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LETTER TO THE EDITOR

Sua5p is required for telomere recombination in *Saccharomyces cerevisiae*

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Dear Editor,

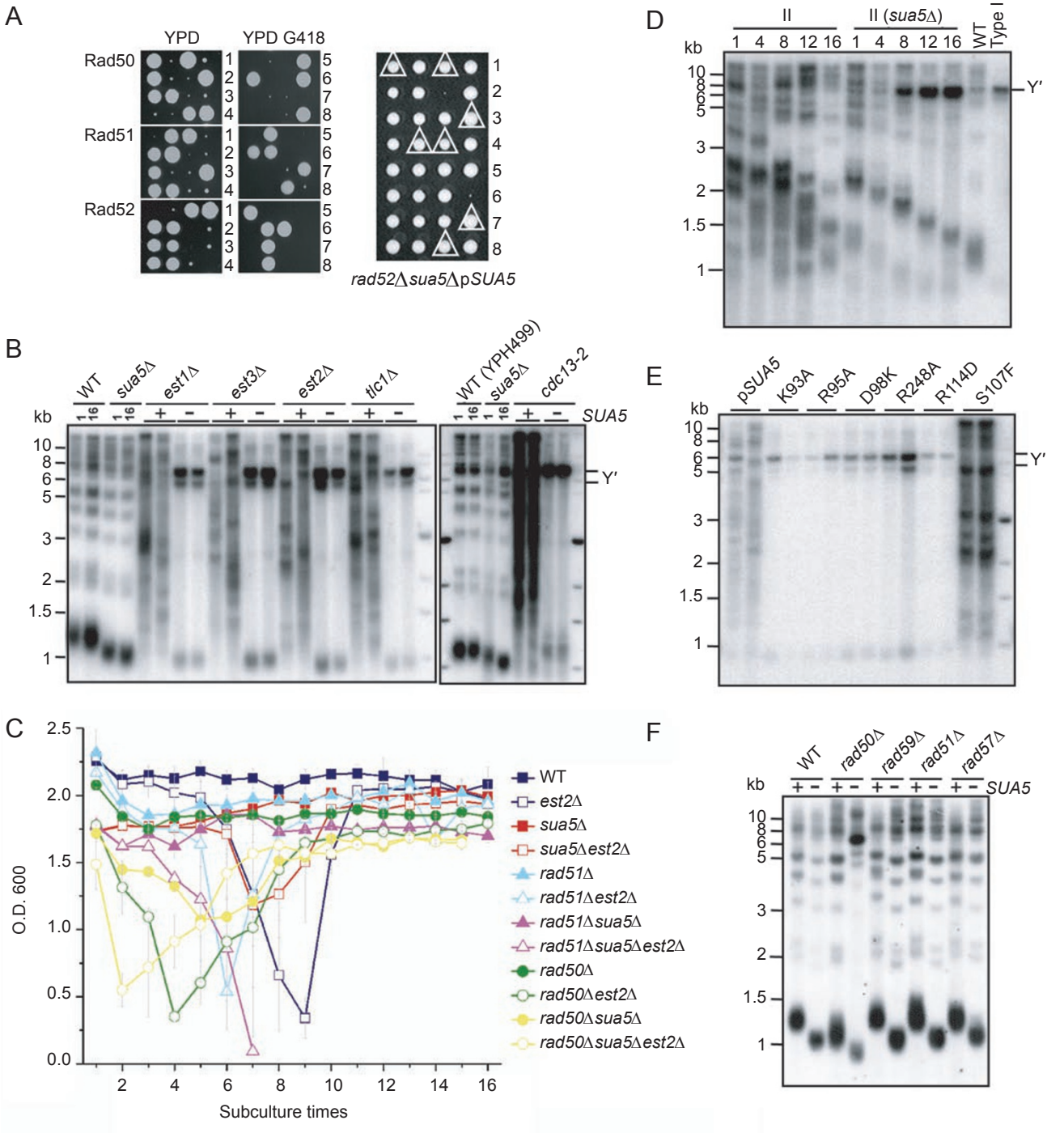
Telomeres are highly organized DNA-protein structures at the ends of linear eukaryotic chromosomes [1]. In budding yeast *Saccharomyces cerevisiae*, each chromosome end has $TG_{1–3}/C_{1–3}A$ double-stranded telomeric DNA and a G-rich single-stranded tail [2]. The replication of telomere involves both telomerase and recombination pathways [3, 4]. When telomerase is absent, telomeric DNA will progressively shorten, and the colony will eventually undergo senescence [4]. Some survivors will escape senescence by maintaining telomeres through a Rad52-dependent homologous recombination mechanism [3]. Our previous work had identified a conserved non-OB-fold single-stranded telomeric (ssTG) DNA binding protein Sua5p, which facilitates telomerase activity [5]. Here we report that Sua5p is also involved in the telomeric DNA recombination in telomerase-negative cells, and type II survivors were completely diminished in the *SUA5* deletion telomerase-negative cells. These two distinct functions of Sua5p are independent of each other, but both require its DNA-binding ability.

We performed a genetic interaction analysis of *SUA5* and other telomere regulation genes including Rad52 epistasis group genes, in which *RAD* genes were replaced by a KanMX4 cassette (conferring G418 resistance to the cells). Notably, *sua5* deletion mutants grew relatively slowly and formed small colonies compared to the wild-type colonies (Figure 1A). Failure of recovering small colonies on YPD-G418 plates indicated that *sua5*Δ and *rad52*Δ are synthetically lethal (Figure 1A). However, double deletion of other genes in the Rad52 epistasis group (i.e. *RAD50*, *RAD51*, *RAD57* and *RAD59*) with *SUA5* did not affect cell viability (Figure 1A left and Supplementary information, Figure S1A), supporting the argument that the genetic interaction of *SUA5* and *RAD52* was not a "sick" phenotype. In addition, a *SUA5* CEN plasmid, containing the Sua5 ORF and Sua5 endogenous promoter and terminator (Supplementary information, Figure S1B), can rescue the synthetic lethality of *rad52*Δ*sua5*Δ (Figure 1A, right). These data suggested that Sua5 may affect recombination pathway(s), e.g. telomere recombination.

In budding yeast, telomerase-deficient cells will undergo senescence after approximately 120 population doublings [4]. Most of the telomerase-deficient cells are subjected to cell cycle arrest at G2/M phase and cell death [4]. Rare survivors, which use homologous recombination to maintain telomere, could recover at a low frequency [4]. Type I survivors have tandem arrays of the subtelomeric Y' element, whereas type II survivors have very long terminal tracts of $C_{1–3}A/TG_{1–3}$ DNA. As Rad52 epistasis group genes are required for telomere recombination maintenance, we wondered whether Sua5 had any role in survivor production. The *est*Δ and *est*Δ*sua5*Δ cells were continuously cultured in liquid medium until the survivor arising. Double deletions of *SUA5* and *EST* genes showed similar senescence rates with the *EST* single-deletion mutants (Supplementary information, Figure S2). As Type II survivors grow much faster than Type I survivors in liquid medium, the *est2*Δ post-senescent survivor cells under this condition were typical Type II according to the telomere pattern (Figure 1B). Interestingly, the *est2*Δ*sua5*Δ post-senescent survivor cells in liquid medium were Type I with amplified Y' subtelomeric elements (Figure 1B). The same results were observed in *est1*Δ*sua5*Δ, *est3*Δ*sua5*Δ and *tlc1*Δ*sua5*Δ post-senescent cells (Figure 1B, left), and *cdc13-2sua5*Δ post-senescent cells of YPH499 background (Figure 1B, right). To confirm the result of liquid senescence assay, we continuously passaged the *est2*Δ*sua5*Δ cells on solid medium. The single-colony survivors were picked at the fifth restreak and subjected to telomere southern blot. Consistent with the result of liquid senescence assay, all the 58 independent *est2*Δ*sua5*Δ post-senescent clones were Type I, while the *est2*Δ post-senescent clones showed a significant fraction of Type II survivors (Supplementary information, Figure S3).

The high-frequency appearance of Type I survivor could be attributed to an elevated rate of Type I survivor formation or a diminished rate of Type II survivor production in *sua5*Δ cells. In the Rad52 epistasis group,

RAD51 and *RAD57* are essential for Type I survivor formation, *RAD50* and *RAD59* are essential for Type II survivor production, and *RAD52* is required for both types arising [6]. Survivors are almost eliminated in *rad50*Δ*rad51*Δ*est2*Δ triple mutant cells similar to *rad52*Δ*est2*Δ cells [7]. If *SUA5* increases Type I survivor production, *rad51*Δ *sua5*Δ *est2*Δ or *rad57*Δ *sua5*Δ *est2*Δ cells will produce Type II survivors. On the other hand, if *SUA5* is essential for Type II survivor production, *rad51*Δ *sua5*Δ *est2*Δ or *rad57*Δ *sua5*Δ *est2*Δ cells will not produce survivors. The isogenic spores were dissected and subjected to senescence assay. The *rad51*Δ *sua5*Δ *est2*Δ and *rad57*Δ *sua5*Δ*est2*Δ triple mutant cells underwent senescence and no survivors were recovered (Figure 1C and Supplementary information, Figure S4). This result suggests that Sua5 is required for Type II sur-



vivor formation in post-senescent cells.

To further address whether Sua5 is essential for the maintenance of Type II survivors, we deleted *SUA5* in diploid Type II survivor cells (*est2*Δ::*LEU2*/*est2*Δ::*URA3*). The *sua5*Δ haploid Type II survivor cells were obtained after tetrad dissection. Continuous passages of *SUA5* Type II survival cells resulted in gradual telomere shortening (Figure 1D) [8]. Notably, the *sua5*Δ Type II survival cells exhibited accelerated telomere shortening and extensive Y' amplification (Figure 1D and Supplementary information, Figure S5). The telomere pattern of the 16th-restreaked *sua5*Δ Type II cells (about 400 generations after sporulation) is similar to that of Type I cells (shown by *Xho*I digestion of Figure 1D, and *Alu*I-*Hae*III-*Hinf*I-*Msp*I digestion of Supplementary information, Figure S5), indicating that *SUA5* deletion abolishes the telomere-telomere recombination in the Type II survivors, and the post-senescent cells have to elongate their telomeres through Y'-element recombination.

Our previous data have shown that Sua5p is a single-stranded telomeric DNA binding protein, and mutations at its DNA-binding region affect its function at telomeres [5]. We then asked whether the recombinational role of Sua5p also requires its DNA-binding activity. Mutant *sua5* alleles that are defective in DNA-binding were transformed into the pre-senescent telomerase-deficient cells, and the cells were continuously passaged until survivors arose. The cells harboring a loss-of-DNA-binding *sua5* allele could only produce Type I survivors in liquid-medium senescence assay (Figure 1E), indicating that the function of Sua5p in recombinational replication also requires its telomeric DNA-binding ability. Our earlier report [5] and the current study showed that Sua5 affected telomerase activity in the wild-type cells and recombination-dependent telomere elongation in telomerase-deficient cells. It was interesting to know whether the telomere shortening phenotype in *sua5*Δ telomerase-positive cells was also caused by the telomere recombination defect. In *rad51*Δ, *rad57*Δ, *rad50*Δ or *rad59*Δ mutant cells, which were defective in either subtelomere or telomere-telomere recombination and could not produce certain type of survivors, *SUA5* deletion further shortened the telomeres (Figure 1F), suggesting that *SUA5* functions independently in telomerase- and recombination-dependent telomere maintenance pathways.

In conclusion, our study on Sua5 function showed that ssTG-binding protein Sua5p also plays a role in telomere recombination in the post-senescent telomerase-negative cells, and its ssTG-binding activity is indispensable for these functions.

(Experimental materials and methods are depicted in the Supplementary information, Data S1)

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Figure 1 (A) Left, Tetrad dissection of *RAD*/*rad*Δ *SUA5*/*sua5*Δ strains on YPD and YPD G418 plates. *Rad52* epistasis group genes were replaced with KanMX4 maker, conferring G418 resistance, and *SUA5* deletion cells grew slowly and formed small colonies. Eight asci labeled as 1 to 8 are shown for each genotype. No small colony was seen in the tetrad dissection result of *RAD52*/*rad52*Δ *SUA5*/*sua5*Δ strains on YPD G418 plate. Right, Tetrad dissection of *RAD52*/*rad52*Δ *SUA5*/*sua5*Δ strain, which harbored a plasmid-borne *SUA5* gene. The genotypes were confirmed by auxotrophic makers. The *rad52*Δ*sua5*Δp*SUA5* clones were indicated by open triangles. Eight asci labeled as 1 to 8 are shown. **(B)** Deletion of *SUA5* eliminates type II survivor production. The survivor types of isogenic strains were determined by telomere southern blot using *Xho*I digested genomic DNAs and hybridized with a TG probe. Individual strains were continuously passaged in YPD liquid medium until survivors arose. Two independent clones of survivors are shown for each telomerase-deficient strain. The *cdc13-2* mutants were constructed in the YPH499 background, and the corresponding wild-type and *sua5*Δ controls in YPH499 were shown in the right panel. The left panel shows strains derived from BY4743. Telomere lengths of first- and sixteenth-passage cultures are shown for wild-type and *sua5*Δ strains. "+" and "−" indicate wild-type *SUA5* and *sua5*Δ, respectively. **(C)** Senescence assay of deletion mutants lacking telomere recombination genes. Serial passages were initiated with O.D600 ~0.05, and the cell numbers were measured by spectrometry. The X-axis is normalized to the passaged times as *sua5*Δ grew slowly. No survivor was recovered in the triple-deletion strain of *est2*Δ*sua5*Δ*rad51*Δ after the seventh subculturing. **(D)** Type II survivor switches to type I survivor upon deletion of *SUA5*. Sibling spores of *SUA5/sua5*Δ type II cells were restreaked on the YPD plate for 16 times. The genomic DNAs were digested with *Xho*I, and telomeres were examined by southern blot with a TG probe. Restreak times are labeled on the top of the panel. The Y' band is indicated on the right. **(E)** Loss-of-DNA-binding mutants (K93A, R95A, D98K, R248A and R114D) cannot produce type II survivor cells. The genomic DNAs were digested with *Xho*I, and telomeres were examined by southern blot with a TG probe. **(F)** Deletion of *SUA5* in telomere-recombination deficient cells further shortens telomere length. Telomere lengths of single- and double-deletion mutants of *RAD51*, *RAD57*, *RAD50* or *RAD59* with *SUA5* are shown. "+" and "−" indicate wild-type *SUA5* and *sua5*Δ, respectively. The genomic DNAs were digested with *Xho*I, and telomeres were examined by Southern blot with a TG probe.

to JQZ.

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References

1 Dubrana K, Perrod S, Gasser SM. Turning telomeres off and on. *Curr Opin Cell Biol* 2001; **13**:281-289.
2 Vega LR, Mateyak MK, Zakian VA. Getting to the end: telomerase access in yeast and humans. *Nat Rev Mol Cell Biol* 2003; **4**:948-959.
3 Lundblad V, Blackburn EH. An alternative pathway for yeast telomere maintenance rescues est1-senescence. *Cell* 1993; **73**:347-360.
4 Lundblad V, Szostak JW. A mutant with a defect in telomere elongation leads to senescence in yeast. *Cell* 1989; **57**:633-643.
5 Meng FL, Hu Y, Shen N, *et al.* Sua5p a single-stranded telomeric DNA-binding protein facilitates telomere replication. *Embo J* 2009; **28**:1466-1478.
6 Le S, Moore JK, Haber JE, Greider CW. RAD50 and RAD51 define two pathways that collaborate to maintain telomeres in the absence of telomerase. *Genetics* 1999; **152**:143-152.
7 Teng SC, Chang J, McCowan B, Zakian VA. Telomerase-independent lengthening of yeast telomeres occurs by an abrupt Rad50p-dependent, Rif-inhibited recombinational process. *Mol Cell* 2000; **6**:947-952.
8 Teng SC, Zakian VA. Telomere-telomere recombination is an efficient bypass pathway for telomere maintenance in Saccharomyces cerevisiae. Mol Cell Biol 1999; **19**:8083-8093.

(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)