Journal of Microbiology (2014) Vol. 52, No. 8, pp. 652–658
DOI 10.1007/s12275-014-4173-2

Growth Phase-dependent Roles of Sir2 in Oxidative Stress Resistance and Chronological Lifespan in Yeast

Woo Kyu Kang[1], Yeong Hyeock Kim[1], Byoung-Soo Kim[2], and Jeong-Yoon Kim[1]*

[1]*Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University, Daejeon 305-764, Republic of Korea*
[2]*Department of Physiology, College of Korean Medicine, Daejeon University, Daejeon 300-716, Republic of Korea*

(Received Mar 20, 2014 / Revised May 7, 2014 / Accepted May 7, 2014)

Silent Information Regulator 2 (Sir2), a conserved NAD^+-dependent histone deacetylase, has been implicated as one of the key factors in regulating stress response and longevity. Here, we report that the role of Sir2 in oxidative stress resistance and chronological lifespan is dependent on growth phase in yeast. In exponential phase, *sir2*Δ cells were more resistant to H_2O_2 stress and had a longer chronological lifespan than wild type. By contrast, in post-diauxic phase, *sir2*Δ cells were less resistant to H_2O_2 stress and had a shorter chronological lifespan than wild type cells. Similarly, the expression of antioxidant genes, which are essential to cope with oxidative stress, was regulated by Sir2 in a growth phase-dependent manner. Collectively, our findings highlight the importance of the metabolic state of the cell in determining whether Sir2 can protect against or accelerate cellular aging of yeast.

Keywords: Sir2, oxidative stress resistance, chronological lifespan, aging, *Saccharomyces cerevisiae*

Introduction

Now more than ever, aging studies are becoming important in biomedical research (Longo *et al*., 2012). Aging can be defined as a progressive decline in the ability of a cell or an organism to resist stress, damage, or disease. Genomic instability, telomere attrition, epigenetic alteration, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication have all been proposed as hallmarks of aging (Lopez-Otin *et al*., 2013). However, due to the complexities of aging, extensive studies during the last few decades have only offered a glimpse of the molecular mechanisms of aging.

The budding yeast *Saccharomyces cerevisiae* has contributed to the identification of genes and genetic and biochemical pathways that regulate lifespan in organisms ranging yeast to mammals. Yeast has two aging models: replicative lifespan (RLS) and chronological lifespan (CLS). RLS is a model of mitotic cell, whereas CLS is a model of post-mitotic cell (Longo *et al*., 2012). RLS is determined by measuring the number of times a mother cell divides and produces a daughter cell before dying (Mortimer and Johnston, 1959). CLS is the length of time that yeast cells survive in a post-mitotic state (Werner-Washburne *et al*., 1993).

Silent Information Regulator 2 (*SIR2*) is one of the best characterized yeast aging genes. It is a class III NAD^+-dependent histone deacetylase and is essential for transcriptional silencing near telomeres, mating type loci, and rDNA locus (Ivy *et al*., 1986; Rine and Herskowitz, 1987; Gottschling *et al*., 1990). Sir2 extends RLS by suppressing homologous recombination in the ribosomal DNA (rDNA) thereby preventing the formation of extrachromosomal rDNA circles (ERCs) (Kaeberlein *et al*., 1999). These circular DNA molecules are self-replicating and asymmetrically segregate to the mother cell during cell division (Sinclair and Guarente, 1997). While Sir2 is thought to be an anti-aging factor in RLS, many studies suggest that Sir2 accelerates ageing in CLS (Wierman and Smith, 2013). In the absence of Sir2, CLS is dramatically increased by induction of uptake and catabolism of ethanol and by up-regulating many stress resistance genes, including oxidative stress resistance genes (Fabrizio *et al*., 2005). Nonetheless, the complex roles of Sir2 in aging are far from being completely understood.

In this study, we show that the roles of *SIR2* in oxidative stress resistance and chronological lifespan vary depending on the growth phase. We also demonstrate that Sir2 affects the transcription of antioxidant genes in a growth phase-dependent manner in yeast.

Materials and Methods

Yeast strains and media

The *S. cerevisiae* DBY746 strain (*MATa, leu2-3, 112, his3D, trp1-289, ura3-52, GAL*$^+$) was used as a wild type strain. The disruption of *SIR2* was performed by replacing the open reading frame with *URA3* through homologous recombination. To confirm the effects of *SIR2* deletion on stress resistance, the fragment including the *SIR2* promoter (-1000 to -1), the entire *SIR2* gene, the *ADH1* terminator, and *URA3* was integrated at the endogenous *SIR2* promoter locus of a *sir2*Δ mutant strain by homologous recombination.

Yeast cells were routinely grown in YPD (1% yeast extract, 2% peptone, and 2% glucose) at 30°C. Synthetic complete

*For correspondence. E-mail: jykim@cnu.ac.kr; Tel.: +82-42-821-6419; Fax: +82-42-822-7367

medium lacking uracil was used for selection of *URA*$^+$ strains. When required, 1 mg/ml 5′-fluoroorotic acid (Fermentas, Germany) was added to solid media for selection of *ura*$^-$ strains.

Stress resistance test

Yeast cells grown to exponential (6 h), post-diauxic growth phase (24 h), or stationary phase (48 h) in YPD were used for stress resistance assay. For oxidative stress resistance assays, cells were washed with sterile distilled water, diluted to an OD_{600} of 1 in potassium phosphate buffer (pH 6.0), and treated with 25–600 mM of hydrogen peroxide (H_2O_2) for 30 min at 30°C. The control or treated cells were serially diluted, spotted onto YPD plates, and incubated at 30°C for 2–3 days. For osmotic stress resistance assay, cells were washed twice with sterile distilled water, serially diluted, and spotted onto YPD or YPD containing 2 M sorbitol plates. Cells were incubated for 3 days at 30°C, and the plates were photographed.

Chronological lifespan measurement

To test chronological lifespan of cells growing in the exponential or post-diauxic growth phase, yeast cells grown in YPD for 6 h or 24 h were harvested, washed with sterile distilled water, diluted to an OD_{600} of 1 in the expired medium, which was obtained by culturing the wild type strain for 5 days in YPD, or sterile distilled water (pH 6.0), and then incubated at 30°C with vigorous shaking (220 rpm). Survival rates were monitored by measuring colony-forming units (CFUs) every 2 or 3 days. The number of CFUs at day 3 was considered as an initial survival (100%).

Intracellular ROS measurement

The oxidant-sensitive probe 2′,7′-dichlorofluorescin diacetate (H_2DCFDA, Sigma, USA) was used to measure the intracellular ROS level in yeast. Briefly, cells were diluted to an OD_{600} of 1 in potassium phosphate buffer (pH 6.0) and exposed to 20 μM H_2DCFDA for 30 min at 30°C in the dark. H_2DCFDA stained cells were then treated with 25–600 mM of H_2O_2 for 30 min at 30°C. The cells were harvested, washed twice with sterile distilled water, and analyzed by flow cytometry (Becton Dickinson, USA). Mean DCF fluorescence intensity was obtained from 20,000 cells.

RNA isolation, cDNA synthesis, and real-time quantitative PCR analysis

Total RNA was purified using the RNeasy Mini kit (Qiagen, USA) and 0.5 μg of total RNA was reverse-transcribed using the First Strand cDNA synthesis kit (Invitrogen, USA) according to the manufacturer's recommendations. Real-time quantitative PCR (qPCR) was performed with SYBR Green PCR mix (Bio-Rad, USA) in the CFX connect system (Bio-Rad). Sequences of the oligonucleotides are as follows: *SOD1*, 5′-ATGGTTGTGTCTCTGCTGG-3′ and 5′-TGGCCACACCATTTTCGTCC-3′; *SOD2*, 5′-GACCAACACAAAGCTAGCAG-3′ and 5′-GACGGTATCCTGGTTGTAGG-3′; *CTT1*, 5′-ACGTTGTTTGCCACGCTTGT-3′ and 5′-GAACTCCCAAGCATTCTG-3′; *CTA1*, 5′-CCAACAGGACAGACCCATTCA-3′ and 5′-GCTTGCACGAAATCTACATC-3′; *TRX1*, 5′-CCCACAAGCTGATTTCTAT-3′ and 5′-GAAGCAAAGTTGGCATAGCG-3′; *TRX2*, 5′-TTCTGACGCTGCTTTTTACAAG-3′ and 5′-GAAGATTAGGGTAGGCATGG-3′; *GSH1*, 5′-GTTACGCCAATCCTATGCC-3′ and 5′-CTCTCGTGTTTGGAAGAATGC-3′; *GSH2*, 5′-GTTTCGCAATAGGCCC-3′ and 5′-CCTCATCTGTCAACAATTGCTG-3′; *GRX1*, 5′-CCATGCAGCCCTAAACACG-3′ and 5′-ATCTCATATAACGCAGCC-3′; *GRX2*, 5′-CCAGGAAACAGTTGCTCA-3′ and 5′-CTTGGAAGAGGGTAGACA-3′; *ACT1*, 5′-AGCCTTCTACGTTTCCATCCA-3′ and 5′-TAACACCATCACCGGAATCCA-3′. Gene expression levels were normalized with *ACT1* and all experiments were performed at least three times independently.

Metabolite measurement and total catalase activity assay

To measure the glucose and ethanol concentration in the culture medium during growth, at designated time-points, aliquots of the culture supernatant were collected by centrifugation and then filtrated with 0.2 μm membrane filter. The glucose and ethanol concentrations in the supernatant were measured using the YSI 2700 biochemistry analyzer (YSI Life Sciences, USA) with enzyme-bound membranes specific for each metabolite.

Total catalase activity was analyzed as described previously (Beers and Sizer, 1952). Briefly, cells were washed, resuspended in cold PBS (pH 7.2), and disrupted using glass beads. Supernatants were collected by centrifugation and then mixed with 30 mM H_2O_2 in PBS (pH 7.2). The rate of H_2O_2 disappearance was monitored at 240 nm. One unit of catalase activity was defined as the amount of protein required to degrade 1 μmole of H_2O_2 in 1 min at 25°C. Protein concentration was determined using the Bradford reagent (iNtRON Biotechnology, Korea). Reported values are averages from at least three independent experiments.

Western blotting

Total yeast cell extracts were prepared using the TCA method (Keogh *et al*., 2006). Proteins were separated by 10% SDS-PAGE gels and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, USA). Membranes were probed with anti-Sir2 antibody (1:200, Santa Cruz, USA) or anti-GAPDH antibody (1:10,000, Acris, Germany), and detected using an enhanced chemiluminescence reagent (Elpis Biotech, Korea). Band density traces and quantification were performed using ImageJ software (National Institutes of Health, USA).

Results

The role of Sir2 in oxidative stress resistance differs with growth phase

Although accumulation of oxidative damage is a well-known aging factor (Pan, 2011), the role of Sir2 in oxidative stress resistance is unclear (Fabrizio *et al*., 2005; Erjavec and Nystrom, 2007; Merksamer *et al*., 2013). Since yeast cells may experience different degrees of intracellular oxidative stress during growth, we hypothesized that there might be

a growth phase when the role of Sir2 in oxidative stress resistance is maximized. Under these conditions Sir2's role could be more easily determined. We therefore measured the H_2O_2 resistance of wild type and *sir2*Δ cells growing in exponential (6 h), post-diauxic (24 h), and stationary phase (48 h). Surprisingly, we found that the role of Sir2 in oxidative stress resistance differs with the growth phase of yeast cells (Figs. 1A and 1B). During exponential phase, *sir2*Δ cells were more resistant to H_2O_2 than wild type cells (Fig. 1B), which is consistent with the previously reported role of Sir2 in stress resistance (Fabrizio *et al.*, 2005). During the post-diauxic and stationary phases, when cells generally become highly resistant to oxidative stress, the wild type cells were more resistant to H_2O_2 than the *sir2*Δ cells (Fig. 1B). We confirmed that the expression of *SIR2* in the *sir2*Δ cells restored the H_2O_2 sensitive level of the *sir2*Δ cells up to that of wild type in all growth phases (Figs. 1B and 1C). In addition, we made an interesting observation that the Sir2 protein level dramatically decreased after the diauxic shift (Fig. 1D). However, the reduction in the Sir2 level cannot account for the growth phase-dependent role of Sir2 in oxidative stress resistance because the sensitivity to H_2O_2 stress was neither directly nor inversely proportional to the amount of Sir2. Collectively, these findings suggest that the roles of *SIR2* in oxidative stress resistance differ depending on the growth phase.

We next examined whether deletion of *SIR2* affects hyperosmotic stress resistance in a similar manner to H_2O_2 resistance because high osmolarity stress is similarly related to lifespan in yeast (Kaeberlein *et al.*, 2002; Murakami *et al.*, 2008). Wild type and *sir2*Δ cells in exponential or post-diauxic growth phase were spotted onto medium containing a high concentration of sorbitol. As shown in Fig. 1E, although cells in post-diauxic phase were more resistant to osmotic stress than cells in exponential phase, *SIR2* was not associated with osmotic stress resistance of the cells, irrespective of growth phase.

Role of Sir2 in chronological lifespan is also dependent on the growth phase

Since H_2O_2 stress resistance is related to the lifespan of yeast cells (Molin *et al.*, 2011), we wondered whether the effect of Sir2 on chronological lifespan also varies with growth phase, especially before and after the diauxic shift. To test this hypothesis, we monitored the survival of wild type and *sir2*Δ cells in exponential or post-diauxic phase in either expired culture medium or distilled water. We noticed that deletion of *SIR2* slightly but clearly increased the chronological lifespan of exponentially growing cells in both the expired medium and distilled water (Figs. 2A and 2C). By contrast, the chronological lifespan of the cells in post-diauxic phase was decreased significantly in the *sir2*Δ cells in both the expired medium and distilled water (Figs. 2B and 2D). These results demonstrate that the role of Sir2 in chronological lifespan, like in H_2O_2 stress resistance, can be positive or negative depending on the growth phase.

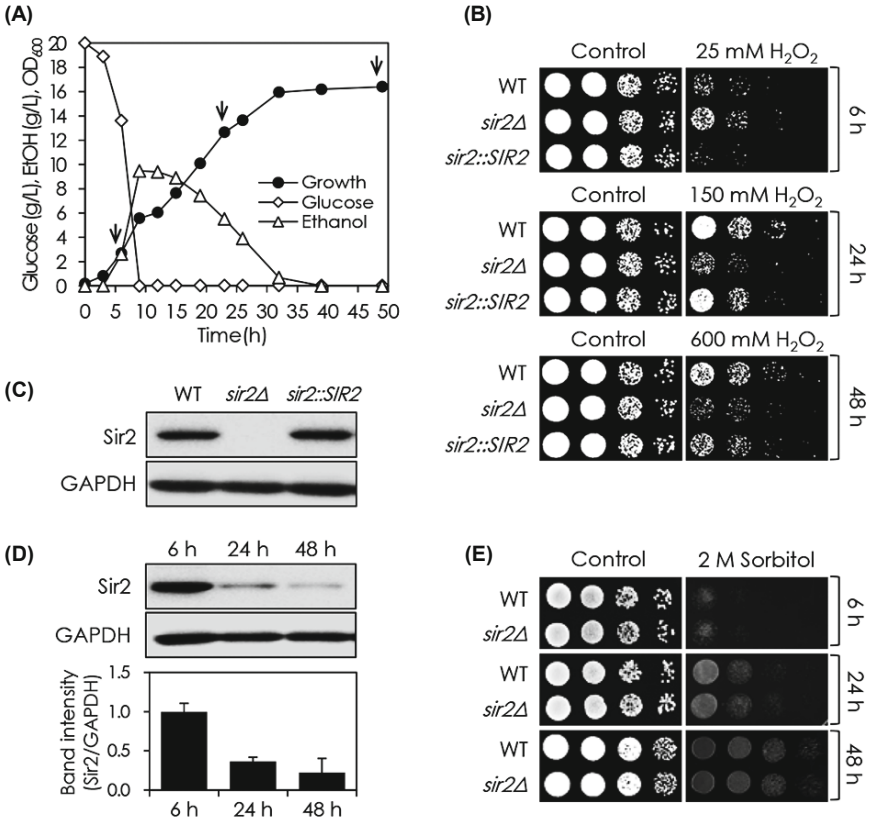

Fig. 1. Effects of *SIR2* deletion on H_2O_2 resistance differ with growth phase. (A) Growth and metabolic profile of DBY746 wild type cells grown in YPD were measured at different time-points. Cells were collected for further analysis in exponential (6 h), post-diauxic (24 h), and stationary phases (48 h), as indicated by the arrows. (B) H_2O_2 resistance of wild type, *sir2*Δ and *sir2*Δ mutant cells carrying the *SIR2* gene were tested duirng different growth phases. Cells were treated with or without the indicated amount of H_2O_2 for 30 min, spotted onto YPD plates, and incubated at 30°C for 2–3 days. (C) Sir2 protein levels of wild type, *sir2*Δ and *sir2*Δ mutant cells carrying the *SIR2* gene were measured by Western blot. GAPDH was used as a loading control. (D) Sir2 protein level of wild type cells growing in different growth phases was measured by Western blot (upper). GAPDH was used as a loading control. Relative expression levels of Sir2 are the average of three independent experiments (lower). (E) Osmotic stress resistance of wild type and *sir2*Δ cells was tested during different growth phases. Cells were spotted onto YPD plates with or without 2 M sorbitol and incubated at 30°C for 3 days.

Growth phase-dependent role of Sir2 is not related with metabolism, but ROS response

The similar effects of *SIR2* deletion on H_2O_2 resistance (Fig. 1) and chronological lifespan (Fig. 2) suggest that the growth phase-dependent role of Sir2 for chronological lifespan results from growth phase-dependent changes in oxidative stress resistance. To test this hypothesis, we examined whether chronological aging factors, such as ethanol accumulation, acidification of culture medium (Fabrizio *et al.*, 2005; Burtner *et al.*, 2009; Murakami *et al.*, 2012), and reduced glucose uptake (Bishop and Guarente, 2007; Smith *et al.*, 2007), are involved in the growth phase-dependent effect of Sir2 on chronological lifespan. We monitored changes in ethanol accumulation, medium pH, and residual glucose concentration in the culture media in wild type and *sir2*Δ cells. During exponential growth, there was no difference in growth or ethanol and residual glucose concentrations between the wild type and *sir2*Δ cells (Fig. 3A). After the diauxic shift, accumulated ethanol concentrations were only marginally lower in the culture medium of wild type than in the medium of *sir2*Δ cells (Fig. 3A), suggesting that ethanol may not be an aging factor responsible for the growth phase-dependent effect of Sir2 on chronological lifespan. Similarly, no difference in medium acidification was seen between wild type and *sir2*Δ; pH gradually decreased from 6.0 to around 4.3 during growth of both strains (Fig. 3B). Thus, extracellular acidification does not appear to be linked to the role of Sir2 in the chronological lifespan of yeast under our experimental condition.

To further elucidate Sir2's role in the oxidative stress response, we compared the intracellular reactive oxygen species (ROS) levels in wild type and *sir2*Δ cells in different growth phases. Intracellular ROS levels were measured using H_2DCFDA, which is oxidized to the fluorophore DCF (2′,7′-dichlorofluorescein) within the cell. Levels of DCF can then be analyzed by flow cytometry. Under normal growth condition, deletion of *SIR2* did not affect ROS levels, regardless of the growth phase (Fig. 3C). However, upon H_2O_2 treatment, *sir2*Δ mutant cells showed lower ROS levels than the wild type cells during exponential growth, but higher intracellular ROS levels in post-diauxic and stationary phase (Fig. 3D). This result suggests that Sir2 decreases expression of genes for intracellular ROS removal during exponential phase but increases expression of genes that protect cells from intracellular ROS in the post-diauxic and stationary phases.

Growth phase-dependent regulation of antioxidant genes by Sir2

All aerobic organisms have evolved defense systems against ROS, which include the transcriptional activation of genes encoding antioxidant proteins such as catalase, superoxide dismutase, thioredoxin, and glutathione-related enzymes (Morano *et al.*, 2012). We investigated whether the expression of antioxidant genes was altered in the *sir2*Δ cells and whether these changes were also growth phase-dependent.

In exponentially growing cells, the transcript levels of all queried genes, except *CTT1* encoding cytosolic catalase T, were similar in wild type and *sir2*Δ cells (Fig. 4A). However, after treatment with a sublethal dose of H_2O_2 (0.5 mM), the mRNA levels of most of these antioxidant genes were significantly higher in the *sir2*Δ cells than in wild type cells (Fig. 4B). By contrast, in cells growing in post-diauxic or stationary phase, when ROS are endogenously generated as

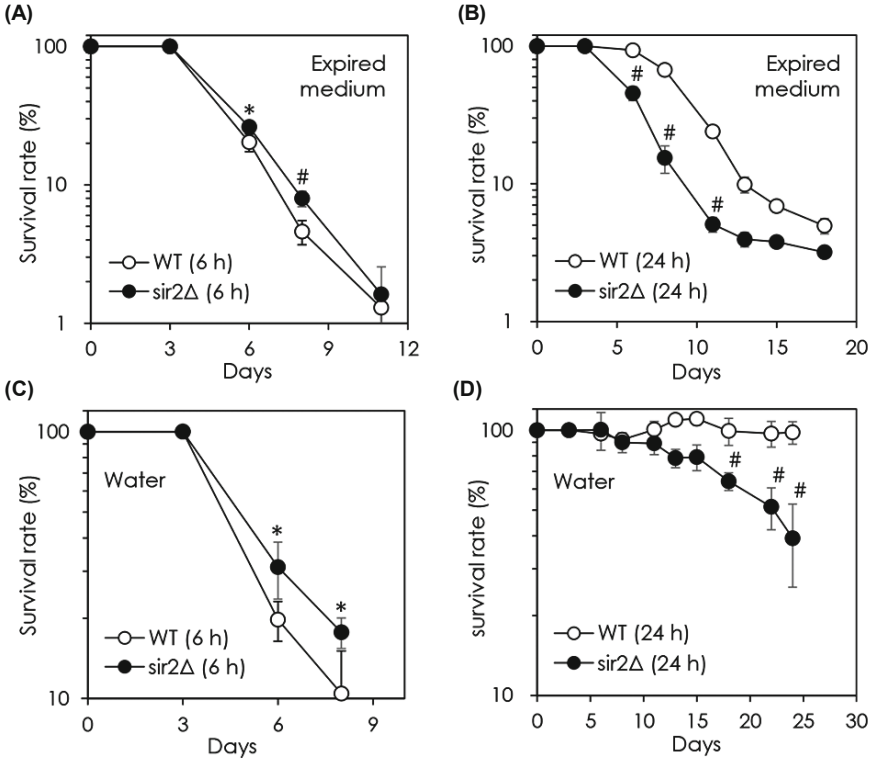

Fig. 2. Effects of *SIR2* deletion on chronological aging are opposite in exponential and post-diauxic phase cells. Wild type and *sir2*Δ cells grown in exponential growth phase (A and C) or post-diauxic growth phase (B and D) were incubated in expired medium (A and B) or water (C and D). Survival rates were monitored by measuring CFUs every 2 or 3 days. The values are the average of at least five independent experiments (± SD), and *p* values were calculated using t-test (*$p < 0.005$, #$p < 0.0001$, versus wild type at an indicated time point).

normal metabolic by-products due to high respiration activity, deletion of *SIR2* decreased the transcription of most antioxidant genes (Figs. 4C and 4D).

To address whether the changed transcript level is reflected in the protein level and enzyme activity, we measured the activity of catalase. We observed that during exponential phase, catalase activity was higher in *sir2*Δ than in wild type cells, but during post-diauxic and stationary phase the wild type cells showed higher catalase activity (Fig. 4E). Taken together, our results suggest that the expression of antioxidant genes in yeast is regulated by Sir2 in a growth phase-dependent manner, which is mirrored in oxidative stress resistance and chronological lifespan.

Discussion

It is generally accepted that Sir2 and ROS are important factors that affect yeast longevity (Merksamer *et al.*, 2013). ROS accumulate in both replicatively and chronologically aged cells (Reverter-Branchat *et al.*, 2004), and deletion of the antioxidant *SOD1* and *SOD2* genes significantly decreases both RLS and CLS (Longo *et al.*, 1996; Laun *et al.*, 2001). However, in contrast to the many studies that support a critical role of sirtuins in regulating ROS levels in mammals (Merksamer *et al.*, 2013), only a few studies have demonstrated that Sir2 is involved in the regulation of oxidative stress response. Despite reports that Sir2 prevented ROS accumulation and cell death caused by Hog1 activation (Vendrell *et al.*, 2011) and reports that it was required for the asymmetric segregation of oxidatively damaged proteins from daughter cells to mother cells during cell division in yeast (Aguilaniu *et al.*, 2003; Erjavec and Nystrom, 2007; Liu *et al.*, 2010), it remained unclear whether Sir2 increases lifespan by increasing oxidative stress resistance. Here, we report that Sir2 regulates oxidative stress resistance and CLS in a growth phase-dependent manner. We find that deletion of *SIR2* decreased H_2O_2 resistance and CLS of yeast cells in exponential phase, but increased H_2O_2 resistance and CLS of the cells growing in post-diauxic and stationary phases (Figs. 1 and 2).

The relevance of several cell-intrinsic and cell-extrinsic factors was reported to be associated with CLS of yeast (Longo and Fabrizio, 2012). In particular, ethanol catabolism seems to be associated with CLS in that deletion of *SIR2* enhanced the ability to deplete ethanol in the culture medium and contributed to CLS extension (Fabrizio *et al.*, 2005). We, however, observed no significant difference in ethanol production and consumption rates between wild type and *sir2*Δ cells under the tested culture condition (Fig. 3A). Moreover, because the expired medium and water used as the chronological aging medium contained no ethanol, it is unlikely that ethanol catabolism is involved in CLS regulation by Sir2 under our experimental conditions. Furthermore, since glucose uptake rate, extracellular acidification, and hyperosmotic stress resistance were not altered in the *sir2*Δ strain (Figs. 1E, 3A, and 3B), we excluded cell-extrinsic factors as the possible causes for the observed difference in CLS between wild type and *sir2*Δ cells.

The most important finding of our study is that Sir2 regu-

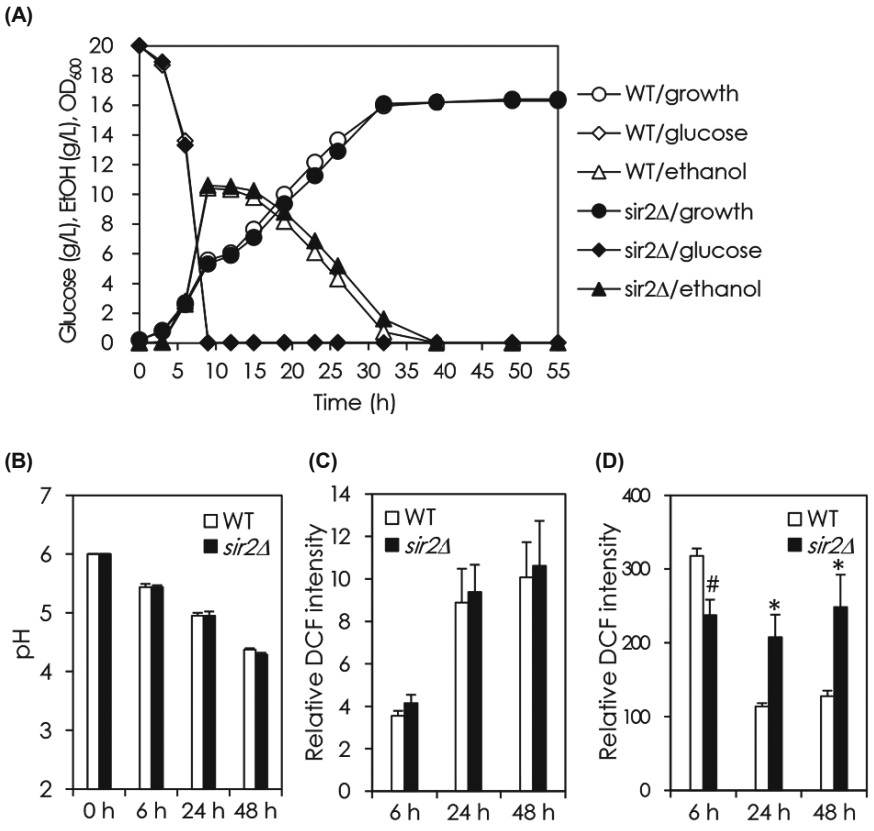

Fig. 3. Growth phase-dependent role of Sir2 is not related with metabolism, but ROS response. (A) Time-course profile of cell growth, glucose consumption, and ethanol metabolism of wild type and *sir2*Δ cells grown in YPD. (B) pH changes in the culture medium of wild type and *sir2*Δ cells were monitored over time. Reported values are the average of at least three independent experiments (± SD). (C) Endogenous ROS levels of wild type and *sir2*Δ cells in exponential (6 h), post-diauxic (24 h), or stationary phase (48 h) were analyzed using the molecular probe H_2DCFDA. Reported values are the average of at least three independent experiments (± SD). (D) Intracellular ROS levels of H_2O_2-treated wild type and *sir2*Δ cells were analyzed using the molecular probe H_2DCFDA. Cells sampled in exponential (6 h), post-diauxic (24 h), or stationary phase (48 h) were treated with 25 mM, 150 mM, or 600 mM of H_2O_2 for 30 min, respectively. Reported values are the average of at least three independent experiments (± SD), and p values were calculated using t-test ($^*p < 0.05$, $^\#p < 0.005$, versus wild type at an indicated time point).

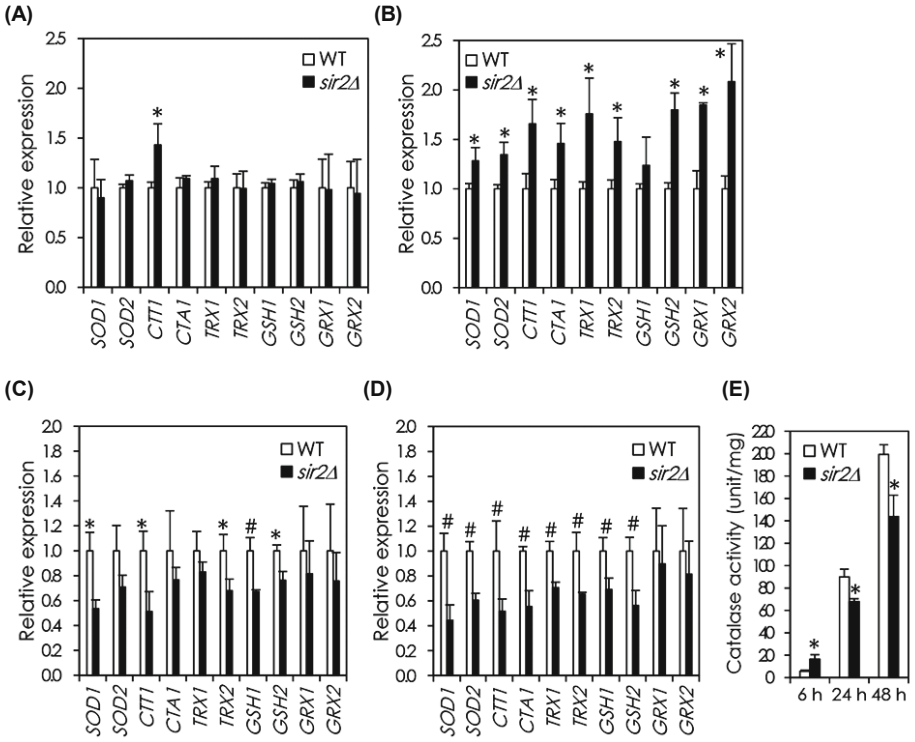

Fig. 4. Deletion of *SIR2* affects the expression of antioxidant genes in a growth phase-dependent manner. (A-B) Exponentially growing wild type and *sir2*Δ mutant cells were untreated (A) or treated with 0.5 mM H_2O_2 for 30 min (B), and mRNA levels of antioxidant genes were analyzed by qRT-PCR. *ACT1* was used as a control. Reported values are the average of at least three independent experiments (± SD), and *p* values were calculated using t-test (*$p < 0.05$, #$p < 0.005$, versus wild type). (C-D) mRNA levels of the antioxidant genes of wild type and *sir2*Δ cells growing in post-diauxic phase (C) or stationary phase (D) were analyzed by qRT-PCR and normalized to *ACT1*. Reported values are the average of at least three independent experiments (± SD), and *p* values were calculated using t-test (*$p < 0.05$, #$p < 0.005$, versus wild type). (E) Total catalase activity of wild type and *sir2*Δ cells growing in exponential (6 h), post-diauxic (24 h), or stationary phase (48 h). The reported values are the average of at least three independent experiments (± S.D.), and *p* values were calculated using t-test (#$p < 0.05$, versus wild type at an indicated time point).

lates oxidative stress resistance and CLS of yeast cells in a growth phase-dependent manner, which strongly suggests that diauxic shift-induced physiological changes may be a key determinant of Sir2's effects on the oxidative stress response and CLS. As glucose becomes depleted, cells pass through the diauxic shift and reprogram their metabolism from fermentation to respiration, which is mainly regulated by protein kinase A (PKA) and TOR signaling pathway (DeRisi *et al*., 1997; Gray *et al*., 2004; Galdieri *et al*., 2010). Interestingly, like the diauxic shift, reducing glucose concentration in the culture medium or inhibition of PKA or TOR signaling has been proposed to increase Sir2 activity (Lin *et al*., 2004; Medvedik *et al*., 2007). Thus, we speculate that the reduced PKA and/or TOR signaling activity as well as an increased NAD^+/NADH ratio and/or mitochondrial ROS signaling might elicit the protective role of Sir2 after the diauxic shift. However, it remains unresolved why and how Sir2 in exponentially growing cells represses the expression of antioxidant genes, which is essential to protect cells from oxidative stress. Further studies focusing on the metabolic state of cells and the physiological function of Sir2 are required to better understand the controversial effects of Sir2 on oxidative stress resistance and CLS.

Acknowledgements

This work was supported by National Research Foundation of Korea Grants funded by the Korean Government (NRF-2010-0013086) and Regional Innovation System (RIS) project funded by the Ministry of Trade, Industry and Energy (R0001989).

References

Aguilaniu, H., Gustafsson, L., Rigoulet, M., and Nystrom, T. 2003. Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. *Science* **299**, 1751–1753.

Beers, R.F., Jr., and Sizer, I.W. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* **195**, 133–140.

Bishop, N.A. and Guarente, L. 2007. Genetic links between diet and lifespan: shared mechanisms from yeast to humans. *Nat. Rev. Genet.* **8**, 835–844.

Burtner, C.R., Murakami, C.J., Kennedy, B.K., and Kaeberlein, M. 2009. A molecular mechanism of chronological aging in yeast. *Cell Cycle* **8**, 1256–1270.

DeRisi, J.L., Iyer, V.R., and Brown, P.O. 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278**, 680–686.

Erjavec, N. and Nystrom, T. 2007. Sir2p-dependent protein segregation gives rise to a superior reactive oxygen species management in the progeny of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **104**, 10877–10881.

Fabrizio, P., Gattazzo, C., Battistella, L., Wei, M., Cheng, C., McGrew, K., and Longo, V.D. 2005. Sir2 blocks extreme life-span extension. *Cell* **123**, 655–667.

Galdieri, L., Mehrotra, S., Yu, S., and Vancura, A. 2010. Transcriptional regulation in yeast during diauxic shift and stationary phase. *Omics* **14**, 629–638.

Gottschling, D.E., Aparicio, O.M., Billington, B.L., and Zakian, V.A. 1990. Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription. *Cell* **63**, 751–762.

Gray, J.V., Petsko, G.A., Johnston, G.C., Ringe, D., Singer, R.A., and Werner-Washburne, M. 2004. "Sleeping beauty": quiescence in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **68**, 187–206.

Ivy, J.M., Klar, A.J., and Hicks, J.B. 1986. Cloning and characterization of four *SIR* genes of *Saccharomyces cerevisiae*. *Mol. Cell.*

Biol. **6**, 688–702.

Kaeberlein, M., Andalis, A.A., Fink, G.R., and Guarente, L. 2002. High osmolarity extends life span in *Saccharomyces cerevisiae* by a mechanism related to calorie restriction. *Mol. Cell. Biol.* **22**, 8056–8066.

Kaeberlein, M., McVey, M., and Guarente, L. 1999. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev.* **13**, 2570–2580.

Keogh, M.C., Mennella, T.A., Sawa, C., Berthelet, S., Krogan, N.J., Wolek, A., Podolny, V., Carpenter, L.R., Greenblatt, J.F., Baetz, K., and *et al.* 2006. The *Saccharomyces cerevisiae* histone H2A variant Htz1 is acetylated by NuA4. *Genes Dev.* **20**, 660–665.

Laun, P., Pichova, A., Madeo, F., Fuchs, J., Ellinger, A., Kohlwein, S., Dawes, I., Frohlich, K.U., and Breitenbach, M. 2001. Aged mother cells of *Saccharomyces cerevisiae* show markers of oxidative stress and apoptosis. *Mol. Microbiol.* **39**, 1166–1173.

Lin, S.J., Ford, E., Haigis, M., Liszt, G., and Guarente, L. 2004. Calorie restriction extends yeast life span by lowering the level of NADH. *Genes Dev.* **18**, 12–16.

Liu, B., Larsson, L., Caballero, A., Hao, X., Oling, D., Grantham, J., and Nystrom, T. 2010. The polarisome is required for segregation and retrograde transport of protein aggregates. *Cell* **140**, 257–267.

Longo, V.D. and Fabrizio, P. 2012. Chronological aging in *Saccharomyces cerevisiae*. *Subcell Biochem.* **57**, 101–121.

Longo, V.D., Gralla, E.B., and Valentine, J.S. 1996. Superoxide dismutase activity is essential for stationary phase survival in *Saccharomyces cerevisiae*. Mitochondrial production of toxic oxygen species *in vivo*. *J. Biol. Chem.* **271**, 12275–12280.

Longo, V.D., Shadel, G.S., Kaeberlein, M., and Kennedy, B. 2012. Replicative and chronological aging in *Saccharomyces cerevisiae*. *Cell Metab.* **16**, 18–31.

Lopez-Otin, C., Blasco, M.A., Partridge, L., Serrano, M., and Kroemer, G. 2013. The hallmarks of aging. *Cell* **153**, 1194–1217.

Medvedik, O., Lamming, D.W., Kim, K.D., and Sinclair, D.A. 2007. MSN2 and MSN4 link calorie restriction and TOR to sirtuin-mediated lifespan extension in *Saccharomyces cerevisiae*. *PLoS Biol.* **5**, e261.

Merksamer, P.I., Liu, Y., He, W., Hirschey, M.D., Chen, D., and Verdin, E. 2013. The sirtuins, oxidative stress and aging: an emerging link. *Aging (Albany NY)* **5**, 144–150.

Molin, M., Yang, J., Hanzen, S., Toledano, M.B., Labarre, J., and Nystrom, T. 2011. Life span extension and H_2O_2 resistance elicited by caloric restriction require the peroxiredoxin Tsa1 in *Saccharomyces cerevisiae*. *Mol. Cell.* **43**, 823–833.

Morano, K.A., Grant, C.M., and Moye-Rowley, W.S. 2012. The response to heat shock and oxidative stress in *Saccharomyces cerevisiae*. *Genetics* **190**, 1157–1195.

Mortimer, R.K. and Johnston, J.R. 1959. Life span of individual yeast cells. *Nature* **183**, 1751–1752.

Murakami, C., Delaney, J.R., Chou, A., Carr, D., Schleit, J., Sutphin, G.L., An, E.H., Castanza, A.S., Fletcher, M., Goswami, S., and *et al.* 2012. pH neutralization protects against reduction in replicative lifespan following chronological aging in yeast. *Cell. Cycle* **11**, 3087–3096.

Murakami, C.J., Burtner, C.R., Kennedy, B.K., and Kaeberlein, M. 2008. A method for high-throughput quantitative analysis of yeast chronological life span. *J. Gerontol. A Biol. Sci. Med. Sci.* **63**, 113–121.

Pan, Y. 2011. Mitochondria, reactive oxygen species, and chronological aging: a message from yeast. *Exp. Gerontol.* **46**, 847–852.

Reverter-Branchat, G., Cabiscol, E., Tamarit, J., and Ros, J. 2004. Oxidative damage to specific proteins in replicative and chronological-aged *Saccharomyces cerevisiae*: common targets and prevention by calorie restriction. *J. Biol. Chem.* **279**, 31983–31989.

Rine, J. and Herskowitz, I. 1987. Four genes responsible for a position effect on expression from HML and HMR in *Saccharomyces cerevisiae*. *Genetics* **116**, 9–22.

Sinclair, D.A. and Guarente, L. 1997. Extrachromosomal rDNA circles-a cause of aging in yeast. *Cell* **91**, 1033–1042.

Smith, D.L.Jr., McClure, J.M., Matecic, M., and Smith, J.S. 2007. Calorie restriction extends the chronological lifespan of *Saccharomyces cerevisiae* independently of the Sirtuins. *Aging Cell* **6**, 649–662.

Vendrell, A., Martinez-Pastor, M., Gonzalez-Novo, A., Pascual-Ahuir, A., Sinclair, D.A., Proft, M., and Posas, F. 2011. Sir2 histone deacetylase prevents programmed cell death caused by sustained activation of the Hog1 stress-activated protein kinase. *EMBO Rep.* **12**, 1062–1068.

Werner-Washburne, M., Braun, E., Johnston, G.C., and Singer, R.A. 1993. Stationary phase in the yeast *Saccharomyces cerevisiae*. *Microbiol. Rev.* **57**, 383–401.

Wierman, M.B. and Smith, J.S. 2013. Yeast sirtuins and the regulation of aging. *FEMS Yeast Res.* doi: 10.1111/1567-1364.12115.