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Original Article

CTT1 overexpression increases the replicative lifespan of MMS-sensitive *Saccharomyces cerevisiae* deficient in *KSP1*

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ABSTRACT

Ksplp is a nuclear-localized Ser/Thr kinase that is not essential for the vegetative growth of yeast. A global gene function analysis in yeast suggested that Ksplp was involved in the oxidative stress response; however, the underlying mechanism remains unclear. Here, we showed that *KSP1*-deficient yeast cells exhibit hypersensitivity to the DNA alkylating agent methyl methanesulphonate (MMS), and treatment of the *KSP1*-deficient strain with MMS could trigger abnormal mitochondrial membrane potential and up-regulate reactive oxygen species (ROS) production. In addition, the mRNA expression level of the catalase gene *CTT1* (which encodes cytosolic catalase) and total catalase activity were strongly down-regulated in the *KSP1*-deleted strain compared with those in wild-type cells. Moreover, the *KSP1* deficiency also leads to a shortened replicative lifespan, which could be restored by the increased expression of *CTT1*. On the other hand, *KSP1*-overexpressed (*KSP1*OX) yeast cells exhibited increased resistance towards MMS, an effect that was, at least in part, *CTT1* independent. Collectively, these findings highlight the involvement of Ksplp in the DNA damage response and implicate Ksplp as a modulator of the replicative lifespan.

1. Introduction

Ksplp is a Ser/Thr kinase that is localized in the nucleus and is not essential for the vegetative growth of yeast (Fleischmann et al., 1996). A previous study has suggested that Ksplp participates in the fine-tuning of TORC1 (target of rapamycin complex 1) activity to regulate pseudohyphal growth (PHG) and autophagy (Bharucha et al., 2008; Umekawa and Klionsky, 2012). *KSP1* deletion mutants exhibited an increased growth rate under the rapamycin-stressed condition but slightly decreased invasive growth ability under the invasive growth condition (Laxman and Tu, 2011). Overexpression of *KSP1* partially suppresses the temperature-sensitive defect of the prp20-10 yeast mutant, and the protein kinase activity is essential for suppressing the activity of Ksplp (Fleischmann et al., 1996). However, the molecular mechanisms governing these processes are largely uncharacterized.

Ksp1p has been identified as an interactor of yeast Nar1p in high-throughput screening (Ptacek et al., 2005). Previously, we have shown that Nar1p plays a role in the oxidative stress response and replicative lifespan modulation (Zhao et al., 2014). A global gene function analysis in yeast has suggested that *KSP1* might participate in the oxidative stress response, but its mechanism remains uncertain (Brown et al., 2006).

Oxidative stress occurs due to the imbalance between antioxidant systems and various prooxidants (Ayer et al., 2014). The oxidative stress theory of aging suggests that ROS cause oxidative damage to several macromolecules, including DNA (Harman, 1956). A growing body of evidence has indicated that mitochondrial and nuclear DNA damage could trigger genomic instability, which has long been implicated as the main causal factor of aging (Burhans and Weinberger, 2011; Bautista-Niño et al., 2016). On the other hand, DNA damage could also induce intracellular ROS pro-

Abbreviations: DCFH-DA, dichloro-dihydro-fluorescein diacetate; MMS, methyl methanesulphonate; PCR, polymerase chain reaction; PHG, pseudohyphal growth; ROS, reactive oxygen species; RLS, replicative lifespan; TORC1, target of rapamycin complex 1.

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duction (Rowe et al., 2008; Kang et al., 2012), suggesting a complex interaction between DNA damage and ROS.

The budding yeast *Saccharomyces cerevisiae* possesses both enzymatic (such as catalase, superoxide dismutase, and glutathione reductase) and non-enzymatic (such as glutathione, thioredoxin, and glutaredoxin) defence systems to detoxify ROS (Jamieson, 1998). Notably, unlike human cells (which possess only one catalase), budding yeast cells possess two different types of catalase enzymes, catalase A (located in the peroxisome) and catalase T (located in the cytosol), encoded by the *CTA1* and *CTT1* genes, respectively (Cohen et al., 1988; Hartig and Ruis, 1986). The main physiological role of catalase A is suggested to be to remove H_2O_2 produced by fatty acid β-oxidation, while catalase T is considered to play a much broader role in the oxidative stress response and aging in yeast (Jamieson, 1998; Martins and English, 2014; Rona et al., 2015).

MMS is an alkylating agent that interacts directly with DNA and has been used to study the intracellular DNA-damage response and ROS generation (Salmon et al., 2004; Rowe et al., 2008; Kim et al., 2011). In this study, we found that *KSP1*-deficiency leads to sensitivity to MMS and a decreased replicative lifespan, and these effects were accompanied by a reduced *CTT1* mRNA expression level and altered catalase activities. By contrast, wild-type yeast that overexpress *KSP1* exhibit an increased resistance towards the DNA damage agent MMS, an effect that was, at least in part, *CTT1* independent. These findings highlight the involvement of Ksplp in the DNA damage response and aging in yeast.

2. Materials and methods

2.1. Yeast strains and culture conditions

The wild-type yeast strain used in this paper was the haploid strain BY4742. The *ksp1Δ* mutant strain, *KSP1* overexpression and *CTT1* overexpression strains were derived from BY4742 (listed in Table 1).

The *ksp1Δ* mutants were generated by polymerase chain reaction (PCR)-mediated gene disruption, and the *KSP1* gene was replaced by the selectable marker *URA3* (Baudin et al., 1993). First, we used the gene-specific primers(5′-GTTAGTGCAATATTTTTTTCTTACAATTTTTTGA AACTCGAGATTGTACTGAGAGTGCAC-3′,5′-AAGAAAATAATAAGCAACATAACAGAGGGAATAGGTGCGCCTG-TGCGGTATTTCACACCG-3′) and the plasmid pRS306 as the template to amplify the *URA3* cassette. Second, the purified PCR products were transformed into the BY4742 strain using the modified LiAc/SS carrier DNA/PEG method. The transformed cells were then plated on SD/-URA (0.67% yeast nitrogen base, 2% glucose, and the appropriate amino acid) selective plates. The grown colonies were further confirmed by PCR.

The *KSP1* overexpression yeast strain (*KSP1OX*) was made by integrating an extra *KSP1* copy with its endogenous promoter in BY4742; thus, the expression of *KSP1* would be driven by its natural promoter (Stearns et al., 1990). First, 535 bp upstream from the *KSP1* ORF and 371 bp downstream from the *KSP1* ORF sequence (containing the entire coding region of *KSP1*) were amplified from yeast genomic DNA using the *SpeI*-tagged primer KSP1-S (5′-CGCACTAGTCACGTGACCCGGATATTGTT-3′) and *SalI*-tagged primer KSP1-A (5′-CGTGTCGACCGACGTATACTGGTACATGA-3′). The PCR reaction products were cloned into the pRS303 vector. Integration of *KSP1* was accomplished by transforming cells with plasmid pRS303KSP1 digested with *EcoRI*. Transformants were selected on SD/-HIS (0.67% yeast nitrogen base, 2% glucose, and the appropriate amino acid) agar media (Clontech). The grown colonies were further confirmed by PCR.

To delete the *CTT1* gene in the *KSP1OX* yeast strain, first, a deletion cassette carrying *KanMX* was obtained by PCR directed on the template plasmid pUG6 using the gene-specific primers 5′-TTGTCTCATGCCAATAAGATCAATCAGCTCAGCTTCACAACAGC-TGAAGCTTCGTACGC-3′ and 5′-GAGATATAATTACGAATAATTATGA-ATAAATAGTGCTGCCGCATAGGCCACTAGTGGATCTG-3′. Second, the purified PCR products were transformed into *KSP1OX* using standard techniques, and then the transformed cells were plated on the SD/-HIS (0.67% yeast nitrogen base, 2% glucose, and the appropriate amino acid) agar media (Clontech) containing the kanamycin derivative G-418 at 200 mg/L. The grown colonies were further confirmed by PCR.

YPD medium containing 1% Difco yeast extract, 2% Difco peptone, and 2% dextrose glucose was used for yeast cell culture, the solid YPD plate containing 2% agar.

2.2. Plasmid construction and yeast transformation

A high-copy yeast expression vector pAUR123 with a strong promoter, *ADH1*, was used to ensure overexpression of *CTT1* or *KSP1*. For the construction of plasmid pAUR123CTT1, the *CTT1* ORF was amplified from wild-type yeast genomic DNA using PCR and primers containing *SalI* sites (5′-TATGTCGACATGAACGTGTTCGGTAAAAAA-3′) and *XbaI* sites (5′-GC GTCTAGATTAATTGGCACTTGCAATGG-3′). For the construction of plasmid pAUR123KSP1, the *KSP1* ORF was amplified from the wild-type yeast genomic DNA and primers containing *SalI* sites (5′-CGCGTCGACATGACTTTAGATTACGAGAT-3′) and *Hpa*I sites (5′-CGCGTT AACTTAGTCTTGTTGCTGTAAAAT-3′). The PCR reaction products were cloned into the empty pAUR123. The recombinant plasmids were transformed into the *E. coli* strain DH5α. DNA sequencing analysis of these plasmids was performed by Sangon (Shanghai, China).

The plasmids pAUR123KSP1 and pAUR123CTT1 were introduced into *ksp1Δ* or wild-type cells, respectively, as reported previously (Ito et al., 1983). The transformants were screened on YPD medium plates containing 0.2 μg/mL Aureobasidin A (AbA) at 30 °C for 2 days. A transformant that introduced pAUR123 was also prepared as a control strain. These overexpression strains were verified by PCR.

2.3. Growth rate determination

The growth rates were determined in the cell culture plates using the Bioscreen C machine (Growth Curves USA) (Tauk-Tornisielo et al., 2007; Delaney et al., 2013). First, a single colony was inoculated into YPD medium and was grown overnight at 30 °C with shaking. Next, 2.5 μl of the culture was inoculated into 147.5 μl of fresh YPD medium or MMS-added YPD medium in culture plates, the plates were constantly shaken at 30 °C for more than 48 h, and finally the optical density (OD) values were recorded at 600_{nm} every 120 min. The experiment was repeated three times, and the averages were used to generate the growth curves. Statistical significance was calculated by the Friedman Test. $p < 0.05$ was considered to be a significant difference.

2.4. Spot assay

The traditional spot assays were used to monitor the resistance of yeast to MMS. Briefly, 5 ml of YPD medium was inoculated with a colony of yeast, followed by culture overnight at 30 °C. Next, the overnight culture was inoculated into 5 ml of fresh YPD and was grown to the exponential phase. Cells were washed once with 1 M sorbitol, and the concentration was adjusted to an OD_{600} of 0.1. The cells were then diluted with sterile PBS in a 5-fold series, and 5 μl of each dilution was spotted onto solid medium with or without