DNA DAMAGE

Understanding nucleotide excision repair and its roles in cancer and ageing

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Abstract | Nucleotide excision repair (NER) eliminates various structurally unrelated DNA lesions by a multiwise 'cut and patch'-type reaction. The global genome NER (GG-NER) subpathway prevents mutagenesis by probing the genome for helix-distorting lesions, whereas transcription-coupled NER (TC-NER) removes transcription-blocking lesions to permit unperturbed gene expression, thereby preventing cell death. Consequently, defects in GG-NER result in cancer predisposition, whereas defects in TC-NER cause a variety of diseases ranging from ultraviolet radiation-sensitive syndrome to severe premature ageing conditions such as Cockayne syndrome. Recent studies have uncovered new aspects of DNA-damage detection by NER, how NER is regulated by extensive post-translational modifications, and the dynamic chromatin interactions that control its efficiency. Based on these findings, a mechanistic model is proposed that explains the complex genotype–phenotype correlations of transcription-coupled repair disorders.

The integrity of DNA is constantly threatened by endogenously formed metabolic products and by-products, such as reactive oxygen species (ROS) and alkylating agents, and by its intrinsic chemical instability (for example, by its ability to spontaneously undergo hydrolytic deamination and depurination). Environmental chemicals and radiation also affect the physical constitution of DNA[1]. In total, damage loads may amount to 10^4–10^5 DNA lesions per mammalian cell per day[2], and it is therefore of prime importance that they are removed in an efficient and timely manner. DNA damage may stall replication and generate single-strand breaks or double-strand breaks (DSBs), which lead to chromosomal aberrations. Lesions may also decrease replication fidelity when dedicated translesion DNA polymerases transiently take over from the regular high-fidelity polymerases to bypass the damage, which results in increased mutagenesis[3]. Mutations and chromosomal aberrations can activate oncogenes or inactivate tumour suppressor genes and thus increase the risk of cancer. Transcription also relies on an intact template, and damage-induced transcription arrest disturbs cellular homeostasis or may cause cell death and thereby promote ageing.

In contrast to other cellular macromolecules, damaged DNA cannot be replaced and solely relies on repair to remain intact. This situation has forced the evolution of an intricate DNA-damage response (DDR), which comprises sophisticated repair and damage signalling processes. The DDR involves DNA-damage sensors and signalling kinases that regulate a range of downstream mediator and effector molecules that control repair, cell cycle progression and cell fate[4]. The core of this DDR is formed by a network of complementary DNA repair systems, each of which deals with a specific class of DNA lesions. The repair systems include the direct reversal of damage, nucleotide excision repair (NER), base excision repair (BER), DSB repair, and interstrand crosslink repair[5].

The mammalian NER pathway can function as a paradigm for the clinical influence of many DDR processes, owing to the striking clinical heterogeneity that is associated with its impairment. This heterogeneity can now be mostly explained by our understanding of the various molecular mechanisms that underlie NER and its crosstalk with other cellular processes. NER is exceptional among the DNA repair pathways in its ability to eliminate the widest range of structurally unrelated DNA lesions, including: cyclobutane–pyrimidine dimers (CPDs) and 6–4 pyrimidine–pyrimidone photoproducts (6–4PPs), which are the major lesions induced by ultraviolet (UV) radiation; numerous bulky chemical adducts (man-made and natural); intrastrand crosslinks caused by drugs such as cisplatin; and ROS-generated cyclopurines. As we explain

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below, the basis of this versatility of NER is that it circumvents recognition of the lesion itself and instead focuses on a set of commonalities shared by many different lesions.

In this Review, we first discuss the two mechanisms of DNA-damage detection by NER, which can occur either through global genome NER (GG-NER) or through transcription-coupled NER (TC-NER). This discussion is followed by a short mechanistic description of the downstream excision and gap-filling DNA synthesis steps. Next, we discuss the regulation of NER by ubiquitylation, its interplay with chromatin and its regulation *in vivo*. Finally, we discuss the variable cancer and premature ageing phenotypes that are caused by mutations in genes involved in NER and suggest a novel, unifying model that links the different NER molecular defects with the complex phenotypes of NER disorders.

Global genome NER

The first and essential step in any DNA repair process is the detection of lesions. In the GG-NER subpathway, the entire genome is examined for helix distortions (that is, disturbed base pairing) associated with structural changes to nucleotides, whereas TC-NER is activated when RNA polymerase II (RNA Pol II) is stalled during transcript elongation by a lesion in the template strand (FIG. 1).

DNa-damage recognition. XPC, which is stabilized and assisted by its association with the UV excision repair protein RAD23B and centrin 2 (CETN2)[6,7], is the main damage sensor in GG-NER[8]. How can a single protein recognize numerous structurally diverse lesions? *In vitro*, XPC binds to different DNA structures that cause local DNA helix destabilization and even binds to nucleotide mismatches that do not activate NER *in vivo*[9]. Based on this property, a two-step recognition model for NER initiation was proposed, in which XPC first binds to the small single-stranded DNA (ssDNA) gap caused by disrupted base pairing. XPC binding is then followed by a lesion-verification step[9,10]. The crystal structure of Rad4 (the fission yeast orthologue of XPC) bound to a DNA molecule containing a helix distortion caused by a CPD showed that it does indeed bind to ssDNA opposite the lesion by inserting its carboxy-terminal double β-hairpin at the junction between the double-stranded DNA (dsDNA) and the ssDNA[11]. This binding mode readily explains the remarkably broad range of damage that is targeted by GG-NER, as the presence of unpaired ssDNA opposite the distortion is common to all NER-inducing lesions. Based on the *in vivo* mobility of a GFP-tagged XPC protein[12], we favour a model in which NER mostly detects damage by DNA probing rather than by DNA scanning.

Although XPC is the main initiator of GG-NER, UV-radiation-induced CPDs are a poor substrate for XPC, mainly because they only mildly destabilize the DNA helix[9,13]. To enable CPD repair, the UV–DDB (ultraviolet radiation–DNA damage-binding protein) complex (FIG. 1), which comprises DDB1 (also known as XPE-binding factor) and the GG-NER-specific protein DDB2[14], directly binds to UV-radiation-induced lesions and functions as an auxiliary damage-recognition factor by stimulating the subsequent binding of XPC[15,16] (BOX 1). UV–DDB forms a larger complex with the CRL (cullin 4A (CUL4A)–regulator of cullins 1 (ROC1) E3 ubiquitin ligase) complex[17]. In this complex, DDB1 forms the linker between the CRL core and the associated protein containing a WD40 domain (DDB2 in this case) that provides substrate specificity (in this case, the WD40 domain of DDB2 enables recognition of UV-radiation-induced lesions). Structural analysis showed that DDB2 binds to UV-radiation-induced lesions, extrudes the lesions into its binding pocket and kinks the DNA[16], thereby creating ssDNA to facilitate XPC binding.

Figure 1 | **Nucleotide excision repair.** In the global genome nucleotide excision repair (GG-NER; left) subpathway, the damage sensor XPC, in complex with UV excision repair protein RAD23 homologue B (RAD23B) and centrin 2 (CETN2), constantly probes the DNA for helix-distorting lesions (step 1, left), which are recognized with the help of the UV–DDB (ultraviolet (UV) radiation–DNA damage-binding protein) complex (step 2, left). Upon binding of the XPC complex to the damage, RAD23B dissociates from the complex (step 3, left). In the transcription-coupled NER (TC-NER; right) subpathway, damage is indirectly recognized during transcript elongation by the stalling of RNA polymerase II (RNA Pol II) at a lesion. During transcript elongation UV-stimulated scaffold protein A (UVSSA), ubiquitin-specific-processing protease 7 (USP7) and Cockayne syndrome protein CSB transiently interact with RNA Pol II (step 1, right). Upon stalling at a lesion, the affinity of CSB for RNA Pol II increases (step 2, right) and the Cockayne syndrome WD repeat protein CSA–CSB complex is formed, which probably results in reverse translocation (backtracking) of RNA Pol II (step 3, right) that renders the DNA lesion accessible for repair. RNA Pol II and the nascent mRNA transcript are not depicted further. After damage recognition, the TFIIH (transcription initiation factor IIH) complex is recruited to the lesion in both GG-NER and TC-NER (step 4). In NER the XPG structure-specific endonuclease, either associated with TFIIH or separately, binds to the pre-incision NER complex (step 4). Upon binding of TFIIH, the CAK (CDK-activating kinase) subcomplex dissociates from the core TFIIH complex. The helicase activity of TFIIH further opens the double helix around the lesion, and 5′–3′ unwinding of the DNA by the TFIIH basal transcription factor complex helicase subunit XPD verifies the existence of lesions with the help of the ATPase activity of the TFIIH XPB subunit and XPA, which binds to single-stranded, chemically altered nucleotides (step 4). In this step the single-stranded DNA binding protein replication protein A (RPA) is also recruited and coats the undamaged strand. XPA recruits a structure-specific endonuclease — the XPF–ERCC1 heterodimer, which is directed to the damaged strand by RPA to create an incision 5′ to the lesion (step 5). Once this 'point of no return' is reached, XPG is activated and cuts the damaged strand 3′ to the lesion, which excises the lesion within a 22–30 nucleotide-long strand (step 6). The trimeric proliferating cell nuclear antigen (PCNA) ring, which is directly loaded after the 5′ incision by XPF–ERCC1, recruits DNA Pol δ, DNA Pol κ or DNA Pol ε for gap-filling DNA synthesis (step 7). Gap filling can begin immediately after the 5′ incision is made. The NER reaction is completed through sealing the final nick by DNA ligase 1 or DNA ligase 3 (step 8).

Direct reversal
A one-step DNA repair process involving an enzyme that reverts the DNA lesion to the original nucleotides. Examples are O^6-methylguanine DNA methyltransferase (MGMT) and photolyases. MGMT specifically transfers the methyl group from guanine methylated at the O^6 position to an internal Cys145 residue in MGMT. This causes a structural change in the enzyme that induces its degradation. Photolyases, which are not found in placental mammals, bind to ultraviolet-radiation-induced photoproducts (either cyclobutane–pyrimidine dimers or 6–4 pyrimidine–pyrimidone photoproducts). With the aid of two light-capturing cofactors, photolyases use the energy of visible light to split these dimers into monomers.

Base excision repair
(BER). This pathway removes oxidative and alkylating DNA lesions. Damaged bases are recognized and cut out from the DNA by lesion-specific glycosylases, which is followed by cleavage of the phosphodiester backbone and gap-filling DNA synthesis of one or a few nucleotides of the resulting single-stranded DNA.

Interstrand crosslink repair
A repair pathway that removes DNA bases from complementary strands that are covalently crosslinked. Defects in this pathway cause Fanconi anaemia.

Cyclobutane–pyrimidine dimers
(CPDs). The most common ultraviolet-radiation-induced DNA lesion, which is formed by covalently linking the C5 and C6 carbon atoms of two adjacent pyrimidines.

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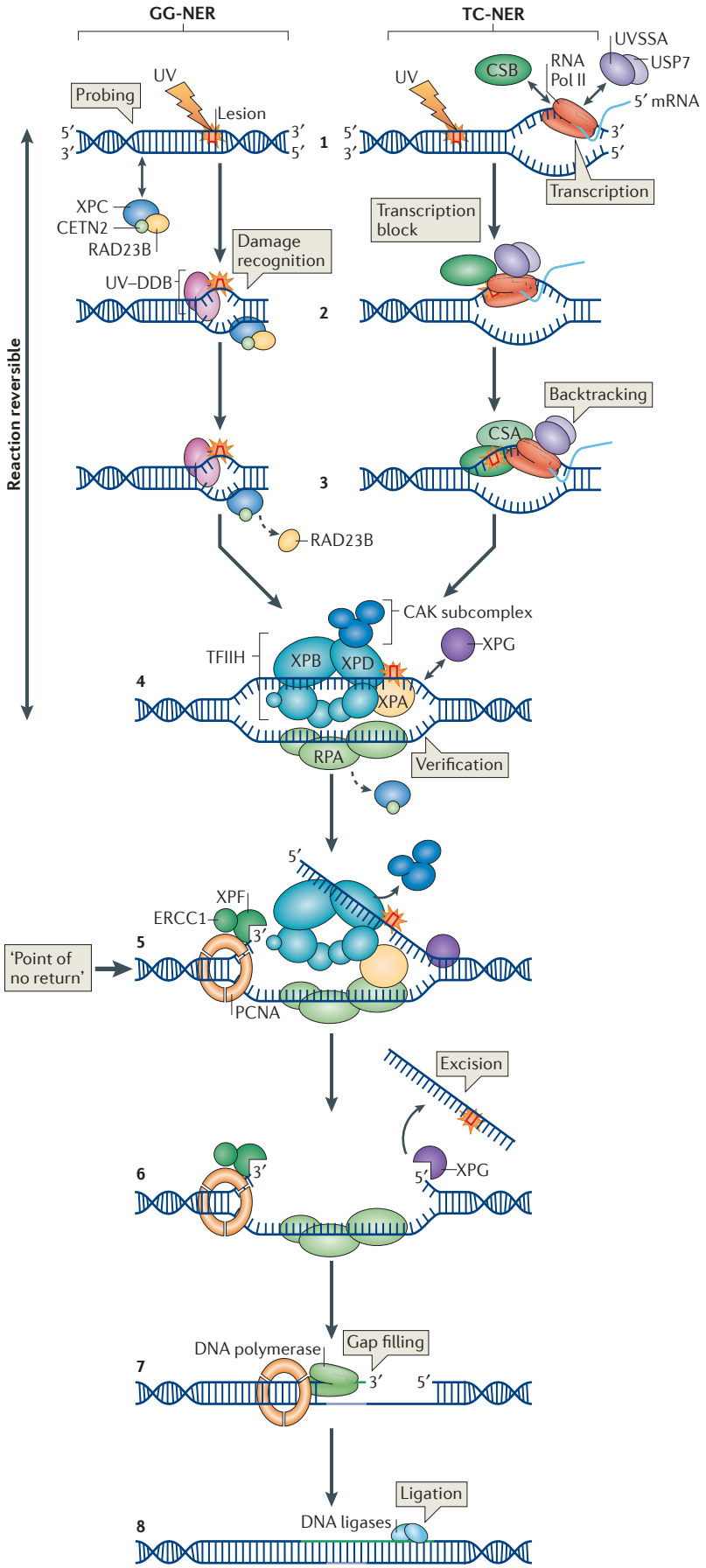

DNA damage verification. Binding of XPC to lesions provides a substrate for the association of the TFIIH (transcription initiation factor IIH) complex[18–20], which is a transcription initiation and repair factor consisting of ten protein subunits. Its two DNA helicases, the two TFIIH basal transcription factor complex helicase subunits XPB and XPD (encoded by *ERCC3* and *ERCC2*, respectively), have opposite polarities and extend the open DNA configuration around the lesion[21,22], which probably verifies the presence of a lesion (FIG. 1). Whereas the ATPase activity of XPB, rather than its helicase activity, is implicated in recruiting TFIIH to DNA damage[23,24], the 5′–3′ unwinding activity of XPD seems to be indispensable for NER[25]. *In vitro* experiments clearly showed that TFIIH itself (assisted by XPA; see below), when loaded by XPC onto a bubble DNA substrate, scans the DNA in a 5′–3′ direction for helicase-blocking lesions[26], suggesting that the XPD helicase is mainly required for damage verification. Structural analysis of archaeal XPD orthologues further suggests that the Arch and Fe-S cluster domains of XPD form an internal channel through which undamaged ssDNA can probably pass but damaged DNA cannot[27–31]. When the XPD helicase does not detect any damage, the repair reaction may be aborted[27–31]. Damage verification also probably involves the XPA protein, which detects nucleotides with altered chemical structures in ssDNA[32]. TFIIH was originally identified as an essential transcription initiation factor, but it can switch between functions in transcription and in NER[22,33]. The trimeric CAK subcomplex (CDK-activating kinase subcomplex) of TFIIH is essential for transcription initiation, but it is not required for its repair function. Upon binding of TFIIH to DNA-bound XPC, the CAK subcomplex dissociates[34]. Conversely, the 8 kD TFIIH basal transcription factor complex TTDA subunit (also known as GTF2H5) seems to be important for the role of TFIIH in NER, but it is dispensable for its transcription activity[35,36].

Dual incision and gap filling. The highly dynamic multistep strategy of lesion detection and verification contains several reversible steps before the actual removal of lesions by dual (5′ and 3′) incision, presumably to prevent the formation of undesirable and irreversible DNA modifications[37,38]. However, the next step is strand incision, after which a 'point of no return' is reached (FIG. 1) and the reaction must be efficiently concluded to avoid leaving potentially dangerous intermediates. Lesion excision is catalysed by the structure-specific endonucleases XPF–ERCC1 and XPG (encoded by *ERCC5*), which incise the damaged strand at short distances 5′ and 3′ from the lesion, respectively[39] (FIG. 1). The excision leaves a single-strand gap of 22–30 nucleotides, which probably triggers a DNA-damage-signalling reaction (BOX 2). Increased damage signalling and genomic instability are indeed observed if the dual incision is improperly coordinated[40]. Accurate coordination of incision involves the assembly of XPA, XPG and replication protein A (RPA) at NER lesions that are marked by XPC and verified by TFIIH.

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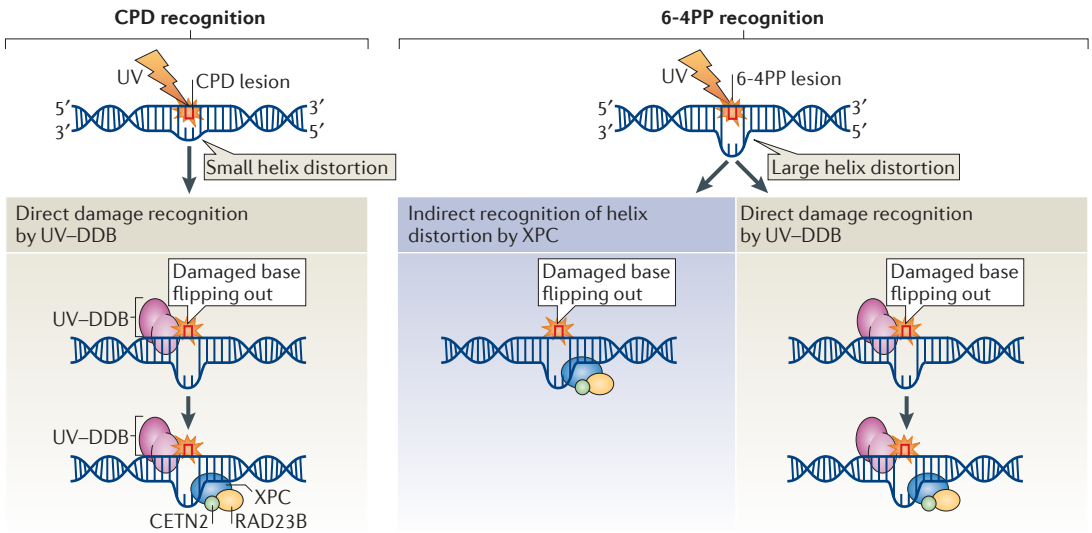
Box 1 | **Different modes of damage recognition by global genome nucleotide excision repair**



The XPC complex, which also contains UV excision repair protein RAD23 homologue B (RAD23B) and centrin 2 (CETN2), recognizes DNA-helix-destabilizing lesions and is crucial for the initiation of global genome nucleotide excision repair (GG-NER). XPC detects the damage in an indirect manner by binding to the undamaged strand opposite the actual lesion, as it has increased affinity for unpaired DNA at the junctions of single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA)[11]. This mode of DNA binding also explains the remarkably broad range of lesions repaired by GG-NER, as all lesions that induce NER are helix-destabilizing and create short unpaired regions. Upon binding of XPC to these unpaired regions its carboxy-terminal β-hairpin domain is inserted between the DNA strands, thereby stabilizing and possibly extending the unpaired region to enable recruitment of TFIIH (transcription initiation factor IIH) for lesion verification (FIG. 1) in the damaged strand. In mammals, NER is the only DNA repair process that can remove the major photoproducts induced by ultraviolet (UV) radiation: cyclobutane–pyrimidine dimers (CPDs) and 6–4 pyrimidine–pyrimidone photoproducts (6–4PPs) (see the figure). Indeed, 6–4PPs sufficiently destabilize the DNA helix to induce small unwound DNA bubbles that can be directly recognized by XPC (see the figure, middle panel). However, CPDs, which are approximately twice as abundant as 6–4PPs after UV irradiation, only poorly influence Watson–Crick base pairing and thus do not create helix distortions that can be directly recognized by XPC. The UV–DDB (UV radiation–DNA damage-binding protein) complex, which comprises DDB1 and DDB2, associates with the CRL (cullin 4A (CUL4A)–regulator of cullins 1 (ROC1) E3 ubiquitin ligase) complex and assists in the recognition of CPDs (see the figure, left panel), as the DDB2 subunit, which is connected through DDB1 to the CRL complex, has high affinity for these lesions. Binding of the UV–DDB complex promotes XPC-mediated damage detection by stabilizing a DNA conformation in which the CPD is 'flipped out', thereby creating unpaired bases that are a suitable substrate for XPC binding. Although 6–4PP lesions can be directly recognized by XPC, the UV–DDB complex also improves the recognition and repair of these lesions[88] (see the figure, right panel).

6–4 pyrimidine–pyrimidone photoproducts
(6–4PPs). The second most common ultraviolet-radiation-induced DNA lesion, formed by a covalent link between the C4 and C6 carbon atoms of two adjacent pyrimidines. This causes a greater distortion of the DNA helix than cyclobutane–pyrimidine dimers (CPDs). It is more efficiently detected and repaired by mammalian global genome nucleotide excision repair than CPDs.

DNA probing
A process in which DNA-binding proteins freely diffuse through the nucleus and detect DNA damage through a repetitive sampling mechanism (that is, transient DNA binding).

DNA scanning
A process in which DNA-binding proteins slide along the DNA over long distances.

UV–DDB
(Ultraviolet radiation–DNA damage-binding protein). A complex formed by the DDB1 and DDB2 proteins, which is part of a larger complex including the CRL (cullin 4A (CUL4A)–regulator of cullins 1 (ROC1) E3 ubiquitin ligase) complex. It has a high affinity for DNA lesions caused by UV radiation and assists XPC-mediated DNA damage recognition during global genome nucleotide excision repair.

CRL
(Cullin 4A (CUL4A)–regulator of cullins 1 (ROC1) E3 ubiquitin ligase). A modular E3 ubiquitin ligase complex consisting of the RING finger protein ROC1 and the CUL4A scaffold protein, which interacts with DNA damage-binding protein 1 (DDB1). Its target specificity is regulated by switching interactions with WD40-domain-containing substrate proteins, such as DDB2 in global genome nucleotide excision repair (GG-NER) and Cockayne syndrome protein CSA in transcription-coupled NER (TC-NER).

XPA is considered to be a central coordinator of the NER complex because of its diverse functions. Besides stimulating lesion verification by TFIIH[26] and binding to altered nucleotides in ssDNA, XPA also interacts with almost all NER proteins[41]. The single-strand-binding protein RPA protects the non-damaged DNA strand from endonucleases and properly orients XPF–ERCC1 and XPG to specifically incise only the damaged strand[42]. XPG is recruited either independently or simultaneously with TFIIH[43–45] and needs to be physically present (but not necessarily nucleolytically active) to enable XPF–ERCC1 to make the 5′ incision. XPF–ERCC1 (REF. 46) is recruited through its interaction with XPA[47,48]. The 5′ incision is sufficient to initiate gap-filling DNA synthesis[46] even before the XPG-mediated 3′ incision is made. The synchronization of lesion excision and gap-filling DNA synthesis, which is coordinated by RPA and XPG[49,50], may prevent the accumulation of ssDNA gaps that induce DNA-damage signalling (BOX 2). Final DNA gap-filling synthesis and ligation are executed by the replication proteins proliferating cell nuclear antigen (PCNA), replication factor C (RFC), DNA Pol δ, DNA Pol ε or DNA Pol κ, and DNA ligase 1 or XRCC1–DNA ligase 3. Which specific proteins are involved depends on the proliferative status of the cell[51,52]. DNA Pol ε-dependent repair and subsequent ligation by DNA ligase 1 mainly occurs in replicating cells, whereas DNA Pol δ and DNA Pol κ are the main NER polymerases in non-replicating cells, in which nucleotide pool concentrations are low. The expression of DNA ligase 1 is also low in non-cycling cells, and under these circumstances the constitutively present XRCC1–DNA ligase 3 complex seals the gap.

Transcription-coupled NER

The removal of poorly recognized lesions such as CPDs is slow and ineffective, despite the activity of UV–DDB, and the persistence of CPDs interferes with replication and transcript elongation. Long-term stalling of replication activates DDR-associated cell cycle checkpoints and may eventually lead to the generation of DSBs. Damage avoidance processes, including lesion

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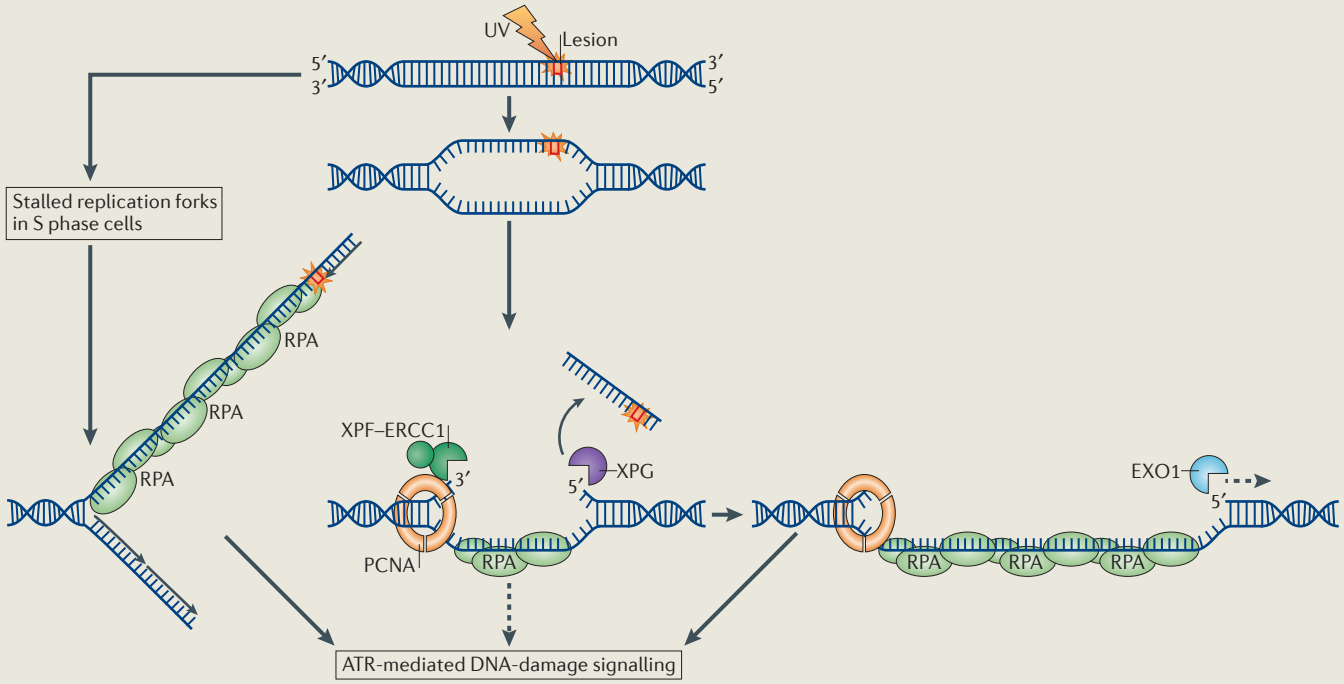
Box 2 | **DNA-damage signalling triggered by nucleotide excision repair substrate lesions**

The most abundant chromatin modification associated with the DNA-damage response (DDR) is the phosphorylation of histone variant H2A.X (γH2A.X). It attracts numerous DDR factors to the vicinity of lesions to accelerate repair and to initiate and sustain a signalling network that, for example, activates cell cycle checkpoints, thereby preventing genomic instability. Initially, γH2A.X was thought to mediate a double-strand break (DSB) repair-specific response, in which the DSB sensor — the MRN (MRE11–RAD50–NBS1) complex — activates ATM to phosphorylate H2A.X[167]. Phosphorylation of H2A.X is also induced by ATR (another DDR-associated protein kinase) during S phase in response to stalled DNA replication forks caused, for example, by ultraviolet (UV)-radiation-induced lesions, which creates long stretches of single-stranded DNA (ssDNA) coated with replication protein A (RPA) (see the figure, left). Binding of the ATR-interacting protein (ATRIP) complex to RPA-coated ssDNA in conjunction with DNA topoisomerase 2-binding protein 1 (TOPBP1), and the cell cycle checkpoint protein RAD17-dependent loading of the checkpoint sensor clamp RAD9–RAD1–HUS1 complex (also known as the 9-1-1 complex; not shown), activates ATR signalling at the site of damage[168,169].

In both replicating and non-replicating cells, the ATR-mediated damage-induced response is dependent on global genome nucleotide excision repair (GG-NER) activity[170–172]. The NER pathway recognizes and processes UV-radiation-induced DNA damage by dual incision with the XPG and XPF–ERCC1 endonucleases; the incision results in a region of ssDNA that is 22–35 nucleotides long and coated with RPA. This region is a potential substrate for triggering GG-NER-dependent ATR activation (see the figure, middle). Indeed, accumulation of these normally short-lived NER intermediates, which can be triggered by the inhibition of gap-filling DNA synthesis using DNA replication inhibitors, strongly increases damage-induced signalling[171,172]. It is not known whether these NER intermediates, which can accommodate only a single RPA heterotrimer, are sufficient to trigger ATR signalling[173] or if they must be extended to do so. Exonuclease 1 (EXO1), a 5′–3′ enzyme, amplifies damage signalling after UV-radiation-induced damage in non-cycling cells, which suggests that longer stretches of ssDNA are generated from NER intermediates by the activity of EXO1 (REFS 174,175) (see the figure, right). EXO1 activity is, however, normally not required for NER. Thus, it is likely to only be involved in special circumstances, such as when the availability of NER factors becomes limited, when the damage load is too high and NER intermediates persist, or during the collision of two opposing NER reactions. Interestingly, ATR activation is not directly triggered by DNA damage-sensing complexes (unlike ATM activation, which requires the DSB sensor MRN), but occurs after a delay when the NER reaction has passed its 'point of no return' on true NER substrates. This feature may enable cells to avoid continuous checkpoint activation by commonly occurring helix distortions that are formed by to intercalating agents or mismatches, which are cleared by genome surveillance mechanisms other than NER. Despite clear mechanistic differences in inducing γH2A.X by either ATM or ATR, the end result is similar: mediator of DNA damage checkpoint protein 1 (MDC1), a DDR scaffold protein, is recruited to γH2A.X. Numerous other DDR-mediating factors bind to MDC1, including the E3 ubiquitin protein ligases RNF8 and RNF168, which are involved in the DNA-damage-dependent ubiquitylation of histone H2A[171,176,177]. This enables the binding of tumour suppressor p53-binding protein 1 (53BP1) and BRCA1 at sites of UV-radiation-induced damage, which is similar to the response to DSBs[167] or replication stress[178].

PCNA, proliferating cell nuclear antigen.



bypass through the use of alternative DNA polymerases and homologous-recombination-dependent template switching, may alleviate this problem. Damage-induced transcription arrest that remains unresolved may trigger cell death[53]. Although bypass of some DNA lesions in the transcribed strand has been reported to cause transcriptional mutagenesis[54,55], no specific translesion RNA polymerases are known to bypass helix-distorting adducts. Hence, a dedicated TC-NER[56] pathway has evolved to selectively repair transcription-blocking lesions and thus enable the resumption of transcription and gene expression. As TC-NER factors, their mode of action and their regulation have been reviewed recently[57], below we only summarize these topics.

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The TC-NER machinery seems to indirectly detect the existence of damage through its ability to sense the blocking of transcript elongation. TC-NER is initiated by lesion-stalled RNA Pol II, which recruits the TC-NER-specific Cockayne syndrome proteins CSA (Cockayne syndrome WD repeat protein A; also known as ERCC8) and CSB (Cockayne syndrome protein B; also known as ERCC6). CSA and CSB are required for further assembly of the TC-NER machinery[58], which includes the core NER factors (except for the GG-NER-specific UV–DDB and XPC complexes) and several TC-NER-specific proteins, such as UV-stimulated scaffold protein A (UVSSA), ubiquitin-specific-processing protease 7 (USP7; also known as ubiquitin C-terminal hydrolase 7), XPA-binding protein 2 (XAB2; also known as pre-mRNA-splicing factor SYF1) and high mobility group nucleosome-binding domain-containing protein 1 (HMGN1; also known as non-histone chromosomal protein HMG14)[58,59] (FIG. 1). The stalling of transcript elongation is probably caused by a broader range of lesions than can be recognized by GG-NER, as TC-NER-deficient cells from patients with Cockayne syndrome are also hypersensitive to oxidative DNA damage[60,61], whereas cells with defects in GG-NER are not. Most oxidized bases do not trigger GG-NER and are repaired by BER. Although it is controversial whether RNA Pol II stalls at oxidative DNA lesions[62–64], Cockayne syndrome proteins are believed to be involved in removing oxidized bases (reviewed in REF. 61). Importantly, CSB (but not downstream core NER factors) accumulates at sites of locally induced oxidative damage *in vivo*, in a transcription-dependent manner[65]. This specific role of Cockayne syndrome proteins in the response to oxidative damage — such as that induced by X-rays and potassium bromate[66] — suggests that a subset of transcription-arresting oxidative lesions are detected by BER and recruit the TC-NER machinery but are subsequently repaired by BER rather than NER. If this model is correct, it argues for the existence of a broad transcription-coupled repair (TCR) mechanism that also includes transcription-interfering lesions that are normally repaired by BER or other repair systems.

A CPD-stalled RNA Pol II covers about 35 nucleotides on the transcribed strand[67], which prevents the NER incision machinery from accessing the lesion. Several mutually non-exclusive models have been proposed for the fate of lesion-stalled RNA Pol II, including dissociation from the template DNA, backtracking to permit access of repair proteins to lesions (FIG. 1) or even degradation. The damage-induced proteolysis of polyubiquitylated RNA Pol II may function as a last resort for template clearance[68] (see below). The main process by which TC-NER functions is thought to be RNA Pol II backtracking[69], which is a common mechanism in transcription proofreading and at natural transcription pause sites. The mechanisms and factors involved in RNA Pol II backtracking and the subsequent assembly of the TC-NER complex at lesions remain elusive. The CSB protein may be involved in reverse translocation or backtracking, which would be in line with the observed translocation activity of CSB in DNA–protein complexes[70,71].

Regulation of NER by ubiquitylation

Complex, multistep processes like NER require strict regulation and coordination, which often involve different types of post-translational modifications of key proteins. Damage-induced phosphorylation[72], poly(ADP-ribosyl)ation (PARylation)[73,74], sumoylation and ubiquitylation[59,75,76] have been shown to regulate the DDR and NER. In particular, ubiquitylation has an important role in controlling NER[77]. The covalent attachment of the 8 kDa ubiquitin protein to a Lys residue of substrate proteins or the formation of polyubiquitin chains on Lys residues within ubiquitin itself (for example, Lys48 and Lys63) can have a great impact on the fate of the targeted proteins in terms of their half-life, conformation, localization or the specificity of their interactions. The wide variety of consequences of protein ubiquitylation can be explained by the diversity of ubiquitin chain structures[78] and types of ubiquitin-binding proteins[79]. In addition, approximately 100 deubiquitylating enzymes can reverse ubiquitylation and many of these enzymes are involved in the DDR[80]. Recent advances in the quantitative, proteome-wide analysis of ubiquitylated proteins[81] have identified numerous ubiquitylation events that are induced by UV radiation. Strikingly, the largest increase in ubiquitylation events was found for known NER damage-recognition factors[59,76], which emphasizes that these are highly regulated proteins and that specific protein–protein interactions are quickly induced to initiate NER and to drive the reaction forwards. The reversible nature of these post-translational modifications facilitates the quick removal of the activity they impart on a protein; for example, NER may need to be aborted once the damage cannot be verified by XPA and TFIIH, or terminated when a lesion has been repaired.

Global genome NER regulation by ubiquitin or ubiquitin-like modifiers. A key E3 ubiquitin ligase involved in the initiation of GG-NER is the UV–DDB and CRL complex[17] (FIG. 2). A central target of ubiquitin-mediated regulation is XPC, which is consistent with its role as a main initiator of GG-NER[82–84]. Upon UV radiation damage, UV–DDB is activated by its conjugation to the ubiquitin-like modifier NEDD8, whereas in normal conditions this complex is kept in an inactive state by the deneddylation activity of the COP9 signalosome[85]. UV–DDB ubiquitylates core histones[86], XPC and DDB2 itself[87]. Strikingly, whereas DDB2 is necessary for efficient NER[15,88], CUL4A seems to inhibit CPD and 6–4PP removal[89], which implies that ubiquitylation is delicately balanced to control GG-NER. Whereas ubiquitylated DDB2 is targeted for proteolytic degradation, ubiquitylated XPC is not degraded but instead gains increased affinity for DNA lesions *in vitro*[84]. The degradation of ubiquitylated XPC might be inhibited by its interaction with RAD23, a protein that contains two ubiquitin-binding domains and is required to stabilize XPC in normal conditions[90,91]. However, RAD23 dissociates from XPC upon its binding to DNA lesions, almost simultaneously with the ubiquitylation of XPC, and it is therefore unlikely that RAD23 stabilizes ubiquitylated XPC that is bound to lesions[92].

WD40 domain
A short structural protein motif with β-propeller architecture that is believed to be involved in protein–protein interactions.

TFIIH
(Transcription initiation factor IIH). An essential transcription initiation complex that is also pivotal for nucleotide excision repair. In both processes it functions to unwind DNA using its two helicase subunits.

CAK subcomplex
(CDK-activating kinase subcomplex). A subcomplex of TFIIH (transcription initiation factor IIH) that consists of cyclin-dependent kinase 7 (CDK7), cyclin H (CCNH) and MNAT1 (also known as MAT1). The CAK subcomplex has an important function in transcription initiation as it phosphorylates the largest subunit of RNA polymerase II, but it is not required for nucleotide excision repair and dissociates from TFIIH.

Damage avoidance
A process that occurs when DNA replication encounters an unrepaired DNA lesion. Such lesions block the regular replication machinery on the damaged strand. However, replication of the undamaged complementary strand can still continue, which generates a daughter strand with the same sequence as the damaged template. The lesion in the original template strand can be bypassed by transiently switching replication to the newly synthesized daughter strand.

Cockayne syndrome
A human disorder characterized by ultraviolet radiation sensitivity, progeria, and neurological and developmental abnormalities. The syndrome is caused by mutations in several genes encoding proteins involved in transcription-coupled nucleotide excision repair (TC-NER).

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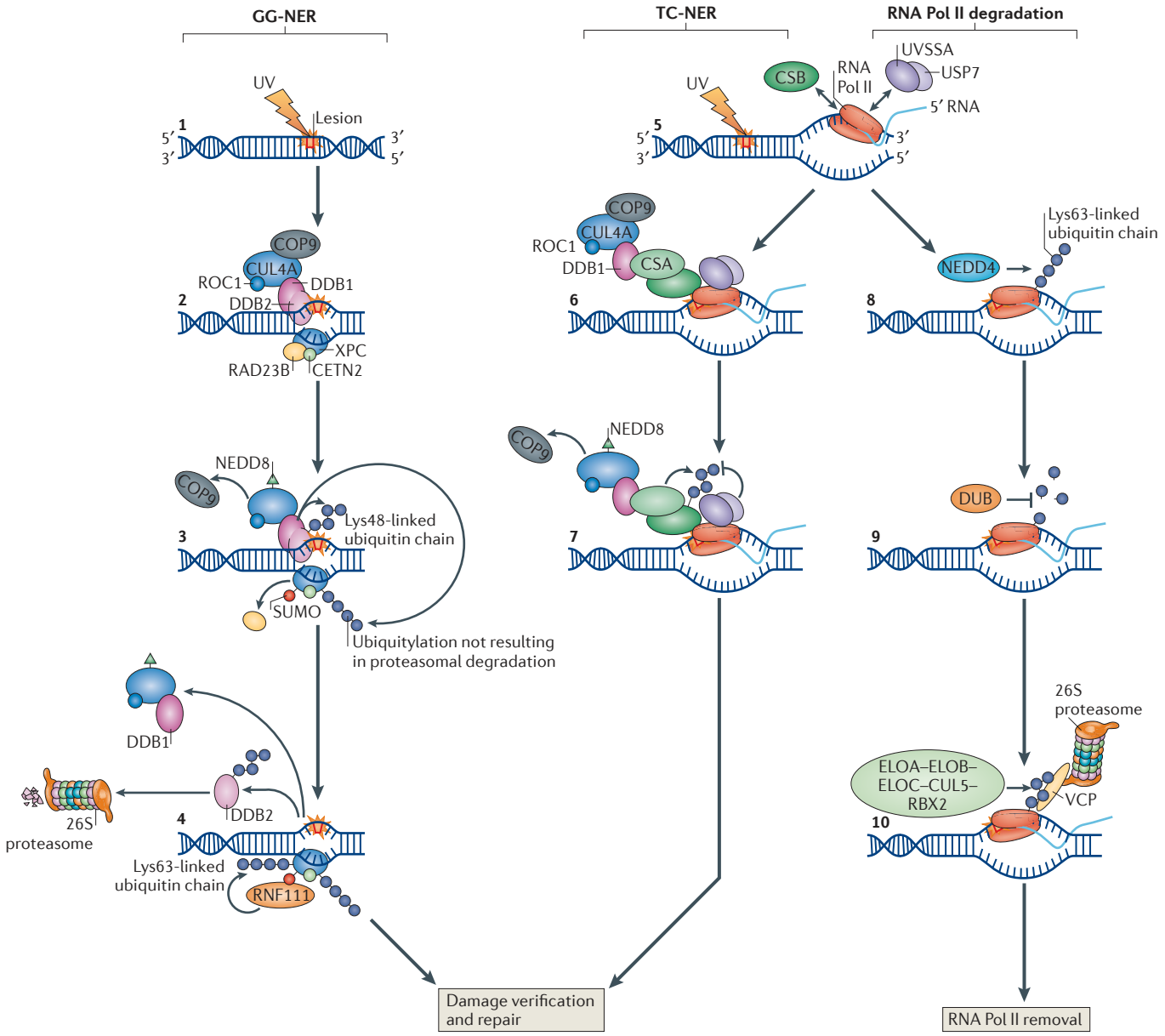

Figure 2 | **Roles of ubiquitylation in nucleotide excision repair.** Helix-destabilizing DNA lesions are detected by the global genome nucleotide excision repair (GG-NER) machinery (steps 1, 2). The UV–DDB (ultraviolet (UV) radiation–DNA damage-binding protein) complex comprises the DDB1 and DDB2 proteins, binds to UV-radiation-induced lesions and also associates with the CRL (cullin 4A (CUL4A)–regulator of cullins 1 (ROC1) E3 ubiquitin ligase) complex, to which the COP9 signalosome also binds (step 2). Upon binding of this complex to the lesion the COP9 signalosome is released. This results in neddylation of CUL4A by the ubiquitin-like modifier NEDD8, which activates the UV–DDB complex and triggers the polyubiquitylation of DDB2 (step 3), and leads to its proteasomal degradation (step 4). The UV–DDB complex also ubiquitylates XPC, which increases its affinity for DNA damage. Upon lesion binding by XPC, its interaction partner UV excision repair protein RAD23 homologue B (RAD23B) is released from the complex (step 3). Following UV damage, XPC also undergoes sumoylation, which results in the recruitment of the E3 ubiquitin ligase RING finger protein 111 (RNF111) to the lesion through its SUMO-interacting motif (SIM). RNF111 subsequently polyubiquitylates XPC at Lys63, which is important for an efficient NER reaction (step 4). DNA damage that stalls transcript elongation (step 5) triggers either the formation of an active transcription-coupled NER (TC-NER) complex (middle) or leads to degradation of RNA polymerase II (RNA Pol II) subunit B1 (RPB1; not depicted) (right). Upon stalling of RNA Pol II at lesions, Cockayne syndrome protein CSB stably binds to RNA Pol II and recruits Cockayne syndrome WD repeat protein CSA, which forms, together with CUL4A and ROC1, an E3 ubiquitin ligase complex (step 6). During the TC-NER reaction the previously bound COP9 signalosome is released and CUL4A is activated by neddylation (step 7). The activated E3 ubiquitin ligase complex probably ubiquitylates CSB. The enzyme ubiquitin-specific-processing protease 7 (USP7), which is recruited to the stalled RNA Pol II by UV-stimulated scaffold protein A (UVSSA), stabilizes CSB during the TC-NER reaction by deubiquitylating it (step 7). If TC-NER fails to repair the lesion, the stalled RNA Pol II can be degraded as a 'last resort' solution (right). The E3 ubiquitin protein ligase NEDD4 adds Lys63-linked polyubiquitin chains to RPB1 (step 8). These chains are shortened to monoubiquitylated forms by deubiquitylating enzymes (DUBs) (step 9) to generate a substrate for the elongin A (ELOA)–ELOB–ELOC–CUL5–RING-box protein 2 (RBX2) E3 ubiquitin ligase complex, which adds Lys48-linked polyubiquitin chains to RPB1 (step 10). The polyubiquitylated RPB1 is extracted from the chromatin-bound RNA Pol II complex by valosin-containing protein (VCP) ATPase and is subsequently degraded by the proteasome (step 10). CETN2, centrin 2.

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Recently, another E3 ubiquitin ligase, RING finger protein 111 (RNF111; also known as Arkadia), was found to modify XPC with Lys63-linked polyubiquitin chains. RNF111 specifically recognizes XPC that has been sumoylated in response to UV radiation damage[82] (FIG. 2). This RNF111-dependent XPC ubiquitylation regulates the binding of XPC to DNA lesions[83]. Crosstalk between different post-translational modifications and ubiquitylation was also described for DDB2, as UV-radiation-induced PARylation of DDB2 inhibits its ubiquitylation and subsequent degradation[74]. Further research is necessary to delineate the precise order of post-translational modification events and how they control the actions of DDB2 and XPC in GG-NER.

Transcription-coupled NER regulation by ubiquitylation. During the initial steps of repairing an RNA Pol II-blocking lesion by TC-NER, another CRL E3 ubiquitin ligase has an important role. In this case, the protein that specifies substrate binding of this complex is CSA (rather than DDB2 as in GG-NER), which probably targets CSB for ubiquitylation and degradation[93] (FIG. 2). Recently, UVSSA was identified as a novel TC-NER factor implicated in ubiquitylation[59,94–96]. Following DNA damage, UVSSA recruits the deubiquitylating enzyme USP7 to TC-NER complexes, thereby counteracting the CSA-dependent ubiquitylation of CSB and stabilizing CSB by inhibiting its degradation[59,95]. Thus, UVSSA and CSA, through different ubiquitin-mediated mechanisms, seem to have opposite roles in determining the fate of CSB. Interestingly, in addition to deubiquitylation, UVSSA-dependent ubiquitylation of RNA Pol II also occurs upon UV-radiation-induced damage, but this modification does not result in the proteasomal degradation of RNA Pol II (REF. 94). Finally, both UVSSA and CSB have ubiquitin-binding domains, which are crucial for efficient TC-NER. This observation further corroborates the importance of NER regulation by ubiquitylation[94,97].

Lesion-stalled RNA Pol II itself may undergo polyubiquitylation and degradation, possibly as a last resort to clear stalled RNA polymerases from lesions when TC-NER fails[68,98] (FIG. 2). UV-radiation-stimulated RNA Pol II ubiquitylation is a prime example of the complex layers of regulation that are offered by ubiquitylation (reviewed in REF. 68). The largest subunit of RNA Pol II, RNA Pol II subunit B1 (RPB1), is monoubiquitylated or polyubiquitylated with Lys63-linked ubiquitin chains by the E3 ubiquitin protein ligase NEDD4. The polyubiquitin chains are shortened by deubiquitylation, and the resulting monoubiquitins are elongated by another E3 ubiquitin ligase complex consisting of elongin A (ELOA; also known as TCEB3), ELOB (also known as TCEB2), ELOC (also known as TCEB1), CUL5 and RING-box protein 2 (RBX2) to form Lys48-linked polyubiquitin chains on RPB1. The ATPase valosin-containing protein (VCP; also known as p79 or TER ATPase) specifically removes Lys48-polyubiquitylated RPB1 from the RNA Pol II complex and directly feeds it into the 26S proteasome for degradation[68].

The exact molecular mechanism of these TCR-associated ubiquitylation events is not known; however, they are probably necessary for the reverse translocation of RNA Pol II, which enables proper repair. This is the most favourable TC-NER strategy, as both RNA Pol II and the nascent transcript are preserved. However, when TC-NER cannot be executed a more destructive strategy is used, in which lesion-stalled RNA Pol II is degraded. If not removed, the stalled RNA Pol II will block transcription and perhaps even trap additional polymerases, which would in effect completely inactivate gene expression from the damaged allele. Preventing transcription may have important clinical implications, as discussed below.

Chromatin dynamics in NER

For optimal genome maintenance, NER should be capable of reaching all lesions in the genome at any time, irrespective of chromatin conformation that may affect repair efficiency. The 'access, repair, restore' model of DNA damage repair[99,100] postulates that chromatin is first modified to enable repair proteins to access it efficiently. Following repair, the original chromatin configuration is restored. Chromatin modifications are thought to promote access of the repair machinery to lesions[101,102], and recent evidence (described below and in BOX 2) suggests that these modifications also actively regulate the DDR.

Chromatin remodelling in global genome NER. The UV–DDB complex plays a central part in organizing the chromatin during GG-NER initiation (FIG. 3). The complex ubiquitylates core histones[86,103], which is thought to stimulate repair by histone displacement[104]. DDB2 also induces PARylation-dependent chromatin decondensation, which is independent of ubiquitylation[105]. PARylation of DDB2 itself suppresses its ubiquitin-dependent degradation and promotes its binding to damaged DNA. Moreover, DDB2 mediates PARP1 (poly(ADP-ribosyl) polymerase 1)-dependent PARylation of chromatin, which aids recruitment of the ATP-dependent chromatin remodelling enzyme ALC1 (amplified in liver cancer protein 1; also known as CHD1L) and stimulates NER[74]. Other ATP-dependent chromatin remodelling complexes implicated in GG-NER have been identified and probably function to promote access of GG-NER-initiating factors to DNA[106]. For example, members of the SWI/SNF family of chromatin-remodelling proteins stimulate NER[107–109], but their precise activity remains unclear. Furthermore, the INO80 chromatin-remodelling complex may facilitate repair by interacting with DDB1 to stimulate the recruitment of XPC and XPA to damaged chromatin[110]. UV–DDB may also induce other histone post-translational modifications, as it associates with the histone acetyltransferase p300 (REFS 111,112) and the chromatin-acetylating STAGA complex, which contains the histone acetyltransferase GCN5[113]. This observation suggests that acetylation has a role in mammalian GG-NER, as has been shown in yeast[114]. Indeed, histone acetylation stimulates repair[101] and the histone acetyltransferases GCN5[115] and p300 (REF. 116) are targeted to UV-radiation-induced damage. These findings suggest

Oxidative DNA damage
A large group of DNA lesions that are mainly caused by reactive oxygen species (ROS) that oxidize nucleotides at several positions. Oxidative DNA lesions are unavoidable, as ROS are natural products of cellular metabolism and the immune system, or are formed by environmental chemicals and radiation.

Poly(ADP-ribosyl)ation
(PARylation). The polymerization of ADP–ribose units from donor NAD^+ molecules on target proteins by enzymes of the poly(ADP-ribosyl) polymerase (PARP) family. PARP enzymes detect single-strand breaks in DNA and regulate the efficiency of several lesion repair mechanisms by PARylation of damaged chromatin and signalling proteins.

COP9 signalosome
A multisubunit protease that regulates the activity of CRL (cullin 4A (CUL4A)–regulator of cullins 1 (ROC1) E3 ubiquitin ligase) complexes by removing the ubiquitin-like protein NEDD8.

Chromatin remodelling
Dynamic alteration of the chromatin structure to regulate access of proteins to DNA, which is induced by post-translational modifications of histone tails and ATP-dependent remodelling complexes that move or restructure nucleosomes.

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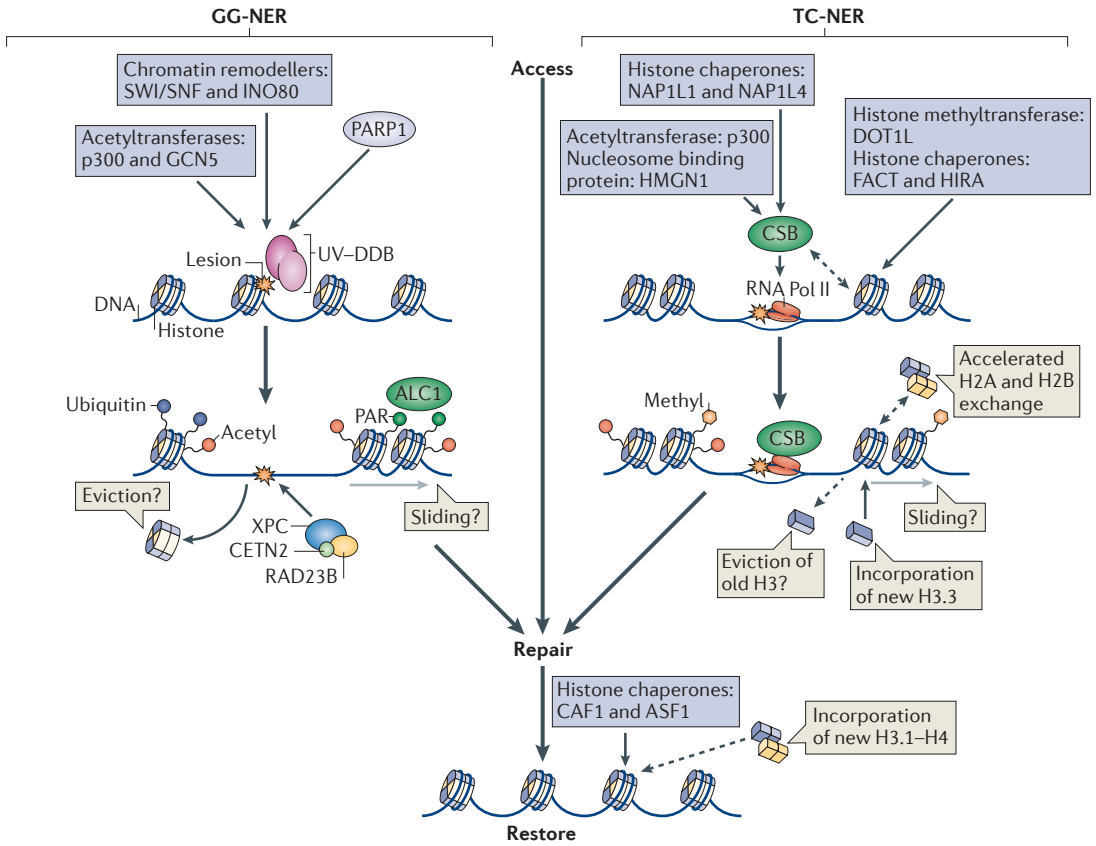

Figure 3 | **Chromatin dynamics in nucleotide excision repair.** According to the 'access, repair, restore' model, chromatin conformation is adapted so that damage detection and repair can efficiently take place, after which the original chromatin configuration is restored. Global genome nucleotide excision repair (GG-NER; left) is stimulated by an open chromatin environment, which is promoted by the activity of several chromatin remodellers and histone modifications. Repair is stimulated by the activity of the UV–DDB (ultraviolet (UV) radiation–DNA damage-binding protein) complex, which ubiquitylates core histones and mediates chromatin poly(ADP-ribosyl)ation (PARylation) through its association with poly(ADP-ribosyl) polymerase 1 (PARP1); these processes result in chromatin decondensation. Furthermore, NER is stimulated by histone acetylation, perhaps induced by histone acetyltransferases p300 and GCN5, which are both recruited to UV-radiation-induced damage. The SWI/SNF and INO80 ATP-dependent chromatin remodelling complexes, which displace nucleosomes by histone eviction or sliding, promote repair by interacting with and stimulating the recruitment of GG-NER initiation factors. Amplified in liver cancer protein 1 (ALC1) is an ATP-dependent chromatin remodelling protein that is recruited to PARylated chromatin and stimulates GG-NER. The modification of chromatin is thought to facilitate recruitment of the XPC complex (which also contains UV excision repair protein RAD23 homologue B (RAD23B) and centrin 2 (CETN2)), which binds to unpaired bases in DNA opposite to the lesion and initiates NER. In transcription-coupled NER (TC-NER; right), the chromatin structure is altered by the activity of histone modifiers, histone chaperones and chromatin-remodelling proteins. Cockayne syndrome protein CSB remodels nucleosomes *in vitro* and this activity is stimulated by the histone chaperones nucleosome assembly protein 1-like 1 (NAP1L1) and NAP1L4, which interact with CSB. It is unclear whether these three proteins have similar activities *in vivo*, but nevertheless the association of CSB with DNA lesions depends on its chromatin remodelling domain. CSB attracts the histone acetyltransferase p300 and the nucleosome binding protein high mobility group nucleosome-binding domain-containing protein 1 (HMGN1) to TC-NER complexes, although the precise function of these activities in TC-NER remains unclear. After lesion removal, chromatin is adapted to enable efficient transcription restart, a process in which the histone methyltransferase DOT1-like protein (DOT1L) is implicated by promoting transcriptionally active chromatin marks. Furthermore, accelerated histone exchange by the histone chaperone FACT (facilitates chromatin transcription) and incorporation of new histone H3.3 by the histone chaperone protein HIRA facilitate transcription restart after lesion-induced transcriptional arrest. Following repair, new histones are incorporated at sites of damage by the histone chaperone complex CCR4-associated factor 1 (CAF1), which is assisted by the histone chaperone alternative-splicing factor 1 (ASF1). RNA Pol II, RNA polymerase II.

that a complex network of chromatin modifications and remodelling assists lesion recognition in GG-NER. It remains unknown, however, whether all these events take place during every GG-NER reaction or only in specifc chromatin environments.

Chromatin remodelling in transcription-coupled NER. It is reasonable to assume that TC-NER involves chromatin-modifying activities that differ from those of GG-NER, as transcript elongation predominantly functions in open (that is, active) chromatin (FIG. 3).

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CSB harbours a DNA-dependent ATPase domain of the SNF2 family that is required for its chromatin-binding and repair functions[117–120]. CSB remodels chromatin *in vitro*[70], an activity that is stimulated by its interactions with the histone chaperones nucleosome assembly protein 1-like 1 (NAP1L1) and NAP1L4 (REF. 121), and attracts other chromatin proteins, including p300 and HMGN1 (REF. 58). Whether CSB also remodels chromatin *in vivo* and what its exact *in vivo* activity and substrates are remain elusive.

Recently, the histone chaperone FACT complex subunit SPT16 was found to facilitate accelerated H2A and H2B exchange at sites of TC-NER[122]. SPT16 promotes restarting of transcription at sites of DNA damage, which suggests that accelerated histone turnover stimulates TC-NER and/or the resumption of transcription after repair. Efficient transcription restart, which coincides with the occurrence of transcriptionally active chromatin marks, is also promoted by DOT1-like protein (DOT1L; also known as histone-Lys *N*-methyltransferase, H3 Lys79-specific)[123] and by the deposition of newly synthesized histone variant H3.3 at UV-radiation-damaged sites by the histone chaperone protein HIRA[124]. These observations imply the involvement of complicated chromatin modifications in TC-NER and transcription restart, although transcription occurs in open chromatin.

In turn, NER itself induces chromatin remodelling. Upon its completion, new H3.1 histones are incorporated at sites of newly synthesized DNA by the coordinated action of the histone chaperones CCR4-associated factor 1 (CAF1; also known as CNOT7) and alternative-splicing factor 1 (ASF1; also known as SRSF1)[125–127]. This *de novo* incorporation of H3.1 raises the question of how the original histone marks are retained and epigenetic imprinting is preserved after DNA damage repair. The fate of other histones, and whether they are recycled or replaced, requires further investigation.

Regulation of NER *in vivo*

Almost all our knowledge of NER was acquired from *in vitro* experiments and studies that used fast-replicating mammalian cells. Interestingly, *in vivo* studies of specific cell types suggest the existence of several modes of NER that differ by cell type and differentiation stage. For instance, real-time imaging of fluorescent TFIIH in tissues of knock-in transgenic mice revealed that this multifunctional complex becomes mostly immobile on chromatin during cellular differentiation. By contrast, TFIIH shows free mobility and fast dynamics in cultured cells and proliferative tissues, which indicates that fundamental spatiotemporal changes exist in the organization of transcription and DNA repair upon differentiation[128]. It is likely that in dividing cells the highest priority is given to avoiding mutagenesis (hence, to GG-NER), whereas in postmitotic cells unperturbed gene expression, and thus TC-NER, is prioritized. Indeed, upon their differentiation, keratinocytes[129,130], neurons[131] and macrophages[132] show decreased removal of UV-radiation-induced lesions at the global genome level, whereas the activity of TC-NER remains mostly unaffected[133,134]. It is currently unknown which factors drive this differentiation-driven switch in NER activity from GG-NER to TC-NER. Surprisingly, in some terminally differentiated cells (for example, neurons and macrophages) damage is repaired not only in the transcribed strand of active genes, which is typical for TC-NER, but also in the non-transcribed strand. This poorly characterized phenomenon is known as transcription domain-associated repair[135]. Presumably, long-term maintenance of crucial transcribed genes requires protection of both strands against the accumulation of DNA damage.

As the germ line, unlike the soma, is transmitted to future generations, germ cells may use different strategies from those of somatic cells for genome maintenance. Indeed, in *Caenorhabditis elegans* GG-NER is the main process used in the maternal germ lineage and in early stages of embryogenesis to maintain the entire genome[136], although the genomes of mature spermatozoa seem to be refractory to repair[137–139], possibly due to the very compact nature of their chromatin. During development, successive somatic tissues increasingly rely on TC-NER, which deals with damage to transcribed genes and ignores lesions in non-transcribed DNA[136]. Similarly, in pluripotent mouse embryonic stem cells, GG-NER seems to be more important than TC-NER for surviving UV-radiation-induced damage, a situation that is reversed in embryonic fibroblasts[140]. These findings support the idea that maintenance of the entire genome is prioritized in the immortal germ cell lineage, whereas in mortal somatic cells the focus is switched to the repair of expressed genes.

Clinical consequences of NER deficiency

Defects in NER provide a paradigm for the diverse clinical consequences of DNA damage. Mutations in genes involved in NER can result in phenotypes that range from normal development with an extreme predisposition to cancer, to neurodevelopmental defects associated with premature ageing abnormalities but without cancer predisposition. These diverse effects are due to the wide range of lesions that are repaired by NER, the existence of two NER subpathways and the multiple functions of several NER factors.

Global genome NER defects and cancer predisposition. Defects in GG-NER cause the genome-wide accumulation of lesions, which can be bypassed by error-prone translesion DNA polymerases. Lesion bypass promotes cell survival but increases mutagenesis, which explains the very strong cancer predisposition observed in the GG-NER disorder xeroderma pigmentosum. Patients of xeroderma pigmentosum complementation group C (XP-C) and XP-E, who harbour defects only in GG-NER (FIG. 1), are only mildly hypersensitive to UV radiation, exhibit sun-induced cutaneous features such as hypopigmentation and hyperpigmentation, and have the highest incidence of cancer of all the xeroderma pigmentosum complementation groups: their susceptibility to sun-induced skin cancer is increased by >1,000-fold and they also have an increased risk of various internal tumours[141]. The increased susceptibility to internal tumours is

Xeroderma pigmentosum
A human disorder caused by defects in genes that encode proteins involved in global genome nucleotide excision repair (GG-NER). It is characterized by ultraviolet radiation hypersensitivity and an increased risk of skin cancer and internal tumours.

Xeroderma pigmentosum complementation group
Cells from patients with xeroderma pigmentosum are classified into eight genetic complementation groups (XP-A to XP-G and XP-Variant), which are based on their respective gene and protein defects.

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presumably due to the accumulation of endogenously induced DNA lesions (for example, cyclopurines that are caused by ROS) that would otherwise be repaired by GG-NER[142,143]. The mutations detected in patients of all other xeroderma pigmentosum complementation groups are in genes that participate in core NER reactions and hence also affect the TC-NER subpathway. For example, patients who have mutations in *XPA* exhibit additional clinical symptoms (such as accelerated neurodegeneration starting in the second decade of life)[144,145], which suggests a link between TC-NER defects and neuronal loss. This form of xeroderma pigmentosum is also known as De Sanctis–Cacchione syndrome. Most of the other proteins participating in the NER core reaction are also involved in other repair mechanisms and DNA-associated processes (TABLE 1), and mutations in these genes also result in additional symptoms (discussed below).

TCR defects and accelerated segmental ageing. A deficient TCR causes the accumulation of transcription complexes that are stalled by lesions, which have either not been detected by GG-NER or are poorly recognized by other repair processes, for example CPDs and Illudin S adducts[146]. Obviously, transcription is vital and a deficiency in TCR compromises cell function, induces premature cell death[147] and, consequently, accelerates ageing[148]. The effects of a TCR defect can vary from tissue to tissue depending on, for example, its metabolism (that is, the level of reactive metabolites), exposure to genotoxins, the activity of antioxidant systems and that of partially complementary repair mechanisms, such as GG-NER and BER. This explains the progeroid phenotype that is associated with TCR deficiency in severe, progressive neurodevelopmental disorders, such as Cockayne syndrome and the even more severe cerebro-oculo-facio-skeletal syndrome (COFS)[149]. These conditions are characterized by early cessation of growth, microcephaly, mental retardation associated with dysmyelination, retinal degeneration, sensorineural deafness, cachexia, photosensitivity and a greatly reduced life expectancy. The reported average life expectancy of patients with Cockayne syndrome is 12 years, whereas that of patients with COFS is often less than 2 years. Ageing in patients with Cockayne syndrome is predominantly accelerated in tissues that are mainly composed of non-proliferating or slowly proliferating cells, such as neurons and Schwann cells, which form the myelin sheath. These observations are consistent with the idea that post-mitotic cells may have less-potent GG-NER, accumulate life-long damage and hence are heavily dependent on active TCR to maintain unperturbed gene expression[136]. However, other organs are also affected in patients with (and mouse models of) Cockayne syndrome and COFS, including the skeleton, kidney and liver[149,150]. The proliferative tissues of affected individuals seem to suffer less from deficient TCR, possibly because the replication machinery may dissociate the stalled transcription machinery when it bypasses it, which could enable repair of the lesion by other mechanisms. Moreover, replication counteracts damage accumulation by diluting the damaged DNA through *de novo* synthesis.

Gene expression profiling in mouse models of NER disorders that include a TCR defect reveal suppression of key hormonal axes that support growth and metabolism; instead, energy resources are redirected to maintenance and defence mechanisms, such as antioxidant defences and stress resistance[151]. This 'survival response' resembles the anti-ageing response elicited by calorific restriction, which promotes longevity[152]. Thus, in this way, these animals presumably attempt to extend their short lifespan, which could explain the profound growth retardation in patients with Cockayne syndrome and Cockayne syndrome-like diseases; reduced growth and improved defence systems may also contribute to the absence of cancer that has been noted in Cockayne syndrome. Hence, GG-NER and TCR defects elicit almost diametrically opposed clinical features in terms of cancer and ageing, which can be explained by the dysfunction of specific molecular mechanisms and the roles of these processes in preventing mutagenesis and cell death, respectively.

Phenotypes of defects in multifunctional NER factors. The phenotype of mutations in genes encoding core NER factors, which affect GG-NER, TC-NER and/or the broader TCR process, depends on the extent to which each of these subpathways is disturbed, on synergism that occurs when more than one subpathway is defective and on the functions of these factors that are beyond DNA-damage repair (TABLE 1). In particular, mutations in genes that encode the subunits of TFIIH, a NER core component that interacts with both GG-NER-specific and TC-NER-specific factors, may differentially influence the degree to which GG-NER and/or TC-NER and TCR are compromised[153,154]. This complexity can explain the striking clinical heterogeneity associated with different mutations in the gene encoding XPD (and to a lesser extent XPB), which encompasses pure xeroderma pigmentosum (when GG-NER is primarily compromised), xeroderma pigmentosum combined with Cockayne syndrome (to various degrees) and very severe COFS phenotypes, in which both GG-NER and TCR are strongly compromised. In addition, specific mutations in the genes encoding the TFIIH subunits XPD, XPB and TTDA can give rise to trichothiodystrophy, which exhibits hallmarks of Cockayne syndrome along with the unique features of brittle hair and nails, and scaly skin[144]. These additional features are a complication derived from the fact that trichothiodystrophy-causing mutations affect the stability as well as the repair functions of TFIIH, thereby interfering with its transcription initiation function during the final stages of terminal differentiation of hair, nail and skin cells[36,155,156].

Also, in the case of the gene encoding XPG, point mutations that only inactivate the endonuclease activity of XPG primarily affect GG-NER and TC-NER in manners similar to that of mutations in *XPA* (see below). However, XPG is also likely to have a structural role in the broader TCR process, BER and other DNA-based processes (reviewed in REF. 157), including in replication[158]. Thus, mutations in the gene encoding XPG that result in a truncated XPG protein are likely to affect NER as well as additional cellular pathways. Thus, such

De Sanctis–Cacchione syndrome
A severe and rare form of xeroderma pigmentosum in which patients display accelerated neurodegeneration, microcephaly, retarded growth and impaired sexual development.

Illudin S
A natural (mushroom-derived) sesquiterpene drug, which causes DNA lesions that block replication and transcription. These lesions are repaired by transcription-coupled nucleotide excision repair (TC-NER) but ignored by global genome nucleotide excision repair (GG-NER).

Progeroid phenotype
A phenotype of accelerated ageing that is exhibited by patients at a young age.

Cerebro-oculo-facio-skeletal syndrome
(COFS). A very severe human disorder resembling Cockayne syndrome. It involves the neurological system, eyes, face, and skeleton, and results in a very short life expectancy of 2–3 years. It is caused by severe mutations in genes encoding proteins involved in transcription-coupled nucleotide excision repair as well as in several other DNA repair processes.

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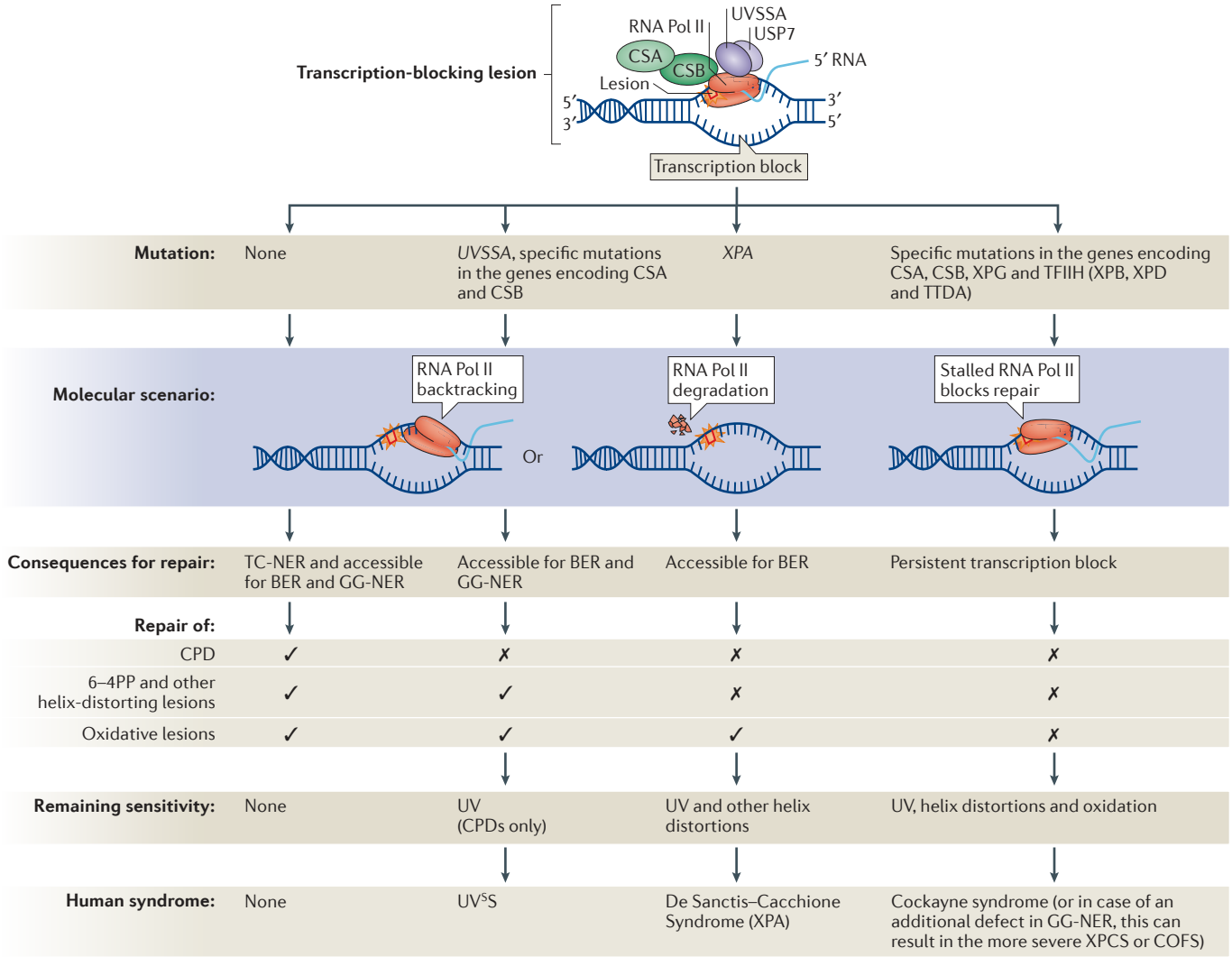

Figure 4 | **Genotype–phenotype correlations in disorders of nucleotide excision repair: a unifying model.** A transcription-blocking lesion is shown, and its outcome is depicted when several different transcription-coupled repair (TCR) proteins are mutated. When TCR functions normally, stalled RNA polymerase II (RNA Pol II) backtracks, enabling the lesion to be repaired, after which transcript elongation can resume. In patients with UV-sensitive syndrome (UV^{S}S), who have inactivating mutations in *UVSSA* (which encodes UV-stimulated scaffold protein A), or specific mutations in the genes encoding Cockayne syndrome WD repeat protein CSA and Cockayne syndrome protein CSB, TCR is impaired and RNA Pol II backtracking is compromised. However, CSB degradation (and perhaps also its absence) and the degradation of arrested RNA Pol II, which would otherwise mask the damage, leave the lesion accessible to other repair systems. Such systems include global genome nucleotide excision repair (GG-NER) (which repairs lesions that distort the DNA helical structure) or base excision repair (BER), which repairs non-distorting lesions resulting from oxidation or alkylation that block transcription. GG-NER can quickly repair 6–4 pyrimidine–pyrimidone photoproducts (6–4PPs), but the repair of cyclobutane–pyrimidine dimers (CPDs), the most common ultraviolet (UV)-radiation-induced DNA lesion, is very slow and inefficient, which explains the specific UV sensitivity of patients with UV^{S}S. In our model, transcription-blocking lesions in cells from patients in xeroderma pigmentosum complementation group A (XP-A) remain accessible to global repair systems, but the defect in XPA also impairs GG-NER. Hence, besides CPDs, 6–4PPs and other helix-distorting lesions, such as cyclopurines, also persist and trap RNA Pol II complexes and thus compromise the recovery of transcription. As oxidative lesions can still be repaired by BER, the phenotypes of patients with mutations in the *XPA* gene (De Sanctis–Cacchione syndrome) are of intermediate severity between those of patients with UV^{S}S and patients with Cockayne syndrome. Cockayne syndrome is caused by mutations in the genes encoding CSA and CSB, or specific mutations in the gene encoding XPG, or in the genes encoding the XPB, XPD and TTDA subunits of the TFIIH (transcription initiation factor IIH) basal transcription factor complex. These mutations mean that TCR is not functional. Furthermore, RNA Pol II cannot be removed from lesions, which renders all transcription-blocking lesions inaccessible to repair. This further accelerates cell death and results in the progressive, devastating phenotypes of Cockayne syndrome. In patients with inactivating defects in the core NER factors XPB, XPD, XPG and TTDA, the additional inactivation of GG-NER, which leaves even more lesions in the genome unrepaired, can explain their severe phenotypes, which combine features of xeroderma pigmentosum and Cockayne Syndrome (XPCS), as well as the even more severe cerebro-oculo-facio-skeletal syndrome (COFS). TC-NER, transcription-coupled NER; USP7, ubiquitin-specific-processing protease 7.

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mutations cause Cockayne syndrome-type features on top of xeroderma pigmentosum symptoms, which results in combined xeroderma pigmentosum and Cockayne syndrome or even severe COFS.

Mutations in genes encoding the multifunctional complex XPF–ERCC1 result in the most complex constellation of clinical symptoms[159]. In addition to its function in NER, this structure-specific endonuclease is implicated in interstrand crosslink repair and in single-strand annealing repair of DSBs (TABLE 1). Depending on which functions are affected, human patients and mouse mutants can exhibit xeroderma pigmentosum, severe Cockayne syndrome, COFS and/or Fanconi anaemia features[151,159–162]. The human disorder Fanconi anaemia is caused by defects in genes related to interstrand crosslink repair that result in chromosomal instability and cell death, particularly in proliferative tissues such as the bone marrow. The phenotype of this disease illustrates three consequences of unrepaired DNA damage: congenital abnormalities, which are probably derived from stochastic DNA damage that causes loss of progenitor cells during embryogenesis; early bone marrow depletion, which is due to accelerated-ageing-like exhaustion of stem cells; and an increased incidence of leukaemia and lymphomas caused by DNA-damage-induced genomic instability. One of the best-studied mouse models is an *Ercc1*$^{\Delta/-}$ mutant, containing one knockout allele and one truncating mutation, in which most DNA repair functions are severely compromised (but not completely absent). This mouse has a lifespan of ~25 weeks and exhibits a remarkably wide range of pathological, physiological and behavioural features related to accelerated ageing, such as progressive neurodegeneration (dementia, ataxia, hearing and vision loss); liver, kidney, vascular and haematological ageing; osteoporosis, cachexia, sarcopenia accompanied by hormonal changes, loss of stem-cell renewal capacity and increased cellular senescence; and gene expression patterns similar to those of natural ageing[151,163,164]. Hence, this mouse mutant combines symptoms of premature ageing in postmitotic tissues (for example, the brain, as in Cockayne syndrome) and in proliferative tissues (early haematopoietic stem cell exhaustion, as in Fanconi anaemia).

UV-sensitive syndrome: a unifying model of TCR disorders. UV-sensitive syndrome (UV^{S}S) is the mildest form of TCR deficiency. Affected patients display sun hypersensitivity, which is due to a cellular inability to restore RNA synthesis in response to UV radiation damage, but lack the severe neurodevelopmental abnormalities of Cockayne syndrome and the strong predisposition to skin cancer and other cancers of xeroderma pigmentosum[165]. In some patients with UV^{S}S, specific mutations in the genes encoding CSB and CSA were found, including mutations in the gene encoding CSB that result in the complete absence of CSB[66,166], which further links the syndrome with TC-NER and TCR. Recently, mutations that cause UV^{S}S were also discovered in the *UVSSA* gene[59,94–96]. The encoded protein associates with active TCR complexes and has a role in stabilizing CSB at sites of DNA damage[59,95].

How do inactivating mutations in *UVSSA* and specific mutations in the genes encoding CSA and CSB account for the mild UV radiation sensitivity of patients with UV^{S}S, who lack all other abnormalities and oxidative-damage-associated sensitivity of patients with Cockayne syndrome? Such a selective UV-radiation-specific sensitivity suggests that these mutations are somehow linked with lesion specificity[66]. To provide an explanation for this observation and for the genotype–phenotype correlations of the different TCR syndromes, we propose the following unifying model (FIG. 4): mutations that cause UV^{S}S compromise TCR because CSB and possibly other proteins of the lesion-stalled transcription complex are degraded, which is consistent with the presumed function of UVSSA in stabilizing CSB and the complete absence of CSB proteins in patients with UV^{S}S[166]. However, degradation of lesion-stalled transcription complexes renders the lesion accessible to other repair systems. If the DNA damage is induced by UV radiation, GG-NER will quickly remove blocking 6–4PPs but will repair CPDs very inefficiently. Thus, only CPDs will persist and arrest any following transcript-elongating RNA polymerase, which hinders the resumption of transcription. This mechanism explains the selective UV sensitivity and mild phenotype of patients with UV^{S}S, and also the finding that cells from patients with this syndrome are hypersensitive to other DNA lesions that are poor substrates for GG-NER, such as illudin S lesions[59,146]. In this unifying model, an XPA defect would make the transcription-arresting lesion available for alternative modes of repair, but this will only enable the repair of lesions that are not substrates for GG-NER (such as substrates for BER), as cells with defective XPA are also defective in GG-NER. Hence, in cells of patients in the XP-A group (De Sanctis–Cacchione syndrome), GG-NER substrates, including endogenously generated helix-distorting lesions such as cyclopurines[143], persist and keep interfering with transcription, which would explain why patients in XP-A have phenotypes that lie between those of UV^{S}S and Cockayne syndrome in terms of severity (including, for example, neurodegeneration). We suggest that the most severe phenotypes of Cockayne syndrome (which are caused by other mutations in genes encoding the Cockayne syndrome proteins, specific TFIIH gene mutations and severe mutations in the gene encoding XPG) might leave the stalling lesion inaccessible for any repair machinery. RNA Pol II complexes that are stuck on DNA lesions render them inaccessible to alternative repair mechanisms and thus permanently inactivate expression of the damaged allele. By contrast, in UV^{S}S and De Sanctis–Cacchione syndrome, proteolytic turnover of lesion-stalled RNA Pol II is permitted by milder mutations in the genes encoding CSA, CSB, UVSSA or XPA that enable lesion repair by other global genome repair systems. Although many tentative hypotheses have been offered to explain some of the features of TCR syndromes, the model presented here unifies all TCR conditions and explains the progressive and heterogeneous symptoms of many patients. Future studies will prove or disprove its overall validity.

Single-strand annealing
An error-prone mechanism that repairs double-strand breaks situated between two repetitive DNA sequences. It functions by resecting the broken ends, which is followed by homologous pairing of the repeats, gap-filling DNA synthesis and ligation. The sequences between the repeats are lost as the consequence of this process.

UV-sensitive syndrome (UV^{S}S). A human disorder characterized by mild ultraviolet radiation sensitivity of the skin. It is caused by inactivating mutations in the *UVSSA* gene (which encodes UV-stimulated scaffold protein A) and specific mutations in the genes encoding Cockayne syndrome proteins CSA and CSB, which are involved in transcription-coupled nucleotide excision repair (TC-NER).

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Table 1 | **Functions of nucleotide excision repair proteins outside nucleotide excision repair***

Mammalian complex or protein	Subunits	HUGO nomenclature	Main function or functions outside nucleotide excision repair
XPF–ERCC1 (excision repair cross-complementation group 1)	ERCC1	*ERCC1*	Interstrand crosslink repair (ICLR), single-strand annealing (SSA) and gene conversion
	XPF	*ERCC4*	ICLR, SSA and gene conversion
XPG	None	*ERCC5*	Base excision repair (BER) and resolving of stalled replication
Cockayne syndrome protein CSB	None	*ERCC6*	BER
Cockayne syndrome WD repeat protein CSA	DNA damage-binding protein 1 (DDB1)–cullin4A (CUL4A)–regulator of cullins 1 (ROC1)	*DDB1–CUL4A–RBX1* (RING-box 1, E3 ubiquitin protein ligase)	Ubiquitylation of many different targets
	CSA	*ERCC8*	Unknown
DNA ligase 1	None	*LIG1*	DNA replication
Proliferating cell nuclear antigen (PCNA)	None	*PCNA*	DNA replication; translesion synthesis (TLS) (by ubiquitylated PCNA)
DNA Polymerase δ (DNA Pol δ)	DNA Pol δ subunit (POLD1–4)	*POLD1–4*	DNA replication
DNA Pol ε	DNA polymerase ε subunit (POLE1–3)	*POLE1–3*	DNA replication
DNA Pol κ	None	*POLK* (polymerase (DNA directed) κ)	TLS and somatic hypermutation
Replication factor C (RFC)	RFC1–5	*RFC1–5*	DNA replication
Replication protein A (RPA)	RPA1–3	*RPA1–3*	DNA replication and homologous recombination
TFIIH (transcription initiation factor IIH) core complex	TFIIH subunit 1 (GTF2H1)–4	*GTF2H1–4*	Transcription
	TFIIH basal transcription factor complex TTDA subunit	*GTF2H5*	Transcription and repair of oxidative lesions
	TFIIH basal transcription factor complex XPB subunit	*ERCC3*	Transcription
	TFIIH basal transcription factor complex XPD subunit	*ERCC2*	Transcription and chromosome segregation
TFIIH CAK (CDK activating kinase) subcomplex	Cyclin H (CCNH)	*CCNH*	Transcription and cell cycle control
	Cyclin-dependent kinase 7 (CDK7)	*CDK7*	Transcription and cell cycle control
	MNAT1	*MNAT1*	Transcription and cell cycle control
UV–DDB	DDB1–CUL4A–ROC1	*DDB1–CUL4A–RBX1*	Ubiquitylation of different targets
	DDB2	*DDB2*	Unknown
UV-stimulated scaffold protein A (UVSSA)	None	*UVSSA*	Unknown
XPA	None	*XPA*	Unknown
XPC	XPC	*XPC*	Repair of oxidative lesions
	UV excision repair protein RAD23 homologue B (RAD23B)	*RAD23B*	Ubiquitin proteasome system
	Centrin 2 (CETN2)	*CETN2*	Centrosome component
DNA repair protein XRCC1 and DNA ligase 3	XRCC1	*XRCC1*	BER
	DNA ligase 3	*LIG3*	BER

*Proteins identified in nucleotide excision repair (NER) deficiency disorders or that are structurally required for NER are listed.

Concluding remarks

Our understanding of the NER reaction now includes the process of DNA damage recognition over successive steps by the GG-NER subpathway, the involvement of post-translational modifications, crosstalk between NER and chromatin dynamics and the *in vivo* regulation of NER activity in the repair process. However, despite intense efforts, the complex TCR pathway has so far resisted revealing its mechanistic secrets. Our knowledge of the NER process in the soma in different organs, tissues and during differentiation is also still rudimentary. Nevertheless, insight into the clinical effect of defects in

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both NER subpathways is emerging: defects in GG-NER predispose to cancer and defects in TC-NER predispose to premature ageing; thus, these defects reflect the two sides of the DNA damage coin. The balance between GG-NER and TCR is crucial for protection from cancer as well as from premature ageing. In TCR deficiency this balance is disturbed, and as a consequence cells with a low DNA damage load die from transcriptional stress, which results in both accelerated ageing and strong protection from cancer. In GG-NER deficiency, the still-functional TCR promotes cell survival and delays ageing at the expense of accumulating DNA damage in non-transcribed sequences, including the non-transcribed strand of actively expressed genes. During replication, these lesions will increase mutagenesis, which results in an increased cancer risk. The two NER subpathways show considerable overlap, and mutations that affect both have synergistic effects on clinical outcomes. For example, the process of accelerated ageing can be greatly increased and restrict life expectancy to the infantile stage in both human patients and mouse models, as in COFS.

The lessons learned about NER undoubtedly apply to the other DDR pathways, including DNA-damage signalling and damage tolerance mechanisms. In fact, in a broader context, the other cellular and environmental processes that influence the accumulation of DNA damage — including metabolism and growth controlled by hormones, antioxidant defence systems and environmental exposure — are all relevant to our understanding of cancer and ageing and their multifunctional nature. Only when we are able to integrate the contribution of the entire DDR and the other processes mentioned above will we be able to grasp the full influence of DNA damage on health.

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Competing interests
The authors declare no competing interests.

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