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The hardwired transcriptional response to DNA damage

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Abstract
There is a complex network of interactions between bulky DNA damages and transcription. Bulky damages block RNA polymerases but also elicit a regulated transcriptional response. At the same time, active transcription enhances the ability to recognize and repair damages. Eventually, transcription is completely shut down until after damages are removed. Recent projects untangle this web of interaction in mammalian cells by applying time-sensitive and high-resolution measurements of damage, repair, and transcription at genome-wide scales. The emerging model indicates the transcriptional response to damage is primarily hardwired in the damaged genomic DNA, and transcription shutdown can be explained almost completely by (1) aborted transcription by blocked RNA polymerases and (2) ubiquitination and degradation of RNA polymerase II after encountering a damage.

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Introduction

The two major roles of a genome are to faithfully transfer the genetic information to progeny and to correctly express genes necessary for cellular and organismal function. Namely, a functional genome must be able to replicate and to be transcribed. Both these processes require genomic integrity to be maintained. DNA damages compromise replication and transcription and can lead to cell death, mutations, and cancer. DNA damaging treatments trigger cellular responses that minimize these adverse consequences by activation of checkpoints and cell cycle arrest and by initiation of DNA repair pathways. These responses occur at every level of regulation — from transcription to post-translational modifications and protein degradation.

In this review, we focus on bulky DNA base damages that distort the DNA helix and block RNA polymerases. These include DNA adducts induced by polyaromatic hydrocarbons such as those released in cigarette smoke and adducts induced by platinum-based chemotherapies. The most studied of these types of damages are cyclopyrimidine dimers (CPDs) induced by ultraviolet (UV) radiation.

In humans, the major mechanism for removal of bulky DNA base damages is nucleotide excision repair (NER) [1–3]. In NER, after damage recognition, damages are excised through incisions on both sides of the damage, releasing an oligomer of 22–30 nt. The resulting gap in the genome is filled in by DNA polymerases and ligated to complete error-free repair. Damage recognition in NER can occur either by the repair factors directly, in what is termed global genome repair, or alternatively by the RNA polymerase that is blocked by the damage, in what is termed transcription-coupled repair (TCR). Thus, bulky DNA base damages not only elicit a transcriptional cellular response but also may rely on transcription for efficient repair.

In global genome repair, damage recognition is initiated by XPC-HR23B, with the assistance of DNA damage-binding 1 and 2 (DDB1 and DDB2). In TCR, the blocked polymerase moonlights as the damage recognition factor and through the action of the Cockayne syndrome A (CSA), Cockayne syndrom B (CSB), and UV-stimulated scaffold protein A (UVSSA) proteins recruits the rest of the core NER machinery. The subsequent steps are similar between the two pathways and shared enzymes include replication protein A (RPA), Xeroderma Pigmentosum group A, G, F (XPA, XPG, XPF), the repair and transcription factor transcription factor IIH (TFIIH), Xeroderma Pigmentosum group B and D (XPB and XPD), DNA polymerase δ and ε, and ligase I and III.

Mutations that inactivate global genome repair genes primarily cause the severe genetic syndrome xeroderma pigmentosum. Mutations in TCR factors all cause TCR deficiency but can have vastly different clinical manifestation. For example, mutations in CSA and CSB genes usually cause severe Cockayne syndrome. At the same time, complete loss of CSB or a specific mutation in CSA will cause the much milder UV sensitivity syndrome (UV^{s}S, mostly caused by UVSSA mutations) [4]. While patients with xeroderma pigmentosum exhibit extreme UV sensitivity and a high risk for skin cancer, patients with Cockayne syndrome suffer from neurological and developmental defects, microcephaly, mental retardation, and reduced life expectancy but no cancer predisposition. There are extensive efforts at understanding the underlying mechanisms explaining the different phenotypes of these genetic diseases reviewed recently in the studies by Geijer and Marteijn [5] and Lans et al [6].

Until recent years, the view on the transcriptional response to damage was that DNA damages induce a shift in gene regulation and through the action of transcription repressors, such as activating transcription factor 3 (ATF3), cause a transcriptional shutdown that lasts approximately 24h in human cells in culture [7–11]. In fact, recovery of transcription after damage, though indirect, has been a gold standard method for measuring efficiency of DNA repair.

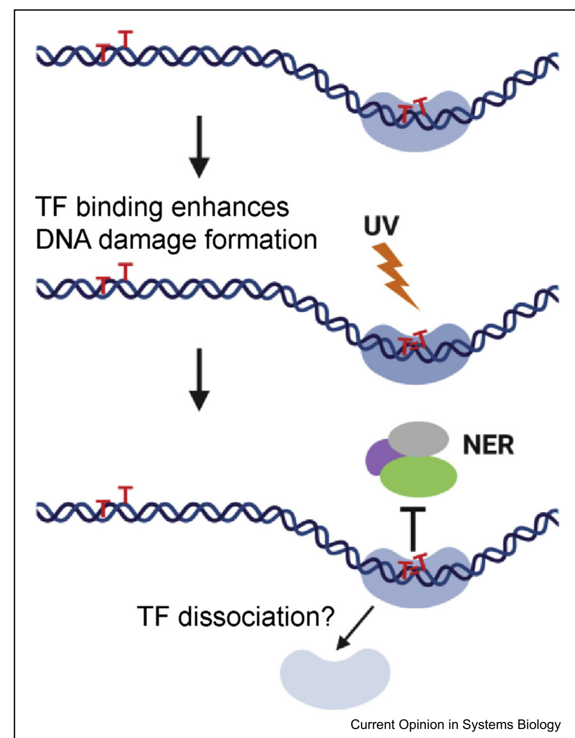
High-resolution genomics studies shifted our understanding of TCR and the transcriptional response to damage. The field of transcription and transcription regulation was first to enter the genomic era. As the field evolved, the sensitivity of RNA sequencing methods improved with the development of high-resolution methods for mapping not just steady-state RNA levels but also nascent transcription in cells [12–16]. In addition, the plunge in sequencing costs has made large, time-course experiments feasible. While the DNA damage field initially lagged behind, in the last five years, high-resolution genome-wide methods for mapping DNA damages and their repair have emerged [17–23]. The ability to map active transcription, protein binding, DNA damage, and DNA repair in parallel across the genome provided a systematic understanding of the transcriptional responses to stress.

New insights into transcription-coupled repair

TCR was first discovered when it was shown that repair of *DHFR*, an expressed gene, and specifically its transcribed strand, is faster than the genomic average [24,25]. Since this discovery, preferential repair of expressed regions was shown in organisms from bacteria to humans [26,27]. The genes studied were almost exclusively protein-coding genes transcribed by RNA polymerase II (RNA pol II) [5,28,29]. Even within this limited set of tested genes, there were different observations pertaining to whether faster repair starts upstream of the gene and to what degree the nontranscribed strand is efficiently repaired.

These questions were resolved with the ability to map TCR in a genome-wide manner. For this purpose, excision repair sequencing was performed to map repair of UV-induced damages in cell lines that were proficient only in TCR [20]. These experiments showed that TCR is indeed exclusive to the transcribed strand and that the levels of repair correlate to the levels of expression of RNA pol II genes. Interestingly, RNA polymerase I does not appear to elicit TCR [30]. Whether RNA polymerase III transcription also elicits preferential repair remains to be determined. These high-resolution measurements uncovered that TCR is not limited to annotated genes but occurs in all RNA Pol II–transcribed regions, including divergent transcription at promoters and bidirectional transcription at enhancers. These results indicate that active transcription at regulatory regions is beneficial in itself after damage, as it will facilitate faster repair and faster recovery of their function.

Figure 1



The relationship between transcription factor binding and DNA damage. Adjacent ‘T’ nucleotides that are potential dimer-forming sites are depicted in red.

Mapping nucleotide excision repair in TCR-deficient Cockayne syndrome group B (CS-B) cells has shown that not only is repair no longer elevated on the transcribed strand but also has slight preferential repair on the nontranscribed strand [20,31,32]. This could be due to stalled RNA polymerases on the transcribed strand blocking access of the repair proteins and could explain some of the severe phenotypes of patients with TCR defects.

The effects of transcription factor binding on damage and repair

An understudied question is how DNA damages directly affect transcription regulation. The recent ability to map UV-induced damages at single-nucleotide resolution indicates that the bending of the DNA by transcription factors could alter the sensitivity to damage formation ([18,22,33,34], Figure 1). Specifically, ETS proto-oncogene 1 (ETS1) sites are associated with higher damage levels and higher UV mutagenesis rates. Another observation has been that transcription factor binding sites are associated with a local decrease in DNA repair efficiency, implying transcription factor binding hinders access of repair proteins to the damage [35,36]. Both higher damage formation and lower repair efficiency could explain the higher cancer mutation rates reported at transcription factor binding sites. However, there is still a very big open question: Do transcription factors stay bound if the DNA template is damaged? We would expect, and some evidence suggests, that a helix-distorting DNA damage such as pyrimidine dimers formed by UV would affect protein binding [37]. If the transcription factor dissociates due to damage — how can it interfere with repair? And would that affect the overall gene expression profile? Today, these questions remain open, but with our improved sensitivity and ability to measure complex networks of interactions, they will likely be addressed in the near future.

A comprehensive understanding of the global transcriptional response to DNA damages

A longstanding question in the field has been the following one: What happens to an RNA pol II after it recruits the TCR proteins? While in vitro, repair can occur in the presence of the RNA polymerase [38,39], the footprint of RNA pol II at damage sites suggest it should be vacated to allow repair. Initially, there were two possible models. The first was that RNA pol II can backtrack to evict the damage site, and transcription is resumed after repair through 3′ cleavage of the nascent transcript by transcription factor IIS (TFIIS) [29,40,41]. The second model was that transcription is aborted, and RNA polymerase is either degraded or released. While both models are not mutually exclusive, the model of aborted transcription was less favored as it resulted in substantial energy loss to the cells. However, evidence of UV-induced degradation of RNA pol II supported the second model [42]. Furthermore, kinetic genomic measurements after UV treatment showed elevated RNA pol II binding and RNA synthesis at the promoters of genes [43,44]. These results strongly suggest that RNA pol II complexes were aborting transcription after encountering damages and were recycled to the gene start. Indeed, the resulting higher RNA pol II density at 5′ regions correlates with faster removal of damages from the 5’ of genes [44,45]. The consequence of abortive transcription would result in an inherent bias toward expression of shorter genes after UV because they would likely harbor less damage and could recover faster. Indeed, differential expression analysis of transcripts after UV showed a higher level of shorter transcripts and even a switch to a shorter alternative last exon isoform [43,46,47]. These shorter transcripts include the immediate early response genes that are induced upon stress and include genes encoding for transcription factors such as Fos Proto-Oncogene (FOS), Jun Proto-Oncogene (JUN), and ATF3 [11,48,49].

Exciting articles in the last two years have greatly enhanced our understanding of the transcriptional response to damage due to the two main findings:

En-mass release of RNA pol II into gene bodies

Under normal conditions, the expression level of genes is regulated by pausing of polymerases ~60bp from the transcription start site and a regulated release of polymerases into active elongation. This release is stimulated by the positive transcription elongation factor b and involves phosphorylation of the C-terminal domain of RNA pol II. The work of Lavigne et al. [50] showed that upon UV, RNA polymerases are released en-masse from the pause site into active elongation, resulting in a wave of transcription that continues along genes, constantly scanning and detecting damages. This was supported by reports that genotoxic stress increases the pools of active positive transcription elongation factor b [51–53]. In a follow-up article, Liakos et al. [54] showed that contrary to reports of repressed initiation, after this initial wave of released polymerases, transcription initiation continues. The observed decrease in initiating (hypophosphorylated) RNA pol II is explained by a swift release into active elongation in the absence of promoter-proximal pausing [54]. These findings point to an abandonment of gene expression regulation after damage. However, the risk in loss of transcription regulation is alleviated due to the fact that RNA pol IIs are blocked by damages in the genes and the high likelihood that transcription will be aborted when the damages are encountered. Such a costly damage-scanning mechanism, involving futile RNA synthesis and degradation of RNA pol II, highlights the importance of damage removal from functional genes for cell survival.

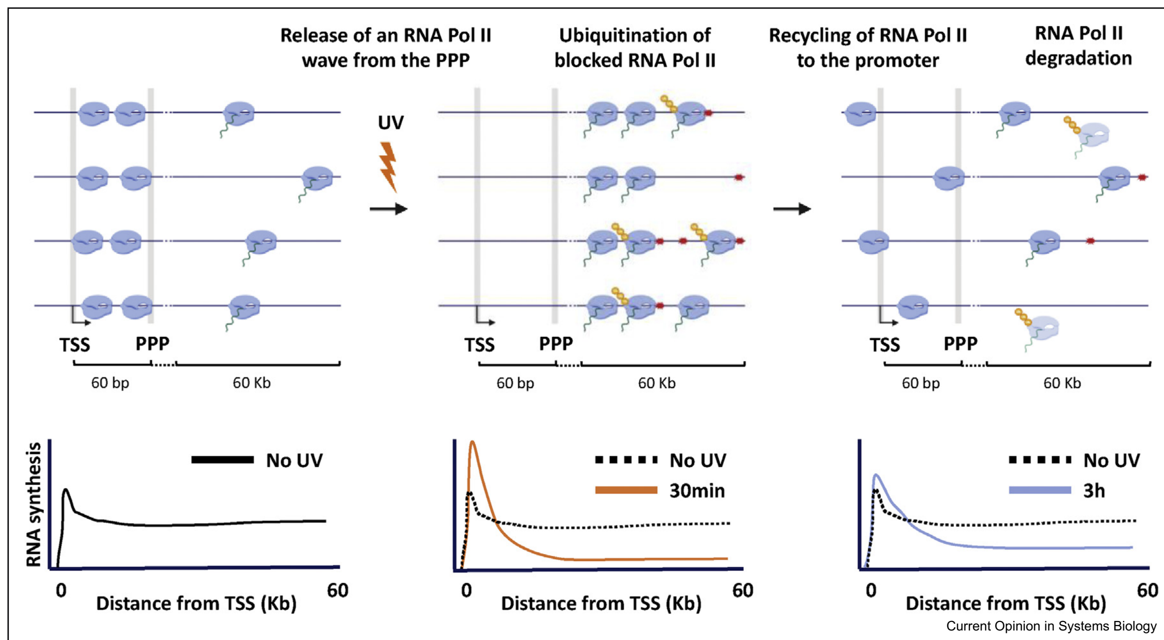
Actively elongating RNA polymerases that encounter damages are subsequently ubiquitinated

It was previously reported that RNA pol II is ubiquitinated after UV and can be degraded. Liebelt et al. [55] recently showed that this UV-induced ubiquitination is dependent on the CSA protein. Two complementary articles published back to back this year provide an unprecedented mechanistic understanding to the process [56–58]. Both articles report that DNA directed RNA polymerase II subunit (RPB1), the large subunit of RNA pol II, is ubiquitinated specifically on K1268 after UV. Human cell lines carrying a K1268R mutation in RPB1 are UV sensitive and display a defect in recovery of RNA synthesis after UV. The work of Nakazawa et al. [56] highlights a role for this ubiquitination in TCR. They report the ubiquitination of RPB1 is necessary for recruitment of the key repair factor TFIIH by UVSSA to the site of damage. To measure damage at RPB1-bound sites, they use an elegant strand-specific chromatin immunoprecipitaion followed by sequencing (ChIP-seq) approach. In the ChIP-seq protocol, after isolation of RPB1-bound DNA, damages in the transcribed strand result in a block of in vitro PCR amplification. Therefore, RPB1 bound to damage sites would yield asymmetric ChIP-seq signal, with less signal on the transcribed vs. nontranscribed strand. Nakawaza et al. [56] nicely show that in RPB1 K1268R mutants, the asymmetry at RPB1 sites persists for longer times, suggesting a delay in damage removal. Tufegdzic Vidakovic et al. [58] showed that the K1268R ubiquitination is necessary and sufficient for degradation of RNA pol II after UV. This degradation is necessary for the subsequent transcriptional shutdown. In fact, a mathematical model of transcription after UV that solely incorporated rates of damage formation, damage removal, dissociation of RNA pol II at damage sites, and RNA pol II degradation was sufficient to almost completely recapitulate in silico the transcriptional response to damage.

The transcriptional shutdown after UV can therefore be explained as a three-step mechanism (Figure 2): First, active elongation is shut down because of damages blocking polymerase. Second, RNA pol II molecules that meet damages get ubiquitinated and degraded. And last, the higher the damage level — the more RNA pol IIs are ubiquitinated and degraded to the point of total initiation shutdown.

Releasing RNA pol IIs into active elongation to scan the genome and facilitate transcription repair comes both at a cost of multiple aborted transcripts and a risk of complete deregulation of gene expression. This risk is

Figure 2



Schematic for the current model of the transcriptional response to bulky DNA base damages. (Top) Before damage, release of RNA pol II from the promoter-proximal pause site (PPP) is regulated, resulting in accumulation of polymerases at the 5′ of genes. After damage (middle panel), paused polymerases are released en-masse resulting in a wave of RNA pol II molecules scanning and detecting damages. Upon encountering a damage, these polymerases are ubiquitinated. With time (right panel), new RNA pol IIs initiate without pausing; however, the pool of RNA pol II is depleted due to degradation, and transcription is eventually shut down until after damage removal. Genes are not drawn to scale. Bottom, schematic graphs summarizing the reported profile of active transcription after UV (mapped by either nascent RNA sequencing or ChIP-seq of elongating RNA pol II [43,47,50,56]). These plots show that 30 min and 3h after UV, transcription is enhanced at 5′ of genes, and with time, proceeds to the 3′ regions. With time, total RNA levels will decrease. To measure absolute RNA synthesis levels an internal spike-in control can be incorporated into the genomic experimental scheme. UV, ultraviolet; RNA pol II, RNA polymerase II.

higher in shorter genes which will harbor less damages and in which full-length transcription would recover first. However, these risks are minimized by the subsequent degradation of RNA pol II, which guarantees transcriptional shutdown. Mapping nascent RNA in cells harboring K1268R RPB1 mutants show how, in the absence of this degradation, short transcripts are indeed overexpressed. These shorter transcripts could include immediate early response genes, transcription factors, and oncogenes — and their imbalanced expression could be deleterious to cell survival and contribute to the UV sensitivity of these mutants.

Conclusions

The beauty in using RNA pol II as a damage-scanning enzyme is that it will inherently prioritize the functional regions of the genome for repair. These include not only gene bodies but also promoter and enhancer elements that are actively transcribed. These higher repair efficiencies protect the cells and organisms from higher mutagenesis and carcinogenesis.

The recent reports reviewed here provide a comprehensive genomic characterization of the response to RNA polymerase blocking damages. While the vast majority of the work was performed with UV damages, they likely apply to additional bulky lesions.

There are still many open questions left unanswered: Is the ubiquitination of RNA pol II necessary for its release from the damage sites? Does ubiquitination of RPB1 play a direct role in repair? The evidence presented by Tufegdzic Vidakovic et al. [58] suggests it does, but it is based on recovery of RNA synthesis, and thus indirect. The ability to measure damage and repair at high resolution in the genome could provide direct evidence for a TCR defect in these cells. Lastly, do we abandon a model of transcription factor–mediated response to damage? Is the entire response mediated by these hardwired effects of damages in DNA? Or are these systemic changes masking specific transcription regulation events? As technologies improve in sensitivity, throughput, and resolution, we will be able to address these questions not only with genomic methods but also with sensitive assays for single-site and single-molecule measurements.

Conflict of interest statement

Nothing declared

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