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Nucleotide excision repair: variations on versatility

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Abstract. Nucleotide excision repair (NER) is one of the most versatile DNA repair systems. It can be subdivided into several, differentially regulated, subpathways: global genome repair (GGR), transcription-coupled repair (TCR), and transcription domain-associated repair (DAR). This review begins with a brief overview of the numerous types of DNA lesions handled by NER, and proceeds to describe in detail the molecular mechanisms of NER. It then addresses heterogeneities in NER activity in physiological situations (e.g. in differentiated cells) and explores the underlying regulatory mechanism. It then reviews several inherited diseases associated with NER deficiencies: xeroderma pigmentosum, Cockayne syndrome, trichothiodystrophy, UV-sensitive syndrome. It concludes by discussing several currently unresolved issues, relating either to the cause of the above diseases or to the mechanistic details of the various NER subpathways and of their regulation. (Part of a Multi-author Review)

Keywords. DNA repair, nucleotide excision repair, transcription-coupled repair, domain-associated repair, xeroderma pigmentosum, Cockayne syndrome, trichothiodystrophy, UV-sensitive syndrome, cellular differentiation.

Introduction

What makes nucleotide excision repair (NER) stand out among the various repair systems is probably its extreme versatility in the type of DNA lesions it can handle. This is achieved via a cunning strategy: rather than having a collection of specific enzymes each recognizing a different lesion, as is the case for base excision repair, NER senses the presence of a lesion through the distortion it causes to the DNA structure. The damaged strand is then identified and a short oligonucleotide spanning the lesion is excised, leaving a gap that can easily be filled by the replicative polymerases. In this way, many different lesions can be handled by a common set of enzymes.

Another interesting property of NER is that it can be coupled to transcription. Transcription-coupled repair (TCR) ensures that the transcribed strand of active genes is repaired with higher priority than the rest of the genome, probably by using RNA polymerase II (RNAPII) as a lesion sensor. TCR is covered in detail in a review by Silvia Tornaletti in this issue.

The term 'global genome repair' (GGR) was coined to design the component of NER that is not TCR. It encompasses NER in the non-coding parts of the genome, in silent genes, and in the non-transcribed strand of active genes. Transcription domain-associated repair (DAR) refers to the persistence of proficient NER in transcribed regions of the genome (i.e. on both strands of active genes), in cells that are otherwise NER-deficient. DAR will be discussed in more detail later in this review.

DNA lesions repaired by NER

As mentioned above, a wide variety of DNA lesions can be recognized and repaired by NER. Many of them are caused by chemicals that covalently bind a DNA base and form a so-called bulky DNA adduct. Examples include benzo[a]pyrene diol-epoxide, aromatic amines such as acetyl-aminofluorene, aflatoxin, nitrosamines such as MNNG, and 4-nitroquinoline oxide [1].

One common property of these chemicals is that they are present in the environment in a relatively harmless form, and absorbed as such with food (nitrosamines, aflatoxin), or in the air especially when polluted with cigarette smoke (benzo[a]pyrenes). These so-called proximate carcinogens are then activated by cellular metabolism, possibly in an attempt to improve their elimination, and unfortunately converted into highly reactive species. For instance, benzo[a]pyrene is turned into a 7,8-diol-9,10-epoxide form, while acetylaminofluorene is activated into an acetoxy form, and aflatoxin B1 into an oxide form. These 'ultimate carcinogens' are highly reactive and have a strong tendency to form bulky adducts on DNA [1].

Another class of chemicals handled by NER is crosslinking agents. These are compounds that have the ability to form two distinct covalent bonds with DNA, either on the same strand (intrastrand crosslinks) or across both strands (interstrand crosslinks) [2]. The latter pose a real challenge to NER since both strands are damaged. NER requires an intact strand to serve as a template to repair the other, and the repair of interstrand crosslinks thus necessitates a combination of NER with translesion synthesis and/or homologous recombination [3]. By contrast, intrastrand crosslinks are no different from bulky adducts, as far as NER is concerned, as long as they can be removed as part of a short oligonucleotide.

A classical example of crosslinking agent is cis-dichloro-diaminoplatin (cisplatin), which can form monoadducts (generally on guanine), intrastrand crosslinks in a GG or GNG context, and interstrand crosslinks [2]. Other examples include nitrogen mustards and psoralens. The latter are planar molecules that can intercalate between the stacked DNA bases. Once activated by ultraviolet (UV) A light, they can form covalent bonds with either DNA strand [4].

But the spectrum of lesions recognized by NER is not limited to chemicals. NER is critically important in the repair of UV-induced lesions. Short-wavelength UV light (UVB 290–320 nm, and UVC <290 nm) can cause the formation of covalent bonds between two adjacent pyrimidines on the same DNA strand. There are two main UV-induced lesions: cyclobutane pyrimidine dimers (CPDs) and (6–4)pyrimidine-pyrimidone photoproducts [(6–4)PPs], which differ in the number and position of these bonds. CPDs result from the formation of two bonds, one between carbons 4 of each pyrimidine and one between carbons 5, which form a 4-carbon ring, hence the name of the compound. Only one bond is formed in the case of (6–4)PPs, between carbon 6 of one pyrimidine and carbon 4 of the other, implying the transfer of the amino group present on position 6 in cytidine to position 5 of the other pyrimidine [5].

NER can also recognize several types of oxidative damage. As discussed by Robertson et al. in this issue, oxidized bases are generally repaired by base excision repair (BER). However, there are some oxidized species that pose a special challenge to BER: cyclopurines. These are characterized by the formation of a second bond between the purine and the deoxyribose in the DNA backbone, which makes them difficult to excise by BER enzymes, whereas they constitute perfectly good substrates for NER [6]. In addition, lipid peroxidation can yield some highly reactive products, such as malondialdehyde, which can form DNA adducts [7]. Again, these would pose a considerable challenge to base excision repair, whereas they are just another bulky adduct for NER.

Molecular mechanism of NER

The mechanism of NER is now well understood and has been reconstituted *in vitro* [8, 9]. It consists of several sequential steps (Fig. 1): lesion sensing, opening of a denaturation bubble, incision of the damaged strand, displacement of the lesion-containing oligonucleotide, gap filling, and ligation.

The XPC complex

There are two prerequisites for NER to be activated [10, 11]: there must be a distortion in the structure of the double helix, and there must be a chemical modification in the DNA (with the exception of rare chemicals, like ditercalinium, that intercalate non-covalently into DNA, and are mistaken for lesions by NER, triggering endless futile repair cycles [12]). The distortion-sensing component of NER in mammals consists of three subunits: XPC, HR23B, and centrin 2 [13]. XPC is a DNA binding protein with a strong preference (two to three orders of magnitude) for damaged DNA [14]. It can also bind to other DNA-distorting structures, such as small bubbles, that are not substrates for NER [10]. Recent data indicate that XPC binds preferentially to the stretch of single-stranded DNA that occurs in the non-damaged strand, opposite a lesion [15]. These two facts supports a model in which XPC performs the distortion-sensing role in NER, whereas another level of discrimination (i. e. verifying the presence of a lesion) is necessary for NER to go on. In addition, XPC is polyubiquitinated upon DNA damage, a reversible process that does not result in its degradation, but rather increases its affinity for DNA, damaged or not [16].

Most mammalian genomes contain two orthologs of the yeast protein Rad23, HR23A and HR23B. Both interact with XPC and increase its activity in NER, but normally it is HR23B which is found in association

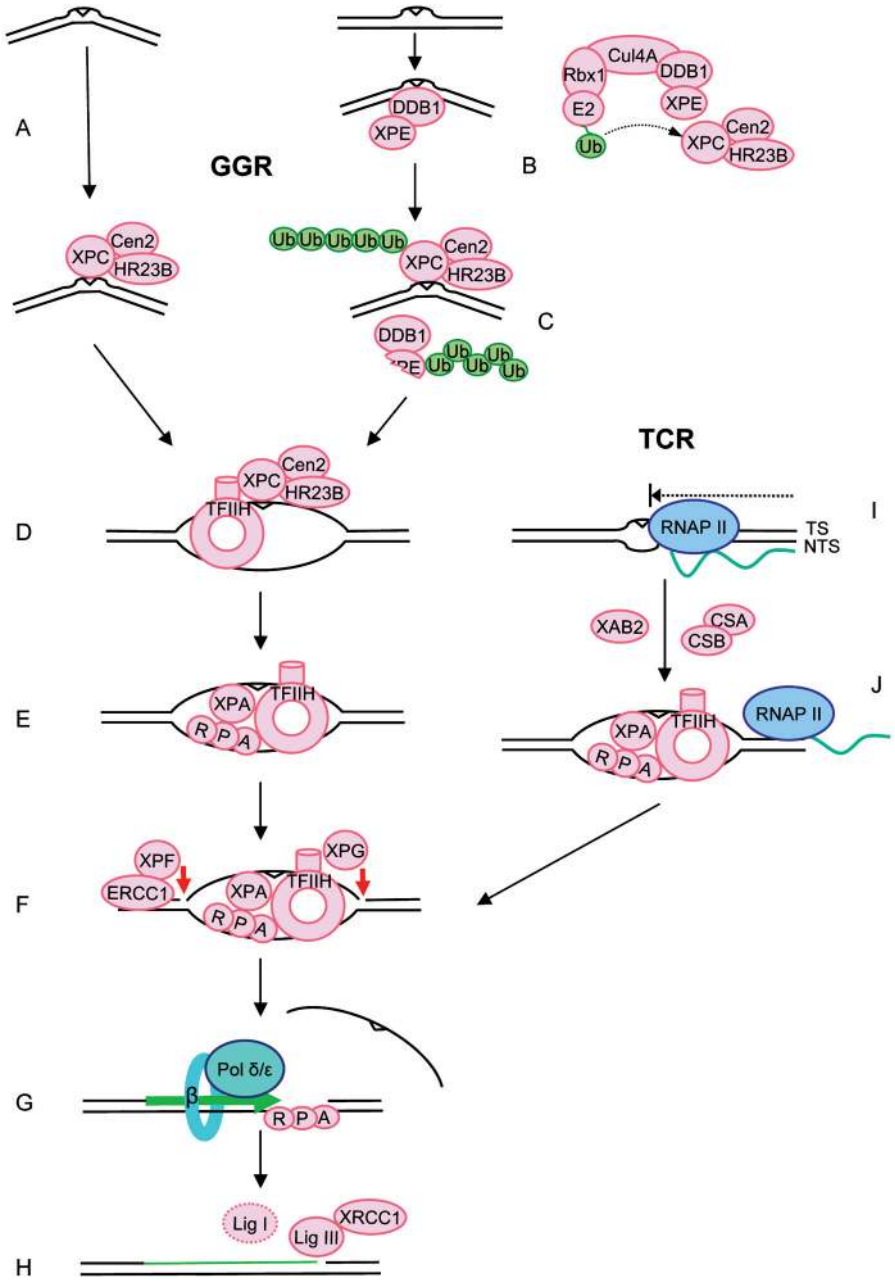

Figure 1. Mechanism of NER. (*A*) Many DNA lesions are detected directly by the XPC-HR23B-Cen2 complex, through the structural distortion that they cause in DNA. (*B*) Lesions that cause little distortion can be recognized by the DDB complex. DDB is also part of an E3 ubiquitin (Ub) ligase that poly-ubiquitinates XPC and XPE. (*C*) Handover mechanism: ubiquitination of XPC increases its affinity for DNA, whereas ubiquitination of XPE leads to its degradation. (*D*) TFIIH opens a denaturation bubble of about 30 nucleotides around the lesion. (*E*) The XPA-RPA complex joins in and displaces the XPC complex. RPA binds to single-stranded DNA; XPA may confirm the presence of a lesion and/or serve to identify the damaged strand. (*F*) XPG incises the damaged strand in 3′ of the lesion, ERCC1-XPF in 5′ of it. (*G*) An oligonucleotide spanning the lesion is displaced and the resulting gap is filled by DNA polymerase delta or epsilon, likely associated to PCNA (β). (*H*) The nick is sealed by ligase III, with a minor contribution of ligase I in replicating cells. (*I*) Transcription-coupled repair. RNA polymerase II (RNAPII) is stalled by lesions in the transcribed strand (TS) of active genes and attracts NER enzymes. (*J*) NER proceeds as for global genome repair (GGR), bypassing the XPC (and DDB) complexes but with a requirement for extra enzymes, CSA, CSB and XAB2, the function of which is not clear. This cartoon shows RNAPII backing up from the lesion to allow for repair, but there are other models.

with XPC [17]. There are about 10 times more HR23B molecules than XPC molecules in a given cell, implying that most of HR23B is not bound to XPC and may have a second function, distinct from NER [18]. Although the exact function of HR23A and HR23B in NER is not known, the fact that both Rad3 orthologs contain ubiquitin-like and ubiquitin-associated domains required for proficient NER [19] suggests that they play a role in controlling XPC ubiquitination.

The XPC-HR23B complex is almost systematically found in association with a third partner, centrin 2 [13]. Although its presence is not strictly required for NER (*in vitro* reactions work well without it), it stabilizes the complex and improves its NER activity.

The DDB complex

One problem with detecting DNA lesions through the distortion they impose to the double-helix is that not all lesions are equally distorting. Take the case of UV-induced lesions for instance. (6–4)PPs cause a strong kink in DNA and thus constitute very good substrates for NER, but this is not true for CPDs, which cause only a modest distortion and are thus very poor NER substrates [20]. This is where the DDB complex, a damaged DNA-binding heterodimer consisting of

DDB1 and DDB2/XPE, comes into play. As implied by its name, this complex has a high affinity for DNA damage, such as (6–4)PPs and CPDs [21]. It was proposed that, upon binding to a lesion, DDB induces a kink in the DNA which allows for the recruitment of the XPC complex [22]. This mechanism is not very important in the case of (6–4)PPs, which are readily recognized by the XPC complex alone, but is critical for proficient repair of CPDs.

A peculiarity of the DDB complex is that it can be part of a multi-subunit E3 ubiquitin ligase [23]. These enzymes comprise a scaffold, in this case the Cul4 protein, associated on one side with an E2 binding subunit (the RBX1 subunit) and on the other with a substrate recognition subunit, in our case the DDB complex. As a matter of fact, DDB1 can be part of two such E3 enzymes, one in association with DDB2/XPE, and one in association with CSA (an enzyme required for TCR) thereby providing different substrate specificities.

The CSA-containing complex has been shown to polyubiquitinate CSB (another TCR-specific enzyme) leading to its degradation [24]. By contrast, the DDB2-containing E3 enzyme polyubiquitinates XPC [16], a process that does not cause XPC degradation, but rather enhances its affinity for DNA. However, DDB2 is also ubiquitinated in the process, and this leads to its rapid degradation within a few hours of DNA damage. This probably constitutes a handover mechanism, which allows for switching from a tightly bound DDB complex, to a lower-affinity XPC complex. The DDB complex ubiquitinates XPC, thereby increasing its affinity for DNA, then self-destructs, ensuring that XPC remains in control [16, 25].

The TFIIH complex

The next step in the NER reaction is the opening of a denaturation bubble around the lesion. This is achieved by the general transcription factor TFIIH, a complex of no fewer than 10 subunits. TFIIH plays an important role in transcription initiation by RNAPII. For one thing, it participates in promoter opening via its XPB helicase subunit, which also facilitates promoter clearance by RNAPII [26]. In addition, three subunits of TFIIH (cyclin H, cdk7 and MAT1) form a so-called cyclin-activated kinase (CAK) complex, which (when part of TFIIH) phosphorylates the large subunit of RNAPII, allowing it to enter elongation mode [27]. The CAK complex works as a phosphorylation cascade: cyclin H being phosphorylated by external kinases phosphorylates Cdk7, which in turn phosphorylates its target. Aside from RNAPII, TFIIH can phosphorylate several nuclear receptors, such are the retinoic acid receptor or the estrogen receptor [28]. It should be mentioned that the CAK complex can exist independently of the rest of TFIIH, in which case it has a very different range of substrates [29]. It is thought that the substrate specificity is controlled by MAT1, which also mediates the interaction between the CAK complex and the core of TFIIH [30]. In the free CAK complex, MAT1 can be polyubiquitinated and partially cleaved, which is thought to be part of a substrate selection mechanism [31].

Aside from XPB, TFIIH contains another helicase subunit, XPD, of opposite polarity. Although XPD is not involved in transcription initiation, it plays a critical role in NER, by opening a bubble of denaturation around the lesion [32]. XPB is also required for this function, but strangely enough, it is the ATPase activity of XPB which is necessary, rather than its helicase activity [33].

XPB and XPD, together with five other subunits (p62, p52, p44, p34, and TTD-A), form a ring-shaped structure, to which the CAK complex is attached like a panhandle [34]. The precise function of all these subunits is not known, aside from the fact that they can stimulate or inhibit each other, and contribute to the stability of the complex. It is worth noting that the yeast ortholog of p44 possesses an E3 ubiquitin ligase activity, which is important in triggering a proper transcriptional response to DNA damage [35]. The mammalian p44 also contains a RING finger motif, typical of E3 enzymes, but it is not known whether it is indeed an E3 ligase.

The XPA complex

XPA is yet another protein that binds DNA with a slight preference for damaged DNA [36]. It is associated with the three subunits of the RPA heterotrimer, which is a single-strand DNA binding protein [37]. The exact role of the XPA complex is still not clear. Originally it was thought that it participated in lesion recognition together with the XPC complex, each complex accounting for part of the recognition process. However, recent data indicate that the XPA complex is recruited after TFIIH, rather than before it [38], and dislodges the XPC complex in the process [39].

A possible role for the XPA complex could be to identify the strand that carries the lesion [14]. This identification is necessary to ensure that it is the damaged strand that will be incised, rather than the undamaged one. However, it is also possible that this discrimination task is accomplished by TFIIH [40]. At any rate, the contribution of the XPA is absolutely required for NER.

The incision complexes

The next step in the NER reaction is the incision of the damaged strand at either end of the denaturation bubble. The cut on the 3′ side of the lesion is performed by the XPG endonuclease [41], whereas that on the 5′ side is carried on by the XPF-ERCC1 heterodimer [42].

XPG is a structure-specific endonuclease, with a preference for the single-strand to double-strand junction found at the end of a denaturation bubble [41]. Interestingly, XPG is structurally related to the FEN1 endonuclease that removes 'flaps' of displaced DNA in long-patch BER [43]. The presence of XPG is necessary for XPF-ERCC1 to incise the other end of the bubble, but the incision by XPG does not necessarily come first [44]. In other words, it is likely that XPG plays a structural role, in addition to its endonuclease activity. In accordance with this notion, it has recently been shown that XPG stabilizes TFIIH, and that its absence leads to the dissociation of the CAK complex and of the XPD subunit from the rest of TFIIH [45].

As for the XPF-ERCC1 complex, it is XPF that harbors the endonuclease activity [46]. The role of ERCC1 in the complex is not very clear, aside from the fact that it stabilizes XPF [47] (and reciprocally, XPF stabilizes ERCC1 [48]). ERCC1 contains a DNA binding domain, and may thus be responsible for bringing XPF into position, at the edge of the denaturation bubble. XPF also contains a DNA binding domain, similar to that of ERCC1, but it appears to be inactive. Conversely, ERCC1 contains a nuclease domain similar to that of XPF, but disrupted [49].

It is worth mentioning that the ERCC1-XPF complex is likely involved in several processes other than NER. Observations with knockout mice suggested that ERCC1 and XPF might play a role in recombination [50] and in telomere maintenance [51]. Experiments in *Saccharomyces cerevisiae* have also suggested that Rad1-Rad10 (the orthologs of ERCC1-XPF) serve as a backup in BER, to remove the 3′ phosphate residues generated by the AP lyase activity of some glycosylases [52]. Finally, the ERCC1-XPF dimer may be involved in the initial steps of repairing interstrand crosslinks, i.e. incising the first DNA strand, as well as in the NER event that eventually repairs the other strand [3].

Gap filling and ligation

The fragment excised by NER in mammals is about 25–30 nucleotides in length, depending on the lesion [53–55]. For any given lesion, there generally is a slight variation in size because the XPG and the ERCC1-XPF endonuclease do not always cut at exactly the same places. The resulting gap is likely filled by either of the replicative DNA polymerases delta and epsilon [56], associated with the 'sliding clamp' PCNA [57, 58].

Finally, the remaining nick needs to be sealed by a ligase. Until recently it was thought that ligase I carried out this task [59]; however, recent data indicate that it is mostly ligase III, together with its partner XRCC1, which concludes the NER process. Ligase I plays a minor role in actively replicating cells, but not in quiescent cells [60].

Heterogeneity of NER

Given the variety of DNA lesions that are handled exclusively by NER, one might think that this repair pathway should be ubiquitous within the organism. This is not the case, however, and it has been known since the 1980s that some cell types display very little NER activity. In human cells, this phenomenon was observed in neuroblastoma [61], neuroteratoma [62, 63], primary neurons [64], macrophages [65], and keratinocytes [66, 67]. It was also demonstrated in various animal models, such as rat striated muscle [68], rat myoblasts [69, 70], and 3T3-derived adipocytes [71] (see [64] for a comprehensive review).

A common characteristic of the above cell types is that they are terminally differentiated. One may thus reason that cells that will never again replicate their genome may dispense with the burden of repairing it. There are several problems with this somewhat naïve explanation, though.

Firstly, NER attenuation has been observed in cells that are quiescent but not terminally differentiated, such as growth-arrested mouse embryo fibroblasts [72], or quiescent B lymphocytes [Nouspikel, unpublished]. Since such cells must retain the ability to replicate their DNA, it would not make sense for them to dispense with repairing it: an accumulation of DNA lesions may prove fatal on re-entering the cell cycle [73].

Second and most importantly, DNA does not exist for the sole purpose of being replicated. It is meant to be transcribed, and an accumulation of lesions in transcribed genes is likely to impair transcription. Depending whether or not RNAPII is able to transcribe through the lesion, this may result in a lack in essential protein (for blocking lesions) or in the production of mutant proteins (for miscoding lesions), both potentially deleterious events. In addition, a stalled RNA polymerase was shown to be a strong signal to trigger apoptosis [74]. Thus, if cells were to attenuate NER at the global genome level, they should at least retain it at the level of active genes.

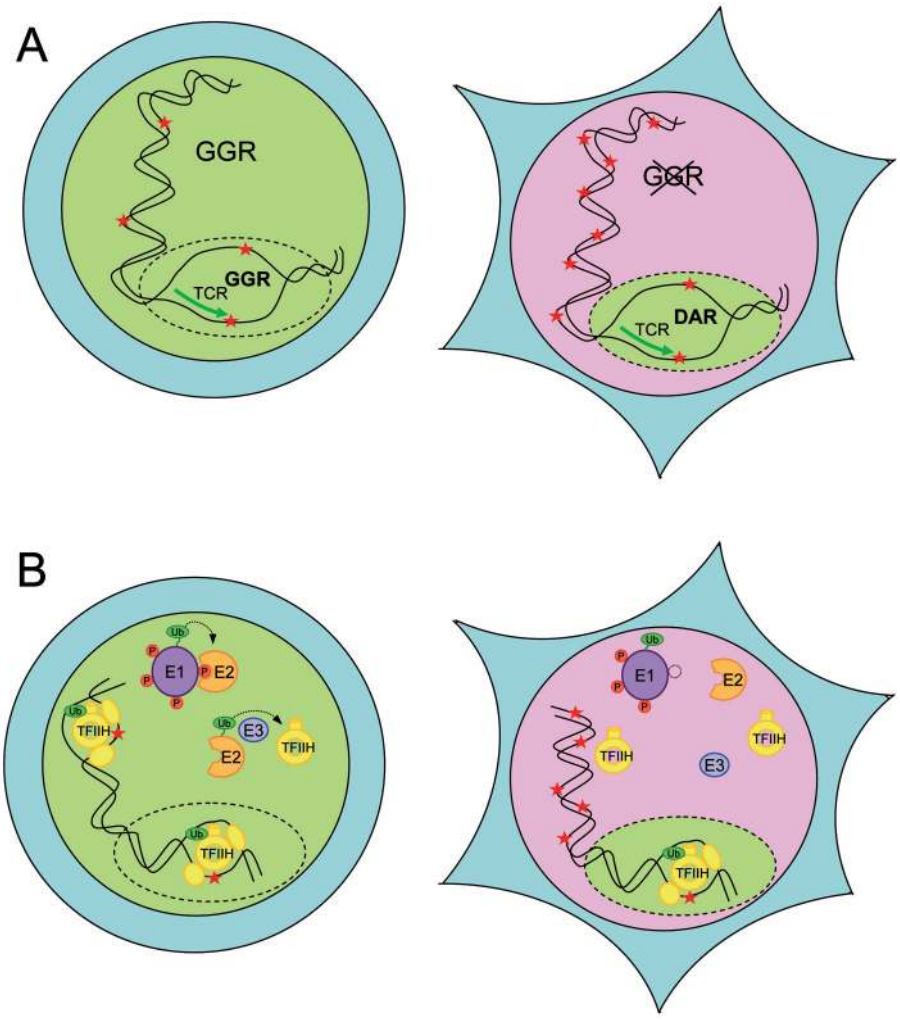

Figure 2. (*A*) The various subtypes of NER. In replicating cells (left) global genomic repair (GGR) occurs in the whole genome, while transcription-coupled repair (TCR) provides extraneous repair to the transcribed strand of active genes. In differentiated cells (right), GGR is downregulated but TCR persists. In addition, transcription domain-associated repair (DAR) ensures proficient repair of both strands within transcription factories (dotted circle). (*B*) Molecular mechanism of the above. In replicating cells, E1 is fully phosphorylated and transfers ubiquitin to the E2 enzyme used to ubiquitinate TFIIH, which renders it NER-proficient. In differentiated cells, E1 is partially dephosphorylated and unable to interact with some E2s. As a result, most of TFIIH is not ubiquitinated and inactive in GGR. DAR (and possibly TCR) is due to the remnants of ubiquitinated TFIIH being concentrated within transcription factories, thereby providing a repair-proficient environment for active genes.

DAR

This is indeed what we [62, 65, 75] and others [72] have observed: transcribed genes remain proficiently repaired in several cell types which display a strong attenuation of GGR (Fig. 2a). Proficient repair of the transcribed strand could be attributed to TCR, but this repair pathway does not operate on the non-transcribed strand. There must thus be another repair pathway that substitutes for GGR in the maintenance of the non-transcribed strand. We named this pathway DAR for transcription domain-associated repair [62]. In the past few years, we have accumulated evidence that DAR operates on both strands in active genes, including in regions of a gene that RNAPII does not reach [75], indicating that it cannot possibly rely on RNAPII to detect lesions in the DNA.

In addition, our small interfering RNA (siRNA) experiments have demonstrated that DAR depends on XPC, like GGR, but not on CSB, unlike TCR [75]. We thus believe that DAR is nothing more than the persistence of active NER in 'transcription factories', i. e. the nuclear subcompartments where transcription takes place. To understand the reasons for this persistence, we first needed to elucidate the mechanism of GGR attenuation.

Mechanism of NER attenuation

Since none of the NER genes appeared to be systematically underexpressed in terminally differentiated cells [62, 65], we postulated that some kind of post-translational modification may downregulate the activity of an NER factor. However, *in vitro* complementation assays between differentiated macrophages and the various XP groups failed to single out the NER enzyme potentially modified in macrophages [76].

We thus resorted to the large-scale purification of an activity from HeLa cells that complemented NER in macrophage extracts. The protein responsible for this complementation was unequivocally identified by mass spectrometry as the E1 ubiquitin-activating enzyme. The importance of E1 for proficient NER

was subsequently confirmed by supplementing macrophage extracts with purified rabbit E1, or with recombinant human E1 [76]. In addition, a mouse cell line bearing a thermosensitive allele of E1 was shown to be UV-sensitive at the restrictive temperature [77], and *in vitro* assays revealed a deficiency in NER that could be complemented by the addition of recombinant E1 [76].

Since E1 does not directly participate in NER, it is likely that an NER factor is activated by mono-ubiquitination, or by poly-ubiquitination with a type of chain (e.g. lysine 63) that does not yield to protein degradation. To identify the NER complex controlled in this way, we performed immunoprecipitation with anti-ubiquitin antibodies. Only the XPC complex and TFIIH were found in the pellets. Addition of TFIIH to macrophage extracts improved their NER capability, whereas addition of XPC-HR23 complex did not [76]. We thus concluded that TFIIH is the NER factor that is activated by ubiquitination. We do not currently know which subunit of TFIIH is ubiquitinated, but we have verified that it is neither XPB nor XPD.

TFIIH is a general transcription factor, and may thus be expected to be present in high amounts in transcription factories. Since differentiated cells retain some level of TFIIH ubiquitination [Nouspikel, unpublished], it is possible that this ubiquitinated TFIIH could be located preferentially in transcription factories. Thus, transcribing a gene would result in bringing it into a 'repair-rich' environment, ensuring proficient repair of either strand, even in regions of the gene that may not be actually transcribed. We believe that this might be the molecular basis for DAR (Fig. 2b).

Diseases caused by NER deficiencies

Not so surprisingly, given the large number of DNA lesions that can be repaired by NER and the number of genes involved, the clinical manifestations of deficits in NER can vary considerably. There are several genetic diseases that have been linked to mutations in genes involved in GGR or in TCR (Table 1).

Xeroderma pigmentosum

Xeroderma pigmentosum (XP) is a hereditary disease characterized by cutaneous symptoms in the sunlight-exposed area of the skin, resulting from the defect in repair of UV-induced lesions: skin atrophy, pigmentation anomalies, and most strikingly a very high incidence of skin cancer. These are mostly squamous cell carcinomas, although there are also basal cell carcinomas, melanomas, angiomas, and sarcomas [78].

In addition, XP patients display a higher propensity to internal cancers, mostly of the lung or gastro-intestinal tract, reflecting the role of NER in dealing with air pollutants and food carcinogens [79].

In addition, about 20 % of all XP patients suffer from neurological symptoms typical of neurodegeneracy [78, 80]. Given that the brain is sheltered from UV light by the skull and from many carcinogens by the blood-brain barrier, it is likely that these symptoms results from a lack in repair of oxidative DNA damage. Since most oxidative lesions, except for cyclopurines, are repaired by base excision repair, it is tempting to assume that the neurological symptoms in XP are due to an accumulation of cyclopurines in transcribed genes [6].

XP results from a deficiency in NER at the global genomic level [81, 82]. That is to say that mutations disabling components of the lesion sensing complexes, XPC or DDB, will also produce XP symptoms (although often milder than in other complementation groups), even though TCR is still active. There are 8 complementation groups in XP: XP-A though XP-G, plus a variant group XP-V. The latter results from the inactivation of the translesion polymerase eta, which is able to bypass CPDs in an essentially error-free manner [83]. It is thought that other, more error-prone, bypass polymerases take over in XP-V cells, resulting in the accumulation of mutations during DNA replication.

Whereas cancer can be attributed to an accumulation of mutations in the genome of actively replicating cells, the degenerative symptoms (e.g. skin atrophy or neuron death) likely result from the accumulation of lesions in transcribed genes, either because they cause the inactivation of essential genes, or because they trigger apoptosis (a stalled RNAPII constitutes a strong signal for apoptosis, if TCR cannot remove the lesion(s) in due time [74]).

Cockayne syndrome

Cockayne syndrome (CS) is a completely different kind of disease, although it may also result from mutations in some of the 'XP' genes. Mostly, it is a developmental disease, with a neurological impairment typical of white matter degeneracy. To be classified as CS a patient must display the following symptoms [84]:

- Profound growth failure, generally beginning in infancy, sometimes already present at birth.
- Neurodevelopmental and later neurological dysfunction, with evidence of white matter involvement.

Table 1. Diseases associated with mutations in NER genes.

Disease	Symptoms	Genes	Defective pathway
Xeroderma pigmentosum (XP)	Photosensitivity, skin and cornea anomalies, skin cancer, risk of internal cancers, neurodegeneracy (sometimes)	*XPC, XPE* *XPV* *XPA – XPG*	GGR Translesion DNA synthesis (Pol eta) NER
Cockayne syndrome (CS)	Growth failure, white matter disease, photosensitivity, ocular anomalies, deafness, dental caries, cachexia	*CSA, CSB* *XPB, XPD, XPG*	TCR NER All: Transcription? Repair of oxidative damage? Recycling?
Cerebro-occulo-facio-skeletal syndrome (COFS)	Resembles CS, with visceral anomalies (e.g. horseshoe kidney)	*CSB* *XPD, XPG* *ERCC1*	TCR NER NER (modest), other?
UV-sensitive syndrome (UVSS)	Photosensitivity	*CSB* unknown gene	TCR
Trichothiodystrophy (TTD), PIBIDS, IBIDS, BIDS	Photosensitivity, ichthyosis, brittle hair, intelligence impairment, decreased fertility, short stature	*TTDA, XPB, XPD*	Transcription? Sulfur-rich protein synthesis.

In addition, the patient should display at least three of the following:

- Cutaneous photosensitivity.
- Ocular abnormalities (such as cataracts, progressive pigmentary retinopathy, decreased lacrimation, mitotic pupils, etc.).
- Sensorineural deafness.
- Dental caries.
- A physical appearance of 'cachectic dwarf', resulting from a greater impairment in weight gain than in longitudinal growth.

The severity of the symptoms can be quite variable, depending on the complementation group and on the nature of the mutation. One can further subdivide CS patients into three groups: type I, the classical CS described above; type II, a very severe form, with major neurological symptoms, generally lethal in infancy; and a milder form of the disease [84, 85].

There also exists a clinical entity known as COFS (cerebro-occulo-facio-skeletal syndrome, sometimes called Pena-Shokeir type II syndrome), which is closely related to CS [86, 87]. The clinical presentation is reminiscent of that of CS, with growth retardation, severe neurological symptoms, ocular abnormalities, and progressive joint contractures, but it also comprises symptoms rarely observed in 'classical' CS, such as horseshoe kidneys and other visceral anomalies. At the cellular level, COFS is indistinguishable from CS, and often caused by mutations in the same genes.

There are five complementation groups in CS [80]. Two result from deficiencies in the *CSA* and *CSB* genes, which are specifically required for TCR but not for GGR. The remaining three, interestingly, are due to mutations in XP genes: *XPB*, *XPD*, and *XPG*. These patients generally suffer from severe (type II) CS. They may also display some concomitant XP symptoms, but the fact that they die in infancy, after spending most of their life in the hospital, does not allow for much sun exposure, nor enough time for the development of skin cancer.

So far, COFS patients have been found to carry mutations in the *CSB* gene [88], in the *XPD* [89] or *XPG* [90] genes, and the only patient with a mutation in the *ERCC1* gene also suffered from COFS [91].

It is interesting that different mutations in the same gene can give rise to clinical presentations that differ so widely. The panel of symptoms seem to closely correlate with the nature of the mutation. In the case of the *XPG* gene for instance, point mutations that inactivate the endonuclease active site give rise to XP, whereas promoter mutations or mutations that yield a severely truncated and unstable XPG protein give rise to CS [92]. What it implies is that the genes in question (*XPB*, *XPD,* and *XPG*) probably function in several pathways, and that different mutations may impair their function in one or the other pathway.

At the cellular level, CS-A and CS-B cells are deficient in TCR, while retaining NER at the global genome level [93, 94]. Cells from XP/CS patients are completely deficient in NER, whether coupled to transcription or not. The unique ERCC1 patient known had hypomorphic mutations disturbing the interaction between ERCC1 and XPF, resulting in reduced amounts of these two proteins (as their interaction stabilizes them), and in reduced NER activity [91]. Although the NER reduction was relatively modest, the symptoms were extremely severe, again suggesting a role for the ERCC1-XPF complex in a process distinct from NER.

In view of the above, one may be tempted to attribute CS to a lack in TCR. This explanation, however, does not hold because most XP patients (aside from the XP-C and XP-E groups) are also deficient in TCR, but not all display CS symptoms. There must thus be another metabolic process that is disturbed by mutations in the CS genes. This is a highly debated topic, and several concurrent models have been proposed, which I shall summarize later in this review.

UV-sensitive syndrome

UV-sensitive syndrome (UVSS) is characterized by mild photosensitivity in sun-exposed areas of the skin, with freckling and telangiectasia, but without the high propensity to skin cancer observed in XP patients [95]. Cells from UVSS patients display a phenotype reminiscent of that of CS cells: proficient NER at the global genomic level, but deficiencies in TCR and in recovery of RNA synthesis (which is inhibited after UV irradiation, but resumes within a few hours in normal cells) [96]. An important difference that may account for the disparity of the symptoms is that UVSS cells are proficient in the repair of oxidative damage, which is impaired in CS cells [97].

There are very few known UVSS patients, possibly because the symptoms are so mild that most patients are not detected. They fall into at least two complementation groups, one of which was shown to bear mutations in the *CSB* gene [98]. Since these patients did not suffer from CS (nor from COFS), we have another case in which different mutations in the same gene give rise to different diseases. Interestingly, UVSS cells display a complete absence of CSB proteins, whereas CS and COFS cells retain a mutated CSB. It thus appears that the presence of dysfunctional CSB disturbs cellular processes to a greater extent than its complete absence, thereby accounting for the severity of the symptoms in CS.

The identity of the gene mutated in the other UVSS complementation group is currently not known.

Trichothiodystropy

This syndrome is characterized by brittle hair, due to a deficiency in high molecular weight sulfur-rich proteins [99]. Under the microscope, the hair has a stripped, 'tiger-tail' appearance, which is typical of trichothiodystrophy (TTD). Other symptoms, such as photosensitivity, can often be present [100].

In fact, several clinical entities have been defined that are closely related to TTD: BIDS, IBIDS, and PIBIDS [101]. These acronyms are coined by combining the initials of those symptoms that can be observed:

– Photosensitivity
– Ichtyosis (scaly skin)
– Brittle hair
– Intellectual impairment
– Decreased fertility
– Short stature

At the molecular level, aside for a deficiency in the synthesis of high molecular weight sulfur-rich proteins, TTD cells also display impairment in NER, the severity of which varies from patient to patient. TTD patients fall into three complementation groups, which all correspond to mutations in subunits of TFIIH: TTD-A [102], XPB [103], and XPD [104].

Given that TFIIH operates in transcription as well as in NER, the above observations have prompted the suggestion that TTD may result from a subtle defect in transcription, rather than from the deficit in NER [105, 106]. This model is supported by the fact that TTD patients generally do not display the characteristics of XP, such as skin atrophy, abnormal pigmentation, or skin cancer (although there are exceptions to this rule). Defects in transcription were indeed observed in some XPD-mutated TTD cells, especially at temperatures higher than normal (41 °C), which correlates well with the fact that TTD patients often lose their hair after an episode of fever [107]. However, no such deficiency in transcription was observed in TTD-A cells.

Current unresolved issues

To conclude this review, I would like to come back and discuss in more detail some of the currently unresolved questions about NER.

Molecular causes of CS and TTD

Although CS and TTD cells display deficiencies in NER (limited to TCR in the case of CS), it is unlikely that this defect is the cause of the disease. For one thing, XP patients are NER-deficient, but most of them do not display the symptoms of CS or of TTD. Conversely, TTD patients and most CS patients do not display the symptoms of XP. This strongly suggests that CS and TTD are caused by defects in (a) pathway(s) not affected in XP. The question is, of course, what this pathway could be.

This is a highly debated topic, and several models have been put forward. I shall only summarize them briefly here; see [108] for a more complete description.

1) CS and TTD may be caused by subtle defects in transcription [105]. Such defects have to be very minor to allow for survival, but could disturb protein synthesis sufficiently to produce the developmental defects observed in CS, or the sulfur deficiency observed in TTD [106]. The basis for this theory is that TTD is caused by mutations in subunits of TFIIH, which is a general transcription factor. One can easily envision that these mutations would perturb the transcriptional activity of TFIIH, as well as inactivating its NER abilities. The situation is less clear in the case of CS, since only two complementation groups have mutations in subunits of TFIIH (interestingly, the same ones that are mutated in TTD: XPB and XPD). The other NER components that can cause CS when mutated, CSA, CSB, XPG, and ERCC1 (if we consider COFS as a variant of CS), are not part of TFIIH. The proponents of this model contend that these proteins interact with TFIIH and that their absence or abnormality may destabilize TFIIH. Indeed, reduced transcriptional activity was observed in CSB cells [109], and it was recently shown that TFIIH falls apart in XPG-null cells, in which the CAK complex and the XPD subunit dissociate from the core of TFIIH [45].

2) Alternatively, CS may be caused be problems with recycling the components used by TCR [110]. For instance, TFIIH might exist in a 'transcription' conformation and in an 'NER' conformation. The CS proteins may be responsible for returning TFIIH into transcription mode after TCR is complete. If this does not happen, TFIIH could become unavailable for transcription.

A variant on this model proposes that CS results from the fact that a stalled RNAPII masks the DNA lesion within a 35 nucleotide 'footprint' [111], and may thereby prevent its repair [112]. The role of the CS proteins could be to cause RNAPII to move back away from the lesion [111], to abort transcription [113], or to change conformation [114], so as to make the site of damage available to NER enzymes.

3) Yet another school of thought postulates that TCR is not limited to NER substrates, but also happens with some forms of oxidative damage (classically handled

by BER) [115]. CS patients would be deficient in both TC-NER and TC-BER, with the latter deficiency causing the CS symptoms. By contrast, XP patients are deficient in GGR and TC-NER, but would remain proficient in TC-BER, giving rise to a very different clinical presentation [116].

We only have indirect evidence in support of this hypothesis, mostly based on *in vitro* experiments [117, 118], or on transfection of plasmids bearing oxidative lesions [97]. We are unfortunately lacking a TCR assay, similar to the one developed in the Hanawalt laboratory for CPDs [119], that would detect oxidative lesions in a gene-specific and strand-specific manner. One major technical obstacle is the difficulty to induce a high enough amount of oxidative lesions in the DNA without killing the cell, and thus precluding repair. Most oxidative agents have a wide spectrum of action, and affect lipids and proteins as well as DNA, which contributes to their toxicity. Some experimental compounds have been shown to have a much narrower activity, and were used for instance to check for TCR of 8-oxoguanine in the genome (no strand bias was observed in that case [120]). Unfortunately these by-products from the pharmaceutical industry exist only in extremely limited supply and often are not available anymore.

Mechanism of TCR

Even though TCR was discovered in the 1980s [121, 122], its molecular mechanism remains largely unclear. We are reasonably confident that lesion detection is performed by RNAPII, in the course of transcribing an active gene. In support of this model, only blocking lesions give rise to TCR, and lesions in the non-transcribed strand, which do not affect RNAPII, are not repaired by TCR [123].

What happens next is less clear. For instance, how does a stalled RNAPII recruit NER enzymes? It is known that TFIIH dissociates upon promoter clearance and does not travel along with RNAPII. It must thus be brought back one way or another.

Another question stems from the observation that the footprint of a stalled RNAPII encompasses the lesion, and protects it against various nucleases [111]. So how do NER enzymes manage to access the lesion to repair it? Does RNAPII back up temporarily [111]? Does it change conformation to unmask the lesion [114]? Does it abort transcription and fall off the DNA [112]? Or is it ubiquitinated and degraded [113]? Each model has its proponents and detractors, and it is quite possible that more than one model is right.

The interested reader will find an in depth discussion of TCR in the review by Silvia Tornaletti in this issue.

Mechanistic details of NER

Although the molecular mechanism of NER has been largely elucidated, there remain a few grey areas. For instance, we do not know how the NER enzymes decide which strand to incise. Since the denaturation bubble opened by TFIIH is symmetrical, XPG could cut it at either end, on opposite strands. The same is true for the XPF-ERCC1 complex.

There must therefore be a mechanism to identify the damaged strand and target the incision complexes to it. We do not know how this mechanism operates, but we can make a number of educated guesses as to which NER sub-complex is involved.

One good candidate is TFIIH, which opens a denaturation bubble around the lesion, although this is not its sole activity since substrates in which a region of mismatch is created around the lesion still require TFIIH to be repaired [124]. It was shown that XPD is the only helicase active in opening the denaturation bubble, although the ATPase activity of XPB is also required [33]. Since helicases work in a processive manner, XPD will have to move across the lesion while opening the denaturation bubble. During this process, it is thus in an ideal position to confirm the presence of a lesion and determine in which strand it sits.

The other candidate is the XPA-RPA complex, mainly because its exact role in NER is not determined. XPA displays an affinity for damaged DNA [36], but so does XPC, which comes into play before XPA [39]. It is thus unlikely that the DNA binding activity of XPA is required for lesion detection. This conclusion is reinforced by the fact the XP-A cells are deficient in TCR, even though lesion detection is performed by RNAPII during TCR [125].

One possible role for XPA may thus be to identify the damaged strand. It may be assisted in the task by its partner RPA, a single-strand DNA binding protein [37] that will thus tend to bind to the denatured DNA of the undamaged strand in the bubble opened by TFIIH.

Another possible, although conceptually similar, role for XPA would be to verify the presence of a lesion. It is known that DNA anomalies such as small bubbles caused by mismatches cause enough distortion in the DNA to be recognized by the XPC complex. Yet, they are not incised by the XPG and ERCC1/XPF complex [10]. It ensues that NER must possess a way to determine whether DNA has indeed been damaged. Good candidates for this 'chemical modification' sensor would be the XPA complex and TFIIH, for the same reasons discussed above.

Regulation of NER

In bacteria, most NER enzymes are inducible by DNA damage [126], but this is not the case in higher organisms. In mammals, *XPE* is virtually the only NER gene to be induced by DNA damage, and the accumulation of the DDB2/XPE protein is slow enough that it only occurs after most of the damage has been repaired [127]. The induction of this regulatory subunit of DDB may thus occur as a preventive mechanism in case of further damage, or it may implement a 'finishing touch' function to make sure that the last remaining lesions will be removed from the genome. It may also be a way of replacing the amount of DDB2/XPE that has been ubiquitinated and degraded in the process of activating XPC.

In mammals NER appears to be mostly regulated by post-translational modifications and by protein-protein interactions. For instance, we have seen that poly-ubiquitination of XPC increases it NER efficiency, whereas poly-ubiquitination of DDB2/XPE leads to its degradation [25]. On the other hand, interaction of XPC with HR23B and Cen2 also increases its efficiency [13, 17], and DDB2/XPE can be found inside a multi-subunit E3 enzyme [23].

Similarly, the activity of TFIIH can be modulated by interactions with other NER enzymes, as well as by post-translational modifications. For instance, phosphorylation of the XPB subunit was shown to inhibit NER, by preventing the incision step by ERCC1-XPF [128]. And we have discussed above our evidence that the ubiquitination of TFIIH is required for GGR, and possibly TCR too [76].

There remain many questions regarding the latter regulatory pathway, though. Is it at work in cell types other than terminally differentiated cells? What is the E2 involved? Are there other E2s sensitive to the phosphorylation state of E1? Are there other proteins, the ubiquitination of which is controlled in this manner? What subunit(s) of TFIIH is/are ubiquitinated? Does ubiquitination (or lack of) affect the function of TFIIH in TCR, and in transcription? Can TFIIH be de-ubiquitinated, and if so by which enzyme? We are currently addressing some of these issues in our laboratory.

Conclusion

At the end of this review, I hope to have convinced the reader of the importance of NER as a DNA repair pathway. Although there remain enough questions to provide us with plenty of work in the forthcoming years and to generate friendly arguments among our colleagues, the mechanistic details of this pathway are largely understood. The generic nature of the NER mechanism accounts for its extreme versatility in terms of the DNA lesions repaired, whereas its various subpathways (GGR, TCR, and DAR) allow for cell-to-cell variations and for differential repair efficiency according to the region of the genome.

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1 Wogan, G. N., Hecht, S. S., Felton, J. S., Conney, A. H. and Loeb, L. A. (2004) Environmental and chemical carcinogenesis. Semin. Cancer Biol. 14, 473–486.
2 Trimmer, E. E. and Essigmann, J. M. (1999) Cisplatin. Essays Biochem. 34, 191–211.
3 Kuraoka, I., Kobertz, W. R., Ariza, R. R., Biggerstaff, M., Essigmann, J. M. and Wood, R. D. (2000) Repair of an interstrand DNA cross-link initiated by ERCC1-XPF repair/recombination nuclease. J. Biol. Chem. 275, 26632–26636.
4 Hearst, J. E., Isaacs, S. T., Kanne, D., Rapoport, H. and Straub, K. (1984) The reaction of the psoralens with deoxyribonucleic acid. Q. Rev. Biophys. 17, 1–44.
5 Pfeifer, G. P. (1997) Formation and processing of UV photoproducts: effects of DNA sequence and chromatin environment. Photochem. Photobiol. 65, 270–283.
6 Brooks, P. J. (2007) The case for 8,5′-cyclopurine-2′-deoxynucleosides as endogenous DNA lesions that cause neurodegeneration in xeroderma pigmentosum. Neuroscience 145, 1407–1417.
7 Johnson, K. A., Fink, S. P. and Marnett, L. J. (1997) Repair of propanodeoxyguanosine by nucleotide excision repair *in vivo* and *in vitro*. J. Biol. Chem. 272, 11434–11438.
8 Aboussekhra, A., Biggerstaff, M., Shijvi, M. K. K., Vilpo, J. A., Moncollin, V., Podust, V. N., Protic, M., Hubscher, U., Egly, J. M. and Wood, R. D. (1995) Mammalian DNA nucleotide excision repair reconstituted with purified protein components. Cell 80, 859–868.
9 Mu, D., Park, C. H., Matsunaga, T., Hsu, D. S., Reardon, J. T. and Sancar, A. (1995) Reconstitution of human DNA repair excision nuclease in a highly defined system. J. Biol. Chem. 270, 2415–2418.
10 Sugasawa, K., Okamoto, T., Shimizu, Y., Masutani, C., Iwai, S. and Hanaoka, F. (2001) A multistep damage recognition mechanism for global genomic nucleotide excision repair. Genes Dev. 15, 507–521.
11 Hess, M. T., Schwitter, U., Petretta, M., Giese, B. and Naegeli, H. (1997) Bipartite substrate discrimination by human nucleotide excision repair. Proc. Natl. Acad. Sci. USA 94, 6664–6669.
12 Gao, Q., Williams, L. D., Egli, M., Rabinovich, D., Chen, S. L., Quigley, G. J. and Rich, A. (1991) Drug-induced DNA repair: X-ray structure of a DNA-ditercalinium complex. Proc. Natl. Acad. Sci. USA 88, 2422–246.
13 Araki, M., Masutani, C., Takemura, M., Uchida, A., Sugasawa, K., Kondoh, J., Ohkuma, Y. and Hanaoka, F. (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, 18665–11672.
14 Sugasawa, K., Ng, J. M., Masutani, C., Iwai, S., van der Spek, P. J., Eker, A.P., Hanaoka, F., Bootsma, D. and Hoeijmakers, J. H. (1998) Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. Mol. Cell 2, 223–232.
15 Maillard, O., Solyom, S. and Naegeli, H. (2007) An aromatic sensor with aversion to damaged strands confers versatility to DNA repair. PLoS Biol. 5, e79.

16 Sugasawa, K., Okuda, Y., Saijo, M., Nishi, R., Matsuda, N., Chu, G., Mori, T., Iwai, S., Tanaka, K., Tanaka, K. and Hanaoka, F. (2005) UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. Cell 121, 387–400.
17 Sugasawa, K., Ng, J. M., Masutani, C., Maekawa, T., Uchida, A., van der Spek, P. J., Eker, A. P., Rademakers, S., Visser, C., Aboussekhra, A., Wood, R. D., Hanaoka, F., Bootsma, D. and Hoeijmakers, J. H. (1997) Two human homologs of Rad23 are functionally interchangeable in complex formation and stimulation of XPC repair activity. Mol. Cell. Biol. 17, 6924–6931.
18 van der Spek, P. J., Eker, A., Rademakers, S., Visser, C., Sugasawa, K., Masutani, C., Hanaoka, F., Bootsma, D. and Hoeijmakers, J. H. (1996) XPC and human homologs of RAD23: intracellular localization and relationship to other nucleotide excision repair complexes. Nucleic Acids Res. 24, 2551–2559.
19 Watkins, J. F., Sung, P., Prakash, L. and Prakash, S. (1993) The *Saccharomyces cerevisiae* DNA repair gene RAD23 encodes a nuclear protein containing a ubiquitin-like domain required for biological function. Mol. Cell. Biol. 13, 7757–7765.
20 Kim, J. K., Patel, D. and Choi, B. S. (1995) Contrasting structural impacts induced by cis-syn cyclobutane dimer and (6–4) adduct in DNA duplex decamers: implication in mutagenesis and repair activity. Photochem. Photobiol. 62, 44–50.
21 Payne, A. and Chu, G. (1994) Xeroderma pigmentosum group E binding factor recognizes a broad spectrum of DNA damage. Mutat. Res. 310, 89–102.
22 Tang, J. and Chu, G. (2002) Xeroderma pigmentosum complementation group E and UV-damaged DNA-binding protein. DNA Repair 1, 601–616.
23 Groisman, R., Polanowska, J., Kuraoka, I., Sawada, J., Saijo, M., Drapkin, R., Kisselev, A. F., Tanaka, K. and Nakatani, Y. (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, 357–367.
24 Groisman, R., Kuraoka, I., Chevallier, O., Gaye, N., Magnaldo, T., Tanaka, K., Kisselev, A. F., Harel-Bellan, A. and Nakatani, Y. (2006) CSA-dependent degradation of CSB by the ubiquitin-proteasome pathway establishes a link between complementation factors of the Cockayne syndrome. Genes Dev. 20, 1429–1434.
25 Sugasawa, K. (2006) UV-induced ubiquitylation of XPC complex, the UV-DDB-ubiquitin ligase complex, and DNA repair. J. Mol. Histol. 37, 189–202.
26 Bradsher, J., Coin, F. and Egly, J. M. (2000) Distinct roles for the helicases of TFIIH in transcript initiation and promoter escape. J. Biol. Chem. 275, 2532–2538.
27 Hirose, Y. and Ohkuma, Y. (2007) Phosphorylation of the C-terminal domain of RNA polymerase II plays central roles in the integrated events of eucaryotic gene expression. J. Biochem. (Tokyo) 141, 601–608.
28 Keriel, A., Stary, A., Sarasin, A., Rochette-Egly, C. and Egly, J. M. (2002) XPD mutations prevent TFIIH-dependent transactivation by nuclear receptors and phosphorylation of RARalpha. Cell 109, 125–135.
29 Rossignol, M., Kolb-Cheynel, I. and Egly, J. M. (1997) Substrate specificity of the cdk-activating kinase (CAK) is altered upon association with TFIIH. EMBO J. 16, 1628–1637.
30 Busso, D., Keriel, A., Sandrock, B., Poterszman, A., Gileadi, O. and Egly, J. M. (2000) Distinct regions of MAT1 regulate cdk7 kinase and TFIIH transcription activities. J. Biol. Chem. 275, 22815–22823.
31 He, Q., Peng, H., Collins, S. J., Triche, T. J. and Wu, L. (2004) Retinoid-modulated MAT1 ubiquitination and CAK activity. FASEB J. 18, 1734–1736.
32 Winkler, G. S., Araujo, S. J., Fiedler, U., Vermeulen, W., Coin, F., Egly, J. M., Hoeijmakers, J. H., Wood, R. D., Timmers, H. T. and Weeda, G. (2000) TFIIH with inactive XPD helicase functions in transcription initiation but is defective in DNA repair. J. Biol. Chem. 275, 4258–4266.
33 Coin, F., Oksenych, V. and Egly, J. M. (2007) Distinct roles for the XPB/p52 and XPD/p44 subcomplexes of TFIIH in damaged DNA opening during nucleotide excision repair. Mol. Cell 26, 245–256.
34 Schultz, P., Fribourg, S., Poterszman, A., Mallouh, V., Moras, D. and Egly, J. M. (2000) Molecular structure of human TFIIH. Cell 102, 599–607.
35 Takagi, Y., Masuda, C. A., Chang, W. H., Komori, H., Wang, D., Hunter, T., Joazeiro, C. A. and Kornberg, R. D. (2005) Ubiquitin ligase activity of TFIIH and the transcriptional response to DNA damage. Mol. Cell 18, 237–243.
36 Jones, C. J. and Wood, R. D. (1993) Preferential binding of the xeroderma pigmentosum group A complementing protein to damaged DNA. Biochemistry 32, 12096–23104.
37 Matsuda, T., Saijo, M., Kuraoka, I., Kobayashi, T., Nakatsu, Y., Nagai, A., Enjoji, T., Masutani, C., Sugasawa, K., Hanaoka, F. Yasui, A. and Tanaka, K. (1995) DNA repair protein XPA binds replication protein A (RPA). J. Biol. Chem. 270, 4152–4157.
38 Volker, M., Mone, M. J., Karmakar, P., van Hoffen, A., Schul, W., Vermeulen, W., Hoeijmakers, J. H., van Driel, R., van Zeeland, A. A. and Mullenders, L. H. (2001) Sequential assembly of the nucleotide excision repair factors in vivo. Mol. Cell 8, 213–224.
39 Hey, T., Lipps, G., Sugasawa, K., Iwai, S., Hanaoka, F. and Krauss, G. (2002) The XPC-HR23B complex displays high affinity and specificity for damaged DNA in a true-equilibrium fluorescence assay. Biochemistry 41, 6583–6587.
40 Wood, R. D. (1999) DNA damage recognition during nucleotide excision repair in mammalian cells. Biochimie 81, 39–44.
41 O'Donovan, A., Davies, A. A., Moggs, J. G., West, S. C. and Wood, R. D. (1994) XPG endonuclease makes the 3′ incision in human DNA nucleotide excision repair. Nature 371, 432–435.
42 Mu, D., Hsu, D. S. and Sancar, A. (1996) Reaction mechanism of human DNA repair excision nuclease. J. Biol. Chem. 271, 8285–8294.
43 Constantinou, A., Gunz, D., Evans, E., Lalle, P., Bates, P. A., Wood, R. D. and Clarkson, S. G. (1999) Conserved residues of human XPG protein important for nuclease activity and function in nucleotide excision repair. J. Biol. Chem. 274, 5637–5648.
44 Wakasugi, M., Reardon, J. T. and Sancar, A. (1997) The noncatalytic function of XPG protein during dual incision in human nucleotide excision repair. J. Biol. Chem. 272, 16030–16034.
45 Ito, S., Kuraoka, I., Chymkowitch, P., Compe, E., Takedachi, A., Ishigami, C., Coin, F., Egly, J. M. and Tanaka, K. (2007) XPG stabilizes TFIIH, allowing transactivation of nuclear receptors: implications for Cockayne syndrome in XP-G/CS patients. Mol. Cell 26, 231–243.
46 Enzlin, J. H. and Scharer, O. D. (2002) The active site of the DNA repair endonuclease XPF-ERCC1 forms a highly conserved nuclease motif. EMBO J. 21, 2045–2053.
47 Houtsmuller, A. B., Rademakers, S., Nigg, A. L., Hoogstraten, D., Hoeijmakers, J. H. and Vermeulen, W. (1999) Action of DNA repair endonuclease ERCC1/XPF in living cells. Science 284, 958–961.
48 Biggerstaff, M., Szymkowski, D. E. and Wood, R. D. (1993) Co-correction of the ERCC1, ERCC4 and xeroderma pigmentosum group F DNA repair defects in vitro. EMBO J. 12, 3685–3692.
49 Nishino, T., Komori, K., Ishino, Y. and Morikawa, K. (2003) X-ray and biochemical anatomy of an archaeal XPF/Rad1/Mus81 family nuclease: similarity between its endonuclease domain and restriction enzymes. Structure 11, 445–457.
50 Sargent, R. G., Meservy, J. L., Perkins, B. D., Kilburn, A. E., Intody, Z., Adair, G. M., Nairn, R. S. and Wilson, J. H. (2000) Role of the nucleotide excision repair gene ERCC1 in

formation of recombination-dependent rearrangements in mammalian cells. Nucleic Acids Res. 28, 3771–3778.
51 Zhu, X. D., Niedernhofer, L., Kuster, B., Mann, M., Hoeijmakers, J. H. and de Lange, T. (2003) ERCC1/XPF removes the 3′ overhang from uncapped telomeres and represses formation of telomeric DNA-containing double minute chromosomes. Mol. Cell 12, 1489–1498.
52 Guillet, M. and Boiteux, S. (2002) Endogenous DNA abasic sites cause cell death in the absence of Apn1, Apn2 and Rad1/Rad10 in *Saccharomyces cerevisiae*. EMBO J. 21, 2833–2841.
53 Moggs, J. G., Yarema, K. J., Essigmann, J. M. and Wood, R. D. (1996) Analysis of incision sites produced by human cell extracts and purified proteins during nucleotide excision repair of a 1,3-intrastrand d(GpTpG)-cisplatin adduct. J. Biol. Chem. 271, 7177–7186.
54 Matsunaga, T., Mu, D., Park, C. H., Reardon, J. T. and Sancar, A. (1995) Human DNA repair excision nuclease. Analysis of the roles of the subunits involved in dual incisions by using anti-XPG and anti-ERCC1 antibodies. J. Biol. Chem. 270, 20862–2089.
55 Svoboda, D. L., Taylor, J. S., Hearst, J. E. and Sancar, A. (1993) DNA repair by eukaryotic nucleotide excision nuclease. Removal of thymine dimer and psoralen monoadduct by HeLa cell-free extract and of thymine dimer by *Xenopus laevis* oocytes. J. Biol. Chem. 268, 1931–1936.
56 Popanda, O. and Thielmann, H. W. (1992) The function of DNA polymerases in DNA repair synthesis of ultraviolet-irradiated human fibroblasts. Biochim. Biophys. Acta 1129, 155–160.
57 Shivji, K. K., Kenny, M. K. and Wood, R. D. (1992) Proliferating cell nuclear antigen is required for DNA excision repair. Cell 69, 367–374.
58 Nichols, A. F. and Sancar, A. (1992) Purification of PCNA as a nucleotide excision repair protein. Nucleic Acids Res. 20, 2441–2446.
59 Nocentini, S. (1999) Rejoining kinetics of DNA single- and double-strand breaks in normal and DNA ligase-deficient cells after exposure to ultraviolet C and gamma radiation: an evaluation of ligating activities involved in different DNA repair processes. Radiat. Res. 151, 423–432.
60 Moser, J., Kool, H., Giakzidis, I., Caldecott, K., Mullenders, L. H. and Fousteri, M. I. (2007) Sealing of chromosomal DNA nicks during nucleotide excision repair requires XRCC1 and DNA ligase III alpha in a cell-cycle-specific manner. Mol. Cell 27, 311–323.
61 Jensen, L. and Linn, S. (1988) A reduced rate of bulky DNA adduct removal is coincident with differentiation of human neuroblastoma cells induced by nerve growth factor. Mol. Cell. Biol. 8, 3964–3968.
62 Nouspikel, T. and Hanawalt, P. C. (2000) Terminally differentiated human neurons repair transcribed genes but display attenuated global DNA repair and modulation of repair gene expression. Mol. Cell. Biol. 20, 1562–1570.
63 Nouspikel, T. (2007) DNA repair in differentiated cells: some new answers to old questions. Neuroscience 145, 1213–1221.
64 Nouspikel, T. and Hanawalt, P. C. (2002) DNA repair in terminally differentiated cells. DNA repair 1, 59–75.
65 Hsu, P.S., Hanawalt, P. C. and Nouspikel, T. (2007) Nucleotide excision repair phenotype of human acute myeloid leukemia cell lines at various stages of differentiation. Mutat. Res. 614, 3–15.
66 Eggset, G., Volden, G. and Krokan, H. (1983) U.v.-induced DNA damage and its repair in human skin *in vivo* studied by sensitive immunohistochemical methods. Carcinogenesis 4, 745–750.
67 Liu, S. C., Parsons, S. and Hanawalt, P. C. (1983) DNA repair in cultured keratinocytes. J. Invest. Dermatol. 81, 179s–183 s.
68 Stockdale, F.E. (1971) DNA synthesis in differentiating skeletal muscle cells: initiation by ultraviolet light. Science 171, 1145–1147.
69 Chan, A. C. and Walker, I. G. (1976) Reduced DNA repair during differentiation of a myogenic cell line. J. Cell. Biol. 70, 685–691.
70 Ho, L. and Hanawalt, P. C. (1991) Gene-specific DNA repair in terminally differentiating rat myoblasts. Mut. Res. DNA Repair 255, 124–141.
71 Tofilon, P. J. and Meyn, R. E. (1988) Influence of cellular differentiation on repair of ultraviolet-induced DNA damage in murine proadipocytes. Radiat. Res. 116, 217–237.
72 Bielas, J. H. (2006) Non-transcribed strand repair revealed in quiescent cells. Mutagenesis 21, 49–53.
73 Nouspikel, T. and Hanawalt, P. C. (2003) When parsimony backfires: neglecting DNA repair may doom neurons in Alzheimer's disease. BioEssays 25, 168–173.
74 Ljungman, M., Zhang, F., Chen, F., Rainbow, A. J. and McKay, B. C. (1999) Inhibition of RNA polymerase II as a trigger for the p53 response. Oncogene 18, 583–592.
75 Nouspikel, T. P., Hyka-Nouspikel, N. and Hanawalt, P. C. (2006) Transcription domain-associated repair in human cells. Mol. Cell. Biol. 26, 8722–8730.
76 Nouspikel, T. and Hanawalt, P. C. (2006) Impaired nucleotide excision repair upon macrophage differentiation is corrected by E1 ubiquitin-activating enzyme. Proc. Natl. Acad. Sci. USA 103, 16188–16193.
77 Ikehata, H., Kaneda, S., Yamao, F., Seno, T., Ono, T. and Hanaoka, F. (1997) Incubation at the nonpermissive temperature induces deficiencies in UV resistance and mutagenesis in mouse mutant cells expressing a temperature-sensitive ubiquitin-activating enzyme (E1). Mol. Cell. Biol. 17, 1484–1489.
78 Kraemer, K. H., Lee, M. M. and Scotto, J. (1987) Xeroderma pigmentosum: cutaneous, ocular, and neurological abnormalities in 830 published cases. Arch. Dermatol. 123, 241–250.
79 Kraemer, K. H., Lee, M. M. and Scotto, J. (1984) DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum. Carcinogenesis 5, 511–514.
80 Rapin, I., Lindenbaum, Y., Dickson, D.W., Kraemer, K. H. and Robbins, J. H. (2000) Cockayne syndrome and xeroderma pigmentosum. Neurology 55, 1442–1449.
81 Cleaver, J. E. (1968) Defective repair replication of DNA in xeroderma pigmentosum. Nature 218, 652–656.
82 Setlow, R. B., Regan, J. D., German, J. and Carrier, W. L. (1969) Evidence that xeroderma pigmentosum cells do not perform the first step in the repair of ultraviolet damage to their DNA. Proc. Natl. Acad. Sci. USA 64, 1035–1041.
83 Masutani, C., Kusumoto, R., Yamada, A., Dohmae, N., Yokoi, M., Yuasa, M., Araki, M., Iwai, S., Takio, K. and Hanaoka, F. (1999) The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta. Nature 399, 700–704.
84 Nance, M. A. and Berry, S. A. (1992) Cockayne syndrome: review of 140 cases. Am. J. Med. Genet. 42, 68–84.
85 Lowry, R. B. (1982) Early onset of Cockayne syndrome. Am. J. Med. Genet. 13, 209–210.
86 Pena, S. D. and Shokeir, M. H. (1974) Autosomal recessive cerebro-oculo-facio-skeletal (COFS) syndrome. Clin. Genet. 5, 285–293.
87 Del Bigio, M. R., Greenberg, C. R., Rorke, L. B., Schnur, R., McDonald-McGinn, D. M. and Zackai, E. H. (1997) Neuropathological findings in eight children with cerebro-oculo-facio-skeletal (COFS) syndrome. J. Neuropathol. Exp. Neurol. 56, 1147–1157.
88 Meira, L. B., Graham, J. M., Jr., Greenberg, C. R., Busch, D. B., Doughty, A. T., Ziffer, D. W., Coleman, D. M., Savre-Train, I. and Friedberg, E. C. (2000) Manitoba aboriginal kindred with original cerebro-oculo- facio-skeletal syndrome has a mutation in the Cockayne syndrome group B (CSB) gene. Am. J. Hum. Genet. 66, 1221–1228.
89 Graham, J. M., Jr., Anyane-Yeboa, K., Raams, A., Appeldoorn, E., Kleijer, W. J., Garritsen, V. H., Busch, D., Edersheim, T. G. and Jaspers, N. G. (2001) Cerebro-oculo-facio-skeletal syndrome with a nucleotide excision-repair defect

and a mutated XPD gene, with prenatal diagnosis in a triplet pregnancy. Am. J. Hum. Genet. 69, 291–300.
90 Patton, M. A., Giannelli, F., Francis, A. J., Baraitser, M., Harding, B. and Williams, A. J. (1989) Early onset Cockayne's syndrome: case reports with neuropathological and fibroblast studies. J. Med. Genet. 26, 154–159.
91 Jaspers, N. G., Raams, A., Silengo, M. C., Wijgers, N., Niedernhofer, L. J., Robinson, A. R., Giglia-Mari, G., Hoogstraten, D., Kleijer, W. J., Hoeijmakers, J. H. and Vermeulen, W. (2007) First reported patient with human ERCC1 deficiency has cerebro-oculo-facio-skeletal syndrome with a mild defect in nucleotide excision repair and severe developmental failure. Am. J. Hum. Genet. 80, 457–466.
92 Nouspikel, T., Lalle, P., Leadon, S. A., Cooper, P. K. and Clarkson, S. G. (1997) A common mutational pattern in Cockayne syndrome patients from xeroderma pigmentosum group G: Implications for a second XPG function. Proc. Natl. Acad. Sci. USA 94, 3116–3121.
93 Venema, J., Mullenders, L. H., Natarajan, A. T., van Zeeland, A. A. and Mayne, L. V. (1990) The genetic defect in Cockayne syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA. Proc. Natl. Acad. Sci. USA 87, 4707–4711.
94 van Hoffen, A., Natarajan, A. T., Mayne, L. V., van Zeeland, A. A., Mullenders, L. H. and Venema, J. (1993) Deficient repair of the transcribed strand of active genes in Cockayne's syndrome cells. Nucleic Acids Res. 21, 5890–5895.
95 Spivak, G. (2005) UV-sensitive syndrome. Mutat. Res. 577, 162–169.
96 Spivak, G., Itoh, T., Matsunaga, T., Nikaido, O., Hanawalt, P. and Yamaizumi, M. (2002) Ultraviolet-sensitive syndrome cells are defective in transcription-coupled repair of cyclobutane pyrimidine dimers. DNA Repair 1, 629–643.
97 Spivak, G. and Hanawalt, P. C. (2006) Host cell reactivation of plasmids containing oxidative DNA lesions is defective in Cockayne syndrome but normal in UV-sensitive syndrome fibroblasts. DNA Repair 5, 13–22.
98 Horibata, K., Iwamoto, Y., Kuraoka, I., Jaspers, N. G., Kurimasa, A., Oshimura, M., Ichihashi, M. and Tanaka, K. (2004) Complete absence of Cockayne syndrome group B gene product gives rise to UV-sensitive syndrome but not Cockayne syndrome. Proc. Natl. Acad. Sci. USA 101, 15410–15415.
99 Gillespie, J. M. and Marshall, R. C. (1983) A comparison of the proteins of normal and trichothiodystrophic human hair. J. Invest. Dermatol. 80, 195–202.
100 Itin, P. H., Sarasin, A. and Pittelkow, M. R. (2001) Trichothiodystrophy: update on the sulfur-deficient brittle hair syndromes. J. Am. Acad. Dermatol. 44, 891–920.
101 Crovato, F., Borrone, C. and Rebora, A. (1983) Trichothiodystrophy – BIDS, IBIDS and PIBIDS? Br. J. Dermatol. 108, 247.
102 Giglia-Mari, G., Coin, F., Ranish, J. A., Hoogstraten, D., Theil, A., Wijgers, N., Jaspers, N. G., Raams, A., Argentini, M., van der Spek, P. J., Botta, E., Stefanini, M., Egly, J. M., Aebersold, R., Hoejmakers, J. H. and Vermeulen, W. (2004) A new, tenth subunit of TFIIH is responsible for the DNA repair syndrome trichothiodystrophy group A. Nat. Genet. 36, 714–719.
103 Weeda, G., Eveno, E., Donker, I., Vermeulen, W., Chevallier-Lagente, O., Taieb, A., Stary, A., Hoeijmakers, J. H., Mezzina, M. and Sarasin, A. (1997) A mutation in the XPB/ERCC3 DNA repair transcription gene, associated with trichothiodystrophy. Am. J. Hum. Genet. 60, 320–329.
104 Stefanini, M., Lagomarsini, P., Arlett, C. F., Marinoni, S., Borrone, C., Crovato, F., Trevisan, G., Cordone, G. and Nuzzo, F. (1986) Xeroderma pigmentosum (complementation group D) mutation is present in patients affected by trichothiodystrophy with photosensitivity. Hum. Genet. 74, 107–112.
105 Bootsma, D. and Hoeijmakers, J.H. (1993) DNA repair. Engagement with transcription. Nature 363, 114–115.
106 Vermeulen, W., van Vuuren, A. J., Chipoulet, M., Schaeffer, L., Appeldoorn, E., Weeda, G., Jaspers, N. G., Priestley, A., Arlett, C. F., Lehmann, A. R., Stefanini, M., Mezzina, M., Sarasin, A., Bootsma, D., Egly, J. M. and Hoejmakers, J. H. G. (1994) Three unusual repair deficiencies associated with transcription factor BTF2(TFIIH): evidence for the existence of a transcription syndrome. Cold Spring Harb. Symp. Quant. Biol. 59, 317–329.
107 Vermeulen, W., Rademakers, S., Jaspers, N. G., Appeldoorn, E., Raams, A., Klein, B., Kleijer, W. J., Hansen, L. K. and Hoeijmakers, J. H. (2001) A temperature-sensitive disorder in basal transcription and DNA repair in humans. Nat. Genet. 27, 299–303.
108 Nouspikel, T. (2008) Nucleotide excision repair and neurological diseases. DNA Repair 7, 1155–1167.
109 Balajee, A. S., May, A., Dianov, G. L., Friedberg, E. C. and Bohr, V. A. (1997) Reduced RNA polymerase II transcription in intact and permeabilized Cockayne syndrome group B cells. Proc. Natl. Acad. Sci. USA 94, 4306–4311.
110 van Oosterwijk, M. F., Versteeg, A., Filon, R., van Zeeland, A. A. and Mullenders, L. H. (1996) The sensitivity of Cockayne's syndrome cells to DNA-damaging agents is not due to defective transcription-coupled repair of active genes. Mol. Cell. Biol. 16, 4436–4444.
111 Tornaletti, S., Reines, D. and Hanawalt, P. C. (1999) Structural characterization of RNA polymerase II complexes arrested by a cyclobutane pyrimidine dimer in the transcribed strand of template DNA. J. Biol. Chem. 274, 24124–24130.
112 Svejstrup, J. Q. (2002) Mechanisms of transcription-coupled DNA repair. Nat. Rev. Mol. Cell. Biol. 3, 21–29.
113 Bregman, D. B., Halaban, R., van Gool, A. J., Henning, K. A., Friedberg, E. C. and Warren, S. L. (1996) UV-induced ubiquitination of RNA polymerase II: a novel modification deficient in Cockayne syndrome cells. Proc. Natl. Acad. Sci. USA 93, 11586–11590.
114 Sarker, A. H., Tsutakawa, S. E., Kostek, S., Ng, C., Shin, D. S., Peris, M., Campeau, E., Tainer, J. A., Nogales, E. and Cooper, P. K. (2005) Recognition of RNA polymerase II and transcription bubbles by XPG, CSB, and TFIIH: insights for transcription-coupled repair and Cockayne Syndrome. Mol. Cell 20, 187–198.
115 Hanawalt, P. C. (2000) DNA repair. The bases for Cockayne syndrome. Nature 405, 415–416.
116 Tsutakawa, S. E. and Cooper, P. K. (2000) Transcription-coupled repair of oxidative DNA damage in human cells: mechanisms and consequences. Cold Spring Harb. Symp. Quant. Biol. 65, 201–215.
117 Sunesen, M., Stevnsner, T., Brosh R. M. Jr, Dianov, G. L. and Bohr, V. A. (2002) Global genome repair of 8-oxoG in hamster cells requires a functional CSB gene product. Oncogene 21, 3571–3578.
118 Tuo, J., Jaruga, P., Rodriguez, H., Dizdaroglu, M. and Bohr, V. A. (2002) The cockayne syndrome group B gene product is involved in cellular repair of 8-hydroxyadenine in DNA. J. Biol. Chem. 277, 30832–30837.
119 Spivak, G. and Hanawalt, P. (1995) Determination of damage and repair in specific DNA sequences. Methods – a companion to Methods Enzymol. 7, 147–161.
120 Thorslund, T., Sunesen, M., Bohr, V. A. and Stevnsner, T. (2002) Repair of 8-oxoG is slower in endogenous nuclear genes than in mitochondrial DNA and is without strand bias. DNA Repair 1, 261–273.
121 Bohr, V. A., Smith, C. A., Okumoto, D. S. and Hanawalt, P. C. (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, 359–369.
122 Mellon, I., Spivak, G. and Hanawalt, P. C. (1987) Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. Cell 51, 241–249.
123 Donahue, B. A., Fuchs, R. P. P., Reines, D. and Hanawalt, P. C. (1996) Effects of aminofluorene DNA adducts on

transcriptional elongation by RNA polymerase II. J. Biol. Chem. 271, 10588–10594.
124 Mu, D., Wakasugi, M., Hsu, D. S. and Sancar, A. (1997) Characterization of reaction intermediates of human excision repair nuclease. J. Biol. Chem. 272, 28971–28979.
125 Hanawalt, P. C. (2002) Subpathways of nucleotide excision repair and their regulation. Oncogene 21, 8949–8956.
126 Van Houten, B. (1990) Nucleotide excision repair in *Escherichia coli*. Microbiol. Rev. 54, 18–51.
127 Nichols, A. F., Itoh, T., Graham, J. A., Liu, W., Yamaizumi, M. and Linn, S. (2000) Human damage-specific DNA-binding protein p48. Characterization of XPE mutations and regulation following UV irradiation. J. Biol. Chem. 275, 21422–21428.
128 Coin, F., Auriol, J., Tapias, A., Clivio, P., Vermeulen, W. and Egly, J. M. (2004) Phosphorylation of XPB helicase regulates TFIIH nucleotide excision repair activity. EMBO J. 23, 4835–4846.