THE BULLETIN OF OKAYAMA UNIVERSITY OF SCIENCE No. 53 A pp. 47-51 (2017)

Effect of a selectable marker *ura4*$^{+}$ gene on survival of stationary-phase cells of the fission yeast cultured in a synthetic defined medium

Takanori SENOO, Naoya KATAYAMA*, Atori NAKAMURA, Shinji KAWANO*, and Shogo IKEDA*

Graduate School of Science,
**Department of Biochemistry, Faculty of Science,*
Okayama University of Science,
1-1 Ridai-cho, kita-ku, Okayama 700-0005, Japan.

(Received October 26, 2017; accepted December 4, 2017)

The chronological lifespan (CLS) in yeast is defined as the survival time of non-dividing cells such as cells in the stationary phase during growth. Deletion mutants of the fission yeast (*Schizosaccharomyces pombe*) have been constructed by replacement of target gene with a selectable marker such as the *ura4*$^{+}$ gene. During CLS assays of various mutants, we noticed that the *ura4*$^{+}$ marker might have affected the survival of stationary-phase cells when mutant cells were cultured in a synthetic defined (SD) medium. In this study, we compared the CLS of *ura4*$^{+}$ strains with that of several *ura4*$^{-}$ mutants and show that the *ura4*$^{+}$ gene may reduce the survival of stationary-phase cells cultured in SD medium. The shortened CLS phenotype of the *ura4*$^{+}$ strain was not observed when cells were cultured in a rich medium YES. Although the reason for this phenomenon is unclear, caution must be taken when the CLS of deletion mutants carrying the *ura4*$^{+}$ marker is measured in SD medium.

Keywords: chronological lifespan; fission yeast; SD medium; stationary phase; *ura4*$^{+}$ gene.

Introduction

Yeast is a popular model organism due to its amenability to genetic, molecular and cell biological analyses, and has been used to study numerous biological processes. The chronological lifespan (CLS) in yeast is defined as the survival time of non-dividing cells such as stationary-phase cells in cell growth and cells in the G_0 phase of the cell cycle. CLS of the budding yeast *Saccharomyces cerevisiae* has been successfully used to identify key pathways responsible for the regulation of aging (Fabrizio and Longo, 2007; Longo et al., 2012). Recently, the fission yeast *Schizosaccharomyces pombe* has been developed for another microbial aging model, and the genes have been found to extend CLS when deleted or overexpressed (Ohtsuka et al., 2008; Chen & Runge, 2009; Roux et al., 2010; Zuin et al., 2010). The *S. pombe ura4*$^{+}$ gene, which encodes orotidine 5'-phosphate decarboxylase, is one of the most widely used selectable markers for gene disruption. During the CLS assays of various *S. pombe* mutants, we noticed that the *ura4*$^{+}$ marker affected the survival of stationary-phase cells when mutant cells were cultured in a synthetic defined (SD) medium. In this study, we compared the CLS of *S. pombe ura4*$^{+}$ strains with that of *ura4*$^{-}$ mutants and showed that the *ura4*$^{+}$ gene may accelerate chronological aging of yeast in SD medium.

2. Materials and Methods

2.1. Strains.

The fission yeast strains used in this study are listed in Table 1. Disruption of the *apn2* gene in ED0665 strain by the *kan*MX6 marker was conducted as follows. The genomic sequence of *apn2* was amplified

Table 1. Yeast strains used in this study.

Strain	Genotype	Source
FY7507 (L972)	*h*⁻	[a]YGRC
FY6843	*h*⁻ *ura4*-D18	[a]YGRC
FY6839	*h*⁻ *ade6*-M210 *leu1*	[a]YGRC
ED0665	*h*⁻ *ade6*-M210 *leu1*-32 *ura4*-D18	ATCC No. 96993
AY01	*h*⁻ *ade6*-M210 *leu1*-32 *ura4*-D18 *apn2*::*ura4*$^{+}$	Sugimoto et al., 2005
KK14	*h*⁻ *ade6*-M210 *leu1*-32 *ura4*-D18 *apn2*::*kan*MX6	This study

[a]YGRC: Yeast Genetic Resource Center

by PCR with the following primers: 5′-CCC CAA TTT ACA TCC TTG TAT CC-3′ and 5′-ACT GTC AAC CCT ATG TCA GAT TC-3′. The gene fragment was then subcloned into pGEM-T vector (Promega) by TA cloning. The *kan*MX6 marker was amplified from pFA6a-kanMX6 (Bähler et al., 1998) and inserted between *Eco*RI and *Bbv*CI sites of *apn2* cloned in pGEM-T. The *apn2*::*kan*MX6 disruption cassette was amplified with the following primers: 5′-GCA CAA CAT GTA TAC TCG TTT AAT GAC-3′ and 5′-GAA GCA GAT ACG TTT TCG CTC TG-3′. The amplified DNA fragment was used to transform haploid strain ED0668 with lithium acetate (Bähler et al., 1998). The genotype of the mutant was verified by PCR.

2.2. Media.

SD medium contains 0.67% yeast nitrogen base without amino acids (BD Difco) and 3% glucose, supplemented with 75 μg/mL each of adenine, leucine, and uracil. YES medium contains 0.5% yeast extract and 3% glucose, supplemented with 225 μg/mL each of adenine, leucine, and uracil.

2.3. CLS assay.

CLS assays of *S. pombe* cells were carried out as follows (Ogata et al., 2016; Senoo et al., 2017). The cells were inoculated at an initial cell optical density (OD) of OD_{600} 0.1 in 10 mL of SD or YES medium in a test tube (25 mm diameter, 200 mm long), and grown at 28°C on a reciprocal shaker (250 rpm). Turbidity of the culture in each growth phase was monitored by measuring OD_{600}, and cell number was counted using a Z1 particle counter (Beckman Coulter). At the time of sampling, aliquots of cultures were taken and serially diluted in YES medium. Multiple dilutions were then plated on YES plates in triplicate and grown at 28°C for 4 days. Colonies were counted and used to calculate the number of colony forming units per mL (CFU/mL) of the culture. Cell viability at each time

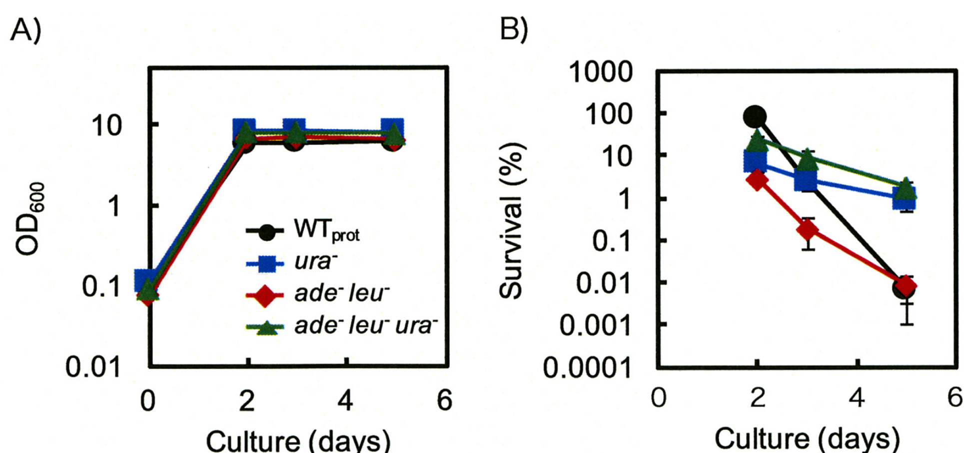

Fig. 1. CLS of various fission yeast strains growing in SD medium. Cell growth (A) and survival in the stationary phase (B) of FY7507 (WT_{prot}, circle), FY6843 (*ura*⁻, square), FY6839 (*ade*⁻ *leu*⁻, diamond), and ED0665 (*ade*⁻ *leu*⁻ *ura*⁻, triangle) were measured as described in the Materials and Methods. CFU of the culture cells at each point was measured by spreading the cells on three YES plates and percentage survival was expressed as the mean ± standard deviation (SD).

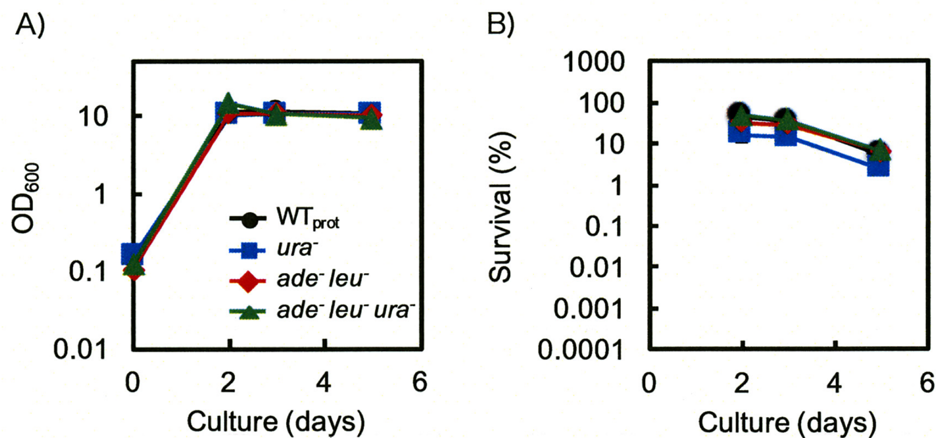

Fig. 2. CLS of various fission yeast strains growing in YES medium. Cell growth (A) and survival in the stationary phase (B) of FY7507 (WT_{prot}, circle), FY6843 (*ura*⁻, square), FY6839 (*ade*⁻ *leu*⁻, diamond), and ED0665 (*ade*⁻ *leu*⁻ *ura*⁻, triangle) were measured. Percentage survival was expressed as the mean ± SD.

point was calculated by dividing the CFU by the total number of cells and plotted on a log scale with error bars.

3. Results and Discussion

We have thus far constructed various gene deletion mutants of *S. pombe* using an auxotrophic mutant ED0665 as the parental strain by displacing the targeted gene with a selectable marker such as the *ura4*⁺ gene (Sugimoto et al., 2005; Tanihigashi et al., 2006; Kanamitsu et al, 2007; Kanamitsu & Ikeda 2011). In the *S. pombe* CLS assay, cells are usually cultured in synthetic medium such as EMM (Edinburgh minimal medium) and SD medium, as well as in a rich medium YES (Chen & Runge, 2009; Roux et al, 2009; Lin & Austriaco, 2014). During the measurement of yeast CLS in SD medium, we noticed that the majority of deletion mutants constructed by displacement using *ura4*⁺ exhibited a shortened CLS phenotype compared with the CLS of parental strain ED0665. This observation hinted at the possibility that the *ura4*⁺ marker affects the survival of stationary-phase cells when these mutants were cultured in SD medium.

To test this hypothesis, we measured CLS in SD medium of a prototrophic strain (FY7507, WT_{prot}) and three auxotrophic mutants with different nutritional requirements. FY6843 is an *ura4*⁻ (*ura4*-D18) mutant that requires uracil. FY6839 is an *ade*⁻ *leu*⁻ (*ad6*-M210 *leu1*-32) mutant that requires adenine and leucine. ED0665 is an *ade*⁻ *leu*⁻ *ura*⁻ (*ad6*-M210 *leu1*-32 *ura4*-D18) mutant that requires uracil, adenine, and

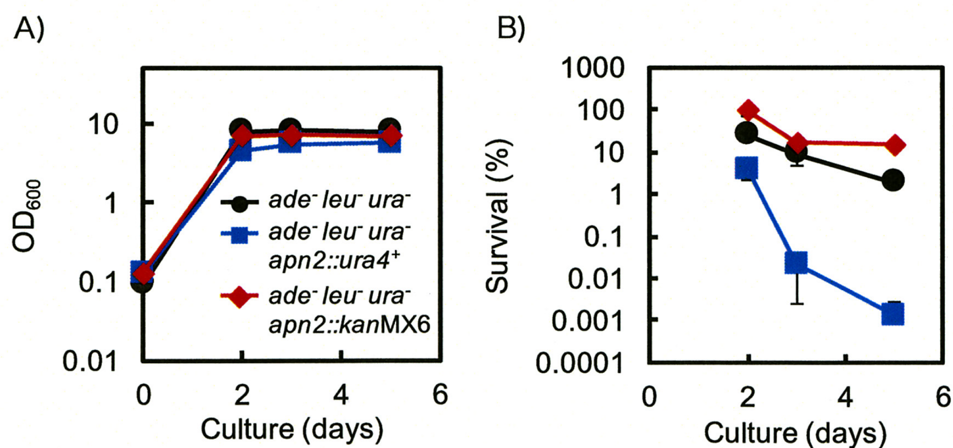

Fig. 3. CLS of various fission yeast strains growing in SD medium. Cell growth (A) and survival in the stationary phase (B) of ED0665 (*ade*⁻ *leu*⁻ *ura*⁻, circle), AY01 (*ade*⁻ *leu*⁻ *ura*⁻ *apn2*::*ura4*⁺, square), and KK14 (*ade*⁻ *leu*⁻ *ura*⁻ *apn2*::*kan*MX6, diamond) were measured. Percentage survival was expressed as the mean ± SD.

leucine. All auxotrophic mutants grew at the same rate as WT_{prot} cells under aerobic conditions in SD medium supplemented with adenine, leucine, and uracil (Fig. 1A). After 2 days of culture, the cell density of these strains reached saturation with an OD_{600} of approximately 10 (3–4 × 10^7 cells/mL, by Coulter counter). In CLS assays, survival of the *ura*⁻ and *ade*⁻ *leu*⁻ *ura*⁻ strains were gradually reduced after entry into the stationary phase (Fig. 1B). The survival percentage of those cells in a 5-day culture declined to one tenth of that in a 2-day culture. In contrast, the cell viability of WT_{prot} and *ade*⁻ *leu*⁻ strains decreased dramatically to approximately 10^{-3} to 10^{-4} after 5 days of culture. These results suggest that *ura4*⁺ may play a negative role in the survival of stationary-phase cells cultured in SD medium.

A rich medium YES contains YE (0.5% yeast extract and 3% glucose) plus nutritional supplements adenine, leucine, and uracil. WT_{prot} and all auxotrophic mutants grew at the same rate in YES medium and ceased cell division at 2 days of culture (Fig. 2A). Unlike in SD medium, the CLS of WT_{prot} and *ade*⁻ *leu*⁻ strains cultured in YES was the same as the CLS of the *ura*⁻ and *ade*⁻ *leu*⁻ *ura*⁻ strains (Fig. 2B). This indicates that the shortened CLS phenotype of the *ura4*⁺ strain is limited to those cells cultured in SD medium.

*kan*MX6 is a selective marker that confers resistance to G18 antibiotics (Bähler et al., 1998). The *ura4*⁺ and *kan*MX6 marker were used separately to displace a DNA repair gene *apn2* in the *ade*⁻ *leu*⁻ *ura*⁻ strain (ED0665), and the CLS of these deletion mutants was compared with that of the parental strain. Auxotrophy of the *apn2*::*kan*MX6 mutant is *ade*⁻ *leu*⁻ *ura*⁻, whereas the *apn2*::*ura4*⁺ mutant is *ade*⁻ *leu*⁻. Both *apn2* mutants grew at the same rate as the parental strain in SD medium supplemented with adenine, leucine, and uracil (Fig. 3A). In CLS assays, survival of the parental strain and the *apn2*::*kan*MX6 mutant was slowly reduced after entry into the stationary phase (Fig. 3B). In contrast, cell viability of the *apn2*::*ura4*⁺ mutant was markedly decreased to approximately 10^{-4} after 5 days of culture. This result supports the hypothesis that *ura4*⁺ may reduce the survival of stationary-phase cells cultured in SD medium.

The *S. pombe ura*⁻ strain is known to undergo drastic cell lysis in medium containing a polypeptone such as YPD, and cell lysis was also induced to a lesser extent when this strain was grown on YE (Matsuo et al., 2013; Nishino et al., 2015). This phenomenon is unlikely to be related to the pro-aging phenotype of the *ura4*⁺ strain in SD medium.

We showed in this study that gene disruption using the *ura4*⁺ selectable marker possibly results in a shortened CLS phenotype of the mutant in SD medium. Although the reason for this phenomenon is unclear, CLS of the mutants constructed with the *ura4*⁺ marker should be carefully estimated when these cells are cultured in SD medium.

Acknowledgements: We would like to thank Mr. Masaaki Ikeda and Dr. Kyoichiro Kanamitsu for construction of the *apn2* mutant KK14. Yeast strains FY7507, FY6843, and FY6839 were provided by the National Bio-Resource Project (NBRP), Japan.

References

Bähler, J., Wu, J. Q., Longtine, M. S., Shah, N. G., McKenzie, A., 3rd, Steever, A. B., Wach, A., Philippsen, P., and Pringle, J. R. (1998) Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. *Yeast* **14**, 943-951

Chen, B. R., and Runge, K. W. (2009) A new *Schizosaccharomyces pombe* chronological lifespan assay reveals that caloric restriction promotes efficient cell cycle exit and extends longevity. *Exp Gerontol* **44**, 493-502

Fabrizio, P., and Longo, V. D. (2007) The chronological life span of *Saccharomyces cerevisiae*. *Methods Mol Biol* **371**, 89-95

Kanamitsu, K., and Ikeda, S. (2011) Fission yeast homologs of human *XPC* and *CSB*, *rhp41* and *rhp26*, are involved in transcription-coupled repair of methyl methanesulfonate-induced DNA damage. *Genes Genet Syst* **86**, 83-91

Kanamitsu, K., Tanihigashi, H., Tanita, Y., Inatani, S., and Ikeda, S. (2007) Involvement of 3-methyladenine DNA glycosylases Mag1p and Mag2p in base excision repair of methyl methanesulfonate-damaged DNA in the fission yeast *Schizosaccharomyces pombe*. *Genes Genet Syst* **82**, 489-494

Lin, S. J., and Austriaco, N. (2014) Aging and cell death in the other yeasts, *Schizosaccharomyces pombe* and *Candida albicans*. *FEMS Yeast Res* **14**, 119-135

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

Matsuo, Y., Nishino, K., Mizuno, K., Akihiro, T., Toda, T., Matsuo, Y., Kaino, T., and Kawamukai, M. (2013) Polypeptone induces dramatic cell lysis in ura4 deletion mutants of fission yeast. *PLoS ONE* **8**, e59887

Nishino, K., Kushima, M., Matsuo, Y., Matsuo, Y., and Kawamukai, M. (2015) Cell lysis in *S. pombe ura4* mutants is suppressed by loss of functional Pub1, which regulates the uracil transporter Fur4. *PLoS ONE* **10**, e0141796

Ogata, T., Senoo, T., Kawano, S., and Ikeda, S. (2016) Mitochondrial superoxide dismutase deficiency accelerates chronological aging in the fission yeast *Schizosaccharomyces pombe*. *Cell Biol Int* **40**, 100-106

Ohtsuka, H., Mita, S., Ogawa, Y., Azuma, K., Ito, H., and Aiba, H. (2008) A novel gene, ecl1(+), extends the chronological lifespan in fission yeast. *FEMS Yeast Res* **8**, 520-530

Roux, A. E., Chartrand, P., Ferbeyre, G., and Rokeach, L. A. (2010) Fission yeast and other yeasts as emergent models to unravel cellular aging in eukaryotes. *J Gerontol A Biol Sci Med Sci* **65**, 1-8

Roux, A. E., Leroux, A., Alaamery, M. A., Hoffman, C. S., Chartrand, P., Ferbeyre, G., and Rokeach, L. A. (2009) Pro-aging effects of glucose signaling through a G protein-coupled glucose receptor in fission yeast. *PLoS Genetics* **5**, e1000408

Senoo, T., Kawano, S., and Ikeda, S. (2017) DNA base excision repair and nucleotide excision repair synergistically contribute to survival of stationary-phase cells of the fission yeast *Schizosaccharomyces pombe*. *Cell Biol Int* **41**, 276-286

Sugimoto, T., Igawa, E., Tanihigashi, H., Matsubara, M., Ide, H., and Ikeda, S. (2005) Roles of base excision repair enzymes Nth1p and Apn2p from *Schizosaccharomyces pombe* in processing alkylation and oxidative DNA damage. *DNA Repair* **4**, 1270-1280

Tanihigashi, H., Yamada, A., Igawa, E., and Ikeda, S. (2006) The role of *Schizosaccharomyces pombe* DNA repair enzymes Apn1p and Uve1p in the base excision repair of apurinic/apyrimidinic sites. *Biochem Biophys Res Commun* **347**, 889-894

Zuin, A., Castellano-Esteve, D., Ayte, J., and Hidalgo, E. (2010) Living on the edge: stress and activation of stress responses promote lifespan extension. *Aging* **2**, 231-237