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REVIEW

Transcription-coupled nucleotide excision repair in mammalian cells: molecular mechanisms and biological effects

Maria Fousteri[1], Leon HF Mullenders[1]

[1]*Department of Toxicogenetics, Leiden University Medical Center, Einthovenweg 20, 2333 ZC, Leiden, The Netherlands*

The encounter of elongating RNA polymerase II (RNAPIIo) with DNA lesions has severe consequences for the cell as this event provides a strong signal for P53-dependent apoptosis and cell cycle arrest. To counteract prolonged blockage of transcription, the cell removes the RNAPIIo-blocking DNA lesions by transcription-coupled repair (TC-NER), a specialized subpathway of nucleotide excision repair (NER). Exposure of mice to UVB light or chemicals has elucidated that TC-NER is a critical survival pathway protecting against acute toxic and long-term effects (cancer) of genotoxic exposure. Deficiency in TC-NER is associated with mutations in the *CSA* and *CSB* genes giving rise to the rare human disorder Cockayne syndrome (CS). Recent data suggest that CSA and CSB play differential roles in mammalian TC-NER: CSB as a repair coupling factor to attract NER proteins, chromatin remodellers and the CSA- E3-ubiquitin ligase complex to the stalled RNAPIIo. CSA is dispensable for attraction of NER proteins, yet in cooperation with CSB is required to recruit XAB2, the nucleosomal binding protein HMGN1 and TFIIS. The emerging picture of TC-NER is complex: repair of transcription-blocking lesions occurs without displacement of the DNA damage-stalled RNAPIIo, and requires at least two essential assembly factors (CSA and CSB), the core NER factors (except for XPC-RAD23B), and TC-NER specific factors. These and yet unidentified proteins will accomplish not only efficient repair of transcription-blocking lesions, but are also likely to contribute to DNA damage signalling events.

***Keywords*:** DNA damage, transcription, nucleotide excision repair, transcription coupled repair, Cockayne syndrome, chromatin remodelling

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Introduction

To warrant genomic stability under conditions of continuous genotoxic stress exerted by endogenous and exogenous sources, a network of DNA damage surveillance systems has evolved. DNA damage not only triggers DNA repair pathways, but also signalling pathways that activate cell cycle checkpoints, apoptosis, transcription, and chromatin remodelling [1]. The mechanisms, by which eukaryotic cells sense DNA damage and activate signalling pathways, are still poorly understood. DNA lesions can interfere with transcription and replication and influence chromatin structure thereby effectuating their toxic effects. Among the possible mechanisms, it has been proposed that cells might sense stalled RNA polymerase or abortive transcripts to monitor DNA integrity and to activate DNA damage signalling [2]. Persistent blockage of transcription has severe consequences for the cells as this event might be a strong signal for P53 dependent apoptosis. To counteract apoptosis and to avoid collapse of replication forks with the stalled transcription machinery, cells remove the transcription-blocking DNA lesions by transcription-coupled nucleotide excision repair (TC-NER), a specialized subpathway of nucleotide excision repair (NER), first identified by Hanawalt and coworkers [3, 4].

Interestingly, the toxic effects of DNA damage-mediated by blockage of either transcription elongation or replication, are relieved by different mechanisms. Interference of replication by DNA lesions is resolved by the recruitment of a special class of translesion synthesis DNA polymerases capable of bypassing damaged DNA templates. As mentioned above, stalled transcription elongation by a DNA lesion is counteracted by the activation of the specialized TC-NER pathway. In this review we focus on recent advances in understanding the molecular mechanism of TC-NER and its biological implications for cells and whole

Correspondence: Leon HF Mullenders
Tel: +31-71-5269603; Fax: +31-71-5268284
E-mail: l.mullenders@lumc.nl

organisms.

Global and transcription-coupled nucleotide excision repair

NER is a multiprotein repair system capable of removing a wide variety of DNA helix distorting lesions such as UV-induced photolesions (cyclobutane pyrimidine dimers (CPD) and pyrimidine 6-4 pyrimidone photoproducts (6-4PP)) and DNA adducts induced by chemicals like aflatoxinB1 and N-acetoxy-2-acetylaminofluorene (NA-AAF). The impact of deficiencies in NER for human health has been best manifested by the existence of rare autosomal recessive human disorders such as xeroderma pigmentosum (XP), Cockayne syndrome, and trichothiodystrophy all associated with sensitivity to sunlight. Cells from XP patients are sensitive to UV-light and chemicals inducing bulky DNA lesions, and complementation studies revealed eight genes involved in the disease (XPA–XPG and the variant form XP-V). NER works through a "cut-and-patch" mechanism by excising and removing a short stretch of DNA containing the lesion and subsequent restoration of the original DNA sequence by polymerization / ligation using the non-damaged strand as a template [5]. Global genome NER (GG-NER) repairs DNA lesions throughout the genome but its repair efficiency varies across the genome most likely influenced by the chromatin environment [6, 7] whereas TC-NER, as mentioned earlier, is confined to repair of DNA lesions in transcribed strands and coupled to active transcription. The hallmark of TC-NER is the accelerated repair of DNA lesions that efficiently block the elongating RNA polymerase II complex (RNAPIIo).

It is generally assumed that RNAPIIo stalled at a DNA lesion efficiently triggers the recruitment of TC-NER

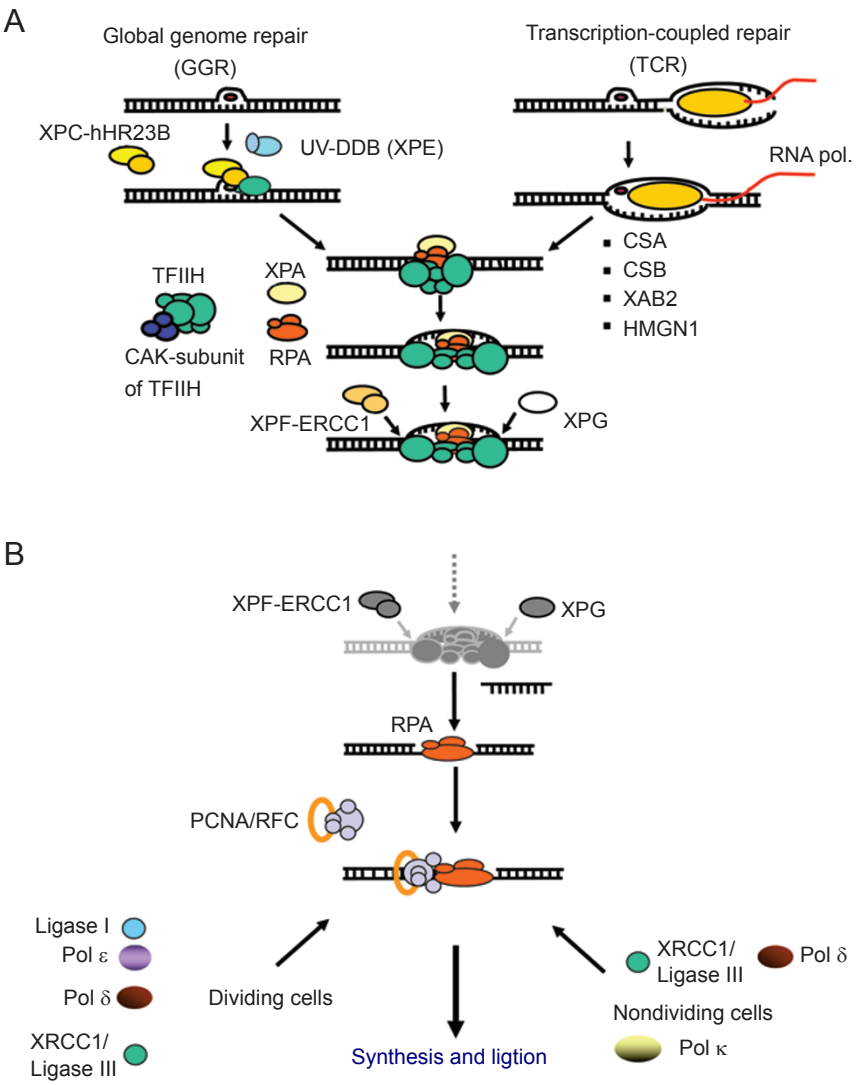

Figure 1 Two subpathways of mammalian NER. **(A)** 1. Damage/distortion recognition in GG-NER and TC-NER. XPC-RAD23B and UV-DDB complexes recognize and bind to DNA damage-mediated helix distortion and initiate GG-NER. TC-NER is triggered by DNA damage-mediated blockage of RNAPIIo. 2. Lesion demarcation. In the next steps, the two sub-pathways converge. The lesion is verified and demarcated as a *bona fide* NER lesion by the concerted actions of helix opening and damage verification provided by TFIIH, XPA and RPA. 3. Dual incision. Within the pre-incision complex, ERCC1-XPF and XPG structure-specific endonucleases incise the damaged strand. **(B)** Gap filling and ligation. After dual incision around the lesion, the single strand gap is filled by DNA polymerase δ, PCNA and RFC, and sealed by DNA ligase III-XRCC1 in both dividing and non-dividing cells, whereas DNA polymerase ε and DNA ligase I are involved in dividing cells in addition to DNA polymerase δ and DNA ligase III-XRCC1. Although the involvement of these proteins has only been demonstrated for GG-NER, it is thought to hold for TC-NER as well.

specific factors and NER proteins whereas in GG-NER the damage-induced DNA distortion is recognized by the UV-DDB (DDB1-DDB2-containing E3-ubiquitin ligase complex) and XPC-RAD23B protein complexes (see also Shuck *et al.* in this issue). Once the lesion has been recognized, all subsequent steps leading to assembly of a functional NER complex, require the same NER core factors in GG-NER and TC-NER (Figure 1). The minimal set of proteins required to perform complete GG-NER (more than 30 polypeptides) has been defined using *in vitro* reconstituted systems [8, 9] and specific roles have been assigned to the various factors involved. The DNA damage recognition protein complexes UV-DDB and XPC-RAD23B are required for the efficient recruitment of all of the following NER proteins to the damaged DNA [10-13]. The basal transcription factor TFIIH has an essential role in NER as two of its components, i.e. the proteins encoded by the XPB and XPD genes exert their DNA-dependent ATPase and DNA-dependent helicase activity, respectively, to open up the DNA helix around the lesion [14-16]. The combined action of XPC-RAD23B and TFIIH creates short stretches of single stranded DNA (ssDNA) around the lesion that facilitates the recruitment of XPA and the ssDNA binding protein RPA to subsequently verify the damage, preventing gratuitous repair by aberrant NER complexes formed on undamaged DNA [17]. Finally, the DNA strand containing the lesion is cut at the single- to double-strand DNA transitions by the structure-specific endonucleases XPG and ERCC1-XPF (which cut at the 3' and 5' side of the lesion, respectively) [18, 19]. Presumably, after the oligonucleotide (25-30 nt in length) containing the lesion has been removed, PCNA is loaded onto the DNA by RFC as is the case in DNA replication [20]. DNA polymerases δ and ε are capable of DNA repair synthesis across the gap using the undamaged strand as a template; the remaining nick can be sealed by DNA ligase I [8, 9]. Recent research developments have suggested that DNA polymerase κ may also play an important role in NER [21], emphasising the need to confirm the roles of these late factors in NER *in vivo*. Similarly, recent evidence [22] points to the XRCC1-Ligase III complex as the principal ligase involved in the ligation step of NER throughout the cell cycle in addition to DNA ligase I that is mainly engaged in NER during the S phase.

Transcription-coupled nucleotide excision repair requires specific factors

TC-NER is a strongly conserved repair pathway identified in a variety of organisms including bacteria, yeast and mammals. However, in some organisms (e.g. the fruitfly *Drosophila* [23] and the archaeon *Sulfolobus solfataricus* [24]) TC-NER could not be demonstrated suggesting that alternative pathways may deal with DNA damage inhibited transcription. Pioneering work by Selby and Sancar [25] has lead to the identification of a 130 kDa protein encoded by the *mfd* gene that is essential for TC-NER in UV-irradiated *E. coli* cells. The Mfd protein also termed TRCF (transcription-repair coupling factor) releases the RNA polymerase and the truncated transcript from the DNA in an ATP dependent manner allowing repair of the DNA damage by attracting NER factors particularly UvrA [26]. In the yeast *S. cerevisiae,* TC-NER involves the mfd counterpart encoded by the Rad26 gene [27]. Similar to the bacterial TC-NER, evidence has been presented in yeast that in some situations, a damage-arrested RNA polymerase might be released from the template by a mechanism that leads to its ubiquitylation and degradation [28]. This process requires the DNA damage dependent interaction of a protein encoded by the *DEF1* gene with the TC-NER specific factor Rad26. However in contrast to the bacterial situation, Rad26 does not promote degradation of the RNA polymerase, but instead it is an inhibitor of its degradation, allowing the transcription block to be first repaired by the TC-NER machinery. As a last resort, when DNA damages such as UV photolesions can not be accessed by TC-NER, Def1 promotes recruitment of the ubiquitylation machinery leading to modification and degradation of the arrested RNA polymerase complexes so that repair can take place by alternative ways.

Specific factors in TC-NER have also been identified in mammalian cells. Strand specific repair measurements of UV-induced CPD in transcriptionally active genes in cells from patients suffering from Cockayne syndrome (CS) revealed impaired TC-NER, i.e. CS cells lack the fast repair of CPD in the transcribed strand of active genes [29]. CS is a rare disorder that is associated with a wide variety of clinical symptoms including dwarfism, mental retardation, cataract and eye abnormalities as well as photosensitivity, but no enhanced susceptibility to cancer. A hallmark of CS at the cellular level is the inability to resume damage-inhibited DNA and RNA synthesis after exposure to UV-light and certain chemical agents that induce bulky DNA adducts [30, 31]. Although it is generally assumed that the inability of CS cells to perform TC-NER underlies the lack of RNA synthesis recovery after the induction of DNA damage, other mechanisms might be involved as well in the transcription response (see the later section). Complementation studies with recovery of RNA synthesis after UV-light exposure as an endpoint have been performed to identify two CS complementation groups, CSA and CSB. A third group encompasses patients with mutations in *XPB, XPD* or *XPG* genes exhibiting both XP and CS symptoms. In addition, patients exist that display a mild UV sensitivity and

a TC-NER defect, with their cells lacking RNA synthesis recovery after UV-light exposure [32]. These patients do not exhibit other characteristics of CS and are diagnosed as UV-sensitive syndrome patients (UVsS). Cell lines from two UVsS patients have a homozygous null mutation in the CSB gene [33], indicating that the truncated CSB polypeptides found in some CS patients, might trigger the more dramatic effects that give rise to the severe CS phenotype. Nonetheless, UVsS is a genetically heterogeneous disease as other unrelated UVsS patients have no mutation in the CSB gene [33].

The CSB gene encodes a 168 kDa protein and is related to the SWI/SNF family of ATP-dependent chromatin remodellers. SWI/SNF family members such as SWI2/SNF2 are putative helicases characterized by seven typical domains. The CSB protein contains helicase domains that show strong homology with similar domains in SNF2-like proteins and as most members of this family, displays DNA-dependent ATPase and DNA binding activity, but not helicase activity. In addition, CSB has nucleosome remodelling activity and binds to core histone proteins *in vitro* [34]. Interestingly, both the bacterial and yeast counterparts of CSB, i.e. Mfd and Rad26 respectively, are DNA-dependent ATPases. Different approaches provide evidence that CSB might have an impact on transcription itself. Transcriptome analysis of CS-B cells revealed deregulation of gene expression similar to that caused by agents that disrupt chromatin structure [35]. When added to a stalled RNAPIIo (at a CPD photolesion) CSB can stimulate transcription elongation by addition of one nucleotide to the nascent transcript, but not bypass of the lesion [36]. In addition, CSB plays a role in initiating the transcriptional program of a subset of genes after UV-irradiation [37]. It is conceivable that these features may underlie some aspects of the complex clinical phenotype of CS patients. However, it is clear that not all clinical features of CS in the combined XP/CS patients can be easily explained based only on the above functions of CSB. A recent study by Tanaka and Egly [38] has provided some clues to understanding the pathogenesis of the XP/CS combined phenotypes. They showed that XPG is a component of TFIIH that functions in preserving the architecture of TFIIH, whereas XPG mutations found in severe XP-G/CS patients disturb the interaction of both XPD and the TFIIH associated CAK complex to the core TFIIH resulting in defective transactivation of nuclear receptors, thus providing a link between the CS features and a defect in basal transcription and transactivation as well as defective TC-NER.

The protein encoded by the CSA gene contains WD-40 repeats [39], a motif known to be involved in protein-protein interactions. Using cells expressing epitope-tagged CSA, it was shown that CSA is part of an E3-ubiquitin ligase (E3-ub ligase) complex consisting of DDB1, Cullin 4A and ROC1/Rbx1 proteins [40]. The ubiquitin ligase activity is stimulated by the covalent attachment of the ubiquitin-like protein Nedd 8 to Cullin 4A. In response to UV, the COP9 signalosome (CSN) was found to associate with the CSA complex resulting in the deneddylation of Cullin 4A and in the inactivation of the ubiquitin ligase activity of the CSA complex at least at the early times after UV irradiation. These data suggest that the CSA (E3-ub ligase) complex when engaged in TC-NER is an inactive ubiquitin ligase [40, 41].

Two other factors, XAB2 and HMGN1, have been identified that play a role in TC-NER although no UV sensitive patients have been associated with mutations in these genes. XAB2 is an essential TPRs- (tetratricopeptide repeats) containing protein involved in pre-mRNA splicing and transcription, and has been identified as an XPA binding protein and might function as a scaffolding factor for protein complex formation in TC-NER [42]. In addition to XPA, XAB2 interacts with chromatin bound stalled RNAPIIo complex in a UV- and CS-dependent manner [41]. In agreement with these data, Tanaka and coworkers [43] showed in a recent study that XAB2 interacts with RNAPIIo and that this interaction is enhanced after DNA damage. Knock-down of XAB2 resulted in hypersensitivity of cells to cytotoxic effects of UV light and led to reduced RNA synthesis recovery. Although it remains to be clarified whether CSA alone or in cooperation with CSB recruits XAB2, it is likely that recruitment is mediated via CSA as proteins with WD-40 domains (CSA) and TRP-repeats (XAB2) are known to interact [44].

HMGN1 is a nucleosome binding protein that competes with histone H1 for binding to nucleosomal linkers and modulates posttranslational modifications of the H3 N-terminal tail [45, 46]. Interestingly, HMGN1 deficiency leads to UV sensitivity in UV-B irradiated *HMGN1* KO mice and impaired TC-NER in UV-C irradiated mouse embryonic fibroblasts [47]. The role of HMGN1 in TC-NER is further highlighted by the recent observation that HMGN1 interacts with UV-stalled RNAPIIo and this interaction depends on CS proteins [41].

TC-NER and transcription response: a complex relationship

Solid evidence exists for a key role of TC-NER in the response to DNA damaging agents that induce lesions that inhibit transcription elongation such as UV photolesions, bulky DNA adducts and DNA-protein complexes induced by topoisomerase I inhibitors [48]. Indirect evidence coming from analysis of mutation spectra, suggests that also less-distorting DNA lesions such as certain types of

alkylation damage, might be processed by TC-NER [49]. However, direct measurement of the repair of N-methylpurines in specific DNA sequences in Chinese hamster ovary cells provided no evidence for involvement of TC-NER [50]. It is unclear whether oxidative damage (e.g. 7,8-dihydro-8-oxoguanine (8-oxoG) and other lesions) is repaired by TC-NER as evidence supporting or being against TC-NER-dependent repair of 8-oxoG has been described [51, 52]. Controversy exists with respect to the transcription-blocking capability of oxidative DNA damage. *In vitro* experiments have shown that 8-oxoG does not block RNAPIIo and that thymine glycols cause only transient pausing [53, 54]. Consistent with these observations, no significant obstruction of transcription was observed *in vivo* employing a luciferase expression vector containing an 8-oxoG lesion [55]. In contrast, both 8-oxoG and thymine glycol reduced the expression of a reporter gene in a host cell reactivation experiment in which the defect appeared to be more pronounced in CS-A and CS-B cells [56]. The situation is further complicated by recent studies, that have implicated a role for CSB in the removal of 8-oxoG from the overall genome, independently of both Ogg1-mediated base excision repair and regular transcription [57]. Taken together, there is no convincing evidence for a role of TC-NER in oxidative damage repair.

The assumption that TC-NER is required to recover RNA synthesis after DNA damage has been challenged by analysis of TC-NER and RNA synthesis recovery following different genotoxic exposures in human cells [31, 58]. CS-A and CS-B cells are hypersensitive to the cytotoxic effects of NA-AAF, a chemical that induces C8-dG-AF and C8-dG-AAF adducts. The kinetics of removal of these DNA adducts is similar in both strands of expressed genes in CS-B and normal cells, suggesting that these lesions are repaired by GG-NER. Curiously, NA-AAF inhibited RNA synthesis does recover in normal cells, but not in CS cells. Several mechanisms may explain the results. One possibility is that the primary defect in CS cells might be related to a defect in transcription initiation rather than a defect in TC-NER. *In vitro* transcription assays revealed that transcription initiation is persistently inhibited in extracts of UV-irradiated CS-B cells due to depletion of the transcription initiating RNA polymerase IIa (RNAPIIa) [59]. Proietti-De-Sanctis *et al.* showed a defective reinitiation of transcription of housekeeping genes in CS-B cells due to impairment of transcription initiation complex formation [37]. This phenomenon was not observed for p53 responsive genes indicating that these genes can be transcribed in the absence of CSB. The role of CSB in initiation of housekeeping genes after UV could be related to CSB-mediated chromatin remodelling events facilitating the recruitment of TBP and other factors to promoters after UV irradiation [37]. These data together indicate that transcription inhibition and recovery is unlikely to be governed by a single mechanism.

TC-NER: a survival pathway with anti-mutagenic properties.

The existence of TC-NER as a defense mechanism to DNA damage implies that fast removal of transcription-blocking lesions is crucial for cells and organisms to escape from lethal effects of transcription inhibition. The blockage of RNAPIIo activates a stress response leading to specific modifications of p53 at Ser 15 and to stabilization of the protein. A persistent block of transcription by UV-light might lead to p53 dependent apoptosis in repair proficient cultured cells and in the epidermis of repair proficient mice [60, 61]. Interestingly, in repair deficient cells including CS-B fibroblasts [62] and in the epidermal keratinocytes of *CSB* deficient mice (Stout *et al.*, unpublished results) UV light induced apoptosis is independent of p53, in spite of the fact that p53 is stabilized by UV. This suggests that in repair proficient cells, the TC-NER pathway protects against UV-induced apoptosis in a p53 dependent manner, but that p53 does not contribute strongly to the induction of apoptosis in TC-NER-deficient fibroblasts and mouse epidermis.

The protective role of TC-NER against genotoxic exposure has been convincingly demonstrated in mouse models with defined mutations in NER genes, i.e. XPE (DDB2), XPA, XPC or CSB deficient mice. *XPA–/–* mice are deficient in both GG-NER and TC-NER, whereas *XPC–/–* and *CSB–/–* mice are defective in GG-NER and TC-NER respectively. *DDB2–/–* mice are deficient in GG-NER of CPD, but are otherwise TC-NER proficient. When hairless mice were exposed to UVB light and the minimal erythema dose (MED) was estimated, it appeared that *XPA–/–* and *CSB–/–* mice were about 10-fold more sensitive to induction of erythema or edema than WT, *XPC–/–* or *DDB2–/–* mice [63, 64]. This difference in sensitivity coincided with a pronounced difference in apoptosis and cell cycle progression: the *XPA–/–* and *CSB–/–* mice appeared to display UVB induced apoptosis at much lower UV dose than the *XPC–/–*, *DDB2–/–* and WT mice [61, 64]. Similar observations were made when mice were exposed to the polycyclic aromatic hydrocarbon DMBA (a potent rodent mutagen and carcinogen) that induces DNA lesions being processed by NER [65].

The faster repair of CPD from the transcribed strand of expressed genes compared to the non-transcribed strand raises questions on the consequences of TC-NER with respect to the frequency and nature of mutations induced by photolesions. Mutation spectrum analysis showed that

almost all mutations induced by UV-light in the *Hprt* gene of rodent cells were found at di-pyrimidine positions in the non-transcribed strand. In contrast, most mutations in CS-B deficient cells were found at di-pyrimidine sites in the transcribed strand demonstrating a strong protecting role of TC-NER against UV induced mutations [66]. Also in tumors isolated from UV-B irradiated mice (with high frequencies of p53 mutations), defective TC-NER (*CSB –/–* and *XPA–/–* mice) resulted in increased mutations in p53 through UV-targeted di-pyrimidine sites in the transcribed DNA strand of the gene. Most strikingly, only *XPA–/–* and *CSB–/–* mice developed initially many benign papillomas before squamous cell carcinomas (SCC) developed at a more explosive rate to outnumber the papillomas [67]. These papillomas carried mutations in the 12th Hras codon with a dipyrimidine site in the transcribed strand; such mutations were not observed in the UV-induced SCCs. Evidently, proficient TC-NER prevents Hras mutagenesis and therefore prevents the development of papillomas.

Roles of CS proteins in TC-NER

This section and Figure 2 summarizes the various steps involved in the assembly of the eukaryotic TC-NER complex.

DNA damage and stalled RNAPIIo

To dissect the mechanisms underlying the various aspects of TC-NER, the majority of experiments have been performed with UV-C irradiated cells or cells treated with cisplatin. The two main photolesions induced by UV-C, CPD and 6-4PP are both severe blocks for RNAPIIo, and in line with this finding, TC-NER removes CPD and 6-4PP (as well as C8-dG-AAF; Mullenders, unpublished results) with equal efficiency from transcribed DNA in human cells in contrast with GG-NER [68]. This suggests that a common structure, i.e. a DNA lesion stalled RNAPIIo or perhaps the distorted transcription bubble, is recognized by the TC-NER machinery and that structural features of the blocking DNA lesions play no or only a minor role.

In vitro transcription experiments showed that RNAPII incorporates nucleotides opposite CPD and 6-4PP [69] and based on this finding and other data, it is hypothesised that the stalled RNAPIIo shields the DNA lesion and prevents access to the NER machinery. Consequently, TC-NER models propose the displacement/degradation of the RNAPIIo to allow access to repair proteins. To prove or disprove this hypothesis, *in vitro* experiments have been performed; however the results are somewhat conflicting as they revealed either an inhibitory effect or no effect of blocked transcription on repair or dual incision [25, 36, 70]. *In vitro* studies by Tremeau -Bravard *et al.* [70] and Laine and Egly [71] also indicate that TC-NER can be carried out without dissociation of the RNAPII (also reviewed in Laine and Egly [72]). However it is conceivable that TC-NER without displacement of RNAPIIo somehow requires conformational changes of the RNA polymerase to allow access to the DNA lesion and to resume transcription.

CS proteins associate with UV-stalled RNAPIIo

Photobleaching experiments [73] revealed that CSB interacts dynamically with the transcription machinery in line with *in vitro* data [74] and cell fractionation experiments showed that a fraction of CSB resides in a large complex, most likely RNAPIIo [75]. The interaction between CSB and the transcription machinery was stabilized by DNA damage [73]. Moreover, recruitment of CSB to chromatin was obvious from fractionation of nuclear extracts prepared from UV-irradiated cells [59, 41]. ChIP analysis of chromatin-bound RNAPIIo isolated from *in vivo* crosslinked cells [41], confirmed the previously reported observations and provided direct evidence for the association of CSB with RNAPIIo in the absence of DNA damage; in contrast, CSA was not found to interact with RNAPIIo consistent with *in vitro* [74] and *in vivo* data [41]. Upon UV-irradiation, interaction of CSB with the chromatin bound-RNAPIIo complex clearly increased. ChIP analysis also revealed a UV- and CSB-dependent association of CSA to RNAPIIo complex consistent with an earlier observation [76]. Thus stalled RNAPIIo at sites of DNA damage attracts CSB and CSA and/or stabilizes their interaction. In addition to CSA, the RNAPIIo/CSB complex was significantly enriched with DDB1 and subunits of the CSN complex in normal cells at early times after UV irradiation [40, 41] suggesting that UV-stalled RNAPIIo associates with a CSA-DDB1/CSN complex inactive for E3-ub ligase activity (Figure 2).

Recent *in vitro* studies suggested that XPG might play an important role in the assembly of the TC-NER complex as XPG interacts with stalled RNAPIIo both independently and cooperatively with CSB [77]. Nonetheless in the context of chromatin, XPG appears to act downstream of CSB in TC-NER complex formation [41].

Recruitment of NER factors to the UV-stalled RNAPIIo

In UV-irradiated *E. coli* cells, the *mfd* gene product recruits NER proteins to the RNA polymerase stalled at a photolesion and acts as the transcription repair coupling factor by disrupting the ternary complex of the damage stalled RNA polymerase [25, 78]. From similar approaches with human cells [36], it was concluded that a coupling mechanism in mammals must be different from that in bacteria as CSB does not disrupt a stalled RNAPIIo *in vitro*. Indeed, ChIP analysis clearly demonstrated that all pre-incision NER core components are recruited to lesion-

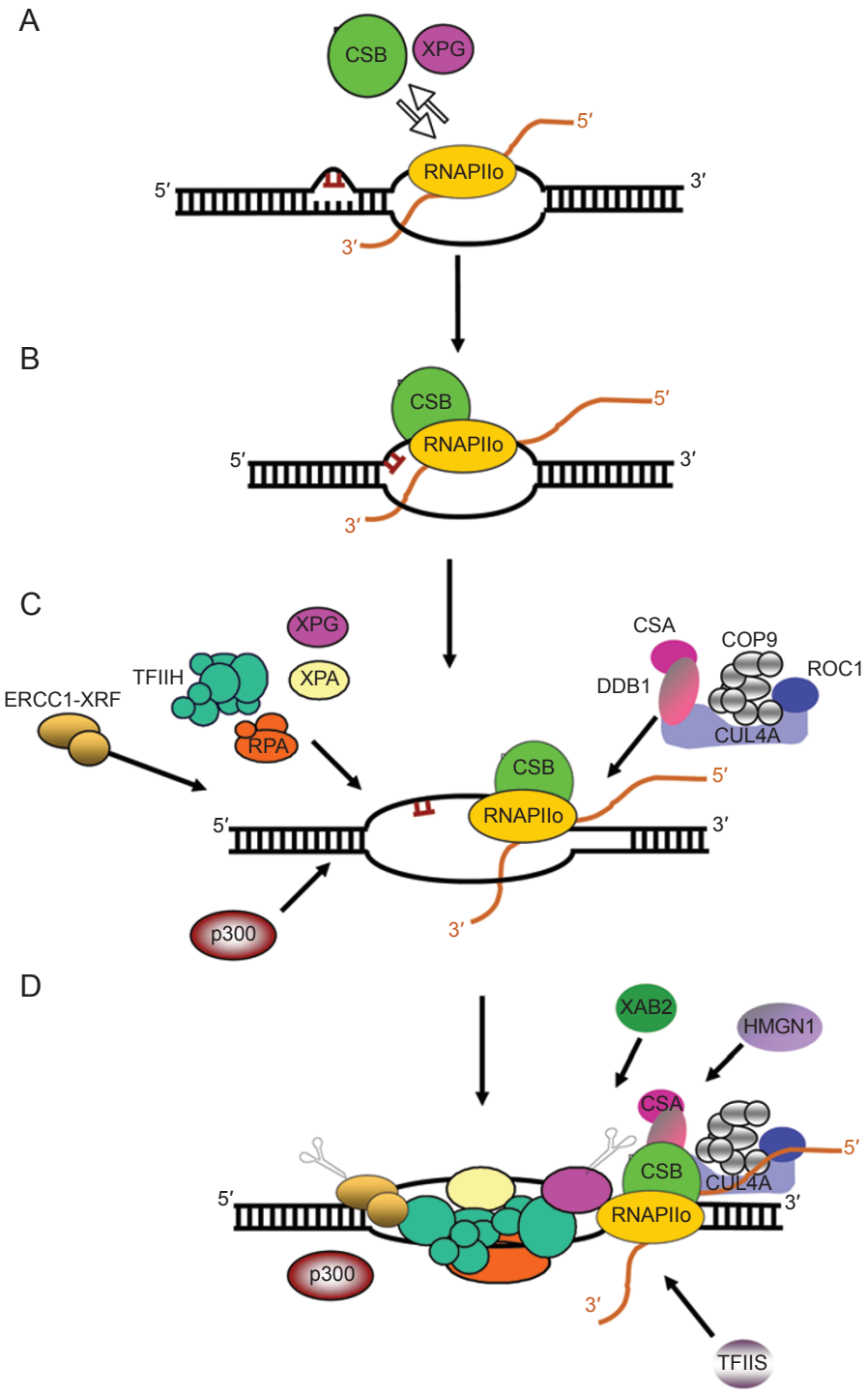

Figure 2 The assembly of a functional TC-NER complex. **(A)** During transcription CSB and XPG dynamically interact with the elongating RNAPIIo. **(B)** Upon encountering transcription-blocking photolesions, the equilibrium is shifted towards a more stable interaction between the arrested RNAPIIo and CSB. **(C)** In this stabilized RNAPIIo/CSB complex, CSB is prerequisite for chromatin remodelling and assessment of the DNA lesion and a key repair coupling factor as it is required to attract the HAT p300 and pre-incision NER core factors. In addition, CSB is required for the recruitment of the CSA-DDB1 E3-ub ligase/CSN complex (inactive for E3-ub ligase activity), which surprisingly is dispensable for the recruitment of NER factors. **(D)** Association of the CSA complex to the lesion site triggers the recruitment of HMGN1, XAB2 and TFIIS to facilitate further chromatin remodelling / signalling events and to enable cleavage of the protruding 3′ mRNA by RNAPIIo for resumption of transcription upon lesion removal.

stalled RNAPIIo in a CSB-dependent manner [41]. This suggests that CSB fulfils a transcription-repair coupling function, while holding back the RNAPIIo to its template, as the stalled RNAPIIo and the NER specific endonucleases can be isolated as a single repair complex.

Surprisingly, although the clinical phenotype of CSB and CSA patients is indistinguishable, CSA works differently from CSB as CSA has no direct function in coupling of the pre-incision NER factors to DNA lesion stalled RNAPIIo. Hence, a deficiency in a mechanism other than repair-transcription coupling must underlie the TC-NER defect in CS-A cells.

Recruitment of chromatin remodellers in TC-NER

Changes in chromatin structure are known to go along with NER and are required for efficient DNA repair in the context of condensed chromatin [79, 80]. Recent studies in yeast revealed an enhanced association of subunits of the SWI/SNF chromatin-remodelling complex with Rad4-Rad23 (XPC-RAD23B in human), the principal DNA damage recognition factor in GG-NER [81]. Also histone acetyltransferases (HATs) are known to stimulate NER. In yeast, the HAT Gcn5 facilitates efficient NER at transcriptionally active and inactive genes by hyperacetylation of histones H3 and H4. The latter occurs however in a NER independent manner [79]. Moreover, the HAT p300 activates transcription through chromatin remodelling and interactions with the basal transcription machinery including RNAPII and interacts with DNA repair proteins, such as DDB1 and PCNA [82, 83]. Evidence has been gained for a role of the nucleosome binding protein HMGN1 in mammalian TC-NER. As mentioned above, HMGN1–/– mice displayed acute skin sensitivity to UVB-irradiation and HMGN1 deficiency leads to impaired repair of CPD in active genes in mouse fibroblasts [47]. ChIP experiments identified HMGN1 as a component of the TC-NER complex and also provided evidence for a CSA-dependent direct interaction between HMGN1 and the UV-stalled RNAPIIo [41]. However, HMGN1 is probably not an essential factor to recruit NER factors for TC-NER as CS-A cells (lacking TC-NER bound HMGN1) are capable of recruiting the pre-incision NER factors to the stalled RNAPIIo. This suggests that HMGN1 has a function beyond incision complex assembly. The protein might activate the preincision complex for dual incision by remodelling chromatin thereby enhancing the action of histone acetyltransferases. Histone deacetylase inhibitors have been shown to induce hyperacetylation of histones leading to stimulation of TC-NER [84, 85]. A candidate HAT for histone hyperacetylation in TC-NER is P300/CBP as *in vitro* HMGN1 enhances the ability of P300/CBP to acetylate nucleosomal, but not free H3 [46]. Indeed, ChIP analysis revealed an association of p300 with RNAPIIo and provided evidence for a strong stimulation of the interaction between p300 and RNAPIIo by UV-irradiation. This UV-stimulated interaction depends on functional CSB. Curiously both p300 and HMGN1 are recruited prior to dual incision in TC-NER (i.e. both factors are recruited to RNAPIIo in XPA cells), but at least HMGN1 is probably not required to recruit the pre-incision NER factors. Interestingly in the yeast *S. cerevisiae,* histone H3 is acetylated in a UV-dependent manner in the absence of functional early damage recognition NER factors (rad4, rad14; XPC, XPA in human) [79], suggesting that post-UV chromatin modifications actually occur and facilitate NER prior to DNA lesion recognition by early NER factors, a situation that might also hold for mammalian TC-NER.

Transcription restart: a crucial role for TFIIS?

As mentioned above, several studies suggest that the mammalian TC-NER complex is built up without displacement of RNAPIIo [41, 70, 71, 77]. Importantly, the stalled RNAPIIo at the lesion may not be a sufficient barrier to prevent the DNA lesion from being accessible to repair proteins [54, 71]. Conformational changes of the RNAPIIo might be required to allow accessibility to repair proteins, and such changes require the CSB protein although *in vitro* the assembly of the NER complex occurs in the absence of CSB [70, 77]. Backtracking of RNAPIIo might be one of the key mechanisms to allow repair and/or transcription restart. During backtracking, RNAPIIo and the transcription-associated DNA bubble shifts backward along the RNA [86] and restart of transcription depends on cleavage of the extruded mRNA to reposition the 3' end of the RNA to the active center of RNAPIIo [87]. The transcription cleavage factor TFIIS implicated in TC-NER [54, 88] can stimulate the cleavage activity of RNAPIIo, allowing arrested RNAPIIo to restart elongation. The interaction of TFIIS with RNAPIIo increased significantly in UV-irradiated repair proficient human cells, but not in TC-NER deficient CS-A or CS-B cells [41]. We speculate that upon UV irradiation and RNAPIIo arrest, assembly of a functional TC-NER complex goes together with or is followed by recruitment of TFIIS thereby facilitating repair and resumption of transcription after removal of the DNA lesion. In contrast to the assembly of pre-incision NER factors, the recruitment of TFIIS is dependent on both CSA and CSB and hence impairment of this step would lead to the defective RNA synthesis recovery observed in CS cells underlying the clinical features of CS patients.

Stability of TC-NER components

Studies in mammalian systems [89, 62] provided evidence that RNAPIIo is a target for ubiquitylation in UV-irradiated human cells and that ubiquitylation requires CSA

and CSB (in contrast to the yeast CSB counterpart Rad26 that inhibits degradation of RNAPII [90]). However, recent results by Svejstrup and co-workers [91] suggest that Nedd4 is the ubiquitin ligase for damage-induced ubiquitylation of RNAPII, and that CS proteins are involved only indirectly. In *in vitro* transcription assays RNAPII undergoes ubiquitylation on DNA containing cisplatin adducts that arrest transcription [92]. Nevertheless, in cisplatin-treated cells, a significant part of ubiquitylated RNAPIIo was not bound to chromatin [70]. Based on these and other data the hypothesis was put forward that degradation of RNAPIIo is required for the efficient recovery of mRNA synthesis but not for TC-NER *per se* [62] and that in response to DNA damage ubiquitylated RNAPIIo dissociates from the template. In agreement with the latter, mammalian RNAPIIo purified from chromatin of UV-irradiated cells did not display modified forms whereas ubiquitylated RNAPIIo was readily detected in the soluble cellular fraction [40, 41]. Thus, removal of RNAPIIo by ubiquitylation and degradation might be the strategy when RNAPIIo becomes prolongedly arrested at DNA damage due to failure to perform repair as suggested by experiments in yeast [93].

The transcription coupling repair factor CSB is another component that has been implicated as a target for ubiquitylation and degradation [94]. Following high dose of UV irradiation, CSB is almost completely degraded 3 h post-UV irradiation in a proteosome- and CSA-dependent manner. Based on these data, Groisman and coworkers [94] proposed that CSB is essential and beneficial for TC-NER during the early hours, but inhibitory at later stages and has to be removed in order to allow recovery of RNA synthesis. However, the degradation of CSB is difficult to reconcile with the linear kinetics of TC-NER measured at high dose and over a time period of more than 30 h [68].

Concluding remarks

Recent studies have greatly improved our understanding of the interplay between DNA damage, transcription and repair. However, much is to be learned about the exact functions of key players in TC-NER, the mechanisms by which eukaryotic cells sense DNA damage that blocks active transcription, and how and which signals activate TC-NER. Elucidation of factors that are involved in resumption of UV-inhibited transcription, and also the fate of the lesion stalled RNAPII and other TC-NER factors, particularly when TC-NER fails to operate, represent important questions that still need to be resolved. Based on several lines of evidence, a picture emerges pointing to the essential role of chromatin dynamics in TC-NER. Nonetheless, it is not clear why chromatin remodelling would be required for TC-NER in addition to fulfilling the structural changes that are needed to allow active transcription of chromatin-embedded DNA substrates.

Increasing evidence points to the RNAPII transcription machinery as a guardian of genomic integrity by sensing DNA damage during all stages of the cell cycle (except mitosis) thereby activating DNA damage signaling, repair pathways, and/or apoptosis. Hence, elucidating the mechanisms by which transcription triggers DNA damage response pathways and the role of defective TC-NER in the aetiology of the progeroid, neurodevelopmental disorder of CS, will be of pivotal importance in our understanding of the mechanisms through which genetic information is safeguarded and genome stability is preserved.

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