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LETTER TO THE EDITOR OPEN

A single circular chromosome yeast

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Dear editor,

Most of the prokaryotic cells contain a single circular chromosome. In contrast, the eukaryotic cells usually contain multiple linear chromosomes. Recently, we artificially created a single linear chromosome yeast strain SY14 from native 16 chromosomes in a haploid *Saccharomyces cerevisiae*, which displays minor fitness defects.[1] In this study, we have created a new yeast strain which contains a single circular chromosome and apparently has not been found in nature.

We used a CRISPR-Cas9 method to induce double-stranded DNA breaks (DSBs) at the regions proximal to two telomeres of the linear chromosome of SY14 (Fig. 1a). Through endogenous homologous recombination, the two DSBs ends were ligated with a donor DNA fragment (Fig. 1a) and this resulted in a new strain designated SY15, which contained a single circular chromosome (Fig. 1a). Immuno-staining of myc-tagged telomere binding protein Sir2[2] showed that one or two telomere signals seen in the SY14 cells were not detected in the SY15 cells (Fig. 1b), suggesting no telomere in SY15. A pulsed-field gel electrophoresis (PFGE) analysis revealed a 1193 kb band in SY15 which was resulted from the fusion of Chr. X and XVI (Supplementary Information, Fig. S1). Both SY14 and SY15 cells showed no detectable changes in the restriction en yme digestion pattern of their genomes compared with their descendant cells at passage 100, suggesting that the single chromosome yeasts are able to maintain stable genomes.

The chromosome conformation capture (3 C)-derived Hi-C assay[3] revealed that the circular chromosome in SY15 displayed similar globular configurations to the linear chromosome in SY14 (Fig. 1c). In SY15, the direct joining of two ends of the single chromosome (Supplementary Information, Fig. S2a) resulted in strong interactions of adjacent regions (Supplementary Information, Fig. S2b). Despite that SY15 lost 46% chromosomal interactions as compared with SY14 and gained 13.5% new interactions (Supplementary Information, Fig. S2c), only 20 genes (0.3% of 5815 genes) were differentially expressed ($\log_2$ (fold change) ≥1 and Padj<0.05) when gene expression profiles of SY15 and SY14 cells were compared (Fig. 1d, Supplementary Information, Table S1). Specifically, 10 genes involved in stress responses were up-regulated in SY15, suggesting that chromosome circular-i ation might have introduced new stresses for yeast cells. Four genes (YPL277C, YPL278C, FEX2, and HSP32) 0002TD(ssi.2024.5((ge

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1.0) of abnormal long-shape cells was observed in SY15 (Supplementary Information, Fig. S3a, b). Phenotype microarray (PM) analysis showed that SY15 and SY14 cells had comparable metabolic activities for 190 carbon sources, 95 nitrogen sources and in 96 pH conditions (Fig. 1f). A modest reduction of metabolic activities under osmolytes conditions (Fig. 1f), e.g., under high concentration (8–10%) of sodium chloride (Supplementary Information, Fig. S4) was detected in SY15 compared to SY14. SY15 cells could undergo cell division as SY14 cells (Fig. 1g), however, SY15 cells displayed a modest reduction of growth rate in both solid (Fig. 1h, higher panels) and liquid media (Supplementary Information, Fig. S5a) and were quickly outcompeted by SY14 cells when they were co-cultured (Supplementary Information, Fig. S5b), indicating a reduced fitness of the single circular chromosome yeast. Notably, when treated with genotoxic chemicals, such as methyl methanesulfonate (MMS), camptothecin (CPT), and phleomycin (Phl), SY15 cells could hardly grow (Fig. 1h, lower panels). These results suggest that the circulari ed chromosome has introduced more hurdles for cell functions. It is known that when subjected to stress some of the yeast chromosomes (i.e. chromosome III) are transiently duplicated in order to increase the expression of genes on these chromosomes.[5] This of course would not be feasible for the yeast strains carrying a single linear or circular chromosome, which may explain the reduced stress tolerance of the single chromosome yeasts reported in this study and in our previous study.[1]

We further examined whether SY15 cells could undergo reproduction sexually. SY15$^{\alpha}$ cells were still capable of mating with the opposite mating type SY15^{a} cells, and formed diploid cells (SY15$^{\alpha}$/ SY15^{a}). However, the mating efficiency of SY15$^{\alpha}$ and SY15^{a} cells was 10 times lower than that of SY14$^{\alpha}$ and SY14^{a} cells. Moreover, the SY15$^{\alpha}$/SY15^{a} diploid cells were unstable, about 15–44% of SY15$^{\alpha}$/ SY15^{a} diploid cells spontaneously converted to haploid cells under normal cultivation conditions. When SY15$^{\alpha}$/ SY15^{a} cells cultured in sporulation medium, no tetrads were detected among 200 examined cells, suggesting that SY15$^{\alpha}$/ SY15^{a} cells have difficulty in meiosis.

Next, we deleted *TLC1* gene, which encodes the RNA template component of telomerase[6] and is essential for telomere replication, in the wide-type BY4742 (32 telomeres), SY14 (2 telomeres) and SY15 (no telomere) strains. The SY14 *tlc1*Δ cells senesced at the fourth re-streak (100 generations) on the plate (Supplementary Information, Fig. S6a) and the eighth passage in liquid medium (Fig. 1i), which was delayed compared to BY4742 *tlc1*Δ cells. SY14 *tlc1*Δ survivors were gradually emerged in both solid and liquid culture (Fig. 1i, Supplementary Information, Fig. S6a). In contrast, the SY15 *tlc1*Δ cells did not show a decline of growth in

Fig. S6a). Telomere Southern hybridi ation revealed that telomeres of SY14 *tlc1*Δ cells shortened along cell passages, and reached to critical length at day 8 when cells were at the senescent state (Supplementary Information, Fig. S6b), indicating that telomere erosion caused cellular senescence. Interestingly, the hybridi ation signals detected in SY14 *tlc1*Δ survivors (passages 9 and 11) were quite similar to those of SY15 *tlc1*Δ cells (Supplementary Information, Fig. S6b), suggesting that the eroded chromosome ends of SY14 *tlc1*Δ cells fused together. These results indicated that yeast cells with a single circular chromosome could bypass the telomerase-dependent senescence. It will be intriguing to

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know whether chromosome circulari ation affects either replicative or chronological aging of yeast cells.

The SY15 strain displays reduced cell growth rate and fitness at conditions tested in this study. The impaired cell growth was also reported in other yeast strains with circulari ation of chromosomes.[7–9] We speculated that the severe reduction of SY15 fitness could be attributed to the difficulties in replicating and/or segregating the circular chromosome. Bacteria with a circular