**REVIEW**

Transcription-associated recombination in eukaryotes: link between transcription, replication and recombination

Ponnari Gottipati¹ and Thomas Helleday¹²*  
¹Gray Institute for Radiation Oncology and Biology, University of Oxford, Oxford OX3 7DQ, UK and ²Department of Genetics Microbiology and Toxicology, Stockholm University, S-106 91 Stockholm, Sweden

Homologous recombination (HR) is an important DNA repair pathway and is essential for cellular survival. It plays a major role in repairing replication-associated lesions and is functionally connected to replication. Transcription is another cellular process, which has emerged to have a connection with HR. Transcription enhances HR, which is a ubiquitous phenomenon referred to as transcription-associated recombination (TAR). Recent evidence suggests that TAR plays a role in inducing genetic instability, for example in the THO mutants (Tho2, Hpr1, Mft1 and Thp2) in yeast or during the development of the immune system leading to genetic diversity in mammals. On the other hand, evidence also suggests that TAR may play a role in preventing genetic instability in many different ways, one of which is by rescuing replication during transcription. Hence, TAR is a double-edged sword and plays a role in both preventing and inducing genetic instability. In spite of the interesting nature of TAR, the mechanism behind TAR has remained elusive. Recent advances in the area, however, suggest a link between TAR and replication and show specific genetic requirements for TAR that differ from regular HR. In this review, we aim to present the available evidence for TAR in both lower and higher eukaryotes and discuss its possible mechanisms, with emphasis on its connection with replication.

Introduction

To maintain genomic stability, cells possess multiple mechanisms of DNA repair that remove damage and in most cases restore the original DNA molecule. DNA repair mechanisms are complex, often interlinked, and their failure can result in tumorigenesis arising from sequential accumulation of unrepaired DNA damage, both at the nucleotide and chromosomal levels (1). DNA repair mechanisms are also associated with cellular processes like transcription and replication to ensure genome integrity at every stage of the cell cycle. DNA repair is more efficient in transcribed regions and it is directly associated with transcription in a process called transcription-coupled repair (TCR) (2,3). Furthermore, specific DNA repair processes, e.g. homologous recombination (HR) and translesion synthesis (TLS), occur at replication forks to ensure proper replication and prevent genomic instability (4). In higher organisms, DNA repair pathways are exploited to generate genetic diversity during development of the immune system. Non-homologous end joining (NHEJ) is involved in V(D)J recombination, an early process in antibody maturation, where as both NHEJ and base excision repair as well as many DNA damage response factors are important for class switch recombination (CSR), a late process in antibody maturation (5). Similarly, error-prone DNA repair processes are suggested to play a role in somatic hypermutation (SHM), another process involved in the late stages of antibody maturation (6).

In this review, we discuss transcription-associated recombination (TAR), a ubiquitous phenomenon that associates HR with the cellular process, transcription. It has long been established that transcription enhances HR (7), though the mechanism remains to be elucidated. Here, we also discuss the possible mechanisms behind this association with emphasis on the involvement of replication-associated lesions as substrates for TAR.

HR and replication lesions

HR is required to repair replication-associated DNA lesions and DNA double-strand breaks (DSBs), is generally error free and occurs mainly during late S and G2 phases of the cell cycle (8,9).

DSBs are produced endogenously by various sources including topoisomerases or exogenously by sources like ionizing radiation and chemicals that generate reactive oxygen species [reviewed in (10)]. DSBs also normally occur during V(D)J recombination and immunoglobulin class-switching processes and also during replication as a consequence of replication fork arrest and collapse.

HR plays an important role in the repair of damage during replication in all organisms (11,12). In mammalian cells, replication forks collapse when they collide with unrepaired DNA single-strand breaks (SSBs) triggering HR (13,14). During normal cell cycle, restarting replication when DNA replication forks stall due to damage in the DNA template can involve generation of a DSB intermediate (15). HR has been suggested to repair such naturally occurring DSBs arising during the S phase of the cell cycle that lead to collapsed replication forks (16,17). There is increasing evidence to show that HR in mammalian cells has an important role in the repair of stalled replication forks caused by a variety of lesions (e.g. breaks, DNA adducts and chromatin-bound proteins) [reviewed in (8)]. Replication may bypass the block either by TLS or by re-priming downstream of the block and the remaining gaps can then be filled by template switching via HR (8). Replication block bypass can also take place by template switching via HR which leads to the uncoupling of leading and lagging strand synthesis and fork regression forming a ‘chicken-foot’ structure. HR is then required to rescue and restart replication [reviewed in (8,18)]. The interesting feature about stalled replication forks is that they may also occur in the absence of detectable DSBs (12), suggesting that HR repairs...
a variety of lesions that occur at replication forks and not just DSBs. Though the substrates for HR in this case remain elusive, it has been proposed that fork reversal and formation of chicken-foot structures as discussed above serve as substrates for HR, which can occur without the cleavage of the Holliday junction with in the chicken foot and can therefore occur in the absence of DSBs (8) as is the case in bacteria [reviewed in (8,19)].

It is possible that transcription may result in such replication-associated lesions due to interference with replication when both cellular processes occur on the same DNA substrate at the same time, perhaps resulting in TAR.

**TAR in yeast**

Evidence for enhanced recombination in transcriptionally active DNA in eukaryotes was first shown in *Saccharomyces cerevisiae* during the search for recombination hot spots. Transcription resulted in a 5- to 10-fold increase in recombination between the nearby duplicated genes in the recombination hot spot *HOT1*, which is present in the ribosomal RNA gene cluster (20). In diploids, transcription driven by RNA polymerase I in the HOT1 region (21,22) stimulates interchromosomal gene conversion (23,24). Several other reports in yeast also demonstrated that gene conversion is the primary recombination event stimulated by transcription in direct repeats (22,25).

Later, RNA polymerase II-dependent transcription was also shown to enhance recombination in yeast indicating that this effect is not specific for RNA polymerase I-mediated transcription (25–27). Mitotic intrachromosomal recombination between non-tandem duplications in *S.cerevisiae* was enhanced by transcription mediated by the inducible Gal10 promoter (26). In addition, meiotic and mitotic interchromosomal recombination in *Schizosaccharomyces pombe* were also enhanced by RNA polymerase II-dependent transcription driven from the highly active ADH1 promoter (25).

Mating type interconversion is a further example of TAR in yeast. It involves a specialized gene conversion event initiated by a HO nuclease-mediated DSB in the MAT locus, which depends on active transcription. The homologous but transcriptionally silent loci are not cleaved by HO nuclease confirming the dependence of mating type switching on transcription (28).

Studies in artificial recombination repeats in yeast have shown that increasing transcription does not further enhance DSB-induced recombination or affect gene conversion tract lengths (29). This indicates that once a recombination event is initiated by a DSB, transcription has no further effect on recombination levels, suggesting that transcription influences recombination by initiating it (29).

The yeast THO complex along with Thp1 protein is another example of TAR in yeast and provides a connection between transcription elongation and recombination (30,31). THO is a conserved eukaryotic complex made of four proteins Hpr1, Tho2, Mfh1 and Thp2 (32). This complex plays a role at the interface between transcription and mRNA export (33–35) and is recruited only to transcriptionally active chromatin (34,36). Mutations in any of the genes in this complex lead to defects in transcription elongation (32,37–39), transcription-dependent hyper-recombination (30,39,40) and nuclear mRNA accumulation (34). Deletion mutants of THO2 and HPR1 showed ~3000-fold increase in recombination between direct repeats compared to the wild-type cells (30) and this hyper-recombination was dependent on transcription elongation (30) as TAR was abolished by terminating transcription elongation by placing a transcription terminator downstream of one repeat (40). The null mutants of THO2 and HPR1 were capable of carrying out all types of recombination in the artificial recombination substrates tested, including both RAD51-independent single-strand annealing events and Rad51-dependent gene conversion involving of strand exchanges (39), unlike spontaneous TAR between direct repeats in yeast which primarily involves gene conversion events (22–25).

THO together with certain RNA export factors forms a larger complex called TREX (34). Mutations in these RNA export factors confer a similar transcription-dependent hyper-recombination phenotype and defect in transcription elongation as THO mutations (33,41). In addition, yeast mutants in proteins involved in various processes associated with transcription like 3′-end cleavage/polyadenylation, components of nuclear exosome (multi-subunit RNA processing and degradation machine), and mRNA export showed a significant transcription-dependent hyper-recombination phenotype though not to the same extent as the THO mutants (42). THO–TREX complex and the hyper-recombination observed in yeast mutants in cleavage/polyadenylation factors and nuclear exosome (42) suggest that there is a strong interdependence between transcription elongation and hyper-recombination and also between mRNP (export competent ribonucleoprotein particle) biogenesis and genetic stability in general (33).

**TAR in mammalian cells**

Nickoloff and Reynolds (43) provided the first direct evidence of TAR in mammalian cells. Transcription of heteroallelic neomycin genes was shown to stimulate recombination 6-fold between transfecting plasmids in Chinese hamster ovary (CHO) cells. Direct evidence that transcription stimulates intrachromosomal recombination was provided by studies on a similar system in CHO cells (44). There was a 2- to 7-fold increase in intrachromosomal recombination between duplicated neomycin genes stably integrated into the CHO cell lines in the presence of transcription. Transcription through only one repeat was enough for stimulating recombination in both direct and inverted repeats (44). Using a similar recombination reporter system containing two non-functional copies of the neomycin gene under the regulation of tetracycline promoter, we showed a quantitative relation between transcription levels and recombination levels. About 60-fold increase in transcription through the construct stimulated recombination by >20-fold (45).

As in yeast, gene conversion was the main spontaneous TAR event between direct repeats in mammals (44,45) but recombination between inverted repeats produced a variety of rearranged products including cross overs and unequal sister chromatid exchanges, suggesting that transcription does not influence the mechanism of recombination (44). Studies performed in our laboratory further revealed that transcription enhances short-tract and long-tract gene conversion events in similar frequencies without preference for either (45). Similar to yeast, transcription in mammalian cells does not seem to influence DSB-induced recombination any further (45,46) and has no effect on the DSB-induced gene conversion spectra (46,47) again confirming that transcription has no influence once recombination is initiated. However, transcription enhanced the use of a donor allele 2- to 3-fold during DSB-induced gene conversion in human cells, as shown using...
a triple neomycin repeat recombination substrate (48). This suggests that TAR may play a role in maintaining genomic stability by preventing the use of non-transcribed pseudogenes as donors during gene conversion (48).

Overall, the available data in mammalian cells suggest that TAR and DSB-induced HR are mechanistically different processes with little influence on each other. Our recent data show that there is a differential genetic requirement for the two processes (49). It was found that the RAD51 parologue XRCC2, which is required for HR induced by a DSB (50), is dispensable for TAR (49). This is interesting as it proves that TAR is carried out in absence of XRCC2 and is genetically distinct from HR induced by a DSB. The XRCC2 protein has been shown to be critical early in HR for RAD51 foci formation following ionizing radiation (51) and is also likely involved in processes late in resolution of HR intermediates, which has been shown to be the case for other RAD51 paralogues (52). If it is the early role of XRCC2 that is dispensable for TAR, it would suggest that TAR may be independent of RAD51-mediated strand invasion. However, this is not likely to be the case as it was shown in the same study that TAR is dependent on BRCA2 (53), which is also involved in the early step of HR, loading RAD51 onto DNA and required for RAD51 foci formation (54,55). Thus, it is plausible that TAR requires RAD51-mediated strand invasion, but is resolved differently than HR induced by a DSB.

TAR may also be linked to the immunoglobulin-diversifying processes in mammals (56). Immunoglobulin V(D)J recombination (57,58), class switching (59,60) and SHM (60,61) in mammalian B cells, though be mediated by specific recombination events, were shown to be dependent on transcription (62–65). During V(D)J recombination, recombination frequencies are higher in transcriptionally active immunoglobulin genes and this is regulated at the level of transcription (62,66). In SHM, there is a high frequency of DSBs in and around the targeted V(D)J region (67) and mutation frequencies correlate with promoter strength and transcriptional activity (64). Many reports suggest the involvement of transcription and recombination in class switching (68–70). Therefore, in mammals, TAR seems to play an important role both in maintaining genomic stability and generating genetic diversity as in the case of immunoglobulin diversification.

**Mechanism of TAR**

In spite of the importance of TAR in all organisms, its mechanism is still elusive. There are many theories postulated over the years to explain TAR (7) and there have been some recent advancements in deciphering its mechanism in both yeast and mammals. All the proposed models to explain the stimulation of spontaneous recombination by transcription are summarized in Figure 1 and can be grouped into one of the two main non-exclusive categories:

(i) Transcription could lead to increased accessibility of a target region to recombination proteins or DNA damage thus stimulating recombination (66)—the accessibility theory.

(ii) TAR could be the consequence of stalled replication forks formed during transcription due to either collision between the transcription and replication machineries or transcription-associated DNA:RNA hybrids obstructing the replication fork movement (7)—the collision theory.

**The accessibility theory**

According to the earliest model proposed to account for TAR in λ phage, transcription leads to strand separation and the single-stranded DNA (ssDNA) thus formed invade homologous regions, stimulating recombination (71). Alternatively, this ssDNA is more accessible to recombinogenic DNA damage (72). This theory was directly tested in yeast by Aguilera and group (73) by studying the effect of different DNA-damaging agents in several different recombination systems that were either transcribed or not transcribed. The DNA-damaging agents and transcription showed a synergistic effect on both intermolecular and intramolecular recombination between direct repeats, suggesting that TAR induced by DNA-damaging agents may be to a large extent due to increased accessibility of DNA to these agents mediated by transcription. For example, while transcription alone or treatment with

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**Fig. 1.** Possible mechanisms of TAR. (A) When RNA polymerase opens up the dsDNA during transcription, the nascent ssDNA on the non-template strand is more susceptible to DNA damage, which may be repaired by HR. The ssDNA may also be more accessible to the recombination machinery, another reason for increase in HR levels. (B) Transcription results in the negative supercoiling of DNA (underwound state of DNA-facilitating unwinding allowing transcription to occur) behind the advancing RNA polymerase. This may facilitate the formation of R-loops by the nascent mRNA as the DNA is underwound. R-loops also result in ssDNA during transcription, which in turn may cause an increase in HR as discussed above. (C) R-loops may form roadblocks to the RNA polymerases behind it or to the replication machinery working on the same DNA substrate, stalling replication forks, which require recombination to be rescued. (D) Stalled forks may also be formed when transcription and replication machineries travelling towards each other on the same DNA substrate run into each other and collide. (E) Finally, TAR may be a parallel pathway to TCR in S phase. When replication is blocked by active transcription, RNA polymerase may recruit recombination machinery, so replication can bypass transcription.
4-nitroquinoline-N-oxide (a carcinogen that forms bulky adducts on DNA) alone increased recombination by ~2- and 40-fold, respectively, both together increased recombination >4000 times. Similarly, all types of recombination events tested in different recombination systems were greatly increased by DNA-damaging agents in actively transcribed DNA (73). In mammals, a similar study evaluated the relationship between DNA damage and TAR by looking at the effect of transcription on ultraviolet (UV)-induced intrachromosomal recombination (74). The study showed that both transcription and UV irradiation alone stimulated recombination as expected. However, both together did not show any synergistic effect. Elevated levels of transcription diminished UV-induced recombination, suggesting that recombination is stimulated by damage and not by repair. On the same lines, UV repair-deficient cells required lower UV doses to stimulate intrachromosomal recombination compared to repair-proficient cells (75). These reports confirm that ssDNA formed during transcription is more accessible to recombinogenic DNA damage and this may be one of the explanations for TAR. Not only does the accessibility of ssDNA to damage increase upon transcription but also the accessibility to nucleases increases upon transcription, which seems to be the case for HO nuclease cleavage of MAT loci in yeast (28) and the cleavage during V(D)J recombination in vertebrates (62,66).

Transcription could alter the repair of already existing damage by recruiting the recombination proteins to damaged regions. This is supported by the fact that there are several mechanistic similarities between transcription and recombination including DNA unwinding and protein recruitment (76). Furthermore, some transcription factors play a dual role in transcription and DNA repair (77,78). This theory suggests that the mechanism of TAR is perhaps parallel to TCR of other types of DNA damage (2) and is further supported by the available biochemical evidence (53). HR protein RAD52 [reviewed in (79)] physically associates with different subunits of transcription factor TFIIH and RNA polymerase II and can either activate or repress transcription (53). In addition, TAR is shown to be dependent on rad52 gene product in yeast (80). Similarly, another recombination protein BRCA1 associates and forms a part of the RNA polymerase II holoenzyme (81). In yeast, Hpr1 component of the THO complex also associates with RNA polymerase II holoenzyme (82) and is shown to participate in transcriptional elongation (30).

Further, there are reports suggesting that proteins required for HR are also involved in TCR. HR proteins BRCA1 and BRCA2 are suggested to be involved in TCR of oxidative DNA damage (83), though this is unconfirmed. Recently, it has been shown that HR is also involved in TCR of UV damage (84). In S. cerevisiae, HR proteins RAD51 and RAD54 were shown to play an important role in the repair of UV damage on transcribed strand only during G2/M phase of the cell cycle, providing evidence for the involvement of HR in the repair of transcribed strands of active genes (84). However, this repair was shown to be transcription dependent but unrelated to replication lesions (84). These reports suggest that the processes of TAR and TCR could be interlinked.

Other topological changes in DNA during transcription may also facilitate the increase in recombination manifesting in TAR. For example, transcription-induced supercoiling of DNA may stimulate recombination by bringing the homologous regions closer together and facilitating strand exchange (85). Similarly, transcription-associated (86,87) or repair-associated (88) remodelling of chromatin could result in increased interactions between transcriptionally active DNA during HR repair of DSBs resulting in TAR. This is supported by the observation that transcription enhances the use of a donor allele during DSB repair (48). Furthermore, transcription elongation results in the accumulation of negatively supercoiled DNA behind the advancing RNA polymerase II. DNA in this underwound state favours for the local unwinding of DNA and this allows the formation of DNA-RNA hybrids or R-loops with the nascent mRNA synthesized during transcription (discussed below in more detail). The non-transcribing ssDNA formed in the process may be more susceptible to DNA-damaging agents, thus forming recombinogenic lesions (7).

The collision theory: dependence of TAR on replication

Many of the theories proposed to explain the mechanism of TAR link TAR to replication-associated lesions and we discuss these theories in the following section.

As transcription and replication can take place on the same DNA substrate at the same time, it is possible that they obstruct each other in more than one way, resulting in stalled or collapsed replication forks, which may be possible substrates for TAR. Transcription may obstruct replication in many different ways. Secondary structures or DNA adducts formed during transcription elongation are one possible form of obstruction to replication. As discussed previously, lesions obstructing replication forks could cause the stalling and collapse of replication forks creating DSBs. Hence, transcription elongation might contribute to the formation of lesions that could be subsequently converted into one-ended DSBs and are then repaired by HR (89). When DSB-induced and spontaneous recombination events were compared in yeast mutants of different recombination proteins under low and high transcription conditions, it was found that the effect of respective mutations was the same in DSB-induced recombination and TAR, suggesting that the initiation events stimulated by transcription might be DSBs or lesions leading to DSBs (89). Our work in mammalian cells further suggests that TAR is not associated with a classical two-ended DSB. We showed that HR repair of DSBs involves short-tract gene conversion in all the phases of the cell cycle in mammalian cells (90) where as transcription induces similar levels of short-tract and long-tract gene conversion events (45); the same pattern of recombinants is formed following thymidine treatment (15,14). Hence, it seems more likely that transcription results in lesions that occur in absence of DSBs.

As explained in the previous section, negative supercoiling of DNA behind the advancing RNA polymerase during transcription may result in the formation of DNA-RNA hybrids or R-loops. These DNA-RNA hybrids might also obstruct replication, stalling replication forks. This in turn might be resolved by recombination (7) resulting in TAR. Cotranscriptional accumulation of DNA-RNA hybrids or R-loops behind the elongating RNA Polymerase II is the major cause of TAR and impairment of transcriptional elongation in the THO mutants (91) and also in mutants of a RNA-splicing factor ASF/SF2 in chicken DT40 cells and human HeLa cells (92). Depletion of ASF/SF2 in these cell lines resulted in high levels of DNA rearrangements due to the formation of R-loops. In addition, DSBs were found around the R-loop region in these mutant cells that led to a G2 arrest. Over-expression of human RNase H-1 in these mutants eliminated the R-loops, DSB-induced G2 arrest and the accompanying genomic instability (92). Similarly, there was a significant reduction in
transcription-dependent hyper-recombination in the THO mutant cells when the resulting transcript during transcription of the direct repeats under study was self-cleaved by an active artificially engineered hammerhead ribozyme or degraded by over-expression of RNase H1 (91). These studies provide a clear mechanistic link between R-loops and hyper-recombination. R-loops could also explain the mechanism of TAR in CSR, though there is no in vivo evidence. In vitro experiments have shown that the transcript of the switch region where the recombination event takes place hybridizes with the template DNA-forming R-loop structures during transcription (93–96), suggesting that R-loops play an important role in TAR.

Another possible criterion where transcription might obstruct replication is when transcription and replication machineries collide with each other. Replication fork pausing (RFP) by RNA polymerases I and III during transcription is a known phenomenon (97,98) and the collision between replication and transcription in the ribosomal DNA in yeast (99) increases recombination (97,100). The link between RFPs and recombination is well established in fission yeast. The specific recombination reaction involved in mating type switching in fission yeast is promoted by a RFP (101) caused by a replication fork barrier (RFB) (a region where particular proteins bind tightly to DNA obstructing replication [see ref. (102) for a review on RFBs]). When this specific RFB is transported to other loci in fission yeast, this led to a stimulation of recombination in the adjacent sequences (103,104) confirming the link between RFPs and recombination. Recently, it has emerged that RFPs also occur during RNA Polymerase II transcription. RFP-led increase in recombination was observed in RNA Polymerase II transcribed leu2 genes (105) and also in the genes highly transcribed by RNA Polymerase II in THO mutants (discussed below) (106), suggesting that hyper-recombination led by RFP is a phenomenon that possibly occurs during transcription of all genes. In S. cerevisiae, TAR in the leu2 direct repeat constructs required head-on oncoming replication (105). TAR occurred only when there was replication fork progression opposite to transcription and it was associated with the appearance of a RFP in the region of homology. Further, the appearance of transcription-associated RFP correlates with TAR (105), indicating that TAR in this construct is a result of the collision between the machineries of transcription and replication causing replication blockage.

Recent work in THO mutants in yeast shed more light on the mechanism of TAR and provided further evidence for the association of TAR with replication impairment. As explained before, mutations in the THO complex in yeast result in transcription-dependent hyper-recombination (30,39) dependent on transcription elongation and also on the type of DNA segment through which transcription elongation took place (40). Long and GC-rich regions are found to be particularly difficult to transcribe in hpr1Δ mutants (30) and the hyper-recombination was shown to be higher in GC-rich regions (40). These THO mutants also showed an impairment in the replication fork progression due to obstruction by DNA:RNA hybrids at the exact same regions which were previously shown to have decreased transcription (106). Ribozyme-mediated cleavage of the nascent mRNA partially suppressed the replication defect and TAR (106), confirming the role of DNA:RNA hybrids in TAR. Moreover, the transcription-dependent hyper-recombination in these mutants occurred only in the S phase of the cell cycle. This further confirmed that TAR in these mutants is associated with replication. One of the mutations in the yeast hpr1 that results in transcription elongation impairment and hyper-recombination phenotype also increases chromosome (107) and plasmid loss (32) in the presence of transcription, also consistent with the notion that replication blockage is associated with TAR. Stalling and collapse of replication forks by transcription can result in DNA breaks and HR-mediated repair or bypassing of these lesions may result in the observed chromosome and plasmid loss. Interestingly, one particular point mutant of the hpr1 gene showed no hyper-recombination phenotype though it showed a strong defect in transcription and a general defect in mRNA export like the other mutants of the THO complex (108). This was due to the absence of replication fork blockage in this mutant, suggesting that stalled replication is a prerequisite for hyper-recombination. In addition, transcription-dependent hyper-recombination observed in mutants with aberrant mRNA processing (42) further supports this theory as incomplete processing of mRNA may result in the DNA:RNA hybrids due to accumulation of nascent mRNA.

Another possibility for stalled replication and transcription to result in TAR is for RNA polymerase to simply push the stalled replication fork backwards, thus forming a Holliday junction, which then requires HR in order to be resolved (7). Hence, the data in yeast suggest that stalled replication forks formed either by collision between transcription and replication machineries or due to RNA:DNA hybrids formed during transcription elongation in the S phase are the substrates for TAR.

In our recent study, we have shown that TAR is S phase associated in mammalian cells also, suggesting that TAR is probably ubiquitously dependent on replication (45). We studied recombination between duplicated neomycin repeats under the regulation of a bi-directional tetracycline promoter. This regulatable bi-directional promoter also controlled transcription through the luciferase gene, which allowed us to quantify both transcription and recombination through the substrate at any given time. Cells stalled in S phase with transcription showed much higher frequencies of HR compared to cells stalled to the same degree in S phase but with no transcription, probably due to more frequent collisions between transcription and replication machineries. Further, inhibiting transcription and replication at the same time abolishes recombination induced by thymidine [a drug that slows the progression of replication forks (12)], suggesting that stalled replication forks are involved in TAR in mammalian cells (45).

In a recent study, we show that both TAR and HR induced at replication forks by thymidine are dependent on BRCA2 protein and at the same time independent of XRCC2 (53). XRCC2 has been shown to be required for most HR events (50,51,109–112), but specifically not for recombination events induced by thymidine (112). This data suggest that HR induced by thymidine and transcription have similar requirements and that a similar HR substrate is formed following thymidine treatments and transcription. The mechanism of action of thymidine is unique, in that it slows down the replication forks through depletion of dCTP levels without causing collapsed replication forks (12). It is possible that transcription similarly slows down replication forks and utilizes a similar recombination bypass pathway (53).

A model for bypassing transcription during replication may involve collision of transcription and replication machineries causing a DNA polymerase block, which can trigger uncoupling of the leading and lagging strand synthesis. Template switching...
may then promote replication by using lagging strand as template until the DNA polymerase bypasses RNA polymerase. Replication may then restart in front of the RNA polymerase using HR.

A B-cell-specific protein activation-induced cytidine deaminase (AID) that mediates immunoglobulin-diversifying processes SHM and class switching (113) provides further proof of the link between TAR and replication-associated lesions. Action of AID is transcription dependent and mutations in THO complex in yeast result in strong and transcription-dependent hyper-recombination induced by AID (114). A recently proposed model for SHM in immunoglobulin genes of vertebrates explains SHM as the result of action of AID and other DNA repair proteins on DSBs created during collision between the transcription apparatus and the replication fork (115). As explained previously, GC-rich S regions form R-loops (93,94,96) which can in turn obstruct the replication machinery leading to the stalling of replication forks, which might further contribute to the high levels of recombination during class switching. Hence, TAR seems to be the result of replication impairment by transcription either by physical obstruction to the replication fork progression or by creating lesions that impair replication.

Concluding thoughts

All the evidence in both lower and higher eukaryotes suggests that TAR is a double-edged sword. While TAR in THO–TREX mutants in yeast suggests that it plays a role in genomic instability, spontaneous TAR in both yeast and mammal seems to prevent genetic instability in more than one way. For example, it appears that TAR prevents genetic instability by preventing the use of pseudogenes during recombination as transcription increases preference for particular donor alleles (48). On the other hand, TAR may help in bypassing transcription during replication thus preventing genetic instability associated with replication blockage (45,53). In addition, by taking advantage of the association between transcription and recombination, immunoglobulin genes seem to utilize TAR in generating genetic diversity. Considering the different manifestations of TAR and the widespread nature of TAR, it is likely that TAR in eukaryotes does not occur by a single mechanism, but by simultaneous involvement of several mechanisms as these are not mutually exclusive.

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References


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