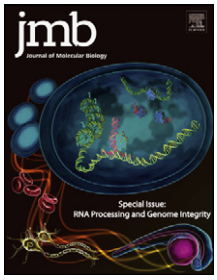

Traveling Rocky Roads: The Consequences of Transcription-Blocking DNA Lesions on RNA Polymerase II

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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

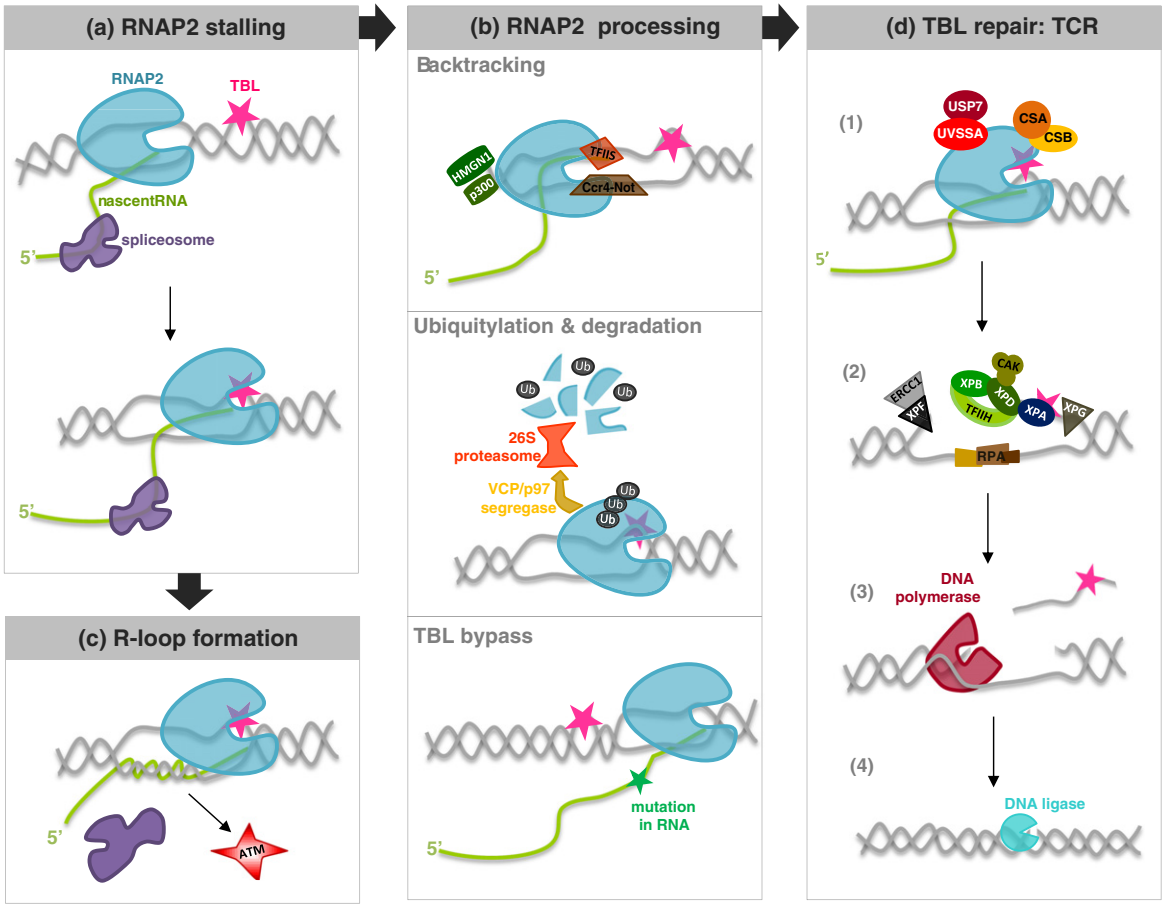

Fig. 1. The arrest of elongating RNA polymerase 2 (RNAP2) on a transcription-blocking DNA lesion (TBL) triggers a series of cellular events. (a) Elongating RNAP2 runs into a TBL and stalls. (b) R-loops can be formed by the hybridization of pre-mRNA with template ssDNA adjacent to the transcription bubble. 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