Molecular Mechanisms of Mammalian Global Genome Nucleotide Excision Repair

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1. Foreword

Over the past few years, media have popularized the story of the "children of the moon" who must remain in the dark and avoid exposure to sunlight.[1−3] These children suffer from a rare genetic disease called xeroderma pigmentosum (XP) characterized by a defect in an essential DNA repair pathway: nucleotide excision repair (NER).

Everyday the DNA of each cell is jeopardized by a variety of modifications arising spontaneously (replication errors), endogenously (reactive metabolites), or exogenously after exposure to environmental mutagens or ultraviolet (UV) light. To counteract the formation of these deleterious DNA lesions and to preserve the integrity of the vital genetic information, all organisms are equipped with a number of DNA repair mechanisms.[4] If lesions in DNA cannot be eliminated, either because the damage load is too high or because the requisite repair pathway is deficient, a cell may be eliminated by apoptosis or will accumulate mutations and transform into a potentially cancerous cell that might proliferate into a tumor.[5] In this regard, the exquisite sensitivity of XP patients to sunlight and their predisposition to skin cancer is a dramatic reminder of the importance of efficient DNA repair for a healthy organism.

DNA repair is commonly divided into five major pathways (direct damage reversal, base excision repair, nucleotide excision repair, mismatch repair, and double strand break repair), each dealing, except for some overlap, with specific types of lesions.[6,7] NER is a particularly intriguing repair pathway because of its extraordinarily wide substrate specificity; it has the ability to recognize and repair a large number of structurally unrelated lesions, such as DNA damage formed upon exposure to the UV radiation from sunlight and numerous bulky DNA adducts induced by mutagenic chemicals from the environment or by cytotoxic drugs used in chemotherapy. NER operates through a "cut-and-patch" mechanism by excising and removing a short stretch of DNA (24- to 32-nucleotides long) containing the damaged base; the original genetic sequence is then restored using the nondamaged strand of the DNA double helix as a template for repair synthesis. Two distinct subpathways have been discerned: global genome NER (GG-NER), which can detect and remove lesions throughout the genome, and transcription-coupled NER (TC-NER), which ensures faster repair of many

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lesions when located on the transcribed strand of actively transcribed genes.

In this review, we will discuss our current knowledge of the molecular mechanisms underlying the GG-NER pathway. We will see how the sequential action of over 30 proteins can perform the repair of an astonishing diversity of DNA lesions. NER is exemplary of how 100 years of research on a rare genetic disorder, XP, together with advances in modern biology, have led to the discovery and the elucidation of a central DNA repair pathway. Therefore, we shall start the review with a brief overview of the history of NER and its associated disorders.

2. History

A number of parallel developments have led to the discovery and subsequent elucidation of the NER system in humans. The first one, in 1964, was the observation of a process that released thymine dimers in the form of short oligonucleotides from UV-irradiated DNA in bacteria.[8,9] Subsequent studies led to the characterization of this process as the NER pathway in bacteria (see the review of Kisker et al. in this issue of *Chemical Reviews*). A second major advance was the observation of de novo DNA synthesis following UV irradiation in human cells.[10,11] This "unscheduled DNA synthesis" (UDS, see below), which reflects the repair synthesis step of NER, was instrumental in the landmark discovery that cell lines derived from XP patients were representative of naturally occurring UV-sensitive mutants with a defect in NER.[12] The subsequent determination of complementation groups of XP and UV-sensitive hamster cell lines paved the way for the cloning of the human NER genes, the characterization of the proteins they encode, and the reconstitution of the NER reaction in vitro.

2.1. Syndromes Associated with Deficiencies in NER Genes

2.1.1. Xeroderma Pigmentosum

The recorded history of XP (literally: "parchment-like skin with pigmentation abnormalities") and hence NER began in 1874, when Moriz Kaposi used this term for the first time to describe the symptoms observed in a patient.[13] XP patients exhibit an extreme sensitivity to sunlight and have more than 1000-fold increased risk to develop skin cancer, especially in regions exposed to sunlight such as hands, face, neck, the anterior part of the eyes or the tip of the tongue. In about 18% of the cases, these symptoms are coupled with primary neuronal degeneration and loss of neurons. The first skin neoplasms appear at a median age of 8 years, that is 50 years earlier than for the general population.[14] XP affects 1 individual in 250,000 in Western countries and up to 1 individual in 40,000 in Japan and North Africa.[4] The heterozygote parents are generally asymptomatic. The symptoms of XP are readily explained by a defect in the repair of DNA damages and in particular of UV lesions. XP therefore exemplifies a prototypical DNA repair syndrome.

2.1.2. Cockayne Syndrome

A second disorder with UV sensitivity was reported by Edward Alfred Cockayne in 1936.[15] Cockayne syndrome (CS) is characterized by additional symptoms such as short stature, severe neurological abnormalities caused by dysmyelination, bird-like faces, tooth decay, and cataracts. CS patients have a mean life expectancy of 12.5 years but in contrast to XP do not show a clear predisposition to skin cancer. CS cells are deficient in transcription-coupled NER but are proficient in global genome NER.[16] In fact, the TC-NER and GG-NER pathways share the same common set of core enzymes (see below) but differ in the way the DNA damage detection takes place. Even though most XP patients are defective in both GG-NER and TC-NER, they do not display several of the symptoms observed in CS. Therefore, the CS phenotype cannot be explained by a defect in

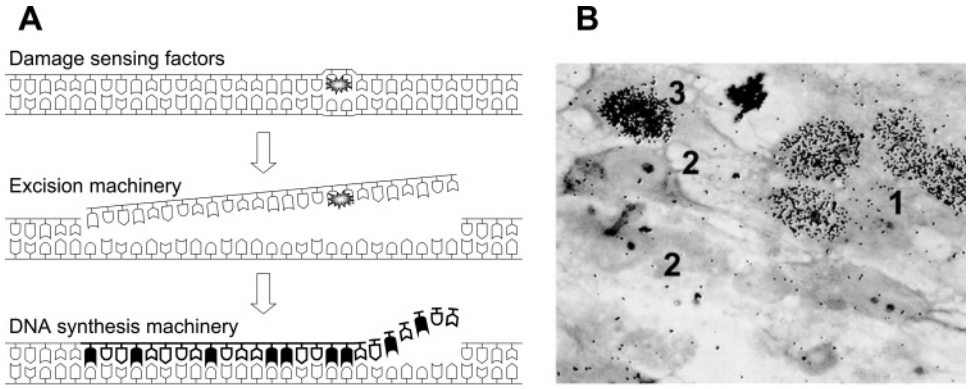

Figure 1. Nucleotide excision repair and unscheduled DNA synthesis assay. (A) Principles of the NER reaction. The repair machinery assembles at sites of UV lesions and performs a dual incision on both sites of the damage. After removal of a 24- to 32-nucleotide stretch of DNA containing the lesion, the DNA repair synthesis machinery fills the gap by using the complementary nondamaged strand as template. Black building blocks on the last scheme represent the ^{3}H-thymine residues incorporated during the course of the UDS assay. (B) Example of an unscheduled DNA synthesis (UDS) assay. Following UV irradiation, fibroblasts are grown for 2−3 h in a medium containing ^{3}H-thymine. The cells in S phase, which are replicating their DNA and are thus incorporating large quantities of nucleotides, display the strongest signal of radioactivity (cell 3) and are not considered for this assay. For the other cells, which are not replicating their DNA, the incorporation of ^{3}H-thymine in their genome represents an "unscheduled" DNA synthesis and reflects the repair synthesis step of nucleotide excision repair system mending the UV damages. UDS is not observed in the NER-deficient XP-A cells (cells 2), but it is restored to wild-type levels upon microinjection of XPA cDNA in XP-A cells (cells 1). Reprinted with permission from ref 124. Copyright 2003 American Society for Microbiology.

TC-NER alone, and it has been suggested that CS cells have a more global mild impairment in transcription.[17]

2.1.3. Trichothiodystrophy

A third genetic disease characterized by UV sensitivity, trichothiodystrophy (TTD, literally: "sulfur-deficient brittle hair"), was reported by Price in 1980.[18] In addition to the symptoms shared with CS patients, TTD patients show characteristic sulfur-deficient, brittle hair and scaling of the skin. This genetic disorder is now known to correlate with mutations in genes involved in NER (*XPB*, *XPD*, and *TTDA* genes). All of these genes are part of the 10-subunit transcription/repair factor TFIIH, and TTD is likely to reflect an impairment of transcriptional transactions rather than a regular defect in DNA repair. This disorder is therefore sometimes referred as a "transcriptional syndrome".[19,20]

2.2. Linking XP to NER and Determination of the Different XP Complementation Groups

Although sharing UV sensitivity as a hallmark, it became evident early on that cultured fibroblast cells from different XP patients were actually exhibiting very different survival capabilities upon exposure to UV irradiation.[14] In all cases, cells from XP patients were more sensitive to UV light than normal cells, with those from patients displaying extra neurological abnormalities being the most severely affected. XP cells were also clearly more sensitive to carcinogenic chemicals generating bulky DNA adducts (see below) but displayed normal sensitivity to DNA methylating agents and X-rays.

The first evidence that XP fibroblasts were unable to perform efficient DNA repair to recover from UV irradiation was provided in 1968 by Cleaver[12] who observed impaired unscheduled DNA synthesis (UDS) in these cells (Figure 1B). This discovery was spurred by a technique developed some years earlier[10,11] and by a newspaper article of the *San Francisco Chronicle* describing the familial skin cancer in XP patients that Cleaver serendipitiously came across as he was thinking about generating human UV-sensitive cell lines.[21] Although additional models were proposed at that time, the UDS phenomenon as observed was already supporting the hypothesis of a "cut-and-patch" mechanism for NER, processing by consecutive (i) incision and removal of a stretch of DNA containing the lesion and (ii) refilling of the resulting gap with the correct DNA sequence using the genetic information contained in the undamaged complementary strand of the double helix (Figure 1A). The incorporation of ^{3}H-thymine observed in UDS is therefore representative of the DNA repair synthesis step, occurring after the excision, and can be used to estimate the level of residual NER activity in XP cells when compared to normal cells (see Figure 1 for details).

The difference in UDS levels between cells from different XP patients corroborated the hypothesis of a genetic heterogeneity for this syndrome and the probable involvement of several genes in the repair process. A classification of the different XP cells was thus endeavored by cell fusion methodology:[22] if a cell A (named XP-A, carrying a mutation in the *XPA* gene and deficient in the corresponding protein XPA) and a cell B (XP-B, mutated in *XPB* and deficient in XPB) are both individually hypersensitive to UV irradiation, fusing their nuclei will result in a cell with normal UV sensitivity (and normal UDS) since it contains now both the XPA and XPB proteins. The cell A and the cell B are thus assigned to two different "complementation groups". By contrast, if two cells do not complement each other after fusing their nuclei, it means that they contain the same genetic deficiency and belong to the same complementation group. If a UV-sensitive cell is complemented by all of the known groups, it represents a new complementation group.

Systematic cell fusion analysis of all the available cell lines from XP patients, has led to the identification of seven complementation groups, ranging from XP-A to XP-G. Additionally, cells from some XP patients, although UV sensitive, displayed a normal UDS response.[23] These cells were designated XP variant (XP-V) and were initially proposed to be defective in postreplication repair.[24] More recently, these cells have been shown to be deficient in a bypass DNA polymerase (Pol η),[25] making them unable to accurately replicate their DNA in the presence of even a few nonrepaired UV lesions.

2.3. From the Complementation Groups to the Genes

The identification of the genes inactivated in the different XP cells has been greatly helped by the use of chinese hamster ovary (CHO) cells specially engineered and selected for their UV sensitivity analogous to that of human XP-deficient cells.[26,27] These UV-sensitive CHO cells were similarly classified into complementation groups[28] and, with the simultaneous advent of modern recombinant DNA techniques, could be screened with random libraries of human DNA fragments in the search for genes allowing for the recovery of UV survival. This strategy successfully resulted in the identification and cloning of the first excision repair cross-complementing (*ERCC*) human genes: *ERCC1*,[29] *ERCC2*,[30] *ERCC3*,[31] *ERCC4*,[32] and *ERCC6*,[33] which were found to correct the corresponding UV-sensitive rodents cells complementation groups. The genes *ERCC2*, *ERCC3*, *ERCC4*, and *ERCC6* were subsequently found to complement the human cells XP-D, XP-B, XP-F, and CS-B, respectively, and their names were thus reassigned (see Table 2 for equivalence). Some genes, such as the first cloned human DNA repair gene *ERCC1*, failed to complement any known XP complementation group and thus retained its rodent name.

A priori, the recourse use to rodent cells to clone human genes may seem unnecessary if one considers the availability of the human repair-deficient cell lines. The far greater capability of rodent cells for transfection and stable integration of foreign DNA sequences has however justified this experimental approach. The considerably higher effort to clone genes by direct complementation of human cells is exemplified by the discovery of the *XPA* gene, identified after a large-scale screening of XP-A cells transfected with mouse genomic libraries.[34,35] In the cases of the *XPC* and *CSA* genes, direct cloning from human cells was achieved using episomally replicating plasmids containing viral replication systems and human cDNA libraries.[36,37] Other genes, such as *XPG*,[38,39] *XPF*,[40,41] *hHR23B*[42] (human homologue of *Saccharomyces cerevisiae RAD23*) and more recently *TTDA*[43] (encoding for the 10th subunit of the TFIIH complex) were identified by chance or by sequence homology with repair genes discovered in other organisms. Interestingly, the cloning of *XPG* was entirely serendipitous—it was discovered as a second open reading frame of a clone isolated in the search for a gene involved in tRNA transcription.[39,44] XPG was then found to be equivalent to ERCC5.[45] In a reverse approach, some groups succeeded in purifying the proteins first, microsequencing of which permitted the deduction of DNA primers and to subsequently cloning of the corresponding genes by PCR. This was the case for the DDB protein which was discovered as a DNA damage binding (DDB) factor missing in XP-E cells[46−48] and for the XPC protein, which was isolated by fractionation of cell extracts complementing the activity of XP-C cell extracts.[42]

2.4. From the Proteins to the Reconstituted NER System

As a next step following the identification of the genes, the purification and characterization of each protein led to preliminary assumption of their roles in NER. XPA,[49,50] RPA,[49,51] XPC-HR23B,[42,52] and DDB[53,54] were found to exhibit affinity for damaged DNA and were thus proposed to be involved in the damage recognition step of NER (Figure 2A, box 1). XPB[55] and XPD[56] were identified as two ATP-dependent helicases (unwinding 3′ → 5′ and 5′ → 3′ duplex DNA, respectively), subunits of the transcription factor IIH (TFIIH) complex involved in basal transcription. This suggested a novel function for the whole TFIIH complex during the NER reaction,[57] in generating an open DNA structure around the lesion (Figure 2A, box 2). ERCC1-XPF[40,58] and XPG[59] were found to be structure-specific endonucleases that incised DNA at single-stranded/double-stranded junctions with a specific polarity (Figure 2A, box 3−4). ERCC1-XPF and XPG were thus proposed as the two nucleases performing the dual incision on the damaged strand, respectively, 5′ and 3′ to the lesion. By compiling the individual biochemical functionalities of the proteins with the identified protein−protein and protein−DNA interactions (Table 2 and summarized in Figure 2A by the arrows), a preliminary sketch of the NER machinery could be outlined (Figure 2B).

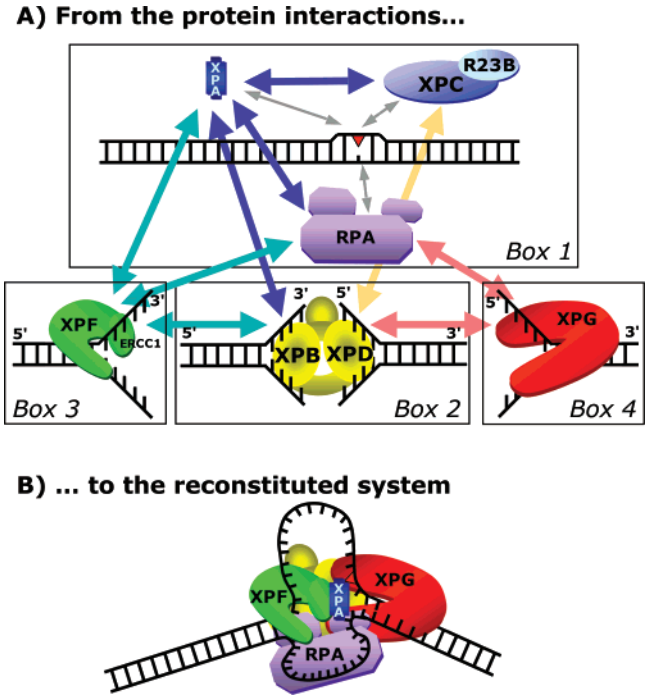

Figure 2. From the protein interactions to the reconstituted system. (A) Individual activities of the NER proteins: XPA, RPA, XPC-HR23B interact with damaged DNA (box 1); TFIIH has helicase activity (XPB: 3′ → 5′, XPD: 5′ → 3′) (box 2); ERCC1-XPF (box 3); and XPG (box 4) have structure-specific endonuclease activity. The identified interactions (Table 2) between these proteins are summarized with arrows. (B) Putative intermediate complex formed prior to dual incision in NER.

In parallel to the characterization of the individual proteins, the development of two types of assays monitoring in vitro the repair of damaged DNA in whole cell extracts led to major advances in the dissection of the molecular mechanisms of the NER reaction. The first assay, developed by Wood and co-workers, was designed to follow the specific incorporation of radioactive dNTPs into damaged plasmids by the polymerase carrying out the repair synthesis step (Figure 3.1 and Figure 3.2).[60,61] A second assay, developed by Sancar and co-workers, allowed direct monitoring of the excision step of the NER reaction by analyzing the release of 24- to 32-nucleotide-long fragments containing an internally radiolabeled phosphate close to the lesion (Figure 3.3).[62,63] A later version of the excision assay, relying on the foreknowledge of the 3′-incision position and on the design of specific primers, consisted of the 3′-end labeling

Table 1. Major DNA Adducts Addressed by the NER System[a]

DNA lesion/adducts	DNA structure	NER repair rates
	UV lesions	
CPD	- bending: 7°-30°[75,247,315,316] - bending towards Mg[316] - unwinding: 9°[316]	+/-[54,79-82]
CPD : GG mismatch	- bending >30°, flexible[77] - Mg windened, mg narrowed	+++[81]
6-4PP	- bending: 44°[75,78]	+++[54,79-82]
	Platinum adducts	
cisplatin 1,2-d(GpG) intra	- bending: 30°-55°[317-319] - bending towards Mg, mg widened - unwinding: 13-25°[320,321]	+/-[87,193]
cisplatin 1,3-d(GpNpG) intra	- bending: 20°-35°[322.323] - bending towards Mg, mg widened - 2 bp local denaturation - unwinding: 19-23°[320,324]	+++[89,218]
transplatin 1,3-intra	- bending: 60°[322] / flexible[323] - hinge joint without directed bend - 4 bp local denaturation	+++[325]
bis-platin (Pt-Pt) 1,3-intra	- flexible nondirectional bend[326] - small local distortion; no ss region	+/-[193]
	Polycyclic Aromatic Hydrocarbons	
(+)-*trans*-B[a]P-dG : dC	- **A**: ring external in mg[327,328] - base pairing conserved	+/-[109]
(-)-*trans*-B[a]P-dG : dC		+/-[109]
(+)-*trans*-B[a]P-dG : del.	- **B**: base displaced/intercalated - dG in mg[329,330] - no disruption of adjacent bp	-[109]
(-)-*trans*-B[a]P-dG : del.		nd
(+)-*cis*-B[a]P-dG : dC	- **B**: base displaced/intercalated - ring in mg(+)/Mg(-); dG flipped in mg(+)/Mg(-)[331,332]	++[109]
(-)-*cis*-B[a]P-dG : dC		+++[109]
(+)-*cis*-B[a]P-dG : del.	- **B**: base displaced/intercalated - dG in Mg[333]	+/-[109]
(-)-*cis*-B[a]P-dG : del.	- nd	+/-[109]
(+)-*cis*-B[a]P-dA : dT	- **B**: base displaced/intercalated - dG in mg[334]	nd
(+)-*trans*-B[a]P-dA : dT	- **B**: base displaced/intercalated - distorting intercalation[335]	+[110]
(-)-*trans*-B[a]P-dA : dT	- **B'**: intercalated/bp conserved[336]	+/-[110]
(+)-*trans*-B[c]Ph-dG : dC	- **B'**: intercalated/bp conserved - intercalation from mg[337]	nd
(-)-*trans*-B[c]Ph-dG : dC		nd
(+)-*trans*-B[c]Ph-dA : dT	- **B**: base displaced/intercalated - intercalation from Mg[338,339]	-[110]
(-)-*trans*-B[c]Ph-dA : dT		-[110]
	Aromatic Amines	
dG-AAF : dC	- **B**: base displaced/intercalated - AAF in mg, dG *syn* in Mg[340]	+++[105,109]
dG-AF : dC	- **A**:**B** equilibrium (90:10 to 50:50) - for **B**: AF in Mg[103]	+/-[105,341]
dG-AF : del.	- **B**-"wedge shapped"[342] - dG syn in Mg in bulge[343,344]	nd
dG-AF : double del.		nd
dG-PhIP : dC	- **A**:**B** equilibrium[345]	nd
dG-IQ : dC	- **A**:**B** equilibrium[346]	nd
dG-APy : dC	- **B**: base displaced/intercalated[347,348]	+++
dG-AB	- **A**: ring external in Mg[349]	nd
	Others	
Cholesterol adduct	- **B**-like: intercalated[350] - opposite dG displaced	+++[80,211]
Psoralen mono-adduct	- bending: 8°, no kink[351] - unwinding: 34°[352]	+[173,353]

[a] Two major conformations can be distinguished. A, ring external in Mg (major groove) or mg (minor groove) with base-pairing conserved and modified dG anti (see Figure 6, middle panel); B, base-displaced, ring-intercalated with the modified dG syn (see Figure 6, right panel); B′, ring-intercalated but base-pairing conserved. Abbreviations: bp: base pair; B[a]P: bay benzo[*a*]pyrene; B[c]Ph: fjord benzo[*c*]phenanthrene; AAF: acetylaminofluorene; AF: aminofluorene; PhIP: 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine; IQ: 2-amino-3-methylimidazo-[4,5-*f*]quinoline; APy: aminopyrene; AB: aminobiphenyl.

Table 2. Repair Genes Involved in Nucleotide Excision Repair and their *ERCC* equivalents[a]

gene (equival.)	protein subunit	protein size (aa)	function	interacting partners	disease
			XPC-HR23B		
hHR23B	HR23B	43 kDa (409)	damage recognition	TFIIH[142,156]	XP
XPC	XPC	106 kDa (940)	assembly platform?	XPA[147]	(GGR)
CEN2	CEN2	20 kDa (172)		DDB[277]	
			DDB		
DDB1 (p127)	DDB1	127 kDa (1140)	CPD recognition?	XPC[277]	mild XP
DDB2 (p48)	DDB2	48 kDa (428)	chromatin remodeling?	RPA[278]	
			XPA		
XPA	XPA	31 kDa (273)	damage binding? architectural role?	XPC[147] RPA[190,204,354] TFIIH[355,356] ERCC1[205,206,357]	XP
			RPA		
RPA1	RPA70	68 kDa (616)	single-stranded DNA binding	XPA[49,50]	not viable
RPA2	RPA32	30 kDa (270)	damaged DNA binding?	XPG[49,185]	
RPA3	RPA14	14 kDa (121)		PCNA/RFC	
			TFIIH		
XPB (ERCC3)	XPB	89 kDa (782)	ATP dep. 3' → 5' DNA helicase	XPC[57,162,358]	XP
XPD (ERCC2)	XPD	87 kDa (760)	ATP dep. 5' → 3' DNA helicase	XPA[358,359]	XP/CS
GTF2H1 (TFB1)	p62	62 kDa (548)	core TFIIH subunit	XPG[162,358,360,361]	TTD
GTF2H2	p44	44 kDa (395)	DNA binding?	XPF[133,358]	
GTF2H3 (TFB4)	p34	34 kDa (308)	DNA binding?		
GTF2H4 (TFB2)	p52	52 kDa (462)	core TFIIH subunit		
GTF2H5 (TTDA)	p8	8 kDa (71)	stabilizing subunit		
MNAT1 (TFB3)	Mat1	36 kDa (309)	CAK subcomplex		
CDK7	Cdk7	39 kDa (346)	phosphorylates RNA Pol II and		
CCNH	CyclinH	38 kDa (323)	other substrates		
			ERCC1-XPF		
ERCC1	ERCC1	33 kDa (297)	5' endonuclease	XPA[204,207]	XP
XPF (ERCC4)	XPF	103 kDa (905)		TFIIH[133]	DSBR?
			XPG		
XPG (ERCC5)	XPG	133 kDa (1186)	3' endonuclease	RPA[185,201]	XP
				TFIIH[198,361]	XP/CS
				PCNA?[362]	ODR?
			RFC		
RFC1	RFC1	128 kDa (1148)	ATP-dep PCNA loading	PCNA[363]	
RFC2	RFC2	39 kDa (354)		RPA[225,234]	
RFC3	RFC3	41 kDa(356)			
RFC4	RFC4	40 kDa (363)			
RFC5	RFC5	38 kDa (340)			
			PCNA		
PCNA	PCNA (trimer)	3 x 29 kDa (3 x 261)	sliding clamp	RFC[363] polδ[226,227] XPG?[236]	
			Pol δ		
p125	p125	124 kDa (1107)	DNA polymerase	PCNA[226,227]	
p66	p66	51 kDa (466)			
p50	p50	51 kDa (469)			
p12	p12	12 kDa (107)			
			Pol ε		
p261	p261	261 kDa (2286)	DNA polymerase	PCNA[223,228]	
p59	p59	60 kDa (527)			
p17	p17	17 kDa (147)			
p12	p12	12 kDa (117)			
			ligase I		
LIG1	ligase I	102 kDa (919)	DNA nick sealing	PCNA[364]	XP-like

[a] Information about the corresponding protein products are given in parallel. The associated syndromes vary in function of the severity of the protein mutation/truncation. When known, the specific repair pathway is given in brackets. Abbreviations: GGR: global genome repair; CAK: CDK-activating kinase; CDK: cyclin dependent kinase; DSBR: double strand break repair; ODR: oxidative damage repair.

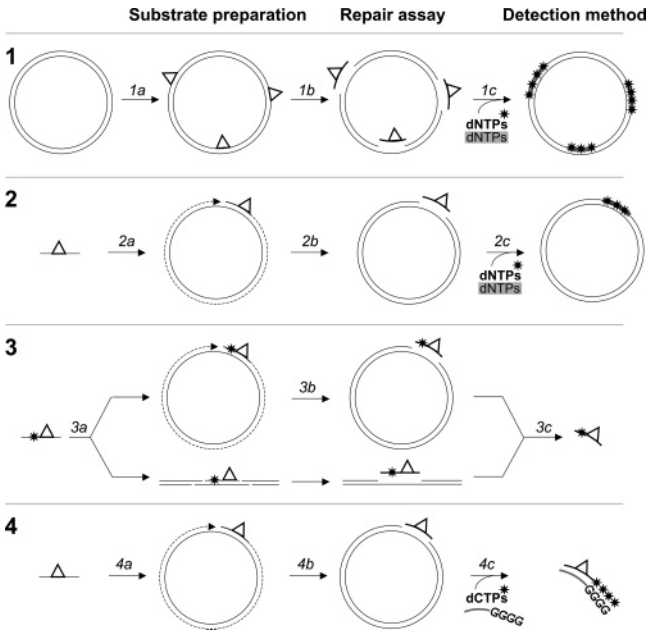

Figure 3. Strategies used for substrate preparation and investigation of the NER reactions in vitro. (1) Detection of the repair synthesis. A plasmid containing multiple UV damages[365] or platin adducts[325,366] is generated by exposing the plasmid to UV irradiation or cisplatin, respectively. Upon incubation with human cell extract, the lesions are excised and the products of the repair reaction are detected by the insertion of radiolabeled deoxy-nucleotides (stars) into the plasmids during the repair synthesis step. (2) Detection of the repair synthesis using plasmids containing a unique lesion at a defined position. The substrates are prepared by annealing a site-specifically modified oligonucleotide with a single-stranded plasmid and formation of the closed circular plasmid by primer-extension and ligation.[79,87,108,353,367] (3) Direct detection of the excision reaction. Short oligonucleotides containing a unique lesion are 5′ radiolabeled with ^{32}P prior to incorporation into a plasmid by primer-extension[62] or by ligation into a longer linear double-stranded DNA.[88] The excised products of the NER reactions containing the radioactive phosphate are directly detectable on sequencing gels. (4) Detection of the excised fragments by a post-labeling method. A plasmid containing a unique lesion is incubated with human cell extract. The repair reaction is then supplemented with an oligonucleotide complementary to the 24−32 nucleotide long expected products of the excision reaction. The annealing of these products to this complementary oligonucleotide yields a template for the incorporation ("fill-in") of radiolabeled dCTP at the 3′-end of the excised products. Note that this particular protocol relies on the foreknowledge of the 3′-incision position for the particular lesion being used.[64]

of the excised damaged oligonucleotides with a DNA polymerase and radiolabeled dNTPs (Figure 3.4).[64]

Both types of in vitro NER assays were used with fractionated cell extracts and led to the identification of additional factors required for the NER reactions such as the single-stranded DNA binding protein RPA and the replication processivity factor PCNA, a deficiency in which does not lead specifically to an XP phenotype as they are essential proteins involved in other aspects of DNA metabolism.[65,66]

The combination of the characterization of the individual NER factors and the development of the repair assays culminated with the in vitro reconstitution of the NER reaction using defined factors and eventually all purified proteins in recombinant form.[67−70] Considering that not less than 20 polypeptides (Table 2) are required for the excision step and another 13 for the repair synthesis step, the in vitro reconstitution of a system of such complexity constituted a real biochemical *tour de force*. These in vitro repair assays with the reconstituted NER system are still widely used today for the elucidation of the detailed mechanisms underlying the NER reaction (see section 4).

3. Lesions Addressed by Nucleotide Excision Repair

One of the most astonishing characteristics of the NER pathway is its ability to recognize and excise an extraordinary diversity of DNA damage. Lesions that are processed by NER involve one or more nucleotides, arise from modifications at different positions of the purine or pyrimidine bases, and are formed by UV irradiation or upon exposure to chemically reactive molecules. In this section, we introduce select DNA lesions, which represent important substrates for NER, and discuss the relationships between structural features of damaged DNA and their propensity to be repaired by NER.

3.1. Damage of the Pyrimidine Bases by UV Light

It is now well established that radiation in the UV range represents the most harmful and mutagenic part of the solar spectrum reaching the surface of the earth. The UV spectrum is subdivided in three wavelength ranges: UVA (320−400 nm), UVB (290−320 nm), and UVC (200−290 nm). Among them, the shortest wavelengths are the most powerful, but fortunately the ozone layer efficiently filters out the UVC and the majority of the shortest UVB. UVA radiation is approximately 1,000 weaker than the UVB, but it reaches the earth in about 100 times greater quantities (see ref 71 and references therein). Furthermore, because of their longer wavelength, UVA can penetrate more deeply within the skin than UVB, although the relationship between depth of penetration and relevance toward photodamage and skin cancer is currently unknown. The formation of DNA photoproducts in human skin is maximal upon exposure up to 300 nm UV light, which correlates with the optimal absorption spectrum of the major DNA chromophores (thymine, cytosine, and the minor 5-methylcytosine).

Prokaryotes and many eukaryotes can remove UV lesions from DNA by NER as well as through the action of specific photolyase enzymes, which use energy from light to revert pyrimidine dimers to the original monomers.[72] In placental mammals, however, NER is the only pathway to remove UV lesions from DNA, and the fact that humans have no backup mechanism for the repair of UV lesion is evidenced by the XP phenotype.[5]

3.1.1. Cyclobutane Pyrimidines Dimers

The cyclobutane pyrimidine dimers (CPDs) constitute the major UV-induced DNA photoproducts. They are formed by a [2+2] cycloaddition reaction of the C5−C6 double bonds of adjacent pyrimidines bases (Figure 4). Theoretically, all combinations of cis/trans (indicating the relative positions of the pyrimidine rings) and syn/anti (indicating the relative orientation of the C5−C6 bonds) diastereoisomers should be considered. Because of steric constraints within DNA however, only syn isomers can be formed with the cis−syn representing the large majority of the CPDs, and the trans−syn occurring exclusively within single-stranded DNA (ssDNA). The majority of CPDs are formed between adjacent thymine residues (TT) but can eventually occur between adjacent TC, CT, or CC, depending on the wavelength, dose

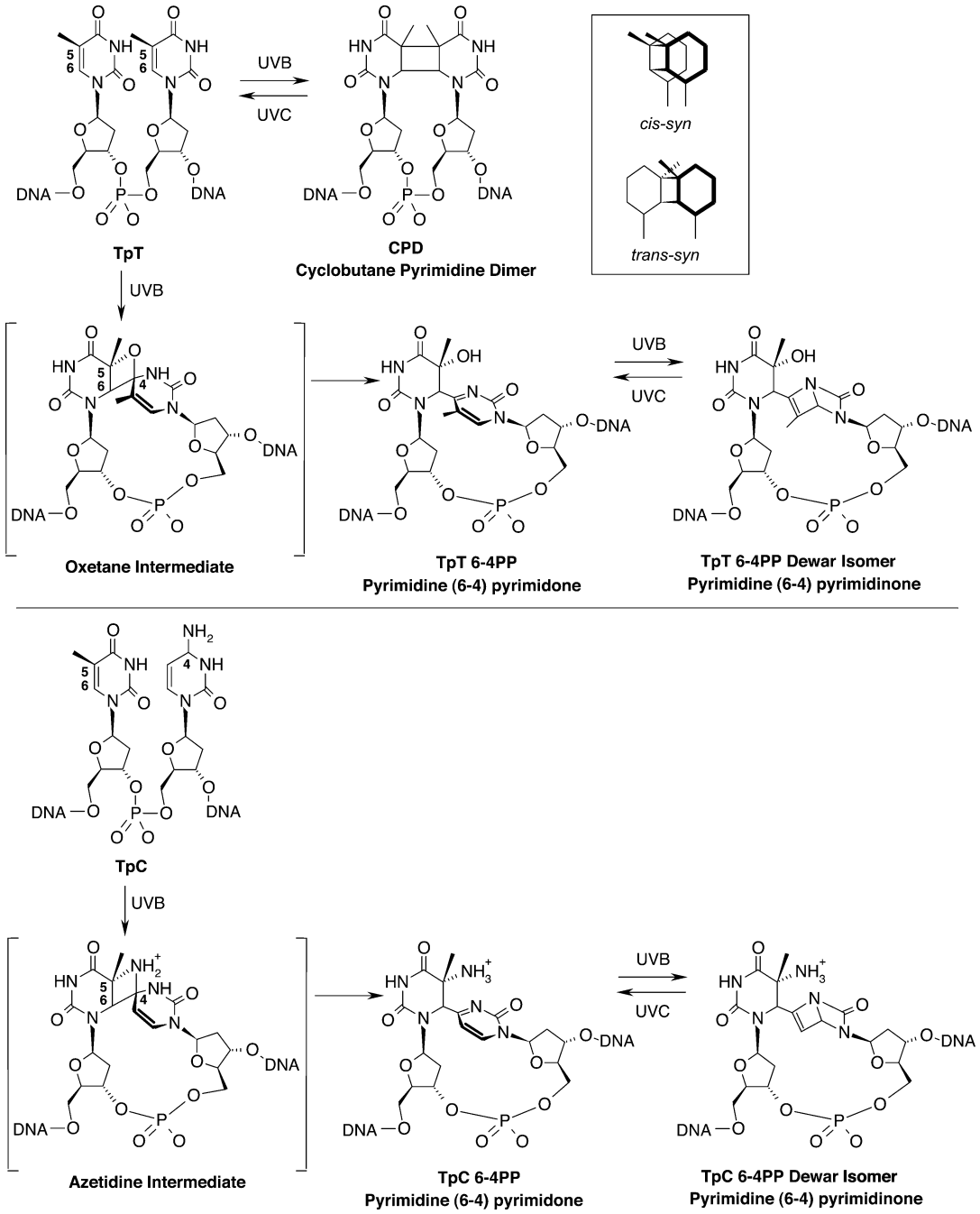

Figure 4. Ultraviolet photoproducts of a TpT in DNA. The structures and formation of the cyclobutane dimer, the pyrimidine (6−4) pyrimidone, and the Dewar pyrimidinone.[368] The inset shows the different diastereoisomers of the CPD within DNA. The different wavelengths for the formation and reversion of the photoproducts are indicated.

of irradiation, and adjacent sequences. The formation of CPDs is reversible upon further irradiation below 250 nm, due to the residual absorption capacity of the dimers at these wavelengths.

3.1.2. 6–4 Photoproducts

The pyrimidine (6−4) pyrimidone adducts (6−4PPs) are the second most prevalent UV lesion with a rate of induction accounting for 25−30% of that of cyclobutane dimers.[73] The [2+2] cycloaddition occurs between the C5−C6 bond of a 5′-pyrimidine residue and the C4 carbonyl group of a 3′-thymine (respectively: the 4-imino group of a 3′-cytosine) and results in the formation of an oxetane (respectively: an azetidine) intermediate, whose spontaneous rearrangement yields to the corresponding pyrimidine(6−4)pyrimidone adducts (Figure 4). The 6−4PPs are induced preferentially at TC, CC, and TT nucleotides, with ratio and yields depending on the irradiation wavelength and adjacent sequences. Further UVB irradiation of a 6−4PP can eventually lead to rearrangement into the corresponding Dewar isomer (Figure 4),[74] although the biological relevance of this lesion has not yet been conclusively shown in mammalian cells.

While CPDs and 6−4PPs are formed at similar wavelengths, they produce very different types of structural distortions within DNA. CPDs induce only a slight bending of the DNA helix with no significant alteration of the Watson−Crick base pairing.[75−77] By contrast, 6−4PPs produce a more pronounced distortion of the DNA backbone (bending and unwinding), which results in loss of base

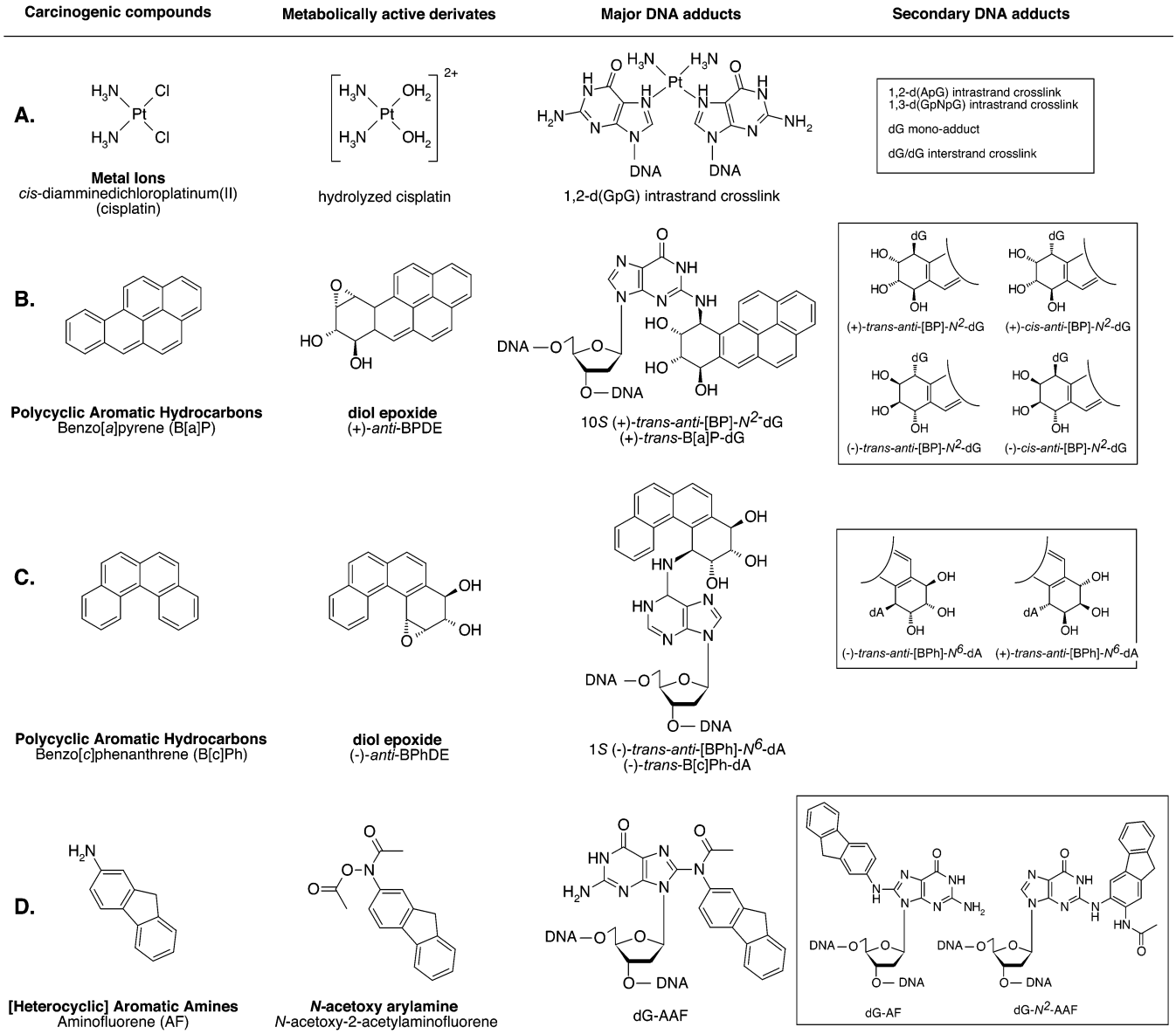

Figure 5. Examples of carcinogenic chemicals and their DNA adducts. Carcinogenic chemicals, their activated derivatives, and the major adducts they form with DNA are shown. The insets show the main secondary DNA adducts formed by B[a]P, B[c]Ph, and AF.

pairing at the site of the lesion (see Table 1 for details and references).[78] These differences in DNA distortion directly reflects the processing of these lesions by NER, and it was observed that 6−4PPs are much better substrates than CPDs (Table 1).[54,79−82] Indeed, the type and extent of the structural distortion induced by a damage on the DNA double helix is thought to be a crucial determinant for the recognition of a lesion by NER (see below).

3.2. DNA Adducts Formed by Electrophilic Molecules

The large majority of carcinogens are electrophilic compounds (often unfortunate products of metabolic activation) that exhibit some affinity for double-stranded DNA (dsDNA) and react with the nucleophilic atoms of the DNA residues: the phosphodiester linkages, N7 of guanine and N3 of adenine, or the exocyclic amino groups of guanine (N^2) and adenine (N^6). The N7 position of guanine is the most reactive position in dsDNA as it combines high electronic density and enhanced accessibility through its localization in the less hindered major groove of the DNA helix (see Figure 6, left panel). Additionally, the negative electrostatic potential of the N7 position of G is further increased when it is flanked by other guanine residues, resulting in zones of mutation "hot spots", which are particularly prone to electrophilic attacks. Even the C8 arylamine adducts of guanine presented hereafter have been proposed to result from the preliminary formation of a transient N7 adduct followed by a rapid rearrangement of this adduct to the C8 position,[83] although this is still a matter of debate.[84]

3.2.1. Cisplatin

cis-Diaminodichloroplatinum (*cis*-DDP or cisplatin) is one the most frequently used chemotherapeutic drug for the treatment of a broad panel of tumors. Cisplatin is a neutral, square planar complex of platinum(II) that is coordinated to two relatively inert ammonia groups and two labile chloride ligands in cis geometry (Figure 5A, reviewed in ref 85). Administered intravenously, cisplatin remains stable in the blood plasma until it diffuses into the cytoplasm of cells, where low salt (chloride) concentration leads to the substitution of the labile chloride ligands by water or hydroxide ions,

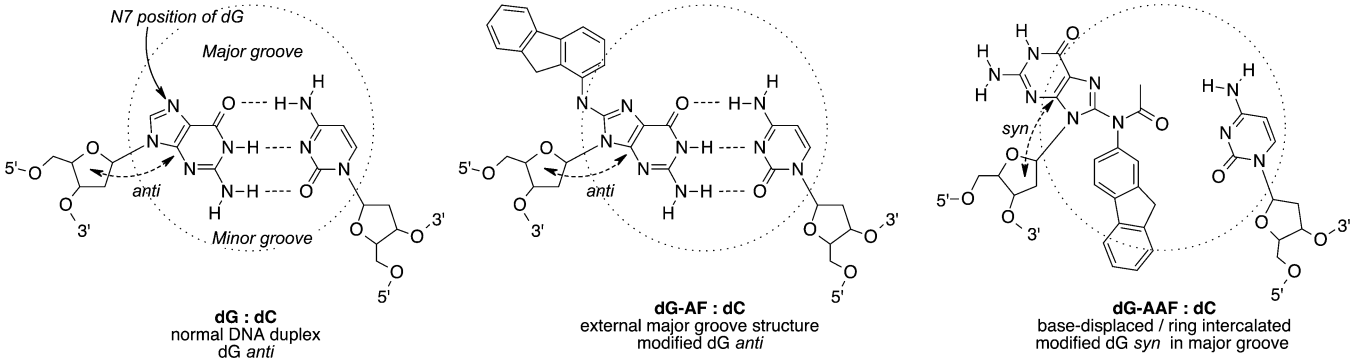

Figure 6. Conformations of dG, dG-AF, and dG-AAF in dsDNA. In a B-form DNA duplex, dG is in anti conformation and fully paired with the complementary dC. Note the accessibility of the nucleophilic N7 position of dG, positioned in the major groove of the DNA. The major conformation of DNA containing the AF modification displays normal Watson−Crick pairing for the modified dG; the fluorene ring is accommodated in the major groove of the DNA helix. The steric bulk at N(8) of dG-AAF causes an anti to syn rotation of the modified guanine, and the fluorene ring is accommodated inside the helix ("base-displaced, intercalated" conformation).[103,340,369] This intercalation produces a severe DNA helix distortion and explains the profound differences in damage recognition, repair efficiency, and mutagenicity of these two adducts.

yielding a charged and activated electrophilic agent (Figure 5A). Subsequent reaction with nucleophilic sites on DNA results in the formation of monoadducts, intrastrand, or interstrand cross-links. Platination of oligonucleotides preferentially yields intrastrand N7−N7 cross-links between neighboring pyrimidine residues: 1,2-d(GpG) (accounting for up to 65% of all cisplatin-induced lesions) or 1,2-d(ApG) intrastrand cross-links (25% of all adducts) between adjacent bases, and intrastrand 1,3-d(GpNpG) adducts (5−10%) with one nucleotide (N) separating the cross-linked guanines.[85,86] Since they involve differently spaced bases, the 1,2- and 1,3-intrastrand cross-linked exhibit very different structural constraints on the DNA double helix:[85] the 1,2-intrastrand cross-link produces a pronounced kink of the DNA but retains almost intact base-pairing. On the other hand, the 1,3-intrastrand adduct induces a much smaller DNA bend but exhibits significant helix unwinding and extended base pairing disruption in the vicinity of the cisplatin adduct (see Table 1).[85] As for the UV lesions, these structural differences are reflected by the corresponding repair rates with the 1,3-intrastrand cross-link being a much better substrate for NER than the 1,2-intrastrand adduct (Table 1).[87−89] Finally, cisplatin interstrand cross-links can also occur between guanines of complementary strands of the DNA but to a much smaller extent (2%). Although these later interstrand cross-links represent only a minority of the adducts formed, they are highly relevant clinically. They are resistant to repair by NER and are addressed by more complex, less well explored repair pathway(s).[89,90]

3.2.2. Aromatic Hydrocarbons

Links between cancer and hydrocarbon exposure were hypothesized more than 200 years ago by correlating cancer predisposition and chimney sweeping.[4] Nowadays, exposure to nonpolar carcinogens remains a major health concern in our societies since these compounds are largely abundant in cigarette smoke and automobile exhausts.[91−93] Benzo[*a*]-pyrene (B[a]P, Figure 5B), initially identified from crude coal tar, has been intensively studied and has served as the prototype potent carcinogenic polycyclic hydrocarbon.[4] Unmodified, it is a chemically unreactive nonpolar compound with a planar configuration. It is now established that metabolic detoxification mechanisms are responsible for the activation of these compounds into potent carcinogens. The function of these detoxifying enzymes is to convert potentially toxic lipophilic chemicals into water-soluble excretable derivatives. Benzo[*a*]pyrene and benzo[*c*]phenanthrene (B[c]-Ph, Figure 5C), like other polycyclic aromatic hydrocarbons, undergo metabolic transformation into phenols or dihydrodiols, which are readily excreted. A fraction of the molecules is converted into electrophilic epoxides (Figure 5B,C),[4,94] which, upon intercalation into DNA, can react with the exocyclic amino group of a guanine. Depending on the stereoisomer formed (Figure 5B,C, third inset), two major conformations can be accommodated within DNA:[94] the apolar ring of the adduct can intercalate into the double helix, forcing the displacement of the modified base from its canonical position, or the aromatic ring can be accommodated in a groove of the DNA double helix and the regular base pairing is conserved (see Figure 6 for the principle and Table 1 for details). Once again, the conformation and thermodynamic stability of the different DNA adducts profoundly influence their propensity to be processed by NER (see below).

3.2.3. Arylamine Carcinogens

The aminofluorene (AF) was first developed as insecticide, but its use was discontinued when it was found to be a powerful mammalian carcinogen. It is nowadays widely used as a model compound to study mutagenesis and DNA repair (for an extensive review, see ref 95). AF belongs to a class of chemicals known as aromatic amines, including the polycyclic aromatic amines found in cooked food and cigarette smoke such as IQ or PhIP (for a review on heterocyclic aromatic amines, see ref 96). As for the nonpolar carcinogens, the aromatic amines can be activated by detoxification mechanisms into *N*-hydroxy, *N*-acetoxy, or *N*-sulfoxy compounds. These derivatives can undergo *N*−O bond heterolysis, yielding a very reactive arylnitrenium intermediate that forms adducts on positions C8 of dG and to a lesser extent on N^2 of dG (Figure 5D). The two C8 adducts are of particular interest: although differing from only one acetyl group, they exhibit very different structural properties within DNA.[97−99] The AF adduct (dG-AF) can adopt two conformations around the glycosidic bond. In the first one, the modified dG remains in the anti conformation retains a normal Watson−Crick base pairing, and the fluorene ring is flipped in the major groove of DNA (Figure 6). In the second one, the fluorene moiety is intercalated inside the DNA helix and the modified dG is displaced in the major groove with a syn conformation of its glycosidic bond (Table

1).[100−103] The acetylaminofluorene adduct (dG-AAF) inevitably assumes a syn conformation for its glycosidic bond and thus a base-displaced conformation because of the steric bulk induced by the acetyl group at C(8) (Figure 6, Table 1). These structural variations profoundly influence how these adducts are processed by NER.[104,105]

3.3. DNA Structure/Repair Relationships

The fact that NER could repair so many structurally different types of DNA damage suggested early on that the system may not recognize the lesion per se but rather some specific conformational features caused by the presence of the lesion within DNA. Superficial inspection of the propensity of a given lesion to be processed by NER suggests that the amount of helical distortion is proportional to the repair rate.[106] A conceptual breakthrough in our thinking about NER substrates came from an observation made by Naegeli and co-workers:[107] small structural modifications of the ribose moiety of one nucleotide were not processed as lesions by NER if present in a fully paired DNA duplex but were converted into efficient substrates when they were placed in a bubble containing three mismatched base-pairs. Mismatch bubbles without chemical modification were intrinsically not substrates for NER. These observations were formulated in the "bipartite substrate discrimination" model, suggesting that only DNA lesions containing a base-pairing disruption *and* a chemical modification trigger NER.[107] This model further implied that the DNA helix distortion was responsible for the recruitment of the NER machinery,[108] and this hypothesis was subsequently corroborated by studies investigating the initial damage recognition step by the XPC-HR23B protein (see section 4.2).[81]

The bipartite discrimination model is also very consistent with observations that lesions inducing higher degrees of DNA helix distortion are more efficiently processed by NER. Thus, the higher repair rates of 6−4PP versus CPD lesions and of 1,3-d(GpNpG) versus 1,2-d(GpG) intrastrand cisplatin cross-links are in agreement with the amount of base pairing disruption and DNA unwinding induced by the adducts (Table 1). The same relationship holds also true for the damages induced by polyaromatic hydrocarbons or aromatic amines. Adducts that can retain base pairing by accommodating the aromatic ring in the major or minor grooves of the DNA (Figure 6, middle panel) are generally poor substrates for NER (Table 1, A; e.g., dG-AF). Conversely, adducts displaying base pairing disruption upon intercalation of the aromatic ring inside the DNA helix (Figure 6, right panel) are generally good substrates for NER (Table 1B; e.g., dG-AAF).

Detailed investigation of the structures and repair of the benzo[*a*]pyrene and benzo[*c*]phenanthrene adducts revealed however that the structure/repair relationships outlined above may be a bit too simplistic. Certain adducts of benzo[*a*]-pyrene with dG and of benzo[*c*]phenanthrene with dA that assumed the base-displaced/intercalated conformation were in fact very poor NER substrates, despite the apparent distortion they induced on the DNA (Table 1).[109−111] This led to the proposal of a multi-partite damage recognition model, which suggested that a number of parameters including base pairing disruption, DNA unwinding, bending, and flexibility should be taken into consideration. All these parameters influence the amount of thermodynamic (de)-stabilization that a given lesion introduces in the DNA helix, and it was found that thermodynamically more stable adducts are also more refractory to processing by NER.[112] To explain the differences in repair rates by NER, another model has proposed that the amount of local conformational flexibility introduced into the dsDNA helix by a lesion is an important determinant for the damage recognition process during NER.[113] Rather than a defined structural feature, both models argue that the amount of thermodynamic stabilization (or destabilization) induced by damage on the DNA is the key parameter that reflects the propensity of a lesion to be repaired by NER.

Intriguingly some adducts, namely, B[a]P or AAF adducts located opposite to a single nucleotide deletion (Table 2), that are not processed by NER have been found to have a "dominant negative" effect on the repair of other adducts, presumably by depleting ("hijacking") some of the NER factors from their regular substrates.[109,114] If these adducts are able to efficiently deplete (and thus attract) some of the NER factors, why are they then not repaired? Apparently, they can bind some of the NER factors in an unproductive configuration that does not allow the proper assembly of the remaining NER factors at the site of the lesion. Clearly, much remains to be done to establish a detailed correlation between the structure of a given DNA lesion and its recognition and repair mechanisms by the many proteins involved in NER. Our present understanding of how the various NER factors recognize damaged sites in DNA, assemble at the site of the lesion, and excise lesion from DNA as part of an oligonucleotide is discussed in detail in the next section.

4. Molecular Mechanism of Nucleotide Excision Repair

Despite the complexity of the NER pathway, its overall molecular mechanisms are now understood in considerable detail. In addition to biochemical studies, recent developments in fluorescence microscopy have allowed the visualization of aspects of NER directly in the nuclei of living cells. These in vivo experiments have brought invaluable answers to questions that were impossible to address with standard in vitro biochemical studies. A convergence of data from in vivo and in vitro studies has resolved some controversial issues, such as the order of recruitment of the damage recognition proteins in NER. In this section, we will discuss, step by step, the molecular mechanisms underlying the NER system, and we will introduce, when relevant for the discussion, some of the recent imaging techniques that have beneficially contributed to the research field.

4.1. A Sequential Assembly Process for the Mammalian NER Machinery

Soon after the initial in vitro reconstitution of the NER reaction, a controversy arose about whether NER operated by sequential assembly of the individual factors at the site of the DNA damage or through the action of a preassembled "repairosome" complex containing the essential factors that would continuously scan the genome for lesions. Indeed, co-purification of several NER factors could be achieved from yeast and later human cells extracts, which argued for the existence of such a preassembled NER holo-complex.[115−118] The concept of "repairosome" is attractive because it circumvents the difficult issue of the rapid and well-concerted assembly of so many proteins at the site of the DNA damage. Nevertheless, the enormous size and reduced mobility of such a repairosome complex would be hard to reconcile with the

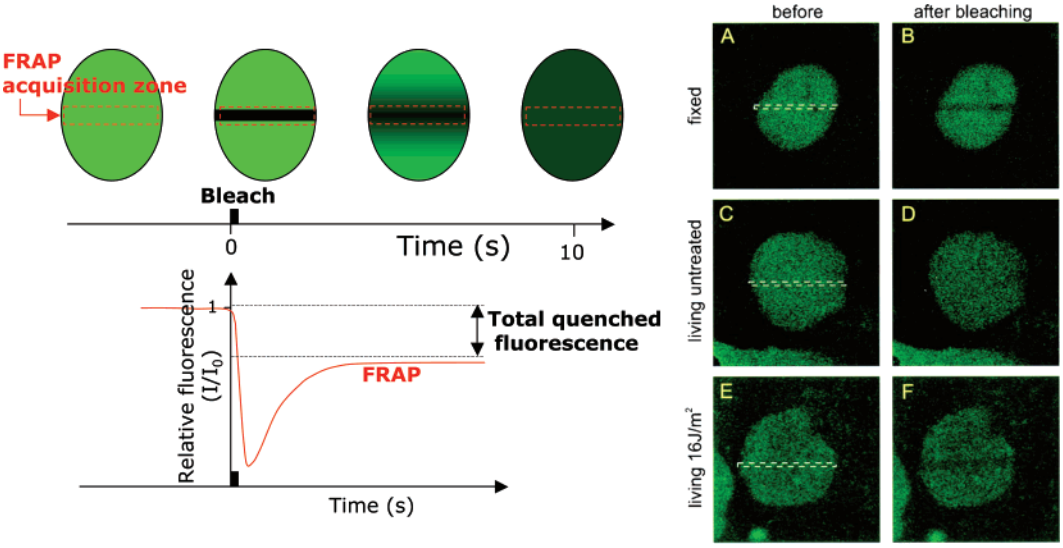

Figure 7. Fluorescence photobleaching techniques used in NER studies. The protein of interest is fluorescently tagged (usually with the green fluorescent protein: GFP), and its distribution in the nucleus is visualized by fluorescence microscopy. At $t = 0$, a laser beam irradiates a defined zone of the nucleus, resulting in definitive quenching of the fluorescent tags of the proteins that were located/diffusing within this zone during the bleaching time. The quenched proteins then diffuse all over the nucleus, while other fluorescently tagged proteins repopulate the bleached zone. The fluorescence redistribution after photobleaching (FRAP) monitors the reappearance of fluorescence at the zone of photobleaching and gives an indication of the diffusion of the fluorescently tagged proteins within the nucleus. If the proteins are chemically fixed, the quenched proteins do not move out of the bleached zone, which remains devoid of fluorescence (panels A and B). In living cells, the quenched proteins diffuse out and are replaced by new fluorescent proteins (panels C and D), yielding a rapid homogenization of the fluorescence (overall decreased) within the nucleus. In irradiated cells, some proteins are sequestered to repair the UV lesions and thus cannot diffuse freely within the nucleus. This results in an intermediate delay of fluorescence recovery at the bleached region (panels E and F) that can be quantified to evaluate the proportion of protein immobilized to perform the repair reactions. The panels A−F represent the diffusion of GFP-tagged XPA proteins during FRAP experiments and demonstrate how XPA diffuses freely through the nucleus and is temporarily immobilized after the cells have been irradiated with UV light. Reprinted with permission from ref 124. Copyright 2003 American Society for Microbiology.

efficient search for lesions in the chromatin-condensed genome of living cells and with any fine regulatory mechanism of the DNA damage response. Other studies have argued for a sequential assembly of the NER machinery based on the fact that the reaction could be reconstituted with individually purified factors and on the observation of stable intermediate "preincision" complexes (see below) containing a subset of the NER factors bound to damaged DNA.[119−121]

The definitive proof of a sequential assembly mechanism for the NER machinery, at least in mammalian cells, was provided only recently by a series of in vivo studies using fluorescently tagged proteins (GFP-ERCC1,[122] GFP-XPB,[123] or GFP-XPA[124]) combined with photobleaching techniques (see Figure 7 for details). It was shown that the NER proteins moved freely through the nuclei of nonirradiated cells with a diffusion coefficient proportional to the expected molecular weight of the individual factors (ERCC1-XPF, TFIIH, or XPA, respectively) and that they were transiently immobilized to perform repair reaction upon UV irradiation. These studies also provided a more quantitative depiction of the NER process; for GFP-ERCC1 for example, 30−40% of the proteins were found to be sequestered to the sites of DNA repair during about 4 min per NER event. These in vivo studies therefore not only provided strong evidence against the existence of a NER holo-complex but allowed also access to quantitative information about the comings and goings of the various proteins during the NER process.

4.2. Damage Recognition by XPC-HR23B

A sequential assembly mechanism of NER is most consistent with the existence of factors responsible for the initial detection of DNA damage and for the subsequent recruitment of all other NER proteins to the site of the lesions. Numerous efforts were aimed at identifying these initial damage recognition factors. Measurements of binding affinities and specificities were inconclusive in this respect because none of the potential candidates (DDB,[53,54,125] XPC-HR23B,[81,126−128] XPA,[50,129,130] RPA,[49,51,131]) exhibited a striking enough preference for binding damaged DNA over nondamaged DNA in the context of a genome. How NER can efficiently recognize one lesion among the enormous background of genomic DNA if none of the factors displays satisfactory affinity and selectivity for damaged DNA has therefore been a vividly debated question. The confusion increased further when, using similar biochemical experiments (reconstituted in vitro NER assays with various order of addition of the factors), either XPC-HR23B[126] or XPA/RPA[132] was proposed as the initial damage recognition factor. Additional studies investigating which factors were required to generate open DNA structures around the lesion supported an early role for XPC and TFIIH.[133] Beyond their apparent contradiction, these results pointed to the limits inherent to in vitro biochemical experiments: some substrates may adopt a conformation, within linear oligonucleotides, that is very different from the one in the context of chromatin in a living cell. Furthermore, proteins may be present in modified forms and localized in specific parts of the nuclei and thus function differently in living cells than in a reconstituted in vitro system.

In hindsight, the observation that XPC-deficient cells were defective only in GG-NER, but not TC-NER, pointed also to a very early role for XPC in the process, as XPC is apparently not required to perform the repair reaction when

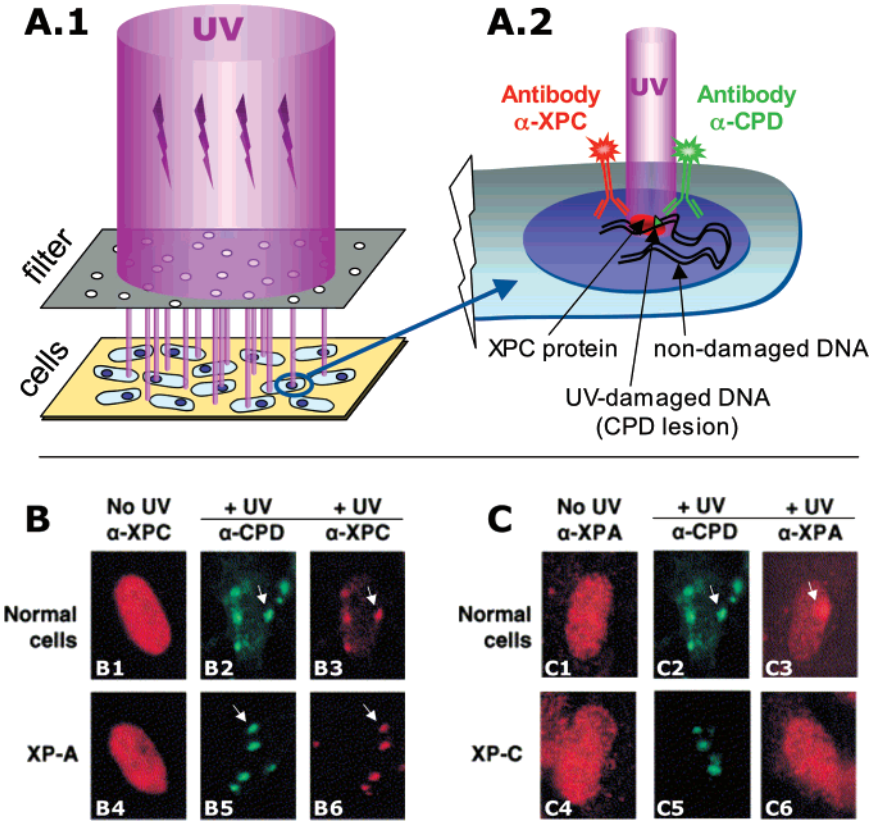

Figure 8. Colocalization of NER proteins with the sites of UV damage. The nuclei of the cells are locally UV irradiated through micropored filters (panel A1) and immunostained with antibodies (α) raised against CPD lesions within DNA (green) and XPC (panels A2 and B, red) or XPA proteins (panels A2 and C, red). The white arrows indicate, when detected, the colocalization between the UV lesion and the proteins. Note that XPC is recruited at the site of the CPD lesions in normal cells (panels B2 and B3) and in XP-A cells (panels B5 and B6). By contrast, XPA is recruited at the site of the CPD lesions in normal cells (pictures C2 and C3) but not in XP-C cells (panels C5 and C6). Reprinted and adapted with permission from ref 134. Copyright 2001 Cell Press.

the damage is recognized by a stalled RNA polymerase. XPA-deficient cells on the other hand are deficient in GG-NER and TC-NER, indicating a role for this factor in a later step of the repair process. Once again, recent in vivo experiments could finally resolve this controversy. The breakthrough came from a technique that allowed the generation of local UV damage at well-defined positions in the nuclei of living cells by irradiating the cells with UV light through the pores of a polycarbonate filter (see Figure 8A).[134−136] The UV lesions inflicted at those specific sites can be detected by antibodies specific against CPDs and 6−4PPs. The subsequent recruitment of NER factors to these sites can similarly be visualized in fixed cells by staining with an appropriate antibody. Using this approach, it was demonstrated that XPC colocalized with the site of UV lesions in XP-A cells, while XPA could not be recruited in XP-C cells.[134] In other words: XPA requires the presence of XPC to be recruited to the sites of the DNA damage and XPC must thus arrive at the lesion before XPA. Subsequent biochemical studies also strongly supported the XPC-first model as XPC was shown to be required for the opening of the DNA around the lesion and for the recruitment of all the other NER factors in a reconstituted system.[137,138]

The XPC protein is found in a tight complex (Table 2) with centrin2[139] and one of the two human homologues of *S. cerevisiae Rad23*, hHR23B or hHR23A.[42] Although HR23A and HR23B seem to be fully redundant for NER,[140] virtually all XPC is complexed with HR23B.[139,141] The binding domains of centrin2 and HR23B have been mapped to the carboxy-terminal half of XPC, with a partial overlap between the HR23B interacting domain and the DNA-binding domain of XPC.[142,143] Although NER is functional in the presence of XPC alone, addition of centrin2[143] and HR23B[140,144,145] substantially stimulates the reaction, and only 56 amino acids of HR23B are necessary and sufficient for this stimulation.[146] Interestingly, the presence of HR23B was also found to significantly enhance XPA/RPA-mediated displacement of XPC from damaged DNA,[147] suggesting a role for HR23B in later steps of NER. An additional role for HR23B was recently pinpointed by in vivo experiments in knockout mice: HR23B stabilizes the heterotrimer by inhibiting polyubiquitylation of XPC,[140] thereby preventing its degradation by the 26S proteosome.[148] Since XPC expression is also upregulated by p53 induced transcription in response to DNA damage,[149−151] it seems that the levels of XPC-HR23B are tightly regulated, probably to overcome potential toxic effects caused by overexpression of this factor.[140]

The binding properties of XPC-HR23B have been the subject of extensive studies: the complex binds efficiently ssDNA[152] and helix distorting lesions.[132,152] It is noteworthy that XPC-HR23B binds helix distortions (3−5 mispaired nucleotide bubbles), irrespective of whether they contain a lesion,[81] although in the absence of a true chemical modification these helix distortions are not repaired by NER.[81,107] This would suggest that recognition of distorted DNA structures by XPC-HR23B is necessary but not sufficient to trigger the repair reaction and that a subsequent damage verification step is required to validate the distortion detected by XPC-HR23B as a legitimate NER substrate.[107] Conversely,

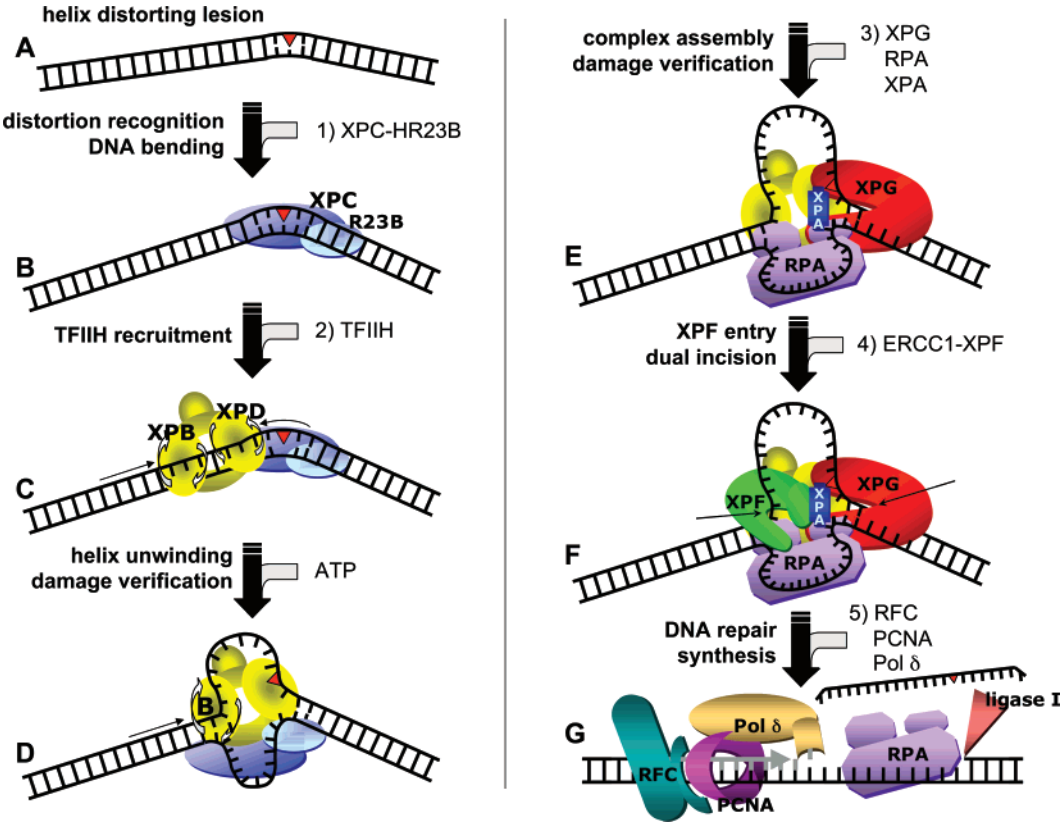

Figure 9. Model of the NER mechanism. (A) A lesion induces DNA helix distortion; (B) XPC-HR23B detects the helix distortion and stabilizes the DNA bend; (C) XPC-HR23B recruits TFIIH at the site of the lesion; (D) upon ATP addition, TFIIH unwinds the DNA helix, until one of its helicase subunit (here XPD) encounters a chemically modified base; the second helicase subunit (here XPB) goes on unwinding the DNA to create a 20-bp opened "bubble" structure; (E) RPA, XPA, and XPG are then recruited to assemble the "preincision" complex; (F) ERCC1-XPF joins the complex and the dual incision (5′ by ERCC1-XPF and 3′ by XPG) occurs; (G) RPA remains bound to the ssDNA and facilitates the transition to repair synthesis by Pol δ (or ϵ) supported by RFC and PCNA; ligase I finally seals the nick.

XPC-HR23B recognizes CPD lesions with hardly any specificity,[80−82] although the CPDs are still (though poorly) repaired by NER. This would suggest the existence of (at least) one additional recognition factor responsible for the recognition of these lesions. Recent in vivo studies have indeed proposed DDB to play an important role for the recruitment of XPC-HR23B at the sites of CPDs.[153,154] Present evidences suggest a role for DDB in making lesions accessible in the context of chromatin; this issue will be discussed in more detail in section 5.2. Finally, XPC-HR23B was found to bind symmetrically to a bubble mismatched oligonucleotide but asymmetrically to a bulged DNA substrate.[81,152] These results suggest that XPC-HR23B might bind in a very specific manner at the site of the DNA damage (Figure 9B), producing a well-defined DNA conformation[155] to direct the productive recruitment of the next NER factor: TFIIH.

4.3. Lesion Demarcation and Verification: TFIIH

Once XPC-HR23B has detected the lesion and has initiated local unwinding of the DNA,[138] it recruits the TFIIH complex by protein−protein interactions to the site of the DNA damage (Figure 9C).[57,134,141,142,156]

TFIIH is a ten-subunit complex (Table 2), arranged in a ring-like structure,[157] which is composed of a core complex (XPB, XPD, p62, p44, p34, p52, p8) and of a cdk activating kinase (CAK) subunit (Mat1, Cdk7, CyclinH). TFIIH is normally involved in RNA polymerase II[158] (and I[159]) transcription but can, upon DNA damage, be recruited within minutes to perform NER reactions.[123] The CAK subunit is required for optimal transcriptional activity but was shown to be dispensable and even inhibitory to NER activity.[69,160−162] More interesting with respect to NER are the two ATP-dependent helicases XPB and XPD, which are responsible for 3′ → 5′ and 5′ → 3′ opening of the DNA around the lesion, respectively (Figure 2, box 2). In transcription, the helicase activity of TFIIH seems to be intrinsically limited to the opening of a 10−20 bp region around the promoter, to allow the priming of the nascent RNA on the template strand.[163] The limited size of this opened intermediate is strikingly reminiscent of the size of the lesion demarcation performed during NER.[133,164] As for Pol II transcription, addition of ATP is necessary during NER for the opening of the DNA double helix by the two helicases XPB and XPD.[137,138,164] Note that, while the activities of both XPB[165] and XPD[166] are required for NER, the XPD helicase activity is dispensable for in vitro transcription.[167] The XPD subunit plays an additional architectural role within the complex by connecting the core TFIIH with the CAK subunit,[157] maybe thereby participating in regulation of the cell cycle.[168]

Importantly, two distinct steps can be identified upon assembly of TFIIH to the damaged DNA. First, TFIIH is recruited to the site of the damage by protein−protein interactions with XPC-HR23B, in an ATP-independent manner.[137,138,142,162] At this stage (Figure 9C), assembly of TFIIH does not greatly modify the DNA opening initially stabilized by XPC-HR23B.[138] In a second ATP-dependent step, TFIIH extends the DNA opening to allow the entry of subsequent NER factors (Figure 9D). It is noteworthy that

this is the first catalytic and likely irreversible step of the core NER reaction, which yields a new structure poised to attract subsequent NER factors. This step has been referred to as "kinetic proofreading", a process invoked in transferring an intermediate of moderate specificity to one of higher specificity.[82,169]

The role of the two helicases may extend even further. An additional role of damage verification or "enzymatic proofreading" for the TFIIH helicases has been proposed.[170,171] According to this model, one of the two helicases would get stalled at the site of the damage when it encounters a chemical modification of the DNA. It was indeed shown that the helicase activity of the XPD homologue in *S. cerevisiae* (Rad3) is inhibited by bulky DNA lesions,[172] and a recent experiment could detect the cross-linking of XPD at the site of a photoactive psoralen lesion.[173] Such a "proofreading" model remarkably corroborates the bipartite damage recognition process proposed by Naegeli and coworkers:[107] the "base-pairing disruption" is sensed by XPC-HR23B and the presence of a "chemical modification" of the DNA immediately confirmed by blockage of a TFIIH helicase at the lesion. This model has also the attractive particularity of performing damage verification at an early step in the process, thereby allowing for the rapid disengagement of the XPC-HR23B and TFIIH factors if a real damage is not actually present, before further assembly of the NER preincision complex occurs.

4.4. Assembly of the Preincision Complex: RPA, XPA, XPG

After preliminary DNA opening by TFIIH, three proteins join the complex: RPA, XPA, and XPG.[121,134,137] This "preincision complex" has been observed in vitro and can be considered as a quasi stable state for the NER reaction[121,161] where all factors, except ERCC1-XPF, are present and stably bound to an open bubble DNA structure (Figure 9E). Note that XPC-HR23B is actually thought to leave this preincision complex upon arrival of XPG.[121,137] It is unclear whether there is an established order of assembly of these proteins. It seems rather that these three factors can join the complex independently of each other: RPA is recruited to the sites of UV lesions early on, without requiring the presence of XPA or XPG.[124] Both XPA and RPA can colocalize with the lesions in absence of XPG,[124] and conversely XPG can also join the damage sites in the absence of XPA.[134] These observations suggest that protein/protein interactions, likely with the central TFIIH complex, play a greater role than detection of secondary DNA structures to attract the NER factors.[162]

RPA is a trimeric protein[174] (Table 2) that binds specifically to ssDNA using four so-called OB-fold motifs.[175] RPA has essential roles in recombination and particularly replication and is thus strictly speaking not an NER protein.[176] RPA was found to be required both for the dual incision[68,177,178] and for the repair synthesis steps of NER.[66,179] RPA displays two intrinsic binding modes by occluding either 8−10 or ∼30-nucleotide-long ssDNA.[180−183] Interestingly, this latter mode is reminiscent of the size of the fully open bubble DNA structure formed during NER.[120,137,164] Consistent with its well-defined DNA binding polarity,[184] it was postulated that RPA binds the nondamaged strand of the opened DNA bubble (Figure 9E), thereby allowing accurate positioning and stimulation of the endonuclease activities of XPG and ERCC1-XPF.[185] RPA has also been proposed as a damage recognition factor,[49,51,173,186] but it is likely that RPA may not recognize the lesion per se but rather locally unwound ssDNA regions induced by the presence of a helix distorting lesion.[51,187] This view is indeed supported by recent contradictory studies reporting the strand-specific photo-cross-linking of RPA to damaged oligonucleotides.[131,188] Its specificity for damaged DNA seems nevertheless to be enhanced by addition of XPA,[49,130,189] and XPA and RPA may work in a cooperative fashion to achieve strand-specific positioning of RPA to the nondamaged strand of the DNA.[188]

XPA is a small protein (Table 2) with a zinc-finger domain[35] that was initially suggested as the DNA binding domain and that was recently also shown to be the RPA interaction domain.[190] XPA was originally thought to be the initial damage recognition factor,[129,191,192] perhaps working in concert with RPA.[49,50,130,132] Now that XPC-HR23B is widely acknowledged to fulfill this role, the actual function of XPA has been readdressed. One study demonstrated that XPA has a much higher affinity for binding unusual kinked DNA structures, such as three-way junctions, than DNA lesions themselves.[193] This suggested that XPA, perhaps together with RPA, may control the proper assembly of the NER preincision complex by probing for appropriately distorted DNA and thereby confirming the existence of the lesion indirectly. The structure of the DNA intermediate that interacts with XPA however remains to be determined. Apart from its interaction with DNA, XPA interacts with a striking number of proteins (RPA, ERCC1-XPF, TFIIH, XPC-HR23B, see Table 2), considering the relatively small size of the protein. Much remains to be done to elucidate the exact roles of XPA in NER, but the protein appears to have a key role in probing the accurate assembly of the NER preincision complex.

XPG is believed to be recruited in the preincision complex (Figure 9E) through its interactions with TFIIH (Table 2).[162] XPG belongs to the FEN-1 (flap endonuclease) family of structure-specific endonucleases.[194] It is able to incise flap or bubble DNA structures with a defined polarity,[59,164,195] cleaving specifically at the junction between the 3′ end of ssDNA and the 5′ end of dsDNA (Figure 2, box 4), consistent with the 3′ incision during the NER reaction.[59] Its active site has been mapped to two conserved domains (an N-terminal domain of ∼100 amino acids and an internal I-region of ∼140 amino acids) that are believed to fold into a globular active site by analogy with known structures of the Fen1 protein.[196,197] The N and I nuclease domains are separated by a "spacer region" of 600 amino acids that is unique within the FEN-1 family.[39] The spacer region has been shown to be important for the interaction with TFIIH and additionally contributes to the substrate specificity of XPG, enabling the cleavage of bubble DNA structures that resemble NER intermediates and that are not processed by FEN-1.[198−200] An interaction between XPG and RPA has also been mapped to the spacer region, but whether a direct interaction between XPG and RPA contributes to the positioning of the XPG protein in the NER incision step is unclear.[49,185,201] XPG is not only involved in performing the 3′ incision in NER; it also plays a structural role in stabilizing the preincision complex. The presence of XPG, but not its catalytic activity, was shown to be required for stabilizing the fully open DNA bubble structure and to permit the 5′ incision by ERCC1-XPF.[202,203] Consistent with a structural role of XPG in NER, the protein has a distinct requirement for binding and cleaving substrates, suggesting that it only becomes catalyti-

cally active once it has reached an appropriate conformation in the preincision complex.[195]

4.5. Dual Incision: ERCC1-XPF, XPG

It has been shown both in vitro[121,137] and *in vivo*[134] that ERCC1-XPF is the last factor joining the complex prior to dual incision (Figure 9F). An interaction between XPA and ERCC1 is essential for NER and it appears that ERCC1-XPF is directly recruited to the NER preincision complex by XPA.[50,204−207]

ERCC1-XPF is an obligate heterodimer (Table 2) and the two subunits are unstable without each other.[208,209] The interaction relies on two helix-hairpin-helix motifs at the very C-terminal end of the two subunits.[210] ERCC1-XPF is a structure specific endonuclease incising DNA with specific polarity at the 5′ side of double-strand/single-strand DNA junctions (Figure 2, box 3), in agreement with its 5′ incision activity during NER.[40,211] It was shown that binding of RPA to ssDNA could stimulate ERCC1-XPF nuclease activity on model substrates when the factors were positioned with a polarity reflective of the one found in NER.[185,201]

The active site of ERCC1-XPF resides in the C-terminal half of XPF and consists of a conserved IERKX_3D motif that is a signature of the XPF family of nucleases which includes also the Mus81 protein and a family of archeal helicases/nucleases.[212,213] Less is known about how ERCC1-XPF interacts with its DNA substrates, but recent structural studies suggest that at least three domains contribute to DNA binding: The helix-hairpin-helix domain that also contributes to the dimer interface, a central domain in ERCC1 and a helicase-like domain in XPF.[214−217]

While the addition of ERCC1-XPF does not appear to induce any particular change in the opened DNA structure,[133,138] the catalytic activity of ERCC1-XPF triggers the transition from the excision to the repair synthesis step. Several studies have addressed the question of whether there is a defined temporal order in which the two incisions to take place. Uncoupled incision 3′ to the lesion by XPG has been observed in the absence of ERCC1-XPF,[40,133,161] while the presence of XPG, but not its catalytic activity, is required for the 5′ incision by ERCC1-XPF.[202,203] In the presence of both proteins, both 5′ and 3′ uncoupled incisions have been observed, suggesting that the two incisions occur in a nearly simultaneous manner.[161,211,218]

The transition from the dual incision to the repair synthesis step should be a tightly coordinated process to avoid the generation of potentially mutagenic and recombinogenic ssDNA intermediates, yet little is known about how this is achieved. This may be accomplished in a number of ways and just two scenarios, which are not mutually exclusive, will be discussed here. The first possibility is that one (or more) factor remains bound to the DNA following dual incision. RPA is an obvious candidate for this scenario, as it is required both for the dual incision and the repair synthesis steps and since it remains stably associated with the gapped DNA formed by dual incision in vitro.[137] A second way to avoid the formation of ssDNA intermediates is through a temporal order of the two incision reactions. If incision by ERCC1-XPF occurred first, the free 3-OH generated could be used to initiate repair synthesis before XPG performs the incision 3′ to the lesion. In this way, repair synthesis could already be well underway before XPG incision, thereby minimizing the time in which ssDNA intermediates exist. Two observations are in line with this hypothesis; first, one study analyzed incision patterns in reconstituted NER reactions using wild-type and catalytically inactive ERCC1-XPF protein and found that efficient 3′ incision occurred only in the presence of catalytically active XPF.[138] Second, because XPG has distinct requirements for binding and cleaving DNA,[195] the incision by ERCC1-XPF in the preincision complex might well bring the conformational change needed to trigger efficient incision activity of XPG.[138]

4.6. Repair Synthesis: RPA, RFC, PCNA, Pol δ/ϵ

Because the repair synthesis and ligation steps in NER are accomplished by the same proteins that are also involved in replication, the mechanistic basis of these steps has been investigated in the context of replication rather than NER. Simultaneously to the in vitro reconstitution of NER excision reaction, it was observed that repair synthesis required the polymerase processivity factor PCNA,[65,219,220] the PCNA-dependent DNA polymerase δ[221−227] or ϵ,[223,228] while RPA was found to be required for both the incision and the repair synthesis steps.[49,66] RPA, PCNA, the pentameric clamp loader RFC, DNA ligase I together with either Pol δ or Pol ϵ were shown to be necessary and sufficient to reconstitute completely the repair synthesis in vitro.[69,228] The detailed mechanism underlying DNA synthesis has been reviewed extensively elsewhere[229−232] and will just be briefly mentioned here. RFC catalyzes the ATP-dependent loading of PCNA to DNA near the 3′ termini of primers. PCNA is a homotrimeric protein (Table 2) that constitutes a ring-shaped clamp,[233] which can slide along the DNA and interacts with the DNA polymerases to ensure that replication occurs processively. Findings that RPA remains bound to the ssDNA intermediates following dual incision,[137] and that it is involved in the recruitment of PCNA[137,234] and RFC[225] again point to a central role for RPA in coordinating the dual incision and the repair synthesis processes. An additional component coordinating the incision and repair synthesis steps may be an interaction between XPG and PCNA.[235,236] XPG contains the classical PCNA interaction motif found in a number of proteins involved in nucleic acid metabolism. Although XPG and PCNA have been shown to interact, conclusive evidence that this interaction is important for NER is presently lacking. Nevertheless, the observation that XPG may dissociate very late from the excised DNA[137] suggests that XPG remains bound to the DNA after excision and could facilitate loading of PCNA to sites of repair synthesis until the DNA polymerase (and thus PCNA) reaches the end of the gap and finally dislodges XPG. Future investigations are required to fully understand this coordination of excision and repair synthesis steps during NER.

4.7. Summing Up: A Model for the Progression through the NER Reaction Pathway

As outlined above, NER accomplishes the removal of damaged sites from DNA through the sequential action of the six main factors involved in dual incision, XPC-HR23B, TFIIH, XPA, RPA, XPG, and ERCC1-XPF, and is completed by repair synthesis and ligation. The sequential assembly model implies the existence of a concerted mechanism to ensure smooth progression through the complete pathway and to avoid side reactions such as, for example, erroneous incisions. In this section, we will briefly summarize the NER pathway with special emphasis on steps that are important for the progression through the pathway.

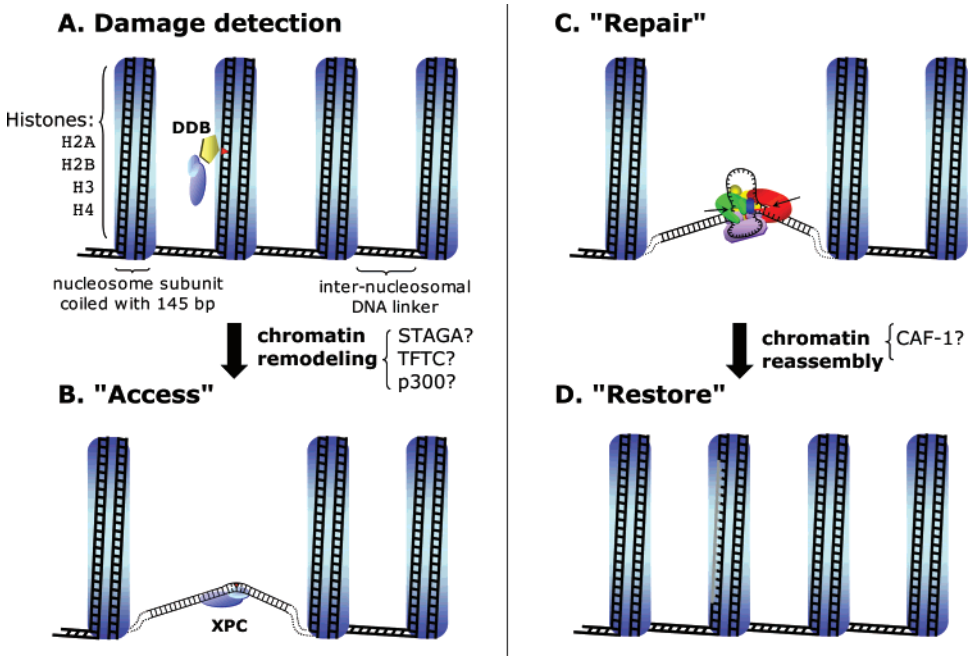

Figure 10. Model for NER in the context of chromatin. (A) The lesion is detected by the combined action of DDB, XPC-HR23B, and maybe some other unknown factors; DDB recruits and activates the factors necessary for the chromatin remodeling (Figure 11); in parallel, histone modifying enzymes (e.g., histone acetyl transferase p300[260]) or other ATP-dependent chromatin remodeling factors (e.g., ACF[250] or SWI/SNF[262]) help to unfold/disassemble one nucleosome. (B) After nucleosome disruption or unfolding, 147 bp of DNA are accessible to perform a standard damage recognition and (C) NER reaction; (D) upon nucleosome reassembly involving CAF-1,[290] the patched DNA is reintegrated in the chromatin structure.

XPC/HR23B recognizes DNA lesions with moderate affinity and specificity in a reversible binding reaction. Following recruitment of TFIIH, which is at least in part mediated by direct interaction with XPC-HR23B, the ATP-dependent opening of the DNA helix around the lesion represents the first step that involves an irreversible chemical reaction, ATP hydrolysis. If one of the helicase subunits of TFIIH, likely XPD, encounters a chemical modification in the DNA, it stalls and, by virtue of the geometrical constraints of the protein/DNA complex, TFIIH becomes immobilized at the site of the lesion. This ternary complex provides a signal for the further assembly of the NER preincision factors. If TFIIH does not encounter a lesion, it is not stalled, no stable intermediate is formed, and NER is abolished. This process involving a first recognition step of moderate affinity and subsequent energy-dependent step verifying and amplifying the recognition step is sometimes referred to as kinetic proofreading.[169,237] The XPC-HR23B/TFIIH/DNA intermediate forms a platform for the recruitment of the XPA, RPA, and XPG proteins, which join the complex independently of one another, again through specific interactions between proteins and proteins/DNA intermediates. The nature of these interactions is crucial; multiple relatively weak and transient interactions rather than strong and irreversible interactions are employed to ensure smooth transition between reaction intermediates.[238] Such interactions make it possible that, upon recruitment of XPA/RPA/XPG, XPC-HR23B is expelled from the complex and a relatively stable preincision complex is formed.[121,137] XPG displays no significant incision activity in this complex, reflecting its distinct requirements for substrate binding and catalysis.[195] Upon binding of ERCC1-XPF, no dramatic further conformational change is induced in the preincision complex,[138] but further progression through the pathway requires the catalytic activity of ERCC1-XPF. We propose that the 5′ incision by ERCC1-XPF occurs first. This will generate a free 3′ hydroxyl end on the DNA, which may be used to initiate repair synthesis and induce a conformational change in the multiprotein/DNA complex that will now allow XPG to exert its catalytic activity. This ensures smooth transition to the repair synthesis stage of NER and error-free completion of the pathway. It is important to note that prior to incision of the DNA, the reaction may be aborted at any stage reflecting the many safeguards incorporated to ensure that nondamaged DNA, or the nondamaged strand opposite a lesion, are not erroneously incised. Although posttranslational modifications of proteins in the NER core reaction have been found,[140,239,240] it is not yet clear to what extent these modifications contribute to the progression through the core NER reaction.

5. Nucleotide Excision Repair in Vivo

So far, our discussion has focused on the core NER reaction, the comings and goings of the various factors on naked DNA. This process is of course much more complex in the nuclei of living cells where the DNA is organized in chromatin structures, themselves packed in highly condensed chromosomes. It is only at this price that more than 2 m of genetic code can be accommodated within the nucleus of each human cell. The minimal structural motif of the chromatin is the nucleosome core (Figure 10A), which is composed of an octamer of H2A, H2B, H3, and H4 histone proteins (two of each) coiled with two turns (147 bp) of DNA double helix.[241−243] Furthermore, in most eukaryotes with the notable exception of yeast, the linker DNA (0−60 bp) connecting two nucleosomes is associated with the additional histone H1, which stabilizes nucleosome structures and further compacts the chromatin fibers.

5.1. Chromatin Remodeling or the Need to Access DNA

Before any processive DNA transaction (replication, transcription, or repair) can occur, the DNA has to be

dislodged from the nucleosome structures. NER does not escape this rule, and the pioneering work of Smerdon and co-workers has shown early on that specific nucleosome rearrangements occur upon excision repair of UV-damaged DNA in human chromatin.[244] It was proposed that NER operates within chromatin through an "access−repair−restore" mechanism,[245,246] where the repair proteins gain access to "linearized" naked DNA strands temporarily after displacement or disruption ("remodeling") of at least one nucleosome subunit (Figure 10). Interestingly, the minimal length required to perform a NER reaction of about ~100 bp[63] is contained within the 147 bp of the DNA coiled around one core nucleosome. In parallel, in vitro NER assays using cell extracts and purified proteins also confirmed that the repair rate of damaged DNA in nucleosomes is reduced compared to that of naked damaged DNA,[70,247−250] suggesting that chromatin remodeling is needed for efficient NER.

Chromatin remodeling, concomitantly with the intrinsic dynamic nature of the nucleosomal structures, occurs actively through two major mechanisms:[243,246] (i) posttranslational modification of histones tails or (ii) ATP-dependent chromatin remodeling. The tails of histones can undergo a large variety of modifications, among which histone acetylation is accepted to confer enhanced DNA accessibility. Increased histone acetylation has been observed following UV irradiation[251] and stabilization of hyperacetylated histones by inhibition of histone deacetylases (HDACs) has been shown to enhance the repair rate of UV lesions.[252−254] More recently, several histone acetyltransferases (HAT) have been suggested as potential candidates of accessibility factors during NER (Figure 10B): STAGA[255,256] (SPT3, TAF_{II}31-GCN5L acetylase), TFTC[257] (TBP-free TAF_{II} complex) or p300[258−260] (see below). Although these observations are not yet supported by in vitro studies,[261] the general increase of histone acetylation observed in response to UV irradiation may well be a part of a global cellular response to DNA damage that facilitates access of the NER machinery to its substrates.

Concerning the second mechanism of chromatin accessibility, in vitro experiments suggested a role for several ATP-dependent remodeling complexes in NER. A recombinant form of ACF (ATP-utilizing chromatin assembly and remodeling factor) has been shown to facilitate excision of 6−4PP in vitro from the linker DNA region between two nucleosomes,[250] suggesting that ACF may assist histone octamer sliding and enhance accessibility to the lesions located within the linker DNA regions. In parallel, yeast SWI/SNF factor has been shown to enhance the human NER repair rates of single 6−4PP or dG-AAF (but not CPD) lesions located within nucleosome cores.[262,263] Presently, there is no genetic or cell biological evidence supporting the specific involvement for any of these factors in NER and further studies are needed to confirm the role of these specific chromatin remodeling factors during NER.

5.2. Damage Recognition in the Chromatin Context and Possible Roles of DDB

Because it seems that histone modifying enzymes or ATP-dependent chromatin remodeling factors do not have any specificity for lesions in nucleosomal DNA, there may be an NER-specific process to mediate localized access to the lesions in chromatin. Indeed how DNA lesions are recognized in the context of chromatin remains an intriguing question. XPA, RPA and XPC-HR23B display a considerably lower ability to bind nucleosomal DNA lesions.[249,264] It is interesting to note that XP-C cells do not show any defect in chromatin remodeling upon UV irradiation but rather present an even faster chromatin reassembly kinetic,[265,266] which would argue a priori against a direct role for XPC in chromatin relaxation. It has been suggested that RNA polymerase blockage at the site of a lesion could act in the absence of XPC to initiate a global chromatin remodeling in living cells.[260] This leads to the paradox of damage recognition in chromatin context: the chromatin has to be remodeled to support efficient damage recognition, but the DNA damage has somehow to be detected to trigger this chromatin remodeling. In search for a damage-sensing factor upstream of XPC-HR23B that could also act in chromatin context, several research groups have started to consider the DDB protein as such a potential candidate.

DDB is a UV-damaged DNA binding factor[46,267] composed of two subunits (DDB1/*p127* and DDB2/*p48*, Table 2). Incorrect expression of the *p48* gene[268−271] results in the very mild XP-E phenotype and in a specific deficiency in global genome repair of CPDs.[272] Interestingly, hamster cells also express DDB1 but not DDB2[269,273] and display a similar deficiency in repairing CPDs.[274,275] DDB is dispensable for the in vitro NER reaction on naked DNA,[68,69,218] and yet it is the NER factor displaying the highest affinity and selectivity for binding damaged DNA.[53,54,82,125,127,276] The requirement for DDB for the efficient repair of the nondistorting CPD lesions in vivo[153,154,267,272−274] suggests a role for DDB in the repair of certain lesions that are not well recognized by XPC-HR23B and perhaps a role in regulating access to chromatin. Similar to the mechanistic study of GG-NER described in section 4 (Figure 8), local UV irradiation techniques have provided some insights into where DDB fits in the NER pathway. DDB was found to be recruited to the sites of DNA damage before all the other NER factors, in particularly prior to XPC-HR23B[153] and to enhance the binding of XPC to CPD lesions.[151,154] One possible role for DDB could therefore be to recognize UV lesions in the context of chromatin and to induce a conformation in the DNA that allows the XPC-mediated assembly of the NER machinery.[125,276,277] A possible role for DDB in chromatin context was further strengthened by the observation that UV irradiation induces a tight association of DDB with chromatin in human cells.[153,259,278−280] Recent studies have started to uncover unexpected complexities surrounding the role of DDB in NER. DDB was found to associate with a number of proteins involved in chromatin remodeling and ubiquitin ligation.[281,282] Using a mild immunopurification protocol, Groisman et al. could isolate DDB as a part of a large complex with Cul4A, Roc1, and COP signalosome (CSN),[280] which are components of a ubiquitin ligase (Figure 11). This complex is recruited to chromatin upon UV irradiation where it displays ubiquitin ligase activity.[280] Recently, XPC has been identified as the main target of this ubiquitin ligase activity, although DDB2 itself and Cul4A were also subject to ubiquitylation.[277] Poly-ubiquitylation is usually a signal to target a protein for proteosomal degradation, and indeed ubiquitylated DDB2 had already been observed in other studies to be degraded rapidly after UV irradiation.[259] By contrast, ubiquitylation of XPC seems to be a reversible process and does not serve as a signal for degradation.[277] Moreover, poly-ubiquitylation of XPC seems to stimulate the binding (but not the specificity) of XPC-HR23B, possibly potentiating the displacement of DDB from the UV damaged DNA and initiating the NER reaction.[277] Finally, DDB1 and

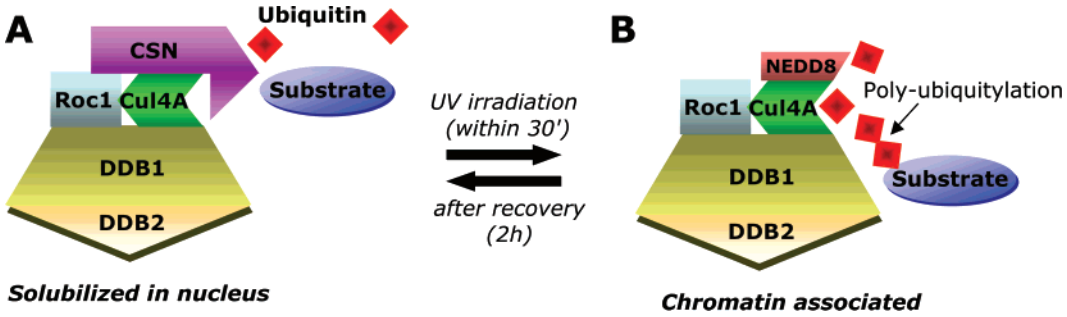

Figure 11. DDB1 complexes. (A) DDB1 is associated with Roc1, Cul4A, and the COP9 signalosome (CSN) as a free complex in the nucleus. (B) Upon UV irradiation, the complex associates with the chromatin. CSN dissociation allows conjugation of NEDD8 (nedylation), which stimulates Cul4A ubiquitin ligase activity.[280] (A) After 2 h, CSN reintegrates the complex from which it deconjugates NEDD8. In the absence of NEDD8, the Cul4A activity is inhibited and CSN intrinsically displays de-ubiquitylation activity. Note that the major targets for this ubiquitylation reaction may be Cul4A itself,[280] DDB2, or XPC.[277]

homologues have also been found in several other complexes involved in histone acetylation: p300,[258,259] STAGA[255] (SPT3, $TAF_{II}31$-GCN5L acetylase) or TFTC[257] (TBP-free TAF_{II} complex), suggesting an even broader role for DDB in triggering histone modification and chromatin remodeling to facilitate the NER reactions in vivo. Whether the UV damage binding activity of DDB allow these different complexes to be recruited to the sites of the DNA lesions in vivo remains to be determined. It is likely however that DDB is the central player linking NER to chromatin and that its role goes far beyond that of an additional damage recognition factor as was initially thought.

5.3. Repair Synthesis and Chromatin Reassembly

Once the NER dual incision and repair synthesis have taken place, the remodeled nucleosomes have to be repositioned on the newly repaired DNA (Figure 10D) to restore the chromatin structure to its original state.[246] The process of chromatin reassembly following NER has been intensively investigated by Smerdon and co-workers and seems to occur in biphasic manner (reviewed in ref 266); the newly repaired DNA patches (initially present in an exposed, nuclease-sensitive form) become rapidly protected (within 20 min) from nuclease digestion upon reassociation with core histones in intact human cells. This rapid phase is followed by a prolonged period (1−24 h) of remaining nuclease sensitivity, likely to reflect the slow nucleosome repositioning onto the newly repaired DNA.[266] Considering in detail the chronological order of events, repair synthesis and patch ligation were shown to precede complete nucleosome formation,[283,284] although it was observed that human DNA ligase I could also seal remaining nicks in nucleosomes.[285] Also, a clear bias for preferential nucleosome assembly at the site of damaged naked DNA has been observed after repair even in the presence of a large excess of undamaged DNA.[286] These results clearly suggest that NER repair synthesis and chromatin reassembly on the newly repaired DNA patches occur in a highly coordinated manner. A concrete link between the two processes was indeed discovered by Almouzni and co-workers in the histone chaperone CAF-1 (chromatin assembly factor 1), which was found to be required for the reassembly of chromatin following NER.[287−289] CAF-1 has been shown to colocalize with sites of UV damages in living cells, but only in cells that are proficient in the NER dual incision reaction.[290] These cell biological observations provide further evidence that CAF-1 is involved in a late stage of NER (Figure 10D). Interestingly, CAF-1 recruitment has been shown to depend on PCNA,[290−292] strengthening the hypothesis of a tight coordination between the repair synthesis and chromatin reassembly steps.

Although much remains to be learned to understand NER in chromatin, the implication of factors such as DDB and CAF-1 in influencing the chromatin state specifically in the context of NER is a starting point for further studies of repair of lesions in their proper cellular context.

6. Concluding Remarks

More than a century of research surrounding XP has served as a paradigm of how studies of rare genetic diseases can lead to unexpectedly broad and general insights into cellular metabolism. In the past decades, XP was linked to NER, the genes involved in NER were identified, and the complete reaction was reconstituted in vitro. Biochemical studies have led to a remarkably detailed understanding of this complex repair system, and NER now serves as a prime example of how over 30 proteins can cooperate in a common pathway. Very recently, advances in fluorescence microscopy have made it possible to directly observe many parameters of the NER reaction in real time in living cells. We expect that the combination of in vitro and in vivo studies will be particularly fruitful and lead to a very detailed understanding of the NER mechanism. NER should therefore emerge as a model of how complex mammalian pathways can be investigated.

In this review, we have focused less on another area that has immensely benefitted from research on NER, namely, the study of carcinogenesis in mammals. It is now known how important NER is for the prevention of skin cancer by repairing DNA lesions caused by UV light.[5,293] In the past decade, mouse genetics have been extensively used to study the physiological consequences of NER deficiency. These knock-out mice constitute invaluable models to study mutagenesis and carcinogenesis at the level of a whole animal and have furthermore uncovered unexpected links to premature aging.[294−296]

The cancer-prone phenotype of XP patients raises the question of whether variation in NER capacity in the general population is associated with an increased risk in the occurrence of sporadic cancers. With the availability of the sequence information of the human genome and the advent of research on single nucleotide polymorphisms (SNPs), this notion can now be investigated. Polymorphisms have been found in a number of NER genes, including ERCC1,[297,298] XPD,[299] XPC,[300] HR23B,[301] XPF,[301,302] XPG,[303] and XPA.[304] Although it remains to be conclusively established that these

polymorphisms influence NER capacity,[305−307] SNPs of NER genes may be used in the future to predict cancer predisposition in the general population.[308−312] Since NER has also been found to counteract the effects of DNA damaging antitumor agents, SNPs may also be used to predict the efficiency of a treatment modality for tumors in individual patients.[313,314] Despite encouraging initial observations, clearly more research is needed to validate the predictive value of SNPs of NER genes and to translate this knowledge to clinical use. In particular, the exact effect of the individual SNPs on NER capacity remains to be established.

We expect that research on NER will continue to thrive on many levels from single molecules to whole animals. As the process will be elucidated in more detail, structural and chemical approaches will increasingly contribute to our understanding of this fascinating repair pathway.

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