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Low doses of DNA damaging agents extend *Saccharomyces cerevisiae* chronological lifespan by promoting entry into quiescence

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ABSTRACT

A variety of mild stresses have been shown to extend lifespan in diverse species through hormesis, which is a beneficial response to a stress or toxin that would cause a negative response at a higher exposure. Whether particular stresses induce hormesis can vary with genotype for a given species, and the underlying mechanisms of lifespan extension are only partly understood in most cases. We show that low doses of the DNA damaging or replication stress agents hydroxyurea, methyl methanesulfonate, 4-nitroquinoline 1-oxide, or Zeocin (a phleomycin derivative) lengthened chronological lifespan in *Saccharomyces cerevisiae* if cells were exposed during growth, but not if they were exposed during stationary phase. Treatment with these agents did not change mitochondrial activity, increase resistance to acetic acid, ethanol, or heat stress, and three of four treatments did not increase resistance to hydrogen peroxide. Stationary phase yeast populations consist of both quiescent and nonquiescent cells, and all four treatments increased the proportion of quiescent cells. Several mutant strains with deletions in genes that influence quiescence prevented Zeocin treatment from extending lifespan and from increasing the proportion of quiescent stationary phase cells. These data indicate that mild DNA damage stress can extend lifespan by promoting quiescence in the absence of mitohormesis or improved general stress responses that have been frequently associated with improved longevity in other cases of hormesis. Further study of the underlying mechanism may yield new insights into quiescence regulation that will be relevant to healthy aging.

1. Introduction

Hormesis is a beneficial response in cells or organisms triggered by a low dose of a toxin or a mild stress that would otherwise have a negative effect at a higher dose/exposure (Calabrese et al., 2015). Extension of lifespan due to hormesis has been observed in many organisms, including *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster*, and mammalian cells (Cypser and Johnson, 2002; Khazaeli et al., 1997; Le Bourg et al., 2000; Lithgow et al., 1995; Mesquita et al., 2010; Moskalev, 2007; Pan et al., 2011; Perez et al., 2008; Rattan, 1998). A variety of stressors have been used to induce hormetic responses, including toxic chemicals, natural compounds in plants, radiation, heat or cold stress, oxidative stress, and hypergravity (Cypser and Johnson, 2002; Fang et al., 2017; Hunt et al., 2011; Khazaeli et al., 1997; Le Bourg et al., 2000; Moskalev, 2007; Pan et al., 2011; Schmeisser et al., 2013). However, genetic background and gender can influence whether lifespan effects are observed (Defays et al., 2011; Henten et al., 2016; Le Bourg et al., 2000; Perez-Benito, 2006; Rodriguez et al., 2012), and lifespan extension is not always consistently observed across multiple studies for a given stressor (Lagisz et al., 2013). Further studies of conditions that induce hormesis, the influences of genotypic variation, and the underlying mechanisms are necessary to better understand the potential for it to be used as a strategy to promote healthy aging.

Hormesis induced by one stressor often leads to improved resistance to other stresses, which may at least partly account for some hormetic effects (Anderson et al., 2016; Cañuelo et al., 2012; Le Bourg, 2011; Rattan, 2004; Schmeisser et al., 2013). There are examples of mild stresses inducing expression of heat shock proteins, oxidative stress response pathways, and autophagy to increase stress resistance and lifespan (Cañuelo et al., 2012; Fang et al., 2017; Hercus et al., 2003; Hunt et al., 2011; Kumsta and Hansen, 2017; Zuo et al., 2013). Calorie

Abbreviations: CFU, colony-forming units; CLS, chronological lifespan; DHE, dihydroethidium; DHR, dihydrorhodamine; $DiOC_6$, 3,3′-dihexyloxacarbocyanine iodide; FOA, 5-fluoroorotic acid; HU, hydroxyurea; MMS, methyl methanesulfonate; NQ, non-quiescent; 4NQO, 4-nitroquinoline 1-oxide; PBS, phosphate buffered saline; Q, quiescent; ROS, reactive oxygen species; SC, synthetic complete medium; YPD, yeast extract-peptone-dextrose (rich) medium

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restriction and physical exercise, considered as mild stresses, stimulate mitochondrial metabolism, production of reactive oxygen species (ROS), and ROS defense mechanisms to promote longevity or characteristics of healthy aging (Ristow and Zarse, 2010). This has led to the development of the mitohormesis concept to explain some forms of hormesis. This theory states that increased mitochondrial respiration and ROS production protect against stresses to extend lifespan (Ristow and Zarse, 2010; Yun and Finkel, 2014). While some studies of hormesis-induced lifespan extension have noted increases in mitochondrial activity, transient increases in ROS, and increased activity of oxidative stress response pathways (De Haes et al., 2014; Fang et al., 2017; Pan et al., 2011; Schmeisser et al., 2013), decreasing mitochondrial function can also increase lifespan in certain contexts (Baumgart et al., 2016; Lee et al., 2003). Mitohormesis or increased overall stress resistance may account for certain examples of hormesis, but there is a lack of evidence that any one particular mechanism is universally required for various stressors to extend lifespan through hormesis. Autophagy, heat-shock proteins, and mitochondrial function are relevant to aging and aging-related diseases independent of hormesis (Goloubinoff, 2016; López-Otín et al., 2013; Schiavi and Ventura, 2014), and further studies of hormesis will provide additional understanding of how stress responses and mitochondrial function are relevant to aging.

Saccharomyces cerevisiae has been a useful model organism for characterizing conserved factors relevant to aging (Longo et al., 2012; Ruetenik and Barrientos, 2015). Yeast chronological lifespan (CLS) is a measure of how long cells remain viable during stationary phase in nutrient-depleted medium (Longo et al., 2012). Yeast stationary phase populations consist of a nonquiescent cell subpopulation that has not appropriately adapted for quiescence and a quiescent cell subpopulation that has arrested in G1/G0 and appropriately adapted for quiescence (Allen et al., 2006; De Virgilio, 2012). Quiescent cells have improved stress-resistance, long-term viability, and re-entry into the cell cycle in the presence of fresh nutrients compared to nonquiescent cells (Allen et al., 2006; De Virgilio, 2012). There are some examples of conditions that extend yeast CLS by triggering hormesis. Calorie restriction or a low dose of hydrogen peroxide extended yeast CLS through hormesis, and both of these effects were associated with higher hydrogen peroxide levels, higher superoxide dismutase activity, and reduced superoxide levels (Mesquita et al., 2010). In another example, extension of CLS by treatment of growing yeast cells with menadione resulted from the generation of mitochondrial ROS that signaled through nuclear DNA damage response kinases to repress subtelomeric DNA transcription (Pan et al., 2011; Schroeder et al., 2013).

Here we report that low doses of three DNA damaging agents and an inhibitor of DNA replication extend yeast CLS when cells are exposed during growth. Three of these four agents decreased ROS levels at late exponential phase, but none of them decreased ROS at the start of stationary phase, and none of the agents changed mitochondrial membrane potential, indicating that mitohormesis may not account for their effect. The treatments did not improve resistance to multiple stresses, but did consistently promote appropriate entry into quiescence during stationary phase. Several mutants that prevented one of these treatments from increasing the proportion of quiescent stationary phase cells also prevented that treatment from extending lifespan. These data indicate that DNA damaging agents can produce a hormetic extension of yeast lifespan that depends on regulation of quiescence, rather than general stress responses or changes in mitochondrial activity.

2. Methods

2.1. Yeast strains and media

Yeast strains were grown in standard rich (YPD) or synthetic complete (SC) medium with 2% (w/v) glucose (Amberg et al., 2005). For some experiments SC medium contained a final concentration of 1 × phosphate-buffered saline (PBS). Treatments with DNA damaging or replication stress agents included chronic exposure beginning on day zero to 200 μM (0.0022%, v/v) methyl methanesulfonate (MMS), 30 mM hydroxyurea (HU), 0.5 μg/ml Zeocin, or 0.2 μg/ml 4-nitroquinoline 1-oxide (4NQO), except where noted otherwise in the results text and figures. Day zero refers to the day that cells were first inoculated into media. All experiments were performed using *S. cerevisiae* strain JC5516, which is a derivative of the BY4741 background (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) that has a partial deletion allele of *kanMX* in place of the *TRP1* open reading frame (*trp1::kanMXΔNsiI*), a partial deletion of *CAN1* at its normal location (*can1Δ1*, lacking positions 84-1427 of the open reading frame), an insertion of *TRP1* on the left arm of chromosome VIII, and insertions of *CAN1*, *HIS3*, and *URA3* on the right arm of chromosome VIII, as previously described (Maxwell et al., 2011). Mutant derivatives of JC5516 with single gene deletions of *ATG1*, *ATP10*, *DCC1*, *ECM27*, *EDC3*, *LSM1*, *MSS51*, *MTL1*, *RAS2*, *SIR2*, *SSD1*, or *TOR1* were generated by one-step gene replacement of the wild type allele with a *kanMX* cassette amplified from the corresponding *S. cerevisiae MATa* deletion collection strain (Thermo Scientific Open Biosystems) using lithium acetate transformation. Presence of the *kanMX* cassette and absence of the wild type allele was verified by PCR in each case, and experiments were conducted with two or three independent transformants for each genotype.

2.2. Chronological aging experiments

Triplicate cultures of each strain were inoculated at 5000 cells/ml in YPD, SC, or buffered SC medium with or without the treatments described in Section 2.1. Cells were grown at 30 °C either in 5 ml of medium in glass culture tubes on rotators or in 50 ml of medium in 250 ml flasks in shaking incubators. Day zero was the day that cells were inoculated into media. Cultures were sampled initially at day two or three, and then every three to seven days thereafter until the viability of the cell populations was < 10%. Viability was determined using trypan blue dye exclusion and examination of cells on hemacytometers by microscopy, as described previously (Maxwell et al., 2011). Trend lines were fit to graphs of viability to determine the days to 50% and 10% viability for each set of triplicate cultures as representations of median and maximum lifespan, respectively.

2.3. Cell doubling times and percentages of budding cells

For measuring doubling times, 5000 cells/ml were inoculated into YPD medium in duplicate with or without DNA damaging agents (Section 2.1) and grown at 30 °C. Cell densities were determined by counting cells on hemacytometers periodically over the course of 20–24 h. Exponential trend lines were fit to plots of cell densities versus the number of hours, and doubling times in hours were calculated as the natural log of two divided by the coefficient for the exponent from the trend line equation. Cells grown in duplicate cultures for three days using the same conditions described in Section 2.2 were diluted into 20 mM EDTA pH 8 and vortexed for 5 min to separate clumped cells prior to determining the percentage of budded cells by microscopy. Approximately 200 cells were examined per sample and categorized as having no, small (< 50% the size of the mother cell), or large (> 50% the size of the mother cell) buds.

2.4. CAN1 and URA3 mutation frequencies

CAN1 and *URA3* mutation frequencies were assayed for cells grown for three days in YPD broth from an initial density of 5000 cells/ml with or without the DNA damaging agents described in Section 2.1. Cells were diluted in water and spread onto YPD medium to determine colony forming units per ml (cfu/ml), and appropriate volumes of cells were pelleted and suspended in water to spread onto SC medium with 1 mg/ml 5-fluoroorotic acid (FOA) or SC medium lacking arginine with 60 μg/ml canavanine to select for cells with loss of function mutations