Topoisomerase 1-dependent deletions initiated by incision at ribonucleotides are biased to the non-transcribed strand of a highly activated reporter

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ABSTRACT

DNA polymerases incorporate ribonucleoside monophosphates (rNMPs) into genomic DNA at a low level and such rNMPs are efficiently removed in an error-free manner by ribonuclease (RNase) H2. In the absence of RNase H2 in budding yeast, persistent rNMPs give rise to short deletions via a mutagenic process initiated by Topoisomerase 1 (Top1). We examined the activity of a 2-bp, rNMP-dependent deletion hotspot [the (TG)2 hotspot] when on the transcribed or non-transcribed strand (TS or NTS, respectively) of a reporter placed in both orientations near a strong origin of replication. Under low-transcription conditions, hotspot activity depended on whether the (TG)2 sequence was part of the newly synthesized leading or lagging strand of replication. In agreement with an earlier study, deletions occurred at a much higher rate when (TG)2 was on the nascent leading strand. Under high-transcription conditions, however, hotspot activity was not dependent on replication direction, but rather on whether the (TG)2 sequence was on the TS or NTS of the reporter. Deletion rates were several orders of magnitude higher when (TG)2 was on the NTS. These results highlight the complex interplay between replication and transcription in regulating Top1-dependent genetic instability.

INTRODUCTION

Ribonucleoside monophosphates (rNMPs) are the most abundant noncanonical nucleotide present in eukaryotic DNA [reviewed in (1)]. During replication, rNMPs can persist as remnants of Okazaki fragment priming or can be directly inserted in place of the corresponding dNMPs. With regard to the latter, rNTP exclusion from the active site pocket of replicative DNA polymerases is efficient but not perfect (2), and rNTP levels in the nucleotide pool are high relative to those of dNTPs (3). The Polɛ leading-strand DNA polymerase of the yeast Saccharomyces cerevisiae is more rNMP-permissive than lagging-strand Polβ in vitro (4), and most likely in vivo as well [reviewed in (5)]. In addition to rNMP incorporation during replication, other types of DNA synthesis, such as damage bypass by rNTP-permissive translesion synthesis DNA polymerases (3,6) or gap-filling during repair/recombination reactions, can introduce rNMPs into DNA. At least some of these reactions likely occur outside of S phase, when ribonucleotide degradase levels are low (7) and rNTP:dNTP ratios are correspondingly elevated. Finally, recent work has shown that RNA transcripts can be used to directly repair DNA double-strand breaks (8), providing another potential source of rNMPs in DNA. If not removed, rNMPs in a DNA template can slow down subsequent DNA synthesis and generate replication stress (9) or can trigger mutagenesis (4).

The heterotrimeric RNase H2 complex is responsible for the removal of one or a few rNMPs embedded in DNA [reviewed in (10)]. In the absence of yeast RNase H2, deletions within short tandem-repeat hotspots accumulate; repeat unit sizes range from 2–5 bp, and hotspots typically contain 2–4 tandem repeats (4,11). rNMP-associated deletions absolutely require the activity of Topoisomerase 1 (Top1) (11), a type IB topoisomerase that transiently forms a covalent, 3′-phosphotyrosyl complex with nicked DNA (12). Top1 is important for removing transcription-associated torsional stress and interacts with the phosphorylated C-terminal domain of RNA polymerase (RNAP) II (13). Accordingly, rNMP-dependent mutagenesis is highly elevated under conditions of robust transcription, and Top1 is the major source of transcription-associated mutagenesis (14,15). When Top1 cleaves at an rNMP embedded in DNA, the 2′-OH of ribose can attack the 3′-phosphotyrosyl linkage with Top1, which releases the enzyme and leaves a nick flanked by a 2′,3′ cyclic phosphate and a 5′-OH (16). We previously proposed that mutagenesis associated with rN-
MPs requires sequential cleavage by Top1, first at an rNMP to generate a non-ligatable nick and then at an upstream (5′) dNMP, which generates a small gap between the cleavage sites (17). The gap is predicted to be the size of the relevant repeat unit and to be flanked by a 3′-linked Top1 cleavage complex and a 5′-OH. Spontaneous misalignment between repeats could then convert the gap to a nick, thereby facilitating enzyme-mediated ligation. Recent in vitro data have provided support for sequential Top1 cleavage during rNMP-dependent deletion formation (18,19).

Understanding the precise mechanism of rNMP-dependent mutagenesis at hotspots requires knowledge of where rNMPs are incorporated into and where Top1 cleaves genomic DNA. Methods that map rNMPs to single nucleotide resolution in vitro have been recently described (20–22), but it is difficult to predict where the enzyme nicks DNA given the very weak consensus site for Top1 cleavage (20–22), but it is difficult to predict where the enzyme nicks DNA. Methodsthat map rNMPstosingle

dependent mutagenesis at hotspots requires knowledge

to specifically examine the effect of transcription-related asymmetry between DNA strands either dictated which strand is cleaved by Top1 and/or determines the genetic outcome of Top1 incision at an rNMP.

**MATERIALS AND METHODS**

**Yeast strains**

Strains SJR2259–SJR2262 were derived by transformation of YPH45 [MATα ura3−52 ade2−101clc lys2−801am trp1Δ1; (27)], a strain congenic to S288C. Strains SJR2259 and SJR2260 contain a pLYS-LYS2 construct in both orientations near ARS306 on chromosome III; in SJR2261 and SJR2262, pLYS was replaced by pTET [for construction details, see (28)]. Transcription and replication forks move in the same direction in strains designated LYS2p; in strains designated LYS2r, transcription and replication forks converge. Strains containing the pLYS-lvs2FΔA746NR,(TG)2 and pTET-lvs2RΔAΔ746NR,(TG)2 alleles were constructed via two-step allele replacement following transformation of SJR2259 and SJR2261 (28), respectively, with Af/I-digested pSR1030. pLYS-lvs2RΔA746NR,(TG)2-inv and pTET-lvs2RΔAΔ746NR,(TG)2-inv strains were constructed via two-step allele replacement following transformation of strains SJR2260 and SJR2262 (28), respectively, with Af/I-digested pSR1031. pSR1030 and pSR1031 were constructed by ligating BglII-digested pSR963 (29) to annealed oligonucleotides 5′-GATCTCCATGGAGGCACAGTTCAGCC and 5′-GATCGCTGAACTGTCCTCCATGGGA; the introduced sequence is from URA3 and the hotspot is underlined. pSR1030 has the (TG)2 sequence on the TS of lys2 and pSR1031 has the (TG)2 sequence on the NTS of lys2. The RNH201, TOP1 or RAD14 gene was deleted by one-step allele replacement using PCR-generated deletion cassettes amplified from a plasmid containing an appropriate selective marker. The pol2-M644L or pol2-M644G allele was introduced by two-step allele replacement using AgeI-digested p173-pol2-M644L or p173-pol2-M644G (4), respectively. Strains with the pol3-L612M allele were constructed via two-step allele replacement using Hpal-digested p170-pol3-L612M (30). Mutant pol2 and pol3 alleles were confirmed by sequencing an appropriate genomic DNA fragment. The mating type of SJR2805 (MATα pTET-lvs2Ftop1Δ strain) was switched to MATα using a pGAL-HO plasmid. This allowed construction of double- and triple-mutant strains by mating, sporulation and tetrad dissection. Supplementary Table S1 provides a complete strain list.

**Mutation spectra and rates**

All growth of yeast was at 30°C. To determine the Lys+ reversion rate, independent cultures were started either by inoculating 2 × 10⁵ cells from an overnight starter culture or by inoculating each culture with an independent colony. Cultures were grown in YEPGE (1% yeast extract, 2% Bacto-peptone, 250 μg/ml adenine hemisulfate supplemented with 2% glycerol and 2% ethanol) until saturated. Appropriate dilutions were plated on YEPE (1% yeast extract, 2% Bacto-peptone, 250 μg/ml adenine hemisulfate supplemented with 2% dextrose) to determine the total number of viable cells and on synthetic complete dextrose medium lacking lysine (SCD-Lys) to determine the total number of revertants in each culture. Mutation rates were calculated using the method of the median (31) and 95% confidence intervals were determined as described previously (32).

Independent Lys+ revertants were selected on SCD-Lys following non-selective growth in YEPGE medium. A portion of lys2 containing the (TG)2
hotspot was PCR-amplified using primers LYSWINF (5’-GCCTCATGATAGTTTTTCTAACAATACG) and LYSWINR (5’-CCCATCACAATACTCATCAATCCAC) and the product was sequenced by the Duke University DNA Analysis Facility, Eurofins MWG Operon or Eton Bioscience INC. The rate of 2-bp deletions was calculated by multiplying the total reversion rate by the proportion of 2-bp deletions in the corresponding mutation spectrum.

**RESULTS**

The rNMP-dependent hotspot used in the current study was initially detected when analyzing URA3 forward mutations isolated in an *rnh201Δ* strain expressing an rNTP- permissive form of Pol [(*pol2*-M644G allele; (4))]. The hotspot was referred to as CACA because this sequence is on the URA3 coding strand, the strand that has the same sequence as the mRNA and hence is the non-transcribed strand (NTS); the complementary TGTG sequence is on the noncoding/transcribed strand (TS). A striking feature of the rNMP-dependent CACA hotspot was its strong dependence on the direction of DNA replication through *URA3*, which had been inserted in both orientations relative to *ARS306* on chromosome III. In *Ori1*, CACA was on the nascent leading strand and synthesized by Pol; in *Ori2*, TGTG was synthesized by Pol. Here, we refer to *Ori1* and *Ori2* as SAME and OPPO, respectively, to indicate the direction of replication fork movement relative to that of the transcription machinery (Figure 1). In the *URA3* system, hotspot activity was estimated to be ~100-fold greater in *Ori2/OPPO* than in *Ori1/SAME*. This indicates that Top1-dependent deletions arise mostly, if not exclusively, when Pol synthesizes and inserts rNMPs into the TGTG-containing strand. The replication-related bias for rNMP-dependent deletions was initially reported in a *pol2*-M644G background, but a similar bias occurs in a *POL2* background (34). Because it is the TGTG-containing strand where the deletion intermediate is generated, we will hereafter refer to this site as the (TG)2 hotspot to reflect the strand cleaved by Top1 to initiate mutagenesis.

The replication bias of the (TG)2 hotspot is recapitulated in a frameshift-reversion assay under low-transcription conditions

We previously demonstrated that small (∼20 bp) DNA fragments containing Top1-dependent, 2-bp deletion hotspots are fully functional when transferred into a LYS2-based, frameshift-reversion assay that detects 2-bp deletions (15).
Figure 2. Effects of transcription on 2-bp deletions in the (TG)$_2$ hotspot. All experiments were performed in an rnh201/Delta1 background. Under low-transcription conditions (panel A), deletion rates are high when (TG)$_2$ is on the nascent leading strand of replication. Under high-transcription conditions (panel B), deletion rates are high when (TG)$_2$ is on the NTS of the reporter.

(TG)$_2$ constructs, the rate of 2-bp deletions at the hotspot was 67-fold higher for (TG)$_2$-inv when it was in the SAME than when it was in the OPPO orientation (Figure 2A). This was evident both in terms of the overall Lys$^+$ reversion rate (6-fold difference) and in the proportion of mutants at the hotspot (31/43 and 3/47 for the SAME and OPPO, respectively). Finally, introduction of the rNTP-restrictive pol2-M644L allele into (TG)$_2$-inv SAME and (TG)$_2$ OPPO strains reduced 2-bp deletions approximately 50-fold (Supplementary Table S1), confirming that the relevant rNMP was introduced by Pol$^+$/H9280 during leading-strand synthesis. Although these experiments were designed to recapitulate the earlier replication-related observations using our specific reporter, it is important to note that, in addition to switching leading- and lagging-strand specificities, inversion of just the hotspot-containing fragment also moves the (TG)$_2$ sequence from the TS to the NTS of the reporter.

High transcription eliminates the replication-associated bias at the (TG)$_2$ hotspot

The effect of transcription on deletions at the (TG)$_2$ hotspot was examined by placing the four lys2 alleles shown in Figure 1 under control of the highly active TET promoter (pTET). Previous analyses demonstrated that the direction of replication through LYS2 has little, if any, effect on pTET activity (28). As observed under low-transcription conditions (pLYS constructs), 2-bp deletions were highly elevated in an rnh201Δ background (Supplementary Table S3), confirming rNMP dependence; all subsequent experiments were done in an rnh201Δ background. Our expectation was that, under high-transcription conditions, the distinctive replication bias for 2-bp deletions would be maintained and elevated transcription thus would have a similar stimulatory effect on 2-bp deletions within each construct. In contrast to this prediction, however, the replication-associated bias observed for the (TG)$_2$ allele was completely eliminated and that for the (TG)$_2$-inv allele was reduced 10-fold under high-transcription conditions (Figure 2B). The net result was that the effect of high transcription varied three orders of magnitude among the four constructs, from only 3-fold for (TG)$_2$ OPPO to 4600-fold for (TG)$_2$-inv OPPO. Importantly, for either direction of replication through the pTET-lys2 reporter, deletion rates in the (TG)$_2$-inv constructs were much higher than in the corresponding (TG)$_2$ constructs. The transcription-associated mutation bias thus correlated with whether the site of Top1 cleavage [(TG)$_2$] was located on the TS or on the NTS of the reporter, with the rate being several orders of magnitude higher when (TG)$_2$ was on the NTS.

Mutagenic rNMPs on the NTS of (TG)$_2$-inv constructs are inserted by Pol$^+$ as well as Pol$^-$

The data in Figure 2B demonstrate that highly elevated transcription eclipses the replication-associated bias in hotspot activity observed under low-transcription conditions. This suggests that, in contrast to low-transcription conditions where only rNMPs inserted by Pol$^+$ are mutagenic, the Pol$^-$ lagging-strand polymerase can be the primary source of mutagenic rNMPs under high-transcription conditions. We confirmed this for the (TG)$_2$-inv alleles using mutant forms of Pol$^+$ and Pol$^-$ that incorporate either fewer or more rNMPs than the WT polymerases (Figure 3). The catalytic subunit of Pol$^+$ is encoded by POL2; the pol2-M644G and pol2-M644L alleles render the en-
zyme more and less rNTP permissive, respectively, than WT during leading-strand synthesis (3). The catalytic subunit of Polô is encoded by POL3; the pol3-L612M allele renders Polô more rNMP-permissive than WT and is associated with increased rNMP-incorporation during lagging-strand synthesis (24). In experiments with the (TG)2-inv SAME reporter (Top1 cleavage site on the NTS/leading strand), where Polé is expected to incorporate rNMPs into the (TG)2-containing strand, 2-bp deletions were elevated ~2-fold in the rNMP-permissive pol2-M644G background and reduced ~50-fold in the rNMP-restrictive pol2-M644L background (Figure 3A). There was no significant increase in the deletion rate in the rNMP-permissive pol3-L612M background. For the (TG)2-inv OPPO allele (Top1 cleavage site on the NTS/lagging strand), however, there was a 13-fold increase in the rate of 2-bp deletions in the rNMP-permissive pol3-L612M background, while altering the rNMP permissiveness of the Polé leading-strand polymerase had no significant effect on mutagenesis (Figure 3B). This confirms that the mutation-initiating rNMPs were inserted into the (TG)2-containing strand of the (TG)2-inv OPPO construct by the Polô lagging-strand polymerase. Thus, in contrast to the strong, Polé-specific observed under low-transcription conditions, hotspot activity is strongly biased to the NTS under high-transcription conditions and either Polô or Polé can be the primary source of the mutagenic rNMPs.

Neither biased repair nor R-loops account for the NTS bias for 2-bp deletions

The data presented above suggest that, relative to the NTS, the TS of a highly transcribed gene is a very poor substrate for the rNMP-dependent mutagenesis initiated by Top1. One possible explanation for this bias is that cleavage of the NTS by Top1 is more efficient than cleavage of the TS. Alternatively, it is possible that the cleavage efficiency of the two strands is similar, but that incision of the NTS is much more likely to produce the 2-bp deletions detected by our reporter constructs. This could reflect either some feature of the NTS strand that promotes mutagenesis and/or some feature of the TS that limits mutagenesis.

There are two well-characterized asymmetries between the DNA strands during transcription: DNA damage on the TS is repaired more efficiently and the TS can stably hybridize to the transcript to form an R-loop (Figure 4). With respect to asymmetric repair, the nucleotide excision repair (NER) pathway is triggered when RNAII and/or RNAPII are black and dotted red lines, respectively. In panel (A), the triangle corresponds to the 2',3' cyclic phosphate that results from Top1 incision at an rNMP. EV, empty vector.

Figure 4. Neither TC-NER nor R-loop formation limits mutagenesis on the TS under high-transcription conditions. In the cartoons shown, the gray and yellow ovals represent RNAII and Top1, respectively; DNA and RNA are black and dotted red lines, respectively. In panel (A), the triangle corresponds to the 2',3' cyclic phosphate that results from Top1 incision at an rNMP. EV, empty vector.

corresponding (TG)2-inv constructs. There was no effect of Rad14 loss on 2-bp deletions in the (TG)2 SAME construct, however, and only a modest, 3.3-fold increase in the rate of 2-bp deletions in (TG)2 OPPO construct (Figure 4A and Supplementary Table S3). With regard to the latter, the rate of 2-bp deletions was still at least 30-fold less than with the corresponding (TG)2-inv constructs, leading us to conclude that TC-NER is not responsible for the transcription-related asymmetry.

Within an R-loop, the TS is base-paired with the transcript and such RNA:DNA hybrids are not a substrate for Top1 (16). Although the single-stranded NTS within an R-loop would also not be a substrate for Top1 (37), it could potentially fold into secondary structures that can be cleaved by the enzyme (38). We examined whether R-loops limit mutagenesis initiated by Top1 cleavage of the TS by over-producing RNase H1, which degrades R-loops in yeast. Overproduction of RNase H1 did not significantly alter the rate of 2-bp deletions in the (TG)2 OPPO construct in either the presence or absence of Rad14 (Figure 4B and Supplementary Table S4). Our data thus indicate that neither R-loops nor biased repair is responsible for the transcription-related strand bias in Top1-dependent mutagenesis.

**DISCUSSION**

Top1 relieves torsional stress associated with transcription and replication, and Top1-dependent deletions that reflect incision at rNMPs are associated with both processes. Although both Polô and Polé insert rNMPs at a low level during replication, only those inserted by the Polé leading-strand DNA polymerase are mutagenic (24). A favored explanation for the replication-associated asymmetry is that Top1 is needed to remove supercoils that accumulate on the leading strand behind the replication fork; nicks that are naturally generated during lagging-strand synthesis can serve a similar role. For the (TG)2 hotspot used here, syn-
the (TG)\textsuperscript{2} sequence between the leading and lagging strands (Figure 1). Data obtained using these constructs are summarized in Table 1 and discussed below.

Under low-transcription conditions, we recapitulated the rNMP-dependent 2-bp deletions rates in the (TG)\textsuperscript{2} hotspot.

### Table 1. Summary of rNMP-dependent 2-bp deletions rates in the (TG)\textsuperscript{2} hotspot

| Construct | (TG)\textsuperscript{2} Location | 2-bp deletion rate relative to (TG)\textsuperscript{2} SAME, leading-strand DNA polymerase | High/low transcriptions |
|-----------|---------------------------------|---------------------------------------------------------------------------------|-------------------------|
| (TG)\textsuperscript{2}, SAME | nascent lagging TS | 1.0X | Low 5X, High 5X |
| (TG)\textsuperscript{2}, OPPO | nascent leading TS | 15X | Low 41X, High 2.7X |
| (TG)\textsuperscript{2}-inv SAME | nascent leading NTS | 64X | Low 15,000X, High 280X |
| (TG)\textsuperscript{2}-inv OPPO | nascent lagging NTS | 0.6X | Low 3700X, High 4600X |

The open arrow corresponds to the LYS2 coding sequence and indicates the direction of transcription. Filled and open boxes correspond to the (TG)\textsuperscript{2} and (CA)\textsuperscript{2} sequence, respectively.

deletion formation, we were able to more accurately quantify the magnitude of the replication effect. With the (TG)\textsuperscript{2} construct, the direction of replication had a 15-fold effect; with the (TG)\textsuperscript{2}-inv construct, the effect was ~70-fold.

Unexpectedly, the replication-associated bias for the (TG)\textsuperscript{2} hotspot was abolished when the reporter was highly transcribed. In contrast to low-transcription conditions, the rate of 2-bp deletions correlated with whether the (TG)\textsuperscript{2} sequence was on the TS or the NTS of the highly transcribed reporter. When the (TG)\textsuperscript{2} sequence was on the NTS, deletion rates were several orders of magnitude higher than when it was on the TS (Table 1). Replacing a leading-strand bias under low-transcription conditions with an NTS bias under high-transcription resulted in a highly variable effect of transcription on mutagenesis. As summarized in the final column of Table 1, the stimulatory effect of transcription on 2-bp deletions was only 3-fold for the (TG)\textsuperscript{2} construct, but almost 5000-fold for (TG)\textsuperscript{2}-inv OPPO. The observation that mutagenesis is driven by the location of the (TG)\textsuperscript{2} sequence on the TS versus NTS of the reporter indicates that the mutagenic rNMP can be inserted by the lagging- as well as the leading-strand DNA polymerase. We confirmed this for the (TG)\textsuperscript{2}-inv OPPO construct, where altering the rNTP permissiveness of Pol\textsubscript{e}b, but not that of Pol\textsubscript{e}, affected the deletion rate. This provides the first example of rNMP-dependent mutagenesis driven by rNMPs inserted by Pol\textsubscript{e} rather than by Pol\textsubscript{e}.

The finding that rNMPs inserted by Pol\textsubscript{e} initiate mutagenesis under high-transcription conditions is consistent with (i) high levels of transcription creating a new, Pol\textsubscript{e}b-dependent rNMP insertion site that does not exist under low-transcription conditions, (ii) high levels of transcription increasing the amount of rNMPs incorporated by Pol\textsubscript{e}b, and/or (iii) high levels of transcription recruiting Top1 to DNA replicated by either DNA polymerase. Unscheduled DNA synthesis that occurs outside of S phase, when the rNTP:dNTP ratio is elevated, is most likely catalyzed by Pol\textsubscript{e}. This does not appear to be the primary source of rNMPs inserted by Pol\textsubscript{e}b, however, as we were unable to detect a change in rNMP levels in our his\textsubscript{2} reporter under high-transcription conditions (data not shown). We also think it is unlikely that high transcription modifies the sites of rNMP insertion because the positions of hotspots in a forward mutation assay are similar under low- and high-transcription conditions (11,17). We suggest that Pol\textsubscript{e}b inserts the same amount of rNMPs irrespective of transcription level, but that Top1 efficiently incises at rNMPs incorporated by Pol\textsubscript{e}b only under high transcription conditions. Increased recruitment of Top1 to highly transcribed genes is a well-established phenomenon and may partially reflect interaction with the phosphorylated C-terminal domain of RNAPII (13).

The most striking aspect of the data reported here is that under high-transcription conditions, Top1-dependent deletions occur much more frequently when its target site is on the NTS than when on the TS of the reporter. It should be noted that this bias is not limited to the (TG)\textsuperscript{2} hotspot, and has been observed for at least one other hotspot following transplantation into the his\textsubscript{2}-based reversion assay (data not shown).
repair occurs more efficiently on the TS and the TS can be extensively paired with the transcript as part of an R-loop. The former might eliminate premutagenic lesions specifically on the TS while the latter would preclude cleavage of the TS by Top1. Neither elimination of an essential NER protein (rad14Δ mutant) nor overexpression of RNase H1, however, stimulated deletions when the (TG)2 target site was on the TS.

The basis of the distinctive NTS bias for hotspot activity under high-transcription conditions remains an enigma. One possibility is that Top1 may have a strong preference for cleaving the NTS relative to TS of a highly active gene. How this might occur is unclear, but one intriguing possibility is that the bias might be related to the interaction of Top1 with elongating RNAPII (13). Two activities have been implicated in limiting rNMP-dependent mutagenesis, and either might limit mutagenesis in a strand-specific manner. First, processing of a trapped Top1 cleavage complex by Tdp1/Top1p promotes error-free removal of rNMPs and precludes deletion formation in vitro (18), but whether a similar mechanism operates in vivo is not known. Second, the Srs2 helicase cooperates with Exo1 to prevent rNMP-dependent mutagenesis by removing the 5′-OH generated by the initial Top1 cleavage (39). An additional possibility is that a Top1-dependent deletion intermediate arising on TS is more efficiently converted into a double-strand break (DSB) during replication. Such a DSB would be repaired via homologous recombination with the sister chromatid, which is a genetically silent event. Finally, the sequential-cleavage model of Top1-dependent mutagenesis requires that the strand covalently bound by Top1 realign with respect to the complementary strand. This realignment converts a 2-npt gap into a nick with a 5′-OH positioned correctly for efficient Top1 ligation. If the Top1cc is attached to the NTS, RNAPII might ‘push’ the Top1cc toward the 5′-OH to drive realignment and ligation. When the Top1cc is on the TS, however, RNAPII would push Top1 away from 5′-OH and thereby preclude the final ligation reaction. Regardless of the underlying molecular mechanism, the results presented here further extend the range of transcriptional effects on genetic stability, and underscore the complexity of factors that together produce a highly dynamic mutation landscape across the eukaryotic genome.

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

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