

Nucleotide excision repair

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All living organisms are equipped with DNA repair systems that can cope with a wide variety of DNA lesions. Among these repair pathways, nucleotide excision repair (NER) is a versatile repair pathway, involved in the removal of a variety of bulky DNA lesions such as UV induced cyclobutane pyrimidine dimers (CPD) and pyrimidine 6-4 pyrimidone photoproducts (6-4PP). NER is a complex process in which basically the following steps can be distinguished:

- (i) recognition of a DNA lesion;
- (ii) separation of the double helix at the DNA lesion site;
- (iii) single strand incision at both sides of the lesion;
- (iv) excision of the lesion-containing single stranded DNA fragment;
- (v) DNA repair synthesis to replace the gap and
- (vi) ligation of the remaining single stranded nick.

The importance of NER for human health is illustrated by the occurrence of rare autosomal recessive disorder [xeroderma pigmentosum \(XP\)](#). Patients characteristically show severe photosensitivity and abnormal pigmentation, often accompanied by mental retardation, and they usually develop skin cancer at very young age (Bootsma et al., 1998). Cells from these patients are also extremely sensitive to UV light and have a defect in NER. Complementation studies revealed that eight genes are involved in XP: XPA through XPG and XPV (XP-Variant). Mutations in the XP genes (except XP-variant) lead to defective NER and hypersensitivity to UV. The XP variant cells are proficient in NER but deficient in lesion bypass when the replication fork encountered a bulky adduct. Normally, the translesion synthesis is carried out by the polymerase η , which is mutated in the XP variant. XP-V patients are more UV-sensitive than normal individuals but less than classical XP. They develop skin cancers around the age of 20-30 and exhibit less neurological abnormalities.

In addition to XP, other UV sensitive syndromes exist. [Cockayne' syndrome \(CS\)](#) is a rare disorder that is associated with a wide variety of clinical symptoms. Beside other symptoms, the patients generally show dwarfism, mental retardation and photosensitivity. In contrast to XP, CS is not associated with an enhanced incidence

of skin cancer. Cells from CS patients are hypersensitive to the cytotoxic effects of UV and are characterized by the inability to resume UV inhibited DNA and RNA synthesis. Two CS complementation groups (A and B) have been established. A third group encompasses patients exhibiting both XP and CS symptoms, they belong to XP groups B, D or G. The progressive neurological abnormalities associated with CS may be due to the inability of CS cells to repair oxidative DNA lesions (LePage et al., 2000).

PIBIDS is a photosensitive variant of [Trichothiodystrophy \(TTD\)](#) and the third syndrome that can be associated with NER defects (PIBIDS is the acronym of the characteristic clinical symptoms of the patients for Photosensitivity, Ichthyosis, Brittle hair, Impaired intelligence, Decreased fertility and Short stature) (Itin et al., 2000). Certain mutations in the XPB and XPD genes have been shown to cause the PIBIDS phenotype, but not in combination with the specific XP characteristics like cancer proneness.

Name when cloned	Usual Name	Other Alias	Location	Disease
XPA	XPA	XPAC	9q22.3 Ð 9q22.3	XP
ERCC3	XPB	XPBC	2q21 Ð 2q 21	XP ; CS ; TTD
XPC	XPC	XPCC	3p25.1 Ð 3p25.1	XP
ERCC2	XPD	XPDC	19q13.2 - 19q13.3	XP ; XP/ CS ; TTD
p48; p125	XPE	XPEC DDB1, DDB2	p48 = 11p12 - p11 p125 = 11q12 - q13	XP
ERCC4	XPF	XPFC	19q13.3 - 19q13.3	XP
ERCC5	XPG	XPGC	13q32 Ð 13q 32	XP ; XP/ CS
ERCC8	CSA		(5pter Ð 5 qter) unapproved	CS
ERCC6	CSB		10q11 Ð 10 q 21	CS
Pol eta	XPV		6p21.1 Ð 6p12	XP variant

It has been shown that NER can operate via two subpathways. The first pathway is global genome repair (GGR) and involves repair activity that acts on DNA lesions across the genome. Although the efficiency of this pathway can be influenced by various parameters, it is not actively targeted to specific regions of the genome. A second NER pathway is coupled to active transcription and is called transcription coupled repair. This pathway involves repair activity that is directed to the transcribed strand of active genes.

The cloning of the XP genes and the isolation of the encoded proteins has led to the elucidation of the core NER reactions and ultimately to the reconstitution of the process in vitro (Aboussekhra et al., 1995 ; Mu et al., 1995).

NER proteins and their functions

DNA damage recognition. Two proteins have been identified and implicated in (one of) the first steps of NER, i.e. the recognition of lesions in the DNA: the XPA gene product and the XPC gene product in complex with HR23B. In addition, the XPE protein has been shown to have a high affinity for damaged DNA, but whether it is required for the damage recognition step of NER remains unclear. Cells from XPA patients are extremely sensitive to UV and have very low nucleotide excision repair activity. In vitro the XPA protein binds preferentially to damaged DNA compared to nondamaged DNA. The XPA protein binds to replication protein A (RPA) which enhances the affinity of XPA for damaged DNA and is essential for NER. The other complex that has been implicated in DNA damage recognition is XPC-HR23B. XPC

cells have low NER repair capacity, but the residual repair has been shown to occur specifically in transcribed genes. It is very likely that the XPC-HR23B complex is the principal damage recognition complex i.e. essential for the recognition of DNA lesions in the genome (Sugasawa et al, 1998). Binding of XPC-HR23B to a DNA lesion causes local unwinding, so that the XPA protein can bind and the whole repair machinery can be loaded onto the damaged site. This would imply that the XPA protein has binding affinity for other repair proteins. Indeed, the XPA protein has been shown to bind to ERCC1 and TFIIH. The XPC-HR23B complex is only required for global genome repair. In case of transcription coupled repair when an RNA polymerase is stalled at a lesion, the DNA is unwound by the transcription complex and XPA can bind independently of XPC- HR23B complex.

XPE patients show mild dermatological symptoms and cells from these patients have a relatively high repair capacity. The function of the gene product is not completely clarified yet. Band shift assays suggested that the XPE gene product acts as a damaged DNA binding protein (DDB), with high affinity to UV-induced 6-4PP. However, defective DDB binding activity is not a common feature of XPE mutant cell lines and in fact two (or even more) proteins may be involved in the binding activity: p48 and p125. In cells from several XPE patient mutations in p48 have been found but so far no mutations have been found in the p125 gene. XPE cells are not necessarily defective in repair: p125 is proposed to play a role in opening up chromatin to make CPD accessible to the NER machinery, but is not required for repair of 6-4PP. Interestingly, cell lines and primary tissues from rodents are fully deficient in the expression of the p48 protein (Tang et al., 2000). This explains the absence of GGR of CPD in these cells. Exogenous expression of p48 in hamster cells confers enhanced removal of CPD from genomic DNA and nontranscribed strand of active genes.

Damage demarcation. The striking discovery that subunits of basal transcription factor TFIIH were involved in NER sheds light on a new aspect of NER : a close coupling to transcription via common use of essential factors. Two repair proteins, encoded by XPB and XPD genes, appeared to be identical to components of the basal transcription factor TFIIH, a large complex involved in the initiation of transcription. The XPB and XPD proteins displayed 3'-5' and 5'-3' helicase activity respectively (Schaeffer et al., 1994). TFIIH fulfills a dual role in transcription initiation and NER and the role of TFIIH in NER might closely mimic its role in the transcription initiation process. In transcription initiation TFIIH is thought to be involved in unwinding of the promoter site and to allow promoter clearance. In the NER process TFIIH causes unwinding of the damage containing region that has been localized by XPC-HR23B and XPA-RPA, enabling the accumulation of NER proteins around the damaged site.

Among the XP patients, XPB patients are extremely rare (only 3 patients known in the world) due to the fact that the XPB gene product is essential for transcription initiation and in all cases, these patients show the double symptoms of XP and CS. The helicase activity of XPD is indispensable for NER but not for transcription initiation. So , there is much more XPD patients, and only two patients have been described as XP and CS.

Incision. The XPF protein and the ERCC1 protein form a complex that exhibits structure specific endonuclease activity that is responsible for the 5' incision during the NER reaction. XPF-ERCC1 also binds to XPA (through ERCC1) and to RPA (through XPF) but not preferentially to damaged DNA. The XPG protein has DNA endonuclease activity without preference for damaged DNA and is responsible for the

3' incision made during NER. At the site of a lesion NER proteins create a DNA bubble structure over a length of approximately 25 nucleotides and the XPG protein incises the damaged DNA strand 0-2 nucleotides 3' to the ssDNA-dsDNA junction. In most studies the 3'-incision made by the XPG protein appeared to be made prior to and independently of the 5'-incision by XPF-ERCC1. Patients belonging to the XP-G complementation group clinically exhibit heterogeneous symptoms, from mild to very severe, sometimes associated with CS. XP-G cells are almost completely repair-deficient and as UV-sensitive as XP-A cells. About half of the described XPG patients exhibit also CS symptoms. In contrast to XPG, XP-F patients have a relatively mild XP phenotype without neurological abnormalities. Cells from XP-F patients are slightly UV-sensitive and exhibit low levels of repair initially after UV-irradiation.

Repair patch synthesis and ligation. Proliferating Cell Nuclear Antigen (PCNA) is required for DNA synthesis by DNA polymerases delta and epsilon. PCNA has also been shown to be required for NER *in vitro* i.e. for the DNA resynthesis step, suggesting that DNA polymerase delta or epsilon is involved in NER. Biochemical analysis and fluorescence microscopy revealed that in quiescent cells upon UV-irradiation PCNA (that usually resides in the cytoplasm) becomes rapidly bound to chromatin. The enzymes involved in these pathways are normal in DNA repair-deficient cells.

Global genome repair (GGR)

GGR acts on DNA lesions throughout the genome, but the kinetics of repair can be influenced by a number of parameters related to DNA lesion structure and chromatin configuration. It is conceivable that the damage recognition step is a rate-limiting step in the repair process and that more efficient recognition of DNA lesions will lead to more rapid repair. The lesion recognition and binding potency of proteins that are involved in damage recognition, depends on the chemical structure of the DNA lesion itself or the way it interferes with the DNA helical structure. Some lesions such as ultraviolet light induced 6-4PP and CPD, are large bulky lesions located in the minor groove of the DNA helix and are recognized by NER proteins as being abnormal structures in the DNA. DNA is thought to be a dynamic molecule subject to an extremely rapid process of bending, twisting, unwinding and rewinding ('breathing'). Lesions that interfere with these dynamic properties of the DNA may be recognized by repair proteins. Lesions that have been shown to be a good substrate for NER often cause local unwinding of a few DNA bases around the damaged site. UV-induced CPD as well as cisplatin-induced intrastrand crosslinks are a better substrate for *in vitro* NER when they are superimposed on a mismatch than in normally base paired DNA. The unwinding of a few basepairs energetically favours bending of the DNA and this may facilitate further unwinding by NER enzymes. Repair of DNA lesions that are substrates for NER by themselves, is strongly stimulated by disruption of base pairing at the site of the lesion. The role of chromatin structure in governing the repair efficiency is indicated by the notion that repair in the nontranscribed strand of active genes or chromatin poised for transcription, is faster than in inactive X- chromosomal genes (Venema et al., 1992). The latter are known to consist of heavily methylated DNA sequences and their chromatin structure is relatively inaccessible to molecular probes such as DNase1. Thus, the efficiency of repair might be influenced by accessibility of DNA lesions to repair proteins. Indeed, when repair was investigated at the nucleotide level, profound differences in repair rate were found due to protein binding in promotor regions.

Transcription-coupled repair

The NER subpathway transcription-coupled repair (TCR) first described by Mellon and Hanawalt for cultured mammalian cells (Mellon et al., 1987), specifically removes DNA lesions from the transcribed strand of an active gene. Subsequently, TCR was shown to operate in a variety of organisms including bacteria and yeast. All data indicate that TCR is directly coupled to active transcription and it is generally assumed that a stalled transcript provides a strong signal to attract the repair machinery. All classical XP cells are deficient in TCR except the group C that is fully deficient in GGR but proficient in TCR (Van Hoffen et al., 1995). However, until now it is not clear how repair is coupled to transcription. A major obstacle that prevents a major breakthrough, is the lack of a cell free system capable to perform TCR. Genetic analysis has put some light on specific factors that play a role in TCR. In an *E. coli* mdf- mutant strain a protein has been identified called transcription-repair coupling factor (TRCF, the mdf gene product), that actively couples repair to a stalled RNA polymerase at the site of a DNA lesion (Selby and Sancar, 1993). In mammalian cells such factor has not been found yet, but it was suggested that the proteins mutated in the Cockayne' syndrome might fulfill such a function. Similarly to the mdf bacteria strain, Cockayne syndrome cells are unable to perform transcription-coupled repair, whereas the global repair pathway is functioning normally. The defect in transcription-coupled repair has been related to the inability of CS cells to restore UV-inhibited RNA synthesis (Mayne and Lehmann 1982). Slow removal of DNA lesions from transcription templates would prevent efficient transcription and this could lead to cell death if essential genes are involved. Moreover, by analogy to bacteria such a factor could attract NER proteins. Indeed, several investigators showed that CSB can be copurified with RNA polymerase II but could not detect interaction of CSB with any other tested NER component. In cells that have been treated with UV, a small fraction of RNA polymerase II becomes ubiquitinated within 15 minutes after treatment and this fraction persists for about 8 hours (Bregman et al., 1996). However, neither in CS-A nor in CS-B cells this specific response was observed. One explanation favoured by several studies, is that the polymerase could be ubiquitinated as a signal for degradation of the protein so that the lesion becomes accessible for repair enzymes. In this model, CS proteins would be required to make lesions (at stalled transcripts) repairable.

References

1. Failure of RNA synthesis to recover after UV irradiation : an early defect in cells from individuals with Cockayne's syndrome and xeroderma pigmentosum. Mayne L.V., Lehmann A.R. *Cancer Res* 1982; 42: 1473-1478. [Medline](#)
2. Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. Mellon I., Spivak G., Hanawalt P.C. *Cell* 1987; 51: 241-249. [Medline](#)
3. Transcription affects the rate but not the extent of repair of cyclobutane pyrimidine dimers in the human adenosine deaminase gene Venema J, Bartosova Z, Natarajan A.T., Van Zeeland A.A., L.H.F. Mullenders, L. *J Biol Chem* 1992; 267: 8852-8856. [Medline](#)

4. Molecular mechanism of transcription-repair coupling Selby C.P., Sancar A. Science 1993; 260: 53-58. [Medline](#)
5. The ERCC2/DNA repair protein is associated with the class II BTF2/TFIIH transcription factor. Schaeffer L., Moncolin V., Roy R., Staub A., Mezzina M., Sarasin A., Weeda G., J.H.J. Hoeijmakers J.H.J., Egly J.M. The EMBO Journal 1994; 13: 2388-2392. [Medline](#)
6. Mammalian DNA nucleotide excision repair reconstituted with purified protein components. Aboussekhra A., Biggerstaf M., Shivji M.K.K., Vilpo J.A., Moncollin V., Podust V.N., Proti M., Hÿbscher U., Egly J-M, Wood, R.D. Cell 1995; 80: 859-868 [Medline](#)
7. Reconstitution of human DNA repair excision nuclease in a highly defined system. Mu D., Park C.H., Matsunaga T., Hsu D.S., Reardon J.T., Sancar A J Biol Chem 1995; 270: 2415-2418. [Medline](#)
8. Transcription-coupled repair removes both cyclobutane pyrimidine dimers and 6-4 photoproducts with equal efficiency and in a sequential way from transcribed DNA in xeroderma pigmentosum group C fibroblasts. Van Hoffen A., Venema J., Meschini R., van Zeeland A.A. and Mullenders L.H.F. EMBO J 1995; 14: 360-367. [Medline](#)
9. UV-induced ubiquitination of RNA polymerase II: a novel modification deficient in Cockayne syndrome cells. Bregman D.B., Halaban R., van Gool A.J., Henning K.A., Friedberg E.C. Warren S.L. Proc Natl Acad Sci USA 1996; 93: 11586-11590. [Medline](#)
10. Nucleotide excision repair syndromes: xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy. Bootsma D., Kraemer K.H., Cleaver J., Hoeijmakers J.H.J. In: B Vogelstein and KW Kinzler (ed) The Genetic Basis of Human Cancer 1998; pp 245-274, McGraw-Hill (New York).
11. Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. Sugawara K., Ng J.M.Y., Masutani C., Iwai S., van der Spek P.J., Eker A.P.M., Hanaoka F., Bootsma D., Hoeijmakers J.H.J. Mol Cell 1998; 2, 223-232. [Medline](#)
12. Trichothiodystrophy: uptake on the sulfur-deficient brittle hair syndromes. Itin P.H., Sarasin A., Pittelkow M.R. (2000) J. Am. Acad. Dermatol. 2000; In press.
13. Transcription-coupled repair and mutation avoidance at 8-oxoguanine: Requirement for XPG, TFIIH, and CSB and implications for Cockayne Syndrome. Le Page F., Kwoh E.E., Avrutskaya A., Gentil A., Ledon S.A., Sarasin A., Cooper P.K. Cell 2000; 101,159-171. [Medline](#)
14. Xeroderma pigmentosum p48 gene enhances global genomic repair and suppresses UV-induced mutations Tang J.Y., Hwang B.J., Ford J.M., Hanawalt P.C., Chu G. Cell 2000; 5, 737-744. [Medline](#)

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