controlling zinc homeostasis at the transcriptional level. *Metallomics Integr. Biometal Sci.* 6:1198–215
26. Choi S, Yoon H, Oh KS, Oh YT, Kim YI, et al. 2011. Widespread effects of nicotinic acid on gene expression in insulin-sensitive tissues: implications for unwanted effects of nicotinic acid treatment. *Metabolism* 60:134–44
27. Cichy SB, Uddin S, Danilkovich A, Guo S, Klippel A, Unterman TG. 1998. Protein kinase B/Akt mediates effects of insulin on hepatic insulin-like growth factor-binding protein-1 gene expression through a conserved insulin response sequence. *J. Biol. Chem.* 273:6482–87
28. Coolon JD, Jones KL, Todd TC, Carr BC, Herman MA. 2009. *Caenorhabditis elegans* genomic response to soil bacteria predicts environment-specific genetic effects on life history traits. *PLoS Genet.* 5:e1000503
29. Couturier A, Keller J, Most E, Ringseis R, Eder K. 2014. Niacin in pharmacological doses alters microRNA expression in skeletal muscle of obese Zucker rats. *PLoS ONE* 9:e98313
30. Covert MW, Knight EM, Reed JL, Herrgard MJ, Palsson BO. 2004. Integrating high-throughput and computational data elucidates bacterial networks. *Nature* 429:92–96
31. Covert MW, Schilling CH, Palsson B. 2001. Regulation of gene expression in flux balance models of metabolism. *J. Theor. Biol.* 213:73–88
32. Covert MW, Xiao N, Chen TJ, Karr JR. 2008. Integrating metabolic, transcriptional regulatory and signal transduction models in *Escherichia coli*. *Bioinformatics* 24:2044–50
33. Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG. 2005. Algae acquire vitamin B12 through a symbiotic relationship with bacteria. *Nature* 438:90–93

34. Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. 1995. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 378:785–89
35. D'Aniello E, Waxman JS. 2015. Input overload: contributions of retinoic acid signaling feedback mechanisms to heart development and teratogenesis. *Dev. Dyn.* 244:513–23
36. David CJ, Chen M, Assanah M, Canoll P, Manley JL. 2010. HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. *Nature* 463:364–68
37. de Oliveira Dal'Molin CG, Quek LE, Palfreyman RW, Brumbley SM, Nielsen LK. 2010. AraGEM, a genome-scale reconstruction of the primary metabolic network in *Arabidopsis*. *Plant Physiol.* 152:579–89
38. di Masi A, Leboffe L, De Marinis E, Pagano F, Cicconi L, et al. 2015. Retinoic acid receptors: from molecular mechanisms to cancer therapy. *Mol. Asp. Med.* 41:1–115
39. Dong XY, Tang SQ, Chen JD. 2012. Dual functions of Insig proteins in cholesterol homeostasis. *Lipids Health Dis.* 11:173
40. Duarte NC, Becker SA, Jamshidi N, Thiele I, Mo ML, et al. 2007. Global reconstruction of the human metabolic network based on genomic and bibliomic data. *PNAS* 104:1777–82
41. Efrat S, Tal M, Lodish HF. 1994. The pancreatic beta-cell glucose sensor. *Trends Biochem. Sci.* 19:535–38
42. Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, et al. 2001. *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* 107:43–54
43. Faria JP, Overbeek R, Xia F, Rocha M, Rocha I, Henry CS. 2014. Genome-scale bacterial transcriptional regulatory networks: reconstruction and integrated analysis with metabolic models. *Brief. Bioinform.* 15:592–611
44. Feist AM, Herrgard MJ, Thiele I, Reed JL, Palsson BO. 2009. Reconstruction of biochemical networks in microorganisms. *Nat. Rev. Microbiol.* 7:129–43
45. Ferguson AA, Roy S, Kormanik KN, Kim Y, Dumas KJ, et al. 2013. TATN-1 mutations reveal a novel role for tyrosine as a metabolic signal that influences developmental decisions and longevity in *Caenorhabditis elegans*. *PLoS Genet.* 9:e1004020
46. Folger O, Jerby L, Frezza C, Gottlieb E, Ruppin E, Shlomi T. 2011. Predicting selective drug targets in cancer through metabolic networks. *Mol. Syst. Biol.* 7:501
47. Fraser DA, Hessvik NP, Nikolic N, Aas V, Hanssen KF, et al. 2012. Benfotiamine increases glucose oxidation and downregulates NADPH oxidase 4 expression in cultured human myotubes exposed to both normal and high glucose concentrations. *Genes Nutr.* 7:459–69
48. Frezza C, Zheng L, Tennant DA, Papkovsky DB, Hedley BA, et al. 2011. Metabolic profiling of hypoxic cells revealed a catabolic signature required for cell survival. *PLoS ONE* 6:e24411
49. Gao J, Kim HM, Elia AE, Elledge SJ, Colaiacovo MP. 2015. NatB domain-containing CRA-1 antagonizes hydrolase ACER-1 linking Acetyl-CoA metabolism to the initiation of recombination during *C. elegans* meiosis. *PLoS Genet.* 11:e1005029
50. Giammona LM, Fuhrken PG, Papoutsakis ET, Miller WM. 2006. Nicotinamide (vitamin B3) increases the polyploidisation and proplatelet formation of cultured primary human megakaryocytes. *Br. J. Haematol.* 135:554–66
51. Goelzer A, Bekkal Brikci F, Martin-Verstraete I, Noirot P, Bessieres P, et al. 2008. Reconstruction and analysis of the genetic and metabolic regulatory networks of the central metabolism of *Bacillus subtilis*. *BMC Syst. Biol.* 2:20
52. Gracida X, Eckmann CR. 2013. Fertility and germline stem cell maintenance under different diets requires nhr-114/HNF4 in *C. elegans*. *Curr. Biol.* 23:607–13
53. Greer EL, Oskoui PR, Banko MR, Maniar JM, Gygi MP, et al. 2007. The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. *J. Biol. Chem.* 282:30107–19
54. Gunther V, Lindert U, Schaffner W. 2012. The taste of heavy metals: gene regulation by MTF-1. *Biochim. Biophys. Acta* 1823:1416–25
55. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, et al. 2008. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol. Cell* 30:214–26

56. Han JM, Jeong SJ, Park MC, Kim G, Kwon NH, et al. 2012. Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway. *Cell* 149:410–24
57. Harada H, Itasaka S, Kizaka-Kondoh S, Shibuya K, Morinibu A, et al. 2009. The Akt/mTOR pathway assures the synthesis of HIF-1α protein in a glucose- and reoxygenation-dependent manner in irradiated tumors. *J. Biol. Chem.* 284:5332–42
58. Hardie DG. 2014. AMPK: positive and negative regulation, and its role in whole-body energy homeostasis. *Curr. Opin. Cell Biol.* 33C:1–7
59. Haussler MR, Haussler CA, Bartik L, Whitfield GK, Hsieh JC, et al. 2008. Vitamin D receptor: molecular signaling and actions of nutritional ligands in disease prevention. *Nutr. Rev.* 66:S98–112
60. Hawley SA, Boudeau J, Reid JL, Mustard KJ, Udd L, et al. 2003. Complexes between the LKB1 tumor suppressor, STRADα/β and MO25α/β are upstream kinases in the AMP-activated protein kinase cascade. *J. Biol.* 2:28
61. Hay N, Sonenberg N. 2004. Upstream and downstream of mTOR. *Genes Dev.* 18:1926–45
62. Helliwell KE, Wheeler GL, Smith AG. 2013. Widespread decay of vitamin-related pathways: coincidence or consequence? *Trends Genet.* 29:469–78
63. Henquin JC. 2011. The dual control of insulin secretion by glucose involves triggering and amplifying pathways in β-cells. *Diabetes Res. Clin. Pract.* 93(Suppl. 1):S27–31
64. Herrgard MJ, Lee BS, Portnoy V, Palsson BO. 2006. Integrated analysis of regulatory and metabolic networks reveals novel regulatory mechanisms in *Saccharomyces cerevisiae*. *Genome Res.* 16:627–35
65. Hinnebusch AG. 2005. Translational regulation of GCN4 and the general amino acid control of yeast. *Annu. Rev. Microbiol.* 59:407–50
66. Hofer A, Crona M, Logan DT, Sjoberg BM. 2012. DNA building blocks: keeping control of manufacture. *Crit. Rev. Biochem. Mol. Biol.* 47:50–63
67. Holz MK, Ballif BA, Gygi SP, Blenis J. 2005. mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. *Cell* 123:569–80
68. Horton JD, Goldstein JL, Brown MS. 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Investig.* 109:1125–31
69. Inoki K, Li Y, Zhu T, Wu J, Guan KL. 2002. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat. Cell Biol.* 4:648–57
70. Inoki K, Zhu T, Guan KL. 2003. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 115:577–90
71. Jerby L, Shlomi T, Ruppin E. 2010. Computational reconstruction of tissue-specific metabolic models: application to human liver metabolism. *Mol. Syst. Biol.* 6:401
72. Jerby L, Wolf L, Denkert C, Stein GY, Hilvo M, et al. 2012. Metabolic associations of reduced proliferation and oxidative stress in advanced breast cancer. *Cancer Res.* 72:5712–20
73. Jewell JL, Kim YC, Russell RC, Yu FX, Park HW, et al. 2015. Differential regulation of mTORC1 by leucine and glutamine. *Science* 347:194–98
74. Jun HJ, Kim S, Dawson K, Choi DW, Kim JS, et al. 2011. Effects of acute oral administration of vitamin C on the mouse liver transcriptome. *J. Med. Food* 14:181–94
75. Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M. 2012. KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Res.* 40:D109–14
76. Kanzaki M. 2006. Insulin receptor signals regulating GLUT4 translocation and actin dynamics. *Endocr. J.* 53:267–93
77. Karagianni P, Talianidis I. 2015. Transcription factor networks regulating hepatic fatty acid metabolism. *Biochim. Biophys. Acta* 1851:2–8
78. Kersten S. 2014. Integrated physiology and systems biology of PPARα. *Mol. Metab.* 3:354–71
79. Kim SG, Buel GR, Blenis J. 2013. Nutrient regulation of the mTOR complex 1 signaling pathway. *Mol. Cells* 35:463–73
80. Kitano H. 2004. Biological robustness. *Nat. Rev. Genet.* 5:826–37
81. Kotas ME, Medzhitov R. 2015. Homeostasis, inflammation, and disease susceptibility. *Cell* 160:816–27
82. Kousteni S. 2012. FoxO1, the transcriptional chief of staff of energy metabolism. *Bone* 50:437–43

83. Landrier JF, Gouranton E, Reboul E, Cardinault N, El Yazidi C, et al. 2010. Vitamin E decreases endogenous cholesterol synthesis and apo-AI-mediated cholesterol secretion in Caco-2 cells. *J. Nutr. Biochem.* 21:1207–13
84. LeBlanc JG, Milani C, de Giori GS, Sesma F, van Sinderen D, Ventura M. 2013. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr. Opin. Biotechnol.* 24:160–68
85. Li Y, Xu S, Mihaylova MM, Zheng B, Hou X, et al. 2011. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab.* 13:376–88
86. Liberles JS, Thorolfsson M, Martinez A. 2005. Allosteric mechanisms in ACT domain containing enzymes involved in amino acid metabolism. *Amino Acids* 28:1–12
87. Lin YW, Wang JL, Chen HM, Zhang YJ, Lu R, et al. 2011. Folic acid supplementary reduce the incidence of adenocarcinoma in a mouse model of colorectal cancer: microarray gene expression profile. *J. Exp. Clin. Cancer Res.* 30:116
88. Liu S, Stromberg A, Tai HH, Moscow JA. 2004. Thiamine transporter gene expression and exogenous thiamine modulate the expression of genes involved in drug and prostaglandin metabolism in breast cancer cells. *Mol. Cancer Res.* 2:477–87
89. Liu Y, Cox SR, Morita T, Kourembanas S. 1995. Hypoxia regulates vascular endothelial growth factor gene expression in endothelial cells. Identification of a 5′ enhancer. *Circ. Res.* 77:638–43
90. Liu YC, Li F, Handler J, Huang CR, Xiang Y, et al. 2008. Global regulation of nucleotide biosynthetic genes by c-Myc. *PLoS ONE* 3:e2722
91. Lushchak OV, Piroddi M, Galli F, Lushchak VI. 2014. Aconitase post-translational modification as a key in linkage between Krebs cycle, iron homeostasis, redox signaling, and metabolism of reactive oxygen species. *Redox Rep. Commun. Free Radic. Res.* 19:8–15
92. Ma H, Sorokin A, Mazein A, Selkov A, Selkov E, et al. 2007. The Edinburgh human metabolic network reconstruction and its functional analysis. *Mol. Syst. Biol.* 3:135
93. Ma W, Trusina A, El-Samad H, Lim WA, Tang C. 2009. Defining network topologies that can achieve biochemical adaptation. *Cell* 138:760–73
94. Machado D, Herrgard M. 2014. Systematic evaluation of methods for integration of transcriptomic data into constraint-based models of metabolism. *PLoS Comput. Biol.* 10:e1003580
95. MacKenzie ED, Selak MA, Tennant DA, Payne LJ, Crosby S, et al. 2007. Cell-permeating α-ketoglutarate derivatives alleviate pseudohypoxia in succinate dehydrogenase-deficient cells. *Mol. Cell. Biol.* 27:3282–89
96. MacNeil LT, Walhout AJ. 2011. Gene regulatory networks and the role of robustness and stochasticity in the control of gene expression. *Genome Res.* 21:645–57
97. MacNeil LT, Walhout AJ. 2013. Food, pathogen, signal: the multifaceted nature of a bacterial diet. *Worm* 2:e26454
98. MacNeil LT, Watson E, Arda HE, Zhu LJ, Walhout AJ. 2013. Diet-induced developmental acceleration independent of TOR and insulin in *C. elegans*. *Cell* 153:240–52
99. Magnuson B, Ekim B, Fingar DC. 2012. Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. *Biochem. J.* 441:1–21
100. Makpol S, Zainuddin A, Chua KH, Mohd Yusof YA, Ngah WZ. 2013. Gamma-tocotrienol modulated gene expression in senescent human diploid fibroblasts as revealed by microarray analysis. *Oxid. Med. Cell. Longev.* 2013:454328
101. Maret W. 2013. Zinc biochemistry: from a single zinc enzyme to a key element of life. *Adv. Nutr.* 4:82–91
102. Marin-Hernandez A, Gallardo-Perez JC, Ralph SJ, Rodriguez-Enriquez S, Moreno-Sanchez R. 2009. HIF-1α modulates energy metabolism in cancer cells by inducing over-expression of specific glycolytic isoforms. *Mini-Rev. Med. Chem.* 9:1084–101
103. McCall KA, Huang C, Fierke CA. 2000. Function and mechanism of zinc metalloenzymes. *J. Nutr.* 130:1437S–46
104. McKenna MC. 2011. Glutamate dehydrogenase in brain mitochondria: Do lipid modifications and transient metabolon formation influence enzyme activity? *Neurochem. Int.* 59:525–33
105. Menard L, Maughan D, Vigoreaux J. 2014. The structural and functional coordination of glycolytic enzymes in muscle: evidence of a metabolon? *Biology* 3:623–44

106. Mihaylova MM, Shaw RJ. 2011. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat. Cell Biol.* 13:1016–23
107. Monk J, Nogales J, Palsson BO. 2014. Optimizing genome-scale network reconstructions. *Nat. Biotechnol.* 32:447–52
108. Mottillo EP, Bloch AE, Leff T, Granneman JG. 2012. Lipolytic products activate peroxisome proliferator-activated receptor (PPAR) α and δ in brown adipocytes to match fatty acid oxidation with supply. *J. Biol. Chem.* 287:25038–48
109. Munday MR. 2002. Regulation of mammalian acetyl-CoA carboxylase. *Biochem. Soc. Trans.* 30:1059–64
110. Mustacich DJ, Gohil K, Bruno RS, Yan M, Leonard SW, et al. 2009. Alpha-tocopherol modulates genes involved in hepatic xenobiotic pathways in mice. *J. Nutr. Biochem.* 20:469–76
111. Nakano E, Mushtaq S, Heath PR, Lee ES, Bury JP, et al. 2011. Riboflavin depletion impairs cell proliferation in adult human duodenum: identification of potential effectors. *Dig. Dis. Sci.* 56:1007–19
112. Nam H, Campodonico M, Bordbar A, Hyduke DR, Kim S, et al. 2014. A systems approach to predict oncometabolites via context-specific genome-scale metabolic networks. *PLoS Comput. Biol.* 10:e1003837
113. Nijhout HF, Reed MC, Ulrich CM. 2008. Mathematical models of folate-mediated one-carbon metabolism. *Vitam. Horm.* 79:45–82
114. Nowicki S, Gottlieb E. 2015. Oncometabolites: tailoring our genes. *FEBS J.* 282:2796–805
115. Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, et al. 1997. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* 389:994–99
116. Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, et al. 2000. Ubiquitination of hypoxia-inducible factor requires direct binding to the β-domain of the von Hippel-Lindau protein. *Nat. Cell Biol.* 2:423–27
117. Pang S, Curran SP. 2014. Adaptive capacity to bacterial diet modulates aging in *C. elegans*. *Cell Metab.* 19:221–31
118. Peterson TR, Sengupta SS, Harris TE, Carmack AE, Kang SA, et al. 2011. mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. *Cell* 146:408–20
119. Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, et al. 2008. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. *Cell Metab.* 8:224–36
120. Ravi S, Schilder RJ, Kimball SR. 2015. Role of precursor mRNA splicing in nutrient-induced alterations in gene expression and metabolism. *J. Nutr.* 145:841–46
121. Robey IF, Lien AD, Welsh SJ, Baggett BK, Gillies RJ. 2005. Hypoxia-inducible factor-1α and the glycolytic phenotype in tumors. *Neoplasia* 7:324–30
122. Roh HC, Dimitrov I, Deshmukh K, Zhao G, Warnhoff K, et al. 2015. A modular system of DNA enhancer elements mediates tissue-specific activation of transcription by high dietary zinc in *C. elegans*. *Nucleic Acids Res.* 43:803–16
123. Rouault TA. 2002. Post-transcriptional regulation of human iron metabolism by iron regulatory proteins. *Blood Cells Mol. Dis.* 29:309–14
124. Ryu JY, Kim HU, Lee SY. 2015. Reconstruction of genome-scale human metabolic models using omics data. *Integr. Biol.* 7:859–68
125. Sakai J, Duncan EA, Rawson RB, Hua X, Brown MS, Goldstein JL. 1996. Sterol-regulated release of SREBP-2 from cell membranes requires two sequential cleavages, one within a transmembrane segment. *Cell* 85:1037–46
126. Sakamoto T, Weng JS, Hara T, Yoshino S, Kozuka-Hata H, et al. 2014. Hypoxia-inducible factor 1 regulation through cross talk between mTOR and MT1-MMP. *Mol. Cell. Biol.* 34:30–42
127. Salceda S, Caro J. 1997. Hypoxia-inducible factor 1α (HIF-1α) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. *J. Biol. Chem.* 272:22642–47
128. Samarasinghe SP, Sutanto MM, Danos AM, Johnson DN, Brady MJ, Cohen RN. 2009. Altering PPARγ ligand selectivity impairs adipogenesis by thiazolidinediones but not hormonal inducers. *Obesity* 17:965–72
129. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, et al. 2008. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* 320:1496–501

130. Schofield CJ, Ratcliffe PJ. 2004. Oxygen sensing by HIF hydroxylases. *Nat. Rev. Mol. Cell Biol.* 5:343–54
131. Schomburg I, Chang A, Ebeling C, Gremse M, Heldt C, et al. 2004. BRENDA, the enzyme database: updates and major new developments. *Nucleic Acids Res.* 32:D431–33
132. Semenza GL. 2013. HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. *J. Clin. Investig.* 123:3664–71
133. Semenza GL, Roth PH, Fang HM, Wang GL. 1994. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J. Biol. Chem.* 269:23757–63
134. Shenefelt RE. 1972. Morphogenesis of malformations in hamsters caused by retinoic acid: relation to dose and stage at treatment. *Teratology* 5:103–18
135. Shlomi T, Benyamini T, Gottlieb E, Sharan R, Ruppin E. 2011. Genome-scale metabolic modeling elucidates the role of proliferative adaptation in causing the Warburg effect. *PLoS Comput. Biol.* 7:e1002018
136. Shlomi T, Cabili MN, Herrgard MJ, Palsson BO, Ruppin E. 2008. Network-based prediction of human tissue-specific metabolism. *Nat. Biotechnol.* 26:1003–10
137. Shlomi T, Eisenberg Y, Sharan R, Ruppin E. 2007. A genome-scale computational study of the interplay between transcriptional regulation and metabolism. *Mol. Syst. Biol.* 3:101
138. Sigurdsson MI, Jamshidi N, Steingrimsson E, Thiele I, Palsson BO. 2010. A detailed genome-wide reconstruction of mouse metabolism based on human Recon 1. *BMC Syst. Biol.* 4:140
139. Supuran CT. 2008. Carbonic anhydrases: an overview. *Curr. Pharm. Des.* 14:603–14
140. Takahashi K, Kishimoto Y, Konishi T, Fujita Y, Ito M, et al. 2014. Ascorbic acid deficiency affects genes for oxidation-reduction and lipid metabolism in livers from SMP30/GNL knockout mice. *Biochim. Biophys. Acta* 1840:2289–98
141. Tanaka H, Arakawa H, Yamaguchi T, Shiraishi K, Fukuda S, et al. 2000. A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. *Nature* 404:42–49
142. Tanaka T, Sohmiya K, Kono T, Terasaki F, Horie R, et al. 2007. Thiamine attenuates the hypertension and metabolic abnormalities in CD36-defective SHR: uncoupling of glucose oxidation from cellular entry accompanied with enhanced protein O-GlcNAcylation in CD36 deficiency. *Mol. Cell. Biochem.* 299:23–35
143. Thiele I, Palsson BO. 2010. A protocol for generating a high-quality genome-scale metabolic reconstruction. *Nat. Protoc.* 5:93–121
144. Thiele I, Swainston N, Fleming RM, Hoppe A, Sahoo S, et al. 2013. A community-driven global reconstruction of human metabolism. *Nat. Biotechnol.* 31:419–25
145. Toya K, Hirata A, Ohata T, Sanada Y, Kato N, Yanaka N. 2012. Regulation of colon gene expression by vitamin B6 supplementation. *Mol. Nutr. Food Res.* 56:641–52
146. Tzameli I, Fang H, Ollero M, Shi H, Hamm JK, et al. 2004. Regulated production of a peroxisome proliferator-activated receptor-γ ligand during an early phase of adipocyte differentiation in 3T3-L1 adipocytes. *J. Biol. Chem.* 279:36093–102
147. Varga T, Czimmerer Z, Nagy L. 2011. PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. *Biochim. Biophys. Acta* 1812:1007–22
148. Vazquez A, Liu J, Zhou Y, Oltvai ZN. 2010. Catabolic efficiency of aerobic glycolysis: the Warburg effect revisited. *BMC Syst. Biol.* 4:58
149. Virk B, Correia G, Dixon DP, Feyst I, Jia J, et al. 2012. Excessive folate synthesis limits lifespan in the *C. elegans*: *E. coli* aging model. *BMC Biol.* 10:67
150. Wang S, Tsun ZY, Wolfson RL, Shen K, Wyant GA, et al. 2015. Metabolism. Lysosomal amino acid transporter SLC38A9 signals arginine sufficiency to mTORC1. *Science* 347:188–94
151. Wang X, Sato R, Brown MS, Hua X, Goldstein JL. 1994. SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. *Cell* 77:53–62
152. Wang Y, Eddy JA, Price ND. 2012. Reconstruction of genome-scale metabolic models for 126 human tissues using mCADRE. *BMC Syst. Biol.* 6:153
153. Watson E, MacNeil LT, Arda HE, Zhu LJ, Walhout AJ. 2013. Integration of metabolic and gene regulatory networks modulates the *C. elegans* dietary response. *Cell* 153:253–66
154. Watson E, MacNeil LT, Ritter AD, Yilmaz LS, Rosebrock AP, et al. 2014. Interspecies systems biology uncovers metabolites affecting *C. elegans* gene expression and life history traits. *Cell* 156:759–70

155. Wegner A, Meiser J, Weindl D, Hiller K. 2014. How metabolites modulate metabolic flux. *Curr. Opin. Biotechnol.* 34:16–22
156. Xiao B, Sanders MJ, Underwood E, Heath R, Mayer FV, et al. 2011. Structure of mammalian AMPK and its regulation by ADP. *Nature* 472:230–33
157. Yilmaz LS, Walhout AJ. 2014. Worms, bacteria, and micronutrients: an elegant model of our diet. *Trends Genet.* 30:496–503
158. Zhang P, Suidasari S, Hasegawa T, Yanaka N, Kato N. 2014. Vitamin B_6 activates p53 and elevates p21 gene expression in cancer cells and the mouse colon. *Oncol. Rep.* 31:2371–76