

REVIEW

Molecular mechanism of nucleotide excision repair

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From its very beginning, life has faced the fundamental problem that the form in which genetic information is stored is not chemically inert. DNA integrity is challenged by the damaging effect of numerous chemical and physical agents, compromising its function. To protect this Achilles heel, an intricate network of DNA repair systems has evolved early in evolution. One of these is nucleotide excision repair (NER), a highly versatile and sophisticated DNA damage removal pathway that counteracts the deleterious effects of a multitude of DNA lesions, including major types of damage induced by environmental sources. The most relevant lesions subject to NER are cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts (6-4PPs), two major kinds of injury produced by the shortwave UV component of sunlight. In addition, numerous bulky chemical adducts are eliminated by this process. Within the divergent spectrum of NER lesions, significant distortion of the DNA helix appears to be a common denominator. Defects in NER underlie the extreme photosensitivity and predisposition to skin cancer observed with the prototype repair syndrome xeroderma pigmentosum (XP). Seven XP complementation groups have been identified, representing distinct repair genes *XPA-G* (discussed in detail below).

In the last decade, all key NER factors have been cloned and the core of the 'cut-and-paste' reaction has been reconstituted in vitro from purified components. Recently, XPC (complexed to hHR23B) has been identified as a DNA-damage sensor and repair-recruitment factor. The general transcription factor complex TFIIH, containing the XPB and XPD helicases, mediates strand separation at the site of the lesion. XPA verifies the damage in an open DNA conformation and is crucial in the assembly of the remainder of the repair machinery. Replication protein A (RPA) stabilizes the opened DNA complex and is involved in positioning the XPG and ERCC1-XPF endonucleases responsible for the DNA incisions around the lesion. After removal of the damage-containing oligonucleotide, typically 24–32 nucleotides in length, general replication factors fill in the remaining gap and close it.

Two modes of NER can be distinguished: repair of lesions over the entire genome, referred to as global genome NER (GG-NER), and repair of transcription-blocking lesions present in transcribed DNA strands, hence called transcription-coupled NER (TC-NER). Most XP groups harbor defects in a common component of both NER subpathways. GG-NER is dependent on the activity of all factors mentioned above, including the GG-NER-specific complex XPC-hHR23B. The rate of repair for GG-NER strongly depends on the type of lesion. For instance, 6-4PPs are removed much faster from the genome than CPDs, probably because of differences in affinity of the damage sensor XPC-hHR23B. In addition, the location (accessibility) of a lesion influences the removal rate in vivo. In TC-NER, damage is detected by the elongating RNA polymerase II complex when it encounters a lesion. Interestingly, a distinct disorder, Cockayne syndrome (CS), is associated with a specific defect in transcription-coupled repair. The identification of two complementation groups (CS-A and CS-B) shows that at least two gene products are specifically needed for fast and efficient repair of transcribed strands. Phenotypically, CS is a very pleiotropic condition characterized by photosensitivity as well as severe neurological, developmental, and premature aging features. Most of these symptoms are not seen even with totally NER-deficient XP patients. The additional symptoms of CS suggest that transcription-coupled repair and/or the CS proteins have functions beyond NER. Also, non-NER-specific lesions (such as oxidative damage) that stall transcription elongation appear to be removed in a transcription-coupled fashion, linking a blocked polymerase to multiple repair pathways. Intriguingly, some XP-B, XP-D, and XP-G patients display CS features combined with XP manifestations. Yet other XP-B and XP-D individuals suffer from the CS-like brittle-hair syndrome trichothiodystrophy (TTD). This clinical conundrum points to additional roles of these NER factors as well. A recent mouse model for TTD has linked mutations in the XPD subunit of the dual functional TFIIH complex with deficiencies in basal transcription underlying at least some of the TTD manifestations. Thus, NER defects are associated with a surprisingly wide clinical heterogeneity due to additional functions of the NER factors involved.

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This review focuses on the core NER components of mammalian cells, integrating recent advances into a detailed model for the molecular reaction mechanism. Various aspects of NER and associated syndromes have been comprehensively summarized in previous reviews (see Hanawalt and Mellon 1993; Hoeijmakers 1994; Friedberg et al. 1996; Sancar 1996; Wood 1996, 1997; Bootsma et al. 1997; de Boer and Hoeijmakers 1999).

XPC-hHR23B

XPC is the sole XP factor dispensable for TC-NER and is restricted to global genome NER (Venema et al. 1990a, 1991; van Hoffen et al. 1995). The 125-kD XPC protein is complexed with the 58-kD hHR23B gene product, one of the two human homologs of the yeast NER factor Rad23 (Masutani et al. 1994) (Table 1) (for proteins and domains, see Fig. 1). hHR23B stimulates XPC activity in vitro (Sugasawa et al. 1996), probably in a structural rather than a catalytic fashion, as the 54-amino acid XPC-binding domain of hHR23B is already sufficient for

XPC stimulation (Masutani et al. 1997). hHR23B is much more abundant than XPC in mammalian cells, and like hHR23A, the other human homolog of Rad23, mostly exists in a free form in the cell (Masutani et al. 1994; van der Spek et al. 1996). hHR23A can substitute for hHR23B in binding and stimulating XPC in vitro, suggesting some functional redundancy (Sugasawa et al. 1997). Yeast Rad23 and its two mammalian derivatives harbor a ubiquitin-like moiety at their amino terminus (Masutani et al. 1994; Fig. 1), pointing to additional engagements in the ubiquitin pathway of protein (in)stability. In *Saccharomyces cerevisiae*, this domain is indispensable for the repair function of Rad23 (Watkins et al. 1993; Mueller and Smerdon 1996).

The specific role of XPC-hHR23B in GG-NER has been obscure for quite some time. XPC was detected in partially purified TFIIH fractions after seven chromatographic steps; and in *S. cerevisiae*, in vitro binding between the XPC homolog Rad4 (Table 1) and yTFIIH was reported (Bardwell et al. 1994; Drapkin et al. 1994), suggesting a direct interaction between these two protein

Table 1. Core NER factors

NER factor			Subunits				
name	function in NER	(additional) engagements	name	protein size (aa)	rodent mutant	<i>S. cerevisiae</i> homolog	remarks
XPC-hHR23B	damage sensor and repair recruitment factor	only in GG-NER; not involved in TC-NER	XPC	940		Rad4	affinity for damaged DNA
			hHR23B	409		Rad23	stimulates XPC activity in vitro
			hHR23A	363		Rad23	can substitute for hHR23B in vitro
TFIIH	catalyzes open complex formation around the lesion and facilitates repair complex assembly	basal RNA Pol II transcription cell cycle regulation (?) additional non-NER-specific role in transcription-coupled repair (?)	XPB	782	ERCC3	Rad25/SSL2	3' → 5' helicase
			XPD	760	ERCC2	Rad3	5' → 3' helicase
			p34	303		TFB4	DNA binding?
			p44	395		SSL1	DNA binding?
			p62	548		TFB1	
			p52	462		TFB2	
			Mat1	309		TFB3	CAK subcomplex
			Cdk7	346		Kin28	CAK subcomplex
Cyclin H	323		CCL1	CAK subcomplex			
XPA	binds damaged DNA and facilitates repair complex assembly		XPA	273		Rad14	affinity for single-stranded and damaged DNA
RPA	stabilizes opened DNA complex and positions nucleases	replication recombination	RPA70	616		Rfa1	ssDNA binding
			RPA32	270		Rfa2	ssDNA binding
			RPA14	121		Rfa3	
XPG	catalyzes 3' incision and stabilizes full open complex	additional non-NER-specific role in transcription-coupled repair (?)	XPG	1186	ERCC5	Rad2	Member of FEN-1 family of structure-specific nucleases
ERCC1-XPF	catalyzes 5' incision	interstrand cross-link repair recombination via single-strand annealing	ERCC1	297	ERCC1	Rad10	structure-specific endonuclease
			XPF	905	ERCC4	Rad1	

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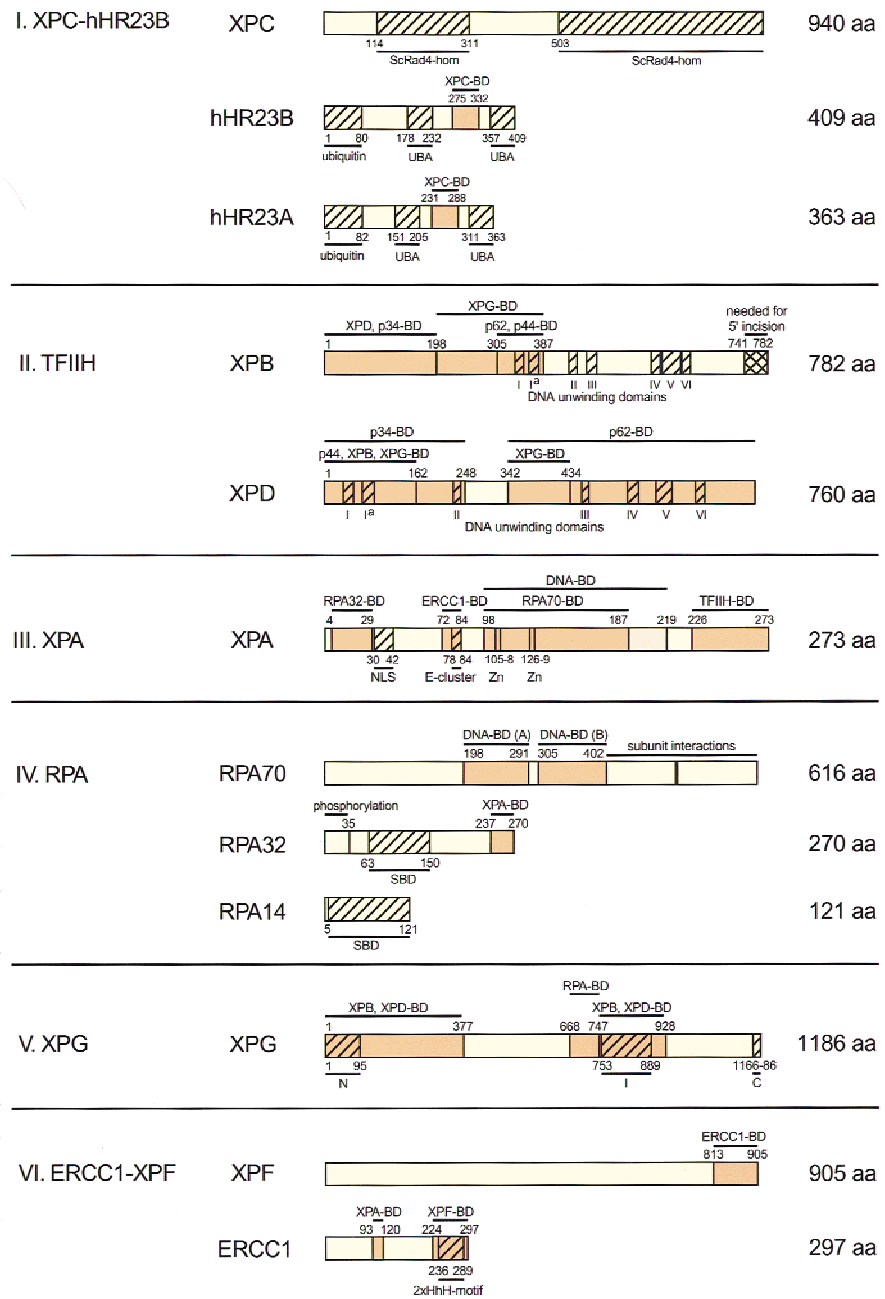


Figure 1. Functional domains in mammalian NER proteins. Schematic presentation of identified functional domains in NER proteins. DNA- and protein-interaction domains are indicated above (brown boxes) (BD) Binding domain; (putative) functional domains based on primary amino acid sequence are indicated below each schematic presentation (hatched boxes). (XPC-hHR23B) ScRad4-hom: Domains within XPC with (limited) homology to *S. cerevisiae* Rad4. Ubiquitin: Ubiquitin-like region. UBA: Ubiquitin-associated domain. (TFIIH) p34, p44, and p62 are other TFIIH subunits. (XPA) NLS: Nuclear localization signal. E-cluster: Glutamic acid-rich region. Zn: Zn²⁺ finger. (RPA) SBD: Putative ssDNA-binding domain. (XPG) N, I, and C are regions conserved in the FEN-1 family of structure-specific endonucleases. (ERCC1-XPF) 2xHhH-motif: Double-HhH motif.

complexes (Table 2). XPC-hHR23B and XPC alone display a similar high affinity for both single-stranded (ss) and double-stranded (ds) DNA and a preference for UV-damaged DNA (Masutani et al. 1994; Shivji et al. 1994; Reardon et al. 1996a). XPC-hHR23B is absolutely required for dual incision as well as for open complex formation during GG-NER (Aboussekhra et al. 1995; Mu et al. 1995, 1997; Mu and Sancar 1997; Evans et al. 1997b). An artificial DNA substrate containing single-stranded bubbles across or 5' of a lesion obviated the requirement of XPC-hHR23B (Mu and Sancar 1997). Furthermore, both the helicase complex TFIIH and XPC-hHR23B were essential for (limited) opening around a damage (Evans et al. 1997b). Also, XPC-hHR23B is dispensable for in vitro

repair of an artificial cholesterol DNA adduct. Apparently, certain DNA structures alleviate the need for XPC-hHR23B (Mu et al. 1996). These data are all in line with a role for XPC-hHR23B in open complex formation and/or stabilization but do not exclude a role even earlier in the reaction.

Evidence for the latter was obtained recently by a series of experiments in which two damaged plasmids of different sizes were separately preincubated, one with purified XPC-hHR23B and the other with all other NER factors. After mixing, initial repair was found focused on the plasmid preincubated with XPC-hHR23B, demonstrating that XPC-hHR23B is the first factor in NER, working even before TFIIH, XPA, and RPA and capable

Table 2. Physical interactions between NER proteins

	TFIIH	RPA	XPG	ERCC1-XPF
XPA	PD (Park et al. 1995a; Nocentini et al. 1997)	PD, CP (He et al. 1995; Li et al. 1995b; Saijo et al. 1996; Stigger et al. 1998)		PD, 2H (Li et al. 1994; 1995a; Park and Sancar 1994; Saijo et al. 1996)
XPC-hHR23B	CP (Drapkin et al. 1994)			
TFIIH			CP, IP (Mu et al. 1995; Iyer et al. 1996)	
RPA			PD, XL (He et al. 1995; de Laat et al. 1998b)	PD, XL (Bessho et al. 1997; de Laat et al. 1998b)

(PD) Pull-down experiments: (GST-, MBP-) affinity chromatography (techniques using large quantities of 'bait' protein). (CP) Copurification or coprecipitation from cell extracts. (2H) Dual hybrid system. (IP) (Immuno-) coprecipitation of *in vitro*-translated gene products (low protein concentrations). (XL) Glutaraldehyde cross-linked at DNA junctions. Relevant references are indicated.

of recruiting the remainder of the repair machinery to the lesion (Sugasawa et al. 1998). DNase I footprinting showed that XPC-hHR23B binds directly to DNA damage and changes the DNA conformation around the lesion (Sugasawa et al. 1998). Thus, XPC-hHR23B initiates GG-NER by sensing and binding lesions, locally distorting the DNA double helix and recruiting the other factors of the system.

TFIIH

TFIIH is a nine-subunit protein complex (Winkler et al. 1998) involved in initiation of RNA polymerase II (Pol II) transcription (Conaway and Conaway 1989; Feaver et al. 1991; Gerard et al. 1991; Flores et al. 1992), NER (Feaver et al. 1993; Schaeffer et al. 1993, 1994; Drapkin et al. 1994; van Vuuren et al. 1994; Vermeulen et al. 1994; Wang et al. 1994, 1995) and possibly in cell cycle regulation (Feaver et al. 1994; Roy et al. 1994a; Serizawa et al. 1995; Shiekhattar et al. 1995). In NER, TFIIH functions both in GG-NER and TC-NER. XP patients with mutations in XPB or XPD may also show manifestations typical of CS or TTD, indicating a central role of TFIIH in transcription-coupled repair (see below). TFIIH has multiple enzymatic activities. XPB and XPD exhibit DNA-dependent ATPase and helicase functions; XPB can unwind DNA in a 3' → 5' direction, and XPD in the opposite direction (Schaeffer et al. 1993, 1994; Roy et al. 1994b). The cdk-activating kinase (CAK) subcomplex, comprising subunits Cdk7, cyclin H, and Mat1, can phosphorylate several cyclin-dependent kinases (cdks), as well as the carboxy-terminal domain of the large subunit of RNA Pol II, activities relevant to cell cycle regulation and transcription initiation, respectively (Feaver et al. 1991; Roy et al. 1994a). The p34 and p44 subunits of TFIIH contain Zn²⁺-finger motifs and putative DNA-binding capacity (Humbert et al. 1994). As yet, no functions have been assigned to the remaining p52 and p62 subunits (Fischer et al. 1992; Marinoni et al. 1997). Several subcomplexes of TFIIH exist in the cell. The core

complex comprises p34, p44, p52, p62, and XPB. CAK is present both as a free heterotrimer and as a component of TFIIH, presumably by binding to XPB and XPD (Rossignol et al. 1997). XPD is found associated with both core TFIIH and the CAK complex (Serizawa et al. 1995; Shiekhattar et al. 1995; Svejstrup et al. 1995; Reardon et al. 1996b; Rossignol et al. 1997). CAK is required for RNA Pol II transcription initiation but is dispensable for *in vitro* NER (Sung et al. 1996; Wang et al. 1995; Mu et al. 1996).

Both in transcription initiation and in NER, TFIIH is needed for melting the DNA double helix (Holstege et al. 1996; Evans et al. 1997b; Mu et al. 1997). Permanganate footprinting studies on the adenovirus major late promoter showed that promoter melting, necessary to activate the RNA Pol II initiation complex, proceeds in three steps (Dvir et al. 1996; Holstege et al. 1996, 1997; Jiang et al. 1996; Yan and Gralla 1997). Initially, 11 bp around the initiator site (from position -9 to +2) are melted. Melting and maintenance of this open complex require the ATP-dependent helicases of TFIIH (Holstege et al. 1995, 1996; for review, see Okhuma 1997). Further opening to position +4 (13 bp unwound) occurs concomitant with the formation of the first RNA phosphodiester bonds and is also TFIIH dependent. Finally, progression to a 17-bp opened complex (-9/+8), which marks the transition to a productive RNA Pol II elongation complex, proceeds independent of TFIIH (Holstege et al. 1997). Thus, TFIIH is actively involved in creating and maintaining an 11- to 13-bp opening during transcription initiation. In NER, the full open complex spans 20–30 bp around the lesion. Footprinting studies on a site-specific lesion with cell-free extracts showed that TFIIH is also indispensable for repair opening. In the absence of ATP, repair unwinding is abolished (Evans et al. 1997a). Accordingly, an ATPase-inactivated mutant form of TFIIH fails to make an open complex (G. Winkler and J.H.J. Hoejmakers, unpubl.). Limited opening (<10 bp) was still observed in extracts lacking RPA, XPA, or XPG but was completely absent in extracts containing mutated XPB, XPD, and XPC protein (Evans et al. 1997b). Possibly, analogous to

opening in transcription initiation, open complex formation in repair occurs in multiple steps and the helicase activity of TFIIH is restricted to initial opening only (discussed more extensively below).

Demarcation of the lesion through local DNA opening provides the ss- to dsDNA transitions required for cleavage by the two structure-specific NER nucleases XPG and ERCC1-XPF (see below). Studies with premelted DNA substrates containing a site-specific lesion revealed that DNA unwinding is not the only function of TFIIH in excision repair. Unlike what was found for transcription initiation, bubbles of 10 nucleotides at the 3' and 5' side, as well as one of 20 nucleotides spanning the lesion, all still require TFIIH for efficient further processing, indicating an additional, probably structural, role for TFIIH in repair (Mu and Sancar 1997). Consistent with this notion, a number of interactions with repair factors have been reported, including XPA and the aforementioned interactions with XPC (Bardwell et al. 1994; Drapkin et al. 1994; Park et al. 1995a; Nocentini et al. 1997) (Table 2). Direct *in vitro* interactions were also described between multiple subunits of TFIIH and XPG (Iyer et al. 1996), consistent with the reported detection of XPG in partially purified TFIIH fractions (Mu et al. 1995). A mutant in the carboxy-terminal domain of XPB was shown to fully support DNA unwinding and allow 3' but not 5' incision, suggesting that TFIIH facilitates the 5' incision by ERCC1-XPF (Evans et al. 1997b). *In vitro* binding was also described between TFIIH and CSA, an interaction perhaps relevant for TCR (Henning et al. 1995). Clearly, in addition to unwinding, TFIIH has other engagements in NER.

XPA

The XPA gene product has a crucial role at an early stage of both TC-NER and GG-NER (Tanaka et al. 1990). It is a DNA-binding protein (Fig. 1) with a marked preference for damaged DNA (Robins et al. 1991; Jones and Wood 1993; Asahina et al. 1994). The Zn²⁺-finger containing minimal region required for DNA binding (Fig. 1) (Tanaka et al. 1990; Kuraoka et al. 1996; Morita et al. 1996; Buchko and Kennedy 1997) is essential for its function (Miyamoto et al. 1992; Asahina et al. 1994). The structure of this domain has been resolved recently (Buchko et al. 1998; Ikegami et al. 1998). Various NER-specific types of damage, including 6-4PPs and CPDs, are recognized by XPA and, in general, the affinity of XPA for a lesion correlates with the extent of helical distortion (Robins et al. 1991; Jones and Wood 1993; Asahina et al. 1994). It has been suggested that the single-strandedness of damaged sites may be the determinant for XPA recognition (Jones and Wood 1993). In addition, XPA maintains an intricate network of contacts with core repair factors (see Table 2). *In vitro* protein-protein interactions were reported with ERCC1 (Li et al. 1994a, 1995a; Park and Sancar 1994), XPF (weak interaction) (Bessho et al. 1997), the p32 and p70 subunits of RPA (He et al. 1995; Li et al. 1995b; Saijo et al. 1996; Ikegami et al. 1998; Stigger et al. 1998), and TFIIH (Park et al. 1995a;

Nocentini et al. 1997) (see Table 2; for mapped interaction domains in XPA, see Fig. 1). Also, *in vitro* binding was claimed for the Cockayne syndrome B (CSB) protein and the p34 subunit of basal transcription factor TFIIE (Park et al. 1995a; Selby and Sancar 1997). In view of its damage preference, XPA has long been considered the damage-sensing and repair-recruitment factor of NER. However, as XPC-hHR23B was shown recently to act first, the function of XPA has to be reconsidered. Given its affinity for damaged DNA and its ability to interact with many (core) repair factors, XPA is anticipated to verify NER lesions and to play a central role in positioning the repair machinery correctly around the injury.

RPA

RPA, originally identified as a factor required for *in vitro* SV40 DNA replication (Wobbe et al. 1987; Fairman and Stillman 1988; Wold and Kelly 1988) has additional roles in NER and recombination (Coverley et al. 1991; 1992; Longhese et al. 1994; for review, see Wold 1997). Human RPA (hRPA) is a ssDNA-binding protein composed of three subunits of 70, 32, and 14 kD (see Fig. 1). Its apparent ssDNA association constant of 10⁹–10¹¹/M is at least three orders of magnitude higher than to dsDNA (Kim et al. 1992, 1994). Binding of an RPA molecule to ssDNA involves the 70-kD subunit (Wold et al. 1989; Kenny et al. 1990; Gomes and Wold 1996; Kim et al. 1996), but single-stranded binding domains are also present in the 32-kD and perhaps in the 14-kD subunit (Philipova et al. 1996; Bochkarev et al. 1997, 1998; Brill and Bastin-Shanower 1998). Two binding modes have been identified; RPA interacts with a minimal region of 8–10 nucleotides (Blackwell and Borowiec 1994) that is thought to precede the almost 100-fold more stable 30-nucleotide binding mode (Kim et al. 1992, 1994; Blackwell et al. 1996). In yeast, binding modes involving larger DNA stretches (90–100 nucleotides) have been reported (Alani et al. 1992). Recently, we showed that RPA binds ssDNA with a defined polarity and that initial DNA binding occurs at the 5'-oriented site of RPA (de Laat et al. 1998b). Possibly, transition to the full 30-nucleotide binding form involves stretching of the RPA molecule along the DNA in the 3' direction. Cooperativity of RPA binding to ssDNA is considered low, but human RPA is still 10–20 times more likely to bind adjacent to an already bound RPA molecule than to naked DNA (Kim et al. 1994, 1995). In RPA-dependent DNA-metabolizing processes, complementary DNA strands are separated at a certain stage and action is required along ssDNA intermediates. By binding to ssDNA, RPA is thought to stabilize such intermediates and remove secondary structures.

In NER, full opening around the lesion requires RPA (Evans et al. 1997b; Mu et al. 1997), which probably binds to the undamaged DNA strand (de Laat et al. 1998b). The size of the fully opened repair intermediate is ~30 nucleotides, which corresponds to the size of the optimal DNA-binding region of a single RPA heterotrimer. RPA may not only stabilize a fully open repair com-

plex, but also facilitate its creation. RPA is also crucial for coordinating the NER nucleases. Interactions were demonstrated with both XPG and ERCC1 (excision repair cross complementation group 1)-XPF (He et al. 1995; Matsunaga et al. 1996; Bessho et al. 1997; de Laat et al. 1998b) (Table 2), and recently it was found that the defined DNA-binding orientation of RPA is particularly relevant for these interactions. Bound to the undamaged strand, the 3'-oriented side of RPA binds ERCC1-XPF, whereas the 5'-oriented side binds XPG. RPA confers strand specificity to ERCC1-XPF by strongly stimulating incisions in the damaged strand and inhibiting cuts in the undamaged strand (de Laat et al. 1998b). RPA and XPA bind cooperatively to damaged DNA (He et al. 1995; Saijo et al. 1996). RPA itself was also found to have affinity for damaged DNA (Clugston et al. 1992; He et al. 1995; Burns et al. 1996), which led to the suggestion that this factor is involved in DNA damage recognition. However, because RPA is anticipated to bind the undamaged strand during NER (de Laat et al. 1998b), RPA probably does not recognize the lesion per se but, rather, has affinity for single-stranded regions exposed by lesion-induced helical distortion. Consistent with this notion, RPA was reported to bind to single-stranded bubbles as small as 4 nucleotides (Wold 1997).

On the basis of its dual involvement in replication and repair, it can be anticipated that RPA not only acts at preincision stages but also during DNA repair synthesis. DNA polymerase δ and ϵ (Pol δ and Pol ϵ) have been implicated in repair synthesis, and both can be stimulated by RPA. Stimulation was not dependent on specific protein-protein interactions, as other SSBs could replace RPA (Kenny et al. 1989, 1990; Tsurimoto et al. 1989; Lee et al. 1991). However, genetic evidence in yeast suggests that RPA and Pol δ do have a direct interaction (Longhese et al. 1994). Possibly, RPA remains bound to the undamaged strand after excision, thereby facilitating gap-filling DNA repair synthesis, which initiates at the 5' incision site.

Finally, the p32 subunit of RPA was found to be phosphorylated in a cell cycle-dependent manner and in response to DNA damage (Din et al. 1990; Fang and Newport 1993; Liu and Weaver 1993; Carty et al. 1994). Whether the phosphorylation status of RPA has any effect on the efficiency of NER in vivo remains to be established (Pan et al. 1995; Ariza et al. 1996).

XPG

The XPG gene encodes a structure-specific endonuclease, which cleaves a variety of artificial DNA substrates, including bubbles, splayed arms and stem-loops (O'Donovan and Wood 1993; Scherly et al. 1993; O'Donovan et al. 1994; Cloud et al. 1995; Evans et al. 1997a). In addition, the XPG homolog of *S. cerevisiae*, Rad2 (Table 1) can remove single-stranded arms protruding from duplex DNA in so-called flap substrates (Habracken et al. 1995). XPG-mediated incisions always occur in one strand of duplex DNA at the 3' side of a junction with ssDNA. One single-stranded arm, protruding in either the 3' or 5' direction, is necessary and sufficient for

correct positioning of XPG incisions (de Laat et al. 1998a). Consistent with this cleavage polarity, XPG makes the 3' incision during NER (O'Donovan et al. 1994) at the border of the open DNA intermediate (Evans et al. 1997a). In bubble substrates, XPG requires a minimal opening of 5 nucleotides for incisions (Evans et al. 1997a). The XPG protein is a member of the FEN-1 family of structure-specific endonucleases, which all cut with similar polarity at junctions of duplex and unpaired DNA (for review, see Lieber 1997). XPG shares three regions of homology (Fig. 1) with the founder of this family, FEN-1, which is implicated in the processing of Okazaki fragments during replication (Waga et al. 1994b; Rumbaugh et al. 1997), the completion of a subpathway of BER (Klungland and Lindahl 1997), and possibly DNA end joining of dsDNA breaks (Lieber 1997). Three aspartic acid and one glutamic acid residue are absolutely essential for FEN-1 cleavage (Shen et al. 1996, 1997). One of these, Asp-181 in FEN-1, is required for cleaving but not for binding DNA. Substitution of the corresponding residue in XPG, Asp-812 by alanine (D812A) induced a selective defect in its nuclease activity (Mu et al. 1997; Wakasugi et al. 1997).

The XPG-mediated 3' incision precedes the 5' incision made by ERCC1-XPF (Mu et al. 1996). Interestingly, XPG is not only required for the 3' incision but also for full open-complex formation, indicating a structural role in the core NER reaction (Evans et al. 1997b; Mu et al. 1997). Evidence for such a role was provided with the D812A active-site mutant of XPG, which had to be present to detect ERCC1-XPF-mediated 5' incisions in an in vitro-reconstituted repair assay with purified factors (Mu et al. 1997; Wakasugi et al. 1997). Furthermore, this same XPG mutant was found to stabilize a preincision complex containing XPC-hHR23B, TFIIH, XPA, and RPA (Mu et al. 1997). Apparently, independent of its cleavage activity, XPG has a structural function in the assembly of the NER DNA-protein complex. The reported interactions of XPG with TFIIH and RPA may be relevant in this respect (Bardwell et al. 1994; He et al. 1995; Iyer et al. 1996; de Laat et al. 1998b). Similar to FEN-1, XPG was found to interact with proliferating cell nuclear antigen (PCNA) (Gary et al. 1997), a factor involved in DNA repair synthesis but dispensable for the incision stage of NER (Shivji et al. 1992). Whereas PCNA stimulates FEN-1 nuclease activity (Wu et al. 1996), no effect was found when PCNA was added to XPG nuclease assays (Evans et al. 1997a). Perhaps, this interaction allows cross talk between the incision and gap-filling stages of NER. An in vitro interaction was also observed with CSB (Iyer et al. 1996), possibly relevant to the mechanism that underlies transcription-coupled repair. Because various XP-G patients show CS features combined with XP manifestations, XPG, like TFIIH, is anticipated to be a key factor in coupling various repair processes to transcription.

ERCC1-XPF

The gene products of *ERCC1* (33 kD; van Duin et al. 1986) and *XPF* (103 kD; Brookman et al. 1996; Sijbers et

al. 1996a) form a stable complex in vivo and in vitro (Biggerstaff et al. 1993; van Vuuren et al. 1993; Park et al. 1995b; Sijbers et al. 1996a; de Laat et al. 1998c), involving amino acid stretches in the carboxyl terminus of both ERCC1 and XPF (de Laat et al. 1998c). Stability of the individual components in the cell is dependent on heterodimer formation (Sijbers et al. 1996b; Yagi et al. 1997). UV-sensitive Chinese hamster cells defective in either ERCC1 or XPF display also a unique, extreme sensitivity to interstrand cross-linking agents (Busch et al. 1989, 1997; Collins 1993). Removal of such cross-links probably involves recombination. Consistent with this idea, the homologous complex in *S. cerevisiae*, Rad10–Rad1 (Table 1) is required for a specific mitotic recombination pathway called single-strand annealing (SSA) (Fishman-Lobell and Haber 1992), possibly via interactions with mismatch repair proteins (Paques and Haber 1997; Sugawara et al. 1997; Bertrand et al. 1998; Bhui-Kaur et al. 1998). Also, its *Schizosaccharomyces pombe* counterpart Swi10–Rad16 is involved in the recombinational events that underlie mating-type switching (Gutz and Schmidt 1985; Rodel et al. 1992, 1997).

The ERCC1–XPF complex is a structure-specific endonuclease (Sijbers et al. 1996a). Like its yeast homolog Rad1–Rad10, ERCC1–XPF incises a variety of DNA substrates, including bubbles, stem-loops, splayed arms, and flaps (Sijbers et al. 1996a; Bessho et al. 1997; de Laat et al. 1998a), with the latter possibly representing recombination intermediates. A minimal loop size of 4–8 nucleotides is required to detect ERCC1–XPF incisions (de Laat et al. 1998a). Incisions are always made in one strand of the duplex at the 5' side of the junction with ssDNA (Sijbers et al. 1996a; de Laat et al. 1998a). One single-stranded arm protruding in either the 3' or 5' direction is necessary and sufficient to correctly position ERCC1–XPF incisions at a DNA junction (de Laat et al. 1998a). In NER, ERCC1–XPF makes the 5' incision, consistent with its cleavage polarity (Matsunaga et al. 1995; Sijbers et al. 1996a).

A hint that ERCC1 may be the subunit catalyzing cleavage comes from the presence of a double helix–hairpin–helix (HhH) motif in its carboxyl terminus (Sijbers et al. 1996b). HhH motifs are shared by other structure-specific endonucleases and have been implicated in DNA binding (Doherty et al. 1996). Deletion of the homologous double HhH motif in the carboxyl terminus of the *E. coli* NER protein UvrC disrupts 5' incisions without affecting 3' cleavage (Moolenaar et al. 1998). Possibly, this DNA-binding domain positions the catalytic cleavage site for 5' incision. Intriguingly, recognizable HhH motifs are not apparent in the yeast homolog of ERCC1 Rad10.

The 5' incision by ERCC1–XPF follows the XPG-mediated 3' incision in NER (Mu et al. 1996). ERCC1–XPF can be omitted for full open complex formation and 3' incision in vitro and can be added to a preformed incision complex, containing all other factors, to make the 5' incision (Mu et al. 1996, 1997; Evans et al. 1997b). Thus, unlike XPG, ERCC1–XPF does not appear to have an architectural function in the NER protein–DNA com-

plex. Several protein interactions have been reported, that may account for positioning of ERCC1–XPF during NER. XPA interacts with the complex (Table 2) (Li et al. 1994a, 1995a; Park and Sancar 1994; Saijo et al. 1996), mainly via ERCC1 (Fig. 1), although a weak affinity for XPF also has been reported (Bessho et al. 1997). RPA and ERCC1 likely bind sequentially to XPA (Saijo et al. 1996). RPA also interacts with ERCC1–XPF (Table 2), presumably via XPF (Matsunaga et al. 1996; Bessho et al. 1997). This interaction seems particularly important for positioning the nuclease. Bound to ssDNA, the 3' oriented side of RPA interacts with ERCC1–XPF and strongly stimulates its nuclease activity, whereas the 5' oriented side of RPA does not interact with the complex and blocks ERCC1–XPF-mediated incisions (de Laat et al. 1998b).

XPE

XPE is dispensable for NER in vitro. However, in vivo it is required, as XP-E patients exhibit XP-like skin abnormalities and reduced repair synthesis (Bootsma et al. 1997). The defect in XP-E cells is not assigned unambiguously yet: a unique candidate for the *XPE* gene is still lacking. Some but not all XP-E patients lack a functional damaged DNA-binding (DDB) factor (Chu and Chang 1988; Hirschfeld et al. 1990; Kataoka and Fujiwara 1991; Keeney et al. 1992, 1994; Nichols et al. 1996). DDB is a heterodimeric protein complex with 127- and 48-kD subunits and with affinity for certain types of DNA lesions (Hwang and Chu 1993; Keeney et al. 1993; Reardon et al. 1993), hinting at a function in damage recognition in NER. Some, but not all, patients carry a mutation in the gene for the small subunit (Nichols et al. 1996), which is under damage-inducible control by p53 (Hwang et al. 1999). This explains the partial defect in GG–NER in p53^{-/-} cells (Ford and Hanawalt 1997), supporting the idea that DDB facilitates the identification of lesions that are poorly recognized by the XPC–hHR23B complex, such as UV-induced CPD dimers (Hwang et al. 1999).

DNA repair synthesis

The incision and DNA synthesis stages of NER can be separated in vitro, and the only factor in common is RPA, which may remain bound to the undamaged strand to facilitate replication. In vitro studies with antibodies and chemical inhibitors revealed that both DNA Pol δ and Pol ϵ function in NER DNA synthesis (Dresler and Frattini 1986; Nishida et al. 1988; Hunting et al. 1991; Coverley et al. 1992;). A similar observation was made in vivo in yeast (Budd and Campbell 1995), but the relative contribution of each remains to be determined. The requirement of PCNA is consistent with repair synthesis by these polymerases (Shivji et al. 1992), as it serves as a processivity factor for both, in conjunction with replication factor C (RF-C). The combination of RPA, PCNA, RF-C (five subunits) and either Pol δ or Pol ϵ was sufficient for repair synthesis in vitro (Shivji et al. 1995).

DNA synthesis by Pol δ and Pol ϵ and their cofactors PCNA and RF-C has been studied extensively (for reviews and original references, see Budd and Campbell 1997; Hindges and Hübscher 1997; Jonsson and Hübscher 1997; Wood and Shivji 1997). Briefly, RF-C preferentially binds to 3' termini of DNA primers and facilitates the loading of PCNA, which forms a homotrimeric ring-shaped clamp that can track along duplex DNA. This complex serves as a docking platform for both Pol δ and Pol ϵ , which upon binding form holoenzymes with the cofactors that efficiently can replicate ssDNA.

PCNA might serve as a mediator between cell cycle control and DNA repair. It interacts with p21, a cdk inhibitor that is up-regulated in a p53-dependent manner upon DNA damage (Waga et al. 1994a). This interaction inhibits DNA replication but does not affect DNA repair, which may contribute to the induction of replicational arrest to allow repair and prevent mutagenesis (Li et al. 1994b).

The final step in NER is ligation of the 5' end of the newly synthesized patch to the original sequence. This step is probably carried out by DNA ligase I. Interestingly, a single case of DNA ligase I deficiency in humans has been described. This patient not only suffered from symptoms probably arising from (mild) defects in semiconservative replication but also showed increased sensitivity to several DNA-damaging agents, including UV light (Barnes et al. 1992; Prigent et al. 1994). (For a review on mammalian DNA ligases, see Tomkinson and Levin 1997.)

Biochemical dissection of NER

Eukaryotic NER removes damage as part of a 24- to 32-nucleotide oligomer (Huang et al. 1992; Moggs et al. 1996), depending on the type of damage and the sequence context. Reconstitution of the NER reaction with purified proteins allowed the definition of a minimal set of proteins required for the entire GG-NER reaction (Aboussekhra et al. 1995; Mu et al. 1995, 1996). The repair synthesis stage merely involves general replication factors, and their action has been discussed above. Here, we will focus on the crucial events prior to repair synthesis. Recent studies on damage recognition, open complex formation and nuclease positioning, together with data obtained from genetic and cell biological studies, have allowed a detailed interpretation of the individual steps that lead to dual incision. All these events are composed into a molecular model shown in Figure 2.

Damage sensing in GG-NER and TC-NER

As discussed above, the XPC-hHR23B complex is the first NER factor to detect a lesion and recruit the rest of the repair machinery to the damaged site in GG-NER (Sugasawa et al. 1998). The complex has affinity for a variety of NER lesions including UV-induced injury and chemical damage, such as cisplatin and N-acetyl-acetoxyminofluorene (AAF) adducts. DNase I footprinting assays revealed specific binding to a 6-4PP (Sugasawa et

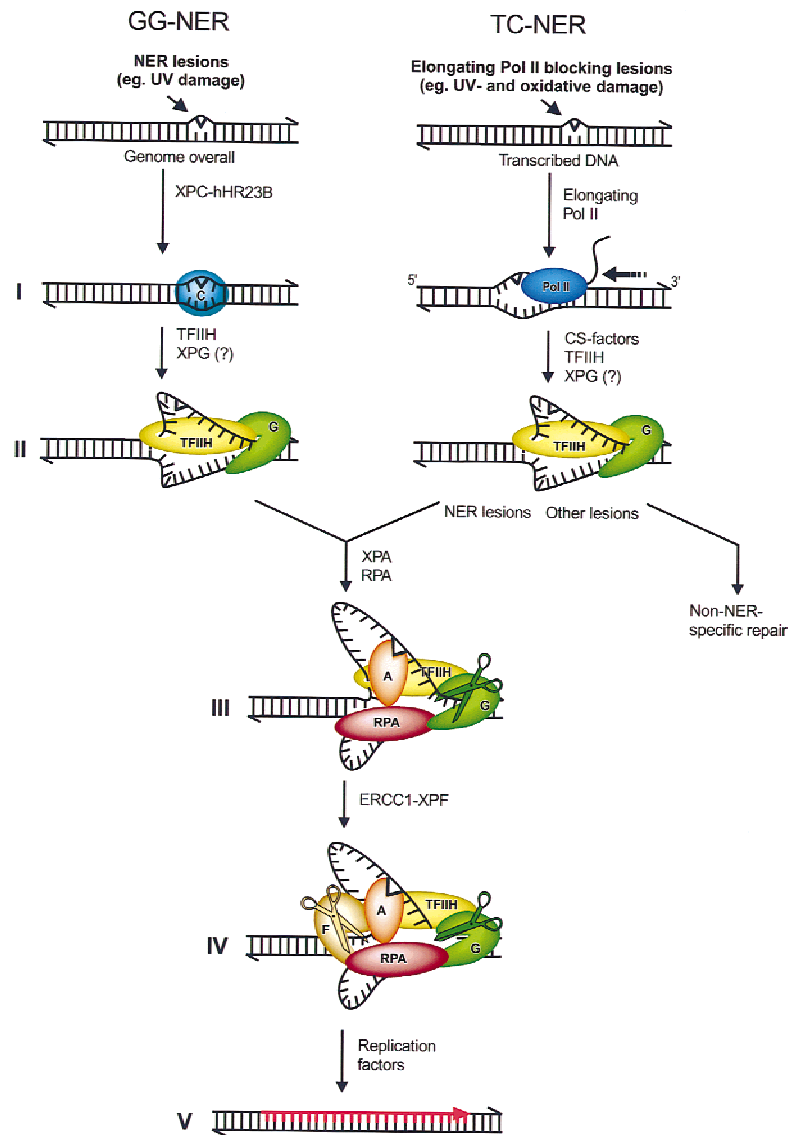
al. 1998). Probably, XPC is the subunit responsible for discerning 'right from wrong' in DNA, but at this moment it is unclear how this protein senses the wide range of structurally unrelated lesions in a vast excess of normal DNA. For some types of damages, such as the poorly repaired CPD lesions, other proteins like the UV-DDB protein complex may assist in lesion detection (see above). Furthermore, XPA, as another NER factor with preferential affinity for several types of injury, likely acts as a damage verifier in subsequent stages in the NER reaction (Sugasawa et al. 1998).

How does XPC-hHR23B recruit other repair factors in GG-NER? Evans et al. (1997b) reported that XPC and TFIIH are the only factors absolutely required for helix distortion around the lesion. This may be sufficient for the rest of the repair machinery to act, as locally premelted lesions are repaired efficiently in the absence of XPC-hHR23B (Mu and Sancar 1997; Mu et al. 1997). Thus, XPC-hHR23B may slightly increase single strandedness at a damaged site to facilitate entering of TFIIH and other repair factors. Perhaps a similar helical distortion underlies the observation that artificial cholesterol lesions bypass the need for XPC-hHR23B (Mu et al. 1996). In addition, XPC-hHR23B may recruit other repair factors through specific protein-protein interactions. The complex has only been reported to interact (weakly) with TFIIH (Drapkin et al. 1994). In yeast, Rad4 (XPC)-TFIIH, Rad23-TFIIH, and Rad23-Rad14 (XPA) interactions have been claimed (Bardwell et al. 1994; Guzder et al. 1995).

XPC-hHR23B is not involved in transcription-coupled repair. This suggests that other factors perform damage detection in TC-NER and provide a DNA substrate that can be processed by the rest of the repair machinery. Elongating RNA Pol II is blocked by many lesions in the transcribed strand. This makes it an efficient damage sensor (Donahue et al. 1994; Hanawalt and Mellon 1993). The transcription bubble present at the lesion can serve as a substrate for XPC-hHR23B-independent repair (Hanawalt and Mellon 1993; Mu and Sancar 1997; Mu et al. 1997). In vivo XPC-hHR23B competes with elongating Pol II for detecting lesions in transcribed strands; depending on their damage detection rates and the intensity of transcription, lesions will be repaired by either GG-NER or TC-NER. In agreement with this model, removal of 6-4PPs by GG-NER is very fast (Mitchell and Nairn 1989), and TC-NER does not contribute significantly to the repair rate (van Hoffen et al. 1995). On the other hand, repair of CPDs by GG-NER is much slower. Consequently, TC-NER accounts for the fast repair of these lesions from actively transcribed strands (Mellon et al. 1987; Mellon and Hanawalt 1989). On the other side of the spectrum, lesions are to be expected that fully depend on TC-NER, due to failing recognition by GG-NER. Such a condition is fulfilled by DNA damage caused by the mushroom drug illudin S (N.G.J. Jaspers, unpubl.). In comparison to UV, illudin-induced NER levels are quite low, even in normal cells, and require the presence of all TC-NER factors, including CSA and CSB, but are independent of XPC and XPE.

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Figure 2. Molecular model for the incision stage of NER. (I) XPC-hHR23B (C) senses DNA helix-distorting NER lesions in global genome NER (GG-NER) leading to conformational alterations of the DNA. In transcription-coupled repair (TC-NER) lesions are detected by elongating RNA Pol II blocked by, e.g., CPDs (NER lesions) and thymine glycols (non-NER lesion). (II) (Left) XPC-hHR23B at lesion attracts TFIIH [and possibly XPG (G)]. TFIIH creates a 10- to 20-nucleotide opened DNA complex around the lesion by virtue of its helicases XPB and XPD; this step requires ATP. XPC-hHR23B may be released at this or one of the subsequent stages. (Right) CSA, CSB, TFIIH, XPG, and possibly other cofactors displace the stalled Pol II from the lesion, which now becomes accessible for further repair processing; depending on the type of lesion, repair is completed by NER or by other repair pathways. (III) XPA (A) and RPA stabilize the 10- to 20-nucleotide opening and position other factors. XPA binds to the damaged nucleotides, RPA to the undamaged DNA strand. Possibly, RPA binds in its 8- to 10-nucleotide binding mode and transition to the 30-nucleotide binding mode (RPA stretching) plays an important role in full open complex formation. XPG stabilizes the fully opened complex. (IV) XPG, positioned by TFIIH and RPA, makes the 3' incision. ERCC1-XPF (F), positioned by RPA and XPA, makes the second incision 5' of the lesion. (V) Dual incision is followed by gap-filling DNA synthesis and ligation. Drawn contacts between molecules reflect reported protein-protein interactions.



Open complex formation

Once lesions have been traced, an open DNA complex is formed by the coordinated activities of XPC-hHR23B, TFIIH, XPA, and RPA. The ATP-dependent helicases of TFIIH have a key role in this process, whereas XPG seems to stabilize the complex (Evans et al. 1997a,b; Mu et al. 1997; Wakasugi et al. 1997). The fully opened intermediate is formed asymmetrically around the lesion, skewed to the 5' site. Permanganate footprinting studies on damaged DNA substrates in the presence of repair-deficient cell extracts suggest that XPC-hHR23B and TFIIH together are required for an initial opening of <10 nucleotides, and that addition of XPA, RPA, and XPG is needed to obtain full opening of ~25 nucleotides (Evans et al. 1997a,b). Other studies using similar techniques but with purified factors suggested that all four preincision factors are necessary and sufficient to obtain an intermediate opening of 10–20 nucleotides positioned rather symmetrically around the lesion; full opening was

only observed in conjunction with dual incision (Mu et al. 1997). These discrepancies may be due to differences in experimental procedures and/or DNA substrates or the limited sensitivity of permanganate acting only on thymine residues. Both studies, however, indicate a two-step unwinding model with an ATP-dependent, TFIIH-mediated initial opening and a subsequent extension of the open complex 5' away from the lesion. Such a mechanism would be analogous to TFIIH-dependent promoter opening in transcription initiation (Holstege et al. 1996, 1997; Yan and Gralla 1997).

How do the various factors contribute to opening? A number of possibilities exist: (1) TFIIH harbors two oppositely directed, ATP-dependent helicase subunits XPB and XPD, and is the motor driving the strand separation. In transcription initiation, TFIIH-dependent opening spans initially ~10–15 nucleotides (Holstege et al. 1996, 1997), which is similar to the size of initial opening in repair (Evans et al. 1997b; Mu et al. 1997). The fact that opening is restricted to 10 to 20 nucleotides may reflect

an intrinsic limitation of TFIIH-mediated strand separation. In addition to unwinding, TFIIH may have a structural role in the preincision complex, as premelted lesions still require TFIIH for repair (Mu and Sancar 1997; Mu et al. 1997). (2) XPA might account for correct positioning of the opened DNA-protein preincision complex, because it can bind the DNA adduct in an open conformation and interacts with both TFIIH and RPA (see above). (3) RPA may stabilize the unwound DNA intermediate. Most likely it binds and protects the undamaged strand in repair (de Laat et al. 1998b), and it is tempting to implicate the ssDNA-binding characteristics of RPA in the creation of a full open repair complex. The 5'-oriented side of RPA contains a strong DNA-binding domain that accounts for initial association to 8- to 10-nucleotide DNA regions (Blackwell and Borowiec 1994; Blackwell et al. 1996; de Laat et al. 1998b). Stable binding of RPA to DNA requires ~30-nucleotide single-stranded regions (Kim et al. 1992; Blackwell and Borowiec 1994). Interestingly, initial opening in NER exposes ~10–20 nucleotides of the undamaged strand, thus creating an ideal docking site for the 5'-oriented side of RPA. We propose that subsequent RPA stretching in the 3' direction contributes to the formation of a fully opened complex, which matches the observed ~30-nucleotide open intermediate.

It is not known whether repair *in vivo* involves sequential assembly of individual factors or loading of a complete 'repairoosome' onto a DNA lesion. In either case, the repair factors are likely to act in a defined order. It is interesting to note that in mammals only TFIIH has been reported to interact with the repair recruitment factor XPC-hHR23B (Drapkin et al. 1994). Because XPC and TFIIH are the only factors indispensable for any conformational change around a lesion, Evans et al. (1997b) proposed that XPC-hHR23B and TFIIH may accomplish initial repair opening. Thus, TFIIH may well be the second factor acting at the site of damage. This would imply that TFIIH facilitates the recruitment of XPA to the lesion, rather than the other way around (Park et al. 1995a; Nocentini et al. 1997). Although merely speculation, TFIIH also seems an attractive candidate to be the first 'repair' factor acting in the XPC-hHR23B-independent TC-NER pathway, given its intimate link with both transcription and repair. Also, TFIIH has been shown to enter early stalled Pol II complexes (Dvir et al. 1997).

Dual repair incision

Following lesion demarcation, the actual incisions are made by the structure-specific endonucleases XPG (3' incision) and ERCC1-XPF (5' incision) (O'Donovan et al. 1994; Matsunaga et al. 1995; Sijbers et al. 1996a). Incisions are made asymmetrically around the lesion, with the 3' incision 2–8 nucleotides and the 5' incision 15–24 nucleotides away from the lesion, corresponding to the borders of the open complex (Huang et al. 1992; Moggs et al. 1996; Evans et al. 1997a). The exact incision positions seem to depend in part on the type of lesion (Matsunaga et al. 1995; Moggs et al. 1996). Although incisions occur

near synchronously, consensus exists that the 3' incision precedes the 5' incision (Mu et al. 1996). In agreement with this order, XPG-mediated cleavage can be detected in the absence of ERCC1-XPF, but ERCC1-XPF incision activity requires the structural presence, but not the catalytic activity, of XPG (Mu et al. 1997; Wakasugi et al. 1997). Also, limited opening of 10–20 nucleotides is sufficient for XPG cleavage, whereas ERCC1-XPF cutting in NER requires full opening of 25–30 nucleotides (Evans et al. 1997b; Mu et al. 1996, 1997).

In principle, XPG and ERCC1-XPF are able to cut both strands of an opened DNA intermediate (O'Donovan et al. 1994; Sijbers et al. 1996a), but during repair the nucleases are directed to the damaged strand only. RPA appears to have a crucial role in nuclease positioning. Each side of this molecule, when oriented on ssDNA, interacts with a distinct nuclease. In fact, bound to the undamaged strand, RPA alone is sufficient to confer strand specificity to ERCC1-XPF-mediated incisions (de Laat et al. 1998b). XPA's interaction with both RPA and ERCC1-XPF may facilitate or stabilize the positioning of ERCC1-XPF and RPA onto the damaged strand (Li et al. 1994a, 1995a,b; Park and Sancar 1994; Saijo et al. 1996). RPA presumably contributes, but is not sufficient, to confer strand specificity to XPG. Despite a specific interaction with RPA, XPG incisions in the damaged strand are not stimulated by RPA, nor does RPA inhibit XPG incisions in the undamaged strand (de Laat et al. 1998b). TFIIH is an attractive candidate to be involved in XPG positioning. Physical interaction between these two NER components has been reported both in yeast and in man (Bardwell et al. 1994; Habraken et al. 1996; Iyer et al. 1996). In addition, strikingly similar CS features are associated with mutations in both factors. XPC-hHR23B, on the other hand, seems not directly involved in coordinating either of the nucleases, as this factor probably leaves the repair complex prior to incisions (Wakasugi and Sancar 1998).

The 5' incision by ERCC1-XPF, which completes the incision stage, leaves a hydroxyl (-OH)-group at the 3' terminus of the primer strand and no additional modifications are required to start DNA synthesis at this side of the gap (Sijbers et al. 1996a). *In vitro*, the oligonucleotide containing the damage can be released by the NER incision factors in the absence of DNA repair synthesis (Mu et al. 1996, 1997). Probably, most NER proteins leave prior to repair synthesis. However, RPA is required for gap-filling DNA synthesis to protect the template strand against nucleases and/or to facilitate DNA replication. Replication does not result in strand displacement beyond the patch but, rather, stops at the 3' cleavage site.

Coupling of transcription to different repair pathways; a central role for TFIIH and XPG

Transcription-coupled repair is well documented for elongation-stalling NER lesions for which GG-NER is too slow. However, evidence is accumulating that also transcription-blocking damage targeted by other repair

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systems is subject to preferential repair, including oxidative damage such as thymine glycols (Leadon and Lawrence 1992; Leadon and Cooper 1993; Cooper et al. 1997). Thus, all lesions that interfere with transcription elongation may well be a substrate for transcription-coupled repair (see also Tijsterman et al. 1997; Tu et al. 1997). Cells from XP-A, XP-F, and XP-G patients, which display only XP features, are defective in transcription-coupled repair of typical NER lesions, but appear normal in transcription-coupled repair of oxidative damage removed by other repair pathways (Cooper et al. 1997). In contrast, cells from CS-A and CS-B patients are defective in transcription-coupled repair of both CPDs and at least some types of oxidative damage lesions (Venema et al. 1990b; Leadon and Lawrence 1992; Leadon and Cooper 1993; van Hoffen et al. 1993; Cooper et al. 1997). This suggests that CS is linked to a more general transcription-coupled repair defect not limited to TC-NER. Thus, it seems that more than one repair pathway utilizes the damage-sensing capacity of elongating Pol II. It should be noted also that mismatch repair proteins have been implicated in the coupling between transcription and repair (Mellon et al. 1996; Leadon and Avrutskaya 1997, 1998).

Interestingly, a subclass of XP-B, XP-D, and XP-G patients displays CS features in combination with XP. Cells from these individuals appear deficient in the transcription-coupled removal of both UV-induced lesions and oxidative damage (Cooper et al. 1997), consistent with the idea that the repair defect in CS involves transcription coupling to multiple repair systems. This distinguishes the factors involved, TFIIH and XPG, from the other core NER proteins and links them with coupling of transcription to other repair pathways as well. One possible explanation is that these factors play a role in a stage of transcription-coupled repair common to different repair processes. Hanawalt and Mellon (1993) argued that for TC-NER, the stalled Pol II complex has to retract or dissociate to allow access of repair proteins to the lesion. Assuming that defects in this process underlie the extensive and perhaps even complete defect in transcription-repair coupling observed in CS cells, we propose that TFIIH and XPG, like CSA and CSB, function in the displacement of Pol II from the damaged site. CSB was recently found to be associated to Pol II, most likely in the elongation mode (Selby and Sancar 1997; Tantin et al. 1997; van Gool et al. 1997a). Possible roles for CSA and CSB in transcription-repair coupling have been discussed recently (van Gool et al. 1997b) and will not be reiterated here. In view of the discussion above, it is interesting to speculate on the role of XPG and TFIIH in this process.

Elongating Pol II complexes track along the template strand in a 3' → 5' direction and are expected to position a transcription bubble 3' of obstructive lesions. In GG-NER, recruitment of TFIIH to the damaged site presumably depends on XPC-hHR23B-mediated changes in DNA conformation and protein interactions, whereas XPG recruitment depends on the formation of an opened DNA complex. In transcription-coupled repair the Pol II-induced DNA opening 3' of the lesion may be acces-

sible to TFIIH and XPG in the absence of other NER factors. Thus, a stalled Pol II complex may attract TFIIH and XPG independent of the type of lesion causing the block. The *in vitro* observed interactions between isolated TFIIH subunits and CSA (Henning et al. 1995), XPG and CSB (Iyer et al. 1996), and XPG and TFIIH (Iyer et al. 1996; Mu et al. 1995) may have a role in this recruitment.

The apparently crucial role of the nuclease XPG in transcription-repair coupling is intriguing. The XP-type XP-G patient XP125LO carries a defect in GG-NER and in TC-NER of UV-induced lesions but still displays transcription-coupled repair of oxidative damage (Cooper et al. 1997). The NER defect is caused by an Ala-792 → Val substitution next to a presumed catalytic residue, Glu-791, in nuclease domain I of XPG (see Fig. 1) (Noussipikel and Clarkson 1994; Shen et al. 1996, 1997). Presumably the mutant protein is inactive in cleavage (Cooper et al. 1997; Noussipikel and Clarkson 1994; Noussipikel et al. 1997; Reardon et al. 1997). On the basis of this assumption, Cooper et al. (1997) suggested that the requirement of XPG for transcription-coupled repair of oxidative damage is independent of its incision activity and may depend on structural properties. Defects in the functions of XPG and TFIIH in transcription-repair coupling are anticipated to interfere with the release of trapped transcription caused by (oxidative) damage (Hanawalt and Mellon 1993; van Gool et al. 1997b).

Excision repair and chromatin

As is apparent from this review, *in vitro* NER is fairly well understood. However, most studies have utilized naked DNA as substrate. A major challenge will be to understand the NER process in the context of chromatin, preferably in a living cell. Compaction of DNA into nucleosomes and higher order structures will certainly affect the accessibility of lesions. Repair on the nontranscribed strand of an active gene was found to be rapid in linker DNA and slow in sequences occupied by nucleosomes, whereas TC-NER of the transcribed strand appeared independent of chromatin organization *in vivo* (Wellinger and Thoma 1997). Two NER components may be important in this context. Purified DDB failed to stimulate repair of naked DNA by XP-E cell extracts, but partially corrected the repair defect upon microinjection in living XP-E cells, suggesting a function of this factor in the repair of UV lesions in chromatin (Rapic Otrin et al. 1998). In yeast, a complex of Rad7-Rad16 (Guzder et al. 1997) functions specifically in GG-NER (Verhage et al. 1994, 1996; Mueller and Smerdon 1995). On the basis of sequence homology, Rad16 belongs to the Swi2/Snf2 subfamily of DNA-dependent ATPases, a group of proteins implicated in chromatin remodeling. Possibly, DDB and as-yet-unidentified human homologs of Rad7-Rad16 are involved in lesion-dependent chromatin remodeling in an early stage of global excision repair *in vivo*.

Repair rates probably depend on both the concentration of repair factors and their affinity for lesions. For

some NER factors we estimated the presence of 10^4 – 10^5 molecules per nucleus. This indicates that one repair molecule or complex still has to guard 10^4 – 10^5 bp of DNA in human cells. As repair in vivo is highly efficient, repair proteins can be anticipated to act in a highly coordinated fashion in the context of chromatin. Transient association of damaged DNA with the nuclear matrix has been reported (Koehler and Hanawalt 1996). It is not known whether repair in vivo involves the sequential assembly of individual factors or loading of a complete repairosome onto a DNA lesion. By bleaching green fluorescent protein (GFP)-tagged ERCC1–XPF in a subcompartment of the nucleus of a living cell and measuring the rate of influx of fluorescent complexes in the bleached region, it was found that ERCC1–XPF diffuses very rapidly through the nucleus. The diffusion constant is compatible with the majority of ERCC1–XPF being free (i.e., not part of a large NER holocomplex). A significant fraction of ERCC1–XPF complexes became temporarily immobilized on UV exposure as the consequence of actual engagement in repair (A. Houtsmuller, W. Vermeulen, and J.H.J. Hoeijmakers, unpubl.). These findings support a model for NER in vivo involving successive assembly of repair factors in which freely diffusing ERCC1/XPF participates in a distributive fashion. It would be interesting to see whether DNA damage-binding factors like DDB, XPC–hHR23B, and XPA, as well as TFIIH display a similar or a different behavior.

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Molecular mechanism of nucleotide excision repair

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