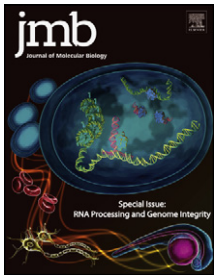

Transcription and DNA Damage: Holding Hands or Crossing Swords?

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Abstract

Transcription has classically been considered a potential threat to genome integrity. Collision between transcription and DNA replication machinery, and retention of DNA:RNA hybrids, may result in genome instability. On the other hand, it has been proposed that active genes repair faster and preferentially via homologous recombination. Moreover, while canonical transcription is inhibited in the proximity of DNA double-strand breaks, a growing body of evidence supports active non-canonical transcription at DNA damage sites. Small non-coding RNAs accumulate at DNA double-strand break sites in mammals and other organisms, and are involved in DNA damage signaling and repair. Furthermore, RNA binding proteins are recruited to DNA damage sites and participate in the DNA damage response. Here, we discuss the impact of transcription on genome stability, the role of RNA binding proteins at DNA damage sites, and the function of small non-coding RNAs generated upon damage in the signaling and repair of DNA lesions.

Introduction

The stability of our genome is continuously challenged by endogenous and exogenous factors [1]. A DNA lesion activates a cellular response, known as DNA-damage response (DDR), that leads to the recruitment of repair proteins to sites of DNA damage and to the activation of checkpoint responses that slow down, or arrest, cell-cycle progression, until repair is fully carried out [2,3]. Among different kinds of lesions, DNA double-strand breaks (DSBs) are recognized by the MRE11–RAD50–NBS1 (MRN) complex, which recruits the ataxia telangiectasia mutated (ATM) protein kinase, responsible for the phosphorylation of the histone variant H2AX (γH2AX). The consequent spreading of γH2AX along the chromosome [4] and the recruitment of additional DDR factors, such as the mediator of DNA-damage checkpoint (MDC1) and the p53-binding protein (53BP1), generate a microscopically detectable focus [2]. The repair of DSBs mainly relies on either non-homologous end joining (NHEJ) or homologous recombination (HR). NHEJ simply stitches back together the broken DNA ends, and functions throughout all cell cycle phases. During the S/G2 phase, resection of broken DNA ends commits repair toward additional mechanisms based on HR [5]. Upon DNA end resection, replication protein A (RPA)-coated ssDNA activates ataxia telangiectasia and Rad3-related protein (ATR)-dependent signaling and checkpoint kinase 1 (Chk1) phosphorylation, by recruiting ATR and ATR interacting protein (ATRIP) [6,7]. Resected DNA ends can invade the homologous sequence from the undamaged sister or homologous chromatid through the RAD51 recombinase, resulting in error-free repair. Alternatively, the exposure of homologous sequences on the resected DNA ends may result in the error-prone DSB repair pathways known as alternative end joining and single-strand annealing [8].

A new component has recently been integrated into the classical DDR cascade: the RNA. Upon damage, small non-coding RNAs (sncRNAs), named DNA-damage response RNAs (DDRNAs) and DSB-induced RNA (diRNA), accumulate near

DSBs in different organisms [9,10,11]. DDRNAs have been discovered in mammals, where they are generated by DROSHA and DICER cleavage of a presumably longer precursor RNA, and participate in DDR signaling [9]. diRNAs have been discovered in plants where they are produced by DICER-mediated cleavage in an ATR-dependent fashion. Differently from DDRNAs, diRNAs have been proposed to participate in DNA repair and not in DDR activation [11–13]. The production of sncRNAs upon DSB generation suggests the transcription of a longer precursor RNA that undergoes further processing. Conversely, however, upon DNA damage, inhibition of canonical transcription has been observed in yeast and mammals [14–18]. Moreover, a role of transcription as a source of DNA damage has also been extensively described [19]. In this review, the detrimental and beneficial effects of transcription on genome stability, the recruitment of RNA binding proteins (RBPs) to DNA-damage sites, and the role of sncRNAs generated from DNA lesions are discussed.

The dual role of transcription at DNA-damage sites

Transcription may be a harmful process for DNA integrity [20]. Collision between the transcription and replication machineries causes replication-fork stalling, which is often associated with DNA damage and recombination [21,22]. During transcription, the pairing of the newly synthetized RNA with the template DNA generates a DNA:RNA hybrid which displaces the non-template ssDNA to form a three-stranded nucleic acid structure known as R loop [23] (Fig. 1a). Short DNA:RNA hybrids form physiologically during DNA replication [19], during transcription, and have a role in transcription regulation [24,25]. Unscheduled R loops formation may be facilitated by several factors. The negative DNA supercoiling associated with transcription favors DNA unwinding and increases DNA accessibly to RNA, with the consequent formation of DNA:RNA hybrids [26]. Negative supercoiling is counteracted by the action of the DNA

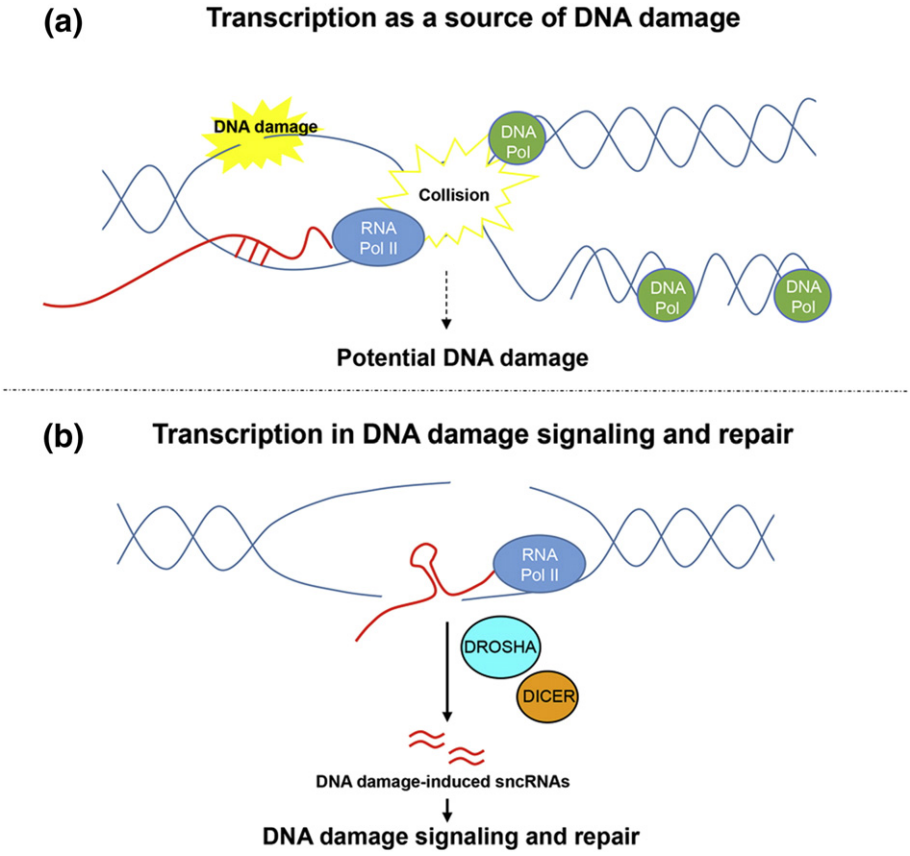

Fig. 1. The dual role of transcription in DNA-damage generation, signaling, and repair. (a) Collision between the transcription and replication machineries and retention of DNA:RNA hybrids may result in DNA damage and recombination. Moreover, the ssDNA displaced in the R loop is exposed to several assaults that may cause genome instability. (b) Upon damage, transcription of a precursor RNA and its processing by the RNAi nucleases DROSHA and DICER generate sncRNAs that participate in DDR signaling and repair.