

exploiting this potent tumor-suppressor network for treating cancer.

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The Enigmatic Role of Sir2 in Aging

In this issue of *Cell*, Longo and colleagues (Fabrizio et al., 2005) examine the role of Sir2, a histone deacetylase, in chronological aging in yeast by measuring the long-term survival of nondividing cells. In contrast to measurements of aging for mitotic cells, cell survival in the nonmitotic state is decreased by Sir2 activity under conditions that mimic calorie restriction.

The replicative aging of the budding yeast *Saccharomyces cerevisiae* has been measured by the number of daughter cells that one mother cell can produce. More than a decade ago, a screen for genes that determine yeast replicative life span identified the SIR complex (including the Sir2 histone deacetylase) as a regulator of replicative aging (Kennedy et al., 1995). Since then, an extensive body of work, largely from Leonard Guarente and his colleagues, has demonstrated that Sir2 activity correlates with yeast replicative life span: *SIR2* deletion strains are short lived, whereas strains that

overexpress *SIR2* are long lived (Kaeberlein et al., 1999).

A second model of yeast aging has been developed, termed chronological aging, in which life span is measured by the length of time that cells can survive in a nondividing state (Fabrizio et al., 2001). Whereas replicative aging may be a useful model for mammalian cells that remain mitotically active, chronological aging may more effectively model postmitotic cells. In this study, Fabrizio et al. (2005) examine the role of Sir2 in chronological aging, finding—in apparent contrast to replicative aging—that Sir2 interferes with the life-span extension conferred by the exposure of cells to hypocaloric media or by mutations that impair the cell's ability to respond to nutrients.

In yeast, Sir2 promotes replicative longevity by repressing the recombination of repetitive ribosomal DNA (rDNA) and the subsequent formation of extrachromosomal rDNA circles (ERCs) (Figure 1A) (Kaeberlein et al., 1999). The accumulation of ERCs is thought to be one cause of replicative senescence; however, there is no reason to believe that ERCs play a role in yeast chronological aging. How then might Sir2 affect aging in nondividing yeast cells? Fabrizio et al. (2005) present evidence for three possible mechanisms (Figure 1A). First, deletion of *SIR2* in nutrient-deprived cells was found to decrease the rate of DNA mutations that accumulate with age in postmitotic conditions. 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. (2005) find that the *SIR2* deletion strain exhibits increased stress resistance, including resistance to both heat shock and oxidative stress, compared to wild-type yeast. Finally, these authors report that the *SIR2* deletion strain has elevated levels of the alcohol dehydrogenase, Adh2. Mitotically active yeast cells generate energy primarily through fermentation, leading to the production of ethanol. As fermentable carbon sources become scarce and ethanol accumulates, yeast undergo a metabolic shift and begin to use ethanol as an energy source, only entering stationary phase after ethanol levels are largely depleted. Fabrizio et al. (2005) speculate that 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.

The finding that Sir2 limits chronological life span under nutrient-restricted conditions runs counter to studies of yeast replicative aging, as well as studies of Sir2 orthologs in worms and flies, where increased Sir2 activity enhances longevity (Blander and Guarente, 2004). However, evidence that the story may be more complicated than previously thought comes as no surprise to those familiar with the enigmatic Sir2. First, the mechanism by which Sir2 slows yeast replicative aging, by inhibiting the formation of ERCs, is almost certainly not relevant in higher organisms. Thus, we are left to ponder how (and why) Sir2-family proteins might have evolved different molecular functions in determining longevity. Second, the question of whether Sir2 plays any role in replicative life-span extension by calorie re-

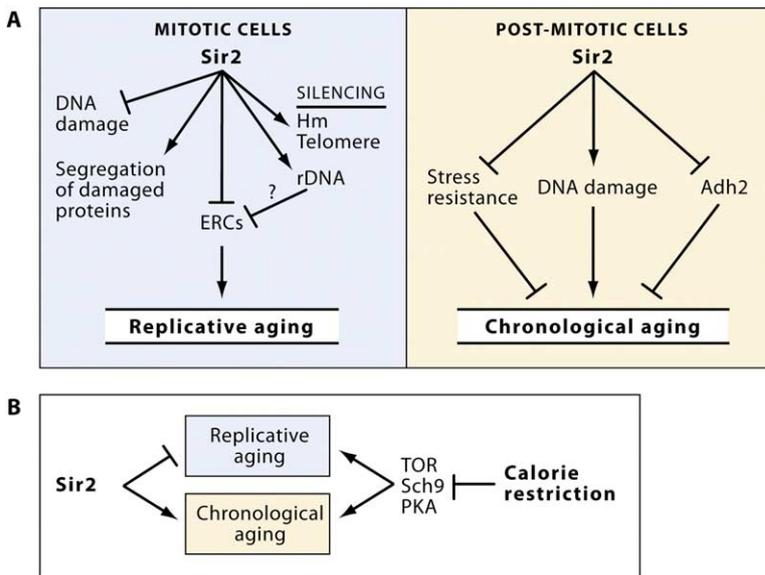


Figure 1. The Enigmatic Role of Sir2 in Aging (A) The figure depicts reported functions of Sir2 in mitotic and postmitotic cells. In yeast, Sir2 promotes replicative longevity by repressing the recombination of repetitive ribosomal DNA (rDNA) and the subsequent formation of extrachromosomal rDNA circles (ERCs). Sir2 decreases chronological longevity, perhaps by promoting DNA damage, inhibiting stress resistance, and/or inhibiting the activation of the alcohol dehydrogenase, Adh2. Sir2 also has other cellular functions that are apparently unrelated to its aging-related roles, such as transcriptional silencing of HM mating-type loci and telomeric genes, DNA damage response in mitotic cells, and its reported involvement in the mother cell-specific retention of oxidatively damaged proteins. (B) This model depicts the factors and pathways that regulate replicative and chronological aging. Although Sir2 appears to affect replicative and chronological aging differently, increasing evidence suggests that the highly conserved kinases Tor, Sch9, and PKA regulate both replicative and chronological aging in response to nutrient availability.

striction also remains a perplexing topic. Sir2 was initially proposed to be an essential downstream mediator of calorie restriction, but more recent findings indicate that Sir2 is not required for calorie restriction as long as ERC levels are kept low (Kaeberlein et al., 2004). In fact, at least in some genetic backgrounds, calorie restriction increases life span to a much greater extent in cells lacking both Sir2 and the rDNA replication fork blocking protein, Fob1, than it does in wild-type cells. Finally, the relationship between Sir2 and calorie restriction in higher eukaryotes remains murky. In flies, dSir2 is reported to be required for life-span extension by calorie restriction (Blander and Guarente, 2004), whereas evidence from studies with worm Sir2.1 suggests otherwise (M.K., unpublished data). Clearly, more studies need to be performed before any conclusions can be drawn concerning direct links between Sir2 orthologs and calorie restriction.

The results of Fabrizio et al. (2005) suggest that, rather than slowing aging, Sir2 proteins might actually accelerate aging in at least some types of mammalian cells and tissues. Although regulation of alcohol dehydrogenase is unlikely to play a direct role in mammalian aging, Fabrizio et al. (2005) speculate that Sirt1, the mammalian Sir2 ortholog, may play a similar role through its reported ability to regulate glucose and fat metabolism (Fabrizio et al., 2005; Picard et al., 2004). Most mice deficient in Sirt1 die during embryonic development; however, those animals that reach adulthood appear largely normal. This begs the question of whether adult mice lacking Sirt1 will have a short life span (as might be predicted from yeast replicative aging) or a long life span (as might be predicted from yeast chronological aging), or neither. Although the life span of these animals has not yet been reported, Sirt1^{-/-} fibroblasts are resistant to senescence induced by oxidative stress (Chua et al., 2005), and Sirt1^{-/-} animals demonstrate several phenotypes consistent with

longer life span, including decreased body weight and fat mass, smaller pituitary size, and lower levels of free IGF-1 (Lemieux et al., 2005). The completion of life span studies on Sirt1 knockout and transgenic animals should be a priority and will likely enhance our understanding of this important gene and its role, if any, in mammalian aging. Of particular interest will be studies that examine whether mice lacking Sirt1 exhibit increased life span under conditions of calorie restriction.

The findings of Fabrizio et al. (2005) regarding Sir2 also underscore how little is known about the genes that influence the aging of both mitotic and postmitotic cells. In yeast, three kinases appear to play a shared role in regulating both types of aging (Figure 1B). Mutations that result in decreased activity of PKA, Sch9, or TOR increase both replicative and chronological life span (Fabrizio et al., 2001, 2004; Kaeberlein et al., 2005; R.W. Powers, M.K., B.K.K. and S. Fields, unpublished data). Moreover, the long replicative life span of TOR and Sch9 deletion strains is not further increased by calorie restriction (Kaeberlein et al., 2005), suggesting that TOR and Sch9 might mediate replicative life-span extension by calorie restriction (Figure 1B). Importantly, mutations that decrease the activity of the orthologous proteins (Tor and Akt) in worms and flies also extend life span, suggesting that these kinases share an evolutionarily conserved role in responding to nutrients and growth factors.

In yeast, as in higher eukaryotes, TOR, Sch9, and PKA coordinate signals from nutrients and growth factors to regulate ribosome biogenesis, stress response, cell size, autophagy, and other cellular processes. Prior work from Longo and colleagues has suggested a model for chronological life-span extension by deletion of SCH9 that involves upregulation of the stress-response transcription factors Msn2 and Msn4 (Fabrizio et al., 2001). However, Msn2 and Msn4 are not required for replicative life-span extension in the SCH9 deletion strain (Fabrizio et al., 2004). Recent evidence

suggests instead that it is a decrease in ribosome biogenesis that accounts for the increase in replicative life span upon deletion of *SCH9* or *TOR1* (Kaeberlein et al., 2005). These studies suggest an intriguing model in which similar nutrient-responsive signaling pathways coordinate aging in mitotic and postmitotic cells, albeit through different downstream effectors (Figure 1B).

A long-standing and still unresolved debate among biogerontologists concerns whether the aging process is controlled by a relatively small number of regulatory pathways or whether aging results from many different and cell-type-specific changes that occur over time. Certainly different types of cells show different aging characteristics. On the other hand, single gene mutations can profoundly delay the rate of aging in all of the well-studied model systems, including mice and rats. A network in which regulatory proteins are used to coordinate a wide range of downstream events important to aging could unite both of these models and may help to explain the wide range of aging phenotypes retarded by calorie restriction. The combined use of both chronological and replicative models of yeast aging will continue to be a powerful approach for dissecting the molecular mechanisms that coordinate longevity.

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