The EMBO Journal Vol.18 No.23 pp.6585–6598, 1999

NEW EMBO MEMBER'S REVIEW

Light and dark in chromatin repair: repair of UV-induced DNA lesions by photolyase and nucleotide excision repair

Fritz Thoma

Institut für Zellbiologie, ETH-Zürich, Hönggerberg, CH-8093 Zürich, Switzerland

e-mail: thoma@cell.biol.ethz.ch

Nucleotide excision repair (NER) and DNA repair by photolyase in the presence of light (photoreactivation) are the major pathways to remove UV-induced DNA lesions from the genome, thereby preventing mutagenesis and cell death. Photoreactivation was found in many prokaryotic and eukaryotic organisms, but not in mammals, while NER seems to be universally distributed. Since packaging of eukaryotic DNA in nucleosomes and higher order chromatin structures affects DNA structure and accessibility, damage formation and repair are coupled intimately to structural and dynamic properties of chromatin. Here, I review recent progress in the study of repair of chromatin and transcribed genes. Photoreactivation and NER are discussed as examples of how an individual enzyme and a complex repair pathway, respectively, access DNA lesions in chromatin and how these two repair processes fulfil complementary roles in removal of UV lesions. These repair pathways provide insight into the structural and dynamic properties of chromatin and suggest how other DNA repair processes could work in chromatin.

Keywords: chromatin/DNA repair/nucleotide excision repair/photolyase/transcription

Chromatin, a dynamic and heterogeneous substrate for DNA repair

Chromatin is the packaged state of DNA in the nucleus and the substrate for all DNA-dependent processes, including transcription and DNA repair (Wolffe, 1995). At its lowest level of structural organization, DNA is folded in an array of nucleosomes called the nucleosome filament. The core of a nucleosome is composed of an octamer of four highly folded histone proteins, H2A, H2B, H3 and H4 (two of each), and 145 bp of DNA wrapped around the octamer. The nucleosome filament is formed by connecting nucleosome cores with linker DNA of variable length and by association of one histone H1 per nucleosome. The nucleosome filament is condensed further into compact 30 nm fibres and higher order structures. These structures are present in interphase and metaphase and are the target for damage formation and repair. There is pronounced structural and functional heterogeneity along chromosomal DNA with respect to DNA sequence, composition and functional activity, which projects into heterogeneity of damage formation and repair. This heterogeneity includes regions required for regulation of gene expression and replication, which associate with sequence-specific proteins and frequently are characterized by enhanced accessibility to nucleases (nuclease-sensitive regions; Workman and Kingston, 1998). Only a minor fraction of genes are transcribed or pre-set for transcription at any moment. The structure of these genes may include histone proteins modified by acetylation, altered or disrupted nucleosomes in the promoter regions, an unfolding of the whole chromatin domain, a loss or rearrangement of nucleosomes in the transcribed part, as well as differential loading of the genes with transcribing RNA polymerases. Moreover, a large fraction of genes are inactivated in heterochromatic regions by epigenetic silencing, which requires a special set of proteins to set up the repressive structure (Grunstein, 1998). Superimposed on this, there is time-dependent heterogeneity reflecting the dynamic properties of chromatin structure and function. To understand the molecular mechanisms of DNA repair, we need to investigate repair at the chromatin level, in particular how protein–DNA interactions affect damage formation, and vice versa, and how DNA lesions can be recognized and processed by repair proteins despite the presence of nucleosomes, transcriptional activity and numerous other proteins. This review is focused on UV damage formation and repair at the nucleosome level and does not include a discussion of repair in higher order chromatin organization. For additional reviews, see Tornaletti and Pfeifer (1996), Smerdon and Thoma (1998), Meijer and Smerdon (1999), Moggs and Almouzni (1999) and Smerdon and Conconi (1999).

Mutual effects of UV damage formation and protein–DNA interactions

The two major classes of mutagenic DNA lesions induced by ultraviolet light (UV-C, 200–280 nm, and UV-B, 280–320 nm) are *cis–syn* cyclobutane–pyrimidine dimers (CPDs) and pyrimidine (6–4) pyrimidone photoproducts (6–4PPs). 6–4PPs are formed at 20–30% of the yields of CPDs (Friedberg *et al*., 1995). Both classes of lesions distort the DNA helix. CPDs and 6–4PPs induce a bend or kink of 7–9° and 44°, respectively (Ciarrocchi and Pedrini, 1982; Wang and Taylor, 1991; Kim *et al*., 1995). The ability of UV light to damage a given base is determined by the flexibility of the DNA. Sequences that facilitate bending and unwinding are favourable sites for damage formation. For example, CPDs form at higher yields in single-stranded DNA (Becker and Wang, 1989), at the flexible ends of poly(dA)·(dT) tracts, but not in their rigid centre (Lyamichev, 1991). Bending of DNA towards the minor groove reduces CPD formation (Pehrson and Cohen, 1992). Proteins and protein complexes that disturb the B-form DNA structure affect both yields and

types of DNA damage formation, as reported for the lac repressor bound to the *lac* operator in *Escherichia coli* (Becker and Wang, 1984) and for several promoter elements that interact with sequence-specific proteins in yeast and mammalian cells (Selleck and Majors, 1987a,b, 1988; Becker *et al*., 1989; Pfeifer *et al*., 1992; Axelrod *et al*., 1993; Rozek and Pfeifer, 1995; Tornaletti and Pfeifer, 1995). One of the transcription factors that has a direct effect on DNA damage formation and repair is the TATA-box binding protein (TBP). TBP promotes the selective formation of 6–4PPs in the TATA-box, where the DNA is bent, but CPDs are formed at the edge of the TATA-box and outside, where DNA is not bent. The observations made *in vitro* and in active yeast genes imply that the structural distortion introduced in DNA by TBP is the same *in vitro* and in the initiation complexes *in vivo* (Aboussekhra and Thoma, 1999). Thus, protein-dependent modulation is a common theme leading to the heterogeneous distribution of DNA lesions in chromosomes.

Significant questions are whether DNA lesions in protein–DNA complexes affect functional properties of the complexes or whether repair is affected by the complexes. Extracts containing transcription factors were unable to band-shift UV-damaged oligonucleotides containing binding sequences for transcription factors (Tommasi *et al*., 1996). UV damage inhibits binding of TFIIIA to 5S rDNA, and irradiation of the TFIIIA–5S rDNA complex displaces TFIIIA, indicating that the complex is unable to accommodate UV photoproducts (Liu *et al*., 1997). On the other hand, the TBP binds damaged DNA (Vichi *et al*., 1997; Coin *et al*., 1998). These examples illustrate that the fate of protein–DNA complexes depends on the individual components. A damage-induced dissociation of the complex could affect function until the site is repaired. On the other hand, slow repair at sites of factor binding indicates that proteins can remain bound after damage formation and inhibit DNA repair (Tu *et al*., 1996; Aboussekhra and Thoma, 1999).

Modulation of DNA damage formation in nucleosomes

The major fraction of genomic DNA is folded in nucleosomes, and the position of nucleosomes on the DNA sequence can play a decisive role in regulation of gene expression and replication. They can serve both a repressive function by limiting access to DNA and an activating function by facilitating binding of transcription factors. Hence, understanding of how damage formation and repair are affected by nucleosomes or how DNA lesions affect nucleosome structure and positioning is of predominant significance.

The crystal structure of a reconstituted core particle (Luger *et al*., 1997) provides insight into how the structural organization could affect damage formation and repair. The DNA is wrapped in 1.65 superhelical turns around the histone octamer to form a pseudo-symmetric particle. Nucleosomal DNA has an inner surface facing the histones and an outer surface facing the solvent (Figure 1). The superhelical path is characterized by significant distortions, compression of grooves facing inside and local twist variations. The relative mobility of the segments of the DNA phosphate chains facing the solvent is high compared with other protein–DNA complexes. The sequence binds the octamer with a central base pair at the particle pseudo 2-fold axis so that the DNA is divided into 73 and 72 bp halves, with one base pair falling on the dyad. The additional base pair is accommodated without disruption of histone–DNA contacts. The structural distortions of nucleosomal DNA, its mobility and its flexibility suggest that nucleosomes are well suited to tolerate interactions with DNA-binding drugs and proteins and to accommodate various DNA lesions without disruption of the fundamental organization of the particle.

How does the structure of nucleosomes affect damage formation by UV light? Nucleosome core particles isolated from irradiated human cells by nuclease digestion revealed a modulation of CPD distribution with a period of 10.3 bases (Gale *et al*., 1987; Gale and Smerdon, 1990). A similar modulation was observed in DNA bent in a loop (Pehrson and Cohen, 1992), but not in linker DNA between nucleosomes (Pehrson, 1989, 1995) nor in unfolded nucleosomes (Brown *et al*., 1993). Apparently, bending DNA around the octamer facilitates CPD formation at sites where the DNA minor grooves face outside. These observations were made in a random population of nucleosomes with mixed sequences. The CPD patterns in individual nucleosomes, however, can deviate substantially, as observed in a reconstituted nucleosome ('HISAT-nucleosome'), which contains a polypyrimidine region and has a defined DNA surface ('rotational setting') (Schieferstein and Thoma, 1996).

In contrast to CPDs, 6–4PP distribution is not modulated in nucleosomes (Gale and Smerdon, 1990). There is apparently no natural distortion in nucleosomal DNA which facilitates 6–4PP formation at any particular site. Moreover, 6–4PPs, but not CPDs, are found predominantly in linker DNA (Niggli and Cerutti, 1982). One explanation for these results can be enhanced formation of 6–4PPs in linker DNA due to a higher flexibility.

Irrespective of the CPD modulation or preferential location of 6–4PPs in linker DNA, CPDs and 6–4PPs are detected throughout the nucleosome core. Hence, there seems to be no strict exclusion from any particular site, which implies that UV lesions can be tolerated even at positions where the damage-induced distortions do not coincide with the natural distortions of nucleosomal DNA. On the other hand, folding of DNA in nucleosomes exerts structural constraints that can disrupt the rigid structure of T-tracts (Hayes *et al*., 1991; Schieferstein and Thoma, 1996). Thus, nucleosomes reveal a substantial degree of DNA flexibility as well as structural constraints, which modulate damage formation and might also be essential for damage recognition.

DNA damage, nucleosome stability and nucleosome positioning

Although nucleosomes are particles with strong histone–DNA interactions, their stability is challenged by various dynamic transitions such as dissociation/reassembly, unfolding or nucleosome mobility (Figure 1; reviewed in Thoma, 1992; Widom, 1998). This raises the question of whether damage to DNA can affect those properties and, thereby, influence its own accessibility to repair enzymes or the functional properties of the nucleosome.

Can UV lesions destabilize or disrupt nucleosomes?

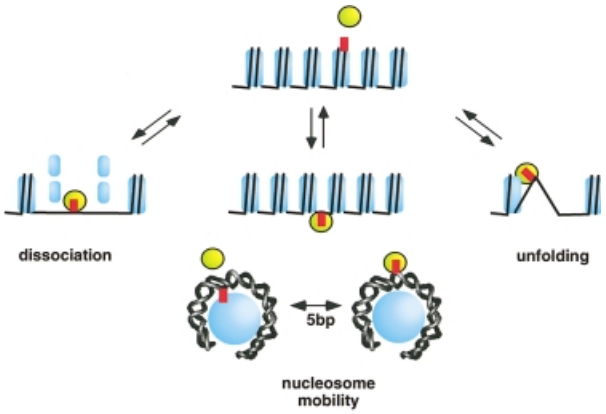

Fig. 1. Structural and dynamic properties of nucleosomes affecting DNA damage accessibility. DNA lesions (red square) can be accessible in linker DNA and on the nucleosome surface or be protected when facing the histones in the nucleosome. Dissociation/reassembly, unfolding/refolding and moving a histone octamer along the DNA (changing nucleosome positions, nucleosome mobility) may affect the accessibility of DNA lesions. Changing a nucleosome position by 5 bp alters the 'rotational setting' and turns the inner surface of DNA outside (one turn of nucleosomal DNA is shown). Vice versa, DNA lesions may affect the positions and stability of nucleosomes to accommodate the damage optimally. Histone octamers are in blue, DNA is in black, a damage recognition protein is in yellow. Arrows indicate a dynamic equilibrium. Remodelling factors may support the destabilization of nucleosomes.

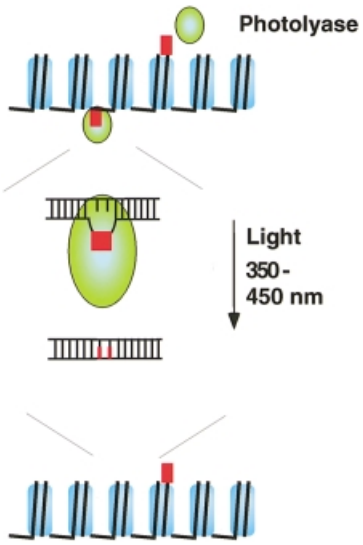

Fig. 2. Schematic illustration of photoreactivation in chromatin. Histone octamers are in blue, DNA is in black. Photolyase binds to cyclobutane–pyrimidine dimers (CPDs), 'flips out' the pyrimidine dimer and restores the native pyrimidines in a light-dependent reaction (Sancar, 1996b). Photolyase preferentially repairs CPDs in linker DNA. Repair in nucleosomes is slow, presumably facilitated by dynamic properties of nucleosomes that move the DNA lesions into linker DNA (see the text, Figure 1).

The fact that UV-damaged nucleosomes can be isolated shows that UV lesions have no dramatic effect on disruption of nucleosomes. However, a few observations *in vitro* may indicate differential stability of individual nucleosomes. The reconstituted HISAT-nucleosome was not destabilized by UV irradiation (Schieferstein and Thoma, 1996), but a loss of nucleosomes was reported after irradiation of plasmid DNA assembled with nucleosomes (Matsumoto *et al*., 1994). A slightly reduced efficiency of nucleosome assembly was observed on UV-damaged 5S rDNA (Mann *et al*., 1997), and nucleosome assembly on plasmid DNA was inhibited preferentially by 6–4PPs compared with CPDs (Matsumoto *et al*., 1994, 1995). We do not yet know whether UV damage formation affects nucleosome stability in living cells. However, when DNA photolyase was used as a tool to measure CPD accessibility in a few yeast nucleosomes (see below), no substantial variation in repair rates was observed (Suter *et al*., 1997), indicating that those nucleosomes had a similar stability.

The effect of DNA lesions could be more subtle and affect nucleosome positioning (Figure 1). *In vitro*, nucleosome positioning is dominated by the bending properties of DNA sequences. *In vivo*, positioning of nucleosomes depends on a combination of DNA sequence, DNA-binding proteins and chromatin folding (Thoma, 1992; Widom, 1998). Nucleosome positions can be altered *in vitro* (Beard, 1978; Pennings *et al*., 1991; Flaus and Richmond, 1998; Whitehouse *et al*., 1999) and *in vivo* (Thoma, 1986). This may occur by sliding histone octamers along the DNA sequence (nucleosome mobility), but a local dissociation and reassembly at a new position may also be possible. *In vivo* footprinting experiments revealed multiple positions for individual nucleosomes, suggesting that those positions reflect a dynamic equilibrium of nucleosome mobility (Thoma, 1986; Buttinelli *et al*., 1993; Thoma *et al*., 1993; Tanaka *et al*., 1996).

Since damage-induced distortions alter the bending properties of the DNA, it is conceivable that nucleosomes can change their positions as a result of damage induction. Bulky lesions and strong distortions that cannot be accommodated easily in the nucleosome might therefore end up in linker DNA. Thus, the preferential location of 6–4PPs in linker DNA could be the result of altered nucleosome positions after damage formation.

Direct evidence for damage-induced nucleosome mobility is missing. A change of a nucleosome position by irradiation of reconstituted HISAT-nucleosomes with UV light was not observed. However, the rotational setting was altered when DNA was extracted from irradiated nucleosomes and used again for a reconstitution (Schieferstein and Thoma, 1996). Similarly, UV-irradiated mixed sequence DNA adopted a preferential rotational setting when reconstituted in nucleosomes (Suquet and Smerdon, 1993). Those examples show that DNA lesions can affect nucleosome positioning, but *in vitro* it requires a disruption of histone–DNA contacts and reassembly in order to optimize the rotational setting and the accommodation of the DNA lesion.

DNA repair by photolyase in chromatin

To remove DNA lesions generated by sunlight, many organisms have enzymes that specifically bind to CPDs (CPD photolyase) or 6–4PPs (6–4 photolyases) and reverse the damage with the energy of light (photoreactivation). CPD photolyases were found in bacteria, fungi, plants, invertebrates and many vertebrates, while 6–4 photolyases were identified in *Drosophila*, silkworm, *Xenopus laevis* and rattlesnakes, but not in *Escherichia coli* or yeast. No photolyase was found in humans (Yasui *et al*., 1994; Sancar, 1996b; Todo, 1999) (Figure 2). Photolyases contain FAD as a catalytic cofactor and a second chromophore as

a light-harvesting antenna. The second chromophores are either 5,10-methenyltetrahydrofolate or 8-hydroxy-5-deazariboflavin, with absorption maxima of ~380 and ~440 nm, respectively. The crystal structures of CPD photolyase of *E.coli* and *Anacystis nidulans* suggest that upon binding to DNA, the enzymes flip the pyrimidine dimer out of the duplex into a hole that contains the catalytic cofactor (Park *et al*., 1995; Tamada *et al*., 1997). The cyclobutane ring is then split by a light-initiated electron transfer reaction. CPD photolyases recognize CPDs with a selectivity similar to that of sequence-specific DNA-binding proteins (Sancar *et al*., 1987), which suggests that they could compete with histones for DNA accessibility in a way similar to transcription factors.

Nucleosomes inhibit photoreactivation in vitro

DNA repair on the nucleosome surface requires that the damage is accessible and that DNA is flexible enough to support binding of the repair enzyme and the repair reaction. While >70% of nucleosome core DNA is accessible to a small probe of 0.4 nm diameter (Luger *et al*., 1997), the accessibility to proteins is much more restricted. DNase I, which binds to the minor groove, cuts nucleosomal DNA only every 10 base pairs, when the minor groove faces outside (Lutter, 1979). CPD accessibility and repair in nucleosomes were tested using reconstituted nucleosomes as model substrates and two damage-specific enzymes, T4 endonuclease V (T4 endoV) and *E.coli* DNA photolyase (Schieferstein and Thoma, 1998; Kosmoski and Smerdon, 1999). T4 endoV is another CPD-specific repair enzyme. It bends DNA at the lesion by 60°, flips-out the base opposite to the lesion and generates a single strand cut at the CPD (Gordon and Haseltine, 1980; Vassylyev *et al*., 1995). Although T4 endoV and photolyase were very efficient in naked DNA, their activity was reduced dramatically on the surface of the reconstituted nucleosomes. Thus, folding of DNA in nucleosomes efficiently protects DNA from being repaired (Figure 2). This is consistent with the observation that a fraction of CPDs (probably nucleosomal DNA) in SV40 minichromosomes or in lysed cells is resistant to cleavage by CPD-specific endonucleases (Wilkins and Hart, 1974; Evans and Linn, 1984).

On the HISAT-nucleosome (Schieferstein and Thoma, 1998), there was weak but clear site-specific repair, which was common for T4 endoV and photolyase, indicating that the structure of the nucleosome, and not the individual properties of the repair enzymes, is responsible for that effect. Taken together, inhibition of photolyase and T4 endoV, but efficient cutting by DNase I, indicate that nucleosomal DNA does not support the structural distortions and/or the base flip-out required for those repair enzymes.

Chromatin structure tightly modulates CPD repair by photolyase in vivo

Photoreactivation is an elegant reaction to investigate DNA damage recognition in chromatin in living cells, since only one enzyme is involved in this process and its activity can be regulated precisely by light. Photoreactivation experiments with chicken embryo fibroblasts (van de Merwe and Bronk, 1981) and injection of photolyases from yeast and *A.nidulans* in human fibroblasts (Zwetsloot *et al*., 1985) documented that a significant fraction of CPDs can be repaired despite packaging of DNA in chromatin. Another study showed that ~75% of the DNA was shielded from photorepair immediately after UV exposure, but all sites became available after 9–12 h (Pendrys, 1983). This was an indication that dynamic properties of chromatin could affect a DNA repair process.

Direct information on how photolyase interacts with nucleosomes, linker DNA and non-nucleosomal regions was obtained by comparison of CPD removal with chromatin structures in yeast (Suter *et al*., 1997). The yeast strains contained minichromosomes with the *URA3* or the *HIS3* gene, and an origin of replication ARS1. Both genes contain positioned nucleosomes flanked by nuclease-sensitive ('open') promoter regions and 3′ ends (Thoma, 1986; Losa *et al*., 1990). NER was inactivated by disruption of the *rad1* gene, which is essential for NER. Exposure of cells to photoreactivating light revealed two classes of repair. Repair was fast in linker DNA and nuclease-sensitive regions (complete in 15–30 min). On the other hand, ~2 h were required to remove CPDs, which mapped within the footprints of positioned nucleosomes. Thus, photoreactivation in living yeast cells is tightly modulated by chromatin structure (Figure 2).

In contrast to its severe inhibition on the nucleosome surface *in vitro* (see above), photolyase finds access to CPDs in nucleosomes *in vivo*. So far, there is no evidence that photolyase requires help from another protein (e.g. nucleosome remodelling activities) for damage recognition. This suggests that dynamic properties of nucleosomes *in vivo* allow repair (Figure 1). The simplest explanation is that nucleosome mobility exposes the lesion in the linker DNA, thus making it accessible to photolyase. This is consistent with the observation of nucleosome mobility (multiple nucleosome positions) in the *URA3* gene (Tanaka *et al*., 1996). Alternatively, instability of nucleosomes by dissociation/reassembly or a (partial) unfolding could also enhance damage accessibility.

Irrespective of how photolyase repairs nucleosomes, photoreactivation sheds light on how an individual repair enzyme interacts with chromatin. Thus, photolyase serves as an example of damage recognition in chromatin. In addition, I would like to emphasize that photolyase has the same accessibility to chromatin as micrococcal nuclease, which is used for chromatin analysis *in vitro*. This tight modulation of photoreactivation by chromatin structure makes photolyase a molecular tool to measure DNA accessibility and structural and dynamic properties of chromatin in living cells (Livingstone-Zatchej *et al*., 1999; Suter *et al*., 1999). The 2 h required for nucleosome repair compared with 15–30 min in linker DNA gives us a rough estimate of the stability of nucleosomes and nucleosome positions in yeast.

Nucleotide excision repair

In contrast to photoreactivation, NER is a much more complex pathway, which removes a wide variety of DNA-distorting lesions, including CPDs and 6–4PPs. It is present in most organisms and is highly conserved in eukaryotes. NER is not essential for viability, but defects in repair genes cause the sun-sensitive, cancer-prone genetic disorders xeroderma pigmentosum (XP), Cockayne's syndrome (CS)

and trichothiodystrophy (TTD) (Friedberg *et al*., 1995). NER is divided into two subpathways. Transcription-coupled repair (TC-NER) refers to the preferential repair of transcribed strands in active genes, while global genome repair (GG-NER) refers to repair in non-transcribed parts of the genome, including the non-transcribed strand of transcribed genes.

The molecular mechanism of the eukaryotic NER pathway has been worked out primarily in yeast and human cells, and the core reactions have been reconstituted *in vitro* from purified components using damaged DNA as a template (for reviews and original references, see Friedberg, 1996; Sancar, 1996a; de Laat *et al*., 1999; Guzder *et al*., 1999; Wood, 1999). Figure 3 summarizes the basic reaction according to recent models of the human pathway (de Laat *et al*., 1999; Wood, 1999) and illustrates how it might work in chromatin. The human and, in analogy, the yeast components are indicated side by side. In the first step, the XPC-hHR23B proteins (and the yeast Rad4–Rad23 complex) act as damage detectors that bind to the DNA distortion and initiate NER (Sugasawa *et al*., 1998). In a second step, XPA (Rad14), RPA (Rfa) and the general transcription factor TFIIH enter the reaction to form an open complex. DNA helicase activities of XPB (Rad25/Ssl2) and XPD (Rad3) in TFIIH are used to unwind the DNA. A preference of XPA and Rad14 for binding damaged DNA (Robins *et al*., 1991; Guzder *et al*., 1993) and the observation that Rad3 helicase activity is inhibited by DNA damage (Naegeli *et al*., 1992) suggest that those proteins are involved in verification of the damage site in the open complex (Sugasawa *et al*., 1998). In a next step, nuclease activity is recruited. The 3′ incision is made by XPG (Rad2), the 5′ incision by a complex of XPF–ERCC1 (Rad1–Rad10). Finally, the gap is filled by DNA synthesis and ligation to form a 'repair patch'.

With respect to the first steps in damage recognition, an alternative order of assembly of the excision complex has been postulated (Wakasugi and Sancar, 1999). In this case, XPA and RPA are the initial damage-sensing factors that bind damaged DNA and then recruit TFIIH, XPC and hHR23B to form an open complex ('pre-incision complex'). In the yeast *in vitro* system, damage recognition is supported by Rad7 and Rad16. Rad7 and Rad16 form a complex which, together with the Rad4–Rad23 complex, binds to UV-damaged DNA synergistically and in a reaction that is dependent on ATP (Guzder *et al*., 1997, 1999).

Nucleotide excision repair in chromatin and the question of chromatin remodelling

The complexity of the NER pathway makes it difficult to imagine how DNA lesions can be recognized and processed in chromatin. The first step (Figure 3, step 1) is damage recognition. It is not known whether the damage recognition complexes can interact with lesions on the nucleosome surface; however, it is likely that they recognize lesions in linker DNA, which is not protected by chromosomal proteins. Thus, in analogy to photoreactivation, exposure of DNA lesions in linker DNA or a disruption or unfolding of nucleosomes by 'natural' dynamic properties would be sufficient to allow damage recognition.

The next steps in NER need more space (Figure 3, steps 2–4). About 25 bp of DNA are unwound in the open complex (Evans *et al*., 1997), and the human excision complex requires ~100 bp of DNA to excise the lesion *in vitro* (Huang and Sancar, 1994). Such a complex appears to be incompatible with the structure of a nucleosome, and the linker DNA between nucleosomes (0–90 bp) is too short to accommodate a repair complex. Hence, nucleosomes need to be disrupted or rearranged or histones need to be removed. Disruption of one nucleosome, or unwrapping of one turn of nucleosomal DNA (80 bp) together with the linker DNA, or sliding of a nucleosome by a few base pairs would be sufficient to provide space for NER. There is no obvious requirement to remove nucleosomes over long distances. A disruption of higher order structures (e.g. by removal of histone H1 and by histone acetylation), however, might be necessary to facilitate the access to nucleosomes or enhance nucleosome dynamics.

Repair patches are nuclease sensitive and not folded into nucleosomes immediately after DNA synthesis (see below; Figure 3, step 4). This implies a removal or destabilization of nucleosomes during an earlier step of NER. It is unknown how this chromatin remodelling happens, but conceptually we have to distinguish two components: the natural dynamic properties of chromatin (Figure 1) and an active chromatin remodelling process driven by enzymatic activities.

Acetylation of histones is a general way by which nucleosomes and chromatin fibres are believed to be destabilized. Consistent with a general effect of acetylation, it was found that sodium butyrate, which inhibits histone deacetylation, stimulates NER in human cells at concentrations where the histones are hyperacetylated maximally (Smerdon *et al*., 1982). This experiment, however, does not tell us whether the damaged nucleosomes are acetylated selectively at the site of and prior to repair.

Given the rare distribution of DNA lesions in the genome, a putative remodelling activity should be targeted to the site of the lesion and therefore be coupled to the damage recognition process. This requirement is reminiscent of the transcriptional activation process, which applies remodelling of nucleosomes to facilitate binding of transcription factors to DNA. Such activities are provided by several complexes that may contain histone acetyltransferases as well as ATP-driven molecular machines that disrupt histone–DNA interactions or promote sliding (e.g. SWI/SNF and NURF complexes) (Imbalzano, 1998; Workman and Kingston, 1998; Whitehouse *et al*., 1999). It is conceivable that similar activities could facilitate NER in chromatin.

Factors that participate in this early step of NER are the human DDB and the yeast Rad7–Rad16 proteins. DDB is a DNA-damage binding protein, which is lacking in some XPE patients (Protic *et al*., 1989). Purified DDB protein did not stimulate repair of naked DNA by extracts of XP-E cells, but microinjection of the protein into XP-E cells could partially correct the repair defect (Rapic Otrin *et al*., 1998), suggesting a role in chromatin repair. In *Saccharomyces cerevisiae*, the *RAD7* and *RAD16* genes are required for efficient repair of transcriptionally inactive chromatin and the non-transcribed strand of transcribed

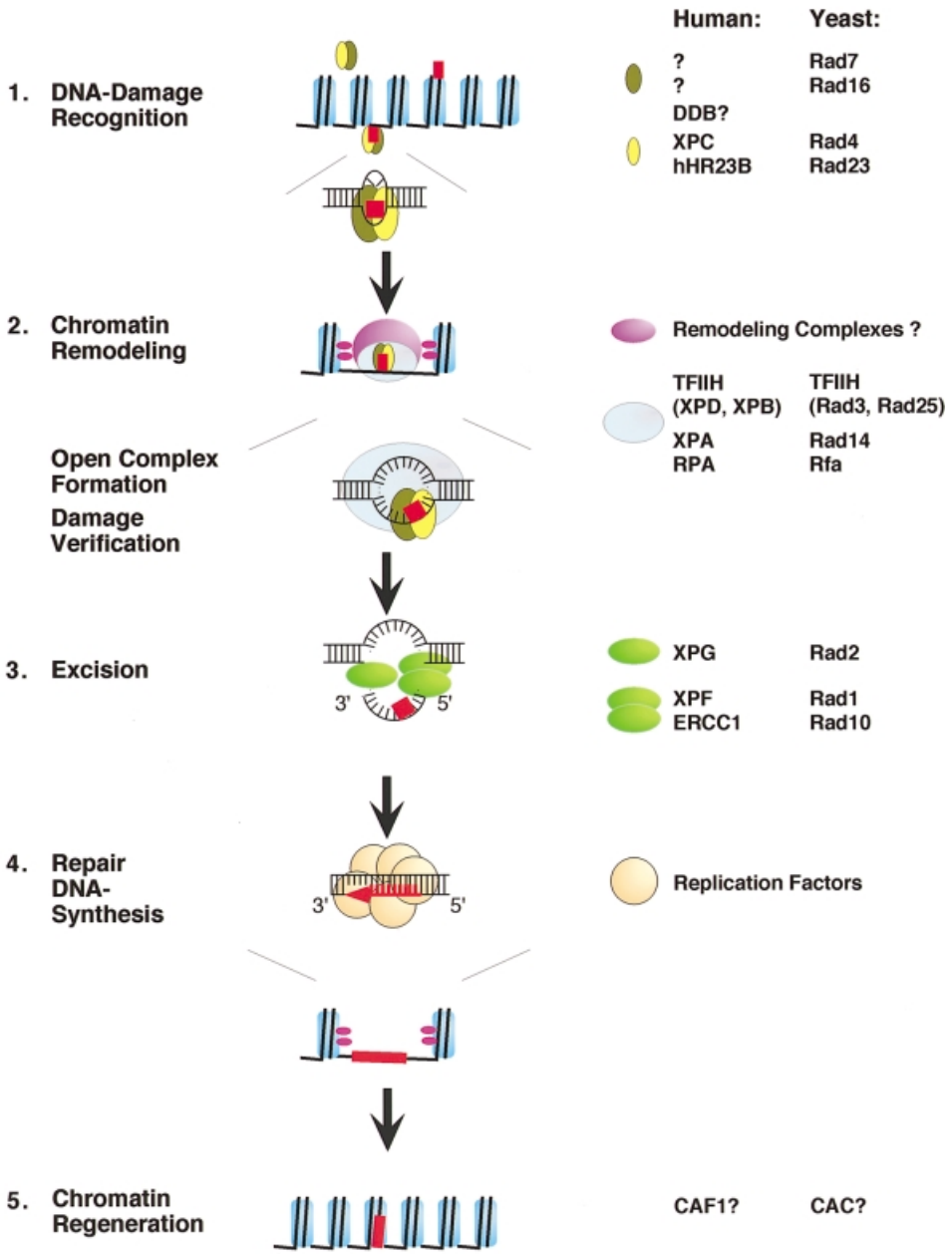

Fig. 3. Schematic illustration of nucleotide excision repair in chromatin. The basic reaction in DNA (inserts) is adopted from de Laat *et al*. (1999) and Wood (1999). The major repair components are indicated for human and *S.cerevisiae*. Damage recognition (1) is followed by open complex formation and DNA damage verification (2). Damage recognition might occur preferentially in linker DNA and could be facilitated by dynamic properties of nucleosomes that shift DNA lesions into linker DNA (see Figure 1). The space required for open complex formation might be provided by chromatin remodelling, possibly with the help of nucleosome remodelling activities. After excision of the damaged nucleotide (3) and DNA synthesis (4), the repair patch (red bar) is not folded in nucleosomes. Regeneration of chromatin (5) requires redistribution of nucleosomes (mobility), and refolding and/or reassembly of nucleosomes, possibly with the help of chromatin assembly factors (CAF1 and CAC). Nucleosomes, DNA and DNA lesions are indicated as in Figures 1 and 2. Indicated are proteins involved in damage recognition (yellow, dark green) and in open complex formation (grey), remodelling complexes (purple), nucleases (green) and replication factors (brown). Putative histone modifications by acetylation are shown as red dots on the histones (blue).

genes, while they are not required for NER of the transcribed strand (Verhage *et al*., 1994, 1996b; Mueller and Smerdon, 1995). Rad7 interacts with Sir3, a protein involved in chromatin organization of silenced genes, and a *sir3* deletion rescues part of the UV sensitivity associated with a *rad7* deletion, thus indicating a chromatin–repair connection (Paetkau *et al*., 1994). Rad7 binds to DNA unspecifically, and the Rad7–Rad16 complex recognizes UV lesions in an ATP-dependent way. This suggests a model in which ATP hydrolysis promotes translocation of the complex on DNA in search of UV lesions (Guzder *et al*., 1998b, 1999). Rad16 has homology to Snf2, a protein of the SWI/SNF nucleosome remodelling complex (Schild *et al*., 1992). Since this complex can slide and remodel nucleosomes in an ATP-dependent reaction (Whitehouse *et al*., 1999), it is conceivable that Rad7–Rad16 directly opens up a chromatin gap by promoting nucleosome sliding or that it recruits nucleosome remodelling activities (SWI/SNF components; Figure 3, step2).

We also need to consider that helicases (T-antigen of

SV40 or the *E.coli* recBCD) can bind to nucleosome substrates, unwind DNA and disrupt nucleosomes (Eggleston *et al*., 1995; Ramsperger and Stahl, 1995). Therefore, it seems conceivable that the helicases of the NER process (XPD/Rad3, XPB/Rad25), which are recruited to the reaction in the process of open complex formation (Figure 3, step 2), could help to clear DNA of histones and open up the repair site. Since each nucleosome stores one negative supercoil of DNA and since melting of DNA generates positive supercoils, the helicase activity during open complex formation might indeed facilitate nucleosome disruption.

Once NER is initiated and space is available, excision and DNA repair synthesis will occur and leave a repair patch that is not folded in nucleosomes. Regeneration of chromatin will need an additional remodelling process (see below).

Nucleotide excision repair at the nucleosome level

What is known about NER at the nucleosome level? Wilkins and Hart discovered that a fraction of UV-induced dimers was resistant to NER for 24–48 h and is possibly shielded by histone proteins (Wilkins and Hart, 1974). The NER reaction was not tested on nucleosomes reconstituted with DNA of defined sequence. However, human cell extracts were unable to repair UV-irradiated plasmid DNA effectively that was reconstituted with nucleosomes (Wang *et al*., 1991), and the reaction was less efficient in SV40 minichromosomes than in naked DNA (Sugasawa *et al*., 1993). Thus, NER appears to be suppressed by the presence of nucleosomes. The chromatin substrates used in those experiments apparently did not provide the dynamic properties required for efficient repair or the extracts missed the putative remodelling activities.

How are DNA lesions removed from nucleosomes *in vivo*? To address this question, nucleosomes were isolated after different repair times and their CPD distribution was analysed. After damage induction, the CPD pattern displays a characteristic 10.3 base periodicity (see above). After different repair times, little or no change in the periodic pattern was observed, suggesting that CPDs are removed at nearly equal rates from the inner and outer surfaces of the DNA on the nucleosome. Moreover, no preferential repair was observed towards the ends of the nucleosomes (Jensen and Smerdon, 1990). Under the assumption that nucleosomes do not rearrange during isolation, this result suggests that the rotational setting of nucleosomal DNA and the location of the lesion in the nucleosome are not critical for damage recognition and processing. This observation would be consistent with the idea that the nucleosome is absent or disrupted at the moment of initiation of NER (e.g. that the lesion is in linker DNA; Figure 3, step 1).

In an alternative approach, NER was investigated by comparison of pyrimidine dimer removal with chromatin structure in yeast minichromosomes as described for photoreactivation (Smerdon and Thoma, 1990; Smerdon *et al*., 1990). The minichromosome, YRpTRURAP, contained the *URA3* gene, which is rarely transcribed and most of the time is in an inactive state (Bedoyan *et al*., 1992). Chromatin analysis revealed nucleosomes, each with multiple positions and with a region of reduced accessibility to DNase I ('internal protected region') (Tanaka *et al*., 1996). PDs (CPDs and 6–4PPs) were mapped by primer extension using *Taq* polymerase, which is blocked at CPDs and 6–4PPs (Wellinger and Thoma, 1996). While repair rates on the transcribed strand were dominated by transcription-coupled repair and showed no correlation with chromatin structure (see below), analysis of the non-transcribed strand revealed pronounced heterogeneity in repair rates. Fast repair correlated with PD locations in linker DNA and towards the 5′ end of a nucleosome. Slow repair correlated with the 'internal protected region' (Wellinger and Thoma, 1997). Similar results were reported for the genomic copy of the *URA3* gene and for removal of 6–4PPs, indicating that modulation by chromatin structure is not damage dependent (Tijsterman *et al*., 1999). These results provide strong evidence that NER is modulated by the arrangement of nucleosomes along the DNA.

There seems to be a discrepancy between the results in human and yeast cells. In contrast to human cells, the yeast experiments revealed enhanced repair towards the end of nucleosomes. One explanation could be differences in the methods. Alternatively, differences in chromatin structure between yeast and human cells should be considered. Although the primary structure of yeast nucleosomes is similar to that of higher eukaryotes (Bavykin *et al*., 1985), they contain hyperacetylated core histones (Davie *et al*., 1981) and differ in some physical properties such as thermal or salt stability (Morse *et al*., 1987; Pineiro *et al*., 1991). Moreover, in contrast to higher eukaryotes, yeast is missing a conventional histone H1, which stabilizes the nucleosomes (Thoma *et al*., 1979) and prevents nucleosome mobility *in vitro* (Pennings *et al*., 1994). Thus, a reduced nucleosome stability and enhanced mobility in yeast could facilitate damage recognition and repair. Moreover, in the absence of H1, the ends of nucleosomal DNA are bound less tightly to the histone octamer and interact more efficiently with transcription factors (Workman and Kingston, 1998). This could also contribute to preferential repair towards the end of a nucleosome.

How are 6–4PPs removed from nucleosomes and linker DNA? Radioimmunoassays showed that 6–4PPs are removed much faster than CPDs from the genome overall (Mitchell *et al*., 1985), as well as from nucleosome cores and chromatosomes [a nucleosome particle containing 166 bp of DNA and histone H1 (Simpson, 1978)] (Mitchell *et al*., 1990; Suquet *et al*., 1995). Repair of 6–4PPs was also faster than repair of CPDs in a nucleosomal region of a yeast gene (Tijsterman *et al*., 1999). One explanation for preferential repair of 6–4PPs could be enhanced damage recognition, since several damage recognition proteins preferentially bind 6–4PPs rather than CPDs, including the NER proteins Rad14 (Guzder *et al*., 1993), the Rad4–Rad23 complex (Guzder *et al*., 1998a) and XPA (Jones and Wood, 1993). On the other hand, it seems more likely that 6–4PPs (due to the strong bend in DNA) are exposed more frequently in linker DNA than CPDs, thus allowing rapid repair.

In view of the observations described above, it seems reasonable to assume that damage recognition occurs predominantly in linker DNA or in an altered nucleosome and that natural dynamic properties provide a transient window of accessibility for the lesions.

Nucleosome rearrangements after DNA repair synthesis

Immediately after repair synthesis, repair patches are sensitive to micrococcal nuclease and do not reveal the 10 bp DNase I periodicity characteristic for nucleosomal DNA. Later, repair patches become nuclease resistant and are found in nucleosome core DNA (Smerdon and Lieberman, 1978, 1980). While there is a preference for repair patch distribution towards the ends of a nucleosome in the early phase of repair, the repair patches become distributed more evenly in the late phase (Jensen and Smerdon, 1990). These experiments demonstrate that immediately after DNA repair synthesis, nucleosomes are disrupted or absent and that chromatin is regenerated and reorganized slowly after DNA repair synthesis (Figure 3, steps 4 and 5).

The details of this chromatin maturation step are unknown. It probably requires refolding or repositioning of pre-existing nucleosomes, nucleosome assembly and packaging into higher order structures (Smerdon and Thoma, 1998; Conconi *et al*., 1999). Slightly enhanced binding of repair patch-containing chromatin fragments to an organomercury column implicates (partially) unfolded or incompletely assembled nucleosomes as a structural intermediate (Baxter and Smerdon, 1998). Biochemical support for a link between nucleosome assembly and DNA repair comes from *in vitro* experiments with extracts from *Xenopus* eggs, human cells and *Drosophila* embryos. It was reported that UV damage-dependent DNA synthesis occurs simultaneously with chromatin assembly and that chromatin assembly factor 1 (CAF1) was necessary for repair-associated chromatin formation (Gaillard *et al*., 1996, 1997). Genetic evidence comes from yeast strains lacking chromatin assembly factor I (CAC). Those strains show enhanced UV sensitivity which is consistent with a DNA repair deficiency (Kaufman *et al*., 1997). However, the CAC-dependent repair deficiency is not restricted to the NER pathway (Game and Kaufman, 1999), indicating that CAC appears to play a more general role. Surprisingly, chromatin assembly *in vitro* was still effective when the DNA synthesis step in the NER process was inhibited (Gaillard *et al*., 1997). This raises the possibility that the CAF1-dependent chromatin assembly operates whenever a nucleosome-free gap is generated.

Search mechanisms for damage recognition in chromatin

How do the damage recognition proteins find and identify a single lesion within thousands of base pairs of DNA in chromatin? Sequence-specific proteins can find their target site by three-dimensional diffusion from solution, which is a distributive mechanism, or by one-dimensional (linear) diffusion, which is a processive mechanism. Linear diffusion requires non-specific binding to DNA followed by moving along the DNA in a search for specific sites (Berg *et al*., 1981; Hanawalt, 1993).

Among the DNA repair enzymes, uracil DNA-glycosylase and T4 endoV are processive in low salt conditions, but distributive in higher salt (Ganesan *et al*., 1986; Gruskin and Lloyd, 1986; Higley and Lloyd, 1993). On naked DNA, NER is achieved via a random diffusion mechanism (Szymkowski *et al*., 1993), but one-dimensional search was proposed for Rad7–Rad16 (Guzder *et al*., 1998b) and for photolyase in low salt (van Noort *et al*., 1998). The mechanisms by which NER and photolyase locate DNA lesions in chromatin *in vivo* are not known, but the high salt conditions in nuclei favour a distributive mechanism. Moreover, the UV damage-dependent intracellular redistribution of the NER endonuclease ERCC1/XPF tagged with green fluorescent protein suggests that ERCC1/XPF participates in repair of DNA damage in a distributive fashion rather than by processive scanning of large genome segments (Houtsmuller *et al*., 1999).

One-dimensional search can be achieved in different ways (Berg *et al*., 1981; Hanawalt, 1993). One way is by sliding along the contour length of DNA or by tracking along a DNA strand [as RNA polymerases (RNAPs) do]. This appears to be possible for short distances in linker DNA or in non-nucleosomal regions. In the nucleosomes, however, this process is sterically restricted and might require disruption of nucleosomes in an energy-consuming reaction. Thus a long distance search through numerous nucleosomes appears to be unlikely.

An alternative mode of linear diffusion is microscopic dissociation–reassociation between closely spaced sites in the DNA molecule ('hopping'), and intersegmental transfer of proteins between different segments of the same DNA molecule (Berg *et al*., 1981; Hanawalt, 1993). These mechanisms would be facilitated by the close proximity of DNA strands in nucleosomes and chromatin fibres. In combination with the nucleosome dynamics, which provide the window of damage accessibility (Figure 1), those mechanisms could indeed facilitate the search for DNA lesions.

It will be important to extend the damage recognition studies to chromatin substrates and to see whether an ATP-dependent translocation of the Rad7–Rad16/Rad4–Rad23 complex is possible in nucleosome arrays. The other class of ATP-consuming proteins of the NER process, the helicases (XPD/Rad3, XPB/Rad25), are less likely to participate in a long distance search, since they belong to the same complex.

Interactions between transcription and repair

Transcription produces additional levels of complexity in the studies of damage formation and repair. First, eukaryotic cells have three systems to transcribe different sets of genes, with different RNAPs and transcription factors. The genes are of variable length and therefore have a different probability of being hit by DNA-damaging agents. Moreover, their distribution, copy number and location in the nucleus vary. Second, transcription leads to transient alterations in chromatin structure which, as discussed above, affect damage formation and repair. NER of transcribed genes has been reviewed extensively (Friedberg, 1996; Sancar, 1996a; Wood, 1996; Hanawalt, 1998). Here, I focus on the contributions of NER and photoreactivation to CPD repair in active genes and their interactions with polymerases and chromatin (Figure 4).

DNA lesions can inhibit transcription and transcription can inhibit DNA repair by photolyase

It is well known that UV lesions inhibit RNA synthesis and that recovery of RNA synthesis is important for cell

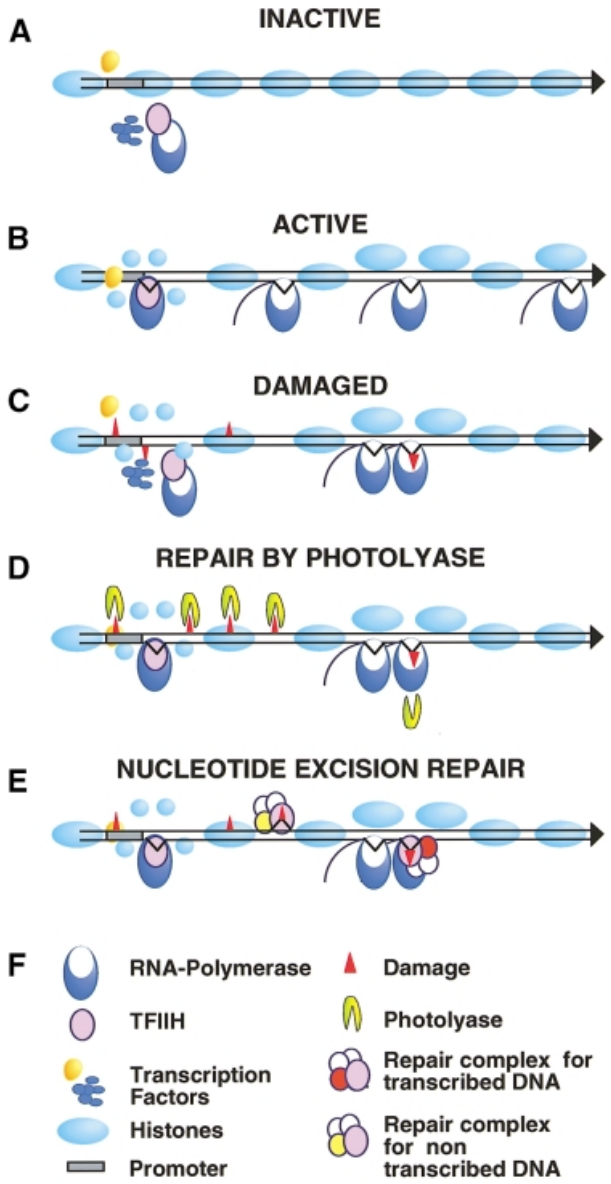

Fig. 4. Transcription-dependent chromatin changes and repair of genes transcribed by RNAP II. (**A**) A transcriptionally inactive gene packaged in nucleosomes. (**B**) A transcriptionally active gene with nucleosome remodelling at the promoter, elongating RNAPs and rearranged nucleosomes. (**C**) A damaged gene. DNA lesions may affect binding of transcription factors thus affecting initiation. DNA damage blocks the elongation of RNAP. Other RNAPs may initiate and line up at the lesion. Downstream of the lesions, the gene becomes depleted of RNAPs. (**D**) Gene repair by photolyase. RNAP II is blocked at a CPD on the transcribed strand and inhibits access of photolyase to the lesion, explaining slow repair of the transcribed strand. In the non-transcribed strand, photoreactivation is fast in linker DNA and open regions, but slow in nucleosomes (see the text and Figure 2). Photoreactivation can be inhibited by factors bound to the lesion. (**E**) Nucleotide excision repair. NER of the non-transcribed strand is modulated by chromatin structure, it is fast in linker DNA, slow in nucleosomes and can be inhibited by factors bound to a lesion. NER of the transcribed strand is initiated by RNAP II blocked at a CPD. It promotes assembly of the NER machinery, explaining preferential repair of the transcribed strand (transcription-coupled repair). Sharing of proteins between the transcription machinery and the NER is indicated (TFIIH). Repair of the transcribed strand requires the human factors CSA and CSB or yeast factor Rad26 (red circles); repair of non-transcribed DNA requires XPC-hHR23B (Rad4–Rad23) and Rad7–Rad16 (yellow circle). (**F**) Legends to symbols.

survival. Inhibition of RNA synthesis occurs by stalling the elongation of RNAP at DNA lesions (Figure 4C). Thus, a central question is how these blocks are removed to allow completion of RNA synthesis (Hanawalt and Mellon, 1993; Hanawalt, 1994). *In vitro*, RNAPs are blocked by pyrimidine dimers on the transcribed strand, but they can bypass lesions on the non-transcribed strand (Selby and Sancar, 1990; Donahue *et al*., 1994). A blocked *E.coli* RNAP inhibits the excision of the photodimer by (A)BC excinuclease (Selby and Sancar, 1990). A blocked human RNAP II inhibits access of CPDs to photolyases (Donahue *et al*., 1994), but neither stimulates nor inhibits excision repair (Selby *et al*., 1997). In yeast genes transcribed by RNAP II and RNAP III, the transcribed strands were repaired more slowly by photolyase than the non-transcribed strands (Figure 4D). In inactive genes, both strands were repaired at similar rates (Livingstone-Zatchej *et al*., 1997; Suter *et al*., 1997). CPDs were also repaired more slowly by NER on the transcribed strand of active RNAP III genes in yeast (Aboussekhra and Thoma, 1998). Thus, RNAP II and III are blocked by lesions and can inhibit repair processes in living cells. To allow repair, RNAP must either fall off or step back. *In vitro*, the complex of RNAP II at a lesion is remarkably stable, with a half-life of ~20 h (Selby *et al*., 1997). In yeast, however, the complex seems to be less stable, since photolyase repairs ~80% of the CPDs in the transcribed strand in 2 h. For RNAP II transcription, it was shown that the transcription elongation factor SII (TFIIS) catalyses cleavage of nascent transcripts at the pause sites and enables the RNAP II to back off without aborting the incomplete transcript (Donahue *et al*., 1994; Tornaletti *et al*., 1999).

Nucleotide excision repair of transcribed genes

While it is readily accepted that a stalled polymerase inhibits repair, it was most surprising to realize that transcription can facilitate NER. This process, referred to as transcription-coupled repair (TC-NER), is ubiquitous from *E.coli* to yeast and humans. It is based on the following observations. (i) Transcribed genes are repaired more quickly than non-transcribed DNA ('gene-specific repair') (Bohr *et al*., 1985). (ii) Lesions are removed more quickly from the transcribed strand than the non-transcribed strand. This 'strand-specific repair' was observed originally in human and hamster cells (Mellon *et al*., 1987), and later in many organisms including *E.coli* (Mellon and Hanawalt, 1989) and yeast (Smerdon and Thoma, 1990; Leadon and Lawrence, 1992; Sweder and Hanawalt, 1992). (iii) The general transcription factor TFIIH plays a dual role in initiation of transcription by RNAP II and in NER (Feaver *et al*., 1993; Schaeffer *et al*., 1993; Drapkin *et al*., 1994). (iv) Mutations in the *CSA* and *CSB* genes of Cockayne's syndrome patients and mutations in the yeast homologue of *CSB*, *RAD26*, lead to a defect in repair of the transcribed strand (Venema *et al*., 1990; Tijsterman *et al*., 1997). The yeast homologue of *CSA*, *RAD28*, is not required for repair of the transcribed strand (Bhatia *et al*., 1996). A current model of transcription repair coupling in human cells is as follows (Friedberg, 1996; Sancar, 1996a; de Laat *et al*., 1999): RNAP II stalls at a lesion. CSA/CSB, possibly with the help of another general transcription factor (TFIIS), bind to the complex. RNAP II backs off the lesion without

releasing the RNA. CSA/CSB recruit XPA and TFIIH and promote the assembly of the repair complex. After excision and repair synthesis, RNAP II resumes transcription (Figure 4E).

In eukaryotic cells, preferential repair of the transcribed strand generally is found in genes transcribed by RNAP II, while the observations on genes transcribed by the other polymerases are controversial and may indicate different properties of transcription complexes. The rRNA genes transcribed by RNAP I showed no repair or very modest repair in human and hamster cells, but no evidence for transcription-coupled repair (Christians and Hanawalt, 1993; Fritz and Smerdon, 1995). Preferential repair of the transcribed strand, however, was observed in yeast, but only in *rad7* and *rad16* mutants that are defective in repair of non-transcribed DNA, and in a *rad4* mutant that otherwise seems completely NER deficient (Verhage *et al*., 1996a). Genes transcribed by RNAP III showed no preferential repair in humans (Dammann and Pfeifer, 1997). In yeast, however, NER of the transcribed strand is even slower than in the non-transcribed strand (Aboussekhra and Thoma, 1998). Thus, the observation that in eukaryotic cells only the RNAP II genes show TC-NER suggests that TC-NER evolved to ensure efficient repair of single copy genes and long genes. The genes transcribed by RNAP III are short, which reduces their chance of being damaged, while RNAP I genes occur in multiple copies, which reduces the chance that all genes are inactivated simultaneously by a DNA lesion.

Chromatin structure and repair of transcribed genes

The dynamic properties of transcription-dependent chromatin transitions make it difficult to assess how chromatin structure in transcribed genes affects DNA repair (Figure 4). Nucleosomes are lost in rRNA genes transcribed by RNAP I (Conconi *et al*., 1989; Dammann *et al*., 1993), but are present in genes transcribed by RNAP II (McKnight *et al*., 1978; De Bernardin *et al*., 1986; Nacheva *et al*., 1989; Daneholt, 1992; Cavalli and Thoma, 1993). The fate of nucleosomes might depend on the RNAP and the rate of transcription. *In vitro*, nucleosomes inhibit initiation of transcription and are an obstacle during elongation. Initiation needs the help of transcription factors and nucleosome remodelling activities (Workman and Kingston, 1998) and elongation requires a factor to release RNAP II from a nucleosome-induced block (LeRoy *et al*., 1998). Experiments with phage polymerases and RNAP III suggest that the histone octamer is transferred from a position in front of the polymerase to one behind (Studitsky *et al*., 1995, 1997), which is also consistent with the nucleosome rearrangement observed in transcribed yeast genes (Figure 4B) (Cavalli and Thoma, 1993; Cavalli *et al*., 1996). Regeneration of the inactive chromatin structure after inactivation of transcription is a fast process that may not require replication (Schmid *et al*., 1992; Cavalli and Thoma, 1993).

Given that chromatin remodels rapidly after inactivation of transcription and that RNAPs can be arrested for many hours, it must be assumed that chromatin rearranges around a blocked polymerase (Figure 4C). Lost nucleosomes could be replaced, disrupted nucleosomes refolded, or displaced nucleosomes repositioned. Downstream of stalled polymerases, the genes might become depleted of transcribing RNAPs and adopt an inactive structure. Upstream, it is conceivable that polymerases continue to initiate at the promoter and queue up behind the stalled polymerase. At the damage site, repair will depend on: (i) the half-life of the stalled complex; (ii) the time required to recruit the NER complex; (iii) the rate of transcription that would allow replacement of a polymerase after the previous polymerase detached from the lesion; and (iv) the time required to generate a nucleosome or rearrange nucleosomes after a polymerase detaches from the lesion.

Several studies on site-specific repair of RNAP II-transcribed genes in different cells and organisms show fast repair rates on the transcribed strand, which is generally consistent with transcription-coupled repair, but variable degrees of heterogeneity in the transcribed and non-transcribed strands (Smerdon and Thoma, 1990; Tornaletti and Pfeifer, 1994; Mueller and Smerdon, 1995; Tijsterman *et al*., 1996; Tu *et al*., 1996; Teng *et al*., 1997; Wellinger and Thoma, 1997; Li *et al*., 1999). It seems likely that this variability is related to the different contribution of nucleosomes and stalled RNAP II. A more precise interpretation is possible in the case of the yeast *URA3* gene where chromatin data and repair data of both stands are available. Only the non-transcribed strand showed a modulation of NER with nucleosome position, while repair rates in the transcribed strands were faster, more uniform and did not correlate with the chromatin structure (Wellinger and Thoma, 1997). Moreover, 6–4PPs, which are repaired more quickly in the genome overall than CPDs, were repaired with similar rates as CPDs in the transcribed strand (Tijsterman *et al*., 1999). These data are consistent with a dominant role of the stalled polymerase in repair initiation.

Interactions between photolyase and NER

Cells that are exposed to sunlight are exposed simultaneously to damage-inducing radiation and photoreactivating light. What are the contributions of each pathway to removal of the major photoproduct, CPDs? In yeast, photolyase is much faster than NER in repair of nucleosome-free regions, such as promoters and origins of replication, which identifies a role for photolyase in regenerating regulatory regions. Since the DNA lesions in nuclease-sensitive regions are readily accessible to photolyase, slow repair by NER indicates that there is either a limitation in damage recognition proteins, or that the assembly of the NER complex is slow. In genes transcribed by RNAP II, photolyase and NER serve complementary roles (Figure 4D and E); they preferentially remove lesions in the non-transcribed and transcribed strands, respectively, and ensure the efficient repair of active genes (Livingstone-Zatchej *et al*., 1997; Suter *et al*., 1997). On the other hand, NER is indispensable for removal of 6–4PPs and can remove CPDs that are not accessible to photolyase, such as CPDs generated in the TATA-box of the yeast *SNR6* gene (Aboussekhra and Thoma, 1999).

A number of organisms and tissues that are never exposed to sunlight express photolyase, suggesting a non-photoreactivation function for this enzyme (Ozer *et al*., 1995). In the dark, photolyase stimulates removal of UV

damage by NER in yeast (Sancar and Smith, 1989) and in *E.coli* (Yamamoto *et al*., 1983). Furthermore, photolyase binds to other lesions (e.g. *cis*-diamminedichloroplatinum adducts) and either inhibits NER of those lesions in *S.cerevisiae* (Fox *et al*., 1994) or enhances it in *E.coli* (Ozer *et al*., 1995). These observations suggest an interaction between NER and photolyase, most likely at the level of DNA damage recognition. Hence, knowing the characteristics of CPD recognition by photolyase in chromatin could provide further insight into the damage recognition process of NER and into the interaction between these two repair mechanisms.

Conclusions

Studies in recent years have established that chromatin plays a central role in modulation of DNA-dependent processes. The studies on NER and photoreactivation of UV lesions provide clear examples for mutual interactions and functional links between chromatin structures, transcription and DNA repair processes. Repair of UV lesions by photolyase and NER is just one example of cellular reactions to environmental mutagens. Defending the genome against other lesions using other repair enzymes and pathways may occur with a similar complexity. Base excision repair (BER) is responsible for repair of the major lesions generated by active oxygen or simple alkylating agents. It is initiated by DNA-glycosylases that specifically recognize the lesion and excise the modified base using a 'flip-out' mechanism similar to that of photolyase. It is therefore surprising that no tight correlation has been found so far between BER and chromatin structure and that repair of thymine glycols is linked to transcription, while BER of other lesions is not (for references, see Ye *et al*., 1998; Li and Smerdon, 1999). This illustrates that different repair proteins may use different methods to cope with chromatin structures and underlines the significance of, and the challenge of investigating, repair processes in the chromatin of living cells.

Acknowledgements

I thank Dr B.Suter and A.Meier for critical comments on the manuscript and Dr U.Suter for continuous support. This work was supported by the Swiss National Science Foundation and by the Swiss Federal Institute of Technology Zürich (ETH).

References

Aboussekhra,A. and Thoma,F. (1998) Nucleotide excision repair and photolyase preferentially repair the nontranscribed strand of RNA polymerase III-transcribed genes in *Saccharomyces cerevisiae*. *Genes Dev.*, **12**, 411–421.

Aboussekhra,A. and Thoma,F. (1999) TATA-binding protein promotes the selective formation of UV-induced (6–4)-photoproducts and modulates DNA repair in the TATA box. *EMBO J.*, **18**, 433–443.

Axelrod,J.D., Reagan,M.S. and Majors,J. (1993) *GAL4* disrupts a repressing nucleosome during activation of *GAL1* transcription *in vivo*. *Genes Dev.*, **7**, 857–869.

Bavykin,S.G., Usachenko,S.I., Lishanskaya,A.I., Shick,V.V., Belyavsky,A.V., Undritsov,I.M., Strokov,A.A., Zalenskaya,I.A. and Mirzabekov,A.D. (1985) Primary organization of nucleosomal core particles is invariable in repressed and active nuclei from animal, plant and yeast cells. *Nucleic Acids Res*., **13**, 3439–3459.

Baxter,B.K. and Smerdon,M.J. (1998) Nucleosome unfolding during DNA repair in normal and xeroderma pigmentosum (group C) human cells. *J. Biol. Chem*., **273**, 17517–17524.

Beard,P. (1978) Mobility of histones on the chromosome of simian virus 40. *Cell*, **15**, 955–967.

Becker,M.M. and Wang,J.C. (1984) Use of light for footprinting DNA *in vivo*. *Nature*, **309**, 682–687.

Becker,M.M. and Wang,Z. (1989) Origin of ultraviolet damage in DNA. *J. Mol. Biol*., **210**, 429–438.

Becker,M.M., Wang,Z., Grossmann,G. and Becherer,K.A. (1989) Genomic footprinting in mammalian cells with ultraviolet light. *Proc. Natl Acad. Sci. USA*, **86**, 5315–5319.

Bedoyan,J., Gupta,R., Thoma,F. and Smerdon,M.J. (1992) Transcription, nucleosome stability and DNA repair in a yeast minichromosome. *J. Biol. Chem*., **267**, 5996–6005.

Berg,O.G., Winter,R.B. and von Hippel,P.H. (1981) Diffusion-driven mechanisms of protein translocation on nucleic acids. 1. Models and theory. *Biochemistry*, **20**, 6929–6948.

Bhatia,P.K., Verhage,R.A., Brouwer,J. and Friedberg,E.C. (1996) Molecular cloning and characterization of *Saccharomyces cerevisiae RAD28*, the yeast homolog of the human Cockayne syndrome A (*CSA*) gene. *J. Bacteriol*., **178**, 5977–5988.

Bohr,V.A., Smith,C.A., Okumoto,D.S. and Hanawalt,P.C. (1985) DNA repair in an active gene: removal of pyrimidine dimers from the *DHFR* gene of CHO cells is much more efficient than in the genome overall. *Cell*, **40**, 359–369.

Brown,D.W., Libertini,L.J., Suquet,C., Small,E.W. and Smerdon,M.J. (1993) Unfolding of nucleosome cores dramatically changes the distribution of ultraviolet photoproducts in DNA. *Biochemistry*, **32**, 10527–10531.

Buttinelli,M., DiMauro,E.D. and Negri,R. (1993) Multiple nucleosome positioning with unique rotational setting for the *Saccharomyces cerevisiae* 5S rRNA gene *in vitro* and *in vivo*. *Proc. Natl Acad. Sci. USA*, **90**, 9315–9319.

Cavalli,G. and Thoma,F. (1993) Chromatin transitions during activation and repression of galactose-regulated genes in yeast. *EMBO J*., **12**, 4603–4613.

Cavalli,G., Bachmann,D. and Thoma,F. (1996) Inactivation of topoisomerases affects transcription dependent chromatin transitions in rDNA but not in a gene transcribed by RNA polymerase II. *EMBO J*., **15**, 590–597.

Christians,F.C. and Hanawalt,P.C. (1993) Lack of transcription-coupled repair in mammalian ribosomal RNA genes. *Biochemistry*, **32**, 10512–10518.

Ciarrocchi,G. and Pedrini,A.M. (1982) Determination of pyrimidine dimer unwinding angle by measurement of DNA electrophoretic mobility. *J. Mol. Biol*., **155**, 177–183.

Coin,F., Frit,P., Viollet,B., Salles,B. and Egly,J.M. (1998) TATA binding protein discriminates between different lesions on DNA, resulting in a transcription decrease. *Mol. Cell. Biol*., **18**, 3907–3914.

Conconi,A., Widmer,R.M., Koller,T. and Sogo,J.M. (1989) Two different chromatin structures coexist in ribosomal RNA genes throughout the cell cycle. *Cell*, **57**, 753–761.

Conconi,A., Liu,X., Koriazova,L., Ackerman,E.J. and Smerdon,M.J. (1999) Tight correlation between inhibition of DNA repair *in vitro* and transcription factor IIIA binding in a 5S ribosomal RNA gene. *EMBO J*., **18**, 1387–1396.

Dammann,R. and Pfeifer,G.P. (1997) Lack of gene- and strand-specific DNA repair in RNA polymerase III-transcribed human tRNA genes. *Mol. Cell. Biol*., **17**, 219–229.

Dammann,R., Lucchini,R., Koller,T. and Sogo,J.M. (1993) Chromatin structures and transcription of rDNA in yeast *Saccharomyces cerevisiae*. *Nucleic Acids Res*., **21**, 2331–2338.

Daneholt,B. (1992) The transcribed template and the transcription loop in Balbiani rings. *Cell Biol. Int. Rep*., **16**, 709–715.

Davie,J.R., Saunders,C.A., Walsh,J.M. and Weber,S.C. (1981) Histone modifications in the yeast *S.cerevisiae*. *Nucleic Acids Res*., **9**, 3205–3216.

De Bernardin,W., Koller,T. and Sogo,J.M. (1986) Structure of *in vivo* transcribing chromatin as studied in simian virus 40 minichromosomes. *J. Mol. Biol*., **191**, 469–482.

de Laat,W.L., Jaspers,N.G. and Hoeijmakers,J.H. (1999) Molecular mechanism of nucleotide excision repair. *Genes Dev.*, **13**, 768–785.

Donahue,B.A., Yin,S., Taylor,J.S., Reines,D. and Hanawalt,P.C. (1994) Transcript cleavage by RNA polymerase II arrested by a cyclobutane pyrimidine dimer in the DNA template. *Proc. Natl Acad. Sci. USA*, **91**, 8502–8506.

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

Eggleston,A.K., O'Neill,T.E., Bradbury,E.M. and Kowalczykowski,S.C. (1995) Unwinding of nucleosomal DNA by a DNA helicase. *J. Biol. Chem.*, **270**, 2024–2031.

Evans,D.H. and Linn,S. (1984) Excision repair of pyrimidine dimers from simian virus 40 minichromosomes *in vitro*. *J. Biol. Chem.*, **259**, 10252–10259.

Evans,E., Moggs,J.G., Hwang,J.R., Egly,J.M. and Wood,R.D. (1997) Mechanism of open complex and dual incision formation by human nucleotide excision repair factors. *EMBO J.*, **16**, 6559–6573.

Feaver,W.J., Svejstrup,J.Q., Bardwell,L., Bardwell,A.J., Buratowski,S., Gulyas,K.D., Donahue,T.F., Friedberg,E.C. and Kornberg,R.D. (1993) Dual roles of a multiprotein complex from *S.cerevisiae* in transcription and DNA repair. *Cell*, **75**, 1379–1387.

Flaus,A. and Richmond,T.J. (1998) Positioning and stability of nucleosomes on MMTV 3′ LTR sequences. *J. Mol. Biol.*, **275**, 427–441.

Fox,M.E., Feldman,B.J. and Chu,G. (1994) A novel role for DNA photolyase: binding to DNA damaged by drugs is associated with enhanced cytotoxicity in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **14**, 8071–8077.

Friedberg,E.C. (1996) Relationships between DNA repair and transcription. *Annu. Rev. Biochem.*, **65**, 15–42.

Friedberg,E.C., Walker,G.C. and Siede,W. (1995) *DNA Repair and Mutagenesis*. ASM Press, Washington, DC.

Fritz,L.K. and Smerdon,M.J. (1995) Repair of UV damage in actively transcribed ribosomal genes. *Biochemistry*, **34**, 13117–13124.

Gaillard,P.H., Martini,E.M., Kaufman,P.D., Stillman,B., Moustacchi,E. and Almouzni,G. (1996) Chromatin assembly coupled to DNA repair: a new role for chromatin assembly factor I. *Cell*, **86**, 887–896.

Gaillard,P.H., Moggs,J.G., Roche,D.M., Quivy,J.P., Becker,P.B., Wood,R.D. and Almouzni,G. (1997) Initiation and bidirectional propagation of chromatin assembly from a target site for nucleotide excision repair. *EMBO J.*, **16**, 6281–6289.

Gale,J.M. and Smerdon,M.J. (1990) UV induced (6–4) photoproducts are distributed differently than cyclobutane dimers in nucleosomes. *Photochem. Photobiol.*, **51**, 411–417.

Gale,J.M., Nissen,K.A. and Smerdon,M.J. (1987) UV-induced formation of pyrimidine dimers in nucleosome core DNA is strongly modulated with a period of 10.3 bases. *Proc. Natl Acad. Sci. USA*, **84**, 6644–6648.

Game,J.C. and Kaufman,P.D. (1999) Role of *Saccharomyces cerevisiae* chromatin assembly factor-I in repair of ultraviolet radiation damage *in vivo*. *Genetics*, **151**, 485–497.

Ganesan,A.K., Seawell,P.C., Lewis,R.J. and Hanawalt,P.C. (1986) Processivity of T4 endonuclease V is sensitive to NaCl concentration. *Biochemistry*, **25**, 5751–5755.

Gordon,L.K. and Haseltine,W.A. (1980) Comparison of the cleavage of pyrimidine dimers by the bacteriophage T4 and *Micrococcus luteus* UV-specific endonucleases. *J. Biol. Chem.*, **255**, 12047–12050.

Grunstein,M. (1998) Yeast heterochromatin: regulation of its assembly and inheritance by histones. *Cell*, **93**, 325–328.

Gruskin,E.A. and Lloyd,R.S. (1986) The DNA scanning mechanism of T4 endonuclease V. Effect of NaCl concentration on processive nicking activity. *J. Biol. Chem.*, **261**, 9607–9613.

Guzder,S.N., Sung,P., Prakash,L. and Prakash,S. (1993) Yeast DNA-repair gene *RAD14* encodes a zinc metalloprotein with affinity for ultraviolet-damaged DNA. *Proc. Natl Acad. Sci. USA*, **90**, 5433–5437.

Guzder,S.N., Sung,P., Prakash,L. and Prakash,S. (1997) Yeast Rad7–Rad16 complex, specific for the nucleotide excision repair of the nontranscribed DNA strand, is an ATP-dependent DNA damage sensor. *J. Biol. Chem.*, **272**, 21665–21668.

Guzder,S.N., Sung,P., Prakash,L. and Prakash,S. (1998a) Affinity of yeast nucleotide excision repair factor 2, consisting of the Rad4 and Rad23 proteins, for ultraviolet damaged DNA. *J. Biol. Chem.*, **273**, 31541–31546.

Guzder,S.N., Sung,P., Prakash,L. and Prakash,S. (1998b) The DNA-dependent ATPase activity of yeast nucleotide excision repair factor 4 and its role in DNA damage recognition. *J. Biol. Chem.*, **273**, 6292–6296.

Guzder,S.N., Sung,P., Prakash,L. and Prakash,S. (1999) Synergistic interaction between yeast nucleotide excision repair factors NEF2 and NEF4 in the binding of ultraviolet-damaged DNA. *J. Biol. Chem.*, **274**, 24257–24262.

Hanawalt,P.C. (1993) Close-fitting sleeves—recognition of structural defects in duplex DNA. *Mutat. Res.*, **289**, 7–15.

Hanawalt,P.C. (1994) Transcription-coupled repair and human disease. *Science*, **266**, 1957–1958.

Hanawalt,P.C. (1998) Genomic instability: environmental invasion and the enemies within. *Mutat. Res.*, **400**, 117–125.

Hanawalt,P. and Mellon,I. (1993) Stranded in an active gene. *Curr. Biol.*, **3**, 67–69.

Hayes,J.J., Bashkin,J., Tullius,T.D. and Wolffe,A.P. (1991) The histone core exerts a dominant constraint on the structure of DNA in a nucleosome. *Biochemistry*, **30**, 8434–8440.

Higley,M. and Lloyd,R.S. (1993) Processivity of uracil DNA glycosylase. *Mutat. Res.*, **294**, 109–116.

Houtsmuller,A.B., Rademakers,S., Nigg,A.L., Hoogstraten,D., Hoeijmakers,J.H. and Vermeulen,W. (1999) Action of DNA repair endonuclease ERCC1/XPF in living cells. *Science*, **284**, 958–961.

Huang,J.C. and Sancar,A. (1994) Determination of minimum substrate size for human excinuclease. *J. Biol. Chem.*, **269**, 19034–19040.

Imbalzano,A.N. (1998) Energy-dependent chromatin remodelers: complex complexes and their components. *Crit. Rev. Eukaryot. Gene Expr.*, **8**, 225–255.

Jensen,K.A. and Smerdon,M.J. (1990) DNA repair within nucleosome cores of UV-irradiated human cells. *Biochemistry*, **29**, 4773–4782.

Jones,C.J. and Wood,R.D. (1993) Preferential binding of the xeroderma-pigmentosum group—a complementing protein to damaged DNA. *Biochemistry*, **32**, 12096–12104.

Kaufman,P.D., Kobayashi,R. and Stillman,B. (1997) Ultraviolet radiation sensitivity and reduction of telomeric silencing in *Saccharomyces cerevisiae* cells lacking chromatin assembly factor-I. *Genes Dev.*, **11**, 345–357.

Kim,J.K., Patel,D. and Choi,B.S. (1995) Contrasting structural impacts induced by *cis–syn* cyclobutane dimer and (6–4) adduct in DNA duplex decamers: implication in mutagenesis and repair activity. *Photochem. Photobiol.*, **62**, 44–50.

Kosmoski,J.V. and Smerdon,M.J. (1999) Synthesis and nucleosome structure of DNA containing a UV photoproduct at a specific site. *Biochemistry*, **38**, 9485–9494.

Leadon,S.A. and Lawrence,D.A. (1992) Strand-selective repair of DNA damage in the yeast *GAL7* gene requires RNA polymerase II. *J. Biol. Chem.*, **267**, 23175–23182.

LeRoy,G., Orphanides,G., Lane,W.S. and Reinberg,D. (1998) Requirement of RSF and FACT for transcription of chromatin templates *in vitro*. *Science*, **282**, 1900–1904.

Li,S. and Smerdon,M.J. (1999) Base excision repair of *N*-methylpurines in a yeast minichromosome. *J. Biol. Chem.*, **274**, 12201–12204.

Li,S., Livingstone-Zatchej,M., Gupta,R., Meijer,M., Thoma,F. and Smerdon,M.J. (1999) Nucleotide excision repair in a constitutive and inducible gene of a yeast minichromosome in intact cells. *Nucleic Acids Res.*, **27**, 3610–3620.

Liu,X., Conconi,A. and Smerdon,M.J. (1997) Strand-specific modulation of UV photoproducts in 5S rDNA by TFIIIA binding and their effect on TFIIIA complex formation. *Biochemistry*, **36**, 13710–13717.

Livingstone-Zatchej,M., Meier,A., Suter,B. and Thoma,F. (1997) RNA polymerase II transcription inhibits DNA repair by photolyase in the transcribed strand of active yeast genes. *Nucleic Acids Res.*, **25**, 3795–3800.

Livingstone-Zatchej,M., Suter,B. and Thoma,F. (1999) Photolyase: a molecular tool to characterize chromatin structure in yeast. *Methods Mol. Biol.*, **119**, 245–259.

Losa,R., Omari,S. and Thoma,F. (1990) Poly(dA)·poly(dT) rich sequences are not sufficient to exclude nucleosome formation in a constitutive yeast promoter. *Nucleic Acids Res.*, **18**, 3495–3502.

Luger,K., Mader,A.W., Richmond,R.K., Sargent,D.F. and Richmond,T.J. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*, **389**, 251–260.

Lutter,L.C. (1979) Precise location of DNase I cutting sites in the nucleosome core determined by high resolution gel electrophoresis. *Nucleic Acids Res.*, **6**, 41–55.

Lyamichev,V. (1991) Unusual conformation of (dA)n·(dT)n-tracts as revealed by cyclobutane thymine–thymine dimer formation. *Nucleic Acids Res.*, **19**, 4491–4496.

Mann,D.B., Springer,D.L. and Smerdon,M.J. (1997) DNA damage can alter the stability of nucleosomes: effects are dependent on damage type. *Proc. Natl Acad. Sci. USA*, **94**, 2215–2220.

Matsumoto,H., Takakusu,A. and Ohnishi,T. (1994) The effects of ultraviolet C on *in vitro* nucleosome assembly and stability. *Photochem. Photobiol.*, **60**, 134–138.

Matsumoto,H., Takakusu,A., Mori,T., Ihara,M., Todo,T. and Ohnishi,T. (1995) Preferential inhibition of nucleosome assembly by ultraviolet-induced (6–4) photoproducts. *Photochem. Photobiol.*, **61**, 459–462.

McKnight,S.L., Bustin,M. and Miller,O.L.,Jr (1978) Electron microscopic analysis of chromosome metabolism in the *Drosophila melanogaster* embryo. *Cold Spring Harbor Symp. Quant. Biol.*, **42**, 741–754.

Meijer,M. and Smerdon,M.J. (1999) Accessing DNA damage in chromatin: insights from transcription. *BioEssays*, **21**, 596–603.
Mellon,I. and Hanawalt,P.C. (1989) Induction of the *Escherichia coli* lactose operon selectively increases repair of its transcribed DNA strand. *Nature*, **342**, 95–98.
Mellon,I., Spivak,G. and Hanawalt,P.C. (1987) Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian *DHFR* gene. *Cell*, **51**, 241–249.
Mitchell,D.L., Haipek,C.A. and Clarkson,J.M. (1985) (6–4) Photoproducts are removed from the DNA of UV-irradiated mammalian cells more efficiently than cyclobutane pyrimidine dimers. *Mutat. Res.*, **143**, 109–112.
Mitchell,D.L., Nguyen,T.D. and Cleaver,J.E. (1990) Nonrandom induction of pyrimidine–pyrimidone (6–4) photoproducts in ultraviolet-irradiated human chromatin. *J. Biol. Chem.*, **265**, 5353–5356.
Moggs,J.G. and Almouzni,G. (1999) Chromatin rearrangements during nucleotide excision repair. *Biochimie*, **81**, 45–52.
Morse,R.H., Pederson,D.S., Dean,A. and Simpson,R.T. (1987) Yeast nucleosomes allow thermal untwisting of DNA. *Nucleic Acids Res.*, **15**, 10311–10330.
Mueller,J.P. and Smerdon,M.J. (1995) Repair of plasmid and genomic DNA in a *rad7*Δ mutant of yeast. *Nucleic Acids Res.*, **23**, 3457–3464.
Nacheva,G.A., Guschin,D.Y., Preobrazhenskaya,O.V., Karpov,V.L., Ebralidse,K.K. and Mirzabekov,A.D. (1989) Change in the pattern of histone binding to DNA upon transcriptional activation. *Cell*, **58**, 27–36.
Naegeli,H., Bardwell,L. and Friedberg,E.C. (1992) The DNA helicase and adenosine triphosphatase activities of yeast Rad3 protein are inhibited by DNA damage—a potential mechanism for damage-specific recognition. *J. Biol. Chem.*, **267**, 392–398.
Niggli,H.J. and Cerutti,P.A. (1982) Nucleosomal distribution of thymine photodimers following far- and near-ultraviolet irradiation. *Biochem. Biophys. Res. Commun.*, **105**, 1215–1223.
Ozer,Z., Reardon,J.T., Hsu,D.S., Malhotra,K. and Sancar,A. (1995) The other function of DNA photolyase: stimulation of excision repair of chemical damage to DNA. *Biochemistry*, **34**, 15886–15889.
Paetkau,D.W., Riese,J.A., Macmorran,W.S., Woods,R.A. and Gietz,R.D. (1994) Interaction of the yeast RAD7 and SIR3 proteins: implications for DNA repair and chromatin structure. *Genes Dev.*, **8**, 2035–2045.
Park,H.W., Kim,S.T., Sancar,A. and Deisenhofer,J. (1995) Crystal structure of DNA photolyase from *Escherichia coli. Science*, **268**, 1866–1872.
Pehrson,J.R. (1989) Thymine dimer formation as a probe of the path of DNA in and between nucleosomes in intact chromatin. *Proc. Natl Acad. Sci. USA*, **86**, 9149–9153.
Pehrson,J.R. (1995) Probing the conformation of nucleosome linker DNA *in situ* with pyrimidine dimer formation. *J. Biol. Chem.*, **270**, 22440–22444.
Pehrson,J.R. and Cohen,L.H. (1992) Effects of DNA looping on pyrimidine dimer formation. *Nucleic Acids Res.*, **20**, 1321–1324.
Pendrys,J.P. (1983) A model of the kinetics of photorepair in chick embryo fibroblasts. *Mutat. Res.*, **122**, 129–133.
Pennings,S., Meersseman,G. and Bradbury,E.M. (1991) Mobility of positioned nucleosomes on 5S rDNA. *J. Mol. Biol.*, **220**, 101–110.
Pennings,S., Meersseman,G. and Bradbury,E.M. (1994) Linker histones H1 and H5 prevent the mobility of positioned nucleosomes. *Proc. Natl Acad. Sci. USA*, **91**, 10275–10279.
Pfeifer,G.P., Drouin,R., Riggs,A.D. and Holmquist,G.P. (1992) Binding of transcription factors creates hot spots for UV photoproducts *in vivo*. *Mol. Cell. Biol.*, **12**, 1798–1804.
Pineiro,M., Puerta,C. and Palacian,E. (1991) Yeast nucleosomal particles: structural and transcriptional properties. *Biochemistry*, **30**, 5805–5810.
Protic,M., Hirschfeld,S., Tsang,A.P., Wagner,M., Dixon,K. and Levine,A.S. (1989) Induction of a novel damage-specific DNA binding protein correlates with enhanced DNA repair in primate cells. *Mol. Toxicol.*, **2**, 255–270.
Ramsperger,U. and Stahl,H. (1995) Unwinding of chromatin by the SV40 large T antigen DNA helicase. *EMBO J.*, **14**, 3215–3225.
Rapic Otrin,V., Kuraoka,I., Nardo,T., McLenigan,M., Eker,A.P., Stefanini,M., Levine,A.S. and Wood,R.D. (1998) Relationship of the xeroderma pigmentosum group E DNA repair defect to the chromatin and DNA binding proteins UV-DDB and replication protein A. *Mol. Cell. Biol.*, **18**, 3182–3190.
Robins,P., Jones,C.J., Biggerstaff,M., Lindahl,T. and Wood,R.D. (1991) Complementation of DNA repair in xeroderma pigmentosum group A cell extracts by a protein with affinity for damaged DNA. *EMBO J.*, **10**, 3913–3921.
Rozek,D. and Pfeifer,G.P. (1995) *In vivo* protein–DNA interactions at the c-*jun* promoter in quiescent and serum-stimulated fibroblasts. *J. Cell. Biochem.*, **57**, 479–487.
Sancar,A. (1996a) DNA excision repair. *Annu. Rev. Biochem.*, **65**, 43–81.
Sancar,A. (1996b) No 'end of history' for photolyases. *Science*, **272**, 48–49.
Sancar,G.B. and Smith,F.W. (1989) Interactions between yeast photolyase and nucleotide excision repair proteins in *Saccharomyces cerevisiae* and *Escherichia coli. Mol. Cell. Biol.*, **9**, 4767–4776.
Sancar,G.B., Smith,F.W., Reid,R., Payne,G., Levy,M. and Sancar,A. (1987) Action mechanism of *Escherichia coli* DNA photolyase. I. Formation of the enzyme–substrate complex. *J. Biol. Chem.*, **262**, 478–485.
Schaeffer,L., Roy,R., Humbert,S., Moncollin,V., Vermeulen,W., Hoeijmakers,J.H., Chambon,P. and Egly,J.M. (1993) DNA repair helicase: a component of BTF2 (TFIIH) basic transcription factor. *Science*, **260**, 58–63.
Schieferstein,U. and Thoma,F. (1996) Modulation of cyclobutane pyrimidine dimer formation in a positioned nucleosome containing poly(dA·dT) tracts. *Biochemistry*, **35**, 7705–7714.
Schieferstein,U. and Thoma,F. (1998) Site-specific repair of cyclobutane pyrimidine dimers in a positioned nucleosome by photolyase and T4 endonuclease V *in vitro. EMBO J.*, **17**, 306–316.
Schild,D., Glassner,B.J., Mortimer,R.K., Carlson,M. and Laurent,B.C. (1992) Identification of *RAD16*, a yeast excision repair gene homologous to the recombinational repair gene *RAD54* and to the *SNF2* gene involved in transcriptional activation. *Yeast*, **8**, 385–395.
Schmid,A., Fascher,K.D. and Horz,W. (1992) Nucleosome disruption at the yeast *PHO5* promoter upon *PHO5* induction occurs in the absence of DNA replication. *Cell*, **71**, 853–864.
Selby,C.P. and Sancar,A. (1990) Transcription preferentially inhibits nucleotide excision repair of the template DNA strand *in vitro. J. Biol. Chem.*, **265**, 21330–21336.
Selby,C.P., Drapkin,R., Reinberg,D. and Sancar,A. (1997) RNA polymerase II stalled at a thymine dimer: footprint and effect on excision repair. *Nucleic Acids Res.*, **25**, 787–793.
Selleck,S.B. and Majors,J. (1987a) Photofootprinting *in vivo* detects transcription-dependent changes in yeast TATA boxes. *Nature*, **325**, 173–177.
Selleck,S.B. and Majors,J.E. (1987b) *In vivo* DNA-binding properties of a yeast transcription activator protein. *Mol. Cell. Biol.*, **7**, 3260–3267.
Selleck,S.B. and Majors,J. (1988) *In vivo* 'photofootprint' changes at sequences between the yeast GAL1 upstream activating sequence and 'TATA' element require activated GAL4 protein but not a functional TATA element. *Proc. Natl Acad. Sci. USA*, **85**, 5399–5403.
Simpson,R.T. (1978) Structure of the chromatosome, a chromatin particle containing 160 base pairs of DNA and all the histones. *Biochemistry*, **17**, 5524–5531.
Smerdon,M.J. and Conconi,A. (1999) Modulation of DNA damage and DNA repair in chromatin. *Prog. Nucleic Acid Res. Mol. Biol.*, **62**, 227–255.
Smerdon,M.J. and Lieberman,M.W. (1978) Nucleosome rearrangement in human chromatin during UV-induced DNA-repair synthesis. *Proc. Natl Acad. Sci. USA*, **75**, 4238–4241.
Smerdon,M.J. and Lieberman,M.W. (1980) Distribution within chromatin of deoxyribonucleic acid repair synthesis occurring at different times after ultraviolet radiation. *Biochemistry*, **19**, 2992–3000.
Smerdon,M.J. and Thoma,F. (1990) Site-specific DNA repair at the nucleosome level in a yeast minichromosome. *Cell*, **61**, 675–684.
Smerdon,M. and Thoma,F. (1998) Modulations in chromatin structure during DNA damage formation and DNA repair. In Hoekstra,M.F. and Nickoloff,J.A. (eds), *DNA Damage and Repair: Molecular and Cell Biology.* The Humana Press Inc., Totowa, NJ, Vol. 2, pp. 199–222.
Smerdon,M.J., Lan,S.Y., Calza,R.E. and Reeves,R. (1982) Sodium butyrate stimulates DNA repair in UV-irradiated normal and xeroderma pigmentosum human fibroblasts. *J. Biol. Chem.*, **257**, 13441–13447.
Smerdon,M.J., Bedoyan,J. and Thoma,F. (1990) DNA repair in a small yeast plasmid folded into chromatin. *Nucleic Acids Res.*, **18**, 2045–2051.
Studitsky,V.M., Clark,D.J. and Felsenfeld,G. (1995) Overcoming a nucleosomal barrier to transcription. *Cell*, **83**, 19–27.
Studitsky,V.M., Kassavetis,G.A., Geiduschek,E.P. and Felsenfeld,G. (1997) Mechanism of transcription through the nucleosome by eukaryotic RNA polymerase. *Science*, **278**, 1960–1963.
Sugasawa,K., Masutani,C. and Hanaoka,F. (1993) Cell-free repair of UV-damaged simian virus-40 chromosomes in human cell extracts.1. Development of a cell-free system detecting excision repair of UV-irradiated SV40 chromosomes. *J. Biol. Chem.*, **268**, 9098–9104.
Sugasawa,K., Ng,J.M., Masutani,C., Iwai,S., van der Spek,P.J., Eker,A.P., Hanaoka,F., Bootsma,D. and Hoeijmakers,J.H. (1998) Xeroderma

pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. *Mol. Cell*, **2**, 223–232.
Suquet,C., Mitchell,D.L. and Smerdon,M.J. (1995) Repair of UV-induced (6–4) photoproducts in nucleosome core DNA. *J. Biol. Chem.*, **270**, 16507–16509.
Suquet,C. and Smerdon,M.J. (1993) UV damage to DNA strongly influences its rotational setting on the histone surface of reconstituted nucleosomes. *J. Biol. Chem.*, **268**, 23755–23757.
Suter,B., Livingstone-Zatchej,M. and Thoma,F. (1997) Chromatin structure modulates DNA repair by photolyase *in vivo*. *EMBO J.*, **16**, 2150–2160.
Suter,B., Livingstone-Zatchej,M. and Thoma,F. (1999) Mapping cyclobutane–pyrimidine dimers in DNA and using DNA-repair by photolyase for chromatin analysis in yeast. *Methods Enzymol.*, **304**, 447–461.
Sweder,K.S. and Hanawalt,P.C. (1992) Preferential repair of cyclobutane pyrimidine dimers in the transcribed strand of a gene in yeast chromosomes and plasmids is dependent on transcription. *Proc. Natl Acad. Sci. USA*, **89**, 10696–10700.
Szymkowski,D.E., Hajibagheri,M.A. and Wood,R.D. (1993) Electron microscopy of DNA excision repair patches produced by human cell extracts. *J. Mol. Biol.*, **231**, 251–260.
Tamada,T., Kitadokoro,K., Higuchi,Y., Inaka,K., Yasui,A., de Ruiter,P.E., Eker,A.P. and Miki,K. (1997) Crystal structure of DNA photolyase from *Anacystis nidulans*. *Nature Struct. Biol.*, **4**, 887–891
Tanaka,S., Livingstone-Zatchej,M. and Thoma,F. (1996) Chromatin structure of the yeast *URA3* gene at high resolution provides insight into structure and positioning of nucleosomes in the chromosomal context. *J. Mol. Biol.*, **257**, 919–934.
Teng,Y., Li,S., Waters,R. and Reed,S.H. (1997) Excision repair at the level of the nucleotide in the *Saccharomyces cerevisiae MFA2* gene: mapping of where enhanced repair in the transcribed strand begins or ends and identification of only a partial rad16 requisite for repairing upstream control sequences. *J. Mol. Biol.*, **267**, 324–337.
Thoma,F. (1986) Protein–DNA interactions and nuclease-sensitive regions determine nucleosome positions on yeast plasmid chromatin. *J. Mol. Biol.*, **190**, 177–190.
Thoma,F. (1992) Nucleosome positioning. *Biochim. Biophys. Acta*, **1130**, 1–19.
Thoma,F., Koller,T. and Klug,A. (1979) Involvement of histone H1 in the organization of the nucleosome and of the salt-dependent superstructures of chromatin. *J. Cell Biol.*, **83**, 403–427.
Thoma,F., Cavalli,G. and Tanaka,S. (1993) Structural and functional organisation of yeast chromatin. In Broda,P.M.A., Oliver,S.G. and Sims,P.F.G. (eds), *The Eukaryotic Genome*. Cambridge University Press, Cambridge, UK, pp. 43–72.
Tijsterman,M., Tasseron-de Jong,J.G., van de Putte,P. and Brouwer,J. (1996) Transcription-coupled and global genome repair in the *Saccharomyces cerevisiae RPB2* gene at nucleotide resolution. *Nucleic Acids Res.*, **24**, 3499–3506.
Tijsterman,M., Verhage,R.A., van de Putte,P., Tasseron-de Jong,J.G. and Brouwer,J. (1997) Transitions in the coupling of transcription and nucleotide excision repair within RNA polymerase II-transcribed genes of *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **94**, 8027–8032.
Tijsterman,M., de Pril,R., Tasseron-de Jong,J.G. and Brouwer,J. (1999) RNA polymerase II transcription suppresses nucleosomal modulation of UV-induced (6–4) photoproduct and cyclobutane pyrimidine dimer repair in yeast. *Mol. Cell. Biol.*, **19**, 934–940.
Todo,T. (1999) Functional diversity of the DNA photolyase/blue light receptor family. *Mutat. Res.*, **434**, 89–97.
Tommasi,S., Swiderski,P.M., Tu,Y., Kaplan,B.E. and Pfeifer,G.P. (1996) Inhibition of transcription factor binding by ultraviolet-induced pyrimidine dimers. *Biochemistry*, **35**, 15693–15703.
Tornaletti,S. and Pfeifer,G.P. (1994) Slow repair of pyrimidine dimers at p53 mutation hotspots in skin cancer. *Science*, **263**, 1436–1438.
Tornaletti,S. and Pfeifer,G.P. (1995) UV light as a footprinting agent: modulation of UV-induced DNA damage by transcription factors bound at the promoters of three human genes. *J. Mol. Biol.*, **249**, 714–728.
Tornaletti,S. and Pfeifer,G.P. (1996) UV damage and repair mechanisms in mammalian cells. *BioEssays*, **18**, 221–228.
Tornaletti,S., Reines,D. and Hanawalt,P.C. (1999) Structural characterization of RNA polymerase II complexes arrested by a cyclobutane pyrimidine dimer in the transcribed strand of template DNA. *J. Biol. Chem.*, **274**, 24124–24130.
Tu,Y., Tornaletti,S. and Pfeifer,G.P. (1996) DNA repair domains within a human gene: selective repair of sequences near the transcription initiation site. *EMBO J.*, **15**, 675–683.
van de Merwe,W. and Bronk,B.V. (1981) Increased efficiency of photoreversal of UV-induced dimers in the DNA of chick embryo fibroblasts with post-UV dark time. *Mutat. Res.*, **84**, 429–441.
van Noort,S.J., van der Werf,K.O., Eker,A.P., Wyman,C., de Grooth,B.G., van Hulst,N.F. and Greve,J. (1998) Direct visualization of dynamic protein–DNA interactions with a dedicated atomic force microscope. *Biophys. J.*, **74**, 2840–2849.
Vassylyev,D.G., Kashiwagi,T., Mikami,Y., Ariyoshi,M., Iwai,S., Ohtsuka,E. and Morikawa,K. (1995) Atomic model of a pyrimidine dimer excision repair enzyme complexed with a DNA substrate: structural basis for damaged DNA recognition. *Cell*, **83**, 773–782.
Venema,J., Mullenders,L.H., Natarajan,A.T., van Zeeland,A.A. and Mayne,L.V. (1990) The genetic defect in Cockayne syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA. *Proc. Natl Acad. Sci. USA*, **87**, 4707–4711.
Verhage,R., Zeeman,A.M., de Groot,N., Gleig,F., Bang,D.D., van de Putte,P. and Brouwer,J. (1994) The *RAD7* and *RAD16* genes, which are essential for pyrimidine dimer removal from the silent mating type loci, are also required for repair of the nontranscribed strand of an active gene in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **14**, 6135–6142.
Verhage,R.A., Van de Putte,P. and Brouwer,J. (1996a) Repair of rDNA in *Saccharomyces cerevisiae*: *RAD4*-independent strand-specific nucleotide excision repair of RNA polymerase I transcribed genes. *Nucleic Acids Res.*, **24**, 1020–1025.
Verhage,R.A., Zeeman,A.M., Lombaerts,M., van de Putte,P. and Brouwer,J. (1996b) Analysis of gene- and strand-specific repair in the moderately UV-sensitive *Saccharomyces cerevisiae rad23* mutant. *Mutat. Res.*, **362**, 155–165.
Vichi,P., Coin,F., Renaud,J.P., Vermeulen,W., Hoeijmakers,J.H., Moras,D. and Egly,J.M. (1997) Cisplatin- and UV-damaged DNA lure the basal transcription factor TFIID/TBP. *EMBO J.*, **16**, 7444–7456.
Wakasugi,M. and Sancar,A. (1999) Order of assembly of human DNA repair excision nuclease. *J. Biol. Chem.*, **274**, 18759–18768.
Wang,C.I. and Taylor,J.S. (1991) Site-specific effect of thymine dimer formation on dAn·dTn tract bending and its biological implications. *Proc. Natl Acad. Sci. USA*, **88**, 9072–9076.
Wang,Z.G., Wu,X.H. and Friedberg,E.C. (1991) Nucleotide excision repair of DNA by human cell extracts is suppressed in reconstituted nucleosomes. *J. Biol. Chem.*, **266**, 22472–22478.
Wellinger,R.E. and Thoma,F. (1996) *Taq* DNA polymerase blockage at pyrimidine dimers. *Nucleic Acids Res.*, **24**, 1578–1579.
Wellinger,R.E. and Thoma,F. (1997) Nucleosome structure and positioning modulate nucleotide excision repair in the non-transcribed strand of an active gene. *EMBO J.*, **16**, 5046–5056.
Whitehouse,I., Flaus,A., Cairns,B.R., White,M.F., Workman,J.L. and Owen-Hughes,T. (1999) Nucleosome mobilization catalysed by the yeast SWI/SNF complex. *Nature*, **400**, 784–787.
Widom,J. (1998) Structure, dynamics and function of chromatin *in vitro*. *Annu. Rev. Biophys. Biomol. Struct.*, **27**, 285–327.
Wilkins,R.J. and Hart,R.W. (1974) Preferential DNA repair in human cells. *Nature*, **247**, 35–36.
Wolffe,A. (1995) *Chromatin*. Academic Press, San Diego, CA.
Wood,R.D. (1996) DNA repair in eukaryotes. *Annu. Rev. Biochem.*, **65**, 135–167.
Wood,R.D. (1999) DNA damage recognition during nucleotide excision repair in mammalian cells. *Biochimie*, **81**, 39–44.
Workman,J.L. and Kingston,R.E. (1998) Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu. Rev. Biochem.*, **67**, 545–579.
Yamamoto,K., Fujiwara,Y. and Shinagawa,H. (1983) Evidence that the phr^+ gene enhances the ultraviolet resistance of *Escherichia coli recA* strains in the dark. *Mol. Gen. Genet.*, **192**, 282–284.
Yasui,A., Eker,A.P.M., Yasuhira,S., Yajima,H., Kobayashi,T., Takao,M. and Oikawa,A. (1994) A new class of DNA photolyases present in various organisms including aplacental mammals. *EMBO J.*, **13**, 6143–6151.
Ye,N., Holmquist,G.P. and O'Connor,T.R. (1998) Heterogeneous repair of *N*-methylpurines at the nucleotide level in normal human cells. *J. Mol. Biol.*, **284**, 269–285.
Zwetsloot,J.C., Vermeulen,W., Hoeijmakers,J.H., Yasui,A., Eker,A.P. and Bootsma,D. (1985) Microinjected photoreactivating enzymes from *Anacystis* and *Saccharomyces* monomerize dimers in chromatin of human cells. *Mutat. Res.*, **146**, 71–77.

Received July 20, 1999; revised September 30, 1999; accepted October 4, 1999