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 showed that one or two telomere signals seen in the SY14 and in 96 pH conditions (Fig.1f). A modest reduction of metabolic (PM) analysis showed that SY15 and SY14 cells had comparable 1.0) of abnormal long-shape cells was observed in SY15 –linear chromosome of SY14 (Fig.1a). Through endogenous breaks (DSBs) at the regions proximal to two telomeres of the single chromosome yeasts are able to maintain stable genomes. Recently, we artificially created a single linear chromosome yeast strain SY14 from native 16 chromosomes in a haploid *Saccharomyces cerevisiae* haploid cells under normal cultivation conditions. When SY15/ SY13 cells cultured in sporulation medium, no tetrads were detected among ~200 examined cells, suggesting that SY15/ SY13 cells have difficulty in meiosis.

Next, we deleted TLC1 gene, which encodes the RNA template component of telomerase6 and is essential for telomere replication, in the wide-type BY4742 (32 telomeres), SY14 (2 telomeres) and SY15 (no telomere) strains. The SY14 tlcΔ cells senesced at the fourth re-streak (~100 generations) on the plate (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 circularized 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. 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. SY15Δ cells were still capable of mating with the opposite mating type SY15Δ cells, and formed diploid cells (SY15Δ/ SY15Δ). However, the mating efficiency of SY15Δ and SY15Δ cells was ~10 times lower than that of SY14Δ and SY14Δ cells. Moreover, the SY15Δ/SY15Δ diploid cells were unstable, about 15–44% of SY15Δ/ SY15Δ diploid cells spontaneously converted to haploid cells under normal cultivation conditions. When SY15Δ/ SY13Δ cells cultured in sporulation medium, no tetrads were detected among ~200 examined cells, suggesting that SY15Δ/ SY13Δ cells have difficulty in meiosis.

SY15 and SY14 cells were similar in both size and shape (Fig. 1e), however, a slightly higher ratio (2.2% vs 0.6% at OD600 = 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 out-competed 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 circularized 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. 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

In this study, we created a new yeast strain which contains a single circular chromosome and apparently has not been found in nature. In conclusion, the SY15 single linear chromosome strain might be a useful tool for the study of the telomere position effect (TPE).4

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know whether chromosome circularization 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 circularization of chromosomes.\textsuperscript{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 chromosome usually replicates its genome from a single replication origin.\textsuperscript{10} But most archaea with circular chromosomes replicate their DNA using multiple origins,\textsuperscript{11} although the controlling mechanism is not well understood. We speculate that the yeast with a single circular chromosome may also replicate its genome using multiple origins, but this speculation awaits future investigations.

From the evolution point of view, the linear chromosomes are thought to facilitate an organism to produce its progenies...
sexually. However, the emerging of telomeres has imposed many difficulties in cell survival, because telomeres have to be protected by specialized protein complex to avoid fusion and degradation of the linear chromosomal ends. Additionally, due to the end replication problem, telomere replication requires specialized enzyme, i.e. telomerase. Therefore, the evolution of linear chromosome, as well as telomeres and telomerase, for an organism might be a trade-off for gaining more fitness to the environmental challenges.

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AUTHOR CONTRIBUTIONS

Z.Q. and X.X. designed and analyzed all experiments. J.Q.Z., G.Z., and Z.Z. contributed to the experiment designs and data evaluation. Y.S. conducted the characterization of the single circular chromosome yeast SY15 strain. Ligation of two chromosome ends via both CRISPR-Cas9 induced DSBs and homologous recombination. The red arrowheads indicated the indicating sites of Cas9. DR: direct repeat. The URA3 selection marker was further deleted via homologous recombination of two DR regions by negative selection. b The myc-tagged telomere binding protein Sir2 was detected with polyclonal anti-myc antibody and Cy3-conjugated (red) secondary antibody. Nop1, a nucleolar protein, was detected with a monoclonal anti-Nop1 antibody and Alexa 488-conjugated (green) secondary antibody. DNA was stained by DAPI (blue). 1 c 3D conformation of the SY15 genome in comparison to that of SY14. d Classification of differentially expressed genes, defined as those with log2 (fold change) ≥ 1 and P<0.05 in SY15 compared to SY14. Data were collected from three biological replicates. e Scanning electron microscopy pictures of SY14 and SY15 cells. Representative images from three independent experiments. f Heatmap of the Phenotype Microarray profiles of SY14 and SY15 cells. Low to high metabolic activities are depicted by a color spectrum from light blue to yellow. Data were collected from two biological replicates. g Cell cycle analysis. The yeast cells were synchronized with hydroxyurea and the progression of the cell cycle was analyzed by flow cytometry. Data are representative of two independent experiments. h Fitness analysis of SY15 cells under various growth conditions. Representative results of three independent experiments. i Senescence assay in liquid medium. The growth of wild type BY4742 (dark brown), SY14 (dark green), SY15 (dark blue), BY4742 tlc1Δ (light brown), SY14 tlc1Δ (light green), SY15 tlc1Δ (light blue) strains were monitored for 16 days. Every 24 h, the growth of the strains was measured in the value of OD600. The diluted cultures were started from OD600 = 0.01. For each strain, two clones were examined

ADDITIONAL INFORMATION

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