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Ageing

Genetic mechanisms of lifespan extension by dietary restriction

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Dietary restriction (DR) is the most robust environmental method of lifespan extension in species as diverse as yeast, worms, fruit flies and rodents. Here we discuss the genetic mechanisms that have been proposed for slowing the rate of aging by DR in various organisms. Understanding these genetic pathways will not only yield excellent research tools for understanding the biological mechanisms of aging but might also serve as potential drug targets for delaying age-related diseases like cancer, cardiovascular diseases and neurodegeneration.

Introduction

Reduction of calories by one-third of that consumed *ad libitum* by rodents increases both maximum and mean lifespan by over 50% [1–3]. The robust effects of lifespan extension by dietary restriction (DR) in numerous model organisms [4–6] have made it one of the most rapidly evolving areas of inquiry in biological research. Given the protective effects of DR against neurodegeneration and tumorigenesis in rodents, a better understanding of pathways related to DR will provide insight into the etiology of age-related diseases. Conservation of signaling pathways and the rapidity of discovery make research in model organisms germane to understanding lifespan and age-related diseases in humans.

Are the beneficial effects of DR universal?

Various studies using rodents have demonstrated the potential applicability of DR to other mammalian species, beginning with the seminal work by McCay and co-workers [7,8] in

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the early part of the past century. Canines are the largest mammals with confirmed life extension when placed on DR regimen [9]. Studies in non-human primates are currently underway but as yet incomplete [7,10]. The question of whether DR can extend human lifespan remains unknown. A 2-year biosphere study in which human participants underwent DR showed physiological changes that were previously demonstrated in rodents under DR [11,12]. Interestingly, the Baltimore Longitudinal Study on Aging in humans observed reduced insulin and body temperature associated with increased lifespan in male participants, parameters similarly modified during DR in rodents [13]. Strong evidence in favor of linking nutrition and lifespan in humans comes from a study involving more than a million subjects relating body-mass index (BMI) to mortality. The results showed that the risk of death from all major diseases including cancer and cardiovascular diseases increases throughout the range of moderately and severely overweight individuals [14].

Why does DR extend lifespan? Natural environments have fluctuations in food availability. It has been proposed that Darwinian fitness will be increased if animals cease breeding during periods of food deprivation and invest resources in maintenance of the soma [15]. This would increase the probability of living long enough to produce viable offspring once nutritional conditions are improved. The evolutionary adaptation which allows the shift in metabolic investment from reproduction to somatic maintenance has been hypothesized to be the basis for the lifespan extension effects of DR [15]. Such

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a metabolic shift upon nutritional manipulation is supported by experimental observations in flies [16] and rodents [7]. However, depending on their ecological niche different species are probable to benefit to varying degrees from DR.

The quest to understand mechanisms of DR-induced lifespan extension has led to intensive studies in the primary genetic model organisms *Saccharomyces cerevisiae* (yeast), *Caenorhabditis elegans* (worms), *Drosophila melanogaster* (flies) and the mammalian model organism *Mus musculus* (mice). Invertebrate models continue to be useful in providing candidates to be tested in mammals owing to their short lifespan and ease of genetic manipulation. Given the conservation of biological processes and signaling pathways, studies in model organisms are probable to make important contributions to our understanding of biological mechanisms of lifespan extension by DR.

The importance of restricting particular nutrients

It was previously believed that total caloric intake was the most important aspect of extending lifespan by manipulating nutrients [7]. Thus, the term 'caloric restriction' (CR) was used initially to describe the field of inquiry. The term is not apt for what numerous studies over the past two decades have demonstrated, namely that the particular nutrients and methods of restriction are as important and have the potential to be more informative than restricting the total number of calories. Results comparable to restricting *ad libitum* feeding have been demonstrated by restricting intake of a particular amino acid, such as methionine [17–20] or tryptophan [21,22] in rodent studies. Although total body weight was reduced in methionine-restricted animals, they actually ate more per unit of body mass than controls [18]. These results suggest that a decrease in energy intake or expenditure on a body weight basis is not necessary for lifespan extension. Another method of DR, 'every other day' (EOD) feeding involves short-term fasting and shows equal life extension benefits compared with rodents restricted to 60% of *ad libitum* feeding [23]. EOD animals consume nearly equal (total) amounts of food and weigh the same as *ad libitum*-fed controls, again demonstrating that the benefits of DR need not be calorie-dependent.

In *D. melanogaster* restriction of total food yields extended lifespan [24–26]. Fly media primarily consists of yeast (the major source of protein, vitamins, lipids and cholesterol) and carbohydrates (sucrose). It has been shown that reducing yeast extract alone is sufficient to extend lifespan [16,27–30]. A recent study took these results further, showing total caloric intake is not crucial for lifespan extension by DR in *D. melanogaster* [30]. Interestingly, mediterranean flies (*Ceratitis capitata*) exhibit two separate nutrient-dependent modes of aging. These animals persist in a nonaging waiting mode when fed sugar only and switch to a reproductive aging mode in the presence of yeast, a scarce resource in the wild [31].

These results further demonstrate the importance of individual macronutrients over calories in determining aging.

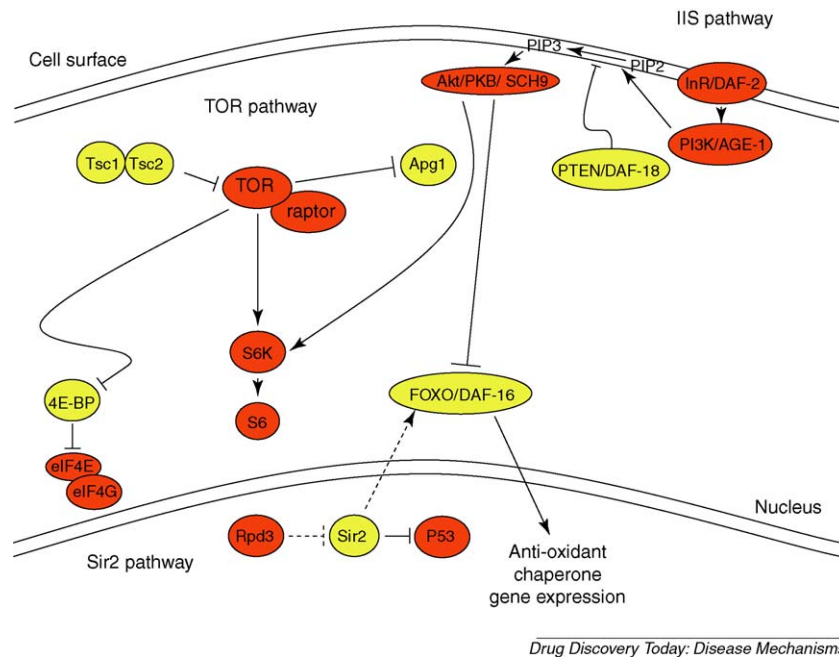
Yet another method of DR involves restriction of a particular micronutrient. *C. elegans* normally feed on bacteria that contain a compound called ubiquinone. When worms are fed bacteria lacking the ability to produce ubiquinone, a significant life extension is observed compared with controls fed normal *Escherichia coli* [32]. Ability to manufacture ubiquinone endogenously is associated with the *clk-1* gene, mutations of which are also associated with extended lifespan [33,34]. The lifespan extension by *clk-1* is not further enhanced by mutation in *eat-2*, which causes DR by reduced feeding, suggesting they are in the same pathway [33]. One possibility is that many nutrients act coordinately to manifest physiological change; hence reduction of a single nutrient is sufficient for lifespan extension. Identification of genetic pathways that mediate the lifespan effects of specific nutrients will provide a more complete understanding of how DR modulates lifespan.

Genetic pathways linked to lifespan extension by DR IIS pathway

Evidence from recent studies implicate the importance of the insulin/IGF-1 signaling (IIS) pathway, the TOR pathway and Sir2 in mediating nutrient modulated lifespan changes (Fig. 1). Modulation of genes in the IIS pathway, known to alter nutrient sensing [35], extends lifespan in various species [4,5,36,37]. Mutants that inhibit the IIS pathway phenocopy the cellular and organismal effects of starvation, leading to a reduction in body size [35,38]. In *C. elegans* the IIS pathway controls the formation of the dauer larva in response to crowding and starvation conditions, an alternative developmental state that is nonreproducing, stress resistant and long lived [39,40].

Mutants in the same pathway, *daf-2* (insulin receptor) and *age-1* (PI3 kinase), lead to a doubling of lifespan and render the animals more prone to dauer formation [41,42]. Both the dauer formation and lifespan extension phenotypes are suppressed by mutations in *daf-16*, a forkhead family transcription factor [41,43,44]. In *D. melanogaster*, mutations in IIS pathway genes *Inr* (insulin-like receptor) and *chico* (phosphatidylinositol 3-kinase) also regulate lifespan [45,46].

The relationship between DR and IIS mutants has been examined in worms, flies and mice. A study on *chico* mutant flies employing varying concentrations of media demonstrates that similar maximal lifespan is observed at a lower concentration of media for controls than for *chico* flies [24]. This result points to an overlapping mechanism of life-extension by DR and IIS pathway mutants. However, a study in *C. elegans* demonstrated lifespan extension on DR in both *daf-2* and *daf-16* mutants suggesting the independence of IIS and DR lifespan extension mechanisms in worms [47]. Interactions between mechanisms of longevity by genetic perturba-



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Figure 1. Signaling pathways that modulate nutrient dependent lifespan extension. TOR, IIS and SIR2 pathways are represented. TOR pathway activation decreases autophagy through Apg1 and enhances translation by phosphorylating ribosomal S6K and 4E-BP1, an inhibitor of eIF4E. eIF4E is part of the eIF4 complex that mediates cap-mediated translation to promote growth in response to nutrients. IIS pathway activation initiates a signaling cascade that blocks FOXO/DAF-16 from translocating to the nucleus and upregulating stress resistance genes including antioxidants and chaperones. The IIS pathway also phosphorylates S6K via Akt/PKB. SIR2 activity inhibits P53 activation, although itself being repressed by Rpd3. Red/yellow indicate factors that increase/decrease aging when activated. Dashed lines indicate that unknown factors might mediate signaling (S6K – S6 kinase, 4E-BP – eIF4E-binding protein, eIF4E – eukaryotic initiation factor 4E, FOXO/DAF16 – forkhead transcription factor/dauer formation, Akt/PKB – protein kinase B).

tion and DR have also been examined in mice. Ames (Prop1df homozygous) dwarf mice are deficient in growth hormone, prolactin and thyroid stimulating hormone, live about 50% longer than their normal siblings, and show phenotypes of stress resistance, reduced insulin secretion, hypersensitivity to insulin and reduced glucose concentration [48]. These mice demonstrate patterns that typify IIS pathway mutants in invertebrates, suggesting a common mechanism of slowed aging functions in mammals [49]. DR confers a further lifespan increase in these dwarf mice, indicating that the two factors might act through different pathways. However, there is a formal possibility that maximal DR longevity responses are not achieved by the genetic perturbations and DR protocols employed, which should be borne in mind while interpreting these studies [6].

TOR pathway

Mutations that affect components of the TOR pathway cause growth defects that point toward its role in nutrient sensing. In *S. cerevisiae*, disruption of TOR1 or treatment with rapamycin leads to a cell arrest at the G1 phase of the cell cycle, reduced protein synthesis, increased levels of autophagy and decreased amino acid transport [50,51]. In *D. melanogaster*, larvae lacking TOR show similarities to amino acid-deprived animals, such as reduced nucleolar size and lipid vesicle aggregation in the larval fat body (a structure that acts as both the liver and the

adipose tissue of the fly) [52]. In *C. elegans*, deletion of the gene encoding TOR leads to developmental arrest at the L3 larval stage; these larvae exhibit an intestinal phenotype characterized by an increase in gut lumen size and a decreased ability to absorb and digest nutrients [53].

Mutations that inhibit the TOR pathway extend lifespan in yeast, worms and flies. TOR1 mutants in *S. cerevisiae* extend lifespan in a manner overlapping with DR but distinct from Sir2 [54]. Downregulation of TOR activity by RNA interference (RNAi) [55] or a heterozygous mutation in *daf-15* mutants [56] displays an extended lifespan as compared to wild type *C. elegans*. We have observed that modulation of various genes that encode components of the TOR signaling pathway, including the products of the tuberous sclerosis complex genes (*Tsc1* and *Tsc2*), TOR and S6K extend lifespan in *D. melanogaster* [27]. The lifespan extension displayed by mutant flies was ameliorated under conditions of DR connecting effects of TOR on lifespan with its previously characterized role in responding to nutrients [57]. The TOR and IIS pathway appear to show both parallel and overlapping interactions which are reviewed elsewhere [57–59].

SIR2

The SIR2 pathway extends lifespan in an overlapping manner with DR in yeast [60,61]. There, a cyclic AMP-dependent protein kinase A (PKA) pathway senses glucose availability.

Lowering glucose concentration extends the replicative lifespan of normal yeast, but this extension does not take place in PKA pathway mutants [62]. Similarly, overexpression of Sir2, a NAD-dependent histone deacetylase gene [63], extends lifespan under rich nutrient conditions, but does not further extend lifespan under DR [62]. The role of Sir2 in mediating the lifespan extension effect of DR is conserved in flies [64]. Mutations in Rpd3, another histone deacetylase gene, have been shown to extend the lifespan of both yeast and flies [65,66]. In *D. melanogaster* the lifespan extension by Rpd3 mutants is not observed under DR [65,66]. Furthermore, lifespan extension of Rpd3 mutants does not take place in a Sir2 null background, suggesting that both genes are in the same pathway [64,67].

In mammals, studies show a connection between sirt1, the ortholog of Sir2 and nutrition. Nutrient withdrawal increased SIRT1 expression that was dependent on the presence of Foxo3a (forkhead transcription factor). Interestingly, SIRT1 expression was found to be mediated by binding of Foxo3a and p53 on its promoter [68]. It was recently shown that enhanced physical activity owing to DR in mice is dependent on Sirt1 [69]. However, this study failed to demonstrate that changes in glucose, triglycerides and IGF-1 upon DR are dependent on Sirt1. SIRT1 also functions as a p53 deacetylase which leads to downregulation of its activity [70]. P53 itself has been shown to have an effect on lifespan in mice and *D. melanogaster*. The Helfand lab has demonstrated that neuronal expression of a dominant-negative form of P53 extends lifespan and resistance to genotoxic stress [71]. In addition, DR did not further increase lifespan in these mutants, suggesting a connection between the life-extending mechanisms of DR and P53 in flies.

Future outlook

The robust life extension of DR along with developments in our understanding of mechanisms that might mediate its effects has led to interest in developing DR mimetics. In attempts to attenuate signaling through the insulin receptor, researchers in the late 1990s focused on glycolytic inhibition and used glucose analogs in rodents such as iodoacetate and 2-deoxyglucose [72–74]. Unfortunately, these measures have been unable to mimic the full range of beneficial effects of DR, most notably and in particular, that of increased lifespan. An interesting compound that enhances insulin sensitivity is metformin, a drug in use today for treatment of type 2 diabetes. A recent screen in rodents using microarray technology demonstrated that metformin produced many of the gene expression profiles associated with long-term DR [75]. Compounds called thiazolidinediones activate peroxisome proliferator activated receptors (PPARs), important regulators of energy metabolism, and are under investigation for treatment in models of obesity and diabetes in non-human primates [76]. One effect of DR is to reduce the age-related

decline in PPARs [77], raising the possibility of their use in the search for DR mimetics. Another class of DR mimetics activate sirtuins like SIR2, referred to as sirtuin-activating compounds (STACS). One of the most potent SIR2 activators comes from a naturally occurring plant polyphenolic found in grapes called resveratrol. Interest in resveratrol continues to grow because this compound has been shown to increase longevity in yeast [78], nematodes and flies [79], and in a short-lived vertebrate species [80].

The genetic pathways that modulate lifespan changes upon nutrient manipulation are beginning to be identified and characterized. Future studies utilizing proteomics will likely provide crucial insight into the translation and post-translational modifications that take place on nutrient manipulations. It is generally believed that aging takes place owing to accumulation of cellular damage and that this is slowed under DR. An interesting study from the Partridge lab questions this idea [26]. Their study in *D. melanogaster* shows that DR extends lifespan entirely by reducing the short-term risk of death and hence DR instigated at any age could generate a full reversal of mortality. Many questions remain regarding the downstream mechanisms that are responsible for the lifespan effects of this important environmental manipulation. Studies relating the pathways discussed to respiration, hormesis, free radical damage, chaperone defenses and protein turnover will help us understand whether these processes are causally linked to nutrient-modulated lifespan changes.

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