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Transcription-Coupled Nucleotide Excision Repair and the Transcriptional Response to UV-Induced DNA Damage

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Abstract

Ultraviolet (UV) irradiation and other genotoxic stresses induce bulky DNA lesions, which threaten genome stability and cell viability. Cells have evolved two main repair pathways to remove such lesions: global genome nucleotide excision repair (GG-NER) and transcription-coupled nucleotide excision repair (TC-NER). The modes by which these subpathways recognize DNA lesions are distinct, but they converge onto the same downstream steps for DNA repair. Here, we first summarize the current understanding of these repair mechanisms, specifically focusing on the roles of stalled RNA polymerase II, Cockayne syndrome protein B (CSB), CSA and UV-stimulated scaffold protein A (UVSSA) in TC-NER. We also discuss the intriguing role of protein ubiquitylation in this process. Additionally, we highlight key aspects of the effect of UV irradiation on transcription and describe the role of signaling cascades in orchestrating this response. Finally, we describe the pathogenic mechanisms underlying xeroderma pigmentosum and Cockayne syndrome, the two main diseases linked to mutations in NER factors.

Contents

1. INTRODUCTION

While DNA is a relatively stable molecule and therefore an excellent carrier of genetic information, it is constantly subject to modification by endogenous and environmental mutagens. In fact, a vulnerability of DNA resides in the chemical structure of the nitrogenated bases: The electrons in the conjugated double bonds are excited by ultraviolet (UV) irradiation, turning the bases into reactive species that can form dimers with their neighboring nucleotides, thus making DNA damage as ancient as DNA. Reactive oxygen species, alkylating agents, metabolites and toxins, reactive metal compounds like cisplatin, gamma irradiation, and even mechanical stress may also induce changes in DNA. There are multiple DNA repair pathways in eukaryotes that remove these different types of damage, but in this review, we focus on the cellular response to bulky DNA lesions, such as those generated by UV irradiation. We summarize the key findings with a historical perspective, provide the current picture of mammalian nucleotide excision repair (NER)

NUCLEOTIDE EXCISION REPAIR IN BACTERIA: NEW PARADIGM-SHIFTING EVIDENCE

The basic NER reaction also occurs in bacteria, through an analogous pathway. The classical view is that GG-NER in bacteria starts with damage detection by the UvrA protein, which in turn recruits the helicase UvrB. UvrB confirms and demarcates the lesion and recruits UvrC, which performs the 5′ and 3′ incisions. Finally, the UvrD helicase is recruited to remove the damaged DNA fragment and allow resynthesis and ligation (reviewed in 189). TC-NER, elicited by RNA polymerase (RNAP)-mediated lesion detection and RNAP removal by the transcription-repair coupling factor (TRCF, also known as Mfd), was traditionally thought to make only a minor contribution to repair. A recent report, however, challenges this view: UvrA and UvrD seem to interact more persistently with RNAP, and their recruitment to DNA lesions is due to RNAP getting stalled, and thus dependent on transcription. Consistent with this finding, transcription inhibition generally impairs NER, implying that RNAP is the main sensor for DNA lesions in bacteria and that virtually all repair is transcription coupled (190).

and the transcription-related response to UV irradiation, and discuss the key unanswered research questions remaining in this exciting field of research.

The beginning of our understanding of DNA repair can be traced back to the first half of the twentieth century and the pioneering findings by Dulbecco (1) and Kelner (2) who independently found that, after exposure to DNA-damaging UV irradiation, bacteria growing on plates would recover more efficiently if also exposed to a source of white light. Such photoreactivation was later shown to be performed by photolyases, which are present across living beings but absent in some domains, such as placental mammals. Subsequent research showed that, in addition to photoreactivation, bacteria also possess a light-independent repair pathway, i.e., NER, in which short fragments of DNA harboring lesions are excised and replaced by newly synthesized DNA (3) (see also the sidebar titled Nucleotide Excision Repair in Bacteria: New Paradigm-Shifting Evidence).

Later, Rasmussen & Painter (4) provided evidence that NER also occurs in human cells. When HeLa cells were UV irradiated and cultured in the presence of modified nucleosides, these were incorporated into the genome independently of replication, i.e., by means of unscheduled DNA synthesis (UDS). Some years later, Cleaver (5) showed that cells derived from individuals with the rare autosomal recessive disease xeroderma pigmentosum (XP) have impaired UDS after UV irradiation, thus suggesting that specific human genes are responsible for NER. Complementation experiments, in which cells from different XP patients were fused and assayed for UDS, showed that some combinations led to a recovery of UDS, suggesting the existence of multiple, distinct NER genes (6). In 1984, Westerveld and colleagues (7) reported the cloning of the first human NER gene, termed excision repair cross-complementation group 1 (*ERCC1*), by transfecting NER-defective Chinese hamster ovary (CHO) cells with a library of human genes and selecting the cells that became resistant to DNA damage. This approach was subsequently used to clone the genes for several other NER factors, often found to be homologs of the *RAD* genes identified in the yeast *Saccharomyces cerevisiae* (8). Then, in 1988, cell-free NER was reconstituted in vitro for the first time by Richard Wood et al. (9), who also showed that NER activity could be recovered in repair-defective patient cell extracts by combining extracts from different NER complementation groups. Later, Wood's group (10) reconstituted NER with recombinant proteins and factors purified from human cells, thus defining the minimal set of factors necessary for the process (11). Similar results were obtained by Sancar and colleagues (12).

A higher rate of NER in actively transcribed regions compared to the rest of the genome in mammalian cells was first shown by the Hanawalt laboratory (13). They observed that in CHO cells, the removal of UV lesions in the active *DHFR* gene is several times faster than in the genome

Xeroderma pigmentosum (XP): autosomal recessive disease associated with mutations in various general NER genes

NER complementation group: a group of patients deficient in NER whose cells fail to rescue NER activity when fused together

overall. Further research showed that faster repair is restricted to the transcribed strand of active genes (14). This process is now termed transcription-coupled NER (TC-NER). These findings, combined with evidence that cells derived from Cockayne syndrome (CS) patients are UV sensitive and have impaired recovery of RNA synthesis despite being proficient in general excision repair [now termed global genome NER (GG-NER)], suggested that CS cells might have defects in TC-NER (15). This was confirmed by the observation that NER of the transcribed strand of an active gene is specifically impaired in cells derived from CS patients (16) but is proficient in cells derived from XP group C (XP-C) patients (17).

Altogether, research over the last several decades has allowed the identification of over a dozen factors involved in NER and provided the foundation for the present understanding of how this process occurs in mammalian cells.

2. NUCLEOTIDE EXCISION REPAIR: THE BASIC REACTION

DNA repair by NER is divided into three stages, for which factors are recruited in a stepwise fashion: (*a*) lesion recognition, (*b*) excision of the damaged DNA, and (*c*) replacement of the excised fragment by synthesis of new DNA.

As already outlined in Section 1, there are two independent subpathways of NER for the damage recognition step: (*a*) GG-NER, in which the genome-surveilling factors damage-specific DNA binding protein 2 (DDB2, also known as XPE) and/or XPC detect the DNA lesion, and (*b*) TC-NER, in which the stalling of RNA Polymerase II (RNAPII) at DNA lesions acts as the sensor of DNA damage and leads to recruitment of the TC-NER-specific factors CS protein B (CSB), CSA, and UV-stimulated scaffold protein A (UVSSA). The two subpathways later converge in the recruitment of transcription factor IIH (TFIIH), XP group A–complementing protein (XPA), replication protein A (RPA) and XPG, which results in the opening of the DNA double helix (by TFIIH) and confirmation of the presence of the DNA lesion. XPF–ERCC1 is then recruited, completing the formation of the NER preincision complex. XPF and XPG, with their endonuclease activities, perform the 5′ and 3′ incisions, respectively. The excised DNA fragment, still bound to TFIIH and XPG, is then removed, replaced by new DNA synthesized using the undamaged DNA strand as template, and ligated to the downstream DNA (**Figure 1**).

3. DAMAGE RECOGNITION: GLOBAL GENOME NUCLEOTIDE EXCISION REPAIR VERSUS TRANSCRIPTION-COUPLED NUCLEOTIDE EXCISION REPAIR

3.1. Global Genome Nucleotide Excision Repair

In GG-NER, lesions are recognized by the distortion they induce in the DNA double helix (18). This is achieved by two different protein complexes with affinity for damaged DNA, UV-damaged DNA-binding protein (UV-DDB), formed by DDB1 and DDB2 (XPE) (19), and XPC–RAD23B–Centrin 2 (XPC–RAD23B–CETN2) (20–23) (**Figure 1*a*,*b***). Both complexes have an ∼400-fold preference for binding to UV-damaged DNA over nondamaged DNA, but UV-DDB has considerably greater affinity for DNA than XPC (24). UV irradiation induces two major types of DNA lesions: cyclobutane pyrimidine dimers (CPDs) and pyrimidine–pyrimidone (6–4) photoproducts [(6–4)PPs]. The latter, which are more helix distorting than CPDs, are readily detected by XPC–RAD23B–CETN2, as demonstrated by the relatively efficient removal of (6–4)PPs in XP-E patient cell lines in comparison to XP-C cells. In contrast, CPDs are poorly removed in both XP-E and XP-C cells (25). These differences likely explain why (6–4)PP repair is efficient, while CPD repair is slow.

Transcription-coupled nucleotide excision repair (TC-NER): a subpathway restricted to repair of bulky DNA lesions in the transcribed strand

Cockayne syndrome (CS): severe neurodevelopmental disorder associated with mutations in the genes encoding CSB and CSA

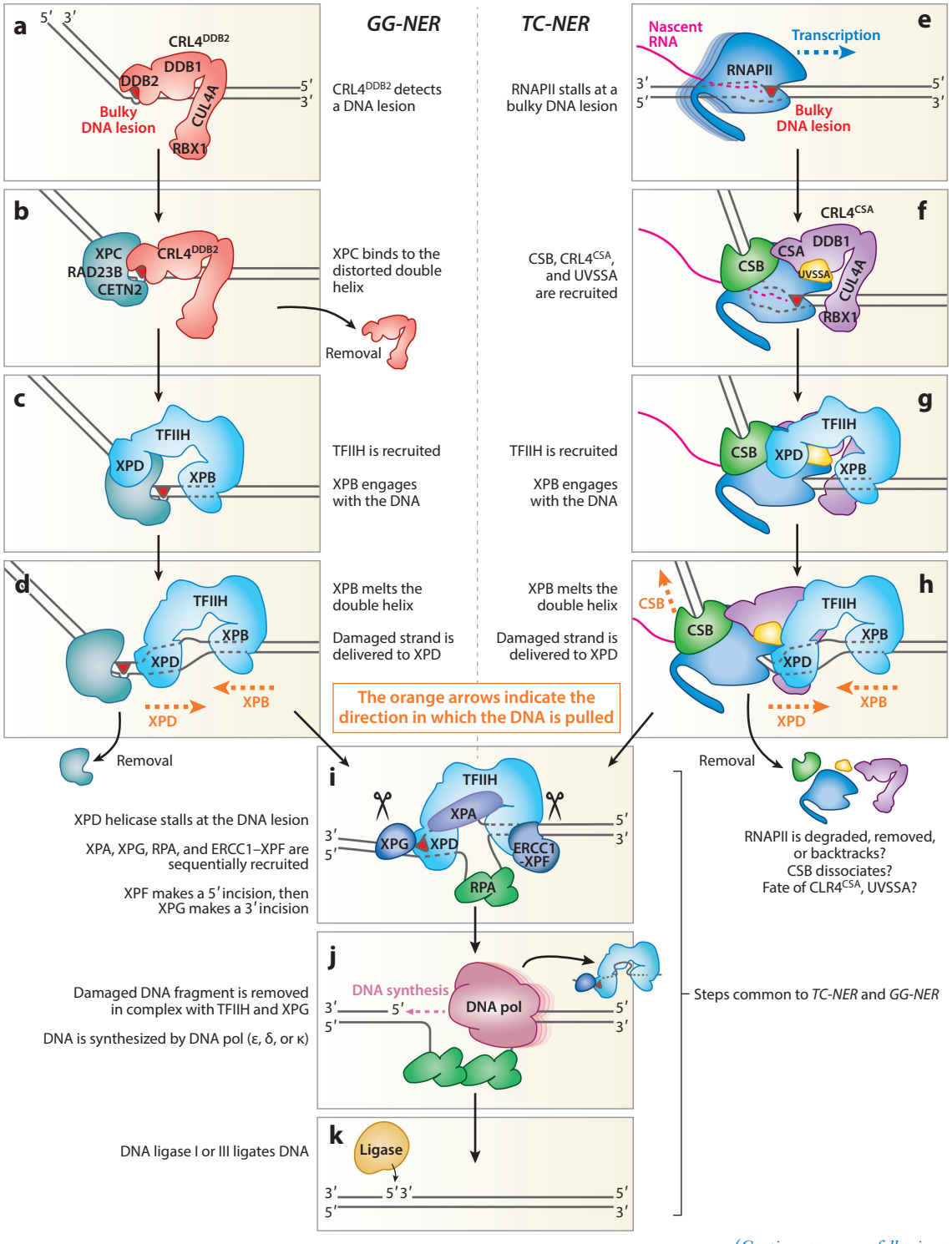
Global genome nucleotide excision repair (GG-NER): a subpathway that repairs bulky DNA lesions genome wide

Damage-specific DNA binding protein 2 (DDB2): a GG-NER-specific DNA damage detection factor, also known as xeroderma pigmentosum group E protein (XPE)

XPC: Xeroderma pigmentosum group C protein, a DNA damage detection factor of the GG-NER subpathway

RNA polymerase II (RNAPII): one of the three human RNA polymerases; RNAPII transcribes all protein-coding and some noncoding genes

CSB: Cockayne syndrome group B protein, has translocase activity and is the first factor recruited to lesion-stalled RNAPII



(Caption appears on following page)

Figure 1 (*Figure appears on preceding page*)

Mechanism of GG-NER (*a–d*) and TC-NER (*e–h*) and the steps shared by both subpathways (*i–k*). (*a*) CRL4^{DDB2} detects a DNA lesion. (*b*) The heterotrimer XPC–RAD23B–CETN2 is recruited and then binds to the undamaged strand (this is also possible for certain lesions in the absence of DDB2). (*c*) TFIIH is recruited via initial interaction with XPC, and the translocase subunit XPB engages with the DNA. (*d*) XPB activity induces repair bubble opening, and the helicase subunit XPD engages with the damaged strand. The mechanism of GG-NER continues in panel *i*. (*e*) Transcribing RNAPII gets stalled at a bulky DNA lesion and cannot continue further. (*f*) CSB, CRL4^{CSA}, and UVSSA are sequentially recruited and, together with RNAPII, form the TC-NER complex. (*g*) TFIIH is recruited via initial interaction with UVSSA, and the translocase subunit XPB engages with the DNA. (*h*) XPB translocation opens the repair bubble and delivers the damaged strand to the helicase subunit XPD active site. From here on, GG-NER and TC-NER converge. (*i*) XPD helicase activity moves away from XPB and toward the lesion; it stops at the lesion. XPA, XPG, RPA, and ERCC1–XPF are recruited sequentially, and XPC–RAD23B–CETN2 (GG-NER) or RNAPII–CSB–CRL4^{CSA}–UVSSA (TC-NER) are removed (see Section 4.2 for details). XPF and XPG perform 5′ and 3′ incisions on the damaged strand. (*j*) The damaged fragment is removed in complex with TFIIH and XPG. DNA polymerase (ε, δ, or κ) fills in the gap left using the undamaged strand as template. (*k*) The nick left by the DNA polymerase is sealed by ligase I or III. Abbreviations: CETN2, centrin 2; CRL4, cullin-RING E3 ubiquitin ligase 4; CSB, Cockayne syndrome protein B; DDB2, damage-specific DNA binding protein 2; ERCC1, excision repair cross-complementation group 1; GG-NER, global genome nucleotide excision repair; RNAPII, RNA polymerase II; RPA, replication protein A; TC-NER, transcription-coupled nucleotide excision repair; TFIIH, transcription factor IIH; UVSSA, UV-stimulated scaffold protein A; XP, xeroderma pigmentosum.

CSA: Cockayne syndrome group A protein, recruited to the RNAPII–CSB complex as the ubiquitin E3 ligase CRL4^{CSA} (CUL4A, RBX1, and DDB1)

UV-stimulated scaffold A protein (UVSSA): a TC-NER-specific factor recruited to RNAPII–CSB–CRL4^{CSA}

Transcription factor IIH (TFIIH): transcription factor involved in both transcription and NER; multi-subunit complex containing XPB and XPD, among others

UV-damaged DNA-binding protein (UV-DDB): dimer formed by DDB1 and DDB2

The structures of XPC–RAD23B (26) and UV-DDB (27, 28) bound to DNA lesions show that upon DNA lesion recognition, both XPC–RAD23B and UV-DDB flip out the damaged dinucleotide and induce an ∼40° kink in the DNA double helix. However, the manner by which each binds is very different: While UV-DDB binds to the damaged dinucleotide and accommodates it in a specific binding pocket (**Figure 1*a***), XPC–RAD23B binds to the undamaged strand, flipping out the damaged base (**Figure 1*b***). XPA, another NER factor, also displays affinity for damaged DNA, but XPC–RAD23B recruitment precedes XPA recruitment (29).

Interestingly, UV-DDB also forms part of a cullin RING E3 ubiquitin ligase (CRL) with CUL4A and RBX1, designated CRL4^{DDB2}. In the absence of DNA damage, CRL4^{DDB2} is mainly in an inactive state in which CUL4A is deneddylated and CRL4^{DDB2} is bound to its allosteric inhibitor, the COP9 signalosome (CSN) (30, 31). Shortly after DNA damage, this complex is activated by the dissociation from CSN and neddylation of CUL4A (27, 31). The structure of CRL4^{DDB2} bound to damaged DNA suggests that the histones surrounding the lesion may be ubiquitylated by CRL4^{DDB2}, which could induce a more open DNA conformation to recruit downstream NER factors. CRL4^{DDB2} also ubiquitylates DDB2 itself (27, 32). In vitro, XPC can be ubiquitylated by CRL4^{DDB2} as well, and this may promote the handover of damaged DNA from CRL4^{DDB2} to XPC (32, 33). XPC is deubiquitylated by the ubiquitin-specific protease USP7 (34). A subsequent, small ubiquitin-like modifier (SUMO)-dependent ubiquitylation of XPC by RNF111 may help promote handover of lesions to downstream NER factors (35), of which TFIIH is the first to be recruited (36, 37) via interaction of the PH domain of its p62 subunit with an acidic string in XPC (38) (**Figure 1*c***). According to recent cryo–electron microscopy (cryo-EM) evidence, the yeast homologs of XPC–RAD23B–CETN2 (i.e., Rad4–Rad23–Rad33) are positioned on the 3′ side of the damaged base, with TFIIH positioned on the 5′ side of the lesion (39). This asymmetry helps specify which DNA strand harbors the lesion.

3.2. Transcription-Coupled Nucleotide Excision Repair

TC-NER is initiated by the stalling of RNAPII at the DNA lesion (**Figure 1*e***) (see also the sidebar titled Are There RNAPI- and RNAPIII-Mediated Transcription-Coupled Nucleotide Excision Repair Pathways?). In the case of CPDs, structures of damage-stalled RNAPII showed that this involves the 5′-thymine of the CPD directing uridine misincorporation, which blocks further translocation (40), while even bulkier DNA lesions, such as cisplatin adducts, block transcription due to their failure to be accommodated in the RNAPII active site (41). Stalled

ARE THERE RNAPI- AND RNAPIII-MEDIATED TRANSCRIPTION-COUPLED NUCLEOTIDE EXCISION REPAIR PATHWAYS?

The critical role played by RNAPII in TC-NER in protein-coding genes begs the question of whether RNAPI and RNAPIII play similar roles. RNAPI transcribes ribosomal DNA (rDNA) in the nucleolus, of which there are multiple copies, whereas RNAPIII transcribes short genes, most notably those encoding transfer RNA (tRNA). Despite their short length, these genes can also accrue DNA damage, which would need to be repaired to allow transcription to continue. If these lesions were repaired via TC-NER, an increased efficiency of lesion repair on the transcribed strand compared to the nontranscribed strand should be detected. Since such a strand bias in repair is not observed for genes encoding rRNA and tRNA (191, 192), it appears unlikely that RNAPI- and RNAPIII-mediated TC-NER exist in mammalian cells. Moreover, the striking disassembly of the nucleolus structure and the inhibition of RNAPI transcription in response to DNA damage, which has been interpreted as RNAPI-mediated TC-NER (193), might just be a consequence of RNAPII transcription inhibition, which has been shown to be essential for maintenance of nucleolus structure and RNAPI transcription (194).

RNAPII recruits the SWI/SNF-like translocase CSB (42) (**Figure 1*f***). While CSB is often thought of as the eukaryotic transcription-repair coupling factor, it also plays a general role in transcription (see also Section 8.1). For example, CSB also enhances transcript elongation in vitro in the absence of DNA damage (43, 44), and fluorescence recovery after photobleaching experiments indicated that it interacts weakly and transiently with RNAPII in untreated cells, and that this interaction is stabilized after DNA damage (45). CSB also allows RNAPII to add an extra base when blocked by DNA lesions (44). More recently, in vitro and structural evidence showed that Rad26, the *S. cerevisiae* homolog of CSB, binds DNA upstream of stalled RNAPII and helps overcome transient stalling at minor obstacles by pulling on the DNA and in effect pushing RNAPII forward. However, when RNAPII progress is blocked by a CPD, Rad26 fails to promote forward translocation past the lesion, and its interaction with the stalled transcription complex is stabilized, inducing an 80° bend in the upstream DNA. Importantly, in such assays, RNAPII backtracks upon stalling, made evident by TFIIS-stimulated transcript cleavage and shortening of the nascent transcript (46). However, if Rad26 or CSB is added to such reactions prior to TFIIS, transcript cleavage is significantly reduced, suggesting stabilization of the stalled RNAPII–CSB complex and inhibition of backtracking (44, 46).

The RNAPII–CSB complex recruits CSA (**Figure 1*f***), which is part of a large family of proteins containing WD-40 repeats that often act as interaction scaffolds for different partners. As such, it was shown early on that CSA interacts with CSB and with a subunit of TFIIH in vitro, thus providing an attractive model to explain the sequential recruitment of TC-NER factors (47). A recent study mapped a 13–amino acid CSA-interaction motif in CSB that is necessary for recruitment of CSA to the RNAPII–CSB complex in vivo (48).

Intriguingly, in this connection, CSA is part of a ubiquitin ligase complex, designated $CRL4^{CSA}$, with CUL4A, RBX1, and DDB1 (31) (**Figure 1*f***) that ubiquitylates CSB (49). Ubiquitylation of RPB1, the major subunit of RNAPII, was found to be decreased in CS-B and CS-A cells (50), giving rise to the idea that $CRL4^{CSA}$ is involved in UV-induced ubiquitylation of RPB1 as well. Such a role for CSA was recently given compelling support by structural characterization of the $CLR4^{CSA}$–RNAPII association (51). Interestingly, however, CSA-dependent poly-ubiquitylation peaks 2 h after damage (52, 53). Consistent with this, $CRL4^{CSA}$ activity is at least partially inhibited in the first few hours after UV irradiation, during which time its interaction with the inhibitory CSN transiently increases, promoting CUL4A deneddylation and thus inactivation. Indeed, association of CSA with the neddylated, active form of CUL4A is not detected at the early time

$CRL4^{DDB2}$: E3 ubiquitin ligase complex formed by CUL4A, RBX1, DDB1, DDB2, and an E2 ubiquitin ligase

Neddylation: a ubiquitin-like posttranslational protein modification characterized by the addition of mono- or poly-NEDD8 chains on lysine residues

Ubiquitylation: posttranslational protein modification, characterized by the addition of mono- or poly-ubiquitin chains on lysine residues

$CRL4^{CSA}$: E3 ubiquitin ligase complex formed by CUL4A, RBX1, DDB1, CSA, and an E2 ubiquitin ligase

RPB1: major and catalytic subunit of RNA polymerase II, subject to ubiquitylation after DNA damage

points after DNA damage (31) when RPB1 ubiquitylation is otherwise at its peak (50, 52, 54). The multi-faceted process of RPB1 ubiquitylation after DNA damage is further described in Section 5.

UVSSA, a TC-NER factor identified only 10 years ago, interacts with CSA as well as USP7, even in the absence of DNA damage. These proteins are likely recruited together to the RNAPII–CSB complex (55–57) (**Figure 1*f***). This is mediated by CSA interacting with CSB, while UVSSA in turn mediates the interaction with, and recruitment of, TFIIH (58) (**Figure 1*g***). UVSSA–USP7 appear to prevent UV-induced CSB poly-ubiquitylation and degradation (56, 57) and might potentially counteract RPB1 ubiquitylation as well.

Two important interaction regions were mapped in UVSSA: a CSA-interacting region that is essential for recruiting UVSSA to RNAPII–CSB–CRL4^{CSA} and a TFIIH-interacting region that allows tethering of TFIIH to the RNAPII–CSB–CRL4^{CSA}–UVSSA complex (48). The interactions described appear to be cooperative, so that if one or more interactions are disrupted, the rest are diminished or even abolished. It is worth noting that general NER factors recruited downstream of TFIIH have not been detected in mass spectrometry analysis of, for example, immunoprecipitated CSB, RNAPII, or UVSSA (48, 59), opening the possibility that neither RNAPII, nor the initial assembly around it, remain at the lesion when the downstream general NER factors are recruited (**Figure 2**) (see further details in Section 4.2).

Immunoprecipitation of CSB specifically after DNA damage coisolates not only RNAPII but also UVSSA, CRL4^{CSA}, and a plethora of positive elongation factors including DSIF (SPT4 and SPT5) (59), indicating that CSB is recruited to lesion-stalled elongation complexes. However, cryo-EM studies of the presumed initial TC-NER complex indicate that stable binding of the transcript elongation factor DSIF and CSB to RNAPII are mutually exclusive (51) and thus suggests that CSB binding promotes a transition from an elongation complex to the TC-NER complex. When CRL4^{CSA} and UVSSA were included in the structural analysis, CSA was found to be positioned over RNAPII, bridging the contact between CSB and UVSSA, the latter of which was positioned close to the downstream DNA, altogether enclosing RNAPII (**Figure 1*f***). The authors also modeled an E2 enzyme–ubiquitin complex binding to the RBX1 subunit of CRL4^{CSA}, with the flexibility of the CUL4A subunit allowing ubiquitylation of CSB, as well as of RPB1 at the key ubiquitylation site at lysine 1268 (K1268) (51). These structures help explain how TFIIH is loaded onto the DNA in front of RNAPII: Like in GG-NER, TFIIH associates with the 5′ side of the lesion, and this asymmetry helps specify the damaged DNA strand.

A new, potential TC-NER factor, STK19, was first identified by multi-omic screening and shown to affect the UV response (59). STK19 was also later identified, along with ELOF1, as a putative TC-NER factor in CRISPR screens for sensitivity to a variety of DNA-damaging compounds (60). While little has been reported about the role of STK19, ELOF1 was first identified in yeast as a factor that interacts directly with RNAPII and promotes transcript elongation (61). Recent reports showed that in the absence of ELOF1, there is reduced UVSSA recruitment to the TC-NER complex, RPB1 ubiquitylation is reduced, and TC-NER is delayed, suggesting an intriguing accessory role for ELOF1 in TC-NER (62, 63). Besides STK19 and ELOF1, the precise role of the XAB2 complex in TC-NER, splicing, and transcription still needs to be resolved (64, 65).

4. CONVERGENCE BETWEEN GLOBAL GENOME NUCLEOTIDE EXCISION REPAIR AND TRANSCRIPTION-COUPLED NUCLEOTIDE EXCISION REPAIR DOWNSTREAM OF DAMAGE RECOGNITION

Although the GG-NER and TC-NER subpathways converge in later stages, the parallels between the pathways even soon after DNA lesion recognition are so striking that their descriptions are

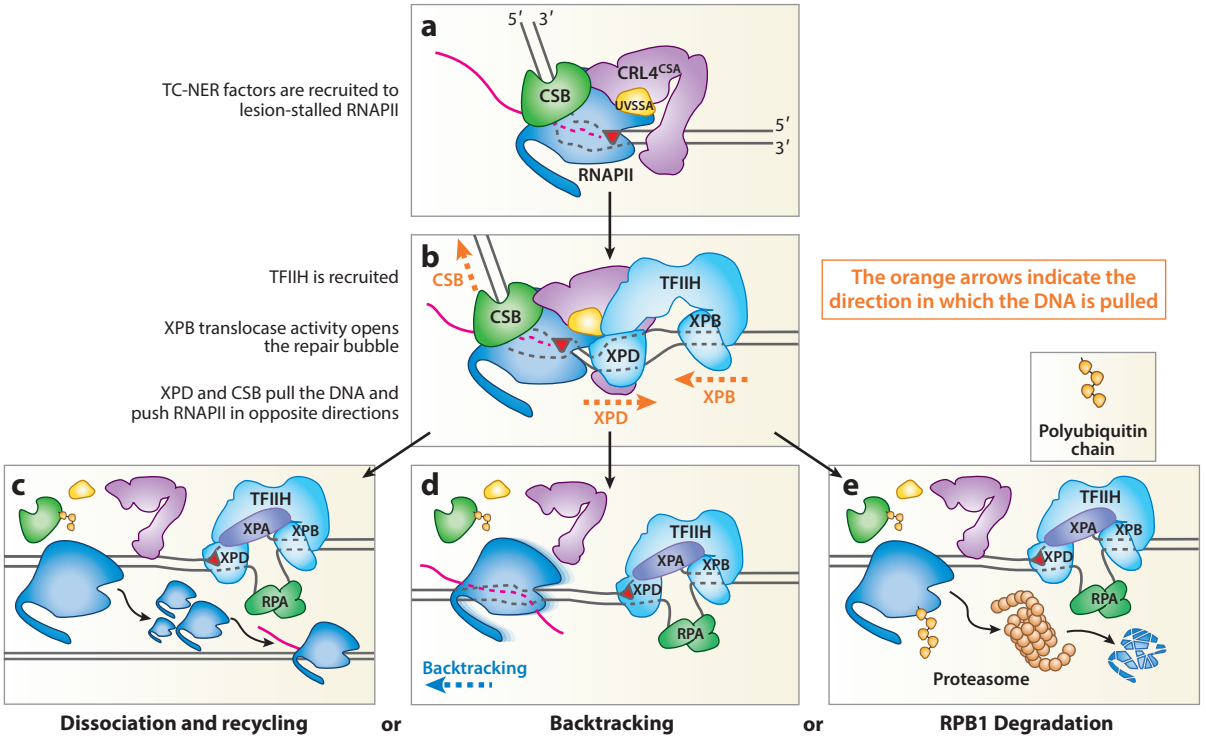


Figure 2

Models for RNAPII removal to allow DNA damage repair. (*a*) RNAPII stalls at a bulky DNA lesion and the TC-NER factors are sequentially recruited. (*b*) Next, TFIIH is recruited and (*c–e*) three alternative outcomes for RNAPII and TC-NER factors are envisioned. In these models, CSB (and sometimes RPB1) is ubiquitylated, and this facilitates destabilization of the TC-NER complex (see Section 5 for details). (*c*) RNAPII is removed from the DNA lesion and recycled, (*d*) RNAPII backtracks a considerable distance and resumes transcription once the DNA damage is repaired, or (*e*) RNAPII is poly-ubiquitylated and degraded by the proteasome, perhaps only when the actions described in panels *c* or *d* are not possible. Abbreviations: CSB, Cockayne syndrome protein B; RNAPII, RNA polymerase II; TC-NER, transcription-coupled nucleotide excision repair; TFIIH, transcription factor IIH.

provided together, as this allows a better side-by-side comparison and support for the idea that they evolved together and share basic characteristics.

4.1. The Dual Role of TFIIH in Transcription and Repair

Besides being essential for NER, the multi-subunit TFIIH complex is a general RNAPII transcription factor that forms part of the preinitiation complex, where it provides two important enzymatic activities: (*a*) translocase activity (XPB), which mediates promoter melting, and (*b*) kinase activity (CDK7), which phosphorylates the carboxy terminal domain (CTD; 52 heptapeptide repeats in mammals with consensus sequence YSPTSPS) of RPB1 at serine 5 residues to signal transcription initiation (66). *XPB* was already known to be mutated in some XP patients and thus to be required for NER, so its identification as a subunit of TFIIH revealed an important and unexpectedly direct connection between transcription and NER (67). Moreover, another NER factor, XPD, which has 5′→3′ helicase activity, is also a TFIIH subunit; its catalytic activity is required only for NER (66). In both yeast and humans, TFIIH exists in two forms: a transcriptionally active holoTFIIH form, which includes the CTD kinase subcomplex (CDK7, Cyclin H, and MAT1), and a core form involved in NER, which does not include this complex (68, 69). In

fact, the presence of the kinase complex in TFIIH inhibits XPD activity, which is essential for NER (70), and XPA binding to the NER complex releases this inhibitory kinase module from TFIIH (68).

4.2. Preparing for DNA Lesion Excision: Sequential Recruitment of the Key Players

When TFIIH is recruited to XPC during GG-NER, it is positioned at the 5′ side of the DNA lesion through interaction of its p62 subunit with an acidic string in XPC (38). In TC-NER, TFIIH is positioned on the DNA in the same fashion, through interaction of p62 with an acidic string in UVSSA as part of the RNAPII–CSB–CRL4^{CSA}–UVSSA complex (48, 71). This orientation places XPB on the DNA to the 5′ side of the lesion (**Figure 1*c*** and ***g***). XPA and XPG can then be recruited to TFIIH (36), and the TFIIH kinase subcomplex is evicted (68).

The structure of the yeast homologs of XPC–RAD23B–CETN2 bound to TFIIH and DNA shows that at the early stage of GG-NER, XPB (Ssl2 in yeast) in TFIIH is engaged with double-stranded DNA while XPC (Rad4 in yeast) is placed over the lesion (**Figure 1*c***) (39). The orientation of XPB is such that if it were not tethered to XPC, TFIIH might simply move away from the lesion. Instead, XPC acts as an anchor so that when XPB translocates, it actually pulls DNA toward itself and induces unwinding of the DNA between these two factors. This in turn allows delivery of the damaged single-stranded DNA (ssDNA) strand to XPD's active site (**Figure 1*d***), analogously to what happens in transcription, where the anchors allowing unwinding and delivery to RNAPII for transcription initiation are the TATA-binding protein (TBP) and other general transcription factors (39). Recent structures of mammalian TFIIH bound to XPA reveal how XPA derepresses XPD by competing with and displacing CAK from TFIIH and inducing rearrangements in TFIIH. Crosslink–mass spectrometry evidence suggested that XPG binding displaces XPC. In addition, a beta-hairpin of XPA is inserted in the edge of the open DNA bubble, likely demarcating its 5′-end and clamping TFIIH to the DNA (72). Furthermore, XPA and XPG binding stimulate the catalytic activities of XPB and XPD (**Figure 1*i***) (72, 73).

As described in the previous paragraph, substantial molecular insight into the action of TFIIH in the GG-NER complex with XPC has been achieved. By contrast, it remains unclear what happens at the equivalent stages in TC-NER. It is obvious that the general mechanism may be shared, with the RNAPII–CSB–CRL4^{CSA}–UVSSA complex acting analogously to XPC, anchoring TFIIH and allowing XPB to unwind DNA with the resulting delivery of the lesion to XPD (**Figure 1*b***). Once XPD is engaged with the damaged strand, two opposing forces would then be acting on RNAPII: CSB translocase activity pushing it forward and XPD helicase activity in effect pushing it backward (**Figure 1*b***). It is easy to imagine how this might destabilize the RNAPII on DNA. Moreover, we note that in GG-NER, disruption of the interaction between TFIIH and XPC is triggered by recruitment of XPA and XPG (39) and helps to evict XPC. Similar to the interaction between XPC and TFIIH, the initial interaction of TFIIH with UVSSA is mediated by the PH domain of the TFIIH p62 subunit and an acidic string in UVSSA (38, 71). It seems likely that XPA, XPG, and ubiquitylation play a similar role to disrupt the TC-NER complex, leading to eviction of RNAPII and its TC-NER-specific cofactors prior to nucleotide excision. The possible mechanisms underlying this event are described in more detail in Section 5 (see also **Figure 2**).

Once TFIIH has been remodeled and activated by interaction with XPA and XPG, the XPD helicase opens the repair bubble by moving toward the lesion (**Figure 1*i***) (73). XPD stalls when encountering the modified base and thus functions as a means of lesion verification (74). In turn, the ssDNA-binding protein RPA binds to the undamaged complementary strand and helps position XPA (75). Together, XPA and RPA stabilize the open repair bubble, position XPG 3′ for incision, and stimulate XPG and XPF incision at later stages of the NER process (**Figure 1*i***) (76).

4.3. Excision of the Damaged DNA and Resynthesis

The last factor to be recruited to the preincision complex is XPF–ERCC1 (36). XPG and XPF–ERCC1 are structure-specific endonucleases that cleave on the 3′ and 5′ sides of an open DNA bubble, respectively (77, 78). The order in which the incisions occur was elegantly determined in vivo using catalytically inactive point mutations of XPF and XPG (79). This work revealed that XPF needs to cut on the 5′ side of the damage before XPG cuts on the 3′ side (**Figure 1*i***). Importantly, the cleavage order (first XPF, then XPG) is the opposite of the recruitment order (first XPG, then XPF); this likely helps ensure that the whole NER complex is correctly assembled before any incision in DNA can take place. This work also showed that after XPF cleavage, but prior to XPG cleavage, the DNA synthesis factor PCNA is recruited. Moreover, partial DNA synthesis was observed in cells bearing an inactive XPG point mutation, suggesting that a segment of new DNA may sometimes already be synthesized prior to XPG-mediated cleavage (79). An alternative interpretation was proposed based on the observation that three different DNA polymerases affect repair DNA synthesis in NER (80): Gap filling can be performed by DNA polymerase ε, recruited to the repair site by PCNA and CTF18–RFC, while in scenarios where there is increased steric hindrance, DNA polymerase δ, or alternatively the error-prone translesion-synthesis DNA polymerase κ, is recruited by RFC1 or ubiquitylated PCNA, respectively (**Figure 1*j***). It is somewhat surprising that translesion-synthesis DNA polymerases are involved in NER in this way, as their action in replication has typically been seen as an alternative to removing the lesion by NER. A likely explanation might be their ability to work at low dNTP concentrations, which allows them to also support efficient synthesis outside of S phase (80). Finally, the newly synthesized DNA fragment is ligated to the downstream DNA by either DNA ligase I or III (**Figure 1*k***) (81).

Translesion-synthesis DNA polymerase: polymerase capable of using damaged DNA as a template for replication

After excision, the damaged ssDNA fragment is dislodged from chromatin in complex with TFIIH and XPG (82, 83). This feature has been used as a starting point for measuring NER activity genome wide (84). A recent report identified the helicase-like transcription factor (HLTF) as a new player in NER and provided evidence that it is recruited to the XPF incision site only after XPG incision, where it aids in the release of the excised, TFIIH-complexed damage fragment and facilitates synthesis of a new DNA fragment (85). While these data appear to contradict previous evidence that DNA synthesis can start prior to XPG incision (79), they might simply reflect that the process requires additional factors in certain chromatin environments or repair site conformations, once again emphasizing the complexity of NER.

Altogether, the concerted and multistep fashion by which the NER machinery is assembled, including an initial damage-recognition step followed by damage verification, positioning of endonucleases, and coordinated cleavage and DNA resynthesis, allows precise and efficient removal of DNA lesions from the genome (**Figure 1**).

5. THE FATE OF RNAPII AND CSB DURING TRANSCRIPTION-COUPLED NUCLEOTIDE EXCISION REPAIR: THE INTRIGUING ROLE OF PROTEIN UBIQUITYLATION

As argued in Section 4.2, it seems a distinct possibility that the TC-NER-specific factors are displaced from sites of DNA damage prior to, or alongside, assembly of the incision-competent core NER complex. Importantly, such displacement is likely brought about by a combination of mechanisms, an important one of which is ubiquitylation.

RPB1 is poly-ubiquitylated and degraded in response to problems during transcript elongation. Importantly, while this was first detected in response to UV irradiation (50), it also occurs in response to a range of other challenging transcription conditions; it is a general transcription stress response, likely triggered by prolonged RNAPII stalling or arrest. It is often termed the last resort pathway (86, 87).

The residue on RPB1 that is poly-ubiquitylated was recently identified to be K1268, which is positioned in an unstructured loop protruding from the surface of RPB1 close to the DNA entry tunnel (53, 88). Mutation of this residue to arginine (K1268R) abrogates damage-induced RPB1 ubiquitylation and degradation (53) and compromises cell viability after UV irradiation (53, 88). While recruitment of CSB, $CRL4^{CSA}$, and UVSSA is not affected by K1268R mutation, recruitment of the downstream NER factor TFIIH to RNAPII is reduced (88). The mutant cells remain somewhat TC-NER proficient, as seen by RNA synthesis recovery assays (53), even though DNA damage–dependent strand-specific RNAPII chromatin immunoprecipitation sequencing experiments show a clear delay in TC-NER (88). It seems likely that while RPB1 ubiquitylation may not be an absolute requirement for all instances of TC-NER, it affects the overall efficiency (62, 63), akin to the recently observed partial contribution of HLTF to NER (85).

An E3 ligase for RNAPII ubiquitylation was first discovered in yeast, in which Rsp5 function is required for ubiquitylation of RPB1 after DNA damage (89). Later, its human E3 ortholog, NEDD4, was found to be involved in RPB1 ubiquitylation in human cells (52). Concurrently, a requirement for Elc1, Ela1 (yeast homologs of the human Elongin proteins), and Cul3 was established (90, 91), and the counterpart, Elongin–CUL5 ($CRL5^{Elongin}$), was found to play a similar role in human cells (92). The conundrum of which E3 ligase is responsible for RPB1 ubiquitylation in yeast was resolved when it was shown that it occurs in two steps, requiring first Rsp5 (or NEDD4 in humans) for RPB1 mono-ubiquitylation and then the Elongin proteins (Cul3–Elongin/$CRL5^{Elongin}$) for poly-ubiquitylation (93). Prior to this, a requirement for yeast Def1 in the ubiquitylation reaction had already been established (94). Def1 was identified through its interaction with Rad26 (yeast CSB). Intriguingly, in the absence of Def1, RPB1 is no longer poly-ubiquitylated and degraded in response to UV irradiation, while it becomes degradable again if Rad26 is missing as well, pointing to a role for Rad26 (CSB) in stabilization of RPB1 (94). Further work showed that an arrested RNAPII elongation complex is the preferred substrate for Def1 (95) and that Def1's function is to recruit the Elongin–Cul3 complex to RNAPII for RPB1 poly-ubiquitylation (96). Recent data show that UBAP2 and UBAP2L are the human orthologs of Def1; they play a role in RPB1 ubiquitylation after DNA damage, although RPB1 ubiquitylation is not completely abolished in their absence (97).

In agreement with this observation, RPB1 K1268 appears to be the target of ubiquitylation by numerous different Cullin E3 ligases. Indeed, only use of the general Cullin inhibitor MLN4924 (Pevonedistat, a neddylation inhibitor) or mutation of RPB1 K1268 is sufficient to more or less completely abrogate RPB1 poly-ubiquitylation. Knockdown or knockout of RPB1 E3 ligases, such as $CRL4^{CSA}$, $CRL5^{Elongin}$, and $CRL2^{VHL}$, has only a partial effect on ubiquitylation levels after UV irradiation (52, 53, 88, 92, 98).

As already alluded to, the connection between RPB1 ubiquitylation and TC-NER has remained a matter of debate. Based on results from yeast, which showed that TC-NER is unaffected by an inability to perform RPB1 ubiquitylation (94, 99), it was suggested that damage-induced RPB1 ubiquitylation/degradation represents an alternative to TC-NER. It would act as a last resort pathway that is activated if a lesion-blocking RNAPII cannot be repaired by TC-NER, for example, to allow damage removal by other, slower repair pathways such as GG-NER (86, 87, 94). Indeed, RPB1 degradation happens more rapidly in cells lacking Rad26 (94). This simple model may have to be modified for mammalian cells. For example, human cells have CSA and UVSSA, which are not found in yeast. Moreover, recent results show that stable recruitment of the $CRL5^{Elongin}$ E3 ligase to sites of DNA damage is actually CSB dependent (100) and that blocking RPB1 ubiquitylation by mutation of K1268 affects the rate of TC-NER (88), together indicating a positive functional relationship between RPB1 ubiquitylation and TC-NER in human cells. Two main scenarios seem likely, which are not necessarily mutually exclusive. In the first, ubiquitylation

FACTORS INDUCING TRANSCRIPTION TERMINATION

Transcription termination factor 2 (TTF2) is an ATP-dependent translocase of the SWI2/SNF2 family, which can induce dissociation of RNAPII from elongation complexes. Its function in cells is particularly clear when cells enter mitosis, where it ensures that chromosomes are free of transcribing RNAPII while being condensed and segregated (199), but it can also dissociate RNAPII stalled at a DNA lesion in vitro (101).

Integrator is an RNAPII-associated complex with subunit homology to the cleavage and polyadenylation specificity factor (CPSF) required for transcript cleavage during transcriptional termination. Integrator has been shown to dissociate and recycle RNAPII paused at the promoter-proximal pause sites (102).

Senataxin is a superfamily 1 helicase, involved in certain forms of transcriptional termination (200). In vitro, the yeast homolog of Senataxin (Sen1) can dissociate RNAPII from an elongation complex (201).

These factors might also play roles in the proposed dissociation of damage-stalled RNAPII during TC-NER (**Figure 2*c***).

of RPB1 at K1268 is recognized as a signal (by an unknown TC-NER factor) to aid, for example, in the recruitment of TFIIH. Thus, when K1268 cannot be ubiquitylated, TFIIH recruitment is less efficient and TC-NER is slower (88). Alternatively, RPB1 poly-ubiquitylation serves as one of several mechanisms employed by cells to remove RNAPII from sites of DNA damage so that the downstream, general NER factors can be recruited to perform the actual excision reaction. In this model, removal of RNAPII, and with it perhaps CSB, $CRL4^{CSA}$, and UVSSA, can occur via ubiquitylation and degradation of RPB1 (**Figure 2*e***). Other pathways to achieve the same removal would coexist: CSB in cooperation with TFIIH, as well as proteins such as TTF2, senataxin, and the Integrator complex, may well be capable of, and contribute to, dissociation of damage-stalled RNAPII as well, with less dramatic consequences than RPB1 proteolysis (**Figure 2*c***) (see the sidebar titled Factors Inducing Transcription Termination) (101, 102). Indeed, even though RPB1 is not ubiquitylated and degraded in RPB1 K1268R cells, RNAPII at DNA lesions is rapidly turned over (53). The idea that RNAPII is dissociated from sites of DNA damage was further supported by experiments in which RNAPII initiation was inhibited, released for a short time window, and inhibited again, resulting in a single discrete wave of RNAPII transcription elongation. This setup, combined with genome-wide sequencing, supported the idea that when RNAPII runs into DNA damage, it dissociates from the chromatin (103). As mentioned previously, the activity of $CRL4^{CSA}$ is somewhat restricted in the first few hours after DNA damage by interaction with the CSN (31). This might help ensure that damage-stalled RNAPII is not degraded by $CRL4^{CSA}$ as the first plan of action, but only if the damage-stalled polymerase is caught in a situation that does not allow dissociation by nondegradative means. This might in turn explain why K1268R mutation merely results in a delay of TC-NER: Only a subset of damage-stalled RNAPII complexes require ubiquitylation for TFIIH recruitment and subsequent RNAPII removal.

It is important to stress that, although it can modify RPB1, the main role of $CRL4^{CSA}$ is likely to be CSB ubiquitylation (49). The precise role of CSB ubiquitylation remains to be shown, but it is likely that ubiquitylation plays analogous roles in the eviction of XPC (GG-NER) and CSB (TC-NER). Indeed, $CRL4^{DDB2}$ ubiquitylates XPC, while $CRL4^{CSA}$ ubiquitylates CSB. Moreover, XPC and CSB are both deubiquitylated by USP7 (34, 56, 58) and regulated by SUMOylation (35, 104, 105). As nature often reuses successful mechanisms, it seems a strong possibility that ubiquitylation is required for the displacement of CSB (**Figure 2*c–e***). Just like XPC ubiquitylation by RNF111 brings about XPC eviction (35) (see Section 3.1), CSB ubiquitylation might help evict CSB from TC-NER complexes. This would happen upon recruitment of downstream NER

SUMOylation: posttranslational protein modification, characterized by addition of mono- or poly-SUMO chains on lysine residues

EVOLUTIONARY REWIRING OF THE NUCLEOTIDE EXCISION REPAIR PROCESS

Model organisms show how the function of NER factors has evolved. In *Saccharomyces cerevisiae*, the XPC homolog Rad4 is required for incision in both GG-NER and TC-NER. By contrast, yeast cells lacking Rad7 or Rad16 (which have no mammalian homologs) have impaired GG-NER but normal TC-NER (195). Also, while the CSB homolog Rad26 contributes to TC-NER, yeast still has some TC-NER without Rad26, mediated by a pathway dependent on the nonessential RNAPII subunit Rpb9 (158). Interestingly, in the absence of Spt4/Spt5 function, Rad26 is not required at all, pointing to an important role for Rad26 in transforming the elongation complex into an NER complex.

In *Drosophila melanogaster*, although some GG-NER factors are missing, the NER process is largely conserved. By contrast, CSA, CSB, or UVSSA homologs have not been found in fruit flies and other insects, suggesting that they lack TC-NER (196). Nevertheless, repair of CPDs in the transcribed strand is more efficient than in the nontranscribed strand in fruit flies, suggesting that they do have transcription-coupled repair of DNA damage (197). This seems to be dependent on XPC, because when XPC is knocked out, no NER excision products are detected at all (198).

factors, like XPC removal depends on XPA and XPG recruitment (39). In support of this, interactome experiments show that while CSB clearly interacts with the RNAPII elongation complex, $CRL4^{CSA}$, UVSSA, and TFIIH after UV-induced DNA damage, NER factors such as XPA, XPG, and XPF invariably fail to be detected in such CSB immunopurifications (59). Moreover, as CSB activity keeps RNAPII pushed up to the lesion (44, 46), eviction of CSB would potentially allow a third, alternative RNAPII fate during repair: Besides degradation (**Figure 2*e***) or dissociation (**Figure 2*c***) of RNAPII, the polymerase might sometimes backtrack and then resume transcription once the DNA damage is repaired (**Figure 2*d***).

Intriguingly, CSB contains a ubiquitin-binding domain (UBD). Without it, CSB can still be recruited to DNA damage and assemble a TC-NER complex, but DNA incision does not take place (106). Like ubiquitylation, the precise molecular role of CSB's UBD remains to be shown, but it must recognize a ubiquitin moiety, most likely added by $CRL4^{CSA}$, either on CSB itself or on another protein such as RPB1. Tellingly, CSB is normally dynamically associated with DNA damage sites, but CSB lacking the UBD is slow to dissociate from such lesions (106). Together, these data support a working model in which $CRL4^{CSA}$-mediated ubiquitylation, and recognition thereof by CSB's UBD, helps to displace CSB so that NER can take place. Intriguingly, in the complex formed by XPC and RAD23B, a UBD is found in the RAD23B partner protein, yet again underscoring the remarkable analogy between GG-NER and TC-NER in this aspect of their mechanism (107). Whether RAD23B's UBD is required for XPC displacement remains to be investigated. We propose that such analogous mechanisms might have allowed the repurposing of several NER factors through evolution (see also the sidebar titled Evolutionary Rewiring of the Nucleotide Excision Repair Process).

6. SIGNALING CASCADES REGULATING GENE INDUCTION IN RESPONSE TO UV

UV irradiation triggers a number of cellular response mechanisms, which involve activation of DNA damage response pathways, cell cycle arrest, and apoptosis. Many of these are regulated at the level of gene expression, i.e., through effects on transcription, splicing, and mRNA translation. The signaling cascades that regulate gene activation in response to UV are conserved among eukaryotes and can be divided into two distinct pathways: a cytoplasmic and a nuclear pathway

(108, 109). These pathways function in parallel and are both needed to yield a full UV response (**Figure 3**).

6.1. Membrane-Associated Receptor Signaling Cascade

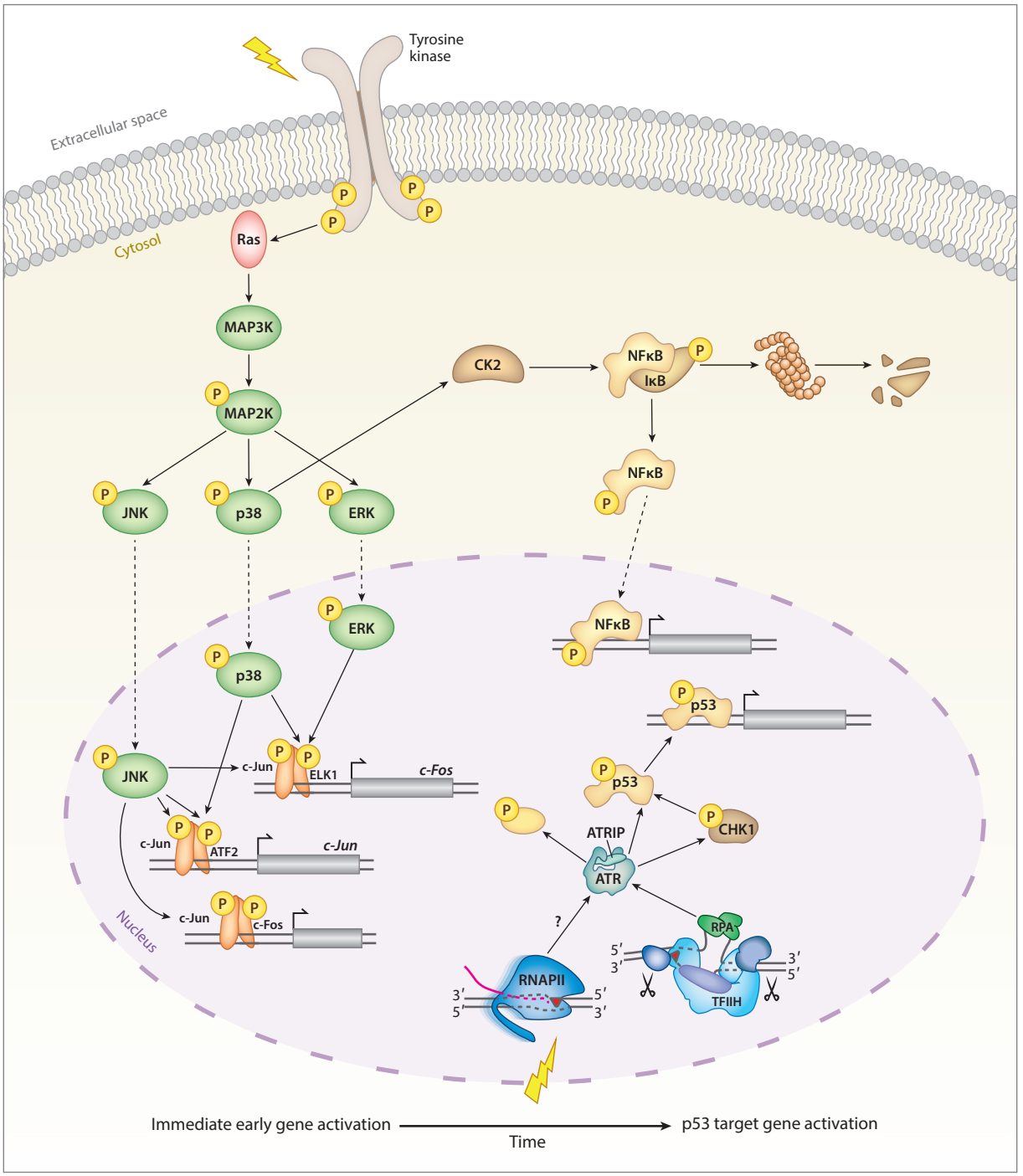
Within 15 min of UV irradiation, the expression of several genes, such as immediate-early genes *JUN* (c-Jun) and *FOS* (c-Fos), is drastically induced (110, 111). Activation of these genes is important for the UV response, as demonstrated by the fact that $FOS^{-/-}$ and $JUN^{-/-}$ cells are hypersensitive to UV irradiation (112). UV-responsive elements were identified in the promoters of the affected genes (110, 111). This in turn led to the identification of several transcription factors that are activated in response to UV irradiation, such as NFκB and Elk-1, or the heterodimers collectively referred to as activator protein 1 (AP-1), which consist of combinations of members of the JUN, FOS, and/or ATF protein families (**Figure 3**) (113, 114). These transcription factors also bind constitutively to the UV-responsive sequence elements in the absence of UV irradiation (115, 116), meaning that it is not the binding to promoters but rather posttranslational modification of the bound transcription factors that activates transcription of UV-responsive genes.

UV-induced phosphorylation of the transcription factors AP-1 and Elk-1 is rapid, occurring within 5 to 10 min, and usually occurs at serine and threonine residues followed by prolines (117, 118). The phosphorylation kinetics and patterns of different UV-activated transcription factors are similar because they are the result of a shared signaling cascade that is initiated by membrane-associated receptors (**Figure 3**). This starts off with the activation of the epidermal growth factor receptor (EGFR) and membrane-associated receptor tyrosine kinases in response to UV irradiation (117, 119–121). These receptors in turn activate Ras, which activates the Erk1–2, JNK/SAPK, and p38 mitogen-activated protein kinase (MAPK) pathways that each phosphorylate their respective substrates (**Figure 3**) (113, 117, 122). Together, these transcription factors contribute to the rapid activation of immediate-early genes, which then orchestrate the cellular response to DNA damage. Given that several immediate-early genes encode transcription factors themselves, one might expect that their respective target genes are also rapidly induced upon UV irradiation. However, some target genes such as collagenase are upregulated during a much later phase of the UV response (8–12 h). The mechanism responsible for this delay is unclear, but it has been suggested to be mediated by an extracellular signaling loop involving growth factors and interleukins (119, 121).

6.2. DNA Damage–Induced Signaling Cascade

While UV irradiation can directly activate cell surface receptors, kinases, and transcription factors to induce immediate-early gene expression, as described in Section 6.1 (123), the predominant molecule that absorbs photon energy is DNA. As such, DNA damage itself is a critical instigator of signaling cascades that activate genes. Cells deficient in CSA and CSB require a much lower UV dose to induce such gene expression than wild-type (WT) cells (124). Since these cells cannot rapidly remove UV-induced lesions from transcribed genes, but have normal GG-NER activity, this led to the idea that transcription-blocking lesions or stalled RNAPII are the starting point of this signaling cascade. In line with this, XPC-deficient cells, which have normal TC-NER, showed normal UV dose-dependence for gene activation (124). The idea that RNAPII might act as a sensor for detecting lesions in transcribed genes was further supported by the fact that inhibition of transcript elongation by RNAPII through microinjection of phospho-CTD-specific antibodies activated a similar signaling cascade even in the absence of UV (125).

It has also been suggested that RNAPII stalling in front of bulky DNA lesions and the formation of R-loops lead to the prolonged exposure of RPA-coated ssDNA around the polymerase, which could also induce a nuclear signaling cascade (**Figure 3**) (126, 127). Of course, ssDNA is



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

UV-induced signaling pathways to activate gene expression. UV irradiation activates membrane-associated tyrosine kinase receptors, which leads to the activation of the MAPK signaling pathway and phosphorylation of transcriptional activators at promoters. These proteins act to upregulate immediate early genes. UV irradiation also induces DNA damage, which leads to the activation of ATR and phosphorylation of its substrates. Ultimately, this leads to the expression of p53-target genes. Abbreviations: ATR, ataxia telangiectasia and Rad3-related protein; MAPK, mitogen-activated protein kinase; UV, ultraviolet.

also generated as an intermediate in the NER pathway (i.e., UDS), as well as during DNA replication, and thus might also be the consequence of stalled replication forks. Somewhat surprisingly, (6–4)PPs but not CPDs have recently been shown to impede replication of DNA sufficiently to activate this signaling cascade (128). After coating of the ssDNA, RPA recruits the ataxia telangiectasia and Rad3-related protein (ATR)–ATR-interacting protein dimer (109), which, upon its activation, phosphorylates hundreds of substrates (**Figure 3**) (129, 130). This includes CHK1, whose phosphorylation controls the G2/M checkpoint of the cell cycle (131). Additionally, CHK1 phosphorylates the sequence-specific transcription factor p53 at serine 20, which in turn leads to its activation (132–135). p53 can also be phosphorylated directly by ATR at serine 15, further enhancing its transactivating activity (**Figure 3**). Additionally, ATR phosphorylates MDM2, resulting in the degradation of this p53-inhibitor, thereby stabilizing p53 (136). Together, such mechanisms increase the amount of active p53 in the cell after UV irradiation, leading to activation of its target genes (**Figure 3**). The importance of inducing these genes during the UV response is underscored by the fact that $p53^{-/-}$ cells display increased UV sensitivity and are deficient in repairing UV-induced DNA lesions. The latter might be explained by the fact that p53 regulates the expression of several key DNA damage repair genes, such as *XPC*, *DDB2* (XPE), and *PCNA* (25). In addition, p53 orchestrates the cellular response to UV damage by inducing the expression of both proapoptotic and prosurvival genes (137), whose functions normally offset each other. However, most proapoptotic genes are short and therefore have a relatively low chance of attaining transcription-blocking DNA damage. In contrast, most p53-induced prosurvival genes are longer, thereby increasing the chance of having DNA lesions that inhibit their transcription. Consequently, high doses of UV and/or deficient NER may tilt the balance toward a proapoptosis signal, resulting in cell death. Indeed, while p53 phosphorylation and expression of its target genes normally decrease within 2–8 h of UV exposure, in NER-deficient cells, high levels of p53 phosphorylation have been observed up to 72 h after UV irradiation (138). The tight connection between DNA damage repair and p53 function also underscores the importance of choosing the right cell line for studying certain DNA damage response mechanisms, as several commonly used cell lines do not have functional p53.

7. THE TRANSCRIPTIONAL RESPONSE TO DNA DAMAGE

While UV irradiation activates a relatively small group of genes, it otherwise generally results in robust repression of transcription across the genome: a dramatic transcription shutdown. Not surprisingly, transcript elongation is inhibited by the presence of DNA lesions. However, inhibition of transcription after UV irradiation is not limited to the area of a gene containing DNA lesions. For example, it was found that nuclear extracts from UV-irradiated cells have reduced transcription capacity in vitro, even of an undamaged DNA template (139). Moreover, undamaged DNA transfected into UV-irradiated cells is transcribed at reduced rates (140). These findings suggest that there is a wider signaling cascade taking place in cells to generally shut down transcription while still allowing activation of the damage-inducible genes.

Measurements of nascent transcription after UV irradiation revealed that the level and kinetics of the transcriptional shutdown are UV-dose-dependent (141) but generally follow the

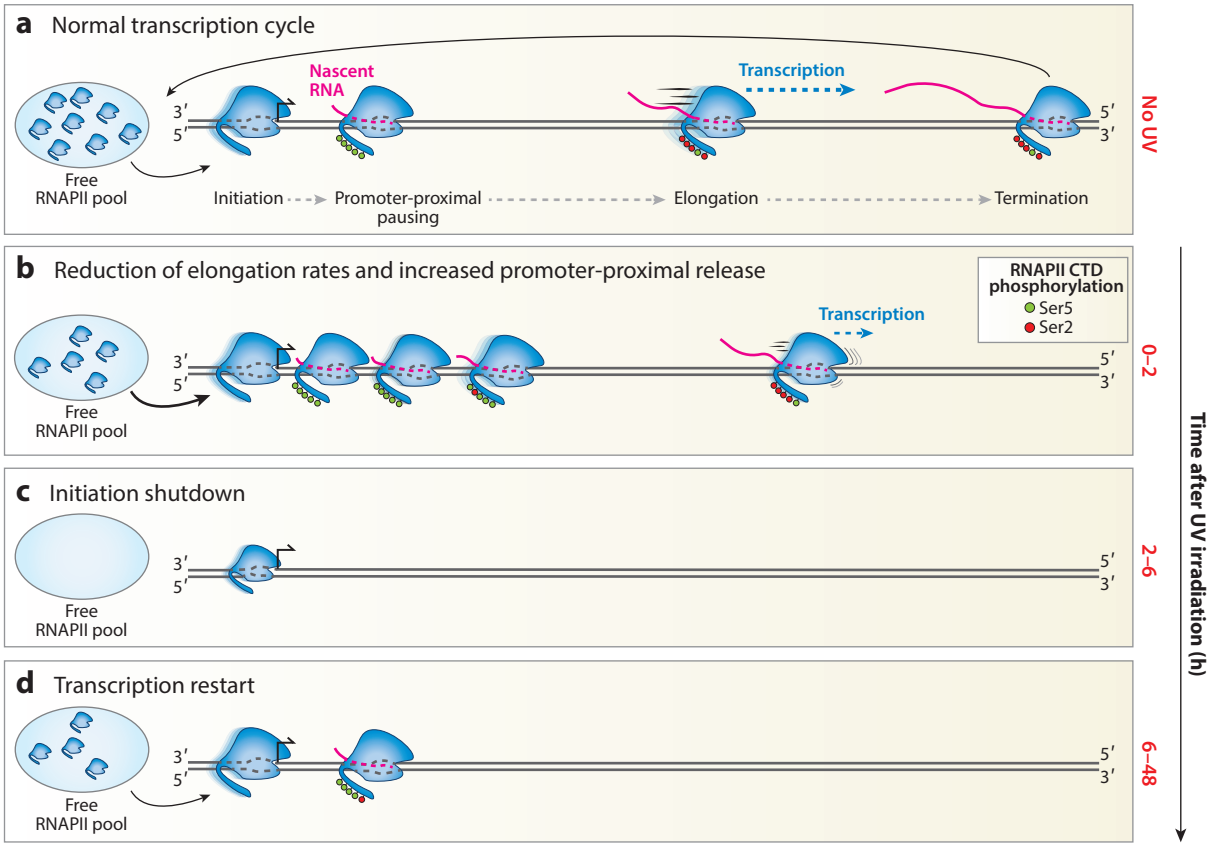


Figure 4

Model for the genome-wide transcriptional response to UV irradiation. (*a*) In the absence of UV irradiation, unphosphorylated RNAPII is recruited from the free pool of RNAPII to the promoters of genes, phosphorylated for elongation, and dephosphorylated and recycled after transcription termination. (*b*) Immediately after UV irradiation, transcription elongation rates decrease, and an increased release of promoter-proximal paused RNAPII into the gene body can be observed. (*c*) RNAPII stalls at lesions. Because lesion-stalled RNAPII is often degraded, the pool of free RNAPII is depleted, and transcription initiation is drastically reduced after a few hours. (*d*) After DNA damage has been repaired and the RNAPII pool has been restored, transcription is restarted from the promoter.

same trend of inhibition within an hour and recovery to baseline after approximately 18–24 h. In NER-deficient patient cells, RNA synthesis recovery rates are much lower (141). More recently, genome-wide studies of nascent transcription have provided temporal and single-gene resolution for the effects of UV irradiation. During the early phase (the first hour or so after UV exposure), transcript elongation rapidly decreases genome wide, except for short genes (53, 142, 143). In addition, while transcription initiation is relatively unaffected, there is increased release of RNAPII from promoter-proximal areas into gene bodies (143–145). After several hours, transcription initiation is also inhibited, resulting in a general transcription shutdown (**Figure 4**) (53, 142, 143).

7.1. Slowing Down of Transcript Elongation

The initial inhibition of transcript elongation is not just the consequence of RNAPII stalling at DNA lesions but is also due to a genome-wide reduction in elongation rates (**Figure 4*b***)

(143, 146). This can also be seen from the fact that mRNA splicing patterns change globally upon UV irradiation in a manner consistent with altered elongation rates (146). The exact mechanism underlying this phenomenon is currently unknown but might be related to altered activity of elongation factors such as PAF1, ELOF1, and FACT (51, 62, 63, 147, 148).

7.2. Promoter-Proximal Release of RNAPII

Normally, RNAPII pauses briefly in the promoter-proximal region (60–150 bp downstream of the transcription start site) before it engages in processive transcript elongation, due to the inhibitory action of proteins such as DSIF and NELF (149). Interestingly, upon UV irradiation, an increase in promoter-proximal pause release is observed (**Figure 4*b***) (143–145). Such release is generally regulated by phosphorylation of DSIF, NELF, and the serine 2 residues of the RNAPII CTD by the CDK9 subunit of p-TEFb kinase (149), which results in the displacement of NELF and release of RNAPII into the gene body. These same proteins play a role in the increased clearance of RNAPII from the promoter-proximal region after UV irradiation. The active form of p-TEFb is usually sequestered by the 7SK snRNP, rendering it inactive (150). UV irradiation results in the activation of p38, which stimulates the promoter-proximal release in two ways (151, 152). On one hand, it stimulates the binding of RBM7 with 7SK, leading to the release of the active p-TEFb and increased phosphorylation of promoter-proximally paused RNAPII (150, 152). On the other hand, active p38 phosphorylates RNA-binding proteins such as NELF (151). This has been shown to promote the recruitment of 14-3-3 and dissociation of NELF from chromatin, thereby counteracting its inhibitory role in pause release (151). Finally, the PAF1 complex has also been shown to play a role in the increased promoter-proximal release of RNAPII after UV irradiation (148). Increased release from promoter-proximal pausing into genes has been proposed to enable cells to promote uniform and accelerated surveillance of the whole transcribed genome and to increase TC-NER upon UV irradiation (144).

7.3. Inhibition of Transcription Initiation

Finally, several hours after the induction of DNA damage, transcription initiation is inhibited genome wide (**Figure 4*c***). Over the years, different models have been proposed to explain this phenomenon, including sequestration of TBP at the site of DNA damage and UV-induced transcriptional downregulation by the general transcription repressor ATF3 (153–155). While multiple different mechanisms likely contribute to UV-induced repression, recent findings point to a critical role for RNAPII abundance (53). It has long been known that free, unphosphorylated RNAPII major subunit RPB1 rapidly disappears upon UV irradiation, followed by a decrease in the hyperphosphorylated RNAPII form, which is engaged in transcript elongation (139, 156). This is not due to more rapid degradation of the unphosphorylated RNAPII form but rather to its gradual conversion to the hyperphosphorylated form under conditions where only relatively few polymerases manage to finish transcription of a gene and get recycled to replenish this pool of free, initiation-competent, unphosphorylated RNAPII (**Figure 4**) (53). In fact, in the first hours after UV irradiation, polymerases initiate transcription normally (and become hyperphosphorylated), but they then reach a DNA lesion, where they are often poly-ubiquitylated at RPB1 K1268 and degraded. Indeed, unless DNA lesions are efficiently removed by TC-NER, this polymerase degradation cycle continues and eventually leads to depletion of free, unphosphorylated RNAPII. Without free polymerase, no new transcription can initiate (**Figure 4*c***). Importantly, in RPB1 K1268R cells, transcription initiation does not become repressed after UV irradiation (53); this is because RPB1 in these cells is not degraded after UV irradiation but is instead recycled from sites of DNA damage by unknown mechanisms (see Section 5). Interestingly, a recent study suggests

that RPB1 ubiquitylation by an unknown E3 ligase and subsequent degradation may occur not only at DNA lesions but also when RNAPII is at the transcription pause site (157). This might represent an example of signaling in trans from DNA damage (or damaged membranes) to E3 ligases regulating RPB1 ubiquitylation.

While the data described support the idea that ubiquitylation of RPB1 triggers downregulation of transcription via proteasome-mediated degradation of RPB1, it cannot be ruled out that ubiquitin-mediated signaling also plays a role independently of RPB1 degradation. Indeed, RPB1 is poly-ubiquitylated not only with proteasome-targeting K48-linked ubiquitin chains but also with K63 chains (88), which are typically responsible for signaling. Nevertheless, the transcriptional response to UV irradiation, including an intriguing and persistent upregulation of short genes in UV-irradiated K1268R mutant cells compared to WT cells, was faithfully modeled in silico simply by introducing into the computational model a requirement that WT cells deplete their RPB1 levels after UV irradiation, while K1268R cells do not (53).

7.4. Transcription Restart

Once transcription-blocking, UV-induced damage is repaired, transcription resumes. Genome-wide analysis of UV-irradiated cells has revealed that transcription recovery occurs as a wave in the 5′→3′ direction, suggesting that much of the transcription restart occurs from the promoters of genes (**Figure 4*d***) (142). Indeed, later analysis of genome-wide repair following synchronized release of RNAPII from promoter-proximal regions resulted in the conclusion that RNAPII molecules are likely removed from the DNA template when they encounter a lesion (103), further suggesting that restart does not occur from the sites of DNA repair. Moreover, even in the absence of RPB1 degradation (in K1268R cells), polymerases do not pile up at sites of DNA damage (53). These new developments in the field are important: They question the previously ingrained idea that RNAPII remains on the template during repair; i.e., it merely backtracks to allow TC-NER (**Figure 2*d***) but stays engaged and then restarts its journey after the lesion has been removed. In this new model, the vast majority of restart occurs from the promoter of the affected gene by newly recruited RNAPII (**Figure 2*c***) rather than from the lesion site by the previously stalled RNAPII.

Regardless of the precise definition of restart, a failure to restart transcription after UV-irradiation is a hallmark of cells deficient in TC-NER (141). It has always been thought that the failure to recover transcription in these cells is caused by a defect in TC-NER. However, recent data suggest that another reason for the lack of transcription recovery in UV-irradiated CSB-deficient cells may in fact be long-lasting depletion of RPB1 levels. Indeed, expression of the nondegradable form of RNAPII (RPB1 K1268R) in CSB knockout cells was enough to rescue the defect of these cells in transcription restart (53). This might explain why, at timepoints when WT fibroblasts have recovered transcription, CSB patients' cells fail to transcribe, even when the DNA template is undamaged (139, 140). This finding implies not only that RNAPII levels play a role in determining the level of restart but also that DNA damage in genes cannot itself be the reason for lack of transcription restart after UV irradiation in CSB-deficient cells. Interestingly, the results with the CSB-deficient K1268R cells also open the possibility that DNA damage removal in genes, which must occur to allow transcription of long genes to recover, might come about at least partly via the mammalian equivalent of the still poorly understood Rad26-independent pathway in yeast, which requires the Rpb9 subunit of RNAPII (53, 158).

A number of additional factors have been shown to affect transcription restart after UV irradiation. One such example is the elongation factor ELL. Depletion of ELL, shown to be an interactor of the TFIIH complex, does not affect repair of damaged DNA, but strongly affects transcription recovery (159). Mechanistic insight into the role of ELL in transcription restart

remains to be reported. Like ELL, the H3.3 histone chaperone HIRA was also found to be necessary for genome-wide transcription recovery after UV irradiation (160, 161). Initially, it was suggested that the deposition of H3.3 marks on damaged DNA by HIRA might act as a signal for other factors to restart transcription once repair is finished (160). However, it has more recently been shown that the function of HIRA in transcription restart is independent of H3.3 deposition and may instead be linked to its role in stabilizing the associated factor UBN2 (161). Several other chromatin remodelers, such as FACT, DOT1L, and SIRT, have also been reported to play a role in transcription recovery (162–164). The exact mechanisms by which they facilitate transcription restart remain unclear. Indeed, whether such factors are required, directly or indirectly, for resetting chromatin structure specifically around repair sites, or in general for allowing pioneer transcription after general transcription shutdown, is unclear.

7.5. UV-Induced Changes in Alternative Splicing

Given the drastic effect of UV-induced DNA damage on transcription, it is not surprising that cotranscriptional processes are also affected. Best characterized is the effect of UV irradiation on alternative mRNA splicing, which in turn has an impact on gene expression and cellular signaling (146). Most notably, UV irradiation affects whether an exon is included or excluded from a mature mRNA, as reflected by increased levels of exon inclusion/skipping and usage of alternative last exons (127, 143, 146). These changes in alternative splicing can have marked consequences for the function of the affected genes. For example, the predominant isoform of Bcl-x is Bcl-xL, but UV irradiation or cisplatin treatment results in a change in its alternative splicing pattern, so that the isoform Bcl-xS is expressed at high levels (146, 165). Importantly, while Bcl-xL promotes cell survival, the shorter Bcl-xS isoform has proapoptotic functions. UV-induced alternative splicing changes can thus result in the differential expression of isoforms with antagonistic functions. Another key example of this is *ASCC3*. ASCC3 is a component of the activating signal cointegrator 1 (ASC1) complex and its protein-coding mRNA isoform functions to somehow repress transcription after UV irradiation. However, UV-induced alternative splicing changes result in the expression of a long noncoding RNA transcript with an alternative last exon, which is essential for transcription restart (143). By modulating the expression of these isoforms with opposite functions, the cell can regulate the transcriptional and cellular response to UV irradiation.

Two kinds of evidence indicate that UV-induced regulation of alternative splicing occurs in trans and not merely because of transcription-blocking damage in the template DNA. First, UV-induced alternative splicing changes also occur in a plasmid-encoded reporter minigene if it is transfected into cells after they are UV irradiated (146). Strikingly, if nonirradiated cells are cotransfected with an untreated alternative splicing reporter minigene and a plasmid DNA that is UV irradiated in vitro, this leads to changes in alternative splicing of the untreated reporter similar to those observed in response to UV irradiation of cells (166). The mechanisms by which UV irradiation induces such splicing changes remain poorly understood. UV-induced alternative splicing has been observed in response to both a reduction in transcript elongation rates (the kinetic coupling model) and changes in the recruitment of spliceosome components and other splicing factors (the recruitment coupling model). The rate of transcript elongation has long been known to influence the decision to include or exclude an exon (167). It is therefore likely that many of the UV-induced alternative splicing changes are a consequence of the reduced elongation rates observed upon UV exposure (146). In addition, components of the activated spliceosome have been shown to be depleted from chromatin after UV irradiation, resulting in the formation of R-loops (127). Transcription rates and spliceosome displacement seem to be dependent on ATR and ATM respectively, though the mechanisms by which they are affected remain unclear

(127, 166). Finally, a minority of UV-induced alternative splicing changes likely stem from alterations in the phosphorylation status and/or subcellular localization of splicing factors such as hnRNPA1, EWS, SRSF10, and hSLU7 (165, 168–170). Given that many of these alternative splicing factors have cell-type enriched expression patterns, their effect on UV-induced alternative splicing might be restricted to specific cell types. Indeed, UV irradiation has been shown to regulate alternative splicing differently in fibroblasts and keratinocytes (166).

8. DISEASES ASSOCIATED WITH MUTATIONS IN NUCLEOTIDE EXCISION REPAIR GENES

Given that DNA damage repair is critical for maintaining genome integrity, it is not surprising that pathogenic variants in genes involved in the NER pathway have been associated with human diseases. This family of autosomal recessive diseases includes XP, CS, cerebro-oculo-facio-skeletal syndrome (COFS), trichothiodystrophy (TTD), and UV-sensitive syndrome (UVSS) (171–175). These are all genetically and clinically heterogeneous, thereby compounding the identification of clear genotype–phenotype relationships. While observed only in a subset of COFS, TTD, and CS patients, photosensitivity, (i.e., an exaggerated response to sunlight exposure, often resulting in skin burns and blisters) is a hallmark phenotype of individuals with XP and UVSS (175). Regardless, cultured fibroblasts of all individuals with NER diseases are sensitive to UV irradiation (171–175). Surprisingly, only XP patients have an increased risk of developing cancer of skin and mucous membranes. The 1,000-fold increased risk of skin cancer observed in patients with mutations in *XPA–XPG* is thus not observed in CS patients carrying mutations in the TC-NER genes *ERCC8* (CSA) and *ERCC6* (CSB) (176), suggesting that elevated risk is the consequence of impaired GG-NER but not TC-NER, i.e., that the more rapid DNA repair enabled by TC-NER is not required to prevent UV-induced, cancer-driving mutations.

8.1. Cockayne Syndrome Is a Transcription Disease

Historically, CS was considered a DNA repair disease, as it correlates with mutations in the genes encoding the TC-NER components CSA and CSB. Hallmark phenotypes of CS include a developmental delay, progressive growth failure, and microcephaly. Additionally, patients frequently present with additional symptoms such as retinal degeneration, cataracts, or sensorineural hearing loss (174). Evidence has been accumulating that the much more severe symptoms observed in CS patients are in fact the result of transcription defects (140, 164, 177, 178). Importantly, while the general NER proteins are absolutely required for TC-NER as well, their mutation does not result in the severe symptoms seen in CS patients but rather in the milder symptoms associated with XP. In itself, this argues that although CS is likely to be caused by problems arising from transcription-blocking lesions, it cannot be the actual removal of these lesions that is problematic; TC-NER in XP-A, XP-F, and XP-G patients is as defective as it is in CS-A and CS-B patients. Moreover, while the neurological abnormalities observed in some XP patients appear to stem from neurodegeneration, in CS they are more likely the result of compromised neuronal development (179) and hypomyelination of neurons (180). Of course, neurons are not exposed to UV irradiation. Thus, transcription-blocking lesions likely stem from endogenous sources of DNA damage, such as oxidative stress (see the sidebar titled Nucleotide Excision Repair Factors and Repair of Oxidative Damage). Importantly, endogenous formaldehyde was recently found to impede transcription, with marked physiological consequences. Indeed, cells deficient in both formaldehyde clearance and CSB function [*ADH5/ERCC6* (CSB) double knockout] show hypersensitivity to formaldehyde and evidence of transcription stress, including decreases in the RPB1 pool upon formaldehyde treatment. Importantly, $Adh5^{-/-}/Csb^{m/m}$ mice develop cachexia and neurological

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Oxidative lesions are assumed to be mostly repaired by glycosylases of the base excision repair (BER) pathway. However, some oxidative lesions such as cyclopurines do not have specialized glycosylases and are repaired by NER. More interestingly, NER factors generally contribute to BER (reviewed in 202). The most abundant oxidative lesion, 8-oxoguanine, is cleaved by the OGG1 glycosylase. The repair intermediate is a single strand break that is further processed by downstream BER factors such as APE1, XRCC1, Pol β, and LigIII. This intermediate blocks transcription, and CSB, UVSSA, and XPA are involved in its repair (203). Furthermore, transcription and CSB promote enhanced recruitment of downstream BER factors, thus pointing to the existence of a TC-BER pathway (204).

defects and succumb to kidney failure, all characteristic of CS (181). This study provides evidence that CSB protects organs such as the kidney and the brain against DNA damage and transcription stress caused by endogenous formaldehyde.

The first indications that CS is a transcription syndrome can be found in the early discovery that CS cells have reduced transcription even in the absence of UV irradiation (43, 182). Purified TFIIH from XP/CS cells also cannot support transcription to the normal level in vitro (183). Moreover, cell culture experiments, animal models, and postmortem tissue from CS patients have revealed that loss of function of CSB results in global gene expression changes even in the absence of DNA damage, resulting in upregulation of inflammatory genes and downregulation of neuronal genes (177, 178, 184, 185). Indeed, CSB-deficient cells cannot be differentiated into neurons, nor can fibroblasts from CS patients be reprogrammed to neurons (177, 178, 186, 187), suggesting that CSB is required for the development and maintenance of neuronal identity. Recent data suggest that the altered transcription programs in CS patients might at least partly be due to destabilization of RPB1, resulting in a relative depletion of the RNAPII pool (53). In this context, it is interesting to note that pathogenic variants in *POLR2A*, the gene that encodes RPB1, also result in neurodevelopmental defects (188).

SUMMARY POINTS

1. Bulky DNA lesions are detected by two distinct repair subpathways, global genome nucleotide excision repair (GG-NER) and transcription-coupled nucleotide excision repair (TC-NER), which converge in downstream repair steps.
2. RNA polymerase II (RNAPII) stalling promotes rapid repair of lesions in the transcribed strand.
3. Ubiquitylation of GG-NER and TC-NER factors increases the efficiency of DNA repair.
4. UV-induced gene activation is mediated by membrane-associated and DNA damage–induced signaling cascades.
5. UV irradiation results in a global transcriptional response that includes a reduction of transcription elongation rates and a genome-wide transcription shutdown.
6. RPB1 degradation plays a central role in the transcription shutdown after UV irradiation.
7. Neurodevelopmental phenotypes in Cockayne syndrome are the result of transcription dysregulation.

FUTURE ISSUES

1. What determines the fate of RNAPII during TC-NER?
2. What is the precise role of RPB1 ubiquitylation in TC-NER? Do mono-ubiquitin or K63-linked chains also play a role?
3. What is the role of CSB ubiquitylation? Can this be analogous to the role of XPC ubiquitylation in GG-NER?
4. How is transcription restart regulated? Which factors are required?
5. How is ATR-mediated gene induction regulated through in trans signaling from damage-stalled RNAPII?
6. What is the role of poorly understood factors such as STK19 in TC-NER?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Dulbecco R. 1949. Reactivation of ultra-violet-inactivated bacteriophage by visible light. *Nature* 163(4155):949–50
2. Kelner A. 1949. Effect of visible light on the recovery of *Streptomyces griseus* conidia from ultra-violet irradiation injury. *PNAS* 35(2):73–79
3. Friedberg EC. 2008. A brief history of the DNA repair field. *Cell Res.* 18(1):3–7
4. Rasmussen RE, Painter RB. 1964. Evidence for repair of ultra-violet damaged deoxyribonucleic acid in cultured mammalian cells. *Nature* 203(4952):1360–62
5. Cleaver JE. 1968. Defective repair replication of DNA in xeroderma pigmentosum. *Nature* 218(5142):652–56
6. De Weerd-Kastelein EA, Keijzer W, Bootsma D. 1972. Genetic heterogeneity of xeroderma pigmentosum demonstrated by somatic cell hybridization. *Nat. New Biol.* 238(81):80–83
7. Westerveld A, Hoeijmakers JHJ, van Duin M, de Wit J, Odijk H, et al. 1984. Molecular cloning of a human DNA repair gene. *Nature* 310(5976):425–29
8. Bootsma D, Hoeijmakers J. 1994. The molecular basis of nucleotide excision repair syndromes. *Mutation Res./Fundam. Mol. Mechanisms Mutagenesis* 307(1):15–23
9. Wood RD, Robins P, Lindahl T. 1988. Complementation of the xeroderma pigmentosum DNA repair defect in cell-free extracts. *Cell* 53(1):97–106
10. Aboussekhra A, Biggerstaff M, Shivji MKK, Vilpo JA, Moncollin V, et al. 1995. Mammalian DNA nucleotide excision repair reconstituted with purified protein components. *Cell* 80(6):859–68

11. Araújo SJ, Tirode F, Coin F, Pospiech H, Syväoja JE, et al. 2000. Nucleotide excision repair of DNA with recombinant human proteins: definition of the minimal set of factors, active forms of TFIIH, and modulation by CAK. *Genes Dev*. 14(3):349–59
12. Mu D, Park C-H, Matsunaga T, Hsu DS, Reardon JT, Sancar A. 1995. Reconstitution of human DNA repair excision nuclease in a highly defined system. *J. Biol. Chem.* 270(6):2415–18
13. Bohr VA, Smith CA, Okumoto DS, Hanawalt PC. 1985. DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. *Cell* 40(2):359–69
14. Mellon I. 1987. Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. *Cell* 51(2):241–49
15. Mayne LV, Lehmann AR. 1982. Failure of RNA synthesis to recover after UV irradiation: an early defect in cells from individuals with Cockayne's syndrome and xeroderma pigmentosum. *Cancer Res.* 42(4):1473–78
16. Venema J, Mullenders LH, Natarajan AT, van Zeeland AA, Mayne LV. 1990. The genetic defect in Cockayne syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA. *PNAS* 87(12):4707–11
17. Venema J, van Hoffen A, Natarajan AT, van Zeeland AA, Mullenders LHF. 1990. The residual repair capacity of xeroderma pigmentosum complementation group C fibroblasts is highly specific for transcriptionally active DNA. *Nucl. Acids Res.* 18(3):443–48
18. Gunz D, Hess MT, Naegeli H. 1996. Recognition of DNA adducts by human nucleotide excision repair. *J. Biol. Chem.* 271(41):25089–98
19. Keeney S, Chang GJ, Linn S. 1993. Characterization of a human DNA damage binding protein implicated in xeroderma pigmentosum E. *J. Biol. Chem.* 268(28):21293–300
20. Araki M, Masutani C, Takemura M, Uchida A, Sugasawa K, et al. 2001. Centrosome protein centrin 2/caltractin 1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair. *J. Biol. Chem.* 276(22):18665–72
21. Legerski R, Peterson C. 1992. Expression cloning of a human DNA repair gene involved in xeroderma pigmentosum group C. *Nature*. 359(6390):70–73
22. Masutani C, Sugasawa K, Yanagisawa J, Sonoyama T, Ui M, et al. 1994. Purification and cloning of a nucleotide excision repair complex involving the xeroderma pigmentosum group C protein and a human homologue of yeast RAD23. *EMBO J.* 13(8):1831–43
23. Nishi R, Okuda Y, Watanabe E, Mori T, Iwai S, et al. 2005. Centrin 2 stimulates nucleotide excision repair by interacting with xeroderma pigmentosum group C protein. *Mol. Cell Biol.* 25(13):5664–74
24. Batty D, Rapic'-Otrin V, Levine AS, Wood RD. 2000. Stable binding of human XPC complex to irradiated DNA confers strong discrimination for damaged sites. *J. Mol. Biol.* 300(2):275–90
25. Hwang BJ, Ford JM, Hanawalt PC, Chu G. 1999. Expression of the *p48* xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair. *PNAS* 96(2):424–28
26. Min J-H, Pavletich NP. 2007. Recognition of DNA damage by the Rad4 nucleotide excision repair protein. *Nature* 449(7162):570–75
27. Fischer ES, Scrima A, Böhm K, Matsumoto S, Lingaraju GM, et al. 2011. The molecular basis of $CRL4^{DDB2/CSA}$ ubiquitin ligase architecture, targeting, and activation. *Cell* 147(5):1024–39
28. Scrima A, Koníčková R, Czyzewski BK, Kawasaki Y, Jeffrey PD, et al. 2008. Structural basis of UV DNA-damage recognition by the DDB1-DDB2 complex. *Cell* 135(7):1213–23
29. Sugasawa K, Ng JMY, Masutani C, Iwai S, van der Spek PJ, et al. 1998. Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. *Mol. Cell* 2(2):223–32
30. Cavadini S, Fischer ES, Bunker RD, Potenza A, Lingaraju GM, et al. 2016. Cullin-RING ubiquitin E3 ligase regulation by the COP9 signalosome. *Nature* 531(7596):598–603
31. Groisman R, Polanowska J, Kuraoka I, Sawada J, Saijo M, et al. 2003. The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially regulated by the COP9 signalosome in response to DNA damage. *Cell* 113(3):357–67
32. Sugasawa K, Okuda Y, Saijo M, Nishi R, Matsuda N, et al. 2005. UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. *Cell* 121(3):387–400

33. Ribeiro-Silva C, Sabatella M, Helfricht A, Marteijn JA, Theil AF, et al. 2020. Ubiquitin and TFIIH-stimulated DDB2 dissociation drives DNA damage handover in nucleotide excision repair. *Nat. Commun.* 11(1):4868
34. He J, Zhu Q, Wani G, Sharma N, Han C, et al. 2014. Ubiquitin-specific protease 7 regulates nucleotide excision repair through deubiquitinating XPC protein and preventing XPC protein from undergoing ultraviolet light-induced and VCP/p97 protein-regulated proteolysis. *J. Biol. Chem.* 289(39):27278–89
35. van Cuijk L, van Belle GJ, Turkyilmaz Y, Poulsen SL, Janssens RC, et al. 2015. SUMO and ubiquitin-dependent XPC exchange drives nucleotide excision repair. *Nat. Commun.* 6(1):7499
36. Volker M, Moné MJ, Karmakar P, van Hoffen A, Schul W, et al. 2001. Sequential assembly of the nucleotide excision repair factors in vivo. *Mol. Cell* 8(1):213–24
37. Yokoi M, Masutani C, Maekawa T, Sugasawa K, Ohkuma Y, Hanaoka F. 2000. The xeroderma pigmentosum group C protein complex XPC-HR23B plays an important role in the recruitment of transcription factor IIH to damaged DNA. *J. Biol. Chem.* 275(13):9870–75
38. Okuda M, Kinoshita M, Kakumu E, Sugasawa K, Nishimura Y. 2015. Structural insight into the mechanism of TFIIH recognition by the acidic string of the nucleotide excision repair factor XPC. *Structure* 23(10):1827–37
39. van Eeuwen T, Shim Y, Kim HJ, Zhao T, Basu S, et al. 2021. Cryo-EM structure of TFIIH/Rad4-Rad23-Rad33 in damaged DNA opening in nucleotide excision repair. *Nat. Commun.* 12(1):3338
40. Brueckner F, Hennecke U, Carell T, Cramer P. 2007. CPD damage recognition by transcribing RNA polymerase II. *Science* 315(5813):859–62
41. Damsma GE, Alt A, Brueckner F, Carell T, Cramer P. 2007. Mechanism of transcriptional stalling at cisplatin-damaged DNA. *Nat. Struct. Mol. Biol.* 14(12):1127–33
42. Troelstra C, van Gool A, de Wit J, Vermeulen W, Bootsma D, Hoeijmakers JHJ. 1992. ERCC6, a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. *Cell* 71(6):939–53
43. Balajee AS, May A, Dianov GL, Friedberg EC, Bohr VA. 1997. Reduced RNA polymerase II transcription in intact and permeabilized Cockayne syndrome group B cells. *PNAS* 94(9):4306–11
44. Selby CP, Sancar A. 1997. Cockayne syndrome group B protein enhances elongation by RNA polymerase II. *PNAS* 94(21):11205–9
45. van den Boom V, Citterio E, Hoogstraten D, Zotter A, Egly J-M, et al. 2004. DNA damage stabilizes interaction of CSB with the transcription elongation machinery. *J. Cell Biol.* 166(1):27–36
46. Xu J, Lahiri I, Wang W, Wier A, Cianfrocco MA, et al. 2017. Structural basis for the initiation of eukaryotic transcription-coupled DNA repair. *Nature* 551(7682):653–57
47. Henning KA, Li L, Iyer N, McDaniel LD, Reagan MS, et al. 1995. The Cockayne syndrome group A gene encodes a WD repeat protein that interacts with CSB protein and a subunit of RNA polymerase II TFIIH. *Cell* 82(4):555–64
48. van der Weegen Y, Golan-Berman H, Mevissen TET, Apelt K, González-Prieto R, et al. 2020. The cooperative action of CSB, CSA, and UVSSA target TFIIH to DNA damage-stalled RNA polymerase II. *Nat. Commun.* 11(1):2104
49. Groisman R, Kuraoka I, Chevallier O, Gaye N, Magnaldo T, et al. 2006. CSA-dependent degradation of CSB by the ubiquitin-proteasome pathway establishes a link between complementation factors of the Cockayne syndrome. *Genes Dev.* 20(11):1429–34
50. Bregman DB, Halaban R, van Gool AJ, Henning KA, Friedberg EC, Warren SL. 1996. UV-induced ubiquitination of RNA polymerase II: a novel modification deficient in Cockayne syndrome cells. *PNAS* 93(21):11586–90
51. Kokic G, Wagner FR, Chernev A, Urlaub H, Cramer P. 2021. Structural basis of human transcription-DNA repair coupling. *Nature* 598(7880):368–72
52. Anindya R, Aygün O, Svejstrup JQ. 2007. Damage-induced ubiquitylation of human RNA polymerase II by the ubiquitin ligase Nedd4, but not Cockayne syndrome proteins or BRCA1. *Molecular Cell.* 28(3):386–97
53. Tufegdžić Vidaković A, Mitter R, Kelly GP, Neumann M, Harreman M, et al. 2020. Regulation of the RNAPII pool is integral to the DNA damage response. *Cell* 180(6):1245–61.e21

54. Tufegdzic Vidakovic A, Harreman M, Dirac-Svejstrup AB, Boeing S, Roy A, et al. 2019. Analysis of RNA polymerase II ubiquitylation and proteasomal degradation. *Methods* 159–160:146–56
55. Nakazawa Y, Sasaki K, Mitsutake N, Matsuse M, Shimada M, et al. 2012. Mutations in *UVSSA* cause UV-sensitive syndrome and impair RNA polymerase IIo processing in transcription-coupled nucleotide-excision repair. *Nat. Genet.* 44(5):586–92
56. Schwertman P, Lagarou A, Dekkers DHW, Raams A, van der Hoek AC, et al. 2012. UV-sensitive syndrome protein UVSSA recruits USP7 to regulate transcription-coupled repair. *Nat Genet* 44(5):598–602
57. Zhang X, Horibata K, Saijo M, Ishigami C, Ukai A, et al. 2012. Mutations in *UVSSA* cause UV-sensitive syndrome and destabilize ERCC6 in transcription-coupled DNA repair. *Nat. Genet.* 44(5):593–97
58. Fei J, Chen J. 2012. KIAA1530 protein is recruited by Cockayne syndrome complementation group protein A (CSA) to participate in transcription-coupled repair (TCR). *J. Biol. Chem.* 287(42):35118–26
59. Boeing S, Williamson L, Encheva V, Gori I, Saunders RE, et al. 2016. Multiomic analysis of the UV-induced DNA damage response. *Cell Rep*. 15(7):1597–1610
60. Olivieri M, Cho T, Álvarez-Quilón A, Li K, Schellenberg MJ, et al. 2020. A genetic map of the response to DNA damage in human cells. *Cell* 182(2):481–96.e21
61. Prather D, Krogan NJ, Emili A, Greenblatt JF, Winston F. 2005. Identification and characterization of Elf1, a conserved transcription elongation factor in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 25(22):10122–35
62. Geijer ME, Zhou D, Selvam K, Steurer B, Mukherjee C, et al. 2021. Elongation factor ELOF1 drives transcription-coupled repair and prevents genome instability. *Nat. Cell Biol.* 23(6):608–19
63. van der Weegen Y, de Lint K, van den Heuvel D, Nakazawa Y, Mevissen TET, et al. 2021. ELOF1 is a transcription-coupled DNA repair factor that directs RNA polymerase II ubiquitylation. *Nat. Cell Biol.* 23(6):595–607
64. Kuraoka I, Ito S, Wada T, Hayashida M, Lee L, et al. 2008. Isolation of XAB2 complex involved in pre-mRNA splicing, transcription, and transcription-coupled repair. *J. Biol. Chem.* 283(2):940–50
65. Nakatsu Y, Asahina H, Citterio E, Rademakers S, Vermeulen W, et al. 2000. XAB2, a novel tetratricopeptide repeat protein involved in transcription-coupled DNA repair and transcription. *J. Biol. Chem.* 275(45):34931–37
66. Compe E, Egly J-M. 2016. Nucleotide excision repair and transcriptional regulation: TFIIH and beyond. *Annu. Rev. Biochem.* 85(1):265–90
67. Schaeffer L, Roy R, Humbert S, Moncollin V, Vermeulen W, et al. 1993. DNA repair helicase: a component of BTF2 (TFIIH) basic transcription factor. *Science* 260(5104):58–63
68. Coin F, Oksenych V, Mocquet V, Groh S, Blattner C, Egly JM. 2008. Nucleotide excision repair driven by the dissociation of CAK from TFIIH. *Molecular Cell*. 31(1):9–20
69. Svejstrup JQ, Wang Z, Feave WJ, Wu X, Bushnell DA, et al. 1995. Different forms of TFIIH for transcription and DNA repair: holo-TFIIH and a nucleotide excision repairosome. *Cell* 80(1):21–28
70. Sandrock B, Egly J-M. 2001. A yeast four-hybrid system identifies Cdk-activating kinase as a regulator of the XPD helicase, a subunit of transcription factor IIH. *J. Biol. Chem.* 276(38):35328–33
71. Okuda M, Nakazawa Y, Guo C, Ogi T, Nishimura Y. 2017. Common TFIIH recruitment mechanism in global genome and transcription-coupled repair subpathways. *Nucleic Acids Res*. 45(22):13043–55
72. Kokic G, Chernev A, Tegunov D, Dienemann C, Urlaub H, Cramer P. 2019. Structural basis of TFIIH activation for nucleotide excision repair. *Nat. Commun.* 10(1):2885
73. Sugasawa K, Akagi J, Nishi R, Iwai S, Hanaoka F. 2009. Two-step recognition of DNA damage for mammalian nucleotide excision repair: directional binding of the XPC complex and DNA strand scanning. *Mol. Cell.* 36(4):642–53
74. Mathieu N, Kaczmarek N, Rüthemann P, Luch A, Naegeli H. 2013. DNA quality control by a lesion sensor pocket of the xeroderma pigmentosum group D helicase subunit of TFIIH. *Curr. Biol.* 23(3):204–12
75. Topolska-Woś AM, Sugitani N, Cordoba JJ, Le Meur KV, Le Meur RA, et al. 2020. A key interaction with RPA orients XPA in NER complexes. *Nucleic Acids Res*. 48(4):2173–88

76. de Laat WL, Appeldoorn E, Sugasawa K, Weterings E, Jaspers NGJ, Hoeijmakers JHJ. 1998. DNA-binding polarity of human replication protein A positions nucleases in nucleotide excision repair. *Genes Dev*. 12(16):2598–609
77. O'Donovan A, Davies AA, Moggs JG, West SC, Wood RD. 1994. XPG endonuclease makes the 3′ incision in human DNA nucleotide excision repair. *Nature* 371(6496):432–35
78. Sijbers AM, de Laat WL, Ariza RR, Biggerstaff M, Wei Y-F, et al. 1996. Xeroderma pigmentosum group F caused by a defect in a structure-specific DNA repair endonuclease. *Cell* 86(5):811–22
79. Staresincic L, Fagbemi AF, Enzlin JH, Gourdin AM, Wijgers N, et al. 2009. Coordination of dual incision and repair synthesis in human nucleotide excision repair. *EMBO J.* 28(8):1111–20
80. Ogi T, Limsirichaikul S, Overmeer RM, Volker M, Takenaka K, et al. 2010. Three DNA polymerases, recruited by different mechanisms, carry out NER repair synthesis in human cells. *Mol. Cell.* 37(5):714–27
81. Paul-Konietzko K, Thomale J, Arakawa H, Iliakis G. 2015. DNA ligases I and III support nucleotide excision repair in DT40 cells with similar efficiency. *Photochem. Photobiol.* 91(5):1173–80
82. Hu J, Choi J-H, Gaddameedhi S, Kemp MG, Reardon JT, Sancar A. 2013. Nucleotide excision repair in human cells. *J. Biol. Chem.* 288(29):20918–26
83. Kemp MG, Reardon JT, Lindsey-Boltz LA, Sancar A. 2012. Mechanism of release and fate of excised oligonucleotides during nucleotide excision repair. *J. Biol. Chem.* 287(27):22889–99
84. Hu J, Adar S, Selby CP, Lieb JD, Sancar A. 2015. Genome-wide analysis of human global and transcription-coupled excision repair of UV damage at single-nucleotide resolution. *Genes Dev*. 29(9):948–60
85. van Toorn M, Turkyilmaz Y, Han S, Zhou D, Kim H-S, et al. 2022. Active DNA damage eviction by HLTF stimulates nucleotide excision repair. *Mol. Cell.* 82(7):1343–58.e8
86. Gregersen LH, Svejstrup JQ. 2018. The cellular response to transcription-blocking DNA damage. *Trends Biochem. Sci.* 43(5):327–41
87. Wilson MD, Harreman M, Svejstrup JQ. 2013. Ubiquitylation and degradation of elongating RNA polymerase II: the last resort. *Biochim. Biophys. Acta Gene Regul. Mech.* 1829(1):151–57
88. Nakazawa Y, Hara Y, Oka Y, Komine O, van den Heuvel D, et al. 2020. Ubiquitination of DNA damage-stalled RNAPII promotes transcription-coupled repair. *Cell* 180(6):1228–44.e24
89. Beaudenon SL, Huacani MR, Wang G, McDonnell DP, Huibregtse JM. 1999. Rsp5 ubiquitin-protein ligase mediates DNA damage-induced degradation of the large subunit of RNA polymerase II in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 19(10):6972–79
90. Ribar B, Prakash L, Prakash S. 2006. Requirement of *ELC1* for RNA polymerase II polyubiquitylation and degradation in response to DNA damage in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 26(11):3999–4005
91. Ribar B, Prakash L, Prakash S. 2007. *ELA1* and *CUL3* are required along with *ELC1* for RNA polymerase II polyubiquitylation and degradation in DNA-damaged yeast cells. *Mol. Cell Biol.* 27(8):3211–16
92. Yasukawa T, Kamura T, Kitajima S, Conaway RC, Conaway JW, Aso T. 2008. Mammalian Elongin A complex mediates DNA-damage-induced ubiquitylation and degradation of Rpb1. *EMBO J.* 27(24):3256–66
93. Harreman M, Taschner M, Sigurdsson S, Anindya R, Reid J, et al. 2009. Distinct ubiquitin ligases act sequentially for RNA polymerase II polyubiquitylation. *PNAS* 106(49):20705–10
94. Woudstra EC, Gilbert C, Fellows J, Jansen L, Brouwer J, et al. 2002. A Rad26–Def1 complex coordinates repair and RNA pol II proteolysis in response to DNA damage. *Nature* 415(6874):929–33
95. Somesh BP, Reid J, Liu W-F, Søgaard TMM, Erdjument-Bromage H, et al. 2005. Multiple mechanisms confining RNA polymerase II ubiquitylation to polymerases undergoing transcriptional arrest. *Cell* 121(6):913–23
96. Wilson MD, Harreman M, Taschner M, Reid J, Walker J, et al. 2013. Proteasome-mediated processing of Def1, a critical step in the cellular response to transcription stress. *Cell* 154(5):983–95
97. Herlihy AE, Boeing S, Weems JC, Walker J, Dirac-Svejstrup AB, et al. 2022. UBAP2/UBAP2L regulate UV-induced ubiquitylation of RNA polymerase II and are the human orthologues of yeast Def1. *DNA Repair* 115:103343

98. Kuznetsova AV, Meller J, Schnell PO, Nash JA, Ignacak ML, et al. 2003. von Hippel-Lindau protein binds hyperphosphorylated large subunit of RNA polymerase II through a proline hydroxylation motif and targets it for ubiquitination. *PNAS* 100(5):2706–11
99. Lommel L, Bucheli ME, Sweder KS. 2000. Transcription-coupled repair in yeast is independent from ubiquitylation of RNA pol II: implications for Cockayne's syndrome. *PNAS* 97(16):9088–92
100. Weems JC, Slaughter BD, Unruh JR, Boeing S, Hall SM, et al. 2017. Cockayne syndrome B protein regulates recruitment of the Elongin A ubiquitin ligase to sites of DNA damage. *J. Biol. Chem.* 292(16):6431–37
101. Hara R, Selby CP, Liu M, Price DH, Sancar A. 1999. Human transcription release factor 2 dissociates RNA polymerases I and II stalled at a cyclobutane thymine dimer. *J. Biol. Chem.* 274(35):24779–86
102. Elrod ND, Henriques T, Huang K-L, Tatomer DC, Wilusz JE, et al. 2019. The integrator complex attenuates promoter-proximal transcription at protein-coding genes. *Mol. Cell.* 76(5):738–52.e7
103. Chiou Y-Y, Hu J, Sancar A, Selby CP. 2018. RNA polymerase II is released from the DNA template during transcription-coupled repair in mammalian cells. *J. Biol. Chem.* 293(7):2476–86
104. Poulsen SL, Hansen RK, Wagner SA, van Cuijk L, van Belle GJ, et al. 2013. RNF111/Arkadia is a SUMO-targeted ubiquitin ligase that facilitates the DNA damage response. *J. Cell Biol.* 201(6):797–807
105. Sin Y, Tanaka K, Saijo M. 2016. The C-terminal region and SUMOylation of Cockayne syndrome group B protein play critical roles in transcription-coupled nucleotide excision repair. *J. Biol. Chem.* 291(3):1387–97
106. Anindya R, Mari P-O, Kristensen U, Kool H, Giglia-Mari G, et al. 2010. A ubiquitin-binding domain in Cockayne syndrome B required for transcription-coupled nucleotide excision repair. *Mol. Cell.* 38(5):637–48
107. Chen L, Shinde U, Ortolan TG, Madura K. 2001. Ubiquitin-associated (UBA) domains in Rad23 bind ubiquitin and promote inhibition of multi-ubiquitin chain assembly. *EMBO Rep.* 2(10):933–38
108. Engelberg D, Klein C, Martinetto H, Struhl K, Karin M. 1994. The UV response involving the ras signaling pathway and AP-1 transcription factors is conserved between yeast and mammals. *Cell* 77(3):381–90
109. Zou L, Elledge SJ. 2003. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* 300(5625):1542–48
110. Herrlich P, Sachsenmaier C, Radler-Pohl A, Gebel S, Blattner C, Rahmsdorf HJ. 1994. The mammalian UV response: mechanism of DNA damage induced gene expression. *Adv. Enzyme Regul.* 34:381–95
111. Stein B, Rahmsdorf HJ, Steffen A, Litfin M, Herrlich P. 1989. UV-induced DNA damage is an intermediate step in UV-induced expression of human immunodeficiency virus type 1, collagenase, c-*fos*, and metallothionein. *Mol. Cell Biol.* 9(11):5169–81
112. Schreiber M, Baumann B, Cotten M, Angel P, Wagner EF. 1995. Fos is an essential component of the mammalian UV response. *EMBO J.* 14(21):5338–49
113. Devary Y, Rosette C, Didonato J, Karin M. 1993. NF-κB activation by ultraviolet light not dependent on a nuclear signal. *Science* 261(5127):1442–45
114. Price MA, Cruzalegui FH, Treisman R. 1996. The p38 and ERK MAP kinase pathways cooperate to activate ternary complex factors and c-*fos* transcription in response to UV light. *EMBO J.* 15(23):6552–63
115. Herr I, van Dam H, Angel P. 1994. Binding of promoter-associated AP-1 is not altered during induction and subsequent repression of the *c-jun* promoter by TPA and UV irradiation. *Carcinogenesis* 15(6):1105–13
116. Rozek D, Pfeifer GP. 1993. In vivo protein-DNA interactions at the c-jun promoter: preformed complexes mediate the UV response. *Mol. Cell Biol.* 13(9):5490–99
117. Devary Y, Gottlieb RA, Smeal T, Karin M. 1992. The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. *Cell* 71(7):1081–91
118. Radler-Pohl A, Sachsenmaier C, Gebel S, Auer HP, Bruder JT, et al. 1993. UV-induced activation of AP-1 involves obligatory extranuclear steps including Raf-1 kinase. *EMBO J.* 12(3):1005–12
119. Krämer M, Sachsenmaier C, Herrlich P, Rahmsdorf HJ. 1993. UV irradiation-induced interleukin-1 and basic fibroblast growth factor synthesis and release mediate part of the UV response. *J. Biol. Chem.* 268(9):6734–41

120. Sachsenmaier C, Radler-Pohl A, Zinck R, Nordheim A, Herrlich P, Rahmsdorf HJ. 1994. Involvement of growth factor receptors in the mammalian UVC response. *Cell* 78(6):963–72
121. Schorpp M, Mallick U, Rahmsdorf HJ, Herrlich P. 1984. UV-induced extracellular factor from human fibroblasts communicates the UV response to nonirradiated cells. *Cell* 37(3):861–68
122. Dérijard B, Hibi M, Wu IH, Barrett T, Su B, et al. 1994. JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* 76(6):1025–37
123. Kulms D, Schwarz T. 2002. Mechanisms of UV-induced signal transduction. *J. Dermatol.* 29(4):189–96
124. Blattner C, Bender K, Herrlich P, Rahmsdorf HJ. 1998. Photoproducts in transcriptionally active DNA induce signal transduction to the delayed U.V.-responsive genes for collagenase and metallothionein. *Oncogene* 16(22):2827–34
125. Derheimer FA, O'Hagan HM, Krueger HM, Hanasoge S, Paulsen MT, Ljungman M. 2007. RPA and ATR link transcriptional stress to p53. *PNAS* 104(31):12778–83
126. Sikorski TW, Ficarro SB, Holik J, Kim T, Rando OJ, et al. 2011. Sub1 and RPA associate with RNA polymerase II at different stages of transcription. *Mol. Cell.* 44(3):397–409
127. Tresini M, Warmerdam DO, Kolovos P, Snijder L, Vrouwe MG, et al. 2015. The core spliceosome as target and effector of non-canonical ATM signalling. *Nature* 523(7558):53–58
128. Hung K-F, Sidorova JM, Nghiem P, Kawasumi M. 2020. The 6–4 photoproduct is the trigger of UV-induced replication blockage and ATR activation. *PNAS* 117(23):12806–16
129. Matsuoka S, Ballif BA, Smogorzewska A, McDonald ER, Hurov KE, et al. 2007. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 316(5828):1160–66
130. Stokes MP, Rush J, MacNeill J, Ren JM, Sprott K, et al. 2007. Profiling of UV-induced ATM/ATR signaling pathways. *PNAS* 104(50):19855–60
131. Liu Q, Guntuku S, Cui XS, Matsuoka S, Cortez D, et al. 2000. Chk1 is an essential kinase that is regulated by Atr and required for the G_2/M DNA damage checkpoint. *Genes Dev.* 14(12):1448–59
132. Hall-Jackson CA, Cross DA, Morrice N, Smythe C. 1999. ATR is a caffeine-sensitive, DNA-activated protein kinase with a substrate specificity distinct from DNA-PK. *Oncogene* 18(48):6707–13
133. Lakin ND, Hann BC, Jackson SP. 1999. The ataxia-telangiectasia related protein ATR mediates DNA-dependent phosphorylation of p53. *Oncogene* 18(27):3989–95
134. Shieh SY, Ahn J, Tamai K, Taya Y, Prives C. 2000. The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. *Genes Dev.* 14(3):289–300
135. Tibbetts RS, Brumbaugh KM, Williams JM, Sarkaria JN, Cliby WA, et al. 1999. A role for ATR in the DNA damage-induced phosphorylation of p53. *Genes Dev.* 13(2):152–57
136. Shinozaki T, Nota A, Taya Y, Okamoto K. 2003. Functional role of Mdm2 phosphorylation by ATR in attenuation of p53 nuclear export. *Oncogene* 22(55):8870–80
137. McKay BC, Stubbert LJ, Fowler CC, Smith JM, Cardamore RA, Spronck JC. 2004. Regulation of ultraviolet light-induced gene expression by gene size. *PNAS* 101(17):6582–86
138. Vrouwe MG, Pines A, Overmeer RM, Hanada K, Mullenders LHF. 2011. UV-induced photolesions elicit ATR-kinase-dependent signaling in non-cycling cells through nucleotide excision repair-dependent and -independent pathways. *J. Cell Sci.* 124(Part 3):435–46
139. Rockx DA, Mason R, van Hoffen A, Barton MC, Citterio E, et al. 2000. UV-induced inhibition of transcription involves repression of transcription initiation and phosphorylation of RNA polymerase II. *PNAS* 97(19):10503–8
140. Proietti-De-Santis L, Drané P, Egly J-M. 2006. Cockayne syndrome B protein regulates the transcriptional program after UV irradiation. *EMBO J.* 25(9):1915–23
141. Nakazawa Y, Yamashita S, Lehmann AR, Ogi T. 2010. A semi-automated non-radioactive system for measuring recovery of RNA synthesis and unscheduled DNA synthesis using ethynyluracil derivatives. *DNA Repair* 9(5):506–16
142. Andrade-Lima LC, Veloso A, Paulsen MT, Menck CFM, Ljungman M. 2015. DNA repair and recovery of RNA synthesis following exposure to ultraviolet light are delayed in long genes. *Nucleic Acids Res.* 43(5):2744–56
143. Williamson L, Saponaro M, Boeing S, East P, Mitter R, et al. 2017. UV irradiation induces a non-coding RNA that functionally opposes the protein encoded by the same gene. *Cell* 168(5):843–55.e13

144. Lavigne MD, Konstantopoulos D, Ntakou-Zamplara KZ, Liakos A, Fousteri M. 2017. Global unleashing of transcription elongation waves in response to genotoxic stress restricts somatic mutation rate. *Nat. Commun.* 8(1):2076
145. Liakos A, Konstantopoulos D, Lavigne MD, Fousteri M. 2020. Continuous transcription initiation guarantees robust repair of all transcribed genes and regulatory regions. *Nat. Commun.* 11(1):916
146. Muñoz MJ, Pérez Santangelo MS, Paronetto MP, de la Mata M, Pelisch F, et al. 2009. DNA damage regulates alternative splicing through inhibition of RNA polymerase II elongation. *Cell* 137(4):708–20
147. Sanchez A, De Vivo A, Uprety N, Kim J, Stevens SM, Kee Y. 2016. BMI1-UBR5 axis regulates transcriptional repression at damaged chromatin. *PNAS* 113(40):11243–48
148. van den Heuvel D, Spruijt CG, González-Prieto R, Kragten A, Paulsen MT, et al. 2021. A CSB-PAF1C axis restores processive transcription elongation after DNA damage repair. *Nat. Commun.* 12(1):1342
149. Noe Gonzalez M, Blears D, Svejstrup JQ. 2021. Causes and consequences of RNA polymerase II stalling during transcript elongation. *Nat. Rev. Mol. Cell Biol.* 22(1):3–21
150. Studniarek C, Tellier M, Martin PGP, Murphy S, Kiss T, Egloff S. 2021. The 7SK/P-TEFb snRNP controls ultraviolet radiation-induced transcriptional reprogramming. *Cell Rep.* 35(2):108965
151. Borisova ME, Voigt A, Tollenaere MAX, Sahu SK, Juretschke T, et al. 2018. p38-MK2 signaling axis regulates RNA metabolism after UV-light-induced DNA damage. *Nat. Commun.* 9(1):1017
152. Bugai A, Quaresma AJC, Friedel CC, Lenasi T, Düster R, et al. 2019. P-TEFb activation by RBM7 shapes a pro-survival transcriptional response to genotoxic stress. *Mol. Cell.* 74(2):254–67.e10
153. Epanchintsev A, Costanzo F, Rauschendorf M-A, Caputo M, Ye T, et al. 2017. Cockayne's syndrome A and B proteins regulate transcription arrest after genotoxic stress by promoting ATF3 degradation. *Mol. Cell* 68(6):1054–66.e6
154. Kristensen U, Epanchintsev A, Rauschendorf M-A, Laugel V, Stevnsner T, et al. 2013. Regulatory interplay of Cockayne syndrome B ATPase and stress-response gene ATF3 following genotoxic stress. *PNAS* 110(25):E2261–70
155. Vichi P, Coin F, Renaud JP, Vermeulen W, Hoeijmakers JH, et al. 1997. Cisplatin- and UV-damaged DNA lure the basal transcription factor TFIID/TBP. *EMBO J.* 16(24):7444–56
156. Heine GF, Horwitz AA, Parvin JD. 2008. Multiple mechanisms contribute to inhibit transcription in response to DNA damage. *J. Biol. Chem.* 283(15):9555–61
157. Steurer B, Janssens RC, Geijer ME, Aprile-Garcia F, Geverts B, et al. 2022. DNA damage-induced transcription stress triggers the genome-wide degradation of promoter-bound Pol II. *Nat. Commun.* 13(1):3624
158. Li S, Smerdon MJ. 2002. Rpb4 and Rpb9 mediate subpathways of transcription-coupled DNA repair in *Saccharomyces cerevisiae*. *EMBO J.* 21(21):5921–29
159. Mourgues S, Gautier V, Lagarou A, Bordier C, Mourcet A, et al. 2013. ELL, a novel TFIIH partner, is involved in transcription restart after DNA repair. *PNAS* 110(44):17927–32
160. Adam S, Polo SE, Almouzni G. 2013. Transcription recovery after DNA damage requires chromatin priming by the H3.3 histone chaperone HIRA. *Cell* 155(1):94–106
161. Bouvier D, Ferrand J, Chevallier O, Paulsen MT, Ljungman M, Polo SE. 2021. Dissecting regulatory pathways for transcription recovery following DNA damage reveals a non-canonical function of the histone chaperone HIRA. *Nat. Commun.* 12(1):3835
162. Dinant C, Ampatziadis-Michailidis G, Lans H, Tresini M, Lagarou A, et al. 2013. Enhanced chromatin dynamics by FACT promotes transcriptional restart after UV-induced DNA damage. *Mol. Cell.* 51(4):469–79
163. Oksenych V, Zhovmer A, Ziani S, Mari P-O, Eberova J, et al. 2013. Histone methyltransferase DOT1L drives recovery of gene expression after a genotoxic attack. *PLOS Genet.* 9(7):e1003611
164. Vélez-Cruz R, Zadorin AS, Coin F, Egly J-M. 2013. Sirt1 suppresses RNA synthesis after UV irradiation in combined xeroderma pigmentosum group D/Cockayne syndrome (XP-D/CS) cells. *PNAS* 110(3):E212–20
165. Shkreta L, Toutant J, Durand M, Manley JL, Chabot B. 2016. SRSF10 connects DNA damage to the alternative splicing of transcripts encoding apoptosis, cell-cycle control, and DNA repair factors. *Cell Rep.* 17(8):1990–2003

166. Muñoz MJ, Nieto Moreno N, Giono LE, Cambindo Botto AE, Dujardin G, et al. 2017. Major roles for pyrimidine dimers, nucleotide excision repair, and ATR in the alternative splicing response to UV irradiation. *Cell Rep*. 18(12):2868–79
167. Kornblihtt AR, Schor IE, Alló M, Dujardin G, Petrillo E, Muñoz MJ. 2013. Alternative splicing: a pivotal step between eukaryotic transcription and translation. *Nat. Rev. Mol. Cell Biol.* 14(3):153–65
168. Paronetto MP, Miñana B, Valcárcel J. 2011. The Ewing sarcoma protein regulates DNA damage-induced alternative splicing. *Mol. Cell.* 43(3):353–68
169. Shomron N, Alberstein M, Reznik M, Ast G. 2005. Stress alters the subcellular distribution of hSlu7 and thus modulates alternative splicing. *J. Cell Sci.* 118(Part 6):1151–59
170. van der Houven van Oordt W, Diaz-Meco MT, Lozano J, Krainer AR, Moscat J, Cáceres JF. 2000. The $MKK_{3/6}$-p38–signaling cascade alters the subcellular distribution of hnRNP A1 and modulates alternative splicing regulation. *J. Cell Biol.* 149(2):307–16
171. Suzumura H, Arisaka O. 2010. Cerebro-oculo-facio-skeletal syndrome. *Adv. Exp. Med. Biol.* 685:210–14
172. Fassihi H, Sethi M, Fawcett H, Wing J, Chandler N, et al. 2016. Deep phenotyping of 89 xeroderma pigmentosum patients reveals unexpected heterogeneity dependent on the precise molecular defect. *PNAS* 113(9):E1236–45
173. Stefanini M, Botta E, Lanzafame M, Orioli D. 2010. Trichothiodystrophy: from basic mechanisms to clinical implications. *DNA Repair* 9(1):2–10
174. Laugel V. 2013. Cockayne syndrome: the expanding clinical and mutational spectrum. *Mechanisms Ageing Dev*. 134(5):161–70
175. Spivak G, Hanawalt PC. 2015. Photosensitive human syndromes. *Mutat. Res.* 776:24–30
176. DiGiovanna JJ, Kraemer KH. 2012. Shining a light on xeroderma pigmentosum. *J. Investig. Dermatol.* 132(3, Part 2):785–96
177. Wang Y, Chakravarty P, Ranes M, Kelly G, Brooks PJ, et al. 2014. Dysregulation of gene expression as a cause of Cockayne syndrome neurological disease. *PNAS* 111(40):14454–59
178. Wang Y, Jones-Tabah J, Chakravarty P, Stewart A, Muotri A, et al. 2016. Pharmacological bypass of Cockayne syndrome B function in neuronal differentiation. *Cell Rep*. 14(11):2554–61
179. Brooks PJ. 2013. Blinded by the UV light: how the focus on transcription-coupled NER has distracted from understanding the mechanisms of Cockayne syndrome neurologic disease. *DNA Repair* 12(8):656–71
180. Itoh M, Hayashi M, Shioda K, Minagawa M, Isa F, et al. 1999. Neurodegeneration in hereditary nucleotide repair disorders. *Brain Dev*. 21(5):326–33
181. Mulderrig L, Garaycoechea JI, Tuong ZK, Millington CL, Dingler FA, et al. 2021. Aldehyde-driven transcriptional stress triggers an anorexic DNA damage response. *Nature* 600(7887):158–63
182. Dianov GL, Houle J-F, Iyer N, Bohr VA, Friedberg EC. 1997. Reduced RNA polymerase II transcription in extracts of Cockayne syndrome and xeroderma pigmentosum/Cockayne syndrome cells. *Nucleic Acids Res*. 25(18):3636–42
183. Hwang JR, Moncollin V, Vermeulen W, Seroz T, van Vuuren H, et al. 1996. A $3' \rightarrow 5'$ XPB helicase defect in repair/transcription factor TFIIH of xeroderma pigmentosum group B affects both DNA repair and transcription. *J. Biol. Chem.* 271(27):15898–904
184. Newman JC, Bailey AD, Weiner AM. 2006. Cockayne syndrome group B protein (CSB) plays a general role in chromatin maintenance and remodeling. *PNAS* 103(25):9613–18
185. Vessoni AT, Herai RH, Karpiak JV, Leal AMS, Trujillo CA, et al. 2016. Cockayne syndrome-derived neurons display reduced synapse density and altered neural network synchrony. *Hum. Mol. Genet.* 25(7):1271–80
186. Ciaffardini F, Nicolai S, Caputo M, Canu G, Paccosi E, et al. 2014. The cockayne syndrome B protein is essential for neuronal differentiation and neuritogenesis. *Cell Death Dis*. 5(5):e1268
187. Wu Z, Zhu X, Yu Q, Xu Y, Wang Y. 2019. Multisystem analyses of two Cockayne syndrome associated proteins CSA and CSB reveal shared and unique functions. *DNA Repair* 83:102696
188. Haijes HA, Koster MJE, Rehmann H, Li D, Hakonarson H, et al. 2019. De novo heterozygous POLR2A variants cause a neurodevelopmental syndrome with profound infantile-onset hypotonia. *Am. J. Hum. Genet.* 105(2):283–301

189. Wozniak KJ, Simmons LA. 2022. Bacterial DNA excision repair pathways. *Nat. Rev. Microbiol.* 20:465–77
190. Bharati BK, Gowder M, Zheng F, Alzoubi K, Svetlov V, et al. 2022. Crucial role and mechanism of transcription-coupled DNA repair in bacteria. *Nature* 604(7904):152–59
191. Dammann R, Pfeifer GP. 1997. Lack of gene- and strand-specific DNA repair in RNA polymerase III-transcribed human tRNA genes. *Mol. Cell Biol.* 17(1):219–29
192. Yang Y, Hu J, Selby CP, Li W, Yimit A, et al. 2019. Single-nucleotide resolution analysis of nucleotide excision repair of ribosomal DNA in humans and mice. *J. Biol. Chem.* 294(1):210–17
193. Daniel L, Cerutti E, Donnio L-M, Nonnekens J, Carrat C, et al. 2018. Mechanistic insights in transcription-coupled nucleotide excision repair of ribosomal DNA. *PNAS* 115(29):E6770–79
194. Abraham KJ, Khosraviani N, Chan JNY, Gorthi A, Samman A, et al. 2020. Nucleolar RNA polymerase II drives ribosome biogenesis. *Nature* 585(7824):298–302
195. Prakash S, Prakash L. 2000. Nucleotide excision repair in yeast. *Mutation Res./Fundam. Mol. Mechanisms Mutagenesis* 451(1–2):13–24
196. Mota MBS, Carvalho MA, Monteiro ANA, Mesquita RD. 2019. DNA damage response and repair in perspective: *Aedes aegypti*, *Drosophila melanogaster* and *Homo sapiens*. *Parasites Vectors* 12(1):533
197. Deger N, Yang Y, Lindsey-Boltz LA, Sancar A, Selby CP. 2019. *Drosophila*, which lacks canonical transcription-coupled repair proteins, performs transcription-coupled repair. *J. Biol. Chem.* 294(48):18092–98
198. Deger N, Cao X, Selby CP, Gulec S, Kawara H, et al. 2022. CSB-independent, XPC-dependent transcription-coupled repair in *Drosophila*. *PNAS* 119(9):e2123163119
199. Jiang Y, Liu M, Spencer CA, Price DH. 2004. Involvement of transcription termination factor 2 in mitotic repression of transcription elongation. *Mol. Cell.* 14(3):375–86
200. Groh M, Albulescu LO, Cristini A, Gromak N. 2017. Senataxin: genome guardian at the interface of transcription and neurodegeneration. *J. Mol. Biol.* 429(21):3181–95
201. Porrua O, Libri D. 2013. A bacterial-like mechanism for transcription termination by the Sen1p helicase in budding yeast. *Nat. Struct. Mol. Biol.* 20(7):884–91
202. Kumar N, Raja S, Van Houten B. 2020. The involvement of nucleotide excision repair proteins in the removal of oxidative DNA damage. *Nucleic Acids Res.* 48(20):11227–43
203. Guo J, Hanawalt PC, Spivak G. 2013. Comet-FISH with strand-specific probes reveals transcription-coupled repair of 8-oxoGuanine in human cells. *Nucleic Acids Res.* 41(16):7700–12
204. Menoni H, Wienholz F, Theil AF, Janssens RC, Lans H, et al. 2018. The transcription-coupled DNA repair-initiating protein CSB promotes XRCC1 recruitment to oxidative DNA damage. *Nucleic Acids Res.* 46(15):7747–56

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Errata

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