Regulation of Longevity and Stress Resistance by Sch9 in Yeast
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The protein kinase Akt/protein kinase B (PKB) is implicated in insulin signaling in mammals and functions in a pathway that regulates longevity and stress resistance in Caenorhabditis elegans. We screened for long-lived mutants in nondividing yeast Saccharomyces cerevisiae and identified mutations in adenylate cyclase and SCH9, which is homologous to Akt/PKB, that increase resistance to oxidants and extend life-span by up to threefold. Stress-resistance transcription factors Msn2/Msn4 and protein kinase Rim15 were required for this life-span extension. These results indicate that longevity is associated with increased investment in maintenance and show that highly conserved genes play similar roles in life-span regulation in S. cerevisiae and higher eukaryotes.

Mutations that extend life-span in C. elegans, Drosophila melanogaster, and mice are associated with increased resistance to oxidative stress (1, 2). However, the mechanisms that regulate aging in these multicellular organisms are poorly understood. As in higher eukaryotes, the unicellular yeast Saccharomyces cerevisiae undergoes an age-dependent increase in cell dysfunction and mortality rates (3, 4). Aging in yeast is associated with an enlargement of the cell and a slowing in the budding rate, and is commonly measured by counting the number of buds generated by a single mother cell (replicative life-span) (5, 6). The replicative life-span of yeast is regulated by the Sir2 protein, which mediates chromatin silencing in a nicotinamide adenine dinucleotide-dependent manner (6, 7). However, yeast can also age chronologically as a population of nondividing cells (2, 4, 6). Saccharomyces cerevisiae grown in complete glucose medium [synthetic complete (SC) medium] stop dividing after 24 to 48 hours and survive for 5 to 7 days while maintaining high metabolic rates (2, 8, 9), a situation more akin to their experience in nature where they are likely to survive as nondividing populations exposed to scarce nutrients. For these reasons, and to avoid extended growth and entry into the hypometabolic stationary phase induced by incubation in the nutrient-richer yeast extract/peptone/dextrose (YPD) medium (10), our studies were performed exclusively in SC medium. The survival of nondividing yeast is shortened by null mutations in either or both superoxide dismutases (SODs) (2, 11, 12) and is modestly extended by overexpressing the antiapoptotic protein Bcl-2 (8).

To understand the molecular mechanism that regulates yeast longevity, we transposon-mutagenized yeast cells and isolated long-lived mutants (13). Because of the association between stress resistance and longevity in higher eukaryotes, we screened for mutants that survived both a 1-hour heat stress at 52°C and a 9-day treatment with the superoxide-generating agent paraquat (1 mM). From 2 billion cells screened, we isolated 4000 thermotolerant colonies and 40 paraquat-resistant colonies carrying transposons. From the 4040 stress-resistant mutants, we isolated nine that were able to survive to day 9, when most of the wild-type cells are dead. The only two long-lived mutants isolated independently in both the paraquat and heat shock selections, designated Tn3-5 and Tn3-24, were also the longest lived (Fig. 1A), suggesting that resistance to multiple stresses is associated with increased longevity. Allele rescue of the mutants revealed that transposons had integrated in the promoter region of the Sch9 protein kinase gene (sch9::mTn) (Tn3-5) (33 base pairs upstream of the start codon) and in the NH1-terminal regulatory region of adenylate cyclase (cyr1::mTn) (Tn3-24) (between codons 208 and 209). The mean life-spans of sch9::mTn and cyr1::mTn were extended by 30 and 90%, respectively. Transformation of Tn3-5 cells with wild-type SCH9, and of Tn3-24 cells with CYR1, abolished the survival extension, strongly suggesting that the decreased expression or activity of Sch9 and Cry1 extends survival (not shown).

To investigate further the role of SCH9 in chronological survival, we deleted the SCH9 gene (14). The sch9Δ mutants grew slowly, but survived three times longer than wild-type cells (Fig. 1B). To determine whether the protein kinase activity of Sch9 accelerates mortality in nondividing yeast, we transformed mutants with either wild-type SCH9 or with forms of SCH9 bearing kinase-inactivating mutations: sch9K441A and sch9D556R (15). Transformation of sch9Δ with wild-type SCH9 reversed the life-span extension, whereas transformation with vector alone wild-type SCH9 or with a mutated sch9 encoding for a catalytically inactive proteins (Sch9K441A or Sch9D556R). Cell viability was measured every 2 days starting at day 3 (14). Experiments were repeated between three and seven times with two or more samples per experiment with similar results. The average of all experiments is shown. The mean life-span increase in cyr1::mTn (90%), sch9::mTn (30%), and sch9Δ (300%) is significant [P < 0.05, analysis of variance (ANOVA)].

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with the genes encoding for the inactive Sch9 K441A or Sch9 D555K kinases did not (Fig. 1C).

Both Sch9 and Cyr1 function in pathways that mediate glucose-dependence signaling, stimulate growth and glycolysis, and decrease stress resistance, glycolgen accumulation, and gluconeogenesis (16). The COOH-terminal region of Sch9 is highly homologous to the AGC family of serine/threonine kinases, which includes Akt/PKB, whereas the N-terminal region contains a C2 phospholipid and calcium-binding motif. The 327–amino acid serine/threonine kinase domain of yeast Sch9 is, respectively, 47 and 45% identical to that of C. elegans AKT-2 and AKT-1, which function downstream of the insulin-receptor homolog DAF-2 in a longevity/diapause regulatory pathway (14, 17, 18). In this domain conserved from yeast to mammals, Sch9 is also 49% identical to human AKT-1/ AKT-2/ PKB, which are implicated in biological functions including insulin signaling, the translocation of glucose transporter, apoptosis, and cellular proliferation (19).

The CYR1 gene encodes for adenylate cyclase, which stimulates cysolic adenosine monophosphate (cAMP)–dependent protein kinase (PKA) activity required for cell cycle progression and growth. The catalytic subunits of PKA are also 35 to 42% identical to C. elegans and human AKT-1/ AKT-2, although PKA belongs to a different family of serine/threonine kinase. The inactivation of the Ras/cAMP/PKA pathway in S. cerevisiae increases resistance to thermal stress, in part, by activating transcription factors Msn2 and Msn4, which induce the expression of genes encoding for several heat shock proteins, catalase (CTT1), and the DNA damage inducible gene DDR2 (14, 16). MnSOD also appears to be regulated in a similar manner (20). To determine whether MSN2/ MSN4 mediate survival extension, we deleted both genes in the cyr1::mTn mutants. The absence of both transcription factors abolished the life-span extension conferred by cyr1::mTn, but did not affect the survival of wild-type cells (Fig. 2A). By contrast, the deletion of MSN2/ MSN4 did not reverse the survival extension in sch9Δ cells (Fig. 2B).

The protein kinase Rim15 regulates genes containing a PDS (postdiauxic shift) element T(T′)AAG3AT involved in the induction of thermotolerance and starvation resistance by a Msn2/Msn4-independent mechanism (21). To test the role of Rim15 in survival, we generated sch9 Δ rim15Δ mutants. The lifespan of the double mutant was decreased compared to sch9Δ (Fig. 2B). The deletion of Rim15 also abolished the life-span extension in cyr1::mTn cells (Fig. 2A). However, it is difficult to establish whether Rim15 mediates the survival extension in these mutants, because rim15 single mutants are short-lived (Fig. 2A).

To test whether the long-lived strains were stress-resistant, we exposed the mutants to hydrogen peroxide, menadione, or heat. All mutants were resistant to a 1-hour heat shock treatment at 55°C (Fig. 3A). Similarly, 3- to 5-day-old mutants were resistant to a 30-min treatment with 100 mM hydrogen peroxide (Fig. 3B) or with the superoxide/H2O2-generating agent menadione (20 μM) (Fig. 3C).

In yeast sod2Δ mutants, superoxide specifically inactivates aconitase and other [4Fe-4S] cluster enzymes and causes the loss of mitochondrial function and cell death (11, 12). To investigate further the role of superoxide toxicity in aging, we monitored the activity and reactivation of mitochondrial aconitase, which can also serve as an indirect measure of superoxide concentration (22). In agreement with the pattern of resistance to superoxide toxicity (Fig. 3C), aconitase specific activity decreased by 50% in wild-type cells, and by 30% in cyr1::mTn mutants, but did not decrease in sch9Δ mTn and sch9Δ mutants at day 7 compared to day 3 (14). The percent reactivation of aconitase was lowest in the long-lived sch9Δ mutants and highest in wild-type cells (Fig. 4A) and correlated with death rates (Fig. 4B), suggesting that cyr1 and sch9Δ mutants increase survival, in part, by preventing superoxide toxicity. However, the overexpression of both SOD1 and SOD2 only increases survival by 30% (9), indicating that additional systems, regulated by Msn2, Msn4, and Rim15, are responsible for the major portion of chronological life-span extension in cyr1::mTn and sch9Δ mutants.

There are many phenotypic similarities between long-lived mutants in S. cerevisiae, C. elegans, Drosophila, and mice (1, 2). Cae...
norhabditis elegans age-1 and daf-2 mutations extend the life-span in adult organisms by 65 to 100%, by decreasing AKT-1/AKT2 signaling and activating transcription factor DAF-16 (14, 18, 23). These changes are associated with the induction of superoxide dismutase (MnSOD), catalase, and the heat shock proteins HSP70 and HSP90 (14, 17). A role for oxidants in the aging of C. elegans was confirmed by the extended survival of wild-type worms treated with small synthetic SOD/catalase mimetics (24). Thus, the yeast Gpr1/Cyr1/PKA/Msn2/4-AKT/DAF16 pathways play similar roles in regulating longevity and stress resistance (14). Analogously, a Drosophila line with a mutation in the heterotrimeric guanosine triphosphate–binding protein (G protein)–coupled receptor homolog MTH gene displays a 35% increase in life-span and is resistant to starvation and paraquat toxicity (25). Furthermore, in flies, aciontase undergoes age-dependent oxidation and inactivation (26), and the overexpression of SOD1 increases survival by up to 40% (27, 28). A mutation in a signal-transduction gene also increases resistance to stress and lengthens survival in mammals. A knockout mutation in the signal transduction p66<sup>Wis</sup> gene increases resistance to paraquat and hydrogen peroxide and extends survival by 30% in mice (29).

We propose that yeast Sch9 and PKA and worm AKT-1/AKT-2 evolved from common ancestors that regulated metabolism, stress resistance, and longevity in order to overcome periods of starvation. Analogous mechanisms triggered by low nutrients may be responsible for the extended longevity of dietary restricted rodents (3). The phenotypic similarities of long-lived mutants ranging from yeast to mice (1, 2), and the role of the conserved yeast Sch9 and PKA and mammalian Akt/PKB in glucose metabolism, raise the possibility that the fundamental mechanism of aging may be conserved from yeast to humans.

References and Notes

Functional Specialization in Rhesus Monkey Auditory Cortex
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Neurons in the lateral belt areas of rhesus monkey auditory cortex prefer complex sounds to pure tones, but functional specializations of these multiple maps in the superior temporal region have not been determined. We tested the specificity of neurons in the lateral belt with species-specific communication calls presented at different azimuth positions. We found that neurons in the anterior belt are more selective for the type of call, whereas neurons in the caudal belt consistently show the greatest spatial selectivity. These results suggest that cortical processing of auditory spatial and pattern information is performed in specialized streams rather than one homogeneously distributed system.

Hearing plays a dual role in the identification of sounds and in their localization. Although it is undisputed that auditory cortex participates in the analysis of spectro-temporal patterns for the identification of complex sound objects, including speech and music, the neural basis of auditory spatial perception remains a matter of controversy. Brainstem structures play a significant role in the processing of binaural cues, which contain important information for sound localization (1). However, lesions of auditory cortex also impair auditory spatial analysis (2, 3). With the recent discovery of multiple cochleotoxic maps in nonprimary auditory cortex of the rhesus monkey (4, 5), the question arises whether neurons in some of these areas show greater specificity for sound source location than in others. This could indicate the existence of a specialized cortical stream for the processing of auditory space, similar to what

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