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Review

Bacterial DNA repair genes and their eukaryotic homologues: 4. The role of nucleotide excision DNA repair (NER) system in mammalian cells★

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The eukaryotic cell encounters more than one million various kinds of DNA lesions per day. The nucleotide excision repair (NER) pathway is one of the most important repair mechanisms that removes a wide spectrum of different DNA lesions. NER operates through two sub pathways: global genome repair (GGR) and transcription-coupled repair (TCR). GGR repairs the DNA damage throughout the entire genome and is initiated by the HR23B/XPC complex, while the CSB protein-governed TCR process removes DNA lesions from the actively transcribed strand. The sequence of events and the role of particular NER proteins are currently being extensively discussed. NER proteins also participate in other cellular processes like replication, transcription, chromatin maintenance and protein turnover. Defects in NER underlay severe genetic disorders: xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD).

Keywords: DNA damage, DNA repair, nucleotide excision repair, xeroderma pigmentosum, Cockayne syndrome, trichothiodystrophy

INTRODUCTION

The genetic material is under constant insult from various exo- and endogenous damaging agents which alter its coding properties. It is estimated that each cell encounters one million DNA damage incidents per day (Lodish, 2004). It has been suggested that a gradual accumulation of DNA damage during the cellular and organismal life-time contributes to normal aging and carcinogenesis (Harman, 1956; Olinski *et al.*, 2007).

Living cells have developed a complex network of DNA repair mechanisms which collectively maintain genome integrity. Among the major DNA repair mechanisms are: nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR) and recombination repair, which is a common term for two repair pathways: homologous recombination (HR) repair and non-homologous end joining (NHEJ) (for more details see accompanying review by Nowosielska, 2007). The first three mechanisms, i.e. NER, BER (for more details see ac-

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Abbreviations: CS A–B, Cockayne syndrome group A–B; CPD, cyclobutane pyrimidine dimers; DDB, DNA-binding protein; ERCC1, excision repair cross complementation group 1; GGR, global genome repair; hHR23, human homolog of yeast RAD23; NER, nucleotide excision repair; LIG 1, ligase 1; PCNA, proliferating cell nuclear antigen; POL δ/ε, polymerase delta/epsilon; RFC, replication factor C; RNA pol II, RNA polymerase II; RPA, replication protein A; TFIIH, transcription factor IIF; TCR, transcription-coupled repair; XP A–G, xeroderma pigmentosum group A–G.

companying review by Krwawicz *et al.*, 2007) and MMR (for more details see accompanying review by Arczewska & Kusmierek, 2007), are involved in the excision of the damaged or mispaired DNA bases, while the HR and NHEJ systems repair DNA double strand breaks (DSBs) (Hoeijmakers, 2001; Lindahl & Wood, 1999). An alternative to the BER mechanism, n*ucleotide incision repair* (NIR), is initiated by one of BER proteins, AP endonuclease 1 (APE1) which is able to incise the DNA strand 5′ to certain oxidized and subsequently fragmented DNA nucleotides (Ischenko & Saparbaev, 2002; Gros *et al.*, 2004; Ishchenko *et al.*, 2006). Additionally, a unique class of low-fidelity DNA polymerases can bypass DNA lesions in the process named translesion synthesis (TLS), enabling cell survival in the case of ineffective DNA repair (Lehmann *et al.*, 2007).

More than 150 proteins are known to be involved in the DNA repair processes (Wood *et al.*, 2005). All these proteins are the products of stability genes (caretakers) which protect the genetic material from the consequences of DNA damage and thereby prevent cancer and pre-mature aging diseases. Mutations in any of the DNA repair genes can result in genome instability (Lindahl & Wood, 1999; Hoeijmakers, 2001).

NUCLEOTIDE EXCISION REPAIR

Overview of NER reactions in eukaryotes

NER is a versatile multi-step DNA repair pathway that serves to remove a broad range of bulky, helix-distorting lesions. It is ubiquitous from simplest organisms such as mycoplasma to well developed ones like, mammals (Wang *et al.*, 1994; Lehmann, 1995; Wood, 1997). The majority of environmental carcinogens, including UV irradiation, antitumor drugs (cis-platin) and food-borne genotoxins, form bulky DNA adducts which may block replication and transcription. Among other helix-distorting lesions, NER is responsible for the repair of UV light-induced photoproducts (e.g. cyclobutane pyrimidine dimers), lipid peroxidation-induced DNA adducts, cigarette smoke-induced benzo[*a*]pyrene DNA adducts, chemical carcinogen-induced 4-nitroquinoline and other DNA adducts (Kraemer *et al.*, 2007a). NER is also involved in the removal of oxidative DNA lesions (Brooks, 2007; Johnson *et al.*, 2004) and in *Escherichia coli*, single strand breaks (SSBs) (by UvrABC enzymes) (Truglio *et al.*, 2006).

NER system was extensively studied in bacteria since 60-ties of 20th century. Damage recognition and consecutive steps of repair were recently described in depth by Van Houten and coworkers (2005) and Mellon (2005). This paper will focus exclusively on mammalian NER system, since sequence of events and the role of participating proteins is still extensively discussed.

NER process in mammals is carried out by a multi-protein complex referred to as the nucleotide excision repairosome, consisting of over 30 proteins which function in a stepwise manner (Table 1). The main stages of mammalian NER are: (1) DNA damage recognition, (2) assembly of the protein complex that carries out excision of damaged DNA, and finally, (3) synthesis and ligation of a stretch of DNA strand for gap filling (Park & Choi, 2006). The key event in eukaryotic NER is excision of an approximately 28-nucleotide (nt) DNA fragment containing the damaged site (Wood, 1997; Leibeling *et al.*, 2006). In mammalian cells, NER consists of two distinct sub-pathways, namely global genome repair (GGR) and transcription-coupled repair (TCR), which are fundamentally identical except for the mode of the DNA damage recognition. In TCR, RNA polymerase II (RNA pol II) stalled at the damaged site constitutes the signal for the recruitment of DNA repair proteins, while in GGR, the DNA damage-induced helical distortion is recognized by a specific protein complex. Consistently, TCR specifically repairs transcription-blocking lesions in actively transcribed DNA regions. In contrast, GGR eliminates DNA lesions from the entire genome (Hanawalt, 2002; Gillet & Scharer, 2006; Saxowsky & Doetsch, 2006).

Global genome repair (GGR)

In GGR, the major DNA damage recognition factor is the XPC–hHR23 complex (Fig. 1). The transcription factor IIH (TFIIH), XPA and replication protein A (RPA) sequentially bind to the site of the damage to form a pre-incision complex. The two helicases XPB and XPD, which are parts of TFIIH, unwind the DNA double helix at the damaged site. The dual incision process is performed by endonuclease XPG and the XPF–ERCC1 complex, which hydrolyse phosphodiester bonds 2–8 nucleotides downstream and 15–24 nucleotides upstream of the damaged site. The resulting gap is filled in by DNA polymerases delta/epsilon (POL δ/ε) which require proliferating cell nuclear antigen (PCNA), RPA and replication factor C (RFC). Finally, the DNA fragments are ligated by DNA ligase I (LIG1) (Friedberg, 2001; Costa *et al.*, 2003; Sancar & Reardon, 2004).

Transcription-coupled repair (TCR)

For the first time, TCR was described in mammalian cells by Bohr and co-workers and by Mellon and Hanawalt who observed that the removal of DNA lesions from the transcribed strands of active genes

is much more efficient than from the whole genome (Bohr *et al.*, 1985; Mellon & Hanawalt, 1989). The TCR specifically repairs transcription-blocking lesions from the actively transcribed DNA regions. RNA polymerase (RNAP) stalled at the damaged site is the signal for the recruitment of TCR proteins (Fig. 2). The lesion is removed only in the presence of CSB in an ATP-dependent manner (Sarker *et al.*, 2005; Laine & Egly, 2006a). Recent models of the TCR pathway propose that the CSB protein interacts with lesion-stalled RNAP and recruits other TCR factors to the damaged site (Sarasin & Stary, 2007). XPG has also been shown to bind cooperatively with CSB (Sarker *et al.*, 2005). Subsequently TFIIH, RPA and XPA arrive at the site of the damage. It appears that TFIIH and XPA stabilize each other's interactions with RNAP. A recent study suggests that an ATP-dependent activity of TFIIH is required to release the stalled RNAP (Laine & Egly, 2006a; 2006b). Afterwards, XPF is bound to the damaged site and the CSB-dependent incision

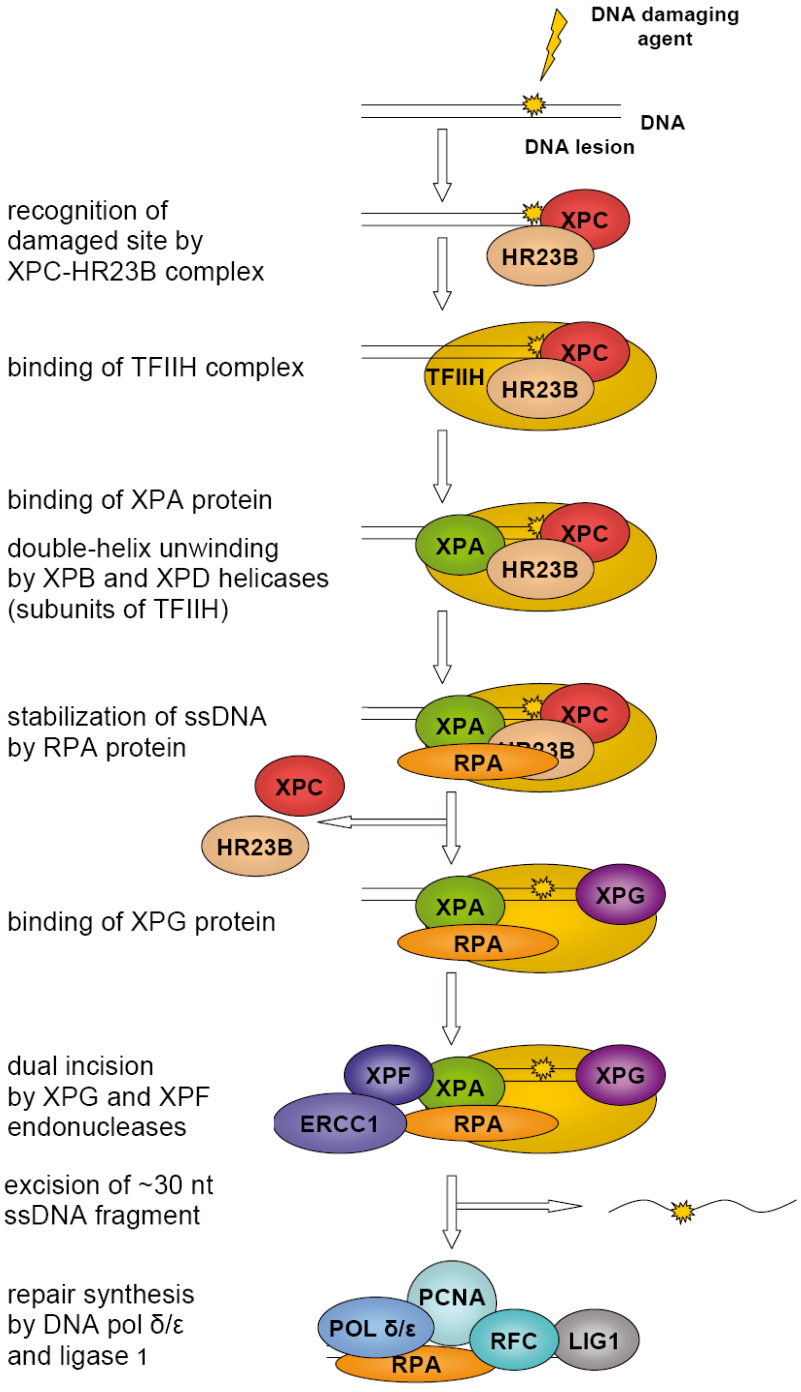

Figure 1. The mechanism of the global genome repair (GGR) subpathway of NER.

Table 1. Human nucleotide excision repair (NER) proteins

NER factor	Function in NER	Gene	Chromosome location	Protein	Size (kDa)	accession number IN NCBI Entrez	*Saccharomyces cerevisiae* homolog	*E. coli* NER factor
XPC/ HR23B	Damage recognition in GGR							UvrA2B complex
	Binds damaged DNA with HR23B and recruits other NER proteins	*XPC*	3p25.1	XPC	125	NM 004628	Rad4	
	Binds damaged DNA with XPC and recruits other NER proteins	*HR23B*	9q31.2	HR23B	58	NM 002874	Rad23 homolog B	
	Substitutes for HR23B	*HR23A*	19p13.13	HR23A	40	NM 005053	Rad23 homolog A	
DDB	Damage recognition in GGR							
	Binds damaged DNA with XPE in GGR and E3 ubiquitin ligase component of TCR	*DDB1*	11q12.2	DDB1	127	NM 001923	Ddb1 (*S. pombe*)	
	Binds damaged DNA with DDB1	*DDB2*	11p11.2	XPE	48	NM 000107	–	
CSB	Stabilization and remodelling of DNA–RNAP interface and recruitment of other repair factors to damaged site in TCR	*ERCC6*	10q11.23	CSB	168	NM 000124	Rad26	Mfd (involved in removal of stalled RNAP from damaged site)
CSA	E3 ubiquitin ligase complex subunit in TCR, Assembly of TCR repairosome	*ERCC8 (CKN1)*	5q12.1	CSA	48	NM 000082	Rad28	
XAB2	Remodeling of RNAP–DNA interface with CSB in TCR	*XAB2 (HCNP)*	19p13.3	XAB2	100	NM 020196	Syf1	
XPA	Damaged DNA binding? open complex formation and stabilization	*XPA*	9q22.33	XPA	36	NM 000380	Rad14	
RPA	Single-stranded DNA binding factor involved in open complex formation and stabilization	*RPA*		RPA (RF-A, HSSB)			Rfa	
	RPA subunit	*RPA1*	17p13.3	RPA70	70	NM 002945	Rfa1	
	RPA subunit	*RPA2*	1p35.3	RPA32	32	NM 002946	Rfa2	
	RPA subunit	*RPA3*	7p21.3	RPA14	14	NM 002947	Rfa3	
TFIIH	Required for both GGR and TCR							
	3′→5′ DNA helicase (DNA unwinding)	*XPB (ERCC3)*	2q14.3	XPB	89	NM 000122	Rad25 (Ssl2)	UvrD

	5′→3′ DNA helicase (DNA unwinding)	*XPD (ERCC2)*	19q13.32	XPD	80	NM 000400	Rad3	UvrD
	Core TFIIH subunit	*GTF2H1(TFB1)*	11p15.1	P62	62	NM 005316	Tfb1	
	Core TFIIH subunit	*GTF2H4 (TFB2)*	6p21.33	P52	52	NM 001517	Tfb2	
	DNA binding?	*GTF2H2*	5q13.2	P44	44	NM 001515	Ssl1	
	DNA binding?	*GTF2H3 (TFB4)*	12q24.31	P34	34	NM 001516	Tfb4	
	Stabilizing subunit	*GTF2H5 (TTD–A)*	6q25.3	P8	8	NM 207118	–	
	CAK sub-complex	*MNATI (TFB3)*	14q23.1	Mat1	36	NM 002431	Tfb3	
	Phosphorylation of RNAP and other subunits	*CDK7*	5q13.2	Cdk7	39	NM 001799	Kin28	
		CCNH	5q14.3	Cyclin H	38	NM 001239	Ccl1	
XPG	3′ incision (endonuclease)	*XPG (ERCC5)*	13q33.1	XPG	133	NM 000123	Rad2	UvrB, UvrC, Cho
XPF/ERCC1	5′ incision (endonuclease)							UvrC
	5′ incision subunit	*XPF (ERCC4)*	16p13.12	XPF	115	NM 005236	Rad1	
	5′ incision subunit	*ERCC1*	19q13.32	ERCC1	38	NM 001983	Rad10	
PCNA	Sliding clamp for Pol δ/ ε	*PCNA*	20p12.3	PCNA	37	NM 002592	Pol30	B–clamp
RFC	PCNA loading	*RFC*		RFC			Rfc	
	RFC subunit	*RFC1*	4p14-p13	RFC140	145	NM 002913	Rfc1	
	RFC subunit	*RFC2*	7q11.23	RFC40	40	NM 181471	Rfc2	
	RFC subunit	*RFC3*	13q12.3-q13	RFC38	38	NM 002915	Rfc3	
	RFC subunit	*RFC4*	3q27	RFC37	37	NM 002916	Rfc4	
	RFC subunit	*RFC5*	12q24.2-q24.3	RFC36	36	NM 181578	Rfc5	
POLδ	DNA resynthesis			POL δ				DNA Pol I
	Pol δ subunit	*POLD1*	19q13.33	POLD1	125	NM 002691	Pol3	
	Pol δ subunit	*POLD3*	11q14	POLD3	68	NM 006591	Pol31	
	Pol δ subunit	*POLD2*	7p13	POLD2	50	NM 006230	Pol32	
	Pol δ subunit	*POLD4*	11q13	POLD4	12	NM 021173	–	
POLε	DNA resynthesis			POL ε				
	Pol ε subunit	*POLE(POLE1)*	12q24.33	POLE	261	NM 006231	Pol2	
	Pol ε subunit	*POLE2*	14q21-q22	POLE2	59	NM 002692	Dpb2	
	Pol ε subunit	*POLE3*	9q33	POLE3	17	NM 017443	Dpb3	
	Pol ε subunit	*POLE4*	2p12	POLE4	12	NM 019896	Dpb4	
POLκ	DNA resynthesis	*POLK*	5q13.3	POLκ	100	NM 016218	–	DNA Pol V DNA Pol IV
LIG1	DNA nick sealing	*LIG1*	19q13.32	Ligase1	102	NM 000234	Cdc9	DNA ligase

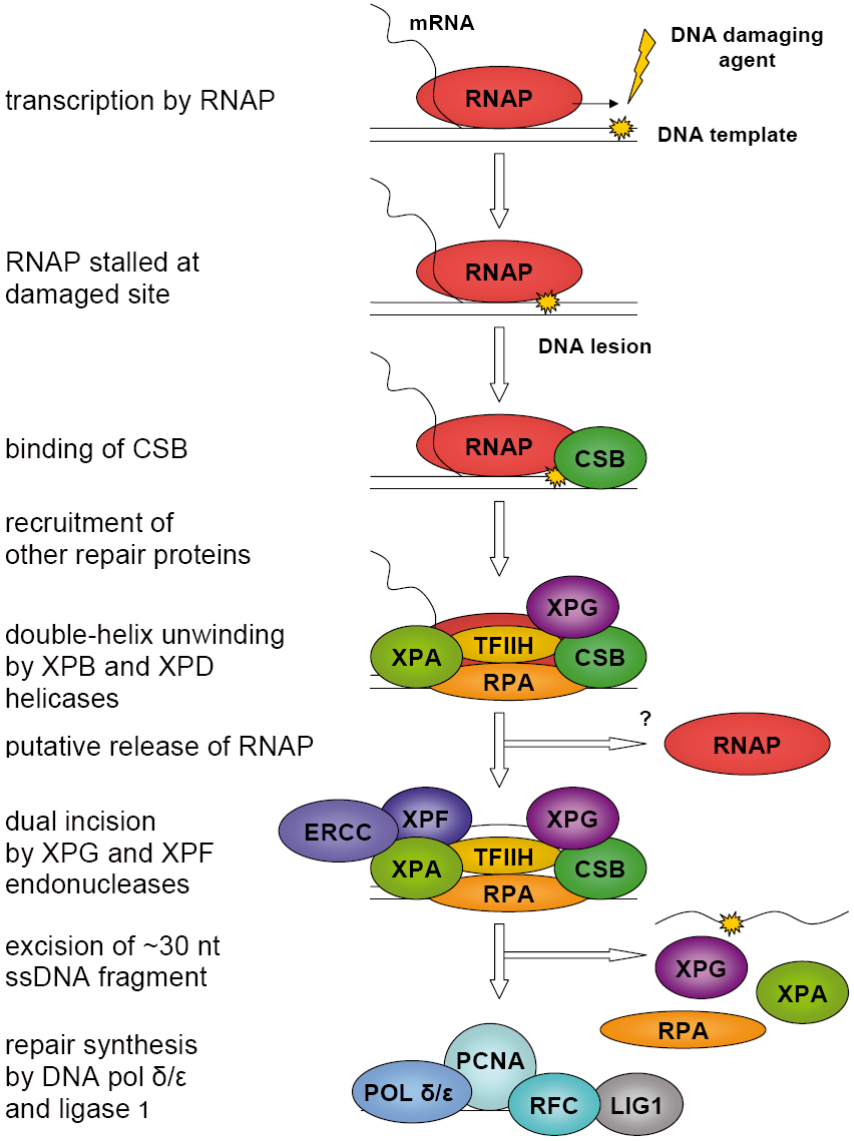

Figure 2. The current view of the transcription-coupled repair (TCR) subpathway of NER.

process, performed by endonuclease XPG and XPF-ERCC1 complex, takes place. Functional CSB is also required for the assembly of the CSA–DDB1 E3–Ub ligase–CSN complex (Fousteri *et al.*, 2006).

DNA damage recognition in GGR

Several proteins are involved in the DNA damage recognition step of GGR, among which the XPC–HR23B complex plays the major role. The human XPC is a 125 kDa protein, the function of which is restricted to GGR (van Hoffen *et al.*, 1995). It is known to form a stable complex with a 58 kDa HR23B protein (human homolog of yeast RAD23 protein) at the damaged site. In this complex, XPC alone is responsible for the binding to the site of the damage, while HR23 stimulates XPC function and is required for the displacement of the complex in the next steps of GGR. HR23B can be substituted by HR23A, suggesting a functional redundancy of these two proteins (Sugasawa *et al.*, 1998; You *et al.*, 2003). The main role of the XPC–HR23B complex is the direct recognition and binding to the damaged DNA (small bubble) which causes a change of the DNA conformation and enables the recruitment of the entire repairosome to the damaged site (Batty & Wood, 2000; Yokoi *et al.*, 2000). Earlier studies implied that the XPC–HR23B complex was also involved in the open complex formation and stabilization (Aboussekhra *et al.*, 1995; Mu *et al.*, 1997). Besides the XPC–HR23B complex, other proteins, namely XPA and XPE, may also be involved in the damage recognition process.

Another DNA damage sensor of GGR is the DNA damage binding (DDB) factor, a heterodimer consisting of subunits of 127 kDa (DDB1) and 48

kDa (XPE or DDB2). The XPE is required only for *in vivo* but not *in vitro* repair process and is characteristic for human and absent in hamster cells that fail to repair CPDs in GGR (Tang *et al.*, 2000; Sancar & Reardon, 2004; Reardon & Sancar, 2005).

DNA damage recognition in TCR

In TCR, the XPC–HR23B complex is not required for the damage recognition step. The transcription arrest caused by stalled RNAP itself is a strong signal for TCR and recruits the repair machinery to the damaged site (Laine & Egly, 2006a; Sarker *et al.*, 2005). Following transcription blockage, the CSB–RNAP interaction is established or a pre-existing complex of the two proteins is further stabilized (van den Boom *et al.*, 2004; Sarasin & Stary, 2007). The CSB is a 168 kDa DNA-dependent ATPase belonging to the SNF2 protein family. One of its main roles is remodeling of the DNA-RNAP interface by interacting with chromatin factors such as XPA-binding protein 2 (XAB2) and histone acetyltransferase (HAT) p300. However, previous reports indicating its participation in the release of RNAP was not confirmed (Fousteri *et al.*, 2006). For intrinsic pause sites, and perhaps small base damage such as 8-oxoguanine, the influence of CSB may allow RNAP to bypass the site of pausing. If the blocking lesion is bulky, such as the UV-induced lesions or cisplatin cross-link, and RNAP bypass is impossible, the polymerase becomes more permanently arrested. At this juncture the presence of CSB induces the recruitment of other TCR factors to the damaged site (Kamiuchi *et al.*, 2002; Saxowsky & Doetsch, 2006).

In vivo studies of Fousteri *et al.* (2006) showed that following UV irradiation CSB recruits the CSA–DDB1 E3–Ub ligase–CSN complex to the damaged site. CSA is a 48 kDa protein with seven WD-40 repeats which is known to function as a protein–protein interaction interface (Henning *et al.*, 1995; Zhou & Wang, 2001). Together with DDB1, COP9 signalosome (CSN) and other subunits CSA forms an E3 ubiquitin ligase complex (Groisman *et al.*, 2003). In CS cells, ubiquitylation of RNAP after UV irradiation is not observed, which implies that in normal cells this modification may depend on CSA and CSB (Bregman *et al.*, 1996). This post-translational modification might be a mechanism of regulation of RNAP function, possibly leading to protein degradation. However, it has not been shown whether this E3-ligase activity can cause polyubiquitination of RNAP and what the molecular function of the ubiquitylated stalled RNAP is (Groisman *et al.*, 2003). Moreover, CSA can also bind to the p44 subunit of TFIIH, suggesting a possible role in the assembly of the TCR repair machinery (Henning *et al.*, 1995).

DNA unwinding in GGR and TCR

The further steps of the NER pathway are similar in both GGR and TCR. The XPC–HR23B complex in GGR, and CSB protein in TCR, recruits other NER factors such as TFIIH, XPA, and RPA and XPF–ERCC1 in a sequential manner to the damaged site (Laine & Egly, 2006a; Park & Choi, 2006).

It has been shown that, in GGR, the damage-recognition XPC–HR23B complex interacts with the p62 subunit of TFIIH to bring it to the site of the damage (Yokoi *et al.*, 2000). TFIIH is a multi-protein complex which consists of ten subunits, including a 6 subunit core of XPB, XPD, p34, p44, p52 and p62, the CDK-activating kinase (CAK) complex (cyclin-dependant kinase CDK7, cyclin H, and the assembly factor MAT1) and TFB5 (Jaitovich-Groisman *et al.*, 2001; Giglia-Mari *et al.*, 2004).

In eukaryotes, TFIIH is functionally conserved and exhibits a dual role. It is involved in the initiation of RNAP transcription and participates in the both NER subpathways (Flores, 1992; Drapkin *et al.*, 1994; Schaeffer *et al.*, 1993; Wang *et al.*, 1994). The CDK complex of TFIIH phosphorylates the carboxy-terminal domain of RNAP during transcription initiation (Coin & Egly, 1998).

The XPB and XPD subunits of TFIIH are helicases which exhibit a DNA-dependent ATPase activity. The 89 kDa XPB enzyme catalyzes DNA unwinding in the 3′→5′ direction while the 80 kDa XPD enzyme functions in the opposite direction, i.e. 5′→3′ (Schaeffer *et al.*, 1994; van Gool *et al.*, 1997; Jaitovich-Groisman *et al.*, 2001; Sugasawa *et al.*, 2001). XPB appears to have a weaker helicase activity than XPD (Schaeffer *et al.*, 1993) and is not involved directly in the unwinding and repair of damaged DNA. The XPD helicase unwind an approximately 30-nt fragment of the DNA double helix around a lesion with the requirement of the XPB ATPase activity (Evans *et al.*, 1997; Coin *et al.*, 2007).

In TCR, these reactions probably lead to a partial release of stalled RNAP. Such incomplete release of RNAP has been observed in the absence of wild-type TFIIH or in the presence of mutated TFIIH, which indicates the importance of TFIIH helicase activity in the release of RNAP (Laine & Egly, 2006a).

The damage-recognition XPC–HR23B complex can also interact with XPA (You, 2003). It is a 36 kDa protein which is required for both GGR and TCR (Hanawalt, 1994; de Laat *et al.*, 1999) and is known to interact with RPA, ERCC1 and TFIIH, as well as with the damaged DNA. An *in vitro* study shows that XPA protein preferentially binds to the damaged DNA through its central loop-rich domain (Ikegami *et al.*, 1998). The zinc finger central region of XPA is also responsible for the binding of the

RPA70 subunit of RPA protein, while the N-terminal domain mediates a strong interaction with RPA32 (Lee & Hurwitz, 1990; Park & Sancar, 1994; Li *et al.*, 1995; Ikegami *et al.*, 1998; Stigger *et al.*, 1998). Interestingly, the ERCC1-binding region of XPA forms a transient intra-molecular interaction with its DNA-binding region (Buchko *et al.*, 2001).

RPA, also known as replication factor A (RFA) or human single-strand binding protein (HSSB), consists of three subunits: RPA14, RPA32 and RPA70 and has an ssDNA-binding activity which is responsible for the stabilization of the ssDNA intermediate produced by the helicase subunits of TFIIH (de Laat *et al.*, 1998). Moreover, in the presence of XPA-minimal DNA binding domain (XPA-MBD), the RPA70 subunit has an affinity for the undamaged DNA strand opposite the DNA lesion. In this way, RPA may protect the intact DNA strand from the inadvertent nuclease attack (Lee *et al.*, 2003). The interaction with ssDNA is mediated by the central domain of the 70 kDa subunit. Other regions of RPA are involved in interactions with its multiple protein partners. It has been shown that the ssDNA- and XPA-binding sites of RPA70 overlap partially, which implies that the stability of the XPA–RPA complex may be modulated by RPA–ssDNA interactions (Daughdrill *et al.*, 2003).

Due to its affinity for ssDNA and the strong interaction with XPA, RPA plays an important role in the open complex formation and stabilization but also participates in the next steps of the NER pathway. In particular, it interacts with XPG and the XPF–ERCC1 complex, coordinating the function of these nucleases in the incision step.

Dual incision in GGR and TCR

Following DNA unwinding, sequential recruitment of the nucleases XPG and the XPF–ERCC1 complex leads to a dual incision in close proximity to the damaged site. XPG hydrolyses a phosphodiester bond 2–8 nt 3′ to the damaged site (Habraken *et al.*, 1993) while XPF–ERCC1 cuts 15–24 nt 5′ to the lesion (Mu *et al.*, 1995; Moggs *et al.*, 1996).

XPG and XPF–ERCC1 are structure-specific nucleases which hydrolyse duplex substrates preferentially near the junction between the ssDNA and dsDNA (Sijbers *et al.*, 1996). The XPF-ERCC1 and XPG binding to XPA (through ERCC1) and RPA (through XPF) proteins (Bessho *et al.*, 1997) not only facilitates the correct positioning of these proteins at the damaged site but also stimulates the junction-cutting endonuclease activity of XPG and XPF–ERCC1 (Matsunaga *et al.*, 1996; Bessho *et al.*, 1997). In TCR, XPG seems to be recruited at an earlier step of the pathway and bind together with CSB to a stalled RNAP (Sarker *et al.*, 2005).

XPG is a 133 kDa protein, a member of the FEN1 family of structure-specific endonucleases which incise a variety of DNA substrates, including bubbles, flap, splayed arms and stem-loops (Scherly *et al.*, 1993; Harrington & Lieber, 1994; Cloud *et al.*, 1995; Matsunaga *et al.*, 1996). XPG possesses two highly conserved nuclease motifs separated by a spacer region which serves as a protein–protein interaction interface and determines the substrate specificity (Dunand-Sauthier *et al.*, 2005).

Binding of XPG to the pre-incision complex induces a conformational change that is required for the recruitment of the XPF–ERCC1 complex, the last protein complex to join the incision complex.

XPF is a 115 kDa protein that forms a tight complex with the 38 kDa ERCC1 protein (Park *et al.*, 1995). The strong interaction between the two proteins is mediated *via* the C-terminal helix-hairpin-helix domains of both proteins. XPF has a nuclease activity while a central region of ERCC1 is similar to the XPF nuclease domain but does not contain the residues crucial for the enzymatic activity (Choi *et al.*, 2005). The XPF–ERCC1 heterodimer incises a variety of DNA substrates such as bubbles, stem-loop and flaps. The XPF and XPG nucleases require Mg^{2+} or Mn^{2+} but not ATP for the specific cleavage activity (Evans *et al.*, 1997).

DNA re-synthesis in GGR and TCR

After incision of the damaged DNA, the resulting gap of about 30 nt is filled in by DNA polymerase δ (POLδ) or ε (POLε) and subsequently the DNA fragments are ligated by DNA ligase I (LIG1) (Wood *et al.*, 2000).

Following dual incision, RPA remains bound to the ssDNA intermediates and is involved in the recruitment of PCNA and RPA to the repair synthesis site (Yuzhakov *et al.*, 1999a; 1999b; Gomes & Burgers, 2001; Gomes *et al.*, 2001; Riedl *et al.*, 2003). The re-synthesis step by these polymerases occurs in a PCNA-dependent manner and with the requirement of RFC. RFC preferentially binds to the 3′-hydroxyl end of DNA primer and assembles PCNA onto the DNA template in an ATP-dependent manner (Fotedar *et al.*, 1996; Waga & Stillman, 1998). In addition, XPG has also been shown to interact with PCNA and facilitate its loading to the repair synthesis site (Gary *et al.*, 1997; Riedl *et al.*, 2003). PCNA is a 37 kDa protein belonging to the DNA sliding clamp protein family (Wyman & Botchan, 1995) which forms a ring-shaped homo-trimeric sliding clamp that encircles DNA. The RFC–PCNA complex serves as a docking platform that links polymerase to the DNA template and initiates chain elongation (Lee & Hurwitz, 1990; Podust *et al.*, 1994; Budd & Campbell, 1997; Wood & Shivji, 1997). PCNA also stabilizes the interaction

of polymerases with the DNA template (Ng *et al.*, 1991). Furthermore, the interaction of polymerase with PCNA and RFC allows an accurate and efficient DNA synthesis (Waga & Stillman, 1998). Interestingly, POLδ but not POLε exhibits low processivity in the absence of PCNA (Burgers, 1991) Recently, a low-fidelity Y family DNA polymerase kappa (POLκ) was found to participate in the re-synthesis step of NER (Ogi & Lehmann, 2006).

Both POLδ and POLε belonging to the B family polymerases show an intrinsic proof-reading exonuclease activity (3′→5′) (Syvaoja *et al.*, 1990; Syvaoja, 1990). Mammalian POLδ is a complex of four subunits: 125, 68, 50 and 12 kDa (Liu *et al.*, 2000) while POLε consists of a large catalytic subunit of 261 kDa and three associated subunits of 59 kDa, 17 kDa and 12 kDa (Syvaoja & Linn, 1989; Li *et al.*, 2000). Both replicative polymerases δ and ε are highly processive and can polymerize long DNA stretches without dissociating from the template and are stimulated by RPA and PCNA (Tan *et al.*, 1986; Tsurimoto & Stillman, 1989).

RFC is a heteromeric protein complex composed of one large subunit, RFC145, and four small subunits: RFC40, RFC38, RFC37 and RFC36 (Uhlmann *et al.*, 1996). The genes encoding these subunits share homology among themselves although each of them is necessary for the proper function of RFC (Bunz *et al.*, 1993; Cullmann *et al.*, 1995).

After the synthesis of a new DNA strand by DNA polymerase, the remaining nick is sealed by LIG1 to complete the repair. The enzyme binds to PCNA, encircles and partially unwinds nicked DNA and catalyzes the estrification of the 3′-hydroxyl and 5′-phosphoryl termini of the nick in DNA. The reaction also requires ATP and a diavalent cation. Human LIG1 is a 102 kDa monomer, composed of a highly conserved C-terminal domain with the active site and an N-terminal domain which contains the nuclear localization signal (NLS) and directs the enzyme to the sites of DNA replication (Modrich *et al.*, 1973; Ranalli *et al.*, 2002; Pascal *et al.*, 2004).

Regulation of NER proteins

The activation of DNA repair towards damage response results in the promotion of protein–DNA and protein–protein interactions. The repair process is further modulated through phosphorylation, ubiquitylation and other post-translational modifications of the proteins engaged (Huang & D'Andrea, 2006).

NER DEFICIENCY DISEASES

The importance of the described DNA repair system is further highlighted by the fact that defects in some NER proteins cause severe genetic disorders: xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD). To date, defects in 11 genes have been associated with these diseases, however, additional but as yet unidentified complementation groups may exist. Inherited mutations in these genes influence functions of the multiprotein complexes which play essential roles in both DNA repair and transcription.

Consistently with their genetic and molecular complexity, the phenotypes of these diseases are very diverse (Kraemer *et al.*, 2007b).

In principle, two distinct phenotypes can be distinguished: (1) a >1000-fold increased UV light-induced skin cancer in XP, and (2) segmental progeria with a normal cancer incidence in CS and TTD. However, in some cases the symptoms of more than one of these diseases are observed simultaneously. On the other hand, defects in one gene may lead to different phenotypes, depending on the type and position of the mutation (e.g., defects in the *XPD* gene can lead either to XP, TTD or XP-CS, depending on the causative point mutation), making the genotype-phenotype relationship more complex (Andressoo *et al.*, 2006).

Xeroderma pigmentosum (XP)

Xeroderma pigmentosum is a genetically diverse syndrome which falls into seven complementation groups: XP A–G which correspond to mutations in seven proteins, the key components of the NER system. An additional complementation group, XPV (XP variant), is caused by a mutation in the low-fidelity Y family DNA polymerase eta (POLη) which is responsible for error-free trans-lesion synthesis (TLS) past UV-induced DNA damage (Subba Rao, 2007).

Among XP patients severe photosensitivity and a >1000-fold increase in susceptibility to UV light-induced skin and eye cancer is observed. About 50% of the patients suffer from acute burning on minimal sun exposure and all develop freckle-like pigmentation on exposed skin. About 30% of XP patients additionally display progressive neurodegeneration characterized by sensorineural deafness, abnormal gait and cerebrum atrophy (Kraemer *et al.*, 2007a); this subtype of XP is referred to as XP neurological disease (Brooks, 2007).

The phenotype is linked to the accumulation of mutations due to inefficient repair of DNA damage by the inactive NER system. The impaired repair of UV-induced DNA lesions is known to be responsible for elevated skin and eye cancer risk in XP patients (Friedberg, 2005). Recent studies indicate that the accumulation of certain types of endogenous oxidative bulky DNA lesions (including cyclo-

deoxyadenine and cyclo-deoxyguanine) in neurons of XP patients might account for their neurological abnormalities (Brooks, 2007). However, why not all XP patients display neurological abnormalities and why the disease affects only certain parts of XP patients brain, remains to be elucidated (Kraemer *et al.*, 2007a).

Interestingly, the most severe proneness to skin cancer is observed in patients with mutations in genes, such as *XPC*, *DDB1* or *POL η*, which are unique to GGR and do not participate in TCR (Subba Rao, 2007).

Cockayne syndrome (CS)

On the contrary, defects in proteins required for efficient TCR, namely CSA and CSB, are the cause of Cockayne syndrome (CS). Approximately 80% of CS cases are due to mutations in CSB and the remaining patients have a mutated CSA protein (Kleppa *et al.*, 2007). CS is a rare autosomal recessive disorder characterized by postnatal physical and mental retardation, cachetic dwarfism, microcephaly, immature sexual development, features of premature aging, sensorineural deafness, white matter hypomyelination, central nervous system calcification, retinal and Purkinje's cell degeneration. In contrast to XP, no increase in skin-cancer susceptibility is observed among CS patients (Subba Rao, 2007).

There is growing evidence that the features of premature aging in CS are linked to the accumulation of endogenous DNA damage in the genetic material of CS cells. Other defects, for instance defective transcriptional activation of nuclear hormone receptors by the TFIIH complex, may also play a role. The TCR defect in NER and the resulting RNAP blockage protect against cancer by triggering cell-cycle arrest and/or inducing apoptosis *via* both p53-dependent and independent pathways. On the other hand, an increased apoptosis rate in the nervous system is believed to be the cause of neurodegeneration in CS brain (Andressoo *et al.*, 2006).

Trichothiodystrophy (TTD)

TTD is a clinically diverse syndrome characterized by severe neurological, somatic developmental and skeletal abnormalities. TTD patients share overlapping progeroid features with CS and XP ones but in addition show the hallmark sulfur-deficient brittle hair, postnatal growth failure, mental and physical retardation and ichthyosis. In contrast to XP, TTD is not a cancer-prone disease (Itin *et al.*, 2001).

To date there is no evidence of progressive neurodegeneration in TTD patients and the neurological abnormalities seen in these patients seem to be due to impaired development and maturation of the nervous system. Dysmyelination of the cerebrum, congenital cataracts, prenatal development abnormalities and short stature appear to confirm the developmental cause of the observed phenotype (Kraemer *et al.*, 2007a).

TTD patients carry mutations in XPD, XPB and TTDA, the three subunits of TFIIH, which, in addition to its role in NER, serves as a basal transcription factor for many genes implicated in normal organism development. It has been suggested that specific mutations in these genes affect the transcriptional rather than DNA repair functions of XPD, XPB and TTD proteins (Dubaele *et al.*, 2003).

Combined NER disorders

The combined NER disorders, XP-CS and XP-TTD display both cancer predisposition and segmental progeria. The coexistence of cancer and progeria in these syndromes in spite of the absence of cancer in CS and TTD makes them interesting models for investigating connections between cancer and aging (Andressoo *et al.*, 2006).

Mutations in XPB, XPD and ERCC5 may lead to combined symptoms of XP and TTD or CS, depending on the mutation type and position. Specific and limited point mutations in XPB and XPD are associated with XP-CS; in the majority of XP-CS patients XPG mutations were observed (Sarasin & Stary, 2007).

Why XP-CS patients are not protected from cancer similarly to CS patients remains unclear. However, it seems that a defect in XPD-XP-CS cells causes a genomic instability more severe than could be caused by UV only and as a result DNA lesions accumulate more quickly and, despite the elevated activity of gatekeepers, lead to cancerous transformation (Andressoo *et al.*, 2006).

SUMMARY

NER is a complex system that serves as a prime example of how more than 30 proteins can cooperate in a common pathway to remove a wide range of DNA lesions. The further detailed research on NER genes can provide the better understanding of the molecular mechanisms involved in NER related diseases such as XP, CS and TTD and may show new preventional and therapeutic strategies especially in skin cancer and premature aging.

REFERENCES

Aboussekhra A, Biggerstaff M, Shivji MK, Vilpo JA, Moncollin V, Podust VN, Protic M, Hubscher U, Egly JM,

Wood RD (1995) Mammalian DNA nucleotide excision repair reconstituted with purified protein components. *Cell* **80**: 859–868.

Andressoo JO, Hoeijmakers JH, Mitchell JR (2006) Nucleotide excision repair disorders and the balance between cancer and aging. *Cell Cycle* **5**: 2886–2888.

Arczewska K, Kusmierek J (2007) Bacterial DNA repair genes and their eukaryotic homologues: 2. Role of bacterial mutator gene homologues in human disease. Overview of nucleotide pool sanitization and mismatch repair systems. *Acta Biochim Polon* **54**: 435–457.

Batty DP, Wood RD (2000) Damage recognition in nucleotide excision repair of DNA. *Gene* **241**: 193–204.

Bessho T, Sancar A, Thompson LH, Thelen MP (1997) Reconstitution of human excision nuclease with recombinant XPF-ERCC1 complex. *J Biol Chem* **272**: 3833–3837.

Bohr VA, Smith CA, Okumoto DS, Hanawalt PC (1985) DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. *Cell* **40**: 359–369.

Bregman DB, Halaban R, van Gool AJ, Henning KA, Friedberg EC, Warren SL (1996) UV-induced ubiquitination of RNA polymerase II: a novel modification deficient in Cockayne syndrome cells. *Proc Natl Acad Sci USA* **93**: 11586–11590.

Brooks PJ (2007) The case for 8,5′-cyclopurine-2′-deoxynucleosides as endogenous DNA lesions that cause neurodegeneration in xeroderma pigmentosum. *Neuroscience* **145**: 1407–1417.

Buchko GW, Isern NG, Spicer LD, Kennedy MA (2001) Human nucleotide excision repair protein XPA: NMR spectroscopic studies of an XPA fragment containing the ERCC1-binding region and the minimal DNA-binding domain (M59–F219). *Mutat Res* **486**: 1–10.

Budd ME, Campbell JL (1997) The roles of the eukaryotic DNA polymerases in DNA repair synthesis. *Mutat Res* **384**: 157–167.

Bunz F, Kobayashi R, Stillman B (1993) cDNAs encoding the large subunit of human replication factor C. *Proc Natl Acad Sci USA* **90**: 11014–11018.

Burgers PM (1991) *Saccharomyces cerevisiae* replication factor C. II. Formation and activity of complexes with the proliferating cell nuclear antigen and with DNA polymerases delta and epsilon. *J Biol Chem* **266**: 22698–22706.

Choi YJ, Ryu KS, Ko YM, Chae YK, Pelton JG, Wemmer DE, Choi BS (2005) Biophysical characterization of the interaction domains and mapping of the contact residues in the XPF-ERCC1 complex. *J Biol Chem* **280**: 28644–28652.

Cloud KG, Shen B, Strniste GF, Park MS (1995) XPG protein has a structure-specific endonuclease activity. *Mutat Res* **347**: 55–60.

Coin F, Egly JM (1998) Ten years of TFIIH. *Cold Spring Harb Symp Quant Biol* **63**: 105–110.

Coin F, Oksenych V, Egly JM (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.

Costa RM, Chigancas V, Galhardo Rda S, Carvalho H, Menck CF (2003) The eukaryotic nucleotide excision repair pathway. *Biochimie* **85**: 1083–1099.

Cullmann G, Fien K, Kobayashi R, Stillman B (1995) Characterization of the five replication factor C genes of *Saccharomyces cerevisiae*. *Mol Cell Biol* **15**: 4661–4671.

Daughdrill GW, Buchko GW, Botuyan MV, Arrowsmith C, Wold MS, Kennedy MA, Lowry DF (2003) Chemical shift changes provide evidence for overlapping single-stranded DNA- and XPA-binding sites on the 70 kDa subunit of human replication protein A. *Nucleic Acids Res* **31**: 4176–4183.

de Laat WL, Appeldoorn E, Sugasawa K, Weterings E, Jaspers NG, Hoeijmakers JH (1998) DNA-binding polarity of human replication protein A positions nucleases in nucleotide excision repair. *Genes Dev* **12**: 2598–2609.

de Laat WL, Jaspers NG, Hoeijmakers JH (1999) Molecular mechanism of nucleotide excision repair. *Genes Dev* **13**: 768–785.

Drapkin R, Reardon JT, Ansari A, Huang JC, Zawel L, Ahn K, Sancar A, Reinberg D (1994) Dual role of TFIIH in DNA excision repair and in transcription by RNA polymerase II. *Nature* **368**: 769–772.

Dubaele S, Proietti De Santis L, Bienstock RJ, Keriel A, Stefanini M, Van Houten B, Egly JM (2003) Basal transcription defect discriminates between xeroderma pigmentosum and trichothiodystrophy in XPD patients. *Mol Cell* **11**: 1635–1646.

Dunand-Sauthier I, Hohl M, Thorel F, Jaquier-Gubler P, Clarkson SG, Scharer OD (2005) The spacer region of XPG mediates recruitment to nucleotide excision repair complexes and determines substrate specificity. *J Biol Chem* **280**: 7030–7037.

Evans E, Moggs JG, Hwang JR, Egly JM, Wood RD (1997) Mechanism of open complex and dual incision formation by human nucleotide excision repair factors. *EMBO J* **16**: 6559–6573.

Fotedar R, Mossi R, Fitzgerald P, Rousselle T, Maga G, Brickner H, Messier H, Kasibhatla S, Hubscher U, Fotedar A (1996) A conserved domain of the large subunit of replication factor C binds PCNA and acts like a dominant negative inhibitor of DNA replication in mammalian cells. *EMBO J* **15**: 4423–4433.

Fousteri M, Vermeulen W, van Zeeland AA, Mullenders LH (2006) Cockayne syndrome A and B proteins differentially regulate recruitment of chromatin remodeling and repair factors to stalled RNA polymerase II *in vivo*. *Mol Cell* **23**: 471–482.

Friedberg EC (2001) How nucleotide excision repair protects against cancer. *Nat Rev Cancer* **1**: 22–33.

Friedberg EC (2005) *DNA repair and mutagenesis*. ASM Press, Washington, DC.

Gary R, Ludwig DL, Cornelius HL, MacInnes MA, Park MS (1997) The DNA repair endonuclease XPG binds to proliferating cell nuclear antigen (PCNA) and shares sequence elements with the PCNA-binding regions of FEN-1 and cyclin-dependent kinase inhibitor p21. *J Biol Chem* **272**: 24522–24529.

Giglia-Mari G, Coin F, Ranish JA, Hoogstraten D, Theil A, Wijgers N, Jaspers NG, Raams A, Argentini M, van der Spek PJ, Botta E, Stefanini M, Egly JM, Aebersold R, Hoeijmakers JH, Vermeulen W (2004) A new, tenth subunit of TFIIH is responsible for the DNA repair syndrome trichothiodystrophy group A. *Nat Genet* **36**: 714–719.

Gillet LC, Scharer OD (2006) Molecular mechanisms of mammalian global genome nucleotide excision repair. *Chem Rev* **106**: 253–276.

Gomes XV, Burgers PM (2001) ATP utilization by yeast replication factor C. I. ATP-mediated interaction with DNA and with proliferating cell nuclear antigen. *J Biol Chem* **276**: 34768–34775.

Gomes XV, Schmidt SL, Burgers PM (2001) ATP utilization by yeast replication factor C. II. Multiple stepwise ATP binding events are required to load proliferating cell nuclear antigen onto primed DNA. *J Biol Chem* **276**: 34776–34783.

Groisman R, Polanowska J, Kuraoka I, Sawada J, Saijo M, Drapkin R, Kisselev AF, Tanaka K, 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.

Gros L, Ishchenko AA, Ide H, Elder RH, Saparbaev MK (2004) The major human AP endonuclease (Ape1) is involved in the nucleotide incision repair pathway. *Nucleic Acids Res* **32**: 73–81.

Habraken Y, Sung P, Prakash L, Prakash S (1993) Yeast excision repair gene RAD2 encodes a single-stranded DNA endonuclease. *Nature* **366**: 365–368.

Hanawalt PC (1994) Transcription-coupled repair and human disease. *Science* **266**: 1957–1958.

Hanawalt PC (2002) Subpathways of nucleotide excision repair and their regulation. *Oncogene* **21**: 8949–8956.

Harman D (1956) Aging: a theory based on free radical and radiation chemistry. *J Gerontol* **11**: 298–300.

Harrington JJ, Lieber MR (1994) The characterization of a mammalian DNA structure-specific endonuclease. *EMBO J* **13**: 1235–1246.

Henning KA, Li L, Iyer N, McDaniel LD, Reagan MS, Legerski R, Schultz RA, Stefanini M, Lehmann AR, Mayne LV, Friedberg EC (1995) The Cockayne syndrome group A gene encodes a WD repeat protein that interacts with CSB protein and a subunit of RNA polymerase II TFIIH. *Cell* **82**: 555–564.

Hoeijmakers JH (2001) DNA repair mechanisms. *Maturitas*, **38**: 17–22; discussion 22–13.

Huang TT, D'Andrea AD (2006) Regulation of DNA repair by ubiquitylation. *Nat Rev Mol Cell Biol* **7**: 323–334.

Ikegami T, Kuraoka I, Saijo M, Kodo N, Kyogoku Y, Morikawa K, Tanaka K, Shirakawa M (1998) Solution structure of the DNA- and RPA-binding domain of the human repair factor XPA. *Nat Struct Biol* **5**: 701–706.

Ischenko AA, Saparbaev MK (2002) Alternative nucleotide incision repair pathway for oxidative DNA damage. *Nature* **415**: 183–187.

Ishchenko AA, Deprez E, Maksimenko A, Brochon JC, Tauc P, Saparbaev MK (2006) Uncoupling of the base excision and nucleotide incision repair pathways reveals their respective biological roles. *Proc Natl Acad Sci USA* **103**: 2564–2569.

Itin PH, Sarasin A, Pittelkow MR (2001) Trichothiodystrophy: update on the sulfur-deficient brittle hair syndromes. *J Am Acad Dermatol* **44**: 891–920; quiz 921–894.

Jaitovich-Groisman I, Benlimame N, Slagle BL, Perez MH, Alpert L, Song DJ, Fotouhi-Ardakani N, Galipeau J, Alaoui-Jamali MA (2001) Transcriptional regulation of the TFIIH transcription repair components XPB and XPD by the hepatitis B virus x protein in liver cells and transgenic liver tissue. *J Biol Chem* **276**: 14124–14132.

Johnson NA, McKenzie R, McLean L, Sowers LC, Fletcher HM (2004) 8-Oxo-7,8-dihydroguanine is removed by a nucleotide excision repair-like mechanism in *Porphyromonas gingivalis* W83. *J Bacteriol* **186**: 7697–7703.

Kamiuchi S, Saijo M, Citterio E, de Jager M, Hoeijmakers JH, Tanaka K (2002) Translocation of Cockayne syndrome group A protein to the nuclear matrix: possible relevance to transcription-coupled DNA repair. *Proc Natl Acad Sci USA* **99**: 201–206.

Kleppa L, Kanavin OJ, Klungland A, Stromme P (2007) A novel splice site mutation in the Cockayne syndrome group A gene in two siblings with Cockayne syndrome. *Neuroscience* **145**: 1397–1406.

Kraemer KH, Patronas NJ, Schiffmann R, Brooks BP, Tamura D, Digiovanna JJ (2007a) Xeroderma pigmentosum, trichothiodystrophy and Cockayne syndrome: A complex genotype-phenotype relationship. *Neuroscience* **145**: 1388–1396.

Kraemer KH, Sander M, Bohr VA (2007b) New areas of focus at workshop on human diseases involving DNA repair deficiency and premature aging. *Mech Ageing Dev* **128**: 229–235.

Krwawicz J, Arczewska KD, Speina E, Maciejewska A, Grzesiuk E (2007) Bacterial DNA repair genes and their eukaryotic homologues: 1. Mutations in genes involved in base excision repair (BER) and DNA-end processors and their implication in mutagenesis and human disease. *Acta Biochim Polon* **54**: 413–434.

Laine JP, Egly JM (2006a) Initiation of DNA repair mediated by a stalled RNA polymerase IIO. *EMBO J* **25**: 387–397.

Laine JP, Egly JM (2006b) When transcription and repair meet: a complex system. *Trends Genet* **22**: 430–436.

Lee SH, Hurwitz J (1990) Mechanism of elongation of primed DNA by DNA polymerase delta, proliferating cell nuclear antigen, and activator 1. *Proc Natl Acad Sci USA* **87**: 5672–5676.

Lee JH, Park CJ, Arunkumar AI, Chazin WJ, Choi BS (2003) NMR study on the interaction between RPA and DNA decamer containing *cis-syn* cyclobutane pyrimidine dimer in the presence of XPA: implication for damage verification and strand-specific dual incision in nucleotide excision repair. *Nucleic Acids Res* **31**: 4747–4754.

Lehmann AR (1995) The molecular biology of nucleotide excision repair and double-strand break repair in eukaryotes. *Genet Eng (N Y)* **17**: 1–19.

Lehmann AR, Niimi A, Ogi T, Brown S, Sabbioneda S, Wing JF, Kannouche PL, Green CM (2007) Translesion synthesis: Y-family polymerases and the polymerase switch. *DNA Repair (Amst)* **6**: 891–899.

Leibeling D, Laspe P, Emmert S (2006) Nucleotide excision repair and cancer. *J Mol Histol* **37**: 225–238.

Li L, Lu X, Peterson CA, Legerski RJ (1995) An interaction between the DNA repair factor XPA and replication protein A appears essential for nucleotide excision repair. *Mol Cell Biol* **15**: 5396–5402.

Li Y, Pursell ZF, Linn S (2000) Identification and cloning of two histone fold motif-containing subunits of HeLa DNA polymerase epsilon. *J Biol Chem* **275**: 23247–23252.

Lindahl T, Wood RD (1999) Quality control by DNA repair. *Science* **286**: 1897–1905.

Liu L, Mo J, Rodriguez-Belmonte EM, Lee MY (2000) Identification of a fourth subunit of mammalian DNA polymerase delta. *J Biol Chem* **275**: 18739–18744.

Lodish H BA, Matsudaira P, Kaiser CA, Krieger M, Scott MP, Zipursky SL, Darnell J (2004) *Molecular Biology of the Cell*. WH Freeman, New York, NY.

Matsunaga T, Park CH, Bessho T, Mu D, Sancar A (1996) Replication protein A confers structure-specific endonuclease activities to the XPF-ERCC1 and XPG subunits of human DNA repair excision nuclease. *J Biol Chem* **271**: 11047–11050.

Mellon I (2005) Transcription-coupled repair: a complex affair. *Mutat Res* **577**: 155–161.

Mellon I, Hanawalt PC (1989) Induction of the *Escherichia coli* lactose operon selectively increases repair of its transcribed DNA strand. *Nature* **342**: 95–98.

Modrich P, Anraku Y, Lehman IR (1973) Deoxyribonucleic acid ligase. Isolation and physical characterization of the homogeneous enzyme from *Escherichia coli*. *J Biol Chem* **248**: 7495–7501.

Moggs JG, Yarema KJ, Essigmann JM, Wood RD (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.

Mu D, Park CH, Matsunaga T, Hsu DS, Reardon JT, Sancar A (1995) Reconstitution of human DNA repair excision nuclease in a highly defined system. *J Biol Chem* **270**: 2415–2418.

Mu D, Wakasugi M, Hsu DS, Sancar A (1997) Characterization of reaction intermediates of human excision repair nuclease. *J Biol Chem* **272**: 28971–28979.

Ng L, Tan CK, Downey KM, Fisher PA (1991) Enzymologic mechanism of calf thymus DNA polymerase delta. *J Biol Chem* **266**: 11699–11704.

Nieminuszczy J, Grzesiuk E (2007) Bacterial DNA repair genes and their eukaryotic homologues: 3. AlkB dioxygenase and Ada methyltransferase in the direct repair of alkylated DNA. *Acta Biochim Polon* **54**: 459–468

Nowosielska A (2007) Bacterial DNA repair genes and their eukaryotic homologues: 5. The role of recombination in DNA repair and genome stability. *Acta Biochim Polon* **54**: 483–4940.

Ogi T, Lehmann AR (2006) The Y-family DNA polymerase kappa (pol kappa) functions in mammalian nucleotide-excision repair. *Nat Cell Biol* **8**: 640–642.

Olinski R, Siomek A, Rozalski R, Gackowski D, Foksinski M, Guz J, Dziaman T, Szpila A, Tudek B (2007) Oxidative damage to DNA and antioxidant status in aging and age-related diseases. *Acta Biochim Polon* **54**: 11–26.

Park CH, Sancar A (1994) Formation of a ternary complex by human XPA, ERCC1, and ERCC4(XPF) excision repair proteins. *Proc Natl Acad Sci USA* **91**: 5017–5021.

Park CH, Bessho T, Matsunaga T, Sancar A (1995) Purification and characterization of the XPF-ERCC1 complex of human DNA repair excision nuclease. *J Biol Chem* **270**: 22657–22660.

Park CJ, Choi BS (2006) The protein shuffle. Sequential interactions among components of the human nucleotide excision repair pathway. *FEBS J* **273**: 1600–1608.

Pascal JM, O'Brien PJ, Tomkinson AE, Ellenberger T (2004) Human DNA ligase I completely encircles and partially unwinds nicked DNA. *Nature* **432**: 473–478.

Podust LM, Podust VN, Floth C, Hubscher U (1994) Assembly of DNA polymerase delta and epsilon holoenzymes depends on the geometry of the DNA template. *Nucleic Acids Res* **22**: 2970–2975.

Ranalli TA, DeMott MS, Bambara RA (2002) Mechanism underlying replication protein A stimulation of DNA ligase I. *J Biol Chem* **277**: 1719–1727.

Reardon JT, Sancar A (2005) Nucleotide excision repair. *Prog Nucleic Acid Res Mol Biol* **79**: 183–235.

Riedl T, Hanaoka F, Egly JM (2003) The comings and goings of nucleotide excision repair factors on damaged DNA. *EMBO J* **22**: 5293–5303.

Sancar A, Reardon JT (2004) Nucleotide excision repair in *E. coli* and man. *Adv Protein Chem* **69**: 43–71.

Sarasin A, Stary A (2007) New insights for understanding the transcription-coupled repair pathway. *DNA Repair (Amst)* **6**: 265–269.

Sarker AH, Tsutakawa SE, Kostek S, Ng C, Shin DS, Peris M, Campeau E, Tainer JA, Nogales E, Cooper PK (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.

Saxowsky TT, Doetsch PW (2006) RNA polymerase encounters with DNA damage: transcription-coupled repair or transcriptional mutagenesis? *Chem Rev* **106**: 474–488.

Schaeffer L, Roy R, Humbert S, Moncollin V, Vermeulen W, Hoeijmakers JH, Chambon P, Egly JM (1993) DNA repair helicase: a component of BTF2 (TFIIH) basic transcription factor. *Science* **260**: 58–63.

Schaeffer L, Moncollin V, Roy R, Staub A, Mezzina M, Sarasin A, Weeda G, Hoeijmakers JH, Egly JM (1994) The ERCC2/DNA repair protein is associated with the class II BTF2/TFIIH transcription factor. *EMBO J* **13**: 2388–2392.

Scherly D, Nouspikel T, Corlet J, Ucla C, Bairoch A, Clarkson SG (1993) Complementation of the DNA repair defect in xeroderma pigmentosum group G cells by a human cDNA related to yeast RAD2. *Nature* **363**: 182–185.

Sijbers AM, de Laat WL, Ariza RR, Biggerstaff M, Wei YF, Moggs JG, Carter KC, Shell BK, Evans E, de Jong MC, Rademakers S, de Rooij J, Jaspers NG, Hoeijmakers JH, Wood RD (1996) Xeroderma pigmentosum group F caused by a defect in a structure-specific DNA repair endonuclease. *Cell* **86**: 811–822.

Stigger E, Drissi R, Lee SH (1998) Functional analysis of human replication protein A in nucleotide excision repair. *J Biol Chem* **273**: 9337–9343.

Subba Rao K (2007) Mechanisms of disease: DNA repair defects and neurological disease. *Nat Clin Pract Neurol* **3**: 162–172.

Sugasawa K, Ng JM, Masutani C, Iwai S, van der Spek PJ, Eker AP, Hanaoka F, Bootsma D, Hoeijmakers JH (1998) Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. *Mol Cell* **2**: 223–232.

Sugasawa K, Okamoto T, Shimizu Y, Masutani C, Iwai S, Hanaoka F (2001) A multistep damage recognition mechanism for global genomic nucleotide excision repair. *Genes Dev* **15**: 507–521.

Syvaoja JE (1990) DNA polymerase epsilon: the latest member in the family of mammalian DNA polymerases. *Bioessays* **12**: 533–536.

Syvaoja J, Linn S (1989) Characterization of a large form of DNA polymerase delta from HeLa cells that is insensitive to proliferating cell nuclear antigen. *J Biol Chem* **264**: 2489–2497.

Syvaoja J, Suomensaari S, Nishida C, Goldsmith JS, Chui GS, Jain S, Linn S (1990) DNA polymerases alpha, delta, and epsilon: three distinct enzymes from HeLa cells. *Proc Natl Acad Sci USA* **87**: 6664–6668.

Tan CK, Castillo C, So AG, Downey KM (1986) An auxiliary protein for DNA polymerase-delta from fetal calf thymus. *J Biol Chem* **261**: 12310–12316.

Tang JY, Hwang BJ, Ford JM, Hanawalt PC, Chu G (2000) Xeroderma pigmentosum p48 gene enhances global genomic repair and suppresses UV-induced mutagenesis. *Mol Cell* **5**: 737–744.

Truglio JJ, Croteau DL, Van Houten B, Kisker C (2006) Prokaryotic nucleotide excision repair: the UvrABC system. *Chem Rev* **106**: 233–252.

Tsurimoto T, Stillman B (1989) Purification of a cellular replication factor, RF-C, that is required for coordinated synthesis of leading and lagging strands during simian virus 40 DNA replication *in vitro*. *Mol Cell Biol* **9**: 609–619.

Uhlmann F, Cai J, Flores-Rozas H, Dean FB, Finkelstein J, O'Donnell M, Hurwitz J (1996) In vitro reconstitution of human replication factor C from its five subunits. *Proc Natl Acad Sci USA* **93**: 6521–6526.

van den Boom V, Citterio E, Hoogstraten D, Zotter A, Egly JM, van Cappellen WA, Hoeijmakers JH, Houtsmuller AB, Vermeulen W (2004) DNA damage stabilizes interaction of CSB with the transcription elongation machinery. *J Cell Biol* **166**: 27–36.

van Gool AJ, van der Horst GT, Citterio E, Hoeijmakers JH (1997) Cockayne syndrome: defective repair of transcription? *EMBO J* **16**: 4155–4162.

van Hoffen A, Venema J, Meschini R, van Zeeland AA, Mullenders LH (1995) 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. *EMBO J* **14**: 360–367.

van Houten B, Croteau DL, DellaVecchiaMJ, Wang H, Kisker C (2005) 'Close-fitting sleeves': DNA damage recognition by the UvrABC nuclease system. *Mutat Res* **577**: 92–117.

Waga S, Stillman B (1998) The DNA replication fork in eukaryotic cells. *Annu Rev Biochem* **67**: 721–751.

Wang Z, Svejstrup JQ, Feaver WJ, Wu X, Kornberg RD, Friedberg EC (1994) Transcription factor b (TFIIH) is required during nucleotide-excision repair in yeast. *Nature* **368**: 74–76.

Wood RD (1997) Nucleotide excision repair in mammalian cells. *J Biol Chem* **272**: 23465–23468.

Wood RD, Shivji MK (1997) Which DNA polymerases are used for DNA-repair in eukaryotes? *Carcinogenesis* **18**: 605–610.

Wood RD, Araujo SJ, Ariza RR, Batty DP, Biggerstaff M, Evans E, Gaillard PH, Gunz D, Koberle B, Kuraoka I, Moggs JG, Sandall JK, Shivji MK (2000) DNA damage recognition and nucleotide excision repair in mammalian cells. *Cold Spring Harb Symp Quant Biol* **65**: 173–182.

Wood RD, Mitchell M, Lindahl T (2005) Human DNA repair genes, 2005. *Mutat Res* **577**: 275–283.

Wyman C, Botchan M (1995) DNA replication. A familiar ring to DNA polymerase processivity. *Curr Biol* **5**: 334–337.

Yokoi M, Masutani C, Maekawa T, Sugasawa K, Ohkuma Y, Hanaoka F (2000) The xeroderma pigmentosum group C protein complex XPC-HR23B plays an important role in the recruitment of transcription factor IIH to damaged DNA. *J Biol Chem* **275**: 9870–9875.

You JS, Wang M, Lee SH (2003) Biochemical analysis of the damage recognition process in nucleotide excision repair. *J Biol Chem* **278**: 7476–7485.

Yuzhakov A, Kelman Z, Hurwitz J, O'Donnell M (1999a) Multiple competition reactions for RPA order the assembly of the DNA polymerase delta holoenzyme. *EMBO J* **18**: 6189–6199.

Yuzhakov A, Kelman Z, O'Donnell M (1999b) Trading places on DNA – a three-point switch underlies primer handoff from primase to the replicative DNA polymerase. *Cell* **96**: 153–163.

Zhou HX, Wang G (2001) Predicted structures of two proteins involved in human diseases. *Cell Biochem Biophys* **35**: 35–47.