Copyright © 2003 by the Genetics Society of America

Recombinogenic Effects of DNA-Damaging Agents Are Synergistically Increased by Transcription in *Saccharomyces cerevisiae*: New Insights Into Transcription-Associated Recombination

M. García-Rubio, P. Huertas, S. González-Barrera and A. Aguilera[1]

Departamento de Genética, Facultad de Biología, Universidad de Sevilla, 41012 Sevilla, Spain

Manuscript received April 3, 2003
Accepted for publication May 16, 2003

ABSTRACT

Homologous recombination of a particular DNA sequence is strongly stimulated by transcription, a phenomenon observed from bacteria to mammals , which we refer to as transcription-associated recombination (TAR). TAR might be an accidental feature of DNA chemistry with important consequences for genetic stability. However, it is also essential for developmentally regulated processes such as class switching of immunoglobulin genes. Consequently, it is likely that TAR embraces more than one mechanism. In this study we tested the possibility that transcription induces recombination by making DNA more susceptible to recombinogenic DNA damage. Using different plasmid-chromosome and direct-repeat recombination constructs in which transcription is driven from either the P_{GAL1}- or the P_{tet}-regulated promoters, we have shown that either 4-nitroquinoline-*N*-oxide (4-NQO) or methyl methanesulfonate (MMS) produces a synergistic increase of recombination when combined with transcription. 4-NQO and MMS stimulated recombination of a transcriptionally active DNA sequence up to 12,800- and 130-fold above the spontaneous levels observed in the absence of transcription, whereas 4-NQO and MMS alone increased recombination 193- and 4.5-fold, respectively. Our results provide evidence that TAR is due, at least in part, to the ability of transcription to enhance the accessibility of DNA to exogenous chemicals and internal metabolites responsible for recombinogenic lesions. We discuss possible parallelisms between the mechanisms of induction of recombination and mutation by transcription.

TRANSCRIPTION of a DNA sequence increases its level of instability. Both spontaneous mutation and recombination of a particular DNA sequence are significantly higher when they are transcribed (Wright 2000; Aguilera 2002). To specifically refer to mutations and recombination events stimulated by transcription, we use the terms transcription-associated mutation (TAM) and transcription-associated recombination (TAR).

TAM was reported first in bacteria (Brock 1971; Herman and Dworkin 1971; Wright *et al.* 1999), but it also exists in yeast (Datta and Jinks-Robertson 1995) and phage (Beletskii *et al.* 2000). Transcription in the presence of alkylating agents (Brock 1971) or ICR-191 (Herman and Dworkin 1971) stimulates mutation rates of the β-galactosidase locus of *Escherichia coli.*

TAR has been reported in prokaryotes (Ikeda and Matsumoto 1979; Dul and Drexler 1988; Vilette *et al.* 1995) and eukaryotes from yeast (Voelkel-Meiman *et al.* 1987; Thomas and Rothstein 1989) to mammals (Nickoloff 1992). Important developmental processes, such as V(D)J recombination and immunoglobulin class switching, are also transcription dependent (Stavnezer-Nordgren and Sirlin 1986; Daniels and Lieber 1995; Sikes *et al.* 2002). However, the molecular basis of the association between transcription and recombination is unclear. It seems likely that there is more than one mechanism by which transcription can stimulate recombination. Thus, yeast mutants of the THO complex, which functions at the interface between transcription and mRNP metabolism, show a transcription elongation impairment that strongly stimulates direct-repeat recombination (Chavez and Aguilera 1997; Prado *et al.* 1997; Piruat and Aguilera 1998; Chavez *et al.* 2000). However, TAR may not be necessarily linked to transcription impairment. It is possible that DNA becomes more susceptible to chemical reactions yielding DNA breaks during proper transcription elongation. The topological change (Sternglanz *et al.* 1981) or chromatin remodeling (Jones and Kadonaga 2000; Orphanides and Reinberg 2000) associated with transcription might increase the probability of occurrence of single-strand DNA (ssDNA) regions that are more susceptible to recombinogenic damage.

In this study we directly tested the possibility that transcription induced recombination by making DNA more accessible to damaging agents. Our rationale was based on the ideas, first, that chemically or UV-damaged bases can be processed into DNA breaks by the incomplete action of base excision repair (BER) or nucleotide excision repair (NER) and replication and, second, that DNA breaks can induce recombination (see Paques and Haber

[1]*Corresponding author:* Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Avd. Reina Mercedes 6, 41012 Sevilla, Spain. E-mail: aguilo@us.es

1999; Kupiec 2000). Thus, in yeast, when a modified base is recognized by BER it is first removed by an *N*-glycosylase such as Ntg1p, Ntg2p (Eide *et al.* 1996; Alseth *et al.* 1999), and Ogg1p (Nash *et al.* 1996; van der Kemp *et al.* 1996), leaving an apurinic or apyrimidinic site (AP site). This is then cleaved by either an Ap-lyase or an Ap-endonuclease such as Apn1p (Ramotar *et al.* 1991). Failures in any of these activities could lead to DNA-break accumulation. Consistent with this view, triple mutants *ntg1 ntg2 apn1* and *ntg1 ntg2 ogg1* have been reported to have little effect on TAM (Morey *et al.* 2000), but they do lead to increased recombination frequencies (Swanson *et al.* 1999). Similarly, DNA lesions that are substrates of NER, such as those produced by UV or 4-nitroquinoline-*N*-oxide (4-NQO; Zaborowska *et al.* 1983; Iijima and Morimoto 1986; Darroudi *et al.* 1989), are also recombinogenic, the recombinogenic effect of these agents being stronger in yeast NER-deficient mutants such as *rad1* in ectopic substrates (Kadyk and Hartwell 1992; Saffran *et al.* 1994).

Using newly developed recombination assays we show in this study that 4-NQO and methyl methanesulfonate (MMS) cause synergistic increases of homologous recombination in a DNA sequence if it is actively transcribed. We provide evidence that TAR, and by extension TAM, might be, at least in part, a consequence of an increased susceptibility of DNA to damaging agents, whether naturally produced during cell metabolism or added exogenously.

MATERIALS AND METHODS

Yeast strains and plasmids: Plasmid-chromosome recombination was determined in the congenic strains BER08-64A (*MAT***a** *his3::leu2-k leu2Δ0 lys2Δ0 met15Δ0 trp1-1 ura3Δ0*), BER07-64C (*MAT***a** *his3::leu2-k leu2Δ0 met15Δ0 ura3Δ0 ntg1Δ::KanMX4 ntg2Δ::KanMX4 apn1Δ::KanMX4*), BER08-39D (*MAT*α *his3::leu2-k leu2Δ0 lys2Δ0 trp1-1 ura3Δ0 ntg1Δ::KanMX4 ntg2Δ::KanMX4 ogg1Δ::KanMX4*), and BEWRI-22C (*MAT***a** *ade2-101 his3::leu2k leu2 lys2Δ0 met2Δ15 trp1-1 ura3 rad1Δ::KanMX4*). The strain BER06-99D (*MAT*α *his3Δ0 leu2Δ0 lys2Δ0 trp1-1 ura3Δ0*) was used for Northern and direct-repeat recombination analyses. *ntg1Δ::KanMX4, ntg2Δ::KanMX4, apn1Δ::KanMX4,* and *ogg1Δ::KanMX4* simple mutants obtained from EUROSCARF (Frankfurt, Germany) were crossed with a wild type harboring the *his3::leu2-k* allele. Single mutants harboring *his3::leu2-k* were then crossed to obtain wild-type and triple-mutant derivatives. Strain WSR1-4C (*rad1Δ::KanMX4* isogenic to W303; González-Barrera *et al.* 2002) was crossed with BER08-64A to obtain BEWRI-22C.

Plasmid pCM184-L2HOr, in which the *leu2-HOr* allele containing an HO cleavage site inserted at the *Eco*RI site is under control of the P_{tet} promoter, was constructed by inserting the 1.34-kb *Bam*HI-*Ssp*I *leu2-HOr* fragment into the pCM184 vector (Gari *et al.* 1997). Plasmid p414-GL2HOr, in which the *leu2-HOr* allele is under control of the *GAL1* promoter, has a 21-bp HO site at the *Eco*RI site of *LEU2* (González-Barrera *et al.* 2002). pRS414-GLB, pSG206, pRS314-GllacZ, and pRS314-GLNA were constructed replacing the 1.22-kb *Sac*I-*Cla*I *LEU2* promoter fragment for the 0.62-kb *Sac*I-*Cla*I P_{GAL1} promoter fragment of p414-GLEU2 in p314LB, pSCh206, p314LlacZ, and p314LNA, respectively (Chavez and Aguilera 1997; Prado *et al.* 1997; Piruat and Aguilera 1998).

Determination of recombination frequencies: 4-NQO was added at a final concentration of 0.1 mg/liter to synthetic medium (SC) on plates, with the exception of the experiments performed with *rad1Δ* and its corresponding wild-type control, in which 0.01 mg/liter of 4-NQO was used. Menadione and MMS were added to final concentrations of 0.1 mm and 0.035% to SC medium, respectively. Gene expression driven from the P_{tet} promoter was repressed by adding doxycycline (dox) at the concentration of 5 mg/liter to synthetic medium on plates (Gari *et al.* 1997). Midlog phase cultures were plated onto the corresponding SC media and cultured for 3–4 days to obtain single colonies. Recombination tests were performed on independent colonies.

Each recombination frequency value was obtained by a fluctuation test as the median value of six independent colonies isolated in SC-trp containing 2% glucose or 2% galactose, as previously described (Prado and Aguilera 1995), in the presence or absence of menadione, 4-NQO (in the dark), or MMS. Recombinants were selected on SC-leu supplemented with either 2% galactose (for p414-GL2HOr, pRS414-GLB, pSG206, pRS314-GllacZ, and pRS314-GLNA transformants) or 2% glucose (for pCM184-L2HOr). For each strain and condition used, two to three different fluctuation tests were performed, each with six independent cultures, and two to three median recombination frequency values were obtained. The final recombination frequency given for each strain and condition tested is the mean and standard deviation of the two to three median values.

RNA analysis: Total yeast RNA was prepared from SC-2% glucose overnight cultures, subjected to electrophoresis on formaldehyde-agarose gels, and hybridized with the appropriate radiolabeled DNA probes. The 597-bp *Cla*I-*Eco*RV *LEU2* internal fragment and the 589-bp 28S rRNA internal fragment obtained by PCR as previously described (Chavez and Aguilera 1997) were used as probes. RNA levels were quantified with a Fuji FLA3000 and were normalized with respect to the 28S rRNA value.

Miscellaneous: Growth conditions, yeast transformation, genetic analysis, and preparation of α^{32}P-labeled DNA probes were performed following standard procedures (see Prado and Aguilera 1995).

RESULTS

Plasmid-chromosome homologous recombination assays to study damage-induced transcription-associated recombination: We constructed two systems to analyze homologous gene conversion under either low or high transcription levels. In both systems, recombination occurring between two different *leu2* alleles, one located in a chromosome and the other in a monocopy plasmid, was assayed. *leu2-k* was used as the chromosomal allele, containing a 6-bp deletion at the *Kpn*I site, whereas as the plasmid-borne allele *leu2-HOr* was used, containing a 25-bp insertion at the *Eco*RI site located 0.4 kb downstream of *Kpn*I. In plasmid pCM184-L2HOr, the *leu2-HOr* allele is under the P_{tet} promoter (Figure 1A); thus, transcription of the *leu2-HOr* sequence was repressed with doxycycline and activated in its absence. In plasmid p414-GL2HOr, *leu2-HOr* was under the P_{GAL1} promoter (Figure 1B), which was repressed with glucose and activated with galactose.

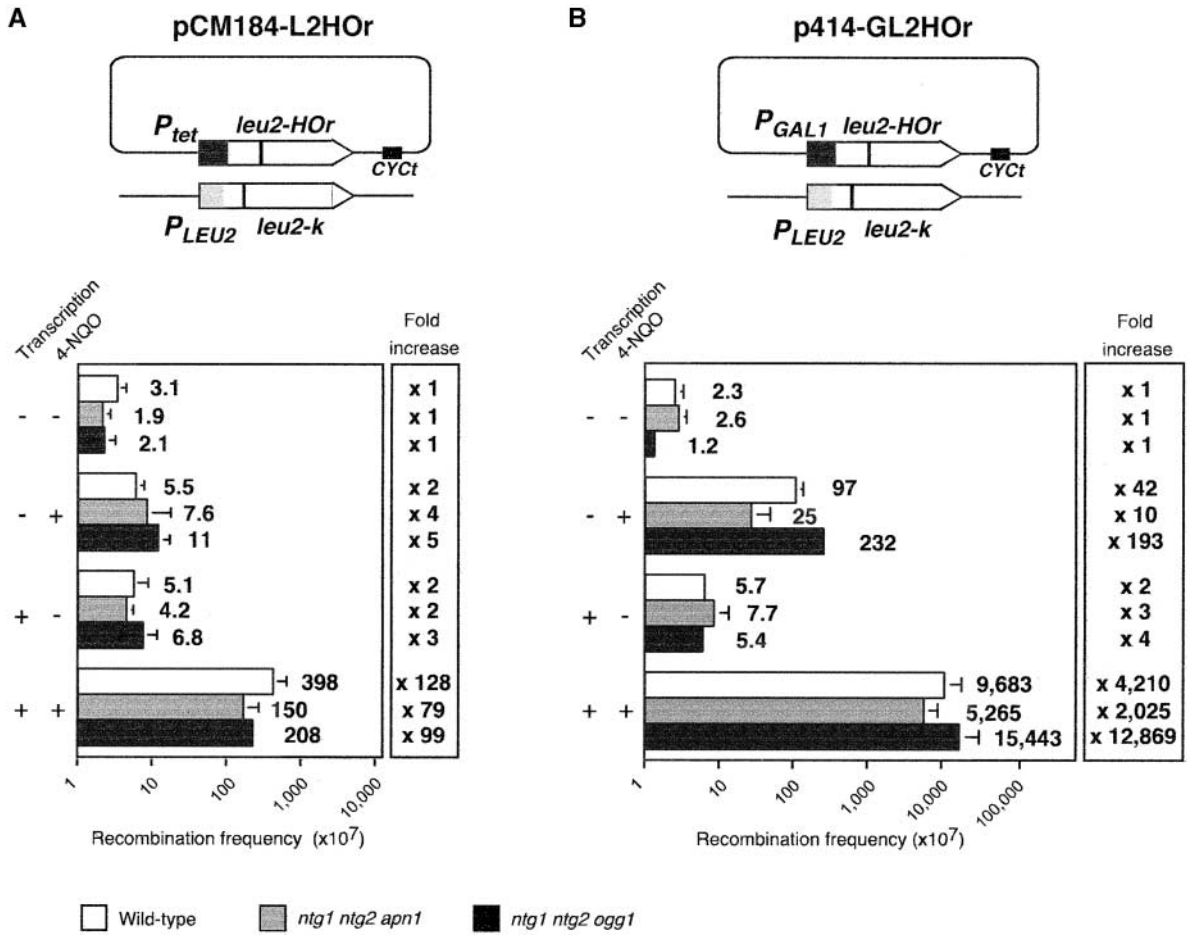

FIGURE 1.—Effect of transcription and 4-NQO on intermolecular recombination. (A) Recombination frequency in the presence and absence of transcription and 4-NQO (0.1 μg/ml) in wild type (BER07-73B), *ntg1 ntg2 apn1* (BER07-64C), and *ntg1 ntg2 ogg1* (BER08-39D) in the plasmid-chromosome recombination assay based on a chromosomal *leu2-k* allele, harboring a 6-bp deletion at the *Kpn*I site, under a P_{LEU2} promoter and a plasmid-borne *leu2-HOr* allele, containing a 25-bp insertion at the *Eco*RI site, under the control of the P_{TET}-regulated promoter. The *LEU2* wild-type allele can arise by gene conversion of either *leu2-HOr* or *leu2-k* alleles. A diagram of the system is shown. (B) Recombination frequency under the same transcriptional and 4-NQO conditions using plasmid p414G-L2HOr carrying the *leu2-HOr* allele fused to the P_{GAL1} promoter and the chromosomal *leu2-k* allele. The mean and standard deviations of two to three median frequency values obtained from two to three different fluctuations tests, each made with six independent colonies, are plotted.

In both cases, we detected gene conversion of either the chromosomal *leu2-k* or the plasmid-borne *leu2-HOr* allele.

Menadione does not increase recombination in the plasmid-chromosome recombination systems: Since we were interested in determining the effect of transcription on damage-induced recombination, the recombinogenic effect of different chemicals was tested. The first chemical we tried was the oxidative agent menadione. It has been shown that mutations abolishing BER—but not *rad1* or *rev3* mutations, which abolish NER and translesion synthesis, respectively—increase spontaneous recombination (SWANSON *et al.* 1999). So we assayed the recombinogenic activity of menadione on the wild-type and BER-deficient yeast strains *ntg1 ntg2 apn1* and *ntg1 ntg2 ogg1* in the P_{tet}-based recombination systems. Using concentrations (0.1 mM) that had no effect on cell growth, we found that menadione had no significant effect on recombination under the conditions tested in either the wild-type or BER mutant backgrounds (Table 1). It is likely that, in contrast to the recombination assay used by SWANSON *et al.* (1999), in which BER mu-

TABLE 1

Effect of menadione on intermolecular recombination (×10^7)

	Spontaneous	Menadione induced
Wild type	5.1	7 (×1.3)
ntg1 ntg2 apn1	4.2	7.2 (×1.7)
ntg1 ntg2 ogg1	6.8	8.4 (×1.2)

Recombination was assayed in the P_{tet}-based plasmid-chromosome recombination system under low-transcription conditions (+dox). Details are as in Figure 1.

tants show a clear spontaneous hyperrecombinogenic effect, our plasmid-chromosome recombination assay was not sensitive enough to detect either spontaneous or induced hyperrecombination in BER-deficient mutants. In addition, it could also be that the oxidative effect of menadione on DNA is strongly dependent on the genetic background and the different intracellular red-ox state of the strains used. Consequently, we decided to not use menadione as a chemical to study TAR, but to use chemicals for which the recombinogenic effects have been reported, such as the UV and X-ray-mimetic chemicals 4-NQO and MMS, respectively.

Synergistic increase of plasmid-chromosome recombination caused by 4-NQO and transcription: The recombinogenic effect of the UV mimetic chemical 4-NQO has been reported previously (Friedman and Yasbin 1983; Iijima and Morimoto 1986; Darroudi *et al.* 1989). For this reason, we decided to use 4-NQO to study the capacity of a chemical to stimulate recombination under conditions of low and high transcription in our recombination assays.

The effect of 4-NQO on recombination under low- and high-transcription conditions was determined in the recombination system under the control of P_{tet}. Figure 1A shows that in wild-type cells Leu$^+$ gene conversions were stimulated 2-fold by 4-NQO. In BER-deficient strains, this increase was 4- to 5-fold under low-transcription conditions, that is, in the presence of doxycycline. When cells were cultured without doxycycline in the media, that is, under high transcription of the *P_{tet}::leu2-HOr* allele, Leu$^+$ gene conversions were stimulated 2-fold by transcription in the absence of 4-NQO. However, under high-transcription conditions, 4-NQO stimulated recombination 128-fold above basal levels. Similar results were observed in the BER mutants (Figure 1A).

To confirm the direct relationship between 4-NQO-induced recombination and transcription, all our experiments were repeated using identical recombination assays but under the control of P_{GAL1}, a stronger and more tightly regulated promoter than P_{tet}. As can be seen in Figure 1B, the basal recombination frequencies (no transcription, no 4-NQO) were similar to those of the recombination assays based on P_{tet}. Recombination was stimulated 42-fold by 4-NQO and 2-fold by transcription. However, the increase in recombination caused by the simultaneous action of transcription and 4-NQO was 4210 times above basal levels in wild-type cells. Similar results were obtained in BER mutants (Figure 1B), an expected result given the lack of evidence that BER deals with UV lesions. This implies that channeling of potential BER substrates into recombinational repair does not occur.

These results confirm that transcription and 4-NQO synergistically increase recombination. Although quantitative differences in the overall levels of recombination are produced by transcription and 4-NQO in each case, both systems behave similarly regardless of using doxycycline, galactose, or glucose in the media. It is worth noting that the induction of recombination caused by 4-NQO under no-transcription conditions was higher in the P_{GAL1}-dependent recombination construct than in the P_{tet}-dependent construct. It is possible that either 4-NQO accesses P_{GAL1} better than P_{tet} or, given the higher strength of P_{GAL1} compared with P_{tet}, a putative leaky transcription of *P_{GAL1}::leu2-HOr* in 2% glucose stimulates the action of 4-NQO. The latter would be consistent with the observation that the recombinogenic effect of 4-NQO increases synergistically with transcription.

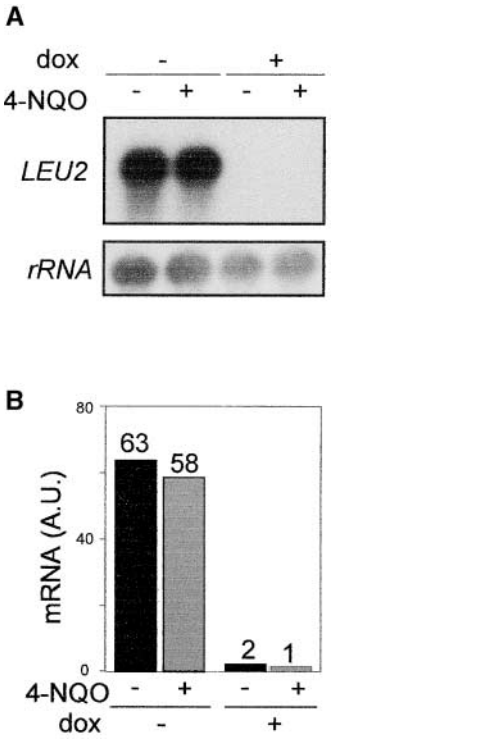

Figure 2.—Transcription analysis of *P_{tet}::leu2-HOr* in the wild-type strain BER06-99D. (A) Northern analysis of *leu2* mRNA driven from the P_{tet} promoter. Total RNA was isolated from overnight cultures in SC-trp containing 4-NQO and/or dox as indicated. (B) Quantification of Northern results in arbitrary units (A.U.) and normalized with respect to the 28S rDNA value.

Impairment of transcription elongation observed as a strong reduction in accumulation of full transcripts can lead to a strong increase of recombination, as we have shown for mutants of the THO complex and other functionally related proteins (Piruat and Aguilera 1998; Chavez *et al.* 2000; Jimeno *et al.* 2002). Therefore, we considered it important to show that 4-NQO did not produce a strong impairment of transcription that could explain the strong induction of recombination, as is the case of mutants of the THO complex. As can be seen in Figure 2, transcript levels in the *P_{tet}::leu2-HOr* construct are not affected by 4-NQO under either high (−dox) or low (+dox) transcription conditions.

Finally, as 4-NQO induced DNA lesions that are sub-

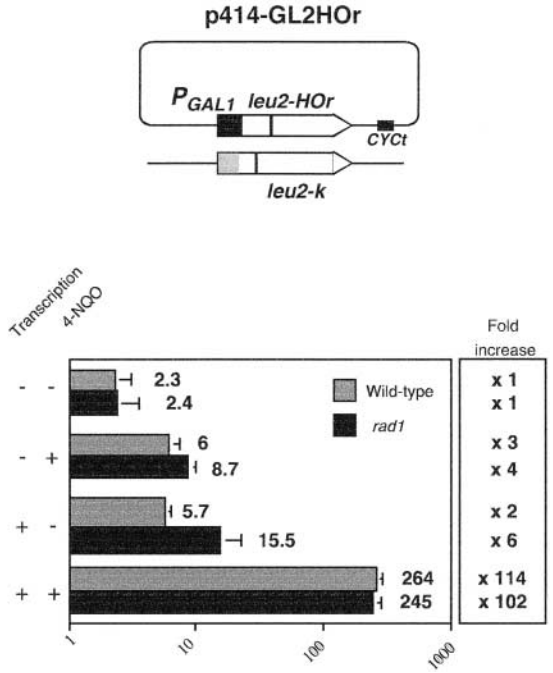

FIGURE 3.—Effect of transcription and 4-NQO on intermolecular recombination in *rad1*Δ strains. Recombination frequencies of wild-type (BER08-64A) and *rad1*Δ (BEWRI-22C) strains in the absence and presence of transcription and 4-NQO (0.01 μg/ml) in the P_{GAL1}-based plasmid-chromosome recombination system. Other details are as in Figure 1.

strates for NER (NAGAO and SUGIMURA 1976; PATERSON *et al.* 1984), we wondered whether the effect of 4-NQO on TAR could also be observed in *rad1*Δ NER-deficient mutants, given that recombinogenic DNA lesions should accumulate in *rad1*Δ strains. As *rad1*Δ mutants were hypersensitive to 4-NQO, we performed these experiments using 4-NQO concentrations (0.01 mg/liter) 10-fold lower than those used before, for which we determined that *rad1*Δ strains form colonies after 4 days. Figure 3 shows that, whereas transcription or 4-NQO alone induced recombination in wild-type and *rad1*Δ cells at similar low levels (2.5- to 6.4-fold above spontaneous level), the simultaneous action of 4-NQO and transcription induced recombination synergistically in both strains (102- to 114-fold). These results confirm that 4-NQO has a synergistic effect on recombination, regardless of the DNA repair mutant background used. The similar hyperrecombination effect of 4-NQO in *rad1*Δ *vs.* wild-type cells may be a consequence of the fact that Rad1p is also required for recombination (PRADO *et al.* 2003), in particular for TAR (MALAGON and AGUILERA 2001; GONZÁLEZ-BARRERA *et al.* 2002). Alternatively, it is possible that in NER-deficient strains a large fraction of 4-NQO-induced DNA lesions could be processed into cytotoxic intermediates that could not be used as substrates of the recombinational repair machinery.

Our recombination results predict that the allele under the control of the strong P_{tet} or P_{GAL1} promoters, *leu2-HOr*, should be the one preferentially converted under high-transcription conditions. Indeed, we have shown that this is the case in the systems based on the weaker P_{tet} promoter. In this system, spontaneously occurring gene conversions of the *leu2-HOr* allele shifted from 87% under low-transcription (+dox) to 100% under high-transcription (−dox) conditions (GONZÁLEZ-BARRERA *et al.* 2002), consistent with the idea that most gene conversion events initiate at the strongly transcribed allele (SAXE *et al.* 2000). Consequently, we could not expect a shift to values >100% by the joint action of 4-NQO and transcription.

Synergistic increase of direct-repeat recombination caused by 4-NQO and transcription: There is cumulative genetic and molecular evidence that, although a DNA break presumably initiates all types of homologous recombination events, they may be processed by different mechanisms. Thus, whereas recombination between a plasmid and a chromosome is strongly Rad51 dependent and occurs by a standard double-strand-break-repair mechanism (GONZÁLEZ-BARRERA *et al.* 2002), deletions between direct repeats can occur by Rad51-independent single-strand annealing as a major mechanism in addition to unequal sister-chromatid exchange (see PAQUES and HABER 1999; SYMINGTON 2002; PRADO *et al.* 2003). To test whether 4-NQO and transcription synergistically increase the frequency of events, regardless of the mechanism of recombination, we tested the effect of 4-NQO and transcription on direct-repeat recombination.

We first developed direct-repeat recombination assays for the analysis of transcription-dependent deletions and then constructed direct-repeat systems based on the same 600-bp internal fragment of *LEU2*, but separated by intervening sequences of different length and nature, all of which were under control of the P_{GAL1} promoter (Figure 4). The results were similar in all direct-repeat assays, regardless of the nature or length of the intervening region. As can be seen in Figure 4, 4-NQO induced deletions 6- to 16-fold above spontaneous levels obtained when transcription was repressed. Transcription by itself caused a low but repetitive and significant increase in deletions of 1.2- to 3-fold above spontaneous levels. When 4-NQO was added to cultures in which fully active transcription was driven from the P_{GAL1} promoter, a strong synergistic increase in the frequency of deletions was observed (84- to 542-fold above spontaneous levels). Therefore, we conclude that 4-NQO and transcription synergistically stimulate the frequency of recombinogenic events, regardless of the mechanism of recombination by which the initiation events are processed.

Synergistic increase of plasmid-chromosome recombination caused by MMS and transcription: To assay whether the synergistic effect of transcription and 4-NQO on recombination was also observed with other DNA-damaging agents, we studied the effect of the X-ray-

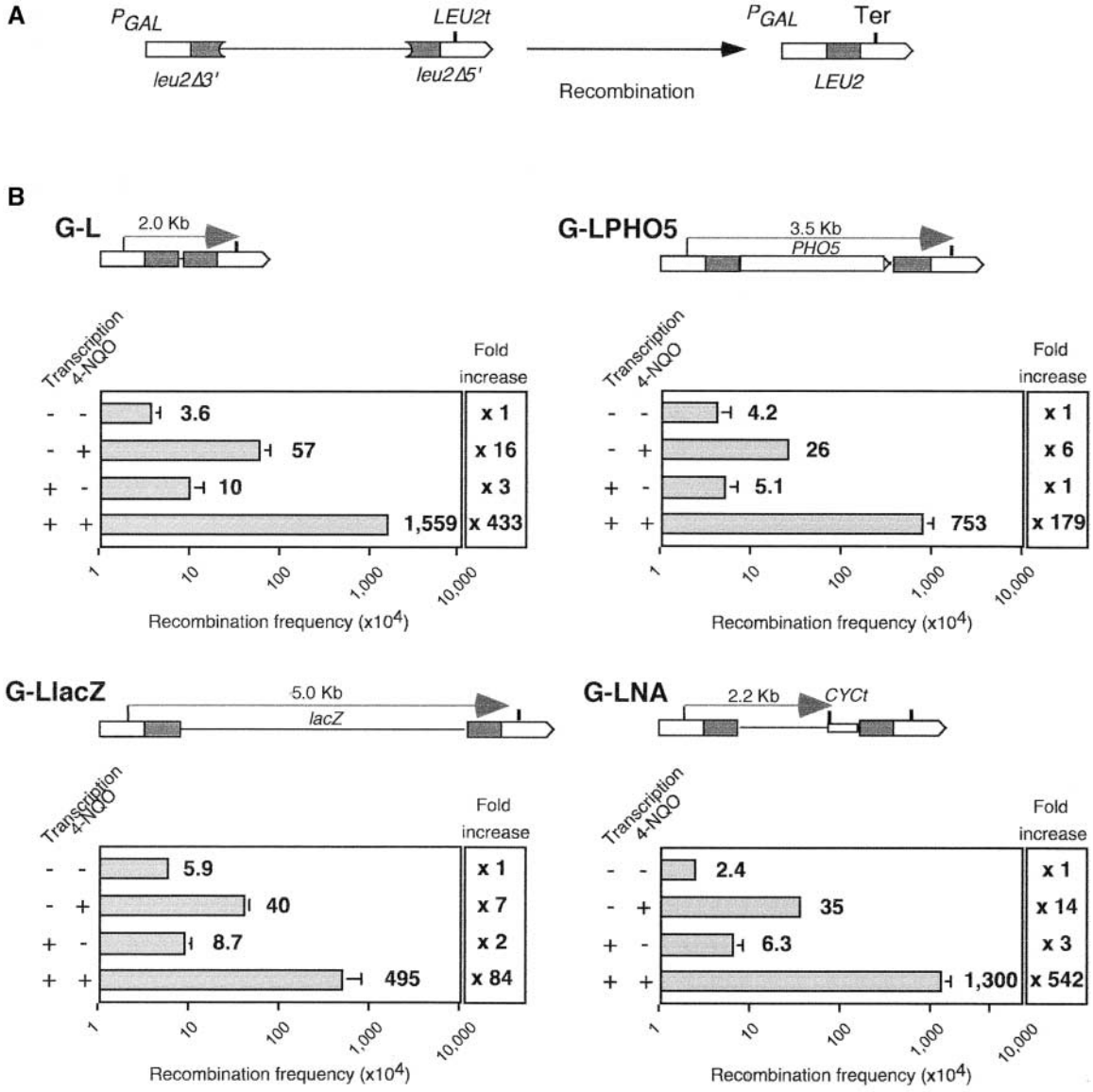

FIGURE 4.—Effect of transcription and 4-NQO on intrachromosomal recombination. (A) Recombination between direct repeats leads to the deletion of the intervening sequence. All systems used are based on a 600-bp truncated *leu2* repeat under the P_{GAL1} promoter. (B) Frequency of recombination in the absence and presence of transcription and 4-NQO using direct-repeat substrates with different intervening sequences that differ in length and nature. Other details are as in Figure 1.

mimetic alkylating agent MMS on TAR. In contrast to 4-NQO, MMS causes base alkylations and DNA breaks that are known to be recombinogenic (SNOW and KORCH 1970; YAN *et al.* 1995; KUPIEC 2000). The effect of MMS on recombination under low- and high-transcription conditions was determined in the plasmid-chromosome recombination system under the control of P_{GAL1}. Figure 5 shows that in wild-type cells Leu$^+$ gene conversions were stimulated 4.5-fold by MMS and 2.5-fold by transcription. However, the increase in recombination caused by the simultaneous action of transcription and MMS was 69 times above basal levels. Therefore, we can conclude that the recombinogenic effect of MMS, like that of 4-NQO, increases synergistically with transcription.

DISCUSSION

The possibility that transcription induces recombination by making DNA more susceptible to recombinogenic damage was tested. Using different plasmid-chromosome and direct-repeat recombination systems in which transcription is driven from either the *GAL1*- or the *tet*-regulated promoters, we showed that transcription causes a synergistic increase of recombination in addition to the UV- and X-ray-mimetic chemicals 4-NQO

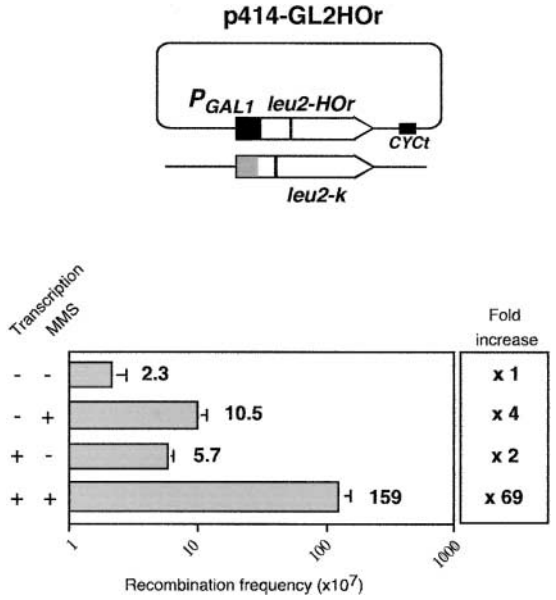

FIGURE 5.—Effect of transcription and MMS on intermolecular recombination. Recombination frequencies of wild-type strain (BER08-64A) in the absence and presence of transcription and MMS (0.035%) in the P_{GAL1}-based plasmid-chromosome recombination system. Other details are as in Figure 1.

and MMS. Our results suggest that transcription increases the accessibility of DNA to chemicals and internal metabolites responsible for recombinogenic lesions.

We analyzed the recombinogenic effect of 4-NQO and MMS in several DNA recombination substrates that were either transcribed or not transcribed. We used, in addition to the wild-type strain, the triple mutants *ntg1 ntg2 apn1* and *ntg1 ntg2 ogg1* lacking BER and the *rad1* strain lacking NER. In these mutants, it has been shown that spontaneous recombination between heterologous chromosomes was increased (SWANSON *et al.* 1999). In our plasmid-chromosome recombination assays, 4-NQO and transcription driven from the *tet* promoter increased recombination 128 times the spontaneous levels when the increases caused by either transcription or 4-NQO alone were <2-fold (Figure 1A). When transcription was driven from the P_{GAL1} promoter, 4-NQO and transcription increased recombination 4210 times the spontaneous levels, whereas these values were 42- and 2-fold for either 4-NQO or transcription alone (Figure 1B). In this same recombination system, MMS and transcription increased recombination 69 times, whereas the values were <5-fold for either transcription or MMS alone (Figure 5). This synergistic effect of transcription and damaging agents on intermolecular recombination was also observed for intramolecular recombination between direct repeats, in which 4-NQO and transcription increased the frequency of deletions 200-fold above spontaneous levels, whereas either 4-NQO or transcription alone stimulated recombination <10-fold. Therefore, all types of recombination events, whether yielding gene conversions or deletions, are synergistically increased by DNA-damaging agents in actively transcribed DNA. This result indicates that DNA-damaging agents and transcription increase the frequency of initiation events. This effect is observed in assays (direct and inverted DNA repeats) that detect recombination events occurring by different mechanisms (see SYMINGTON 2002; PRADO *et al.* 2003) or when using chemicals that cause different types of DNA lesions (4-NQO and MMS cause lesions that are substrates for NER and recombinational repair, respectively). Consequently, we believe that these chemicals cause more recombination initiation events in transcriptionally active DNA than in nontranscribed DNA.

Our result can be interpreted as if the accessibility of 4-NQO or MMS to transcribed DNA is higher in transcriptionally active DNA. In this sense, the results indicating that transcription induces mutation are particularly interesting. It was shown that the mutation rates of the β-galactosidase locus of *E. coli* were increased 4- to 7-fold by alkylating agents under induced transcription conditions *vs.* noninduced conditions. In addition, ICR-191 reverted *lac*$^-$ mutations in the presence of the *lac* inducer isopropyl thiogalactoside at a rate 2-fold higher than the rate in the absence of the inducer (HERMAN and DWORKIN 1971). It is worth noting that many mutations occur as a consequence of a previous lesion in the DNA, which is subject to attack by internal metabolites or external compounds. Many chemical reactions work more efficiently on ssDNA than on double-strand DNA (dsDNA). This is the case with bisulfite, which works efficiently on ssDNA but not on dsDNA (PINE and HUANG 1987), or the spontaneous deamination of cytosine, which has been shown to be 140-fold more efficient on ssDNA than on dsDNA (FREDERICO *et al.* 1990). Along these lines, in the *E. coli tac* region it has been reported that transcription causes a 4- to 5-fold increase in C-to-T mutation (BELETSKII and BHAGWAT 1996). During transcription the DNA could remain single-stranded and unprotected and could be the substrate for nucleotide modification (FIX and GLICKMAN 1987; SKANDALIS *et al.* 1994).

Our observation that 4-NQO and MMS increase recombination synergistically with transcription is consistent with the idea that damage susceptibility of transcribed DNA is higher than that of nontranscribed DNA. One possibility is that the stimulation of mutation and recombination by transcription are two different outcomes of the same phenomenon. Depending on the chemical used, whether causing mutagenic or genotoxic lesions (4-NQO and MMS are examples of the latter), an increase in mutation or recombination can be detected. The use of external chemicals to detect a higher susceptibility of DNA to damage provides a rational explanation for TAR and, by extension, TAM. DNA is continu-

ously subject to attack by internal metabolites, including hyperoxide and other compounds that can create recombinogenic lesions. During transcription, there is a transient accumulation of highly negatively supercoiled DNA behind the advancing RNA polymerase. This negatively supercoiled DNA might facilitate strand separation. Therefore, an open DNA structure can be formed transiently to be more susceptible to attack by internal metabolites that are reactive with ssDNA yielding recombinogenic lesions. Additionally, transcription is accompanied by chromatin remodeling (Jones and Kadonaga 2000; Orphanides and Reinberg 2000), which can transiently lead to an open chromatin structure that could make DNA more susceptible to attack by recombinogenic DNA-damaging agents. Increases in recombination associated with chromatin alterations have indeed been reported (Malagon and Aguilera 2001). Therefore, we provide genetic evidence suggesting that transcription makes DNA less protected against the attack by DNA-damaging agents, whether internal metabolites or externally added compounds. This hypothesis has the advantage of explaining that both TAM and TAR occur naturally, that is, in the absence of any exogenous DNA-damaging agent.

A second alternative to explain the synergistic effect of 4-NQO and MMS with transcription on recombination is worth discussing. The DNA damage caused by 4-NQO, which is mimetic to UV, will likely block progression of the RNAPII, as shown for UV light (Reagan and Friedberg 1997; Mullenders 1998). Consequently, a DNA being transcribed may contain an RNAPII blocked at the DNA lesions whereas nontranscribed DNA would not. The possibility that these blocked structures could be recombinogenic, because they could act as roadblocks to oncoming replication forks, cannot be discarded. However, we do not favor this possibility because it is known that a RNAPII encountering a DNA lesion makes this an appropriate substrate for transcription-coupled repair (TCR), therefore, providing an additional alternative mechanism for the repair of the lesion that would impede the accumulation of recombinogenic structures. Indeed, we have observed that the recombinogenic effect of UV on transcribed DNA in TCR-defective mutants (*i.e.*, *rad26*Δ) is no higher than that in wild-type cells, even though we would expect a higher accumulation of stalled RNAPIIs at UV-induced DNA lesions (data not shown). Our observation that MMS, a chemical that has not been shown to induce TCR, also induces recombination synergistically with transcription is consistent with our proposal that transcription enhances the accessibility to DNA-damaging agents.

We do not believe that our results can be explained by the idea that transcription could interfere with nonrecombinogenic repair pathways, because the major repair pathway for 4-NQO-induced lesions is NER, which is known to be enhanced by transcription via TCR (Tijsterman *et al.* 1997; Prakash and Prakash 2000). In addition, the possibility that transcription could channel DNA lesions into the recombinogenic repair pathway by, for example, promoting the formation of DNA breaks, is unlikely to be a major source of TAR. This hypothesis would not easily explain the strong synergistic effect of the break-inducing agent MMS with transcription or the similarity of results of BER- and NER-deficient mutants as compared to wild-type cells. The strong effect caused by damaging agents and transcription on recombination, which goes beyond a multiplicative relationship between the two, cannot easily be explained without an increase in the frequency of DNA lesions caused by damaging agents on highly transcribed DNA sequences.

TAR is a complex phenomenon with multiple manifestations. In this study we provide evidence for an explanation for probably the basic manifestation of TAR. Our results suggests that TAR induced by DNA-damaging lesions may to a large extent be due to an increase of the accessibility of DNA to damaging agents mediated by transcription. At least part of spontaneous TAR events may occur by a similar mechanism. However, our results do not exclude additional alternatives to explain TAR in the absence of exogenous DNA-damaging agents. There are other cases of TAR, such as those observed in yeast mutants of the THO complex and functionally related factors (Chavez *et al.* 2000; Gallardo and Aguilera 2001; Jimeno *et al.* 2002) or in class switching of Ig genes in vertebrates (Daniels and Lieber 1995), in which other mechanisms coupled to transcription yet to be deciphered may play an important role. In summary, our study suggests that a major and basic mechanism by which transcription induces recombination is by increasing the accessibility of DNA to chemicals and internal metabolites that damage DNA yielding recombinogenic lesions.

We thank F. Prado for critical reading of the manuscript and D. Haun for style supervision. Research was funded by grants from the Spanish Ministry of Science and Technology (BMC2000-0439), the Human Frontier Science Program (RG1999/0075), and the Regional Government of Andalusia (CVI0102). P.H. and S.G-B. were recipients of predoctoral training grants from the Regional Government of Andalusia and from the Spanish Ministry of Education and Culture, respectively.

LITERATURE CITED

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

Alseth, I., L. Eide, M. Pirovano, T. Rognes, E. Seeberg *et al.*, 1999 The *Saccharomyces cerevisiae* homologues of endonuclease III from *Escherichia coli*, Ntg1 and Ntg2, are both required for efficient repair of spontaneous and induced oxidative DNA damage in yeast. Mol. Cell. Biol. **19:** 3779–3787.

Beletskii, A., and A. S. Bhagwat, 1996 Transcription-induced mutations: increase in C to T mutations in the nontranscribed strand during transcription in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **93:** 13919–13924.

Beletskii, A., A. Grigoriev, S. Joyce and A. S. Bhagwat, 2000 Mutations induced by bacteriophage T7 RNA polymerase and their effects on the composition of the T7 genome. J. Mol. Biol. **300:** 1057–1065.

Brock, R. D., 1971 Differential mutation of the beta-galactosidase gene of *Escherichia coli*. Mutat. Res. **11:** 181–186.

Chavez, S., and A. Aguilera, 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.

Chavez, S., T. Beilharz, A. G. Rondon, H. Erdjument-Bromage, P. Tempst *et al.*, 2000 A protein complex containing Tho2, Hpr1, Mft1 and a novel protein, Thp2, connects transcription elongation with mitotic recombination in *Saccharomyces cerevisiae*. EMBO J. **19:** 5824–5834.

Daniels, G. A., and M. R. Lieber, 1995 RNA:DNA complex formation upon transcription of immunoglobulin switch regions: implications for the mechanism and regulation of class switch recombination. Nucleic Acids Res. **23:** 5006–5011.

Darroudi, F., A. T. Natarajan and P. H. Lohman, 1989 Cytogenetical characterization of UV-sensitive repair-deficient CHO cell line 43-3B. II. Induction of cell killing, chromosomal aberrations and sister-chromatid exchanges by 4NQO, mono- and bi-functional alkylating agents. Mutat. Res. **212:** 103–112.

Datta, A., and S. Jinks-Robertson, 1995 Association of increased spontaneous mutation rates with high levels of transcription in yeast. Science **268:** 1616–1619.

Dul, J. L., and H. Drexler, 1988 Transcription stimulates recombination. II. Generalized transduction of *Escherichia coli* by phages T1 and T4. Virology **162:** 471–477.

Eide, L., M. Bjoras, M. Pirovano, I. Alseth, K. G. Berdal *et al.*, 1996 Base excision of oxidative purine and pyrimidine DNA damage in *Saccharomyces cerevisiae* by a DNA glycosylase with sequence similarity to endonuclease III from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **93:** 10735–10740.

Fix, D. F., and B. W. Glickman, 1987 Asymmetric cytosine deamination revealed by spontaneous mutational specificity in an Ung− strain of *Escherichia coli*. Mol. Gen. Genet. **209:** 78–82.

Frederico, L. A., T. A. Kunkel and B. R. Shaw, 1990 A sensitive genetic assay for the detection of cytosine deamination: determination of rate constants and the activation energy. Biochemistry **29:** 2532–2537.

Friedman, B. M., and R. E. Yasbin, 1983 The genetics and specificity of the constitutive excision repair system of *Bacillus subtilis*. Mol. Gen. Genet. **190:** 481–486.

Gallardo, M., and A. Aguilera, 2001 A new hyperrecombination mutation identifies a novel yeast gene, *THP1*, connecting transcription elongation with mitotic recombination. Genetics **157:** 79–89.

Gari, E., L. Piedrafita, M. Aldea and E. Herrero, 1997 A set of vectors with a tetracycline-regulatable promoter system for modulated gene expression in *Saccharomyces cerevisiae*. Yeast **13:** 837–848.

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

Herman, R. K., and N. B. Dworkin, 1971 Effect of gene induction on the rate of mutagenesis by ICR-191 in *Escherichia coli*. J. Bacteriol. **106:** 543–550.

Iijima, K., and K. Morimoto, 1986 Cell-stage dependence of mutagen-induced sister-chromatid exchanges in human lymphocyte cultures. Mutat. Res. **162:** 121–129.

Ikeda, H., and T. Matsumoto, 1979 Transcription promotes recA-independent recombination mediated by DNA-dependent RNA polymerase in Escherichia coli. Proc. Natl. Acad. Sci. USA **76:** 4571–4575.

Jimeno, S., A. G. Rondon, R. Luna and A. Aguilera, 2002 The yeast THO complex and mRNA export factors link RNA metabolism with transcription and genome instability. EMBO J. **21:** 3526–3535.

Jones, K. A., and J. T. Kadonaga, 2000 Exploring the transcription-chromatin interface. Genes Dev. **14:** 1992–1996.

Kadyk, L. C., and L. H. Hartwell, 1992 Sister chromatids are preferred over homologs as substrates for recombinational repair in *Saccharomyces cerevisiae*. Genetics **132:** 387–402.

Kupiec, M., 2000 Damage-induced recombination in the yeast *Saccharomyces cerevisiae*. Mutat. Res. **451:** 91–105.

Malagon, F., and A. Aguilera, 2001 Yeast *spt6-140* mutation, affecting chromatin and transcription, preferentially increases recombination in which Rad51p-mediated strand exchange is dispensable. Genetics **158:** 597–611.

Morey, N. J., C. N. Greene and S. Jinks-Robertson, 2000 Genetic analysis of transcription-associated mutation in *Saccharomyces cerevisiae*. Genetics **154:** 109–120.

Mullenders, L. H., 1998 Transcription response and nucleotide excision repair. Mutat. Res. **409:** 59–64.

Nagao, M., and T. Sugimura, 1976 Molecular biology of the carcinogen, 4-nitroquinoline 1-oxide. Adv. Cancer Res. **23:** 131–169.

Nash, H. M., S. D. Bruner, O. D. Scharer, T. Kawate, T. A. Addona *et al.*, 1996 Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision DNA-repair protein superfamily. Curr. Biol. **6:** 968–980.

Nickoloff, J. A., 1992 Transcription enhances intrachromosomal homologous recombination in mammalian cells. Mol. Cell. Biol. **12:** 5311–5318.

Orphanides, G., and D. Reinberg, 2000 RNA polymerase II elongation through chromatin. Nature **407:** 471–475.

Paques, F., and J. E. Haber, 1999 Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **63:** 349–404.

Paterson, M. C., N. E. Gentner, M. V. Middlestadt and M. Weinfeld, 1984 Cancer predisposition, carcinogen hypersensitivity, and aberrant DNA metabolism. J. Cell. Physiol. **3** (Suppl.): 45–62.

Pine, R., and P. C. Huang, 1987 An improved method to obtain a large number of mutants in a defined region of DNA. Methods Enzymol. **154:** 415.

Piruat, J. I., and A. Aguilera, 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., and A. Aguilera, 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., J. I. Piruat and A. Aguilera, 1997 Recombination between DNA repeats in yeast *hpr1delta* cells is linked to transcription elongation. EMBO J. **16:** 2826–2835.

Prado, F., F. Cortes-Ledesma, P. Huertas and A. Aguilera, 2003 Mitotic recombination in *Saccharomyces cerevisiae*. Curr. Genet. **42:** 185–198.

Prakash, S., and L. Prakash, 2000 Nucleotide excision repair in yeast. Mutat. Res. **451:** 13–24.

Ramotar, D., S. C. Popoff, E. B. Gralla and B. Demple, 1991 Cellular role of yeast Apn1 apurinic endonuclease/3′-diesterase: repair of oxidative and alkylation DNA damage and control of spontaneous mutation. Mol. Cell. Biol. **11:** 4537–4544.

Reagan, M. S., and E. C. Friedberg, 1997 Recovery of RNA polymerase II synthesis following DNA damage in mutants of *Saccharomyces cerevisiae* defective in nucleotide excision repair. Nucleic Acids Res. **25:** 4257–4263.

Saffran, W. A., R. B. Greenberg, M. S. Thaler-Scheer and M. M. Jones, 1994 Single strand and double strand DNA damage-induced reciprocal recombination in yeast. Dependence on nucleotide excision repair and *RAD1* recombination. Nucleic Acids Res. **22:** 2823–2829.

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

Sikes, M. L., A. Meade, R. Tripathi, M. S. Krangel and E. M. Oltz, 2002 Regulation of V(D)J recombination: a dominant role for promoter positioning in gene segment accessibility. Proc. Natl. Acad. Sci. USA **99:** 12309–12314.

Skandalis, A., B. N. Ford and B. W. Glickman, 1994 Strand bias in mutation involving 5-methylcytosine deamination in the human *hprt* gene. Mutat. Res. **314:** 21–26.

Snow, R., and C. T. Korch, 1970 Alkylation induced gene conversion in yeast: use in fine structure mapping. Mol. Gen. Genet. **107:** 201–208.

Stavnezer-Nordgren, J., and S. Sirlin, 1986 Specificity of immunoglobulin heavy chain switch correlates with activity of germline heavy chain genes prior to switching. EMBO J. **5:** 95–102.

Sternglanz, R., S. DiNardo, K. A. Voelkel, Y. Nishimura, Y. Hirota *et al.*, 1981 Mutations in the gene coding for *Escherichia coli* DNA topoisomerase I affect transcription and transposition. Proc. Natl. Acad. Sci. USA **78:** 2747–2751.

Swanson, R. L., N. J. Morey, P. W. Doetsch and S. Jinks-Robertson, 1999 Overlapping specificities of base excision repair, nucleotide excision repair, recombination, and translesion synthesis pathways for DNA base damage in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **19:** 2929–2935.

Symington, L. S., 2002 Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. Microbiol. Mol. Biol. Rev. **66:** 630–670.

Thomas, B. J., and R. Rothstein, 1989 Elevated recombination rates in transcriptionally active DNA. Cell **56:** 619–630.

Tijsterman, M., R. A. Verhage, P. Van De Putte, J. G. Tasseron-De Jong and J. Brouwer, 1997 Transitions in the coupling of transcription and nucleotide excision repair within RNA polymerase II-transcribed genes of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **94:** 8027–8032.

van der Kemp, P. A., D. Thomas, R. Barbey, R. de Oliveira and S. Boiteux, 1996 Cloning and expression in Escherichia coli of the OGG1 gene of *Saccharomyces cerevisiae*, which codes for a DNA glycosylase that excises 7,8-dihydro-8-oxoguanine and 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine. Proc. Natl. Acad. Sci. USA **93:** 5197–5202.

Vilette, D., S. D. Ehrlich and B. Michel, 1995 Transcription-induced deletions in *Escherichia coli* plasmids. Mol. Microbiol. **17:** 493–504.

Voelkel-Meiman, K., R. L. Keil and G. S. Roeder, 1987 Recombination-stimulating sequences in yeast ribosomal DNA correspond to sequences regulating transcription by RNA polymerase I. Cell **48:** 1071–1079.

Wright, B. E., 2000 A biochemical mechanism for nonrandom mutations and evolution. J. Bacteriol. **182:** 2993–3001.

Wright, B. E., A. Longacre and J. M. Reimers, 1999 Hypermutation in derepressed operons of *Escherichia coli* K12. Proc. Natl. Acad. Sci. USA **96:** 5089–5094.

Yan, Y. X., R. H. Schiestl and L. Prakash, 1995 Mating-type suppression of the DNA-repair defect of the yeast rad6 delta mutation requires the activity of genes in the *RAD52* epistasis group. Curr. Genet. **28:** 12–18.

Zaborowska, D., Z. Swietlinska and J. Zuk, 1983 Induction of mitotic recombination by UV and diepoxybutane and its enhancement by hydroxyurea in *Saccharomyces cerevisiae*. Mutat. Res. **120:** 21–26.

Communicating editor: S. Lovett