Cell. Mol. Life Sci. 64 (2007) 1323 – 1328
1420-682X/07/111323-6
DOI 10.1007/s00018-007-6470-y


Cellular and Molecular Life Sciences

# Visions & Reflections (Minireview)

# Ruminations on dietary restriction and aging

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Received 1 November 2006; received after revision 15 December 2006; accepted 27 February 2007
Online First 31 March 2007

**Abstract.** Calorie restriction has been known for many decades to extend the life span of rodents. Since the more recent discovery that a long-term reduction in nutrient intake also extends life span in nearly every invertebrate model organism used for aging research, the mechanisms behind the longevity benefits of this intervention have been under intense scrutiny. While models have been developed in yeast, worms, and flies, the molecular mechanisms governing life span extension by calorie restriction remain controversial, resulting in great anticipation of mammalian studies testing these models. Here we discuss the links between nutrient reduction and enhanced longevity with emphasis on evolutionarily conserved nutrient response signaling.

**Keywords.** Aging, calorie restriction, dietary restriction, nutrient sensing, TOR kinase, insulin, PKA.

Aging in mammals is often defined as a physiologically degenerative process that culminates in mortality. Within an organism, different tissues and cell types age at different rates and show different pathologies. For example, skeletal muscle and other tissues are infiltrated by adipose cells, contributing to age-related declines in muscle mass and function [1]; neurons are sensitive to protein aggregation leading to neurodegenerative diseases [2]; and adult stem cell populations undergo declining regenerative potential [3]. There is little doubt that diseases of aging can be influenced by both genetic and environmental factors. Polymorphisms in multiple genes have been identified that alter the likelihood of cancer, neurodegenerative decline, type II diabetes, cardiovascular disease, and a number of other diseases. Likewise, many different environmental parameters are known or suspected to play a role in these same diseases. Completely eliminating any one of these diseases, however, would be expected to have only a limited effect on the average life span of a population [4, 5].

Nevertheless, several different single-gene mutations have been found to significantly increase the life span of mice and rats, at least under laboratory conditions [6]. Moreover, mutation of orthologs of many of these genes lead to long life span in invertebrate model organisms used for aging studies. Therefore, single gene mutations profoundly affect aging and conserved aging pathways likely exist among disparate eukaryotic species, although no quantitative assessments of the extent of conservation have been ventured. How can these mutations combat the tissue and cell-type specific decline elegantly described by studies on the physiology of aging?

One environmental intervention, calorie restriction (CR), was discovered to significantly increase life span

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**Table 1.** Various CR/DR protocols extend life span in model organisms.

| Model | Protocol | Lifespan extension | Reference |
|---|---|---|---|
| Yeast RLS | reduced glucose levels in media | 20–30 % | [12, 63] |
| | reduced amino acids levels in media | 50–100 % | [13] |
| | genetic mutations (e.g., *hxk2Δ*, *gpa2Δ*, *sch9Δ*, *tor1Δ*) | 20–30 % | [12, 37], reviewed in [64] |
| Yeast CLS | transfer of cells from medium to water | 50–100 % | reviewed in [24] |
| | reduced asparagine or glutamate levels in the media | 50–100 % | (36) |
| | genetic mutations (e.g., *cyr1Δ, ras2Δ, sch9Δ*, *tor1Δ*) | 50–100 % | [32, 36] |
| Worms | reduced bacterial food (solid medium) | 50 % | [11, 65, 66], reviewed in [67] |
| | reduced bacterial food (liquid medium) | 60 % | |
| | axenic medium | 100 % | |
| | genetic mutations (e.g., *eat-2*) | 50 % | |
| Flies | reduced availability of live yeast | 40 % | reviewed in [15] |
| | dilution of nutrients in food medium | 40 % | |
| Mammals (mice and rats) | reduced food or nutrient (e.g., methionine) intake | 20–60 % | [8, 68] |
| | alternate day feeding | 20–30 % | reviewed in [16] |

of rodents over 7 decades ago [7]. CR delays the onset of multiple age-associated pathologies, including cancer, cognitive decline, diabetes, and cardiovascular disease [8]. Although a variety of different CR protocols have been reported, in general they amount to a 30–50 % reduction in dietary intake relative to *ad libitum* fed controls, resulting in a 20–50 % increase in mean and maximum life span [8].

CR also significantly increases life span in many simpler eukaryotes, including the common model organisms *Drosophila melanogaster* [9, 10], *Caenorhabditis elegans* [11] and *Saccharomyces cerevisiae* [12–14]. In flies, researchers have discovered that life span can be extended by altering the nutrient content of the food without changing the calorie content, thus leading to adoption of the more general term DR (dietary restriction) rather than CR [15]. Alternate day feeding in mice also is reported to extend longevity even though these mice likely consume the same number of calories [16]. Although the protocols for limiting nutrient intake vary in different organisms (Table 1), the percent effect on life span from DR is remarkably similar in each case.

No longevity data have been generated for humans practicing DR and primate studies are still underway; however, short-term studies in both humans and other primates have generally led investigators to conclude that DR produces physiological, metabolic, and hormonal effects that in many ways parallel those seen in rodents [17–23]. Taken together, these findings suggest that DR represents an evolutionarily conserved mechanism for modulating longevity in response to decreased nutrient availability in the environment.

The ability of DR to increase life span of such diverse organisms is particularly impressive when one considers the dramatic differences in how aging is measured in different organisms, as well as the varied age-associated changes that occur. Mice and rats, for example, are highly complex animals that show a broad spectrum of age-associated phenotypes and diseases, compared to worms or flies. Consider the case of cancer, which shows a clear age-associated increase in mammals, but is not relevant in the aging of worms and flies, which (with the exception of the germ line) are largely post-mitotic as adults. This divergence is even more striking in yeast, where longevity is defined separately for dividing and non-dividing cells. Aging of non-dividing yeast cells is termed chronological aging and is defined by the ability of cells to survive in a quiescent-like stationary phase [24]. Aging of dividing yeast cells, on the other hand, is termed replicative aging and is defined by the number of daughter cells a mother cell can produce before senescence [25]. Yet DR is able to increase life span in all of these different organisms and aging paradigms. There is much interest among biogerontologists in understanding the genetic and molecular mechanism(s) linking DR to longevity. The nearly universal ability of DR to slow aging has led to speculation that DR may have similar effects on longevity and age-associated disease in humans. While most people would be unable or unwilling to voluntarily reduce their food consumption by 30–50 %, the genes involved in the response to DR might make viable drug targets for treating a variety of age-associated pathologies. Small molecules that phenocopy the effects of DR, termed 'DR mimetics', are a hot

commodity among entrepreneurial biogerontologists [26].

How does DR work? Insight may come from single-gene mutations that, like DR, increase life span across evolutionarily divergent species. Consistent with this idea, a majority of such mutations result in reduced signaling through conserved nutrient-responsive kinases (Fig. 1). For instance, mutations which cause decreased insulin/IGF-1-like signaling (IIS) increase life span in mice, worms and flies [27–30]; mutations in AKT-family kinases (*SCH9* in yeast) increase life span in worms and increase both replicative (RLS) and chronological (CLS) life span in yeast [31–33]; hypomorphic mutations in TOR increase life span in flies and worms [34, 35], and increase both RLS and CLS in yeast [36, 37], and mutations in the protein kinase A (PKA) pathway increase RLS and CLS in yeast [12, 33]. These proteins and the pathways in which they function have both overlapping and distinct roles in ensuring the appropriate cellular response to nutrient and growth factor cues in each of these organisms.

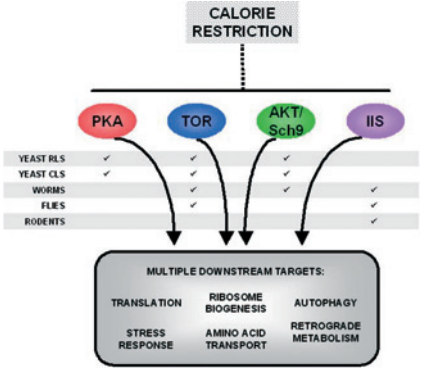


**Figure 1.** Highly conserved nutrient-responsive kinases regulate longevity in evolutionarily disparate organisms. PKA, TOR, AKT/Sch9, and Insulin/IGF-1-like signaling (IIS) pathways coordinately regulate multiple downstream targets. Decreased signaling through these pathways, resulting from calorie restriction (or dietary restriction), leads to enhanced longevity in multiple model organisms (indicated by check marks).

AKT/Sch9, TOR, and PKA activity together regulate a large number of downstream processes that might impinge on aging. These include ribosome biogenesis and translation, metabolic changes involved in amino acid and carbon source utilization, autophagy, and stress response pathways [38–41]. In response to mutations or environmental conditions (such as DR) that decrease the activity of one or more of these kinases, it would be expected that cells will increase the activity of stress response components. These include chaperones important for the heat shock response and enzymes like superoxide dismutase and catalase that combat oxidative damage. In addition to elevating stress response enzymes, decreased signaling through these nutrient responsive pathways may enhance autophagy and/or decrease protein synthesis. Enhanced protein turnover by autophagy could likely lead to reduced accumulation of damaged proteins, whereas a decrease in protein synthesis would likely result in energy savings and/or reduced production of potentially damaged proteins in old cells. Any or all of these effects can be viewed as favorable to long life span; it is this pleiotropy that may reconcile the conundrum of how single-gene changes can extend organismal longevity when cells and tissues within the organism are undergoing differential modes of decline. By mediating different pro-longevity responses in different cells and tissues, the signaling pathways responsive to DR may be at the nexus of aging regulation.

Studies examining the aging effects of altering downstream processes regulated by AKT, TOR, and PKA have been performed in some contexts. For example, mutations leading to reduced translation in some cases have been found to result in life span extension. Flies expressing a dominant negative allele of S6 kinase experience a 20 percent increase in mean life span [34]. Moreover, S6 kinase null mice have some properties associated with long life span, although aging studies have not been done [42, 43]. In yeast, mutations that lead to reduced 60S ribosomal subunit biogenesis lead to long replicative life span [37]. Epigenetically, these mutants map to the same pathway as DR, but it remains to be determined what the specific benefits of reduced 60S biogenesis are to aging yeast cells [K.K.S., V. MacKay, B.K.K., and M.K., unpublished].

Enhanced response to oxidative and other stressors correlates with increased life span in some cases but not others. For instance, catalase overexpression in mitochondria leads to a 15% increase in mouse life span [44], and superoxide dismutase overexpression can lead to long life span in flies [45, 46] as well as increased CLS in yeast [33]. However, reduced superoxide dismutase levels do not necessarily lead to reduced life span in yeast RLS and mouse models [47, 48]. Elevated levels of small heat shock proteins can extend life span in flies [49, 50] and worms [51, 52], and many interventions that increase life span enhance thermotolerance in *C. elegans* [53, 54].

Induced by nutrient deprivation, autophagy has also been implicated as a potential mediator of longevity [55]. For instance, life span extension caused by mutation of the insulin/IGF-like receptor, daf-2, is partially dependent on autophagy [56]. Although it remains to be determined whether down regulation of translation, increased stress resistance, or increased

autophagy are involved in life span extension from DR, the evidence to date supports a model whereby reduced signaling through these highly conserved nutrient-responsive kinases delays the onset of age-related phenotypes through multiple downstream targets that are organism and cell-type specific.

The protein deacetylase Sir2 is one of the few other genes known to regulate aging in multiple eukaryotic organisms. Elevated Sir2 activity leads to increased RLS in yeast [57], and increased life span in worms and flies [58, 59]. Furthermore, the putative SIRT1 (Sir2)-activator resveratrol has been shown to increase life span of mice fed a high-calorie diet [69] as well as extending the life span in the fish *Nothobranchius furzeri* [60]. Paradoxically, Sir2 impedes CLS life span extension when coupled with a genetic mimic of DR (deletion of *SCH9*) [61]. There is currently little consensus regarding mechanisms underlying the pro-longevity effects of Sir2 among different organisms. Consistent with the hypothesis that genetic interventions that extend life span in disparate species are likely involved in nutrient signaling, SIR.2-1 (the worm Sir2 ortholog) has been shown to regulate some of the same downstream targets as the insulin receptor DAF-2 [58]. Evidence is conflicting as to whether Sir2 function is required for life span extension by DR (see [62] and references therein).

Given that there is little selective pressure for organisms to maintain fitness beyond the reproductive period, how could nutrient-responsive pathways that regulate a number of cellular activities which influence longevity have evolved? When nutrients are plentiful, organisms are geared to grow, develop, and reproduce quickly to take advantage of a favorable environment. In times of scarcity, perhaps replicated in lab settings by DR protocols, organisms reduce growth rate and concentrate on prolonged viability to survive until more favorable conditions return. Central to this adaptation is the elevation of stress responsive pathways to offset the accumulation of damage in cells with reduced proliferation rates. This type of growth regulation is likely to be under evolutionary selection in a wide range of eukaryotes. By controlling the critical decision to grow or maintain, nutrient-responsive kinases are ideally positioned to regulate the aging process as well.

Over the last 2 decades, studies of aging have become increasingly tractable, leading to rapid increases in understanding. The development of invertebrate aging models has been central to this advancement. These models are being successfully employed to develop hypotheses concerning aging and, more specifically the mechanisms behind the beneficial effects of calorie restriction, which can then be tested in mammals. Although the pathways by which calorie restriction regulate aging are still poorly defined and largely controversial, better understanding seems near at hand. Whether this knowledge can be employed to generate DR mimetics that effectively combat aging and age-related disease remains to be determined.

*Acknowledgements.* K.K.S. has been supported by NIH training grant P30 AG013280. Aging research in the lab of B.K.K. is funded by awards from the Ellison Medical Foundation and by National Institutes of Health grant R01 AG024287. Aging research in the lab of M.K. is funded by an award from the American Federation for Aging Research.

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