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DROSOPHILA NUTRIGENOMICS CAN PROVIDE CLUES TO HUMAN GENE-NUTRIENT INTERACTIONS

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■ **Abstract** Nutrigenomics refers to the complex effects of the nutritional environment on the genome, epigenome, and proteome of an organism. The diverse tissue- and organ-specific effects of diet include gene expression patterns, organization of the chromatin, and protein post-translational modifications. Long-term effects of diet range from obesity and associated diseases such as diabetes and cardiovascular disease to increased or decreased longevity. Furthermore, the diet of the mother can potentially have long-term health impacts on the children, possibly through inherited diet-induced chromatin alterations. *Drosophila* is a unique and ideal model organism for conducting nutrigenomics research for numerous reasons. *Drosophila*, yeast, and *Caenorhabditis elegans* all have sophisticated genetics as well as sequenced genomes, and researchers working with all three organisms have made valuable discoveries in nutrigenomics. However, unlike yeast and *C. elegans*, *Drosophila* has adipose-like tissues and a lipid transport system, making it a closer model to humans. This review summarizes what has already been learned in *Drosophila* nutrigenomics (with an emphasis on lipids and sterols), critically evaluates the data, and discusses fruitful areas for future research.

CONTENTS

INTRODUCTION

Nutrigenomics is a new discipline that examines nutrient-gene interactions on a genome-wide scale. Nutrigenomics is the intersection of three areas: health, diet, and genomics (Figure 1, see color insert). First, nutrition is a well-established field that involves the intersection of health and diet. Second, the intersection of diet and genomics is illustrated by the fact that improper diets are risk factors for disease (61, 63–66). New tools that take advantage of the numerous available whole-genome sequences to study diet-gene interactions include microarrays (transcriptomics), proteomics, metabolomics, and epigenomics. Microarrays can measure the changes in mRNA expression of every gene in the genome in a dose-dependent manner for a particular nutrient or drug. Proteomics measures the changes in the whole proteome, such as post-translational modifications. Metabolomics measures the changes in the entire metabolome, which is generally defined as metabolites with molecular weights less than 2000 Daltons, in a dose-dependent manner for a particular nutrient or drug. Epigenomics measures the changes in the epigenome, the histone post-translational modifications and DNA methylation pattern, in a dose-dependent manner for a particular nutrient or drug (for more information, see http://www.epigenome.org). Finally, the intersection of health and genomics relates to the identification of markers of aging, disease predisposition, and behavioral genomics.

The fruit fly *Drosophila* is ideally suited for conducting many types of nutrigenomics studies. For example, *Drosophila* has a fat body filled with adipocytes, and conserved metabolic and signaling pathways involved in fat metabolism, adipocyte development, and insulin signaling (6, 19–21). The major advantages of *Drosophila* as a model for nutrigenomics are its sophisticated genetics, small genome size, high fecundity, low cost, and short generation time. Genetic screens

have already been conducted to isolate mutations and identify natural polymorphisms involved in triglyceride and metabolite levels. Isogenic deficiencies and other types of overlapping deficiencies have been generated that uncover more than 90% of the *Drosophila* genome (96). More than 50% of the genes in *Drosophila* already have been knocked out with transposition insertions and chemical-induced point mutations (for more information, see http://www.flybase.org). RNA microarrays and overlapping 1 kb tiling arrays have been developed to analyze gene expression changes and chromatin changes cause by altered genotypes and environments (116). Sophisticated proteomics analyses have been developed that can analyze global protein expression changes and post-translational modifications (4, 97). The challenge for the future is to use newly developed bioinformatics algorithms to combine all of these nutrigenomics approaches to provide clues to human gene-nutrient interactions (83).

Fruit flies have a life cycle that is very different from that of mammals, yet many developmental and cellular pathways are conserved. When the *Drosophila* genome sequence was initially released in early 2000, 177 out of 289 (∼61%) human genes implicated in disease were found to have strong homologs or orthologs in the fly genome (107). Furthermore, two thirds of the genes implicated in human cancers have a counterpart in the fly genome (3). For example, entire signal transduction pathways are present in both organisms, including decapentaplegic (Dpp)/transforming growth factor-beta (TGF-β), Wingless/WNT, Toll/interleukin1 (IL1), Janus kinase/cytokine, Notch/Lin-12, Hedgehog (Hh), and the receptor tyrosine kinases and their intracellular adapter and serine-threonine kinase cascades (106). The insulin-signaling mechanisms in *Drosophila* are conserved with that of humans, as evidenced by the fact that human insulin activates the *Drosophila* insulin receptor (106). Mechanisms for regulating post-translational modification and trafficking of transcription factors from inert cytoplasmic forms to active nuclear forms are also conserved, as demonstrated by Cubitus interruptus (Ci)/Gli (controlled by Hh) and dorsal (dl)/nuclear factor kappa B (NFκB) [controlled by Toll/ interleukin 1(IL1)], among others. It is important to recall that many of the members of these pathways were first discovered by virtue of their mutant phenotypes in *Drosophila*, and further, that the genetic screens in which they were identified had nearly saturated the fly genome, leaving few to be discovered solely by genome sequence annotation (106).

The goals of this review are to summarize diverse types of nutrigenomics research already performed in *Drosophila* and to stimulate new research. Most of the publications in *Drosophila* nutrigenomics are in the subfield of caloric restriction and its effect on life-span extension. However, we only briefly mention caloric restriction research because several recent reviews have highlighted the contributions of model organisms to this field (9, 15, 28, 57). Instead, we focus on areas of nutrigenomics research that are less well studied, such as the genetics of triglyceride storage and steroid signaling, and the effects of dietary composition on longevity. We also promote new areas of studies in *Drosophila* that are poorly developed, such as the effects of diet on proteomics and metabolomics.

FORWARD GENETIC SCREENS FOR IDENTIFYING OBESITY GENES

Obesity is a multifactorial metabolic disorder that has both genetic and environmental components. Both human and mammalian model organism genetics have been used to identify genes that affect obesity (10). However, the identified genes that affect mammalian obesity represent a small fraction of obesity genes, so non-mammalian models need to be further developed (10). As discussed above, most of the human disease genes are present in the genome of *Drosophila* (107). Therefore, *Drosophila* genetic screens potentially could be used to identify candidate human disease genes that affect obesity.

Classical Genetic Screens

"Forward genetic screens" refer to classical genetic screens in which mutations in the genome are induced by a mutagen, and mutant organisms are analyzed for a particular phenotype. To our knowledge, no mutagenesis screen has been conducted in *Drosophila* with the specific purpose of looking for genes that affect obesity or triglyceride levels in *Drosophila*. However, quantitative trait loci (QTL) analyses and multigenerational selection experiments have been performed in *Drosophila* to determine the genetic basis for the natural variation in triglyceride levels (24, 25, 90). Also, mutations that affect lipid storage as secondary phenotypes were identified in massive screens for mutations that cause patterning defects in *Drosophila* embryos, such as those conducted in the early 1980s and before for which Edward Lewis, Eric Wieschaus, and Christiane Nüsslein-Volhard shared the 1995 Nobel Prize in Physiology or Medicine (67, 93, 139). In these classic embryonic patterning genetic screens, some mutations later have been shown to affect development of the fat body, the main lipid storage organ in larvae, as well as the development of other organs and tissues. For example, as described below and in the "Fat Body Development and Nutrient Signaling" section, Serpent was initially identified because it causes a defect in head involution during embryonic development (67), but later it was shown to be required also for fat body development (1, 2) and the immune response (99).

Serpent encodes a GATA transcription factor, a family of regulatory molecules that is conserved in mammalian cells (1, 2). The consensus DNA-binding sequence for Serpent and other GATA family members is 5′-(A/T)GATA(A/G)–3′ (41, 86, 102, 129). To determine whether mammalian GATA transcription factors have a conserved function in mammalian adipogenesis, Tong et al. (127) expressed GATA-2 and GATA-3, the family members that are expressed in adipocytes, in human 3T3-F442A preadipocytes and treated them with insulin for one week to stimulate differentiation (127). The adipocytes differentiated normally in the control "vector-alone" transfection, but constitutive GATA-2 and GATA-3 expression blocked adipocyte differentiation, as revealed by the absence of Oil-Red-O lipid staining (Figure 2, see color insert) (127). The authors concluded that

downregulation of these transcription factors is required for terminal differentiation of adipocytes, which is consistent with the observation that GATA-2 and GATA-3 are downregulated in obese animals (127). These experiments demonstrate the usefulness of the *Drosophila* forward genetics to identify key mammalian candidate genes involved in adipocyte development. They also demonstrate that the adipocyte differentiation assay is a rapid means to validate candidate adipocyte genes that are first identified in *Drosophila* genetic screens (Figure 2).

The *Adipose* Gene

Another type of forward genetic screen is to survey a large population for natural variation in a phenotype or a spontaneous mutation in a gene. The *adipose*^{60} (*adp*^{60}) mutation of *Drosophila* was isolated in 1960 from a natural population in Kaduna, Nigeria, and is, to our knowledge, the first example of a *Drosophila* genetic model for obesity (34, 35). In 1986, triglycerides, which serve as lipid stores, were shown to be increased by ~twofold in *adp*^{60} flies (121). The *adp*^{60} model is interesting because it demonstrates a balance between short-term fitness and long-term survival (34, 35). For example, *adp*^{60} flies are less fit when analyzed on flight-endurance tests (35). However, when well-fed *adp*^{60} flies are starved on a water-only diet, they significantly outlive wild-type flies, presumably because they utilize their larger fat reserves (34, 35, 55).

The *adp* gene was recently identified in *Drosophila* and shown to have a highly conserved mammalian cognate (55). Although the mammalian Adp cognate protein has not yet been demonstrated to have a similar function in mammalian models or in humans, it has a high degree of amino acid sequence homology throughout its structure with the mouse and human orthologs (55). One can argue that the *adp* variants in *Drosophila*, and similar types of natural polymorphisms in other genes, such as those that cause diabetes mellitus in humans, are maintained in populations that have unstable food resources. Unfortunately, as pointed out by Neel in 1962 (92), progress in eliminating famine has led to stable and unlimited food supplies in developed countries, so that the "thrifty genotype" now has detrimental effects. Because all natural populations of *Drosophila* have unstable food supplies, as did early human populations, the *Drosophila* genetic model promises to be of great use to identify further "thrifty genes."

REVERSE GENETIC SCREENS FOR IDENTIFYING OBESITY GENES

"Reverse genetics" refers to knocking out an already cloned candidate gene and then determining whether it has an expected phenotype. In this review, we call the knocking out of a single gene "reverse genetics," and the systematic knocking out of most or all of the genes in an organism a "reverse genetic screen." Examples of both types of knockout analyses are described below.

Perilipin Knockouts

Reverse genetics has recently been used to analyze the mechanism of lipid storage in *Drosophila*. Perilipins are proteins that surround and encapsulate lipids inside the cell, and *Drosophila* has two perilipin genes, *Lsd1* and *Lsd2* (87). By mobilizing a nearby transposable element to generate small deletions, two laboratories recently knocked out *Lsd2* in *Drosophila* to determine whether the mechanism for triglyceride storage is evolutionarily conserved (53, 122). As was found in mouse perilipin knockouts (119), *Drosophila* lacking one of the perilipin genes has a decrease in total triglyceride levels (53, 122). Kuhnlein's laboratory also made *Lsd2*-GFP transgenic flies and showed that this protein surrounds the lipid droplets in fat bodies (53). These studies demonstrate that components regulating homeostasis of lipid-based energy storage are evolutionarily conserved between invertebrates and vertebrates. The *Lsd2*-GFP transgenic flies will also be useful tools in future genetic screens to identify components that regulate the size and assembly of lipid droplets (53).

Whole-Genome Mutant Screens

In a reverse genetic screen, one constructs a panel of mutations in all or most of the cloned genes in an organism and then screens the collection of mutant organisms for a particular phenotype. The ultimate reverse genetic screen would be not only to knock out every gene in an organism, but also to determine the phenotype of each knockout line in a dose-dependent manner for a particular nutrient or drug. The closest to an ultimate screen was done in yeast, where site-specific recombination allowed the systematic knockout of every candidate gene in the organism (48, 140). The collection of knockouts is used by the yeast research community to systematically screen for a phenotype or to search for genetic interactions with other genes or pharmaceuticals (47, 49). A large-scale yeast genetic interaction screen identified a network containing 1000 genes and 4000 interactions by crossing mutations in 132 query genes into a set of deletion mutants and scoring the double-mutant progeny for growth defects (126). In this study, only 132 query genes were made double mutant for all of the other genes (126), but high-throughput technology should make it feasible to analyze the entire genome in this manner.

Whole-Genome RNA-Mediated Interference Screens

Although not yet conducted in whole *Drosophila* animals, a reverse genetic screen of most of the *Caenorhabditis elegans* genome for defects in lipid storage has been completed by Gary Ruvkun's laboratory (7). In this study, they used RNA-mediated interference (RNAi) to disrupt the expression of 16,757 *C. elegans* genes. They found 305 disruptions that lead to an increase in fat storage and 112 disruptions that lead to a decrease in fat storage. Some of the disruptions are in genes with conserved functions in mammalian cells, such as those in the insulin and serotonin-signaling

pathways. However, most of the genes identified in this screen are conserved between mammals and worms, but have no known function in fat storage (7). Verification of the significance of the "pioneer genes" could be accomplished in the future by identifying novel adipocyte differentiation genes using the preadipocyte assay, described above, and by making knockout mice.

Although RNAi-type reverse genetic screens are not yet feasible in *Drosophila* because of the labor involved, RNAi screens knocking out 91% of the predicted fly genome have been performed using *Drosophila* tissue culture cell lines in Norbert Perrimon's laboratory (17). They analyzed cell growth and viability with an automated fluorescent assay that measures ATP levels and found that 438 RNAi constructs cause defects in these processes (17). Partial RNAi screens have also been performed in *Drosophila* cell lines by transfecting the cells with smaller collections of RNAi. For example, Lum and colleagues (75, 76) identified genes that affect the Hh-signaling pathway using a collection of 43% of the fly genome in cells containing an Hh-transcriptional reporter.

It would be extremely informative to conduct whole-genome RNAi screens with *Drosophila* adipocyte cell lines to identify genes involved in fat storage. Unfortunately, to our knowledge, *Drosophila* preadipocyte or adipocyte lines have not yet been developed. However, three recent reports have been published on large-scale RNAi-type screens in mammalian cells. Because long double-stranded RNA in mammalian cells induce the interferon response and shut down translation (40), short inverted RNAs (siRNAs) must be used in mammalian cells. The first large-scale siRNA screen in mammalian cells used the transfection of siRNAs targeted against 8000 individual genes to identify regulators of tumor necrosis factor-α signaling (142). The other two screens involved retroviral-expressed siRNA for 15,000 genes (10,000 human and 5000 mouse) and 8000 human genes (12, 95); the first screen identified modulators of proteosome function, whereas the second screen identified modulators of p53-induced apoptosis and cell cycle arrest (12, 95).

Whole-Genome Deficiency Screens

Whole-genome reverse genetic screens can also be performed in *Drosophila* using the large collection of existing mutations and deficiencies that have been generated in this organism over the past century (http://www.flybase.org). However, the extreme strain-to-strain variation in this collection makes them less useful for nutrigenomics research. Recently, Exelixis, Inc. has released to the *Drosophila* community a large collection of isogenic insertion lines and deficiencies that all have the same genetic background (96, 123). The Exelixis collection includes over 29,000 isogenic insertion lines that tagged or knocked out over 7200 different genes. The Exelixis isogenic deficiency collection, together with those generated by Kevin Cook and colleagues at Indiana University, includes 519 deficiencies that uncover 56% of the *Drosophila* genome (Figure 3, see color insert) (96, 123). Because these deficiencies generally uncover relatively small regions of the

genome (100,000 to 300,000 base pairs), there is a lower chance of deleting multiple genes in the same pathway, which was a problem with previously generated deficiencies.

A type of experiment that could be conducted, for instance, would be to screen the isogenic deficiency lines for modulators of triglyceride levels. A secondary screen of genes uncovered by the deficiencies could be conducted by testing the isogenic insertion knockout lines that are uncovered by deficiencies that either raise or lower the triglyceride levels. Nonisogenic mutations in genes uncovered by the deficiencies can be made mostly isogenic by backcrossing the mutation to the nearest isogenic insertion line for 10 generations or more, as we described previously (46). Through this and similar types of experiments, the isogenic deficiency lines promise to be of great use in future nutrigenomics research. A caveat to performing screens using the isogenic deficiency lines is that single gene mutations often have very different phenotypes, depending on genetic backgrounds (see, for example, Reference 73). This suggests that focusing on a single isogenic strain will give some false negatives, and QTL-type approaches are valid and essential alternatives (24, 90).

PROTEIN-PROTEIN INTERACTION SCREENS

Two types of interaction screens are used to detect protein-protein interactions. The first type is the so-called two-hybrid screen, which is based on the first hybrid protein containing a DNA binding (DB) domain fused to one protein and an activating domain (AD) fused to a second protein (Figure 4, see color insert, top) (42). The second type of protein-protein interaction screen involves biochemical purification of interacting proteins. These two types of screens, and their relevance to nutrigenomics research, are described in this section.

Detecting Interactions Between Proteins with the Two-Hybrid Assay

In the two-hybrid assay, when both interacting hybrids are brought together in yeast cells, they activate expression of a reporter gene, such as β-galactosidase, which turns the yeast blue when grown on indicator plates containing X-GAL. Typically, if both hybrid proteins interact, they also activate expression of an prototrophic selection gene, such as for His3 or Leu2. Although not often used for this purpose, the two-hybrid assay can also potentially detect the effects of nutrients on protein-protein interactions (72). We note that endogenous GAL4 in yeast interacts with its repressor, GAL80, and that this repression is released when the sugar galactose is present in the medium. It is possible to transfect yeast with appropriate genes expressing enzymes that allow proteins to interact only when nutrients induce post-translational modifications, such as phosphorylation (72), mono-ubiquitin (18), mono-sumo (118), or O-GlcNAc (135–137). O-GlcNAc modification of proteins has been proposed to be a "nutrient sensor" (135–137),

and the O-GlcNAc modifying enzymes, such as OGT and OGN, are conserved in *Drosophila* (http://www.flybase.org), but, to our knowledge, no laboratory has yet pursued this system in flies.

Genome-Wide Two-Hybrid Screens

In a classic two-hybrid screen, yeast cells containing the first DB-bait hybrid protein are transfected with a library of vectors that contain the AD-prey hybrid protein, where the "prey" is a library of random cDNAs (44). Proteins that interact with the "bait" are identified by colonies that are blue and grow on plates lacking histidine, for instance. Large-scale two-hybrid screens have recently been completed in yeast, *C. elegans*, and *Drosophila* (100). In the most extensive *Drosophila* two-hybrid screen (51), called interactive mating, a collection of DBs fused to full-length cDNAs for most of the genes from an organism is transfected, one at a time, into yeast cells. Next, a collection of ADs fused to the same collection of full-length cDNAs is transfected into yeast cells of the opposite mating type, again one at a time (44). Finally, robots are used to mate every AD-fusion protein containing yeast strain to every DB-fusion protein yeast strain (Figure 4, bottom). In the *Drosophila* large-scale screen conducted by CuraGen, Inc. and collaborators, 10,623 cDNA were screened against each other and a high-confidence refined map of 4679 proteins and 4780 interactions was produced (51). The authors noted that not all ($10{,}623^2$) possible interactions were analyzed, for several technical reasons (51). For example, a significant percentage of DB-fusion proteins activated transcription in the absence of the AD-fusion protein and therefore were not used further in the screen (51).

The results of the large-scale two-hybrid screens are of great use to the field of nutrigenomics. For example, one can find potential interacting proteins of a gene of interest by querying the CuraGen website, http://portal.curagen.com/. One could then test mutations in the candidate interacting proteins for similar phenotypes caused by mutations in the gene of interest (51).

Genome-Wide Protein Interaction Screens

The second type of interaction screen is based on a biochemical interaction in a test tube. In this screen, all of the proteins from a proteome are purified with an epitope tag and immobilized on a column. Next, whole cell extracts are added to the column, washed, and the bound proteins are eluted and identified with tandem mass spectrometric (MS/MS) approaches. So far, because of the incredible labor involved in this type of interaction screen, a biochemical interaction screen has only been published for a small subset of the *C. elegans* proteome (74), but yeast, *Drosophila*, and *Escherichia coli* proteomes are in progress. When this type of protein-protein interaction screen is fully developed in *Drosophila*, it could be used in nutrigenomics research to identify interactions that occur only in the presence of a particular nutrient, for example.

DROSOPHILA LESSONS CONCERNING STEROL BIOSYNTHESIS

Biosynthesis of a complex set of lipophilic signaling molecules, including steroids, isoprenoids, eicosanoids, and prostaglandins, among others, begins with stepwise enzymatic condensation of acetyl-CoA residues to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). The first committed step in the synthesis of the above lipophilic signaling molecules is catalyzed by HMG-CoA reductase to form mevalonate. Six enzymatic reactions then ensue to generate farnesyl diphosphate (Far-PP), which is the last molecule that serves as precursor for both steroids and isoprenoids. On the sterol branch, nine sequential enzymatic reactions convert Far-PP into cholesterol, which is the precursor of vitamin D3, various steroid hormones, and the bile acids that are the ultimate degradation products of sterols. Far-PP is also used to form geranylgeranyl-diphosphate (Ger-Ger-PP). Far-PP and Ger-Ger-PP can both be covalently attached to proteins, and serve as lipophilic tethers to retain these proteins in close apposition to cellular membranes. Far-PP and geranyl-PP are also precursors for terpenes, vitamins E and K, and, in plants, an extensive series of secondary metabolic pathways that generate (among other products) carotenoids.

Cholesterol Biosynthesis

Mammalian cells closely regulate the de novo biosynthesis of cholesterol in response to dietary uptake of cholesterol and the need to maintain proper membrane fluidity. A family of sterol-sensing proteins, including the endoplasmic reticulum (ER) sterol regulatory element binding protein (SREBP) cleavage-activating protein (SCAP), is involved in measuring sterol concentrations. When sterol concentrations are low, SCAP escorts the inactive form of SREBP from the ER to the Golgi, where proteolytic release of the bHLH-Zip domain of SREBP occurs (52, 108). The bHLH-Zip fragment of SREBP then translocates to the cell nucleus, where it acts as a transcription factor inducing expression of about 35 genes involved in unsaturated fatty acid biosynthesis and cholesterol biosynthesis including HMG-CoA reductase (58). Recently, Radhakrishnan and colleagues (101) showed that cholesterol directly binds SCAP, inducing a conformational change so that SCAP dissociates from the membrane-bound form of SREBP and instead binds to Insig proteins. The inability to escort membrane-bound inactive SREBP to Golgi when sterol concentrations have risen permits this transcription factor to be depleted from cell nuclei. In combination with ubiquitinylation and degradation of HMG-CoA reductase, cholesterol biosynthesis is downregulated both transcriptionally and post-transcriptionally, closing the feedback loop required for cholesterol homeostasis.

In contrast with mammalian cells, arthropods including insects such as *Drosophila* cannot synthesize cholesterol de novo from acetyl-CoA (103). In the case of *Drosophila* this metabolic inability was recently shown to be due to the

absence from the genome of coding regions for seven enzymes in the sterol branch, including lanosterol synthase, which catalyzes the concerted cyclization reactions required to fabricate the four-fused-ring steroid nucleus, and 7-dehydrocholesterol reductase, which synthesizes the definitive cholesterol molecule (88). *Drosophila* is thus dependent upon provision of exogenous cholesterol or closely related sterols, and this dependency contributes to some of the ecological niches various *Drosophila* species inhabit.

Flies contain a single gene each for SCAP and SREBP (114). The subcellular localization, translocation, and proteolytic processing of these proteins are conserved between *Drosophila* and mammalian cells. Quite remarkably, though, SREBP proteolysis and nuclear translocation in *Drosophila* cultured cells is inhibited by palmitate levels rather than sterol levels (114), which operate through metabolic conversion of palmitate to phosphatidylethanolamine (36). Inactivation of either SREBP or SCAP in *Drosophila* cultured cells using RNA interference showed that these proteins are required for saturated fatty acid biosynthesis (114) rather than unsaturated fatty acid biosynthesis as found in mammals. Consistent with the severance of the association of cholesterol levels from HMG-CoA reductase activity, *Drosophila* transcription of the HMG-CoA reductase gene is not affected by inhibition of SREBP or SCAP activity (114). An interesting avenue for future research would be testing the SCAP/SREBp/palmitate network in flies fed different fatty acids, for example.

Ecdysone Biosynthesis

A principal use arthropods make of dietary cholesterol is for biosynthesis of the major molting hormone, 20-hydroxyecdysone (20-HE). This hormone is synthesized in the prothoracic glands during larval and pupal life, during which times it regulates growth and the onset of metamorphosis. In adult insects, 20-HE is additionally synthesized in ovaries, playing a crucial role in oogenesis. Maternal supplies of ecdysteroids are deposited into eggs; mobilization of these stores is required for successful completion of embryonic development. Akin to biosynthesis of steroid hormones such as testosterone, estradiol, and progesterone in mammals, arthropods synthesize 20-HE by mitochondrial cytochrome-P450 family members that catalyze position-specific hydroxylation reactions. Considerable recent progress has been made in cloning and characterizing the genes for the P450 enzymes from *Drosophila* (23, 26, 134) and for a putative cytosol-mitochondria cholesterol transporter (105) that has homology to the steroid acute-regulatory protein-related lipid transfer domain (115).

Sterols and Germ Cell Migration

Although *Drosophila* has deficits in the de novo cholesterol biosynthesis pathway, enzymes downstream of Far-PP required for prenylation of proteins and for isoprenoid biosynthesis are intact. These observations have led to an unexpected role for this pathway in the developing fly. In all animals, primordial germ cells

form early during embryonic development and then migrate to somatic gonadal tissues before engaging in mitotic proliferation, gamete differentiation, and meiosis. In *Drosophila*, mutations that cause primordial germ cells to lose their way during migration and fail to arrive at the somatic gonad map to the genes encoding HMG-CoA reductase, farnesyl-diphosphate synthase (FPPS), geranylgeranyl-diphosphate synthase (GPPS), and the geranylgeranyl transferase type I (109). Ectopic expression of either FPPS or GPPS in the *Drosophila* embryonic nervous system is sufficient to cause germ cell migration to be inappropriately directed toward the nervous system, indicating a role for the isoprenoid pathway in synthesis of a germ cell chemoattractant. Application of either of two commonly prescribed statin drugs that inhibit HMG-CoA reductase activity caused similar defects in germ cell migration in developing zebra fish embryos, as did a drug inhibitor of geranylgeranyl-transferase activity but not of farnesyl-transferase activity (124). "Dietary" supplementation of drug-treated zebra fish embryos with farnesol, geranylgeraniol, or mevalonate, small metabolites downstream of the pharmacological blockades, partly restored germ cell migration (124). Thus, a role for isoprenoid pathway products, possibly a geranylgeranylated protein, in chemoattraction of germ cells is conserved between insects and vertebrates. These studies raise interesting questions regarding dietary and pharmacological interventions that perturb the mevalonate, sterol, or isoprenoid pathways.

DROSOPHILA STEROID RECEPTORS

The steroid receptor superfamily of transcription factors regulates diverse cellular processes, generally in response to binding of small lipophilic molecules. These lipophilic ligands are synthesized, for the most part, from the sterol and isoprenoid branches mentioned above. With the completion of various genome sequences it is possible to arrive at complete lists of superfamily members found in a variety of taxonomic groups and draw inferences about the evolutionary history of the superfamily in each lineage. Maglich and colleagues (77, 78) examined the then-available human, *Drosophila*, and nematode genome sequences for members of the steroid receptor superfamily. Although humans (and by extension, other mammals) contain about 50 functional steroid receptor family members, *Drosophila* contains only 21 steroid receptor family members.

Retinoid-X Receptor and Ecdysone Receptors

As is the case with vertebrate steroid receptors, *Drosophila* superfamily members come in three varieties: those that bind DNA as monomeric proteins, those that bind DNA as homodimers, and those that bind DNA as heterodimers partnered with the retinoid-X receptor (RXR). The molecular phylogenetic analysis of Maglich and colleagues (77, 78) enabled strong association of nine *Drosophila* steroid receptor superfamily members with their corresponding cognate, or orthologous, genes in human. For example, the *Drosophila* ultraspiracle (usp) gene, which encodes ultraspiracle (USP) protein, is the cognate of RXR. The *Drosophila* ecdysone receptor

(EcR) gene, which encodes ECR protein, is the cognate of vertebrate farnesoid-X receptor (FXR). Because of these cognate relationships and the critical importance of USP and ECR to *Drosophila* development, we shall use them as illustrations of the potential future directions of *Drosophila* nutrigenomics research.

First, and quite striking, *Drosophila* uses its EcR cognate as a 20-HE-activated regulator of development, whereas mammals use FXR as a bile-activated regulator of cholesterol metabolism. How the ligand-binding domains came to differ in binding specificity is an outstanding problem of structural biochemistry. More intriguing is how evolution in vertebrate and invertebrate lineages caused the deployment of the FXR/EcR cognates for such different roles. Perhaps the connection is nutritional regulation during development. If this is the case, then studying the nutrigenomics of EcR regulation should yield insights into FXR regulation.

Pathways Regulated by 20-Hydroxyecdysone

In *Drosophila* and other arthropods, larval-to-larval molting, the onset of metamorphosis (larval-to-pupal molting), adult emergence, and adult fertility are controlled principally by two hormones, 20-HE and juvenile hormone (JH). Whereas 20-HE, a steroid, stimulates molting, the nature of the molt is influenced by the presence of several structurally similar JHs, which are methyl-epoxy derivatives of farnesoate. Late in larval life, when JH and ecdysterone concentrations are low, ECR is cytosolic and binds heat-shock protein 90 (HSP90), a molecular chaperone, in an inactive complex. When the concentration of 20-HE rises, it binds to ECR, triggering dissociation from HSP90 (5). Ligand-bound ECR then binds to USP, translocates to the cell nucleus, binds DNA at specific sites, and recruits coactivator proteins that stimulate target gene transcription (5). ECR-USP activates several immediate-ecdysone-inducible genes in all tissues, whereas later-inducible genes are tissue- and developmental stage-specific (5). ECR-USP can also recruit corepressor proteins and inhibit target gene transcription in disparate tissues.

Ecdysone-regulated gene hierarchies have been studied by a variety of methods over the past three decades, most recently by microarrays (45, 85, 125, 138). Gene activation by ECR-USP can involve poly-ADP-ribosylation of histones (110), which has the effect of facilitating access to the chromatin by RNA polymerase II, and by recruitment of the histone H3-lysine-4-methyltransferase encoded by the *Drosophila* trithorax-related gene (113), among other mechanisms. If 20-HE and JH are both present in a cell-culture assay system, JH binds to the USP protein found in ECR-USP heterodimers (81). Ligand occupancy on USP stimulates the recruitment of transcriptional corepressor proteins, which results in a reduced ability of 20-HE to stimulate transcription of target genes. A similar finding was recently reported concerning human RXR agonists and their ability to prevent FXR from recruiting coactivators (69).

Endocrine Disruptors

Many insects are voracious herbivores, and numerous natural products have been isolated from plants that disrupt either ecdysone signaling or JH signaling and

thus are lethal to the insects that consume them. Ecdysone antagonists from plants include oligostilbenes (84) and flavones (94). Ecdysone agonists include phytosteroids, such as makisterones (8), and nonsteroidal diacylhydrazines (32, 33). These natural products are being used to design, test, and eventually deploy novel insecticides. A growing body of molecular modeling, X-ray crystallographic, and structure-reactivity studies are being performed to take advantage of the evolutionary divergences among ECR and USP genes present in various insect taxonomic groups so as to minimize the harmful effects that synthetic insect endocrine disruptive agents have on beneficial insect species (13, 14). Likewise, X-ray structural studies have examined the correspondence between how the *Drosophila* ECR-USP heterodimer and the mammalian FXR-RXR each bind DNA sites (31).

In some crop plants, naturally occurring insect endocrine disruptors accumulate to high concentrations, and thus are nontrivial components of human diets. An important question, therefore, is the extent to which these phytoecdysteroids might act as ligands that bind either the human FXR and RXR cognates of insect ECR and USP, or perhaps other members of the human steroid receptor superfamily repertoire. Reviews of the literature indicate relatively little mammalian toxicity (32, 33). In contrast, there is good evidence of estrogenic effects of plant compounds (including, for example, genistein), and the recent computational discovery of a *Drosophila* ortholog of the vertebrate estrogen-receptor-related gene (77, 78) suggests that fly-derived assays may be useful in identifying natural ligands for this receptor and in determining whether plant products mimic (or antagonize) their effects.

OBESITY AND THE IMMUNE RESPONSE

Recent data have revealed a tight link between inflammation and metabolic conditions (27). The relevance of this relationship became evident upon the demonstration by Hotamisligil and colleagues (60) in 1993 that inflammatory pathways are critical in the mechanisms underlying insulin resistance and type 2 diabetes in mice. More recent data showed that human obesity is associated with a state of chronic inflammation characterized by abnormal production of cytokines, such as interleukin-6 and tumor necrosis factor-α, increased acute-phase reactants, such as C-reactive protein, and other stress-induced molecules (59). An interesting feature of the inflammatory response that emerges in the presence of obesity is that many of these alterations are initiated and reside within the adipose tissue (59). Recently, Hotamisligil (59) suggested that this tight relationship might be explained by evolutionarily conserved genetic pathways that regulate metabolic and immune functions by using common regulatory molecules.

Fat Body Development and Nutrient Signaling

It is interesting that the fat body in *Drosophila* functions not only in energy storage and intermediary metabolism but also in the innate immune response. Experimental

results suggest that there is a close parallel between the molecular mechanisms functioning in the regulation of metabolic and immune functions in *Drosophila*. As discussed in the "Forward Genetic Screens for Identifying Obesity Genes" section, the developmental control of the *Drosophila* fat body specification, like mammalian white adipose tissue (127), is regulated by Serpent, a member of the GATA-binding family of transcription factors (56). Previously, Petersen and colleagues (99) had shown that Serpent regulates *Drosophila* immunity genes in the larval fat body. In particular, they reported that Serpent acts as a positive regulator in a GATA-dependent manner of the transcription of Cecropin A1 gene, which encodes for an antimicrobial peptide (99).

In an attempt to identify the mechanisms underlying nutrient signaling in *Drosophila*, Zinke et al. (143) used Affymetrix microarrays to identify genes regulated by starvation and sugar conditions. One of the genes that were regulated upon both conditions is *Thor* (143). Thor is a member of the 4E-binding protein (4E-BP) family, which in mammals has been defined as a critical regulator in a pathway that controls initiation of translation through binding eukaryotic initiation factor 4E. In accord with the studies in *Drosophila*, studies in mice have shown that the phosphorylation state of the 4E-BP1 changes in response to starvation and refeeding (117). In *Drosophila*, Thor also participates in host immune defense (11). The *Thor* promoter has the canonical nuclear factor-B and associated GATA recognition sequences that have been shown to be essential for immune induction, and becomes upregulated upon bacterial infection (11).

Dopamine and Serotonin

In mammals, the neurotransmitters norepinephrine, dopamine, and serotonin are well known to be involved in central energy balance circuits (111, 112). Dopamine and serotonin are also present in the central nervous system of insects. In *Drosophila* larvae, the presence of serotonergic nerves in several tissues involved in digestion, such as the pharyngeal muscles, the proventriculus, the midgut, and the ring gland, suggest that serotonin also modulates feeding behavior and neuroendocrine activity in this species (54, 89). In insects, dopamine acts as a neurotransmitter in the brain and as a precursor molecule in the synthesis of melanin used by the animal during the immune response. Notably, microarray expression studies have found that genes encoding for enzymes in the dopamine and serotonin pathways are upregulated following bacterial infection (30).

Fatty Acid–Binding Proteins

Recent data have shown that fatty acid–binding proteins, a family of cytoplasmic proteins, play an important role in transporting free fatty acids to various intracellular compartments, regulating cellular lipid metabolism and expression of inflammatory cytokines in mammals (16). Vierstraete and colleagues recently reported the results of two proteomic studies for the analysis of instantly released immune proteins in hemolymph of *Drosophila* third-instar larvae after challenge

with lipopolysaccharide or pricking with sterile needles (132) and after induction with a suspension of Gram-positive bacteria or yeast (133). In both studies, the homologous *Drosophila* retinoid and fatty acid–binding protein was found to be significantly differentially expressed (132, 133). These findings highlight the notion that *Drosophila* is a powerful model to identify molecular pathways regulating both metabolic and immune functions.

HIGH-FAT DIET AND AGING STUDIES IN *DROSOPHILA*

In contrast to caloric restriction, which retards aging in *Drosophila* (43, 62, 79, 80, 104, 120, 141), and is not discussed in this review, the effects of saturated fats in the diet of *Drosophila* have been shown to play a role in the acceleration of aging in this species. However, the specific mechanism that is involved in promotion of aging has been difficult to identify. One of the first studies that attempted to make a connection between *Drosophila* diet and aging found that diets consisting of high fat and low carbohydrate concentrations would shorten, on average, the life span of *Drosophila* (39). This study, however, fell short of identifying the specific aging process or the multiple aging processes that were negatively affected by the consumption of fat. Instead, it laid the groundwork for future studies since some irreversible damage was sustained by the flies on a palmitic acid medium that was characteristic of an aging process.

Further studies of diet and aging in *Drosophila* focused on the fact that a greater stress was placed on older flies fed a high-fat diet than on flies fed a normal low-fat diet. Researchers began to look at metabolic parameters of aging *Drosophila* and went on to test the effects of a high-fat diet on these parameters (37). An interesting finding was that older flies consume more food than younger flies regardless of the diet they are fed. However, the increased consumption was greater in the flies fed a high-fat/low-carbohydrate diet. Further probing suggested that the efficiency of metabolism in *Drosophila* drops significantly with age. Therefore, the flies eat more to compensate for this impaired energy metabolism. It was also hypothesized that older flies are able to generate energy more efficiently from carbohydrates to obtain the needed energy to survive. This explained the greater stress seen in older flies on the high-fat diet mentioned above as well as in the first study. It also suggested that the shorter life span of flies on a high-fat diet could be attributed to this increased need for carbohydrates in energy metabolism.

Subsequent studies led researchers to conclude that some metabolite of fat must be the significant factor that links nutrition and aging (38). Still, the question remains as to what that metabolite could be. One suggestion is that a free-radical mechanism may be involved. Free radicals can be generated during normal fat metabolism as well as through hydrogen peroxide. Significant amounts of hydrogen peroxide are produced by peroxisomes during fat metabolism. Many other possibilities exist, however. In fact, very few studies other than CR have been done on the effects of diet on aging in *Drosophila*, and most of the studies that have been done were conducted more than 20 years ago. With the greater knowledge

and improved technology we have today (especially in relation to genetics), we can further delve into solving for this unidentified mechanism of aging.

FUTURE PROSPECTS

Nutrigenomics, a subject of numerous recent reviews (22, 29, 50, 68, 70, 71, 82, 91, 98, 128, 130, 131), is an emerging field with great prospects for understanding the effect of nutrition on health and disease. Nutrigenomics requires a multidimensional understanding of how the diet affects gene expression, post-translational modifications of proteins, and changes in the chromatin. *Drosophila* is an ideal model to study nutrigenomics and, as outlined in this review, has already contributed a great deal to the field. Candidate pathways influenced by nutrients can be identified first in *Drosophila*, and later verified in mouse models and finally in humans. We hope that this review will stimulate more research in the field of *Drosophila* nutrigenomics.

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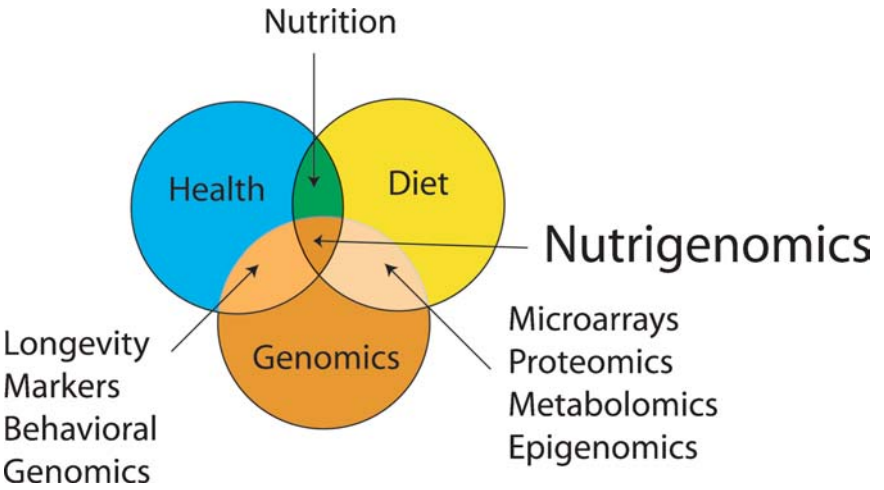

Figure 1 Venn diagram illustration of nutrigenomics as the intersection between health, diet, and genomics.

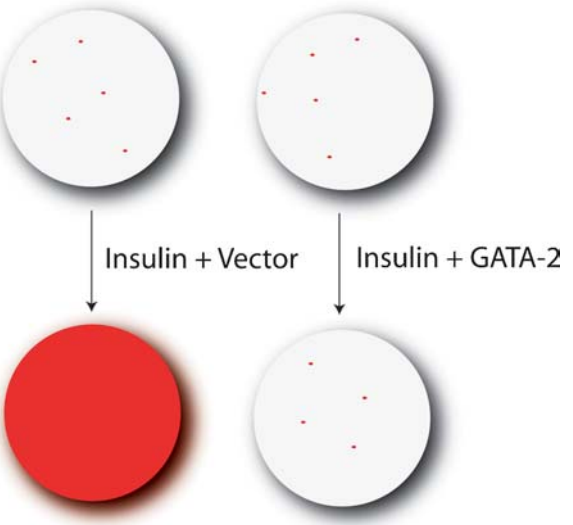

Figure 2 Verification that mammalian GATA-2 inhibits preadipocyte differentiation in a cell culture system. On the left, vector alone has no effect on differentiation of preadipocytes to adipocytes. The red color is Oil-Red-O staining of triglycerides. On the right, GATA-2 prevents differentiation of preadipocytes into adipocytes, as indicated by the lack of Oil-Red-O staining. Cartoon illustration of data from (127).

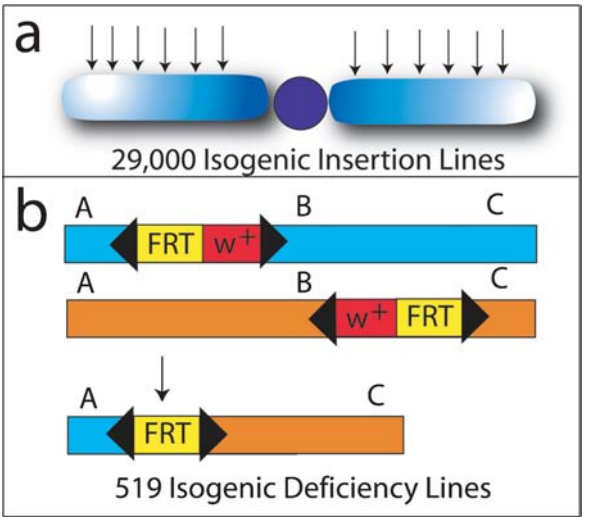

Figure 3 Isogenic insertion and deficiency lines in *Drosophila.* (*a*) Over 29,000 isogenic insertions tag or disrupt over 7200 genes in *Drosophila* (123). (*b*) A total of 519 isogenic deficiencies uncover over 56% of the *Drosophila* genome (96). The deficiencies are generated by recombining FLP-recombinase target (FRT) sites on homologous chromosomes containing two nearby insertions. Deficiency chromosomes are selected by the absence of the white (w+) gene, which causes the eyes to become white in color (96).

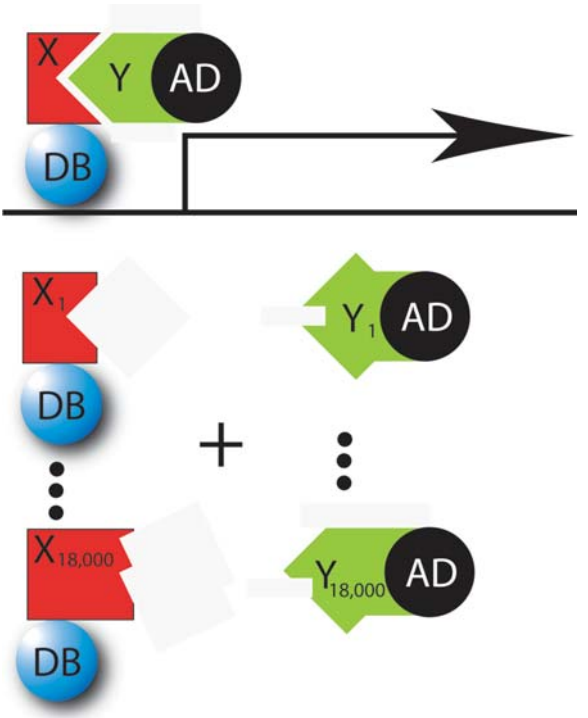

Figure 4 A whole-genome two-hybrid screen identifies interacting proteins. (*Top*) A DNA binding domain (DB) is fused to the cDNA for gene X. An activating domain (AD) is fused to the cDNA of an interacting protein Y. Together the two hybrids form a complex and activate transcription of a reporter gene (*arrow*). (*Bottom*) In a two-hybrid screen, all of the cDNAs of an organism are fused to the DB (X_1, … $X_{18,000}$) and to the AD (Y_1, … $Y_{18,000}$). The DB fusions are transformed one by one into one mating type of yeast (+ or −), and the AD fusions are transformed into the opposite mating type of yeast. Using robots, all of the 18,000 DB yeast are then mated to all of the 18,000 AD yeast, for a total of $18{,}000^2$ (324 million) mating events (51). The data from this experiment can be accessed at http://portal.curagen.com/.

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