The EMBO Journal (2005) 24, 1267–1276 | © 2005 European Molecular Biology Organization | All Rights Reserved 0261-4189/05
www.embojournal.org

THE EMBO JOURNAL

Impairment of replication fork progression mediates RNA polII transcription-associated recombination

Félix Prado and Andrés Aguilera*

Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Sevilla, Spain

Homologous recombination safeguards genome integrity, but it can also cause genome instability of important consequences for cell proliferation and organism development. Transcription induces recombination, as shown in prokaryotes and eukaryotes for both spontaneous and developmentally regulated events such as those responsible for immunoglobulin class switching. Deciphering the molecular basis of transcription-associated recombination (TAR) is important in understanding genome instability. Using novel plasmid-borne recombination constructs in *Saccharomyces cerevisiae*, we show that RNA polymerase II (RNAPII) transcription induces recombination by impairing replication fork progression. RNAPII transcription concomitant to head-on oncoming replication causes a replication fork pause (RFP) that is linked to a significant increase in recombination. However, transcription that is codirectional with replication has little effect on replication fork progression and recombination. Transcription occurring in the absence of replication does not affect either recombination or replication fork progression. The Rrm3 helicase, which is required for replication fork progression through nucleoprotein complexes, facilitates replication through the transcription-dependent RFP site and reduces recombination. Therefore, our work provides evidence that one mechanism responsible for TAR is RNAP-mediated replication impairment.

The EMBO Journal (2005) **24,** 1267–1276. doi:10.1038/sj.emboj.7600602; Published online 3 March 2005
Subject Categories: genome stability & dynamics
Keywords: genetic instability; homologous recombination; replication; transcription

Introduction

The maintenance of genome integrity is essential to safeguard genetic information and to prevent the loss of cell fitness that is generally associated with cancer and a number of genetic disorders (Lengauer *et al*, 1998; Kolodner *et al*, 2002). In mitosis, homologous recombination is a major mechanism of DNA repair that uses as template an intact homologous DNA sequence. Depending on the template used, whether or not an allelic sequence is in the sister chromatid or in the homologous chromosome, homologous recombination can either safeguard genetic integrity or cause DNA rearrangements.

Transcription has been shown to induce homologous recombination from bacteria to humans, thus affecting genome stability (Aguilera, 2002). This phenomenon, termed transcription-associated recombination (TAR), is also linked to the generation of genetic diversity during developmentally regulated processes such as class switching of immunoglobulin (Ig) genes (Jung *et al*, 1993; Peters and Storb, 1996). Despite its importance in genome stability and programmed genome rearrangements, the mechanisms by which TAR occurs are poorly understood.

Transcription elongation introduces local changes in DNA topology and chromatin structure that could lead to a transient accumulation of single-stranded DNA (ssDNA) regions (Gangloff *et al*, 1994). The observation that transcription increases synergistically the hyper-recombinogenic effect of 4-nitroquinoline (4-NQO) and methyl methane-sulphonate (MMS) suggests that transcription makes DNA more accessible to genotoxic agents (Garcia-Rubio *et al*, 2003), possibly by generating transient ssDNA regions. Studies performed with the *hpr1* mutant of the THO complex in *Saccharomyces cerevisiae*, which shows a strong increase of recombination linked to transcription (Chavez and Aguilera, 1997; Prado *et al*, 1997), have revealed that TAR in these mutants is mediated by the nascent mRNA. Cotranscriptionally formed DNA:RNA hybrids accumulate in *hpr1* mutants and are responsible for TAR (Huertas and Aguilera, 2003), suggesting that an ssDNA sequence might contribute to TAR. Interestingly, DNA:RNA hybrids also accumulate during class switching of Ig genes (Yu *et al*, 2003).

Homologous recombination is a major DNA repair pathway of breaks occurring during DNA replication (Cox *et al*, 2000; Rothstein *et al*, 2000). Indeed, replication seems to be a major source of spontaneous genetic instability, as suggested by the accumulation in yeast to humans of DNA breaks and chromosomal rearrangements in S-phase checkpoint mutants (Myung *et al*, 2001; Casper *et al*, 2002; Cha and Kleckner, 2002). The inhibition of replication by physical obstacles (DNA–protein complexes or DNA lesions), chemical inhibitors or mutations may lead to replication fork breakages that require homologous recombination to resume replication (Horiuchi and Fujimura, 1995; Zou and Rothstein, 1997; Seigneur *et al*, 1998; Ivessa *et al*, 2000, 2003; McGlynn and Lloyd, 2000; Sogo *et al*, 2002; Courcelle *et al*, 2003; Torres *et al*, 2004a). In this regard, a number of *in vitro* and *in vivo* studies suggest that transcription could occasionally inhibit replication fork progression. Bacteriophage T4 and ϕ29 replication machineries are transiently paused *in vitro* by collisions with the bacterial RNAP ternary transcription

*Corresponding author. Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Avd. Reina Mercedes 6, 41012 Sevilla, Spain. Tel.: +34 95 455 7107; Fax: +34 95 455 7104;
E-mail: aguilo@us.es

Received: 16 September 2004; accepted: 7 February 2005; published online: 3 March 2005

complex (Liu *et al*, 1993; Liu and Alberts, 1995; Elias-Arnanz and Salas, 1997, 1999). Replication fork progression is inhibited by transcription *in vivo* in bacteria and yeast (French, 1992; Krasilnikova *et al*, 1998; Takeuchi *et al*, 2003), and natural replication fork pauses (RFPs) have been detected at yeast transfer RNA (tRNA) genes that depend on RNA polymerase III (RNAPIII)-mediated transcription (Deshpande and Newlon, 1996).

Here we have tested the possibility that RNAPII-mediated transcription induced recombination by impairing replication fork progression in *S. cerevisiae*. Using plasmid-borne direct-repeat constructs under the control of regulated promoters, we showed that a head-on and, to a much lesser extent, codirectional encounter between transcription and replication promotes TAR. Besides, TAR was associated with the appearance of an RFP at the recombining region that was partially suppressed by the Rrm3 helicase. These results indicate that TAR is a consequence of the impairment of replication fork progression caused by RNAPII-mediated transcription, and provides a molecular link between TAR and DNA replication.

Results

TAR requires head-on oncoming replication

To determine whether TAR is the result of a conflict between RNAPII-mediated transcription and replication, novel *in vivo* recombination constructs were designed (Figure 1A). These constructs were made in centromeric plasmids and were based on two direct repeats of a 0.6-kb internal fragment of the *LEU2* gene, which generate a selectable wild-type copy of *LEU2* by recombination. Transcription through the *leu2* repeats is driven by the regulated *GAL1* promoter, which is repressed in 2% glucose (GAL-IN (Glu)) and activated in 2% galactose (GAL-IN (Gal)). The *leu2* repeats are oriented according to their transcription either inward (IN) or outward (OUT) with respect to the unique *ARSH4* replication origin contained in the plasmid, and are replicated by the proximal replication fork (shown later). Northern analysis showed that the level of transcription through the repeats was similar in the GAL-IN and GAL-OUT constructs (Figure 1B). The constructs permit the analysis of recombination caused by the encounter—either head-on or codirectional—between the replication fork and RNAPII-mediated transcription.

As shown in Figure 1C, in the absence of transcription, the frequency of recombination is the same regardless of the orientation of the *leu2* repeats relative to *ARSH4* (GAL-IN versus GAL-OUT in glucose). When transcription was active, the frequency of recombination was dependent on the orientation of the *leu2* repeats relative to *ARSH4*. In GAL-OUT (Gal), the frequency of recombination was 1.6-fold higher than in GAL-OUT (Glu). This indicates that transcription by itself had little effect on recombination if it advanced in the same direction as the replication fork. In contrast, transcription through the *leu2* repeats increased the frequency of recombination 5.5-fold in the GAL-IN construct, in which

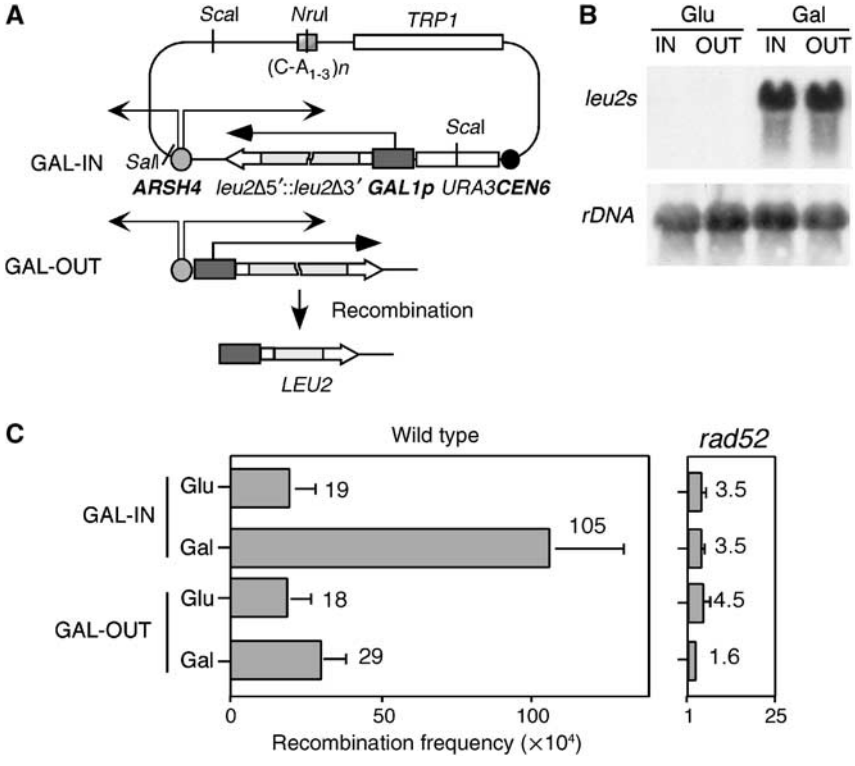

Figure 1 Homologous recombination is induced by RNAPII-mediated transcription if this occurs in the opposite direction to an oncoming replication fork. (**A**) Schemes of the centromeric plasmids harbouring the recombination constructs GAL-IN and GAL-OUT, and of the *LEU2* recombination product. The arrows indicate the progression orientation of RNAPII transcription driven from the *GAL1* promoter and of the replication forks initiated at *ARSH4*. The distance that each fork has to traverse from *ARSH4* to the promoter are approximately 2.5 and 6 kb for the rightward- and leftward-advancing forks, respectively. (**B**) Northern analysis of transcripts emerging from the direct-repeat constructs in wild-type cells grown either in glucose (Glu; transcription OFF) or galactose (Gal; transcription ON). (**C**) Recombination frequencies in wild-type and *rad52*Δ strains. The average and standard deviation are indicated.

transcription and replication are convergent (Figure 1C, compare GAL-IN (Gal) versus GAL-IN (Glu)). This increase in recombination was dependent on *RAD52*, indicating that the events occurred by homologous recombination.

The results suggest that an impairment of replication fork progression caused by the oncoming transcription could be responsible for the transcription-mediated increase in recombination. If this were the case, transcription should induce recombination only during the S phase. To test this hypothesis, three new constructs were generated by replacing the *GAL1* promoter in the IN construct by cell-cycle-specific promoters whose cell-cycle-specific regulation was known to be maintained in centromeric plasmids: CLN-IN, in which transcription is driven by the promoter of the *CLN2* G1-cyclin gene, is expressed in G1 (Wittenberg *et al*, 1990; Stuart and Wittenberg, 1994; Spellman *et al*, 1998), and HHF-IN and HHO-IN, in which transcription is driven from the promoters of the H4- and H1-like histone genes *HHF2* and *HHO1*, respectively, are expressed in late G1/S phase (Hereford *et al*, 1981; Freeman *et al*, 1992; Spellman *et al*, 1998) (Figure 2A). Consequently, whereas transcription and replication would take place at nonoverlapping times in the CLN-IN construct and should not affect recombination, they would occur concomitantly in the HHF-IN and HHO-IN constructs and are expected to increase recombination. As can be seen in Figures 1C and 2B, the frequency of recombination in the CLN-IN construct was similar to that observed in GAL-IN (Glu). In contrast, transcription driven from the S-phase-induced promoters in the HHF-IN and HHO-IN constructs increased recombination 5- and 2.5-fold, respectively. To determine whether the increase in recombination caused by S-phase transcription required a head-on encounter of transcription and replication, recombination was determined in the HHF-OUT construct carrying the OUT repeat system under the control of the *HHF2* promoter (Figure 2A, HHF-OUT). As can be seen in Figures 1C and 2B, S-phase-induced transcription in the HHF-OUT construct increased recombination two-fold above the GAL-OUT levels in the absence of transcription.

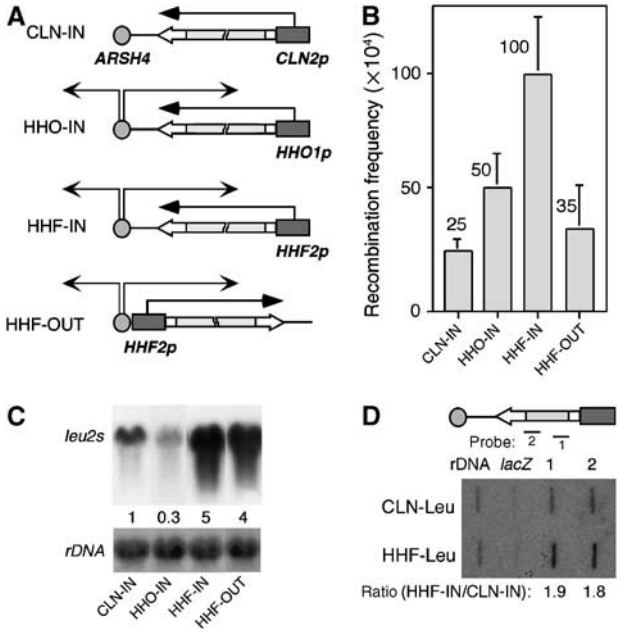

Figure 2 Recombination is induced by transcription only if this is active during S phase. (**A**) Schemes of the CLN-IN, HHO-IN, HHF-IN and HHF-OUT constructs. The *leu2* direct repeats are under the control of the *CLN2*, *HHO* and *HHF2* promoters, respectively, which are activated in G1 phase in the CLN-IN construct and in late G1/S phase in the HHF-IN and HHO-IN constructs. (**B**) Recombination frequencies of each construct in the wild-type strain. The average and standard deviation are indicated. (**C**) Northern analysis of *leu2* transcripts emerging from each system (*leu2s*). RNA levels are normalized with respect to the CLN-IN values, taken as 1. (**D**) Run-on analysis of *leu2* transcripts emerging from the CLN-Leu and HHF-Leu constructs. A scheme of these constructs, which are driven by the *CLN2* and *HHF2* promoters, respectively, is shown on top. The amount of mRNA bound to each probe is normalized with respect to the CLN-IN values, taken as 1. *lacZ* was used as negative control.

Since transcription levels have a direct effect on the frequency of recombination in yeast (Thomas and Rothstein, 1989; Saxe *et al*, 2000; Gonzalez-Barrera *et al*, 2002), we determined whether our results could be explained as a consequence of different transcription rates. As shown in Figure 2C, the levels of recombination in the HHO-IN and HHF-IN constructs correlated with transcript levels, as determined by Northern. However, transcripts in the non-hyper-recombinant CLN-IN construct accumulated at levels three-fold higher than in HHO-IN, whereas transcription levels were similar in HHF-IN and HHF-OUT (Figure 2C). To confirm that transcript levels determined by Northern correlated with transcription rates, we performed run-on analysis of the HHF-Leu and CLN-Leu constructs (Figure 2D), which were obtained directly from yeast Leu$^+$ recombinants derived from HHF-IN and CLN-IN transformants, respectively. As expected, the rate of transcription in the HHF-Leu construct was higher (two-fold) than in the CLN-Leu construct. Altogether, these results confirm that transcription by itself is not sufficient to induce recombination. Instead, the increase in recombination mediated by transcription requires concomitant replication.

TAR is linked to the appearance of an RFP

To further explore the molecular nature of TAR, we determined whether replication fork progression was negatively affected by RNAPII-mediated transcription in our constructs. For this, replication intermediates were analysed by 2D-gel electrophoresis (Figure 3). Two overlapping restriction fragments covering the *leu2* repeats in the IN constructs were analysed: a *Sal*I–*Sca*I fragment that leads to the formation of an arc of Y-shaped replication intermediates (Figure 3B), and a *Sca*I fragment in which the internal position of *ARSH4* leads to the formation of an additional bubble arc (Figure 3C). The absence of termination arcs—double Y- and X-shaped replication intermediates—in both fragments suggested that the proximal replication fork replicated the *leu2* repeats, validating the interpretation concerning orientation of replication relative to transcription.

As can be seen in Figure 3B and C, a region of intense hybridization in the arc corresponding to the Y-shaped replication intermediates was detected in the HHF-IN construct, which is specifically transcribed during the S phase. The mapping of this hybridization signal in the two overlapping restriction fragments revealed that there was an accumulation of replication forks at the *leu2*Δ*3′* repeat, at approximately 350 bp from the translation initiation site. Despite this accumulation of replication forks, a complete arc of Y-shaped

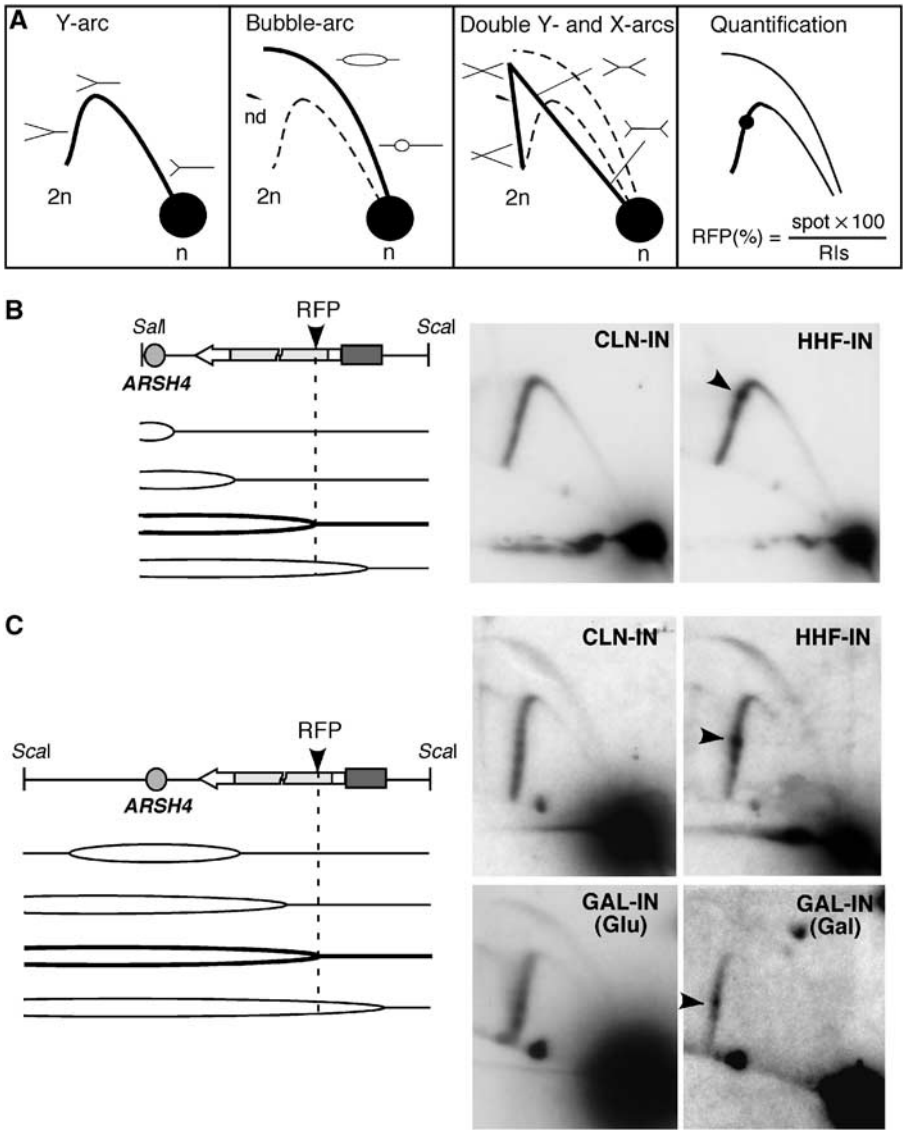

Figure 3 Converging transcription and replication leads to a 2D-gel-detectable RFP. (**A**) Schematic representation of the migration pattern of the Y-, bubble-, double Y- and X-shaped replication intermediates in 2D-gel electrophoresis. n indicates unreplicated molecules, RIs replication intermediates and nd not determined molecules. (**B**) 2D-gel analysis of the *Sal*I–*Sca*I restriction fragment covering the *leu2* repeats in the CLN-IN and HHF-IN constructs. Note that bubble molecules are not detected due to the terminal location of *ARSH4* in the *Sal*I–*Sca*I fragment. (**C**) 2D-gel analysis of the *Sca*I restriction fragment covering the *leu2* repeats in the GAL-IN (either from cells grown in glucose (Glu) or galactose (Gal)), CLN-IN and HHF-IN constructs. The location of *ARSH4* in an internal position leads to the detection of a bubble arc in addition to the Y-arc. A scheme of the replication intermediates expected for each restriction fragment and the position of the transcription-dependent RFP in the HHF-IN and GAL-IN constructs (solid arrow) is shown on the left.

replication intermediates was formed, indicating that most of the replication forks at the *leu2*Δ*3′* repeat were not blocked, but paused or slowed down at a specific RFP site.

To determine whether the RFP at the HHF-IN construct was a consequence of transcription through the *leu2* repeats during S phase, we analysed replication intermediates in the CLN-IN, which is specifically transcribed during G1, and the GAL-IN constructs. As can be seen in Figure 3B and C, the RFP was not detected in CLN-IN. In addition, the RFP was not detected in GAL-IN (Glu), while it appeared in GAL-IN (Gal) at the same position in the *leu2*Δ*3′* repeat as in HHF-IN (Figure 3C). Therefore, the formation of this RFP requires the presence of active transcription during the S phase. Quantification of the hybridization signal revealed a significant accumulation of replication intermediates at this RFP in HHF-IN ($24 \pm 2.5\%$ of replication intermediates) and GAL-IN (Gal) ($21 \pm 2\%$). Such intermediates were not observed in CLN-IN ($13 \pm 1.5\%$) or in GAL-IN (Glu) ($14 \pm 1\%$).

The appearance of an RFP in HHF-IN and GAL-IN (Gal) but not in CLN-IN or GAL-IN (Glu) is consistent with a link of the RFP to TAR. If this were the case, active transcription should not lead to a significant RFP in the GAL-OUT (Gal) or HHF-OUT constructs, as the codirectional progression of transcription and replication hardly affected recombination (Figures 1 and 2). As shown in Figure 4, the GAL-OUT construct did not show detectable RFPs both in glucose and galactose, whereas the HHF-OUT construct seems to show a weak transcription-dependent RFP at the *leu2*Δ*5′* repeat at a similar distance

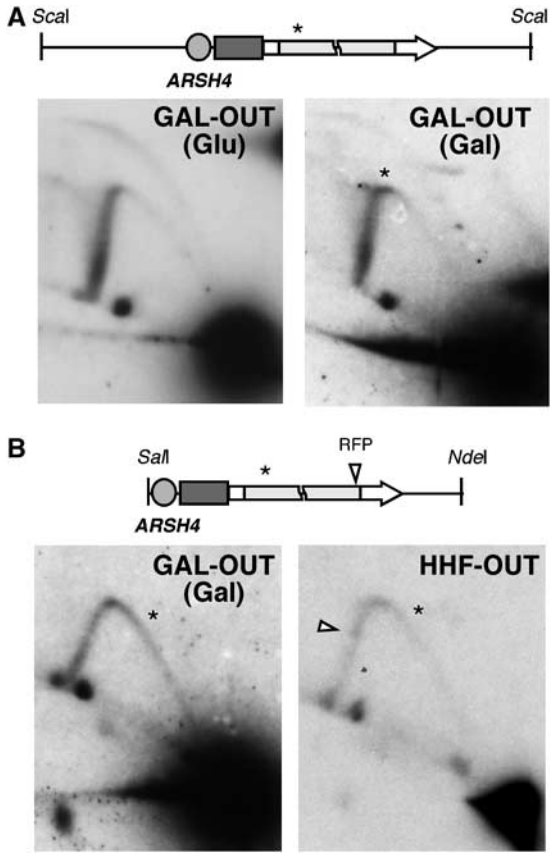

Figure 4 Analysis of the progression of replication forks that are codirectional with transcription. (**A**) 2D-gel analysis of the *Sca*I restriction fragment covering the *leu2* repeats in the GAL-OUT construct isolated from cells grown in glucose (Glu; transcription OFF) or galactose (Gal; transcription ON). (**B**) 2D-gel electrophoresis of the *Sal*I–*Nde*I restriction fragment covering the *leu2* repeats in the GAL-OUT and HHF-OUT constructs isolated from cells grown in galactose or glucose, respectively. An open arrow indicates a weak transcription-dependent RFP in the HHF-OUT construct. The asterisks indicate the expected position corresponding for the RFP at the *leu2*Δ*3′* repeat as detected in the GAL-IN and HHF-IN constructs (Figure 3). Note that the signal accumulated in the inflection points of the Y-arcs in the GAL-OUT construct is the consequence of the overlapping left and right arcs, and does not correspond to a true RFP, since it does not change its position in the overlapping fragments.

from *ARSH4* as the RFP observed in HHF-IN. Altogether, these results indicate that TAR is linked to a transcription-dependent RFP, suggesting that the increase in recombination may result from the impairment of replication fork progression caused by transcription.

RFP is independent of the direct repeats

We reported the first evidence of an RFP associated with RNAPII-mediated transcription. We accordingly wondered whether this RFP could be due to the presence of direct repeats in our constructs that lead to pairing intermediates able to pause replication fork progression. Influence of the repeats in the formation of the transcription-dependent RFP was determined by 2D-gel analysis of the HHF-Leu construct, which differs from the HHF-IN construct by the presence of just one repeat unit. As shown in Figure 5, in two overlapping restriction fragments, an RFP of similar intensity and at the same position in the *LEU2* gene as in the HHF-IN construct was detected. This indicates that the RFP was independent of the presence or absence of direct repeats.

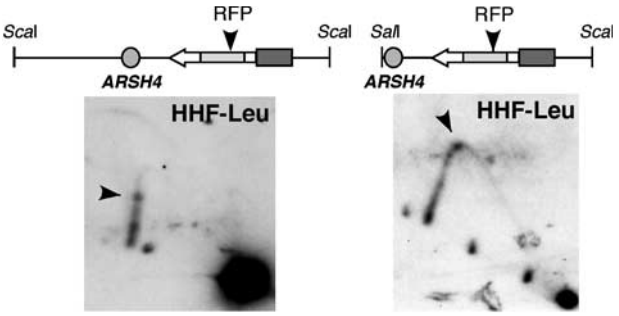

Figure 5 The transcription-dependent RFP is independent of direct repeats. 2D-gel analysis of Leu$^+$ recombinants from the HHF-IN construct containing just one repeat unit (HHF-Leu construct). The overlapping *Sca*I (left) and *Sal*I–*Sca*I (right) restriction fragments are shown on top of each panel. A solid arrow indicates the RFP.

The helicase Rrm3 facilitates the progression of the replication fork through the transcription-dependent RFP site

To get further insight into the molecular nature of the transcription-dependent RFP associated with TAR, replication intermediates were analysed by 2D-gel electrophoresis in mutants affected in recombination and/or replication fork progression. Genetic evidence suggests that the rescue of replication forks in yeast requires the coordinated activity of the recombination proteins Rad51 and Rad52, together with at least two 3′- to -5′ DNA helicases, Srs2 and Sgs1, with roles in replication fork progression (Lee *et al*, 1999; Cobb *et al*, 2003; Versini *et al*, 2003) and recombination (Ira *et al*, 2003; Krejci *et al*, 2003; Veaute *et al*, 2003). Srs2 and Sgs1 seem to prevent the accumulation of genotoxic recombination intermediates, as suggested by the observation that the synthetic lethality of the *srs2 sgs1* double mutant is rescued by the absence of Rad51 and Rad52 (Gangloff *et al*, 2000). These helicases are genetically related to Rrm3, a 5′- to -3′ DNA helicase that facilitates both replication fork progression through natural RFPs at centromeres, tRNA genes, inactive replication origins, telomeres and ribosomal DNA (rDNA) (Ivessa *et al*, 2000, 2002, 2003), and resolution of convergent replication forks at the replication fork barrier (RFB) in the rDNA (Ivessa *et al*, 2000). As shown for *srs2 sgs1*, the absence of Rad51 and Rad52 suppresses the synthetic growth defects of the *srs2 rrm3* and *sgs1 rrm3* double mutants (Ooi *et al*, 2003; Schmidt and Kolodner, 2004; Torres *et al*, 2004b). Thus, replication intermediates were analysed in *rad51*Δ, *rad52*Δ, *srs2*Δ, *sgs1*Δ and *rrm3*Δ strains.

As shown in Figure 6A, the transcription-dependent RFP at the *leu2*Δ*3′* repeat in the HHF-IN construct (solid arrow) was significantly increased in *rrm3*Δ cells (37±2% of replication intermediates versus 24±2.5% in the wild type). This increase was accompanied by an accumulation of convergent forks (X-shaped replication intermediates) at the transcription-dependent RFP site, suggesting that the pause lasted long enough as to allow a fraction of the opposite replication forks to reach the RFP site. To determine whether the increase in this RFP detected in *rrm3*Δ depended on transcription during

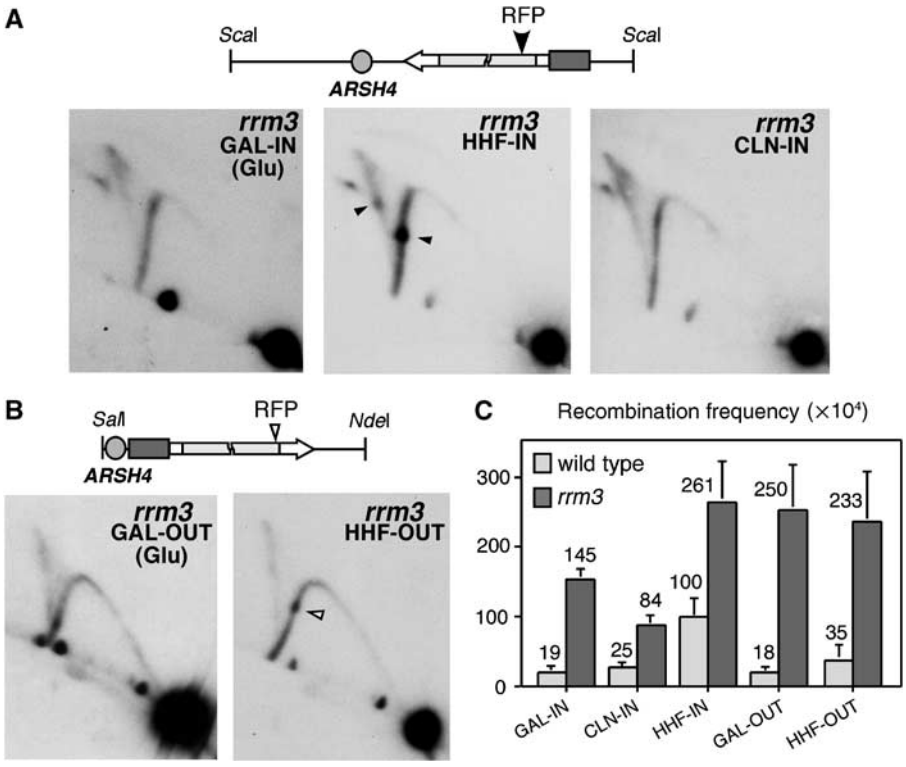

Figure 6 Increase of transcription-dependent RFP and recombination in the absence of the Rrm3 helicase. (**A**) 2D-gel analysis of the *Sca*I restriction fragment covering the *leu2* repeats in the GAL-IN, HHF-IN and CLN-IN constructs isolated from *rrm3*Δ cells grown in glucose. Solid arrows indicate the transcription-dependent RFP in the HHF-IN construct. (**B**) 2D-gel analysis of the *Sal*I–*Nde*I restriction fragment covering the *leu2* repeats in the GAL-OUT and HHF-OUT constructs isolated from *rrm3*Δ cells grown in glucose. Open arrows indicate the transcription-dependent RFP in the HHF-OUT construct. (**C**) Recombination frequencies of the GAL-IN, CLN-IN, HHF-IN, GAL-OUT and HHF-OUT constructs in the wild-type and *rrm3*Δ strains grown in glucose. The average and standard deviation are indicated.

the S phase, replication intermediates in the GAL-IN and CLN-IN constructs were analysed. The transcription-dependent RFP at the *leu2*Δ*3′* repeat was not detected in *rrm3*Δ cells in the absence of transcription (GAL-IN (Glu)), when transcription occurred in G1 (CLN-IN) (Figure 6A) or when transcription and replication were codirectional (Figure 6B). However, the transcription-dependent RFP at the *leu2*Δ*5′* repeat in the HHF-OUT construct was also increased in *rrm3*Δ cells (Figure 6B, open arrow, 20±2% of replication intermediates). These results indicate that the helicase Rrm3 facilitates replication fork progression through an RNAPII transcription-dependent RFP site. Since Rrm3 has been shown to be required for the advance of the replication fork through non-nucleosomal protein–DNA complexes (Ivessa *et al*, 2003), this suggests that the RNAPII-dependent RFP detected in our constructs is due to the presence of a transcription-dependent nucleoprotein complex. In addition to the transcription-dependent RFP detected in the Y arc, the absence of Rrm3 led to an accumulation of intermediates in the cone formed by the arcs of the X- and double Y-shaped molecules in all constructs (Figure 6A and B), consistent with a general replication impairment. As expected from these results, recombination was increased in all constructs in *rrm3*Δ (3- to 10-fold above the wild type) (Figure 6C).

The intensity of the signal at the transcription-dependent RFP site in the HHF-IN construct did not change in *rad51*Δ, *rad52*Δ, *srs2*Δ and *sgs1*Δ mutants with respect to wild-type

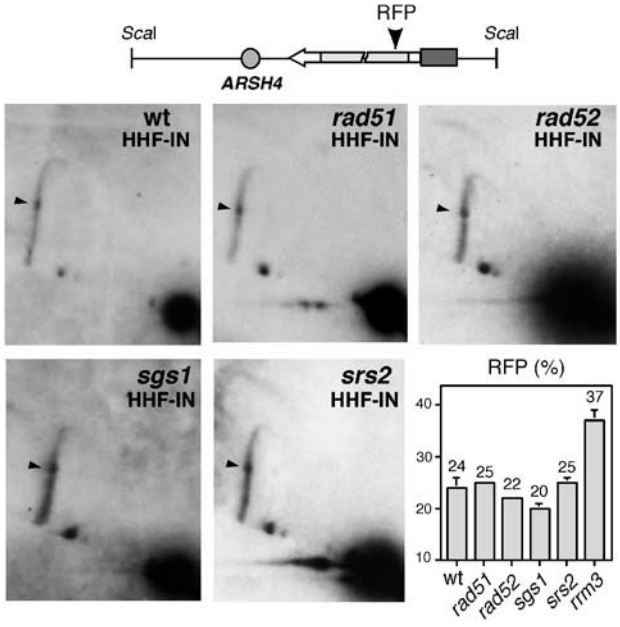

Figure 7 The transcription-dependent RFP in the HHF-IN construct is not affected by *rad51*Δ, *rad52*Δ, *sgs1*Δ and *srs2*Δ. 2D-gel analysis of the *Sca*I restriction fragment covering the *leu2* repeats in the HHF-IN construct in wild type, *rad51*Δ, *rad52*Δ, *sgs1*Δ and *srs2*Δ. Solid arrows indicate the transcription-dependent RFP at the *leu2*Δ*3′* repeat. Quantification data of the RFPs relative to the replication intermediates, taken as 100, are shown.

cells (Figure 7). In these strains, the proportion of paused replication forks at the transcription-dependent RFP site represented 20–25% of replication intermediates.

Discussion

Using specifically designed plasmid-borne constructs, we have analysed the effect in yeast that transcription has on recombination, depending on whether or not the transcribed DNA sequence is simultaneously replicated either codirectionally or in a head-on orientation. We show that transcription by itself is not sufficient to induce recombination. TAR requires replication fork progression opposite to transcription and is associated with the appearance of an RFP. The codirectional advance of transcription has little effect on replication fork progression and recombination. The helicase Rrm3 facilitates the advance of the replication fork through the transcription-dependent RFP, suggesting that an RNAPII-dependent nucleoprotein complex participates in the impairment of replication. These results indicate that TAR can be mediated by impairment of replication fork progression.

The effect on genomic integrity of the potential collisions between transcription and replication is poorly understood. Here, we observed that transcription driven from the *GAL1* promoter along two direct repeats increased recombination between the repeats if these were simultaneously replicated by an oncoming replication fork. In addition, transcription driven from the *HHF2* and *HHO1* promoters, which are activated in S phase, but not from the *CLN2* promoter, which is activated in G1, increased recombination in the head-on orientation. In contrast, transcription occurring codirectionally with replication had little effect on recombination (Figures 1 and 2). These results indicate that transcription by itself is not sufficient to induce recombination. A head-on and, to a lesser extent, a codirectional encounter of RNAPII-mediated transcription and DNA replication leads to recombinogenic DNA damage. Consistent with this conclusion, transcription of a DNA fragment that is simultaneously replicated by an oncoming replication fork led to the appearance of a transcription-dependent RFP, while transcription occurring codirectionally led to a much weaker transcription-dependent RFP. Transcription occurring in the absence of replication did not affect replication fork progression (Figures 3 and 4).

The appearance of a transcription-dependent RFP indicates that DNA replication is impaired by RNAPII-mediated transcription in our constructs. This impairment is more pronounced when the encounter between transcription and replication is head-on than when it is codirectional. Replication impairment could generate recombinogenic intermediates. In accordance with this possibility, defective DNA replication caused by either replication inhibitors or mutations affecting components of the replication machinery accumulates recombination intermediates in yeast (Zou and Rothstein, 1997; Sogo *et al*, 2002), and a number of studies have suggested a tight connection between inefficient DNA replication and homologous recombination from bacteria to humans (Rothstein *et al*, 2000).

A general impairment of replication fork progression along the whole DNA repeat region that would not be detected in the form of RFPs could contribute to TAR. Nevertheless, the transcription-dependent RFP detected in this study could be associated with the initiation of recombination, because it lies in the region of homology and because its appearance correlates with TAR. Indeed, RFPs have been shown to promote recombination. Thus, RFPs generated either by the binding of the Tus protein to the replication termination *Ter* site in *Escherichia coli* (Horiuchi *et al*, 1994; Horiuchi and Fujimura, 1995) or by the absence of the helicase Rrm3 in yeast (Ivessa *et al*, 2003; Torres *et al*, 2004a) are associated with an increase in homologous recombination. Also, yeast mutants lacking the RFB-binding protein Fob1, required for RFB activity, display slowed down replication fork progression and repeat instability at the rDNA locus as a consequence of head-on collisions between RNAPI transcription and replication (Takeuchi *et al*, 2003).

Rrm3 promotes the advance of the replication fork through non-nucleosomal nucleoprotein complexes (Ivessa *et al*, 2003). Therefore, the observation that the helicase Rrm3 facilitates replication through the transcription-dependent RFP sites (Figure 6) suggests that a transcription-dependent nucleoprotein complex could be responsible for the RFP in our constructs. A transiently arrested RNAPII ternary transcription complex could impair the advance of the replication fork. This is consistent with the observation that natural RFPs found at tRNA genes—which are also polar and increased in the absence of Rrm3 regardless of the transcript length (see below)—require the assembly of the RNAPIII transcription initiation complex (Deshpande and Newlon, 1996; Ivessa *et al*, 2003). Along the same line, studies using the *in vitro*-purified bacteriophage T4 and ϕ29 systems have shown that head-on collisions between the replication fork and the RNAP transcription ternary complex are more disadvantageous than codirectional collisions (Liu *et al*, 1993; Liu and Alberts, 1995; Elias-Arnanz and Salas, 1997, 1999). In the head-on collision, the ϕ29 DNA polymerase was blocked by a halted RNAP complex and resumed replication once the RNAP was allowed to move (Elias-Arnanz and Salas, 1999), suggesting that an arrested, in contrast to an elongating, RNAPII ternary transcription complex could impose a physical hindrance to the advance of the replication fork. The more complex replication apparatus of the bacteriophage T4 was shown to pass the RNAP complex, either halted or elongating, after a pause of a few seconds. Interestingly, the T4 replication apparatus required the activity of the gene 41 DNA helicase to solve the head-on, but not the codirectional, collision (Liu *et al*, 1993; Liu and Alberts, 1995). The DNA helicase Rrm3 might help to detach the RNAPII transcription ternary complex from DNA, as proposed for other nucleoprotein complexes that inhibit replication fork progression (Ivessa *et al*, 2003). This function is specific of Rrm3 in our constructs, since the transcription-dependent RFP was not affected by the absence of the DNA helicases Sgs1 or Srs2 (Figure 7).

Transcription could also impair replication either by increasing the torsional stress of the DNA or by facilitating the binding of sequence-specific proteins that could act as roadblocks for replication fork progression. The torsional stress is expected to be higher in a head-on than in a codirectional arrangement because the former would accumulate positive supercoiling at the converging region. A head-on, but not a codirectional, collision between transcription and replication has been shown to increase the knotting of the sister chromatids behind the fork in *E. coli* (Olavarrieta *et al*, 2002). However, the observations that the transcription-dependent

RFP is independent of the transcript length (1.8 kb in the HHF-IN versus 1.2 kb in the HHF-Leu) (Figure 5), and is not spread along the transcribed region, make replication pauses unlikely as a consequence of torsional stress in our constructs. Also unlikely is that a sequence-specific DNA-binding protein pauses replication because the transcription-dependent RFP in the IN constructs was detected at the *leu2*Δ*3*′ repeat but not at the same DNA sequence at the *leu2*Δ*5*′ repeat. Finally, it is worth noticing that the detection of transcription-dependent RFPs may depend on topological constraints, because such RFPs are not easily detected in linearized plasmids (our preliminary observations with the pARSGLB-IN plasmid, containing GAL-IN, linearized at the telomeric sequences). In this regard, RFPs are not detected downstream of the endogenous *GAL1* and *GAL10* promoters (Ivessa *et al*, 2003). Further molecular analyses would be required to determine the importance of supercoiling stress and/or other DNA structural parameters in the formation of transcription-dependent RFPs.

In summary, the link between a transcription-dependent RFP and recombination observed here sheds light on understanding the mechanism by which transcription induces recombination and underlines the relevance of homologous recombination as a DNA repair mechanism connected with DNA replication. Our work raises the question of whether other cases of TAR, such as recombination associated with cotranscriptionally formed RNA:DNA hybrids of yeast THO mutants (Huertas and Aguilera, 2003) or the generation of genetic diversity during class switching of Ig genes (Jung *et al*, 1993; Peters and Storb, 1996), are also linked to impairment of replication fork progression. Our results provide evidence that head-on collisions between transcription and replication can be a source of genomic instability. This might explain the preferential positioning in the leading strand of essential genes in bacteria (Rocha and Danchin, 2003), and the presence of polar RFBs that prevent head-on collisions between RNAPI transcription and replication at the rDNA locus in eukaryotes (Brewer and Fangman, 1988; Little *et al*, 1993; Wiesendanger *et al*, 1994; Gerber *et al*, 1997). A tight control of transcription during DNA replication may be, therefore, essential for the prevention of genetic instability and for proper control of developmentally programmed chromosomal rearrangements.

Materials and methods

Yeast strains and plasmids

Yeast strains used in this study were BY4741 (a *his3*Δ0 *leu2*Δ0 *ura3*Δ0) and its isogenic Y10540 (*rad52*Δkan), Y16401 (*rad51*Δkan), Y01331 (*srs2*Δkan), Y10775 (*sgs1*Δkan) and Y00994 (*rrm3*Δkan). Yeast cells were grown in synthetic complete (SC) medium as described (Kaiser *et al*, 1994). Plasmids pARSGLB-OUT, pARSGLB-IN, pARSHLB-OUT, pARSHLB-IN, pARSCLB-IN and pARSXLB-IN are yeast centromeric plasmids containing the GAL-OUT, GAL-IN, HHF-OUT, HHF-IN, CLN-IN and HHO-IN recombination constructs, respectively. They are based on plasmid pFERNU, which was constructed by cloning *ARSH4*, *URA3*, *CEN6* and the 83 bp $(C\text{-}A_{1\text{-}3})_n$ telomeric sequences in pRS304 (Sikorski and Hieter, 1989) lacking the *Eco*RI site at the polylinker. The PCR-amplified *ARSH4* sequence was inserted at the *Cla*I–*Sal*I site; the *URA3* marker obtained by *Hin*dIII digestion from YEp24 and made blunt ended was inserted at the *Sma*I site; and the PCR-amplified *CEN6* sequence was inserted at the *Sac*II site. The telomeric sequences were cloned in two steps: (1) two *Bam*HI (made blunt ended)–*Eco*RI $(C\text{-}A_{1\text{-}3})_n$ fragments were inserted as inverted repeats at the *Aat*II site (made blunt ended) and (2) an *Nru*I-containing linker was inserted at the resulting *Eco*RI site. pARSGLB-IN and pARSGLB-OUT were constructed by inserting the *Sac*I–*Apa*I (made blunt ended) fragment of p314GLB (Piruat and Aguilera, 1998), containing the *leu2*Δ*3*′::*leu2*Δ*5*′ direct-repeat recombination system (Prado and Aguilera, 1995) under control of the *GAL1* promoter, at the *Cla*I (made blunt ended) site of pFERNU. The fragment was cloned either inward (pARSGLB-IN) or outward (pARSGLB-OUT) of the *ARSH4* sequence with respect to the direction of transcription. pARSHLB-IN and pARSHLB-OUT were cloned in two steps: (1) the *Sac*I–*Bam*HI fragment from pRS314-GLB, containing the *GAL1* promoter, was replaced by a PCR fragment that contained the *HHF2* promoter (pRS314-HLB) and (2) the *Sac*I–*Apa*I fragment (made blunt ended) from pRS314-HLB, containing the *HHF2pr*::*leu2*Δ*3*′::*leu2*Δ*5*′ recombination system, was inserted at the *Cla*I (made blunt ended) site of pFERNU either in the IN (pARSHLB-IN) or the OUT orientation (pARSHLB-OUT). pARSCLB-IN and pARSXLB-IN were constructed by replacing either the *Aat*II or the *Nar*I–*Sph*I fragment of pARSHLB-IN, containing the *HHF2* promoter, with a PCR fragment containing the *CLN2* or *HHO1* promoter, respectively. PCR amplifications were made with oligos 5′-attcatcgattatatgtaaagtacgctttt-3′ and 5′-tcaggtcgactaataatggtttcttaggac-3′ (*ARSH4*), 5′-cattaccgcggcttttcatcacgtgctataa-3′ and 5′-tgaatccgcggttttacatcttcggaaaaca-3′ (*CEN6*), 5′-cattagagctcgacgtcgcatgcgttatcacgcaaactatgttttgac-3′ and 5′-gcgtaggatccggcgccgacgtctattttattgtattgattgttgttt-3′ (*HHF2* promoter), 5′-cattagacgtcgcatgccgaactaaagcaactatacattg-3′ and 5′-gcgtaggcgccgacgtctgtctgtcgttaaatttaatgaa-3′ (*CLN2* promoter) and 5′-cattagacgtcgcatgctataactgatatgtaaactgtgc-3′ and 5′-gcgtaggcgccgacgtcgttgctttagttgtattataatt-3′ (*HHO1* promoter). Plasmids pARSHLB-Leu and pARSCLB-Leu are yeast centromeric plasmids containing the HHF-Leu and CLN-Leu constructs, respectively. They resulted by homologous recombination between the *leu2* repeats of the HHF-IN and CLN-IN constructs, respectively.

Recombination analysis

Spontaneous recombination frequencies were obtained as the average value of median frequencies obtained by 6–10 fluctuation tests performed with 2–3 independent transformants. For each fluctuation test, six independent colonies were analysed as previously described (Prado and Aguilera, 1995). Note that the entire endogenous *LEU2* gene was completely deleted in all strains used in this study, so that Leu + recombinants could only arise by recombination between the *leu2* repeats of the plasmid-borne recombination constructs.

RNA level analysis

Total RNA from 2–4 independent transformants was extracted and analysed by Northern hybridization as previously published (Chavez and Aguilera, 1997). mRNA was probed with the 598 bp *Cla*I–*Eco*RV internal *LEU2* fragment, quantified in a Fuji FLA3000 and normalized with respect to the 25S rRNA value. Run-on analysis was performed as previously described (Chavez and Aguilera 1997). As probes we used PCR fragments obtained with primers 5′-gttccacttccagatgaggc-3′ and 5′-ttagcaaattgtggcttgat-3′ (*leu2*-1), 5′-gttttggcctcttcaagatt-3′ and 5′-gttcttgtctggcaaagagg-3′ (*leu2*-2), 5′-ttggagagggcaactttgg-3′ and 5′-caggatcggtcgattgtgc-3′ (rDNA) and 5′-tcgttgctgcataaaccg-3′ and 5′-tcgataatttcaccgccg-3′ (*lacZ*). A 1 μg portion of each DNA probe was immobilized in Hybond-N membranes. Filters were hybridized with *in vivo* α^{32}P-UTP-labelled total RNA extracted from strains harbouring either the CLN-Leu or HHF-Leu construct. The run-on was performed twice with similar results.

Analysis of replication intermediates

Total DNA from 0.5 l of mid-log-phase cells was isolated according to Allers and Lichten (2000) with the modifications of Wellinger *et al* (2003) in the absence of hexamine cobalt trichloride (HCC). After digestion with the appropriate restriction enzymes, DNA was enriched in replication intermediates by selective adsorption to BND-cellulose (Huberman *et al*, 1987). DNA molecules were resolved by neutral/neutral 2-D gel electrophoresis as described previously (Brewer and Fangman, 1987) and probed with the 598 bp *Cla*I–*Eco*RV *LEU2* fragment. Quantification of the RFP signal was determined relative to the total intensity of the replication intermediates, and the average and standard deviations of two (*rad51*Δ, *rad52*Δ, *sgs1*Δ, *srs2*Δ) or three (wild type and *rrm3*Δ) independent quantifications are plotted.

Acknowledgements

We thank M Nieto and J Escalante for technical assistance, RE Wellinger for help on the replication intermediate analysis, F Antequera for critical reading of the manuscript and D Haun for style supervision. The research was funded by the Spanish Ministry of Science and Technology (BMC2000-0439 and SAF2003-00204 grants).

References

Aguilera A (2002) The connection between transcription and genomic instability. *EMBO J* **21:** 195–201

Allers T, Lichten M (2000) A method for preparing genomic DNA that restrains branch migration of Holliday junctions. *Nucleic Acids Res* **28:** e6

Brewer BJ, Fangman WL (1987) The localization of replication origins on ARS plasmids in *S. cerevisiae*. *Cell* **51:** 463–471

Brewer BJ, Fangman WL (1988) A replication fork barrier at the 3′ end of yeast ribosomal RNA genes. *Cell* **55:** 637–643

Casper AM, Nghiem P, Arlt MF, Glover TW (2002) ATR regulates fragile site stability. *Cell* **111:** 779–789

Cha RS, Kleckner N (2002) ATR homolog Mec1 promotes fork progression, thus averting breaks in replication slow zones. *Science* **297:** 602–606

Chavez S, Aguilera A (1997) The yeast *HPR1* gene has a functional role in transcriptional elongation that uncovers a novel source of genome instability. *Genes Dev* **11:** 3459–3470

Cobb JA, Bjergbaek L, Shimada K, Frei C, Gasser SM (2003) DNA polymerase stabilization at stalled replication forks requires Mec1 and the RecQ helicase Sgs1. *EMBO J* **22:** 4325–4336

Courcelle J, Donaldson JR, Chow KH, Courcelle CT (2003) DNA damage-induced replication fork regression and processing in *Escherichia coli*. *Science* **299:** 1064–1067

Cox MM, Goodman MF, Kreuzer KN, Sherratt DJ, Sandler SJ Marians KJ (2000) The importance of repairing stalled replication forks. *Nature* **404:** 37–41

Deshpande AM, Newlon CS (1996) DNA replication fork pause sites dependent on transcription. *Science* **272:** 1030–1033

Elias-Arnanz M, Salas M (1997) Bacteriophage phi29 DNA replication arrest caused by codirectional collisions with the transcription machinery. *EMBO J* **16:** 5775–5783

Elias-Arnanz M, Salas M (1999) Resolution of head-on collisions between the transcription machinery and bacteriophage phi29 DNA polymerase is dependent on RNA polymerase translocation. *EMBO J* **18:** 5675–5682

Freeman KB, Karns LR, Lutz KA, Smith MM (1992) Histone H3 transcription in *Saccharomyces cerevisiae* is controlled by multiple cell cycle activation sites and a constitutive negative regulatory element. *Mol Cell Biol* **12:** 5455–5463

French S (1992) Consequences of replication fork movement through transcription units *in vivo*. *Science* **258:** 1362–1365

Gangloff S, Lieber MR, Rothstein R (1994) Transcription, topoisomerases and recombination. *Experientia* **50:** 261–269

Gangloff S, Soustelle C, Fabre F (2000) Homologous recombination is responsible for cell death in the absence of the Sgs1 and Srs2 helicases. *Nat Genet* **25:** 192–194

Garcia-Rubio M, Huertas P, Gonzalez-Barrera S, Aguilera A (2003) Recombinogenic effects of DNA-damaging agents are synergistically increased by transcription in *Saccharomyces cerevisiae*. New insights into transcription-associated recombination. *Genetics* **165:** 457–466

Gerber JK, Gogel E, Berger C, Wallisch M, Muller F, Grummt I, Grummt F (1997) Termination of mammalian rDNA replication: polar arrest of replication fork movement by transcription termination factor TTF-I. *Cell* **90:** 559–567

Gonzalez-Barrera S, García-Rubio M, Aguilera A (2002) Transcription and double-strand-breaks induce similar mitotic recombination events in *Saccharomyces cerevisiae*. *Genetics* **162:** 603–614

Hereford LM, Osley MA, Ludwig II TR, McLaughlin CS (1981) Cell-cycle regulation of yeast histone mRNA. *Cell* **24:** 367–375

Horiuchi T, Fujimura Y (1995) Recombinational rescue of the stalled DNA replication fork: a model based on analysis of an *Escherichia coli* strain with a chromosome region difficult to replicate. *J Bacteriol* **177:** 783–791

Horiuchi T, Fujimura Y, Nishitani H, Kobayashi T, Hidaka M (1994) The DNA replication fork blocked at the Ter site may be an entrance for the RecBCD enzyme into duplex DNA. *J Bacteriol* **176:** 4656–4663

Huberman JA, Spotila LD, Nawotka KA, el-Assouli SM, Davis LR (1987) The *in vivo* replication origin of the yeast 2 microns plasmid. *Cell* **51:** 473–481

Huertas P, Aguilera A (2003) Cotranscriptionally formed DNA:RNA hybrids mediate transcription elongation impairment and transcription-associated recombination. *Mol Cell* **12:** 711–721

Ira G, Malkova A, Liberi G, Foiani M, Haber JE (2003) Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. *Cell* **115:** 401–411

Ivessa AS, Lenzmeier BA, Bessler JB, Goudsouzian LK, Schnakenberg SL, Zakian VA (2003) The *Saccharomyces cerevisiae* helicase Rrm3p facilitates replication past nonhistone protein–DNA complexes. *Mol Cell* **12:** 1525–1536

Ivessa AS, Zhou JQ, Schulz VP, Monson EK, Zakian VA (2002) *Saccharomyces* Rrm3p, a 5′ to 3′ DNA helicase that promotes replication fork progression through telomeric and subtelomeric DNA. *Genes Dev* **16:** 1383–1396

Ivessa AS, Zhou JQ, Zakian VA (2000) The *Saccharomyces* Pif1p DNA helicase and the highly related Rrm3p have opposite effects on replication fork progression in ribosomal DNA. *Cell* **100:** 479–489

Jung S, Rajewsky K, Radbruch A (1993) Shutdown of class switch recombination by deletion of a switch region control element. *Science* **259:** 984–987

Kaiser C, Michaelis M, Mitchell A (1994) *Methods in Yeast Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press

Kolodner RD, Putnam CD, Myung K (2002) Maintenance of genome stability in *Saccharomyces cerevisiae*. *Science* **297:** 552–557

Krasilnikova MM, Samadashwily GM, Krasilnikov AS, Mirkin SM (1998) Transcription through a simple DNA repeat blocks replication elongation. *EMBO J* **17:** 5095–5102

Krejci L, Van Komen S, Li Y, Villemain J, Reddy MS, Klein H, Ellenberger T, Sung P (2003) DNA helicase Srs2 disrupts the Rad51 presynaptic filament. *Nature* **423:** 305–309

Lee SK, Johnson RE, Yu SL, Prakash L, Prakash S (1999) Requirement of yeast *SGS1* and *SRS2* genes for replication and transcription. *Science* **286:** 2339–2342

Lengauer C, Kinzler KW, Vogelstein B (1998) Genetic instabilities in human cancers. *Nature* **396:** 643–649

Little RD, Platt TH, Schildkraut CL (1993) Initiation and termination of DNA replication in human rRNA genes. *Mol Cell Biol* **13:** 6600–6613

Liu B, Alberts BM (1995) Head-on collision between a DNA replication apparatus and RNA polymerase transcription complex. *Science* **267:** 1131–1137

Liu B, Wong ML, Tinker RL, Geiduschek EP, Alberts BM (1993) The DNA replication fork can pass RNA polymerase without displacing the nascent transcript. *Nature* **366:** 33–39

McGlynn P, Lloyd RG (2000) Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression. *Cell* **101:** 35–45

Myung K, Datta A, Kolodner RD (2001) Suppression of spontaneous chromosomal rearrangements by S phase checkpoint functions in *Saccharomyces cerevisiae*. *Cell* **104:** 397–408

Olavarrieta L, Hernandez P, Krimer DB, Schvartzman JB (2002) DNA knotting caused by head-on collision of transcription and replication. *J Mol Biol* **322:** 1–6

Ooi SL, Shoemaker DD, Boeke JD (2003) DNA helicase gene interaction network defined using synthetic lethality analyzed by microarray. *Nat Genet* **35:** 277–286

Peters A, Storb U (1996) Somatic hypermutation of immunoglobulin genes is linked to transcription initiation. *Immunity* **4:** 57–65

Piruat JI, Aguilera A (1998) A novel yeast gene, *THO2*, is involved in RNA pol II transcription and provides new evidence for transcriptional elongation-associated recombination. *EMBO J* **17:** 4859–4872

Prado F, Aguilera A (1995) Role of reciprocal exchange, one-ended invasion crossover and single-strand annealing on inverted and

direct repeat recombination in yeast: different requirements for the *RAD1*, *RAD10*, and *RAD52* genes. *Genetics* **139:** 109–123

Prado F, Piruat JI, Aguilera A (1997) Recombination between DNA repeats in yeast hpr1delta cells is linked to transcription elongation. *EMBO J* **16:** 2826–2835

Rocha EP, Danchin A (2003) Essentiality, not expressiveness, drives gene-strand bias in bacteria. *Nat Genet* **34:** 377–378

Rothstein R, Michel B, Gangloff S (2000) Replication fork pausing and recombination or 'gimme a break'. *Genes Dev* **14:** 1–10

Saxe D, Datta A, Jinks-Robertson S (2000) Stimulation of mitotic recombination events by high levels of RNA polymerase II transcription in yeast. *Mol Cell Biol* **20:** 5404–5414

Schmidt KH, Kolodner RD (2004) Requirement of Rrm3 helicase for repair of spontaneous DNA lesions in cells lacking Srs2 or Sgs1 helicase. *Mol Cell Biol* **24:** 3213–3226

Seigneur M, Bidnenko V, Ehrlich SD, Michel B (1998) RuvAB acts at arrested replication forks. *Cell* **95:** 419–430

Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122:** 19–27

Sogo JM, Lopes M, Foiani M (2002) Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects. *Science* **297:** 599–602

Spellman PT, Sherlock G, Zhang MQ, Iyer VR, Anders K, Eisen MB, Brown PO, Botstein D, Futcher B (1998) Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Mol Biol Cell* **9:** 3273–3297

Stuart D, Wittenberg C (1994) Cell cycle-dependent transcription of *CLN2* is conferred by multiple distinct *cis*-acting regulatory elements. *Mol Cell Biol* **14:** 4788–4801

Takeuchi Y, Horiuchi T, Kobayashi T (2003) Transcription-dependent recombination and the role of fork collision in yeast rDNA. *Genes Dev* **17:** 1497–1506

Thomas BJ, Rothstein R (1989) Elevated recombination rates in transcriptionally active DNA. *Cell* **56:** 619–630

Torres JZ, Bessler JB, Zakian VA (2004a) Local chromatin structure at the ribosomal DNA causes replication fork pausing and genome instability in the absence of the *S. cerevisiae* DNA helicase Rrm3p. *Genes Dev* **18:** 498–503

Torres JZ, Schnakenberg SL, Zakian VA (2004b) *Saccharomyces cerevisiae* Rrm3p DNA helicase promotes genome integrity by preventing replication fork stalling: viability of rrm3 cells requires the intra-S-phase checkpoint and fork restart activities. *Mol Cell Biol* **24:** 3198–3212

Veaute X, Jeusset J, Soustelle C, Kowalczykowski SC, Le Cam E, Fabre F (2003) The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. *Nature* **423:** 309–312

Versini G, Comet I, Wu M, Hoopes L, Schwob E, Pasero P (2003) The yeast Sgs1 helicase is differentially required for genomic and ribosomal DNA replication. *EMBO J* **22:** 1939–1949

Wellinger RE, Schar P, Sogo JM (2003) Rad52-independent accumulation of joint circular minichromosomes during S phase in *Saccharomyces cerevisiae*. *Mol Cell Biol* **23:** 6363–6372

Wiesendanger B, Lucchini R, Koller T, Sogo JM (1994) Replication fork barriers in the *Xenopus* rDNA. *Nucleic Acids Res* **22:** 5038–5046

Wittenberg C, Sugimoto K, Reed SI (1990) G1-specific cyclins of *S. cerevisiae*: cell cycle periodicity, regulation by mating pheromone, and association with the p34CDC28 protein kinase. *Cell* **62:** 225–237

Yu K, Chedin F, Hsieh CL, Wilson TE, Lieber MR (2003) R-loops at immunoglobulin class switch regions in the chromosomes of stimulated B cells. *Nat Immunol* **4:** 442–451

Zou H, Rothstein R (1997) Holliday junctions accumulate in replication mutants via a RecA homolog-independent mechanism. *Cell* **90:** 87–96

