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Mechanistic Insights into Transcription Coupled DNA Repair

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Abstract

Transcription-coupled DNA repair (TCR) acts on lesions in the transcribed strand of active genes. Helix distorting adducts and other forms of DNA damage often interfere with the progression of the transcription apparatus. Prolonged stalling of RNA polymerase can promote genome instability and also induce cell cycle arrest and apoptosis. These generally unfavorable events are counteracted by RNA polymerase-mediated recruitment of specific proteins to the sites of DNA damage to perform TCR and eventually restore transcription. In this perspective we discuss the decision-making process to employ TCR and we elucidate the intricate biochemical pathways leading to TCR in *E. coli* and human cells.

Keywords

DNA repair; TCR; transcription elongation; backtracking; UvrD; ppGpp; Mfd

1. Introduction

The integrity of the DNA molecules comprising our genetic material is not innately secure. Our genome is constantly threatened by exogenous factors such as radiation (e.g. UV) and chemical products from smoke, pollution, and drugs, as well as natural byproducts of cellular metabolism, such as reactive oxygen species (ROS) [1]. These threats can cause DNA to undergo various modifications, including strand breaks, base damage, helix distortions and interstrand crosslinks [2]. Various DNA damages lead to lesions that block the elongating RNA polymerase (RNAP). A serious threat arises when an advancing replication fork encounters an arrested transcription complex at a damaged site in the template DNA [3]. Cells resolve these DNA lesions appropriately in order to survive and function. To maintain the balance required for survival and diversity, evolution has equipped

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Conflicts of interest

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all life forms with mechanisms to either eliminate or tolerate DNA damage. Basic strategies for DNA repair include removing individual damaged nucleotides via the base excision repair (BER) mechanism [4] or by excising longer sections containing helix-distorting bulky lesions through the nucleotide excision repair (NER) mechanism[5].

Repair of DNA damage in active genes occurs more rapidly than in non-transcribed regions of the genome [6]. This rapid repair is not simply due to the higher accessibility of DNA in actively transcribed regions, as it is specifically the transcribed strand that is preferentially repaired [7][8]. The non-transcribed strand is repaired with a rate similar to that of non-transcribed regions of the genome. Preferential repair of the transcribed strand of active genes, or transcription-coupled repair (TCR), is evolutionary conserved from prokaryotes to eukaryotes [9]. TCR is a sub-pathway of nucleotide excision repair (NER), which can detect a variety of structurally unrelated lesions in DNA. The TCR process begins with the recognition of a DNA lesion, followed by incisions of the damaged DNA strand on either side of the lesion. The lesion-bearing oligonucleotide is then expelled and new DNA is synthesized using the undamaged strand as a template. Finally, the new DNA is ligated to the adjacent strand [10][11][12]. Blockage of the RNAP at the damaged site is the general trigger for TCR [13][14][15]. As prolonged stalling of RNAP is detrimental to the genome stability and maintenance, it is important that the path of the elongating RNAP be cleared of obstructions. The preferential repair of active genes can be vital for cellular viability and prevention of cancer. While mutations in some NER genes causes predisposition to cancer, those in some TCR genes (e.g. CSA and CSB) typically result in resistance to cancer [16]. An intriguing observation is that different mutations in a particular TCR gene can lead to different syndromes with varied clinical symptoms [17][18][19]. Several of these mutations have also been linked to cancer and aging [20][21][22]. The severe developmental problems that characterize Cockayne syndrome do not occur in UV-sensitive syndrome even though the causal mutations can be in either CSA or CSB [10]. However, non-productive engagement of TCR at or near naturally occurring non-canonical DNA structures can be mutagenic [12][23]. Therefore, cells must make a delicate but stringent decision to resolve natural DNA sequences from damaged ones, and to employ TCR to avoid both cytotoxicity and mutagenesis.

Although numerous studies have demonstrated extensive coupling between transcription and DNA repair, but one key observation marked the earliest indications of the existence of TCR. When mammalian cells are exposed to UV light, RNA synthesis resumes before the majority of UV-induced adducts have been removed from the genome [24]. In mammalian cells, in which the expressed genes constitute only a small portion of the genome, TCR facilitates recommencement of transcription prior to the completion of global DNA repair. How do cells coordinate the assembly of TCR complexes? And how does this molecular complex accurately recognize DNA damage to carry out the repair process? Insights into answers to these questions have been gained over the last three decades. In this review, we focus on recent advances in understanding the molecular details of TCR, and the recently established connections between transcription and DNA repair machinery. We describe insights into the assembly and dynamics of the TCR complexes, and the execution of the TCR mechanism in response to DNA damage. We discuss the process by which DNA

damage is evaluated to engage TCR in order avoid genome instability and to prevent subsequent detrimental effects on cells.

2. TCR in E. coli

When transcription complexes encounter a DNA lesion they stall and mask the DNA damage from recognition and removal by repair systems [25]. The stalled RNAP must be moved away from the lesion before DNA repair enzymes can obtain access. During TCR, repair enzymes are recruited to the stalled RNAP where they not only rescue and repair the DNA damage occluded by RNAP, but also lead to preferential repair of DNA damage within the transcribed strand. In *E. coli* two distinctive TCR pathways have been described that involve two different DNA helicases that remove the blocking RNAP. The first pathway relies on mutation frequency decline protein (Mfd) [26], which displaces RNAP by pushing it forward, using its helicase fold and ATP hydrolysis, to unmask the lesion [27][28][29]. In the second pathway, UvrD pulls RNAP backward in coordination with alarmone ppGpp and the transcription elongation factor NusA [30][31][32]. This second pathway appears to conceptually resemble the process of TCR in mammalian cells.

2.1. Damage surveillance and recognition

In *E. coli*, damage recognition and processing is carried out by the UvrABC system [11][33][34]. TCR and global genomic repair (GGR) are the two sub-pathways of NER that share the downstream repair components but differ in their mode of detection of DNA damage. GGR initiation involves a two-step mechanism in which UvrA scans the genome and locates DNA damage. UvrA then recruits UvrB to form the $UvrA_2B$ complex. These events are directed by ATP binding and hydrolysis in the ‘proximal’ and ‘distal’ UvrA ATP-binding sites [35]. After the initial damage-recognition step, UvrA dissociates from the complex leaving an unwound pre-incision complex and UvrB translocates to replace UvrA at the lesion [12]. Part of UvrB is then inserted into the DNA helix to verify the lesion. The crystal structure of a bacterial UvrAB complex and single-molecule in vivo imaging of UvrAB recruitment provide important molecular details of lesion verification [35][36]. This complex is recognized and bound by UvrC. UvrBC produces an asymmetric incision on each side of the lesion: the first incision is made at the 4th or 5th phophodiester bond 3′ to the lesion and the second incision is made at the 8th phosphodiester bond 5′ to the lesion. The catalytic sites for the 3′ and 5′ incisions are located in separate regions of the N-terminal and C-terminal regions of UvrC [37]. After both incisions are made, UvrB dissociates from the DNA. However, the sequence of events leading to the formation of the incision complex is not well understood because of the observation that UvrC can facilitate the binding of UvrB to DNA in the absence of UvrA [38][39] New evidence with respect to the copy numbers of UvrC and UvrB has led to the proposal that they always exist as a complex [38]. Following the incision, UvrD and DNA polymerase I are thought to work together to remove the oligonucleotide containing the lesion and the missing bases are replaced by DNA synthesis using the undamaged complementary strand as a template. However, the actual involvement of UvrD in this process has not been demonstrated *in vivo*. Finally, ligase I joins the newly synthesized sequence to the contiguous DNA strand [37].

2.2. RNA polymerase as a damage sensor

In TCR, RNAP, instead of UvrA, acts as a DNA damage sensor. When transcribing RNAP encounters a damaged DNA base, it halts because the chemically damaged base at active center cannot serve as a template for RNA synthesis. Bulky DNA lesions, such as pyrimidine dimers (e.g. T-T dimers) or chemical adducts (such as benzopyrenes), and abasic sites are damages to DNA that can promote RNAP stalling [25][40]. As the stalled RNAP is stably associated with the DNA template, it doesn't spontaneously dissociate from the damaged base. This stalled RNAP poses not only a threat to the integrity of the DNA [41], but also a signal for recognition of the damage; the high stability of the stalled RNAP makes it a particularly effective marker of DNA damage [1], presenting the repair components an opportunity to identify the lesions without the need to scan the entire genome by a less efficient mechanism dependent on random diffusion. As a marker of DNA damage, RNAP can recruit either UvrD or Mfd to initiate the process of TCR.

2.3. Mfd-dependent TCR: Forward translocation model

Mfd is an ATP-dependent DNA translocase that binds to stalled transcription complexes and removes RNAP from DNA by pushing it forward [29][11] (Fig. 1). Mfd also interacts with UvrA [13]. In the absence of DNA damage, Mfd functions as an anti-backtracking factor. When the transcription elongation complex (EC) encounters a pause site, it backtracks and becomes transiently arrested; Mfd can reactivate it and allow it to continue transcribing if nucleotides are available [42] Reactivation of the EC is achieved by its forward translocation to realign the 3′ end of RNA in its active site. If a DNA lesion blocks the EC, such Mfd-dependent forward translocation can result in transcription termination, i.e. dissociation of RNAP from DNA [43][44]. Mfd is an eight-domain monomeric protein [13]. Domain 4 is the RNAP-binding domain, and domains 5 and 6 constitute a helicase superfamily 2 DNA translocation element. Domains 1a, 2, and 1b are structurally homologous to three of the five domains of UvrB. In UvrB, these domains are involved in the interaction with UvrA and UvrD during damage recognition, and in ATP hydrolysis [44]. Mfd is proposed to undergo a large conformational change during its interaction with stalled RNAP (Fig. 1B). This exposes the UvrB homology domain, which is presumed to recruit UvrA to the damage location [45]. It has been speculated that one monomer of UvrA interacts with Mfd, whereas the other monomer binds UvrB [46] (Fig. 1B). The Mfd:UvrA interaction has been characterized by mutagenic studies. Mutants in which the UvrB homology domain of Mfd has been modified to compromise its interaction with UvrA lose the preferential repair of UV induced damage in the transcribed strand, even though the mutant proteins are capable of dissociating the EC from DNA [47]. Several questions still remain regarding the structure/functional organization of the Mfd, UvrAB, and DNA complex. The temporal and spatial association of these interactions remains to be elucidated. As the highly stable stalled EC serves as a lesion marker, it would be logical for the cells to recruit repair factors before this marker is removed from the DNA by Mfd. Therefore, it is important to understand the sequence of events during Mfd-mediated TCR. As fortuitous TCR is likely to be mutagenic [48][49][50] an intriguing question relates to how Mfd distinguishes RNAP stalling at a lesion from natural pauses at various DNA sequences during elongation.

Mfd had also been proposed to promote repair of lesions in the template strand located downstream of damage-independent stalled RNAP [51], suggesting that Mfd tracks along the transcribed strand as it searches for DNA damage. Placing a protein roadblock can obstruct this downstream repair. Several interesting questions arise from this observation: How does Mfd reposition from the rear of RNAP to the leading face to scout for downstream DNA damage? Are there any signals relayed by the lesion itself that impels Mfd to scan downstream of the paused RNAP instead of pushing it forward to reactivate it or to dissociate it from DNA? Does Mfd-associated UvrA also reposition for lesion verification? What is the range covered by Mfd while it scans downstream of a paused RNAP? Using a tethered RNAP assay, a recent single molecule study showed that recruitment of UvrA and UvrAB leads to arrest of Mfd–RNAP complex on DNA, which rapidly dissociates the complex from UvrAB. Mfd-RNAP complex slides along DNA for a short length before dissociation from DNA [29] (Fig. 1C). It is important to determine which of these processes take place under physiological conditions in the cell.

In vivo, the EC comprises a number of regulatory proteins, including NusA, NusG, and Rho, which modulate the behavior of the EC by DNA/RNA sequence-guided assembly and disassembly of higher hierarchy complexes [52] NusA binds to the RNAP as it leaves the promoter region and the σ subunit dissociates. NusA contacts the RNA exit channel by binding to the β flap tip helix of the β flap region of RNAP [53][54][46]. NusA might compete with Mfd, which also interacts with the RNAP β subunit [27][45][42]. Moreover, NusA has been proposed to interact with UvrA to facilitate an alternative pathway of TCR, independent of Mfd [44][45]. As the inactivation of Mfd doesn't affect cell viability or sensitivity to UV significantly, it is apparent that other robust TCR pathways exist. Another fundamental issue regarding Mfd-mediated TCR is the repair of highly transcribed genes. As RNAP are stacked back to back on the DNA of the actively transcribed genes [46], there is little room for Mfd to act. Moreover, the relatively inefficient process of Mfd-mediated transcription termination, which takes minutes in vitro [42][28], appears to be too slow to account for the rapid TCR observed in vivo. As a result, questions arise regarding the prevalence of Mfd-mediated repair in the cell and how it acts in concert with other modes of TCR?

2.4. UvrD mediated TCR: Backtracking model

In recent years, UvrD has emerged as a major component of TCR in E. coli. The initial clue to its pathway came from the observation that UvrD interacts directly with RNAP in similar abundance as several other well-studied transcription factors [30][55]. When the repair zones were tracked in UV treated cells, they were repaired more rapidly in the absence of the anti-backtracking transcript cleavage factors GreA and GreB. Furthermore, deletion of *greAB* greatly suppressed the sensitivity of *uvrD*-deficient cells to UV. In vitro, UV induced lesions, such as thymine dimers, impeded RNAP progression and suppressed UvrABC-mediated cleavage at the lesion site. UvrD-mediated backtracking of RNAP was observed at these locations, which facilitated UvrABC-mediated processing of the lesions [30]. Thus a new model for TCR emerged in which UvrD pulls the blocked EC backwards away from the lesion site (Fig. 2). Backtracking of the EC exposes the lesion to UvrABC, thereby promoting repair [32][56][10]. This action of UvrD may be succeeded by its function in

dissociating the oligonucleotide resulting from the double incision of the DNA. A TCR deficient mutant of UvrD still capable of acting in GGR has been reported [57], corroborating the finding that UvrD participates in a major TCR pathway. NusA and ppGpp assist UvrD in this mode of TCR (section 2.5).

UvrD is a SF1 DNA helicase-translocase, with a typical Walker-motif common to all members of this helicase family. It translocates in a 3′ to 5′ direction via ATP hydrolysis [58]. The function of the UvrD translocase or helicase depends on different oligomeric states acquired by the protein. Monomeric UvrD functions as a translocase, but to unwind DNA it must dimerize [59]. The high affinity of monomeric UvrD for RNAP and the high abundance crosslinking between UvrD and RNAP in vivo suggest that it remains associated with actively transcribing ECs and co-migrates with RNAP during processive transcription [30] (Fig. 2A). In addition to its ability to effectively clear the lesion site of RNAP, UvrD may also serve as a recruitment factor to expedite DNA damage recognition, as evident from its direct interaction with UvrB [60]. Transcription factor NusA, which facilitates the pro-backtracking activity of UvrD [61] (Fig. 2), may also contribute to UvrA/UvrB recruitment to the lesion site by directly interacting with UvrA [62]. Interactions of UvrD with the EC were mapped near the upstream fork of the transcription bubble with the help of protein-protein and protein-DNA crosslinking [30]. The non-transcribed strand in the transcription bubble is exposed to solution [63][64] and interacts with UvrD [30] (Fig. 2A). The mechanism for UvrD-mediated backtracking is likely to involve unwinding of the upstream dsDNA and the active pulling of RNAP in reverse. If the upstream part of the transcription bubble unwinds, it would create torsion, leading to the concurrent rewinding of the transcription bubble at its leading edge, forcing RNAP backwards. Moreover, the energy generated from ATP hydrolysis by UvrD can generate the mechanical force to thrust RNAP backwards. It is also possible that UvrD renders RNAP backtracking-prone via direct allosteric interaction. Future structural studies will shed light on the exact mechanistic details of UvrD-induced RNAP backtracking.

How do cells control such a robust TCR pathway to avoid unwanted EC backtracking and futile attempts of repair at naturally occurring pause sites? UvrD is an abundant protein, with approximately 3,000 copies per exponentially growing cell [32]; RNAP is approximately equimolar compared to UvrD. Considering the high affinity of UvrD for RNAP (~35 nM), it is likely to remain associated with the elongating RNAP at all times under normal growth condition. Why then does UvrD exert its potentially harmful pro-backtracking activity only during genotoxic stress? Most likely the appropriate control is provided by the ‘SOS’ response, which coordinates the bacterial response to DNA damage via the LexA repressor. Relieve of repression leads to the increase in cellular levels of UvrD by approximately three-fold [65]. This should favor UvrD dimerization and promote its potent helicase activity, forcing RNAP to backtrack at DNA lesions. In addition to the increasing concentration of UvrD, the SOS response also causes the concentration of ppGpp to increase rapidly [66]. This bacterial alarmone orchestrates the global reprogramming of transcription [66][67]. Recent studies show that ppGpp- mediated changes in the cellular environment allow for a fine regulation of RNAP backtracking and TCR [31].

2.5. Cooperation between ppGpp and UvrD in TCR

Even during the SOS response UvrD dimerization is likely to occur only transiently because UvrD dimers are highly unstable (Kd~470nM) [68], creating only a narrow window of opportunity for UvrD to act as a pro-backtracking factor. ppGpp provides a critical boost for UvrD in this capacity by inducing a conformational change in RNAP that renders it backtracking-prone [69] (Fig. 2B). Tracking the front edge of RNAP by exonuclease III footprinting shows that ppGpp shifts the equilibrium of RNAP toward the pre-translocated and backtracking states. The backtracking promoting activity of ppGpp was also confirmed *in vivo* by in situ DNA footprinting [31]. Induction of ppGpp and the stringent response by treating cells with serine hydroxamate (SHX) caused the roadblocked EC to backtrack. Cells overexpressing UvrD displayed substantially higher backtracking, whereas UvrD-deficient cells did not show increased backtracking upon SHX treatment. Additionally, SHX-pretreated cells became more resistant to genotoxic stress, which further corroborating the role of ppGpp in TCR [31].

DksA is a bacterial transcription factor that binds RNAP in the secondary channel and augments the action of ppGpp during the stringent response [70][71][72]. It was observed that DksA-deficient cells are sensitive to genotoxic agents, such as 4-nitroquinoline 1-oxide (4NQO), nitrofurazone (NFZ), and mitomycin C, whereas GreA inactivation completely suppressed this sensitivity [73][31]. Likewise, DksA overexpression suppressed the sensitivity of ppGpp-deficient cells to genotoxic stress, suggesting that DksA aids in UvrD/ppGpp-mediated TCR. Together, these findings advocate for a model in which DksA competes with the anti-backtracking factors GreA and GreB for the same binding site (in the secondary channel) on RNAP and stabilizes it in the backtracking-prone (TCR competent) state induced by ppGpp [31][74].

2.6. Relative involvement of UvrD and Mfd in TCR in vivo

The above two proposed models of TCR in *E.coli* are not necessarily mutually exclusive, although their mechanism of action appears to be competing. Hence the question arises, which one of these two mechanisms dominates during genotoxic stress?

The UvrD-mediated (backtracking) model of TCR is strongly supported by both *in vitro* and *in vivo* evidences. The deletion of anti-backtracking transcript cleavage factors GreA and GreB suppresses sensitivity of Δ*uvrD* to genotoxic stress, arguing that most of the activity of UvrD associated with NER can be attributed to its pro-backtracking function, irrespective of its other potential roles in NER [30]. As transcription is coupled to translation in bacteria, the leading ribosome acts as a major anti-backtracking factor. Decelerating ribosomes with sublethal doses of chloramphenicol (Cm) promotes RNAP backtracking [75]; and it partially suppresses the sensitivity of Δ*uvrD* to genotoxic stress [29], further supporting the role of UvrD in backtracking-mediated TCR. The sensitivity of ppGpp-deficient cells (*ΔrelA ΔspoT*) to genotoxic stress is suppressed by deletions of *greAB*, by Cm, or by the mutation in RNAP that renders it backtracking-prone (*rpoB**35) [31], demonstrating the role of ppGpp in backtracking-mediated TCR. These results corroborate the *in vitro* observations that ppGpp facilitates UvrD-mediated backtracking. In addition, NusA mutants highly sensitive to genotoxic stress are epistatic with general NER mutants like *ΔuvrA* and

synergistic with *Δmfd*, indicating that NusA acts in an Mfd-independent TCR pathway [62]. The sensitivity of NusA mutants to genotoxic stress can be suppressed by *greAB* deletions, by Cm, or by the pro-backtracking mutation in RNAP, also demonstrating the important role of NusA in backtracking-mediated TCR [31].

The Mfd mediated TCR has been supported largely by *in vitro* evidences. The very weak sensitivity of Mfd-deficient cells to UV or genotoxic chemicals [26][13][31] argue that the role of Mfd in DNA repair is modest. The only *in vivo* evidence in favor of Mfd-mediated TCR shows that UV-treated *E. coli* deficient in *mutS*, *mutL*, or *mfd*, do not repair the segment of the template strand (TS) of the *lacZ* gene at a higher rate than that of the non-template strand (NTS) [76]. The role of mismatch repair proteins or Mfd in TCR *in vivo* has never been confirmed. A recent paper by Adebali et al, examined the relevance of Mfd in TCR in *E. coli* [77]. The authors used NGS method XR-seq (excision repair sequencing) [78] to argue that Mfd plays the central role in TCR. In the XR-seq methodology the excised oligonucleotide from NER is isolated and subjected to high-throughput sequencing. The evidence used to support the authors' claim is based on the ratio of TS (template strand) and NTS (non-template strand) oligos that map to selected genes. The absence of an alleged TCR factor is expected to lower the TS/NTS ratio due to deficiency in TS. Indeed, the authors show that *mfd* deletion lowers the TS/NTS ratio in a majority of genes, but do not acknowledge the actual reason of this change. Reanalysis of their raw data indicates that the differences in ratios of TS vs NTS in Δ*mfd* is primarily due to increased repair of the NTS, and not the diminished repair of TS [79], suggesting that Mfd not only does not play an obvious role in TCR, but rather interferes with DNA repair under selected conditions. Indeed, deletion of Mfd partially suppresses the sensitivity of UvrD to genotoxic stress [30]. Overexpression of Mfd renders cells more sensitive to UV and genotoxic agents [31]. As Mfd is an anti-backtracking factor [42], these results reinforce the idea that Mfd interferes with UvrD-mediated TCR *in vivo*, at least for a majority of genes under genotoxic stress conditions.

Using XR-seq and the Δ*uvrD* strain, Adebali et al also argued that UvrD has no role in TCR, acting only in the removal of the excised oligo at the later step of NER [77]. However, the use of a complete deletion of *uvrD* for XR-seq cannot unambiguously address its role in TCR, because the functioning of UvrD at the downstream stage of NER is compromised as well. Moreover, the authors elected to challenge cells with a very high dose of UV (120 J/m^2) and let them to recover for only 5 minutes. Such conditions do not support SOS-mediated accumulation of UvrD [80][81] and the formation of backtracking-competent UvrD dimers [29]. Surprisingly, even under these conditions, obviously biased against UvrD-mediated TCR, after examining ~150 genes with the highest propensity for TCR in the wild type cells (High TS, low NTS), it was shown that the deletion of *uvrD* preferentially decreases TS repair of these genes [79], providing a strong *in vivo* evidence for UvrD-mediated TCR.

3. TCR in human cells

Tightly packed chromatin poses a major obstacle for DNA repair in eukaryotes. In addition to marking DNA damage, RNAP provides another advantage to TCR by transiently opening

the nucleosome structure during transcription [10]. Although the sequence of events in human TCR is similar to that in bacteria, the process is more complicated with respect to the number of proteins involved and their interplay [12][82]. Human cells have three RNAPs whereas *E. coli* has only one. A notable difference in human TCR is that it can take place in the absence of the global genomic NER (GGR) factors Xeroderma Pigmentosum Complementation Group E Protein (XPE), Xeroderma Pigmentosum Complementation Group C Protein (XPC), or hRAD23b, unlike in *E. coli* where all the NER factors are necessary for both GGR and TCR [83].

In placental mammals, major UV-induced DNA lesions, such as cyclobutane-pyrimidine dimers (CPD) and 6–4 pyrimidinepyrimidone photo products (64PP) are efficiently removed only by NER [83]. Persistent CPDs can interfere with replication and transcript elongation. Long-term stalling of replication activates the DNA damage response (DDR) associated cell cycle checkpoints and may eventually lead to the generation of double strand breaks (DSB) [84]. Pathways of lesion bypass through the use of alternative DNA polymerases and homologous-recombination-dependent template switching have evolved for damage avoidance during DNA replication [40]. However, unresolved damage-induced transcription arrest triggers cell death [85]. To make matters worse, bypass of DNA lesions in the transcribed strand can cause transcriptional mutagenesis [86][87]. No specific translesion RNAPs are known to bypass helix distorting adducts. Hence, over the course of evolution a highly specialized TCR pathway has developed to selectively repair transcription-blocking lesions, thus enabling the resumption of transcription and gene expression.

3.1 Components and general organization of TCR in human cells

RNAP II (Pol II) trapped at the DNA lesion site constitutes the signal for recruitment of TCR factors [88][15]. To overcome persistent Pol II stalling and to facilitate the access of the DNA repair machinery, cells must combine or choose among different mechanisms: Pol II displacement, backtracking [89], degradation [90], and lesion bypass [91] for template clearance [83][92][93]. There have been no reports of Pol I (transcribing ribosomal RNA in the nucleolus) and Pol III (located outside the nucleolus) participating in TCR in human cells.

TCR is initiated when a lesion-stalled Pol II recruits the TCR specific factors to the damage site. At first, Cockayne syndrome proteins CSA (Cockayne syndrome WD repeat protein A) and CSB (Cockayne syndrome protein B) are recruited which is required for further assembly of the TCR components [94]. Subsequently, the core NER factors and several TC-NER-specific proteins, such as UV-stimulated scaffold protein A (UVSSA), ubiquitin-specific-processing protease 7 (USP7) [95], XPA-binding protein 2 (XAB2) and high mobility group nucleosome-binding domain-containing protein 1 (HMGN1) are engaged. In the next steps, the two sub-pathways of NER, GGR and TCR, converge. The lesion is verified and established as a bona fide NER lesion by the rigorous actions of helix opening and damage verification carried out by Transcription factor II Human (TFIIH), Xeroderma pigmentosum group A-complementing protein (XPA) and Replication protein A (RPA) [96]. Short stretches of single stranded DNA (ssDNA) are created around the lesion by mutual action of XPC-RAD23B and TFIIH. This facilitates the recruitment of XPA and the ssDNA

binding protein RPA to verify the damage, preventing unnecessary repair by aberrant NER complexes formed on undamaged DNA [97]. Finally, the DNA strand containing the lesion is cut by the structure-specific endonucleases XPG and ERCC1-XPF at the 3′ and 5′ side of the lesion, respectively [98]. When the lesion containing oligonucleotide of about 25–30 nucleotides in length is removed, PCNA is loaded onto the DNA by RFC. DNA repair synthesis across the gap is carried out by the DNA replication machinery pol δ/ε/κ-PCNA-RFC-RPA. It is presumed that pol ε is the primary polymerase in replicating cells whereas non-replicating cells mainly utilize pol δ and κ [99][100]. Ligation is also performed by cell cycle specific ligases; DNA ligase I is primarily engaged in NER during the S phase whereas XRCC1- Ligase III complex is the principal ligase involved in the ligation step of NER throughout the cell cycle [101].

3.2 Backtracking model of mammalian TCR

Backtracking of Pol II occurs at the lesion sites in DNA that interfere with the progression of the EC and also at DNA sequences that are difficult to transcribe [102][103] In higher eukaryotes, the employment of a backtracking mechanism similar to that of *E. coli* TCR has been proposed [104][12] although the direct experimental evidence supporting this mechanism is lacking. In order to resume transcription, the protruding nascent RNA in the backtracked EC must be cleaved to reposition the 3′ end of the RNA in the active center of RNAP. In eukaryotes, transcription factor IIS (TFIIS), stimulates the intrinsic transcript cleavage activity of Pol II [105][106]. This activity is analogous to Gre factors in bacteria. Ccr4–Not complex is shown to assist TFIIS recruitment to elongating Pol II during reactivation of arrested EC [107][89]. Pol II backtracking from the lesion would provide the space needed for the TCR machinery to repair the DNA damage. One of the earliest evidences of Pol II backtracking at DNA lesion came from using photolyases, which specifically bind UV-induced DNA lesions and block Pol II. TFIIS induced transcript shortening occurred at the block sites [108]. Moreover, TFIIS is essential for efficient recovery of transcription following UV irradiation and is recruited to sites of damage by CSA [97]. Although there is evidence of backtracking by Pol II during TCR, no bona fide pro-backtracking factors have been identified to date.

The backtracking mode of TCR would be cost effective for cells especially during transcription of very long genes like dystrophin, which span more than 2 megabases and require 16 hours to be transcribed [109]. If backtracking is indeed the predominant pathway of mammalian TCR, then what factors promote Pol II backtracking in human cells? Several mechanistic questions arise regarding this model. How does Pol II manage the trailing nucleosomes while backtracking? The chromatin factors, p300 and HMGN1, which remain associated with stalled RNAPII complexes, might facilitate nucleosome sliding upstream of Pol II to assist backtracking [110].

3.3. When there is no going back

Persistent stalling of Pol II can have dire consequences, such as cell cycle arrest and apoptosis. Therefore, the polymerase must be evicted from the DNA damage site. If backtracking TCR fails, the arrested Pol II is tagged for degradation as a fallback strategy. This leads to transcription abortion and perhaps transcript degradation. Stalled Pol II is first

mono-ubiquitylated by Nedd4, followed by poly-ubiquitylation by the ElonginA/B/C-Cullin5 complex, routing the polymerase for proteasomal degradation [111][90].

Furthermore, the key TCR protein CSB may participate in the displacement of stalled Pol II, as it contains a SWI/SNF2 ATPase domain and has chromatin remodeling activity that is stimulated by the histone chaperone NAP1. Although CSB can in principle push Pol II forward, its ability to dislodge Pol II from DNA has not been demonstrated [112][113].

Another model of Pol II eviction from the lesion site proposes that TFIIH-dependent remodeling of stalled Pol II without release may be sufficient to allow repair. TFIIH induces a phosphorylation-dependent conformational change in the polymerase by reversal clamp module locking, consequently allowing access of XPG to the upstream segment of the transcription bubble [114].

4. Future challenges

The mechanistic understanding of TCR in *E. coli* has formed a basis for the conceptual framework of the design and interpretation of TCR models in higher organisms. In each case, TCR has evolved to handle a broad range of substrates. Both bacteria and humans utilize specialized sets of helicases and ATP hydrolysis to carry out TCR. The strategies to manage lesion in transcribed DNA strand also appears to be conserved with respect to two major mechanisms; RNAP reverse translocation that saves the transcript, and RNAP removal that aborts RNA synthesis. Do the two organism have parallels in decision making about which mode of repair is preferred under a specific physiological state? In the past, CSB was thought to be an analog of Mfd, but its ability to dislodge Pol II from DNA has not been confirmed. Could CSB be an UvrD analog and promote backtracking, or are there other specialized backtracking factors in human cells yet to be identified?

Both bacteria and mammals with compromised TCR display significant phenotypes in response to DNA damaging agents. However, nearly all our knowledge of NER was gained from *in vitro* experiments and studies using cells growing in the laboratory. There is no doubt that cells grow in a different physical and chemical environment within an organism. Remarkably, several modes of cell specific TCR are observed that differ between cell types and throughout differentiation. Important spatiotemporal regulation appears to exist in the organization of transcription and DNA repair with respect to differentiation. Several studies demonstrate that differentiated keratinocytes, neurons and macrophages show decreased removal of UV-radiation-induced lesions at the global genome level, whereas the activity of TC-NER remains mostly unaffected [115][116][117]. The differentiation-driven switch in prioritizing TC-NER over GG-NER is still a mystery. Curiously, in various terminally differentiated cells, such as neurons and macrophages, damage is repaired with equal efficiency in both the transcribed strand of active genes and the non-transcribed strand [118][119][120]. This phenomenon, known as ‘transcription domain-associated repair’ is not yet understood. This pathway is presumed to maintain lesion-free transcription templates and their complementary strands, which are needed for error-free DNA synthesis during repair. Highly specialized mechanisms seem to be employed for long-term maintenance of crucial

transcribed genes that require protection of both strands against the accumulation of DNA damage. All these mechanistic enigmas and questions still remain open for exploration.

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Abbreviations

TCR	Transcription coupled repair
RNAP	RNA polymerase
EC	Elongation complex
NER	Nucleotide excision repair
BER	Base excision repair
CPD	Cyclobutane pyrimidine dimer
6-4PP	6-4 pyrimidine–pyrimidine photoproduct
dsDNA	double stranded DNA
SHX	Serine hydroxamate
4NQO	4-nitroquinoline 1-oxide
NFZ	Nitrofurazone

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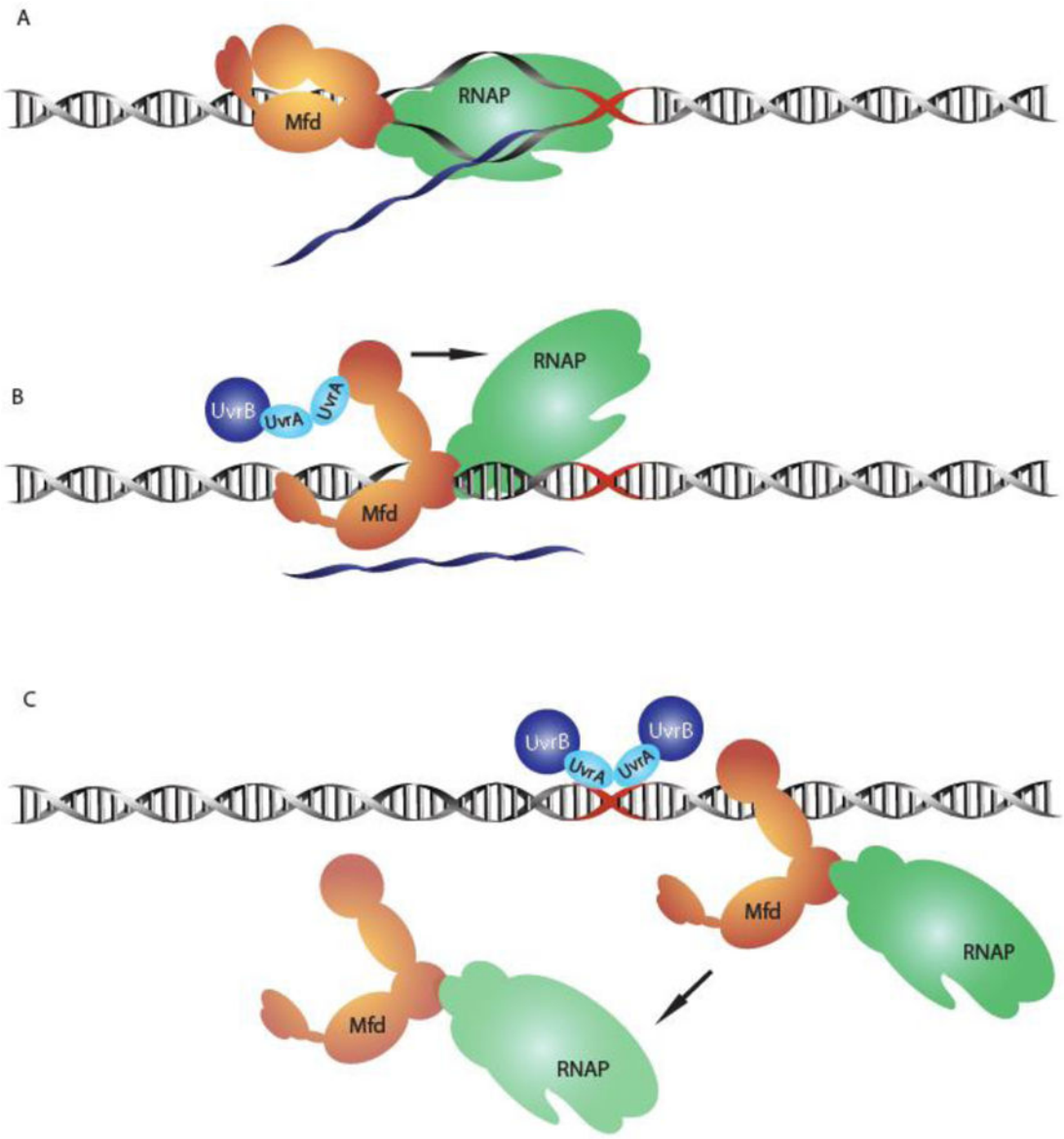

Figure 1. Schematic representation of Mfd mediated TCR

A. Elongating RNAP (green) stalls at DNA lesions in the template strand (Red) and recruits Mfd (Orange).

B. Mfd undergoes a conformational change, recruits the $UvrA_2B$ complex (Cyan, Blue), and promotes forward translocation of RNAP via ATP hydrolysis. The upstream region of the transcription bubble rewinds, resulting in bubble collapse.

C. The elongation complex dissociates. UvrAB initiates the repair process. Mfd remains attached to RNAP, tracks along the DNA, and subsequently dissociates.

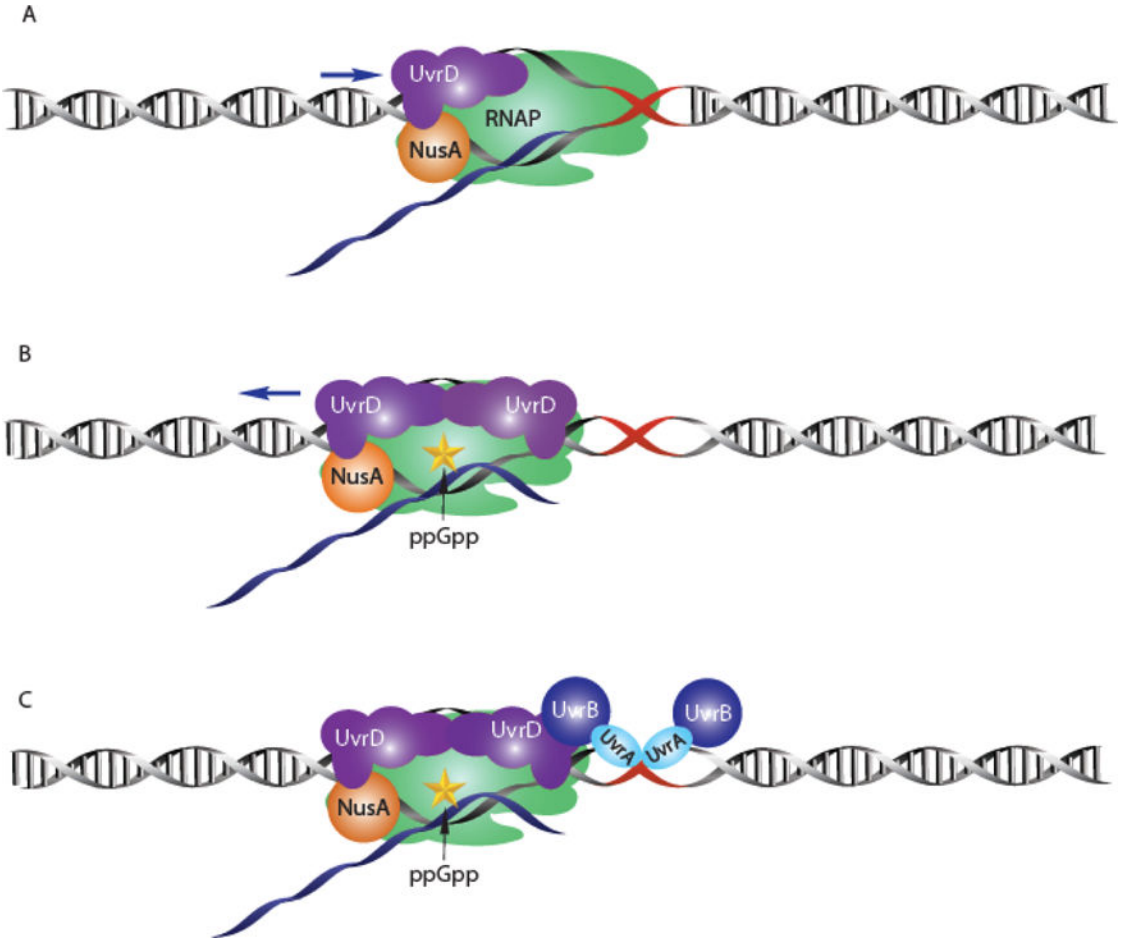

Figure 2. Schematic representation of UvrD mediated TCR

A. UvrD (purple) bound elongating RNAP (green) stalls at DNA lesions in the template strand (Red).

B. The intracellular levels of UvrD and ppGpp increase during genotoxic stress. UvrD forms a helicase competent dimer, which is capable of pulling RNAP backwards by unwinding the upstream fork of transcription bubble. ppGpp (yellow star) binds RNAP and induces backtracking-favoring conformation. NusA also facilitates UvrD-mediated RNAP backtracking to expose the DNA lesion.

C. The UvrAB complex is recruited via UvrD and NusA to initiate the repair process.

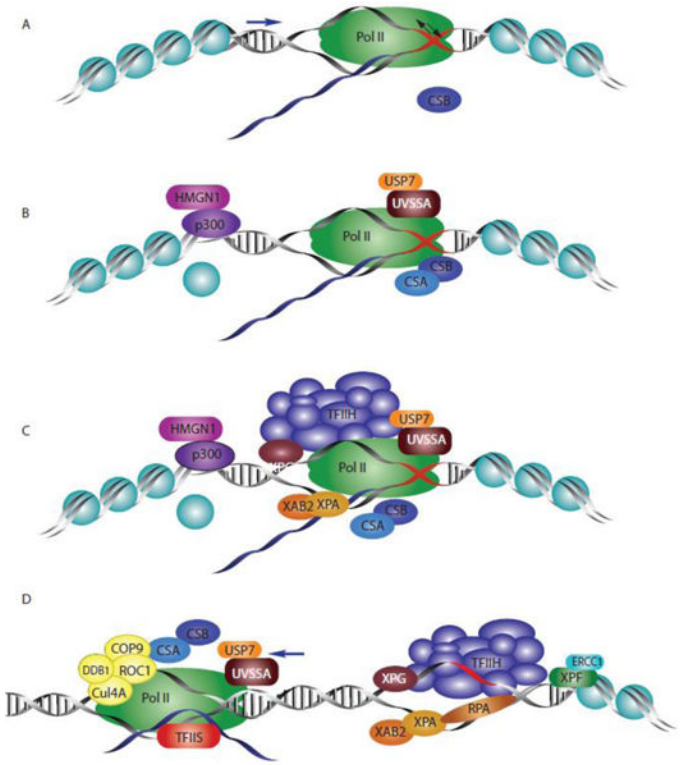

Figure 3. Schematic representation of TCR in mammalian cells

A. During transcription elongation Pol II travels along with CSB. Pol II stalls and masks the DNA lesion (red).

B. Pol II stalling stabilizes the CSB/Pol II interaction. The UVSSA/USP7 complex is recruited to Pol II. CSA is recruited to CSB, Chromatin remodeling factors HMGN1 and p300 clear the upstream nucleosome to make space for Pol II backtracking;

C. TFIIH is recruited to the arrested elongation complex with XPG and XPA is recruited by the XPA-binding protein-2 (XAB2); Pol II backtracks;

D. Replication protein A (RPA) is recruited in the meantime. TFIIH with associated XPG, XPA and RPA remain at the lesion site, the bubble of denatured DNA may be kept open without the RNA–DNA hybrid. XPA and RPA bind the single-stranded DNA near the lesion, lesion verification and strand specificity is determined. After Pol II backtracking, TFIIH denatures about 30 nucleotides around the lesion, DNA nicking is performed by the structure-specific endonucleases XPG and the XPF–ERRC1 complex. TFIIS is recruited to the backtracked Pol II, stimulating its cryptic 3′–5′ exonuclease activity. CSA remains as a component of a cullin-containing ubiquitylation E3 ligase complex. This may help transcription restart after the repair. The factors and their positions are not drawn to scale.