Chromatin Remodeling Activities Act on UV-damaged Nucleosomes and Modulate DNA Damage Accessibility to Photolyase*

Received for publication, January 23, 2003, and in revised form, March 6, 2003. Published, JBC Papers in Press, March 11, 2003. DOI 10.1074/jbc.M300770200

Hélène Gaillard‡§, Daniel J. Fitzgerald‡, Corey L. Smith†, Craig L. Peterson‡, Timothy J. Richmond‡, and Fritz Thoma‡**

From the ‡Institut für Zellbiologie and §Institut für Molekularbiologie, Departement Biologie, ETH-Hönggerberg, CH-8093 Zurich, Switzerland and ¶Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts 01605

Nucleosomes inhibit DNA repair in vitro, suggesting that chromatin remodeling activities might be required for efficient repair in vivo. To investigate how structural and dynamic properties of nucleosomes affect damage recognition and processing, we investigated repair of UV lesions by photolyase on a nucleosome positioned at one end of a 226-bp-long DNA fragment. Repair was slow in the nucleosome but efficient outside. No disruption or movement of the nucleosome was observed after UV irradiation and during repair. However, incubation with the nucleosome remodeling complex SWI/SNF and ATP altered the conformation of nucleosomal DNA as judged by UV photo-footprinting and promoted more homogeneous repair. Incubation with yISW2 and ATP moved the nucleosome to a more central position, thereby altering the repair pattern. This is the first demonstration that two different chromatin remodeling complexes can act on UV-damaged nucleosomes and modulate repair. Similar activities might relieve the inhibitory effect of nucleosomes on DNA repair processes in living cells.

Folding of eukaryotic DNA into nucleosomes and higher order structures restricts its accessibility to proteins, thereby repressing DNA-dependent processes like transcription and DNA repair. Dynamic properties of nucleosomes and chromatin remodeling activities contribute to relieve the repressive role of chromatin. Although remodeling has been extensively studied in the context of transcriptional regulation, its contribution to DNA repair remains unclear (1–5). Here we show that two different nucleosome remodeling activities can act on UV-damaged nucleosomes and modulate repair by photolyase.

The basic unit of chromatin is the nucleosome core particle. It consists of 147 bp of DNA wrapped in 1.65 left-handed superhelical turns around an octamer of core histones. The octamer itself consists of a histone (H2A–H4) tetramer, which binds the central six turns of DNA, and two H2A–H2B dimers, which primarily bind distal regions of the core DNA. The structure of DNA changes upon folding into nucleosomes (6).

In principle, all reactions that involve DNA can be regulated by changing DNA packaging. A contribution to the regulation of DNA accessibility comes from intrinsic properties of nucleosomes, such as nucleosome mobility, unfolding, or partial disruption (7). Another contribution is made by protein complexes that remodel chromatin structures. One class consists of histone-modifying complexes that add or remove covalent modifications from histone tails. Another class utilizes the energy of ATP hydrolysis to modify chromatin structure in a non-covalent manner (2, 3, 8, 9). The ATP-dependent chromatin remodeling complexes contain an ATPase subunit that belongs to the SNF2 superfamily of proteins. Based on the identity of this subunit, they have been divided into the SWI2/SNF2 family, the ISWI family, and the Mi-2 family (10). Although functional analysis of these complexes has been focused mainly on transcription, there is increasing evidence that similar activities may assist recombination and repair (1–3, 11–13).

Cyclobutane pyrimidine dimers (CPDs)† are the major class of DNA lesions produced by UV light. The recent crystal structure of a CPD in DNA showed that the overall helical axis bends ~30° toward the major groove and unwinds ~9° (14). CPDs appear to have only minimal effect on nucleosome stability but may affect nucleosome positioning during chromatin assembly. CPD formation is modulated by the structure of nucleosomes and by other protein-DNA interactions (4, 5). In most organisms, pyrimidine dimers are removed by the multi-step nucleotide excision repair (NER) pathway (15). As an alternative or additional pathway, a wide variety of organisms can specifically revert photoproducts to their native bases by DNA photolyase in the presence of light (photoreactivation) (16).

Both DNA repair mechanisms are modulated by chromatin structure (4, 5). Nucleosomes exert a repressive role on NER and photoreactivation because repair is slow in regions of positioned nucleosomes and fast in linker DNA of yeast (17–19). However, the repression is not tight, since all lesions need to be repaired to prevent mutagenesis. In vitro, reconstituted nucleosomes also exert an inhibitory effect on repair by photolyase or T4-endonuclease V (20, 21) and by NER (13, 22–25). Repair of SV40 minichromosomes by NER was also reduced compared with naked DNA (26). However, Xenopus extracts proficient for NER can repair a lesion placed in a reconstituted nucleosome (27), suggesting that factors responsible for modulating nucleosome structure may be present in the extract.

Several proteins that are involved in DNA repair pathways

* This work was supported by grants from the Swiss National Science Foundation, the ETH Zurich, and the Roche Research Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Present address: Dept. de Genética, Facultad de Biología, Universidad de Sevilla, Avenida Reina Mercedes 6, 41011 Sevilla, Spain.

‡ To whom correspondence should be addressed: Institut für Zellbiologie, ETH-Hönggerberg, CH-8093 Zurich, Switzerland. Tel: 41-1-633 9° (14).

§ Present address: Dept. de Genética, Facultad de Biología, Universidad de Sevilla, Avenida Reina Mercedes 6, 41011 Sevilla, Spain.

¶ To whom correspondence should be addressed: Institut für Zellbiologie, ETH-Hönggerberg, CH-8093 Zurich, Switzerland. Tel: 41-1-633 10 69; E-mail: thomas@cell.biol.ethz.ch.

1 The abbreviations used are: CPDs, cyclobutane pyrimidine dimers; NER, nucleotide excision repair; T4endoV, T4-endonuclease V; CSB, Cockayne’s syndrome B; m.o.i., multiplicity of infection; ACF, ATP-using chromatin assembly factor; y, yeast.
belong to the SNF2 superfamily of ATPases and thereby might provide chromatin remodeling activities to facilitate DNA repair (1, 28). Nucleosome remodeling activity was shown on undamaged nucleosomes for the Cockayne’s syndrome B (CSB) protein, which is involved in transcription coupled repair (29), and for the INO80 chromatin remodeling complex of yeast. Mutations in INO80 cause sensitivity to hydroxyurea, methyl methane sulfonate, and ultraviolet and mitomycin C. On the other hand, the ACF remodeling complex was reported to facilitate NER of a lesion placed in linker DNA of a dinucleosome but not of a lesion in the nucleosomes (25). In contrast to ACF, the SWI/SNF remodeling complex was most recently reported to stimulate excision nuclease activity on a nucleosome containing a bulky acetylamino-fluorene-guanine adduct (13). Thus, SWI/SNF and ACF might act differently on damaged nucleosomes, or their remodeling activities might be dependent on the nature and/or location of the damage within the nucleosome. We show that two different chromatin remodeling activities (SWI/SNF and ISW2) can act on UV-damaged nucleosomes and facilitate repair by photolyase. In addition, the photoreactivation pattern of remodeled nucleosomes reflected differences in the activities of SWI/SNF and ISW2.

**MATERIALS AND METHODS**

**Preparation of DNA for Reconstitution**—The HindIII/BamHI fragment of p8ATDED (31) was subcloned in either orientation into the SacI site of pUC18 to generate p8ATDED and p8ATDED-h for top and bottom strand labeling, respectively. The 226-bp fragments generated by cleavage with Smal and EcoRI were purified and the 3’ ends labeled by filling the recessed ends with [α-32P]dATP and dTTP.

**Nucleosome Reconstitution**—Reconstitution was done by histone octamer transfer from chicken erythrocytes core particles (20). 200 ng of end-labeled DNA was incubated with a 40-fold excess of core particles (8 μg) in 200 μl of 10 mM Tris (pH 7.5), 1 mM EDTA (pH 7.5), 50 μM phenylmethylsulfonfluor fluoride, and 2 μl of NaCl at 37 °C for 30 min and at 4 °C for another 30 min. The samples were dialyzed at 4 °C in a microdializer (Pierce, molecular weight cut-off 8000) overnight against dialysis buffer 1 (0.6 μl NaCl, 10 mM Tris (pH 7.5), 1 mM EDTA (pH 7.5), 50 μM phenylmethylsulfonfluor fluoride), 4–5 h against dialysis buffer 2 (10 mM Tris (pH 7.5), 50 mM NaCl, 1 mM EDTA (pH 7.5), 50 μM phenylmethylsulfonfluor fluoride), and finally 2–3 h against fresh dialysis buffer 2.

**Yeast SWI/SNF Remodeling**—Yeast SWI/SNF was added to nucleosomes (160 ng for the DNase I footprint, 100 ng for the UV footprint, and 800 ng for the photoreactivation experiments), and the buffer was adjusted to final concentrations of 8 mM Tris (pH 7.5), 100 mM NaCl, 1 mM dithiothreitol, 5 mM MgCl2, 3% glycerol (in final volumes of 30, 20, and 80 μl, respectively). Where indicated, ATP was added to a final concentration of 0.5 or 1 mM. The photoreactivation experiment contained in addition 46 μg/ml insulin brought in by a new yeast SWI/SNF batch. The molar ratios of ySWI/SNF to nucleosomes were 1 (10 ng of complex/ng of nucleosome) for the DNase I footprinting (Fig. 3, A and B), 0.8 (8 ng of complex/ng of nucleosome) for the UV photo-footprinting (Fig. 3, B and C), and 0.6 (6 ng of complex/ng of nucleosome) for the photoreactivation experiments (Fig. 4). The reactions were incubated 30 min at 30 °C. For competition, 2-μl aliquots were removed, and an excess of linear plasmid DNA (pBSFP79; 1 μg in 10 mM Tris (pH 7.5), 100 mM NaCl, 3% glycerol) was added, resulting in a final volume of 5 μl. The reaction was continued for 40 min at room temperature.

**Expression and Purification of the Yeast ISW2 Complex**—The yISW2 complex was expressed using the Bac to Bac baculovirus expression system (Invitrogen). SF21 cells were grown in suspension culture to a density of 1 x 10^6 cells/ml in SF900 media and infected with viral m.o.i. of 0.1 or 1.0 using a C-terminally His6-tagged version of ISW2. Cells were harvested by centrifugation (4800 × g, 10 m.o.i.) or 7200 × g, 1 m.o.i.) post-infection. All subsequent steps were performed at 4 °C. Lysis and binding to Talon metal affinity resin was carried out according to the manufacturer’s instructions (Clontech) except that the binding buffer was adjusted to pH 7.4. Elution was carried out with a linear gradient to 100 mM imidazole. Eluted material was loaded directly onto the HPO column (Beckman Instruments) and eluted with a linear gradient to 0.6 M KCl. Combined fractions were loaded on a Sepharose G-400 gel filtration column run in 100 mM KCl, 20 mM Tris (pH 7.5). Peak fractions were concentrated using Vivaspun concentrators (Sartorius).

The yISW2 remodeling activity was added to nucleosomes (2 μg), and the buffer was adjusted to final concentrations of 10 mM Tris (pH 7.5), 90 mM NaCl, 5 mM MgCl2, 1 mM dithiothreitol, 8% glycerol, 30 μg/ml bovine serum albumin (in a final volume of 100 μl). Where indicated, ATP was added to a final concentration of 0.5 mM. The molar ratio of ISW2 to nucleosomes was 0.7 (1 ng of complex/ng of nucleosome). The reactions were incubated for 30 min at 30 °C.

**UV Irradiation**—Nucleosomes (40 ng/μl), remodeled nucleosomes (5 ng/μl), and DNA (5–40 ng/μl) samples were split in 20–40-μl droplets and irradiated on parafilm strips placed on ice at a fluence of 15 watts/m² using germicidal lamps (G15T8S, Sylvania) emitting predominantly at 254 nm.

**Photoreactivation**—Remodeled nucleosomes or naked DNA isolated from irradiated nucleosomes were mixed with *Escherichia coli* photolyase (BD Biosciences) to yield a ratio of 70–75 ng of photolyase/μg of DNA. Photoreactivation was performed by irradiating the samples at 30 °C with six fluorescent lamps (15 watts, F15T8BBL, Sylvania, a peak emission at 375 nm) with a fluence of 17 watts/m². In the SWI/SNF and ISW2 remodeling experiments, samples were photoreactivated in remodeling buffer.

**CPD Analysis**—SDS was added to the repair samples (0.5% final concentration), followed by proteinase K digestion. DNA was purified through QiAquick columns (Qiagen) and eluted in 50 mM Tris, 5 mM EDTA. DNA was incubated at 37 °C; T4-endonuclease V (Epicentre) was added (0.06 units/μg DNA), and incubation was continued for 3 h. DNA was purified by Stratagene Clean resin (Stratagene) extraction, precipitated, and analyzed on denaturing 8% acrylamide gels.

**Ntice Nucleoprotein Gels**—Reconstitution products were analyzed on 4% acrylamide gels run in 0.5× TBE. Where indicated, the nucleoprotein gels also contained 10% glycerol (see figure legends). Prior to loading, the samples were mixed with glycerol to yield a final concentration of 6% glycerol and electrophoresed at 4 °C for 3–4 h at 12 mA.

**DNase I Footprinting**—DNase I digestion of nucleosomal and naked DNA samples was performed in parallel. The samples were adjusted to 5 mM MgCl2 and incubated with DNase I (Roche Applied Science; 2 units of DNase I/μg of nucleosomal DNA and 0.2 units of DNase I/μg of naked DNA) at room temperature between 30 s and 6 min. The reaction was stopped by adjusting the samples to 10 mM EDTA. The DNA was purified and analyzed on 8% denaturing acrylamide gels.

**Restriction Enzyme Digestions**—1.8 μg of nucleosomes or naked DNA was incubated with 50 units of restriction enzyme (AflII, Hhal, and XhoI) at 30 °C for 3 h in a buffer adjusted to 100 mM NaCl, 10 mM MgCl2, 1 mM dithiothreitol, 50 mM Tris (pH 7.5), and 0.1 mg/ml bovine serum albumin for the restriction enzymes AflII and Hhal. Purified DNA was analyzed on 8% denaturing acrylamide gels.

**Quantification**—The gels were dried on Whatman DE81 and 3MM papers and quantified using a PhosphorImager (Amersham Biosciences).

**RESULTS**

The “ATTED-long Nucleosome,” a Chromatin Substrate to Study CPD Repair—We reconstituted a nucleosome at the end of a 226-bp DNA fragment. This “ATTED-long” fragment originates from the natural yeast DED1 promoter and contains several polypryrimidine tracts (31), which allowed us to monitor DNA structure by UV footprinting and DNA damage accessibility by photolyase (Fig. 1A). This nucleosome has the space to reassemble or slide to alternative positions as a consequence of DNA damage formation and interactions with DNA repair enzymes and remodeling complexes.

The DNA was end-labeled with 32P on either strand separately, and nucleosomes were reconstituted by histone octamer transfer from chicken erythrocytes core particles. Nucleoprotein gels showed that reconstitution was efficient, with over 90% of the DNA folded into nucleosomes. Only one preferential product was observed (see examples below). DNase I digestion produced a 10-bp repeat pattern between map units 1360 and 1497, which is characteristic for nucleosomes occupying a single rotational orientation (Fig. 1B, lanes 2 and 3; Fig. 3B, lanes 5 and 6). No such patterns were generated in naked DNA (Fig. 1B, lanes 4). The translational position of the nucleosome was verified by digestion with restriction enzymes. Reconstituted DNA was efficiently cleaved by *AflII* (84%) and *Hhal* (79%), whereas cleavage by *XhoI* was strongly inhibited (2% cut; Fig. 1C). Thus, DNase I and restriction analyses demonstrate that...
the nucleosome is positioned at the right end of the DNA and adopts a preferential rotational setting.

For repair experiments, reconstituted nucleosomes were irradiated with UV light at a dose of 750 J/m² to generate about 1.5 CPDs per fragment and exposed to *E. coli* DNA photolyase and photoreactivating light for up to 120 min at 30 °C. Nucleoprotein gels revealed that the nucleosomal fraction remained unchanged after UV irradiation and during photoreactivation (Fig. 1A, lanes 3–9). Hence, neither UV irradiation nor photolyase disrupted the nucleosome.

To assess the CPD distribution and their removal by photolyase, DNA of UV-irradiated and photoreactivated nucleosomes was purified and cut at CPDs with T4-endonuclease V, therefore might indicate an unusual structure at the end of the nucleosome (Fig. 1B, bottom strand).

In conclusion, CPDs in nucleosomes are resistant to photoreactivation, whereas CPDs outside are efficiently repaired. The results imply that the nucleosome was not displaced either by UV damage formation or by incubation with photolyase despite the length of the fragment. Thus, UV lesions and *E. coli* photolyase are unable to displace the histone octamer.

*Remodeling by ySWI/SNF Alters the DNA Structure of Nucleosomes*—In contrast to the results obtained in *vitro*, DNA lesions are completely removed from nucleosomes in *vivo*. Therefore, we tested whether nucleosome remodeling activities can act on UV-damaged nucleosomes and facilitate CPD accessibility and repair. We first tested ySWI/SNF, SWI/SNF is known to generally enhance accessibility of nucleosomes to DNase I, restriction endonucleases, and transcription factors in *vitro*, without disruption of the histone octamer (32–35).

Reconstituted nucleosomes were incubated with ySWI/SNF either in the absence or in the presence of ATP. In the nucleoprotein gel, all labeled DNA was found in the well after addition of ySWI/SNF (Fig. 3A, lanes 3 and 4) and ATP (lane 4), indicating that the nucleosomes were bound to the remodeling complex. DNase I footprinting revealed that the rotational setting of the nucleosome was maintained when complexed with ySWI/SNF in the absence of ATP and lost after incubation with ySWI/SNF and ATP (Fig. 3B, lanes 5–10). The resulting cutting pattern was similar to naked DNA (lanes 2–4). Thus the complex apparently remodels the ATDED-long nucleosome as described for other substrates (32, 34, 36).

CPD formation depends on the structure of DNA and can be...
altered by folding of DNA in nucleosomes (37–39). ATTED-long nucleosomes were analyzed by UV photo-footprinting during remodeling (Fig. 3, C and D). Naked DNA, nucleosomes, and nucleosomes treated with ySWI/SNF in the presence or absence of ATP were irradiated with UV light at a dose of 500 J/m² to generate about one CPD per fragment. The nucleoprotein gel confirmed that prior to irradiation, all nucleosomes were complexed with ySWI/SNF in the presence and absence of ATP (Fig. 3C, lanes 3 and 4). In naked DNA, the CPD formation pattern was heterogeneous, depending on the sequence and the unusual structure of T-tracts which forms characteristic damage patterns (38, 40) (Fig. 3D, lane 5) demonstrating that folding of ATTED-DNA in a nucleosome alters its DNA structure. In particular, folding of DNA into nucleosomes induced enhanced CPD formation in cluster 16 and at the 5’ end of cluster 13. The nucleosome pattern was maintained when nucleosomes were irradiated in the presence of ySWI/SNF (Fig. 3D, lane 6), but irradiation in the presence of ySWI/SNF and ATP generated a pattern similar to that of naked DNA (lane 7). These UV photo-footprinting results demonstrate a change in the structure upon folding of DNA into nucleosomes and upon remodeling by ySWI/SNF.

To test whether DNA might have been released from nucleosomes by ySWI/SNF under our conditions, aliquots were incubated with an excess of plasmid DNA to compete for ySWI/SNF and analyzed by nucleoprotein gel electrophoresis (Fig. 3C, lanes 5–8). After competition with plasmid DNA, only a small fraction of material was observed as free DNA, whereas most of the material was found in nucleosomal fractions (Fig. 3C, lanes 7 and 8). This is in agreement with previous work (34, 36, 41, 42), where no or very little increase in naked DNA was observed in similar competition experiments. Two major nucleosomal bands (Fig. 3C, N and N’) indicate that some nucleosomes might have joined to form dimers following interactions with ySWI/SNF. In summary, the nucleoprotein gel demonstrates that ySWI/SNF did not promote a release of free DNA from nucleosomes. Thus, the UV damage pattern observed by footprinting reflects the pattern of a nucleosome interacting with ySWI/SNF or being remodeled by ySWI/SNF.

Nucleosome Remodeling by ySWI/SNF Facilitates Repair—To address a possible contribution of nucleosome remodeling activities to DNA repair, nucleosomes were irradiated with UV light at a dose of 500 J/m², incubated with ySWI/SNF either in the absence or presence of ATP, and exposed to photolyase in the presence of photoreactivating light. The nucleoprotein gel (Fig. 4A) showed that the nucleosomes were stable over the course of the experiment (lanes 2–7). Addition of ySWI/SNF resulted in complexes that appeared as a smear or remained stuck in the wells (Fig. 4A, lanes 8–15). Thus, ySWI/SNF can bind to damaged nucleosomes both in the presence and absence of ATP. Photolyase does not disrupt the complexes or may disrupt the complexes only transiently.

To address the state of the nucleosome during the repair experiment, the ySWI/SNF complex was removed by competition with plasmid DNA (Fig. 4B). In all samples, addition of plasmid DNA resulted in the recovery of the nucleosomal band (lanes 8–15) indicating that most of the DNA remained incorporated into nucleosomes after remodeling by ySWI/SNF and over the course of photoreactivation.

Repair analysis is shown in Fig. 4, C and D. Repair of naked DNA was fast and complete after 30 min, whereas repair of the nucleosomal substrate was fast outside and slow inside of the nucleosome as observed above. Incubation with ySWI/SNF in the absence of ATP did not dramatically alter the repair pat-
tern. Only clusters 12 and 19 were more slowly repaired than in nucleosomes. Thus, binding of ySWI/SNF did not substantially inhibit the accessibility of CPDs, neither outside nor inside the nucleosome. Substantial changes in repair were observed in the presence of ySWI/SNF and ATP (Fig. 4, C, lanes 10–12, and D). Repair of clusters 10 and 11 was reduced, and repair of clusters 12–18 was enhanced, resulting in a more homogeneous repair pattern over the DNA fragment. Only cluster 19 remained poorly repaired. Since the nucleosomes were not disrupted, we can conclude that in the presence of ATP, ySWI/SNF facilitates CPD accessibility and repair by remodeling the chromatin substrate.

yISW2 Remodels ATDED-long Nucleosomes—To investigate whether other ATP-dependent remodeling complexes can promote CPD repair in nucleosomes, His-tagged-yISW2 was tested in our model system.

The nucleosomes were irradiated with UV light at a dose of 500 J/m², incubated with yISW2 either in the absence or in the presence of ATP, and exposed to DNA photolyase and photoreactivating light. The nucleoprotein gels (Fig. 5A) showed that incubation of UV-irradiated nucleosomes with yISW2 in the absence of ATP did not change the migration of the nucleosomes (lanes 7–10), indicating that the majority of the nucleosome substrate was not firmly bound to yISW2 in our conditions. In the presence of ATP, nucleosome migration was retarded, and the altered mobility was maintained over the course of photoreactivation (Fig. 5A, N2, lanes 11–14) indicating that yISW2 was actively remodeling the substrate. Since nucleosomes located at a fragment end migrate faster than those that are positioned more centrally in non-denaturing gels (43), it is likely that yISW2 and ATP shifted the nucleosome to a more central position. This behavior was not dependent on UV irradiation, because similar results were obtained on non-damaged nucleosomes (data not shown). This part of the experiment demonstrates that yISW2 can remodel UV-damaged nucleosomes and that the remodeled nucleosomes remain stable over the course of the experiment.

The DNA repair gels and the fraction of CPDs repaired in 30 min are shown in Fig. 5, B and C. Repair of naked DNA was complete after 30 min, whereas repair of nucleosomes was modulated as described above. Although incubation with yISW2 had no dramatic effect on CPD repair, yISW2 in the presence of ATP altered repair on both strands. Repair was inefficient in the central clusters 4–7 (top strand) and 12–17...
of material in the well (W). The samples described in the text were incubated with an excess plasmid DNA for 45 min at room temperature. The fraction of material in the well (W), the nucleosomal bands (N), and the naked DNA bands (D) is indicated for each lane (bottom). C, photoreactivation of naked DNA, nucleosomes, and remodeled nucleosomes. Description is as in Fig. 2B. Lane 1, initial CPD distribution; lanes 4–6, photoreactivation of nucleosomes and ySWI/SNF; lanes 10–12, photoreactivation of nucleosomes, ySWI/SNF and ATP; lanes 13–15, photoreactivation of naked DNA isolated from irradiated nucleosomes; lanes 1 and 3, damaged DNA treated with no T4-endonuclease V and an excess of T4-endonuclease V, respectively; ellipse, the position of the histone octamer; black bars 10–19, pyrimidine clusters. D, fraction of CPD repaired in 30 min in clusters 10–19. Nucleosomes (open bars), nucleosomes incubated with ySWI/SNF (gray bars), nucleosomes incubated with SWI/SNF and ATP (black bars). (Data of Figs. 3 and 4 are from three independent experiments reproducing ATP-dependent modulation of DNA accessibility (Fig. 3B and Fig. 4, C and D) and DNA structure (Fig. 3D and Fig. 4C).)

Fig. 4. Enhanced CPD repair on nucleosomes remodeled by ySWI/SNF. DNA was end-labeled on the bottom strand, reconstituted in nucleosomes, irradiated with 500 J/m² UV, incubated with ySWI/SNF (0.6 complex per nucleosome), and exposed to photolyase and photoreactivating light for up to 60 min. A, nucleoprotein gel. D, naked DNA; N and Nucl., nucleosomes; W, wells. B, nucleoprotein gel analysis after competition of ySWI/SNF. The samples described in A were incubated with an excess plasmid DNA for 45 min at room temperature. The fraction of material in the well (W), the nucleosomal bands (N), and the naked DNA bands (D) is indicated for each lane (bottom). C, photoreactivation of nucleosomes, ySWI/SNF, and remodeled nucleosomes. Description is as in Fig. 2B. Lane 2, initial CPD distribution; lanes 4–6, photoreactivation in nucleosomes; lanes 7–9, photoreactivation of nucleosomes and ySWI/SNF; lanes 10–12, photoreactivation of nucleosomes, ySWI/SNF and ATP; lanes 13–15, photoreactivation of naked DNA isolated from irradiated nucleosomes; lanes 1 and 3, damaged DNA treated with no T4-endonuclease V and an excess of T4-endonuclease V, respectively; ellipse, the position of the histone octamer; black bars 10–19, pyrimidine clusters. D, fraction of CPD repaired in 30 min in clusters 10–19. Nucleosomes (open bars), nucleosomes incubated with ySWI/SNF (gray bars), nucleosomes incubated with SWI/SNF and ATP (black bars). (Data of Figs. 3 and 4 are from three independent experiments reproducing ATP-dependent modulation of DNA accessibility (Fig. 3B and Fig. 4, C and D) and DNA structure (Fig. 3D and Fig. 4C).)

DNA accessibility in eukaryotes is affected by intrinsic properties of nucleosomes, their higher order organization, and by chromatin modifying activities. Here we show that neither DNA damage formation by UV light nor interaction with photolyase disrupted a preformed nucleosome. However, two different chromatin remodeling activities were shown to remodel UV-damaged nucleosomes and modulate damage accessibility for a repair enzyme. Thus, our results suggest that remodeling activities could be engaged in cells to relieve the inhibitory effect of nucleosomes in DNA repair processes.

UV Damage Formation and Nucleosome Stability—UV-induced DNA lesions represent distortions in the DNA structure. It is therefore conceivable that DNA lesions interfere with the stability of nucleosomes and may promote their disruption or promote sliding to alternate positions. The currently available data are somewhat controversial. Generally, UV-damaged nucleosomes appear to be quite stable, since they can be purified from irradiated cells (e.g. Ref. 44). On the other hand, irradiation was shown to destabilize nucleosomes reconstituted on 5 S rDNA (45) and plasmids (46), whereas nucleosomes reconstituted on HISAT DNA and 5 S rDNA were not similarly affected (20, 38, 39). In the examples shown in this study, UV lesions were not sufficient to disrupt the nucleosomes. Thus, structural distortions introduced by UV lesions were accommodated by both nucleosomes and nucleosome remodeling complexes. This is consistent with the observation that crystallized nucleosomes can accept the deficit of a base pair in one turn of the superhelix (6) and therefore could as well accept a DNA lesion.

**Inhibition of Photoreactivation by a Positioned Nucleosome**—Previous work (20, 21) reported a strong inhibition of CPD repair by photolyase and T4-endonuclease V in nucleosomes in vitro. Here we demonstrate that the inhibition of photolyase is restricted to nucleosomal DNA, whereas the nucleosome-free “linker” DNA was rapidly repaired. Thus, it appears that binding and/or processing of CPDs is strongly inhibited on the nucleosome surface. Moreover, there is no obvious correlation of site-specific photoreactivation with the rotational setting as determined by DNase I. In contrast to DNase I, which binds to the minor groove and generates single strand cuts, photolyase bends DNA and flips out the pyrimidine dimer into its active site (47, 48). Despite the flexibility of nucleosomal DNA with respect to damage accommodation, such a flip-out mechanism appears to be severely inhibited by the structural constraints of nucleosomes. Consequently, efficient repair of nucleosomal DNA requires disruption or displacement of nucleosomes with or without the help of remodeling activities.
Remodeling by ySWI/SNF Alters the Structure of Nucleosomal DNA and Facilitates CPD Accessibility—SWI/SNF generally increases the accessibility of nucleosomal DNA to transcription factors, DNase I, and restriction endonucleases (32–35) and induces octamer sliding (49, 50). The UV photofootprint experiments show that the DNA structure changes upon remodeling by ySWI/SNF. In naked DNA, CPD formation is influenced by the DNA structures, in particular by the unusual rigid structure of T-tracts. The CPD pattern changed, when DNA was reconstituted in nucleosomes, indicating that the histone octamer exerts a dominant constraint on those sequences (38, 51). Binding of ySWI/SNF alone had no effect on the CPD formation pattern, which suggests that the nucleosome remained intact. However, addition of ySWI/SNF and ATP changed the CPD pattern, which appeared similar to that of naked DNA, although the complex remained bound. Thus, DNA appears to be relaxed or extended enough in the nucleosome-SWI/SNF-ATP complex to allow formation of the T-tract structure.

The repair data also support a generally better accessibility of remodeled DNA. In contrast to the modulation of repair observed in nucleosomes or nucleosomes with ySWI/SNF, ySWI/SNF and ATP allowed more uniform repair along the reconstituted DNA fragment. Only cluster 19 at the end was relatively poorly repaired. Therefore, ySWI/SNF activity appears to generally facilitate the accessibility of photolyase to nucleosomal DNA.

Remodeling apparently destabilizes the structure of the histone-DNA complex to such an extent that the photo-footprint reveals a naked DNA-like structure, and the activity of a base flip-out enzyme can be accommodated. The details of that structure are not known at present. If nucleosome sliding were involved, the octamer must move to and even overlap the DNA end (8) to allow repair of cluster 18 but inhibit repair of cluster 19.

Nucleosome Mobilization by yISW2 Influences CPD Repair—ISWI containing complexes have been shown to induce octamer
sliding (52–55). Yeast ISW2 is a two-subunit complex belonging to the ISWI group of ATP-dependent remodeling factors, which influences nucleosome positioning in vitro (56) and nucleosome spacing in vitro (57). Here we provide two lines of evidence that His-tagged yISW2 can act on UV-damaged nucleosomes and move the nucleosome from the end to a more central position in an ATP-dependent manner. First, an altered migration was observed in nucleoprotein gels. Second, the photoreactivation revealed an altered repair pattern, in particular enhanced repair at sites toward the end of the fragment and inhibition in the center. It has to be pointed that, in contrast to the ySWI/SNF, yISW2 was not firmly bound to nucleosomes. Thus, the repair data do not reflect the accessibility in the complex but rather the accessibility in the remodeled products. Very recently, ISW2 has been shown to move undamaged nucleosomes from an end position to a central position on a DNA fragment (58), thus indicating that the ISW2 complex behaves similarly on undamaged and damaged nucleosomes.

Several mechanisms are discussed how nucleosomes are mobilized by ATP-dependent remodeling complexes: (i) disruption and reformation of all histone-DNA contacts, (ii) formation of a DNA bulge, or (iii) local twist of DNA that propagates on the histone surface (reviewed in Ref. 2). The results shown here demonstrate that ySWI/SNF and yISW2 in the presence of ATP can remodel UV-damaged nucleosomes. Thus, irrespective of the underlying mechanism, UV lesions do not inhibit those structural transitions.

DNA Damage Accessibility in Vivo—To defend the cells against extensive mutagenesis of the genome, all DNA lesions need to be repaired efficiently (15). Although there is a pronounced modulation by nucleosomes and other protein-DNA interactions, both nucleotide excision repair and photolyase almost completely remove UV-induced DNA lesions (4, 5). High resolution repair analyses on positioned nucleosomes of the \textit{URA3} gene in yeast showed similar repair patterns for NER and photolyase: fast repair in the linker and a decrease toward the center of the nucleosomes (17, 19, 59). In combination with multiple positions found by nuclease footprinting, those studies (59) suggest that intrinsic mobility of nucleosomes might place a lesion in the linker DNA, thus providing a window of accessibility for damage recognition.

Alternatively, there is an increasing number of repair-related proteins with potential roles in chromatin remodeling (1). CSB and its yeast homologue Rad26 belong to the SNF2 family and are involved in the transcription-coupled repair rather than in the repair of non-transcribed DNA (28, 60). Recombinant CSB was shown to remodel undamaged nucleosomes and nucleosome arrays in vitro, thereby being the first repair enzyme demonstrated to possess remodeling activity (29). Rad7-Rad16 is a complex of the NER pathway of yeast \textit{Saccharomyces cerevisiae} that is essential for repair of nontranscribed, nucleosomal chromatin (61) and recognizes UV lesions in an ATP-dependent manner in vitro (62). Rad16 has homology to SNF2 (63) and therefore might play a role in nucleosome remodeling to generate space for the other NER proteins (4). ACF, on the other hand, is a chromatin assembly and remodeling factor containing ISWI and ACF1, which was shown to facilitate NER of a specific lesion located in linker DNA but not of a lesion in the nucleosome (25). The observation made here that two remodeling activities, ySWI/SNF and yISW2, can act on damaged nucleosomes and alter the accessibility for a base flip-out enzyme suggests that similar activities could play a role in vivo. This hypothesis is further supported by the recent findings that ySWI/SNF stimulates the human excision reaction on a nucleosome containing a bulky adduct (13) and that binding of transcriptional activators to their cognate sequences in the absence of transcription stimulates NER by inducing a local chromatin remodeling mediated by ATP-driven chromatin remodelers and acetyltransferases (11).

For transcriptional regulation, nucleosome remodeling complexes are recruited to the promoter regions of specific genes by transcription factors. The situation is different for DNA repair, because DNA lesions are generated almost randomly all over the genome. This implies that, in principle, a DNA lesion needs to be recognized first, before nucleosome remodeling activities can be recruited. Therefore, damage accessibility depends on the structural properties of the region containing the DNA lesion (e.g. nucleosome, linker, and nucleosome-free region) and on the genetic activity, e.g. whether it is transcribed or replicated. We might speculate about a more general role of remodeling activities in chromatin organization. Since all ATP-dependent remodeling complexes apparently can change the structure or translational positions of nucleosomes, it seems conceivable that these complexes might also act randomly on the chromatin substrate in order to enhance the intrinsic dynamic properties of nucleosomes and keep chromatin in a “fluid” state. This would facilitate any DNA sequence recognition in transcriptional regulation and repair and, in addition, adjust packaging constraints imposed by chromosome metabolism. Thus, remodeling complexes might perform a rather general role in the maintenance of chromosome structure.

In this study, \textit{E. coli} photolyase was used as a tool to assess CPD accessibility and repair in nucleosomes. However, \textit{E. coli} photolyase has many properties in common with the yeast \textit{S. cerevisiae} photolyases. Both photolyases share a high sequence homology, require the same co-factors, and can partially cross-complement each other in photoreactivation-deficient mutants (reviewed in Ref. 16). Photolyase repair CPDs via a “flip-out” mechanism (47, 48) as do many other DNA-processing enzymes, including methyltransferases and DNA glycosylases involved in base excision repair (64). It is conceivable that those flip-out enzymes have similar structural requirements for DNA accessibility. Indeed, as observed for photolyase and T4-endonuclease V (20), human uracil-DNA glycosylases, SMUG1 and SMUG2, were able to remove uracil from nucleosomes, but the efficiency of uracil excision from nucleosomes was severely reduced when compared with naked DNA (65). With our model system we have tested a possible contribution of nucleosomes and remodeling activities toward DNA repair of UV lesions. Thus, it will be important to investigate whether similar observations can be made with other enzymes and DNA lesions and whether a contribution of remodeling activities can be detected as a requirement or help for repair processes in living cells.

Acknowledgments—We thank Dr. R. E. Wellington for discussions and Dr. U. Suter for continuous support.

REFERENCES

1. Green, C. M., and Almouzni, G. (2002) \textit{EMBO Rep.} 3, 28–33
2. Narlikar, G. J., Fan, H. Y., and Kingston, R. E. (2002) \textit{Cell} 108, 475–487
3. Pyndyk, D. V., and Kadonaga, J. T. (2001) \textit{Cell} 106, 523–525
4. Thoma, F. (1999) \textit{EMBO J.} 18, 6585–6588
5. Smerdon, M. J., and Conconi, A. (1999) \textit{Proc. Natl. Acad. Sci. USA} 96, 327–332
6. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) \textit{Nature} 389, 251–260
7. Widom, J. (1998) \textit{Annu. Rev. Biophys. Biomol. Struct.} 27, 285–327
8. Peterson, C. L. (2000) \textit{FEBS Lett.} 476, 68–72
9. Vignali, M., Hassan, A. H., Neeley, K. E., and Workman, J. L. (2002) \textit{Mol. Biol. Cell} 13, 1899–1910
10. Boyer, L. A., Logie, C., Bonte, E., Becker, P. B., Wade, P. A., Wolffe, A. P., Wu, Y., Imbalzano, A. N., and Peterson, C. L. (2000) \textit{J. Biol. Chem.} 275, 18864–18870
11. Frit, P., Kwon, K., Coin, F., Auriol, J., Dubaule, S., Salles, B., and Egly, J. M. (2002) \textit{Mol. Cell} 10, 1281–1301
12. Alexandiadis, V., and Kadonaga, J. T. (2002) \textit{Genes Dev.} 16, 2767–2771
13. Har, R., and Sancar, A. (2002) \textit{Mol. Cell. Biol.} 22, 6779–6787
14. Park, H., Zhang, K., Ren, Y., Nadj, S., Sinha, N., Taylor, J. S., and Kang, C.
