hpr1Δ Affects Ribosomal DNA Recombination and Cell Life Span in Saccharomyces cerevisiae

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Multiple genetic pathways have been shown to regulate life span and aging in the yeast Saccharomyces cerevisiae. Here we show that loss of a component of the RNA polymerase II complex, Hpr1p, results in a decreased life span. Although hpr1Δ mutants have an increased rate of recombination within the ribosomal DNA (rDNA) array, this is not accompanied by an increase in extrachromosomal rDNA circles (ERCs). Analyses of mutants that affect replication of the rDNA array and suppressors that reverse the phenotypes of the hpr1Δ mutant show that the reduced life span is associated with increased genomic instability but not with increased ERC formation. The hpr1Δ mutant acts in a pathway distinct from previously described mutants that reduce life span.

Cellular or organismal life span is determined by genetic and extrinsic factors. Toward the end of the life span, cells often deteriorate, giving rise to the phenotypes associated with aging. Recently, experimental systems have been developed that now permit the identification of genes that influence life span. Often, although not always, mutations that reduce life span result in the phenotype of an early appearance of aging features (32). The ability to tolerate stress and the effects of caloric restriction are correlated with increased life span in Caenorhabditis elegans, Drosophila melanogaster, and Saccharomyces cerevisiae (for reviews, see references 18, 23, 33, 51, and 57). Recognition of the contribution that these processes have on life span has led to an identification of some of the genetic components underlying life span and senescence in various experimental organisms (12, 19).

In addition to the above genetic factors, in the yeast S. cerevisiae life span is influenced by gene silencing. This is thought to act by silencing within the ribosomal DNA (rDNA) array, which is composed of 100 to 200 tandem repeats. The Sir2 protein, encoding an NAD-dependent histone deacetylase, is localized to the silenced genomic regions that are the telomeres (16), the HML and HMR mating-type genes (40), and the rDNA array to form a silenced state of chromatin (4, 25, 50, 52). The silenced rDNA array is repressed for recombination (15). The products of rDNA recombination, excised circles with rDNA repeats, which are termed ERCs (for extrachromosomal rDNA circles), have been suggested to be correlated with aging, but this metric of aging has been challenged in recent studies (21, 27, 28). Nonetheless, instability within the rDNA has been correlated with reduced life span in a number of mutants, including the sgs1 and sir2 mutants (9, 24, 31, 42, 48). Sgs1p is the yeast homolog of the Escherichia coli RecQ helicase and the human BLM and WRN proteins. Since the topoisomerase genes TOP1, TOP2, and TOP3 function in maintaining wild-type levels of recombination within the rDNA and the Top2 and Top3 proteins interact with the Sgs1 protein (13, 56), other mutants that affect rDNA stability may have an interaction with the topoisomerase genes. To further explore the relationship between rDNA instability and life span, we examined the hpr1Δ mutant of S. cerevisiae.

The yeast hyperrecombination mutant hpr1Δ increases intrachromosomal recombination between non-rDNA direct repeats by almost 1,000-fold (2). In those studies we noted a slight enhancement of recombination within the rDNA array in the hpr1Δ mutant strain (2). Other phenotypes of the hpr1Δ mutant also suggested that Hpr1p had a function in the stabilization of the rDNA array. First, a full complement of topoisomerase activities is necessary for viability in an hpr1Δ background (2). Second, hpr1Δ cells have aberrant ring-shaped nucleoli and general defects in rRNA processing when shifted to a nonpermissive growth temperature (37°C) (46). Although the exact biochemical function of Hpr1p remains unclear, it has a functional role in RNA polymerase II transcription, and most genetic suppressors of hpr1Δ-stimulated hyperrecombination are mutations in components of the RNA polymerase II transcription machinery (10, 11, 37, 38, 45, 54). Furthermore, the hyperrecombination phenotype of hpr1Δ is dependent on RNA polymerase II transcription (10), specifically transcriptional elongation (6). Hpr1p has recently been identified as a component of at least two unique protein complexes, each with both biochemical and genetic associations with RNA polymerase II transcription. One biochemically defined complex containing Hpr1p, Paflp, Ccr4p, and Cdc73p appears to be a subunit of an alternative RNA polymerase II holoenzyme with distinct regulatory functions (5), while another biochemically defined complex is composed of Hpr1p, Tho2p, Mft1p, and Thp2 and functions in transcriptional elongation (7).

In this study we have reinvestigated the effect of the hpr1Δ mutation on rDNA recombination. We demonstrate that hpr1Δ does indeed affect the stability of the rDNA repeat array and life span in yeast in an ERC-independent process. The
hpr1Δ effect on life span is independent of other mutants known to affect yeast life span and suggests that transcription effects have an important role in cellular life span.

MATERIALS AND METHODS

Strains and growth media. The S. cerevisiae strains used in this study are listed in Table 1. All strains are isogenic and have the W303 RAD56 background. The W303R strain contains a single copy of ADE2 inserted into the rDNA repeat array and is described elsewhere (24). The strains with the ADE2 marker inserted into the rDNA array were used for obtaining rDNA recombination rates. The non-ADE2 marker strains were used for life span analysis. For ADE2 strains, the foB1::URA3 disruption was generated by a targeted PCR method described earlier (3, 41). The foB1::URA3 marker was lost after the first cell division after plating. Fully red colonies can only be the result of marker loss prior to plating and are not used in the analysis. The recombination rate is determined by considering only the first cell division after plating and is calculated by dividing the total number of half-sectored colonies by the total number of colonies (white plus half-sectors plus partial sectors). The Student’s t test was used to analyze significance between rDNA recombination rates.

Segregation of old cells. The segregation of old cells from the majority of young cells in a mid-log phase culture was accomplished by using the techniques described in detail elsewhere (47, 49). Briefly, cells were cultured at 30°C in liquid YPD medium to mid-log phase (optical density at 600 nm [OD600] = 0.7). Cells were collected by centrifugation, washed twice in phosphate-buffered saline (PBS) buffer, and resuspended in 1 ml of PBS. Approximately 10 mg of sulfo-NHS-LC-biotin (Pierce) was added to the cells, and the mixture as gently shaken at room temperature for 15 min. Cells were washed eight times in 1 ml of PBS and then resuspended in 1 ml of YPD liquid medium. Then, 10 mg of biotinylated culture were resuspended into 1 liter of YPD containing 3% glucose, followed by growth at 30°C overnight for ca. 12 to 13 h (cultures were not allowed to exceed an OD600 of 1.0). Cells were collected by centrifugation and resuspended in 40 ml of cold PBS. Next, 300 μl of streptavidin-coated magnetic beads (PerSeptive Biosystems; 5 μg/μl, washed and equilibrated with PBS) was added to the cells, and the cells were kept on ice for 4 h with occasional mixing. Cells were collected by 10 ml test tubes and placed in a magnetic sorber for 15 min at 4°C. Free cells were carefully removed with a pipette. The bead-bound cells were then resuspended in 10 ml of ice-cold YPD and placed back into the magnetic sorber at 4°C. This washing procedure was repeated eight times before the cells were pooled and stored on ice prior to DNA isolation. An aliquot of cells was used for staining with Calcofluor and examination with a fluorescence microscope to count the number of bud scars per cell. Cell from the old cell fraction contained 8 to 10 generations.

Old and young cell DNA isolation and ERC quantitation. DNA preparations from old cells were performed immediately after sorting. Old and young cells were washed in 1 ml of double-distilled water, centrifuged, and resuspended in 0.5 ml of sorbitol solution (1 M sorbitol, 0.1 EDTA [pH 8.0]) containing 30 μg of zymolyase 10140 U (U.S. Biological)/ml. Then, 0.05 ml of 0.26 M β-mercaptoethanol was added, and the cells were incubated at 37°C for 25 min. The resulting spheroplasts were gently centrifuged and resuspended in 0.5 ml of 50 mM Tris (pH 7.8)–20 mM EDTA. The spheroplasts were lysed by adding 0.05 ml of 10% sodium dodecyl sulfate and incubating them at 65°C for 20 min. Then, 0.2 ml of 5 M potassium acetate was added, followed by gentle mixing and incubation on ice for 30 min. Tubes were centrifuged at top speed for 5 min, the supernatant was collected, and the DNA was precipitated with an equal volume of isopro-

### Table 1. Yeast strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>580-10D</td>
<td>MATa RAD5 in W303 background</td>
<td>H. Klein</td>
</tr>
<tr>
<td>HFY824-1A</td>
<td>MATa hpr1Δ::HIS3 RAD5 W303</td>
<td>H. Fan</td>
</tr>
<tr>
<td>RMY212-12D</td>
<td>MATa cdc73Δ::TRP RAD5 W303</td>
<td>This study</td>
</tr>
<tr>
<td>RMY059-2B</td>
<td>MATa sgs1Δ::URA3 RAD5 W303</td>
<td>This study</td>
</tr>
<tr>
<td>RMY058-5A</td>
<td>MATa hpr1Δ::HIS3 sgs1Δ::URA3 RAD5 W303</td>
<td>This study</td>
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<td>H. Fan</td>
</tr>
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</tr>
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</tr>
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<tr>
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<td>MATa sir2Δ::HIS3 RAD5 W303</td>
<td>This study</td>
</tr>
<tr>
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</tr>
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<td>W303R</td>
<td>MATa RDN1::ADE2 RAD5 W303</td>
<td>L. Guarente</td>
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<td>RMY184-8C</td>
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</tr>
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<td>RMY175-11C</td>
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<td>RMY172-5C</td>
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<td>MATa sir2Δ::HIS3 RDN1::ADE2 RAD5 W303</td>
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</tr>
<tr>
<td>RMY193-12C</td>
<td>MATa hpr1Δ::HIS3 sir2Δ::HIS3 RDN1::ADE2 RAD5 W303</td>
<td>This study</td>
</tr>
</tbody>
</table>

* All strains possess the W303 genetic background (ura3-1 leu2-3,112 his3-11,15 trp1-1 ade2-1 can1-100).
The rDNA recombination rates was measured by determining the loss of an ADE2 marker (see Materials and Methods). Average rates were calculated from at least four independent experiments totaling at least 20,000 colonies.

**RESULTS**

**hpr1Δ** cells have an increased rate of rDNA mitotic intrachromosomal recombination. Loss-of-function mutations in the HPR1 gene result in a dramatic increase in mitotic intrachromosomal recombination between direct repeats in non-rDNA chromosomal locations (2). At the time, we had a suggestive effect of a hpr1 mutation on recombination within the rDNA repeat array, but the recombination reporter was not optimal. To more accurately determine whether a hpr1Δ mutant had an increased recombination rate in the rDNA, we measured rDNA recombination by monitoring the loss of an ADE2 marker inserted into the repeat array, by using a half-sector protocol that measures recombination only in the first cell division after plating (see Materials and Methods). We observed that the hpr1Δ strain has a significant increase in rDNA recombination compared to the HPR1 control strain (Table 2). hpr1Δ loses the ADE2 marker at a rate that is threefold higher than the rate with HPR1 (P < 0.005).

The average longevity of hpr1Δ cells is reduced. Since the hpr1Δ mutant has an increased level of rDNA recombination, we next determined whether hpr1Δ had an effect on life span. Yeast life span is measured by counting the number of daughter cells generated from an individual mother cell (see Materials and Methods). The average life span for the W303-RAD5 strain background is 20.1 generations, while the average life span for the hpr1Δ mutant in this background is 13.7 generations (Fig. 1A), which is a significant reduction in life span (P < 0.001) (Table 3). It is interesting that at least some of the difference in the average life span derives from an increased mortality rate in young hpr1Δ cells. Since Hpr1p has been identified as part of a complex containing Cdc73p, Paflp, and Ccr4p interacting with RNA polymerase II, we examined additional mutants to see whether there was a consistent effect on life span. We chose a cdc73Δ strain since this null allele mutant has a greater hyperrecombination phenotype than mutants of other components in this complex and we wanted to determine whether there was a correlation between the hyperrecombination phenotype and life span (5). Similar to hpr1Δ, cdc73Δ has a decreased average life span compared to wild type (P < 0.001). However, the life span curve differs from that of the hpr1Δ strain, particularly in the susceptibility of young cells to an early death.

Since hpr1Δ cells display an increase in rDNA recombination and a decrease in life span, we investigated whether these phenotypes could be attributed to an overabundance of ERCs.

**TABLE 2**. rDNA recombination rates

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Recombination rate (10³) (SD)</th>
<th>Fold difference vs wild type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>1.3 (0.5)</td>
<td></td>
</tr>
<tr>
<td>hpr1</td>
<td>4.0 (0.9)</td>
<td>3.1</td>
</tr>
<tr>
<td>cdc73</td>
<td>1.5 (0.03)</td>
<td>1.1</td>
</tr>
<tr>
<td>fob1</td>
<td>0.5 (0.2)</td>
<td>0.4</td>
</tr>
<tr>
<td>hpr1 fob1</td>
<td>5.6 (1.6)</td>
<td>4.3</td>
</tr>
<tr>
<td>sos1</td>
<td>7.4 (2.6)</td>
<td>5.8</td>
</tr>
<tr>
<td>hpr1 sos1</td>
<td>11.9 (2.6)</td>
<td>9.2</td>
</tr>
<tr>
<td>soh1</td>
<td>1.6 (0.4)</td>
<td>1.2</td>
</tr>
<tr>
<td>hpr1 soh1</td>
<td>1.7 (0.5)</td>
<td>1.3</td>
</tr>
<tr>
<td>sir2</td>
<td>6.3 (0.3)</td>
<td>4.8</td>
</tr>
<tr>
<td>hpr1 sir2</td>
<td>8.3 (0.9)</td>
<td>6.4</td>
</tr>
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</table>

FIG. 1. hpr1Δ cells have a reduced average life span that is FOB1 independent. (A) The life spans for wild-type (WT; 580-10D), hpr1Δ (HFTY824-1A), and cdc73Δ (RMY212-12D) cells were determined. The mean life spans for the wild-type (n = 82), hpr1Δ (n = 53), and cdc73Δ (n = 56) strains were 20.1, 13.7, and 15.2 generations, respectively. The life span curve differs from that of the hpr1Δ strain, particularly in the susceptibility of young cells to an early death.
To accomplish this, we tested both unsorted logarithmically growing cells (young cells) and aged mother cells that had been sorted away from younger cells. We examined ERCS in young cells because, thus far, any factor suggested to have a positive or negative effect on rDNA circle formation in aged cells also shows the same effect in young unsorted cells (9, 21, 24). ERCS accumulation in young cells is a simple measure to determine the relative abundance of ERCS, regardless of whether or not they accumulate prematurely in old cells. Figure 2A shows the levels of ERCS in unsorted young cells. There is no obvious difference in the abundance of the two visible ERCS species observed in young wild-type and young hpr1Δ cells. Quantitating these results by normalizing ERCS levels against a single-copy chromosomal sequence, ACT1, confirmed this observation (Fig. 2B). We found no significant differences between ERCS present in wild-type and hpr1Δ cells when the levels of the two visible ERCS species were combined and averaged from three independent experiments.

We also examined aged wild-type and hpr1Δ cells and observed slight differences in the relative distribution between the two observable ERCS species (Fig. 2A). The lowest-molecular-weight ERCS species is enriched twofold in hpr1Δ cells at the expense of the higher-molecular-weight ERCS. However, there is no indication of major ERCS accumulation in hpr1Δ. Therefore, although hpr1Δ is hyperrecombinogenic for rDNA recombination, this recombination does not produce detectable ERCS, and the shortened life span does not seem to be a direct result of an overabundance of ERCS.

**hpr1Δ-mediated rDNA hyperrecombination and life span is FOB1-independent.** FOB1 is required for the replication fork block that is observed within the rDNA array during replication (29, 30). A fob1Δ mutation results in a decreased rDNA recombination rate, a decreased amount of ERCS, and an increased life span (9). This indicates that the replication block mediated by Fob1p is necessary for the majority of the rDNA recombination events and consequently for ERCS formation in wild-type cells. We used the hpr1Δ fob1Δ double mutant to determine whether the rDNA recombination events observed in hpr1Δ cells are dependent on the Fob1p-mediated replication fork block. The rDNA recombination rate in the hpr1Δ fob1Δ double mutant is statistically the same as that obtained for the hpr1Δ single mutant (Table 2). This suggests that the majority of rDNA recombination events in hpr1Δ are independent of FOB1.

Since the hpr1Δ fob1Δ double mutant has levels of rDNA recombination similar to those of the hpr1Δ strain, we examined whether the double mutant could generate ERCS in a FOB1-independent manner. The hpr1Δ fob1Δ double mutant has almost no detectable ERCS in unsorted young cells and is indistinguishable from the fob1Δ single mutant (Fig. 2A). Quantitation of the ERCS levels in these two strains confirms this observation (Fig. 2B). Therefore, the formation of ERCS in hpr1Δ cells is dependent on FOB1, in spite of rDNA recombination being FOB1 independent. This reinforces the obser-

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**TABLE 3. Average life spans**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Avg life span (no. of generations)*</th>
<th>% Wild-type life span</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>20.1</td>
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</tr>
<tr>
<td>hpr1</td>
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<tr>
<td>cdc73</td>
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<td>fob1</td>
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<td>hpr1 fob1</td>
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<td>sgs1</td>
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<tr>
<td>hpr1 sgs1</td>
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<td>25</td>
</tr>
<tr>
<td>soh1</td>
<td>15.4</td>
<td>77</td>
</tr>
<tr>
<td>hpr1 soh1</td>
<td>15.2</td>
<td>76</td>
</tr>
<tr>
<td>sir2</td>
<td>12.8</td>
<td>64</td>
</tr>
<tr>
<td>hpr1 sir2</td>
<td>8.2</td>
<td>41</td>
</tr>
</tbody>
</table>

*Average life spans for all the strains in the present study.

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![FIG. 2. ERC accumulation in young and old cells.](http://mcb.asm.org/)

(A) DNA was isolated from young cells (logarithmically growing cultures) and old cells (ca. 8 to 10 generations old) to determine the levels of ERCS in unsorted young cells. There is no obvious difference in the abundance of the two visible ERCS species observed in young wild-type and young hpr1Δ cells. Quantitating these results by normalizing ERCS levels against a single-copy chromosomal sequence, ACT1, confirmed this observation (Fig. 2B). We found no significant differences between ERCS present in wild-type and hpr1Δ cells when the levels of the two visible ERCS species were combined and averaged from three independent experiments.

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**hpr1Δ-mediated rDNA hyperrecombination and life span is FOB1-independent.** FOB1 is required for the replication fork block that is observed within the rDNA array during replication (29, 30). A fob1Δ mutation results in a decreased rDNA recombination rate, a decreased amount of ERCS, and an increased life span (9). This indicates that the replication block mediated by Fob1p is necessary for the majority of the rDNA recombination events and consequently for ERCS formation in wild-type cells. We used the hpr1Δ fob1Δ double mutant to determine whether the rDNA recombination events observed in hpr1Δ cells are dependent on the Fob1p-mediated replication fork block. The rDNA recombination rate in the hpr1Δ fob1Δ double mutant is statistically the same as that obtained for the hpr1Δ single mutant (Table 2). This suggests that the majority of rDNA recombination events in hpr1Δ are independent of FOB1.

B. ERC levels relative to WT. The circle indicates chromosomal rDNA; the arrows indicate two identifiable ERC species. (B) ERC levels of each mutant relative to the wild type in young cells. The levels indicated are the averages from three additional independent experiments. The error bars represent the standard deviations from each set of experiments.
served in the levels of the two detectable ERC species when

/h9004

hpr1

reduction in average life span of the
significantly decreased life span compared to the life spans of

examined the
array (13, 55), as well as a decrease in life span (48). We
genomic instability both within the rDNA array and in non-
hpr1

The decreased life span of
rDNA recombination and life span.
The effect of both mutations on the rDNA recombination include expansions and contractions of repeat sequence tracts during replication slippage events and changes in the number of rDNA array units through gene conversion events (14).

As fob1Δ suppresses the formation of ERCs in hpr1Δ cells, we examined whether a fob1Δ mutation would suppress the decreased life span of the hpr1Δ mutant. Figure 1B and Table 3 show that the life span of the hpr1Δ fob1Δ double mutant is not significantly different from that of the hpr1Δ single mutant. Taken together, these data indicate that the increased rDNA recombination rate and the decreased life span observed in hpr1Δ are independent of FOB1 and are not correlated with ERC formation.

hpr1Δ has additive effects with both sgs1Δ and sir2Δ for rDNA recombination and life span. The decreased life span of the hpr1Δ mutant is most likely due to the combined effects of genomic instability both within the rDNA array and in non-rDNA chromosomal regions. Another mutant, sgs1Δ, also displays genetic instability within and outside of the rDNA repeat array (13, 55), as well as a decrease in life span (48). We examined the hpr1Δ sgs1Δ double mutant to determine if these two genes acted together or separately in determining life span. The effect of both mutations on the rDNA recombination rate of the hpr1Δ sgs1Δ double mutant was additive (P < 0.05) (Table 2). The hpr1Δ sgs1Δ double mutant also has a significantly decreased life span compared to the life spans of the sgs1Δ and hpr1Δ single mutants (P < 0.001) (Fig. 3). The reduction in average life span of the hpr1Δ sgs1Δ mutant is the product of the reductions in life span of the hpr1Δ and sgs1Δ mutants, showing that these genes act independently on life span (Table 3).

ERCs in young unsorted cells were also examined in the hpr1Δ sgs1Δ double mutant. No significant difference was observed in the levels of the two detectable ERC species when hpr1Δ sgs1Δ was compared to wild type (Fig. 2). We also examined ERC levels in old (8 to 10 generations) sgs1Δ and hpr1Δ sgs1Δ strains. The levels of ERCs in these strains were not in excess compared to age-matched wild-type and hpr1Δ strains (data not shown). Therefore, similar to hpr1Δ, we can find no evidence that suggests sgs1Δ leads to the accumulation of an overabundance of ERCs at any stage in its life span. These data are consistent with recently published results that also failed to reveal an increase in the levels of ERCs in old sgs1Δ strains compared to age-matched wild-type strains (21).

The sir2Δ mutant has increased genetic instability within the rDNA as well as a decreased life span. In addition, the sir2Δ mutant displays a general decrease in transcriptional silencing of RNA polymerase II-transcribed genes when inserted into the rDNA repeat array. Since HPR1 has a role in transcription and in the maintenance of rDNA stability and wild-type life spans, the relationship between HPR1 and SIR2 in controlling rDNA stability, life span, and transcriptional silencing was examined. The results of hpr1Δ sir2Δ mutant analyses show that HPR1 functions independently of SIR2 in maintaining rDNA stability and normal life span. The hpr1Δ sir2Δ double mutant displays a small but significant (P < 0.05) increase in rDNA recombination over the sir2Δ single mutant (Table 2), indicating separate contributions of hpr1Δ and sir2Δ to rDNA instability. These data suggested that the hpr1Δ sir2Δ double mutant would have a shorter life span than either single mutant. Figure 4 shows that this is indeed the case. The hpr1Δ sir2Δ life span is significantly shorter than both the sir2Δ life span (P < 0.001) and the hpr1Δ life span (P < 0.001), indicating that HPR1 and SIR2 promote wild-type life spans through different pathways. Table 3 shows that the reduction in the average life span of the hpr1Δ sir2Δ double mutant is the product of the reduction in average life spans of the hpr1Δ and sir2Δ mutants, indicating independent influences on life span.

We have not observed any effect of a hpr1Δ mutation on silencing of the HML or HMR loci or subtelomeric reporter genes. Nevertheless, since hpr1Δ displays significant effects on rDNA recombination, we tested whether hpr1Δ had any effect

FIG. 3. Additive effects of the sgs1Δ and hpr1Δ mutations on life span. The life spans for the sgs1Δ (RMY059-2B) and hpr1Δ sgs1Δ (RMY058-5A) strains were determined and are shown with the hpr1Δ data from Fig. 1. The mean life spans of the sgs1Δ (n = 78), sgs1Δ hpr1Δ (n = 64), and hpr1Δ (n = 53) strains were 8.3, 5.0, and 13.7 generations, respectively.

FIG. 4. Additive effects of the sir2Δ and hpr1Δ mutations on life span. The life spans for wild-type (WT; 580-10D), hpr1Δ (HFY824-1A), sir2Δ (RMY206-5B), and hpr1Δ sir2Δ (RMY207-3A) strains were determined. The mean life spans for the wild-type (n = 50), hpr1Δ (n = 56), sir2Δ (n = 58), and hpr1Δ sir2Δ (n = 60) strains were 21.6, 13.8, 12.8, and 8.2 generations, respectively.
on transcriptional silencing within the rDNA. Using a CAN1 reporter (42), we have observed that hpr1Δ strains show no discernible difference in transcriptional silencing compared to wild-type strains. In addition, the hpr1Δ sir2Δ double mutant displays the same rDNA silencing characteristics as that of the sir2Δ single mutant (data not shown).

Old hpr1Δ cells display terminal phenotypes characteristic of old wild-type cells. The terminal morphology of the dividing yeast mother cell can give information on whether cell death is stochastic and age independent or whether it is related to the aging processes (34). Mother cells from wild-type yeast strains usually cease division as unbudded cells in G1 at the end of their life span. This unbudded terminal morphology comprises the majority of cell deaths for both short-lived (young, ≤75% of the maximal life span) and long-lived (old, >75% of the maximal life span) yeast cells (34) (Fig. 5). Analysis of the terminal morphologies of young and old hpr1Δ cells showed that the majority of terminal phenotypes of young hpr1Δ cells were as budded cells. Since this terminal G2 arrest is not as common in young wild-type cells, young hpr1Δ cells may be dying due to stochastic and age-independent mechanisms. However, the majority of old hpr1Δ cells display an unbudded terminal phenotype similar to that of old wild-type cells. Therefore, two different factors may be operating in the determination of the average hpr1Δ life span. The first may be age independent and more related to DNA damage or blocked transcription that leads to a G2 arrest-like phenotype, while the second factor seems to be age dependent and related to the wild-type aging process.

While there is an increased fraction of hpr1Δ young cells that cease division as budded cells, this does not imply that growing hpr1Δ cells exhibit an obvious cell cycle defect. Indeed, the distribution of unbudded, small budded, and large budded cells in a log-phase hpr1Δ population is indistinguishable from that of the wild type and is not altered by mutations in the cell cycle checkpoint functions RAD17, RAD24, and MEC3 (H. Klein, unpublished observations).

soh1Δ suppresses hpr1Δ-mediated rDNA hyperrecombination and increased mortality rate of young cells. The soh1Δ mutation was identified in a selection for hpr1Δ suppressor mutants. soh1Δ suppresses 90% of hpr1Δ-mediated recombination events of non-rDNA origin (10, 11). soh1Δ also suppresses the hpr1Δ requirement for all three functional topoisomerase genes. Soh1p is connected to the RNA polymerase II machinery, and the human counterpart is part of the mediator complex (17). We wanted to determine whether the soh1Δ mutation also suppresses hpr1Δ-mediated rDNA recombination and the decrease in life span. The soh1Δ mutant has a wild-type rate of rDNA recombination (Table 2). The rDNA recombination rate of the hpr1Δ soh1Δ double mutant is indistinguishable from the wild-type and soh1Δ rates, showing that the soh1Δ mutation also suppresses the hpr1Δ-mediated increase in rDNA recombination.

Although the soh1Δ mutant has a wild-type rDNA recombination rate, the life span in this strain is slightly decreased compared to the wild type (Fig. 6A) (p < 0.01). The effect of the soh1Δ mutation is most acutely observed in the viability of older cells, a finding similar to what we observed in the cdc73Δ mutant. The soh1Δ hpr1Δ double mutant has an average life span of 15.2 generations, which is not significantly different from that of the hpr1Δ average life span (13.7 generations) (Fig. 6A and Table 3). By this measure, it cannot be said that soh1Δ suppresses the overall life span decrease of the hpr1Δ mutant. However, the shape of the curves shows that the soh1Δ mutation affects the mortality rates of young hpr1Δ cells. The mortality rate at each generation for hpr1Δ, and hpr1Δ soh1Δ cells is shown in Fig. 6B. A comparison of the mortality rates of the first six generations shows that the hpr1Δ strain has a significantly increased mortality compared to the hpr1Δ soh1Δ double mutant strain (p < 0.001). After the sixth generation, the mortality rates between hpr1Δ and hpr1Δ soh1Δ cells are not significantly different. We conclude that the soh1Δ mutation suppresses the increased early mortality rate of hpr1Δ strains but has no effect on the mortality rate during the later phase of the life span.

**DISCUSSION**

We have found that the hpr1Δ mutant increases recombination within the rDNA array but that this increase is not accompanied by an increase in the accumulation of ERCs, a product predicted to result from a conservative reciprocal recombination that gives two recombination products. ERC accumulation in old cells have been associated with the later stages of the yeast life span, including the onset of features characteristic of aging cells. In spite of the lack of early ERC accumulation in hpr1Δ mutants, this strain has a reduced life span, which we believe is the result of increased genomic instability. We believe that Hpr1p acts in transcription elongation to ensure unimpeded elongation through DNA regions that are prone to inducing RNA polymerase II (RNAPII) stalling. In the absence of Hpr1p, transcription is stalled, and this in turn impedes the passage of a DNA polymerase complex. Through some type of template switching or slippage event, the DNA polymerase passes through the regions where
the RNAPII complex has stalled but can result in a genomic deletion event when repeated DNA sequences are close to the region of the stalled RNAPII complex. Frequent RNAPII stalling events or the accumulated genomic instability may contribute to the cell life span. The linkage of RNAPII stalling, DNA replication, and the ensuing genomic instability may explain why young hpr1Δ cells die as large budded cells, a result expected if cells die in late S phase or G₂ phase. The cells do not appear to be arrested at a cell cycle arrest since DNA damage or whether the deaths can be attributed to wild-type cell deaths are due to age-independent processes such as DNA damage, which is manifested as hyperrecombination and increased cell death. Recent studies on the life span of the sgs1Δ mutant have led to the conclusion that the early part of the life span curve is the result of DNA damage, while the late part of the curve is the result of age-related death (34). However, the hpr1Δ mutant has no DNA damage-sensitive phenotype (1), while the sgs1Δ mutant is sensitive to DNA-damaging agents (43). This reinforces the conclusion that each mutant contributes independently to the rDNA hyperrecombination and reduced life span and shows that there are independent mechanisms that contribute to life span that are related to spontaneous DNA damage.

The terminal phenotypes of mother cells after they have ceased dividing may provide clues as to whether the majority of cell deaths are due to age-independent processes such as DNA damage or whether the deaths can be attributed to wild-type aging processes (34). hpr1Δ cells that die at a young generation tend to die as G₂-arrested budded cells. This is opposite of the terminal phenotypes that are observed for young wild-type cells, which have a greater tendency to die as G₁-arrested unbudded cells. This suggests that young hpr1Δ cells die from different causes than those experienced by young wild-type cells. As we have argued above, we believe that this is the genomic instability that results from DNA replication complexes passing through stalled RNAPII complexes. In contrast, the distribution of unbudded to budded cells among old dying hpr1Δ cells is more similar to the distribution observed for old wild-type cells. This indicates that young dying hpr1Δ cells may occur through homologous recombination, they are not conservative and do not produce the reciprocal product which is an excised circle containing the deleted DNA (44). In terms of recombination within the rDNA this type of recombination would not give an ERC product. This is precisely what we have observed; hpr1Δ strains have an increase in rDNA recombination without an increase in ERCS in young or old cell populations. The fact that there is a reduction in life span without an increase in ERCS argues that, in the hpr1Δ mutant, life span reduction is not related to ERC production. This is further substantiated by the finding that a fob1Δ mutation eliminates all detectable ERCs and yet has no effect on the reduced life span of hpr1Δ cells. This does not mean that increased instability within the rDNA array and at other genomic sites is not related to the reduction in life span. The soh1Δ mutation suppresses hpr1Δ-induced recombination between rDNA repeats and suppresses 90% of the hpr1Δ-induced recombination events between non-rDNA direct repeats (11). soh1Δ also suppresses the increased early mortality of hpr1Δ cells. This correlation suggests that in hpr1Δ cells, reduced life span may be linked to genomic instability. The fact that the hpr1Δ soh1Δ double mutant still has a reduced average life span suggests that this reduction in life span could primarily be due to genomic instability that is not suppressed by soh1Δ.

The hpr1Δ life span curve is distinct in that young cells are particularly at risk for death. It is this increased mortality rate in young cells that is suppressed by the soh1Δ mutation. Since we have argued that soh1Δ suppresses genomic instability, this would suggest that young cells have an increased sensitivity to the rate of genomic instability. The mortality rate of young hpr1Δ cells is similar to that of the sgs1Δ mutant (Fig. 3). Both mutant strains may experience early sensitivity to spontaneous DNA damage, which is manifested as hyperrecombination and increased cell death. The terminal phenotypes of mother cells after they have ceased dividing may provide clues as to whether the majority of cell deaths are due to age-independent processes such as DNA damage or whether the deaths can be attributed to wild-type aging processes (34). hpr1Δ cells that die at a young generation tend to die as G₂-arrested budded cells. This is opposite of the terminal phenotypes that are observed for young wild-type cells, which have a greater tendency to die as G₁-arrested unbudded cells. This suggests that young hpr1Δ cells die from different causes than those experienced by young wild-type cells. As we have argued above, we believe that this is the genomic instability that results from DNA replication complexes passing through stalled RNAPII complexes. In contrast, the distribution of unbudded to budded cells among old dying hpr1Δ cells is more similar to the distribution observed for old wild-type cells. This indicates that young dying hpr1Δ cells may
be dying from mechanisms related to DNA damage, while old dying hpr1Δ cells may be dying from mechanisms more related to wild-type aging. Therefore, at least one component of the hpr1Δ life span curve seems to be related to wild-type aging mechanisms.

**Transcription and life span.** Hpr1p is part of an RNA polymerase II complex in a subcomplex with Cdc73p, Paflp, and Ccr4p (5). Mutants in any one of the genes encoding these proteins are fully viable under normal growth conditions, but double mutants grow poorly or are inviable. We examined the cdc73Δ mutant and found that it has a reduced life span. This reduction is not readily apparent from inspection of colonies grown on rich medium. The life span curve is unusual in that reduction is not readily apparent from inspection of colonies grown on rich medium. The life span curve is unusual in that the viability of successive generations rapidly declines. We do not know the source of the inviability, but we suggest that it reflects the accumulation of some global damage to the cell.

A similar, but not so accentuated, life span curve was observed with the soh1Δ mutant. Soh1p has been found in the human mediator complex of RNA polymerase II (17), and in yeasts soh1Δ has been related genetically to the RNA polymerase II complex and in particular to the CTD tail (10). Hpr1p is part of RNA polymerase II complexes, and in the mutant transcription elongation is affected (6). Our results suggest that modest transcription mutants can have a significant impact on cellular life span and that defects arising first as impairments in transcription can set off a cascade of events that leads to genomic instability and reduced generative potential of a cell. This highlights the multiple mechanisms that can compromise the cellular life span.

Transcriptional regulation through Sir2 has been linked to aging in yeast, as well as in *C. elegans*, and may function to couple life span to nutrient availability in these organisms (31, 53). Our results suggest that additional transcriptional components influence life span.

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