integral step in the termination mechanism.

To confirm the cross-linking result and to define the location of the Nun-template interaction, *N*-(1,10-phenanthrolin-5-yl) iodacetamide (IOP) was conjugated to Nun C110, creating Nun-OP (*13*). Coordination of Cu^{2+} and treatment with 3-mercaptoproprionic acid and H_2O_2 converts IOP to a chemical nuclease that will cleave template DNA upon contact (*14*, *15*).

To perform the cleavage reaction, a TEC was paused at +15 on a 250–base pair (bp) λ DNA template that was ^{32}P-labeled at both 5′ ends in the same manner described above. Although the template alone was resistant to cleavage by Nun-OP, TEC treated with Nun-OP yielded two major cleavage products of about 100 and 150 bp (Fig. 4, compare lanes 1 and 3). The recovery of two bands of equivalent intensity from the reaction, with a combined size of template, indicates that Nun-OP cleaved both template strands with equal efficiency. This implies that the Nun COOH-terminus contacts double-stranded DNA. The major site of DNA cleavage correlated well with the location of RNAP on the template, as determined by a transcription arrest assay with Nun-OP (Fig. 4, lane 2) (*12*) (note that transcription initiates 77 bp from the template end). The sizes of the major cleavage products are consistent with a contact between the Nun COOH-terminus and template about 8 bp promoter-distal to the active center of RNAP and to the RNA:DNA hybrid. Assuming that the paused TEC did not undergo upstream translocation, Nun contacted double-stranded template in the DNA binding site of RNAP (*8*). This observation supports our findings that Nun requires double-stranded template for transcription arrest in vitro (*9*). The contact between Nun and template may involve the intercalation of W108 into double-stranded DNA. As shown, W108 can be substituted with tyrosine, another planar aromatic amino acid, but not with leucine or alanine (Fig. 1B). It has been postulated that movement of nascent RNA and template DNA through a "double-sliding clamp" in the β′ subunit of RNAP is coupled (*16*). As a result of this coupling, the disruption of either motion would compromise the processivity of RNAP.

Like polymerase, Nun binds by different domains to both RNA and DNA. Although not proven, Nun probably contacts both transcript and template simultaneously. Nun is the first auxiliary transcription factor with such properties to be described.

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6. *Escherichia coli* N99 was grown to confluence on TB plates alongside strains carrying plasmids containing WT Nun, NunW108A, NunW108Y, and parent vector pET23d (Novagen) alone at 37°C. At this time, 1 × 10^2, 1 × 10^4, and 1 × 10^6 plaque-forming units of λ phage were spotted on the plates in a volume of 10 μl. The plates were incubated at 37°C overnight and plaques were counted.
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11. Nun was purified as described (*5*). The purified protein was then dialyzed against 0.1 M sodium borate (pH 8.4) and treated with 50 mM DTT at 37°C for 30 min to reduce C110. The reduced protein was then passed through a G25 desalting column to remove the DTT. All of the following procedures were performed in the dark under red light illumination. Intramolecular cross-linking was performed by incubating 200 pmol of ^{125}I-AET-Nun at 4°C for 30 min in the presence or absence of 100 μM $ZnCl_2$. After incubation, the reaction mixtures were irradiated with a hand-held UV lamp for 1 min at the short-wavelength setting. Where indicated, cross-linked ^{125}I-AET-Nun protein was reduced by treatment with 50 mM DTT at room temperature for 10 min followed by removal of DTT by centrifugation through a G25 desalting column. Nun-NusA cross-linking was performed by incubating 300 pmol of NusA with 200 pmol of ^{125}I-AET-Nun in the presence of 100 μM $ZnCl_2$. Irradiation was performed as described above. Cross-linking reactions in the context of the transcription reaction were performed as follows: Transcription was allowed to proceed to the +15 position of the λ pL template by incubating RNAP at 32°C with a mixture of ribonucleoside triphosphates (rNTPs) lacking UTP for 15 min (*3*, *4*, *8*); ^{125}I-AET-Nun (200 pmol) was added to the paused elongation complex. After a 30-min incubation, the reaction mixture was irradiated as described above and stopped with 5 mM EDTA. The DNA was purified with the Qiaquick PCR purification kit (Qiagen) and divided into two samples, one of which was treated with 50 mM DTT to reduce the disulfide bond and cleave the cross-linker from Nun. As a control, ^{125}I-AET-Nun was incubated with template DNA in the absence of RNAP and rNTPs and irradiated with UV light in the same manner described above. The two samples were then heated to 95°C for 5 min to melt template DNA, and resolved on a 12% polyacrylamide–8 M urea denaturing gel after addition of one volume of 2× formamide loading buffer. As the marker, ^{32}P-labeled template, also melted at 95°C, was subjected to electrophoresis alongside the two samples.
12. Sepharose G100 exclusion chromatography was performed as described (*5*) with respect to protein concentrations, incubation times, and centrifugation conditions.
13. Purified, reduced Nun protein was conjugated to IOP by incubation at room temperature in 50 mM sodium phosphate (pH 7.0) for 1 hour in the dark. 3-Mercaptoproprionic acid (3 mM) was then added to inactivate any unreacted IOP. The reaction mixture was then passed through a G25 desalting column. Template DNA was treated with alkaline phosphatase and labeled with ^{32}P at both 5′ ends with T4 polynucleotide kinase. The transcription complex was allowed to proceed to the +15 site on the template in the same manner described above. Nun-OP was then added to the reaction along with 40 μM $CuSO_4$ and incubated at 32°C for 30 min (*11*). To induce scission, 3-mercapto-proprionic acid and H_2O_2 were then added to the reaction at a final concentration of 3 mM. The reaction was allowed to proceed for 45 min at 32°C. EDTA was then added to 5 mM to stop the transcription reaction, and the DNA was purified by ethanol precipitation. As a control, 3-mercapto-proprionic acid and H_2O_2 were withheld, and 150 μM ATP, UTP, GTP, and CTP were added to the reaction to determine whether Nun-OP was still termination competent. The purified DNA and RNA from the transcription reaction were then analyzed in the same manner as the cross-linked complexes. The size markers were determined on the basis of a combination of DNA markers of known length and the products of a Cycle Sequencing Reaction (Perkin-Elmer) of the pL template.
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18. We thank E. Nudler for his helpful comments and suggestions.

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Requirement of Yeast *SGS1* and *SRS2* Genes for Replication and Transcription

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The *SGS1* gene of the yeast *Saccharomyces cerevisiae* encodes a DNA helicase with homology to the human Bloom's syndrome gene *BLM* and the Werner's syndrome gene *WRN*. The *SRS2* gene of yeast also encodes a DNA helicase. Simultaneous deletion of *SGS1* and *SRS2* is lethal in yeast. Here, using a conditional mutation of *SGS1*, it is shown that DNA replication and RNA polymerase I transcription are drastically inhibited in the *srs2*Δ *sgs1-ts* strain at the restrictive temperature. Thus, *SGS1* and *SRS2* function in DNA replication and RNA polymerase I transcription. These functions may contribute to the various defects observed in Werner's and Bloom's syndromes.

Patients with Bloom's syndrome (BS) and Werner's syndrome (WS) suffer from growth retardation and a high incidence of cancers. In addition, individuals with WS age prematurely (*1*). Both syndromes display hyperrecombination and an increased incidence of karyotypic abnormalities (*1*). The *SGS1* gene of *Saccharomyces cerevisiae* is a member of the RecQ family of DNA helicases (*2*), and it shares extensive homology with the human Bloom's syndrome gene *BLM* and the Werner's syndrome gene *WRN* (*3*). The helicases

encoded by *SGS1*, *BLM*, and *WRN* function in the 3′ → 5′ direction (*4*). A 3′ → 5′ DNA exonuclease activity is also present in the WRN protein (*5*). Sgs1 protein interacts physically with DNA topoisomerases II and III, and the deletion of *SGS1* results in a reduction in growth rate, an elevation in the rate of mitotic recombination, and a decrease in the fidelity of chromosome segregation (*2*). As in WS, the *sgs1* deletion (*sgs1*Δ) decreases the average life-span of cells and accelerates aging (*6*). The WRN and Sgs1 proteins are concentrated in the nucleolus (*6*, *7*), and *sgs1*Δ cells display premature fragmentation of their nucleoli (*6*). In addition, *sgs1*Δ cells accumulate large numbers of extrachromosomal ribosomal DNA (rDNA) circles (ERCs), and ERC accumulation in yeast leads to senescence (*8*). Here we elucidate the cellular function of *SGS1*.

Like *SGS1*, the *S. cerevisiae SRS2* gene encodes a DNA helicase with 3′ → 5′ polarity, and mutations in *SRS2* result in hyper-recombination (*9*). To examine whether the Sgs1 and Srs2 proteins function redundantly in a biological process such as DNA replication, we tried to generate the *sgs1*Δ *srs2*Δ double mutant strain by deleting the *SRS2* gene from the *sgs1*Δ strain or by deleting the *SGS1* gene from the *srs2*Δ strain (*10*). All our attempts, however, were unsuccessful, which suggested that the *sgs1*Δ *srs2*Δ combination was lethal. To verify this, we crossed a *MAT*α yeast strain, which carried the *sgs1*Δ mutation marked with the *LEU2* gene, to a *MATa* strain, which carried the *srs2*Δ mutation marked with *URA3* (*10*). From analysis of 100 tetrads, we obtained 239 spores, none of which carried both the *URA3* and *LEU2* markers. Thus, deletion of both *SGS1* and *SRS2* is lethal.

Next, we isolated a recessive mutation of *SGS1* that confers a temperature-sensitive (ts) conditional lethal phenotype in the *srs2*Δ *sgs1*Δ mutant strain (*11*). The *srs2*Δ *sgs1*Δ strain carrying this *sgs1-ts* mutation in plasmid pPM980 (*srs2*Δ *sgs1-ts*) grew at the permissive temperature (25°C) but did not grow at the nonpermissive temperature (39°C), whereas the *srs2*Δ *sgs1*Δ strain carrying the wild-type *SGS1* gene in plasmid pPM914 (*srs2*Δ *SGS1*) grew at 39°C (Fig. 1A). Sequence analysis has revealed four mutational alterations in the *sgs1-ts* mutant gene that consist of a GAA (Glu) to AAA (Lys) change in codon 171, two consecutive AGA (Arg) to AAA (Lys) changes in codons 1048 and 1049, and a CGC (Arg) to TGC (Cys) change in codon 1267. The Arg 1048 and 1049 codons are invariant among the yeast *SGS1* and human *BLM* and *WRN* genes.

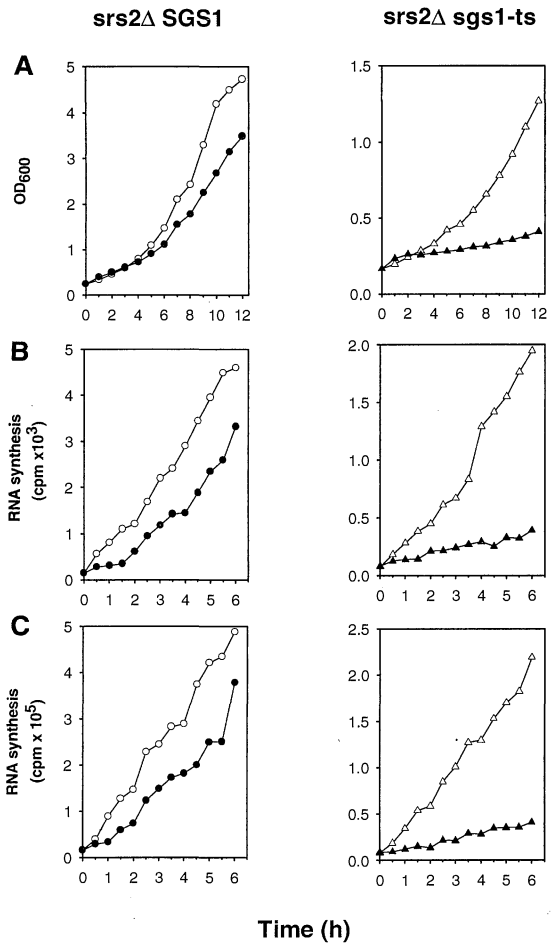

Fig. 1. DNA and RNA synthesis in the *srs2*Δ *SGS1* and *srs2*Δ *sgs1-ts* mutant strains. (**A**) Growth of *srs2*Δ *SGS1* and *srs2*Δ *sgs1-ts* strains. Strains were grown at 25°C in YPD medium. At time 0, the cultures were divided. Half of the culture was left at 25°C, the other half was shifted to 39°C, and the cell density was determined (OD_{600}) at the indicated time points. (**B** and **C**) DNA and RNA synthesis in the mutant strains. [^{3}H]uracil was added to cultures grown at 25°C, and cultures were incubated for another 20 min at 25°C to allow the nucleotide pools to equilibrate. Cultures were then split (time 0). Half of the culture was incubated at 25°C and the other half at 39°C. Radioactivity at each time point was determined by liquid scintillation counting. cpm, counts per minute. ○, *srs2*Δ *SGS1* strain at 25°C and ●, 39°C ; △, *srs2*Δ *sgs1-ts* strain at 25°C and ▲, 39°C.

To determine whether the *srs2*Δ *sgs1-ts* mutant was defective in DNA or RNA synthesis, we examined the incorporation of radioactive label into these macromolecules (*12*, *13*). DNA and RNA synthesis were severely inhibited in the *srs2*Δ *sgs1-ts* cells incubated at 39°C (Fig. 1, B and C) but were not affected in the *srs2*Δ *sgs1-ts* strain at 25°C or in the *srs2*Δ *SGS1* strain at 25° and 39°C (Fig. 1, B and C). Growth and DNA and RNA synthesis were not affected in the *sgs1*Δ or the *sgs1-ts* mutant strains at 39°C (*14*).

To verify the defect in RNA synthesis in the *srs2*Δ *sgs1-ts* mutant, we measured the rate of total RNA synthesis by pulse-labeling cells with [^{3}H]uracil after a shift to the restrictive temperature (*12*, *13*). Both the *srs2*Δ *sgs1-ts* and *srs2*Δ *SGS1* strains showed a rapid drop in RNA synthesis after a shift to 39°C (Fig. 2A). This inhibition was transient and was caused by heat shock (*15*). The *srs2*Δ *SGS1* strain recovered from heat shock after about 1 hour and resumed RNA synthesis. The *srs2*Δ *sgs1-ts* mutant, however, displayed a reduced rate of RNA synthesis (Fig. 2A).

Results of RNA analysis suggested that the synthesis of large ribosomal RNAs (rRNAs) was affected in the *srs2*Δ *sgs1-ts* strain (*16*). The synthesis of RNA polymerase I–dependent rRNA was specifically analyzed by pulse-labeling RNA with [^{3}H]uracil and subjecting the RNA to gel electrophoresis and fluorography (*12*, *13*). Upon transfer to 39°C, the *srs2*Δ *SGS1* strain showed a transient decrease in the synthesis of all large rRNA species (27*S*, 25*S*, 20*S*, and 18*S*) and was followed by nearly full recovery after 2 hours (Fig. 2B). In contrast, the *srs2*Δ *sgs1-ts* strain showed a dramatic decrease in all large rRNA species upon shift to 39°C, with no recovery to normal amounts even after 5 hours at 39°C (Fig. 2B). Thus, inactivation of both *SRS2* and *SGS1* leads to a defect in RNA polymerase I transcription.

To determine the effect of the *srs2*Δ *sgs1-ts* mutation on RNA polymerase II transcription, we used Northern (RNA) blot analysis to examine the amounts of *HIS3*, *MET19*, *ACT1*, *POL1*, and other mRNAs (*12*, *13*) in the *srs2*Δ *sgs1-ts* and *srs2*Δ *SGS1* strains before (sample at 0 min) and after shifting the cultures to 39°C (Fig. 2C) (*17*). However, we observed no decrease in the amounts of the seven mRNAs examined in the *srs2*Δ *sgs1-ts* strain over the 6-hour period at 39°C (Fig. 2C) (*17*). We next examined the effect of the *srs2*Δ

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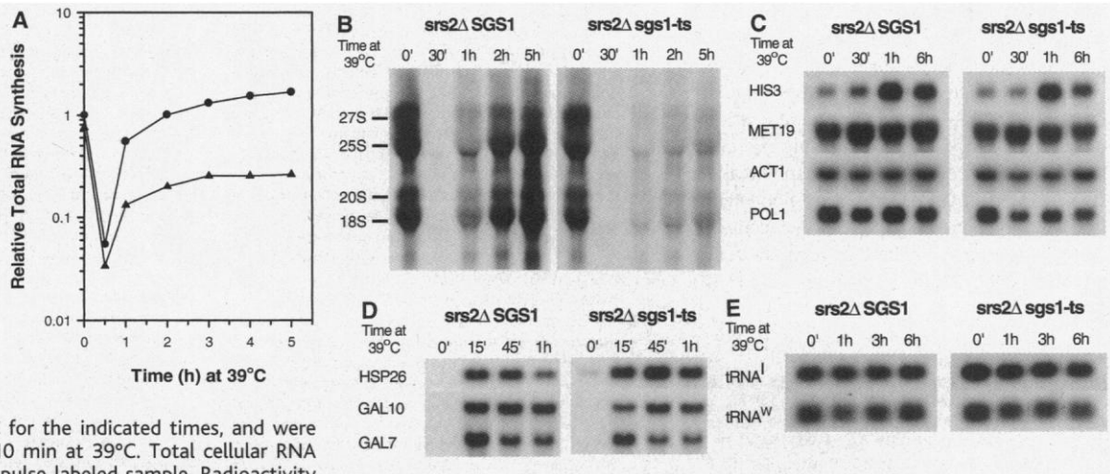

Fig. 2. RNA polymerase I, II, and III transcription in the *srs2Δ SGS1* and *srs2Δ sgs1-ts* mutant strains. (**A**) Total RNA synthesis in the *srs2Δ SGS1* and *srs2Δ sgs1-ts* strains. Strains were grown at 25°C in YPD medium. Cultures were divided into several aliquots, [^{3}H]uracil was added to one aliquot, and cells were pulse-labeled at 25°C for 10 min (sample at time 0). The other aliquots were incubated at 39°C for the indicated times, and were then pulse-labeled for 10 min at 39°C. Total cellular RNA was isolated from each pulse-labeled sample. Radioactivity in the same amount of total RNA of each sample was determined by liquid scintillation counting, and incorporation of [^{3}H]uracil (counts per minute per microgram of total RNA) was normalized by the counts present in the *srs2Δ SGS1* sample that was pulse-labeled at 25°C (time 0). ●, *srs2Δ SGS1*; ▲, *srs2Δ sgs1-ts*. (**B**) Amounts of rRNAs transcribed by RNA polymerase I in the *srs2Δ SGS1* and *srs2Δ sgs1-ts* strains after shift to 39°C. Total RNA obtained from cells pulse-labeled with [^{3}H]uracil as in (A) was subjected to electrophoresis and fluorography. (**C**) Amounts of *HIS3*, *MET19*, *ACT1*, and *POL1* mRNAs in the *srs2Δ SGS1* and *srs2Δ sgs1-ts* strains after shift to 39°C. (**D**) Heat shock-inducible synthesis of *HSP26* mRNA and galactose-inducible synthesis of *GAL10* and *GAL7* mRNAs in the *srs2Δ SGS1* and *srs2Δ sgs1-ts* strains. (**E**) Amounts of intron-containing precursors of tRNA^{Ile} (tRNA^I) and tRNA^{Trp} (tRNA^W)in the *srs2Δ SGS1* and *srs2Δ sgs1-ts* strains after shift to 39°C. For (C), (D), and (E), cultures were transferred from 25° to 39°C, and mRNA amounts (C and D) and amounts of intron-containing precursors of tRNAs (E) were examined at the indicated times by Northern hybridization.

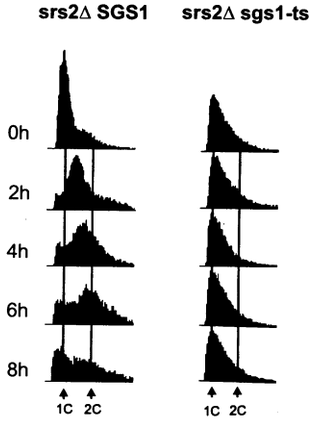

Fig. 3. Inhibition of DNA replication in the *srs2Δ sgs1-ts* mutant strain. Strains grown at 25°C were synchronized in G_1 with mating pheromone α factor, released from α-factor arrest, and then shifted to 39°C (time 0). Samples were taken at the indicated time points, and their DNA content was determined by flow cytometry.

sgs1-ts mutation on the inducible synthesis of *HSP26*, *GAL7*, and *GAL10* mRNAs (*12*, *13*). Transfer of *srs2Δ SGS1* and *srs2Δ sgs1-ts* strains from 25° to 39°C greatly increased heat shock–inducible *HSP26* mRNA in both strains (Fig. 2D). To examine the induction of *GAL7* and *GAL10* mRNAs, galactose was added and cultures were transferred immediately to 39°C. The synthesis of *GAL7* and *GAL10* mRNAs also was not affected in the *srs2Δ sgs1-ts* mutant strains (Fig. 2D). Thus, RNA polymerase II transcription was not affected in the *srs2Δ sgs1-ts* strain at the restrictive temperature.

To determine the effect of the *srs2Δ sgs1-ts* mutation on RNA polymerase III transcription, we examined the synthesis of Ile tRNA (tRNA^{Ile}) and Trp tRNA (tRNA^{Trp}). We used hybridization probes that were complementary to the intron sequences present in these tRNA genes to avoid the problem of high stability of tRNAs. Because tRNA introns are processed rapidly, with a half-life of <3 min (*18*), the amount of precursor tRNA containing the intron sequences reflects the rate of transcription of these genes. The amounts of precursor tRNA^{Ile} or tRNA^{Trp} were not affected in the *srs2Δ sgs1-ts* cells incubated at 39°C for up to 6 hours (Fig. 2E).

To verify the effect of the *srs2Δ sgs1-ts* mutations on DNA synthesis, the *srs2Δ SGS1* and *srs2Δ sgs1-ts* strains were synchronized in G_1 with the use of the yeast mating pheromone α factor (*19*). Cells were shifted to either the permissive or restrictive temperature after removal of the α factor, and their DNA content was monitored by flow cytometry (*19*). The kinetics of DNA synthesis were similar in both strains at the permissive temperature (*14*). At the restrictive temperature, there was no DNA synthesis in the *srs2Δ sgs1-ts* strain even after at least 8 hours (Fig. 3). In contrast, DNA synthesis continued in the *srs2Δ SGS1* strain at the restrictive temperature (Fig. 3). These observations were further confirmed by releasing these strains from α-factor arrest into medium containing [^{3}H]uracil at 25° or 39°C and by determining the amount of radioactivity incorporated into DNA (*20*).

Thus, yeast *SGS1* and *SRS2* genes function in RNA polymerase I transcription and in DNA replication. Sgs1 and Srs2 proteins must play redundant roles, because the absence of either *SGS1* or *SRS2* is not lethal, and the effects on rRNA transcription and DNA replication are not seen in the *sgs1Δ* or *srs2Δ* single mutants but are evident only in the *srs2Δ sgs1-ts* double mutant. In humans, the *BLM* and *WRN* genes and the putative *SRS2* counterpart may function redundantly in RNA polymerase I transcription and DNA replication. The effects of the *srs2Δ sgs1-ts* mutations are similar to the inhibition of RNA polymerase I transcription and DNA replication in the yeast *top1 top2-ts* double mutant at the restrictive temperature (*21*). It has been proposed that topoisomerases I and II act as a swivel to relax the torsional stress that arises during DNA replication, and which may also result from the high rate of transcription of rDNA (*21*).

Sgs1 and Srs2 may function in the unwinding of double-stranded DNA during movement of the replication fork. A role for Sgs1 and Srs2 in the initiation of DNA replication is also possible and is suggested by the observation that the Sgs1-WRN counterpart foci-forming activity–1 (FFA-1) from *Xenopus laevis* is a component of replication foci, which are the initiation sites of DNA replication (*22*). *SGS1*, *SRS2*, and their human counterparts may also function in the unwinding of rDNA during transcription and be responsible for the high rate of rRNA synthesis.

The involvement of Sgs1 in DNA replication and in RNA polymerase I transcription may help to explain the various defects observed in Bloom's and Werner's syndromes. A subtle deficiency in DNA replication may cause increased recombination, chromosome loss,

and other karyotypic abnormalities. These manifestations, in turn, could account for the increased frequency of cancers in BS and WS patients. Ribosomal RNA chain elongation may be slowed in WS cells, which may render RNA polymerase I more prone to pausing that could trigger the formation of double strand breaks in rDNA. Repair of such breaks by nonhomologous end-joining could result in the accumulation of deletions within the genomic rDNA array and contribute to premature aging in WS patients.

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11. To generate mutations in the *SGS1* gene, we cloned the wild-type *SGS1* gene into the low-copy (*TRP1*, *CEN/ARS*) vector YCplac22, which generated plasmid pPM914. Ten micrograms of pPM914 were incubated with 1 M hydroxylamine at 75°C for up to 3 hours before precipitation with ethanol in the presence of 50 μg of bovine serum albumin and 33 mM NaCl. Precipitated DNAs were repeatedly washed with 70% ethanol and then transformed into the *srs2*Δ *sgs1*Δ double mutant strain YRP349, which carries the wild-type *SGS1* gene on the low-copy (*URA3*, *CEN/ARS*) vector YCplac33–based plasmid pPM915. Strains carrying mutant alleles of *SGS1* in pPM914 were isolated by plasmid shuffle. Trp$^+$ Ura$^+$ transformants were replica-plated onto synthetic complete medium lacking Trp and containing 5-fluoro-orotic acid (FOA) to select for the loss of the wild-type *SGS1* gene on the *URA3* plasmid. The FOA-resistant Trp$^+$ Ura$^-$ transformants were then analyzed for temperature-sensitive growth. One of the isolates lacked the ability to grow at 39°C and was analyzed further. The entire *sgs1-ts* mutant gene from the plasmid conferring the ts phenotype was cloned into another plasmid that had not been treated with hydroxylamine and was shown to confer the ts phenotype in the *srs2*Δ *sgs1*Δ strain. The *sgs1-ts* mutant gene in plasmid pPM980 was then sequenced with the Thermo Sequenace kit (Amersham Pharmacia Biotech).
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19. For cell cycle synchronization, cells were grown in medium containing yeast extract, peptone, and dextrose (YPD). When the density reached an optical density at 600 nm (OD_{600}) of ~0.5, the cultures were harvested and resuspended in fresh YPD medium (pH 4.0). Cells were treated with α factor to a final concentration of 10 μg/ml and held for 1.5 doubling times. After confirmation of cell cycle arrest in G_1 under the microscope, α factor was washed away and the cells were resuspended in fresh YPD medium. The cultures were then split (time 0). Half of the culture was incubated at 25°C and the other half at 39°C. DNA content was determined as described [R. Nash, G. Tokiwa, S. Anand, K. Erickson, A. B. Futcher, *EMBO J.* **7**, 4335 (1988)]. Samples were taken at the indicated time points and fixed overnight at 4°C in 1 ml of 70% ethanol. The fixed cells were washed twice, resuspended in 1 ml of 50 mM sodium citrate (pH 7.0), and then treated with ribonuclease A (0.25 mg/ml) for 3 hours at 50°C. Cells were then washed and resuspended in 1 ml of 50 mM sodium citrate (pH 7.0), propidium iodide (16 μg/ml) was added and cells were incubated overnight at 4°C. The DNA content of cells was analyzed by a Becton-Dickinson FACScan flow cytometer.
20. α-factor–arrested cells were released from G_1 arrest into YPD medium containing [^{3}H]uracil. The cultures were then split and placed at 25° or at 39°C, and DNA synthesis was measured by the incorporation of radioactivity into DNA. Upon transfer to 39°C, DNA synthesis ceased rapidly in the *srs2*Δ *sgs1-ts* strain but was not affected in the *srs2*Δ *SGS1* strain.
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Posttranscriptional Gene Silencing in *Neurospora* by a RecQ DNA Helicase

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The phenomenon of posttranscriptional gene silencing (PTGS), which occurs when a transgene is introduced into a cell, is poorly understood. Here, the *qde-3* gene, which is required for the activation and maintenance of gene silencing in the fungus *Neurospora crassa*, was isolated. Sequence analysis revealed that the *qde-3* gene belongs to the RecQ DNA helicase family. The QDE3 protein may function in the DNA-DNA interaction between introduced transgenes or with an endogenous gene required for gene-silencing activation. In animals, genes that are homologous to RecQ protein, such as the human genes for Bloom's syndrome and Werner's syndrome, may also function in PTGS.

Posttranscriptional gene silencing as a consequence of transgene introduction is a broadly diffused phenomenon in plants and fungi (*1*, *2*). Introduction of double-stranded RNA (dsRNA) induces a similar phenomenon in animals (*3*). The wide occurrence of gene silencing among different organisms indicates that these phenomena may have evolved from an ancestral mechanism involved in genome protection from invading DNA (*4*) and viruses (*5*). Several models have been proposed to explain PTGS on the basis of the notion that the introduced transgenes result in the production of aberrant RNAs (aRNAs) (*2*) that are recognized as a template by host RNA-dependent RNA polymerase (RdRP). The RdRP enzyme may synthesize antisense RNA that can bind to mRNA and form dsRNAs that are targets for sequence-specific RNA degradation (*6*). These models have received experimental support. The *qde-1* gene, which encodes a cellular component of PTGS in the fungus *Neurospora crassa*, is homologous to RdRP (*7*). Moreover, the accumulation of small antisense RNA molecules correlates with the occurrence of gene

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