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Minireview

Longevity Genes: Insights from Calorie Restriction and Genetic Longevity Models

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In this review, we discuss the genes and the related signal pathways that regulate aging and longevity by reviewing recent findings of genetic longevity models in rodents in reference to findings with lower organisms. We also paid special attention to the genes and signals mediating the effects of calorie restriction, a powerful intervention that slows the aging process and extends the lifespan in a range of organisms. An evolutionary view emphasizes the roles of nutrient-sensing and neuroendocrine adaptation to food shortage as the mechanisms underlying the effects of CR. Genetic and non-genetic interventions without CR suggest a role for single or combined hormonal signals that partly mediate the effect of CR.

Longevity genes fall into two categories, genes relevant to nutrient-sensing systems and those associated with mitochondrial function or Redox regulation. In mammals, disrupted or reduced growth hormone (GH)–insulin-like growth factor (IGF)-1 signaling robustly favors longevity. CR also suppresses the GH–IGF-1 axis, indicating the importance of this signal pathway. Surprisingly, there are very few longevity models to evaluate the enhanced anti-oxidative mechanism, while there is substantial evidence supporting the oxidative stress and damage theory of aging. Either increased or reduced mitochondrial function may extend the lifespan. The role of Redox regulation and mitochondrial function in CR remains to be elucidated.

Keywords: calorie restriction, longevity gene, mitochondria, neuroendocrine, nutrients

INTRODUCTION

Moderate restriction of food intake with essential nutrients reduces morbidity and mortality in aging animals compared with animals with abundant food supply (Masoro, 2003). This paradigm is often referred to as the anti-aging effect of calorie restriction (CR). Studies to date have identified a wide variety of physiological and pathological age-related changes are inhibited partially or almost completely by CR, and that the effect is induced mostly by reduced energy intake and not reduction of specific nutrients or toxic substances (Masoro, 2003). Since this phenomenon of lifespan extension in rats was first reported in 1935 (McCay et al., 1989), many laboratories have confirmed the effects of CR in rodents and attempted to elucidate the mechanisms

by which CR slows aging. However, our knowledge remains incomplete, particularly with regard to the key genes or signals that mediate the effect of CR.

Studies over the past 70 years have also demonstrated that lower organisms such as nematodes, flies, and even yeast have increased lifespan in response to CR. These findings suggest the presence of evolutionary conserved mechanisms that regulate aging and the lifespan in response to shortages of energy resources.

In 1988, Friedman and Johnson (Friedman and Johnson, 1988) reported that the longer lifespan in a strain of *Caenorhabditis elegans* was due to the mutation of a single gene, later named age-1. Thereafter, genes favoring longevity have been increasingly identified in lower organisms by analyses of long-living mutants and by genetic engineering. In 1996, Ames dwarf mice, in which pituitary hormones are deficient due to a loss-of-function mutation of the prop-1 gene, were reported to live longer than wild-type counterparts (Brown-Borg et al., 1996). That is the first report indicating that a single gene mutation can extend the lifespan in mammals. Since then, over 20 genes have been reported to increase the lifespan of rodents, as identified by spontaneously mutations or genetic engineering (Table 1).

Investigators soon noticed the homology of longevity genes and signals between lower organisms and rodents. Some of the phenotypes are also similar to those in CR animals. These findings suggest the presence of conserved signal pathways that regulate aging and lifespan in a range of organisms (Longo and Finch, 2003), and some of these signals may mediate the effects of CR. Ongoing molecular and genetic dissection of the signal pathways that regulate lifespan, whether CR-related or not, will inevitably aid the development of molecules or compounds that will affect aging and the lifespan, which was once considered inevitable. A comprehensive review of longevity signals in organisms is beyond our scope. Instead, in this mini-review, we

have focused on longevity genes and signals in rodents and discuss their relevance to CR.

Neuroendocrine hypothesis of calorie restriction

Organisms retain the nutrient-sensing system to adapt to times of famine for survival. If not, they cannot survive as a species. In the process of adaptation, organisms suppress physiological functions that cost energy, for example, reproduction and growth, and promote mechanisms involved in the stress response or cellular protection. The effect of CR is hypothesized to be imminent in the nutrient-sensing and adapting process (Holliday, 1989).

In mammals, the hypothalamic arcuate nuclei contain two groups of neurons (Schwartz et al., 2000). One expresses NPY and/or AGRP (see the legend of Fig. 1 for abbreviations); the other expresses POMC and/or CART. These neurons respond to nutrient signals, such as leptin, insulin, IGF-1 and ghrelin, and competitively regulate GnRH, GHRH, TRH and CRH. Reduced food intake or fat storage in the body decreases plasma levels of leptin, insulin and IGF-1, and simultaneously increases ghrelin (Ahima and Lazar, 2008; Camina et al., 2003). These changes of peripheral nutrient signals activate the NPY/AGRP neurons and attenuate the activity of the POMC/CART neurons, leading to reduced activity of GnRH, GHRH and TRH neurons, and activation of CRH neurons (Shimokawa and Higami, 2001a). These hypothalamic changes result in secretion of pituitary and adrenal hormones. Most of these neuroendocrine changes have been confirmed in CR rodents (Nelson, 1994).

Overexpression of NPY, a primary regulator for neuroendocrine adaptation to CR and fasting, increases the lifespan of rats (Michalkiewicz et al., 2003). Ames and

Snell mice, in which pituitary GH, PRL and TSH are almost deficient owing to a loss-of-function mutation of the *prop-1* and *pit-1* genes, respectively, are reported to live longer than wild-type counterparts (Brown-Borg et al., 1996; Flurkey et al., 2001). Disruption or suppression of the GHRH–GH–IGF-1 signal pathway at any level extends lifespan (Table 1). Chemical ablation of the thyroidal axis in young rats and thus reduced serum T4 levels in adulthood also increases lifespan (Ooka and Shinkai, 1986). The life-prolonging effect of CR is reported to be absent in GHR knockout (KO) mice (Bonkowski et al., 2006), although CR further extended lifespan in Ames mice (Bartke et al., 2001). These findings suggest that CR affects the lifespan in rodents, partly through the suppression of pituitary hormones. This emphasizes the importance of the GH–IGF-1 axis in the effect of CR.

CR elevates the plasma concentration of adiponectin, which is secreted from white adipose tissue (Yamaza et al., 2007). Adiponectin sensitizes insulin action in peripheral tissues but also promotes glucose uptake in the skeletal muscle in an insulin-independent manner (Yamauchi et al., 2002). Overexpression of human adiponectin in the mice liver has been reported to extend lifespan (Otabe et al., 2007). Thus, adiponectin is also suggested to be involved in the effect of CR.

CR inhibits chemically induced carcinogenesis in mice, but adrenalectomy ablates the effect of CR (Pashko and Schwartz, 1992; Stewart et al., 2005). In CR rats, the free fraction of corticosterone, which is biologically more potent, is reported to increase although the total concentration did not differ from control rats (Sabatino et al., 1991). Thus, it is also suggested that the adrenal axis is involved in the effect of CR.

As described above, the life-prolonging effect of CR could be reproduced partly if a single or multiple neuroendocrine signaling pathways are modulated in the same directions as those induced by CR. Conversely, removal of specific organs

weakens the effect of CR on neoplasm. These findings support the neuroendocrine hypothesis of CR. Long-term reductions in food intake and thus decreased body fat as the energy store leads to altered synthesis and secretion of relevant hormones and elicits neuroendocrine adaptations through the hypothalamic nutrient sensing system. These nutrient sensing and adaptation systems also subsequently regulate aging and longevity.

Longevity genes and signals relevant to calorie restriction

Some of the long-lived animals do not show an additional increase in lifespan under CR conditions, while others do show an increased lifespan. In the former cases, genes or signals are considered to be implicit in the effect of CR. However, most of these studies have been conducted in yeast and nematodes. In rodents, there are relatively few experiments owing to the demands on time. Nevertheless, phenotypic similarities between genetic models and CR rodents suggest the importance of genes or signals in the effect of CR. Longevity genes fall into two categories, genes related to nutrient-sensing and neuroendocrine systems and those associated with mitochondrial function or Redox regulation.

Nutrient sensing system models

In *C. elegans*, a transcription factor, Daf-16 (equivalent to the mammalian FoxO family of transcription factors) is phosphorylated and inactivated by Daf-2-Age-1 (mammalian insulin or IGF-1 receptor (IR or IGF-1R) and phosphatidylinositol 3-kinase (PI3K)) signaling. Reduction-of-function mutations in *daf-2* or *age-1* gene extend the lifespan, an effect that requires Daf-16 (Dorman et al., 1995; Kenyon et al.,

1993). CR conditions are often induced in *C. elegans* by crossing with mutants of *eat-2*, the gene product of which regulates a pharyngeal function, and mutation of which leads to reduced food intake and increased lifespan (Lakowski and Hekimi, 1998). Lifespan extension by mutation of the *daf-2* or *age-1* gene was initially reported to be independent of mutations of *eat-2* (Lakowski and Hekimi, 1998). However, a recent CR study using a solid plate culture system containing diluted numbers of bacteria as food has shown that the effect of CR partially depends on Daf-16 (Greer et al., 2007). In this experiment, *aak-2* (mammalian AMP-activated protein kinase) was found to phosphorylate and activate Daf-16. The study indicates that the sites of phosphorylation are different from those phosphorylated by the Daf-2-Age-1 signal. Thus, it is suggested that Daf-16 is regulated by dual signals, depending on nutrient states.

As shown in Table 1, rodent longevity genes are clustered in GH–IGF-1 and/or insulin axes. Reduction or disruption of the GH–IGF-1 axis at the hypothalamus, pituitary or peripheral tissues, extends the lifespan in rodents. Our transgenic dwarf rat strain, in which GH is moderately suppressed by overexpression of antisense GH gene and thus plasma IGF-1 concentrations are modestly reduced similar to those in CR rats (Shimokawa et al., 2002), exhibit phenotypes similar to CR rats including glucose-insulin homeostasis and plasma adiponectin levels (Yamaza et al., 2004; 2007). As already described, CR does not additionally increase lifespan in GHR/BP KO mice (Bonkowski et al., 2006). In parallel with the neuroendocrine hypothesis, the GH–IGF-1 pathway is considered an evolutionary conserved signal that regulates lifespan as well as development and growth in organisms. CR could exert the effect partly by affecting the GH–IGF-1 axis.

A role for FoxO transcription factors, mammalian orthologs of Daf-16, in the

GH-IGF-1 models has not been fully evaluated. In the liver of long-lived dwarf rats, inactivation of FoxO1-DNA binding activity by glucose-stimulated insulin is minimized as is in CR rats (Hayashi et al., 2008), although basal activity levels are unaffected. This finding is consistent with that for Daf-2-Age-1-Daf-16 in *C. elegans*. Triple KO of FoxO1, FoxO3a, and FoxO4 shortens the lifespan in mice (Paik et al., 2007), although individual KO of FoxO genes does not significantly affect lifespan. Although overexpression of FoxO1 induces sarcopenia (Kamei et al., 2004), the lifespan of these mice has not been reported. Moreover, alterations in the effect of CR on lifespan in FoxO KO mice models have not been published. Because FoxO transcription factors are reported to regulate the expression of stress response genes and metabolic genes (Nakae et al., 2008), further analyses are needed to evaluate the potential role of FoxO in the aging process in mammals.

In *C. elegans*, a transcription factor, Pha-4 (mammalian FoxA transcription factors), was recently reported to be more important than Daf-16 with regard to the effect of CR (Panowski et al., 2007). *pha-4* mutants show no change in lifespan in response to CR, which is induced by bacterial dilution in the culture system. Meanwhile, the longer lifespan in *eat-2* mutant animals is shortened if they are fed *pha-4* RNAi bacteria. In contrast, Pha-4 is not required for the long lifespan of *daf-2* mutant animals or animals with reduced mitochondrial electron transport chain activity. Thus, Pha-4 is considered one of the CR-specific transcription factors. Daf-16 and Pha-4, both of which require the transcriptional cofactor SMK-1, differentially regulate subtypes of Mn-SOD genes (Mair and Dillin, 2008). The difference in expression of Mn-SOD subtypes may lead to the altered responses to CR for longevity. Since the FoxA family is known to regulate glucagon production and glucose homeostasis, particularly in response to fasting (Kaestner, 2000), the potential role of the FoxA family member in

the effects of CR in mammals should be investigated.

Insulin and IGF-1 are not clearly separated in *C. elegans*, in which multiple insulin-like ligands bind to the specific receptor Daf-2. In mammals, severe reduction of the insulin signaling mostly induces diabetic conditions and shortens lifespan (Nandi et al., 2004). Only fat-specific KO of the insulin receptor gene (FIRKO) in mice has been reported to extend lifespan (Bluher et al., 2003). An exact mechanism for lifespan extension in this model is unknown. However, these mice show reduced fat content and increased plasma adiponectin concentrations (Bluher et al., 2002), which are well-known characteristics of CR mice. In addition, an increase in the fraction of small-sized adipocytes has been reported in FIRKO and CR mice (Bluher et al., 2004; Zhu et al., 2007). Specific changes in whole-body metabolism, caused by the alterations of the adipose tissue, may direct animals toward longevity.

Reduction or modulation of expression of intracytoplasmic signal molecules in the IGF-1 or insulin signaling pathways also lead to extended lifespan. The binding of IGF-1 and insulin to their cell membrane receptors (IGF-1R and IR, respectively) activates receptor tyrosine kinase that phosphorylates insulin receptor substrate (IRS) proteins (Taguchi and White, 2008). In turn, the tyrosine-phosphorylated IRS proteins activate (PI3K) and its downstream kinases, including protein kinase B (PKB/Akt). The IGF-1R/IR–IRS–PI3K pathway mainly mediates the metabolic effects of insulin and IGF-1. Deletion of chico, the only IRS protein in *Drosophila*, extends the lifespan (Clancy et al., 2001). In mice, the lifespan of females but not males is also extended by homozygous KO of the *Irs1* gene (Selman et al., 2008). Long-lived female *Irs1* (-/-) mice are resistant to aging-related pathologies including dermatitis. They also showed good scores in motor coordination evaluated by the Rotarod test. In contrast, *Irs2* (-/-) mice, which have diabetic phenotypes, had shortened life spans and died earlier.

Meanwhile, the *Irs1* (+/-) and *Irs2* (+/-) mice had normal life spans.

Taguchi et al (Taguchi et al., 2007) have published some controversial but intriguing findings for *Irs2*. Heterozygous KO of the *Irs2* gene (+/-) or brain-specific double KO of the *Irs2* gene (b*Irs2* (-/-)) in mice extended lifespan by up to 18%. However, those mice tended to become overweight, hyperinsulinemic, and glucose intolerant in older ages. In the b*Irs2* (-/-) mouse brain, SOD2 and FoxO1 protein levels are maintained during old age, but are reduced in control mice of similar age. The authors speculated that attenuation of brain *Irs2* signaling shields the aging brain from the negative effects of hyperinsulinemia on the brain that can reduce lifespan. This discrepancy in the role of *Irs2* between these two studies remains to be elucidated. However, these findings suggest that appropriate suppression of IRS functions also favors longevity in mammals.

The binding of insulin or IGF-1 to their receptors leads to autophosphorylation of the receptor, which generates binding sites for Shc proteins (Taguchi and White, 2008). The association of Shc with the receptor activates the RAS mitogen-activated protein (MAP) kinase pathway, which mediates the mitogenic effects of insulin and IGF-1. Targeted mutation of the p66shc gene, a splice variant of shcA, induces stress resistance and prolongs lifespan in mice (Migliaccio et al., 1999). Although p66shc is not directly involved in activation of the RAS-MAPK pathway (Migliaccio et al., 1997), the study suggests the presence of a pathway separate to that of IRS-PI3K in insulin/IGF-1 signaling that controls lifespan in mammals. Although CR decreases the plasma levels of insulin and IGF-1, the effect of CR on signal transduction of Shc-RAS-MAPK remains to be elucidated.

In *Saccharomyces cerevisiae*, nutrient-sensing pathways controlled by Sch9 (mammalian Akt or S6K), Tor (target of rapamycin; mTor in mammals), and PKA-Ras,

negatively regulate the serine/threonine kinase Rim15 (Wei et al., 2008). Under conditions of abundant nutrients, these pathways are activated to promote cell growth and division (Jorgensen et al., 2004; Martin et al., 2004). Inactivation of Sch9, Tor, or Ras extends the chronological lifespan of yeast (Wei et al., 2008). In these situations, Rim15 is required and up-regulates the expression of genes of superoxide dismutase (SOD) and heat shock proteins through Gis1, Msn2 and Msn4, which are downstream transcription factors of Rim15 that are involved in the stress response and cellular protection. Experiments using extreme CR or starvation, in which cells in the stationary phase are switched from medium to water, or commonly used CR protocols that involve a reduction of glucose concentration have indicated that Rim15 and the stress response transcription factors Gis1, Msn2, and Msn4 are essential for CR-induced lifespan extension (Wei et al., 2008).

Deletion of type 5 adenylate cyclase (Adcy5 or AC5), located upstream of PKA, is reported to extend lifespan of mice by 30% compared with wild-type mice (Yan et al., 2007). In the KO mice, the aging-related reduction of bone mineral density and cardiac dysfunction and pathology are ameliorated. Fibroblasts derived from KO mice also exhibit ERK-dependent resistance to oxidative stress. As indicated, the importance of the Ras–Adcy/cAMP–PKA pathway in CR-induced lifespan extension of yeast and the AC–PKA pathway also remain to be elucidated to better understand the mechanisms of CR.

Genetically reduced TOR signaling has been shown to extend the lifespan of yeast, *C. elegans*, and *Drosophila* (Kaeberlein et al., 2005; Kapahi et al., 2004; Vellai et al., 2003). TOR is known to regulate protein synthesis and growth in response to nutrient intake, particularly amino acids (Tokunaga et al., 2004). In mammals, mTOR signaling may play a role in regulation of aging and lifespan, because mTOR is

negatively regulated by AMPK, which is known to be activated by fasting (Hardie, 2003). Because a failure to inhibit mTOR results in increased neoplasm (Shaw and Cantley, 2006), CR may suppress neoplastic processes by activating mTOR. However, at present, evidence supporting the role of mTOR signaling in the effects of CR is lacking. Evidence for consistent upregulation of AMPK activity in mammals under CR conditions is also limited (Gonzalez et al., 2004). In our study, phosphorylated (p; activated) AMPK levels were lower in the CR rat liver compared with control rats fed ad libitum (AL) (To, 2007 #241). In CR white adipose tissue, p-AMPK levels are increased (Park et al., 2008). Therefore, in rodents, AMPK activity could be regulated in a tissue-dependent manner. However, a potential role for the AMPK–mTOR pathway in the effect of CR remains to be elucidated.

CR increases the replicative lifespan of yeast. The effect of CR is also reported to be dependent on SIR (silent information regulator)-2p (Lin et al., 2002). However, recent studies have indicated that CR additionally increases the lifespan of sir2-deficient yeast and sir2.1-deleted *C. elegans*, casting doubt on the necessity of Sir2p in the effect of CR (Hansen et al., 2007; Kaeberlein and Powers, 2007). In SIRT1-KO mice, however, the beneficial effect of CR on motor functions is minimized (Chen et al., 2005), while overexpression of SIRT1 in mice reduces serum insulin and glucose as seen in CR rodents (Pfluger et al., 2008). SIRT1 is a NAD⁺-dependent histone deacetylase and its activity is altered in response to ATP production in mitochondria (Neugebauer et al., 2008). FoxO transcription factors are substrates of the enzyme. In mammals, seven subtypes of SIRT have been identified; thus, biological functions of these subtypes in response to CR should be elucidated.

As described above in the neuroendocrine hypothesis of CR, altered nutrient-sensing signals are predicted to enhance the stress response and cellular

protection. Many, if not all, longevity models or cells derived from those models exhibit resistance to stressors (Table 1), supporting this hypothesis. However, the role of stress resistance in longevity remains elusive, as discussed below.

Mitochondrial functions or Redox regulation

It is surprising to note that only a few genes of Redox regulation, particularly genes for anti-oxidative enzymes, have been reported to successfully extend lifespan, despite the wealth of evidence supporting the oxidative stress and damage theory of aging and the stress resistance in longevity models.

Overexpression of thioredoxin (TXN), a small Redox-active protein, extended the lifespan in mice (Mitsui et al., 2002). Bone marrow cells exhibit resistance to ultraviolet C-induced cytotoxicity, mostly induced by oxidative stress. The authors correlate oxidative stress resistance with longer lifespan in these TXN-transgenic mice. In the kidney of CR rats, cytoplasmic TXN protein is not increased at 6 and 12 months of age, although the subsequent aging-related decrease of TXN is minimized (Cho et al., 2003). In contrast, in adulthood, CR elevates nuclear TXN, which interacts with Ref-1 to form a regulatory TXN/Ref-1 complex that modulates the transcriptional activities of Redox-sensitive transcription factors such as NF- κ B and AP-1. Thus, TXN may play a role in the oxidative stress response induced by CR.

Overexpression of SOD-2 (Mn-SOD) in mice elicited a marginal increase in lifespan (Hu et al., 2007), although the authors do not describe the formal statistics for longevity, indicating uncertainty over the effect on lifespan. In contrast, SOD2-KO (+/-) mice show increased production of superoxide in tissues, greater DNA damage and increased neoplasm (Van Remmen et al., 2003). However, unexpectedly, lifespan is not shortened. Although many trials have been conducted, albeit unsuccessfully, to

test the effect of anti-oxidative agents on lifespan of rodents, only one recent study has shown that a carboxylfullerene compound, C3, a mitochondrially active SOD mimetic, successfully increased lifespan (an 11% increase in mean lifespan) in normal mice when fed from 12 months of age (Quick et al., 2008). The treatment also attenuated the aging-related increase in production of superoxide radicals in the brain. In tissues from CR rodents, however, Mn-SOD was not consistently upregulated (Gong et al., 1997). In the *daf-2* mutant of *C. elegans*, deletion of SOD3 or double deletion of SOD2 and SOD3 additionally extended lifespan (Honda et al., 2008), although the single SOD2 deletion slightly shortened the lifespan of *daf-2* mutants. Either deletion of SOD2 or SOD3 or double deletion of SOD2 and SOD3 reduces or ablates the resistance to oxidative stress in *daf-2* worms. These findings suggest that damage induced by oxidative stress or stress resistance is not primarily a causal factor that determines lifespan; rather, signals modified by changes in the mitochondrial Redox state seem to be important. Lifespan extension in mitochondrial-targeted catalase overexpression in mice also supports the notion (Schriner et al., 2005).

Mitochondrial functions are important for aging and longevity, because it is the main source of energy as well as its byproducts, including reactive oxygen species. In *C. elegans*, deletion of the *clk-1* gene, which encodes a hydroxylase required for biosynthesis of ubiquinone (Stenmark et al., 2001), lengthens the lifespan (Lakowski and Hekimi, 1996). Ubiquinone is a lipid found in biological membranes and is a co-factor in many Redox processes including the mitochondrial respiratory chain. Double mutants of *eat-2; clk-1* do not exhibit an additional increase in lifespan; this finding suggests the importance of *clk-1* in the effect of CR (Lakowski and Hekimi, 1998).

In mice, KO of the mouse ortholog of *clk-1* (*mclk-1*) resulted in embryonic

lethality if embryos are homozygous (-/-) (Levavasseur, 2001 #253); however, *mclk* (+/-) mice show longer lifespan as compared with wild-type counterparts (Liu et al., 2005). *mclk* (+/-) mice also exhibit reduced mitochondrial function including decreased ATP synthesis, total amounts of NAD, and activities of key enzymes of the TCA cycle (Lapointe and Hekimi, 2008). Surprisingly, although *mclk* (+/-) mice have increased mitochondrial oxidative stress, this is accompanied by decreased oxidative damage of cytoplasmic proteins and plasma isoprostanes, systemic biomarkers of oxidative stress and aging.

A recent study also indicated that mild dysfunction of mitochondrial respiration favors longevity. *Surf1*, a gene encoding a putative cytochrome c oxidase (COX) assembly factor, was reported to be involved in extending the lifespan of mice, if it is knocked-out, and induced a mild defect of COX activity (Dell'agnello et al., 2007).

There is little evidence indicating that CR mildly suppresses mitochondrial functions; in contrast, promotion of mitochondrial biogenesis could substantiate the effect of CR, in which a key molecule is PGC-1 (Lopez-Lluch et al., 2008). In yeast, CR is suggested to activate Sir2p to extend lifespan by shunting carbon metabolism towards the mitochondrial tricarboxylic acid cycle resulting in a concomitant increase in respiration (Lin et al., 2002). A non-genetic approach to modulate mitochondrial respiration has been shown to extend the lifespan of rodents; treatment of mice with low doses of the protonophore 2,4-dinitrophenol, a mitochondrial uncoupler, was found to increase lifespan (Caldeira da Silva et al., 2008). The treatment enhanced tissue respiratory rates, improved serological glucose, triglyceride and insulin levels, decreased reactive oxygen species levels and tissue DNA and protein oxidation. Most of these effects are also seen in CR rodents, except for the enhanced respiration rate. In

some of the nutrient sensing gene models, FIRKO mice showed elevated oxygen consumption at the whole-body level and increased expression of the nuclear-encoded mitochondrial genes for energy metabolism in white adipose tissue (Katic et al., 2007). Replacement of C/EBP α with C/EBP β in mice was also reported to increase mitochondrial oxidative respiration and energy expenditure (Chiu et al., 2004).

Both mild suppression and activation of respiratory function in the mitochondria could be attributable to extension of lifespan, although CR does not appear to affect the metabolic rate if normalized for unit body mass or weight in rats, mice and *Drosophila* (Faulks et al., 2006; Hulbert et al., 2004; McCarter and Palmer, 1992). Altered mitochondrial respiration affects the ratios of ATP/AMP and NAD/NADH and production of oxygen radicals. A key issue regarding the regulation of aging and lifespan could be the identification of signals that are modulated by fluctuations of these mitochondrial molecules. Signals elicited by AMPK and SIRT, both of which are activated by alterations in ATP/AMP and NAD/NADH ratios respectively, have been extensively investigated, whereas our understanding is incomplete regarding the regulation of aging and lifespan.

CONCLUSIONS

The neuroendocrine hypothesis of CR predicted the importance of nutrient-sensing systems and subsequent alterations of the neuroendocrine system in the anti-aging effect of CR. Genetic longevity models have provided direct evidence supporting this hypothesis. Genetic longevity models also indicate the importance of mitochondrial respiratory and Redox states in the regulation of lifespan and aging. Many of the longevity models also demonstrate resistance to oxidative stress; however, lifespan

extension is not solely dependent on resistance to stress or attenuation of tissue damage. We believe that one of the crucial issues for future study regarding the regulation of aging is the identification of longevity signals that respond to changes in mitochondrial respiration and subsequent alterations in ATP/AMP, NAD/NADH, and oxygen radicals as signal molecules. The connection between nutrient-sensing signals and mitochondrial functions also needs to be elucidated.

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Figure Legend

Fig. 1. Neuroendocrine hypothesis of the anti-aging effect of calorie restriction (CR). CR reduces daily energy intake as well as body fat content and these changes lead to alterations in plasma concentrations of hormones (e.g., ghrelin, insulin, IGF-1, leptin, adiponectin). These peripheral signals provide information regarding energy intake and storage states to the hypothalamic arcuate nuclei where neuropeptide Y (NPY) and agouti-related peptide (AGRP) neurons are activated, while proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) neurons are attenuated (Schwartz et al., 2000). These neuronal changes suppress the activity of Gonadotropin-releasing hormone (GnRH), GH-releasing hormone (GHRH), and thyrotropin-releasing hormone (TRH) neurons, and concurrently activate corticotropin-releasing hormone (CRH) neurons. These hypothalamic alterations lead to the suppression of reproduction, growth and thermogenesis, and activation of the adrenal axis. The effect of CR is hypothesized to be induced during the process of nutrient sensing and adaptation (Shimokawa and Higami, 2001b).

Table 1. Longevity genes in rodents focusing on the metabolic rate and stress resistance.

Gene	Symbol	Context of gene modification/animals	Mean or median survival (vs control group)	Metabolic rate or oxygen consumption /unit body weight	Stress resistance	References
<u>Nutrient-sensing/ neuroendocrine adaptation</u>						
Neuropeptide Y	Npy	OE/ rat	698 d vs 633 d in M	n/a	Yes (mice; acute physical restraint stress)	Michalkiewicz et al., 2003; Carvajal et al., 2004
Paired like homeodomain factor 1	Prop	Mut (homo)/ mouse	1076 d vs 723 d in M/ 1206 d vs 718 d in F	n/a	Yes (skin fibroblasts; hydrogen peroxide, paraquat, and UV)	Brown-Borg et al., 1996; Salmon et al., 2005
POU domain, class 1, transcription factor 1 (Pit1, growth hormone factor 1)	Pou1f1 or Pit1	Mut (homo)/ mouse	1178 d vs 832 d in M and F combined.	n/a	Yes (skin fibroblasts; hydrogen peroxide, paraquat, and UV)	Flurkey et al., 2001; Murakami et al., 2003
Growth hormone releasing hormone receptor	Ghrhr	Mut (homo)/ mouse	1093 d vs 886 d in M/ 1070 d vs 857 d in F	n/a	n/a	Flurkey et al., 2001
Growth hormone	Gh1 or Gh	OE of antisense-gh gene (hetero)/ rat	138 w vs 126 w in M	n/a	Yes (rats; endotoxin)	Shimokawa et al., 2002; Shimokawa, 2006
Growth hormone receptor	Ghr	KO (homo)/ mouse	975 d vs 629 d in M/ 1031 d vs 749 d in F	n/a	No (mice; more sensitive to paraquat)	Coschigano et al., 2000
Insulin-like growth factor I receptor	Igf1r	KO (hetero)/ mouse	679 d vs 585 d in M / 756 d vs 568 d in F	Similar	Yes (mice; paraquat/ embryonic fibroblasts; oxidative stress)	Holzenberger et al., 2003
Pregnancy-associated plasma protein A	Pappa	KO (homo)/ mouse	960 d vs 698 d in M and F combined.	n/a	n/a	Conover and Bale, 2007
Insulin receptor	Insr	KO (fat-specific, homo)/ mouse	887 d vs 753 d in M and F combined	Increased	n/a	Bluher et al., 2003

Insulin receptor substrate 1	Irs1	KO (homo)/ mouse	900 d vs 760 d in M and F combined	Similar		Selman et al., 2008
Insulin receptor substrate 2	Irs2	KO (hetero)/ mouse	925 d vs 789 d in M and F combined	Similar	n/a	Taguchi et al., 2007
Insulin receptor substrate 2	Irs2	KO (brain-specific, homo & hetero)/ mouse	bIrs2(+/-): 936 d vs 791 d, bIrs2 (-/-): 901 d vs 791 d in M and F combined	Decreased	n/a	Taguchi et al., 2007
src homology 2 domain-containing transforming protein C1	Shc1 or p66Shc	KO (homo)/ mouse	973 d vs 761 d (sexes unspecified)	n/a	Yes (embryonic fibroblasts; oxidative stress/ mice; paraquat)	Migliaccio et al., 1999
Adenylate cyclase 5	Adcy5	KO (homo)/ mouse	33 mo vs 25 mo in M and F combined	n/a	Yes (embryonic fibroblasts; oxidative stress)	Yan et al., 2007
Klotho	Kl	OE/ mouse	858 d or 936 d vs 715 d in M/ 829 d or 830 d vs 697 d F (Two lines of Kl overexpression mice are used)	n/a	Yes (mice; paraquat)	Kurosu et al., 2005; Yamamoto et al., 2005
Adiponectin	Adipoq	OE/ mouse	137 w vs 100 w in M	Increased	n/a	Otabe et al., 2007

Mitochondria/Redox

Thioredoxin	TXN	OE/ mouse	23 m vs 18 m (sexes unspecified)	n/a	Yes (bone marrow cells; UV)	Mitsui et al., 2002
Catalase	Cat	OE targeted to mitochondrial/ mouse	32 m vs 26 m in M/ 30 m vs 26 m in F	n/a	Yes (fibroblasts)	Schriner et al., 2005

superoxide dismutase 2, mitochondrial	Sod2 or MnSOD	OE (hetero)/ mouse	28.8 m vs 27.6 m (sexes unspecified)	n/a	n/a	Hu et al., 2007
Glutathione peroxidase 4	Gpx4	KO (hetero)/ mouse	964 d vs 915 d in M	n/a	No (mice; sensitive to Diquat-induced apoptosis in the liver)	Ran et al., 2007
Surfeit gene 1	Surf1	KO (homo)/ mouse	793 d vs 654 d in M and F combined.	n/a	Yes (mice; kainic acid induced neurotoxicity)	Dell'agnello et al., 2007
Demethyl-Q 7	Coq7/ clk-1 or melk-1	KO (hetero)/ mouse	825 d vs 720 d in F of the 129Sv/J genetic background. 980.4 d vs 749.8 d in M and F combined of the 129Sv/J x Balb/c.	Decreased in some tissues	Yes (ES cells, liver cells; oxidative stress)	Liu et al., 2005
Uncoupling protein 2	Ucp2	OE in hypocretin neuron in the hypothalamus/ mouse	23 m vs 18 m in F; 27 m vs 23 m in M	n/a (Core body temperature is lower)	n/a	Conti et al., 2006
<u>Unspecified</u>						
CCAAT/enhancer binding protein (C/EBP), beta	Cebpb	Replacement of C/EBPa with C/EBPb/ mouse	28.9 m vs 23.7 m (sex unspecified)	Increased	n/a	Chiu et al., 2004
urokinase-type plasminogen activator	plau	OE, brain specific/ mouse	33 m vs 28 m in F	Increased	n/a	Miskin and Masos, 1997; Miskin et al., 2005

Names and symbols for each gene are as described in the NCBI "Gene" database. The usual abbreviations are also added for some genes. In the context of gene modification/animals, OE, Mut, and KO represent overexpression, mutation, and knockout, respectively. For the mean or median survivals, d, w and m represent day, week and month, respectively. M, males. F, females. n/a, data are not available/published to our knowledge.

Calorie restriction

