Histone H3 Lysine 14 (H3K14) Acetylation Facilitates DNA Repair in a Positioned Nucleosome by Stabilizing the Binding of the Chromatin Remodeler RSC (Remodels Structure of Chromatin)*

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Ming-Rui Duan and Michael J. Smerdon
From Biochemistry and Biophysics, School of Molecular Biosciences, Washington State University, Pullman, Washington 99164-7520

Background: Histone H3K14 acetylation is induced by UV damage.
Results: Nucleosomes with acetylated H3K14 show greater CPD repair than unacetylated nucleosomes in the presence of the chromatin remodeling complex RSC.
Conclusion: H3K14ac acts as a “docking” site to retain RSC on nucleosomes and facilitate DNA repair.
Significance: DNA repair is accelerated by an orchestrated action between UV-induced histone acetylation and chromatin remodeling.

Histone H3 acetylation is induced by UV damage in yeast and may play an important role in regulating the repair of UV photodensities in nucleosome-loaded genomic loci. However, it remains elusive how H3 acetylation facilitates repair. We generated a strongly positioned nucleosome containing homogeneously acetylated H3 at Lys-14 (H3K14ac) and investigated possible mechanisms by which H3K14 acetylation modulates repair. We show that H3K14ac does not alter nucleosome unfolding dynamics or enhance the repair of UV-induced cyclobutane pyrimidine dimers by UV photolyase. Importantly, however, nucleosomes with H3K14ac have a higher affinity for purified chromatin remodeling complex RSC (Remodels the Structure of Chromatin) and show greater cyclobutane pyrimidine dimer repair compared with unacetylated nucleosomes. Our study indicates that, by anchoring RSC, H3K14 acetylation plays an important role in the unfolding of strongly positioned nucleosomes during repair of UV damage.

UV damage buried in condensed regions of chromatin is highly inaccessible to DNA repair factors. Nucleosomes at damaged sites must undergo rearrangement to allow the access of repair machinery, as described in the “access-repair-restore” model (1, 2). The access step in UV damage repair is regulated by several different mechanisms, including histone posttranslational modification and chromatin remodeling by ATP-dependent remodeling complexes (3, 4). Previous studies have documented the involvement of histone acetylation in UV damage repair. Early studies indicated that DNA repair of UV lesions was stimulated in human fibroblasts treated with sodium butyrate, an inhibitor of histone deacetylase (5). More recently, it has been shown that histone H3 is hyperacetylated at Lys-9 and Lys-14 after UV irradiation in yeast (6). Indeed, deletion of Gcn5, the histone acetyltransferase responsible for H3K142 acetylation, results in deficient nucleotide excision repair (NER) and photoreactivation of cyclobutane pyrimidine dimers (CPDs) by UV photolyase in the nucleosome-containing MFA2 gene locus (6, 7).

Although histone acetylation is linked to DNA repair of UV damage, the molecular mechanisms involved are not clear. One proposed mechanism is that modification of nucleosome structural dynamics may influence the accessibility of NER enzymes (8, 9). Nucleosomes are not static particles but exist in a dynamic equilibrium in which the DNA unwraps and rewraps from the histone octamer spontaneously (10–12). This transient DNA site exposure may result in increased accessibility to repair proteins. Indeed, as reported by Poirier and coworkers (13), histone acetylation can shift the dynamic equilibrium in nucleosome unwrapping and disassembly. These authors showed that acetylation at histone octamer sites near the DNA entry-exit region of the nucleosome regulates DNA unwrapping, whereas acetylation at sites near the dyad center regulate nucleosome disassembly. Furthermore, single-molecule FRET experiments revealed that acetylation at H3 Lys-56 increases DNA breathing 7-fold (9).

An alternative mechanism for the effect of histone acetylation on DNA accessibility is for acetylation acting as a signal for ATP-dependent chromatin remodeling factors. Previous studies have demonstrated that these remodeling complexes regulate chromatin structure and participate in various DNA repair pathways (14–19). To date, however, there is no direct evidence showing that chromatin remodeling complexes and histone acetylation act in a coordinated manner to facilitate repair following UV damage.

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We dedicate this work to the memory of our colleague Dr. Jonathan Widom, William Deering Professor of Molecular Biosciences, Northwestern University.

1 To whom correspondence should be addressed. Tel.: 509-335-6853; Fax: 509-335-4159; E-mail: smerdon@wsu.edu.

2 The abbreviations used are: H3K14, histone H3 lysine 14; NCP, nucleosome core particle; NER, nucleotide excision repair; CPD, cyclobutane pyrimidine dimer; H3K14ac, histone H3 acetylated at Lysine 14; CcPhr, C. crescentus photolyase; HRPF, high-resolution photo-footprinting; TAP, tandem affinity purification.
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Among the chromatin remodeling complexes, RSC (Remodels the Structure of Chromatin), an essential and abundant ATP-dependent chromatin remodeling complex, directly interacts with H3K14 acetylation (20, 21). This remodeling complex has been shown to function in DNA double strand break repair, where RSC-mediated chromatin remodeling assists in the access of repair proteins to the damaged site in early stages of double strand break repair (22). In addition, RSC is known to enhance restriction endonuclease digestion, DNase I accessibility, and transcription elongation in nucleosomal DNA in vitro by altering the structure and positioning of nucleosomes (23–25). Whether RSC can act on UV-damaged nucleosomes to promote CPD removal is yet unknown. Moreover, a major difficulty in determining whether RSC remodeling activity is influenced by H3K14 acetylation during repair has been the inability to generate homogenously acetylated nucleosome substrates.

UV light preferentially generates CPDs and 6,4 pyrimidine-pyrimidone photoproducts in DNA (26). DNA photolyase is a single protein that contains a reduced flavin adenine dinucleotide and uses blue light to restore CPDs to the normal bases by splitting the cyclobutane ring (27, 28). Thoma and co-workers have shown that photolyase activity is greatly impaired in nucleosomes and is modulated by chromatin structure (29, 30). Interestingly, in many organisms, including Saccharomyces cerevisiae, DNA photolyase repairs CPDs effectively on a time scale of seconds, implying the use of additional mechanisms for gaining access to CPDs in cells (31). Thus, compared with the stepwise repair of CPDs during NER, DNA photolyase provides a direct analysis of DNA accessibility in a positioned nucleosome in vitro.

In this study, we measured the activity of DNA photolyase on a strongly positioned nucleosome using the Widom 601 nucleosome positioning sequence (32). Nucleosomes were reconstituted with either unmodified histone octamers or octamers with homogeneously acetylated H3K14 to compare the influence of this acetylation on nucleosome stability and photolyase repair activity. As expected, we find that this single acetylation at H3K14 is not sufficient to promote nucleosome instability or CPD reversal. However, it markedly strengthens the binding of RSC to nucleosomes in the presence of a competitor and facilitates repair by DNA photolyase. These results reveal a potential link between a chromatin remodeling complex and histone acetylation during repair of UV lesions.

EXPERIMENTAL PROCEDURES

Histone Preparation and Nucleosome Reconstitution—Acetylated histone H3 (H3K14ac) was generated from Escherichia coli (BL21) cells transformed with plasmids pAcKRS-3 and pCDF PytT-1 carrying the histone H3 ORF with amber codon at Lys-14 (a gift from Dr. Jason Chin, Medical Research Council Laboratory of Molecular Biology) and purified using the method of Neumann and coworkers (9). Unmodified histone octamer and 147-bp 601 DNA labeled with Cy3 and Cy5 were prepared as described previously (33, 34). The 257-bp DNA fragment containing the 601 nucleosome positioning sequence was prepared by PCR using the primers 5’-ATGATTCTTCACACCAGGTTCTACC-3’ and 5’-GGGAGCTCGGAACACATCCGA with the plasmid pGEM3Z.601 (a gift from Dr. Jonathon Widom, Northwestern University) as template. Reconstitution was done by salt gradient dialysis (35) and analyzed on 5% native polyacrylamide gels (33).

FRET Assay—FRET experiments were carried out on a Photon Technology International Quantamaster UV-visible steady-state fluorometer as described previously (33). FRET efficiencies were measured by the sensitized emission of the acceptor and calculated as described by Yang et al. (36).

UV Irradiation and Photoreactivation—The 257-bp naked DNA was dissolved in water and irradiated on Parafilm with 254-nm UV light under two low-pressure mercury lamps (Sylvania, model no. G30T8) at a dose of 500 J/m². The UV dose was measured with a Spectroline DM-254N UV meter (Spectronics Corp., Westbury, NY). UV-damaged DNA was subjected to nucleosome reconstitution with either unmodified or H3K14ac-modified histone octamers. Irradiated naked DNA or nucleosomes (0.15 pmol) were mixed with 0.7 pmol of Caulobacter crescentus photolyase (CcPhr; a gift from Dr. Aziz Sanz-Camara, University of North Carolina) in repair buffer (50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, and 10 mM DTT). The mixture was irradiated at 365 nm of light with a Spectroline UV lamp (model ENF-240C, Westbury, NY) through a Pyrex filter for different time points. For RSC remodeling experiments, samples were photoreactivated in remodeling buffer (see below). Photoreactivation was stopped by phenol chloroform extraction prior to ethanol precipitation of DNA.

High-resolution Photo-footprinting Gel—Pellets of repaired DNA were washed with 70% ethanol and resuspended with T4 endonuclease V digestion buffer (100 mM NaCl, 50 mM Tris-HCl (pH 7.5), 10 mM EDTA, and 0.05 mg/ml BSA) followed by incubation with 0.4 units of T4 endonuclease V (Epicenter) at 37 °C for 2 h. Digestion products were isolated and analyzed on 8% denaturing polyacrylamide DNA sequencing gels as described previously (37).

Quantification of CPDs—Band intensities were determined using ImageQuant (GE Healthcare) and PeakFit deconvolution software (Systat Software). The sum of signal intensities of all bands within each lane was used as the DNA loading signal, which was used for normalization. The amount of CPDs in a sample was calculated as a fraction of the initial damage at the sites. Peaks in the gel scans were deconvoluted using PeakFit, and intensities of bands (i.e. areas of the deconvoluted peaks) in each cluster were analyzed (37).

Purification of the RSC Complex—The RSC complex was purified from yeast (strain BCY211, a gift from Dr. Bradley Cairns, University of Utah) as described previously (38). Briefly, yeast cells (9-liter cultures) were harvested, washed, and suspended in lysis buffer containing 50 mM HEPES (pH 7.5), 250 mM potassium acetate, 10 mM EDTA, 0.5 mM DTT, 20% glycerol, and protease inhibitors. Cells were disrupted by 425–600 μm glass beads in a bead beater (Biospec, catalog no. 11079105), followed by centrifugation at 15,000 rpm for 10 min at 4 °C. The potassium acetate concentration in the supernatant was increased to 400 mM, and the nucleic acids were precipitated by addition of 10% polyethyleneimine to a final concentration of 0.1%. The sample was subjected to centrifugation in a Beckman L8–70 M ultracentrifuge at 45,000 rpm in a Ti-50.2
rotor for 45 min at 4 °C. The clear lysate was incubated with IgG-Sepharose beads (GE Healthcare), and the TAP-tagged RSC complex was captured and then released from the IgG beads by tobacco etch virus protease (Invitrogen). The supernatant was bound to calmodulin beads in the presence of calcium and eluted in the presence of EGTA. Purification was monitored by Western blotting, using anti-TAP antibody (Open Biosystems) to detect the Rsc2 subunit. Finally, the purified RSC complex was verified by SDS-PAGE followed by silver staining (Fig. 6).

**RSC Remodeling**—Purified RSC (20 ng) was added to unmodified or H3K14ac nucleosomes in remodeling buffer (0.075 mg/ml BSA, 15 mM HEPES (pH 7.5), 3 mM MgCl$_2$, 20 mM potassium acetate, and 1 mM ATP where indicated). For the repair assay with competitors, 0.09 μg or 0.45 μg (3.6-fold or 18-fold excess competitor) of NCPs (primarily 147-bp DNA), prepared from chicken erythrocytes as described (39), was added simultaneously with the RSC complex. The remodeling reaction was carried out at 30 °C for 20 min prior to the addition of CcPhr for photoreactivation.

**RSC Retention Assay**—A 257-bp 601 DNA fragment was generated by PCR using biotin-labeled primer (Integrated DNA Technologies) to biotin-label the DNA at one 5’ end. The biotinylated DNA was reconstituted with WT or H3K14ac histone octamer to form nucleosomes. Nucleosomes (250 ng) were bound to streptavidin paramagnetic beads (Dynabeads M-280 Streptavidin, Invitrogen) in binding buffer containing 10 mM HEPES (pH7.5), 50 mM KCl, 5 mM DTT, 5 mM PMSF, 5% glycerol, 0.25 mg/ml BSA, 2 mM MgCl$_2$, and 300 mM KCl. After incubation at 30 °C for 20 min, the beads were washed twice in washing buffer (10 mM HEPES (pH 7.5), 50 mM KCl, 5 mM DTT, 5 mM PMSF, 5% glycerol, 0.25 mg/ml BSA, 2 mM MgCl$_2$, and 0.5% Nonidet P-40). RSC (5 ng) and competitor NCPs (0.27 μg) were added simultaneously to the remodeling buffer and incubated at 30 °C for another 20 min. The beads were collected using a magnet rack, and the supernatant was removed. The beads were washed three times with washing buffer before being subjected to Western blot analysis. The blots were probed against TAP antibody (Open Biosystems) to detect RSC binding. Western blot signal intensities were quantified using ImageQuant TL software.

## RESULTS

**H3K14 Acetylation Does Not Affect Nucleosome Unwrapping Dynamics and Salt Stability**—To investigate the role of H3K14 acetylation, a modification that is induced by UV irradiation and is important for NER (6), we prepared both unmodified histone octamers (35) and histone octamers homogenously acetylated at H3K14. The modified histone H3 was generated in a genetically modified *E. coli* expression system in which there are aminoacyl-tRNA synthetase and tRNA$_{CUA}$ pairs that direct the incorporation of acetyl lysine in response to an amber codon in the H3 gene (9). As expected, Western blot analyses using anti-H3K14ac antibody demonstrated that H3K14 was indeed acetylated during H3 preparation (Fig. 1A). MS/MS confirmed that virtually all H3 molecules were acetylated at Lys-14 and that no other sites were acetylated (data not shown).

In addition, histone octamer formation and nucleosome reconstitution were unaffected by this modification (data not shown). To examine the effect of H3K14ac on nucleosome unwrapping dynamics, NCPs were generated with or without H3K14ac.
and a 147-bp DNA fragment containing the 601 nucleosome positioning sequence (32). Furthermore, NCPs were generated with undamaged or UV-damaged DNA to examine the impact of H3K14ac on nucleosome dynamics in the presence of UV damage (33). As shown in Fig. 1B, nucleosome formation is not significantly affected by H3K14 acetylation or the presence of UV damage. The DNA was tagged with the fluorescent cyanine dyes Cy3 and Cy5 at positions 33 and 113 on opposing strands (33) (Fig. 2A), where both dyes are located in the internal region of the NCP DNA. Both undamaged and UV-damaged NCPs were assembled with these labeled DNAs for FRET analyses (33). In the folded nucleosome state, the close proximity of donor (Cy3) and acceptor (Cy5) permits efficient FRET, where greater emission occurs from Cy5 (670 nm peak) and concurrent reduced emission from Cy3 (570 nm peak).

Initially, we analyzed the FRET efficiency at a low salt concentration (50 mM NaCl) to compare the unwrapping dynamics between NCPs with or without H3K14ac. Emission spectra of the donor-accepter pair were comparable upon excitation of the donor (Cy3) (Fig. 2B), and UV damaged NCPs showed lower FRET efficiency, as expected, in each case (33). In addition, the FRET efficiency as a function of salt concentration yielded dissociation profiles that were indistinguishable with or without UV damage (Fig. 2, C and D). These results suggest that the single acetylation at H3K14 is not sufficient to alter nucleosome site exposure dynamics or stability.

Because the location of H3K14 acetylation is on the N-terminal tail of histone H3 and the local effect of this modification may not be readily detectable by FRET between dyes in the “internal” DNA positions (i.e. away from the DNA ends), we also tested a label pair in which Cy3 was positioned just 6 bp from the end of the DNA and Cy5 was close to the dyad center on the opposite strand (called “end-labeled” dye pairs (34) (Fig. 2A). The end-labeling strategy enabled us to determine whether the acetylation at H3K14 influences the spontaneous unwrapping of DNA at the entry-exit position of nucleosomes (13). Similar to the internal labeling data (Fig. 2), no change was observed with H3K14ac NCPs in FRET efficiency for either nucleosome site exposure dynamics or salt stability (Fig. 3, A and B). These results demonstrate that H3K14ac alone does not change nucleosome structure by influencing the unwrapping dynamics and stability.

The Rotational Setting of CPDs in Nucleosomes Modulates CPD Removal by DNA Photolyase—To evaluate how structural and dynamic properties of nucleosomes facilitate DNA damage recognition and repair in unmodified and H3K14ac nucleosomes, we used CcPhr (40) to study CPD accessibility in nucleosomes. Photolyase activity is markedly inhibited by nucleosome structure and is modulated by the orientation of CPDs on the histone surface (or “rotational setting”) in nucleosomes in vitro (41, 42). However, buried sites may become more accessible to proteins by dynamic transitions (43–46), and the unwrapping-
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rewrapping dynamics of nucleosomes may facilitate their own “invasion” (10). Therefore, a 257-bp DNA fragment containing the 601 positioning sequence was irradiated with a dose of 500 J/m² UV light to generate an average of 1 CPD/601 fragment and distributed among the various CPD sites with different yields (calculated as described previously (47)) (Fig. 4A). The UV damaged DNA was reconstituted with recombinant unmodified (WT) or H3K14ac histone octamers to generate CPD sites along nucleosome DNA is very similar between H3K14ac nucleosomes and unmodified nucleosomes (Fig. 5). These data are consistent with the FRET measurements (Fig. 2), supporting the notion that acetylation at H3K14 does not markedly change nucleosome structure or unwrapping dynamics, and indicate that H3K14ac alone is insufficient to change access to DNA repair proteins.

RSC Acts on UV-damaged Nucleosomes in an ATP-dependent Manner—Because H3K14ac does not play a direct role in destabilizing nucleosome structure to increase DNA damage accessibility, we hypothesized that this modification may function as a signal for bromodomain-containing complexes to facilitate DNA repair. Indeed, H3K14ac can be recognized by the tandem bromodomains on Rsc2 of the RSC complex in vitro (20). Thus, we tested whether RSC is retained by H3K14ac to assist photolyase in overcoming the nucleosome barrier during photocrosslinking. Purified RSC complex was prepared by tandem affinity purification from a yeast strain expressing TAP-tagged Rsc2 (52) (Fig. 6). The remodeling activity of the purified RSC complex was verified by restriction enzyme accessibility, as described by Cairns and colleagues (52). As shown in Fig. 6, the accessibility of Hhal in 601 nucleosomes is clearly stimulated by adding both RSC and ATP. This result demonstrates that remodeling activity is associated with our purified RSC complex on nucleosomes in the presence of ATP.

We next carried out HRPF to measure the CPD removal efficiency in unmodified or H3K14-acetylated nucleosomes. As shown in Fig. 7, incubation with RSC in the absence of ATP does not change the repair efficiency at any of the poorly repaired CPD clusters. These results indicate that RSC binding, and not remodeling, has little (or no) effect on nucleosome accessibility, similar to the chromatin remodeler SWI/SNF (switch/sucrose nonfermenting) (42). However, in the presence of ATP, RSC greatly enhances the removal of CPDs located in the nucleosome core region (i.e. clusters 3, 5, and 7–10), where repair is markedly inhibited (Fig. 7A). The overall repair efficiency in these clusters increases significantly (Fig. 7B), indicating that RSC remodeling of nucleosomes facilitates CPD accessibility to CcPhr and repair in vitro. Furthermore, under these reaction conditions, RSC activity appears to be independent of the acetylation state of H3K14 because both nucleosome substrates show similar repair enhancement stimulated by RSC in the presence of ATP (Fig. 7).

RSC Preferentially Stimulates CPD Repair in H3K14ac Nucleosomes in the Presence of Competitor Nucleosomes—The RSC complex in yeast contains multiple subunits with bromodomains or protein domains that interact with acetylated lysine residues (21). Moreover, previous results showing that RSC recognizes H3K14 in yeast (20) suggest that there is a func-
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FIGURE 4. Reconstitution of nucleosome substrates with the long (257-bp) 601 positioning sequence. A, schematic of the pyrimidine cluster locations and sequences on the nucleosome substrate. The oval represents histone octamer where the strong positioning sequence is located. The pyrimidine clusters are numbered, and the horizontal bars show the locations of the clusters relative to the histone octamer. Vertical lines indicate cleavage efficiencies by hydroxyl radicals (an indication of orientation relative to the histone surface), where longer lines indicate more efficient cleavage and more outward-facing orientations. B, nucleosome positions of the reconstitution products. Unmodified or H3K14ac histone octamer was assembled with the 257-bp 601 DNA and irradiated at a UV dose of 500 J/m². NCPs were run on a 5% native polyacrylamide gel followed by staining with SYBR Gold. M denotes DNA size markers, and minus sign (−) denotes DNA only.

FIGURE 5. Photoreactivation in naked DNA and nucleosomes. A, high-resolution gel of CPDs and their removal in naked DNA and nucleosomes. The pyrimidine clusters are marked with bars on the left of the gel, and the ellipse represents the position of the histone octamer. Lanes 1–4, naked DNA; lanes 5–8, unmodified nucleosomes (WT); lanes 9–12, H3K14ac nucleosomes (H3K14ac). B, percentage of CPDs repaired in 60 min in each cluster. Naked DNA is shown in black, unmodified in white, and H3K14ac in gray.

Additional connection between RSC and H3K14 acetylation. However, RSC has a high affinity to nucleosomes in general (24), which could mask the preference of RSC for acetylated nucleosomes. Therefore, to more accurately assess the binding of RSC to the two NCP populations in this study, and repair of these NCPs by CcPhr, photoreactivation was carried out with a fixed amount of RSC in the presence of increasing amounts of undamaged competitor nucleosomes.

As shown in Fig. 8A, HRPF measurements indicate that H3K14ac NCPs are repaired more efficiently than unmodified NCPs at individual sites in the presence of competitors. Indeed, the addition of competitor nucleosomes strongly inhibits the...
FIGURE 6. Purification and remodeling activities of RSC. A, purified RSC was separated on a SDS-PAGE gel and silver stained with a Pierce silver stain kit. B, RSC enhances restriction enzyme accessibility of nucleosome DNA in the presence of ATP. The location of the restrict enzyme cut site (HhaI) used in this study is shown in the top panel. The oval represents the nucleosome position. Nucleosomes were incubated with RSC in the presence or absence of ATP at 30 °C for 20 min, and then HhaI was added and incubated for another hour. The reaction was stopped by phenol-chloroform extraction, and the DNA fragments were separated on a 12% native PAGE gel followed by staining with SYBR Gold.

FIGURE 7. Enhanced CPD repair of nucleosomes following remodeling by the RSC complex. A, high-resolution gel of CPD removal in unmodified or H3K14ac nucleosomes in the presence or absence of RSC and/or ATP. RSC was added to the nucleosomes and incubated at 30 °C for 20 min prior to addition of UV photolyase for 60 min. B, percentage of CPDs repaired at clusters 3, 5, and 7–10. Unmodified nucleosome substrate is shown in white and H3K14ac in gray. The columns represent the mean of at least three independent experiments, and error bars represent ± 1 S.D.
repair efficiency in unmodified NCPs, whereas acetylated NCPs show a much smaller reduction in repair (Fig. 8B). Notably, the presence of competitors does not change the repair efficiency of DNA outside of the histone binding region of the UV-damaged nucleosomes (Fig. 8B, clusters 1, 2, 3, 5, 7–10, 13, 14, and 15), making the repair efficiency at these sites “internal controls” for comparing CPD removal between NCPs. These data illustrate that RSC enhances the accessibility to CcPhr in both unmodified and H3K14 acetylated NCPs to promote repair but preferentially increases CPD accessibility in acetylated NCPs when competitor nucleosomes are present.

Acetylation at H3 Lysine14 Enhances RSC Retention on Nucleosomes—To investigate the mechanism of RSC enhancement of DNA repair in H3K14 acetylated nucleosomes, we employed an RSC pulldown assay (53). The 257-bp 601 DNA used for the HRPF repair assay was end-labeled with biotin on one strand and reconstituted into nucleosomes with unmodified or H3K14 acetylated NCPs to promote repair but preferentially increases CPD accessibility in acetylated NCPs when competitor nucleosomes are present.

FIGURE 8. Preferential repair of H3K14-acetylated nucleosomes in the presence of RSC and competitor nucleosomes. A, high-resolution gel of CPD removal in unmodified or H3K14ac nucleosomes in the presence or absence of ATP. RSC was added to the nucleosomes and incubated at 30 °C for 20 min prior to addition of UV photolyase for 60 min. B, the percentage of CPD repaired in nucleosomes incubated with RSC at 30 °C for 20 min prior to addition of UV photolyase for 60 min at clusters 1, 2, 3, 5, 7–10, 13, 14, and 15 in the absence or presence of a competitor. The columns represent the mean of at least three independent experiments, and error bars represent ± 1 S.D.

DISCUSSION

H3K14 Acetylation Acts as a Signal for Repair of UV Damage Sites in Nucleosomes—Acetylation of histone H3 at lysines 9 and 14 has been shown to play an important role in NER and photoreactivation of UV damage in yeast (6, 7). However, little...
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is known about how this modification facilitates the activity of UV damage repair in chromatin. In this report, we used purified type III DNA photolyase from C. crescentus (40) to study the access of UV damage in a well positioned nucleosome with (or without) homogeneously acetylated H3K14. This simple, purified system allows for well controlled reactions with a specifically acetylated nucleosome template. We focused on two possible mechanisms: the influence of H3K14ac on nucleosome unwrapping dynamics and retention of the chromatin remodeling complex RSC for repair at UV damaged sites. Regarding the first mechanism, we used FRET to show that H3K14ac does not enhance nucleosome unwrapping to expose damaged sites or destabilize nucleosomes (Figs. 2 and 3). Consistent with this observation, H3K14ac alone does not improve DNA repair in vitro (Fig. 5). These results most likely reflect the position of H3K14ac being on the H3 tail (i.e. out of the nucleosome core), where it may have little, if any, direct impact on nucleosomal structure.

Retention of RSC was tested with purified TAP-tagged complex. RSC is an abundant nucleosome remodeling complex in yeast shown in vitro to specifically recognize H3K14ac via its tandem bromodomains (20). We found that the addition of RSC and ATP to the repair reactions significantly increased both the photoreactivation activity of photolyase on nucleosomes (Fig. 7) and cleavage at an internal restriction site by HhaI (Fig. 6). This indicates that the purified RSC complex is able to remodel nucleosomes and improve the access of photolyase to CPDs. Importantly, in the absence of competitors, the stimulatory effect of RSC on CPD repair is comparable between unmodified and H3K14-acetylated nucleosomes (Fig. 7). Conversely, the enhancement of RSC retention by H3K14ac is clearly seen in the presence of competitors (i.e. isolated, undamaged nucleosomes), an effective way to reduce “back-ground nucleosome binding” of chromatin remodelers (53). Our data show that nucleosomes containing H3K14ac bind RSC ~2.5-fold stronger in the presence of competitors (Fig. 9), demonstrating that RSC can differentiate between unacetylated and H3K14 acetylated nucleosomes. Consistent with this observation, RSC stimulates CPD repair more efficiently on H3K14ac nucleosomes when competitor nucleosomes are present (Fig. 8), a situation more relevant to intact cells. This biased remodeling activity of RSC toward H3K14ac-containing nucleosomes provides a mechanistic link for directing RSC, and, possibly, other H3K14ac recognition proteins, to UV-damaged sites in chromatin.

Role of the ATP-dependent Chromatin Remodeling Complex RSC during Repair—Although RSC has not been implicated in UV damage repair in vivo, previous studies showed that yeast cells lacking RSC activity are defective in double strand break repair. In this study, we demonstrated that RSC significantly enhances CPD removal in nucleosome DNA in vitro. Because RSC repositions histone octamers along DNA in an ATP-dependent manner (24), models have proposed that DNA translocation occurs on the nucleosome surface by the complex “pulling” DNA in from one side of the dyad and moving it to the opposite side while being fixed to an internal site on the nucleosome (54). We showed that the remodeling activity is robust on both UV-damaged and undamaged nucleosomes, suggesting that UV lesions do not affect these structure transitions. The enhanced accessibility of CPDs in nucleosomes shown by HRPF in this study is consistent with the model that the RSC-nucleosome complex allows the formation and propagation of a DNA bulge (55). The DNA loop generated by RSC could then lead to attenuated histone-DNA interactions that are crucial for exposure of buried damaged sites to DNA repair proteins.

Histone Acetylation Coordinates Chromatin Remodeling with DNA Repair—A number of studies have demonstrated that cross-talk occurs between histone modifications and chromatin remodeling during transcription regulation (23, 53, 56). It is generally believed that remodeling complexes and histone acetyltransferases are recruited by transcriptional activators (57, 58). However, little is known about the cross-talk between histone modifications and chromatin remodelers during repair of UV damage. Because this damage can occur in DNA within all levels of compaction in chromatin (59, 60), the mechanism for retention of remodeling complexes and histone acetyltransferase at DNA damage sites poses an intriguing question on mechanism. Studies have shown that the Rad4-Rad23 heterodimer, the damage recognition complex for NER in yeast, interacts with the chromatin remodeling complexes Swi/Snf and Ino80 to dock these complexes at repair sites on the transcriptionally silent HML locus (61, 62). In addition, molecular links between DNA damage and chromatin modifications are well established. For example, the histone acetyltransferase p300 colocalizes with NER sites shortly after UV irradiation in cultured human fibroblasts (63). These data imply that UV damage recognition is closely connected with histone modification. How chromatin remodeling and histone modification complexes are retained at damaged sites likely depends on the requirements for a particular “chromatin landscape” in eukaryote cells.
Our results suggest that histone H3K14 acetylation and the chromatin remodeler RSC act in concert to facilitate the process of repair of UV damage in DNA. We note that UV damage is repaired by NER in human cells (although photolyase plays an important role in other species), but it is difficult to establish efficient NER reactions in vitro (64, 65). Moreover, previous studies have demonstrated that the impact of nucleosome structure on UV photolyase accessibility is similar for NER recognition factors (42, 66) and that UV-induced H3 acetylation is important for both repair mechanisms (7). Therefore, UV photolyase is a single protein “nucleosome sensor” that mimics the accessibility of the early steps of NER in cells, and our results likely reflect the impact of “first-responder” NER proteins in chromatin. Upon UV damage in vivo, we propose that Gcn5-dependent H3 acetylation provides a signal for, and retains, RSC binding may also apply to other proteins, such as the bromodomain-containing protein complex Swi-Snf. The function of RSC binding may also apply to other proteins, such as the bromodomain-containing protein complex Swi-Snf. The function of H3K14ac in facilitating chromatin remodeling activity shown in this study provides new insight into the interaction of epigenetic marks and proteins (or protein complexes) in promoting accessibility for prompt and precise recognition of DNA lesions in the genome.

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