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Transcription-coupled DNA repair: directing your effort where it's most needed

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Abstract. Arrest of transcription at sites of DNA damage represents a strong signal for apoptosis. To overcome the impasse represented by transcription complexes arrested at sites of damage, cells have evolved a specialized nucleotide excision repair (NER) pathway called transcription-coupled DNA repair (TCR), dedicated to removal of transcription-blocking lesions from the genome. This repair pathway was discovered in Phil Hanawalt's laboratory 20 years ago, but the mechanistic details are still subject of intense research. This article will review the recent literature on the subject with emphasis on how lesions affect the elongation step of transcription and how the initial steps of TCR occur in human cells. (Part of a Multi-author Review)

Keywords. Transcription, RNA polymerase II, Nucleotide excision repair, DNA damage, transcription arrest, Cockayne syndrome.

Introduction

Cellular DNA is continuously exposed to damage inflicted by endogenous metabolism and environmental sources. This damage can have mutagenic and cytotoxic effects, posing a remarkable threat to genomic integrity and proper functioning of cells. Not surprisingly, multiple DNA repair processes have evolved in all organisms to provide a major protection mechanism against the deleterious effects of DNA damage. The relevance of efficient repair processes in genome maintenance is clearly indicated by the existence of several genetic disorders characterized by defects in DNA repair processes, including xeroderma pigmentosum (XP), Cockayne syndrome (CS), Fanconi anemia, ataxia telangiectasia, Bloom syndrome, Werner syndrome and hereditary non-polyposis colon cancer [1–5].

A unique problem arises when DNA damage is located in transcribed regions of the genome (Fig. 1). If RNA polymerase (RNAP) continues transcribing its product past a lesion, mistakes may be introduced at a miscoding or non-informational damage site, potentially leading to transcriptional mutagenesis in a non-dividing cell [6]. A lesion may alter the rate of transcription, without affecting the fidelity of the process. In this case the level of expression of a gene might be reduced or increased. Some lesions may transiently arrest the elongation process while others may pose an insurmountable obstacle to the translocating polymerase that causes the RNAP complex to be arrested. For some lesions, the arrested complex may not be stable, resulting in release of the arrested polymerase and nascent RNA product. For other lesions, the ternary complex may be very stable, and persist in the arrested state for a long time. An arrested RNAP at a site of a lesion represents a strong signal for accumulation of p53 and apoptosis [7–9]. Therefore, to prevent the deleterious effects caused by persistence of RNAP complexes arrested at lesions, dedicated mechanisms have evolved to remove them.

Transcription-coupled DNA repair

Several DNA repair pathways function in the cell to maintain genome integrity. The most versatile repair mechanism is nucleotide excision repair (NER), a

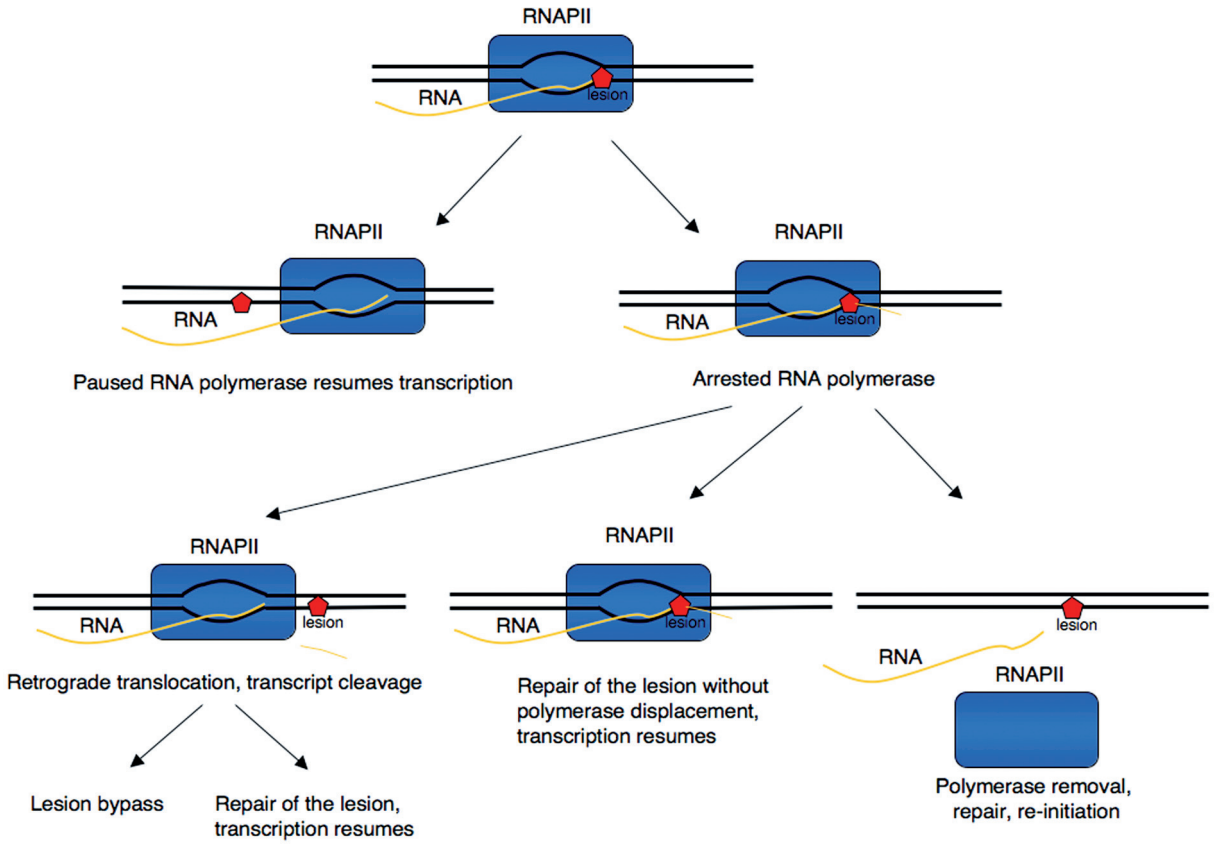

Figure 1. Transcriptional encounters with DNA damage: What are the options? The presence of a lesion in the transcribed strand may cause different transcription outcomes depending on whether the lesion transiently pauses or permanently arrests the elongating polymerase.

ubiquitous repair pathway that recognizes and removes a wide variety of structurally unrelated lesions from DNA, including ultraviolet-induced cyclobutane pyrimidine dimers (CPDs) and 6–4 pyrimidine pyrimidone photoproducts [(6–4)PPs], cigarette smoke-induced benzo[a]pyrene DNA adducts and lesions formed by chemical carcinogens like cisplatin [10–12]. In NER, the lesion is removed as part of a short oligonucleotide followed by synthesis of the resulting gap using as template the undamaged complementary strand (see accompanying review article by T. Nouspikel). The relevance of this repair pathway is indicated by the observation that deficiencies in NER result in rare autosomal recessive human disorders such as xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodistrophy. Transcription-coupled DNA repair (TCR) is a specialized excision repair pathway that operates on lesions in the transcribed strands of expressed genes [13] (for recent review articles on TCR see also [14–19]). NER and TCR differ in their mode of damage recognition; all subsequent steps are common to both repair pathways. The heterodimer XPC/HR23B appears to be the major damage recognition factor in NER in human cells. The UV DNA damage binding protein UV-DDB is additionally required for NER of CPDs, so that XPC can be more efficiently recruited to the damage site [20]. Interestingly, a recently reported crystal structure of a CPD-containing-DNA bound to yeast Rad4, a homologue of human XPC, has revealed that both the CPD and the complementary undamaged nucleotides on the opposite strand are flipped out of the DNA helix. The CPD is not in contact with the Rad4 protein [21], which instead recognizes the two flipped out nucleotides opposite the CPD in the undamaged DNA strand. These results suggest that destabilization of the double helix induced by the lesion and subsequent flipping out of the two base pairs opposite the lesion are key elements in the mechanism of damage recognition in NER [22]. In TCR, lesion recognition occurs through arrest of the elongating RNAP when it encounters the damage. This necessary step initiates the subsequent recruitment of repair factors followed by removal of the lesion. However, the molecular events of the coupling reaction are still largely unknown. TCR operates on bulky lesions like UV-induced CPDs [23] and helix distorting DNA damage like cisplatin DNA crosslinks

Table 1. Transcription arrest *in vitro* and TCR *in vivo* of bulky DNA adducts.

DNA lesion	TCR	Transcription arrest	RNA polymerase	Reference
CPD	+	+	T7, E. coli, yeast, rat liver, human	[23, 44, 50, 68, 70, 78, 99]
(6–4)PP	+	+	human	[78, 100]
cis 1,2-d(GG)	+	+ + –	T7, *E. coli*, yeast, wheat germ rat liver human	[69, 80, 95, 101, 102] [103] [81]
cis 1,3–d(GTG)	+	+ +	T7, *E. coli*, rat liver human	[80, 89, 95, 101–103] [81]
Psoralen monoadduct	–	+	T7, human	[104–106]
Psoralen interstrand crosslink	+	+	T7, *E. coli*, human	[104–107]
BPDE (human cells)	+	+[a]	T7, human	[25, 66, 108, 109]
BPDE (rodent cells)	–			[110]
AAF	+	+/–	T7, rat liver	[95, 111]
AF	–	+	T7, rat liver	[95, 111, 112]
Aflatoxin B1	+	N. D.		[30]
CC-1065	+	N. D.		[113]

[a] The extent of transcription arrest is dependent on the BPDE diastereomer (reviewed in [66]).

[24] and DNA adducts formed by benzopyrene-diol-epoxide [25] (Table 1). Historically, the initial observation was that UV-induced CPDs in an expressed gene in Chinese hamster ovary cells were much more efficiently repaired than were those in a silent sequence downstream [26]. Then it was revealed that this efficient repair was due to the preferential repair of CPD in the transcribed DNA strand [23].

For some lesions, like CPDs, TCR results in more rapid repair of the transcribed strands compared to the non-transcribed strands of expressed genes. This strand bias has usually been taken as the operational definition of TCR. However, for other lesions, like the more distorting (6–4)PPs, which are very efficiently repaired by NER, the effect of TCR may not be seen as a differential rate of repair between the two DNA strands.

Several lines of evidence indicate that initiation of TCR requires that RNAP be in elongation mode. For example, TCR of the *lac operon* of *Escherichia coli* can only be observed when the operon is induced [27]. In eukaryotes TCR operates on RNAPII-transcribed genes and the polymerase must be in the actively elongating mode to initiate TCR. Treatment of mammalian cells with α-amanitin, a specific inhibitor of RNAPII, abolishes the preferential repair of CPDs in expressed genes [28–30]. In yeast with temperature-sensitive mutations in the gene encoding a subunit of RNAPII, a loss of TCR is observed at the non-permissive temperature [31, 32].

TCR does not vary in efficiency through the normal cell cycle in mammalian cells for a gene that is expressed continuously throughout the cycle [33]. It is maintained in several differentiated cell types where the GGR pathway is markedly attenuated [34]. TCR does not appear to be inducible by UV. However, in human cells, efficient global genomic repair of CPDs requires activation of the p53 tumor suppressor [35]. Furthermore, growing evidence indicates that arrested transcription (e. g. at CPDs) provides a sensitive signal for the activation and stabilization of p53 [7, 8, 36].

Although TCR was originally documented for DNA damage induced by UV light [23], it has also been reported that oxidative damage is repaired in a transcription-dependent manner in *E. coli*, using 8-oxoguanine (8-oxoG)-containing plasmids transfected in TCR-proficient or -deficient cells [37]. In support of a link between repair of oxidative damage and transcription, it was recently shown that host cell reactivation of plasmids containing randomly positioned 8-oxoG is defective in CSA and CSB cells [38]. In agreement with these findings, using shuttle vectors containing a single 8-oxoG in the transcribed strand transfected into mouse embryonic fibroblasts lacking Ogg1, required for base excision repair, CSB, required for TCR, or both, it was shown that both Ogg1 as well as CSB were required for expression of the reporter gene [39]. However, when 8-oxoG was located in a different sequence context and using a weaker promoter, Larsen and colleagues (2004) [40] did not observe the same effect, suggesting that factors such as promoter strength, sequence context and position of the lesion with respect to the promoter may affect transcription past a single 8-oxoG in mouse cells [39]. Furthermore, in Chinese hamster ovary cells repair of oxidative damage in the 'housekeeping gene' dihydrofolate reductase did not reveal a strand bias [41]. In addition, several key papers supporting transcription-coupled

repair of oxidative damage have been retracted, making this subject a matter of intense debate [42].

RNA polymerase arrest and initiation of TCR

The original and still current TCR model proposes that RNA polymerase stalled at a lesion directs repair enzymes to the transcribed strand of an active gene [43]. This model assumes that the polymerase must be removed from the site of the lesion to provide access of the repair complex to the lesion site and to allow reannealing of the DNA strands to form a proper substrate for repair. In *E. coli*, the *mfd* gene product, encoding a 130-kDa monomeric protein, participates in this process [44]. Using an *in vitro* system with purified proteins it was shown that the Mfd protein can promote the release of the RNAP and of the incomplete transcript from the DNA template and to target components of NER to the site of transcription blockage. Recent evidence has shown that this is accomplished by a forward movement of the polymerase promoted by Mfd in an ATP-dependent fashion until the polymerase dissociates from the DNA [45]. This forward movement is accompanied by a progressive rewinding of the upstream end of the single-stranded transcription bubble within the transcription complex until the bubble is sufficiently rewound to release the RNAP and the nascent transcript from the DNA, making the lesion accessible for repair [46, 47]. In mammalian cells, the *ERCC6*/*CSB* gene product may be such a coupling factor [48] although the reactions are probably more complex than those in *E. coli.* The CSB gene encodes a 168-kDa protein that is related to the SWI/SNF family of ATP-dependent chromatin remodeling factors. SWI/SNF family members such as SWI2/SNF2 are DNA helicases. The CSB protein, like most members of this family, displays DNA-dependent ATPase and DNA binding activity, but not helicase activity. Similarly, both the bacterial and yeast counterparts of CSB, Mfd and Rad26, are also DNA-dependent ATPases. CSB has also nucleosome remodeling activity and binds to core histone proteins *in vitro* [49]. Furthermore, when added to RNAP arrested at a CPD, CSB can stimulate transcription elongation by addition of one nucleotide to the nascent transcript [50]. It remains unclear whether the polymerase is ubiquitinated and degraded [18, 51, 52], translocated away from the site of damage without dissociating from the template [2, 53], or simply stalled at the site of damage while repair of the lesion occurs, as recently suggested [54–56]. Once the template is repaired, the arrested RNAP might then resume elongation without releasing the incomplete transcript.

Following the initiation of transcription the template DNA strand is preferentially repaired in the region just upstream of the point at which RNAPII clears the promoter and releases TFIIH in normal cells, in CSA and CSB cells, and in the yeast homologue of CSB [57]. 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 RNAPII is arrested at a lesion while in the elongation mode [58, 59]. In support of this model it was shown that the RNAPII/CSB complex can interact with subunits of TFIIH [60]. Using an *in vitro* system consisting of an artificial transcription bubble-like structure it was found that CSB and XPG can bind *in vitro* in a cooperative manner to RNAPII arrested at a cisplatin intrastrand crosslink [54]. Furthermore, the lesion became accessible to XPG incision only in the presence of wild-type TFIIH and required ATP hydrolysis. It was speculated that ATP hydrolysis by TFIIH would promote a conformational change of the RNA polymerase that would render the damaged DNA accessible for incision by XPG. Taking advantage of a reconstituted *in vitro* transcription assay with purified RNA polymerase and initiation factors, Lainé and Egly [56] determined the order of assembly of NER factors at arrested RNAPII complexes. They found that TFIIH is recruited first and that RPA is recruited as soon as single-stranded DNA is formed. Then, XPA is recruited at the damage site followed by XPG and XPF. In this system, CSB appeared to be required for the incision activity. *In vivo* crosslinking assays on RNAPII complexes arrested at UV lesions followed by chromatin immunoprecipitation with specific antibodies revealed that following UV irradiation, RNAPII stalled at UV damage sites appeared to be associated with CSB. The presence of CSB was also necessary to attract NER factors specific for TCR at sites of RNAPII arrest [55]. The CSA gene product, which is also required for TCR, is a member of the WD-40 repeat family of proteins, which is implicated in protein-protein interactions, but its role in TCR is unknown. It was recently shown that CSA is part of an E3-ubiquitin ligase complex consisting of DDB1, Cullin4A, ROC1/Rbx1 and the COP9 signalosome (CSN) [61]. The association of the CSA-DDB1/CSN complex with the UV-stalled RNAP complex is dependent on functional CSB protein [55]. Recent evidence indicates that the CSA complex is responsible for CSB ubiquitination and degradation following UV irradiation, establishing a functional link between CSA and CSB proteins [62].

Two other factors, XAB2 and HMGN1, have also been identified and play a role in TCR. XAB2 is a

TPR (tetratricopeptide repeat) containing protein involved in pre-mRNA splicing and transcription [63], and might function as a scaffold for protein complex formation in TCR [63, 64]. HMGN1 is a nucleosome binding protein that was recently shown to interact with UV-stalled RNAPII complexes in a CS-dependent manner [55] and may be required for nucleosome removal behind the arrested polymerase so that the RNAPII can translocate backward from the lesion.

RNA polymerase arrest at DNA lesions

To understand the potential roles of the various gene products implicated in the initial steps of TCR, it is necessary to characterize the properties of the transcription complex when it encounters a lesion in its path. The analysis of different types of arrested complexes should help us to understand how an arrested RNA polymerase may signal the repair proteins to initiate a repair event. The original TCR model suggests that RNAPII arrest at a lesion is a prerequisite for initiation of TCR [43]. In support of this model, a strong correlation between transcription arrest by a lesion *in vitro* and TCR of that lesion *in vivo* has been found in most cases analyzed [65–67]. Several studies have characterized the behavior of RNAP when it encounters a bulky lesion during the transcription elongation process (Table 1). Here we will focus on the mechanism of transcription arrest at two bulky lesions repaired by TCR, CPDs and cisplatin-induced intrastrand crosslinks, based on the recent resolution of the crystal structure of *Saccharomyces cerevisiae* RNAPII arrested at a T<>T CPD or at a cisplatin-induced (1,2 GG) intrastrand crosslink [68,69]. Review articles describing the behavior of prokaryotic and eukaryotic RNA polymerases at several different adducts have recently been published [6, 15, 67].

Cyclobutane pyrimidine dimers

CPDs are the most abundant DNA lesions produced after DNA exposure to UV light. TCR of CPDs was first documented in Phil Hanawalt's laboratory in UV-irradiated CHO cells [26], followed by the finding that the efficient repair of CPDs was due to the preferential repair in the transcribed strand [23].

Using a reconstituted transcription system with RNAPII and initiation factors we have shown that a CPD is a complete block to mammalian RNAPII progression [70]. The stability of the arrested complex was indicated by its ability to be subject to the transcript cleavage reaction mediated by elongation factor TFIIS [70–72]. TFIIS 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 of the 3′ end of the transcript [73]. Transcript shortening is required to restore the association of the 3′ end of the transcript with the catalytic site in the polymerase after arrest. Chromatin immunoprecipitation studies have recently shown that following UV irradiation TFIIS is associated with the arrested RNAPII [55].

Current models of transcriptional arrest propose that at certain template locations RNAP fails to continue nucleotide addition, resulting in backward translocation of the polymerase along the DNA template, and as a result, misalignment of the transcript 3′ end from the catalytic site [74]. The arrested complex remains stably associated with the DNA, maintaining a 7–8 bp RNA-DNA hybrid upstream of the transcript 3′ end [75,76]. The strength of the RNA:DNA hybrid, and in particular the last few base pairs at the 3′ end of the transcript, is a critical feature of the elongation complex in preventing arrest. This is consistent with arrest generally occurring at T-rich sequences in the non-transcribed strand, where the dA:dT hybrid at the leading edge of the transcription bubble is energetically favored over the dA:rU hybrid [77]. Furthermore, arrest sites are often characterized by helix distortions [74]. A stable interaction in the last base pair is necessary to properly orient the transcript 3′ end with the incoming nucleotide to form a phosphodiester linkage with the nascent transcript. If the hybrid is weak, bond formation is delayed, allowing the polymerase time to translocate upstream on the template. Similarly, formation of a CPD causes a small deformation of the double helix consisting of unwinding by ~15° and bending of at least 7° relative to the B form. The neighboring pyrimidines must rotate from their usual B form DNA alignment with overlapping of the 5,6 bonds. It is likely that the presence of a CPD in the transcribed strand affects formation of the RNA:DNA hybrid, and this in turn may shift the equilibrium from nucleotide addition toward arrest.

With the resolution of the crystal structure of *Saccharomyces cerevisiae* RNAPII arrested at a T<>T CPD located in the template strand, new details on the mechanism of transcription arrest at a CPD have been revealed [68] (Fig. 2a). These studies have shown that RNAPII can correctly incorporate AMP opposite the 3′ thymine of the CPD [68,78], but incorporation of the next nucleotide, opposite the 5′ thymine of the CPD is a very slow process that can only occur if UMP is misincorporated. This, in turn, causes RNAP to become arrested [68]. The CPD 5′ thymine adopts a wobble position with respect to the base in the undamaged complex, where base pairing can only occur with the wrong nucleotide, due to the unusual position of this base in the catalytic site of the polymerase [68]. In fact, the CPD 3′ thymine occupies

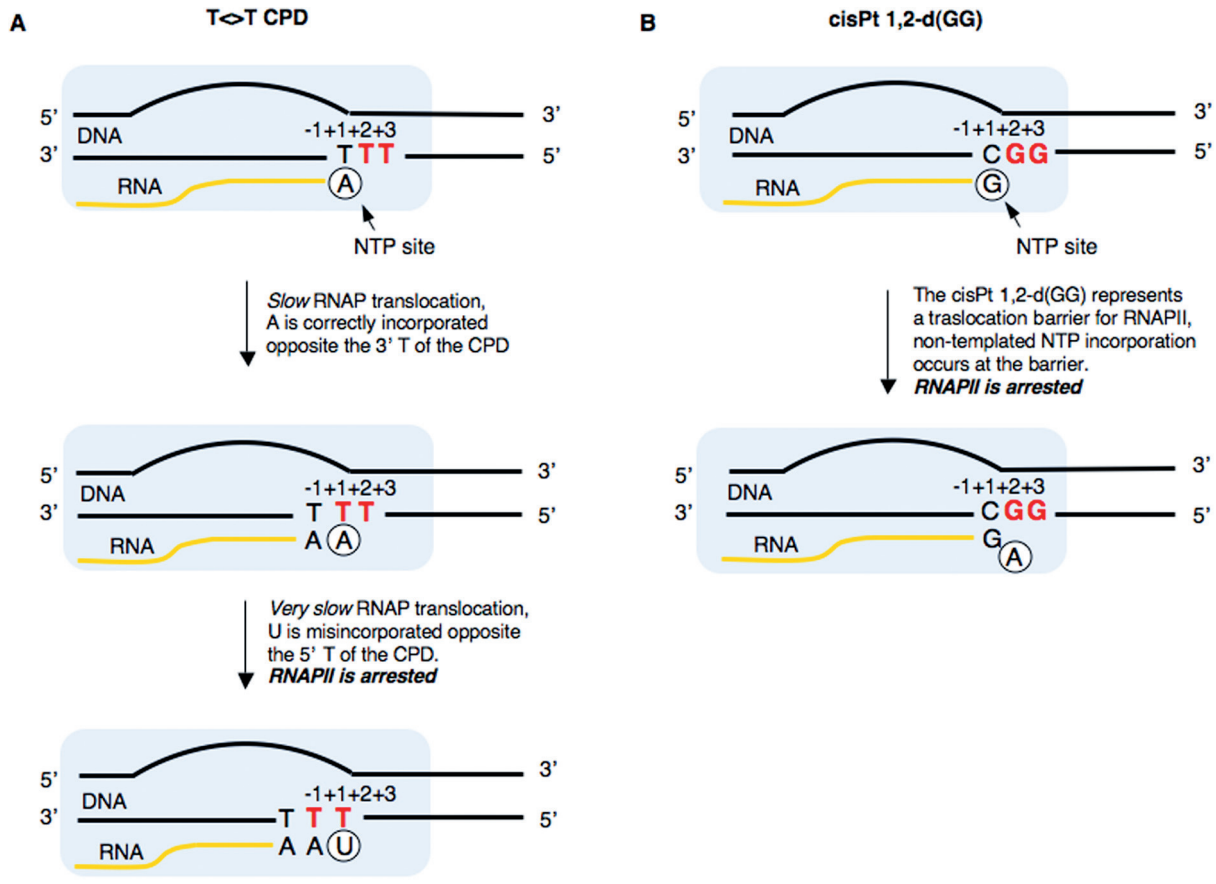

Figure 2. Different mechanisms of RNAPII arrest at bulky lesions. (*a*) T<>T CPD. (*b*) *cis*-Pt 1,2-d(GG). Shaded box: RNAPII.

the same position as in the undamaged elongation complex, whereas the CPD 5′ thymine is tilted by ~40° with the O4 atom at the location normally occupied by the N3 atom. RNAPII becomes arrested because further translocation of the CPD 5′ thymine-uracil mismatch from position +1 to position –1 is strongly disfavored: the positioning of the damage-containing mismatch into the –1 location of the DNA-RNA hybrid would result in a distortion that would likely destabilize the elongation complex.

Cisplatin-induced intrastrand crosslinks

Cis-diamminedicloroplatinum(II) (cisplatin) is a widely used antitumor drug that preferentially reacts with purine bases to form DNA crosslinks. These adducts may impose a more serious problem for an elongating RNA polymerase compared to a CPD, since they cause substantial unwinding and bending of the DNA helix (reviewed in [79]).

Using our reconstituted transcription system, we found that a single *cis* 1,2-d(GG) crosslink (*cis*-Pt-$(NH_3)_2${d(GpG)-N7(1),N7(2)}) or a single *cis* 1,3-d(GTG) crosslink (*cis*-Pt-$(NH_3)_2${d(GpTpG)-N7(1),N7(3)}) located in the transcribed strand is a strong block to both T7 RNAP and RNAPII [80]. Furthermore, the efficiency of the block at a *cis* 1,2-d(GG) is not affected by the sequence context around the lesion. The arrested RNAPII complex was stable, as indicated by the ability of elongation factor TFIIS to induce transcript cleavage, producing a population of transcripts up to 30 nucleotides shorter than those arrested at the lesion, which could then be re-elongated up to the lesion when the nucleotide triphosphate precursors were added. Interestingly, we also observed partial blockage when a single *cis* 1,3-d(GTG) was located in the non-transcribed strand. A *cis* 1,3-d(GTG) in the transcribed strand also blocks RNAPII transcription in extracts of human cells [81]. In addition, the presence of cisplatin-induced lesions in plasmids transfected into human or hamster cells almost completely inhibits RNAPII transcription of a reporter gene [82].

We have speculated that the bulky nature of cisplatin-induced intrastrand crosslinks and the DNA structural changes induced by their presence in the double helix may cause RNAP arrest. Both lesions unwind the DNA, the *cis* 1,2-d(GG) by 13–25°, the *cis* 1,3-d(GTG) by 19–23° [83]. They also cause the DNA to

Table 2. Transcription arrest *in vitro* and TCR *in vivo* of oxidative lesions and non-bulky DNA adducts.

DNA lesion	TCR	Transcription arrest	RNA polymerase	Reference
5-Hydroxyuracil	N. D.	+	human	[89]
2-Hydroxyadenine	N. D.	+	human	[98]
8-Oxo-adenine	N. D.	+	human	[98]
8-Oxoguanine	+/–	+/–	T7, *E. coli*, rat liver, mouse	[37,39–41,89,91,95,96,98,114]
Thymine glycol		+	T7	[89, 91, 96, 98]
	–	+	rat liver, human	[115, 116]
Abasic sites	N. D.	+	T7, rat liver	[93, 95]
2-Ribonolactone	N. D.	+	T3, SP6, rat liver	[93, 94]
	–	–	SP6, E. coli	[117]
Single-strand breaks	–	–	T7	[95, 118]
		+	SP6, E. coli	[117]
		+	human	[91, 92]
Malondialdehyde	N. D.	+	T7, rat liver	[119]
N-Ethylpurines	+	N. D.		[120]
7 Me-guanine	–	–	T7	[121]
				[122]
3 Me-adenine	–	–	T7	[122]
1,N2-Ethenoguanine	N. D.	+	T7, human	[123]

bend toward the major groove, the *cis* 1,2-d(GG) by 32–78° [84], the *cis* 1,3-d(GTG) adduct by 25–35° [84, 85]. In addition, NMR analysis of oligonucleotides containing a single *cis* 1,3-d(GTG) adduct has shown that the overall structure of the DNA is more distorted than that of DNA containing a single *cis* 1,2-d(GG) [86]. This might explain why the *cis* 1,3-d(GTG) adduct also has an effect on transcription when located in the non-transcribed strand, while the *cis* 1,2-d(GG) does not.

It is likely that the structural changes induced by the *cis* 1,2-d(GG) and the *cis* 1,3-d(GTG) would affect the formation and/or the stability of the RNA:DNA hybrid, an essential component of the elongation complex [87]. A weak RNA:DNA hybrid has been proposed as a primary determinant of the arrest modality, as it promotes backward translocation of RNAP along the DNA template. This in turn can result in the displacement of the 3′ end of the RNA from the catalytic site, leading to polymerase arrest [88].

More insights on the mechanism of RNA polymerase arrest at a *cis* 1,2-d(GG) were obtained with the resolution of the crystal structure of a *Saccharomyces cerevisiae* RNAPII arrested at this lesion [69] (Fig. 2b). Different from the mechanism of transcription arrest at a CPD, a *cis* 1,2-d(GG) blocks RNAP transcription because of a translocation barrier that prevents the adduct from entering the catalytic site. This occurs because the two adjacent bases in the *cis* 1,2-d(GG) are covalently linked and as a result cannot twist by approximately 90° as required for the incoming base to enter the polymerase active site.

Oxidative DNA damage

Several *in vitro* studies aimed at characterizing the behavior of 8-oxoG and thymine glycol (Tg) on RNAP transcription have shown various levels of RNAP bypass at these lesions (Table 2). Elongation factors have been recently shown to have a significant role in modulating readthrough past these lesions, suggesting that the differences in the *in vitro* transcription results previously observed may have derived from differences in the cell extract used, and/or on the presence of elongation factors among components of the transcription system [89, 90]. Elongin and CSB, which affect the rate of RNAPII elongation, but not TFIIS, which helps arrested complexes to bypass transcriptional blocks, were shown to stimulate bypass of Tg, whereas Elongin, CSB and TFIIS could all enhance readthrough of an 8-oxoG lesion. Furthermore, transcription past these lesions caused misincorporation opposite the lesion, suggesting that elongation factors, by promoting readthough past 8-oxoG or Tg, could contribute to the transcriptional mutagenesis induced by these lesions [89].

If 8-oxoG and Tg do not represent a significant barrier to RNAP transcription *in vitro*, how can they elicit TCR *in vivo?* It was initially proposed that RNAP pausing at 8-oxoG could be sufficient to initiate TCR [37]. Another possibility is that intermediates in the repair of oxidative damage such as natural abasic sites, or strand breaks, might be sufficient to block transcription and initiate TCR. Indeed, Kathe et al. (2004) [91] have reported that strand breaks located in the transcribed strand are strong blocks to RNAPII elongation *in vitro.* However, due to the rapid repair of these lesions *in vivo*, it is unlikely that single-strand

breaks will have a sustained inhibitory effect on transcription in cells [92].

Our *in vitro* studies aimed at addressing this question have shown that abasic sites, 2-deoxyribonolactone, an oxidized form of the AP site, and its caged precursor, when located in the transcribed strand of template DNA were strong blocks to T7 RNAP and mammalian RNAPII [93, 94]. In agreement with our findings, previous studies have shown that a natural AP site located in the transcribed strand inhibited T7 RNAP transcription [95, 96]. Furthermore, this effect was modulated by the sequence context around the lesion. AP sites have also been shown to block RNAPII transcription *in vivo* [97]. Surprisingly, tetrahydrofuran, a stable AP site analogue, did not affect human RNAPII elongation when transcription was initiated from a C-tailed template in the absence of general transcription factors [98], suggesting that other factors such as the sequence context, the source of RNAP and/or of transcription systems might play a role in the extent of lesion bypass. Based on the current TCR model postulating that only lesions that block RNAP will be subject to TCR, our findings suggest that the abasic site and its oxidative derivative may be sufficient to initiate TCR *in vivo*.

Perspectives

Twenty years after the discovery of TCR, several questions remain unanswered. Although we have a better understanding of the behavior of RNAPII at different lesions and of the order of assembly of the proteins that effect repair, we still do not know how the coupling between transcription arrest and initiation of TCR occurs. What determines whether a lesion will block transcription? What features of an RNAP arrested at a lesion makes it a substrate for repair with respect to a polymerase arrested at a natural arrest site or at a non-canonical DNA structure? Is the polymerase released from the DNA translocated backward from the site of the lesion before repair can take place or is it simply stalled at the site of damage while repair occurs? In this case, how does the arrested polymerase restart transcribing after repair has occurred? Is repair of oxidative damage coupled to transcription? The development of novel approaches for studying TCR will be essential to address some of these challenging questions.

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