DNA DAMAGE

The DNA damage response to transcription stress

Hannes Lans[1], *Jan H. J. Hoeijmakers*[1,2,3], *Wim Vermeulen*[1]* and *Jurgen A. Marteijn*[1]*

Abstract | The spatiotemporal control of RNA polymerase II (Pol II)-mediated gene transcription is tightly and intricately regulated. In addition, preservation of the integrity of the DNA template is required so as to ensure unperturbed transcription, particularly since DNA is continually challenged by different types of damaging agents that can form transcription-blocking DNA lesions (TBLs), which impede transcription elongation and cause transcription stress. To overcome the highly cytotoxic effects of TBLs, an intricate cellular response has evolved, in which the transcription-coupled nucleotide excision repair (TC-NER) pathway has a central role in removing TBLs specifically from the transcribed strand. Damage detection by stalling of the transcribing Pol II is highly efficient, but a stalled Pol II complex may create an even bigger problem by interfering with repair of the lesions, and overall with transcription and replication. In this Review, we discuss the effects of different types of DNA damage on Pol II, important concepts of transcription stress, the manner in which TBLs are removed by TC-NER and how different tissues respond to TBLs. We also discuss the role of TBLs in ageing and the complex genotype–phenotype correlations of TC-NER hereditary disorders.

Translesion synthesis (TLS). DNA polymerization by specialized polymerases past an obstructive DNA modification or damage, which comes at the cost of fidelity and mutagenesis.

[1]*Department of Molecular Genetics, Oncode Institute, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands.*

[2]*Princess Máxima Center for Pediatric Oncology, Oncode Institute, Utrecht, The Netherlands.*

[3]*CECAD Forschungszentrum, University of Cologne, Cologne, Germany.*

**e-mail: w.vermeulen@erasmusmc.nl; j.marteijn@erasmusmc.nl*

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Faithful and timely expression of genes is essential for all organisms. Gene transcription by RNA polymerase II (Pol II) is tightly controlled by intricate epigenetic and signalling-mediated regulatory networks at each step of the transcription process[1–3]. Despite our detailed knowledge of transcription regulation, the importance of preserving an intact DNA template is commonly overlooked. However, since the integrity of DNA is continually challenged by DNA-damaging processes[4], it is essential to understand the consequences of DNA damage for transcription and how cells respond to genomic injuries that interfere with transcription.

Environmental, DNA-reactive chemical agents and electromagnetic radiation, such as ionizing radiation and ultraviolet (UV) light, cause a broad range of DNA lesions[5]. Furthermore, several endogenous cellular processes inherently lead to DNA damage, such as aerobic metabolism, which produces reactive oxygen species (ROS) that directly modify DNA[6]. Many DNA lesions constitute barriers to the translocation of DNA and RNA polymerases on the DNA template. Replication is strongly impeded by damaged DNA when DNA repair systems cannot remove genome-wide injuries in a timely manner[7]. Some subtle base modifications may induce base mispairing, resulting in increased mutagenesis. Bulkier lesions can block replication, which may lead to DNA breaks and cause chromosomal aberrations. Some of the bulky lesions can be bypassed by specific translesion synthesis (TLS) DNA polymerases, but this process increases mutagenesis owing to the reduced fidelity of TLS polymerases[8]. As mutagenesis is a key driver of oncogenesis, much attention is devoted to understanding the cellular responses to DNA-damage-induced replication stress[7].

Importantly, DNA lesions also affect transcription, by slowing down Pol II progression, thereby compromising the fidelity of transcription via Pol II or even fully blocking its forward translocation[9] (FIG. 1). In addition, lesion-blocked transcription can lead to the formation of R-loops[10,11]. The unpaired DNA strand in R-loops is more vulnerable to chemical or enzymatic attack than normal double-strand DNA, thereby decreasing genetic integrity, which is even further decreased in rapidly dividing cells, owing to collisions of stalled Pol II or R-loops with advancing replication forks[10,12]. We propose to name the combined impact of DNA damage on transcription accuracy and efficiency, including genome-wide transcription regulation (discussed below), 'transcription stress'. DNA-damage-induced transcription stress produces mutant transcripts or decreases the abundance of vital mRNAs and increases genome instability, which may result in cellular dysfunction, senescence or even premature cell death, all majorly contributing to ageing[13] (discussed below). Since DNA lesions are inherent to life, it is vital for cells to adequately deal with DNA-damage-induced transcription problems.

a

Type of lesion	Transcription-blocking lesion	Causes	Refs
Structural lesions	6–4PP and CPD photoproducts	Ultraviolet light	28, 220
	Abasic sites	Depurination, BER intermediate	18
DNA crosslinks	Platinum–DNA crosslinks	Chemotherapy	146
	Psoralen–DNA crosslinks	Phototherapy	221
Bulky adducts	BPhDE adducts	Pollutant BPhDE	222
	BPDE adducts	Smoke from organic matter	29
	AAF adducts	Carcinogen AAF	30
	4NQO–DNA adducts	4NQO	223
	DNA–protein crosslinks	Aldehydes, topoisomerase inhibitors	224–226
Oxidative lesions	Cyclopurines	Oxidative stress	31
Toxins	Illudin S adducts	Mushroom toxin	52
	Aristolactam–DNA adducts	Herbal toxin aristolochic acid	53

b

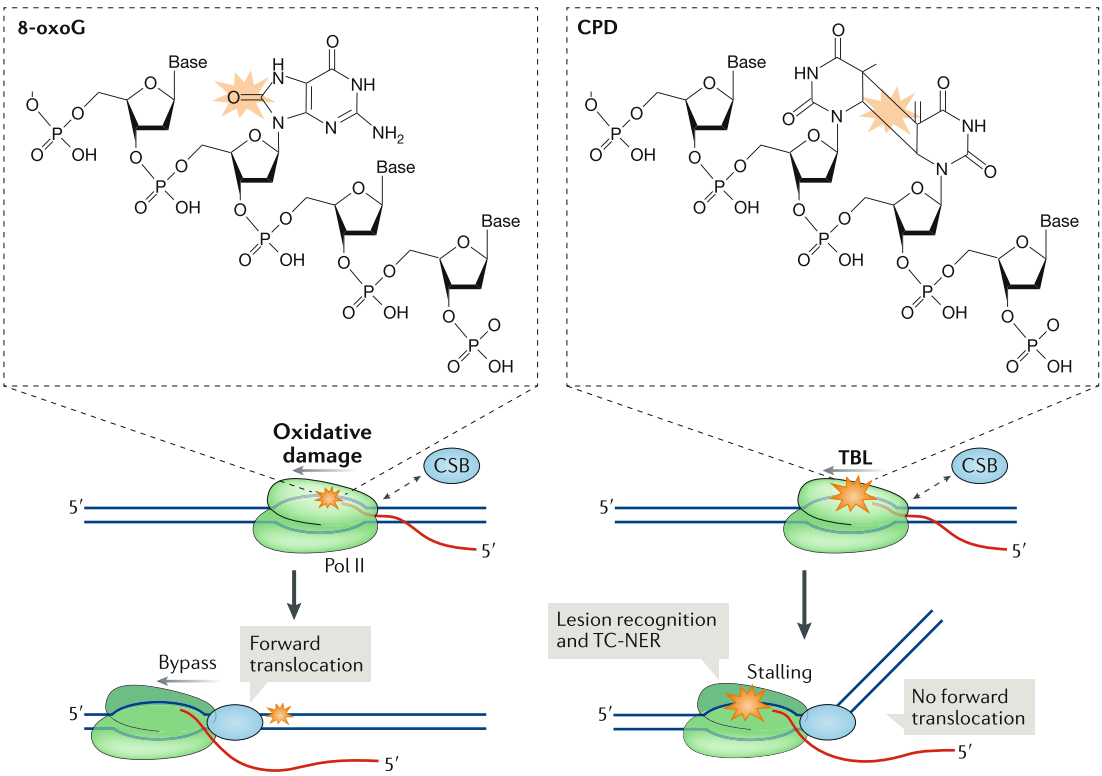


Fig. 1 | **Transcription-blocking lesions and overview of DNA damage sensing by RNA polymerase II. a** | Examples of known transcription-blocking lesions (TBLs) and their causes of induction. The ultraviolet (UV) light-induced photoproducts are cyclobutane pyrimidine dimers (CPDs) and 6–4 pyrimidine–pyrimidone photoproducts (6–4PPs)[28,220]. Abasic sites, where a DNA base is removed from the sugar–phosphate backbone, either originate from spontaneous hydrolysis (depurination) or are formed as a base excision repair (BER) intermediate[18]. Platinum-containing chemotherapeutic drugs such as cisplatin, carboplatin or oxaliplatin[146], as well as psoralens combined with UV radiation[221], induce both intra-strand DNA crosslinks (covalently linked adjacent bases) and inter-strand crosslinks (covalently linked bases on opposite DNA strands). Benzo[c]phenanthrene diol epoxide (BPhDE) covalently binds to adenine to form various bulky DNA adducts[222], whereas benzo[a]pyrene diol epoxide (BPDE)[29] and 2-acetylaminofluorene (AAF)[30] react with guanine to form various other bulky lesions. 4-Nitroquinoline 1-oxide (4NQO) also forms bulky adducts on purines[223]. Aldehydes and drugs like topoisomerase inhibitors cause DNA–protein crosslinks[224–226]. Oxidative stress induces oxidated bases such as 8-oxoguanine (8-oxoG), which are targeted by base excision repair (BER), as well as transcription-blocking cyclopurine deoxynucleosides (cyclopurines)[31]. The mushroom toxin illudin S[52] and the herbal toxin aristolochic acid[53] form bulky, but non-helix-disturbing, transcription-blocking lesions. **b** | DNA damage sensing by RNA polymerase II (Pol II) and Cockayne syndrome group B (CSB). During transcription elongation, Pol II may encounter structures that inhibit its forward translocation, including oxidative damage (left panel) and TBLs such as UV-induced lesions (right panel). Stalled Pol II is recognized by CSB, which binds DNA upstream of Pol II and uses its ATPase activity to push Pol II forward, by translocating 3′ to 5′ over the transcription template strand. CSB is capable of moving Pol II over natural pause sites and less bulky lesions such as the oxidative lesion 8-oxoG (a process known as 'bypass'). However, CSB cannot push Pol II over bulkier TBLs such as CPDs ('stalling'), and in such cases its translocation activity induces strong bending of the upstream DNA and tighter association of CSB with Pol II, which leads to the initiation of transcription-coupled nucleotide excision repair (TC-NER). This mechanism of probing whether arrested Pol II is still able to forward-translocate, by pulling the upstream extruding DNA, is an elegant mechanism to discriminate lesion-stalled from naturally paused Pol II.

R-loops
Three-stranded nucleic acid structures composed of an RNA–DNA hybrid and the complementary single-stranded DNA.

Transcription-blocking DNA lesions
(TBL). Any type of DNA modification, crosslink or damage that strongly impedes or blocks the elongation of RNA polymerases.

Abasic sites
Sites in DNA that lack a purine or pyrimidine base, arising either by spontaneous depurination or by cleavage of the *N*-glycosidic bond by base excision repair glycosylases.

Cyclobutane pyrimidine dimers
(CPDs). The most frequent type of ultraviolet-induced photolesion; formed by covalent linkage of the C5 and C6 carbon atoms of two adjacent pyrimidines.

Cyclopurines
A form of oxidative DNA damage that is repaired by nucleotide excision repair (NER) but not by base excision repair (BER); cyclopurines are formed by linkage of the C5 carbon atom of 2-deoxyribose and the C8 carbon atom of purine.

Transcription pause sites
Promoter-proximal sites where transcription is stalled, to maintain chromatin open and allow 5′ capping of the nascent RNA, as well as to regulate the timing of transcription.

The identification of dedicated transcription-coupled DNA repair machineries, across all kingdoms, that specifically remove transcription-blocking DNA lesions (TBLs), clearly illustrates the biological importance of unperturbed transcription[14–17].

In this Review, we discuss what happens when Pol II encounters different types of DNA lesion and how they are sensed and removed. Next, we discuss the effect of transcription stress on our health, as exemplified by several human hereditary syndromes. Finally, we discuss how transcription stress leads to reorganization of gene expression to prevent additional cytotoxicity by lesion-stalled Pol II complexes.

DNA-damage-induced transcription stress

The impact of DNA damage on transcription accuracy and efficiency depends largely on the type of lesion encountered by Pol II, but likely it is also influenced by the genomic context, including chromatin compaction, the frequency of transcription initiation, the density of lesions, cell cycle phase and cell type. Lesions originating from normal endogenous cellular processes are very abundant and include depurinations and non-bulky single-base modifications, such as spontaneous deamination, alkylation and oxidized nucleotides[6]. Most of these lesions, except abasic sites (which are formed by spontaneous depurination[18]), do not block Pol II[19,20] and can be bypassed by the transcription machinery. Since Pol II is not stalled, transcription-coupled repair is not initiated (see below) and these lesions will be accessible for removal by alternative DNA repair pathways. However, lesion bypass decreases the fidelity of Pol II and therefore may induce transcriptional mutagenesis[21–23]. As long as lesions persist, mutant transcripts will accumulate and can influence cell function[24]. A recent study, using a p53 transcript reporter carrying a site-specific alkylated guanine, showed that DNA-damage-induced transcriptional mutagenesis can produce dominant-negative mutant p53 in sufficient quantities to impede the tumour-suppression function of wild-type p53 (REF.[25]). These results illustrate the immediate threat of single-base or small non-bulky lesions and the potentially severe effects of transcriptional mutagenesis.

In comparison with non-bulky DNA damage, strong helix-distorting DNA lesions and bulky adducts cause an even bigger problem for transcription, as these lesions can completely obstruct Pol II's forward translocation[26,27]. Many different TBLs exist that are induced by exposure of cells to diverse environmental agents, including UV irradiation[28] and various carcinogens[29,30], or to intracellular metabolites[31] (FIG. 1a). A direct consequence of TBLs is that expression of the damaged alleles is interrupted, thereby disturbing cellular homeostasis. Recent studies suggest that TBLs also influence transcription regulation genome-wide (discussed below).

There are different structural causes for lesion-induced Pol II stalling, depending, amongst other factors, on the 'bulkiness' of the lesion. More bulky injuries, such as cisplatin-induced intra-strand crosslinks, cause steric blocks that prevent entry of the damaged base into the active site of Pol II[32]. Other lesions, such as UV-induced cyclobutane pyrimidine dimers (CPDs), which cause moderate helical distortions, stall Pol II by reducing the flexibility of the phosphodiester backbone of the DNA. Reduced DNA flexibility does not allow correct positioning of the incoming ribonucleotide, and therefore leads to arrest of forward translocation[9,26,28].

Furthermore, the same lesion can have variable effects on transcription. For example, cyclopurines and CPDs can be bypassed or can induce Pol II stalling[33–36]. It is unknown which factors determine this difference. Interestingly, experiments with a mutant yeast Pol II suggest that its structure influences its ability to bypass lesions. A specific amino acid substitution (G730D) in the largest yeast Pol II subunit, which is known to enhance processivity and reduce fidelity, was shown to increase bypass of CPDs and simultaneously to improve UV resistance[34,37]. Although lesion bypass by Pol II comes at the expense of increased transcriptional mutagenesis, and thus may seem an undesired option, it might be beneficial for cell survival in specific circumstances — for example, in rapidly dividing cells confronted with acute, high loads of DNA damage, to avoid collisions between stalled Pol II and replication forks.

The mechanism of TBL removal

Nucleotide excision repair (NER) is the main repair pathway that removes DNA helix-distorting lesions, either in the transcribed strand, by its sub-pathway transcription-coupled nucleotide excision repair (TC-NER), or genome-wide, by the sub-pathway global genome nucleotide excision repair (GG-NER; Supplementary Box 1). Currently, Pol II stalling is thought to trigger TC-NER (FIG. 2).

Damage sensing by Pol II stalling. Since Pol II stalling also occurs at undamaged DNA, such as at natural transcription pause sites, cells should be able to discriminate between pausing and TBL-induced stalling. The DNA-dependent ATPase Cockayne syndrome group B (CSB; also known as ERCC6)[38], which is an essential TC-NER factor, was originally suggested to have a primary role in TBL detection. CSB, which transiently interacts with elongating Pol II, becomes more tightly associated with Pol II that is stalled by DNA damage[39–41]. Recently, a plausible molecular mechanism was proposed for how CSB discriminates between paused and lesion-stalled Pol II (FIG. 1b), based on insightful cryo-EM studies of yeast Pol II and RAD26 (the orthologue of human CSB) stalled on different types of DNA damage[9,42]. CSB was shown to bind upstream of Pol II and to push the polymerase forward by translocating 3′ to 5′ over the DNA template strand, which is consistent with the translocation reported for human CSB[43]. A similar RNA polymerase forward-translocating activity was observed in bacterial TC-NER, where the SF2-like ATPase transcription-repair-coupling factor Mfd binds upstream of bacterial RNA polymerase and was proposed either to release the stalled polymerase or to forward-translocate it over the lesion[44,45]. This translocating force of CSB likely facilitates bypassing of natural pause sites and of less obstructing lesions such as oxidative DNA damage[20]. However, in the case of larger

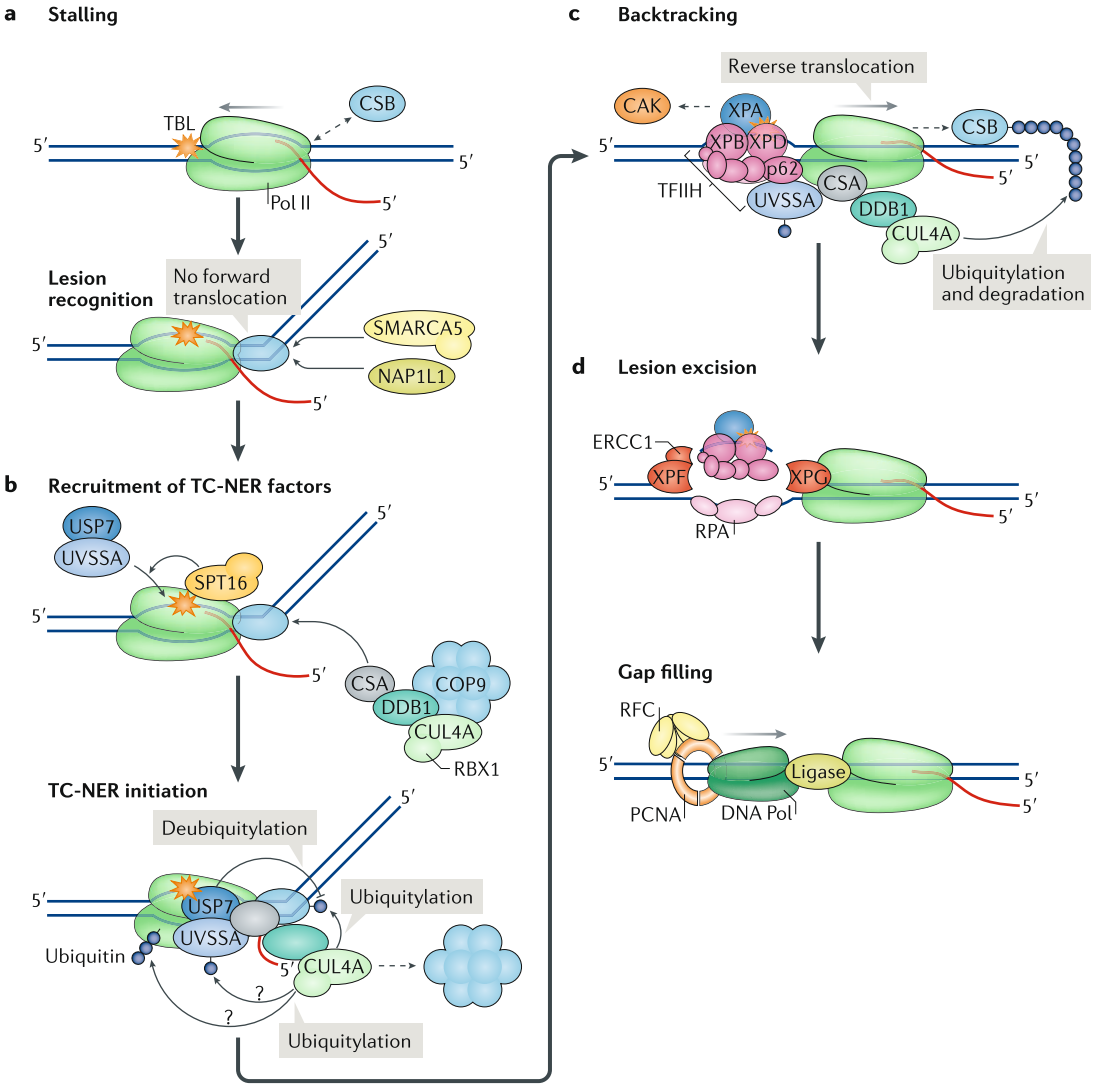


Fig. 2 | **Transcription-coupled nucleotide excision repair.** Model of transcription-coupled nucleotide excision repair (TC-NER), consisting of tightly controlled, consecutive steps from the stalling of RNA polymerase II (Pol II) to lesion excision and gap-filling DNA synthesis. **a** | Cockayne syndrome group B (CSB) transiently interacts with Pol II during transcription and probes whether Pol II can forward-translocate on the DNA. Upon encountering a transcription-blocking lesion (TBL), Pol II stalls, and CSB, unable to push Pol II forward, associates more stably with Pol II and bends the DNA, thereby triggering TC-NER (lesion recognition). CSB recruitment and activity are stimulated by the chromatin remodellers SMARCA5 and NAP1L1. **b** | The $CRL4^{CSA}$ complex, which consists of CSA, DNA damage-binding protein 1 (DDB1) and the cullin 4a (CUL4A)–RBX1 ubiquitin E3 ligase, is recruited to the lesion by CSB and is activated upon dissociation from the COP9 signalosome complex (TC-NER initiation). UV-stimulated scaffold protein A (UVSSA) and ubiquitin C-terminal hydrolase 7 (USP7) are also recruited to the lesion, facilitated by the chromatin remodeller FACT subunit SPT16, and stably associate with CSA. CSB is ubiquitylated by $CRL4^{CSA}$, but this is counteracted by USP7-mediated de-ubiquitylation in order to prevent CSB degradation. UVSSA and Pol II are also ubiquitylated upon DNA damage, but whether $CRL4^{CSA}$ is responsible for this is unclear. **c** | Transcription factor IIH (TFIIH) is recruited through interaction of its p62 (also known as GTF2H1) subunit with UVSSA. This may coincide with the dissociation of USP7 from UVSSA (not shown), so that CSB is no longer deubiquitylated and is degraded to allow Pol II backtracking. TFIIH forward-translocates on the DNA using its 5′–3′ XPD helicase until blocked by the lesion, which may stimulate Pol II backtracking. The other TFIIH helicase, XPB, facilitates TFIIH recruitment and promotes DNA unwinding and lesion verification. Upon TFIIH binding to the DNA, its trimeric CDK-activating kinase (CAK) sub-complex dissociates, which is facilitated by the NER-organizing protein XPA. **d** | XPA binds to TFIIH at DNA lesions, and together with TFIIH verifies the lesion and recruits the structure-specific endonucleases ERCC1–XPF and XPG. The RPA complex is also recruited, coats the single-stranded DNA opposite the lesion and mediates stabilization of the open repair intermediate, DNA damage signalling and orientation of the endonucleases. ERCC1–XPF and XPG incise the DNA 5′ and 3′ of the lesion, respectively, releasing a 22- to 30-nucleotide-long DNA oligomer containing the lesion. The resulting gap is filled by DNA synthesis, which is mediated by proliferating cell nuclear antigen (PCNA), replication factor C (RFC) and DNA polymerase δ, ε or κ, and finally is sealed by either DNA ligase 1 or XRCC1–DNA ligase 3.

Oxidative DNA damage
A type of DNA damage formed by oxidation of nucleotides, which is caused mainly by reactive oxygen species. 8-Oxo-2′-deoxyguanosine is the most common type of oxidative DNA lesion.

Base excision repair
(BER). A DNA repair pathway initiated by lesion-specific glycosylases that recognize and remove small base modifications such as oxidative and alkylating DNA lesions.

6–4 pyrimidine–pyrimidone photoproduct
(6–4PP). The second most frequent type of ultraviolet-induced photolesion; formed by covalent linkage of the C4 and C6 carbon atoms of two adjacent pyrimidines.

obstacles, such as UV-induced CPDs, Pol II cannot be pushed forward, and the interaction of CSB with Pol II is prolonged, which is most likely the actual trigger of TC-NER (FIG. 1b). Pol I is also blocked by UV-induced DNA lesions, and for both RNA polymerases, the canonical TC-NER machinery (see below) is required in order to resume transcription[46], which corroborates the conclusion that impeded translocation of RNA polymerases is the cue to activating TC-NER.

The combination of the pushing force of CSB and the blocking of elongating Pol II represents a unique mode of DNA damage recognition, in which not the lesion itself but rather its consequence is sensed (FIG. 1b; FIG. 2a). This is principally different from damage recognition in some other DNA repair processes, such as base excision repair (BER) and double-strand break repair, in which dedicated proteins directly recognize and bind a specific type of damage in order to initiate repair[47,48]. This indirect manner of damage detection resembles the indirect damage detection in GG-NER (Supplementary Box 1). Despite this resemblance, damage detection by TC-NER — that is, by threading DNA through the Pol II complex — is probably even more versatile than damage detection by GG-NER, in terms of the wide variety of structurally different lesions that can be recognized and removed. A striking example of this is the sensing of CPDs by TC-NER. CPDs, which are the most abundant UV-induced lesions, are actually poor substrates for GG-NER and require the auxiliary UV-DDB complex for their detection (Supplementary Box 1). Even in the presence of UV-DDB, the kinetics of CPD removal by GG-NER are at least six to ten times slower than those of 6–4 pyrimidine–pyrimidone photoproduct (6–4PP) removal[49], whereas both photolesions are repaired with similar kinetics from the transcribed strand of active genes[50]. Some platinum-drug-induced crosslinks may also be more efficiently recognized by TC-NER than by GG-NER[51]. Furthermore, lesions induced by illudin S[52] or aristolochic acid[53] (FIG. 1a) are repaired by TC-NER but are not efficiently sensed by GG-NER. This is likely because these lesions do not sufficiently disrupt base pairing to trigger damage detection by GG-NER, but they do create a block for Pol II translocation. Moreover, CSB is implicated in transcription-coupled repair pathways other than NER[54–57], which advocates for the existence of a broader type of transcription-coupled repair, extending beyond NER, which repairs Pol II-blocking lesions (BOX 1).

Initiation of TC-NER. TC-NER is a highly coordinated, multistep process that excises TBLs and the surrounding nucleotides from the template strand and synthesizes new DNA to fill the single-stranded DNA (ssDNA) gap (FIG. 2). To allow downstream NER factors access to the lesion, removal or remodelling of the lesion-stalled Pol II, which shields the lesion[9,42] (FIG. 2a), is required. To this end, Pol II was proposed to reverse-translocate (that is, backtrack). Backtracking normally happens at transcription pausing and termination sequences to control the fidelity and timing of transcription[58]. As the yeast CSB orthologue RAD26 stimulates forward translocation rather than backtracking of Pol II[9,42], it seems likely that another DNA-translocating factor is required for backtracking. In light of the established 5′-to-3′ translocation activity of the transcription initiation and repair complex transcription factor IIH (TFIIH), it has been suggested that TFIIH performs the backtracking of Pol II[9,59–61] (FIG. 2c). Recently, it was shown that a considerable fraction of lesion-stalled Pol II dissociates from DNA in conjunction with repair[62], which may occur as an alternative or in addition to backtracking in order to provide access for repair factors.

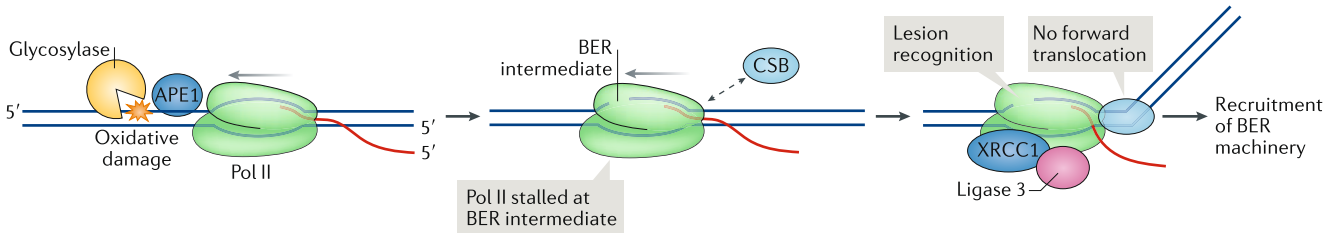
CSB is a central regulator of TC-NER, by organizing the recruitment and/or the stable association of TC-NER factors[63–67] (FIG. 2b). CSB facilitates the recruitment of CSA (also known as ERCC8)[63,64,68,69], which associates with DDB1, RBX1 and CUL4A, and as such serves as the substrate recognition factor in the cullin 4–RING ubiquitin E3 ligase (CRL4) complex ($CRL4^{CSA}$)[70]. CSA requires the TriC chaperonin for proper folding, which in turn is needed for CSA's incorporation into the $CRL4^{CSA}$ complex and targeting of the complex to the nucleus[71]. The ubiquitylation activity of $CRL4^{CSA}$ is suppressed by binding of the de-neddylating COP9 signalosome complex[72]. However, following DNA damage, the COP9 signalosome dissociates and $CRL4^{CSA}$ is neddylated and thereby activated, leading to the polyubiquitylation and subsequent proteasome-dependent degradation of CSB[70,73]. $CRL4^{CSA}$ possibly also ubiquitylates other factors at lesion-stalled transcription sites, which may include the Pol II subunit RPB1 (REF.[74]) and the TC-NER-specific factor UV-stimulated scaffold protein A (UVSSA)[75–77], which are both modified by ubiquitin in response to UV-induced DNA damage (FIG. 2c). However, the exact substrates of $CRL4^{CSA}$ during TC-NER remain elusive and under debate[78].

CSB degradation is counteracted by ubiquitin C-terminal hydrolase 7 (USP7), which is recruited to stalled-transcription sites through its interaction with UVSSA[65,67,76,77] (FIG. 2b). Like CSB, UVSSA also transiently interacts with elongating Pol II[77] and becomes stably bound to a lesion-stalled Pol II through the interaction of Pol II with CSA[65–67]. UVSSA recruitment is stimulated by the histone chaperone FACT subunit SPT16, which promotes histone H2A and H2B turnover at lesion-stalled transcription sites in order to facilitate TC-NER and the subsequent transcription restart[79,80]. Other chromatin-modifying factors also facilitate TC-NER — for example, SMARCA5-containing complexes and NAP1L1, which regulate CSB binding and function, respectively[81,82]. It is conceivable that these and other chromatin remodellers are necessary for creating a chromatin environment suitable for repair-factor access and transcription resumption[83–85]. However, transcription (and thus TC-NER) already takes place in an open chromatin environment, and the recruitment of UVSSA and of CSB is facilitated by different chromatin remodellers[79,82]. It is therefore likely that the chromatin-modifying factors above have a specific role in TC-NER beyond chromatin opening — for example, in adjusting chromatin structure in order to allow specific TC-NER steps, or in promoting TC-NER by facilitating direct protein interactions.

Box 1 | **From transcription-coupled nucleotide excision repair to transcription-coupled repair**

Evidence is accumulating that, in addition to the repair of bulky lesions by transcription-coupled nucleotide excision repair (TC-NER), repair of other types of DNA damage can also be coupled to transcription. This transcription-coupled repair (TCR) response extends beyond canonical TC-NER, although it is unclear precisely which mechanisms are involved. The preferential and transcription-dependent removal of oxidative lesions from the transcribed strand indicates that base excision repair (BER), which is the common repair pathway of oxidative lesions, can be transcription-coupled[121,122]. Moreover, the essential TC-NER factor Cockayne syndrome group B (CSB) appears to have a role in this process[123]. However, in vitro transcription reconstitution assays have shown that most oxidative lesions do not cause a strong block of RNA polymerase II (Pol II)[18,20,191,192], and consequently, most oxidative lesions will be bypassed (FIG. 1b), suggesting that the transcription-associated response to oxidative DNA lesions is mechanistically different from canonical TC-NER. Indeed, although live-cell imaging studies have shown that CSB is recruited to oxidative DNA damage in a transcription-dependent manner, the downstream core NER factor xeroderma pigmentosum group A-complementing protein (XPA) is not[56], clearly differentiating this response from TC-NER. BER is initiated by the glycosylase-mediated removal of oxidized bases from the sugar–phosphate DNA backbone (see the figure). The resulting apurinic–apyrimidinic (AP) site is incised by AP-endonuclease 1 (APE1), leaving a single-nucleotide gap that is filled and sealed by DNA polymerase β (not shown) and by XRCC1–ligase 3, respectively[47]. Since AP sites and single-strand breaks do block Pol II elongation[18,193–195], it is likely that these BER intermediates, rather than the oxidative lesion itself, trigger TCR of oxidative lesions[18,193–195]. This hypothesis is supported by the recent observation that recruitment of the late BER factor XRCC1 to oxidative DNA damage is dependent on transcription and CSB[196] as well as on 8-oxoguanine DNA glycosylase (hOGG1)[51], whereas hOGG1 recruitment itself is independent of transcription and CSB. These results suggest that TC-BER is initiated by glycosylase-mediated removal of oxidized bases from DNA, followed by stalling of Pol II on the BER intermediate structures and subsequent binding of CSB in order to stimulate recruitment of the BER machinery. CSB possibly exerts this function by interacting with BER proteins[197–200] and/or by initiating the remodelling of the stalled Pol II complex so that the BER machinery gains access. The different roles of CSB in TC-NER and TCR might be regulated by specific post-translational modifications, as a specific ubiquitylation site within CSB has been implicated in repair of oxidative DNA damage but not in TC-NER[201].

Besides bulky DNA lesions and BER intermediates, Pol II is stalled upon encountering uncommon secondary DNA structures, RNA–DNA hybrids and lesions such as double-strand breaks (DSBs), inter-strand crosslinks and DNA–protein crosslinks. Although TC-NER and TC-BER are likely not involved in repair of these transcription blocks, other dedicated repair pathways involved in their removal, such as homologous recombination and inter-strand crosslink repair, might possibly act in a transcription-dependent manner. For example, transcription may be even more strongly blocked by the presence of DSB repair enzymes than by the break itself[202,203]. For the repair of DSBs in active genes, homologous recombination appears to be preferentially used over non-homologous end joining[204]. CSB has been implicated in promoting transcription-coupled homologous recombination[54,55], thereby possibly revealing a universal role for CSB in regulating TCR of many different types of lesion. As not much is yet known about the interplay between the transcription machinery and these repair pathways, more work will be needed in order to better understand the molecular mechanism involved.



TC-NER-mediated excision of TBLs. UVSSA bound to lesion-stalled Pol II promotes the recruitment of TFIIH[86] through direct interaction with the p62 (also known as GTF2H1) subunit of TFIIH[87] (FIG. 2c). TFIIH includes two DNA helicases, xeroderma pigmentosum group B-complementing protein (XPB) and XPD, with opposite directionality. The XPD-mediated 5′–3′ translocation is blocked by lesions, and this serves as a vital lesion verification step in GG-NER (Supplementary Box 1)[88,89]. This damage authentication is important, to prevent other helical distortions caused, for example, by mispairing from erroneously triggering NER. It is not known whether this lesion verification is necessary for TC-NER, as damage is already confirmed by the stalling of Pol II. However, as we discussed above, TFIIH-mediated translocation might provide the force for pushing back stalled Pol II in order to allow NER-complex assembly[9]. Interestingly, p62 binds to the same region in UVSSA as USP7 does[76,87]. It is thus likely that through competitive binding of TFIIH to UVSSA, USP7 dissociates, which will change the balance between $CRL4^{CSA}$-mediated ubiquitylation and the antagonizing de-ubiquitylation activity of USP7. A timely regulated ubiquitylation balance may be required in order to allow step-wise control over the intricate TC-NER reaction, in which CSB ubiquitylation at first is suppressed by USP7 in order to prevent its degradation. Once TC-NER is initiated, the hypothesized USP7 dissociation could result in CSB degradation, to facilitate Pol II backtracking by TFIIH. Dynamic ubiquitylation for efficient TC-NER progression is also observed on another level, as mutations in the ubiquitin-binding domain of CSB prolong its binding to lesion-stalled Pol II and strongly inhibit transcription resumption, indicating that controlled removal of CSB is important for efficient TC-NER[90]. The mechanisms of TFIIH recruitment, as well as the intricate regulation by $CRL4^{CSA}$ and USP7, bear a striking resemblance to, respectively, the p62-mediated TFIIH recruitment by XPC and the CRL4- and ubiquitin-mediated regulation of initiation factors in GG-NER (Supplementary Box 1).

Although our understanding of the subsequent repair steps is mostly based on studies of GG-NER, it is generally assumed that these steps are mechanistically similar in TC-NER. Following TFIIH, the DNA-binding factors XPA and RPA are recruited to the lesion (FIG. 2c,d). The NER organizer XPA facilitates release of the CDK-activating kinase (CAK) sub-complex from TFIIH[91], possibly promotes lesion verification by

TFIIH (as in GG-NER)[89] and binds to the DNA lesion in a single-stranded configuration[88]. RPA binds the ssDNA coding strand and likely has a role in DNA damage signalling by activating the DNA damage response kinase ATR, which may involve the generation of longer ssDNA stretches by exonuclease 1 (REFS[92–95]). Together, TFIIH, XPA and RPA promote the recruitment and positioning of the endonucleases ERCC1–XPF and XPG, which incise the DNA 5′ and 3′ areas of the lesion, respectively (FIG. 2d). Following excision of the damaged DNA, the resulting 22–30 nucleotide gap is filled by DNA synthesis and ligation, mediated by proliferating cell nuclear antigen (PCNA); replication factor C (RFC); DNA polymerase δ, ε or κ; and DNA ligase 1 or XRCC1–DNA ligase 3 (FIG. 2e). The precise steps of the general NER reaction have been reviewed in more detail in REFS[96,97].

Repair in differentiated cells. In non-proliferating, highly differentiated cells, which constitute a significant part of the mammalian soma, accurate genome-wide lesion removal is not required in order to prevent replication-associated mutagenesis. It thus seems wasteful to invest much energy in repairing large parts of the genome that have no obvious function for cell viability and function. Instead, focusing on removing TBLs through strand-specific TC-NER appears sufficient to assure unperturbed expression of the subset of genes required by these cells. Indeed, GG-NER is downregulated in terminally differentiated cells[98], including in neurons[99] and keratinocytes[100]. In addition, in *Caenorhabditis elegans*, GG-NER activity appeared less important than TC-NER for the survival of differentiated somatic cells following DNA damage[101,102]. However, impairing GG-NER still strongly accelerates DNA-damage-driven premature ageing features in TC-NER-deficient mice, most notably in postmitotic cells[103,104], and aggravates the UV hypersensitivity of TC-NER-deficient postmitotic cells in *C. elegans*[102]. These findings suggest that lesion removal by GG-NER may reduce the chance of Pol II stalling at lesions. It is also conceivable that GG-NER is required for lesion removal from the non-transcribed strand, as an intact non-transcribed strand is used as the template for faithful repair by TC-NER. Indeed, in terminally differentiated cells, DNA damage removal is almost completely abolished in inactive genes, while it is maintained on both strands in active genes[105]. This GG-NER activity in differentiated cells is concentrated towards the non-transcribed strand of active genes and is called transcription domain-associated repair.

Clinical consequences of TC-NER defects

The biological significance of the TC-NER system is reflected clinically by a conglomerate of rare genetic conditions of specific repair deficiencies. Interestingly, their clinical manifestations display great variation, ranging from the mild UV-sensitive syndrome (UV^{S}S), which is characterized by mild cutaneous phenotypes such as photosensitivity[106,107], to the extremely severe manifestations of cerebro-oculo-facio-skeletal syndrome (COFS), with multi-morbidity and early infant death[108]. In between are the skin-cancer-prone disorder xeroderma pigmentosum (XP), with severe cutaneous features and in some cases association with growth retardation and accelerated neurodegeneration[109]; the premature-ageing disease Cockayne syndrome (CS), with early cessation of development and a wide variety of progressive deficits but an intriguing absence of cancer predisposition[108]; rare cases of XP–CS complex disease, in which patients exhibit both the ageing phenotype of CS and the cutaneous features and cancer-proneness of XP[110]; and the repair-deficient form of trichothiodystrophy (TTD), which is characterized by strikingly brittle hair and nails, but otherwise shares many symptoms with CS[111,112]. Here we discuss different models that explain the differences in the principal disease-specific clinical features and their progressive nature.

From UV^{S}S to CS. Striking phenotypic differences are found in individuals with mutations in the TC-NER-initiating proteins CSA, CSB and UVSSA. The mildest TC-NER disorder is UV^{S}S, whose characteristics are mainly cutaneous, including photo-hypersensitivity, freckling and telangiectasia. UV^{S}S is very rare, but in view of its mildness, it is likely to be underdiagnosed. Except for a few particular cases with specific mutations in *CSA*[113] and *CSB*[114], the main gene mutated in UV^{S}S is *UVSSA*[65,66,77,106]. The mild UV^{S}S phenotype is in sharp contrast to the severe clinical manifestations commonly associated with CS, which involve, besides the UV sensitivity common to all NER syndromes, premature cessation of physical and neurological development; severe progressive neurodysfunction, such as demyelination, calcification, retinal degeneration and hearing loss; and progressive multi-organ decline, including vasculopathy, liver and kidney dysfunction, and osteoporosis[108]. As a consequence, the average life expectancy under CS is only 12 years, but with a wide range.

Interestingly, although most DNA repair syndromes include cancer-prone phenotypes, in UV^{S}S and CS, cancer has never been reported[97,109]. This can be explained by the fact that in the absence of TC-NER, GG-NER still removes the bulk of lesions throughout the genome and induces DNA damage signalling through ATR in order to dampen replication of damaged DNA[92,93]. Moreover, TC-NER deficiency causes cell death even with low levels of DNA damage, owing to transcription stress[115], which protects from cancer because it sets a lower threshold for apoptosis and thus prevents mutagenesis, and thereby carcinogenesis. On the other hand, the elevated cell death and transcription stress cause premature organ atrophy and functional decline, which translates clinically as accelerated ageing.

The transcription-associated removal of UV-induced TBLs is completely absent in both CS and UV^{S}S, as shown by a complete deficit in TC-NER activity[79] and transcription restart[77,116]. This raises the puzzling question of why apparently similar cellular TC-NER deficiencies can cause such a dramatic phenotypic difference. One way to explain this difference is to attribute it to the additional functions of CS proteins not shared with UVSSA. Such functions, mostly reported for CSB, include the preservation of mitochondrial function[117,118], driving specific transcription programmes for neuronal

development[119] and controlling the cellular redox balance[120]. However, in line with the current understanding of transcription-coupled repair mechanisms, we favour the hypothesis that defective genome maintenance is the common denominator of the TC-NER-linked disorders and that the gradual accumulation of DNA damage is the main driver of the progressive nature of many of their symptoms (FIG. 3).

First, CSB is active in other transcription-coupled repair pathways, including in the removal of oxidative lesions from the transcribed strand[56,121–123] (BOX 1), in transcription-coupled homologous recombination of double-strand breaks[54,55] and in inter-strand crosslink repair[124,125]. Such a universal role of CSB in regulating transcription-coupled repair of many different types of lesions might explain the more severe phenotype of CS compared with that of UV^{S}S, owing to greater accumulation of DNA damage, including damage derived from endogenous metabolites. Furthermore, as CSB has an important role in the forward translocation of Pol II over oxidative lesions and natural pause sites[9,42] (FIG. 1), the absence of CSB activity may result in diminished lesion bypass and thus prolonged stalling of Pol II at lesions, which would shield the lesions from repair by other pathways. However, in view of the similar phenotypes caused by mutations in the *CSA* gene, it is important to investigate whether CSA is also involved in the transcription-coupled processes described above. An additional function, reported for both CSB and CSA, is promoting the clearance of lesion-stalled Pol II — for example, by proteasomal degradation[74]. Inability to remove Pol II from a TBL will shield TBLs from other

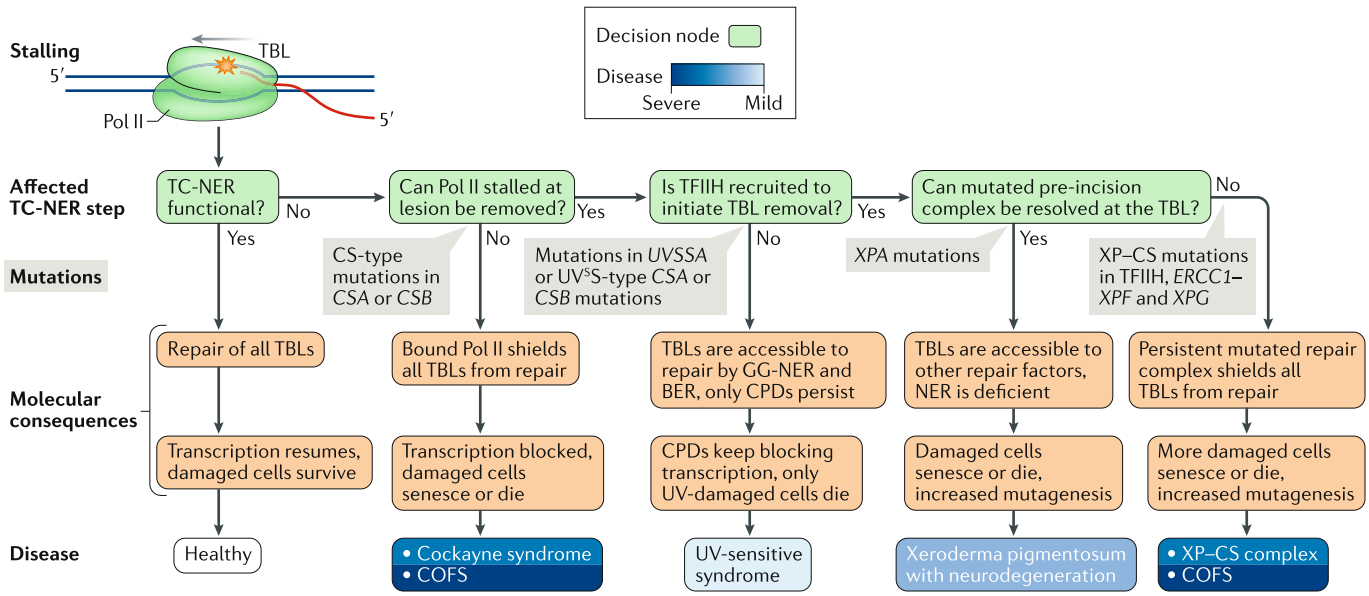


Fig. 3 | **Genotype–phenotype correlations of transcription-coupled nucleotide excision repair disorders.** Tentative flowchart of genotype–phenotype relationships of transcription-coupled nucleotide excision repair (TC-NER) disorders. The events indicated in the flowchart are in part speculative, but they can explain the severity of the main clinical features based on DNA-damage-driven transcription stress. If TC-NER can resolve lesion-stalled RNA polymerase II (Pol II) and repair the transcription-blocking lesion (TBL), transcription resumes and no phenotype is observed. TC-NER impairment causes various disorders with striking phenotypic differences, which are in this model tentatively linked to the ability of other repair pathways to access and repair the TBL. If, owing to mutations in Cockayne syndrome group A (CSA) or CSB, lesion-stalled Pol II cannot be removed from the TBL, the damage becomes inaccessible to repair by any alternative repair pathway, such as global-genome nucleotide excision repair (GG-NER) or base excision repair (BER). Such persistently stalled Pol II will not only inactivate the affected genes, but also induce continuous genome-wide transcription interference by damage-induced trans-acting processes, thereby causing persistent transcription stress and resulting in decreased cellular fitness and increased apoptosis and cell senescence, which are the basis of the severe, progeroid phenotypes of Cockayne syndrome (CS) and cerebro-oculo-facio-skeletal syndrome (COFS). In cases of mutations in UV-stimulated scaffold protein A (UVSSA) or specific mutations in CSA or CSB, lesion-stalled Pol II can be cleared from the chromatin, but transcription factor IIH (TFIIH) cannot be recruited by the mutated UVSSA to initiate TC-NER. In this scenario, the TBL is still accessible to alternative repair pathways such as GG-NER or BER. Because transcription-blocking cyclobutane pyrimidine dimers (CPDs) are not repaired or are very poorly repaired by GG-NER, these ultraviolet (UV)-induced lesions will remain and stall transcription, resulting in the mild UV-sensitive syndrome phenotype. If xeroderma pigmentosum (XP) group A-complementing protein (XPA) is mutated, both TC-NER and GG-NER are impaired, but the formation of a stable pre-incision NER complex is prevented, and TBLs remain accessible to other repair systems. In XPA deficiency, all NER-type TBLs will persist because of the GG-NER defect and hence will continue to impede transcription, but non-NER-type TBLs will be accessible to and removed by other repair systems, such as BER. This might explain the milder and different phenotype of XPA deficiency — XP with neurodegeneration — compared with either CS or COFS. If Pol II stalling can be resolved by the activity of CSA and CSB, TBLs can still be shielded by the persistence of defective NER intermediates — for example, lesion-bound TFIIH — when core NER factors such as TFIIH, ERCC1–XPF or XPG are mutated. This would prevent any repair by either TC-NER or GG-NER and cause persistent transcription stalling. Moreover, the transcription stress would be augmented, because these mutations would also impair GG-NER at least in part, resulting in many more lesions that would interfere with transcription or cause mutations. This would lead to XP–CS complex or to COFS. The severity of the disease depends on the specific mutation and could be determined by the rate of turnover of stalled Pol II or NER intermediates. When the arrest of transcription or repair machinery can be resolved relatively quickly, the clinical manifestations could be milder (for example in mild forms of CS) than when transcription is more severely interrupted, as it is in COFS.

repair processes and lead to a gradual increase of persistently stalled Pol II complexes. This will not only paralyse the expression of damaged genes, but also create additional cytotoxicity — for example, by causing transcription–replication conflicts and R-loops (discussed below). However, CS-protein-dependent degradation of Pol II is being challenged[78] and requires further investigation. Thus, the relatively mild phenotype of UV^{S}S might be explained by the notion that Pol II can still be removed by the CS proteins, thereby preventing gradual accumulation of stalled Pol II complexes, which allows the removal of low levels of TBLs by alternative repair pathways, such as GG-NER, as has been previously hypothesized[97] (FIG. 3). The UV sensitivity of UV^{S}S is then explained by the fact that although UV-induced 6–4PPs are still removed by GG-NER, CPD lesions are not and will keep interfering with transcription. In the case of CS, variation in the rates at which arrested transcription complexes are resolved and the culprit lesion is repaired by other repair systems might determine the severity of the phenotype. It should be noted that various relatively rare DNA lesions, which are produced by endogenous cellular processes, such as oxidative cyclopurines[31] and cellular aldehyde-derived DNA–DNA and DNA–protein crosslinks[126], also cause transcription stalling. Thus, in TC-NER-deficient cells, even in the absence of exposure to environmental toxins, lesions will accumulate, thereby explaining the severe progressive phenotype of CS.

XP–CS complex. In addition to mutations in *CSA* and *CSB*, the CS phenotype can be caused by specific point mutations in the TFIIH helicase genes *XPD* and *XPB*, and in the NER endonuclease genes *XPG* and *ERCC1–XPF*[110,127]. Individuals carrying *XPD*, *XPB*, *XPG* or *ERCC1–XPF* mutations have deficient GG-NER and TC-NER, because these factors function in both sub-pathways, and consequently they will display both CS and XP features, which is designated as XP–CS complex. The fact that in XP–CS complex CSA and CSB proteins are functional — as is the case in UV^{S}S — shows that CS features can be caused by CSA-independent and CSB-independent mechanisms. It is also difficult to envision that CS would be caused by defects in any of the additional, non-TC-NER functions of CSB, as it is unlikely that XPD, XPB, XPG and ERCC1–XPF all share these functions. Because XP–CS cells are deficient in both sub-pathways of NER, all base-pair-disrupting NER lesions, including 6–4PPs and cyclopurines, will persist. Therefore, even though TBLs will still be accessible owing to the activity of CSB and CSA, only non-NER-type transcription-blocking injuries may be repaired, whereas the wide class of NER substrates will continue to cause severe transcription stress, cell death and functional decline, and consequently, premature ageing. This will be particularly problematic in postmitotic tissues (see above), which could explain the accelerated neurodegeneration observed in XP–CS complex. In addition, we recently found that XP–CS complex-causing mutations in TFIIH, XPF and XPG cause persistent presence of NER factors — including TFIIH — at lesions that are refractory to repair[128]. These persistent NER intermediates could very well shield the damage from other repair processes, and, consequently, form permanent roadblocks for transcription, and possibly for replication. A similar persistence of repair intermediates was found in mouse cells carrying XP–CS complex-specific mutations in *XPD*[129,130]. These findings are also in line with structural analysis of the archaeal XPD orthologue, which suggested that XP–CS-causing mutations affect the flexibility and dynamic dissociation of TFIIH[131]. Thus, like persistently stalled Pol II in CS cells, persistent binding of TFIIH and other NER factors to DNA damage in XP–CS cells could lead to functional decline and cell death (FIG. 3). Some mutations in *XPA* that also disrupt GG-NER and TC-NER cause one of the most severe forms of XP, which like CS includes accelerated neurodegeneration, but which is limited to neuron-specific degeneration and therefore has a different phenotype from CS[132]. This slightly milder phenotype, compared with the XP–CS complex, can most likely be explained by the idea that in the absence of XPA activity, the pre-incision NER complex cannot be stably formed, thereby preventing the formation of deleterious persistent NER intermediates (FIG. 3).

Accumulated damage as a disease driver. Even though the exact molecular mechanisms underlying the major differences in clinical features between the different TC-NER-linked disorders are not completely clear, the evidence summarized above strongly indicates that accumulating DNA damage and the resulting transcription stress are the main drivers towards the severe features of CS (FIG. 3). Interestingly, this also suggests that the persistency of Pol II stalling or NER intermediates, the consequences of CS-causing mutations or XP–CS complex-causing mutations, respectively, is more detrimental to cells than is the accumulated DNA damage owing to NER deficiency. This likely occurs because these persistent complexes more strongly interfere with transcription and replication than does the DNA damage itself. The mild phenotype of UV^{S}S is in line with this model, because CSB and CSA are functional in UV^{S}S, and Pol II is thought not to persistently stall. Furthermore, as UVSSA is essential for the recruitment of TFIIH[87] (FIG. 2c), TFIIH will not be recruited to TBLs in individuals with UV^{S}S, and thus, detrimental persistent NER intermediates will not form. This model is supported by our recent discovery that in progeroid DNA-repair-deficient mouse mutants, DNA-damage-induced transcription stress correlates with the observed ageing-dependent, genome-wide decline of expression of genes in a gene-length-dependent manner[133]. The latter finding is consistent with the idea that stochastically accumulating DNA damage interferes with transcription. Long genes are more likely to be damaged than short genes, and thus will have a higher chance of acquiring persistent Pol II stalling or NER intermediates. Strikingly, in our TC-NER-deficient progeroid mouse mutants, as well as in normal human ageing, damage-induced transcription stress is specifically evident in organs with no or slow cell renewal, which lack the ability to dilute or remove both DNA damage and stalled transcription complexes by replication[133].

Progeroid
An adjective to indicate resemblance to accelerated ageing.

Crossing CS mice with NER-deficient mice — all with mild phenotypes as single mutants — markedly aggravates their premature-ageing features. For example, the offspring of *Csa*$^{-/-}$ or *Csb*$^{-/-}$ mice crossed with *Xpc*$^{-/-}$ or *Xpa*$^{-/-}$ mice exhibit extremely severe, CS-like multi-organ degeneration and shortening of the lifespan from 2 years to 3–6 weeks[104,134]. As XPA and XPC have well characterized, distinct roles in GG-NER, the dramatic aggravation in the double mutants of the premature-ageing phenotype of *Csa*$^{-/-}$ or *Csb*$^{-/-}$ mice should be attributed to the contribution of endogenous unrepaired NER-type DNA damage. The phenotype of the double mutants is very similar to that of *Xpg*$^{-/-}$ mice[135], which recapitulate the very severe phenotype of individuals with XPG–COFS, and to that of the *Ercc1*$^{\Delta/-}$ mice, the phenotype of which resembles the premature-ageing features of an individual with XPF–ERCC1 progeroid syndrome (XFE)[136]. Both NER endonucleases (XPG and ERCC1–XPF) are implicated in multiple DNA repair pathways[137,138], which explains additional symptoms observed in, for example, the *Ercc1* mouse mutants, such as Fanconi anaemia features, which are attributed to the role of ERCC1–XPF in DNA crosslink repair[139]. These findings corroborate the concept that the severity of a phenotype correlates with the type of repair defect and its severity, which together determine the spectrum of clinical manifestations and point to causal relationships.

The direct causal link between DNA damage and the symptoms of CS is further substantiated by findings that DNA damage induction by cisplatin or ionizing radiation severely aggravates neuropathy in CS and in NER-deficient or BER-deficient mouse models, in which the accumulation of neuronal DNA damage is directly correlated with the capacity for DNA repair[140–143]. DNA-damage-induced transcription stress is also relevant in the context of cancer therapy. Platinum-based chemotherapy is associated with strong side effects, particularly peripheral neurotoxicity and the induction of premature ageing and frailty[144,145]. The dose and duration of treatment have to be limited in order to reduce the impact on quality of life, which hampers the efficacy of platinum drugs. These drugs exert their cytotoxic effect mainly through the induction of various DNA crosslinks, which kill cancer cells by interfering with DNA replication[146]. Transcription is also strongly inhibited by these drugs[27], in particular in peripheral neurons, in which cisplatin and oxaliplatin have been found to accumulate in the DNA[147–149]. Importantly, TC-NER and, unexpectedly, also BER (BOX 1) — due to the formation of oxidative DNA damage — are of major importance in protecting cells against platinum drug exposure[51,150,151]. This protection, together with the fact that hereditary TC-NER deficiency is particularly associated with neuropathy, indicates that the transcription stress produced by platinum–DNA adducts is a very plausible cause of platinum-drug-induced neurotoxicity, which underlies many of the detrimental side effects of chemotherapy.

Therapeutic options through nutrition. Hitherto, no effective therapy has been developed for TC-NER syndromes. However, several recent studies provide clues to what could be promising interventions. These include the targeting of nicotinamide adenine dinucleotide (NAD) metabolism[152], which may be effective through the activation of sirtuins, known to be involved in longevity, or through disrupting interaction between the transcription factors FOXO and p53, which is implicated in cell senescence and can alleviate several premature-ageing features of NER mouse models[153]. Interestingly, we recently found that a strong overall health improvement was achieved by dietary restriction[133]. Reducing food intake by 30%, without malnutrition, in *Ercc1*$^{\Delta/-}$ and in *Xpg*$^{-/-}$ mice markedly improved their overall condition by systemically delaying their accelerated-ageing features in all investigated organs, and tripling their lifespan[133]. Most notably, severe neurodegeneration, which is the most problematic clinical symptom of TC-NER-deficient CS, was significantly postponed. The rationale for this remarkable effect is that dietary restriction lowers metabolism and thus lowers (endogenously produced) DNA damage loads, thereby reducing transcription stress, to which TC-NER mutants are exquisitely sensitive. This study points to unexpected, counterintuitive treatment options — namely, simply reducing food intake. This is the opposite of the current treatment, which usually aims to increase nutritional intake in order to enhance growth and body weight. Future research will further characterize this surprising nutritional response and the safety of the treatment for genome instability syndromes, and will seek to identify additional anti-ageing interventions. These findings also open unexpected avenues for the treatment and prevention of neurotoxicity side effects of DNA-damaging chemotherapy and of neurodegeneration disorders such as Alzheimer disease and other dementias, for which ageing is the most important risk factor.

Transcription regulation by DNA damage

In addition to the direct transcription interference from Pol II stalling at TBLs, transcription is regulated genome-wide following DNA damage. Intriguingly, this type of regulation includes both transcription stimulation — to increase TBL detection — and transcription inhibition, most likely to diminish Pol II stalling at lesions. In addition, transcription needs to be properly restarted following damage removal, which is the last crucial step in the DNA damage response to transcription stress.

Increased lesion detection by Pol II. Stalling of Pol II is a highly efficient way of detecting a wide variety of structurally different lesions[9,17,42]. It is thus conceivable that through adaptive response mechanisms, de novo transcription could temporarily be increased so as to maximize the chance that DNA lesions will be detected by Pol II (FIG. 4a). Indeed, the positive transcription elongation factor b (p-TEFb) complex is released from its inhibitory 7SK small nuclear ribonucleoprotein (7SK snRNP) complex following exposure to UV light to stimulate transcription elongation[154,155]. Upon UV exposure, p38 MAPK signalling is activated and stimulates association of the RNA-binding protein RBM7 with 7SK snRNP, which results in chromatin localization and the activation of p-TEFb[156]. Furthermore, p38 MAPK

signalling promotes the dissociation of the negative elongation factor (NELF) complex from chromatin following UV-induced DNA damage[157]. Collectively, these actions stimulate a wave-like release of promoter-proximal paused Pol II into productive elongation, possibly to promote lesion detection[156–158].

Even though damage-induced transcription regulation by p38 MAPK signalling is important for cell

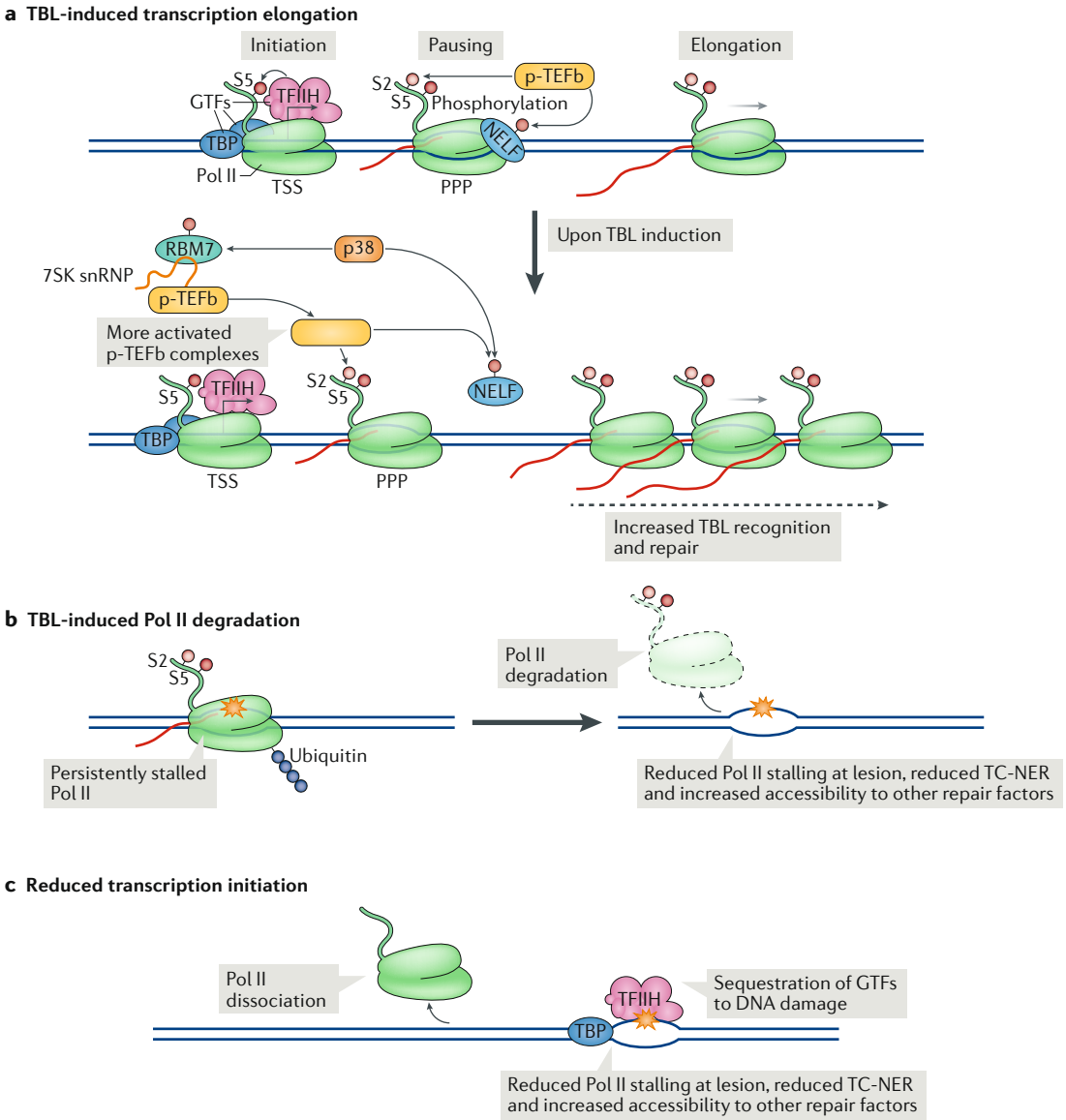
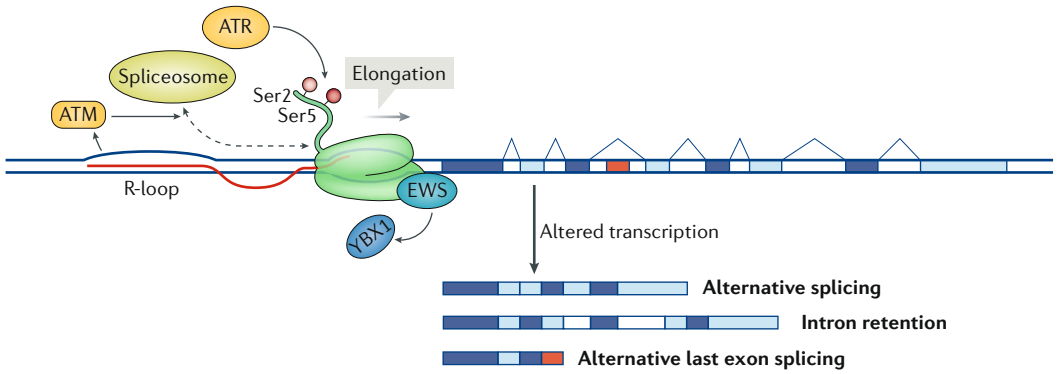


Fig. 4 | **DNA-damage-induced transcription regulation.** Transcription can be regulated at different stages in response to DNA damage. Different models are presented and compared with the unperturbed (no DNA damage) situation. **a** | Transcription-blocking lesion (TBL)-induced transcription elongation. Unperturbed transcription is initiated by loading of RNA polymerase II (Pol II) at the transcription start site (TSS), which is mediated by general transcription factors (GTFs), including TATA-box-binding protein (TBP) and transcription factor IIH (TFIIH). Upon initiation, Ser5 (S5) of the C-terminal domain (CTD) of RPB1 — the largest subunit of Pol II — is phosphorylated by the CDK7 activity of TFIIH. Pol II pauses at the promoter-proximal pause site (PPP) and is released into productive elongation upon phosphorylation of negative elongation factor (NELF) and of Ser2 (S2) of the CTD by positive transcription elongation factor b (p-TEFb). DNA damage could lead to the release of Pol II pausing, which is mediated by p38 MAPK signalling that results in p-TEFb activation and NELF dissociation from the chromatin. These processes promote the release of Pol II into productive elongation, which might stimulate lesion recognition and subsequent transcription-coupled nucleotide excision repair (TC-NER). **b** | TBL-induced Pol II degradation. Ubiquitylation and proteasomal degradation of persistently stalled Pol II prevents prolonged Pol II presence at the lesion, thereby minimizing the chance for replication and transcription collisions and making the lesion accessible to alternative repair mechanisms. This degradation of lesion-stalled Pol II has been referred to as the 'last resort' pathway, which may be required when the TC-NER machinery cannot properly handle TBLs[164]. **c** | Reduced transcription initiation. Following DNA damage, transcription initiation can be reduced by the sequestration of GTFs such as TBP and TFIIH to DNA damage, resulting in reduced Pol II stalling at lesions, reduced TC-NER and increased accessibility of other repair factors to the TBL. 7SK snRNP, 7SK small nuclear ribonucleoprotein; RBM7, RNA-binding protein 7.

Box 2 | **DNA-damage-induced changes in splicing**

Transcription-blocking lesions (TBLs) are expected to affect not only the amount of transcripts generated, but also the nature of the transcripts produced, because inhibition of the forward translocation of RNA polymerase II (Pol II) influences co-transcriptional mRNA maturation processes, including pre-mRNA splicing. Evidence is accumulating that DNA damage affects the highly orchestrated splicing process[205–208]. Importantly, this damage-induced alternative splicing appears to be a tightly regulated cellular response rather than a mere nonspecific bystander effect of Pol II stalling. For example, after DNA damage induction, the ratio between the anti-apoptotic Bcl-X_L and the pro-apoptotic Bcl-X_S splicing isoforms shifts towards Bcl-X_S[176]. The E3 ubiquitin ligase MDM2 is also alternatively spliced following UV-induced DNA damage into an inactive protein isoform, which leads to the stabilization of p53 (REFS[209,210]). Both alternative-splicing events are expected to increase ultraviolet (UV)-induced cell death. Recently, a more general UV-induced splicing response was uncovered, in which genes are transcribed into shorter splice variants by the incorporation of proximal, alternative last exons[160]. Transcribing shorter transcripts from the more proximal parts of genes may reduce the likelihood of Pol II running into a downstream TBL, which may explain why the more proximal parts of genes are preferentially repaired by TC-NER[188,189]. Interestingly, some of these shorter transcripts appear to have additional functions in the cellular transcription stress response. For example, a short isoform (25 kb, 4 exons) instead of a long isoform (>370 kb, 42 exons) of *ASCC3* is predominantly produced following TBL induction. Intriguingly, this short isoform does not encode a protein but a non-coding RNA that is important for proper transcription recovery following UV-induced DNA damage[160].

DNA-damage-induced alternative splicing is hypothesized to be induced by two non-mutually exclusive processes: by changes in the Pol II elongation rate, referred to as 'kinetic coupling'[160,176,211], or by altered interactions between the transcription machinery and splicing factors, called 'recruitment coupling'[11,210,212] (see the figure). In support of the kinetic-coupling model, increased damage-dependent phosphorylation of the C-terminal domain of the largest subunit of Pol II, which is likely mediated by the DNA damage response (DDR) kinase ATR, was shown to slow down transcription elongation, which induces alternative splicing[176,211,213,214]. An example of the recruitment-coupling model is provided by the observed DNA-damage-induced loss of interaction between the RNA-binding protein Ewing's sarcoma proto-oncoprotein (EWS) and the splicing factor YBX1 and its target RNAs, resulting in alternative splicing[210,212,215]. In addition to these splicing regulators, the activity of the core spliceosome itself is affected by transcription stress. TBLs induce the chromatin eviction of late-stage spliceosomes, resulting in increased intron retention and other types of alternative splicing[11,216]. This DNA-damage-induced alternative splicing occurs in a genome-wide manner[11,160,176,217], arguing for the existence of a highly regulated trans-acting process extending beyond the genes directly affected by TBLs. In line with this view, the two main DDR kinases ATR and ATM have been implicated in controlling damage-mediated alternative splicing[11,217,218]. ATR, which is known to be activated by single-strand DNA repair intermediates generated by GG-NER[92,93] (Supplementary Box 1), phosphorylates the C-terminal domain of Pol II to slow down elongation, as described above[217]. Furthermore, TBL-induced spliceosome release[11,219] and subsequent R-loop formation activates ATM, which controls genome-wide alternative splicing[11,216]. These data demonstrate that TBLs and splicing are tightly linked and suggest that this link is important for the transcription stress response.



survival through transcription stress[156,157], this enhanced damage recognition mechanism is also its own Achilles heel. This is because this process promotes the formation of lesion-stalled Pol II complexes, which are potentially even more cytotoxic than the DNA lesion itself when not resolved properly, for several reasons. Firstly, the stalling of transcription not only inactivates the transcribed gene, it may also cause the formation of R-loops, which are deleterious for the cell, as they trigger genome instability[12,159] and genome-wide signalling and transcriptional responses, including changes in splicing[11,160] (BOX 2). Secondly, persistently stalled Pol II may cause chain collisions of successive Pol II complexes transcribing the same gene, thereby precluding alternative lesion bypass by the successive Pol II. Thirdly, ongoing Pol II stalling at lesions may result in trapping more and more factors of the transcription machinery, thereby reducing transcriptional output. Finally, lesion-stalled Pol II molecules form roadblocks for advancing replication forks and thus create replication stress, which will increase genome instability (see below)[10,115,161–163].

Reducing Pol II stalling at lesions. To prevent the detrimental effects of prolonged Pol II stalling, cells have evolved mechanisms to remove lesion-stalled Pol II complexes and to reduce the chance of their appearance. Pol II removal can occur through proteolysis (FIG. 4b). The largest Pol II subunit, RPB1, is degraded by the 26S proteasome following UV-induced stalling[74,164–166] (FIG. 4b). This involves ubiquitylation by the sequential activity

of several E3 ubiquitin ligases and de-ubiquitylation enzymes (reviewed in REFS[164,167]). Ubiquitylated RPB1 is recognized and removed from chromatin by the ubiquitin-selective segregase valosin-containing protein (VCP; also known as p97) and is targeted for proteasomal degradation[167–169]. This degradation has been hypothesized to be a 'last resort' pathway, which happens if TC-NER fails or when the damage load is too high for the TC-NER machinery to properly handle[164].

In addition to transcription impediment in cis, through direct stalling at TBLs[26,170], transcription is also inhibited in trans in a genome-wide manner in response to DNA damage, by impinging on different steps of the transcription process. This regulation in trans includes inhibition of initiation, either by reducing the availability of the transcription-initiating form of Pol II[171] or by sequestering the general transcription initiation factors TATA-box-binding protein (TBP)[172] and TFIIH[173] to DNA lesions (FIG. 4c). Sequence-specific transcription initiation factors also contribute to trans-acting transcription inhibition. For example, upon DNA damage, the expression of the transcriptional repressor activating transcription factor 3 (ATF3) is induced and inhibits the expression of ~5,000 genes by binding to promoters at specific binding sites[174,175]. Transcription repression is resolved when ATF3 is degraded following its ubiquitylation, which is promoted by CSB and the $CRL4^{CSA}$ E3 ligase complex[174]. In addition to damage-induced inhibition of transcription initiation, productive elongation by Pol II is severely slowed down following TBL induction[160,176,177]. This is most likely not only caused by blockage at TBLs, but is expected to occur in a regulated, genome-wide fashion[160]. For example, the BMI1-containing Polycomb repressive complex 1, together with the E3 ubiquitin ligase UBR5, regulates damage-induced transcription inhibition by repressing Pol II elongation at DNA lesions[177]. Another genome-wide response to TBLs that may reduce Pol II stalling at lesions is the extensive TBL-induced changes in co-transcriptional splicing and the associated signalling, which strongly affect genome-wide transcription (BOX 2).

Transcription restart after stress. Successful TC-NER is complemented by proper resumption of transcription. Transcription may be resumed directly at the repair site by utilizing the backtracked Pol II, or it may be reinitiated from the promoter if Pol II was dissociated or degraded, or when transcription was inhibited in trans. Resumption of transcription elongation from backtracked Pol II requires cleavage of the nascent RNA by the intrinsic exonuclease activity of Pol II, which is stimulated by the elongation factors TFIIS[26,178] and CCR4–NOT[179,180]. Moreover, several factors have been identified that are specifically involved in transcription restart but not in repair, including the elongation factor ELL[181], the histone methyltransferase DOT1L[182] and the histone chaperone HIRA[183], but it is unclear whether these are needed for transcription restart at lesions or at promoters. Interestingly, the identification of these factors suggests that the removal of TBLs by TC-NER and the subsequent restart of transcription are not necessarily linked, thereby providing the cell with an additional layer of control to cope with transcription stress. It is likely that many other transcription initiation and elongation factors are required for transcription restart. The interested reader is referred to recent comprehensive reviews for details on transcription recovery[59,60,83].

Cell-type-specific responses to TBLs

The processes described above indicate that TC-NER alone is not sufficient for cells to fully cope with DNA-damage-induced transcription stress, and that additional repair and transcription regulation mechanisms are required. As transcription is not uniform across the genome and in different cell types, it is likely that in addition to generic responses to transcription stress, tailor-made, locus-specific and cell-type-specific response mechanisms exist. Interfering with transcription in non-dividing, highly differentiated cells has severe consequences for cellular homeostasis and eventually results in senescence or even apoptosis[115]. In proliferating cells, transcription interference could be even more detrimental, as TBLs may disturb the synchronization between transcription and DNA replication, which could lead to collisions between the machineries of the two processes and result in interfering with cell proliferation and in genome instability[184,185]. Therefore, the cellular response to TBLs in differentiated cells may be focused mainly on TC-NER and the removal of lesions, whereas in proliferating cells additional regulation of transcription is needed to limit the number of lesion-stalled Pol II complexes and the chance of transcription–replication conflicts (FIG. 5a). When transcription is inhibited in a regulated manner, fewer lesions will be recognized by TC-NER, and cells will therefore depend more on alternative repair pathways. On the other hand, replication might also help dismantle or resolve arrested transcription complexes that otherwise might continually interfere with gene expression, and thus might facilitate repair, which would provide dividing cells in proliferative tissues with a better capacity to deal with transcription stress than in postmitotic cells. Moreover, replication will dilute DNA damage, thereby reducing transcription stress. These considerations may explain why transcription-coupled repair deficiencies preferentially affect largely postmitotic organs and tissues, such as the neuronal system (as seen in CS), liver and kidney[108].

Recently, the view that transcription is a continual process has been challenged, as analysis of the transcription of individual genes has shown that transcription takes place in stochastic bursts, during which each gene is transcribed several times in a relative short time[186]. This implies that multiple Pol II complexes transcribe the gene as a convoy[187], such that stalling of a Pol II complex at a TBL would also affect the Pol II complexes that are trailing behind it. The resulting chain collision might be especially harmful and could be prevented by swift inhibition of subsequent transcription initiation or by slowing down transcription elongation.

In addition, the length of a gene may influence the mechanisms employed by cells to repair TBLs (FIG. 5b). For example, transcription restart at more distal parts of long genes relies on GG-NER rather than on TC-NER[188]. Moreover, sequencing of TC-NER-excised DNA patches

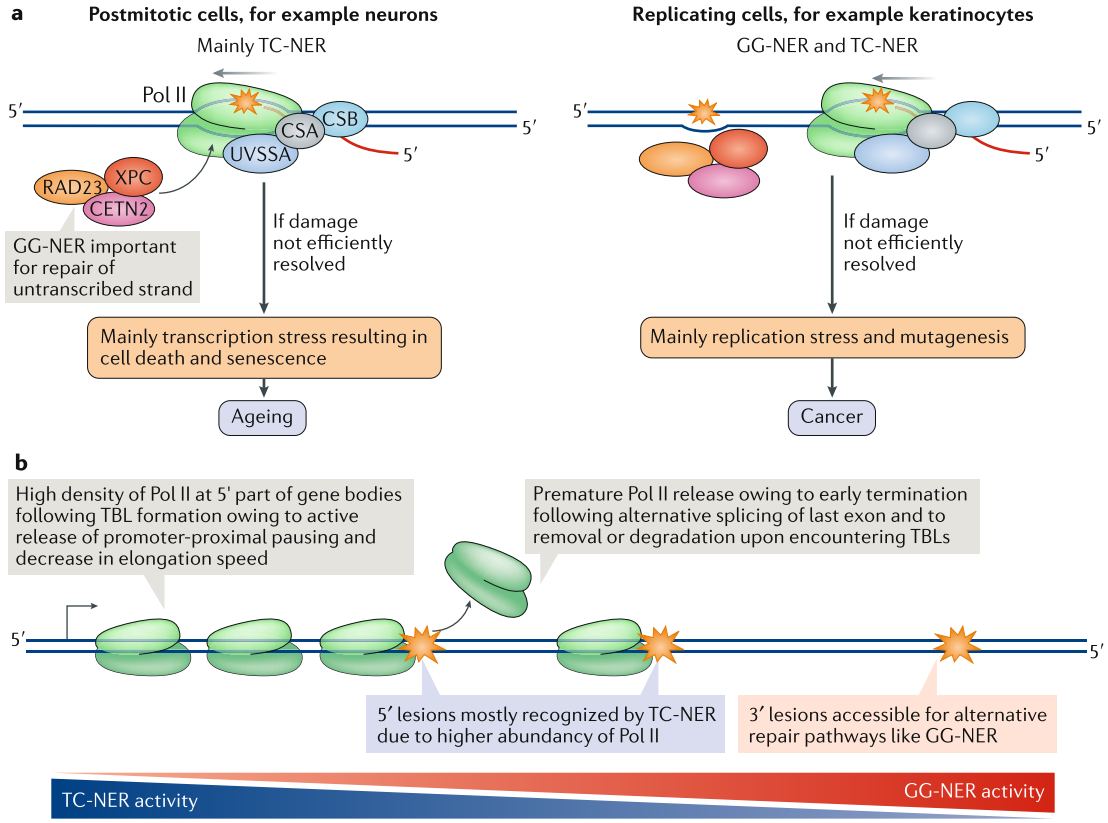


Fig. 5 | **The activity of TC-NER and GG-NER depends on cell type and the location of transcription-blocking lesions.** **a** | It is assumed that in postmitotic cells, such as neurons, DNA damage repair is mainly focused on transcribed genes, as shown by the diminished activity of global-genome nucleotide excision repair (GG-NER). Therefore, transcription-coupled nucleotide excision repair (TC-NER) is expected to be the predominantly active NER sub-pathway in these cells, as it is essential for unperturbed transcription and for preventing transcription stress and the associated cell senescence and death, which would accelerate ageing. However, GG-NER still has a role in these cells, possibly by contributing to the repair of the transcribed strand or by preserving the integrity of untranscribed strands in order to provide a correct template for gap-filling DNA synthesis. In replicating cells, such as basal-layer keratinocytes of the skin, GG-NER is the predominant NER sub-pathway, to avoid damage-induced replication stress and mutagenesis, which could contribute to cancer development. However, TC-NER is also required for repairing active genes in order to maintain cellular homeostasis. **b** | A higher density of elongating RNA polymerase II (Pol II) complexes at the 5′ end of genes can be attributed to reduced elongation rates and to DNA-damage-induced release of promoter-proximal paused Pol II into gene bodies, as well as to premature release of elongating Pol II owing to alternative last exon splicing, or to chromatin eviction or degradation upon encountering transcription-blocking lesions (TBLs). By contrast, because the density of Pol II is reduced at more distal parts of genes, in particular of long genes, 3′-end lesions will more likely be repaired by GG-NER. CETN2, centrin 2; CSA/CSB, Cockayne syndrome group A/B; UVSSA, UV-stimulated scaffold protein A; XPC, xeroderma pigmentosum group C-complementing protein.

by XR-seq showed that TC-NER mostly takes place at the beginning of genes[158,189]. This suggests that, even though TBLs in the 3′ end of a gene will block its expression to the same extent as those in the 5′ end, TC-NER preferentially removes TBLs at the 5′ end of genes. These observations could be explained by the fact that the speed of Pol II elongation decreases following DNA damage[160,176], which would provide additional time for other repair pathways, such as GG-NER, to repair distally located TBLs. In addition, the reduced density of Pol II at more distal parts of damaged gene bodies could be explained by the release of lesion-stalled Pol II before the normal transcription termination site due to alternative last-exon splicing[160] (BOX 2). Alternatively, Pol II may be released from the DNA template during NER-mediated excision[62] or owing to its DNA-damage-induced degradation[164]. These events would result in reduced recognition of distal TBLs by TC-NER. Of note, transcription inhibition through reducing transcription initiation[172–175] will increase the opportunity for other repair pathways, such as GG-NER, to remove TBLs. It is likely that transcription inhibition mechanisms are preferred by cells over the highly inefficient and energy-consuming degradation of lesion-stalled Pol II. Together, these examples show that differences in transcription or cell proliferation will influence the mechanism used by the cell to remove TBLs.

XR-seq
Method for sequencing excised oligomers generated during nucleotide excision repair, which allows genome-wide mapping of repair sites.

Conclusions and future perspective

Although many questions remain unanswered concerning the multi-layered responses to TBLs, including transcription-coupled repair, the contours of this intricate molecular reaction and its cellular and clinical importance are beginning to emerge. The degree of

accessibility of the TBL to repair machineries is likely a crucial determinant of the severity of the phenotypes displayed in TC-NER disorders. However, further clarification of the exact pathogenic mechanisms underlying CS and UV^{S}S will undoubtedly lead to a better understanding of the genotype–phenotype correlations and to an improved appreciation of the exact molecular mechanism of TBL removal by TC-NER. Strikingly, in TC-NER disorders the most severely affected tissues consist of non-replicating cells. Nevertheless, even in proliferating cells and tissues, transcription stress due to blocked Pol II complexes also causes replication stress, which contributes to a wide range of ageing-associated diseases and to carcinogenesis[7,10]. Hence, it will be of the utmost importance to further improve our understanding of these vital genome maintenance pathways, their crosstalk and their putative contributions to the cellular responses to transcription stress or to symptoms observed in TC-NER disorders.

Although so far multiple proteins have been implicated in TC-NER, many of their enzymatic activities are still not fully understood. A recent conceptual breakthrough was the discovery of the forward-translocating activity of CSB on Pol II[42], but as CSB was previously thought to promote Pol II backtracking, this finding raises the next question of how Pol II backtracking is regulated. It is tempting to speculate that this is mediated by TFIIH, but direct evidence is lacking, and other factors could be involved as well. It is furthermore expected that multiple, as-of-yet unidentified TC-NER factors await discovery. Mutations in these factors could lead to CS, UV^{S}S or related diseases. For example, some transcription elongation factors promote Pol II bypass of oxidative lesions in vitro[20], but their precise function and involvement in counteracting transcription stress is unknown.

More research will also be needed to elucidate how transcription regulation in cis and in trans in response to TBL induction is organized, and whether disturbance of the regulation in trans might contribute to any of the symptoms of TC-NER disorders. Another question still largely unexplored is how transcription is restarted after TC-NER — that is, whether this happens at the promoter or at the site of the removed TBL. Whereas the latter option appears to be the most efficient, Poll II has been suggested to be removed from chromatin during TC-NER[62], arguing for new transcription initiation from the promoter. Both options possibly exist in cells, which is why future research should focus on whether the differences in restart mechanisms might depend on gene length or transcription rates, or whether transcription takes place in stochastic bursts. Furthermore, transcription restart might be influenced by both genomic location and cell type. Recently developed molecular genetic techniques, such as single-cell-sequencing approaches, nascent RNA sequencing[188] and XR-seq[189], will help answer these questions and provide a more detailed dissection of the cellular responses to DNA-damage-induced transcription stress.

An important gap in our knowledge is what happens to the nascent RNA being produced when Pol II stalls. If Pol II resumes transcription from the TBL site, the protruding RNA should be cleaved, but if Pol II is removed from chromatin, the nascent RNA is most likely also removed, to prevent the formation of deleterious R-loops. Even though the exonuclease activity of Pol II and of factors that stimulate it, like TFIIS and CCR4–NOT, has been implicated in processing the protruding nascent RNA during Pol II backtracking[26,178–180], the exact proteins and mechanisms involved are still unknown. Interestingly, the nascent RNA may be actively involved in signalling and repair, as it was shown that adenosine in RNA is rapidly methylated in response to UV damage, which has been suggested to promote the recruitment of polymerase κ in order to facilitate repair[190].

Finally, given the recent promising and exciting progress in identifying novel means of alleviating the clinical symptoms of transcription stress — for example, by targeting NAD metabolism[152], inhibiting the FOXO–p53 interaction[153] or reducing food intake[133] — the biggest challenge ahead will be the successful translation of the gained fundamental knowledge into effective therapeutic approaches to counteract DNA-damage-induced diseases.

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Competing interests
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