Nucleotide excision repair : a multi-step mechanism required to maintain genome integrity

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NUCLEOTIDE EXCISION REPAIR

NER is activated by a wide-range of helix-distorting DNA lesions, including UV-induced photoproducts (CPDs and 6-4PPs) (see Fig.2), bulky chemical adducts and certain oxidative lesions. In human cells, the NER reaction requires around 30 core factors and comprises of the following steps: 1) DNA damage recognition, 2) DNA unwinding and lesion verification, 3) dual incision around the lesion, 4) excision of the single-stranded DNA fragment containing the DNA lesion and 5) repair synthesis and ligation of the nick. NER can be subdivided into 2 distinct subpathways, global genome repair (GGR) and transcription-coupled repair (TCR) which differ with respect to lesion detection and some of the factors involved. The GGR factors UV-DDB and XPC-HR23B are responsible for detecting lesions throughout the genome. Various TCR-specific factors such as CSA and CSB are involved in the repair of lesions in transcribed regions (Tornaletti and Hanawalt, 1999). Following recognition of the damaged DNA, common NER factors are recruited in both sub-pathways of NER to complete the repair process. A detailed description of damage recognition and the subsequent NER processes will be described later in this chapter.

THE CONSEQUENCES OF A DEFECT IN NER

Skin cancer is the most common form of cancer in the United States, more than 1 million skin cancers are diagnosed annually (National Cancer Institute, 2007 SEER Database) and 1 in 3 Caucasians are likely to develop skin cancer in the course of his or her lifetime (American Cancer Society 2007). NER represents the only repair pathway which removes mutagenic photolesions induced by sunlight. A defect in NER therefore leads to extreme sun sensitivity and an elevated risk of developing skin cancer. The average age of a patient with skin cancer is approximately 60 years yet the age of onset is reduced by approximately 50 years in individuals with a defective NER pathway. Defects in NER are associated with 3 major autosomal recessive disorders, namely xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD) (Fig. 3). At the clinical level XP

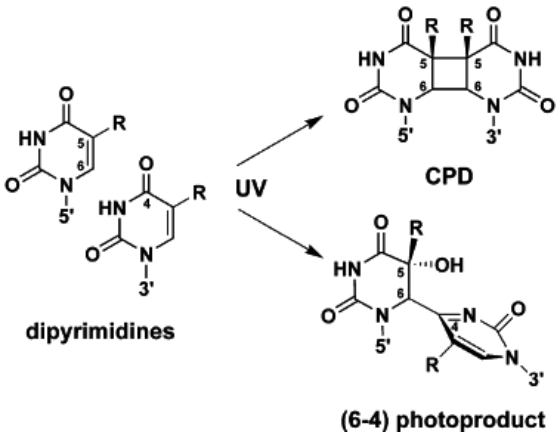

Fig. 2. UV-induced photoproducts. The UV component of sunlight can cause bulky lesions in DNA as a result of dimers forming between thymine bases. The two main types are cyclobutane pyrimidine dimers (CPD) and (6-4) photoproducts (Adpated from Li et al., 2006).

Fig 3. Diseases associated with mutations in NER genes.

Disease	Symptoms	Malignant (skin) lesions	Affected genes
Xeroderma Pigmentosum (XP)	Sunburn, hyper- and hypopigmentation, atrophy in sun-exposed regions, risk of internal cancers, neurological symptoms	Extremely high incidence of skin cancer. Basal cell carcinomas, melanomas (UV-induced skin tumours) in childhood	XPA XPB XPC XPD XPE XPF XPG XPV
Cockayne Syndrome (CS) (XP/CS combined effects of XP and CS)	Sunburn, growth failure, hyperpigmentation, physical and mental retardation, bird-like face, cachexia, neuronal degeneration, loss of retinal cells	No increased (skin) cancer risk	CSA CSB XPB XPD XPG
UV-sensitive syndrome ($UV^{S}S$)	Photosensitivity, mild skin abnormalities	No increased (skin) cancer risk	CSB CSA unknown genes
Cerebro-Oculo-Facio-Skeletal syndrome (COFS)	Symptoms similar to CS	No increased (skin) cancer risk	CSB XPD XPG ERCC1
Trichothiodystrophy (TTD)	Sunburn, erythema, ichthyosis, characteristic brittle nails and short, brittle sulfur-deficient hair, neurologic and skeletal degeneration, cachexia	No increased (skin) cancer risk	TTD-A XPB XPD

is characterized by a highly increased incidence of tumors in sun-exposed areas of the skin (reviewed in Cleaver et al., 2009). In contrast, CS and TTD are disorders without enhanced cancer susceptibility characterized by developmental and neurological abnormalities and premature aging. (reviewed in Lehmann, 2003). (Fig. 3)

Xeroderma Pigmentosum (XP)

NER defects cause mutagenic damage to persist across the entire genome giving rise to the cancer prone syndrome xeroderma pigmentosum (XP). Patients who suffer from XP exhibit severe sun-sensitivity, photophobia, freckling and a 1000-fold elevated sun-induced skin cancer risk and accelerated skin aging (Kraemer and Slor, 1985; Berneburg and Lehmann, 2001). Apart from UV-induced skin cancers, XP-patients have a 10-20 fold increased risk to develop internal cancers before the age of 20 years (Kraemer et al., 1984). An XP-phenotype can result from defects in any one of seven genes involved in the NER cascade (XP-A through to XP-G) (Keeney et al., 1994; Legerski and Peterson, 1992; Masutani et al., 1994; Flejter et al., 1992; Scherly et al., 1993; Sijbers et al., 1996; Weeda et al., 1990).

Cells from all these complementation groups are deficient in GGR and with the exception of XPC and XPE also in TCR (Venema et al., 1991; Hwang et al., 1999). Recent findings with XPE, XPC and XPG suggest that they may also be involved in other processes such as the repair of oxidative damage (Lee et al., 2006; D'Errico et al., 2006; Shimizu et al., 2003; Schärer, 2008). XP patients belonging to complementation groups XP-A, XP-B, XP-D and XP-G, exhibit progressive neurological abnormalities in addition to their XP symptoms. These neurological symptoms include reduced tendon reflexes, deafness, and speaking and walking disability because of primary neuronal degeneration (Mimaki et al., 1986; Robbins et al., 1991). The observation that individuals with XPC and XPE abnormalities are virtually free of neurological defects suggests that TCR of lesions in the transcribed strand of genes is important for the development of neurological symptoms and that functional TCR is sufficient to prevent these symptoms from occurring. Cells from XP-V (XP variant) individuals are NER proficient but lack Polymerase eta which is able to bypass CPDs in an error free manner. More error-prone DNA polymerases take over in XP-V cells, resulting in the accumulation of mutations during DNA replication (Kannouche and Stary, 2003). The first indications of XP are a strong increase in sunsensitivity freckling in sun-exposed areas, followed by other pigmentation changes, loss of elasticity and multiple skin cancers including basal and squamous cell carcinomas as well as malignant melanomas. However, if protected from sunlight at an early age, individuals can remain completely free of skin lesions which accordingly increase their lifespan.

Cockayne Syndrome (CS)

Cockayne syndrome (CS) is a rare, autosomal, recessive disease characterized by severe and diverse clinical symptoms such as mental abnormalities, brain degeneration and pigmentary degeneration of the retina followed by pronounced cachexia leading to early death (Nance and Berry, 1992). In CS patients, growth is retarded and a prominent feature is a bird-like face, characterized by sunken eyes, a beaked nose and a narrow mouth. Patients have reduced subcutaneous fat and skeletal abnormalities such as kyphosis (Cleaver et al., 2009). As seen for XP, CS patients are UV-sensitive and show atrophy of sun-exposed skin. Despite a DNA repair defect, CS is not associated with an increased skin cancer risk (Nance and Berry, 1992). Classic CS is caused by mutations in *CSA* or *CSB* (Cockayne syndrome complementation groups A and B respectively). CSA and CSB are essential for the function of the transcription machinery and defects in either CSA or CSB cause a disruption in the recovery of DNA damage inhibited transcription (Mayne and Lehmann, 1982; Venema et al., 1990). In addition, XP-B patients and certain patients belonging to XP-D or XP-G show features of CS in addition to symptoms of XP (XP-B/CS, XP-D/CS and XP-G/CS). XP-CS cells display both impaired GGR and TCR together with the inability to recover RNA synthesis following DNA damage (van Hoffen et al., 1999). Both CSA and CSB primary skin cells were found to be hypersensitive to the lethal effects of oxidizing agents suggesting that the CSA and CSB proteins have additional functions beyond their roles in TC-NER (D'Errico M, et al. 2007). The involvement of CS proteins in the removal of oxidative damage may explain the neurological and aging features typical of CS. This notion has been supported by several independent observations (reviewed in

Cleaver et al., 2009; Bohr et al., 2007; Kyng et al., 2005) as well as a recent study of eye pathology in CS mouse models, implicating accumulation of endogenous oxidative DNA lesions in the retina in pigmentary retinopathy, a feature of CS-specific premature aging (Gorgels et al., 2007). The mean age of death of CS patients is 12.5 years and the most common cause of death is pneumonia as a result of atrophy and cachexia.

UV-sensitive syndrome (UV^{S}S)

A few years ago a new photosensitive disorder was identified called UV-sensitive syndrome (UV^{S}S). UV^{S}S was found to be distinct from XP (including XP-V) and CS since patients show only mild clinical manifestations (Itoh et al., 1994; Itoh et al., 1995). Cells derived from individuals with UV^{s}S showed UV sensitivity and a failure of recovery of RNA synthesis after UV irradiation, in spite of having a normal level of unscheduled DNA synthesis. Two complementation groups have been identified among UV^{S}S patients, defined by mutations in an as-yet-unidentified gene in 4 cases and in the *CSB* gene in 2 individuals (reviewed by Spikak, 2005). Recently, a UV^{S}S patient with a novel mutation in the CSA gene has been identified which represents the third complementation group of UV^{S}S (Nardo et al., 2009). The expression of the mutant CSA cDNA was found to increase the resistance of cells from a CSA patient to oxidative stress, but did not correct their photosensitivity. These observations imply that some mutations in the *CSA* gene may interfere with the TC-NER-dependent removal of UV-induced damage without affecting its role in the oxidative stress response. The differential sensitivity toward oxidative stress might explain the difference between the range and severity of symptoms in CS and the mild manifestations in UV^{s}S patients that are limited to skin photosensitivity without precocious aging or neurodegeneration (Nardo et al., 2009).

Cerebro-Oculo-Facio-Skeletal Syndrome (COFS)

Similar to CS, Cerebro-Oculo-Facio-Skeletal syndrome (COFS) is a recessive disease with symptoms including growth retardation, severe mental retardation, facial features such as deep-set eyes, prominent nose and large ears, cataracts, joint contractures and microcephaly (Longman et al., 2004; Meira et al., 2000; Del Bigio et al., 1997). Since COFS shares several features also associated with CS it is difficult to distinguish between these syndromes. However, COFS patients generally only live up to the age of 3 years and is therefore often described as an early infantile form of CS (Laugel et al., 2008). Similar to CS, mutations have been found in the CSB, XPD and XPG genes (Meira et al., 2000; Graham, Jr. et al., 2001; Hamel et al., 1996; Laugel et al., 2008). The first case of human inherited ERCC1 deficiency has been recently reported. The observed clinical features were very severe and compatible with a diagnosis of COFS (Jaspers et al., 2007).

Trichothiodystrophy (TTD)

Trichiothiodystrophy (TTD) patients include features such as shortened lifespan, small stature, mental retardation, decreased fertility, skeletal abnormalities and skin photosensitivity without decreased pigmentation or increased cancer sucessptibility and immunodeficiency (Cleaver, 2005; Stefanini et al., 1993). Although some TTD patients

show photosensitivity, they do not have cutaneous lesions and a skin cancer predisposition. The disease is however distinct from CS due to the typical appearance of sulfur-deficient brittle hair which has characteristic "tiger-tail" banding when examined in polarized light. This is due to a reduction of cysteine-rich matrix proteins that normally provide strength to the hair shaft by cross-linking the keratin filaments (Itin and Pittelkow, 1990; Stefanini et al., 1993). Since, most of the symptoms are quite similar to that of CS, TTD has likewise been classified as a segmental premature aging syndrome (de Boer and Hoeijmakers, 2000). Three complementation groups have been identified XPB, XPD and TTDA. In TTD patients, basal transcription is altered leading to decreased transcription of specific genes (Lehmann, 2003; Bohr et al., 2005). In fact, certain XPD point mutations cause TFIIH instability, interfering with a number of processes including the completion of terminal differentiation of hair and nails (Botta et al., 2002; Vermeulen et al., 2000) which explains the brittle hair and nails characteristics of TTD patients.

NUCLEOTIDE EXCISION REPAIR

Transcripton-coupled repair

An arrested RNA polymerase at a site of a lesion represents a strong signal for apoptosis (Ljungman, 2005; Ljungan and Zhang, 1996). To counteract apoptosis and to resume transcription, cells possess the capacity to remove transcription blocking lesions by a specialized sub-pathway of NER termed transcription coupled repair (TCR). TCR is initiated by a bulky lesion blocking the elongating RNA polymerase (RNAPIIo) located on the transcribed strand of an active gene (Brueckner and Cramer, 2007). CSB interacts loosely with the elongating polymerase (Selby and Sancar, 1997) but becomes more tightly bound following transcription arrest (van Gool et al., 1997). The CSB gene encodes a 168kDa protein which is related to the SWI/SNF family of ATP-dependent chromatin remodeling factors. CSB displays DNA-dependent ATPase and DNA binding activity, but not helicase activity (Citterio et al., 2000). Furthermore, when added to an RNAPII arrested at a CPD, CSB can stimulate transcription elongation by addition of one nucleotide to the nascent transcript in vitro (Selby et al., 1997). In vitro transcription assays using purified RNA polymerase and initiation factors determined the order of assembly of NER factors at arrested RNA pol IIo complexes. The CSB/RNAPII complex is able to interact with subunits of the TFIIH complex (Tantin, 1998) and may therefore have a role in recruiting TFIIH to the repair complex. In support of this model, it was shown that CSB and XPG can bind in a cooperative manner to RNAPII arrested at a cisplatin lesion *in vitro* (Sarker et al., 2005). Others have also shown XPG recruitment to the stalled RNAPIIo (Tantin et al., 1997; van den Boom, et al., 2004). ChIP analysis of chromatin-bound RNAPIIo isolated from in vivo crosslinked cells provided direct evidence for the interaction of CSB with RNAPIIo in the absence of damage (Fousteri et al., 2006). Upon UV-irradiation, the interaction of CSB with chromatin-bound RNAPIIo complex was enhanced. The fate of the polymerase remains unclear. In vitro results suggest that the RNAPIIo is released on the arrival of XPF with CSB being required for incision activity (Laine and Egly, 2006a; Laine and Egly, 2006b). However, recent findings suggest that the polymerase may stay stalled at the site

of damage while repair of the lesions occurs since XPG is able to interact with RNAPIIo allowing incision without the removal of the elongating polymerase (Sarker et al., 2005). Furthermore, *in vivo* evidence was presented that the stalled RNAPIIo and the NER specific endonucleases can be isolated as a single repair complex (Fousteri et al., 2006; van den Boom et al., 2004). In vivo studies also revealed a CSB-dependent recruitement of CSA to the RNAPIIo complex consistent with previous findings (Fousteri et al., 2006; Kamiuchi et al., 2002). CSA is part of a multiprotein complex with E3 ubiquitin-ligase activity and the RNAPIIo/CSB complex was shown to facilitate the assembly of not just CSA alone but the CSA-DDB1 E3-ubiquitin ligase/CSN complex (Fousteri et al., 2006) which then allowed further recruitment of HMGN1, HAT p300, XAB2 and TFIIS proteins (Fousteri et al., 2006). Recruitment of p300 required CSB alone whereas HMGN1, XAB2 and TFIIS required both CSB and CSA for binding at the stalled polymerase (Fousteri et al., 2006). Conformational changes of the RNAPIIo may be required to allow accessibility to repair proteins. Backtracking of the RNAPIIo may be one of the key mechanisms to allow repair and/or transcription restart. It was shown that backtracking could proceed far enough that a small DNA repair enzyme photolyase could bind and directly reverse the CPD (Tornaletti et al., 2001). Following backtracking, TFIIS enables cleavage of the protruding 3′ mRNA by RNAPIIo so that transcripton can restart upon lesion removal (Kalogeraki

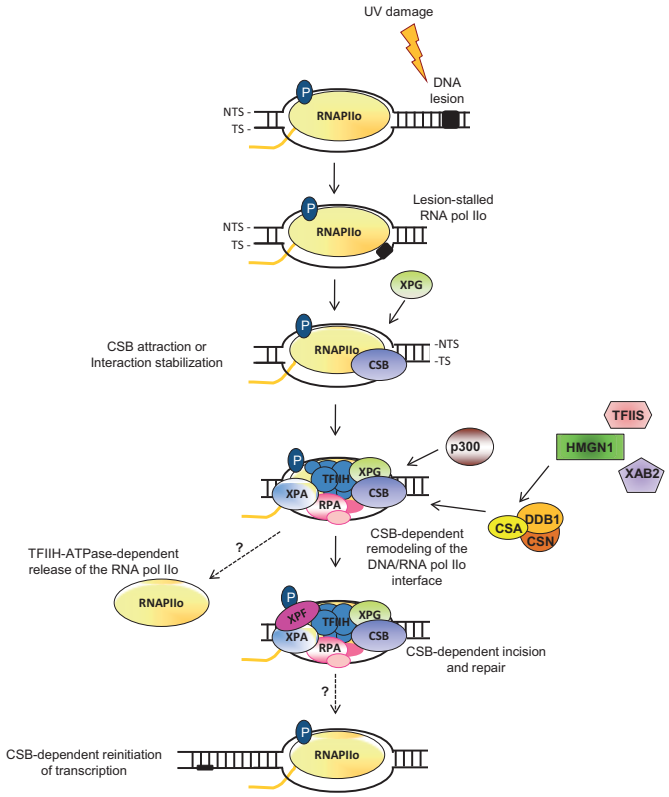

Fig. 4. Transcription-coupled repair. Transcription is arrested when the RNAPII encounters a lesion. CSB becomes tightly bound and facilitates the recruitment of TCR factors. XPG, TFIIH, XPA and ERCC1-XPF localize to the arrested elongation complex with RPA arriving once single-stranded DNA is formed. The chromatin remodeling factors p300 and HMGN1 loosen the nucleosome structure behind the polymerase. The RNAPII reverses direction, backtracking from the lesion allowing cleavage of the 3′ mRNA which is activated by TFIIS. After backtracking, TFIIH extends the denatured region around the lesion in order to allow subsequent incision by XPG and ERCC1-XPF. (Fig adapted from Fousteri et al., 2006; Sarasin and Stary, 2007)

et al., 2005; Fousteri et al., 2006). The backtracking process might require chromatin remodeling events, presently involving the high mobility group protein HMGN1 (Hanawalt and Spivak, 2008). *Hmgn1*-knockout mice exhibit reduced repair of CPDs in active genes implying a role in TCR (Birger et al., 2003). The assembly of HMGN1 may function to help displace nucleosomes that have become re-established behind the translocating transcription complex, so that the RNAPII can regress from the lesion. p300 is also known to have a role in chromatin remodeling during both transcription and DNA repair (Hasan et al., 2001; Cazzalini et al., 2008). XAB2 may have a pivotal role in TCR as well as in transcription. XAB2 is known to interact with XPA which suggests that it may be a key protein at the juncture of both GGR and TCR for which all subsequent steps are shared (Kuraoka et al., 2007) Once TFIIH and other factors have been recruited, the TCR pathway is identical to that of GGR (Bowman et al., 1997; M. Fousteri, unpublished results) and the corresponding repair patch sizes are similar (Bowman et al., 1997).

Global Genome Repair

In the past, numerous models have been proposed to describe the assembly and disassembly of the various NER proteins at the site of damage. One theory proposed that all of the core NER proteins (XPC-hHR23B, TFIIH, XPA, RPA, XPG and ERCC1-XPF) were together in a pre-assembled complex capable of carrying out the excision step. A series of studies showed that the core NER factors were associated in a repair holocomplex in *S. cerevisiae* (Svejstrup et al., 1995; Rodriguez et al., 1998), and in human cells (He and Ingles, 1997). However, several studies provide data against this notion (Araujo and Wood, 1999; Park and Choi, 2006), while others present evidence that the NER factors assemble sequentially at DNA damage (Araujo et al., 2001; Riedl et al., 2003; Mone et al., 2004; Volker et al., 2001). This model is now widely accepted and Chapter 8 describes the assembly and disassembly of NER factors at DNA lesions in detail.

DNA DAMAGE RECOGNITION

UV-DDB and XPC

In GGR, DNA damage recognition proteins are able to distinguish the difference between damaged and undamaged DNA by identifying DNA that has undergone helical distortions. The initial damage recognition factors involved in NER have been the subject of some debate in the past. Some in vitro studies suggested that XPA or a RPA-XPA complex binds first to the damage, subsequently recruiting XPC-hHR23B and the other core NER factors (Wakasugi and Sancar, 1999; Evans et al., 1997). In contrast, other in vitro findings clearly provide evidence that the XPC-hHR23B complex is the primary damage recognition factor (Sugasawa et al., 1998). Finally, in vivo analysis showed conclusive evidence that XPC-hHR23B is the principal damage recognition complex and initiator of NER (Volker et al., 2001). XPC exists in bound form with hHR23B and Centrin 2 in cells (Araki et al., 2001; Shivji et al., 1994). hHR23B protein contains two ubiquitin-associated domains and one ubiquitin-like domain which can stabilize XPC and enhance the binding between XPC and damaged DNA (Sugasawa et al., 2005). Loss of hHR23B results in decreased XPC

levels (Ng et al., 2003). However, recent studies carrying out live cell FRET measurements suggest that hHR23B dissociates when XPC binds to damaged DNA indicating that it is not directly involved in repair (S. Bergink, unpublished data). Centrin 2 is thought to stimulate XPC (Nishi et al., 2005), although its exact function is not yet clear. Although the XPC-hHR23B complex is considered as the primary damage recognition factor, there is considerable evidence for another DNA damage recognition factor i.e., UV-DDB that preceeds XPC-hHR23B.

UV-DDB is a heterodimer of the DDB1 (p127) and DDB2 (p48) proteins. Mutations in DDB2 cause the XPE phenotype. CPD repair is UV-DDB dependent (Fitch et al., 2003b; Tang et al., 2000) which explains why cells from XPE patients are deficient in CPD repair (Nichols et al., 2000; Rapic-Otrin et al., 2003). We also provide data showing that DDB2 significantly stimulates the repair of 6-4PPs especially at low doses (Chapter 6). As already mentioned XPC-hHR23B has a high affinity for DNA lesions such as 6-4PPs, suprisingly it's binding to CPDs is less efficient and in fact repair of CPDs requires DDB2 and XPC-hHR23B (Tang et al., 2000). DDB2 has a binding affinity for a number of different lesions such as mismatches, AP sites and compound lesions (Chu and Chang, 1988; Fujiwara et al., 1999; Payne and Chu, 1994; Wittschieben et al., 2005) but an extremely high affinity for 6-4PPs (Nichols et al., 2000; Reardon et al., 1993; Treiber et al., 1992; Keeney et al., 1993). UV-DDB is not essential for in vitro NER reactions suggesting that it is required for the repair of lesions within chromatin (Aboussekhra et al., 1995; Bessho et al., 1997; Araujo et al., 2000; Kulaksiz et al., 2005). DDB2 is incorporated into a functional CUL4A E3 ubiquitin ligase through its interaction with DDB1 (Groisman et al., 2003; He et al., 2006) and live cell imaging revealed that DDB2, DDB1 and Cul4A are rapidly recruited to UV lesions with similar association kinetics (Chapter 7). DDB2 undergoes ubiquitin mediated proteolysis following UV irradiation and the timing of this degradation determines the arrival of XPC to the damage site (El Mahdy et al., 2006). UV-DDB breakdown increases the binding of XPC to the damaged DNA using a reconstituted NER system (Sugasawa et al., 2005), and interfering with DDB2 proteolysis was shown to compromise the removal of CPDs in vivo (El-Mahdy et al., 2006). These results suggest that the recruitment of UV-DDB to UV damage and the degradation of DDB2 is crucial for XPC recruitment and the repair of photolesions. In line with these observations, we and others found that DDB helps to recruit XPC to both CPDs and 6-4PPs which accelerates the repair of these lesions (Fitch et al., 2003a; Chapters 5 and 6).

It is known that XPC is modified upon UV-irradiation, the modifications include ubiquitylation and sumoylation (Sugasawa et al., 2005). The UV-induced XPC ubiquitylation is regulated by DDB-Cul4A E3 ubiquitin ligase complex comprised of DDB1, DDB2, Cul4A, Roc1 and COP9 signalosome (Groisman et al., 2003). DDB–Cul4A complex can ubiquitylate both DDB2 and XPC, but the fates of ubiquitylated DDB2 and XPC appear to be quite different: ubiquitylated DDB2, but not XPC, is subjected to proteasomal degradation (Sugasawa et al., 2005). XPC is monoubiquitinated after DNA damage which increases the binding of XPC to damaged DNA as well as promoting its interaction with hHR23B (Araki et al., 2001; Ng et al., 2003; Ortolan et al., 2004). The role of DDB2 therefore facilitates prompt recruitment of XPC to UV lesions as well as promoting XPC degradation (Wang et

al., 2007). It has already been reported that during assembly of NER factors, XPC–hHR23B and XPG cannot simultaneously exist in the repair complex and that the entry of XPG into the complex coincides with XPC–hHR23B leaving the complex (Riedl et al., 2003). Although dissociation of XPC from the complex prior to incision has been observed in vitro experiments (Riedl et al., 2003; Wakasugi and Sancar, 1998), recent competition experiments (Chapter 8) in XP-A cells employing a local UV dose that saturates NER, demonstrated that XPC is stably assembled at the initial damage sites in the absence of incision.

HELIX UNWINDING

TFIIH

The later steps of GGR and TCR are conducted by a common mechanism that involves several core repair factors. After DNA damage recognition specific for each subpathway, the DNA duplex must be unwound around the lesion in order to allow access for the subsequent repair proteins. Helix unwinding is accomplished by the basal transcription factor TFIIH. TFIIH is the basal transcription initiation factor, which has an important role in RNA pol I and RNA pol II transcription, promoter escape, and is essential for NER throughout genomic DNA, whether transcriptionally active or not (Coin et al., 1999; Friedberg et al., 2006). In NER, TFIIH unwinds the duplex DNA around the lesion allowing the recruitment of the other core NER components, XPA, RPA, XPG and ERCC1-XPF (Evans et al., 1997). XPC recruits TFIIH to the damage site in GGR (Araujo et al., 2001), whereas CSB mediates the recruitment of TFIIH to TCR complexes (Fousteri et al., 2006). TFIIH is composed of 10 subunits making it the largest factor required for NER. The subunits consist of XPB/p89, XPD/p80, p62, p52, p44, p34, p8/TTDA (Giglia-Mari et al., 2004; Ranish et al., 2004; Coin et al., 2006) and a cdk-activating kinase (CAK) subcomplex; cdk7, cyclin H and MAT 1 (Roy et al., 1994). Certain mutations found in XPD patients disrupt the interaction of XPD with the p44 subunit resulting in dissociation of the CAK subcomplex from the core TFIIH complex (Coin et al., 1998; Dubaele et al., 2003). Both subcomplexes are therefore linked by XPD/p80 which promotes functional transcription (Drapkin et al., 1996; Reardon et al., 1996; Tirode et al., 1999) and DNA damage opening (Coin et al., 1998; Dubaele et al., 2003). The XPB subunit of TFIIH is an ATP-dependent helicase that mediates unwinding of promoter DNA in a 3′ -5′ orientation during transcription initiation (Douziech et al., 2000). The XPD subunit of TFIIH is a 5′ -3′ ATP dependent helicase that is required for strand separation during NER. In NER, XPD requires the ATPase activity of XPB and the helicase activity of p44 (Coin et al., 2007). TTDA/p8 is the smallest subunit of TFIIH which is shown to be essential for open helix formation (Giglia-Mari et al., 2006) and the maintenance of cellular TFIIH concentrations (Coin et al., 2006). TTDA/p8 stimulates the ATPase activity of XPB; this stimulation is probably mediated by the p52 subunit of TFIIH which is known to bind both TTDA/p8 and XPB (Coin et al., 2007). Recent data show that the C-teriminal domain of XPC is required for the recruitment of TFIIH through interactions involving the XPB and p62 subunits (Bernades de Jesus et al., 2008). Once TFIIH associates with XPC/damaged DNA it facilitates structural changes in the damage complex which liberates the

space for damage verification by XPA and the arrival of the other NER factors (Tapias et al., 2004). Recent studies demonstrate that TFIIH is also implicated in the transactivation of genes through a phosphorylation activity resident in a cdk-activating kinase (CAK) subcomplex (Ito et al., 2007).

It was thought that TFIIH remained a stable complex when switching between different cellular processes (Hoogstraten et al., 2002) yet, recent studies have shown that the composition of TFIIH changes. A dynamic dissociation/reassociation of the CAK complex with the core TFIIH was observed, which coincided with the recruitment/release of the NER factors during the NER process (Coin et al., 2008). Release of the CAK from the core TFIIH complex was catalyzed by XPA and coincided with the arrival of the other NER factors promoting incision and excision of the damaged oligonucleotide. The departure of NER factors coincided with the return of the CAK complex to the TFIIH core complex and resulted in transcription (Coin et al., 2008). The C-terminal region of XPA interacts with TFIIH (Park et al., 1995) and is required for the release of the CAK. Similarly, previous data showed that CAK negatively regulates the XPD DNA unwinding activity (Sandrock and Egly, 2001) and that the recruitment of XPA to the XPC/TFIIH pre-incision complex led to full opening of the damaged DNA (Andressoo et al., 2006). C-terminal truncated XPA, which was almost inactive in the presence of a whole TFIIH, was shown to catalyze dual incision in the presence of a TFIIH lacking CAK (Coin et al., 2008). Together, these data show that the release of the CAK complex from the core TFIIH complex is essential for the initiation of the incision/excision step of NER through stimulating TFIIH helicase/ATPase activity allowing enlargement and stabilization of the DNA open structure.

XPA

After unwinding of the DNA duplex by TFIIH, additional proteins are associated forming a complex containing fully opened DNA, called the pre-incision complex. XPA is a 36 kDa scaffold protein which has no enzymatic activity on its own but is nevertheless indispensable for DNA incision. XPA contains a zinc finger domain which is part of the minimal DNA binding domain and consists of zinc complexed to four cysteine residues; substitution of any of the four cysteines leads to severe reduction in NER activity (Miyamoto et al., 1992). Structural studies have revealed that the zinc finger binding motif is important for the protein-protein interaction with RPA (Ikegami et al., 1998). Both XPA and RPA bind to damaged DNA with some specificity (Lao et al., 2000), which is enhanced by the interaction between the two proteins (He et al., 1995; Li et al., 1995). The XPA-RPA complex was originally thought to be responsible for DNA damage recognition, yet substantial in vivo and in vitro evidence shows that recruitment of XPA to DNA lesions occurs later than TFIIH recruitment (Volker et al., 2001; Riedl et al., 2003; Coin et al., 2006) and that the recruitment of XPA is strictly XPC-dependent (Rademakers et al., 2003). XPA has been reported to have binding specificities for certain distorted DNA structures (Missura et al., 2001) which suggests that XPA may recognize certain intermediate conformations of DNA that could emerge during the action of the TFIIH helicase activity. At the same time, the recruitment and assembly of XPA and RPA into the pre-incision complex may occur (Li et al., 1998; Park et al., 1995; You et al.,

2003). As mentioned above, evidence suggests that the detachment of the CAK from the core TFIIH is catalyzed by XPA (Coin et al., 2008) which facilitates enlargement and stabilization of the DNA open structure.

RPA

RPA protein is composed of 3 subunits, 70kDa, 34kDa and 17kDa and has an important role in various processes such as replication, recombination and various DNA repair pathways (Fanning et al., 2006; Wold, 1997). RPA displays an extremely high affinity for single-stranded DNA (ssDNA) which is around 3 orders of magnitude higher than its affinity for double-stranded DNA (Kim et al., 1992). RPA is able to bind to ssDNA through 2 mechanisms; first RPA binds to a region of 8-10 nucleotides which is created by the helicase action of TFIIH, and then elongates to cover a region of around 30 nucleotides which acts as a wedge to separate the DNA strands around the lesion (Blackwell and Borowiec, 1994; de Laat et al., 1998b; Hermanson-Miller and Turchi, 2002). RPA also has an important role in stimulating endonuclease activity by facilitating the correct positioning of the endonucleases (He et al., 1995; Matsunaga et al., 1996) as well as facilitating the open complex formation. RPA interacts with a number of other core NER factors such as XPA (as mentioned above), the endonucleases XPG, ERCC1-XPF and is required for dual incision (Coverley et al., 1992). RPA associates with the undamaged DNA strand and is indispensable for open complex formation. *In vitro* experiments revealed that RPA initially binds 8-10 nucleotides at the 5′ side of the lesion after which RPA stretches along the DNA in the 3′ direction (de Laat et al., 1998a; Kolpashchikov et al., 2001) allowing correct positioning of XPG and ERCC1-XPF. With the 3′-oriented side of RPA facing a duplex ssDNA junction, RPA interacts with and stimulates ERCC1-XPF, whereas the 5′-oriented side of RPA at a DNA junction allows stable binding of XPG (de Laat et al., 1998c).

It was originally thought the XPA and RPA co-exist as a complex in the cell nucleus. However, evidence from GFP-tagged XPA diffusion experiments showed no interaction between XPA and RPA (Rademakers et al., 2003). Furthermore, the binding of RPA to UV lesions was shown to be independent of XPA, suggesting that RPA-XPA interactions only occur on damaged DNA (Rademakers et al., 2003). Recent ChiP results however suggest that XPA and RPA may interact transiently since we found co-precipitation of RPA and XPA in cells containing no DNA damage (Chapter 8).

RPA also has an important function in the latter stages of NER such as DNA repair synthesis and ligation (Shivji et al., 1995). In vitro experiments examining the assembly and disassembly of NER proteins on immobilized DNA damaged templates reveal that RPA remains bound after dual incision, and initiates the assembly of DNA synthesis factors such as PCNA (Riedl et al., 2003). In vivo results support this notion, since the disassembly of RPA after UV irradiation was inhibited in the presence of DNA synthesis inhibitors (Chapter 9). Together, these results support a role for RPA in both pre- and post-incision events in which it may remain localized to ssDNA after excision of the damaged nucleotide and remain bound until DNA synthesis and ligation are completed (Chapter 8). The multi-functional role of RPA in NER is discussed in detail in Chapter 8.

DUAL INCISION

XPG

After the assembly of the pre-incision complex, single-strand breaks are introduced to the damaged DNA strand by XPG and ERCC1-XPF. XPG is a 133kDa structure-specific endonuclease of the FEN-1 family which is indispensable for NER (Friedberg et al., 2006). During NER, XPG functions as a junction-specific endonuclease that specifically incises the damaged single-stranded DNA strand 2-8 nucleotides from the 3′ side of the lesion undergoing repair (O'Donovan et al., 1994a; O'Donovan et al., 1994b). Additionally, XPG is required structurally for formation of the fully opened DNA conformation, since the 5′ incision carried out by ERCC1-XPF endonuclease is dependent on the physical presence of XPG at the damage site (Wakasugi et al., 1997; Constantinou et al., 1999). The XPG endonuclease has been reported to bind to the NER complex in XPA-deficient cells yet was not capable of incision in the absence of XPA (Evans et al., 1997). In contrast, both XPA and XPG are incorporated into the NER complex in XPF-deficient cells (Volker et al., 2001). In this situation, Evans et al (1997) reported that XPG was capable of making the 3′ incision yet other authors have reported that the presence of XPF is required for XPG incision activity (Tapias et al., 2004). Studies using XPA deficient cells show that the DNA binding domain is needed for the action of XPA as a processivity factor and for its ability to enable XPF and XPG to incise damaged nucleosomal DNA (Bartels and Lambert, 2007).

Truncation mutations in the *XPG* gene give rise to individuals with the combined clinical features of XP and Cockayne syndrome (CS), a disorder associated with developmental and neurological abnormalities (Nouspikel et al., 1997). Furthermore, while NER is not essential for the viability of an organism, complete loss of XPG function is incompatible with life in knockout mice (Shiomi et al., 2004) suggesting that XPG is required for additional functions necessary for normal development such as transcription. Recent studies found that certain XPG mutations present in severe XPG and XPG/CS patients were found to disturb the interaction of both CAK and XPD with the core TFIIH complex (Ito et al., 2007). TFIIH-XPG interaction therefore functions to maintain the architecture of TFIIH which also highlights an important role for XPG in transcription. These findings suggest that XPG may be recruited to damaged sites as a pre-assembled complex with TFIIH. However, in vivo (Volker et al., 2001) and in vitro (Riedl et al., 2003) studies propose a sequential assembly model. Moreover, XPG association was shown to be TFIIH dependent (Zotter et al., 2006), suggesting that TFIIH recruits XPG to the NER complex. In addition, live cell imaging studies show that XPG and TFIIH are 2 distinct complexes and only interact on damaged DNA (Hoogstraten et al., 2002; Zotter et al., 2006). Despite these findings, transient interactions between the 2 proteins which would stabilize TFIIH cannot be ruled out.

XPG also contains a PCNA-binding domain which could function to attract PCNA to the repair complex linking the incision and the repair resynthesis steps of NER (Gary et al., 1997). Studies demonstrate that the simultaneous presence of XPG and RPA at the damage site are required for transition between the incision and DNA synthesis steps of NER (Mocquet et al., 2008). Furthermore, recent data found that partial DNA synthesis

was detectable *in vitro* in the presence of catalytically inactive XPG, thereby demonstrating that the incision 5′ to the lesion by ERCC1-XPF is both necessary and sufficient for the initiation of repair synthesis, whereas the 3′ incision by XPG is needed for the completion, but not the initiation of repair synthesis (Staresincic et al., 2009). See section 'DNA repair resynthesis and ligation' for more details regarding the role of XPG in the late stages of NER.

ERCC1-XPF

ERCC1 (33 kDa) and XPF (103 kDa) form a heterodimer which cleaves DNA at the 5′ boundary of the open complex between single-stranded and double-stranded DNA (Matsunaga et al., 1995). ERCC1 is also known to interact with XPA (Bessho et al., 1997; Li et al., 1995). The interaction between ERCC1 and XPA seems to be crucial for its recruitment to the damage site (Li et al., 1994; Park and Sancar, 1994; Saijo et al., 1996). ERCC1-XPF makes an incision in the DNA 15-24 nucleotides away from the 5′ side of the lesion (de Laat et al., 1998c; Matsunaga et al., 1995; Sijbers et al., 1996). Although, XPF contains the nuclease domain which is able to cleave the DNA the ERCC1 subunit is required for nuclease activity (Enzlin and Scharer, 2002). The nuclease domain in ERCC1 is unable to incise DNA (Gaillard and Wood, 2001). The physical presence but not the catalytic activity of XPG is required for ERCC1-XPF incision (Mu et al., 1996; Wakasugi et al., 1997; Constantinou et al., 1999; Tapias et al., 2004; Staresincic et al., 2009).

In theory, both endonucleases are capable of incising both DNA strands of an open complex substrate which highlights the importance of verification of the damaged strand in order to avoid cleavage errors. Damage verification is carried out by TFIIH together with XPA-RPA which is also likely to assemble the pre-incision complex in the correct orientation. As already mentioned RPA binds to the undamaged strand and may guide the endonucleases to their correct positions (de Laat et al., 1998a; Matsunaga et al., 1996). Our studies on the stability of the NER complex suggest that dual incision is the key determinant for the release of the pre-incision proteins (Chapter 8). Similarly, ERCC1-XPF is both necessary and sufficient for the initiation of repair synthesis (Staresincic et al., 2009).

DNA Repair Resynthesis and Ligation

After excision of the damage-containing oligonucleotide, the resulting single-strand gap is filled by DNA synthesis and ligation. In vitro (Shivji et al., 1995) and in vivo (Chapter 9) results show that RPA is involved not only in dual incision but also in subsequent repair synthesis. RPA is likely to bind to the undamaged strand and, upon dual incision, may help recruit PCNA and RFC (Gomes and Burgers, 2001; Yuzhakov et al., 1999). In fact, recent studies demonstrate that the simultaneous presence of XPG and RPA at the damage site are required for transition between the incision and DNA synthesis steps of NER (Mocquet et al., 2008). In vitro experiments revealed that DNA resynthesis is dependent on PCNA (Nichols and Sancar, 1992; Shivji et al., 1995). PCNA forms a homotrimeric clamp (Krishna et al., 1994) which is loaded onto the 3′ end of primers on template strands and supports chain elongation by interacting with replicative DNA polymerases (reviewed in

Maga and Hubscher, 2003). In vitro experiments also show that PCNA loading requires a heteropentameric, DNA-dependent ATPase complex called RFC (Ellison and Stillman, 1998; Tsurimoto and Stillman, 1989). The loading of PCNA by RFC is ATP dependent and occurs at ds- to ssDNA transitions with a 3'-OH end, a structure characteristically formed after 5' incision by ERCC1-XPF (Balajee et al., 1998; Essers et al., 2005). Both endogenous and GFP-tagged RFC and PCNA accumulate at local damage sites after UV irradiation in living cells confirming in vitro observations (R. Overmeer and A. Gourdin, unpublished results; Essers et al., 2005). XPG also contains a PCNA-binding domain which could function to attract PCNA to the repair complex linking the incision and the repair resynthesis steps of NER (Gary et al., 1997). Using XP-A and XP-F cells with mutations that completely abolish UDS but still allow recruitment of XPG (Volker et al, 2001), we found weak accumulation of PCNA (Chapter 9). These results are consistent with a possible assisting role for XPG in the recruitment of PCNA, however it must be noted that XP-G cells (lacking functional XPG) showed similar weak accumulation of PCNA suggesting that XPG is not the only factor involved in recruitment of PCNA prior to incision. Previous work suggests that XPG is recruited together with RFC to the vicinity of the gap (Gary et al., 1999; Miura and Sasaki, 1999). RPA is thought to attract RFC at the same time as XPG recruitment of PCNA (Yuzhakov et al., 1999). Together, loading of RFC and PCNA is associated with the release of the ERCC1-XPF endonuclease (Mocquet et al., 2008). As mentioned above, the incision 5' to the lesion by ERCC1-XPF is both necessary and sufficient for the initiation of repair synthesis, whereas the 3' incision by XPG is needed for the completion, but not the initiation of repair synthesis (Staresincic et al., 2009). Our recent findings suggest that RPA is the only NER protein to remain stably associated at the site of repair following dual incision and remains associated until ligation is complete preventing further strand breaks from accumulating throughout the genome (Chapter 8).

In the past, only in vitro experiments have provided data on the late stages of NER which suggested that polymerase δ and ε are both involved in DNA resynthesis. Similarly, only DNA ligase I was thought to ligate the newly synthesized strand (Aboussekhra et al., 1995; Araujo et al., 2000; Shivji et al., 1995). Our recent in vivo studies show that pol δ is recruited to repair patches in both quiescent and proliferating cells where as pol ε is only recruited to repair patches in proliferating cells after UV irradiation (Chapter 7). We found no accumulation of pol β after UV irradiation which suggests that it plays no or a minor role in NER (Chapter 7). In addition, DNA polymerase κ has been implicated in the repair synthesis step (Ogi and Lehmann, 2006). After resynthesis, the 5' end of the newly synthesized DNA has to be ligated to the original sequence. We found contrary to *in vitro* data that Ligase I alone was not sufficient to rejoin the in repair nicks in chromosomal DNA and only played a role in proliferating cells (Chapter 7). FEN-1 has also been implicated in the ligation process and is thought to function together with Lig1 (Shivji et al., 1995; Araujo et al., 2000; Mocquet., 2008). However, we were unable to identify a role for FEN-1 in our in vivo experiments (J. Moser, unpublished results). We identified the XRCC1-Ligase3 complex as the principle DNA ligase in NER since accumulation of this complex was visualized in both quiescent and proliferating cells (Moser et al., 2007). Repair experiments confirmed the role of XRCC1-Lig3 in NER by demonstrating its requirement for the efficient

removal of lesions and rejoining of nicks in chromosomal DNA (Chapter 7). Together these results show that pol δ and XRCC1-Lig3 are the dominant DNA polymerase/ligase in NER-mediated gap-filling in both quiescent and proliferating cells whereas Pol ε and Lig1 have a role in proliferating cells (Chapter 7). See Chapters 7 and 8 for more details regarding the role of the polymerases and ligases in the late stages of NER.

A MODEL OF GG-NER

Probable sequence of events in mammalian GGR

We propose the following model for global genome repair based on our knowledge of the core NER factors. (See Figure 5)

1. DNA damage recognition

A crucial step in DNA repair is the initial recognition of damaged sites. DNA damage recognition is carried out by UV-DBB and XPC-hHR23B complexes. UV-DDB arrives at the damage site which induces a kink in the DNA allowing recruitment of the XPC-hHR23B complex (Wakasugi et al., 2002; Fitch et al., 2003a). The binding of XPC results in slight opening of the DNA surrounding the lesion (Tapias et al., 2004). DDB2 likely creates a local chromatin environment around lesions that facilitates the assembly of repair complexes (Chapter 5 and 6). UV-DDB physically interacts with XPC and the associated E3 ligase containing cullin 4A and Roc1 which ubiquitylates XPC after UV damage, (Sugasawa et al., 2005) a process that does not cause XPC degradation, but rather enhances its affinity for DNA. DDB2 is also ubiquitylated after UV which leads to rapid degradation after DNA damage (El-Mahdy et al., 2006; Sugasawa et al., 2005). Therefore both ubiquitylated DDB2 and XPC may be important for the efficient transfer of lesions between the two damage recognition factors, switching from tightly-bound UV-DDB, to a lower-affinity XPC-hHR23B complex (Sugasawa et al., 2005).

2. Helix unwinding and damage verification

After DNA damage recognition the DNA duplex is unwound around the lesion, a process that is accomplished by the basal transcription factor TFIIH. The XPC-hHR23B complex recruits TFIIH to the damage site in GGR (Araujo et al., 2001; Yokoi et al., 2000), which allows unwinding of the duplex DNA around the lesion, and promotes the subsequent incision and excision by facilitating the recruitment of the other core NER components, XPA, RPA, XPG and ERCC1-XPF (Evans et al., 1997; Sijbers et al., 1996; O'Donovan et al., 1994b). The detachment of the CAK from the core TFIIH is catalyzed by XPA, in the presence of ATP and is essential for the initiation of the incision/excision step of NER through stimulating TFIIH helicase/ATPase activity allowing enlargement and stabilization of the DNA open structure (Coin et al., 2008). XPA may recognize certain intermediate conformations of DNA that could emerge during the action of the TFIIH helicases. At the same time, the recruitment and assembly of XPA and RPA into the pre-incision complex may occur (Li et al., 1998; Park et al., 1995; You et al., 2003). XPA and TTDA stimulate the

ATPase activity of TFIIH in the presence of lesions, which is required for full opening around the lesion (Coin et al., 2006; Giglia-Mari et al., 2006). XPA also acts as a processivity factor which enables XPF and XPG to incise damaged nucleosomal DNA (Bartels and Lambert, 2007). RPA binds to a region of 8-10 nucleotides which is created by the helicase action of TFIIH, and then elongates to cover a region of around 30 nucleotides which acts as a wedge to separate the DNA strands around the lesion (Blackwell and Borwiec, 1994; Kim et al., 1992; de Laat et al., 1998; Hermanson-Miller and Turichi, 2002). RPA also has an important role in stimulating endonuclease activity by facilitating the correct positioning of the endonucleases (de Laat et al., 1998b; He et al., 1995; Matsunaga et al., 1996; Chapter 8). XPC therefore likely remains associated to the damage site until the complete pre-incision complex is formed or until dual incision is performed.

3. Dual Incision

After the assembly of the pre-incision complex, single-strand breaks are introduced to the damaged DNA strand by endonucleases XPG and ERCC1-XPF complex. The simultaneous presence of XPG and RPA at the damage site is thought to be required for the transition between the incision and DNA synthesis steps of NER (Mocquet et al., 2008). First the 5' cleavage by ERCC1-XPF takes place which is dependent on the physical presence of XPG at the damage site (Wakasugi et al., 1997; Constantinou et al., 1999; Tapias et al., 2004; Staresincic et al., 2009). Recruitment of the repair synthesis factors such as polymerase, clamp loader RFC and PCNA are loaded to the damage site, and repair sythesis is initiated (Staresincic et al., 2009). RPA plays an important role in the transition between pre-incision and post-incision events (Riedl et al., 2003; Chapter 8), by recruiting recruit PCNA and RFC (Riedl et al., 2003; Gomes and Burgers, 2001; Yuzhakov et al., 1999). XPG is likely to recruit PCNA alone or together with RFC to the vicinity of the gap (Gary et al., 1999; Miura, 1999; Chapter 8). RPA is thought to attract RFC at the same time as XPG recruitment of PCNA (Yuzhakov et al., 1999). After incision, all of the pre-incision proteins are free to associate with other sites of damage with the exception of RPA. RPA does not dissociate after incision but likely remains bound to the undamaged strand until ligation is complete (Chapter 8). DNA synthesis is thought to proceed about half way through the repair patch. The stalling of the polymerase at this point might trigger the XPG endonuclease activity, allowing the repair synthesis to be completed (Staresincic et al., 2009). These results suggest that the 5'incision by ERCC1-XPF is both necessary and sufficient for the initiation of repair synthesis, whereas the 3' incision by XPG is needed for the completion of repair synthesis. Late NER factors including PCNA, DNA pol δ and CAF-1 have been found to localise to UV damage sites in cells expressing catalytically inactive XPG, but not in cells expressing catalytically inactive XPF (Staresincic et al., 2009). Therefore, after 5'incision the repair synthesis machinery consisting minimally of polymerase δ, RFC and PCNA and repair synthesis is initiated. XPG specifically incises the damaged single-stranded DNA strand 2-8 nucleotides from the 3' side of the lesion undergoing repair (O'Donovan et al., 1994a; O'Donovan et al., 1994b).

4. DNA repair synthesis and Ligation

With regard to DNA synthesis, we find that DNA pol δ is recruited to repair patches in both quiescent and proliferating cells where as pol ε was only found to be recruited to repair patches in proliferating cells after UV irradiation (Chapter 7). In addition, DNA polymerase κ may also have a role in the repair synthesis step (Ogi and Lehmann, 2006). It is possible that two polymerases, pol δ and pol κ, act at different steps of repair synthesis however further studies are required to elucidate exactly how the various phases in NER are regulated, also with regard to cell cycle status. For years Ligase I was thought to be the ligase responsible for the sealing of NER-mediated gaps. We were the first to provide direct evidence that the XRCC1-Ligase3 complex is the principle DNA ligase in NER. XRCC1-Ligase 3 was found to be functioning in both quiescent and proliferating

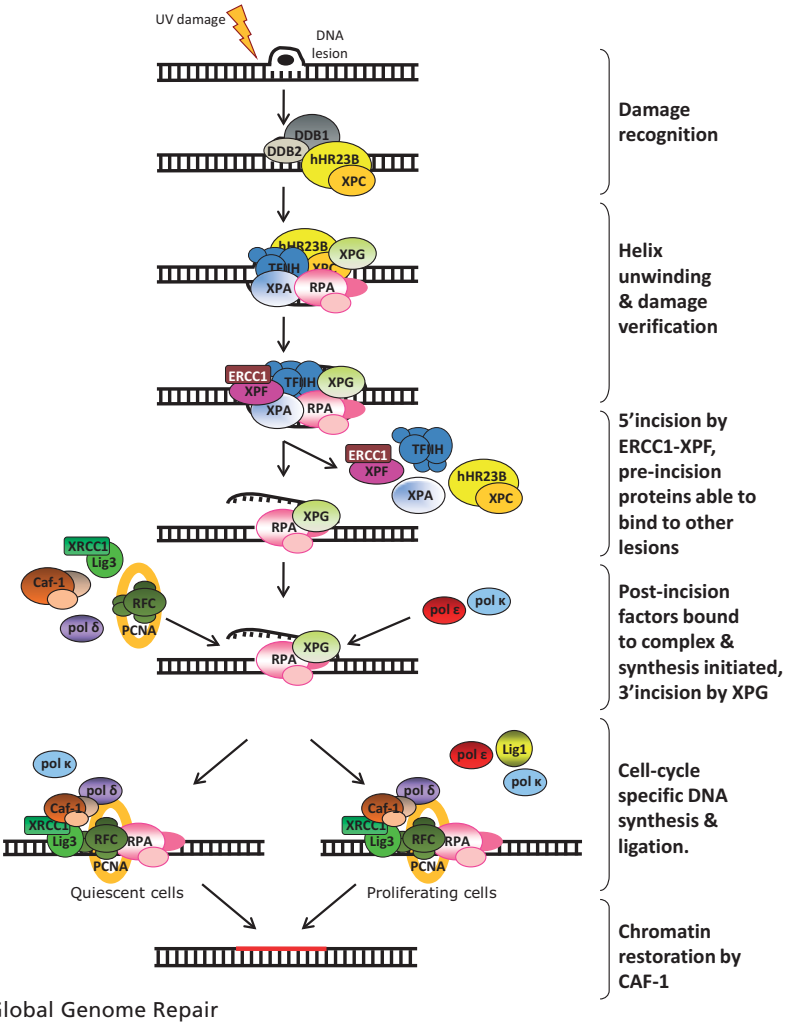

Fig 5. Global Genome Repair

cells and is essential for the efficient removal of lesions and rejoining of chromosomal DNA nicks (Chapter 7). Ligase I was only found to have a role in proliferating cells and partly compensates for a Lig3 deficiency in highly proliferating cells, further supporting a cell-cycle-dependent involvement of Lig1 in NER (Chapter 7). Together these results suggest that pol δ and XRCC1-Lig3 are the dominant DNA polymerase/ligase in NER-mediated gap-filling in both quiescent and proliferating cells whereas Pol ε and Lig1 may only have a role in proliferating cells (Chapter 7).

5. Chromatin restoration

Following successful completion of NER the repaired region needs to be to restored to the pre-existing chromatin structure, and checkpoint signaling switched off, as described in the Access-Repair-Restore model (Smerdon, 1991). Our own in vivo studies as well as other show that CAF-1 is recruited to damage sites within 30 min after UV (Chapter 8; Green and Almouzni., 2003; Staresincic et al., 2009) and also in the presence of DNA synthesis inhibitors HU and AraC (Chapter 8). Similarly, CAF-1 has also recently been found to localise to UV damage sites in cells expressing catalytically inactive XPG, but not in cells expressing catalytically inactive XPF (Staresincic et al., 2009) suggesting that CAF-1 accumulates at UV-induced lesions before the 3'incision by XPG. Together, these results suggest that CAF-1 is recruited to UV damage, presumably via PCNA, prior to completion of DNA synthesis and ligation and therefore cannot carry out nucleosome reassembly when it is first bound (Chapter 8).