

# Sirtuins in Aging and Age-Related Disease

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Sirtuins have been the focus of intense scrutiny since the discovery of Sir2 as a yeast longevity factor. Functioning as either deacetylases or ADP ribosylases, Sirtuins are regulated by the cofactor NAD and thus may serve as sensors of the metabolic state of the cell and organism. Here we examine the roles of Sirtuins in diverse eukaryotic species, with special emphasis on their links to aging and age-related diseases including cancer, diabetes, and neurodegenerative disorders.

The study of aging in invertebrate model organisms has produced fundamental new observations about mechanisms of eukaryotic aging. Among the genes that have been shown to regulate aging in different species are *SIR2* and its functional orthologs that make up a family of protein deacetylases termed Sirtuins. It has been known for four decades that histones can be acetylated (Roth et al., 2001), although the enzymes that acetylate and deacetylate lysine residues on histones and other proteins have only been discovered more recently. Histone and protein deacetylases fall into four classes with the yeast proteins Rpd3 (class I), Hda1 (class II), and Sir2 (class III) serving as charter members of the three major classes (Blander and Guarente, 2004; Sengupta and Seto, 2004). Human HDAC11, the sole member of class IV, is conserved in mice and *D. melanogaster* but not *C. elegans* and yeast (Gao et al., 2002). Class III deacetylases, the Sirtuins, are unique in that they require NAD as a cofactor (Blander and Guarente, 2004; Denu, 2003). In a complicated two-step reaction, Sirtuins couple lysine deacetylation to NAD hydrolysis, yielding O-acetyl-ADP-ribose and nicotinamide (Denu, 2003). As such, Sirtuin activity may be controlled by cellular [NAD]/[NADH] ratios and respond to changes in cellular metabolism (Lin et al., 2000, 2002, 2004).

Both class I and III deacetylases have been linked to aging (Bitterman et al., 2003), although most studies have focused on the class III Sirtuins. Increased Sir2 activity has been reported to enhance yeast replicative life span (described below) (Kaeberlein et al., 1999), as well as the life span of *C. elegans* and *D. melanogaster* (Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001). In contrast, reduced Sir2 activity extends yeast chronological life span (described below) under nutrient poor conditions or in combination with mutations in genes such as *RAS2* and *SCH9*, which function in glucose-responsive signal transduction pathways (Fabrizio et al., 2005). Numerous studies of Sirtuin function have been performed in mammalian cells, although it remains unknown whether Sirtuins directly regulate life span. In

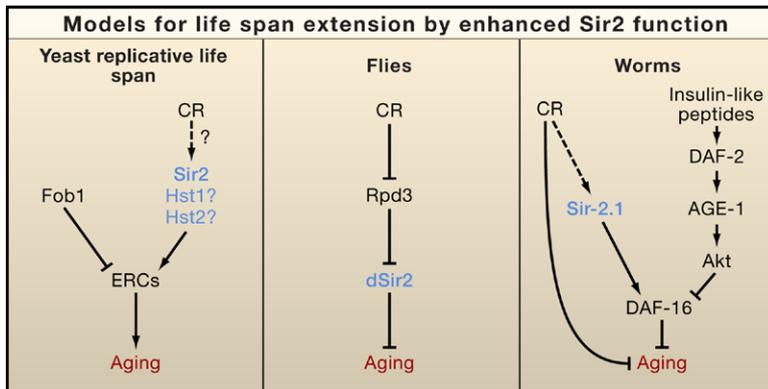
this study, we examine the role of Sir2 and other Sirtuins in invertebrate aging. In addition, we discuss Sirtuin functions in mammals and how they may affect human longevity and age-related disease.

## Sirtuins and Yeast Replicative Life Span

The asymmetrical division characteristic of the yeast *S. cerevisiae* is the basis for replicative life-span measurements. Mother cells give rise by budding to smaller, easily distinguishable daughter cells. Micromanipulation is used to remove successive daughters, which are counted as generations and tabulated (Mortimer and Johnston, 1959). Considerable effort has been directed toward identifying genes that regulate yeast replicative aging. Researchers have placed emphasis on mutations and interventions that lengthen yeast life span, reasoning that interventions reducing life span may not be directly linked to the aging process. To date, approximately 50 mutations, mostly gene deletions, have been reported to result in increased replicative life span (Bitterman et al., 2003; Kaeberlein et al., 2005b).

The first link between *SIR2* and aging came from the finding that specific mutations in a component of the yeast *SIR* complex resulted in extension of replicative life span (Kennedy et al., 1995). At the time, the *SIR* complex (Sir2, Sir3, and Sir4) was known to repress transcription of two yeast silent mating-type loci (which contain untranscribed copies of mating determination genes) and of genes placed near yeast telomeres (Rusche et al., 2003). Initially, it was speculated that *SIR* activity at telomeres may be regulating yeast aging, but followup analysis indicated that enhanced life span correlated with relocalization of the *SIR* complex to the nucleolus (Kennedy et al., 1997), the subnuclear location of ribosomal DNA (rDNA) genes and a major site of ribosome biogenesis.

Homologous recombination within rDNA repeats can lead to the formation of extrachromosomal rDNA circles (ERCs). Sir2 inhibits rDNA recombination and can also repress transcription of inserted genes that are



**Figure 1. Models for Life-Span Extension by Enhanced Sir2 Function**

Life-span extension by enhanced Sir2 function has been reported in three invertebrate models systems. In yeast, elevated Sir2 activity has been reported to extend replicative life span by inhibiting recombination in the rDNA repeats and therefore inhibiting the formation of extrachromosomal rDNA circles. Whether calorie restriction (CR) extends yeast replicative life span by enhancing Sir2 function remains a topic of debate (see text for discussion). Further, the ability of two other yeast Sirtuins (Hst1 and Hst2) to compensate for Sir2 and inhibit rDNA recombination remains controversial. In *D. melanogaster*, enhanced dSir2 expression leads to long life span although the longevity-enhancing effects of dSir2 have not been reported. CR is reported

to enhance dSir2 activity. Finally, in *C. elegans*, elevated SIR-2.1 expression leads to an increase in life span that is dependent on DAF-16, a FOXO transcription factor that is regulated by insulin signaling. Life-span extension by CR is either partly abrogated or not affected by *sir-2.1* deletion, depending on the chosen method of CR (see text for discussion).

transcribed by PolII (Rusche et al., 2003). Since ERCs contain a site of replication initiation but no centromere, they undergo replication during S phase but remain in the mother cell nucleus during mitosis. Accumulation of ERCs within a mother cell is one cause of yeast replicative aging (Sinclair and Guarente, 1997). In the absence of *SIR2*, the rate of ERC formation is enhanced and cells are short lived (Kaeberlein et al., 1999). Reduced recombination mediated by *SIR2* overexpression or deletion of the *FOB1* gene leads to lower ERC levels and results in extended yeast replicative life span (see Figure 1 for longevity extending roles of Sir2 orthologs) (Defossez et al., 1999; Kaeberlein et al., 1999). Fob1 has replication fork blocking activity and also promotes recombination within the rDNA (Kobayashi and Horiuchi, 1996). Although increased activity of Sir2 orthologs is associated with life-span extension in worms and flies (Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001), there is no evidence of a link between ERCs and aging in any organism other than yeast.

### Calorie Restriction and Yeast Replicative Aging

Calorie restriction (CR), defined as a reduction in organismal energy intake, has been shown to enhance longevity of organisms ranging from yeast to mammals. Yeast is a valuable system in which to study calorie restriction because nutrient-responsive pathways regulating cell growth are relatively well understood. CR in yeast can be induced by a reduction of glucose in the growth media or by mutations (such as the deletion of hexokinase 2) that reduce the metabolism of glucose (Lin et al., 2000). The role of Sir2 in the calorie restriction response is controversial (Guarente, 2005; Kennedy et al., 2005). Importantly, it remains undisputed that increased Sir2 activity leads to replicative life-span extension; only the proposed link between Sir2 and CR has been called into question.

Initially, it was reported that *SIR2* was required for life-span extension by calorie restriction in yeast. This conclusion was based on an also undisputed finding

that short-lived strains lacking *SIR2* did not exhibit life-span extension under CR conditions. In the absence of *SIR2*, ERCs accumulate. Thus, the finding that CR fails to increase life span in a strain lacking *SIR2* can be interpreted in one of two ways. Either CR directly leads to a reduction in ERC levels by enhancing Sir2 activity (Lin et al., 2000) or yeast strains lacking *SIR2*, which have an approximate 50% reduction in mean replicative life-span potential, prematurely succumb to elevated ERCs and do not live long enough to respond to CR.

It has been reported that CR causes robust life-span extension in the absence of *SIR2* as long as ERCs are kept at low levels by deletion of *FOB1* (Kaeberlein et al., 2004), a finding that has since been supported by another group (Lamming et al., 2005) but not supported in an earlier study using a different yeast strain background (Lin et al., 2002). This was interpreted by Kaeberlein et al. to indicate that *SIR2* is required not as a direct effector of calorie restriction but indirectly to maintain low ERC levels and thus enable the cells to live long enough to respond to CR (Kaeberlein et al., 2004). To reiterate, there is general agreement that CR can extend replicative life span in a strain lacking both *SIR2* and *FOB1* but not in a strain that lacks only *SIR2*. However, followup experiments and interpretations thereof have led different groups to different conclusions regarding the importance of Sir2 for the CR response (see below). CR implemented by a reduction in amino acids is also reported to extend yeast replicative life span (Jiang et al., 2002). Extension in this case is independent of *SIR2*.

One of the authors (B.K.K.) is at the center of the dispute regarding the importance of Sir2 in the CR response. However, we will present both views to the best of our ability. The debate currently rests on the extent of glucose limitation used to implement the CR response. Guarente, Lin, Sinclair, and colleagues believe that reducing the glucose concentration from 2% to 0.5% (4× reduction) is ideal since it has a minimal effect on yeast growth rate and may be more physiologically similar to levels of calorie restriction employed in other

model organisms (Lamming et al., 2006; Lin and Guarente, 2006). They propose that CR mediated by a reduction in glucose concentration to 0.05% may be *SIR2* independent and mediated through a different mechanism. Kaeberlein, Kennedy, and colleagues have largely, but not exclusively, used 0.05% glucose (40× reduction) because it maximizes life-span extension (Kaeberlein et al., 2004, 2005a, 2006b), permitting easier interpretation of epistasis analysis (Clancy et al., 2002). They support a model whereby life-span extension by CR is Sirtuin independent and not fundamentally different at either level of glucose limitation (Kaeberlein et al., 2005a, 2006a, 2006b). At either 0.5% or 0.05% glucose, CR extends life span in a manner dependent on the nutrient-responsive kinases, TOR, PKA, and *SCH9* (the Akt ortholog) (see brief discussion below) (Fabrizio et al., 2001; Kaeberlein et al., 2005d; Lin et al., 2000). These nutrient-responsive kinases control numerous downstream responses including ribosome biogenesis and cell growth, stress responses, and autophagy. Which of these are important for life-span extension remains to be determined. Although deletion of *SCH9* does reduce recombination in the rDNA (Prusty and Keil, 2004), it is clear from a variety of epistasis studies that this phenotype does not explain replicative life-span extension. For example, double mutants lacking both *SCH9* and *FOB1* survive much longer than mutants lacking only *FOB1*, which already have extremely low levels of ERCs (Kaeberlein et al., 2005d).

Life-span extension by CR at 0.5% or 0.05% glucose can occur in the absence of *SIR2*, as long as *FOB1* is also deleted (Kaeberlein et al., 2004; Lamming et al., 2005). Two findings led Lamming et al. to propose that other Sirtuins were, together with Sir2, acting in a redundant fashion to mediate the calorie restriction response under these conditions (Lamming et al., 2005). First, nicotinamide, a known inhibitor of Sirtuin enzymatic activity, can block at least some of the life-span extension by CR even in the absence of Sir2 (Kaeberlein et al., 2005a; Lamming et al., 2005). Second, a screen for genes that when overexpressed led to enhanced rDNA silencing resulted in the identification of *HST2*, another Sirtuin (Lamming et al., 2005). Although Hst2 is normally cytoplasmic (Perrod et al., 2001), under CR conditions it is reported to relocate to the nucleus (Lamming et al., 2005). Further, Lamming et al. report that, analogously to strains lacking *SIR2*, yeast lacking only *HST2* exhibit elevated rDNA recombination and a short life span. The finding regarding rDNA recombination contrasts with a previous report by Gasser and colleagues, who found that a yeast strain lacking *HST2* exhibits reduced rDNA recombination (Perrod et al., 2001).

At 0.5% glucose, Lamming et al. report that CR fails to extend life span in a strain lacking both *SIR2* and *HST2*, leading to the conclusion that CR extends life span by reducing rDNA recombination and ERC formation in a *SIR2*- and *HST2*-dependent fashion (Lamming et al., 2005). Hst1, another Sirtuin, may also compensate for

*Sir2* in some strains. Another group has found using the same genetic background that CR extends life span in yeast strains that lack *SIR2*, *HST2*, and *FOB1* or in yeast that also lack *HST1* (Kaeberlein et al., 2006b). The reason(s) for these disparate findings are being actively debated (Kaeberlein et al., 2006b; Lamming et al., 2006).

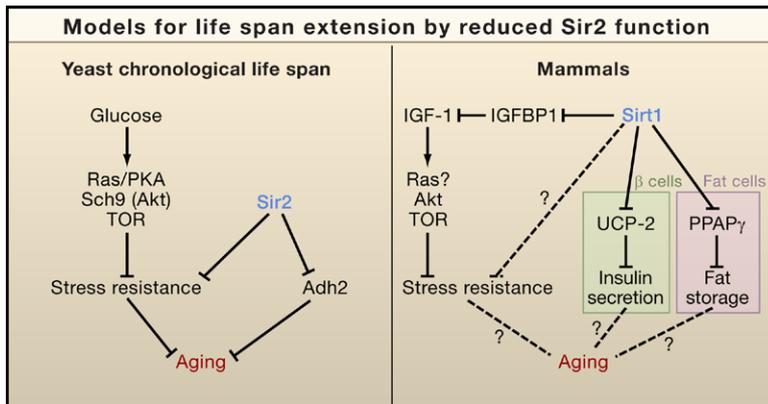
Finally, longevity extension by CR has been linked to cellular respiration. Work from Guarente, Sinclair, and colleagues has led to the model that CR leads to elevated NAD levels (Lin et al., 2002), reduced NADH levels (Lin et al., 2004), and/or reduced nicotinamide levels (Anderson et al., 2003; Bitterman et al., 2002), which in turn lead to Sir2 activation. Reducing glucose levels in yeast enhances the respiratory rate of the facultative anaerobe, and it has been proposed that this enhanced rate of respiration leads to increased [NAD]/[NADH] ratios. Furthermore, CR fails to increase replicative life span of respiratory-deficient yeast (Lin et al., 2002), although this finding has also been disputed (Kaeberlein et al., 2005a). Whether differential levels of glucose deprivation underlie the disparate findings of these studies also continues to be debated (Kaeberlein et al., 2006a; Lin and Guarente, 2006).

### Sir2 and Yeast Chronological Life Span

The chronological life-span models aging in the natural environment because it is a measure of the survival of yeast populations in a nondividing state (Longo et al., 1997). The median chronological life span of *S. cerevisiae* wild-type DBY746 or SP1 yeast grown in glucose medium (SDC) is 6 to 7 days. Under these conditions wild-type DBY746 cells have excess ethanol available for energy production and maintain high metabolic rates for the majority of the life span. A form of severe calorie restriction, achieved by switching cells from ethanol/glucose-containing medium to water between days 1 and 5, causes a decrease in metabolic rates and extends survival by 2- to 3-fold (Fabrizio et al., 2004a; Longo et al., 1997).

Yeast strains with reduced activity of Ras2 or any of the nutrient-responsive kinases Sch9, PKA, and TOR have extended chronological life span (Fabrizio et al., 2001, 2003, 2004b; Powers et al., 2006). Importantly, Sch9 is a functional homolog of Akt/PKB, a component of the conserved pro-aging pathways of worms, flies, and mice (Longo and Finch, 2003).

In contrast to the role of ERCs in yeast replicative aging, superoxide appears to play a central role in *S. cerevisiae* chronological aging and death. In fact, the superoxide-sensitive 4Fe-4S cluster enzyme aconitase is inactivated preceding the high mortality phase (Fabrizio et al., 2001), and overexpression of the superoxide dismutases *SOD1* or *SOD2* extends chronological life span (Fabrizio et al., 2003). Spontaneous DNA mutation frequency also increases with chronological age, although its role in aging and death remains poorly understood (Fabrizio et al., 2004a, 2005).



**Figure 2. Models for Life-Span Extension by Reduced Sir2 Function**

In the yeast chronological life-span assay, deletion of *SIR2* has been shown to enhance survival in a postmitotic state either in low nutrient conditions or when combined with mutations in nutrient-responsive kinases such as Ras, Sch9 (Akt), and TOR. Strains lacking Sir2 show elevated stress resistance and enhanced alcohol dehydrogenase activity under these conditions, although the mechanisms by which Sir2 regulates these processes remain largely unknown. In mammals, the longevity effects of Sirt1 have not been reported. However, Sirt1 is reported to have a number of functions that may regulate longevity. The pathway in green is reported to occur specifically in pancreatic  $\beta$  cells, and the pathway in purple is reported specifically for adipocytes.

The role of Sir2 in the chronological survival of non-dividing yeast cells has only recently been examined (Fabrizio et al., 2005) and appears to be quite different from its role in the yeast replicative life span. Deletion of *SIR2* increases stress resistance but has no effect on the chronological life span of wild-type yeast grown and maintained in medium. However, deletion of *SIR2* extends further the chronological life span caused by severe CR (incubation in water) (Fabrizio et al., 2005) or caused by mutations that decrease PKA or Sch9 activity (see Figure 2 for possible longevity-limiting roles of Sir2 orthologs). Furthermore, the overexpression of *SIR2* has no effect on the chronological life span of wild-type cells and reduces the life span of cells lacking Sch9 activity (Fabrizio et al., 2005). Other Sirtuins have not been examined in this aging assay. Evidence exists for two possible mechanisms by which deletion of *SIR2* results in life-span extension when coupled with reduced nutrient-responsive kinase activity or CR. First, deletion of *SIR2* in combination with reduced PKA or Sch9 activity was found to increase the expression of many stress-resistance and sporulation genes and to decrease the rate of DNA mutations that accumulate with age in postmitotic conditions (Fabrizio et al., 2005). This contrasts with the role of Sir2 in mitotically active cells, where it promotes genome stability by repressing recombination (Blander and Guarente, 2004). Second, Fabrizio et al. report that cells lacking *SIR2* have elevated levels of the alcohol dehydrogenase Adh2 (Fabrizio et al., 2005). Mitotically active yeast cells primarily generate energy through fermentation, which leads to the production of ethanol. As fermentable carbon sources become scarce and ethanol accumulates, yeast undergo a metabolic shift and begin to utilize ethanol as an energy source, only entering stationary phase after ethanol levels are largely depleted. In the absence of Sir2, increased alcohol dehydrogenase activity leads to more rapid ethanol degradation and entry into a more stable postmitotic state. The mechanism by which Sir2 negatively regulates Adh2 levels as cells enter a postmitotic state is yet to be determined. Increased ethanol uptake may represent an attempt by cells lacking *SIR2* to prepare for long periods of starvation.

In summary, although the deletion of *SIR2* decreases and its overexpression increases the yeast replicative life span, changing Sir2 levels does not significantly affect the chronological life span. However, when combined with severe CR- or life-span-extending mutations that reduce the activity of the Ras and Sch9 pathways, mutations in *SIR2* do extend the chronological life span and Sir2 overexpression limits life-span extension in mutants lacking *SCH9*. Loss of *SIR2* may promote entry into a state that protects cells against aging during starvation. These results suggest that Sir2 plays very different roles in the regulation of replicative and chronological life span.

### One Model Organism, Two Measures of Aging?

The replicative life span of yeast is in many respects analogous to the replicative life span of mammalian fibroblasts and lymphocytes, which undergo a limited number of population doublings in culture. Thus, replicative aging in yeast may be a model to study replicative aging in mitotically active mammalian cells. By contrast, chronological aging in yeast may serve to model aging in postmitotic mammalian cells (e.g., neurons) and organisms. To what extent do similar pathways regulate the mechanisms that lead to replicative and chronological aging in the same model organism?

Whereas earlier studies showed similarities but also major differences between the regulation of replicative and chronological life span, more recent studies are beginning to suggest that there may be only one form of "aging" in *S. cerevisiae* with two ways to measure it. A large-scale search for mutants with an extended replicative life span led to the identification of deletion mutants in *sch9* and *tor1*, also implicated in chronological life-span extension (Fabrizio et al., 2001; Kaeberlein et al., 2005d; Powers et al., 2006). Thus, nutrient-responsive kinases control aging of both mitotic and postmitotic cells. Whether the effectors that regulate each type of aging downstream of these kinases are the same remains to be determined. Notably, reduced activity of orthologs of these kinases and/or of the pathways in which they function lead to life-span extension in worms, flies, and mammals (Kenyon, 2005; Longo and Finch, 2003).

We suspect that inhibition of these nutrient-responsive kinases leads to life-span extension in mammals because it provides distinct, beneficial effects in both mitotic and postmitotic populations of cells. Support for this comes from yeast replicative and chronological life-span studies but also from longevity experiments in *C. elegans*, where it was shown that reduced insulin/IGF-1 signaling can slow a variety of age-related phenotypes in distinct tissues (Garigan et al., 2002). Similar effects have been observed for CR in mice.

For yeast replicative life span, double mutants lacking *FOB1* and either *SCH9* or *TOR1* are extremely long lived, suggesting that the accumulation of ERCs caused by *Fob1* may, to a degree, mask the effect of loss of *SCH9* or *TOR1* on replicative life-span extension. Thus, ERCs may be responsible for one discrepancy between the effect of Sir2 on replicative and chronological life span. If Sir2 is absent, ERCs accumulate early and either kill the cell or prevent its division. If Sir2 is overexpressed, ERC levels are decreased and replicative life span is enhanced. ERCs may then be viewed as an agent that hastens death specifically in yeast and obscures other more universal aging processes. However, deletion of *SIR2* does not extend the replicative life span of strains lacking *SCH9* or *TOR1* even in the absence of *FOB1*, so there must be other underlying differences as well.

The force of natural selection against the appearance of “defects” is very high in young organisms, but it declines at advanced ages. Yeast mother cells do not need to produce 25 daughters to ensure colony survival or even a maximal colony growth rate, and, therefore, deleterious events that are specific to old mother cells (such as ERC formation or reduced growth rates) may not be subject to counterselection. Thus, ERCs appear to be a major cause of aging only in yeast subjected to conditions not normally encountered in natural environments. In contrast, other mechanisms of aging may be conserved, and evidence not only from yeast chronological and replicative aging studies, but also from higher eukaryotes, is beginning to show that the Sch9 (Akt), Ras, and Tor may be universal regulators of these mechanisms. Sir2 is clearly important for life-span regulation but it may play either a pro- or an anti-aging role in different organisms depending on the availability of nutrients and on the activity of glucose signaling pathways.

### Sir2 and Aging in Other Invertebrates

Two other model organisms where aging has been studied extensively are *C. elegans* and *D. melanogaster*. In this section we summarize findings regarding the role of Sir2 orthologs in aging in these invertebrates. Other Sirtuins are present in both organisms, but their roles in aging have not been ascertained. In *C. elegans*, increased dosage of the *SIR2* ortholog, *sir-2.1*, increases the mean life span by up to 50% (Figure 1) (Tissenbaum and Guarente, 2001), and this extension requires the FOXO transcription factor DAF-16, which is known to be regulated by the insulin/IGF-1 pathway. Rather than acting directly

in the insulin/IGF-1 signaling pathway, two recent studies suggest that SIR-2.1 may act in a parallel pathway that converges at the point of DAF-16 regulation (Wang et al., 2006) (Berdichevsky et al., 2006). In both studies, both *C. elegans* 14-3-3 proteins were identified as SIR-2.1 interactors, an intriguing finding because mammalian 14-3-3 proteins are known to bind FOXO transcription factors and sequester them in the cytoplasm (Brunet et al., 1999). Although life-span extension by SIR-2.1 overexpression can be blocked by reducing expression of 14-3-3 proteins, this is not the case for mutations that disrupt the insulin pathway (Wang et al., 2006) (Berdichevsky et al., 2006). For instance, the long life span of a *daf-2* mutant is not affected by a reduction in 14-3-3. In fact, deletion of *sir-2.1* does not reduce the life span of a *daf-2* mutant and instead appears to slightly increase it, consistent with the longer chronological life span observed for yeast lacking both *SIR2* and the Akt homolog *SCH9* (Berdichevsky et al., 2006; Fabrizio et al., 2005; Wang and Tissenbaum, 2006).

Berdichevsky et al. propose that SIR-2.1 is part of a stress-response pathway that regulates DAF-16 activity in a manner dependent on 14-3-3 proteins (Berdichevsky et al., 2006). Consistent with this idea, a null mutant of *C. elegans sir-2.1* not only has a slightly shorter life span but exhibits sensitivity to a variety of stresses including hydrogen peroxide, UV irradiation, and heat shock (Wang and Tissenbaum, 2006). These results only partially agree with those observed for nondividing yeast. In fact, in yeast lacking SIR2 the chronological life span is either normal or slightly shorter, DNA mutations are more frequent, but sensitivity to heat shock and oxidative stress is reduced (Fabrizio et al., 2005). SIR-2.1 is also reported to be important for silencing of transgenes inserted into genomic repeat elements, perhaps in a manner analogous to Sir2-dependent silencing in yeast (Jedrussik and Schulze, 2003). Whether this activity is important for longevity regulation remains to be determined.

Different assays have been proposed for calorie restriction in *C. elegans*, including use of *eat-2* mutants that eat less than wild-type worms, growth in axenic media that lacks a bacterial food source, and reduced exposure to food source or dietary restriction (Walker et al., 2005). The dependence of *sir-2.1* has been determined for *eat-2* mutants and dietary restriction, with somewhat different results. Life-span extension by *eat-2* mutants is partially suppressed by deletion of *sir-2.1* (Wang and Tissenbaum, 2006). In contrast, life-span extension by dietary restriction is not affected by *sir-2.1* deletion (M. Kaerberlein, personal communication). Also, whereas life-span extension by *sir-2.1* overexpression is *daf-16* dependent (Tissenbaum and Guarente, 2001), life-span extension by CR is not (Lakowski and Hekimi, 1998).

Small molecule activators and inhibitors of Sirtuins have been reported. Resveratrol has been proposed as a small molecule agonist of yeast Sir2 and of its orthologs

in worms, flies, and mammals and has been studied in the context of longevity (Wood et al., 2004). Resveratrol was identified in a screen for Sirtuin activators using a nonbiological substrate suitable for high-throughput fluorescent analysis (Howitz et al., 2003). However, other groups have shown that the stimulatory effects of resveratrol on Sirtuin enzyme activation are specific to this nonnative substrate (Borra et al., 2005; Kaeberlein et al., 2005c). No enhancement of enzyme activity was seen with native acetylated peptides. One possibility is that resveratrol only stimulates Sirtuin activity toward specific substrates *in vivo*. Alternatively, the effects of resveratrol may be largely Sirtuin independent, an assertion consistent with observations that the polyphenol compound has a plethora of other activities. Reports conflict as to whether resveratrol extends yeast life span (Howitz et al., 2003; Kaeberlein et al., 2005c).

In *C. elegans*, *sir-2.1*-dependent life-span extension has been observed for resveratrol (Wood et al., 2004). A recent report however has suggested that, rather than enhance SIR-2.1 activity in worms, resveratrol may extend life span by antagonizing it (Viswanathan et al., 2005). Also, unlike *sir-2.1* overexpression, resveratrol extends life span in a *daf-16*-independent manner. The key target for life-span extension due to resveratrol treatment appears to be *abu-11*, an endoplasmic reticulum (ER) stress-family gene. Either addition of resveratrol or, curiously, *sir-2.1* deletion leads to enhanced *abu-11* expression, which is in turn required for life-span extension. The authors put forth a model to explain these seemingly paradoxical findings by proposing that (1) resveratrol may bind to SIR-2.1 and change its specificity toward substrates such that deacetylation of some substrates may be enhanced whereas deacetylation of other substrates is impeded and (2) SIR-2.1 has multiple functions that impinge on longevity regulation with the net effect being that overexpression extends life span. Thus, as with Sir2 in yeast, SIR-2.1 in worms may have some activities that promote longevity and others that limit it. Perhaps the most interesting finding from this study is that overexpression of *abu-11* extends worm life span, implicating ER stress as a limiting factor in worm longevity.

In flies, the Sir2 ortholog dSir2 has been reported to extend life span as well (Rogina and Helfand, 2004). In addition, life-span extension by CR is blocked in strains lacking dSir2 (Figure 1). These findings suggest that CR works through a Sir2-dependent mechanism in this organism. Mutant flies with reduced Rpd3 (class I deacetylase) activity exhibit extended life span that is dependent on dSir2, indicating that the two deacetylases are in a single pathway regulating aging with Rpd3 upstream (Rogina and Helfand, 2004; Rogina et al., 2002). It has not been determined whether Rpd3 regulates [NAD]/[NADH] ratios in flies. Life-span extension by resveratrol is also reported to be dSir2 dependent (Wood et al., 2004). Little is known about dSir2 function in flies, especially regarding aging-specific func-

tions. This deacetylase has been reported to be involved in transcriptional repression (Newman et al., 2002), at least in part through interactions with Hairy, a transcription factor involved in developmental regulation (Rosenberg and Parkhurst, 2002). Whether dSir2 regulates the insulin pathway in flies remains to be determined.

In summary, overexpression of Sir2 orthologs increases fly and worm life span, and the links between Sir2 orthologs and calorie restriction appear inconsistent between organisms and remain to be fully understood. It seems paradoxical that the impetus for examining the life span of worms and flies overexpressing their Sir2 orthologs derived from yeast replicative aging studies, given that the proposed role of Sir2 in longevity regulation, reducing ERC production, seems not to be conserved. We offer three possible explanations to resolve this paradox. First, Sir2 in yeast may have other functions aside from reduced ERC production that promote replicative longevity, and this activity may be conserved. Second, worm and fly Sir2 orthologs may extend life span by repressing recombination at other chromosomal regions that are more sensitive (e.g., repetitive DNA) and that do not exist in yeast or influence yeast life span. It is hard to interpret this model in the context of *C. elegans* where *daf-16* is required for the longevity effects of *sir-2.1* overexpression. Finally, Sir2 orthologs may have evolved to couple metabolic signals to specific longevity-enhancing functions in different organisms, a model promoted by Guarente and colleagues (Guarente and Picard, 2005). How this last model would fit with evolutionary theories of aging remains to be resolved. We will have to await further experiments in each of these organisms to begin to differentiate between these models, or devise a different one. Further adding to the incongruity is the finding that loss of *SIR2* in some contexts enhances yeast chronological life span and that its overexpression does not affect or reduces chronological longevity (Fabrizio et al., 2005).

### Sirtuins in Mammals

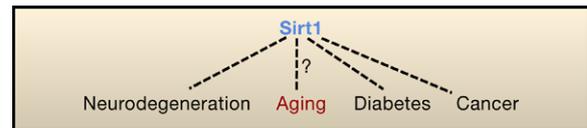
The aging phenotype of mice overexpressing Sirt1, and the effects of CR on longevity in a mouse lacking Sirt1, has not yet been determined. Although a majority of Sirt1 null animals succumb to developmental defects during early postnatal development (Cheng et al., 2003; McBurney et al., 2003), the remaining mice survive and have phenotypes resembling mice overexpressing IGFBP-1 or lacking IGF-I (Longo and Finch, 2003) (see below). In Sirt1-deficient adult survivors, Chen et al. report that calorie restriction fails to induce increased physical activity (Chen et al., 2005a), a common CR-induced behavioral change in wild-type mice. However, physiologic changes similar to those caused by CR, such as reduced blood glucose, triglycerides, and insulin levels, occur normally in these animals. Although these latter phenotypes might be expected to extend longevity, the effect of Sirt1 deficiency alone or in combination with CR remains to be established. Nevertheless, a number of

studies have reported in mammals intriguing functions of Sirtuins that may pertain to aging. In the following sections, we outline some of these findings, focusing on results that relate to aging and age-related disease.

### Sir2/Sirt1, Genomic Instability, and Oncogenesis

Initial links between Sir2 function and the response to DNA damage came from studies in *S. cerevisiae*. In proliferating yeast, several reports indicate that Sir2 protects against DNA damage by inducing nonhomologous end joining and homologous recombination pathways, a function which appears to depend on the derepression of silent mating-type genes (Fabrizio et al., 2005; Lee et al., 1999). Sir2 also mediates the asymmetric inheritance of oxidatively damaged proteins during cell division (Aguilaniu et al., 2003). Mother cells have a limited life span, but the colonies they produce will continue proliferating as long as sufficient nutrients are available. This asymmetric inheritance restricts damaged proteins to the mother cell, likely ensuring the generation of relatively damage-free daughters and continued colony proliferation. In contrast, Sir2 appears to promote chronological age-dependent genomic instability in long-lived mutants. *S. cerevisiae* cells accumulate spontaneous DNA mutations with chronological age, and this accumulation is drastically reduced in strains lacking both *SCH9* and *SIR2* (Fabrizio et al., 2005). Although this effect of Sir2 deficiency appears to be independent of the derepression of silent mating-type genes, the mechanisms by which Sir2 promotes age-dependent genomic instability are not known.

In mammals, considerable evidence exists to suggest that the tumor suppressor p53 is a target for deacetylation by Sirt1 (Cheng et al., 2003). Under normal cellular conditions for proliferation, p53, a transcriptional activator, is highly unstable (Gu et al., 2004). p53 stabilization occurs as part of a checkpoint response to cellular stress (including DNA damage) and results in proliferative arrest, followed by either reentry into the cell cycle, senescence, or apoptosis. Acetylation of p53 leads to enhanced transcriptional activation as part of the checkpoint response. By deacetylating p53, Sirt1 may turn off p53 after recovery from stress to allow cell proliferation to resume. Although it has been shown that increased Sirt1 activity leads to hypoacetylation of p53 and repression of p53 target genes (Luo et al., 2001; Vaziri et al., 2001), the cellular consequences of this remain in question. Whereas Sirt1 activity was originally reported to inhibit apoptosis through deacetylation of p53 (Langley et al., 2002; Luo et al., 2001), more recent reports suggest that although Sirt1 can deacetylate p53 there is little effect on p53-mediated biological outcomes (Kamel et al., 2006; Solomon et al., 2006). Other stress response-related proteins linked to Sirt1 are Ku70 and NF- $\kappa$ B (see below) (Cohen et al., 2004; Yeung et al., 2004). Ku70, a DNA repair factor, is reported to be a target for deacetylation by Sirt1, which is another mechanism by which Sirt1 might inhibit cell death (Cohen et al., 2004).



**Figure 3. Sirt1, Aging, and Age-Related Disease**

Although preliminary, Sirt1 is postulated to protect against neurodegeneration and diabetes but to promote tumorigenesis. However, its role in disease progression and aging in mammals remains to be fully determined. Future studies at the organismal level will be needed to address the relationships between Sirt1, aging, and age-related disease and will likely provide mechanistic insights into the modes of action of this important protein deacetylase.

Expanding on links between *Sir-2-1* and *daf-16* in worms, several studies have tested the hypothesis that Sirt1 can regulate mammalian FOXO transcription factors through direct binding and/or deacetylation. Sirt1 deacetylation can either lead to activation or repression of FOXO-dependent transcription depending on the context (Greer and Brunet, 2005). Current thinking is that deacetylation of FOXO factors by Sirt1 may lead to activation of a set of stress-resistant factors, tipping the balance toward stress resistance and away from apoptosis (Brunet et al., 2004). There also exists an intricate interplay between FOXOs, Sirt1, and p53, with each being able to regulate the other two in at least some cellular contexts (Greer and Brunet, 2005). Because all three of these proteins have been linked to aging, it is imperative that future studies delineate these complex interactions.

Sirt1 function has also been examined in the context of cellular senescence in cell culture. Chua et al. report that mouse embryo fibroblasts lacking Sirt1 are resistant to senescence in the presence of sublethal, chronic oxidative stress (Chua et al., 2005). Interestingly, oncogene-induced senescence occurs normally in these cells. Consistent with this result, nicotinamide, a sirtuin inhibitor, has been reported in another study to extend the replicative life span of primary human fibroblasts (Lim et al., 2006), although no experiments were presented that directly link the actions of nicotinamide to Sirt1 or other Sirtuins in this context. Preliminary studies examining links between Sirt1 function and cancer suggest that it is enhanced Sirt1 activity that may be oncogenic (Figure 3). For instance, treatment of human breast and lung cancer cells with Sirtinol, another Sirt1 inhibitor, has been found to induce a senescent-like growth arrest (Ota et al., 2006). Related findings were reported by Ford et al. (Ford et al., 2005), who employed RNAi to silence expression of Sirt1. In this study, enforced reduction of Sirt1 led to growth arrest and/or apoptosis in human epithelial cancer lines but not primary epithelial cells.

Inhibitors of class I and class II histone deacetylases are under clinical trials as chemotherapeutic agents. The antitumor effects of these inhibitors likely stem from their ability to reactivate transcription of tumor suppressor genes in combination with DNA-demethylating

agents. A similar role has recently been proposed for the Sirt1 inhibitors, which were found to promote the reactivation of tumor suppressor gene transcription in human breast and colon cancer lines (Pruitt et al., 2006). In this case, re-expression could be achieved in the absence of inhibitors of DNA methylation even though the promoters in question remained highly methylated. Although there is no general agreement concerning the mechanisms by which Sirt1 might promote oncogenesis, these studies collectively point to the need for further investigation and the potential role of Sirtuin inhibitors in cancer treatment. These results may be consistent with the effect of *SIR2* deletion mutations in increasing resistance to oxidative damage, reducing DNA damage, and extending the yeast chronological life span in combination with mutations in nutrient-responsive kinases (Fabrizio et al., 2005). Thus, both yeast Sir2 and mammalian Sirt1 can promote DNA damage or oncogenesis, although they also play important roles in protecting against damage during cell growth and organismal development.

Less closely related to the other Sirtuins, the primary activity of Sirt6 is ADP-ribosylation rather than deacetylation (Liszt et al., 2005). A recent report finds that mice lacking Sirt6 exhibit enhanced genome instability leading to a wide range of chromosomal anomalies as well as phenotypes resembling premature aging, including cachexia, kyphosis, and osteopenia (Mostoslavsky et al., 2006). Many other mutations leading to progeria in mammals are associated with genome instability. Mostoslavsky et al. find that mice lacking Sirt6 have a range of sensitivities to DNA-damaging agents including MMS and ionizing radiation that are consistent with defects in base excision repair (Mostoslavsky et al., 2006). The exact role of Sirt6 in this repair process remains to be determined.

### Sirtuins and Mammalian Metabolism

Decreased insulin/IGF-I signaling is intimately linked to enhanced longevity in worms, flies, and mice (Longo and Finch, 2003). Further, deletion of the yeast orthologs of Akt and Ras, which function downstream of mammalian insulin/IGF-I, results in extended replicative and chronological life span in yeast (Fabrizio et al., 2001, 2003, 2004b; Lin et al., 2000). Because insulin signaling links extracellular glucose levels to cell metabolism, and increased life span in worms overexpressing *sir-2.1* is dependent on *daf-16*, considerable effort has been devoted to uncovering the connections between Sirt1 function and metabolism in mammals. To date, the results of these studies have not generated a clear picture of the role of Sirt1 in these processes.

Two studies have examined the function of Sirt1 in the release of insulin by pancreatic  $\beta$  cells. Bordone et al. reported that an siRNA-enforced reduction of Sirt1 expression in  $\beta$  cell lines leads to an increase in the expression of uncoupling protein 2 (UCP2) and a reduc-

tion in insulin secretion (Bordone et al., 2006). This finding may phenocopy food deprivation, which is known to induce UCP2 expression and reduce insulin secretion. In a second study, Sirt1 expression was elevated specifically in the mouse pancreas, leading to reduced UCP2 expression and enhanced insulin secretion during glucose stimulation (Moynihan et al., 2005). Sirt1 also induces gluconeogenic genes and hepatic glucose output through the transcriptional coactivator PGC-1  $\alpha$  (Rodgers et al., 2005). At least with regard to  $\beta$  cell function, reduced Sirt1 may mimic rather than impede the effects of either CR or low-plasma IGF-I, in agreement with the other phenotypes described below for Sirt1 null mice, including small body size and increased levels of IGFBP-1 (Figure 2).

Sirtuins also have functions in adipose tissue. For instance, Picard et al. show that Sirt1 promotes fat mobilization in mammalian adipocytes by repressing PPAR $\gamma$  (Picard et al., 2004), and reduced fat content has been linked to extended longevity (Bluher et al., 2003). However, it is storage and not the mobilization of fat that has been consistently associated with longevity extension in worms, flies, and mice, apparently as part of a program aimed at surviving long periods of starvation (Longo and Finch, 2003). Notably Sirt3, a mitochondrial Sirtuin, is expressed largely in brown fat, suggesting a role for this deacetylase in adaptive thermogenesis (Shi et al., 2005). Links between aging and Sirt3 have yet to be reported.

Are the metabolic consequences of altering Sirt1 levels pro- or anti-aging (or both)? Elevated Sirt1 expression may extend life span by decreasing fat storage. On the other hand, reduced Sirt1 levels cause changes analogous to those observed in the long-lived IGF-I-deficient mice (McBurney et al., 2003). The role for Sirt1 in reducing the expression of the IGF binding protein, IGFBP-1, in increasing hepatic glucose output, in increasing pancreatic insulin secretion, in mobilizing fat, and in preventing a dwarf phenotype raises the possibility that Sirt1 may play both a pro- and anti-aging role in mammals as has been shown in *S. cerevisiae*, *C. elegans*, and mammalian cells (Fabrizio et al., 2005; Kaeberlein et al., 1999).

It will be important to determine whether the role of Sirt1 in decreasing IGFBP-1 and fat storage and increasing hepatic glucose output and pancreatic insulin secretion is evidence for entry into a pro-aging mode and/or whether Sirt1 regulates systems that may promote physical activity during periods of starvation for the purpose of finding food or avoiding predation. In fact, the increase in activity of mice during CR requires Sirt1 (Chen et al., 2005a). One possibility is that, during CR, mammalian Sirt1 plays a role in inhibiting entry into a nonreproductive and reduced physical activity phase that may be characterized by a slower aging rate (McBurney et al., 2003; Chen et al., 2005a), as shown for the role of Sir2 in the *S. cerevisiae* chronological life span (Fabrizio et al., 2005).

### Sirtuins and Neurodegeneration

Both in *C. elegans* and mammals, specific neurological functions have been attributed to Sir2 orthologs (Figure 3). To study neuronal cytotoxicity in worms, Parker et al. developed a system in which a fragment of the human Huntington disease-associated protein, htt, is expressed in touch receptor neurons (Parker et al., 2005). Either increased dosage of *sir-2.1* or exposure to resveratrol rescued neuronal dysfunction. The authors speculated that SIR-2.1-dependent activation of stress-responsive *daf-16* targets may be promoting cell survival. Nothing to date has been reported that directly links Sirt1 with htt in mammals. However, Sirt1 does appear to inhibit axonal degeneration, a process that often precedes neuronal death in neurodegenerative diseases such as Parkinson's and Alzheimer's (Araki et al., 2004). This activity of Sirt1 was discovered because slow axonal degeneration in a strain of mutant mice was linked to overexpression of a NAD biosynthetic enzyme. Of note, another elegant study examining axonal degeneration attributed the benefits of increased NAD biosynthesis to Sirt1-independent alterations in local bioenergetics (Wang et al., 2005). These authors pinpointed the deteriorative decline in NAD levels to axons and not the nucleus where Sirt1 activity is presumably regulated. A final study related to neurodegeneration examined NF- $\kappa$ B signaling in microglia, an activity linked to amyloid- $\beta$  neuronal death and Alzheimer's disease, finding that overexpression of Sirt1 repressed NF- $\kappa$ B signaling by reducing RelA/p65 acetylation (Chen et al., 2005b). Together, these findings call for increased examination of Sirt1 as a possible protective agent in neurons.

### Sir2 Deacetylases: Anti-aging, Pro-aging, or Both?

In summary, the results in *S. cerevisiae* and invertebrate model systems, coupled with similarities between Sirt1-deficient and long-lived IGF-I-deficient dwarf mice (Longo and Finch, 2003), suggest that the safest bet at this point is that Sir2 deacetylases play both pro- and anti-aging roles in different contexts. One hypothesis to be tested is that reduced Sir2/Sirt1 activity can promote life-span extension by causing entry into a slow aging starvation response phase whereas Sir2/Sirt1 overexpression can promote life-span extension by inducing other changes such as fat breakdown and enhanced physical activity.

The field critically awaits studies of mouse longevity. Will mice overexpressing Sirt1, mice deficient in Sirt1, or neither enjoy extended longevity? Will CR extend life span in mice lacking Sirt1? Will Sirt1 deficiency further extend the life span of mice with mutations in the IGF-1/Akt pathway? Other critical mechanistic questions also remain to be answered. What are the key targets of the Sirt1 deacetylase? Does resveratrol stimulate Sirtuin activity toward biological substrates and, if so, which ones? These results and others will no doubt begin to clarify the links between Sirtuins and mammalian longevity.

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