CMLS, Cell. Mol. Life Sci. 59 (2002) 903–908
1420-682X/02/060903-06 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Visions & Reflections

Regulation of longevity and stress resistance: a molecular strategy conserved from yeast to humans?

V. D. Longo* and P. Fabrizio

Division of Biogerontology, Andrus Gerontology Center, University of Southern California, 3715 McClintock Avenue, Los Angeles, California 90089-0191 (USA), e-mail: vlongo@usc.edu

Received 18 March 2002; received after revision 22 April 2002; accepted 22 April 2002

Abstract. Recent studies implicate similar proteins in the regulation of longevity in organisms ranging from yeast to mice. Studies in yeast and worms suggest that inactivation of glucose or insulin/insulin-like growth factor-1 (IGF-1) signaling pathways extends longevity by causing a shift from a reproductive phase to a non-reproductive maintenance phase involving the expression of many genes. These stress resistance pathways appear to have evolved to induce maintenance systems and promote longevity during periods of starvation. In yeast, mutations that decrease the activity of glucose signaling pathways extend longevity by activating stress resistance transcription factors that regulate the expression of genes involved in antioxidant and heat protection, glycogen storage, protein degradation, DNA repair, and metabolism. A remarkably similar set of proteins regulated by growth factors that control glucose metabolism is implicated in life span extension in worms, and possibly in flies and mice. Studies in worms and flies point to secondary hormones as mediators of the effect of insulin/IGF-1 signaling on longevity, whereas studies in yeast and mammalian cells indicate that glucose or insulin/IGF-1 may decrease longevity by directly down-regulating stress resistance genes. In yeast, longevity mutations postpone superoxide toxicity and mitochondrial damage. However, the small life span extension caused by the overexpression of superoxide dismutases and catalase in yeast and flies indicates that increased antioxidant protection alone cannot be responsible for the major life span extension caused by signal transduction mutations. Although we are only beginning to understand the molecular mechanisms that mediate life span extension, the similarities between longevity regulatory pathways in organisms ranging from yeast to mice suggest that insulin/IGF-1 signaling pathways may also regulate cell damage and longevity in humans.

Aging is a complex process that has been studied for centuries. The theories that have attempted to explain the mechanisms of aging range from those focused on free radicals and oxidative damage [1] to those based on the pro-senescence role of genes important for fitness early in life [2]. Although biologists began to lay the foundation for the discovery of the mechanisms of aging many years ago, the molecular and genetics studies performed during the past 10 years have begun to provide the much awaited ‘evidence’ for a universally accepted theory of aging. The parallel genetic studies in yeast, worms, flies, and mice have recently converged and have merged with some of the most widely accepted theories of aging and also with an established but unexplained intervention that extends longevity in many organisms: caloric restriction [3–5]. In particular, the many similarities between the well-studied pathways that regulate longevity in yeast and worms provide strong evidence for the existence of a ‘conserved regulation of longevity.’

* Corresponding author.

The replicative and chronological life span of yeast

The genes that regulate chronological longevity and starvation resistance were identified at approximately the same time in two simple eukaryotes: yeast and worms [6–11]. However, studies of chronological longevity in yeast have gained attention only recently [3, 12] because the 'replicative life span' was believed to be the only paradigm to study aging in this unicellular eukaryote [13, 14]. The replicative life span of yeast, measured by counting the number of buds generated by a single mother cell [15], is similar to the replicative life span of mammalian fibroblasts and lymphocytes, which undergo a limited number of population doublings in culture [16]. However, the replicative life span paradigm can also serve as a model system for organismal aging in higher eukaryotes, since a yeast mother cell that generates a high number of buds must also have a long chronological life span. What is not clear is the relationship between the replicative life span and the post-reproductive survival time. In fact, a mother cell may have a short replicative life span but may live a long chronological life in the post-reproductive period, after generating the last bud.

Chronological longevity in yeast is determined by measuring the mean and maximum survival time (days) of a yeast population [9]. Yeast cells grow rapidly by utilizing the nutrients available and then spend the majority of their chronological life span in either a dormant spore state, a low-metabolism stationary phase, or a high-metabolisms post-diauxic phase [9, 17]. During these phases, extracellular nutrients are depleted but yeast survive by breaking down glycogen and utilizing other nutrients stored at the end of the growth phase, analogously to hibernating mammals. In these non-dividing phases, most of the energy is obtained from respiration and appears to be invested in stress resistance systems that prevent cellular damage [18]. Therefore, the yeast chronological life span is a valuable paradigm to study the role of starvation response genes in the regulation of longevity.

Most chronological life span studies in yeast were performed by measuring the high-metabolism post-diauxic life span, in which the loss of the ability to reproduce is followed by mitochondrial damage and death [6, 7, 9, 12; Fabrizio et al., unpublished data]. Mean chronological life span, which is approximately 6 days in wild-type yeast, is extended by mutations that decrease the activity of the protein kinase Sch9 and of adenylate cyclase (Cyr1) [12]. Sch9 and Cyr1 function in pathways that mediate glucose-dependent signaling, stimulate growth and glycolysis, and decrease stress resistance. The 327-amino acid serine/threonine kinase domain of yeast Sch9 is 47 and 45% identical to that of worm AKT-2 and AKT-1, respectively, which function downstream of the insulin receptor homolog DAF-2 in a longevity/diapause regulatory pathway [19, 20]. In this domain conserved from yeast to mammals, Sch9 is also 49% identical to human Akt-1/Akt-2/PKB, implicated in biological functions including insulin signaling, the translocation of glucose transporter, apoptosis, and cellular proliferation [21]. The yeast *CYR1* gene encodes adenylate cyclase, which stimulates cAMP-dependent protein kinase (PKA) activity required for cell cycle progression and growth. The catalytic subunits of PKA are also 35–42% identical to worm and human Akt-1/Akt-2, although PKA belongs to a different family of serine/threonine kinases. Mutations that block the activity of Ras2, which functions upstream of Cyr1 and PKA, also double the chronological life span and increase resistance to heat and oxidative stress [9; Fabrizio et al., unpublished data].

In addition to its effect on chronological survival, the inactivation of the PKA pathway increases the replicative life span [22]. However, stress resistance transcription factors Msn2 and Msn4, which mediate chronological life span extension, are not required to increase replicative life span, suggesting that the two yeast life span paradigms are regulated by common signal transduction pathways but distinct downstream mechanisms [22]. In fact, chronological aging in yeast is associated with superoxide toxicity and loss of mitochondrial function whereas replicative aging is associated with the accumulation of extrachromosomal ribosomal DNA (rDNA) circles and is attenuated by overexpression of the NAD-dependent histone deacetylase Sir2 [23].

Conserved regulation of stress resistance and longevity in eukaryotes

There are remarkable similarities between the genes and pathways involved in the regulation of longevity in yeast and worms. In yeast, the down-regulation of glucose signaling by *ras2*, *cyr1*, and *sch9* mutations increases longevity and resistance to oxidative stress and heat shock (fig. 1) [9, 12; Fabrizio et al., unpublished data]. In the *cyr1* mutants, chronological life span extension is mediated by stress resistance transcription factors Msn2 and Msn4, which induce the expression of genes encoding several heat shock proteins, catalase (*CTT1*), the DNA damage-inducible gene *DDR2*, and *SOD2* (fig. 1) [12, 24]. Analogously, in worms, mutations in the signal transduction genes *daf-2* and *age-1* extend survival by 65–100% [25, 26] and increase thermotolerance and antioxidant defenses [27–29], apparently through stress resistance transcription factor DAF-16 (fig. 2) [11]. In yeast, chronological life span extension is associated with decreased superoxide generation and aconitase inactivation in the mitochondria [12]. Furthermore, life span extension in *ras2, cyr1,* and *sch9* mutants requires SOD2 and the overexpression of superoxide dismutases (SODs) extends

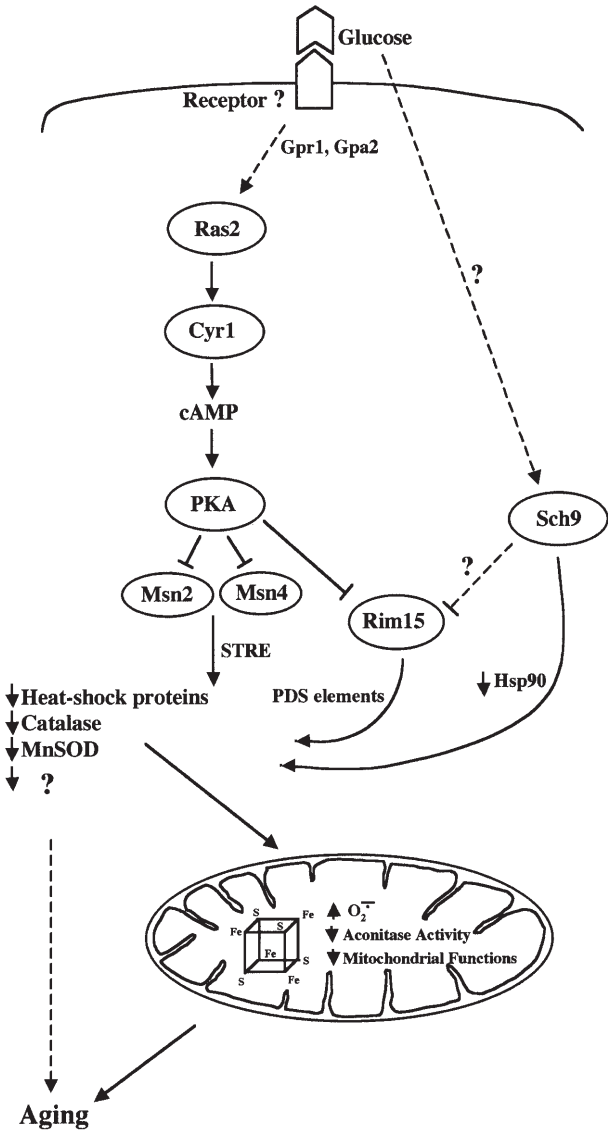

Figure 1. Regulation of stress resistance and chronological longevity in yeast. Glucose and other nutrients activate the Ras2/Cyr1/cAMP/PKA and Sch9 pathways via the G-protein-coupled receptor Gpr1 and by other unidentified mechanisms. Ras2/Cyr1/cAMP/PKA negatively regulate stress resistance transcription factors Msn2/Msn4, which induce the expression of heat shock proteins, catalase, MnSOD, and other maintenance proteins. Activation of Sch9 results in a major decrease in stress resistance either via Rim15 or via an unidentified effector. Mutations in *RAS2*, *CYR1*, and *SCH9* increase multiple stress resistance systems, decrease mitochondrial superoxide levels, delay aconitase inactivation, and extend longevity via an Msn2/Msn4- and Rim15-dependent pathway.

longevity [Fabrizio et al., unpublished data]. In worms, among the genes regulated by the *daf-2* pathway are mitochondrial MnSOD and several heat shock proteins [30, 31]. The yeast Ras/Cyr1/PKA pathway down-regulates glycogen storage and genes involved in the switch to the hypometabolic stationary phase and to the dormant spore state [17, 32]. The worm *daf-2* pathway also down-regulates the storage of reserve nutrients (fat and glycogen) and the switch to the hypometabolic dauer larvae state (fig. 2) [10, 25, 27]. Thus, in addition to the high sequence similarities between the yeast *SCH9* and the worm AKT-1/AKT-2 genes, these unrelated organisms appear to regulate stress resistance and longevity by modulating the activity of similar proteins and pathways (fig. 2).

Thanks to studies by many laboratories, a significant portion of the genes that function in longevity pathways in yeast and worms has been characterized. Although, only a portion of the genes are conserved, these two aging model systems appear to use a remarkably similar 'molecular strategy' to delay aging and death in response to starvation. In these organisms, glucose or a growth factor/hormone activates a receptor, which, in turn, causes the activation of serine/threonine kinases that down-regulate stress resistance transcription factors and consequently SODs, catalase, and HSPs. Whether decreasing metabolic rates is also necessary to extend longevity is not yet clear. In yeast, the threefold increase in the life span caused by the deletion of *sch9* appears to be associated with normal metabolism during the first 4 days and hypometabolism during the rest of the life span [Fabrizio et al., unpublished data]. Although a threefold life span extension may also require a reduction in metabolic rates in higher eukaryotes, more modest extensions of longevity can be uncoupled from hypometabolism [3].

Recent studies suggest that conserved genes also regulate longevity in fruit flies. Mutations in the insulin/insulin-like growth factor-1 (IGF-1) pathway, which regulates growth, extend the longevity of fruit flies by up to 85% [33, 34]. Analogously to yeast and worms, flies with mutations in the insulin/IGF-1 pathway increase storage of nutrients and SOD expression (fig. 2) [34]. Furthermore, the overexpression of *SOD1* increases survival in fruit flies by up to 40% [35–37] and a fruit fly line with a mutation in the G-protein-coupled receptor homolog *MTH* gene displays a 35% increase in life span and resistance to starvation and superoxide toxicity [38]. The mitochondrial enzyme aconitase is oxidatively modified and inactivated in old flies, analogously to its age-dependent inactivation in yeast [39].

The insulin/IGF-1 pathway may also regulate longevity in mammals. Homozygous mice with mutations in the Prop-1 gene show developmental growth defects but live for over 50% longer than wild-type mice [40]. Homozygous Prop-1 mice lack the cells that produce growth hormone (GH) and consequently also lack plasma IGF-1, which is secreted by liver cells upon stimulation with GH. The plasma GH deficiency appears to be responsible for the effects of Prop-1 mutations on longevity since mice that cannot release GH in response to GH-releasing hormone also live longer [41]. IGF-1 appears to regulate antioxidant defenses in mammalian cells, because the activities of SODs and catalase decrease in mice hepatocytes ex-

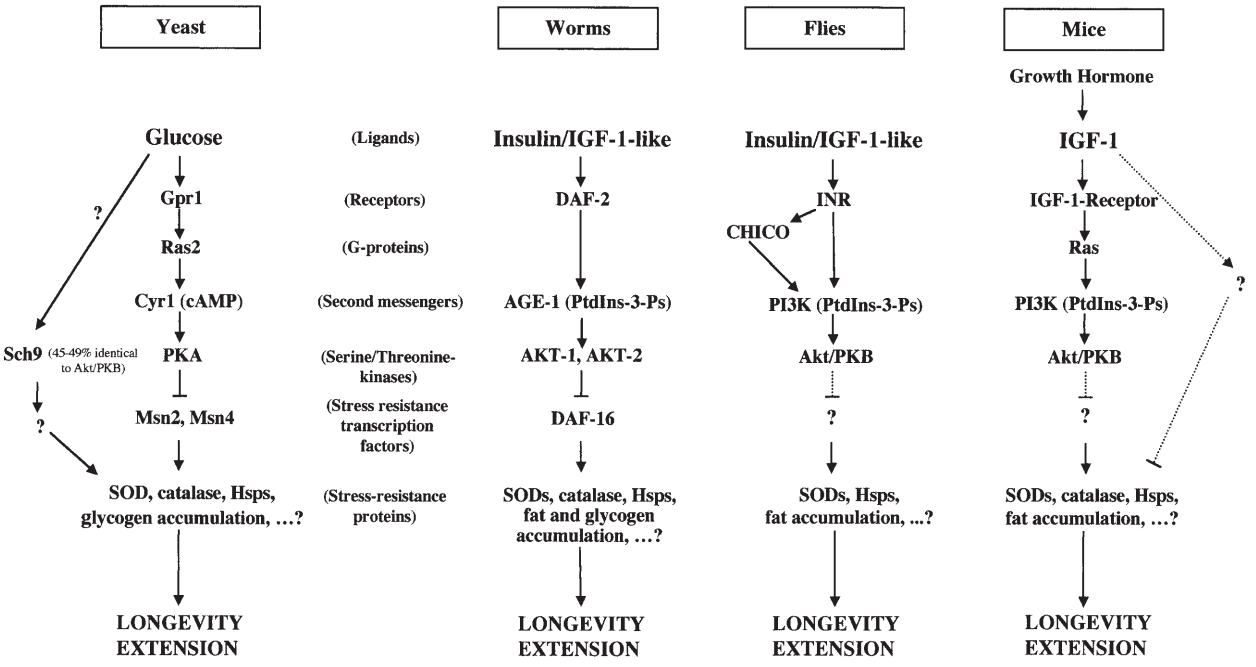

Figure 2. Conserved regulation of longevity. In yeast, worms, flies, and mice, the down-regulation of partially conserved glucose or insulin/insulin-like growth factor-1 (IGF-1)-like pathways induces the expression of superoxide dismutases and heat shock proteins, promotes the accumulation of either glycogen or fat, and extends longevity. Yeast *SCH9* is 45–49% identical to Akt/PKB in higher eukaryotes (in the 327-amino acid kinase domain). The catalytic subunits of PKA are 35–42% identical to worm and human AKT-1/AKT-2, although PKA belongs to a different family of serine/threonine kinases. In mammals, the intracellular mediators of the effect of growth hormone and IGF-1 on life span have not been identified. In worms and flies, a secondary hormone has been proposed to mediate longevity extension downstream of the insulin/IGF-1 receptor [3]. Studies in yeast and mammalian cells instead suggest that the glucose or insulin/IGF-1 signaling pathways may decrease longevity by directly down-regulating stress resistance genes and diverting energy to functions such as growth (see text).

posed to GH or IGF-1 and in transgenic mice overexpressing GH [42, 43]. IGF-1 also attenuates the cellular stress response and the expression of a heat shock protein (HSP) in rats [44]. Furthermore, dwarf mutations cause fat accumulation, which is reversed by dietary administration of GH [45]. In yeast, the down-regulation of the Ras2/PKA or Sch9 pathway results in the accumulation of glycogen, the major carbon and energy source catabolized during periods of starvation [46]. By contrast, in higher eukaryotes, down-regulation of the insulin/IGF-1-like pathways results in the accumulation of fat (fig. 2). Notably, in mammals, fat is the major carbon source during long periods of starvation (hibernation) whereas glycogen provides glucose only during short periods of fasting. Therefore, the switch between glycogen storage in yeast and fat storage in higher eukaryotes is consistent with the role of longevity regulatory pathways in inducing accumulation of the carbon source that would maximize long-term survival.

Recently, a mutation of the mouse signal transduction $p66^{SHC}$ gene was shown to induce stress resistance and to prolong life span [47]. $p66^{SHC-/-}$ mice are resistant to paraquat and survive 30% longer than controls. $p66^{SHC}$ forms stable complexes with Grb2, which activates Ras when bound to the guanine nucleotide exchange factor SOS [48]. Since Grb2 and Ras function downstream of the IGF-1 receptor, the effect of dwarf and $p66^{SHC-/-}$ mutations on longevity may be mediated by common downstream effectors. Notably, the yeast glucose- and the mammalian IGF-1-signaling pathways share the highly homologous RAS and serine/threonine kinase genes (*SCH9* and Akt/PKB) and several stress resistance genes (fig. 2). However, the intracellular mediators of the effect of GH and IGF-1 on life span have not been identified and, therefore, the role of Ras/Akt/PKB in longevity extension in mammals is unknown.

More than free radicals

In 1956, Harman proposed that oxygen species with one unpaired electron (free radicals) may cause aging. The free radical theory of aging became one of the most widely accepted theories after the overexpression of antioxidant enzymes was shown to extend longevity and after most long-lived model organisms were shown to be resistant to oxidative stress [9, 49]. Although superoxide toxicity contributes to aging and death in model organisms, mutations in the yeast Sch9/Cyr1 and in the worm and fly insulin/IGF-1 pathways appear to extend long-

evity by regulating the expression of many genes. In fact, the overexpression of SOD1, SOD2, and catalase in yeast and flies can extend longevity by up to 30%, whereas mutation in signal transduction genes can extend longevity by up to threefold [12, 33, 34]. Therefore, increasing antioxidant protection appears to be important but not sufficient for the major longevity extension caused by mutations in glucose or insulin/IGF-1 signaling pathways.

Are longevity regulatory pathways conserved from yeast to humans?

The analogous role of glucose or hormone/growth factor signaling in stress resistance and longevity in the major genetic model systems suggests that longevity can be extended by similar mechanisms in many organisms (fig. 2). Life span extension appears to be caused by a shift from a reproductive to a non-reproductive maintenance phase, accompanied by changes in the expression of many genes normally induced by starvation. Although the insulin/IGF-1 pathway probably influences mortality and longevity in humans, it is too early to predict whether pharmacological modulation of the insulin/IGF-1 pathway will have a major impact on human diseases and life span. In fact, the long-lived yeast *sch9* mutants, and the dwarf flies and mice have major growth defects. Furthermore, GH is actually prescribed by some doctors to postpone aging, based on its effect in increasing body mass and decreasing adipose tissue in elderly people [50]. Although based on studies in model organisms, long-term GH treatment may accelerate aging and age-related diseases, the temporary beneficial effects of GH underline the difficulties associated with extending longevity in humans while avoiding major side effects. Thus, a great challenge of the 21st century will be to develop drugs that postpone aging and age-related diseases without affecting normal biological functions.

Acknowledgements. This work was supported by NIH Grant AG 01028 and by a Paul Glenn Chair fund (VDL).

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