Review

CellPress

Nutrient control of *Drosophila* longevity

Marc Tatar[1], Stephanie Post[1], and Kweon Yu[2]

[1] Department of Ecology and Evolutionary Biology, Brown University, Providence RI 02912, USA
[2] Neurophysiology Research Group, Korea Research Institute of Bioscience and Biotechnology, Daejon, 305-806, Korea

Dietary restriction (DR) extends the lifespan of many animals, including *Drosophila melanogaster*. Recent work with flies shows that longevity is controlled by the ratio of consumed protein relative to carbohydrates. Given that reduced insulin and/or insulin-like growth factor (IGF) and target of rapamycin (TOR) signaling increase *Drosophila* lifespan, these pathways are candidate mediators of DR. However, this idea has ambiguous experimental support. The Nutritional Geometric Framework (NGF), which dissects the impact of nutrient protein relative to carbohydrates, may provide an approach to resolving the roles for these pathways in DR. Nutrient sensing of protein and carbohydrate may occur in the fat body through signals to hypothalamic-like neurons in the fly brain and, thus, control secretion of insulin-like peptides that regulate longevity.

A long life by eating less

A long-standing problem in the biology of aging is to understand how DR (see Glossary) extends life expectancy [1]. In one branch, research has aimed to uncover the cellular mechanisms whereby DR improves survival, for instance through increasing stress resistance, genomic maintenance, or protein homeostasis. By contrast, these cell-centric mechanisms must be integrated across tissues to assure longevity. The need for such coordination motivates work to understand how nutrients contribute to physiological signaling that affects survival. This question has progressed using *Drosophila* to uncover the nutrient components that modulate longevity assurance, and to analyze the roles of sensory and hormonal systems that systemically integrate longevity control. These data together reveal mechanisms of nutrient sensing that could stimulate new hypotheses to identify the metabolic pathways responsible for DR-induced longevity [2] and can suggest endocrine interventions that emulate the benefits of DR. [3]

Corresponding author: Tatar, M. (Marc_Tatar@Brown.edu).
Keywords: dietary restriction; aging; geometric framework; lifespan extension; insulin/IGF signaling; TOR.

1043-2760/$ – see front matter
 http://dx.doi.org/10.1016/j.tem.2014.02.006

The nutrient lever of restricted diet

Restricting food intake without malnutrition was reported to extend lifespan in rats some 80 years ago [4]. Research has since focused on deciphering which aspect of restricted-diet induces longevity, how this dietary feature is sensed, and what the mechanisms are by which it regulates aging [5]. Likewise, more than 40 years ago David *et al.* [6] reported that longevity of laboratory *Drosophila melanogaster* was extended when adults were maintained on diluted yet freely available diets, that is, diets made in agar-based media with different combinations of yeast, sugar or molasses, cornmeal, and other carbohydrates [7,8]. As with rodents, for *Drosophila*, there is keen interest to understand the nutrient effectors of DR, how they are sensed and integrated, and how this affects the molecular mechanisms of survival. The order of these goals is important: before we can understand the cellular or molecular

Glossary

Dietary restriction (DR): consumption of less food (without malnutrition) resulting in increased life expectancy. Historically attributed to the effect of consuming fewer calories ('caloric restriction'), many data now demonstrate that DR is stimulated by restricting specific nutrients rather than by limiting total energy intake.
***Drosophila* Forkhead box type O transcription factor (dFOXO):** the homolog of mammalian FOXO1, FOXO3a, and FOXO4.
***Drosophila* insulin-like peptides (DILP):** *Drosophila* has eight recognized loci encoding peptides with sequence and putative processing homologous to mammalian insulin. DILPs signal through the insulin-like receptor and variously regulate growth, metabolism, aging, reproduction, and other phenotypes.
***Drosophila* target of rapamycin (dTOR):** the homolog of mTOR, a kinase that responds to cellular import of amino acid to regulate various processes including translation and autophagy.
Holidic diet: nutritional media fed to *Drosophila* comprising entirely of chemically specified ingredients.
Insulin/insulin-like growth factor (IGF) signaling (IIS): *Drosophila* has a single (recognized) insulin-like receptor that regulates traits commonly attributed to both insulin and IGF signaling of mammals.
Life expectancy: from a given age, the duration of time until half the current cohort dies. Life expectancy from the onset of adulthood (eclosion in the case of *Drosophila*) is informally referred to as lifespan or longevity.
Longevity reaction norm: life expectancy plotted as a function of diet for a specific genotype. Reaction norms feature in gene-by-environment analysis, a tool of evolutionary genetics used to study phenotypic plasticity.
Median neurosecretory cells (MNC): neurosecretory cells in the medial region of the insect brain that contains the IPCs, among other neurons.
Nutritional rail: a line (vector) representing a give food (or diet) mixture in nutrient space.
Nutritional response landscape: a 3D topographic form of longevity reaction norm in the context of the NGF where life expectancy, or any other response variable, is a function of two dietary variables: calories consumed as protein and as nonprotein (carbohydrates and fat). Fitted isoclines on the topographic surface on the z-plane connect values of life expectancy observed among the experimental cohorts.

mechanisms of DR, we must first clearly define what nutrients modulate lifespan when flies eat less.

Reduced caloric intake *per se* was long thought to be the longevity determinant of DR for all animals, contributing to theories whereby DR modulates aging by reducing metabolic rate and associated costs of oxidative stress [1]. This interpretation was based on mouse studies where food consumption was measured directly [9]. However, in *Drosophila*, the usual DR protocol is to provide adults a diluted but *ad libitum* diet. By reducing all dietary components or by maintaining adults on media with a fixed concentration of sugar with reduced amounts of autolyzed yeast, longevity can be extended 50% or more [7,10]. Nevertheless, because ingestion rates are not typically measured or controlled, whether adults consumed fewer calories was assumed rather than observed. Techniques to track ingestion eventually revealed that adults partially compensated for dilute diets by eating more [11,12]. Nonetheless, these data did not determine whether flies compensated by consuming more yeast and carbohydrates, or by selectively increasing their intake of one nutrient relative to the other. Overall, diet dilution extends lifespan, but it remained unknown whether longevity assurance is modulated by a reduction in net calories, protein, carbohydrates, or other nutrient features, such as essential fatty acids and micronutrients.

Contribution of specific nutrients

To understand the impact of caloric intake relative to specific nutrients in *Drosophila*, studies have simultaneously manipulated the quantity of carbohydrate (sugar) and protein (provided in hydrolyzed yeast) available from *ad libitum* diets, while directly measuring food intake or net energetics [13–15]. Lee *et al.* [12] provided adults with diets comprising sugar and yeast at seven different protein:carbohydrate ratios, each at four concentrations. Consumption of each nutrient and lifespan were measured for individually held females. The lifespan of each fly was plotted on the z-axis as a function of its intake of yeast-derived protein (P) and of sugar-derived carbohydrate (C), plotted on the x- and y-axis, respectively. The resulting landscape surface revealed isoclines of lifespan where the maximum elevation indicated the P:C ratio of greatest survival (Figure 1). Notably, *Drosophila* lifespan was greatest along a topological ridge with 1P:16C, and this diet ratio maximizes lifespan at any level of total food

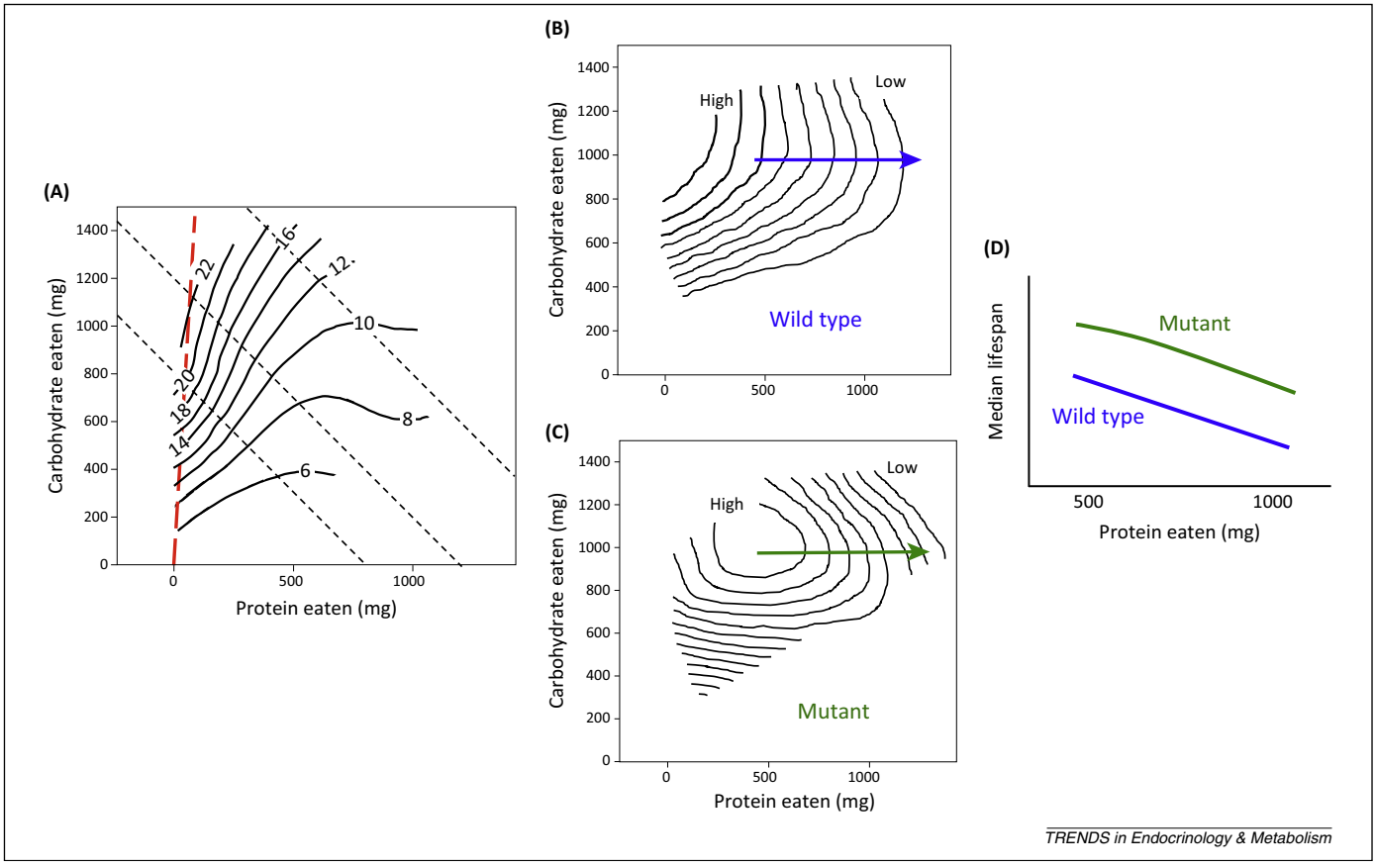

Figure 1. Nutritional response landscapes of median lifespan. **(A)** Topographic representation of *Drosophila melanogaster* median lifespan response landscapes as functions of consumed protein and carbohydrates [12]. Isoclines represent life expectancy fit to a spline surface estimated from median lifespan from 28 female cohorts maintained on different diet combinations of protein (P) and carbohydrate (C). A diet that yields consumption of 1P:16C produces the maximum lifespan (red dash line). Transects of isocaloric intake (black dashed lines): lifespan increases along an isocaloric transect as the consumed P:C ratio decreases; caloric intake itself can vary without affecting lifespan. Hypothetical response landscapes for a wild type **(B)** and a mutant **(C)** that represent different total elevation and topography. The differences among the topographic shape of these landscapes represent an underlying mechanistic interaction between the mutated gene and longevity in response to diet; the gene participates in how DR modulates lifespan. Arrows represent potential vectors of diets in a hypothetical experiment to determine how lifespan responds to DR when diet is manipulated by dilution (here with carbohydrates fixed at 1000 mg and protein diluted from approximately 1500 mg to 400 mg.) **(D)** Observed median lifespan that would be obtained in the gene-by-diet analysis with the diet dilution vectors of (B) and (C). Although the mutant (green) is longer lived than wild type (blue) on all tested diets, its reaction norm to dietary protein has the same response (slope) as that of wild type controls. Such data would inadvertently suggest that the mutated gene does not contribute to the mechanism by which DR modulates lifespan. Modified from [12] (A) and [31] (B,C).