Traveling Rocky Roads: The Consequences of Transcription-Blocking DNA Lesions on RNA Polymerase II

Barbara Steurer and Jurgen A. Marteijn

Department of Molecular Genetics, *Erasmus MC, Wytemaweg 80, Rotterdam 3015 CN, The Netherlands*

Correspondence to Jurgen A. Marteijn: *J.Marteijn@erasmusmc.nl*
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Abstract

The faithful transcription of eukaryotic genes by RNA polymerase II (RNAP2) is crucial for proper cell function and tissue homeostasis. However, transcription-blocking DNA lesions of both endogenous and environmental origin continuously challenge the progression of elongating RNAP2. The stalling of RNAP2 on a transcription-blocking lesion triggers a series of highly regulated events, including RNAP2 processing to make the lesion accessible for DNA repair, R-loop-mediated DNA damage signaling, and the initiation of transcription-coupled DNA repair. The correct execution and coordination of these processes is vital for resuming transcription following the successful repair of transcription-blocking lesions. Here, we outline recent insights into the molecular consequences of RNAP2 stalling on transcription-blocking DNA lesions and how these lesions are resolved to restore mRNA synthesis.

Introduction

DNA damage compromises the fidelity of DNA transcription and replication, threatening cell viability and genome integrity. Many different DNA-damaging agents, of both endogenous and environmental origin, can cause DNA injuries that block or strongly hinder RNA polymerase II (RNAP2) transcription elongation. Furthermore, in cycling cells, advancing replication forks can collide with stalled RNAP2 complexes, as reviewed by Stirling and Hieter in this issue [1]. The arrest of RNAP2 on transcription-blocking lesions (TBLs) leads to a lack of newly synthesized RNA molecules or may result in mutant mRNAs. Not only these effects on RNA expression but also the prolonged arrest of RNAP2 itself are both highly cytotoxic. The stalling of RNAP2 on lesions for extended periods of time can arrest cell cycle progression and lead to apoptosis [2,3], and so, if TBLs remain unrepaired, this blocked transcription can cause severe cellular dysfunction, eventually resulting in DNA-damage-induced aging [4–6]. The structural complexity of lesion-stalled RNAP2 requires an intricate protein network that needs to be activated to ensure the removal of genomic roadblocks and to overcome blocked transcription.

The stalling of elongating RNAP2 on DNA lesions initiates transcription-coupled DNA repair (TCR), which is a multistep pathway that efficiently removes DNA lesions specifically from actively transcribed genes. Only upon completion of TCR will the stalled transcription restart [7]. The biological relevance of this DNA repair pathway is best demonstrated by the severe phenotypes of human disorders that are related to defective TCR [6–8]. However, even though the concept of TCR was discovered almost 3 decades ago [7,9], many questions remain unanswered about how cells coordinate transcription arrest and TBL repair and subsequently restart mRNA synthesis. Here, we discuss the multifaceted cellular response that is triggered following the stalling of RNAP2 on TBLs.

Fates of Lesion-Stalled RNAP2

To repair TBLs, TCR faces a significant steric problem: RNAP2 may be trapped near to or right on the top of a TBL, severely obstructing the access of

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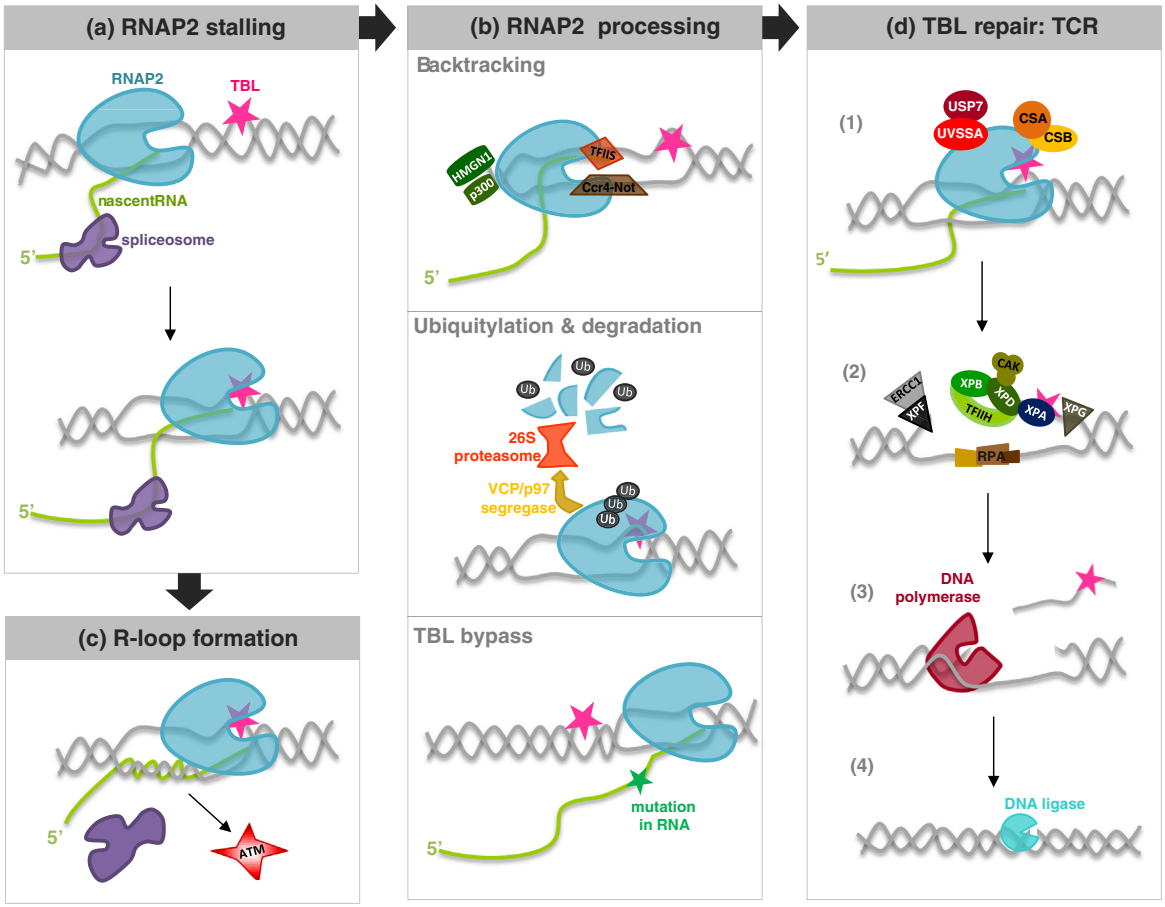

Fig. 1. The arrest of elongating RNA polymerase 2 (RNAP2) on a transcription-blocking DNA lesion (TBL) triggers a series of cellular events. (a) Elongating RNAP2 runs into a TBL and stalls. (b) R-loops can be formed by the hybridization of pre-mRNA with template ssDNA adjacent to the transcription bubble. TBL-induced R-loop formation activates non-canonical ataxia-telangiectasia mutated (ATM) protein kinase signaling, which in turn results in the eviction of co-transcriptional spliceosomes. (c) To allow the repair machinery to access the TBLs, the damage-stalled RNAP2 needs to be removed from the lesion. RNAP2 processing may occur via transcription factor IIS (TFIIS)- and Ccr4–Not-mediated backtracking (top panel). Alternatively, RPB1, the largest subunit of the RNAP2 complex, may be ubiquitylated and proteasomally degraded. Segregase activity is needed to extract RPB1 from chromatin (middle panel). Lesion bypass of RNAP2 may also make the lesion accessible, but this may result in mutant RNA (bottom panel). (d) Transcription-coupled DNA repair (TCR) is initiated when RNAP2 stalls at a TBL during transcription elongation. It is not clear whether TCR stimulates backtracking or whether backtracking is needed to initiate TCR. During transcription elongation, UV-stimulated scaffold protein A (UVSSA), ubiquitin-specific-processing protease 7 (USP7), and Cockayne syndrome protein B (CSB) transiently interact with RNAP2. Upon stalling at a TBL, the affinity of CSB for RNAP2 increases, and the CS WD repeat protein CSA–CSB complex is formed (step 1). Following damage recognition, the transcription initiation factor IIH (TFIIH) complex is recruited to the lesion, and the structure-specific endonuclease xeroderma pigmentosum group G (XPG) binds to the pre-incision nucleotide excision repair (NER) complex. The helicase activity of TFIIH further opens the double helix around the lesion via XPD, which unwinds the DNA in a 5′–3′ direction and verifies the existence of lesions with the help of the ATPase activity of XPB and XPA. XPA and replication protein A (RPA) then recruit the endonuclease XPF/excision repair cross-complementing 1 (ERCC1), which creates an incision 5′ to the TBL. This results in the activation of XPG, which cuts the damaged strand 3′ to the lesion, excising the lesion within a 22- to 30-nt-long strand (step 2). Immediately after the 5′ incision has been made, gap-filling synthesis by DNA Pol δ, DNA Pol κ, or DNA Pol ε can begin (step 3). The NER reaction is completed when the final nick is sealed by DNA ligase 1 or DNA ligase 3 (step 4).

repair factors to the lesion [6,7] (Fig. 1a). Different types of TBLs differentially inhibit the forward translocation of the transcription machinery [7,10]. For example, UV-induced cyclobutane-pyrimidine dimers cause the arrest of RNAP2 on the top of the TBL. The 35-nt footprint of the stalled RNAP2 is asymmetrically located around the lesion, covering 10 nt downstream and 25 nt upstream of the UV lesion [11–13]. By contrast, cisplatin-induced interstrand crosslinks stall RNAP2 before the lesion can enter the polymerase's active site [14]. Oxidative DNA lesions, such as 8-oxo-7,8-dihydroguanine (8-oxo-G), which

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are induced by endogenous reactive oxygen species, also interfere with transcription. However, the damage-induced transcription stalling of RNAP2 does not appear to be caused by 8-oxo-G itself, but rather indirectly by base excision repair intermediates [15,16].

To overcome persistent RNAP2 stalling and to facilitate the access of the DNA repair machinery, cells have evolved three different mechanisms to displace lesion-stalled RNAP2: reverse translocation, degradation, and lesion bypass (Fig. 1c).

Reverse translocation, or backtracking, of RNAP2 not only occurs in the presence of TBLs but also when RNAP2 encounters DNA sequences that are difficult to transcribe [17]. In bacteria, the DNA helicase UvrD travels along with elongating RNAP2 and moves the complex backwards upon encountering a DNA lesion [18]; a similar backtracking mechanism has also been suggested for higher eukaryotes, although this has not yet been confirmed [17–20]. To resume transcription after backtracking, the protruding nascent RNA needs to be cleaved to reposition the 3′ end of the RNA in the active site of the polymerase [21]. In eukaryotes, this reaction is mediated by transcription factor IIS (TFIIS), which stimulates the intrinsic 3′–5′ exonuclease activity of RNAP2 [11,22–24]. A recent study showed that TFIIS recruitment to elongating RNAP2 is increased by the Ccr4–Not complex, and consequently, the authors suggested that TFIIS and Ccr4–Not work together to reactivate arrested RNAP2 [25]. In addition, Ccr4–Not may promote the resumption of elongation by binding to the emerging transcript protruding from the polymerase [26].

RNAP2 backtracking upon collision with a TBL would provide the space needed for the TCR machinery to repair the TBL. This principle was elegantly demonstrated by researchers in the Hanawalt laboratory [11,27], who showed that photolyases, which specifically bind UV-induced DNA lesions, could only recognize TBLs following the TFIIS-mediated backtracking of arrested RNAP2 [27]. Furthermore, TFIIS was shown to be involved in the efficient recovery of transcription following UV irradiation, emphasizing its role in TCR [28]. While little is still known about the factors that mediate RNAP2 backtracking, the process may be facilitated by the sliding of the upstream nucleosomes by the histone acetyltransferase p300 and the nucleosome-binding protein HMGN1, both of which interact with stalled RNAP2 [7,29]. In addition, the key TCR protein CSB may be involved in the displacement of stalled RNAP2, as it contains a SWItch/sucrose non-fermentable (SWI/SNF2) ATPase domain and has chromatin remodeling activity that is stimulated by the histone chaperone NAP1 [30–32].

If backtracking fails, arrested RNAP2 may be degraded instead, most likely to prevent genomic roadblocks caused by its persistent stalling. Ubiquitylation and degradation of RPB1, the largest and core catalytic subunit of RNAP2, also occurs during basal transcription elongation [33,34]; however, it is greatly increased following genotoxic stress [35,36]. After a decade of discovering the individual factors that are involved in RPB1 degradation [37–40], Harreman and colleagues clarified the pathway in yeast by ordering the actions of distinct and sequentially acting ubiquitin ligases and de-ubiquitylating enzymes (DUBs) [41].

In yeast, the HECT ubiquitin ligase Rsp5 binds to the C-terminal domain of RPB1 [37] and modifies the subunit with a K63-linked polyubiquitin chain, which by itself does not trigger proteolysis. This K63-polyubiquitin chain is then trimmed by the DUB Ubp2 [41]. The residual monoubiquitin on RPB1 can be either hydrolyzed by Ubp3, rescuing RPB1 from degradation [42], or extended to K48-linked polyubiquitin by the Elc1/Cul3 ligase complex, marking RPB1 for proteasomal degradation [41]. Finally, the ring-like AAA+ ATPase CDC48/p97 is required to segregate the K48-polyubiquitylated yeast Rpb1 from chromatin and to facilitate RPB1 degradation by the 26s proteasome [43]. Remarkably, RPB1 is the only subunit of the 12-subunit RNAP2 complex that is degraded following UV exposure [44,45].

It is currently unknown whether the DNA-damage-induced degradation of RNAP2 by the successive action of different ubiquitin ligases is conserved in mammals. The mammalian RSP5 homolog Nedd4 was found to ubiquitylate RPB1 in human cells, resulting in its degradation upon genotoxic stress [46]. However, Nedd4-depleted cells are not sensitive to UV light [46], indicating that it is not the only factor required to modify RPB1 upon UV exposure. It has also been shown that the von Hippel–Lindau tumor suppressor protein (pVHL) can bind RPB1 in a proline-hydroxylation-dependent manner and functions as an E3 ligase that targets elongating RPB1 for ubiquitylation and degradation in response to UV light. pVHL-negative cells were shown to accumulate elongating RPB1 and undergo apoptosis in response to UV, whereas cells expressing pVHL did not [47]. These results clearly indicate that pVHL plays a role in eukaryotic RPB1 degradation. pVHL is a crucial component of the VHL–E3 ubiquitin ligase complex, which consists of Elongin BC, Cullin2, and Rbx1. In this complex, pVHL serves as substrate recognition unit, and the Cullin/Rbx module functions as a ubiquitin-activating enzyme [48]. The mammalian Elongin A–Elongin BC–Cul5/Rbx2 complex can also efficiently ubiquitylate RPB1 *in vitro* [49]. However, rather than the pVHL-induced degradation of elongating RPB1 (Ser2-phosphorylated), Elongin A and Cul5 interact with initiating RPB1 (Ser5-phosphorylated) upon exposure to UV light. Furthermore, Ser5-phosphorylated RPB1 has also been shown to be a substrate for the BRCA1/BARD1 ligase complex [50]. Interestingly, BRCA1 also ubiquitylates the RNAP2 subunit RPB8 in response to UV irradiation, but this ubiquitylation does not result in RPB8 degradation [51].

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The observation that initiating Ser5-phosphorylated RPB1 is targeted by specific E3 ligases raises the exciting possibility that the collision of RNAP2 with TBLs in the gene body may also have consequences for transcription-initiating RNAP2 complexes at the promoter. Since the regulation of transcription is far more complex in eukaryotes than in yeast, including, for example, promoter-proximal pausing [52–54], it is tempting to speculate that RNAP2 stalling initiates a much more sophisticated cellular response in mammalian cells compared with yeast. In support of this speculation, it was recently shown that UV irradiation results in the loss of RNAP2 at the promoters of many transcribed genes [55], suggesting a genome-wide mechanism that regulates transcription initiation in response to TBLs.

It is unclear whether the valosin-containing protein VCP/p97, which is the human homolog of the yeast ATPase CDC48/p97, is required for chromatin extraction of mammalian RPB1. Even though several key players in the ubiquitylation of mammalian RPB1 have been identified, our understanding of RPB1 degradation in mammals is incomplete. The identification of many distinct ligases that are involved in the degradation of mammalian RPB1 highlights the importance of RNAP2 regulation by ubiquitin, but further research is needed to fully understand the precise interplay of all of the factors involved.

Finally, DNA lesions that are encountered by RNAP2 may be bypassed, although this occurs infrequently [7,56]. If the helix distortion of a TBL is minimal, such as at abasic sites or single-strand breaks, it might translocate into the RNAP2 active site. The subsequent translocation is disfavored but not totally blocked [56]. Lesion bypass can be stimulated by various transcription factors, such as CSB [57] or TFIIF [58], but this is at the cost of transcriptional mutagenesis [59]. Nucleotide misincorporation due to lesion bypass can have serious consequences for the cell if the faulty nucleotide leads to changes in the amino acid coding and the expression of mutant proteins.

Which type of RNAP2 processing ultimately occurs upon RNAP2 stalling at a TBL (backtracking, degradation, or bypass) and how these options are regulated in the cell remains largely unknown. The pathway choice is probably influenced by the nature of the TBL and the chromatin environment, but it may also be affected by cell type, cell cycle stage, or gene-specific regulation of transcription.

TBL Arrest of RNAP2 Induces R-Loops, Spliceosome Eviction, and Non-Canonical ATM Signaling

The association of multimegadalton spliceosomes with nascent RNA may pose another steric challenge to the repair of TBLs. It was recently reported that late-stage spliceosomes, composed of U2, U5, and U6 small nuclear ribonucleoproteins, are rapidly excluded from DNA damage sites in response to UV-induced TBLs [60]. This displacement of co-transcriptional spliceosomes from arrested RNAP2 most likely results in an increase in R-loop formation through the hybridization of pre-mRNA with template ssDNA adjacent to the transcription bubble [60,61] (Fig. 1b). Persistent R-loops are genotoxic, as they can interfere with transcription and replication, increase the probability of replication fork collapse following collisions with stalled transcription complexes, and promote unscheduled replication by transcription-associated recombination. Furthermore, the ssDNA in the R-loop poses a further threat to genome fidelity, as it is sensitive to mutagens, can undergo spontaneous hydrolysis, and is prone to the formation of secondary structures such as G-quadruplexes. To counteract R-loop toxicity, cells are equipped with specialized RNA hydrolases (RNaseH1 and H2) or helicases (e.g., Pif1, DHX9, and senataxin) that unwind the RNA:DNA hybrid [62–65]. In the context of TBLs, R-loop formation leads to non-canonical activation of the ataxia-telangiectasia mutated (ATM) protein kinase, which signals the further mobilization of spliceosomes from elongating polymerases and those that are located distal to RNAP2-blocking DNA lesions. The exact molecular mechanism by which TBLs activate ATM remains unclear [60,61]. Interestingly, ATM, via R-loop formation, relays the local (*cis*) event of RNAP2 arrest to the genome-wide (*trans*) modulation of alternative splicing, adapting global gene expression and shaping the proteome in response to TBLs [60,66].

Initiation of TCR

To counteract the fatal implications of lesion-stalled RNAP2, TCR has evolved to specifically remove a wide range of helix-distorting lesions that impede the elongation of RNAP2 from actively transcribed genes. TCR is a subpathway of nucleotide excision repair (NER) and is initiated by the recruitment of Cockayne syndrome A (CSA) and CSB [7,19] and the UV-stimulated scaffold protein A (UVSSA) [67–69] to lesion-stalled RNAP2 (Fig. 1d). CSB has ATPase-dependent chromatin remodeling activity and may locally modify the DNA conformation [30,31,70]. CSB recruits CSA, which is part of a Cullin–RING ubiquitin E3 ligase complex, which was described to target CSB for ubiquitylation and degradation [71]. CSB degradation is counteracted by UVSSA, which recruits the DUB USP7 and thereby stabilizes CSB at the site of damage [67,69]. Although CSA is dispensable for the attraction of the excision repair machinery, in combination with CSB, it is essential for the recruitment of xeroderma pigmentosum group A (XPA)-binding protein (XAB), a pre-mRNA splicing factor that is

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involved in TCR [71–73]. Following damage detection, the transcription factor II H (TFIIH) complex unwinds a stretch of approximately 30 nt surrounding the damage site. XPA and replication protein A (RPA) then stimulate the damage-verification activities of TFIIH and orient the XPF/excision repair cross-complementing 1 (ERCC1) and xeroderma pigmentosum group G (XPG) endonucleases, which subsequently excise the damaged DNA. The resulting gap is filled by DNA synthesis and sealed by DNA ligases [74,75].

Restarting Transcription upon Repair of TBLs

Although the successful repair of a TBL is necessary, this in itself is not sufficient for transcription restart following genotoxic stress, which is essential for cell survival. Several factors that have explicit roles in TCR-associated transcription restart, but not in repair itself, have been identified over the past few years. For instance, the eleven-nineteen lysine-rich leukemia (ELL) protein, which interacts with TFIIH via the Cdk7 subunit of the CDK-activating kinase (CAK) complex, was found to be essential for transcription resumption following the removal of TBLs, and yet, it was not involved in the repair of TBLs [76]. Moreover, the downregulation of ELL increased RNAP2 chromatin retention in a UV-dependent manner. Together, these findings suggest that ELL serves as a docking site for proteins involved in the regulation of RNAP2-mediated transcription restart once repair has been completed [76].

The chromatin environment (i.e., histone chaperones, histone variants, and post-translational modifications of histones) also plays an important role during the restart of transcription following DNA damage. For example, knockdown of the histone chaperone HIRA impairs the recovery of RNA synthesis following UV damage to an extent that is comparable to that seen in TCR-deficient cells, but it does not affect the recruitment of repair factors. HIRA accumulates at sites of DNA damage, where it deposits the histone variant H3.3, which is crucial for facilitating the transcription recovery upon the repair of TBLs [77]. In addition, H2A/H2B dimer exchange has also been found to increase at sites of UV-induced DNA damage [78]. This damage-induced histone exchange is mediated by the histone chaperone facilitates chromatin transcription (FACT). FACT is a heterodimer consisting of the SPT16 and SSRP1 subunits and is a known H2A/H2B chaperone [79]. Although both FACT subunits are recruited to sites of UV damage, only SPT16 depletion results in a loss of damage-induced H2A/H2B exchange. Spt16 is required for the efficient restart of RNA synthesis following UV damage. This suggests that the FACT subunit SPT16 plays a specific role in damage-induced chromatin dynamics and transcription recovery [78]. In addition, knockdown of the methyltransferase disruptor of telomeric silencing 1-like (DOT1L) results in UV sensitivity, whereas DNA damage is removed normally [80]. Thus, the activities of HIRA, FACT, and DOT1L are thought to generate the proper chromatin environment or provide the correct chromatin plasticity needed for efficient transcription recovery following the removal of TBLs [81]. Interestingly, transcription restart following the treatment with the transcription inhibitor 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole (DRB) occurs independently of DOT1L and HIRA [77,80]. This indicates that transcriptional restart following DNA damage removal and basal transcription initiation is distinctly regulated, providing the cell with an additional level of control. Together, these findings highlight that the repair of the transcribed strand alone is not sufficient for the cell to restore mRNA expression. Transcription restart requires the synergy of many factors, including not only the discussed chromatin remodelers but most likely also transcriptional regulators [81,82].

Activating transcription factor 3 (ATF3) is one example of a regulator that is involved in transcription restart upon DNA damage but not repair [83]. ATF3 expression is dramatically upregulated by various stress signals, including UV damage. The binding of ATF3 to its target genes usually silences them [84]. However, although the transcription of ATF3 target genes recovers 12–24 h following UV damage in TCR-proficient cells, the ATF3 target gene repression is prolonged in CSB-deficient cells, likely due to ATF3 impeding RNAP2 access to the promoter. Supporting this, silencing ATF3 rescues the transcription restart defect in CSB-deficient cells. These findings allocate a new role to CSB besides its key function in sensing the lesion arrest of RNAP2; CSB may also be involved in overcoming the silencing of ATF3-dependent genes. Furthermore, these results imply that there is a direct link between the stalling of RNAP2 in the gene body and the inhibition of transcription that is regulated via the promoter [83].

This raises an interesting question: does the restart of RNAP2 transcription upon TBL only occur locally at the site of damage, or does it also occur genome-wide at non-arrested polymerases? (see Fig. 2). Although the suggested TFIIS- and Ccr4–Not-mediated backtracking of RNAP2 would allow to resume the elongation of the same transcript, there is currently no experimental proof for this mechanism. Interestingly, recent genome-wide analyses of nascent RNA sequencing data suggest that transcription recovers in a wave from the 5′-end of genes upon TBL induction by either UV irradiation or treatment with the topoisomerase 1 inhibitor camptothecin [85–87]. A wave-like recovery of transcription following genotoxic stress would implicate two interesting new concepts: (1) a significant part of transcription restarts at the beginning of genes, rather than at

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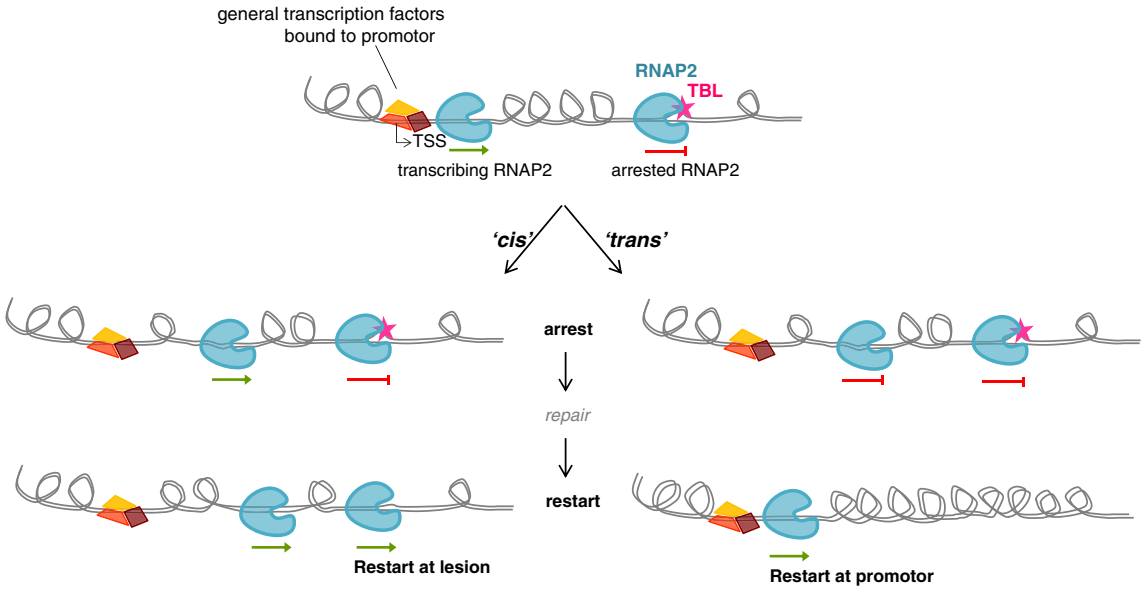

Fig. 2. The potential mechanism of transcription arrest and restart locally ('*cis*') or globally ('*trans*') following the stalling of RNA polymerase 2 (RNAP2) on a transcription-blocking lesion (TBL). '*Cis*' regulation implies that only those RNAP2 that hit a TBL will stall and cause transcription inhibition (indicated by ⊣), while other RNAP2 that are transcribing the same or other genes are not affected and continue to transcribe (indicated by →); upon repair of the TBLs, the arrested RNAP2 may resume transcription at the site of stalling (left panel). '*Trans*' regulation would also arrest other polymerases on the damaged gene, perhaps including both initiating and elongating RNAP2, and may even include the arrest of RNAP2 on other undamaged genes (not shown); the restart of transcription upon TBL repair may occur at the site of arrest (not shown) and also by the re-initiation of RNAP2 at the promoter. If the latter scenario occurs in a regulated manner at many promoters, transcription would recover in a wave from the 5′ start sites of genes (right panel).

the sites where RNAP2 initially stalled; and (2) transcription does not restart stochastically upon repair of individual genes, but rather simultaneously in a regulated manner in most genes [10].

Perspective

Over the past few decades, we have acquired an impressive body of knowledge about the cellular response to transcription-blocking DNA damage. However, to further improve our understanding of the post-repair transcription restart process, several questions remain to be answered.

RNAP2 processing upon DNA damage has been thoroughly studied, with RNAP2 displacement by backtracking, degradation, or lesion bypass now being widely accepted mechanisms. However, what guides this choice of pathways remains largely unknown. The degradation of arrested RNAP2 is assumed to be a last resort mechanism that occurs only when lesion-stalled RNAP2 cannot be resolved, as occurs, for example, in the absence of TCR proteins [88]. However, although preserving RNAP2 and its transcript from degradation by means of RNAP2 backtracking intuitively seems to be the most favorable scenario, experimental evidence to support such regulation is scarce. It is possible that RNAP2 degradation is favored over backtracking above a certain threshold of damage. Alternatively, the pathway choice to process the stalled RNAP2 may be guided by the complexity of the lesion or the chromatin environment or may even be gene specific.

A better insight into the fate of lesion-stalled RNAP2 may also improve our understanding of TCR-associated phenotypes. TCR defects in humans cause Cockayne syndrome (CS) or UV sensitivity syndrome (UVsS). CS and UVsS cells are equally deficient in TCR *in vitro*, and yet, the patients exhibit strikingly distinct clinical symptoms: CS patients display severe developmental, neurological, and premature aging features, whereas UVsS individuals present with a much milder phenotype that is mostly restricted to UV hypersensitivity [7,8,89]. How molecular defects within the same pathway can lead to such strikingly diverse phenotypes remains unresolved, but it may be associated with the specific functions of the CS proteins

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outside TCR [90,91], such as transcription initiation [92], the maintenance of mitochondrial DNA stability [93,94], or the regulation of specific transcriptional programs [95]. Another hypothesis suggests that aberrant processing of lesion-stalled RNAP2 may explain the differences between the UVsS and CS phenotypes. Here, it is proposed that in CS cells, which lack functional CSB, RNAP2 cannot be degraded or displaced [7], and so the lack of TCR combined with the persistent arrest of RNAP2 leads to apoptosis and senescence, causing the severe CS phenotype. By contrast, in UVsS cells, which lack functional UVSSA, stalled RNAP2 may still be ubiquitylated or displaced in a CSA/CSB-dependent manner, making the lesion accessible for alternative DNA repair mechanisms, including global genome NER or base excision repair, thus resulting in the milder UVsS phenotype [19,96].

To date, no study has investigated whether damage-induced R-loop formation and non-canonical ATM activation contribute to the phenotypes of these TCR syndromes. It has been reported that CSB is required to resolve R-loops, whereas XPC (the protein that initiates global genome NER) is not. However, CSB-mediated R-loop removal results in DNA breaks [64]. CSB may promote R-loop removal not only by excision [64] but also by resolving lesion-stalled RNAP2, which is an important source of R-loops [64,97]. Furthermore, R-loop-induced spliceosome displacement may promote TBL repair by facilitating RNAP2 backtracking or removal [60,66]. Alternatively, the loss of the co-transcriptional splicing machinery may be linked to a regulated inhibition of transcription via non-canonical ATM signaling. However, the role of R-loop-induced ATM activation as a new mechanism of transcription-stress signaling requires further investigation.

TBLs may have strikingly different outcomes in different organs and cell types [19,98–101]. A clear example of this is the extreme damage sensitivity of photoreceptor cells in the retina of TCR-deficient mice and the neurodegeneration in CS patients [98,102,103]. This suggests that DNA damage induction, recognition, repair, and signaling also differ between tissues and cell types, which would result in respective changes in the level of damage-induced mutagenesis, senescence, or cell death. Several factors may influence the differential cellular consequences to TBL exposure, including transcription levels, chromatin states, or differential activity of the DNA-repair pathways [104]. Finally, differences in replication rates may also lead to strong differences in the cellular consequences of TBLs. In contrast to post-mitotic differentiated cells in replicating cells, advancing replication forks may collide with TBL-stalled RNAP2 complexes, which can have severe cellular outcomes [62,105]. However, comprehensive studies on tissue-specific regulation of lesion-stalled RNAP2 and its underlying mechanisms are currently lacking.

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RNAP2, RNA polymerase II; TBL, transcription-blocking lesion; TCR, transcription-coupled DNA repair; TFIIS, transcription factor IIS; CS, Cockayne syndrome; CSB, Cockayne syndrome B; DUB, de-ubiquitylating enzyme; pVHL, von Hippel–Lindau tumor suppressor protein; ATM, ataxia-telangiectasia mutated; NER, nucleotide excision repair; CSA, Cockayne syndrome A; XPA, xeroderma pigmentosum group A; TFIIH, transcription factor II H; XPG, xeroderma pigmentosum group G; ELL, eleven-nineteen lysine-rich leukemia; FACT, facilitates chromatin transcription; DOT1L, disruptor of telomeric silencing 1-like; ATF3, activating transcription factor 3; UVsS, UV sensitivity syndrome.

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