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Effect of DNA lesions on transcription elongation

Silvia Tornaletti, Philip C. Hanawalt*

Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020, USA

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Abstract — Some types of damage to cellular DNA have been shown to interfere with the essential transactions of replication and transcription. Not only may the translocation of the polymerase be arrested at the site of the lesion but the bound protein may encumber recognition of the lesion by repair enzymes. In the case of transcription a subpathway of excision repair, termed transcription-coupled repair (TCR) has been shown to operate on lesions in the transcribed strands of expressed genes in bacteria, yeast, mammalian cells and a number of other organisms. Certain genes in mammalian cells (e.g., *CSA* and *CSB*) have been uniquely implicated in TCR while others (e.g., *XPC-HR23* and *XPE*) have been shown to operate in the global genomic pathway of nucleotide excision repair, but not in TCR. In order to understand the mechanism of TCR it is important to learn how an RNA polymerase elongation complex interacts with a damaged DNA template. That relationship is explored for different lesions and different RNA polymerase systems in this article. © Société française de biochimie et biologie moléculaire / Elsevier, Paris

RNA polymerase / transcription-coupled repair / ultraviolet light

1. Introduction

An intact DNA template is a prerequisite for the accurate replication of the genome or the transcription of its genes to produce functional RNA copies. A variety of enzymatic repair systems have evolved to clear the genome of the different sorts of damage produced by environmental or endogenous sources. However, a unique problem may arise when a translocating polymerase encounters a lesion before it has been recognized and repaired. The arrested polymerase and associated proteins may block access to the lesion by repair enzymes. It is likely that the polymerase must be displaced, both to permit verification that a lesion caused the arrest and also to allow the repair enzymes to operate on the lesion. In this article we will focus on the specific situation of an elongating RNA polymerase and we will evaluate the different possibilities for the effect of a lesion in its path.

Some lesions may alter the information content and result in a faulty RNA copy without having any detectable effect upon the progression of the polymerase complex along the DNA template. Other lesions might affect the rate of transcription without affecting the fidelity of the process. In that case the level of expression of a gene might be reduced, or increased, depending upon the sequence context of the lesion. Obviously, the question of whether the lesion is in the transcribed or the non-transcribed DNA strand is important in this regard. Some lesions in the transcribed strands may transiently arrest the elongation process while others may pose absolute blocks to translocation of the RNA polymerase. In the latter case there may be a subset of lesions that actually cause the polymerase and nascent RNA product to be released from the DNA, while for other lesions the ternary complex may be very stable and persist in the arrested state for a long time. A mammalian RNA polymerase II (RNAP II) arrested at a ultraviolet light (UV)-induced cyclobutane pyrimidine dimer (CPD) in the transcribed DNA strand prevents the repair of that lesion by photolyase, a small repair enzyme [1]. The ternary complex of RNAP II at a CPD has been reported to have a half-life of about 20 h [2]. It should come as no surprise that mechanisms have evolved to displace the polymerase in order to facilitate repair of the lesion.

2. Transcription-coupled DNA repair

The most versatile DNA repair mechanism is nucleotide excision repair, that can recognize and remove many sorts of structural alterations from DNA [3–5]. There are two general pathways for initiating nucleotide excision repair: a global genomic repair pathway that recognizes and repairs lesions throughout the genome and a pathway termed ‘transcription-coupled repair’ (TCR), that operates on lesions in the transcribed strands of expressed genes [6]. It is likely that the process of TCR evolved for

* Correspondence and reprints

Abbreviations: RNAP II, RNA polymerase II; CPD, cyclobutane pyrimidine dimer; TCR, transcription-coupled repair; UV, ultraviolet light; AF, N-2 aminofluorene; AAF, N-2-acetylaminofluorene; CS, Cockayne syndrome; XP, xeroderma pigmentosum; CHO, Chinese hamster ovary cells.

the dedicated purpose of resolving the impasse of an RNA polymerase arrested at a lesion. Historically the initial observation was that UV-induced CPDs in an expressed gene in Chinese hamster ovary (CHO) cells were much more efficiently repaired than were those in a silent sequence downstream [7]. Then it was revealed that this efficient repair was due to the preferential repair of CPDs in the transcribed DNA strand [8]. TCR was also documented in human cells but the difference in repair efficiency between transcribed and non-transcribed strands was less striking. The large differential seen in repair efficiency of the two strands in the CHO cells was more a consequence of the poor repair of CPDs in the non-transcribed strand than the unusually rapid repair in the transcribed strand. In fact, when another UV-induced lesion, the 6-4 photoproduct, is studied the effect of TCR is largely masked by the very high efficiency of global genomic repair of this lesion. (Repair rates in the overall genome are generally reflected by the repair rates seen in non-transcribed DNA strands.) Rodent cells and human cells exhibit similar TCR responses but the rodent cells are typically deficient in global genomic repair of some lesions (such as CPDs) compared to that in human cells.

In eukaryotes TCR operates only on RNAP II transcribed genes and the polymerase must be in the actively elongating mode. Ribosomal genes transcribed by RNA polymerase I are not subject to TCR [9–11]. Genes transcribed by RNA polymerase III are also not subject to TCR [12]. Perhaps the persistence of these polymerases at arresting lesions is not as great as that for RNAP II. TCR is eliminated by treating cells with alpha amanitin, an inhibitor of RNAP II elongation [13–15] and abolished at the restrictive temperature in a temperature sensitive RNAP II mutant in yeast [16, 17]. TCR operates equally well on an active gene on a plasmid as when that gene is chromosomal in yeast [17]. TCR does not vary in efficiency through the normal cell cycle in mammalian cells for a gene that is expressed continuously throughout the cycle [18]. TCR does not appear to be inducible by UV but in human cells it has been shown that efficient global genomic repair of CPDs requires activation of the p53 tumor suppressor [19]. The mechanism of this inducible response has been shown to involve p53 dependent upregulation of the XPE-related p48 gene that is required for expression of a UV-damaged DNA binding activity [20]. Interestingly, evidence has been accumulating to suggest that arrested transcription (e.g., at CPDs) provides a sensitive signal for the activation and stabilization of p53 [21, 22].

XPE cells, like XPC cells, have been shown to be proficient in TCR but deficient in the global repair of CPDs [20]. However, the XPC cells are additionally deficient in 6-4 photoproduct repair, and in fact recent evidence suggests that the XPC-HR23B complex operates as an essential lesion-recognition factor prior to the involvement of XPA that is essential for both global repair and TCR [23]. Since the lengths of the repair patches have been shown to be similar for TCR and for global genomic repair [24], the determination of incision sites is probably made by the basal transcription initiation factor TFIIH, following upon the initial lesion recognition by XPC or by an arrested RNAP II. It has been suggested that the determination of which strand contains the lesion is accomplished by the ATP dependent scanning activities of the helicases, XPB and XPD, that are essential components of TFIIH [25]. An implication of that model, if the model proves to be correct, is that the arrest of RNAP II is not necessarily the event that determines that the lesion to be repaired is in the transcribed strand. Rather, that determination may be made by TFIIH subsequent to the arrest of transcription and displacement of the polymerase.

It was originally proposed that the arrest of transcription at lesions and the subsequent release of RNA polymerase from the template could serve as a specific signal to accelerate repair in active genes [26]. Following upon the documentation of TCR in *E. coli* [27] it was shown that a transcription-repair coupling factor binds to and releases the RNA polymerase blocked at a lesion [28]. This factor (the product of the *mfd* gene) then interacts with the excision repair complex in some manner to remove the lesion [29]. In human cells, the product of the CSB gene has been implicated in the coupling process [30]. CSB is a member of the SNF2 family of proteins with helicase motifs, although it is not a helicase but a DNA dependent ATPase. Members of the SNF2 family have been found to function in chromatin remodeling and the removal of bound proteins from DNA [31]. RNAP II transcription termination factors, such as the *Drosophila* factor 2, have been shown to be members of the SWI2/SNF2 family, with strong double-stranded DNA-dependent ATPase activity [32].

Following the initiation of transcription the template DNA strand is preferentially repaired in the region just upstream of the point at which the RNAP II clears the promoter and releases TFIIH in normal cells, in CSA and CSB cells, and in the yeast homolog of CSB [33]. Preferential repair of the transcribed strand beyond the point of TFIIH release requires CSA and CSB or *rad26*, in mammalian cells or yeast, respectively. This suggests that CSB and *rad26* might have a role in re-recruiting TFIIH to the repair complex when the RNAP II is arrested at a lesion while in the elongation mode [34, 35]. In support of this model recent work suggests that the RNAP II/CSB complex can interact with subunits of TFIIH [36]. The CSA gene product that is also required for TCR, is a member of the WD-repeat family of proteins, that are implicated in chromatin remodeling, but its role in TCR is not known [6]. Mutations in the mismatch repair genes *mutS* or *mutL* in *E. coli* or their homologs in human cells have been shown to inhibit TCR but the mechanism of this effect is not yet understood [37, 38]. In yeast, mismatch repair mutants do not appear to be defective in TCR [39].

Table I. Correlation between TCR of a lesion and arrest of transcription in vitro by the lesion in the transcribed strand.

Lesion	*TCR*	*Arrest of transcription*[a]	*RNA polymerase*	*References*
N-ethylpurines	+	N.D.		[63]
7 Me–guanine	–	–	T7	[62]
3 Me–adenine	–	+	T7	[62]
8 Oxo–guanine	N.D.	+	T7	[42]
		–	*E. coli*	[46]
CPD	+	+	*E. coli*, rat liver, human	[1, 8, 29]
Thymine glycol	+	+	T7	[42, 64, 65]
Aflatoxin B1	+	N.D.		[15]
CC-1065	+	N.D.		[55]
Psoralen monoadduct	–	+	T7, human	[40, 59, 60]
Psoralen interstrand cross-link	+	+	T7, *E. coli*, human	[40, 41, 59, 60]
BPDE (human cells)	+	+	T7	[45, 57]
BPDE (rodent cells)	–	+		[45, 56]
N-acetoxy-acetylaminofluorene	+/–	+	rat liver	[53]
Aminofluorene	–	–	T7, rat liver	[44, 53, 54]
Single-strand breaks	N.D.	–	T7	[44, 47]
		+	SP6, *E. coli*	[43]
Abasic sites	N.D.	+	T7	[44]
		–	SP6, *E. coli*	[43]
Gaps	N.D.	–	T7, SP6, *E. coli*	[47–49]

[a] N.D., not determined.

In order to understand the potential roles of the various genes implicated in TCR we need to study the behavior of RNA polymerases on DNA templates containing lesions.

3. Prokaryotic RNA polymerases

The role of RNA polymerase in TCR has been examined more directly by comparing the extent of RNA polymerase arrest in vitro by different lesions with TCR of these lesions in vivo *(table I)*.These studies have shown that various types of base damage in the template strand of DNA can act as blocks to transcription catalyzed by different prokaryotic RNA polymerases, and a correlation between extent of polymerase arrest and extent of TCR has been found in several cases. Bulky lesions such as UV-induced CPDs, psoralen interstrand cross-links, as well as less distorting lesions, such as oxidized bases, strand breaks and abasic sites, in the transcribed strand of the DNA template interfere to different extents with the progression of T7 and *E. coli* RNA polymerases [29, 40–44]. Furthermore, the stereochemistry of the lesion and the base incorporated opposite the lesion can affect the relative amounts of prematurely terminated versus bypassed transcripts, as shown for the effect of anti-BPDE-modified guanine adducts on T7 RNA polymerase elongation [45]. Other DNA lesions such as 8-oxo-guanine and abasic sites, that are efficiently bypassed by *E. coli* RNA polymerase [43, 46], have been shown to be miscoding lesions for RNAP in vitro, causing base substitutions in the resulting transcripts [46]. This so-called 'transcriptional mutagenesis' could lead to production of mutant proteins, with possible deletereous effects on cell physiology.

Remarkably, it has been shown that gaps of various sizes in the template DNA strand are also bypassed by prokaryotic RNA polymerases and the resulting runoff transcripts are internally deleted for the gapped region [47–49]. These findings have revealed a critical role for an RNAP interaction with the non-template strand during the normal process of transcription elongation. However, an elongating mammalian RNAP II is a much more complex enzyme than a prokaryotic polymerase, so a detailed analysis of the effect of DNA lesions specifically on mammalian RNAP II is essential to understand the role of RNAP II arrest in TCR.

4. RNA polymerase II

To study the interactions of an RNAP II elongation complex with a damaged DNA template, we have developed an in vitro transcription system using DNA templates containing a site-specific lesion located in the transcribed or in the non-transcribed strand downstream of the major late promoter of adenovirus and purified RNAP II and

!initiation factors to carry out transcription. Using this system we have shown that a CPD in the transcribed strand in different sequence contexts is a strong block to RNAP II [1, 50]. When a CPD was located in the non-transcribed strand, its effect on transcription arrest was dependent upon the sequence context of the CPD. A site-specific CPD was inserted in the first T run of the histone H3.3 arrest site, a well characterized transcription arrest site for RNAP II in vitro. The H3.3 sequence contains two runs of T in the non-transcribed strand that cause DNA to be bent. A CPD in this sequence context only transiently paused RNAP II and this effect correlated with the reduction of DNA bending caused by the CPD. This result demonstrates the potential importance of the sequence context for the effect of a CPD in transcribed sequences. A CPD can interfere with regulation of transcription through modulation of natural arrest sequences. Thus, there can be the seemingly anomalous situation of enhanced transcription in the presence of an appropriately positioned lesion in the non-transcribed strand. CPDs can also interfere with binding of cell cycle regulatory proteins, DNA-damage responsive transcription factors [51] and nucleosomes [52], suggesting that interference with DNA-protein interactions by CPDs may be another relevant effect of UV-induced DNA damage on cellular metabolism.

The same in vitro system was used to study the effect of DNA lesions formed by the potent carcinogen N-2-acetylaminofluorene (AAF). An AAF adduct located in the transcribed strand was a strong block to RNAP II elongation [53] whereas an aminofluorene (AF) adduct located in the transcribed strand was only a weak pause, and given time RNAP II transcription could read through this lesion. The different effects of AF and AAF adducts on transcription elongation correlate with the different extent of helix distortion that these lesions cause to DNA structure. Both AF and AAF adducts form predominantly at the C-8 position of guanine. The fluorene moiety of the AAF adduct is inserted between base pairs within the helix and the guanine switches from the anti to the syn conformation. This causes a significant helix distortion in the DNA. DNA helix distortions are components of several natural arrest sites [58], and it is likely that the AAF adduct induced distortion combined with the non coding nature of the adduct may contribute to the termination of RNAP II at a AAF-dG. In contrast, the fluorene moiety of the AF adduct remains extrahelical, producing little distortion. When either of these adducts was positioned in the non-transcribed strand it did not cause transcription arrest at the lesion but it enhanced arrest at a sequence about 15 bp downstream from the adduct that was coincident to a natural arrest site for the polymerase. Removal of the sequence specific arrest site eliminated the adduct-specific arrest, suggesting that an AAF lesion increased pause time at this site. The transcriptional arrest induced by AF and AAF located in the non-transcribed strand again reveals the importance of sequence context on polymerase arrest and possibly in turn on TCR.

A different approach has been used to study the effect of psoralen adducts on transcription elongation. Triple-helical DNA structures were produced to deliver site-specific psoralen adducts in the DNA template and HeLa cell nuclear extracts were used to carry out transcription [59]. A stable triple helix structure destabilized the elongation complex causing termination 4–8 nucleotides before RNAP II reached the triple helix, and the presence of a psoralen monoadduct did not have any further effect on pol II elongation. On the other hand an interstrand psoralen cross-link disrupted the triple helix structure and caused transcription arrest 1–2 nucleotides from the lesion site. These in vitro results are consistent with the in vivo report that a psoralen interstrand cross-link elicits TCR but the psoralen monoadduct does not [60].

Most of the early studies on TCR have been carried out with agents that produce lesions subject to nucleotide excision repair. The methyl purines that are efficiently repaired by the base excision repair pathway, initiated by a glycosylase, were evidently not repaired by TCR in CHO cells [61]. This result might have been predicted since the primary lesion produced by DMS in these experiments was 7-Me-guanine that is readily bypassed by the T7 RNA polymerase [62]. On the other hand the N-ethyl purines that are subject both to nucleotide and base excision repair pathways were also shown to exhibit TCR [63]. In that case it is likely that the larger adduct contributes to more encumberence of RNAP II translocation, thereby eliciting TCR. Thus, there can be the situation in which a lesion, normally repaired by base excision repair, becomes subject to nucleotide excision repair if it arrests RNAP II. However, the recent evidence that thymine glycols are subject to a TCR pathway that does not involve nucleotide excision repair – it does not require XPA – suggests that base excision repair can also be coupled to transcription, at least for some lesions [64, 65].

5. Effect of SII transcription elongation factor upon transcription arrest at lesions

During the normal process of transcription elongation RNAP II may encounter impediments that include transcriptional pauses, transcriptional arrest and transcript termination [58]. At a pause site RNAP II can resume transcription after temporarily stopping. In contrast, an arrested elongation complex is unable to resume transcription without the aid of transcription elongation factors. In some cases, arrest occurs at a bend in the helix axis of template DNA [66]. Examples include sites within the first intron of the human *c-myc*, the first intron of the human histone H3.3 gene as discussed earlier, and an early transcription unit of SV40. Polymerase arrest can also be

induced by nucleotide depletion [67], DNA binding drugs [68] and sequence-specific DNA-binding proteins [69]. Factors that facilitate elongation through transcriptional arrest sites have been identified. One such protein is the eukaryotic transcription factor SII. This activity has been demonstrated in yeast [70], *Drosophila* [71], rat [72] and human cells [73, 74], and it is thought to be involved in regulation of transcriptional elongation in vivo. Evidence for such an activity in vivo is the observation that overexpression of SII can overcome sensitivity to a uracil analog, 6-azauracil, which is known to lower intracellular UTP and GTP pools, caused by mutant alleles of the polymerase [75]. SII mediates readthrough at arrest sites in vitro by activation of a cryptic endonuclease function that resides in the polymerase. This results in nascent transcript shortening by hydrolysis near the 3' end of the transcript. Transcript shortening appears to be required to restore the association of the 3' end of the transcript with the catalytic site in the polymerase after arrest.

When RNAP II complexes arrested at the site of a CPD were treated with SII, shortened transcripts were produced, indicating that RNAP II complexes at a CPD are substrates for SII induced transcript shortening, and that the shortened transcripts can be reelongated up to the point of blockage [1]. Importantly, this observation demonstrates that the transcript is not released from the CPD arrested complex, a result more recently confirmed by Selby et al. [2]. However, unlike the case of RNAP II complexes arrested at natural arrest sites, SII did not facilitate readthrough beyond the CPD. Furthermore, SII did not cause a change in polymerase conformation sufficient to allow repair of the dimer by the *A. nidulans* photolyase, indicating that the polymerase must be removed from the lesion to allow a repair complex to access the DNA damage [1].

In contrast to the results with CPDs, SII did not induce transcript cleavage by RNAP II blocked by a AAF adduct in the transcribed strand, suggesting that transcription has been terminated [53]. This different effect of SII may be explained by differences in the stability of the arrested complex. It seems likely that the steric interference of the bulky AAF-dG adduct with the RNA-DNA hybrid and with DNA and RNA binding sites in the complex may affect the stability of the complex. Alternatively, the AAF adduct may simply inhibit the cleavage reaction, perhaps influencing the conformation or recognition of RNAP II by SII.

It is possible that the 3'-5'nuclease of RNAP II on activation by SII can also serve in an editing capacity, as recently documented by studies in which RNAP II quantitatively removed misincorporated nucleotides from the nascent RNA transcript during rapid chain extension [76].

6. Models for transcriptional arrest and the initiation of TCR

An essential question about the mechanism of TCR is how an arrested RNAP II is recognized by the repair proteins as a signal to initiate a repair event. How is a natural ‘arrest site’ distinguished from a lesion site that arrests transcription? Some clues to this question are provided by the process of transcriptional arrest. Current models of transcriptional arrest propose that at certain template locations RNAP fails to continue nucleotide addition resulting in a decreased distance from the 3' end of the transcript to the leading edge of the polymerase and misalignment of the transcript 3' end from the catalytic site [77–80]. A discontinuous model proposes that this strained configuration can be resolved by a discontinuous forward movement of the front edge of the enzyme [78]. A more recent model (monotonous model) proposes that a rigid RNA polymerase can slide backward along the RNA and DNA chains when it approaches pause, arrest and termination sites, shifting the RNA:DNA hybrid and the transcription bubble with it, displacing the RNA 3' end from the active site [81].

A change in RNAP II conformation similar to that resulting from naturally occurring impediments may occur at the site of a lesion and this may be part of the signal required to recruit repair proteins to the damage site. This hypothesis is supported by our observation that elongation complexes arrested at a site specific CPD are substrates for SII induced transcript cleavage and that the shortened transcripts can be re-elongated up to the lesion [1]. If this reaction were to be of importance in the TCR mechanism, however, the RNAP II backup would have to be sufficient to permit access of repair enzymes to the lesion. In more recent studies we have found conditions in which higher concentrations of SII were used that have resulted in RNAP II backup of roughly 25 nucleotides, thereby providing access of photolyase to the arresting CPD (to be published).

Unlike the situation in *E. coli* the proposed RNAP II reverse translocation mechanism would avoid the necessity of aborting partially completed transcripts of the large genes (such as the 2.5 megabase dystrophin gene) in human cells every time the polymerase encounters a lesion [82]. However, in experiments designed to test that model in yeast it was shown that disruption of the SII gene did not affect TCR [83]. Also, evidence has been presented in UV irradiated human cells that CSA and CSB are required for the ubiquitination and targeting for proteosomal degradation of RNAP II [84, 85]. The resultant model directly contradicts experiments reporting that in an in vitro repair system the arrested RNAP II neither stimulated nor inhibited repair and that CSB protein is not even necessary for the repair [2]. Furthermore it was shown that the CSB gene product, when present in sufficient quantity, stimulated RNAP II elongation on an undamaged template

up to three-fold. Although the addition of CSB to an RNAP II arrested at a CPD resulted in the addition of a single nucleotide to the nascent transcript, it inhibited the action of SII [86]. Unfortunately, we still lack a cell free system that carries out TCR so it is difficult to interpret mechanistically many of the recent experiments that have been reported.

It is possible that any of several possible biochemical scenarios may ensue when RNAP II is arrested at a lesion. Depending upon the nature of the lesion and its sequence context, the polymerase might either reverse translocate or be released from the DNA. In spite of a published claim to the contrary [2], it is a priori unlikely that the excision repair process can be initiated and completed while the arrested polymerase is positioned directly over the lesion.

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