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Linking sirtuins, IGF-I signaling, and starvation

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ABSTRACT

Our studies in yeast have shown that the down-regulation of major signal transduction mediators increases stress resistance and causes an up to 10 fold chronological life span extension. Whereas other laboratories have proposed that sirtuins (Sir2 and its homologs), a family of conserved proteins which are NAD^+-dependent histone deacetylases, can extend longevity in various model organisms, we propose that one sirtuin, i.e., Sir2, can also accelerate cellular aging and death. In *Saccharomyces cerevisiae* (yeast), the deletion of Sir2 increases DNA damage but in combination with longevity mutations in principal intracellular signal transduction mediators, or in combination with calorie restriction it causes a further increase in the chronological lifespan as well as an increase in the stress resistance and a major reduction in age-dependent genomic instability. Our recent results also provide evidence for a role of the mammalian Sir2 ortholog SirT1 in the activation of a highly conserved neuronal pathway and in the sensitization of neurons to oxidative damage. However, the mean lifespan of the $SirT1^{+/-}$ mice is not different from that of wild type animals, and the survival of $SirT1^{-/-}$ mice was reduced under both normal and calorie restricted conditions. Here, I review the studies linking SirT1, IGF-I signaling and starvation in various model organisms with a focus on the post-mitotic cells, which indicate that sirtuins can play both protective and pro-aging roles.

1. Introduction

Sirtuins, or Sir2 family proteins, are conserved NAD^+-dependent histone deacetylases (Frye, 2000) that have been shown to extend the lifespan of *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila* (Kaeberlein et al., 1999; Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001). Though earlier studies proposed that Sir2 is required for the effect of calorie restriction (CR) on the lifespan of lower eukaryotes (Guarente and Picard, 2005), later studies found that CR can increase the yeast replicative lifespan (Kaeberlein et al., 2004) or the worm lifespan (Hansen et al., 2007; Kaeberlein et al., 2006; Lee et al., 2006) independently of Sir2. Our results with the chronological lifespan of non-dividing yeast cells indicated that Sir2 can also have the opposite effect on the longevity since the lack of Sir2 further extended the lifespan of calorie restricted cells (Fabrizio et al., 2005b). Sir2 deficiency also further extended the lifespan of long-lived mutants lacking SCH9, homologous to both mammalian S6 kinase and Akt (Geyskens et al., 2000; Urban et al., 2007), and of mutants with deficiencies in the Ras/cAMP pathway (Fabrizio et al., 2005b). Here, I review the connection between sirtuins, insulin like growth factor 1 (IGF) IGF-I-like signaling and calorie restriction with focus on non-dividing yeast and neurons.

2. Conserved regulation of lifespan

Genetic manipulations which reduce insulin/IGF-I-like signaling extend the lifespan of *C. elegans*, *Drosophila* and mammals (Kenyon, 2001; Longo and Finch, 2003). The reduction of insulin/IGF-I signaling also extends the lifespan of mice (Bluher et al., 2003; Holzenberger et al., 2003; Taguchi et al., 2007). Work in *C. elegans* and *Drosophila* points to one major longevity regulatory pathway which includes the IGF-I-like receptor, Akt and forkhead stress resistance transcription factors (Hwangbo et al., 2004; Kenyon et al., 1993). These studies and others have shown that reduced insulin/IGF-I-like signaling protects against oxidative damage and other forms of stress in simple model systems and mice (Holzenberger et al., 2003; Kenyon, 2001; Longo and Finch, 2003). Sir2/SirT1 (SirT1, the mammalian ortholog of yeast Sir2) has also been linked to the insulin/IGF-1 signaling pathway: in *C. elegans* Sir2.1 interacts with 14-3-3 proteins to activate DAF-16, which is a major stress resistance transcription factor in the IGF-like pathway (Berdichevsky et al., 2006; Brunet et al., 2004; Wang and Tissenbaum, 2006). Our studies of the chronological lifespan of yeast have revealed a similar longevity and stress resistance regulatory pathway in which glucose, instead of IGF-I, causes the activation of the serine threonine kinase Sch9 which results in the down-regulation of the downstream stress resistance kinase Rim15 (Cheng et al., 2007; Fabrizio et al., 2001; Wei et al., 2008). Others have shown a similar pro-aging effect of Sch9 in the regulation of the

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replicative lifespan (Kaeberlein et al., 2005). Our work has also pointed to a second pro-aging pathway which includes Ras, adenylate cyclase, PKA and the stress resistance transcription factors Msn2/Msn4 (Fabrizio et al., 2003, 2004), which are also implicated in the regulation of the yeast replicative lifespan (Kaeberlein et al., 2005; Medvedik et al., 2007). Recently, the down-regulation of the adenylate cyclase/PKA pathway by the omission of five adenylyl cyclase was shown to extend the lifespan of mice and to protect them from reduced bone density and aging-induced cardiomyopathy (Yan et al., 2007). Analogously to our findings in yeast (Fabrizio et al., 2001, 2003), the five adenylyl cyclase deficient mice displayed increased levels of MnSOD and stress resistance (Yan et al., 2007). These data support the hypothesis that the mechanisms of lifespan regulation are conserved and that the studies in *S. cerevisiae* can point to additional pathways and mechanisms important for mammalian aging and diseases.

3. Regulation of oxidative stress by mammalian signal transduction pathways

Ras, Akt, and S6K are among the principal intracellular signal transduction mediators of the many growth, survival and metabolic effects of IGF-I (Fig. 1). These proteins are also homologs or orthologues of yeast Ras2 and Sch9. Before introducing the potential link between SirT1, IGF-I signaling and oxidative stress in mammals, I will briefly review some of the studies on the role of proteins in the Ras and Akt pathways in the production of oxidants and in the regulation of stress resistance. Oxidants have recently gained attention as mediators of growth factor signaling. In PC12 cells, the generation of nitric oxide (NO) is required for NGF-dependent differentiation, whereas EGF stimulates the generation of superoxide by a Ras-dependent mechanism (Mills et al., 1998; Peunova and Enikolopov, 1995). The small G protein p21Ras plays a critical role in transmitting growth factor signals in many cell types through the activation of Raf, MEK, and ERK. In PC12 cells, EGF induces the generation of high levels of superoxide by a Ras- and MEK-dependent mechanism (Mills et al., 1998). Superoxide generation in EGF-treated PC12 cells is blocked by the inhibitors of the superoxide-generating enzyme lipoxygenase (Mills et al., 1998). A constitutive active form of p21Ras stimulates the generation of high levels of superoxide and mitogenesis by a Rac1-dependent mechanism in 3T3 fibroblast cells (Irani and Goldschmidt-Clermont, 1998; Irani et al., 1997). This mitogenic activity of Ras is blocked by the antioxidant enzymes (Irani et al., 1997). Down-regulation of mox1, a homologue of the catalytic subunit of a superoxide-generating NADPH oxidase, decreases superoxide generation and growth in smooth muscle cells and 3T3 cells (Suh et al., 1999). As shown in other cell types, in 3T3 cells NADPH oxidase is activated in cells with a constitutively active Ras (Benhar et al., 2001). Ras can induce the generation of NO in neuronal cell lines and astrocytes. The Ras–ERK pathway is required for the activation of neuronal NO synthase in PC12 cells and for the microglial-dependent stimulation of cholinergic neurons, which is attenuated by antioxidants (Jonakait et al., 2000; Schonhoff et al., 2001). A dominant negative form of p21Ras also inhibits the induction of NO synthase in LPS-stimulated primary astrocytes (Pahan et al., 2000) and 3T3 cells expressing a constitutively active Ras increase the levels of hydrogen peroxide, which induce apoptosis (Liou et al., 2000). In embryonic cortical neurons brain-derived neurotrophic factor (BDNF) accelerates NO induced cell death by a mechanism that requires p38 or MAPK/ERK kinase 1 activation, suggesting that these proteins, which function downstream of Ras, can increase the sensitivity of neurons to oxidative damage (Ishikawa et al., 2000). The free radical nitric oxide (NO) plays a role in synaptogenesis and IGF-I signaling (Contestabile, 2000; Schini-Kerth, 1999). The protein kinase Akt is also involved in the generation of oxidants (Erlich et al., 2001). However, Akt is better known for its induction of anti-apoptotic genes: it blocks apoptosis induced by oxidants and serum withdrawal in many cell types, including B cells and 3T3 cells (Ding et al., 2000; Wang et al.,

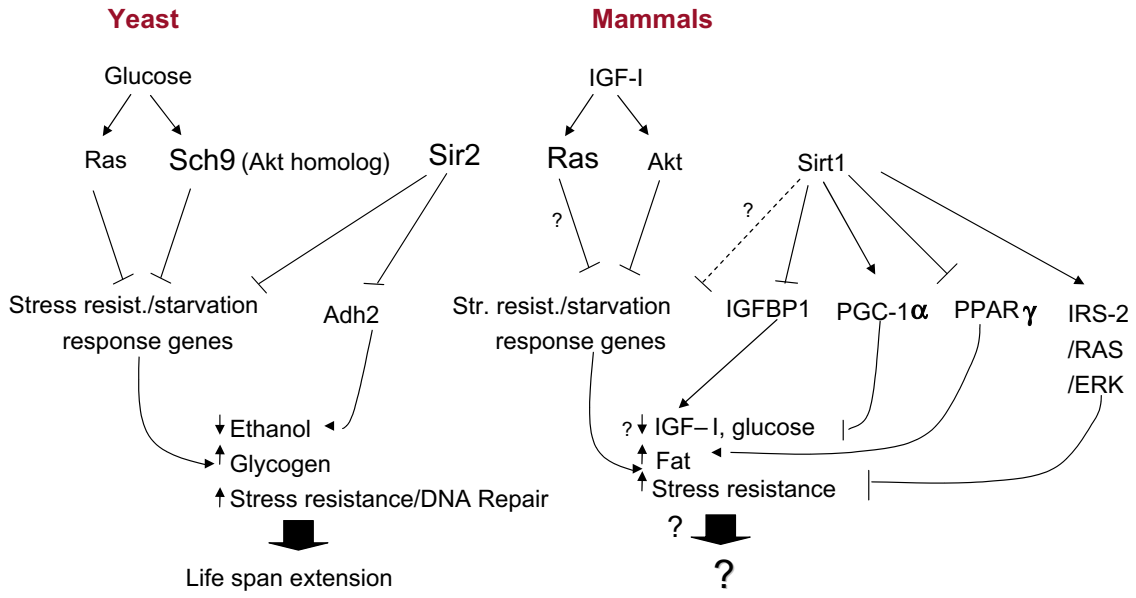

Fig. 1. Models for the role of sirtuins on stress resistance and metabolic pathways in yeast and mammals. Deletion of *SIR2* has been shown to decrease the replicative lifespan and to cause some genomic instability during growth. However, deletion of *SIR2* combined with calorie restriction/starvation or with longevity mutations in nutrient responsive pathways such as the Ras or Tor/Sch9 (Akt, S6K) pathways further enhances the chronological survival of non-dividing cells. *S. cerevisiae sir2Δ* strains show elevated stress resistance and enhanced alcohol dehydrogenase activity, which results in the depletion of extracellular ethanol during the early phases of starvation. In contrast, mice heterozygote for SirT1 display a normal lifespan whereas homozygote SirT1$^{-/-}$ null mutants are short-lived under both normal and calorie restricted conditions. The reduced systemic glucose levels in SirT1 knockdown mice is reminiscent of the reduced ethanol level in *sir2* deficient yeast. At least in rat neurons, inhibition of sirtuins activity increases resistance to oxidative stress but not to other stresses. Note that the many functions of SirT1 in mammals, are occurring in different organs/cell types.