DNA Repair 10 (2011) 743–750

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Mini review

DNA damage response and transcription

Saskia Lagerwerf[a], Mischa G. Vrouwe[a], René M. Overmeer[a], Maria I. Fousteri[a,b], Leon H.F. Mullenders[a,*]

[a] Department of Toxicogenetics, Leiden University Medical Center, 2333 RC Leiden, The Netherlands
[b] Institute of Molecular Biology & Genetics, Biomedical Sciences Research Center Alexander Fleming, 16672 Athens, Greece

ARTICLE INFO

Article history:
Available online 31 May 2011

Keywords:
NER
RPA
DNA damage signaling
Mutagenesis
Cockayne syndrome
TC-NER complex assembly

ABSTRACT

A network of DNA damage surveillance systems is triggered by sensing of DNA lesions and the initiation of a signal transduction cascade that activates genome-protection pathways including nucleotide excision repair (NER). NER operates through coordinated assembly of repair factors into pre- and post-incision complexes. Recent work identifies RPA as a key regulator of the transition from dual incision to repair-synthesis in UV-irradiated non-cycling cells, thereby averting the generation of unprocessed repair intermediates. These intermediates could lead to recombinogenic events and trigger a persistent ATR-dependent checkpoint signaling. It is now evident that DNA damage signaling is not limited to NER proficient cells. ATR-dependent checkpoint activation also occurs in UV-exposed non-cycling repair deficient cells coinciding with the formation of endonuclease APE1-mediated DNA strand breaks. In addition, the encounter of elongating RNA polymerase II (RNAPIIo) with DNA damage lesions and its persistent stalling provides a strong DNA damage signaling leading to cell cycle arrest, apoptosis and increased mutagenesis. The mechanism underlying the strong and strand specific induction of UV-induced mutations in NER deficient cells has been recently resolved by the finding that gene transcription itself increases UV-induced mutagenesis in a strand specific manner via increased deamination of cytosines. The cell removes the RNAPIIo-blocking DNA lesions by transcription-coupled repair (TC-NER) without displacement of the DNA damage stalled RNAPIIo. Deficiency in TC-NER associates with mutations in the CSA and CSB genes giving rise to the rare human disorder Cockayne syndrome (CS). CSB functions as a repair coupling factor to attract NER proteins, chromatin remodelers 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 molecular mechanisms by which these proteins bring about efficient TC-NER and trigger signaling after transcription arrest remain elusive; particularly the role of chromatin remodeling in TC-NER needs to be clarified in the context of anticipated structural changes that allow repair and transcription restart.

Contents

1. DNA damage and genome integrity

Maintenance of genomic integrity in an environment of genotoxic stress is a prerequisite for proper cell function and of prime clinical importance in relation to the development of cancer and age-related disease. The mammalian genome is protected against genotoxic insults by a network of DNA damage response (DDR) mechanisms triggered by the detection of DNA lesions through

* Corresponding author. Tel.: +31 71 526 9600; fax: +31 71 526 8284.
E-mail address: L.Mullenders@lumc.nl (L.H.F. Mullenders).

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doi:10.1016/j.dnarep.2011.04.024

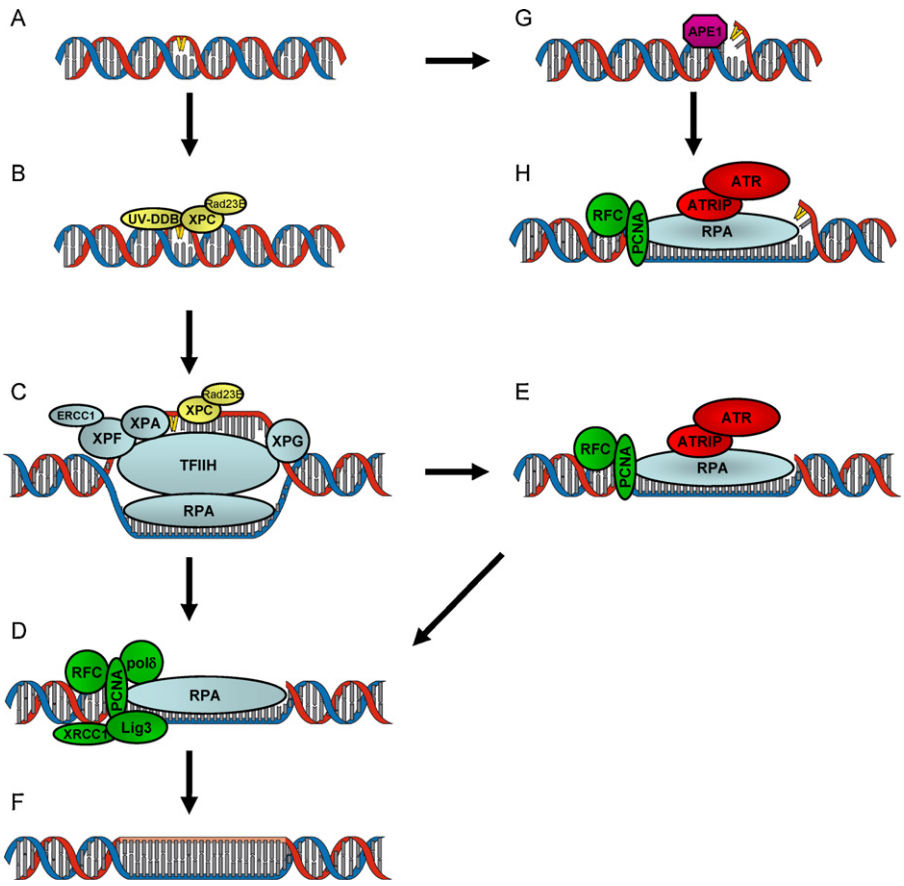

Fig. 1. Global genome NER. UV induces DNA photolesions, most notably CPD and 6-4PP (A). Repair is initiated by recognition of these DNA lesions by UV-DDB and XPC/RAD23B (B). XPC is essential for GG-NER whereas UV-DDB stimulates 6-4PP removal, but is indispensable for the removal of CPD lesion. Following demarcation of the damage, binding of additional pre-incision factors is facilitated (C). The helicase activity of TFIIH serves to open up the DNA whereas XPA is required for recruitment and activation of XPF/ERCC1. RPA is essential to activate the XPF/ERCC1 and XPG endonucleases that incise the damaged DNA (started by XPF/ERCC1-mediated 5′ incision) followed by removal of the damage containing oligo. Once removed a single stranded gap containing RPA remains. By utilizing proteins that also perform normal replicative DNA synthesis, such as RFC, PCNA and polymerase δ, the gap will be filled (D). Note that DNA polymerases such as polymerase ε and κ can also perform this task. Restoration of the DNA is completed by sealing the remaining nick using ligase3/XRCC1 or, alternatively, by ligase1 (F). If resynthesis is delayed, an intermediate gapped DNA structure is formed that activates the ATR kinase (E). Additional factors required for ATR activation such as TopBP1 and the 9-1-1 complex (not shown) are also expected to participate. Eventually these gaps will be filled by repair synthesis and ligated (F). If UV lesions are not readily removed, either due to high damage load or GG-NER deficiency, APE1 can incise directly 5′ of the 6-4PP. It is assumed that also in this APE1-dependent process, 6-4PP are not removed (G). This structure will be converted into a form that supports ATR signaling, possibly by resection of DNA 5′ of the lesion (H). Note that the exact nature of the structure is not known.

specific sensors. The subsequent step is the initiation of a signal transduction cascade including effector molecules which activate various genome-protection pathways i.e. DNA repair, cell cycle control, apoptosis, transcription and chromatin remodeling.

The multiprotein nucleotide excision repair (NER) system removes a wide variety of helix-destabilizing DNA lesions including those induced by UV-irradiation. This broad substrate recognition is achieved by two distinct subpathways of NER, which are triggered by DNA damage mediated structural alterations in the genome rather than direct recognition of the lesion itself. The first subpathway, global genome NER (GG-NER), is able to repair lesions throughout the entire genome by sensing the reduced rigidity of DNA imposed by the helix distortion [1]. The second subpathway, transcription-coupled NER (TC-NER), specifically repairs DNA lesions in genes that block the actively transcribing RNA polymerases II (RNAPIIo).

GG-NER and TC-NER differ by the mechanism of DNA lesion recognition in chromatin. GG-NER is initiated by UV-DDB and XPC/RAD23B mediated recognition of DNA helix distortions inflicted by DNA injuries, whereas RNAPIIo stalled at a DNA lesion efficiently triggers the recruitment of TC-NER specific factors. After recognition of the damage, both pathways utilize identical components for the assembly of the repair complex (pre-incision step); subsequently, the removal of the damage is accomplished by excising a short DNA fragment encompassing the lesion thereby maintaining the capacity to repair a broad spectrum of DNA damage [2]. The remaining gap is filled by DNA repair patch synthesis using the undamaged strand as template (post-incision step) (Figs. 1 and 2). The biological relevance of NER is underscored by severe clinical consequences including premature ageing, developmental abnormalities and extreme cancer-susceptibility associated with inherited NER defects. These defects lead to the rare autosomal inherited diseases xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD) [3]. Eight complementation groups have been found in XP patients, the first 7 (XP-A to XP-G) are either defective in GG-NER exclusively, or in both GG-NER and TC-NER. The last complementation group (XP-V) is defective in DNA polymerase η (Polη), a Y family translesion polymerase that can carry out translesion synthesis past UV-induced DNA lesions. Despite detailed insights into the core NER reaction mechanism, relatively little is known about the molecular events that initiate and regulate this process.

Several lines of evidence suggest that GG-NER and TC-NER operate through coordinated assembly of repair factors into pre- and post-incision complexes; however, how this coordination is regulated in vivo is poorly understood. Although unwinding of the DNA

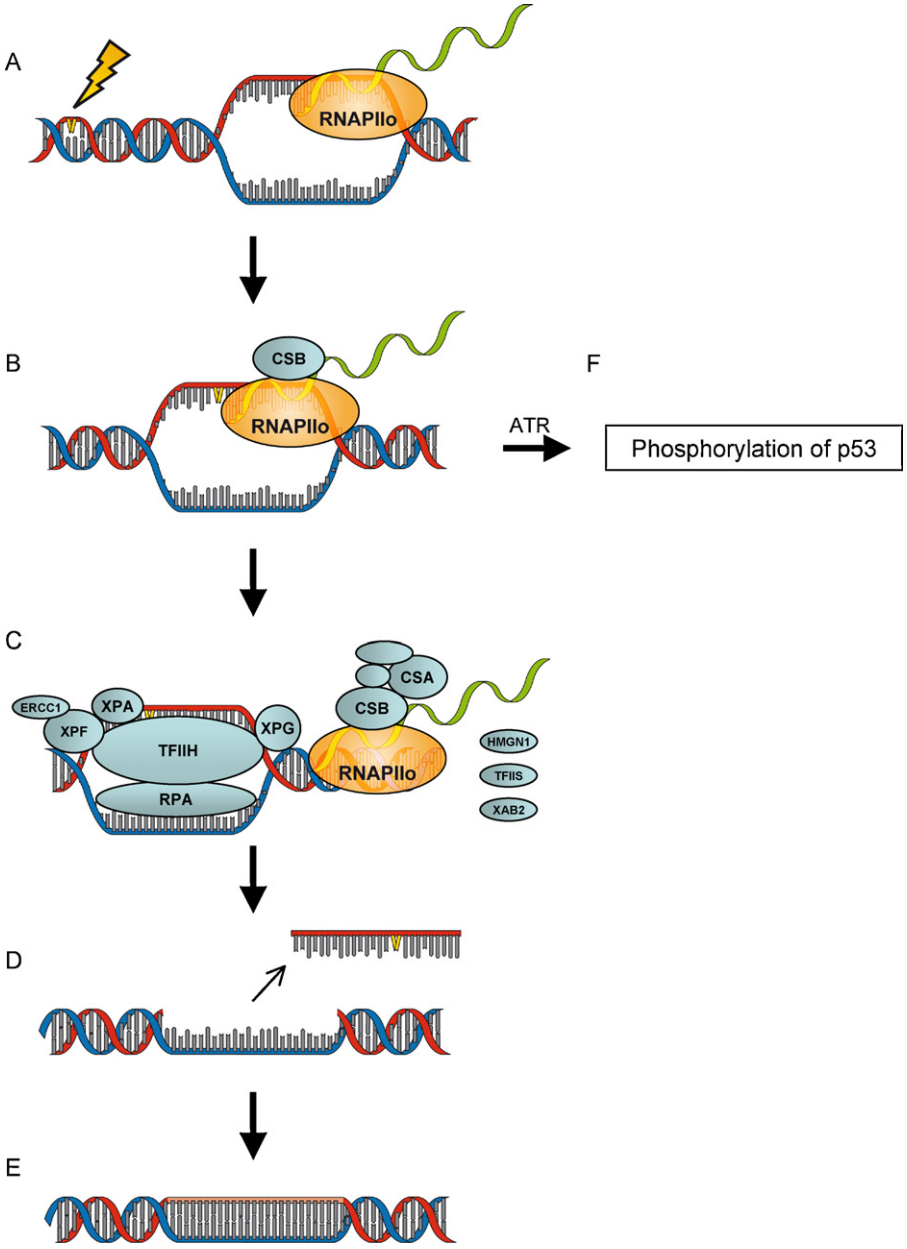

Fig. 2. Processing of UV-lesions by TC-NER. A photolesion in the actively transcribed strand of a gene (A) will pose a block for the elongating RNAPII which needs to be resolved to resume transcription. Damage-arrested RNAPII has a strong interaction with the CSB protein (B) and serves as a trigger to assemble TC-NER core proteins as well as an inactive CSA-ubiquitin ligase complex in vicinity of the photolesion. The presence of CSA is a prerequisite to recruit HMGN1, XAB2 and TFIIS to damage arrested RNAPII/CS complexes. The assembled TC-NER proteins act to verify the lesion, create a stable bubble structure and subsequently incise the DNA at both sides of the lesion (C). A single stranded stretch of DNA of about 30 nucleotides containing the photolesion is released (D) and the resulting gap is filled presumably by the DNA synthesis and ligation factors involved in NER (E). When the transcription blocking photolesion is not removed yet in repair proficient cells, the transient transcriptional arrest induces phosphorylation of p53 (F). Moreover, failure to fill the gap is likely to induce DNA damage signaling as shown for GG-NER (Fig. 1).

helix by the two TFIIH helicases, XPB and XPD, following DNA damage recognition has been suggested to be the first irreversible step of NER [2], incision is an obvious 'point of no return' as this step creates a gap which has to be filled. In a recent study [4] we show that in the absence of RPA, all pre-incision factors (XPC, XPA, TFIIH, XPG, XPF/ERCC1) associate with sites of UV-damage; this association is however dynamic in its nature. Only the arrival of RPA stabilizes/correctly orients the pre-incision complex and therefore commits the complex to excise the DNA lesion and complete the repair process by repair patch synthesis and ligation. Notably, only upon completion of the post-incision step RPA is released from the repaired DNA sites and can associate with pre-incision factors to initiate new incision events. This mechanism provides an explanation for the observation that inhibition of repair synthesis abrogates removal of UV-photolesions in human cells [5]. In a broader perspective, this regulation is especially important as uncoupling incisions from repair synthesis and ligation could lead to an accumulation of ssDNA gaps which would be highly recombinogenic and persistently activate the cellular DNA damage response [2,4].

2. DNA damage signaling

Recognition of aberrant DNA structures by cellular surveillance proteins initiates DNA damage signaling. It has long been recognized that the checkpoint protein p53 becomes upregulated

in response to DNA damaging agents such as UV-light. Although cycling cells are particularly efficient in their p53 response, also non-cycling cells display p53 activation, especially in the presence of DNA replication inhibitors [6,7]. A better understanding of the factors that contribute to UV-mediated checkpoint signaling has come from studies using cells with genetic defects in various NER genes. Notably, impairment of TC-NER results in high p53 expression following treatment with either UV or inhibitors of transcription elongation [6] supporting the concept that persistent arrest of RNAPIIo initiates a signaling response [8–10]. In addition, experimental evidence suggests that processing of UV-photolesions by GG-NER triggers a checkpoint signaling [7,11–14]. ATR together with ATM are considered to be the key kinases that orchestrate DNA damage signaling [15] and each is activated by a distinct DNA structure. ATR is activated by single stranded DNA (ssDNA) containing ssDNA/dsDNA junctions [16]. ATR forms a dimer with ATRIP and it is the latter subunit of the complex that can interact with ssDNA bound RPA, one of the essential components of the NER pathway [17]. It has been proposed that during NER RPA containing single stranded DNA gaps are formed which are substrates for the ATR/ATRIP complex thereby initiating damage signaling (Fig. 1E). This is manifested among others by H2AX phosphorylation (γH2AX) and ubiquitylation of histone H2A [12,18]. However, recent research reveals a defined order of dual incision and repair synthesis in human cells: using catalytically inactive mutants of ERCC1/XPF and XPG Schärer and coworkers [19] demonstrated that the 5′ incision to the damage by ERCC1/XPF precedes the 3′ incision by XPG and that the initiation of DNA repair synthesis although it does not require the catalytic activity of XPG, requires its binding. We examined γH2AX signaling at NER sites in cells expressing the catalytically inactive XPG protein that can execute 5′ incision only and found that even in the absence of DNA repair synthesis (attained by DNA synthesis inhibitors) a profound H2AX phosphorylation is observed. These data demonstrate that a single 5′ incision at a site undergoing NER can provoke ATR-dependent signaling and γH2AX formation even when repair synthesis is inhibited [4].

The question remains whether these structures would be sufficient to result in checkpoint activation when the repair synthesis stage is impeded and there is no "proofreading" by the replication machinery or whether further processing is required. It is conceivably that signaling requires extended regions of single stranded DNA, as under optimal conditions of repair, NER intermediates are only 30 nucleotides in size. Indeed, a recent study [20] in non-cycling yeast cells shows that the Exo1 nuclease is involved in either recruiting or activating checkpoint sensors since direct Mec1-targets (homolog of the human ATR gene) are not targeted after UV-exposure when this kinase is lacking. The proposed trigger for DNA-damage checkpoint activation is Exo1 processing of the NER intermediates resulting in long ssDNA gaps (See also article by Novarina et al. in this issue of DNA Repair).

On the other hand, there is evidence in non-dividing cells that even in the absence of both GG-NER and TC-NER (i.e. persistent RNAPIIo arrest), DNA damage checkpoints are activated. Early work by Miura and coworkers [21] showed that quiescent NER deficient XP-A cells accumulated PCNA foci late (24 h) after UV-irradiation. Recent experiments [7] extend this observation and show that NER proficient and repair deficient cells display a similar, but temporally different damage response after UV-irradiation. Non-cycling NER proficient cells initiate a rapid but transient activation of the damage response proteins p53 and H2AX; in contrast, repair deficient cells display delayed (starting 8 h after UV) but persistent signaling. Similar to repair proficient cells, the response in GG-NER deficient cells depends on ATR and results in activation of the G1 checkpoint that ultimately prevents the transition of UV-irradiated cells from G1 to S phase upon stimulation. Also in *Saccharomyces cerevisiae* UV-induced checkpoint activation can occur independently of replication or NER and is thought to be initiated by breakage of DNA strands [22,23]. In repair deficient cells, the UV-induced checkpoint activation temporally coincides with the formation of single strand DNA breaks (ssDNA breaks) as well as damage specific recruitment of the chromatin binding factors PCNA, phospho-H2AX and TopBP1 [24]. Interestingly, this process depends, at least in part, on an alternative mechanism that involves APE1. In the base excision repair process the endonuclease APE1 incises abasic sites and oxidized bases [25,26] thereby facilitating removal of the lesion. Similarly, when 6-4 photoproducts are not efficiently removed these lesions are targeted by APE1 [7]. The mechanism of lesion processing by APE1 requires 5′ incision, PCNA and the Fen1 5′ to 3′ exonuclease activity to subsequently remove the damaged nucleotide. However, in contrast to single oxidized bases, this step would require the removal of two covalently linked bases in the case of 6-4PP. Hence, it is more likely that APE1 initiates processing of UV-lesions by creating single stranded DNA nicks that are converted into DNA gaps which cannot be repaired and that activate the ATR pathway [7] (Fig. 1G and H).

Recent experiments have implicated ATR in the DDR following transcription blockage through exposure to UV or transcription blocking chemicals [27]. When exposed to UV, p53 phosphorylated at serine 15 rapidly accumulates in normal cells and this is much more pronounced in cells that lack TC-NER such as CS-B and XP-A cells [8] (Fig. 2F). The ability of XPA deficient cells to promote this damage response demonstrates that the process is not initiated by NER-induced incisions. Stalled RNA polymerase complexes might adopt a chromatin configuration facilitating RPA-mediated activation of the ATR kinase. It is surprising that although the DNA damage checkpoint can be induced through distinctly different mechanisms, they all depend on the ATR kinase to transduce the signal [27,28]. In TCR deficient CS-B cells, the early UV-response (i.e. γH2AX and ssDNA breaks) appears to be nearly indistinguishable from normal human cells proficient in GG- and TC-NER. However, and in contrast to normal human cells, phosphorylation of p53 in CS-B cells accumulates in time and persists for up to 72 h, but without an increase in γH2AX levels and ssDNA breaks [7]. Also, ChIP-on-Western analysis with RNAPIIo in extracts from UV-irradiated CS-B cells did not show any UV-induced binding of RPA with the damage-stalled RNAPII complex (Lagerwerf, unpublished results). This raises questions on the mechanism involved: whereas phosphorylation of p53 due to transcription stalling in repair proficient human cells is mediated by ATR [27], the late but massive p53 response in UV-irradiated CS-B cells without increased levels of ssDNA breaks and γH2AX suggests that p53 phosphorylation is mediated by a different mechanism possibly independent of the 9-1-1 clamp and ATR [29].

3. Transcription-coupled NER

Several types of DNA damage, particularly bulky chemical-induced DNA lesions and UV-photoproducts, pose severe blocks to the elongating transcription machinery that can potentially affect gene expression thereby challenging development, cell growth and survival. The cytotoxic threat of blocked transcription is normally counteracted by TC-NER that allows accelerated repair of transcription-blocking damages and rapid resumption of transcription. A damage stalled RNAPIIo molecule is the initiator of TC-NER in which the CS factors (CSA and CSB) play an essential, though partly defined role [30]. It is assumed that the stalled RNAPIIo needs to be either displaced or backtracked from the damage site to provide access to the repair machinery. Release of RNAPII from damage sites has been described in bacteria [31] whereas poly-ubiquitination and proteasomal degradation of RNAPIIo has been observed in yeast

and human cells after high doses of UV-irradiation. This strategy might be the 'only remaining solution' of the cell to remove persistently arrested transcription machineries when there is high DNA damage load and/or repair is not effective [32,33]. On the other hand, in mammals backtracking of RNAPII to allow resumption of transcription after damage removal in repair proficient situations has been suggested and shown in vitro [34,35]. In agreement, it has been demonstrated that in human cells RNAPII remains on the damaged chromatin in close proximity to the TC-NER machinery [36]. Insights into the role of the CSB protein in TC-NER have been provided in vitro [37] and in vivo [36] demonstrating that CSB is the key factor in TC-NER essential for functional RNAPIIo/TC-NER complex assembly. Initially, a stable RNAPIIo/CSB complex is formed [36] followed by the assembly of NER pre-incision factors TFIIH, RPA, XPA and the two structure specific endonucleases XPG and XPF/ERCC1 to the damage site. This pre-incision TC-NER complex creates a stable open bubble structure surrounding the lesion and excises the damaged DNA fragment. Although a role for XPG in recognizing the stalled RNAPIIo complex and stimulating CSB binding has been suggested by elegant in vitro experiments [38], it is obvious that in vivo XPG assembly on the chromatin bound TC-NER complex depends on CSB [36].

CSB is also required for the recruitment of histone acetyltransferase p300 and the CSA containing E3-ubiquitin ligase complex to the UV-damaged chromatin. Assembly of the CSA complex is required to recruit three additional TC-NER specific factors; the nucleosomal binding protein HMGN1, the transcription cleavage factor TFIIS and the pre-mRNA splicing factor XAB2 to the vicinity of damage induced RNAPIIo/CSB complexes [36]. Although experimental evidence is lacking, it has been suggested that recruitment of p300 and HMGN1 might facilitate chromatin remodeling and reverse translocation of RNAPIIo thus allowing removal of the blocking lesion by the repair machinery and resumption of transcription [30]. Notably, in contrast to CSB, CSA is not required for the assembly of NER pre-incision factors TFIIH, XPA, RPA and XPF/ERCC1. In GG-NER, excision of the damaged DNA is followed by the assembly of the post-incision NER factors which fill in the ssDNA gap by DNA repair synthesis and ligation of the resulting nick. PCNA is loaded on the DNA after 5′ incision by ERCC1/XPF and forms a clamp to which different DNA polymerases (δ, κ, or ε) can bind in the presence of the RFC complex or RFC-like complexes to perform the repair synthesis step [4,17,39]. Subsequent ligation will occur by either DNA ligase 3/XRCC1 or DNA ligase 1 [17,40]. Although it is generally accepted that the same proteins perform the post-incision step in TC-NER [30], limited direct evidence exists to support their involvement in vivo (Fig. 2).

The CSB protein plays a crucial role in TC-NER as the transcription-repair coupling factor. CSB protein contains seven conserved helicase-like ATPase motifs, which are a characteristic feature of all members of the SWI2/SNF2 family of ATP-dependent chromatin remodelers. It has been shown that CSB has intrinsic chromatin remodeling activities in vitro that require the hydrolysis of ATP and can directly interact with core histones [41]. The ability of CSB protein to alter chromatin structure and actively change DNA conformation could result in disruption of DNA-protein interactions [41,42]. Based on this, an ATP-dependent chromatin remodeling function of CSB has been predicted in TC-NER since the stalled RNAPIIo might hamper the assembly of the repair machinery at the site of the lesion. However, the molecular mechanism of CSB ATPase activity in TC-NER and possible other cellular processes is still not known.

In vitro studies with synthetic oligonucleotides show that CSB ATPase activity is stimulated by DNA structures of double and/or partially single stranded nature or that nucleosomal DNA can serve as a cofactor [41,43,44]. Furthermore, dephosphorylation of CSB in response to UV-irradiation results in increased ATPase activity, at least in vitro, indicating a phosphorylation-controlled mechanism of CSB ATPase function [44]. To delineate further the role of CSB ATPase activity, phenotypical consequences of different mutations in the ATPase domains of CSB have been addressed. Studies in human cell systems, where a point mutation in an ATPase domain of CSB is introduced, consistently show enhanced UV-sensitivity in clonogenic survival assays, increased percentage of apoptotic cells after UV-exposure as well as impaired resumption of transcription, although the severity of the phenotype differs dependent on the exact mutation site [41,45,46]. CSB ATPase deficiency also renders mammalian cells sensitive towards 4-NQO. However, there is no indication that the CSB ATPase activity is required for the repair of oxidative damages, even though the presence of CSB protein itself is critical for the incision of 8-oxoguanine lesions [45–47]. The proposed role of CSB in transcription regulation [48–50] might lead to deficient transcription of the hOGG1 gene, although microarray analysis of a limited set of genes suggest that a CSB ATPase mutant does not affect expression levels [46]. Furthermore, a recent study revealed [51] that the N-terminal region of CSB protein negatively regulates the UV-induced binding of CSB to chromatin and alleviation of this effect depends on ATP hydrolysis by CSB. Therefore assembly of CSB at gene damages that block transcription probably requires a major conformational change of CSB protein itself. Interestingly, the C-terminal part of CSB protein has been found to contain an ubiquitin-binding domain (UBD) that is absolutely required for TC-NER and recovery of UV-inhibited RNA synthesis in vivo [52]. Although the ubiquitinated target of CSB is currently unknown, the data clearly suggest that the UBD domain of CSB is essential for CSB function, as cells expressing a mutant CSB protein carrying a deletion of the UBD domain (CSB^{del}) show similar features as cells lacking CSB completely and are unable to remove transcription-blocking lesions.

After UV-irradiation CSB protein becomes more stably associated with chromatin [36,51] but might undergo degradation. Nakatani and coworkers [53] show a CSA- and proteasome-dependent ubiquitination and degradation of the CSB protein within 4 h after treatment of cells with high UV doses. The authors suggest that CSB has to be removed from the DNA template in order to facilitate repair or to allow transcription to resume at a normal rate. However, a more recent study reveals that under comparable conditions of high UV dose the CSB re-localizes from the chromatin to the soluble fraction [51]. The apparently conflicting results might be explained by UV-induced posttranslational modifications of CSB protein that leads to biased protein detection due to the use of different antibodies. Nonetheless, both observations do not fit well with repair kinetics when considering the linear removal rate of UV-lesions in a TC-NER fashion at high UV doses over at least 24 h [54] and the essential role of CSB in TC-NER complex assembly.

4. TC-NER deficiency: human disease and genome integrity

As pointed out, human syndromes such as xeroderma pigmentosum (XP), triochothiodystrophy (TTD) and Cockayne syndrome (CS) are all associated with defects in NER. However the clinical features of CS have little in common with XP. CS patients have many developmental defects including mental retardation, microcephaly, a bird-like face, progressive growth failure, severe neurological dysfunction, progeria-like features, retinal degeneration and sun-sensitivity but they do not develop skin cancers as seen in XP patients (see also article by Diderich et al. in this issue of DNA Repair).

In classical CS (type I), the symptoms usually appear within the first few years of life, but early-onset cases with more severe symptoms (type II) and late-onset cases with milder features (type III) have also been described [55]. Moreover, the extremely

severe COFS syndrome regarded as a prenatal form of CS and the very mild UV-sensitive syndrome (UVSS) that is characterized by photosensitivity without any neurological or growth defect have the same cellular TC-NER defect. The majority of CS patients have mutations in either CSA or CSB genes, however no obvious genotype–phenotype correlation has been observed in the patients. Based on the sensitivity of CS cells to oxidative damage, it has been hypothesized that interference of oxidative damage with transcription in highly transcribed cell types such as neurons might underlie the different CS-associated phenotypes [56,57]. Apart from these already complex phenotypes, TC-NER has many further ramifications. Rare cases of mixed XP/CS phenotype have been linked to mutations in the XPD, XPB, and XPG genes, while mutations in XPD, XPG, ERCC1 and CSB genes can give rise to COFS syndrome. XP/CS patients show an extreme clinical photosensitivity, cancer-proneness and have a combined defect in both TC-NER and GG-NER pathways.

Understanding the complex clinical phenotype of CS requires more insights in the biological consequences of arrested RNA polymerase and the DNA lesions engaged. As abovementioned, induction of UV-photolesions in CSB deficient cells leads to profound and persistent accumulation of phosphorylated p53, an event that can induce cells to undergo apoptosis [58]. Indeed CSB deficient mice and human cell lines rapidly undergo apoptosis and severe cell cycle arrest at much lower dose than their TC-NER proficient counterparts following genotoxic stress [59] but surprisingly this apoptosis is independent of p53 both in cells [58] and $CSB^{-/-}$ mice (Mullenders, unpublished results). The protective role of TC-NER against the acute toxic effects of genotoxic exposure has been dissected in CSB deficient mice: $CSB^{-/-}$ mice are 10-fold more sensitive to UVB-light induced erythema/edema of the skin and lethality induced by the polycyclic aromatic hydrocarbon DMBA compared to TC-NER proficient mice [60,61]. These experiments highlight TC-NER as a critical survival pathway [59]. However, the role of TC-NER in skin carcinogenesis in the mouse is complex. Disruption of the CSB gene impairs spontaneous tumorigenesis in cancer-predisposed Ink4a/ARF knockout mice [62]. This antineoplastic effect may result from apoptosis secondary to endogenous DNA damage, similarly as what is reported for spontaneous mutagenesis in mouse lymphocytes [60] and this mechanism might contribute to the absence of enhanced skin cancer in (sunlight sensitive) CSB patients. Surprisingly, CSB deficient mice are susceptible to UVB-induced skin cancer. Recent work shows that the latter is due to inefficient GG-NER in mice indicating that GG-NER can compensate for defective TC-NER in humans [63].

The increased survival of TC-NER proficient cells and tissues after genotoxic insults occur at expense of increased mutagenesis manifested for example by the fast appearance of mutant p53 patches in the epidermis of UVB-irradiated TC-NER proficient mice lacking GG-NER [64]. Analysis of p53 mutations in UVB-induced squamous cell carcinomas in CSB-deficient mice reveals that mutations occur as expected at di-pyrimidine positions but almost exclusively from photolesions in the transcribed strand. The mechanism underlying this strong bias of mutations has been obscure for almost two decades but is discovered now. Recent studies by de Wind and co-workers reveal that gene transcription itself increases UV-induced mutagenesis (transcription associated mutagenesis, TAM) in mouse embryonic stem cells in a strand specific manner. This process occurs via increased deamination of cytosines to uracil within photolesions in the transcribed strand probably due to enhanced single strandedness at arrested transcription complexes [65]. These data provide a mechanistic explanation for the TC-NER mediated strand bias of UV-induced base substitutions and reveals a key role of TC-NER in preventing the mutagenic consequences of TAM, also in non-dividing cells. TAM might hold for other lesions as well. Yeast *S. cerevisiae* strains that are defective in repair of AP sites and that lack RAD26 (homolog of the human CSB gene) display a dramatic increase in toxicity and mutagenesis. This infers that also arrest of transcription at AP sites is a highly mutagenic and toxic event [66]. Additionally it was found that transcription of UV-damaged DNA in mouse ES cells induces a second class of TAM mutations i.e. intragenic deletions, also mediated by photolesions in the transcribed strand. It is conceivable that in S-phase cells replication forks may encounter arrested RNA polymerase complexes; such an encounter might lead to collapsed forks provoking transcription associated recombination (TAR) [67,68] and resulting in DNA strand break formation and subsequently deletions following error prone DNA break repair.

5. Conclusions

Multiple gene products have been implicated in TC-NER but we lack knowledge of the precise function of key components in TC-NER and the signals that regulate TC-NER. Although differential roles for the CSB and CSA proteins have been established in assembly of TC-NER complex, the molecular mechanisms by which these proteins bring about efficient TC-NER remain elusive as well as the possible posttranslational modifications required to carry out their functions. The role of chromatin remodeling in TC-NER needs to be understood, particularly in the context of anticipated structural changes that allow repair and transcription restart. Of particular interest to resolve is the fate of key proteins (RNA polymerase, CS proteins) when TC-NER fails to operate, the mechanisms that trigger signaling after transcription arrest in the absence or presence of CS proteins and the longstanding question which types of DNA damage (induced by metabolic processes) can inhibit transcription and trigger signaling in vivo. There is now evidence that transcription itself is mutagenic and that during S-phase collisions of replication forks with transcription complexes stalled at DNA lesions might affect genomic integrity. Hence, cells deal with two key processes that are affected by TC-NER deficiency and that differentially determine the biological outcome: at the one hand, arrest of transcription is highly mutagenic, at the other hand it is a potent inducer of apoptosis. It is a challenge to understand these consequences of impaired TC-NER as well as transcription defects in the aetiology of the progeroid, neurodevelopmental disorder of CS.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported by grants from ZonMw (912-08-031), ALW (855-01 074) and NTC (050-060-510).

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