Available online at www.sciencedirect.com
ScienceDirect
Ageing Research Reviews 6 (2007) 128–140
ageing research reviews
www.elsevier.com/locate/arr

Review

Sir2 and calorie restriction in yeast: A skeptical perspective

Matt Kaeberlein [a,*], R. Wilson Powers III [b]

[a] *Department of Pathology, University of Washington, Seattle, WA 98195, USA*
[b] *National Institute of Neurological Disorders and Stroke, Bethesda, MD 20892, USA*

Received 20 December 2006; received in revised form 4 April 2007; accepted 9 April 2007

Abstract

Activation of Sir2-family proteins in response to calorie restriction (CR) has been proposed as an evolutionarily conserved mechanism for life span extension. This idea has been called into question with the discovery that Sir2-family proteins are not required for life span extension from CR in yeast. We present here a historical perspective and critical evaluation of the model that CR acts through Sir2 in yeast, and interpret prior reports in light of more recent discoveries. Several specific cases where the Sir2 model of CR is inconsistent with experimental data are noted. These shortcomings must be considered along with evidence supporting a role for Sir2 in CR in order to fully evaluate the validity of this model.
© 2007 Elsevier Ireland Ltd. All rights reserved.

Keywords: Sir2-family; Calorie restriction; Life span; Sirtuins; Dietary restriction; Caloric restriction; Aging

1. Introduction

Calorie restriction (CR) is the only intervention known to enhance longevity in yeast, worms, flies, and mammals; however, the mechanistic details of this process remain largely unknown. A large body of evidence supports the idea that CR slows aging in multicellular eukaryotes, at least in part, by modulating the activity of key nutrient and growth factor responsive genes (such as those regulated by insulin/IGF-1-like signaling and TOR activity) and by decreasing the accumulation of oxidative damage (Bartke et al., 2003; Kenyon, 2005; Merry, 2004; Warner, 2005). An alternative, but not necessarily mutually exclusive, model for CR posits that limiting calories results in activation of the Sir2-family of protein deacetylases (sirtuins), and that this activation accounts for the reduced rate of aging and increased life span observed in response to CR (Guarente and Picard, 2005; Masoro, 2004; Sinclair, 2005).

Although accepted by many biogerontologists and supported by data from multiple studies, aspects of the models proposing a link between sirtuins and CR (referred to hereafter as ‘‘Sir2/CR models’’) are lacking in key experimental verification, and, in a growing number of instances, are inconsistent with recent observations. This perspective-review attempts to provide a more critical analysis of the data regarding the relationship between sirtuins and CR, specifically as it applies to life span extension in the budding yeast. The genetic and molecular models for activation of sirtuins in response to CR were developed largely based on work carried out in yeast. Additionally, yeast offer a facile means of conducting experiments which expose the Sir2/CR models to experimental scrutiny. Thus, we believe that a critical

* Corresponding author. Tel.: +1 206 543 4849; fax: +1 206 543 3644.
E-mail address: kaeber@u.washington.edu (M. Kaeberlein).

1568-1637/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.arr.2007.04.001

evaluation of the primary literature in yeast is an appropriate place to begin a discussion on the validity of these models.

2. A historical perspective of Sir2 and CR in yeast

A role for sirtuins as ''master regulators'' of eukaryotic aging arose from work on replicative aging in the budding yeast, *Saccharomyces cerevisiae*. Replicative life span in yeast is defined as the number of daughter cells produced by a mother cell prior to senescence, and has been used as a model for the aging of dividing cells for more than 50 years (Mortimer and Johnston, 1959). A second model of aging has been described in yeast, termed chronological aging, in which the survival of cells in a non-dividing state is monitored (Fabrizio and Longo, 2003; Kaeberlein, 2006). For the remainder of this review, unless otherwise stated, the term ''life span'' when used in the context of yeast cells refers to replicative life span.

2.1. Sir2 and extrachromosomal rDNA circles

One cause of yeast replicative aging is believed to be the age-associated accumulation of extrachromosomal rDNA circles (ERCs) in the mother cell nucleus (Sinclair and Guarente, 1997). The yeast rDNA is a tandem array of several dozen copies of a 9.1 kb repeat, and ERCs can be formed by homologous recombination between adjacent rDNA repeats (Sinclair and Guarente, 1997). Two factors are likely to contribute to the toxicity of ERCs. First, once an ERC has formed, it has the potential to self-replicate due to the presence of an autonomously replicating sequence (ARS) within the rDNA repeat. Second, ERCs (similar to other CEN-lacking circular DNA molecules) are asymmetrically inherited by the mother cell at cell division. This combination of self-replication and asymmetric segregation leads to an increase in ERC abundance with replicative age, a phenomenon that can be observed by quantifying ERC levels in a sorted population of aged cells (Sinclair and Guarente, 1997).

The evidence for ERCs as a cause of aging in yeast is correlative, but reasonably well-supported. Mutations that increase ERC formation tend to shorten life span, while a subset of mutations that decrease ERC formation increase life span. One such example is deletion of the rDNA replication fork block protein Fob1. Deletion of Fob1 reduces rDNA recombination and the abundance of ERCs in both young and aged cells (Defossez et al., 1999). Deletion of Fob1 also increases life span in many yeast strains (Defossez et al., 1999; Kaeberlein et al., 1999, 2005b), although this is not universal (discussed below). Additional support for the ERC model of replicative aging was provided by the observation that release of an ERC in a young yeast cell is sufficient to shorten life span (Sinclair and Guarente, 1997).

To date, the role that Sir2 plays in modulating replicative longevity in yeast has been assumed to be by affecting the rate of rDNA recombination and thus ERC formation (Guarente, 2000). Deletion of *SIR2* increases rDNA recombination by ~5–10-fold (Gottlieb and Esposito, 1989), increases ERC levels (Kaeberlein et al., 1999), and reduces life span by about 50% (Kennedy et al., 1995). These phenotypes are partially suppressed by deletion of *FOB1* in *sir2Δ* cells, which restores the short life span of a *sir2Δ* mutant to the level of wild type cells (Kaeberlein et al., 1999). Also consistent with this model is the finding that overexpression of Sir2 fails to further increase the long life span of *fob1Δ* mutants (Kaeberlein et al., 1999).

The idea that Sir2 modulates longevity solely by repressing ERC formation may be too simplistic, however, as evidenced by the incomplete suppression of the *sir2Δ* life span defect by deletion of *FOB1*. When ERCs are quantitated in aged cells, both *fob1Δ* and *sir2Δfob1Δ* cells have a dramatic reduction in ERCs relative to wild type cells (Defossez et al., 1999; Kaeberlein et al., 1999). If *sir2Δfob1Δ* cells have a lower level of ERCs than wild type cells, why is their life span not longer than wild type and comparable to that of *fob1Δ* cells? A key piece of data that is missing from the literature is quantification of ERCs in long-lived cells overexpressing *SIR2*. One possibility is that, although ERCs limit the life span of *sir2Δ* mutants, due to hyper-recombination at the rDNA, perhaps ERCs do not limit life span in wild type cells (or in *sir2Δfob1Δ* cells). In support of this idea, we note that, although deletion of *fob1* increases life span, several other mutants with comparably low levels of rDNA recombination do not show increased life span (Defossez et al., 1999; Lin and Keil, 1991). Alternatively, ERCs may limit the life span of some wild type cells but Sir2 may have a novel longevity-promoting role that is necessary for long life span. Given that ERCs are not thought to cause aging in higher eukaryotes, any novel function of Sir2 in yeast could be highly relevant (discussed further in Section 4.2).

2.2. Linking Sir2 to metabolism

At the time Sir2 was first implicated in yeast longevity, little was known about the mechanism by which Sir2 protein was able to promote transcriptional silencing (Kennedy et al., 1995, 1997). Later, three groups independently solved this mystery and reported that Sir2 catalyzes an NAD-dependent histone deacetylation reaction (Imai et al., 2000; Landry et al., 2000; Smith et al., 2000). The unique NAD-consuming catalytic mechanism of Sir2 results in the production of deacetylated lysine, 1-*O*-acetyl-ADP-ribose, and nicotinamide (Tanner et al., 2000).

The NAD-dependent nature of Sir2 catalysis immediately suggested a potential link between Sir2 activity and the metabolic state of the cell (Guarente, 2000). Lin et al. (2000), found that reducing the glucose concentration of the media from 2 to 0.5% increased replicative life span by 20–30%. This protocol of glucose reduction was proposed as a yeast model for CR (Lin et al., 2000). The magnitude of life span extension from this CR protocol is comparable to that observed upon overexpression of Sir2 (Table 1). Furthermore, in cells where the gene coding for Sir2 has been deleted, life span is shortened by approximately 50%, and reducing the glucose concentration fails to increase life span in this short-lived mutant (Lin et al., 2000). This latter finding was interpreted to suggest that life span extension by CR is mediated through activation of Sir2 (Lin et al., 2000).

Building on this model, and using knowledge about the catalytic mechanism of histone deacetylation by Sir2, competing hypotheses were developed to explain how CR might enhance Sir2 activity in yeast (Couzin, 2004). The first hypothesis, proposed by Guarente and co-workers, centers around the metabolic shift from fermentation to respiration that occurs in response to CR (Lin et al., 2002). This shift has been suggested to result in either increased levels of NAD^+ (a substrate of the Sir2 reaction) (Lin et al., 2002) or decreased levels of NADH (an inhibitor of Sir2) (Lin et al., 2004) (referred to hereafter as the ‘‘Sir2/CR respiration model’’). An alternative hypothesis, developed largely from work carried out by Sinclair and co-workers, proposes that CR results in a decreased concentration of nicotinamide (an inhibitor of Sir2) in the cell, which is accomplished by up-regulation of the gene coding for nicotinamidase, *PNC1* (Anderson et al., 2003a) (referred to hereafter as the ‘‘Sir2/CR nicotinamide model’’).

2.3. Strain-specificity: the skeleton in the Sir2 closet

The Sir2/CR models described above provide a reasonable explanation for how Sir2 might be activated in response to CR; however, a significant complication in interpreting the early data relating to Sir2, CR, and aging in yeast is the use of two different strain backgrounds, W303AR5 (also called W303R or W303AR) and PSY316 (and variants PSY316AR, PSY316AT, and PSY316AUT), for different types of experiments. Close examination of the studies proposing a link between Sir2 and CR leads to an interesting observation. In general, experiments involving either rDNA recombination or the effect on life span resulting from overexpression of *SIR2* or deletion of *FOB1* were carried out in W303AR5 (Defossez et al., 1999; Kaeberlein et al., 1999). In contrast, studies on the longevity effects of CR were carried out almost exclusively in PSY316 (Anderson et al., 2003a; Bitterman et al., 2002; Lin et al., 2000, 2002,

Table 1
Summary of reported effects on replicative life span for calorie restriction (CR) at two different glucose concentrations, Sir2-overexpression (Sir2-ox), and deletion of *FOB 1* in three commonly used yeast strains

	BY4742	PSY316	W303AR5
CR (0.5% glucose)	13 ± 1 (2)	26 ± 3 (5)	−8 (1)[a]
CR (0.05% glucose)	22 ± 2 (9)	39 ± 9 (3)	−23 (1)
Sir2-ox	29 ± 4 (2)	0 ± 3 (2)	30 ± 3 (3)
fob1Δ	37 ± 6 (6)	−2 (1)	29 ± 1 (3)
CR + Sir2-ox	62 (1)	–	–
CR + *fob1Δ*	78 ± 18 (2)	–	–
Sir2-ox + *fob1Δ*	–	–	32 (1)

Data shown as the average percent life span extension ± standard error with the number of experiments available for comparison shown in parentheses.

[a] Disputed by Lamming et al. (2005) who report that 0.5% glucose significantly increases life span in W303AR5. Table 1 was constructed from data derived from multiple studies (Defossez et al., 1999; Kaeberlein et al., 1999, 2002, 2004a,b, 2005a,b,c,d, 2006b; Kaeberlein and Kennedy, 2005; Lin et al., 2002; Tsuchiya et al., 2006a).

2004). This dichotomy is particularly evident in cases where both strains were used for different types of experiments within the same study (e.g. see Anderson et al., 2002, 2003a; Bitterman et al., 2002; Howitz et al., 2003).

A possible explanation for why PSY316 was used for studies of CR but not Sir2-overexpression is that, although CR increases life span in PSY316, overexpression of Sir2 does not (Kaeberlein et al., 2004b). Sir2 is activated in these cells, as evidenced by increased silencing near telomeres, yet life span is not increased, thus uncoupling Sir2 activation from longevity in this strain (Kaeberlein et al., 2005c). Deletion of *FOB1* also does not increase life span in PSY316, suggesting that either ERCs are not limiting for life span in this background or Sir2 and Fob1 do not regulate ERC formation in PSY316. Thus, we are left with an equivocation: the experiments supporting life span extension by CR through activation of Sir2 were largely carried out in a genetic background where longevity is responsive to CR but is not responsive to activation of Sir2 (Table 1).

What about W303AR5? Prior to 2005, this strain was used extensively for longevity studies of Sir2 and Fob1, but the effect of CR on life span was not reported (Anderson et al., 2002, 2003a,b; Bitterman et al., 2002; Defossez et al., 1999; Kaeberlein et al., 1999; Lin et al., 2000). One study has since described life span extension from CR at 0.5% glucose in W303AR5 (Lamming et al., 2005). In contrast, we have observed that CR does not increase life span in W303AR5 at 0.5% glucose, and further reduction in the glucose concentration dramatically shortens life span in this strain (Kaeberlein et al., 2006b).

3. Evidence suggesting CR does not act via Sir2

In 2003, we began to quantitatively measure both replicative and chronological life span for ~4800 single-gene deletion strains (Kaeberlein and Kennedy, 2005). It was our opinion that there were several benefits to such an approach, one of which is the unbiased nature of a genome-level longevity screen. Since no predetermined notions of which genes and pathways should modulate longevity entered into the equation, at least one source of potential experimental bias was removed. A second benefit of using the yeast deletion collection for longevity screens was that we would be able to sample a different genetic background than those used by other labs. The yeast deletion collection was constructed in the BY4742 (*MATα*) strain background, which is closely related to S288C, the strain of *S. cerevisiae* for which the genome has been sequenced (Brachmann et al., 1998; Cherry et al., 1997). BY4742 and its sister strains BY4741 and BY4743 have been used for the vast majority of recent genomic studies in yeast, including genome-wide studies of protein localization (Huh et al., 2003) and expression (Ghaemmaghami et al., 2003). We felt that working in the most widely characterized genetic background would prove valuable for placing the results of genome-wide studies of yeast aging into context within the larger framework of eukaryotic cell biology.

As a prelude to the large-scale replicative aging screen, it was important to first determine the replicative aging phenotypes in BY4742 for several mutations previously studied in other yeast backgrounds. In general, single-gene deletion mutations reported to be long-lived or short-lived in prior studies showed similar longevity effects in BY4742 (Kaeberlein et al., 2005b). One important difference, however, was that, unlike either W303AR5 or PSY316, both Sir2-overexpression and CR increased life span in BY4742 (Table 1).

3.1. Sir2 and CR map to parallel genetic pathways

The observation that CR alone or overexpression of Sir2 alone increased the life span of BY4742 mother cells by 20–30% allowed us to carry out a key experiment missing from prior studies: measuring the life span of long-lived Sir2-overexpressing cells under CR. If CR was increasing life span by activating Sir2, as proposed in the Sir2/CR models, then CR of Sir2-overexpressing cells should fail to further increase life span. Surprisingly, a combination of CR plus Sir2-overexpression resulted in an additive longevity effect, increasing life span by approximately 60% relative to wild type cells on 2% glucose (Kaeberlein et al., 2005b). CR combined with deletion of *FOB1* also increased life span by approximately 60% (Kaeberlein et al., 2005b). These results suggested that CR may function to slow aging in yeast through a pathway parallel to Sir2 and Fob1.

The evidence that CR and Sir2 might modulate longevity in parallel genetic pathways seemed at odds with the prior observation that CR fails to increase the life span of *sir2Δ* cells (Kaeberlein et al., 2004b; Lin et al., 2000). Two potential explanations suggested themselves. First, if the Sir2/CR models were correct, the additive effect of combining Sir2-overexpression and CR might simply be an indication that the standard CR protocol does not

optimally activate Sir2. Second, since deletion of *SIR2* shortens life span by about 50% (Kaeberlein et al., 1999, 2004b; Lin et al., 2000), the defect(s) causing this shortened life span could mask the beneficial effects of CR that occur through a Sir2-independent mechanism(s). To differentiate between these two possibilities, we wished to create a situation where Sir2 is absent from cells, but life span is approximately wild type, and test whether CR could increase life span in this context. Fortuitously, deletion of *FOB1* (which reduces formation of ERCs) suppresses the life span defect of *sir2Δ* cells, allowing us to carry out just such an experiment. BY4742 mother cells lacking both Sir2 and Fob1 were grown under standard conditions (2% glucose), as well as at several different reduced glucose concentrations ranging from 0.5 to 0.05% (in order to assure an optimal CR protocol). On 2% glucose, wild type and *sir2Δfob1Δ* cells had median and maximum life spans that were not significantly different (Kaeberlein et al., 2004b). In every case of CR tested, however, the life span extension observed in *sir2Δfob1Δ* cells in response to a particular CR regimen was greater than the life span extension seen in wild type cells (Kaeberlein et al., 2004b). These findings demonstrated unequivocally that Sir2 is not required for CR-induced life span extension.

These epistasis experiments led to a model in which two pathways cooperate to cause senescence, one involving the Sir2 and Fob1, and the other responsive to CR (Fig. 1). In *sir2Δ* cells where Fob1 is present, elevated rDNA recombination (Gottlieb and Esposito, 1989) results in hyper-accumulation of ERCs (Kaeberlein et al., 1999), causing many or all of the cells to senesce prematurely, rendering the CR pathway irrelevant. Deletion of *FOB1* in the *sir2Δ* mutant suppresses the hyper-accumulation of ERCs and, in fact, reduces ERCs to a level much lower than seen in wild type cells (Kaeberlein et al., 1999). Thus, in the *sir2Δfob1Δ* double mutant, the CR pathway has become predominant, which may explain why CR is more effective at enhancing longevity in these cells.

One criticism of this model was that the mechanism accounting for Sir2-independent life span extension from CR was uncharacterized (Guarente and Picard, 2005). Fortunately, this criticism can now be addressed. Accumulating

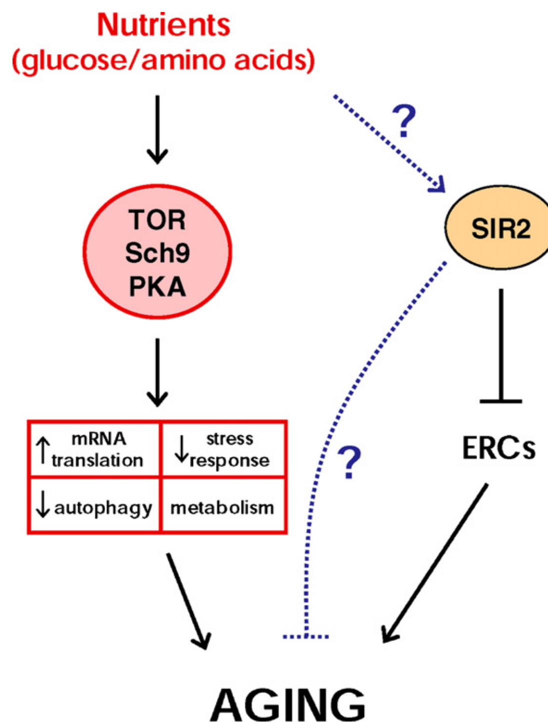

Fig. 1. Factors regulating replicative life span in yeast. Reduction of glucose or amino acids in the growth media is known to increase life span and decrease the activity of a nutrient-responsive aging pathway mediated by TOR, Sch9, and PKA. The precise mechanism by which these kinases modulate aging remains unknown, but may involve regulation of translation, stress resistance, or autophagy. Reduced glucose has also been proposed to activate the Sir2-mediated longevity pathway; however, it remains unclear whether Sir2 is involved in life span extension from calorie restriction. Sir2 regulates the formation of extrachromosomal rDNA circles (ERCs), but there is evidence that Sir2 may also have additional longevity-promoting roles which have yet to be characterized.

evidence suggests that CR may be mediated through an evolutionarily conserved signaling pathway involving the nutrient-responsive kinases TOR, Sch9, and PKA (Fabrizio et al., 2004; Kaeberlein et al., 2005d; Lin et al., 2000). Like CR, these kinases regulate yeast chronological aging (Fabrizio et al., 2001; Powers et al., 2006), as well as aging in worms and flies (Hertweck et al., 2004; Jia et al., 2004; Kapahi et al., 2004; Vellai et al., 2003). Future efforts will clarify which components of these pathways are most relevant for modulating longevity in response to nutrient availability.

3.2. Sir2 is not globally activated by CR

Several groups have reported that CR can increase yeast life span independently of Sir2 (Jiang et al., 2000; Kaeberlein et al., 2004b, 2006b; Lamming et al., 2005, 2006). An important question that remains to be answered, however, is whether CR has any effect on Sir2 activity and whether there exist conditions under which a subset of the effects of CR are mediated by Sir2.

A central premise of both the respiration and nicotinamide Sir2/CR models (see Section 2.1) is that CR increases Sir2 activity. In yeast, Sir2 promotes transcriptional silencing at three loci: telomeres (Aparicio et al., 1991), the silent mating loci (Ivy et al., 1986; Rine and Herskowitz, 1987), and the rDNA (Bryk et al., 1997; Smith and Boeke, 1997), where it also represses recombination between rDNA repeats (Gottlieb and Esposito, 1989). Methods have been developed for assaying Sir2 activity *in vivo* based the insertion of marker genes at one or more of the Sir2-regulated loci (e.g. see Gallo et al., 2004; Imai et al., 2000; Kaeberlein et al., 2005c; Roy and Runge, 2000; Sandmeier et al., 2002; Smith and Boeke, 1997). Several interventions have been reported that influence silencing at one or more of these loci through both Sir2-dependent and Sir2-independent pathways (e.g. see Bryk et al., 2002; Olaharski et al., 2005; Ray et al., 2003; Smith et al., 2000). Thus, if CR activates Sir2 *in vivo*, it should be straightforward to detect.

The effect of CR on Sir2-dependent silencing at telomeres has been examined in the PSY316 strain background using a strain with both *ADE2* (required for adenine biosynthesis) and *URA3* (required for uracil biosynthesis) integrated sub-telomerically (PSY316AUT). Both marker genes are transcriptionally silenced by Sir2 in this strain, and the degree of silencing can be monitored by measuring the viability of cells plated onto media lacking either adenine, uracil or both. A second quantitative measure of *URA3* silencing is also available by plating cells onto media containing 5-fluoroorotic acid, a chemical toxic to cells expressing the Ura3 enzyme, while *ADE2* silencing can also be monitored qualitatively by plating cells onto media low in adenine and monitoring the formation of a red pigment. Using each of these assays, it has been shown that, while overexpression of the Sir2 enzyme significantly increases Sir2 activity *in vivo*, CR does not (Kaeberlein et al., 2005a,d).

In contrast to the lack of effect from CR on Sir2-dependent telomere silencing, CR has been reported to have effects on Sir2-related phenotypes within the rDNA. Specifically, growth on 0.5% glucose media increased transcriptional silencing of a *MET15* gene inserted into the rDNA repeat (Lin et al., 2000), using a qualitative color formation assay (Cost and Boeke, 1996). CR was also reported to reduce rDNA recombination in W303AR5, as measured by loss of an *ADE2* marker from the rDNA (Lamming et al., 2005). Interpretation of these observations is complicated, however, by the fact that TOR, Sch9, and PKA also influence both rRNA transcription and rDNA recombination in a manner consistent with the hypothesis that the observed effects of CR could mediated by these Sir2-independent nutrient-response pathways (Li et al., 2006; Lin et al., 2000; Martin et al., 2004; Prusty and Keil, 2004; Tsang et al., 2003). In unpublished experiments, we have observed that decreased TOR activity dramatically decreases rDNA recombination in W303AR cells in a Sir2-independent manner (M. Kaeberlein and B. Kennedy, unpublished data). Deletion of at least three different TOR- and Sch9-regulated ribosomal proteins has been reported to increase life span in yeast (Chiocchetti et al., 2007; Kaeberlein et al., 2005d), suggesting that regulation of ribosome biogenesis and, perhaps, rRNA levels by TOR may be an important aspect of Sir2-independent life span extension from CR. Thus, activation of Sir2 need not be invoked to explain effects of CR at the rDNA, and it remains unclear whether the rDNA phenotypes associated with CR are Sir2-mediated, TOR/Sch9/PKA-mediated, or both.

The inability of CR to phenocopy overexpression of Sir2, at least with respect to telomere silencing, is clearly inconsistent with the predictions of the Sir2/CR models. This finding alone, however, cannot rule out these models. For example, it is possible that activation of the Sir2 enzyme could result in different phenotypes from overexpression of the enzyme. Perhaps Sir2 enzyme is relocalized to telomeres when overexpressed, but the Sir2 present at telomeres is

not activated by CR. Given the central nature of this question to the Sir2/CR models, a more thorough analysis of the effect (if any) that CR has on Sir2 activity *in vivo* seems warranted.

3.3. Respiration is not required for CR

In yeast, a reduction in the glucose concentration of media results in a metabolic shift from fermentation toward respiration (Lin et al., 2002). Guarente (2000) proposed that CR increases Sir2 activity by increasing cellular NAD levels in response to this metabolic shift. This hypothesis was challenged, however, by subsequent work from the Sinclair lab indicating that NAD levels are not altered by CR (Anderson et al., 2003b). Since then, the respiration Sir2/CR model has been modified to posit that a shift from fermentation to respiration leads to decreased NADH rather than increased NAD levels, and that this decrease in NADH leads to activation of Sir2 (Lin et al., 2004).

A central prediction of both variants of the respiration Sir2/CR model is that CR should not increase life span in cells that are incapable of undergoing a metabolic shift from fermentation to respiration. In contrast to this prediction, CR has been reported to dramatically increase the life span of respiratory deficient mutants in either the PSY316 or BY4742 strain backgrounds. Deletion of the nuclear gene *CYT1*, which codes for cytochrome *c*1, does not significantly alter life span but completely blocks respiratory metabolism (Kaeberlein et al., 2005a,b); CR increases the life span of *cyt1Δ* cells at either 0.5 or 0.05% glucose (Kaeberlein et al., 2005a). Rho^0 cells are respiratory defective and lack mitochondrial DNA, which codes for three cytochrome *c* oxidase subunits (*COX1*, *COX2*, and *COX3*), three ATP synthase subunits (*ATP6*, *ATP8*, and *ATP9*), and apocytochrome *b* (*CYTB*) (de Zamaroczy and Bernardi, 1986; Tzagoloff and Myers, 1986). CR significantly increases the life span of rho^0 cells at either 0.5 or 0.05% glucose (Kaeberlein et al., 2005a). We have also determined that CR increases the life span of more than 10 additional respiratory-deficient single-gene deletion strains (M. Kaeberlein and B. Kennedy, unpublished data). Thus, multiple counter-examples to the respiration Sir2/CR models have been provided where CR is effective at increasing the life span of respiratory deficient yeast cells.

Although a metabolic shift from fermentation to respiration is not necessary for life span extension from CR, this does not imply that respiration and mitochondrial activity are not important for longevity in general. Overexpression of the *HAP4* transcription factor (Lin et al., 2002; Piper et al., 2006), which promotes transcription of many different respiratory genes, or overexpression of mitochondrial NAD-dependent dehydrogenases (Lin et al., 2004) is reported to increase yeast life span. Deletion of the mitochondrial NAD-dependent isocitrate dehydrogenase *IDH2* also increases life span (Kaeberlein et al., 2005d), and recently a component the pyruvate dehydrogenase complex has been suggested to play a role in life span extension from CR (Easlon et al., 2007). It remains to be determined whether the effects of these mitochondrial proteins on life span are directly related to respiration and carbon utilization or whether they modulate longevity via indirect mechanisms.

3.4. Nicotinamide affects life span independently of Sir2

The Sir2/nicotinamide model posits that, rather than resulting from increased respiration, life span extension from CR is due to Sir2 activation in response to a reduction in intracellular nicotinamide (Anderson et al., 2003a). This model was based upon the finding that CR results in increased expression of *PNC1*, the gene coding for nicotinamidase, which catalyzes the breakdown of nicotinamide to nicotinate and ammonia (Anderson et al., 2003a). Since nicotinamide is an inhibitor of Sir2, a reduction in intracellular nicotinamide could, in theory, result in activation of Sir2 and increased life span. Further support for this idea was provided by the report that increased expression of *PNC1* is sufficient to increase life span (Anderson et al., 2003a).

Interpretation of the relationship between *PNC1* and *SIR2* in the context of CR is difficult in light of the fact that all of the experiments reporting increased longevity from *PNC1* overexpression were carried out in PSY316 (Anderson et al., 2003a), the strain background in which enhanced Sir2 expression fails to increase life span (Kaeberlein et al., 2004b). This raises the question that, if *PNC1* overexpression increases life span in PSY316 by activating Sir2, why does not increasing Sir2 activity by overexpression of the enzyme also increase life span in PSY316. As discussed above, it is possible that increased expression of the Sir2 enzyme fails to recapitulate Sir2 activation through decreased nicotinamide levels; however, Sir2-overexpression is known to increase life span in other strain backgrounds. An alternative possibility is that *PNC1*-overexpression increases life span through a Sir2-independent mechanism, similar

to CR. It would be interesting to know whether overexpression of *PNC1* increases life span in strain backgrounds where overexpression of *SIR2* has a similar effect. If so, this would allow more careful genetic testing of the relationship between these two enzymes with respect to longevity in yeast.

More recent studies suggest that nicotinamide, at least, plays a role in mediating longevity that is distinct from its sirtuin-inhibitory function. Treating yeast cells with 5 mM nicotinamide phenocopies deletion of *SIR2*, reducing life span by about 50% (Bitterman et al., 2002; Tsuchiya et al., 2006a). This short life span is suppressed by deletion of *FOB1* (Kaeberlein et al., 2005a). Interestingly, nicotinamide partially blocks life span extension from CR in *sir2Δfob1Δ* double mutant cells (Kaeberlein et al., 2005a). Since Sir2 is absent from these cells, nicotinamide levels must influence the response to CR through a Sir2-independent mechanism(s).

3.5. Sir2 paralogs are not required for Sir2-independent CR

One possible explanation for the observation that nicotinamide partially blocks life span extension from CR in *sir2Δfob1Δ* cells is that Sir2-independent CR is mediated by other nicotinamide-inhibited Sir2-family members. Yeast has four homologs of Sir2, the sirtuins: Hst1, Hst2, Hst3, and Hst4 (Brachmann et al., 1995; Derbyshire et al., 1996).

All five sirtuins are thought to carry out a similar catalytic reaction as Sir2, although with varied cellular localization and substrates.

If Sir2-independent CR were mediated by one of the Sir2 paralogs, then CR should not increase life span when both Sir2 and the relevant Hst protein are deleted. Indeed, this initially appeared to be the case, with the report that CR does not increase the life span of *sir2Δfob1Δ hst2Δ* triple mutants (Lamming et al., 2005). We have independently examined each of the Sir2 homologs, however, to determine their role, if any, in CR. In contrast to Lamming et al. (2005), in our studies CR was found to increase life span by greater than 50% in all four of the *sir2Δfob1Δ hstXΔ* triple mutants (Kaeberlein et al., 2006b; Tsuchiya et al., 2006a).

Although no single Sir2 paralog is sufficient to account for Sir2-independent life span extension from CR, it is formally possible that more than one Sir2 paralog could redundantly function to promote life span extension from CR in the absence of Sir2. Simultaneous deletion of all five sirtuins results in a severe growth defect, precluding interpretation of an experiment directly examining CR in sirtuin-less yeast (Tsuchiya et al., 2006a). Hst1 and Hst2 are the two sirtuins most closely related to Sir2, however, and CR increases life span to a similar extent in *sir2Δfob1Δ hst1Δ hst2Δ* cells as in *sir2Δ fob1Δ* cells (Kaeberlein et al., 2006b; Tsuchiya et al., 2006a). These experiments were carried out using multiple CR regimens, including 0.5, 0.05, and 0.005% glucose. Life span extension from CR was observed at each level of restriction, with optimal extension at glucose levels below 0.5% (Kaeberlein et al., 2006b). In addition, CR increases the life span of *sir2Δfob1Δ hst1Δ hst2Δ hst4Δ* cells, and this life span extension is reduced by 5 mM nicotinamide (Tsuchiya et al., 2006a). Since Hst3 is not significantly inhibited *in vivo* by 5 mM nicotinamide (Tsuchiya et al., 2006a), it seems likely that nicotinamide inhibits life span extension from CR by a mechanism that is independent of the entire family of yeast sirtuins.

4. Searching for clarity from controversy

4.1. "Moderate" versus "extreme" CR

One response to the accumulating data suggesting that CR is independent of sirtuins in yeast and *C. elegans* (see below) has been a proposal that two different pathways for life span extension are activated by CR at different levels of restriction (Sinclair et al., 2006). One pathway could be Sir2-dependent and responds to "moderate CR", whereas the other pathway could be Sir2-independent and responds to "extreme CR". Although it is generally accepted that a level of restriction providing maximum life span extension should be used in genetic studies of CR, it is formally possible that different mechanisms of life span extension could occur at different levels of restriction. Thus, this hypothesis deserves consideration.

In the case of yeast replicative life span, it has been proposed that 0.5% glucose represents "moderate CR" while lower concentrations (such as 0.05% glucose) represent "extreme CR" (Sinclair et al., 2006). Do the data support the hypothesis that life span extension at 0.5% glucose is Sir2-dependent while life span extension at 0.05% glucose is Sir2-independent? There is little experimental evidence indicating that, under the conditions used for measuring yeast replicative life span, cells grown at glucose concentrations lower than 0.5% show substantial physiological

differences from cells grown at 0.5% (Kaeberlein et al., 2006a). Indeed, microarray studies indicate that yeast grown at 0.5% glucose undergo many of the gene expression changes associated with an absence of glucose (Lin et al., 2002). Thus, the available data support the idea that, relative to growth at 2% glucose, ‘‘extreme’’ and ‘‘moderate’’ CR are likely to induce similar gene expression changes, although this needs to be more carefully examined experimentally.

The best evidence supporting the hypothesis that 0.5% glucose increases life span by a mechanism different from 0.05% glucose was provided by Lamming et al. (2006) who reported that 0.05% glucose significantly increases the life span of *fob1Δ sir2Δ hst1Δ hst2Δ* cells, but 0.5% glucose does not. This report has been disputed, however (Kaeberlein et al., 2004b). In contrast to Lamming et al. (2006), our data clearly argue that life span extension from CR occurs independently of Sir2, regardless of the level of restriction used. CR significantly increased the life span of *sir2Δfob1Δ* double mutants at 0.5, 0.1, and 0.05% glucose, with 0.05% glucose providing the maximal benefit (Kaeberlein et al., 2004b). Further, both ‘‘moderate’’ and ‘‘extreme’’ CR also increased the life span of *sir2Δ fob1Δ hst1Δ hst2Δ* cells (Kaeberlein et al., 2006b). The discrepancy between our data and that of Lamming et al. remains unresolved, and may be due to differences in experimental conditions or background mutations present in control strains.

In addition to finding that life span extension at 0.5 and 0.05% glucose are Sir2-independent, we have also observed that life span extension at both glucose levels is independent of respiration (see Section 3.3). This observation has recently been verified by Easlon et al. (2007), who report that both 0.5 and 0.05% glucose increase the life span of *cyt1Δ* cells, with 0.05% glucose providing a maximum life span extension (Easlon et al., 2007). Based on their findings, Easlon et al. (2007) propose a complicated model suggesting that 0.5% glucose and 0.05% glucose increase life span by partially distinct but interacting pathways (Easlon et al., 2007).

Clearly, the current data do not allow for a definitive answer to the question of whether ‘‘moderate’’ and ‘‘extreme’’ CR act by different mechanisms. We do not favor this model because (1) in our opinion, it is not necessary to account for the observed data and (2) in our hands neither ‘‘moderate’’ nor ‘‘extreme’’ CR require Sir2 to increase life span (Kaeberlein et al., 2004b, 2006b).

4.2. Evolutionary considerations

One argument made in support of Sir2 as a mediator of CR is based on apparent evolutionary conservation. Since both CR and Sir2-overexpression increase life span in yeast, worms, and flies, this has been construed as evidence that CR acts through Sir2 (Lamming et al., 2006). The same can be said of other interventions, however, such as decreased TOR activity (Jia et al., 2004; Kaeberlein et al., 2005d; Kapahi et al., 2004; Powers et al., 2006; Vellai et al., 2003) and increased expression of antioxidant enzymes (Fabrizio et al., 2003; Parkes et al., 1998; Phillips et al., 2000; Schriner et al., 2005; Sun and Tower, 1999). The apparent evolutionary conservation of sirtuins as longevity determinants is also perplexing for two reasons. First, Sir2 antagonizes life span extension from CR in the yeast chronological aging model, which may be a more relevant system for modeling the aging of post-mitotic cells and tissues in higher eukaryotes (Fabrizio et al., 2005). In contrast, decreased TOR activity increases both replicative and chronological life span (Powers et al., 2006). Second, Sir2 is thought to modulate yeast longevity by regulating ERC formation (Kaeberlein et al., 1999). Yet, there is no evidence to indicate that ERCs accumulate with age or slow aging in multicellular eukaryotes.

The link between Sir2 and CR in higher eukaryotes is also ambiguous. Published data from studies in flies is, thus far, consistent with a requirement of dSir2 for life span extension from CR, and genetic epistasis places dSir2 and CR in the same pathway (Rogina and Helfand, 2004). The situation in worms, however, is less clear. One report indicated that life span extension in a genetic model of CR, mutation of *eat-2*, is largely blocked by deletion of *sir-2.1* (Wang and Tissenbaum, 2006), but a second study found that deletion of *sir-2.1* does not alter life span extension from mutation of *eat-2* (Hansen et al., 2007), and two groups have independently reported that life span extension from CR achieved through a reduction in bacterial food is unaffected by deletion of *sir-2.1* (Lee et al., 2006; Tsuchiya et al., 2006b). In mammals there are several suggestive findings consistent with the possibility that sirtuins play roles in metabolic processes related to CR (Guarente and Picard, 2005), and mice lacking SirT1 fail to show increased activity in response to CR (although other measured phenotypes were unchanged; Chen et al., 2005). As yet, however, it has not been demonstrated that any mammalian sirtuin is either necessary or sufficient for life span extension in response to CR (Longo and Kennedy, 2006). Thus, definitive statements regarding the mechanistic details of life span extension by CR in any organism seem premature.

5. Conclusion

There are many intriguing links between Sir2, CR, and longevity. Like CR, Sir2 orthologs modulate longevity in yeast, worms, and flies, and plausible molecular models have been suggested by which sirtuin activity could be altered in response to nutrients. Experimental evidence has accumulated in yeast, however, suggesting that these models should be re-evaluated. For example, three specific predictions that arise from the Sir2/CR models are (1) optimal CR combined with overexpression of Sir2 should not result in an additive or synergistic effect on life span, (2) CR should never increase life span in cells lacking Sir2 (and/or sirtuins), and (3) CR should activate Sir2 *in vivo*. In each case, counter-examples have been demonstrated that are inconsistent with these predictions.

Others and we have begun to critically evaluate inconsistencies in the Sir2/CR models, and it is our hope that this trend will continue. If correct in part or whole, the hypothesis that CR increases life span through activation of sirtuins will be strengthened by this type of critical examination and follow-up experiments; if not, then aging-related research will benefit from a more accurate picture of the (different) mechanisms by which sirtuins and CR affect longevity.

Acknowledgements

We would like to acknowledge support to M.K. from the American Federation for Aging Research, the Ellison Medical Foundation, and the University of Washington Nathan Shock Center of Excellence for the Basic Biology of Aging. R.W.P. was supported by NIH training grant P30 AG013280.

References

Anderson, R.M., Bitterman, K.J., Wood, J.G., Medvedik, O., Cohen, H., Lin, S.S., Manchester, J.K., Gordon, J.I., Sinclair, D.A., 2002. Manipulation of a nuclear NAD+ salvage pathway delays aging without altering steady-state NAD+ levels. J. Biol. Chem. 277, 18881–18890.

Anderson, R.M., Bitterman, K.J., Wood, J.G., Medvedik, O., Sinclair, D.A., 2003a. Nicotinamide and PNC1 govern lifespan extension by calorie restriction in *Saccharomyces cerevisiae*. Nature 423, 181–185.

Anderson, R.M., Latorre-Esteves, M., Neves, A.R., Lavu, S., Medvedik, O., Taylor, C., Howitz, K.T., Santos, H., Sinclair, D.A., 2003b. Yeast life-span extension by calorie restriction is independent of NAD fluctuation. Science 302, 2124–2126.

Aparicio, O.M., Billington, B.L., Gottschling, D.E., 1991. Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. Cell 66, 1279–1287.

Bartke, A., Chandrashekar, V., Dominici, F., Turyn, D., Kinney, B., Steger, R., Kopchick, J.J., 2003. Insulin-like growth factor 1 (IGF-1) and aging: controversies and new insights. Biogerontology 4, 1–8.

Bitterman, K.J., Anderson, R.M., Cohen, H.Y., Latorre-Esteves, M., Sinclair, D.A., 2002. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1. J. Biol. Chem. 277, 45099–45107.

Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P., Boeke, J.D., 1998. Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14, 115–132.

Brachmann, C.B., Sherman, J.M., Devine, S.E., Cameron, E.E., Pillus, L., Boeke, J.D., 1995. The SIR2 gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. Genes Dev. 9, 2888–2902.

Bryk, M., Banerjee, M., Murphy, M., Knudsen, K.E., Garfinkel, D.J., Curcio, M.J., 1997. Transcriptional silencing of Ty1 elements in the RDN1 locus of yeast. Genes Dev. 11, 255–269.

Bryk, M., Briggs, S.D., Strahl, B.D., Curcio, M.J., Allis, C.D., Winston, F., 2002. Evidence that Set1, a factor required for methylation of histone H3, regulates rDNA silencing in *S. cerevisiae* by a Sir2-independent mechanism. Curr. Biol. 12, 165–170.

Chen, D., Steele, A.D., Lindquist, S., Guarente, L., 2005. Increase in activity during calorie restriction requires Sirt1. Science 310, 1641.

Cherry, J.M., Ball, C., Weng, S., Juvik, G., Schmidt, R., Adler, C., Dunn, B., Dwight, S., Riles, L., Mortimer, R.K., Botstein, D., 1997. Genetic and physical maps of *Saccharomyces cerevisiae*. Nature 387, 67–73.

Chiocchetti, A., Zhou, J., Zhu, H., Karl, T., Haubenreisser, O., Rinnerthaler, M., Heeren, G., Oender, K., Bauer, J., Hintner, H., Breitenbach, M., Breitenbach-Koller, L., 2007. Ribosomal proteins Rpl10 and Rps6 are potent regulators of yeast replicative life span. Exp. Gerontol. 42, 275–286.

Cost, G.J., Boeke, J.D., 1996. A useful colony colour phenotype associated with the yeast selectable/counter-selectable marker MET15. Yeast 12, 939–941.

Couzin, J., 2004. Scientific community. Aging research's family feud. Science 303, 1276–1279.

de Zamaroczy, M., Bernardi, G., 1986. The primary structure of the mitochondrial genome of *Saccharomyces cerevisiae*. Gene 47, 155–177.

Defossez, P.A., Prusty, R., Kaeberlein, M., Lin, S.J., Ferrigno, P., Silver, P.A., Keil, R.L., Guarente, L., 1999. Elimination of replication block protein Fob1 extends the life span of yeast mother cells. Mol. Cell. 3, 447–455.

Derbyshire, M.K., Weinstock, K.G., Strathern, J.N., 1996. HST1, a new member of the SIR2 family of genes. Yeast 12, 631–640.

Easlon, E., Tsang, F., Dilova, I., Wang, C., Lu, S.P., Skinner, C., Lin, S.J., 2007. The dihydrolipoamide acetyltransferase is a novel metabolic longevity factor and is required for calorie restriction-mediated life span extension. J. Biol. Chem. 282, 6161–6171.

Fabrizio, P., Gattazzo, C., Battistella, L., Wei, M., Cheng, C., McGrew, K., Longo, V.D., 2005. Sir2 blocks extreme life-span extension. Cell 123, 655–667.

Fabrizio, P., Liou, L.L., Moy, V.N., Diaspro, A., SelverstoneValentine, J., Gralla, E.B., Longo, V.D., 2003. SOD2 functions downstream of Sch9 to extend longevity in yeast. Genetics 163, 35–46.

Fabrizio, P., Longo, V.D., 2003. The chronological life span of *Saccharomyces cerevisiae*. Aging Cell 2, 73–81.

Fabrizio, P., Pletcher, S.D., Minois, N., Vaupel, J.W., Longo, V.D., 2004. Chronological aging-independent replicative life span regulation by Msn2/Msn4 and Sod2 in *Saccharomyces cerevisiae*. FEBS Lett. 557, 136–142.

Fabrizio, P., Pozza, F., Pletcher, S.D., Gendron, C.M., Longo, V.D., 2001. Regulation of longevity and stress resistance by Sch9 in yeast. Science 292, 288–290.

Gallo, C.M., Smith Jr., D.L., Smith, J.S., 2004. Nicotinamide clearance by Pnc1 directly regulates Sir2-mediated silencing and longevity. Mol. Cell. Biol. 24, 1301–1312.

Ghaemmaghami, S., Huh, W.K., Bower, K., Howson, R.W., Belle, A., Dephoure, N., O'Shea, E.K., Weissman, J.S., 2003. Global analysis of protein expression in yeast. Nature 425, 737–741.

Gottlieb, S., Esposito, R.E., 1989. A new role for a yeast transcriptional silencer gene, SIR2, in regulation of recombination in ribosomal DNA. Cell 56, 771–776.

Guarente, L., 2000. Sir2 links chromatin silencing, metabolism, and aging. Genes Dev. 14, 1021–1026.

Guarente, L., Picard, F., 2005. Calorie restriction—the SIR2 connection. Cell 120, 473–482.

Hansen, M., Taubert, S., Crawford, D., Libina, N., Lee, S.J., Kenyon, C., 2007. Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. Aging Cell 6, 95–110.

Hertweck, M., Gobel, C., Baumeister, R., 2004. *C. elegans* SGK-1 is the critical component in the Akt/PKB kinase complex to control stress response and life span. Dev. Cell. 6, 577–588.

Howitz, K.T., Bitterman, K.J., Cohen, H.Y., Lamming, D.W., Lavu, S., Wood, J.G., Zipkin, R.E., Chung, P., Kisielewski, A., Zhang, L.L., Scherer, B., Sinclair, D.A., 2003. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. Nature 425, 191–196.

Huh, W.K., Falvo, J.V., Gerke, L.C., Carroll, A.S., Howson, R.W., Weissman, J.S., O'Shea, E.K., 2003. Global analysis of protein localization in budding yeast. Nature 425, 686–691.

Imai, S., Armstrong, C.M., Kaeberlein, M., Guarente, L., 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 403, 795–800.

Ivy, J.M., Klar, A.J., Hicks, J.B., 1986. Cloning and characterization of four SIR genes of *Saccharomyces cerevisiae*. Mol. Cell. Biol. 6, 688–702.

Jia, K., Chen, D., Riddle, D.L., 2004. The TOR pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span. Development 131, 3897–3906.

Jiang, J.C., Jaruga, E., Repnevskaya, M.V., Jazwinski, S.M., 2000. An intervention resembling caloric restriction prolongs life span and retards aging in yeast. FASEB J. 14, 2135–2137.

Kaeberlein, M., 2006. Longevity and aging in the budding yeast. In: Conn, P.M. (Ed.), Handbook of Models for Human Aging. Elsevier Press, Boston, pp. 109–120.

Kaeberlein, M., Andalis, A.A., Fink, G.R., Guarente, L., 2002. High osmolarity extends life span in *Saccharomyces cerevisiae* by a mechanism related to calorie restriction. Mol. Cell. Biol. 22, 8056–8066.

Kaeberlein, M., Andalis, A.A., Liszt, G., Fink, G.R., Guarente, L., 2004a. *Saccharomyces cerevisiae* SSD1-V confers longevity by a Sir2p-independent mechanism. Genetics 166, 1661–1672.

Kaeberlein, M., Hu, D., Kerr, E.O., Tsuchiya, M., Westman, E.A., Dang, N., Fields, S., Kennedy, B.K., 2005a. Increased life span due to calorie restriction in respiratory-deficient yeast. PLoS Genet. 1, e69.

Kaeberlein, M., Hu, D., Kerr, E.O., Tsuchiya, M., Westman, E.A., Dang, N., Fields, S., Kennedy, B.K., 2006a. PLoS Genet. 2, e34 (author's reply).

Kaeberlein, M., Kennedy, B.K., 2005. Large-scale identification in yeast of conserved ageing genes. Mech. Ageing Dev. 126, 17–21.

Kaeberlein, M., Kirkland, K.T., Fields, S., Kennedy, B.K., 2004b. Sir2-independent life span extension by calorie restriction in yeast. PLoS Biol. 2, E296.

Kaeberlein, M., Kirkland, K.T., Fields, S., Kennedy, B.K., 2005b. Genes determining yeast replicative life span in a long-lived genetic background. Mech. Ageing Dev. 126, 491–504.

Kaeberlein, M., McDonagh, T., Heltweg, B., Hixon, J., Westman, E.A., Caldwell, S.D., Napper, A., Curtis, R., Distefano, P.S., Fields, S., Bedalov, A., Kennedy, B.K., 2005c. Substrate-specific activation of sirtuins by resveratrol. J. Biol. Chem. 280, 17038–17045.

Kaeberlein, M., McVey, M., Guarente, L., 1999. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. Genes Dev. 13, 2570–2580.

Kaeberlein, M., Powers 3rd, R.W., Steffen, K.K., Westman, E.A., Hu, D., Dang, N., Kerr, E.O., Kirkland, K.T., Fields, S., Kennedy, B.K., 2005d. Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. Science 310, 1193–1196.

Kaeberlein, M., Steffen, K.K., Hu, D., Dang, N., Kerr, E.O., Tsuchiya, M., Fields, S., Kennedy, B.K., 2006b. Comment on ''HST2 mediates SIR2-independent life-span extension by calorie restriction''. Science 312, 1312 (author's reply).

Kapahi, P., Zid, B.M., Harper, T., Koslover, D., Sapin, V., Benzer, S., 2004. Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. Curr. Biol. 14, 885–890.

Kennedy, B.K., Austriaco Jr., N.R., Zhang, J., Guarente, L., 1995. Mutation in the silencing gene SIR4 can delay aging in *S. cerevisiae*. Cell 80, 485–496.

Kennedy, B.K., Gotta, M., Sinclair, D.A., Mills, K., McNabb, D.S., Murthy, M., Pak, S.M., Laroche, T., Gasser, S.M., Guarente, L., 1997. Redistribution of silencing proteins from telomeres to the nucleolus is associated with extension of life span in *S. cerevisiae*. Cell 89, 381–391.

Kenyon, C., 2005. The plasticity of aging: insights from long-lived mutants. Cell 120, 449–460.

Lamming, D.W., Latorre-Esteves, M., Medvedik, O., Wong, S.N., Tsang, F.A., Wang, C., Lin, S.J., Sinclair, D.A., 2005. HST2 mediates SIR2-independent life-span extension by calorie restriction. Science 309, 1861–1864.

Lamming, D.W., Latorre-Esteves, M., Medvedik, O., Wong, S.N., Tsang, F.A., Wang, C., Lin, S.J., Sinclair, D.A., 2006. Response to comment on "HST2 mediates SIR2-independent life-span extension by calorie restriction". Science 312, 1312.

Landry, J., Sutton, A., Tafrov, S.T., Heller, R.C., Stebbins, J., Pillus, L., Sternglanz, R., 2000. The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. Proc. Natl. Acad. Sci. U.S.A. 97, 5807–5811.

Lee, G.D., Wilson, M.A., Zhu, M., Wolkow, C.A., de Cabo, R., Ingram, D.K., Zou, S., 2006. Dietary deprivation extends lifespan in *Caenorhabditis elegans*. Aging Cell 5, 515–524.

Li, H., Tsang, C.K., Watkins, M., Bertram, P.G., Zheng, X.F., 2006. Nutrient regulates Tor1 nuclear localization and association with rDNA promoter. Nature 442, 1058–1061.

Lin, S.J., Defossez, P.A., Guarente, L., 2000. Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. Science 289, 2126–2128.

Lin, S.J., Ford, E., Haigis, M., Liszt, G., Guarente, L., 2004. Calorie restriction extends yeast life span by lowering the level of NADH. Genes Dev. 18, 12–16.

Lin, S.J., Kaeberlein, M., Andalis, A.A., Sturtz, L.A., Defossez, P.A., Culotta, V.C., Fink, G., Guarente, L., 2002. Calorie restriction extends *Saccharomyces cerevisiae* life span by increasing respiration. Nature 418, 344–348.

Lin, Y.H., Keil, R.L., 1991. Mutations affecting RNA polymerase I-stimulated exchange and rDNA recombination in yeast. Genetics 127, 31–38.

Longo, V.D., Kennedy, B.K., 2006. Sirtuins in aging and age-related disease. Cell 126, 257–268.

Martin, D.E., Soulard, A., Hall, M.N., 2004. TOR regulates ribosomal protein gene expression via PKA and the forkhead transcription factor FHL1. Cell 119, 969–979.

Masoro, E.J., 2004. Role of sirtuin proteins in life extension by caloric restriction. Mech. Ageing Dev. 125, 591–594.

Merry, B.J., 2004. Oxidative stress and mitochondrial function with aging—the effects of calorie restriction. Aging Cell 3, 7–12.

Mortimer, R.K., Johnston, J.R., 1959. Life span of individual yeast cells. Nature 183, 1751–1752.

Olaharski, A.J., Rine, J., Marshall, B.L., Babiarz, J., Zhang, L., Verdin, E., Smith, M.T., 2005. The flavoring agent dihydrocoumarin reverses epigenetic silencing and inhibits sirtuin deacetylases. PLoS Genet. 1, e77.

Parkes, T.L., Elia, A.J., Dickinson, D., Hilliker, A.J., Phillips, J.P., Boulianne, G.L., 1998. Extension of *Drosophila* lifespan by overexpression of human SOD1 in motorneurons. Nat. Genet. 19, 171–174.

Phillips, J.P., Parkes, T.L., Hilliker, A.J., 2000. Targeted neuronal gene expression and longevity in *Drosophila*. Exp. Gerontol. 35, 1157–1164.

Piper, P.W., Harris, N.L., MacLean, M., 2006. Preadaptation to efficient respiratory maintenance is essential both for maximal longevity and the retention of replicative potential in chronologically ageing yeast. Mech. Ageing Dev. 127, 733–740.

Powers 3rd, R.W., Kaeberlein, M., Caldwell, S.D., Kennedy, B.K., Fields, S., 2006. Extension of chronological life span in yeast by decreased TOR pathway signaling. Genes Dev. 20, 174–184.

Prusty, R., Keil, R.L., 2004. SCH9, a putative protein kinase from *Saccharomyces cerevisiae*, affects HOT 1-stimulated recombination. Mol. Genet. Genom. 272, 264–274.

Ray, A., Hector, R.E., Roy, N., Song, J.H., Berkner, K.L., Runge, K.W., 2003. Sir3p phosphorylation by the Slt2p pathway effects redistribution of silencing function and shortened lifespan. Nat. Genet. 33, 522–526.

Rine, J., Herskowitz, I., 1987. Four genes responsible for a position effect on expression from HML and HMR in *Saccharomyces cerevisiae*. Genetics 116, 9–22.

Rogina, B., Helfand, S.L., 2004. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. Proc. Natl. Acad. Sci. U.S.A. 101, 15998–16003.

Roy, N., Runge, K.W., 2000. Two paralogs involved in transcriptional silencing that antagonistically control yeast life span. Curr. Biol. 10, 111–114.

Sandmeier, J.J., Celic, I., Boeke, J.D., Smith, J.S., 2002. Telomeric and rDNA silencing in *Saccharomyces cerevisiae* are dependent on a nuclear NAD(+) salvage pathway. Genetics 160, 877–889.

Schriner, S.E., Linford, N.J., Martin, G.M., Treuting, P., Ogburn, C.E., Emond, M., Coskun, P.E., Ladiges, W., Wolf, N., Van Remmen, H., Wallace, D.C., Rabinovitch, P.S., 2005. Extension of murine life span by overexpression of catalase targeted to mitochondria. Science 308, 1909–1911.

Sinclair, D.A., 2005. Toward a unified theory of caloric restriction and longevity regulation. Mech. Ageing Dev. 126, 987–1002.

Sinclair, D.A., Guarente, L., 1997. Extrachromosomal rDNA circles—a cause of aging in yeast. Cell 91, 1033–1042.

Sinclair, D.A., Lin, S.J., Guarente, L., 2006. Life-span extension in yeast. Science 312, 195–197 (author's reply).

Smith, J.S., Boeke, J.D., 1997. An unusual form of transcriptional silencing in yeast ribosomal DNA. Genes Dev. 11, 241–254.

Smith, J.S., Brachmann, C.B., Celic, I., Kenna, M.A., Muhammad, S., Starai, V.J., Avalos, J.L., Escalante-Semerena, J.C., Grubmeyer, C., Wolberger, C., Boeke, J.D., 2000. A phylogenetically conserved NAD+-dependent protein deacetylase activity in the Sir2 protein family. Proc. Natl. Acad. Sci. U.S.A. 97, 6658–6663.

Sun, J., Tower, J., 1999. FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult *Drosophila melanogaster* flies. Mol. Cell. Biol. 19, 216–228.

Tanner, K.G., Landry, J., Sternglanz, R., Denu, J.M., 2000. Silent information regulator 2 family of NAD-dependent hi stone/protein deacetylases generates a unique product, 1-*O*-acetyl-ADP-ribose. Proc. Natl. Acad. Sci. U.S.A. 97, 14178–14182.

Tsang, C.K., Bertram, P.G., Ai, W., Drenan, R., Zheng, X.F., 2003. Chromatin-mediated regulation of nucleolar structure and RNA Pol I localization by TOR. EMBO J. 22, 6045–6056.

Tsuchiya, M., Dang, N., Kerr, E.O., Hu, D., Steffen, K.K., Oakes, J.A., Kennedy, B.K., Kaeberlein, M., 2006a. Sirtuin-independent effects of nicotinamide on life span extension from calorie restriction in yeast. Aging Cell 5, 505–514.

Tsuchiya, M., Dang, N., Kerr, E.O., Hu, D., Steffen, K.K., Oakes, J.A., Kennedy, B.K., Kaeberlein, M., 2006b. Sirtuin-independent effects of nicotinamide on lifespan extension from calorie restriction in yeast. Aging Cell 5, 505–514.

Tzagoloff, A., Myers, A.M., 1986. Genetics of mitochondrial biogenesis. Annu. Rev. Biochem. 55, 249–285.
Vellai, T., Takacs-Vellai, K., Zhang, Y., Kovacs, A.L., Orosz, L., Muller, F., 2003. Genetics: influence of TOR kinase on lifespan in *C. elegans*. Nature 426, 620.
Wang, Y., Tissenbaum, H.A., 2006. Overlapping and distinct functions for a *Caenorhabditis elegans* SIR2 and DAF-16/FOXO. Mech. Ageing Dev. 127, 48–56.
Warner, H.R., 2005. Longevity genes: from primitive organisms to humans. Mech. Ageing Dev. 126, 235–242.