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RNA Polymerase Encounters with DNA Damage: Transcription-Coupled Repair or Transcriptional Mutagenesis?

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1. Introduction

The inner workings of the cell require thousands of proteins, the blueprints for which reside in the millions of DNA basepairs that comprise the genome. This DNA is subjected to a constant barrage of insults, both by exogenous environmental sources and endogenous byproducts of normal cellular metabolism. To ensure the integrity of its genome, the cell has evolved numerous mechanisms to neutralize dangerous species before they can damage the DNA, as well as multiple, often overlapping, pathways to remove the offending lesions once they are formed (reviewed by Friedberg[1]). However, persistent DNA damage could lead to deleterious consequences for the cell. If a significant amount of damage persists, the cell can trigger signaling

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pathways that lead to its ultimate demise. Alternatively, in dividing cells, the damage in the DNA may direct the incorporation of erroneous bases during replication, leading to heritable coding changes that could alter the phenotype of the cell.

The interactions of DNA polymerases with DNA damage during replicative processes, and the contributions of these interactions to mutagenesis and genomic instability, have been extensively studied. However, it is also important to consider how DNA damage affects the other major nucleic acid transaction essential to the cell—transcription. While most cells utilized in the laboratory setting grow continuously in rich media, the majority of cells growing in nature are far less proliferative. In slowly growing or nondividing cells, where DNA replication is greatly diminished or altogether absent (terminally differentiated mammalian cells, for example[2]), transcription must continue to provide the cell with the proteins necessary for normal physiological processes. As such, the transcription machinery is likely to encounter DNA damage much more frequently than the replication apparatus, and would also lead to deleterious consequences for the cell.

With respect to transcription, as with replication, DNA lesions can be generally classified into two distinct categories, cytotoxic and mutagenic, with distinct effects on the cell if left unrepaired.[3] Cytotoxic lesions are those that block the progression of RNA and DNA polymerases. Included in this group are lesions that are bulky and helix-distorting, such as UV-induced bipyrimidine photoproducts and cisplatin—DNA cross-links. Accumulation of this type of damage and the blockages they present are highly toxic to the cell, often leading to cell death. It has long been known that this type of DNA damage is preferentially repaired in actively transcribed genes, and particularly in the transcribed template strand, alleviating the cytotoxic effects of these lesions with respect to transcription. This process is known as transcription-coupled repair (TCR). Mutagenic lesions, on the other hand, typically represent small changes to the normal DNA bases that often alter their base-pairing properties, such as deamination and oxidation products of normal cellular metabolism. In many instances, this latter type of damage does not block RNA polymerase (RNAP) but can lead to incorrect nucleotide incorporation in the RNA during transcription through a process referred to as transcriptional mutagenesis (TM).[4] There seems to be some overlap in these classifications, however. Certain types of oxidative damage, such as 8-oxoguanine, that fall into the mutagenic category and do not block RNAP in vitro, have been shown to be repaired in a transcription-coupled manner, in both bacteria[5] and mammalian cells.[6,7]

This review will focus on recent advances in understanding the mechanisms and implications of TCR and TM, primarily in eukaryotic cells. Where appropriate, lessons learned from prokaryotic systems will be described, although recent and comprehensive reviews on both TCR[8] and TM[4] in prokaryotes are available.

2. Transcription-Coupled Repair

2.1. Insights from Bacterial Systems

2.1.1. Phenomenon of Mutation Frequency Decline

Nearly 50 years ago, initial studies on the mutagenic potential of UV irradiation using auxotrophic *E. coli* strains revealed an interesting phenomenon. After exposure to UV light, cells were plated to select for reversion to prototrophy. However, when cells were first incubated in media containing an energy source (glucose) but not supporting protein synthesis, the number of revertants was reduced.[9] This process became known as mutation frequency decline (reviewed in refs 10 and 11). Mutant strains that were unable to undergo mutation frequency decline fell into two distinct classes. One class was characterized by mutations in the *uvr* genes required for nucleotide excision repair of UV-induced damage.[12] The second class was confined to a single gene, *mfd* (for mutation frequency decline), the protein product of which would remain unidentified for several decades.[12]

There was some inkling early on that repair of the UV-induced DNA lesions occurred preferentially on the transcribed strand. Nearly all of the mutations leading to reversion to prototrophy in the original experiments were suppressor mutations in glutamine tRNA genes rather than true back-mutations.[13] Further, analysis of the sequence giving rise to these mutations following UV treatment revealed that mutation frequency decline only affected those mutations that arose in the template (transcribed) strand. These data led to the conclusion that this phenomenon somehow involved the excision of potentially mutagenic lesions from only the transcribed strand of the DNA,[14] although this would not be proven for several more years. Results of similar experiments using ethane methylsulfonate to introduce DNA damage yielded the same bias, adding support to this hypothesis.[15]

Although strand-specific repair was first directly demonstrated in mammalian cells,[16] it was not long before *E. coli* was also shown to preferentially repair the transcribed strand of an active gene.[17] This discovery brought revision to the existing model that the extensive chromatin organization in mammalian cells was influencing this type of preferential repair. Thus, during transcription, the DNA would be more accessible to DNA repair factors, and this availability was producing the strand bias observed. The revised model hypothesized that the RNAP stalled at a DNA lesion was recognized specifically by the excision repair machinery, thus targeting repair to the transcribed strand. However, reconstituting this system in vitro with purified components indicated that the stalled RNAP actually blocked repair by the UvrABC excinuclease rather than enhancing it, indicating there were probably other components present in the context of the whole cell necessary for coupling RNAP arrest with DNA repair.[18] Selby and Sancar ultimately isolated this additional component by adding back fractionated proteins to their reconstituted system until they had identified a protein with the desired effect of removing the RNAP and stimulating transcription.[19] This protein was termed transcription repair coupling factor (TRCF). The investigators subsequently demonstrated that this protein was the product of the gene mutated in the original *mfd* mutants.[20] Thus, the protein is referred to as TRCF and Mfd interchangeably.

2.1.2. Mfd, The Coupling Factor

Adding back the purified Mfd protein to a defined in vitro assay system demonstrated that the protein was both necessary and sufficient to remove an RNAP stalled at a DNA lesion and to specifically enhance the repair of the transcribed strand by the UvrABC excinuclease complex.[21] Cloning and sequence analysis of the *mfd* gene, as well as detailed biochemical dissection of the Mfd protein, revealed consider-

able information about its function.[21] The gene encodes a protein of 1148 amino acids with domains of sequence homology to UvrB (part of the DNA repair machinery) and a section of RecG (a protein required for branch migration of Holliday junctions) that includes putative helicase motifs.

The Mfd protein is capable of binding nucleic acids independent of sequence context. It prefers a duplex structure (either DNA:DNA or DNA:RNA) over either single-stranded DNA or RNA. Binding to duplex DNA is stable, as assessed by gel mobility shift assays, in the presence of ATPγS, a nonhydrolyzable ATP analogue, or when a mutation is introduced into the Walker A motif required for ATPase activity. These properties indicate that binding to DNA requires ATP but that subsequent hydrolysis allows release. Analysis with a series of deletion mutants localized the DNA binding and ATP hydrolysis activities to the helicase domain.[22]

The Mfd protein can interact with the RNAP, in both the presence and the absence of DNA.[22] The RNAP interaction domain (RID) of Mfd has been identified as a ~200 amino acid region just upstream of the helicase domain[22] and has been shown to associate with the first 142 amino acids of the β subunit of the polymerase.[23] Mutations in the RNAP β subunit that abolish this interaction have recently been identified.[24] The RID is required for RNAP binding, but it is not sufficient for release of a stalled polymerase from the DNA.[22] While deletion analysis only identified the domain necessary for release to the C-terminal 210 amino acids, it is likely that the TRG domain (see below) is the required component.[25] Interestingly, although Mfd can bind both the initiating and elongating RNA polymerase complexes, it is only capable of removing the elongating form of the enzyme.[22] Recent data indicate that removal requires simultaneous interaction of Mfd with the RNAP and with ~25 basepairs of upstream DNA, and that this configuration is at least partially blocked by the presence of the σ_{70} subunit in the initiating complex.[23]

Despite its characteristic helicase motifs, no strand separation activity was ever shown for Mfd.[26] Rather, the sequence that includes these motifs is homologous to RecG, a protein that can translocate double-stranded DNA and facilitate branch migration of Holliday junctions in an ATP-dependent fashion.[27] In addition to the putative helicase domain, which is required for DNA binding and ATP hydrolysis as described above, Mfd also shares with RecG a short sequence just downstream of the helicase motifs (the TRG domain, for translocation in RecG) that is required for coupling ATP hydrolysis with DNA translocation.[25,28] Point mutations in this region of the protein have been shown to disrupt the ability of Mfd to displace RNAP but have no effect on DNA binding or ATP hydrolysis.[25]

Finally, Mfd was shown to associate with the UvrA protein,[21,22] and as might be expected, this interaction occurred via the N-terminal region of Mfd with homology to UvrB.[22] Interestingly, UvrA binding to Mfd precludes its ability to bind UvrB, likely by binding at the same site.[21] This fits well with the known mechanism of the UvrABC excinuclease. An A_2B_1 complex binds at the site of DNA damage through targeting by the UvrA protein. Upon binding to Mfd, UvrA releases UvrB, depositing it at the site of damage. UvrB then recruits UvrC, which cleaves 3′ and 5′ to the lesion,[29,30] excising a 12 to 13 residue oligonucleotide fragment and allowing repair synthesis to occur. Thus, Mfd can actively recruit the $UvrA_2B_1$ complex to the transcribed strand,[26] accomplishing one of the necessary functions of a transcription−repair coupling factor.

2.1.3. Mechanism of Bacterial TCR

Mfd-dependent release of RNAP is not specific to the polymerase stalled at DNA damage but rather is general to RNAP arrested for any reason, including nucleotide starvation[23] or blockage by a protein bound to the DNA.[26] When the elongating RNAP encounters a block, it can change conformation and backtrack along the DNA, extruding the 3′ end of the mRNA.[31] These backtracked polymerases are in a state of arrest, and their active sites are no longer aligned with the 3′-hydroxyl ends of the growing mRNA chains. The RNAP in this state is a target for the GreA and GreB proteins that can stimulate cleavage of the mRNA at the RNAP active site, aligning it with a newly created 3′ end and restoring productive elongation.[32]

A backtracked polymerase can also be rescued to resume elongation by Mfd, but via a very different mechanism (Figure 1). Mfd approaches RNAP from the 5′ side, interacting with both the upstream DNA and the β subunit of the polymerase. Its translocase activity provides a little push to return the polymerase to the forward position and realign its active site with the 3′ end of the primer. If conditions are such that elongation can resume once the roadblock is removed, then the transcription complex advances (Figure 1, bottom left). If, however, the roadblock is still present (e.g. an unrepaired DNA lesion), continued forward motion imposed by the translocating Mfd will eventually disrupt the transcription complex, leading to release of the RNA and polymerase.[8,23] Perhaps then, in the case of DNA damage, Mfd remains bound to the DNA following RNAP release, recruiting UvrA and the repair machinery to the site of damage (Figure 1, bottom right).

2.2. Transcription-Coupled Repair in Eukaryotes

While the mechanism of TCR in bacteria has been elucidated in some detail, the process is significantly more complicated in a eukaryotic setting, and many of the steps in the pathway remain unclear. In eukaryotic cells, a pathway that couples transcription with DNA repair must carry out three important steps. First, it must recognize and remove an RNAP arrested at a site of DNA damage. Second, it must effectively recruit the repair machinery appropriate to resolve the lesion causing the block. And finally, it must have a mechanism to reset the transcription machinery, allowing the recovery of RNA synthesis. Defects in this coupling pathway can lead to the hereditary human disease Cockayne syndrome (CS), characterized by photosensitivity, growth retardation, skeletal and retinal abnormalities, and progressive neural degeneration.[33] Interestingly, however, this disorder does not lead to an increased risk of skin cancer or any other malignancy.[34]

2.2.1. Recognition and Removal of Stalled RNA Polymerase

The vast majority of mutations causing CS occur in genes encoding the proteins CSA or CSB. At the cellular level, defects in these proteins lead to an almost complete inability to repair lesions in the transcribed strand, yet their ability to repair DNA damage in the genome overall is unaffected. Additionally, cells with mutated CSA or CSB are severely impaired in the recovery of RNA synthesis after exposure to DNA damaging agents.[35]

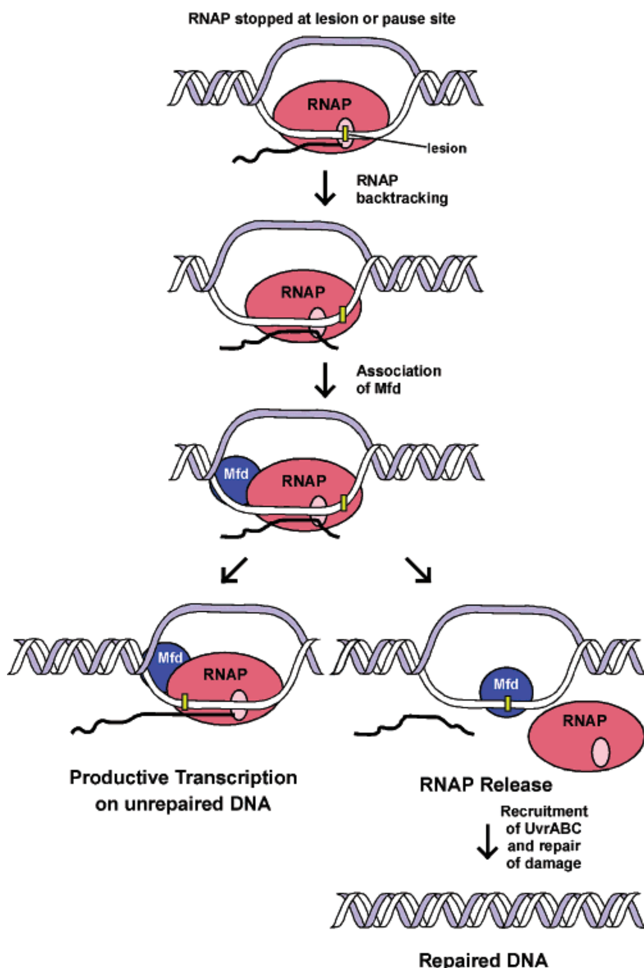

Figure 1. Transcription-coupled repair in bacteria. When RNAP is hindered by a DNA lesion or natural pause site (yellow box), it can backtrack along the DNA, causing misalignment between the polymerase active site and the 3′ end of the mRNA. By virtue of its DNA translocase activity, the association of the Mfd protein with the arrested polymerase stimulates forward motion of the RNAP relative to the DNA, realigning the active site and the mRNA 3′ end. If conditions are favorable (damage has been repaired or can be bypassed), transcription is resumed (left). If transcription cannot resume because the blocking lesion remains, the RNAP and cognate mRNA are disengaged from the template (right). Mfd is then able to recruit the UvrABC excinuclease and promote repair of the lesion via nucleotide excision repair.

Cloning and characterization of the CSB gene revealed a protein of 168 kDa with a region of homology to the Swi2/Snf2 family of helicases involved in chromatin remodeling.[36] Although there is limited sequence homology between them at the protein level, CSB shares many characteristics of the bacterial transcription repair coupling factor Mfd, suggesting it is a functional homologue. CSB binds nonspecifically to DNA, and this binding is stabilized by the addition of nonhydrolyzable ATP analogues.[37] Conversely, it can hydrolyze ATP in a DNA-dependent manner,[37] preferring dsDNA to ssDNA.[38] Also, despite the presence of helicase motifs, CSB also has no classical helicase activity in in vitro assays.[37] It has been suggested that, like Mfd, this portion of the protein renders it a DNA translocase.[39] Although this activity has not been shown for CSB, another Swi2/Snf2 family member RSC has been shown to translocate along DNA in an ATP-dependent manner,[40] implicating translocation as a general mechanism used by these enzymes to move proteins relative to the DNA. Recent data using scanning force microscopy offers an alternative, however, indicating that CSB alters DNA structure by wrapping the DNA around itself.[41] Wrapping requires binding of ATP, whereas ATP hydrolysis results in unwrapping. It is unclear how the presence of other interacting proteins might modulate this activity in vivo.

CSB has also been shown to interact with RNAP II,[42,43] and this interaction can be enhanced by UV exposure.[44] Like Mfd, CSB, as well as its yeast counterpart Rad26p, has been ascribed a function in general transcription[42,45] even in the absence of DNA damage, facilitating bypass of natural transcriptional pause sites.[42] Additionally, Rad26p has been shown to enhance transcription in cells treated with the alkylating agent MMS,[46] indicating that it may stimulate bypass of alkylated bases that might cause the elongating RNAP to pause but likely do not lead to complete arrest. For lesions that do lead to RNAP arrest, such as cyclobutane pyrimidine dimers (CPDs), CSB also influences nucleotide addition, although this is limited to a single nucleotide opposite the first T of the dimer before the polymerase is permanently arrested.[42] In stark contrast to Mfd, the addition of purified CSB to an in vitro reaction containing a transcription complex arrested at a DNA lesion is not sufficient to release the RNAP and nascent mRNA from the DNA template.[47] Interestingly, in mammalian cells the presence of RNAP arrested at the lesion site does not preclude DNA repair, at least the excision step, from taking place.[47,48] Perhaps RNAP remains poised at the lesion site, reactivated by CSB to resume transcription once the block has been removed. In support of this idea, the presence of CSB can counteract the effect of transcription factor IIS (TFIIS),[42] which, similar to GreA and GreB in bacteria, causes backtracking and mRNA cleavage to generate a new 3′ end from which the RNAP can extend. Alternatively, in the context of the cell, CSB may require the concerted effort of other proteins to effectively remove the RNAP from the site of arrest. One could envision both scenarios being useful in different situations.

CSB has also been shown to interact with components of the repair machinery, potentially recruiting them to sites of RNAP-stalling DNA lesions. Work from several laboratories has identified associations between CSB and the nucleotide excision repair proteins XPA[37] and XPG[49,50] as well as with TFIIH, a transcription and repair complex containing the helicases XPB and XPD.[37,49,51] The specific roles of these proteins in the repair process will be discussed below. With the exception of XPG, for which a direct interaction with CSB has been demonstrated using co-immunoprecipitation (co-IP) of in vitro translated proteins,[50] the other associations were identified by either pull-down or co-IP from whole cell extracts and probably represent associations with larger complexes.[49] Certainly, the compositions of these complexes are likely to be dynamic, as size exclusion chromatography indicates that, at least under some conditions, these proteins do not copurify as a stable complex.[43] These interactions are likely to be important to the cell, as certain mutations in XPB, XPD, and XPG also lead to defects in TCR and symptoms of CS.

The role of the gene product representing the other major complementation group in CS, CSA, in the process of TCR is much more obscure. Cloning of the CSA gene revealed that it encoded a 44 kDa protein with multiple WD repeats.[52] Proteins containing WD motifs are functionally diverse and implicated in numerous cellular processes, and these domains

are thought to mediate protein−protein interactions. Thus, proteins with multiple WD repeats could serve as platforms for coordinating assembly of multiprotein complexes. While interactions between CSA and CSB have been demonstrated with in vitro translated proteins and using a yeast two-hybrid system,[52] in the context of the cell, a stable interaction between these two proteins has never been observed. Indeed, each can be purified in large molecular weight complexes lacking the other.[43,53] Despite these observations, transient interactions between these proteins may exist and be very important in the mechanism of TCR. For example, upon exposure of cells to UV irradiation, CSA is translocated from the nucleoplasm to the nuclear matrix, where it colocalizes with RNA polymerase, presumably at sites of DNA damage.[54] This translocation is dependent upon the presence of a functional CSB protein.

CSA has been shown to interact with a number of other interesting proteins. It can bind the p44 subunit of TFIIH,[52] suggesting a possible role in the assembly of the repair machinery. CSA can also bind to XAB2, a newly characterized protein identified by its ability to bind the NER protein XPA.[55] XAB2 consists of several tetratricopeptide repeats, a motif also implicated in protein−protein interactions. In addition to CSA, XAB2 can bind both CSB and RNAP II, and anti-XAB2 antibodies can inhibit TCR when microinjected into cells, indicating that this protein might have a scaffolding function important for protein assemblies involved in the preferential repair of the transcribed strand. Recently, CSA has also been purified as part of a large complex containing DDB1, cullin 4A, and Roc1 that has ubiquitin ligase activity.[53] Interestingly, immediately following UV irradiation, this complex also includes the COP9 signalosome, a negative regulator of ubiquitin ligase activity. Although physiological substrates of this complex remain to be identified, purification of the CSA complex does include a small amount of RNAP II, and this amount is increased in the soluble chromatin fraction after UV exposure. However, it is unclear what events are occurring that might involve these proteins in the insoluble nuclear matrix following UV.

2.2.2. Recruitment of Repair Machinery and Removal of Lesion

2.2.2.1. Nucleotide Excision Repair. TCR has long been considered to be a subpathway of nucleotide excision repair (NER), while global genome repair (GGR) is responsible for excising lesions from the remainder of the genome. NER is a very versatile repair pathway, recognizing and removing numerous types of bulky DNA lesions that share the common feature of distorting the structure of the DNA double helix (reviewed by Dip et al.[56]). Included in this class of damage are CPDs and pyrimidine (6−4) pyrimidone photoproducts (6−4PPs) generated by exposure to UV light, DNA interstrand cross-links generated by chemotherapeutic agents such as cisplatin, and bulky polycyclic aromatic hydrocarbon adducts induced by compounds in cigarette smoke and other combustion products. Repair of these lesions is accomplished through the concerted and highly choreographed efforts of nearly 30 polypeptides which effectively identify the damage, unwind a small region of DNA around the lesion, and make incisions on either side, removing a 24−32 nt oligonucleotide patch of ssDNA containing the damage.[57] The resulting gap is filled by the DNA repair synthesis machinery and religated, resulting in the restoration of the DNA to its original sequence. Mutations in the core NER genes result in xeroderma pigmentosum (XP), a disease characterized by extreme UV sensitivity, parchment skin, freckling, and predisposition to skin cancer largely confined to sun-exposed areas of the body.[58]

The intiation of NER begins with recognition of the lesion by the XPC/HHR23B complex and subsequent assembly of the incision complex.[59,60] For some lesions, such as CPDs, this step is greatly facilitated by the presence of the DDB complex comprised of the XPE and DDB1 proteins.[61,62] Upon lesion binding, XPC/HHR23B recruits TFIIH, a multiprotein complex containing two DNA helicases with opposing polarity, XPB and XPD, which can unwind the DNA locally around the site of DNA damage. TFIIH serves a similar role in transcription initiation, melting the DNA around the promoter.[63] Also recruited to the injury are XPA and RPA. XPA can bind specifically to damaged DNA[64] and probably serves a role in lesion verification, as it has been shown that XPC/HHR23B can bind a bubble structure, but no incisions are made unless damage is present.[65] The DNA bubble generated during NER (~30 nt) corresponds to the optimal binding site of RPA, a single-stranded DNA binding protein complex.[66] RPA binds to the undamaged strand and is likely excluded from the damaged strand by the damage recognition proteins. The polarity with which this complex binds DNA directs the subsequent positioning of the endonuclease components XPG and XPF/ERCC1.[67] XPG is recruited to the complex, likely via its interaction with TFIIH, and the XPF/ERCC1 complex is added via its interaction with XPA. Once the full incision complex is assembled, XPG[68] and XPF[69] make incisions at the 3′ and 5′ edges of the bubble, respectively, removing a 24−32 nt oligonucleotide fragment containing the damage. Subsequent gap filling and ligation require the activities of DNA polymerase δ or ϵ, RPA, RF-C, PCNA, and DNA ligase I.[70]

The XPC protein[71,72] and likely the XPE protein[73] have been shown to be dispensable for TCR, reinforcing the notion of the existence of a damage-recognition mechanism for this process, distinct from that of NER, which most likely involves the stalled RNAP. This is in contrast to the situation in bacteria in which the proteins required for GGR and TCR are likely to be the same. Additionally, a subset of mutations in XPB, XPD, and XPG proteins lead to combined symptoms of XP and CS as well as defective TCR, indicating these proteins have distinct, specialized roles in TCR in addition to their functions in GGR.[58]

2.2.2.2. Base Excision Repair and TCR of Oxidative Damage. Although TCR was long thought to be confined to substrates of NER, evidence is mounting that oxidative base damage, such as 8-oxoguanine (8-OG) and thymine glycol (Tg), generally targets of base excision repair (BER), can also be removed in a transcription-coupled manner.[6,7] BER is mechanistically distinct from NER, recognizing base modifications that do not cause significant distortions in the DNA helix. The damaged base is recognized by a specific DNA glycosylase that hydrolyzes the glycosylic bond, releasing the base but leaving the sugar−phosphate backbone intact and creating an abasic (AP) site. How the repair pathway proceeds from here depends largely upon the nature of the glycosylase. If the glycosylase is monofunctional, such as those that recognize uracil, the AP site is processed by an AP endonuclease, an enzyme that cleaves 5′ of the AP site, generating a 3′-hydroxyl terminus competent for nucleotide addition and a 5′-deoxyribose phosphate (dRP) moiety.

DNA polymerase β (pol β) both adds a nucleotide to the 3′ end of the DNA and removes the dRP,[74] paving the way for DNA ligase III to seal the repaired strand.[75] Some glycosylases, particularly those recognizing oxidative damage, are bifunctional, also possessing a lyase activity that can sever the backbone on the 3′ side of the AP site (reviewed in ref 76). The lyase activity leaves a 3′ unsaturated dRP moiety that must be trimmed by the AP endonuclease[77] to generate a single-nucleotide gap which is subsequently filled by pol β and ligated as above. Regardless of the initiating glycosylase, both arms of BER so far described result in the incorporation of a single nucleotide and are referred to as short-patch BER. If the AP site is either reduced or oxidized and is refractory to dRP removal by pol β, a third pathway exists, known as long-patch BER, in which strand displacement is facilitated by the replication machinery (pol δ/ϵ, PCNA, and RF-C), generating a 2−8 nucleotide flap that can be subsequently cleaved by the flap endonuclease FEN-1.[78] In this instance, ligation is thought to be carried out by DNA ligase I.[79,80] Reviews of BER are included in refs 81 and 82.

TCR of oxidative damage, similar to TCR of UV damage, requires CSB, the XPB and XPD components of TFIIH, and XPG.[7] Other general NER factors, such as XPA and XPF, are not required, however, indicating that these lesions are not simply repaired by NER in this circumstance. TCR via utilization of BER components appears to have different requirements than BER in the global genome. For example, OGG1, the major glycosylase recognizing 8-OG, is not required for TCR, although its absence in the cell has a drastic effect on the repair of this lesion in the genome overall.[6] Candidate enzymes for transcription-coupled BER include the recently described NEIL (nei-like) enzymes that exhibit a preference for excising 8-OG from bubble structures rather than fully duplex DNA,[83] consistent with a role in the excision of damage from a transcription bubble. Additionally, it has been shown that the XPG protein stimulates the activity of human Nth in recognizing oxidized pyrimidines, increasing its affinity for substrate. Interestingly, the endonuclease activity of XPG is not required in this role, as a catalytically inactive mutant of XPG is still able to interact with Nth.[84]

Many questions remain as to how TCR of oxidative damage is accomplished. In vitro, neither Tg or 8-OG effectively block transcription by a mammalian RNAP,[85−88] so how is the signal to initiate TCR delivered? There has been some speculation that other proteins bound tightly to these lesions cause the polymerase to arrest at the site of damage.[7] However, this remains to be proven, and certainly an MSH2/MSH6 complex (involved in DNA mismatch repair) bound at an 8-OG lesion is easily displaced by an elongating RNAP in vitro.[88] It has also been proposed that perhaps it is not the oxidative lesion itself but some subsequent BER intermediate that blocks the RNAP.[89] This is potentially supported by the fact that single-strand breaks are reasonably strong blocks to transcription in HeLa extracts.[85]

In addition to the factors shared by TCR of both oxidative damage and UV damage, there are potentially some factors specific to TCR of one or the other. For example, there seems to be some requirement for BRCA1 and BRCA2 specifically for TCR of oxidative damage, although their exact role(s) remains to be elucidated.[90,91] Interestingly, in BRCA1 or 2 deficient cells, as in CS cells, repair of 8-OG in the transcribed strand of a gene encoded on a transfected shuttle vector is not just reduced to the level of the nontranscribed strand, as is typical of TCR of UV damage, but is completely absent.[91] This would suggest that a stalled polymerase at the site of an oxidative lesion is able to exclude the BER machinery and that possibly RNAP stalled at a single-base lesion is qualitatively different than the case when the same molecule is stalled at a bulky NER-recognized lesion.

While TCR of oxidative damage is an intriguing idea, several important papers reporting data supplied by Steven Leadon have been retracted, and some of the remaining data supporting the current model[7,92] have also recently been called into question.[93] Certainly, independent corroboration of previous findings, as well as new experimental approaches are required to clarify the role and validity of this process as a bona fide DNA repair pathway for certain types of oxidative DNA damage.

2.2.3. Recovery of RNA Synthesis

Following extensive DNA damage, transcription is globally down-regulated, presumably to allow the cell to repair the lesions without having to contend with stalled RNA polymerases. In CS cells, the recovery of RNA synthesis after the damage is severely impaired.[35] After exposure to UV or cisplatin, the initiating hypophosphorylated form of RNAP II (II_a) is rapidly converted to the hyperphosphorylated form (II_o) by kinases that target the C-terminal domain (CTD), presumably preventing reinitiation.[94] Ubiquitinated forms of RNAP can be detected within 15 min of UV irradiation, but this modification is absent in CSA and CSB cells.[95] The half-life of RNAP decreases dramatically under these circumstances, but the protein level can be stabilized by the addition of proteasome inhibitors, indicating that ubiquitination targets RNAP for degradation.[96,97] In this case, the II_o form accumulates at the expense of the II_a form. CTD kinase inhibitors will also slow the degradation of RNAP by blocking the conversion of II_a to II_o.[96,97] These findings, in conjunction with Western blot data using antibodies specific for phosphorylated CTD epitopes, indicate that the hyperphosphorylated form of the polymerase is preferentially ubiquitinated. Finally, experiments using cycloheximide to block new protein synthesis show that, following UV exposure, the level of II_a in the cell never returns to pre-UV levels, indicating that initiation-competent RNAP is not restored by dephosphorylation but rather is generated by a new round of protein synthesis.[96,98]

In the yeast system *Saccharomyces cerevisiae*, the components of the RNAP ubiquitination machinery have been elucidated, and this ubiquitination reaction can be reconstituted in vitro.[99] The E3 ubiquitin ligase Rsp5p has been shown to ubiquitinate RNAP in vitro and is required for the induction of this modification by UV in vivo.[100,101] An additional degradation factor Def1p has been shown to influence the ubiquitination of RNAP. This glutamine-rich protein associates with Rad26p in the salt-stable chromatin fraction.[102] While Def1p-deficient cells are only mildly sensitive to UV, the absence of this protein contributes substantially to the UV sensitivity of strains with mutations in NER proteins, indicating that Def1p has some cellular function during DNA damage. Although its association with the CSB homologue Rad26p might suggest a role for this protein in TCR, the rate of repair of UV damage in the transcribed strand of an active gene is unaffected by *DEF1* deletion. Cells lacking Def1p are unable to ubiquitinate

RNAP, and UV-induced degradation is abolished.[102] This result can be reversed in vitro with the addition of purified Def1p protein to the ubiquitination reaction, showing a direct involvement of this factor in the ubiquitination of RNAP.[99] Unexpectedly, deletion of *RAD26* caused cells to degrade RNAP much more efficiently than wild-type cells in response to UV damage.[102] This finding suggests that Rad26p and Def1p have opposing and counterbalancing properties with respect to RNAP ubiquitination and degradation. Perhaps in the context of a stalled transcription complex, Rad26p protects RNAP from degradation to allow time for repair and potentially resumption of transcription. If transcription cannot resume, Def1p targets the polymerase for ubiquitination and degradation.[102]

The situation in mammalian cells is much less clear and is likely to have significant differences from the case for yeast. A Def1p homologue has yet to be identified, and a functional CSB appears to be required for RNAP ubiquitination, at least in response to UV. However, most CSB-deficient cells used for this type of analysis have point mutations or small deletions in the CSB genes but still express a portion of the CSB protein. A newly described patient with UV sensitive syndrome (UVsS) has been shown to carry homozygous null mutations at the CSB locus.[103] It would be interesting to know if this type of mutation has an effect similar to a *RAD26* knockout in yeast. Also, an RNAP-specific ubiquitin ligase remains elusive, although several candidates have been named.

One attractive ubiquitin ligase for RNAP is the von Hippel−Lindau protein (pVHL)-associated complex known to ubiquitinate the α subunits of hypoxia-induced transcription factors (HIFs) under normoxic conditions, keeping the levels of these transcription factors low and suppressing hypoxia-inducible genes (recently reviewed in refs 104 and 105). Interaction of the pVHL complex with HIF-α subunits is dependent upon hydroxylation of conserved proline residues, and the absence of this modification under hypoxic conditions stabilizes these transcription factors and allows them to upregulate their target genes. Recently, a region of the large subunit of RNAP II with sequence similarity to the pVHL-binding domain of HIF-1α has been identified.[106] An RNAP−pVHL interaction has been demonstrated, and this is dependent on both hyperphosphorylation of the CTD and proline hydroxylation within the pVHL binding domain. It is also greatly enhanced by UV exposure. Additionally, the level of UV-induced RNAP ubiquitination in cells correlates well with the level of pVHL protein.[106]

While early experiments using low doses of H_2O_2 (0.25−2 mM) to induce oxidative DNA damage failed to induce ubiquitination of RNAP,[95] recent evidence suggests that at higher doses of H_2O_2 (10 mM) ubiquitinated RNAP is indeed evident.[107] Of interest, the requirements for this modification in response to oxidative damage are mechanistically quite different from those necessary for the response to UV. While the characteristic and rapid conversion of the hypophosphorylated II_a form of the polymerase to the hyperphosphorylated II_o form occurs, the kinases responsible for phosphorylation of the CTD are clearly different. Following UV exposure, inhibitors of P-TEFb and JNK kinases reduced the phosphorylation status of the CTD, while they had no effect on the response to H_2O_2. Rather, an inhibitor of MEK1/2 (the upstream activator of the MAP kinase ERK1/2) specifically reduced phosphorylation of the CTD and subsequent ubiquitination of RNAP in response to H_2O_2, yet it had no effect when cells were exposed to UV. Additionally, although H_2O_2-directed ubiquitination of RNAP ultimately leads to its degradation in a manner similar to that following exposure to UV, the protein banding pattern of the ubiquitinated RNAP species on SDS PAGE differs between the UV and H_2O_2 cases,[107] suggesting that the polymerase may be modified differently depending upon the lesion that causes its arrest. These differences may be important in recruiting BER vs NER-specific factors to the site of DNA damage. The ubiquitin-containing RNAP species were induced in CSA-, CSB- and pVHL-deficient cells to levels similar to those in wild-type cells, indicating that these proteins are not required for the H_2O_2-induced ubiquitination reaction.[107] It is possible that, following exposure to very high doses of H_2O_2, RNAP ubiquitination is no longer linked to TCR. The identity of the ubiquitin ligase required in this circumstance is still a mystery, although one potential candidate is the BRCA1/BARD1 complex. This complex has ubiquitin ligase activity[108] and associates with RNAP,[109−111] and the absence of BRCA1 has already been shown to be detrimental to the TCR of oxidative lesions.[90,91] That no appreciable ubiquitination of RNAP was observed at lower doses of H_2O_2 suggests that there may be a threshold above which certain signal transduction molecules are activated. This might explain why 8-OG in the transcribed strand on a shuttle vector was not observed to be repaired in CS cells. At levels of damage below the threshold for signal transduction-induced RNAP degradation, and in the absence of any mechanism for bypass, the RNAP is never removed from the lesion.

Adding to the complexity of RNAP ubiquitination in mammalian cells is the recent observation that the CSA protein exists within a multi-protein assembly with potential ubiquitin ligase activity.[53] Although this complex has not been shown to ubiquitinate RNAP, it does associate with the polymerase in a CSA-dependent way. Interestingly, immediately after exposure to UV, within the time frame where ubiquitination of RNA polymerase is occurring, the CSA complex is rendered inactive by the association of the COP9 signalosome, suggesting that CSA may not contribute to RNAP ubiquitination immediately following UV.[53] However, these observations were made using nuclear extracts and solubilized chromatin fractions. Thus, it is unclear how these factors are interacting within the insoluble nuclear matrix.[53] Furthermore, a truncated human homologue of the yeast ubiquitin ligase Rsp5, Rpf1/Nedd4, can bind to and ubiquitinate human RNAP in vitro, adding yet another potential candidate to the list.[100]

Certainly, it is plausible that different RNAP-specific ubiquitin ligases are activated under different cellular circumstances, and it is possible that they target different sites for ubiquitination. In any case, sites of RNAP ubiquitination have yet to be mapped. It is possible that ubiquitination in different sites, or of different linkage types, affects the outcome for both the polymerase and the cell in different ways. There is recent evidence that RNAP polyubiquitination in response to α-amanitin,[112] an inhibitor of elongating RNAP that causes stalling of the polymerase on the DNA, is linked via lysine 63.[113] This linkage is not thought to signal degradation (although α-amanitin treatment causes degradation of RNAP in cells[114]) but may serve as recruitment signals for necessary downstream factors or may modulate the activity of the target protein to which they are attached (reviewed in ref 115). It is also interesting to

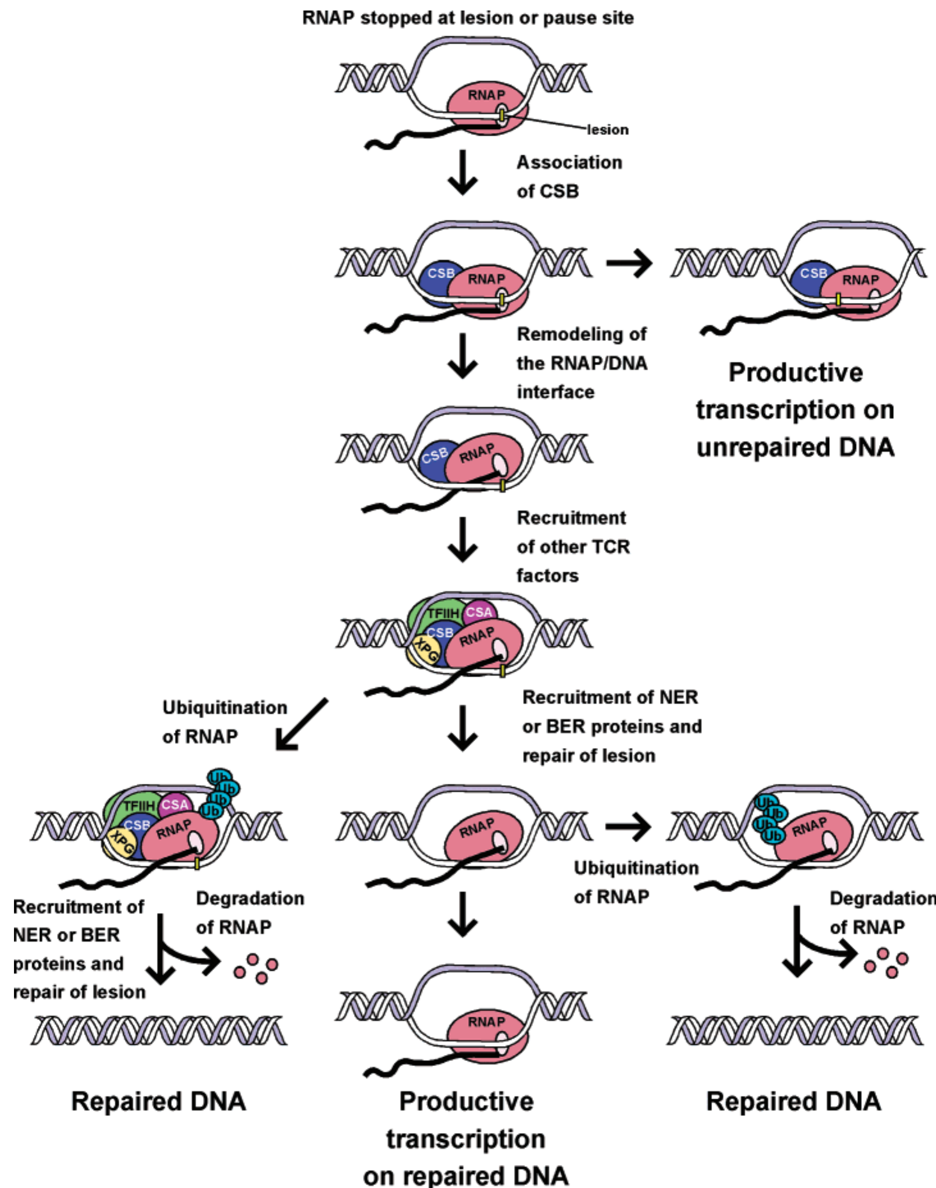

Figure 2. Transcription-coupled repair in eukaryotes. CSB association with an RNAP stopped at a lesion or pause site (yellow box) can provide forward force relative to the DNA. If the site can be bypassed, transcription resumes. If bypass is impossible, CSB potentially remodels the RNAP/DNA interface, allowing for recruitment of other TCR proteins. At this stage the RNAP may be ubiquitinated and removed from the lesion (left), and components of the relevant excision repair machinery are recruited to repair the DNA. Alternatively, the lesion could be repaired in the presence of the RNAP (center), leaving the stalled polymerase poised to resume transcription once the lesion has been repaired. If, after repair of the lesion, the polymerase is still arrested, ubiquitination and degradation at this point represent the last resort for removing the RNAP (right) so that transcription can be resumed by upstream RNAPs.

consider the role that ubiquitination and degradation of RNAP plays in TCR. Perhaps, as suggested in yeast, ubiquitination serves as a final alternative used only when attempts by the TCR machinery to resume productive elongation have failed.[102] Certainly, only 5−10% of the total RNAP is detected as a ubiquitin conjugate following DNA damage or transcription arrest.[95,112]

2.2.4. Model

Taking into account all of the studies discussed above, a potential model can be generated to describe the mechanism of eukaryotic TCR (Figure 2). When an elongating RNAP encounters a DNA lesion or intrinsic pause site in the template DNA, it temporarily ceases forward motion, recruiting the CSB protein to the site of transcription. Stable CSB association is dependent upon ATP hydrolysis,[116] suggesting that CSB can provide movement of the RNAP relative to the DNA (as has been shown for the bacterial Mfd[23]) or that it can somehow remodel the DNA, altering the RNAP/DNA interface.[117] For intrinsic pause sites, and perhaps small base damage such as 8-OG, the influence of CSB may allow RNAP to bypass the site of pausing (Figure 2, top right). If the blocking lesion is bulky, such as a UV-induced lesion or cisplatin cross-link, and RNAP bypass is impossible, the polymerase becomes more permanently arrested.

At this juncture, the presence of CSB induces the recruitment of CSA[54] as well as TFIIH.[51] The XPG protein is also recruited to the complex, likely due to its interaction with TFIIH.[50] The recent discovery of a patient who is homozygous null for the CSB gene is intriguing.[103] This individual suffers not from the severe symptoms of CS but from UVsS, a syndrome characterized by UV sensitivity, but no enhanced predisposition to skin cancer or growth and developmental abnormalities.[118] This observation suggests that abnormal

formation of the TCR complex is clinically more detrimental than no complex formation at sites of DNA damage. It is possible that in the complete absence of CSB, but not in the presence of a mutant version, the ability of THIIS to promote backtracking of the RNA polymerase makes sites of DNA damage more accessible to repair by other means. Indeed, inclusion of TFIIS in an in vitro transcription system allowed subsequent repair of a CPD by a heterologous photolyase and resumption of transcription.[119] Upon assembly of the TCR components, either BER or NER proteins (depending on the type of lesion) will be subsequently engaged in repair and resolution of the lesion.

It is unclear from the data where RNAP ubiquitination fits into this process. One possibility is that RNAP is ubiquitinated after the recruitment of TCR-specific proteins (Figure 2, bottom left). As discussed above, this could require different ubiquitin ligases, could occur at different sites depending upon the lesion to be repaired, and could serve some signaling purpose for the differential recruitment of BER or NER proteins. Ubiquitination may target the RNAP for removal from the DNA and degradation, and this step may be prerequisite for repair of certain lesions. Alternatively, ubiquitination and degradation of RNAP may be the last resort after all other options have been exhausted. Repair of the lesion may not require absolute removal of the RNAP, and rather the polymerase remains bound and ready to resume transcription once the template DNA is restored (Figure 1, bottom center). Perhaps ubiquitination and degradation of RNAP occurs if it is unable to resume productive transcription once repair has taken place (Figure 2, bottom right).

2.2.5. TCR vs GGR

It has puzzled scientists for many years that the developmental and neurological symptoms of CS, thought to be a deficiency in one subpathway of NER, are substantially more biologically severe than the UV sensitivity and cancer predisposition characteristic of XP, caused by a complete deficiency in NER. Several hypotheses have been offered to explain this situation. Of course, none of these are mutually exclusive, and all may play some role in the clinical phenotype of CS.

Some investigators have suggested that it is the inability to repair oxidative damage in a transcription-coupled manner that leads to the severity of CS.[7,120,121] The XP genes with mutations that contribute to CS symptoms have been reported to be required for TCR of oxidative damage.[7] This hypothesis may explain the spectrum of neurological problems associated with CS, as neurons are thought to provide a very metabolically active and highly oxidative cellular environment, potentially contributing to the endogenous levels of oxidative DNA damage that must be repaired for normal cellular function. However, CSA- and CSB-deficient cells have been demonstrated to possess different sensitivities to oxidative stress, with CSA cells being intermediate with respect to their sensitivity (between CSB and WT).[92,122]

An overall defect in general transcription has also been proposed to underlie the severity of CS, particularly because XPD and XPB are components of TFIIH, a basal transcription factor for RNAP II.[123,124] In support of this idea, CSB has been shown to stimulate transcription in vitro, possibly by assisting the transcriptional machinery to push past intrinsic pause sites in the DNA template.[42,116] Additionally, CSB-deficient cells have been shown to have a greatly reduced transcriptional capacity in vivo.[125] Recent studies have shown that CSB also promotes transcription through genes encoding highly structured RNAs including U1, U2, small nuclear RNAs, and 5S RNA.[126] Finally, although TCR was long thought to only be active on RNAP II-transcribed genes, CSB, TFIIH, and XPG have been found in a large complex containing RNA polymerase I. These factors influence the efficiency of rRNA transcription, and mutations in CSB, XPB, and XPD that confer symptoms of CS negatively affect the RNA pol I−TFIIH interaction.[49] Extracts of CSA cells have been shown to have reduced levels of RNAP II transcription in vitro;[127] however, it is unclear what specific effect CSA might have on the other processes related to transcription mentioned above.

One final hypothesis for the significant clinical differences between XP and CS is that a lack of TCR and persistent stalling of RNAP during transcription skews the cellular balance between survival (at the cost of increased mutagenesis) and apoptosis.[3,128] The tumor suppressor p53 is induced at substantially lower UV doses in CSB-deficient cells compared with normal cells,[129] and CSB-deficient cells are much more likely to undergo apoptosis in response to such treatment.[129,130] This observation can be extended to include other NER proteins required for removal of UV damage, although XPC-deficient cells, which are proficient in TCR but not GGR, behave similarly to wild-type cells with respect to their apoptotic potential after UV exposure.[131,132] Furthermore, the trigger for apoptosis and p53 accumulation does not seem to be repair-induced DNA strand breaks but rather persistent lesions in the transcribed strand of active genes.[130,132−134] This hypothesis of increased apoptosis is supported by the fact that, in humans, CS is a multisystem disorder with some features of premature aging, yet these patients have no predisposition to the development of cancer. Thus, premutagenic cells are probably eliminated by DNA damage-induced apoptosis before they have an opportunity to initiate tumorigenesis.

3. Transcriptional Mutagenesis

Up to this point we have discussed the fate of DNA lesions that block the elongating RNAP; however, it is also important to consider transcriptional encounters with DNA damage that result in bypass of the lesion by RNAP. Such events could have serious repercussions on the cell, particularly if the DNA damage that is bypassed has miscoding properties, resulting in the insertion of incorrect nucleotides into the nascent mRNA and generation of a mutant transcript. Until the damage is repaired, this process, referred to as transcriptional mutagenesis (TM), would result in a large population of mutant transcripts that could lead to a pool of mutant proteins with potentially altered functions in the cell (Figure 3).

3.1. Lessons Learned from Prokaryotic Systems

3.1.1. In Vitro Studies

Studies of TM have largely relied on in vitro transcription systems using purified components from either bacteriophage systems (SP6 or T7) or *E. coli* (reviewed in ref 4). Both single-initiation events and multiple-round transcription conditions have been explored, providing a reasonably detailed picture of the types of DNA lesions that can be bypassed by RNAP. In several cases, not only was the

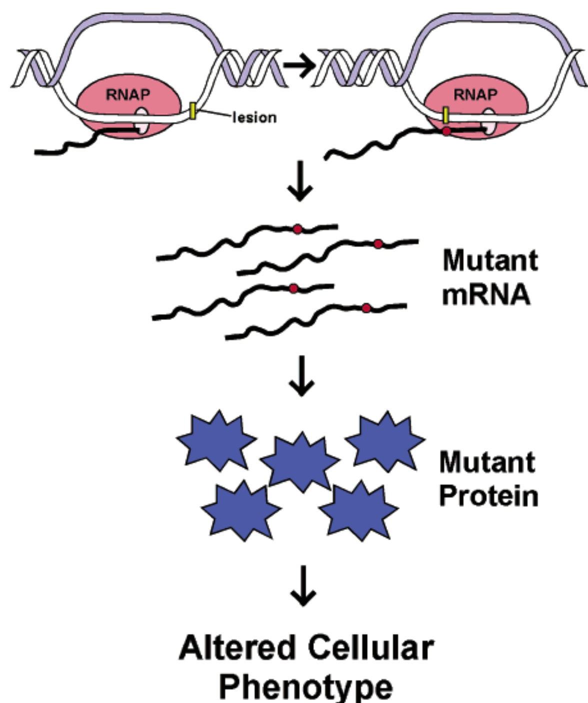

Figure 3. Transcriptional mutagenesis. Transcription past a DNA lesion (yellow box) with altered base pairing properties may lead to the production of a population of mutant transcripts. These transcripts can, in turn, be translated into mutant proteins that could alter the phenotype of the cell.

potential for bypass established, but the sequences of the resulting transcripts were determined to ascertain the mutagenic potential of these lesions. Table 1 presents a compilation of the data from numerous studies by several groups indicating which lesions are bypassed, the relative efficiency with which this bypass occurs, the nature of the RNAP insertion event (if available), and the type of mutation that results.

Bacterial and phage RNAPs readily bypass many types of small base damage that are typically substrates of BER in vitro. These include deamination products such as uracil,[135−139] methylated bases such as O^6-methylguanine,[136] and oxidative base damage including 8-OG,[88,136,140] Tg,[87,141] and dihydrouracil.[135,142] These base modifications miscode at the level of transcription to varying degrees (Table 1). While uracil and O^6-methylguanine are always miscoding (for A and U, respectively),[135−139] dihydrouracil preferentially directs the addition of A,[135,142] although occasionally G is inserted by bacteriophage RNAPs.[135] 8-OG incorrectly directs the incorporation of an A only about half the time, correctly coding for C in other cases.[136,140] No sequence information has been generated regarding the miscoding properties of thymine glycol, but it should be noted that sequence context may contribute to the ability of this lesion to be bypassed.[143] Abasic sites, as well as the abasic site analogue tetrahydrofuran, are also efficiently bypassed by prokaryotic RNAPs.[137,139,140] Adenine was most often incorporated opposite a template abasic site,[137,139,140] although a small fraction of G was incorporated opposite the tetrahydrofuran by T7 RNAP.[140] This pattern of insertion events could be highly mutagenic at the level of transcription, given that depurination at G residues is the most frequent event leading to spontaneous abasic site formation.[144]

Interestingly, transcriptional bypass has been demonstrated for some unlikely bulky adducts as well, albeit with much lower efficiency. N^6-Benzo[*a*]pyrene diol epoxide (BPDE) adducts of both adenine and guanine are bypassed by T7 RNAP.[145,146] While the adenine adducts direct the misincorporation of either A or G,[146] sequence analysis of bypassed G adducts indicates that nonmutagenic C is inserted.[145] However, this study also demonstrated that truncated transcripts resulting from arrest at this lesion contained mutagenic nucleotide insertions, indicating that in some cases RNAP arrest may result from the structural strain of incorrect base-pairing opposite the lesion site. Guanine C-8 aminofluorene (AF) and acetylaminofluorene (AAF) adducts are also subject to some level of bypass, with the bulkier AAF moiety more effective at blocking the transcription machinery.[140] Both lesions were found to be nonmutagenic at the level of transcription, directing the correct incorporation of C.[140]

Remarkably, single-strand breaks and gaps have also been demonstrated to be bypassed by prokaryotic RNAPs.[138,147,148] This event occurs with varying levels of efficiency and depends highly on the size of the gap, the flanking DNA termini, and the type of RNAP assayed. Small gaps are bypassed with higher efficiency than larger gaps,[148] termini containing hydroxyl groups are negotiated better than those containing phosphates or modified sugars,[138] and bacteriophage polymerases are more efficient at bypass than RNAP from *E. coli*.[147] Analysis of the transcripts generated by transcription across single-strand gaps indicates that they contain correctly templated nucleotides on both sides of the gap, but the site of the gap shows up as a deletion in the transcript (a 1-nt deletion for a 1-nt gap, a 2-nt deletion for a 2-nt gap, and so on).[138,147,148]

3.1.2. In Vivo Studies

Although lesions that block RNAP in vitro are likely to elicit TCR in vivo, it was unknown whether bypass in vitro would translate to a similar situation in vivo. An experimental system devised to address this question in *E. coli* had to meet two requirements.[149,150] First, it utilized a reporter plasmid (in this case encoding luciferase) containing a site-specific DNA lesion positioned in the template strand such that if the damage is repaired, the sequence encodes a stop codon, and a truncated protein with no luciferase activity is generated. However, if the damage is misread during transcription, a full-length protein is generated and luciferase activity can be measured to ascertain the extent of bypass. The second requirement for this in vivo system was a method for holding the *E. coli* in a nongrowth state where transcription but not DNA replication is occurring, accomplished by incubation of the cells in novobiocin. This ensures that the luciferase signal measured is the result of transcription across the lesion and not permanent fixation of potential base sequence changes via DNA replication into a heritable mutation. With these tools in hand, the potential for some DNA lesions to cause TM in vivo could be assessed.

Using the experimental design described above, transcriptional bypass of uracil was observed in vivo, giving rise to a phenotypic change characterized by luciferase expression.[5,149] Further, this bypass was enhanced by disruption of the genes encoding the major uracil glycosylases (*ung* and *mug*) in *E. coli*, thereby prolonging the half-life of this inappropriate base in the cellular DNA.[5,149] Interestingly, during the course of the experiment, some uracil is still repaired, indicating the existence of other cellular pathways that remove uracil.[5] Sequence analysis of luciferase transcripts generated in vivo confirm the in vitro result that

Table 1. RNAP Bypass Efficiencies and Insertion Events at Sites of DNA Damage

DNA damage type	RNAP type	rel bypass efficiency[a]	nucleotide(s) inserted[a,b]	outcome
abasic site	phage (SP6, T7)	high[137,139]	A[137,139]	transition or transversion
	E. coli	moderate[137,139]	A[137,139]	transition or transversion
	mammalian RNAP II	high[86]	C[86]	transition or tranversion
	transition or tranversion			
tetrahydrofuran	phage (T7)	high[140]	A, G[140]	transition or transversion
uracil	phage (SP6, T7)	high[135,137,138]	A[135,137,138]	transition
	E. coli	high[**5**,136,137,139,**149**]	A[**5**,136,137,139]	transition
	mammalian RNAP II	high[86]	A, G[86]	transition or nonmut
dihydrouracil	phage (SP6, T7)	high (pauses)[135]	A, G[135]	transition or nonmut
	E. coli	high (pauses)[142]	A, G[142]	transition or nonmut
5-hydroxycytosine	mammalian RNAP II	high[85]	ND	
thymine glycol	phage (T7)	moderate[87,141]	ND	
	mammalian RNAP II	high[85,87]	ND	
8-oxoguanine	phage (T7)	high[88,140]	A, C[140]	transversion or nonmut
	E. coli	high[**5**,136]	A, C, del[**5**,136]	transversion or nonmut
	mammalian RNAP II	high (pauses)[85,86,88,**158**]	C > A[86]	nonmut or transversion
O^6-methyl guanine	*E. coli*	high[136]	U[136]	transition
AF−guanine	phage (T7)	moderate[140]	C[140]	nonmut
	mammalian RNAP II	high (pauses)[155]	ND	
AAF−guanine	phage (T7)	low[140]	C[140]	nonmut
BPDE−adenine (−)	phage (T7)	moderate[146]	A, G, del[146]	transversion
BPDE−adenine (+)	phage (T7)	low[146]	A, G, del[146]	transversion
BPDE−guanine adducts	phage (T7)	low[145]	C[145]	nonmut
single-strand breaks/gaps	phage (SP6, T7)	variable[138,147,148]	del[138,147,148]	frame shift
	E. coli	low[147]	del[147]	frame shift
	mammalian RNAP II	low[85]	ND	

[a] References of studies done in vivo are in bold type. [b] ND = not determined.

RNAP incorporates A opposite to uracil during transcription.[5] Similar studies have also been carried out to address the extent of TM induced by an 8-OG lesion.[5] As with uracil, 8-OG is efficiently bypassed in vivo, giving rise to a measurable luciferase signal. A deficiency in the formamidopyrimidine-DNA glycosylase (Fpg), the major glycosylase responsible for the removal of 8-OG during BER, greatly enhances this bypass. Absence of the *mfd* gene also leads to an increase in bypass, and interestingly, eliminating both Fpg and Mfd has a synergistic effect with respect to the luciferase signal. These data suggest that although 8-OG is not an absolute block to transcription in *E. coli*, it is subject to some level of repair that is coupled to transcription, and that TCR and BER may compete for this lesion. While transcript sequence analysis again confirmed the in vitro data that 8-OG directs the incorporation of A or C, a small fraction of transcripts with a single-nucleotide deletion opposite the lesion site were also identified.[5] This could represent transcription past a single-nucleotide gap generated as an intermediate in the repair pathway, reflecting the results of previous in vitro experiments with *E. coli* RNAP and templates containing single-nucleotide gaps.[138]

3.1.3. Adaptive Mutagenesis

TM induced by DNA damage can potentially generate a substantial pool of mutant mRNAs that could result in the production of mutant proteins that would alter the cellular phenotype. In some cases, these mutant proteins give the cell a growth advantage, or the ability to escape growth suppression, and ensuing DNA replication past the lesion could convert it into a heritable mutation, giving rise to a mutant cell population now permanently expressing this advantageous protein (Figure 4). This process has been called retromutagenesis to reflect the fact that a transcriptional event could lead to a permanent DNA sequence change.[151] Certainly, comparative analysis of the miscoding properties of many lesions indicates that they will miscode similarly during both replication and transcription.[152] Such a process has been proposed to explain "adaptive mutagenesis" induced by starvation in *E. coli*, a setting in which mutations arise rapidly and are confined to those that allow the cells to grow.[153,154] In nonproliferating cells, the contribution of TM to the mutant protein pool, and thus the cellular phenotype, is likely to be much more apparent, especially because the capacities of certain DNA repair pathways are diminished in nondividing cells.[154,155]

3.2. TM in Eukaryotes

3.2.1. In Vitro and in Vivo Studies

While most TM studies to date have utilized prokaryotic systems, data addressing the action of mammalian transcription complexes at nonblocking DNA lesions is beginning to accumulate (see Table 1). The emerging picture suggests that mammalian RNAP can bypass a similar subset of lesions when compared to prokaryotic RNAPs, including the bulky AF−guanine adduct.[156] Accordingly, this lesion is not preferentially repaired on the transcribed strand,[157] reinforcing the requirement to provide at least some block to RNAP for TCR to occur. While abasic sites, uracil, and 5-hydroxycytosine are readily bypassed, single-strand breaks are bypassed by RNAP with a lower efficiency.[85,86] Thymine glycol is also bypassed at high frequency,[85,87] although one study found a small portion of transcripts truncated at the lesion site that are not elongated at later timepoints, indicating that Tg has the potential to arrest the RNAP under certain conditions.[85]

Given the current uncertainties regarding TCR of oxidative DNA damage, a number of recent studies have focused on the interactions of RNAP at sites containing 8-OG. These studies have utilized minimal transcription complexes on C-tailed templates,[86] purified RNAP and transcription factors engaged in promoter-initiated transcription,[88] transcription-competent HeLa nuclear extracts,[85,158] and transfection studies in mouse embryonic fibroblasts.[158] The major conclusion

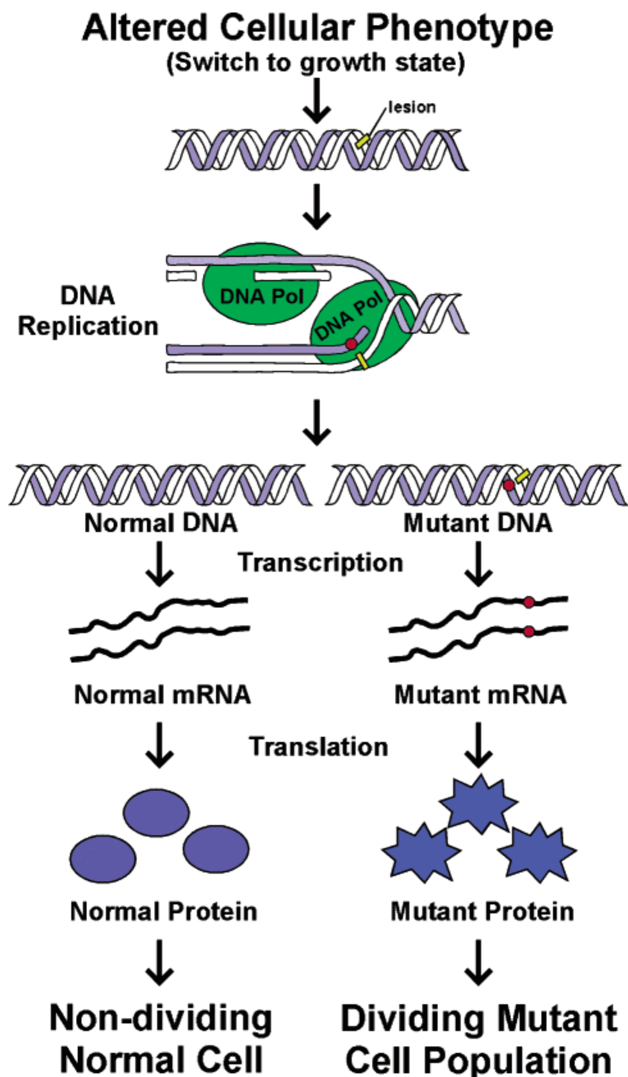

Figure 4. Retromutagenesis. If the mutant protein generated by transcriptional mutagenesis (shown in Figure 3) alters the phenotype of the cell in such a way as to promote growth and initiate a round of replication, then the DNA lesion (if left unrepaired) will be encountered by the replication machinery (top strand, lagging strand synthesis; bottom strand, leading strand synthesis). The lesion will likely cause similar miscoding during DNA synthesis, thus permanently fixing the mutation into the genome of one progeny cell. Subsequent rounds of replication in this progeny will lead to a dividing cell population harboring the mutation that conferred the growth advantage.

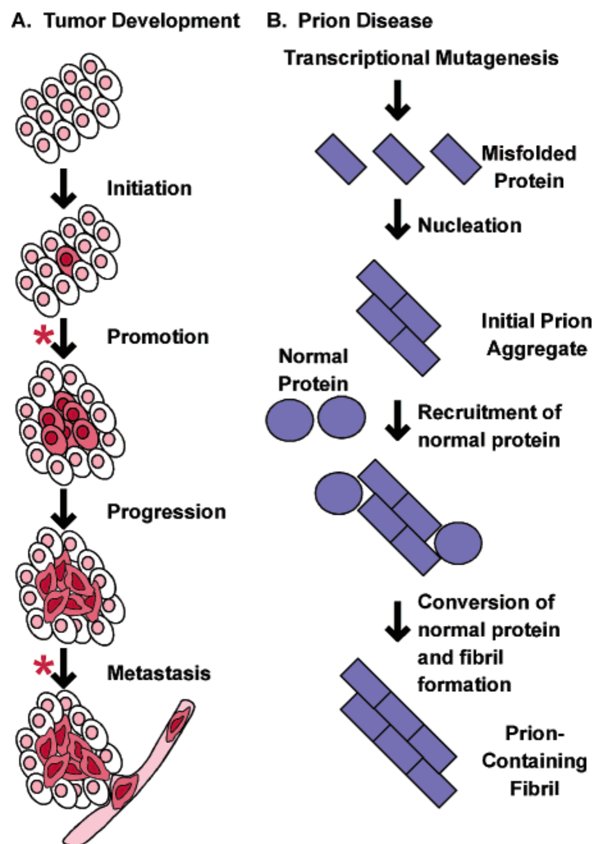

Figure 5. Transcriptional mutagenesis in human disease. (A) A simplified model of tumor development. A role for TM can be envisioned at several steps, especially in tumor promotion (stimulation of cell growth) and metastasis (asterisks). (B) A model for prion formation in neurodegenerative disease. If the mutant protein generated by transcriptional mutagenesis has increased affinity for the prion conformation, it could provide fibril nucleation such that normal proteins are also depleted by way of recruitment to the fibril and conversion to the prion conformation.

from these studies indicates that 8-OG is not a strong block to mammalian RNAP, either in vitro or in vivo. While most of the transcripts generated from 8-OG containing templates were full-length, in some cases a small percentage of shortened transcripts indicative of RNAP paused or stalled at the lesion site was evident.[86,88,158] Pausing at the lesion site is modulated by the relative concentrations of CTP and ATP in the reaction, with higher levels of CTP leading to an increase in the paused species.[86,88]

Sequence analysis of the full-length transcripts generated by transcription past the lesion in mammalian systems has uncovered some interesting differences from prokaryotic systems. While prokaryotic RNAPs insert A opposite an abasic site, purified HeLa RNAP prefers the addition of C.[86] This could greatly reduce the mutagenic potential of this lesion in vivo, as most spontaneous abasic sites occur as a result of depurination at G residues. Uracil directs the insertion of either A or G into the transcript,[86] partially reducing the ability of this lesion to affect TM, with A being mutagenic but G being the correct nucleotide assuming the uracil resulted from cytosine deamination. Similarly, insertions opposite 8-OG are preferentially nonmutagenic C residues, although some A could be detected.[86] It is somewhat puzzling, then, how increasing the CTP concentration during transcription past 8-OG increases pausing. One proposed explanation is that when the 8-OG base is in the *anti* position about the glycosylic bond, it can effectively base pair with C, allowing bypass to occur. Likewise, when the 8-OG base is in the preferred *syn* conformation, base pairing with A allows bypass. However, insertion of C opposite 8-OG when in the *syn* conformation causes errant base pairing and RNAP stalling.[86] A similar scenario has recently been suggested for arrest at UV-induced CPDs and 6−4PPs: that insertion of one or two nucleotides opposite the lesion is necessary to induce arrest and potentially invoke TCR.[159]

3.2.2. Implications for TM in Human Disease

While TM could be viewed as an advantageous property for single-celled organisms, such a cell-selfish process could prove highly detrimental for a multicellular organism (reviewed in ref 160). For example, TM could have a role in the etiology of human cancers (Figure 5A), potentially contributing to various stages of tumor development. One reasonable hypothesis is that the transient expression of

mutant proteins via TM could play a major role in tumor promotion, either stimulating proliferation of an initiated, precancerous cell or allowing it to escape growth-inhibitory signals and undergo clonal expansion. TM might also play a role in the switch to tissue invasiveness that is a hallmark of metastasis.[161]

TM also provides a mechanism for the generation of prions in neuronal cells (Figure 5B). If the mutant protein generated by way of transcription past a DNA lesion is more stable in the β-sheet prion conformation, this TM event could allow the generation of adequate levels of protein to promote prion fiber nucleation, subsequently leading to the conversion of normal protein to the prion conformation and causing fibril formation. One could also envision a role for TM in the generation of other neurodegenerative diseases characterized by aggregates of misfolded proteins, including Alzheimer's and Parkinson's diseases.[162]

4. Concluding Remarks and Future Directions

It is clear from this review of the literature that there are still many gaps in our current knowledge of the interaction between transcription and DNA damage. In light of the recent data, however, perhaps we can revise the encompassing question "Are lesions repaired by TCR or are they bypassed by the RNAP?" to rather ask "What proportion of each lesion is repaired by TCR and what proportion is bypassed?" Certainly, 8-OG and Tg have some potential to stall the RNAP, even if this is not the most common result of an encounter of the transcription machinery with the DNA damage. The ability of these lesions to stall the RNAP may be dependent on sequence context, the conformation of the base when it is met by RNAP, the relative levels of nucleotides or other metabolites in the cell, and the presence of DNA damage-binding proteins. Likewise, traditionally blocking lesions may be bypassed at some minimal rate under some circumstances. However, lesions that are only infrequently bypassed will statistically favor eventual TCR with little effect on the cell. Even if one RNAP bypasses the lesion, the chance of bypass of each subsequent RNAP is statistically reduced. So while one, or even a few, mutant mRNA is made, once the lesion stalls a transcription complex, it will be subjected to TCR.

While our understanding of the outcomes of the encounters of RNAP with DNA damage is expanding, many integral questions regarding TCR and TM remain to be answered. First, it is clear that TCR of oxidative damage needs to be rigorously revisited. Second, the role (or roles) of RNAP ubiquitination in the mechanism of TCR has yet to be fully elucidated. When does this modification occur, and how does it influence the removal of RNAP from the lesion site, recruitment of the appropriate repair machinery, or recovery of RNA synthesis following repair? Also, ubiquitination has only been studied under conditions of induced DNA (and presumably other cellular) damage. Do these events proceed in an analogous way with respect to spontaneously occurring DNA damage? In the absence of a system reconstituted with defined components, our understanding of the TCR mechanism will be somewhat lacking. Of course, the reconstitution of this system will be understandably difficult. In addition to the vast complexity of necessary components, many of the proteins involved are likely to undergo important posttranslational modifications, making it impossible to reconstruct the process with components purified from recombinant systems.

While the few existing studies of TM have begun to shed light on the process, many issues have yet to be addressed. First, the extent to which TM occurs in mammalian cells, and the role this might play in disease processes, is currently unknown. It is interesting that the mammalian RNAP has less of a tendency than prokaryotic RNAPs to misread certain damaged bases (see Table 1). It would be interesting to know the structural basis for this difference and whether it arose via evolutionary pressure selecting against the potentially "cell-selfish" behavior that TM represents.[160] Second, elucidation of all the cellular proteins with an ability to influence TM, such as DNA repair proteins, would be of great utility. In vivo bacterial studies suggest additional, uncharacterized pathways exist for the repair of uracil and 8-OG.[5] Finally, the generation of an experimental system with which to explore the potential of TM for causing retromutagenesis in bacteria or other organisms would be crucial to our understanding of the biological implications of DNA damage bypass during transcription. Future studies addressing these questions will provide clarity to the mechanisms of TCR and TM and further establish the roles of these processes in the context of the cell.

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6. Abbreviations

6−4PP	pyrimidine (6−4) pyrimidone photoproducts
8-OG	8-oxoguanine
AAF	acetylaminofluorene
AF	aminofluorene
BER	base excision repair
BPDE	*N*⁶- benzo[*a*]pyrene diol epoxide
CPD	cyclobutane pyrimidine dimer
CS	Cockayne syndrome
CTD	C-terminal domain
GGR	global genome repair
IP	immunoprecipitation
NER	nucleotide excision repair
RID	RNA polymerase interaction domain
RNAP	RNA polymerase
TCR	transcription-coupled repair
Tg	thymine glycol
TM	transcriptional mutagenesis
UV	ultraviolet
WT	wild-type
XP	xeroderma pigmentosum

7. Note Added in Proof

Reference 7 (Le Page, et al, (2000) Cell 101, 159) has been recently retracted (Le Page, et al, (2005) Cell 123, 711) casting further doubt on the published data from Leadon's group supporting TCR of oxidative DNA damage in mammalian systems.

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