MECHANISMS OF TRANSCRIPTION-COUPLED DNA REPAIR

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Several types of helix-distorting DNA lesions block the passage of elongating RNA polymerase II. Surprisingly, such transcription-blocking lesions are usually repaired considerably faster than non-obstructive lesions in the non-transcribed strand or in the genome overall. In this review, our knowledge of eukaryotic transcription-coupled repair (TCR) will be considered from the point of view of transcription, and current models for the mechanism of TCR will be discussed.

TRANSCRIPTION

In theory, a single irreversibly stalled RNA polymerase II (RNAPII) molecule in an essential gene is enough to result in the eventual death of the affected cell. It is therefore not surprising that a large number of factors and mechanisms have evolved to ensure that this event is exceedingly rare. One of the most common causes of prolonged RNAPII stalling is DNA damage in the transcribed strand of active genes[1]. A variety of well-known DNA lesions hinder the passage of a transcribing polymerase *in vitro*[2–6] and several have also been shown to block RNAPII transcription *in vivo*[7,8].

Hanawalt and co-workers[9,10] first investigated whether the rate of repair in active genes is significantly different from that in non-transcribed regions over 15 years ago (BOX 1). The results were clear-cut: repair of DNA damage in active genes occurs much faster than in non-transcribed regions of the genome. And this faster repair is not simply due to the higher accessibility of DNA in actively transcribed regions, as it was subsequently shown that it is specifically the transcribed strand that is preferentially repaired[11]. The non-transcribed strand of the same gene is repaired with a rate that is similar to that of non-transcribed regions.

Preferential repair of the transcribed strand of active genes, or transcription-coupled repair (TCR), is now known to be conserved not only in eukaryotes, but also in prokaryotes[12]. So, the importance of keeping the path of elongating polymerases clear is evident across species. Moreover, preferential repair of the transcribed strand might occur not only in protein-coding genes transcribed by RNAPII, but also in ribosomal DNA genes transcribed by RNA polymerase I (although this has been much less studied and probably occurs by a different mechanism[13,14]). The fundamental importance of preferential repair is also evident from the finding that people who are compromised in their ability to perform the reaction due to mutations in TCR genes are afflicted with a severe hereditary disorder called Cockayne's syndrome (BOX 2).

In this review, the mechanisms that together constitute the cellular response to damage-stalled RNA polymerases are described, with particular emphasis on the RNAPII transcription machinery and the factors that enable TCR. For updates on general repair mechanisms, the reader is encouraged to read other recent reviews[12,15–18].

Pathways for transcription-coupled repair

TCR was first thought to be a specific sub-pathway of the nucleotide excision repair (NER) pathway (see below), which means that most studies have focused on factors and mechanisms that are intrinsic to this pathway. However, over the past few years, it has become clear that several lesions removed by base excision repair (BER; see below) are also repaired through TCR[8,19,20]. Recent results from the Cooper and Sarasin laboratories[8] have even provided evidence that Cockayne's syndrome might result from a failure to repair oxidative lesions in the transcribed strand of active genes by BER.

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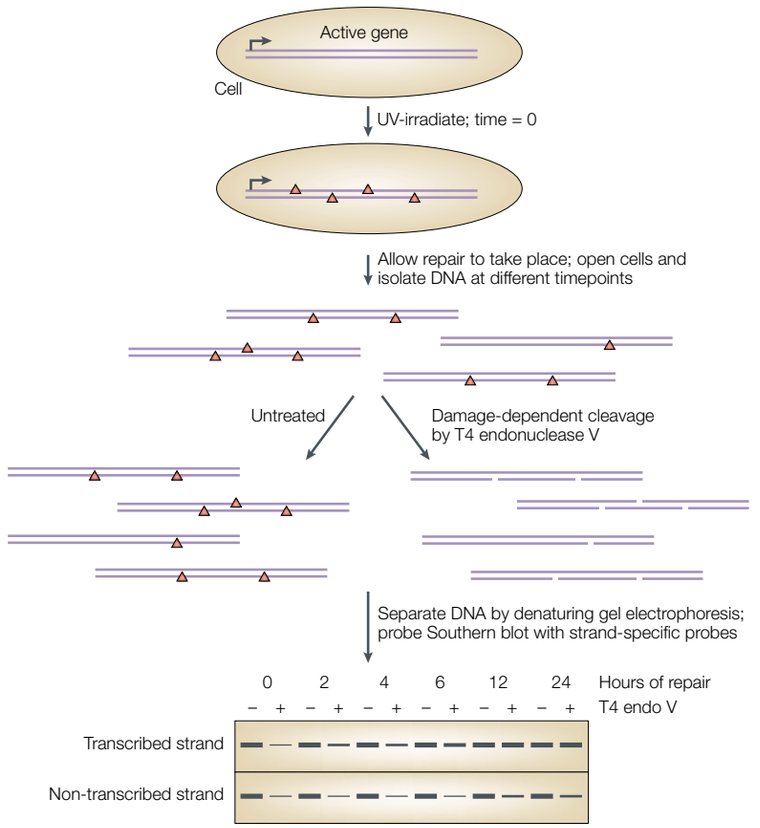
Box 1 | **Measurement of strand-specific DNA repair**

After irradiation of growing cells at time zero, repair is allowed to take place for various periods before the cellular DNA is harvested. Half the DNA is treated with the damage-specific endonuclease, T4 endonuclease V, which will digest DNA fragments that contain unrepaired lesions (red triangles). The DNA is then digested with restriction endonucleases, which yields DNA fragments of defined length that can be visualized by Southern blotting. These fragments are subjected to electrophoresis in alkaline agarose gels, which separate the strands of the fragment, and repair in the individual strands is visualized using radioactive strand-specific probes (generated from M13 single-stranded DNA clones). The proportion of lesions that are repaired at a certain time-point is calculated by comparing T4 endonuclease V-treated DNA with untreated DNA. (Lesions do not significantly affect the mobility of the untreated DNA fragments, while treatment with T4 endonuclease V removes fragments that contain lesions.)



DNA LIGASE
Seals DNA breaks containing a 5′-phosphorylated end and a 3′-OH end. Several different DNA ligases are found in eukaryotic cells.

TFIIH
(Transcription factor IIH). One of the factors that are generally required for RNA polymerase II-dependent transcription. Also has an essential role in nucleotide excision repair.

Nucleotide excision repair. The NER pathway is the most versatile of all the DNA-repair pathways[15,16]. It removes various structurally unrelated lesions, with an apparent preference for bulky base adducts, such as pyrimidine dimers, which cause significant helix distortion. NER occurs by dual, single-stranded incisions on either side of the adduct and removal of a damaged oligonucleotide, 24–32 nucleotides long, before gap filling by DNA polymerase and ligation of the nicks by DNA LIGASE (FIG. 1a). NER requires the concerted action of around 30 polypeptides, which act through the stepwise assembly of several sub-complexes at the site of damage[21–23], or (at least in the budding yeast, *Saccharomyces cerevisiae*) through a pre-assembled 'repairosome'[24,25].

In the first step, the lesion is recognized by binding of the xeroderma pigmentosum C XPC/HHR23B complex (Rad4/Rad23 in *S. cerevisiae*)[22,26,27]. The next step is the formation of an open complex, which requires local unwinding of the DNA helix and demarcation of the DNA lesion. The unwinding of the DNA helix is brought about by the activity of the basal transcription/repair factor TFIIH[28,29]. The ATP-dependent HELICASE activity of the XPB (Rad25) subunit of TFIIH is responsible for helix unwinding during RNAPII transcription initiation, whereas the helicase activities of both the XPB and the XPD (Rad3) subunits are required for NER.

Other basic NER factors that are required for the formation of a functional repair complex are the damage-binding factor XPA (Rad14) and the single-stranded DNA-binding protein, replication protein A (RPA). The association of these proteins with the pre-incision complex enables recruitment of the structure-specific DNA ENDONUCLEASES XPG (Rad2) and XPF/ERCC1 (Rad1/Rad10), which make the single-stranded DNA breaks that enable the removal of an oligonucleotide that contains the DNA lesion. Finally, a DNA polymerase fills the gap and the nicks are sealed by DNA ligase.

Base excision repair. A variety of damaged bases, such as spontaneously formed (hypoxanthine, for example), oxidized (8-oxo-guanine), alkylated (3-methyladenine) or mismatched (T:G) bases are normally repaired by BER[17,18], although many of these lesions are also substrates for NER. A fundamental difference between NER and BER is that NER uses a limited number of proteins to recognize many different types of damage, whereas BER uses various damage-recognition proteins, each specific for a very limited number of lesions.

In the first step of BER, the damaged base is removed by a DNA GLYCOSYLASE (FIG. 1b). In humans, several DNA glycosylases have been identified, and each excises an overlapping subset of spontaneously formed, oxidized, alkylated or mismatched bases, such as those mentioned above. The ABASIC SITE is then recognized by an apurinic/apyrimidinic (AP) endonuclease, which cleaves the phosphodiester bond that is 5′ to the lesion, and leaves behind a strand break with a normal 3′-hydroxyl group and an abnormal 5′-abasic terminus. Replacement of the abasic residue with the correct nucleotide relies on both the endonuclease and polymerization activities of DNA polymerase β (and in certain situations the 'FLAP' ENDONUCLEASE FEN1), and is followed by ligation of the break.

Factors enabling strand-specific repair

The protein factors that are required for TCR and general genome repair (GGR) of DNA damage fall into two broad groups. The first contains factors that are thought to be uniquely involved in directing the repair machinery to lesions in the relevant strand or genome region. The second includes factors that have a well-defined function in DNA repair but also have a distinct role in allowing TCR or GGR (TABLE 1).

The classical TCR factors are the mammalian Cockayne's Syndrome A (CSA) and CSB proteins[30,31]. Cell lines expressing defective forms of these proteins

Box 2 | **Cockayne's syndrome**

Cockayne's syndrome is an autosomal recessive disease, which is characterized by growth retardation, skeletal and retinal abnormalities, progressive neural retardation and severe photosensitivity[98]. Cells derived from patients with Cockayne's syndrome are hypersensitive to ultraviolet (UV) light, and have almost completely defective TCR[33] and a severe deficiency in recovery of RNA synthesis after DNA damage[99]. Cockayne's syndrome is related to several DNA-repair-deficiency disorders, such as xeroderma pigmentosum and the photosensitive form of brittle hair disorder, trichothiodystrophy[98,100,101]. Xeroderma pigmentosum is caused by mutation in genes encoding nucleotide excision repair proteins, such as xeroderma pigmentosum A (*XPA*), *XPB*, *XPC*, *XPD* and *XPF* (see main text for details).

Symptoms observed in patients with xeroderma pigmentosum, such as photosensitivity, parchment skin (xeroderma), freckles (pigmentosum) and a 1,000-fold increased risk of skin cancer, can be explained by their inability to repair DNA damage through nucleotide excision repair. By contrast, although patients with Cockayne's syndrome are deficient in removal of DNA lesions in the transcribed strand of active genes, they are not generally repair-deficient. And as cells from patients with xeroderma pigmentosum show an almost complete general inability to repair UV damage (also in the transcribed strand), yet have much milder symptoms than patients with Cockayne's syndrome, Cockayne's syndrome is unlikely to merely be a repair disorder. Rather, Cockayne's syndrome is now believed to be due to the severely mutagenic and chromosome-destabilizing consequences of stalled RNAPII (REFS 8,55,102,103).

are almost completely unable to repair lesions in the transcribed strand of active genes, but have normal GGR[32,33]. The inability to carry out TCR is expressed at the cellular phenotypic level, in that Cockayne's syndrome cells are sensitive to ultraviolet (UV) light[34]. Although the phenotypes of CSB and CSA cells are the same, the CSA and CSB proteins do not form a stable complex[35]. These factors therefore function in the same process but as discrete molecular entities. Homologues of CSA and CSB have been isolated in *S. cerevisiae*[36,37]. Here, deletion of the *RAD26* gene (encoding the yeast homologue of CSB) also results in TCR defects, although not to the pronounced extent that is observed in human CSB-mutant cell lines. Moreover, *rad26* cells are not UV-sensitive[36].

Interestingly, CSB/Rad26 are DNA-dependent ATPases in the SNF2 PROTEIN FAMILY[38]. Given that most SWI/SNF-LIKE COMPLEXES can alter histone octamer–DNA contacts in an ATP-dependent manner, it would not be surprising if other proteins in the Snf2 protein family were also capable of chromatin remodelling — or at least of altering protein–DNA contacts. Indeed, recombinant CSB protein can remodel chromatin[39], and can also stimulate transcript elongation by RNAPII and enable it to add an extra nucleotide when stalled at a transcription-blocking DNA lesion[40].

In contrast to yeast *rad26* cells, which have an easily detectable defect in TCR, cells lacking *RAD28* (the closest yeast homologue of CSA) are not defective for TCR[37]. However, deletion of *RAD28* does increase UV-sensitivity in certain strain backgrounds, which indicates some role in DNA repair. Rad28 and CSA have multiple WD40 REPEATS covering their entire length. This makes it hard to be certain that the proteins are actually ORTHOLOGUES[37]. As in mammalian cells[35], Rad26 and Rad28 also fail to form a stable complex (E. Woudstra *et al.*, unpublished observations).

Recently, a TCR factor named XPA-binding protein 2 (XAB2) was isolated[41] as an XPA-interacting protein in a yeast two-hybrid screen. XAB2 also interacts with CSA, CSB and RNAPII. Injection of antibodies that are directed against XAB2 into cells results in inhibition of recovery of RNA synthesis after UV irradiation and general inhibition of transcription in the absence of irradiation[41].

TFIIH is unique among all the factors that are involved in TCR in that it acts in several distinct cellular processes. First, it is an essential component of the basic RNAPII transcription machinery[28,29]. Second, it is a basic DNA-repair factor, which is required for all repair by the NER pathway[42–46]. And third, it has a role in TCR of oxidative damage by the BER pathway[8]. The nine subunits of TFIIH contain multiple domains and activities of relevance to these functions, such as two helicases, a protein kinase and a RING FINGER motif[29].

XPG (*S. cerevisiae* Rad2) is another multifunctional entity that is involved in TCR. Like TFIIH, it is required for NER, but also has a distinct role in TCR of oxidative damage by the BER pathway[8]. XPG/Rad2 is a structure-specific DNA endonuclease, which is responsible for the first of the DNA incisions during NER[47,48], but this activity is probably not required for its function in BER-mediated TCR[19,49].

Although the molecular mechanism of TCR is conserved from yeast to man, the way in which DNA lesions on the non-transcribed strand and the genome overall are removed might differ considerably between species. Whereas most of the proteins so far shown to be involved in TCR in man have a functional homologue in yeast, the protein machinery that is dedicated to GGR is much less conserved (TABLE 1). For example, functional homologues of the yeast GGR proteins RAD7 and RAD16 have not been identified in higher eukaryotes. Although the existence of such orthologues cannot be ruled out, they are probably absent, or their role in GGR is fulfilled by other proteins.

Interestingly, whereas human cells have substantial GGR, this process is severely defective in rodents. Significantly, rodents express very low levels of the *p48* gene, which encodes the DDB2 subunit of the UV-damaged DNA-binding factor (UV-DDB). However, transfection of a plasmid expressing DDB2 is sufficient to activate GGR in cultured hamster cells[50]. These, and other, data indicate that the human *p48* gene product (which does not have a yeast homologue) has an important function in GGR in higher eukaryotes.

A further difference between human and yeast GGR mechanisms is that the *XPC* gene product is only required for GGR in human cells[51], whereas its homologue Rad4 is required for both GGR and TCR in yeast[52]. This difference is reminiscent of the case with human CSA and yeast Rad28, and could reflect quantitative rather than qualitative differences between the mode of repair in these organisms.

From yeast to man

Progress in our understanding of TCR (and repair in general) over the past decade has been made possible by a

HELICASE
An enzyme that uses the energy of ATP hydrolysis to unwind/separate the two strands of DNA.

ENDONUCLEASE
Cuts DNA internally. Often, but not always, cuts at specific recognition sequences.

DNA GLYCOSYLASE
Binds specifically to a target base and hydrolyses the *N*-glycosylic bond. This releases the inappropriate base while keeping the DNA backbone intact. Several distinct glycosylases exist in eukaryotic cells.

ABASIC SITE
A nucleotide site that has lost the base.

'FLAP' ENDONUCLEASE
A structure-specific endonuclease, which cleaves off a single-stranded piece of DNA that is not base-paired owing to, for example, mismatched bases.

SNF2 PROTEIN FAMILY
A family of proteins that have helicase-like ATPase domains, yet are not helicases. Rather than unwinding DNA strands, these proteins remodel protein–DNA contacts, such as histone–DNA contacts.

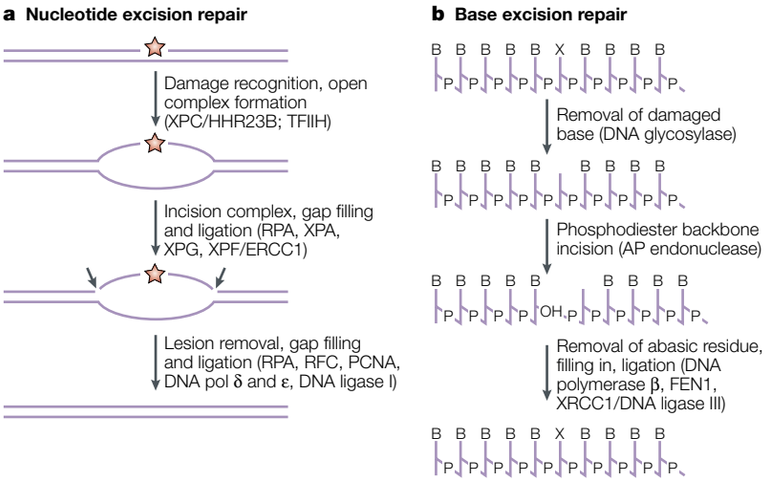


Figure 1 | **Mechanism of nucleotide excision repair and base excision repair.** **a** | Nucleotide excision repair. XPC/HHR23B complex first recognizes the DNA lesion, and enables recruitment of the repair/transcription factor, TFIIH. TFIIH locally unwinds the DNA helix around the DNA lesion. Following helix unwinding, the damage-binding factor XPA and the single-strand binding protein (RPA) become associated with the repair complex. Now the structure-specific DNA endonucleases XPG and XPF/ERCC1 can be recruited. XPG and XPF/ERCC1 make the single-stranded DNA breaks that enable the removal of a damage-containing oligonucleotide. Finally, DNA polymerase (pol δ and ε) fills the gap with the help of its accessory replication proteins (RPA, RFC, PCNA) and the nicks are sealed by DNA ligase I. PCNA, proliferating cell nuclear antigen. **b** | Base excision repair. The damaged base moiety is first recognized and removed by a DNA glycosylase. The abasic site is subsequently recognized by an apurinic (AP) endonuclease, which cleaves the phosphodiester bond that is 5′ to the lesion, and leaves behind a strand break with a normal 3′-hydroxyl group and an abnormal 5′-abasic terminus. DNA polymerase β then uses its endonuclease and polymerization activities to replace the abasic residue with the correct nucleotide. In certain situations the 'flap' endonuclease FEN1 is also involved in removing a patch that contains the abasic site. Finally, XRCC1/DNA ligase III seals the break. RFC, replication factor C; PCNA, proliferating cell nuclear antigen; AP, apurinic/apyrimidinic.

combination of research in yeast and human cells. Because data on basic molecular mechanisms of TCR in yeast can probably be extended directly to the human system, it is important to appreciate the differences between the results in these systems and why they might arise. As mentioned above, human CSA and CSB cells are UV-sensitive, whereas yeast cells lacking *RAD26* are not[36]. This difference is probably due to the presence of very efficient GGR in yeast. So, yeast cells lacking GGR (owing to mutation of the *RAD7* or the *RAD16* genes) are UV-sensitive[53], although much less sensitive than strains that are completely defective for NER (cells that are mutated in a gene encoding a basic NER factor, such as *RAD14*).

In agreement with the idea that yeast survives UV irradiation without TCR because of functional overlap between the TCR and GGR pathways, cells lacking both *RAD26* and *RAD16* are significantly more UV-sensitive than *rad16* single mutants[53]. Moreover, these double mutants are also more defective in damage removal in the transcribed strand of active genes than *rad26* single mutants[53]. These data indicate that although yeast has mechanisms and factors to enable preferential repair of the transcribed strand of active genes, these are not essential for viability during DNA damage because the GGR pathway of repair is so efficient. Similarly, loss of NON-HOMOLOGOUS END-JOINING results in severe phenotypes in mammalian cells but very mild phenotypes in yeast, because homologous recombination is so efficient in the latter[54].

TCR through the BER pathway

The first significant indication that TCR can also occur through the BER pathway was the finding that oxidative damage (normally repaired by BER) can also be removed by TCR[20]. TCR of thymine glycols hence occurs even in cells from xeroderma pigmentosum (XP)-A, XPF and XPG patients (see BOX 2), who have NER defects, but does not occur in cells from XP patients who additionally have severe Cockayne's syndrome.

A recent study[8] expanded these findings and showed that, more generally, Cockayne's syndrome mutations lead to an inability to repair thymine glycols and 8-oxo-guanine by TCR. Such cells cannot remove 8-oxo-guanine in a transcribed sequence despite its proficient removal when the DNA is not transcribed. Moreover, the mutation frequency at 8-oxo-guanine lesions in Cockayne's syndrome cells is 30–40% compared with the normal 1–4%, and unrepaired 8-oxo-guanine blocks transcription by RNAPII in these cells[8]. Cockayne's syndrome mutations also cause metaphase fragility of certain gene loci that contain tandemly repeated, very active genes, which raises the possibility that multiple stalled elongation complexes might locally block chromatin condensation and thereby result in chromosome fragility[55].

The transcriptional response to DNA damage

Cells that have mutations in *CSA* or *CSB* cannot recover RNA synthesis following UV-irradiation. Moreover, there have been reports that transcription is generally affected by mutation of CSB, in both the presence[56] and absence of exogenous induction of DNA damage[57].

Intriguingly, the inability of Cockayne's syndrome cells to recover RNA synthesis after DNA damage is not due solely to the failure of these mutant cells to remove lesions in the transcribed strand of active genes. So, when Cockayne's syndrome cells are treated with the UV-mimetic agent *N*-acetoxy-2-acetylaminofluorene (NA-AAF), RNA synthesis is not resumed, yet NA-AAF-induced DNA adducts are repaired at similar rates, and without strand specificity, in normal and Cockayne's syndrome cells[58]. These data indicate that although NA-AAF-induced DNA adducts are not removed any more slowly in Cockayne's syndrome cells than in normal cells, their induction severely affect the ability of Cockayne's syndrome cells to recover transcription after repair is completed. Similarly, extracts that are prepared from normal and Cockayne's syndrome cells 1 hour after UV-irradiation cannot support *in vitro* transcription, even of undamaged plasmid DNA[59]. Extracts from wild-type cells that are harvested several hours after irradiation have recovered the ability to support transcription, but such recovery is not observed with extracts from Cockayne's syndrome cells. This indicates that cells globally downregulate transcription in response to DNA damage, and that Cockayne's syndrome cells are defective not only for fast repair of the transcription-obstructing

SWI/SNF-LIKE COMPLEXES
Protein complexes that contain a subunit that is homologous to SNF2.

WD40 REPEAT
A protein motif used by a variety of proteins for protein–protein interactions.

ORTHOLOGUE
A homologue whose sequence and function has been conserved during evolution.

RING FINGER
Protein motif that is found in ubiquitin ligases.

RAD7 AND RAD16
Proteins that are required for global genome repair in yeast. They are very important for nucleotide excision repair of the non-transcribed strand of an active gene, but have only a minor role for repair on the transcribed strand/transcription-coupled repair.

Table 1 | **Factors required for TCR and GGR**

Human	Yeast	Function/protein motif
TCR		
CSA	Rad28	WD40 repeats
CSB	Rad26	Swi/Snf-like ATPase
XAB2	Syf1	Tetratricopeptide repeats
TFIIH	TFIIH	Kinase, 2 x helicase, RING finger
XPG	Rad2	DNA endonuclease
GGR		
?	Rad16	Snf2-like ATPase/RING finger
?	Rad17	Damaged DNA binding
UV-DDB	–	Damaged DNA binding
XPC	Rad4	Damaged DNA binding

?, homologue not identified; –, obvious homologue is absent.

lesion, but also for making the switch back to transcription after repair is completed.

Several different mechanisms might account for the observation that transcription is downregulated during DNA damage (FIG. 2). Mullenders and co-workers[59] found that the protein levels of most basal transcription factors are not affected by UV irradiation, but that the HYPOPHOSPHORYLATED RNAPII A-FORM specifically disappears from inactive extracts, and leaves only the hyperphosphorylated form (FIG. 2a). A non-phosphorylated RNAPII is required to enter the pre-initiation complexes that are formed at promoters, while hyperphosphorylation of the carboxy-terminal domain of the largest RNAPII subunit occurs at the subsequent transition to transcriptional elongation[60]. Significantly, RNAPII remains hyperphosphorylated in Cockayne's syndrome cells, whereas wild-type cells quickly recover normal levels of non-phosphorylated RNAPII (REF. 59). This implies that inactivation of RNAPII by phosphorylation causes the observed downregulation of transcription.

Another line of evidence indicates that the TATA-BINDING PROTEIN (TBP) might have a role in downregulating transcription in response to DNA damage[61]. *In vitro* experiments using UV-damaged DNA to compete with the transcription of non-damaged DNA templates showed that TBP can be sequestered by binding directly to damaged DNA, which reduces its availability for transcription. This inhibitory effect of damaged DNA on transcription can be overcome by adding extra TBPs, which indicates that TATA boxes and damaged DNA might compete for limiting amounts of the protein (FIG. 2b).

In a yeast *in vitro* system, NER and transcription were shown to compete for limiting amounts of the transcription/repair factor TFIIH[62]. The ability of NER to reduce the transcription efficiency of extracts was relieved by mutation of genes encoding basal NER factors, such as *RAD14*. Significantly, competition was lost if the TCR factor — *RAD26* — was absent, even though *rad26* extracts (in contrast to *rad14* extracts) repair damage efficiently. In this system, inhibition of transcription by repair was specifically relieved by the addition of purified holo-TFIIH — the transcription-proficient form of TFIIH[24] — but not by the addition of core TFIIH. These data indicate that Rad26 might enable the repair-proficient form of TFIIH to become available for transcription (FIG. 2c). A similar model has been proposed for the action of CSB[58].

NON-HOMOLOGOUS END-JOINING
Repair pathway in which severed DNA ends are re-joined. The prominent pathway for repairing double-stranded breaks in higher eukaryotic cells.

HYPOPHOSPHORYLATED RNAPII A-FORM
The carboxy-terminal domain (CTD) of the largest RNAPII subunit comprises 26–52 heptapeptide repeats (consensus Tyr-Ser-Pro-Thr-Ser-Pro-Ser). The RNAPII form that is hypophosphorylated on the CTD is called RNAPII-A, whereas the form that is multiply phosphorylated (hyperphosphorylated) on Ser2 and Ser5 of the repeat is called RNAPII-0.

TATA-BINDING PROTEIN
The TATA-binding protein (TBP) binds directly to the TATA sequence in the core promoter of a gene to initiate assembly of the general transcription factor machinery that enables RNAPII to transcribe the gene.

Mechanisms of TCR

To understand the mechanism of TCR, we must consider the molecular events that might occur during the process. Any model for TCR needs to explain how damage-stalled RNAPII can be dealt with in a manner that enables the obstructing lesions to be removed faster than non-obstructing lesions in the non-transcribed strand and elsewhere in the genome. Moreover, the models proposed for the mechanism of TCR are rarely mutually exclusive.

TCR is triggered by stalled RNAPII. Several lines of evidence indicate that a stalled polymerase is required to

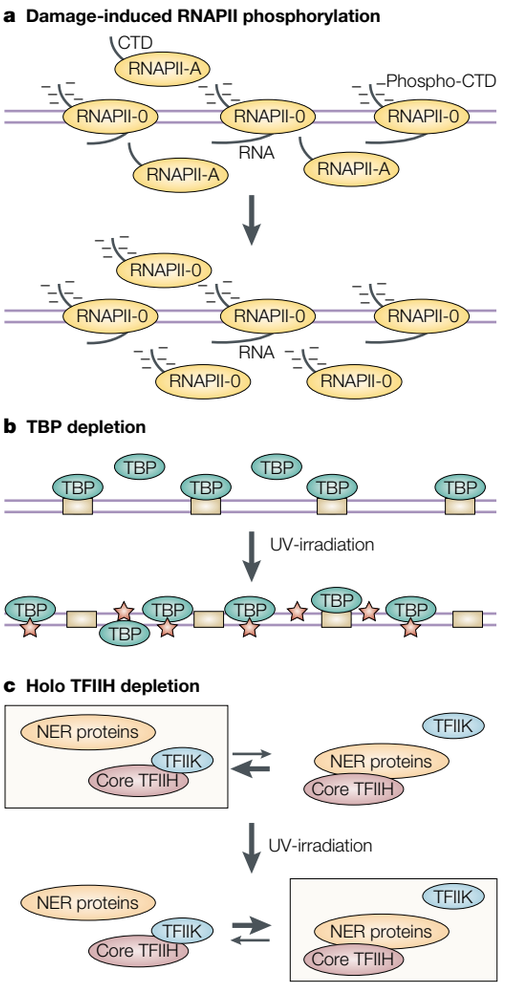


Figure 2 | **Models for the mechanism of transcriptional downregulation in response to DNA damage. a** | DNA damage results in hyperphosphorylation of RNA polymerase II (RNAPII), which prevents its entry into new pre-initiation complexes. **b** | DNA damage sequesters the TATA-binding protein (TBP), depleting it from use in transcription. **c** | DNA damage repair converts all the available transcription/repair factor TFIIH into the repair-proficient form, and precludes its use in transcription. NER, nucleotide excision repair pathway; TFIIK, the kinase component of holo TFIIH[24]; RNAPII-A, hypophosphorylated RNAPII form; RNAP-O, hyperphosphorylated RNAPII form. Stars and rectangles denote DNA lesions and TATA boxes, respectively.

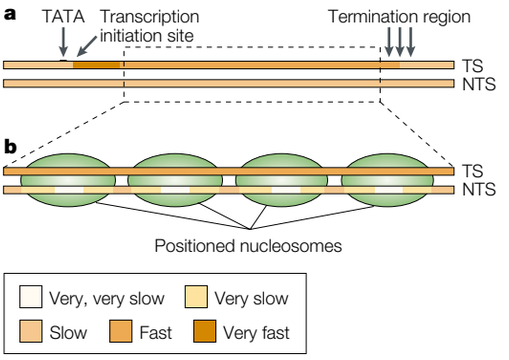


Figure 3 | **Speed of repair in an active gene.** Repair is invariably faster on the transcribed strand (TS) than on the non-transcribed strand (NTS), but variations in the rate of repair are evident between regions of the transcribed strand, as well as in the non-transcribed strand. **a** | In the transcribed strand, repair is very fast close to the transcription-initiation site. **b** | In the non-transcribed strand, but not in the transcribed strand, repair is markedly affected by the presence of nucleosomes. Note that the colour code is different between (**a**) and (**b**).

elicit TCR. In mammalian cells, TCR ceases immediately after the addition of the RNAPII inhibitor, α-amanitin[63]. A similar dependence of TCR on active RNAPII transcription was shown in yeast using a strain expressing a temperature-sensitive allele of *RPB1* (encoding the catalytic RNAPII subunit), which immediately ceases RNAPII transcription on changing to the non-permissive temperature[64].

The most persuasive evidence comes from work on the rates of TCR and GGR at nucleotide-level resolution in and around active genes[65–72] (FIG. 3a). These experiments showed that TCR is specifically observed in the transcribed region of an active gene. In the region immediately upstream from the transcription-initiation site (in the promoter and over the TATA-box), however, the repair rates are similar in the two complementary strands.

Interestingly, TCR in the yeast *URA3* gene first becomes Rad26-dependent 30–40 bases downstream from the site of transcription initiation. In the promoter, by contrast, repair depends on the GGR factor Rad7 (REF. 68). Similar results were obtained in human cells: here, rapid repair was observed close to the promoter of the *JUN* gene, whereas slower CSB- and CSA-dependent TCR was only observed further into the coding region of the gene[70–72]. TFIIH is thought to be released from RNAPII as the polymerase clears the promoter 30–60 bases downstream from the initiation site[73,74]. This might explain why Rad26/CSB are not required for fast TCR close to the point of transcriptional initiation, but only in the coding region further downstream where re-loading of TFIIH onto stalled RNAPII would be required. Indeed, *in vitro* binding studies with stalled RNAPII complexes, CSB and TFIIH indicate that CSB can bind to a stalled polymerase and recruit TFIIH to this complex[75,76]. CSB also associates with a proportion of RNAPII in cell extracts[35].

UBIQUITYLATION
The covalent linkage of the small protein ubiquitin to other proteins is essential for a variety of cellular processes. Ubiquitylation can mark proteins for rapid intracellular degradation, but can also have other consequences for the modified substrate protein.

UBIQUITIN LIGASE
The last enzyme in a cascade of ubiquitin-mobilizing proteins that together enable ubiquitylation. First, ubiquitin is activated by formation of a thioester bond to a ubiquitin-activating enzyme (E1). After transfer to a ubiquitin-conjugating enzyme (E2), ubiquitin is ligated to a protein substrate with the help of a ubiquitin protein ligase (E3). Several ubiquitin moieties can be added to the substrate, each time forming an isopeptide bond to an internal lysine residue.

Nucleosomes and stalled RNAPII inhibit repair. The nucleotide-resolution studies in yeast also uncovered other information about strand-specific NER. First, the rate of repair in the non-transcribed strand is independent of Rad26, but depends on the GGR factors Rad7 and Rad16, and, furthermore, is significantly affected by nucleosome structure[67] (FIG. 3b). The rate of repair on the non-transcribed strand is much slower at the cores of positioned nucleosomes than it is in inter-nucleosomal regions[67,77]. By contrast, the rate of repair on the transcribed strand is largely independent of the presence and position of these same nucleosomes[67].

By comparing the half-life of nearby lesions on the complementary strands (same position, opposite strands), Tijsterman and Brouwer showed[66] that, in the absence of the TCR factor Rad26, certain lesions in the inter-nucleosomal regions are repaired more slowly in the transcribed strand than in the non-transcribed strand. This indicates that NER at these (in principle) more accessible sites is obstructed by RNAPII in the transcribed strand — that is, that Rad26 (and, by extension, CSB) might overcome the obstacle to NER posed by the stalled polymerase itself.

If Rad26/CSB is indeed required to allow the displacement of stalled RNAPII from sites of DNA damage, this function might be bypassed if transcript elongation by RNAPII were less efficient, so that fewer polymerases became stalled at DNA lesions. Interestingly, deletion of the gene encoding the elongation factor Spt4 (REFS 78,79) reactivates TCR in cells lacking Rad26 and Rad16, and results in suppression of the UV-sensitivity of these cells[80]. Such suppression is also seen by mutation of genes encoding subunits of another elongation factor, Elongator[81] (unpublished observations from the Brouwer and Svejstrup laboratories), which indicates that efficient elongation might be counter-productive during DNA damage[82].

RNAPII displacement from sites of DNA damage. Almost all models for TCR incorporate the displacement of RNAPII from the lesion. Conceptually, such displacement could be achieved by several different mechanisms (FIG. 4). In *Escherichia coli*, the transcription-coupling repair factor (TCRF) dissociates the stalled polymerase from DNA using its DNA-dependent ATPase activity[83]. This has sometimes been used to argue that the eukaryotic CSB and Rad26 counterparts should work in the same manner (FIG. 4a). However, there is little sequence homology between TCRF and CSB/Rad26 (the homology is primarily in the ATPase domains).

Moreover, given that elongation is more complex in eukaryotes than prokaryotes (because, for example, of chromatin and co-transcriptional messenger RNA processing), there is no reason to expect that dissociation of damage-associated RNAPII elongation complexes is a conserved feature of TCR. Indeed, CSB cannot dissociate a damage-stalled RNAPII elongation complex *in vitro*[84]. It is also questionable whether factors such as human transcription release factor 2 — which can release both RNAPI and RNAPII from stalls at sites of

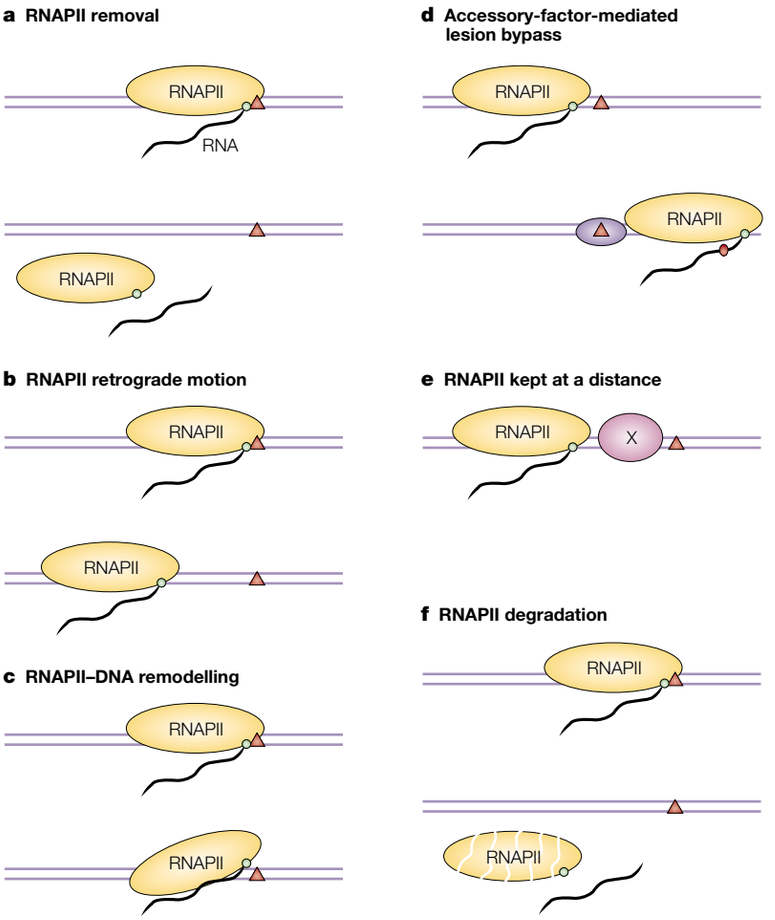


Figure 4 | **Models for transcription-coupled repair.** The models involve different modes of RNAPII displacement from the damaged DNA by CSB and other transcription-coupled repair (TCR) factors. **a** | RNAPII dissociation from the site of damage. Such dissociation could be achieved by use of the Swi/Snf-like activities of CSB or a transcription release factor. **b** | RNAPII is moved away from the damage. Again, such displacement could conceivably be achieved by use of the Swi/Snf-like activities of CSB, and/or the use of other activities, such as that of TFIIS. **c** | Remodelling of the damaged DNA–RNAPII interface by CSB. **d** | At certain lesions, bypass might be promoted by accessory factors. Again, the Swi/Snf-like activity of CSB is a candidate activity. **e** | Damage-binding factors arriving prior to RNAPII might ease repair by keeping the polymerase at a distance. **f** | Degradation of RNAPII stalled at a lesion is known to occur, but the importance for TCR has been questioned. Green circles indicates the active site of RNAPII. Red triangles denote a DNA lesion.

DNA damage *in vitro*[85] — and TFIIS, which can promote retrograde motion of damage-stalled RNAPII *in vitro*[5], are involved in TCR *in vivo*[86] (FIG. 4a and 4b).

By contrast, CSB/Rad26 probably use their Snf2-like ATPase activity to alter the molecular architecture of the damage-stalled RNAPII–DNA complex (FIG. 4c). CSB can enable RNAPII to add an extra nucleotide when stalled at a transcription-blocking DNA lesion[40], which indicates remodelling of the polymerase–DNA interface at the lesion. So these proteins might rearrange interactions between RNAPII and damaged DNA, in much the same way that Swi–Snf complexes do at the histone–DNA interface[87]. Alternatively, CSB/Rad26 might enable a more substantial displacement of the polymerase from the DNA lesion in cooperation with other factors.

The idea of CSB-mediated modification of the RNAPII–DNA interface as an important biochemical event during TCR could also explain why TCR is faster than GGR. A DNA lesion that is exposed in the context of DNA duplex pre-melted by RNAPII might be an excellent substrate for the repair machinery, which could explain why the general initiator of NER, XPC, is not required for TCR[88]. Alternative modes of RNAPII displacement from the site of damage, such as accessory-factor-mediated lesion bypass, and keeping RNAPII at a distance through damage-binding factors (FIG. 4d and e), might also be relevant, at least in certain situations.

Degradation of RNAPII in response to DNA damage. One consequence of UV irradiation is that RNAPII becomes hyperphosphorylated and, probably, incapable of transcriptional initiation. Another damage-induced modification with dramatic consequences is the UBIQUITYLATION of RNAPII (REF. 89). Ubiquitylation of the largest RNAPII subunit requires the highly conserved Rsp1 UBIQUITIN LIGASE[90,91] and results in rapid degradation of this polymerase subunit by the proteasome[92,93]. So, cells that are heavily UV-irradiated temporarily lose a considerable proportion of their RNAPII molecules[90,92].

Significantly, ubiquitylation and degradation of RNAPII do not occur efficiently in CSB or CSA cells[93,94], which prompted the suggestion that TCR might involve the degradation of RNAPII to allow its replacement by the repair machinery at sites of damage[92] (FIG. 4f). A recent study[95] has questioned the importance of RNAPII degradation for preferential repair by showing that the Rsp5 ubiquitin ligase activity does not seem to be required for TCR in yeast. Data from my laboratory indicate that degradation of damage-stalled RNAPII might be an alternative to TCR, rather than a prerequisite for it (E. Woudstra *et al.* unpublished observations).

Owing to its potentially detrimental effect, cells might consequently have evolved a dual response to persistently damage-stalled RNAPII: first, they attempt to quickly repair the obstructing damage by TCR so that transcription can immediately resume; or, alternatively, the stalled polymerase is degraded. Such RNAPII degradation would abort the transcription complex, but would at least allow other repair mechanisms to remove the lesion subsequently, and maintain genome integrity.

Concluding remarks

The cellular response to DNA damage at the level of the basal transcription reaction is remarkably complicated. Moreover, the molecular mechanism of TCR has remained elusive despite the many years since the process was discovered. So, it is probable that not all the proteins involved in TCR have been identified yet, and we certainly do not fully understand the molecular function(s) of CSA and CSB/Rad26.

Besides the precise molecular mechanism of TCR, remaining questions include: what are the mechanisms and factors that are required for RNAPII restart after the

obstructing lesion is removed? Does RNAPII simply bypass certain DNA lesions, perhaps with the help of accessory factors that transform it into a 'trans-lesion RNA polymerase', similar to the recently discovered trans-lesion DNA polymerases[96]? What are the molecular details of the mechanism that shuts down general transcription when there is DNA damage, yet allows transcriptional activation of damage-inducible genes[97]? Finding answers to these and other important questions should be a priority in the years to come.

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Online links

DATABASES

The following terms in this article are linked online to:
Encyclopedia of Life Sciences: http://www.els.net
DNA-repair-deficiency disorders
OMIM: http://www.ncbi.nlm.nih.gov/OMIM
trichothiodystrophy | XPA | XPF | XPG
***Saccharomyces* Genome Database:** http://genome-www.stanford.edu/Saccharomyces/
Rad1 | Rad2 | Rad3 | Rad4 | *RAD7* | Rad10 | Rad14 | *RAD16* | Rad23 | Rad25 | RAD26 | RAD28 | Rsp5 | Spt4 | URA3
Swiss-Prot: http://www.expasy.ch/
CSB | DDB2 | ERCC1 | FEN1 | HHR23B | RPB1 | T4 endonuclease V | XAB2 | XPA | XPB | XPC | XPD | XPF | XPG

FURTHER READING

Human DNA repair genes:
http://www.cgal.icnet.uk/DNA_Repair_Genes.html
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