DNA damage response induces structural alterations in histone H3–H4

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ABSTRACT

Synchrotron-radiation circular-dichroism spectroscopy was used to reveal that the DNA damage response induces a decrement of α-helix and an increment of β-strand contents of histone H3–H4 extracted from X-ray-irradiated human HeLa cells. The trend of the structural alteration was qualitatively opposite to that of our previously reported results for histone H2A–H2B. These results strongly suggest that histones share roles in DNA damage responses, particularly in DNA repair processes and chromatin remodeling, via a specific structural alteration of each histone.

KEYWORDS: chromatin remodeling, post-translational modification, DNA repair, histone dynamics, synchrotron radiation, circular dichroism

INTRODUCTION

DNA wraps around core histone proteins in eukaryotic nuclei. The core histone is an octamer composed of two H2A–H2B dimers and an H3–H4 tetramer. These molecules are partly replaced by their variants, such as H2AX, which is a variant of H2A. It has been revealed that histones play important roles in DNA damage repair processes [1–5]. Post-translational modifications of histones are essential for binding to certain DNA repair proteins and/or recruiting them to sites of DNA damage. For example, when an H2AX is phosphorylated by an ATM kinase after inducing a DNA double-strand break (DSB), the phosphorylated H2AX binds to an MDC1 protein and recruits DNA repair proteins to the DSB site [2]. An alternative mechanism assumes that DNA repair processes involve dramatic alterations in chromatin structure, which makes DNA repair proteins more accessible to damaged sites [6–8], and that structural changes in chromatin basically occur via post-translational histone modifications [4]. However, structural changes in chromatin and histones induced by DNA damage or relationships between such structural changes and post-translational modifications in vitro are scarcely reported, although a few research groups have reported that the acetylation of histones induces structural changes therein in vitro [9, 10].

Our recent uses of a commercial circular-dichroism (CD) spectrometer [11] and a synchrotron-radiation beamline for CD spectroscopy [12] revealed that a DNA damage response (DDR) induces an increment of α-helix and a decrement of β-strand structures of histone H2A–H2B in cultured human cells irradiated with X-rays at 40 Gy. However, the role in DDR and the mechanisms of structural alterations of H2A–H2B during DNA damage repair processes [1–5], because these modifications would alter steric barriers and/or electrostatic interactions between modified amino acid residues and other residues, and possibly change the stable structures as a result. Indeed, it is known that the α-helix content of the histone H4 peptide is increased by acetylation [10]. To understand the structural alteration mechanisms and clarify the role of structural alterations, it is important to improve our knowledge about structural changes in other core histones, namely H3 and H4, in X-ray-irradiated cells, because it is also well known that the
many kinds of modifications, methylation, acetylation, phosphorylation, and ubiquitination, of H3 and H4 occur in DDR and play important roles [4, 5]; for example, methylation of H3K79 and H4K20 are linked with damage signaling; acetylation of H3K9 and H4K16 is linked with chromatin opening; and acetylation of K14, K23 and K56 of H3 and that of K5, K12 and K91 of H4 are associated with chromatin restoration [13]. In this study, we investigated a structural alteration of histone H3–H4 in X-ray–irradiated human cells using synchrotron-radiation circular-dichroism (SRCD) spectroscopy in the region spanning from ultraviolet (UV) to vacuum ultraviolet (VUV). CD, sometimes called ‘molar CD’, \( \Delta \varepsilon \) is defined as the difference between the molar absorption coefficient for left circularly polarized light (LCPL) \( \varepsilon_L \) and that for right circularly polarized light (RCPL) \( \varepsilon_R \): \( \Delta \varepsilon \equiv \varepsilon_L - \varepsilon_R \). SRCD spectra data show susceptibility in the secondary structures of proteins, namely the α-helix, β-strand, turn, and unordered structures. Although the structural information obtained from SRCD spectra is limited in the secondary structural level compared with that obtained by X-ray crystallography or nuclear magnetic resonance (NMR), which provide well-detalled atomic-level structures, SRCD spectroscopy nevertheless has several notable advantages [14]. These include the following: (i) liquid samples can be used; (ii) data collection is rapid; (iii) the required sample amount is small; and (iv) most importantly, SRCD spectroscopy is highly sensitive to structural changes in proteins. These advantages allow for the detection of even subtle structural changes of histones extracted from irradiated cells. One of the advantages of SRCD beamlines over the commercial CD spectrometers is that the wide measurable region spreads to ~200 nm. Since additional CD peaks of proteins can be observed in the UV region, the structural information obtained by using SRCD spectroscopy is more precise than that obtained by using the commercial CD spectrometers.

Here, we report that the DDR induces a decrement of the α-helix and an increment of the β-strand content of the H3–H4 in X-ray–irradiated human cells, which differs from the reported alterations of H2A–H2B [11, 12].

**Materials and Methods**

**Sample preparation**

Sample preparation was performed in a manner similar to that used in our previous study [11]. Briefly, human cancer cells (HeLa.S-FUCCI cells, provided by RIKEN Cell Bank) were cultured in 100-mm cell culture dishes (Corning Inc., Corning, NY). Dulbecco’s modified Eagle medium (Wako Pure Chemical Industries Ltd, Osaka, Japan) supplemented with 10% fetal bovine serum (Biological Industries Ltd, Beit Haemek, Israel) and 1% antibiotic-antimycotic solution (Life Technologies, Grand Island, NY) was used for cell culturing. The cells were incubated at 37°C in a humidified atmosphere of 5% CO\(_2\) and 95% air. The final density of the cultured cells was \( \sim 1 \times 10^7 \) cells/dish.

Cells were irradiated in the culture dishes with X-rays for 20 min using an X-ray generator equipped with a tungsten anode (M-150WE, SOFTEX Co. Ltd, Kanagawa, Japan). A Fricke dosimeter indicated that the dose rate was ~2 Gy/min. An X-ray dose of 40 Gy is thought to produce ~1600 DSBs per cell nucleus [15]. Assuming that such DSBs are randomly spaced in DNA, an average DNA fragment length is ~2 Mbp (because the human genome is ~3000 Mbp). Since phosphorylation of H2AX spreads along ~2 Mbp from the DSB sites in the early stage of DNA repair processes [16], it is expected that most of the histones in the X-ray-irradiated cells are involved in DDRs. After irradiation, cells were incubated for 30 min in the culture conditions to progress DNA repair.

H3–H4 histones were extracted using the Histone Purification Kit (Active Motif Inc., Carlsbad, CA). This kit is designed to extract H3–H4 and/or H2A–H2B from the core histones in chromatin, while maintaining the post-translational modifications of the histones. Core histones were first extracted from X-ray–irradiated or unirradiated HeLa cells. H2A–H2B was eluted and removed from each core histone fraction using a resin column and an elution buffer supplied in the kit. After that, the remaining fraction on the resin column, which contained H3–H4, was also eluted using the other elution buffer supplied in the kit. The eluted fractions containing H3–H4 were collect and dried as per the protocol provided by Active Motif. These dried pellets were dissolved in sterile water, and centrifugal filtration of the aqueous solution was carried out to remove the remaining nucleic acids using the NanoSerp centrifugal devices (OD010C34, Pall Corp., Port Washington, NY), with a molecular weight cut-off of 10 000. The aqueous solution remaining on the filter after centrifugation was collected. We measured the concentrations of the proteins and remaining DNA (with a molecular weight of >10 000) in the collected solutions using the Qubit dsDNA BR Assay Kit, the Qubit ssDNA Assay Kit, and the Qubit Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA). This revealed that the amount of DNA remaining was much less than the amount of protein remaining (~0.5% w/w). Hereafter, the samples obtained from unirradiated and irradiated cells are referred to as ‘unirradiated samples’ and ‘irradiated samples,’ respectively.

**Electrophoresis analysis**

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis was performed to confirm the purity of histone protein samples using NuPAGE 12% Bis–Tris gels in MES running buffer (Life Technologies). Protein bands were stained using Oinole Fluorescent Gel Stain (Bio-Rad Laboratories Inc., Hercules, CA). Images were obtained using an EttaN DIGE Imager (GE Healthcare, Buckinghamshire, UK). Densitometric analysis of the gel image was carried out using imaging analysis software (ImageQuant TL, GE Healthcare).

**Absorption spectroscopy**

Absorption spectroscopy was carried out to double-check the molecular ratio of the histones contained in the samples, since the used kit could not completely remove H2A–H2B from the core histones (as detailed in ‘SDS–PAGE analysis’ in the Results and Discussion). An ultraviolet spectrophotometer (GENESYS 10S UV Vis Spectrophotometer; Thermo Scientific, Boston, MA) was used. The samples were diluted by adding sterile water to fill an SiO\(_2\) cuvette (path length = 1 cm) before absorption measurements and used.
**SRCD spectroscopy**
Prior to SRCD spectroscopy, Tris-HCl buffer (pH = 8.0) and sodium fluoride (NaF) were added to unirradiated and irradiated samples to induce the formation of higher-order structures. The final concentrations of Tris-HCl and NaF were 10 and 250 mM, respectively. SRCD spectroscopy was carried out in the BL12 beam line at the Hiroshima Synchrotron Radiation Center (HiSOR) in Japan [17]. The sample solution was encapsulated in a calcium fluoride (CaF₂) sample cell [18]. The path length of the CaF₂ cell was 12 mm, and the sample was kept at 25°C. CD spectra of unirradiated and irradiated samples were measured between 175 and 260 nm. The CD spectrum of the solvent (10 mM Tris-HCl and 250 mM NaF), which should be zero in the ideal case, was also measured as a baseline. We subtracted this baseline from the CD spectra of unirradiated and irradiated samples to remove artificial CD signals that might have originated from optical systems, the CaF₂ cell, and so on.

**CD spectra analysis**
Since the used kit could not completely remove H2A–H2B from the core histones (as detailed in ‘SDS–PAGE analysis’), we removed CD signals originating from the remaining H2A–H2B from those of unirradiated and irradiated samples using the analytical approach described below and then determined the CD signals from H3–H4.

We assumed that each sample contained only H2A, H2B, H3 and H4, for which the molecular ratio of H2A:H2B:H3:H4 was 13:13:37:37, as determined by SDS-PAGE analysis (detailed in ‘SDS–PAGE analysis’ and ‘Absorption spectroscopy’ in the Results and Discussion). Using Beer–Lambert’s law, we can describe the CD of the sample \( \Delta \) as follows:

\[
\Delta = 0.13 \Delta_{E_{H2A}} + 0.13 \Delta_{E_{H2B}} + 0.37 \Delta_{E_{H3}} + 0.37 \Delta_{E_{H4}},
\]

where \( \Delta_{E_X} \) is the intrinsic CD of protein X \( X = H2A, H2B, H3 \) or \( H4 \), and coefficients 0.13 and 0.37 reflect the molecular ratio. Using Equation (1), we can obtain

\[
\Delta_{E_{H3}} + \Delta_{E_{H4}} = (\Delta - 0.13(\Delta_{E_{H2A}} + \Delta_{E_{H2B}}))/0.37.
\]

The CD of the sample \( \Delta \) is defined as:

\[
\Delta = \frac{\theta \text{MRW}}{32980cL},
\]

where \( \theta \) and L are the ellipticity (in millidegrees; obtained as raw data using the measurement system of the HiSOR BL12), the concentration of the sample (which contains H2A, H2B, H3 and H4; in milligrams per milliliter) and the path length (in centimeters). Ellipticity \( \theta \) is one of the expression methods of CD intensities used frequently and defined as \( \theta = \tan^{-1}(\Delta A / (\ln 10) / 4) \), where \( \Delta A \) is the subtraction difference between absorbance for LCPL \( (A_{LCPL}) \) and that for RCPL \( (A_{RCPL}) \); \( \Delta A = A_{LCPL} - A_{RCPL} \). MRW is the mean residue weight, defined as the molecular weight of a protein divided by its number of amino acid residues. In this work, we defined MRW as

\[
\text{MRW} = \frac{0.13(M_{H2A} + M_{H2B}) + 0.37(M_{H3} + M_{H4})}{0.13(N_{H2A} + N_{H2B}) + 0.37(N_{H3} + N_{H4})} \approx 110.9,
\]

where \( M_X \) and \( N_X \) are the molecular weight and number of amino acid residues of protein X \( X = H2A, H2B, H3 \) or \( H4 \), respectively. It is noted that \( \epsilon/\text{MRW} \), which corresponds to mol concentration divided by number of residues of sample protein, is often used for the CD spectroscopy of proteins instead of normal mol concentration \( C \) to show the concentration of a sample.

Equations (2)–(4) can be used to determine the CD intensity of H3–H4 \( (\Delta_{E_{H3}} + \Delta_{E_{H4}}) \). The concentration of the sample \( c \) was measured using the Qubit Protein Assay Kit (Thermo Fisher Scientific), as mentioned above. Literature data were used for the CD intensity of H2A–H2B \( (\Delta_{E_{H2A}} + \Delta_{E_{H2B}}) \) [12].

**Secondary-structure analysis**
The contents of \( \alpha \)-helices in regular and distorted regions, \( \beta \)-strands in regular and distorted regions, and turn and unordered structures of H3–H4 were analyzed using CD spectra and the SELCON3 program [19, 20], based on reference data measured at HiSOR [21, 22]. The \( \alpha \)-helix and \( \beta \)-strand contents in distorted regions correspond to two residues at each end of the \( \alpha \)-helix segments and one residue at each end of the \( \beta \)-strand segments, respectively. Regular regions are segments other than the distorted regions. The SELCON3 program was applied over a wavelength range from 178 to 260 nm.

The average numbers of \( \alpha \)-helices (\( N_\alpha \)) and \( \beta \)-strands (\( N_\beta \)) were calculated using the following equations [19]:

\[
N_\alpha = \alpha_D N/4,
\]

and

\[
N_\beta = \beta_D N/2,
\]

where \( \alpha_D / \beta_D \) and \( N \) are the contents of \( \alpha \)-helix and \( \beta \)-strands in distorted regions and the total number of amino acid residues of proteins, respectively.

**RESULTS AND DISCUSSION**
**SDS–PAGE analysis**
Figure 1 shows a representative SDS–PAGE result for the unirradiated and irradiated samples (lanes ‘U’ and ‘T’). In both samples, three bands were observed around similar molecular weights. The lowest bands (between 10 and 15 kDa) originated from H4, the bands at 15 kDa originated from the remaining H2A, and the highest bands, between 15 and 20 kDa, originated from both H3 and the remaining H2B. This indicated that the used kit could not completely separate pure H3–H4 from the core histones. To avoid denaturation of the samples, which may change the structures of the histones (such as demodification or additional modification in vitro) during purification, the samples were used for CD spectroscopy without further purification in this work, because the effect of H2A and H2B in CD spectra can be removed analytically.

To subtract the CD signal of H2A–H2B from the observed CD spectra, we quantified a molecular ratio between H3–H4 and impurity H2A–
H2B using densitometric analysis of the gel image. When we assumed that the amount of H2B should be the same as that of H2A, since H2A and H2B form a dimer in the core histone, the molecular ratios in both samples were determined to be H2A:H2B:H3:H4 = 13:13:37:37.

Absorption spectroscopy

Figure 2 shows a representative absorption spectrum for the diluted unirradiated samples. In the case of the irradiated samples, similar spectra were observed, because absorption spectra do not reflect the protein structures. The spectrum showed a peak at ~275 nm. The spectrum was similar in superposition to that of H2A, H2B, H3 and H4 [23]. According to Beer–Lambert’s law, absorbance of the diluted sample (ABS) is described as:

\[
\text{ABS} = \left( \frac{\varepsilon_{H2A} 0.13n}{V} + \frac{\varepsilon_{H2B} 0.13n}{V} + \frac{\varepsilon_{H3} 0.37n}{V} + \frac{\varepsilon_{H4} 0.37n}{V} \right) l,
\]

where we assumed the molecular ratio of H2A:H2B:H3:H4 was 13:13:37:37 as determined by SDS–PAGE analysis. Using the values of \(\varepsilon_X [24]\) and ABS at 275.5 nm in Fig. 2, and that of \(l (=1 \text{ cm})\), we can determine that \((n/V) = 5.5 \times 10^{-5} \text{ M}\). Thus, we can calculate the total histones concentration (c) to be:

\[
c = \frac{0.13n}{V} (M_{H2A} + M_{H2B}) + \frac{0.37n}{V} (M_{H3} + M_{H4}) = 0.73 \text{ mg/ml}.
\]

This calculated concentration based on the molecular ratio determined by SDS–PAGE analysis was quite similar to the concentration of the diluted sample determined by using the Qubit Protein Assay Kit, 0.75 mg/ml. This showed that the molecular ratio determined by SDS–PAGE was a reasonable value. Thus, we used the ratio to analyze the CD spectra as shown in ‘CD spectra analysis’ in the Materials and Methods.

SRCD spectra

Figure 3a shows CD spectra of unirradiated and irradiated samples containing the effect of the remaining H2A–H2B, which correspond to \(\Delta \varepsilon\) in Equation (1). Figure 3b shows CD spectra of H2A–H2B extracted from unirradiated and irradiated cells reproduced from the literature data [12], which correspond to \(\Delta \varepsilon_{H2A} + \Delta \varepsilon_{H2B}\) in Equation (2). Similar CD spectra were observed at HisOR BL12. All spectra in Fig. 3a and b show a positive peak at ~190 nm and two negative peaks at around 208 and 222 nm, which are the characteristic CD peaks of \(\alpha\)-helix structures [25]. These results are reasonable because a previous X-ray crystallographic study found that the \(\alpha\)-helix is the main secondary structure of histone proteins [26]. The variability could be larger for irradiated samples than for unirradiated samples for various reasons, including cell-to-cell variations in DNA damage and the cellular responses to DNA damage. To obtain
the CD spectra of pure H3–H4, the effect of the remaining H2A–H2B was subtracted from the CD spectra of unirradiated and irradiated samples using Equation (2). The obtained spectra are shown in Fig. 3c.

In the case of H3–H4 (Fig. 3c), apparent differences in both samples were not observed in the cases of negative peaks, but the CD intensities of the positive peaks around 190 nm were lower for irradiated samples than for unirradiated samples. In contrast, in the case of H2A–H2B (Fig. 3b), the absolute values of the CD peak intensities were larger when it was extracted from irradiated cells than from unirradiated cells. Since CD spectral shapes reflect the contents of secondary structures of proteins, the change in the CD spectra in Fig. 3b and c shows that the structures of H2A–H2B and H3–H4 extracted from irradiated cells differed from those from unirradiated cells; that is, structural alterations of H2A–H2B and H3–H4 were induced by irradiating the cells with X-rays. In addition, the spectral change of H3–H4 induced by X-irradiation to cells differed from that of H2A–H2B (Fig. 3b and c). This shows that different structural changes were induced in H2A–H4 compared with H2A–H2B by X-irradiation to cells. When protein decomposition or breakage of peptide bonds is induced by radiation damage or other causes, CD peaks originating from α-helix structures tend to shift toward shorter wavelengths [27]. Indeed, direct irradiation of the purified H2A–H2B solution caused a significant blue shift in the CD spectrum relative to the H2A–H2B extracted from unirradiated or X-ray-irradiated cells [11]. Since no peak shift was observed in the CD spectra of H2A–H2B and H3–H4 (Fig. 3b and c), it was concluded that the structural alterations of H2A–H2B and H3–H4 observed in this study were not induced by X-ray damage to H2A–H2B and H3–H4 themselves, but rather by other cellular functions, such as the DDR.

**Secondary-structure alterations of H3–H4**

The contents of secondary structures of H3–H4 extracted from unirradiated and irradiated cells, as analyzed using the SELCON3 program, are listed in Table 1. The contents of the secondary structures of H2A–H2B reported by Izumi et al. [12] are also shown in Table 1. The total α-helix content of H3–H4 in unirradiated samples was 61.6 ± 0.6% [mean ± standard deviation (SD)], which is higher than the summation of the α-helix contents of H3 and H4 in a nucleosome as determined by X-ray crystallography (~50%) [26]. This difference may be caused by differing experimental conditions; for example, the histones were obtained from Xenopus laevis, and in crystal form the nucleosome was composed of an H3–H4 tetramer, two H2A–H2B dimers and a 146-bp palindromic DNA fragment.

The α-helix content in the distorted region was slightly decreased after exposing the cells to X-rays (21.7 ± 0.5% → 19.7 ± 0.8%). When we used the mean number of amino acid residues of H3 and H4 \( [N = 118.5 \text{ in Equation (3)}] \), the mean numbers of α-helices \( (N_{\alpha}) \) of H3–H4 in unirradiated and irradiated samples were calculated to be 6.4 ± 0.1 and 5.8 ± 0.2, respectively. The closeness of the mean numbers of α-helices in the H3–H4 samples suggests that they were unchanged by irradiating the cells with X-rays. On the other hand, the α-helix content of H3–H4 in regular regions was 28.6 ± 0.2% in irradiated samples and 39.9 ± 0.4% in unirradiated samples. This suggests that the α-helix segments of H3–H4 were on average shorter in irradiated samples than in unirradiated samples, because the average numbers of α-helices were almost the same.

The content of β-strand structures in the distorted region was increased by exposing the cells to X-rays (1.2 ± 0.2% → 4.1 ± 0.7%). The mean number of β-strands \( (N_{\beta}) \) of H3–H4 was higher in irradiated samples (2.4 ± 0.4) than in unirradiated samples (0.7 ± 0.1). The β-strand content in the regular region was also increased by irradiating the cells with X-rays (0.7 ± 0.7% → 3.9 ± 2.4%), although with a rather large variability. The content of turn structures did not differ significantly between the unirradiated and irradiated samples. The content of unordered structures was greater in irradiated samples than in unirradiated samples.

Alpha-helix and β-strand structures show negative CD peaks, and the unordered structure shows a positive CD peak at ~210–240 nm [22]. Therefore, a decrease of the α-helix structures and an increase of the unordered structures observed in H3–H4 induce a

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**Fig. 3.** CD spectra of (a) unirradiated (black) and irradiated samples (red) before subtraction of the effect of remaining H2A–H2B, (b) H2A–H2B extracted from unirradiated (black) and irradiated cells (red) reproduced from the literature data [12], and (c) pure H3–H4 extracted from core histones in unirradiated (black) and X-ray–irradiated (red) cells. Data are mean and SD values.
A hypothesis for the structural alteration mechanism and its contribution to DNA repair processes

The mechanisms underlying the structural alterations of H3–H4 and H2A–H2B have not been identified yet. However, we assume that the post-translational modifications of histones during DNA damage repair processes [1–5] relate to the structural alterations as mentioned above. Different structural alterations observed in H3–H4 and H2A–H2B might be caused by the different types of post-translational modifications and/or different modification sites. Cyclopedic CD spectroscopy of specific modified histones is warranted in the future in order to understand the relationship between the kinds of modifications and the structural alterations.

It is also unclear how the structural alterations of histones contribute to DDRs, such as DNA repair processes. Altered histones might be landmarks for DNA repair enzymes and proteins for distinguishing sites of DNA damage from undamaged ones. In view of the lock-and-key model of enzymes and substrates, altered histones might be the substrates of DNA repair enzymes, which would allow the enzymes to work specifically around DNA damage sites. Structural alterations of histones might also contribute to chromatin remodeling, such as the eviction and sliding of histones [6], because the electrostatic interactions between DNA and an altered histone would change from those in the normal condition.

In the future it will be important and interesting to study the mechanisms that alter the structures of H3–H4 and H2A–H2B independently and how the various structural changes in histones induced by DDRs play roles in chromatin remodeling and/or the recruitment of DNA repair proteins to sites of DNA damage.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

1. Price B, D’Andrea A. Chromatin remodeling at DNA double-strand breaks. Cell 2013;152:1344–54, 10.1016/j.cell.2013.02.011.
2. van Attikum H, Gasser SM. Crosstalk between histone modifications during the DNA damage response. Trends Cell Biol 2009;19:207–17, 10.1016/j.tcb.2009.03.001.
3. Pandita TK, Richardson C. Chromatin remodeling finds its place in the DNA double-strand break response. *Nucleic Acids Res* 2009;37:1363–77, 10.1093/nar/gkn1071.

4. Hunt CR, Ramnarain D, Horikoshi N, et al. Histone modifications and DNA double-strand break repair after exposure to ionizing radiations. *Radiat Res* 2013;179:383–92, 10.1667/RR3308.2.

5. Gong F, Miller KM. Mammalian DNA repair: HATs and HDACs make their mark through histone acetylation. *Mutat Res* 2013;750:23–30, 10.1016/j.mrfmmm.2013.07.002.

6. Smerdon MJ. DNA repair and the role of chromatin structure. *Curr Opin Cell Biol* 1991;3:422–8, 10.1016/0955-0674(91)90069-B.

7. Soria G, Polo S, Almouzni G. Prime, repair, restore: the active role of chromatin in the DNA damage response. *Mol Cell* 2012;46:722–34, 10.1016/j.molcel.2012.06.002.

8. Polo SE. Reshaping chromatin after DNA damage: the choreography of histone proteins. *J Mol Biol* 2015;427:622–36, 10.1016/j.jmb.2014.05.025.

9. Adler AJ, Fasman GD, Wangh LJ, et al. Altered conformational effects of naturally acetylated histone 2a(IV) in 2a-deoxyribonucleic acid complexes. Circular dichroism studies. *J Biol Chem* 1974;249:2911–4.

10. Wang X, Moore SC, Laszczak M, et al. Acetylation increases the α-helical content of the histone tails of the nucleosome. *J Biol Chem* 2000;275:35013–20, 10.1074/jbc.M004998200.

11. Izumi Y, Yamamoto S, Fujii K, et al. Secondary structure alterations of histones H2A and H2B in X-irradiated human cancer cells: altered histones persist in cells for at least 24 hours. *Radiat Res* 2015;184:554–8, 10.1667/RR14071.1.

12. Izumi Y, Fujii K, Wien F, et al. Structure change from β-strand and turn to α-helix in histone H2A–H2B induced by DNA damage response. *Biophys J* 2016;111:69–78, 10.1016/j.bpj.2016.06.002.

13. Rossetto D, Truman AW, Kron SJ, et al. Epigenetic modifications in double-strand break DNA damage signaling and repair. *Clin Cancer Res* 2010;16:4543–52, 10.1188/1078-0432.CCR-10-0513.

14. Miles AJ, Wallace BA. Synchrotron radiation circular dichroism spectroscopy of proteins and applications in structural and functional genomics. *Chem Soc Rev* 2006;35:39–51, 10.1039/B316168B.

15. von Sonntag C. Free-radical-induced DNA Damage and its Repair. Berlin/Heidelberg: Springer-Verlag, 2006.

16. Rogakou EP, Pilch DR, Orr AH, et al. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem* 1998;273:5858–68.

17. Sawada M, Namatame H, Taniguchi M. Optical design of a compact and practical UV beamline at HiSOR-BL12. *J Phys: Conf Ser* 2013;425:162010, 10.1088/1742-6596/425/16/162010.

18. Matsuo K, Sakai K, Matsushima Y, et al. Optical cell with a temperature-control unit for a vacuum–ultraviolet circular dichroism spectrophotometer. *Anal Sci* 2003;19:129–32, 10.2116/analsci.19.129.

19. Seereama N, Venyaminov SY, Woody RW. Estimation of the number of α-helical and β-strand segments in proteins using circular dichroism spectroscopy. *Protein Sci* 1999;8:370–80, 10.1100/ps.8.2.370.

20. Seereama N, Woody RW. Estimation of protein secondary structure from circular dichroism spectra: comparison of CONTIN, SELCON, and CDSSTR methods with an expanded reference set. *Anal Biochem* 2000;287:252–60, 10.1006/abio.2000.4880.

21. Matsuo K, Yonehara R, Gekko K. Secondary-structure analysis of proteins by vacuum–ultraviolet circular dichroism spectroscopy. *J Biochem* 2004;135:405–11, 10.1093/jb/mvh048.

22. Matsuo K, Yonehara R, Gekko K. Improved estimation of the secondary structures of proteins by vacuum–ultraviolet circular dichroism spectroscopy. *J Biochem* 2005;138:79–88, 10.1093/jb/mvi101.

23. Oh YH. Spectroscopic studies of five purified histones from calf thymus. *J Biol Chem* 1970;245:6404–16.

24. D’Anna JA, Jr, Isenberg I. Histone cross-complexing pattern. *Biochemistry* 1974;13:4992–7, 10.1021/bi00838a031.

25. Greenfield NJ, Fasman GD. Computed circular dichroism spectra for the evaluation of protein conformation. *Biochemistry* 1969;8:4108–16, 10.1021/bi00838a031.

26. Davey CA, Sargent DF, Luger K, et al. Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 Å resolution. *J Mol Biol* 2002;319:1097–113, 10.1016/S0022-2836(02)00386-8.

27. Wien F, Miles AJ, Lees JG, et al. VUV irradiation effects on proteins in high-flux synchrotron radiation circular dichroism spectroscopy. *J Synchrotron Radiat* 2005;12:517–23, 10.1107/S0909049505006953.