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Analysis of DNA damage-induced histone H3 acetylation during nucleotide excision repair of *Schizosaccharomyces pombe*

Miyuki SUSUKI, Shinji KAWANO*, and Shogo IKEDA*

Graduate School of Science,
**Department of Biochemistry, Faculty of Science,*
Okayama University of Science,
1-1 Ridai-cho, kita-ku, Okayama 700-0005, Japan.

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Nucleotide excision repair (NER) is a robust defense mechanism to remove UV-damaged lesions in DNA. In *Saccharomyces cerevisiae*, the Rad7p/Rad16p NER complex mediates histone H3 acetylation (H3Ac), which results in chromatin remodeling required for efficient NER. UV-induced H3Ac occurs via Gcn5p histone acetyltranferase (HAT). Although *Schizosaccharomyces pombe* possesses NER genes homologous to those of *S. cerevisiae*, the NER pathway has not been extensively investigated. To examine the role of chromatin remodeling in *S. pombe* NER, we measured H3Ac of the whole genome after UV irradiation. The effect of HAT inhibitors CPTH2 and MB-3 on the repair activity of UV damage was also measured. Gcn5p and Ada2p are components of a SAGA chromatin remodeling complex. We examined the involvement of *ada2* in NER and UV-dependent H3Ac. Unlike *S. cerevisiase*, H3Ac did not play a crucial role in NER in *S. pombe* cells under our experimental conditions. These results imply differences in the initial step of NER between *S. cerevisiase* and *S. pombe*.

Keywords: chromatin remodeling; histone H3 acetylation; nucleotide excision repair; *Schizosaccharomyces pombe*; UV-damaged DNA.

Introduction

Nucleotide excision repair (NER) is a versatile DNA repair pathway that removes a broad spectrum of DNA lesions that tend to distort the double helix, and is well known for its role in the repair of UV-induced photodamage (Friedberg *et al.*, 2006; Naegeli & Sugasawa, 2011). NER has two distinct sub-pathways: global genome NER (GG-NER) and transcription-coupled NER (TC-NER). GG-NER can remove lesions throughout the genome while TC-NER is specialized to repair lesions from the transcribed strand of active genes. Recently, we found that both GG- and TC-NER activities in *Schizosaccharomyces pombe* not only target bulky DNA helix-distorting lesions, but can also effectively remove AP sites and subtle base modifications (Kanamitsu & Ikeda, 2011; Sakurai *et al.*, 2015).

The *Saccharomyces cerevisiae RAD7* and *RAD16* genes are essential for GG-NER (Verhage *et al.*, 1994; Reed *et al.*, 1999). Deletion of the *RAD16* gene decreased the acetylation levels of histone H3 at Lys9 and Lys14, indicating that Rad16p-dependent histone H3 acetylation (H3Ac) represents an important stage during GG-NER (Teng *et al.*, 2008). Rad7p and Rad16p form a protein complex with Abf1p and probably mediate UV-induced H3Ac, which results in chromatin remodeling required for efficient GG-NER (Yu *et al.*, 2011; Waters *et al.*, 2012). UV-induced H3Ac occurs via Gcn5 histone acetyltranferase (HAT), which is essential for efficient NER at some yeast genes (Waters *et al.*, 2012). *S. pombe* possesses *rhp7* and *rhp16* genes, which are homologs of *S. cerevisiae RAD7* and *RAD16* genes, respectively. The Rhp7p-Rhp16p complex detects UV photolesions throughout the genome and transfers the lesion site to the Rhp41p-Rhp23p complex in the initial step of GG-NER (Lombaerts *et al.*, 1999; Latypov *et al.*, 2012). However, the precise function of the NER complex in chromatin remodeling has not yet been

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Table 1. Yeast strains used in this study.

Strain	Genotype	Source
ED0665	*h⁻ ade6*-M210 *leu1*-32 *ura4*-D18	ATCC No. 96993
FY18796	*h⁻ ade*-M387 *leu1*-32 *ura4*-D18 *rhp7::kanMX6*	[a]YGRC
FY19692	*h⁻ ada2::kanMX ura4*-A13 *ura4*-D18	[a]YGRC
MS04	*h⁻ ada2::kanMX ura4*-A13 *ura4*-D18 *rhp7::ura4*	This study
BY4742	*Mata, his3Δ1, leu2Δ 0, lys2Δ0, ura3Δ0*	[b]Thermo

[a]YGRC: Yeast Genetic Resource Center
[b]Thermo: Thermo Fisher Scientific

ascertained.

To examine the role of chromatin remodeling in *S. pombe* NER, we examined the acetylation of histone H3 during GG-NER of UV photolesions in *S. pombe* cells. The effect of HAT inhibitors on repair activity for UV damage was also measured. Ada2p and Gcn5p are components of a SAGA (Spt-Ada-Gcn5 acetyltransferase) chromatin remodeling complex which is well conserved among eukaryotic cells (Koutelou *et al.*, 2010). We examined the involvement of *ada2* in GG-NER and UV-dependent H3Ac. Unlike in *S. cerevisiase*, the crucial role of H3Ac was not observed during GG-NER of *S. pombe* under our experimental conditions.

2. Materials and Methods

2.1. Strains and media.

Yeast strains used in this study are listed in Table 1. *S. pombe* strains were routinely grown on YEA medium (0.5% yeast extract, 3% glucose, and 60 μg/mL adenine). *S. cerevisiae* cells were cultured in YPD medium (1% yeast extract, 2% polypeptone, 2% glucose).

2.2. Construction of *ada2Δ/rhp7Δ* double mutant.

The genomic sequence of *rhp7* was amplified with the following primers: 5′- TCT CAA GGT ATC AAT GCT TCA GCA T -3′ and 5′- TTA TTG AAC TTC ACG CCC TAT CAG A -3′. The gene fragment was then subcloned into pGEM-T vector (Promega) by TA cloning. A 1.7-kb *ura4* gene was also amplified from the pUR18 plasmid (ATCC no. 77297) using a PCR primer set with *Hpa*I or *Kpn*I restriction sites (5′- AAC GGC ACC TAT ATG TAT GCA TTT GTG -3′ and 5′- GAG AGG TAC CAC CAA TGT TTA TAA CCA AG -3′) and subcloned into pGEM-T-rhp7 digested with *Hpa*I and *Kpn*I. The *rhp7::ura4* DNA fragment was amplified by PCR and used to transform the *ada2Δ* haploid strain using the Wako *S. pombe* Direct Transformation Kit (Wako Pure Chemical Industries). Genotypes of resulting progeny were analyzed by PCR.

2.3. Immunological detection of acetyl-histone H3 in the overall genome.

Histone proteins were extracted form yeast with alkaline solution. Yeast cells were collected, suspended in 0.1 N NaOH (10 μL/mg wet weight of cells) with vigorous mixing, and incubated for 10 min at room temperature. After centrifugation at 12,000 rpm for 5 min, the pellet was resusupended in sampling buffer (0.25 M sucrose, 2% SDS, 6.3 mM Tris-HCl, pH 6.8, and 0.725 M 2-mercaptoethanol; 3 μL/mg wet weight of cells). Proteins were fractionated by 12.5% SDS-polyacrylamide gel electrophoresis, and transferred onto a Hybond-P blotting membrane (GE Healthcare). The membrane was incubated in blocking solution containing 1×TBS (10 mM Tris-HCl, pH 7.5, 0.15 M NaCl), 0.1% Tween 20, and 5% nonfat dry milk (Difco) with gentle shaking for 1 h. Acetyl-histone H3 (Lys9/Lys14) antibody (Cell Signaling Technology) was diluted 1:1,000 with blocking solution, and incubated with the membrane at 4°C overnight. The membrane was washed with TBS/T (1×TBS containing 0.5% Tween 20), and incubated with the HRP-labeled anti-rabbit Ig antibody (GE Healthcare; 1:5,000) for 1 h. After washing the membrane with TBS/T, acetyl-histone H3 was visualized using the ECL Plus Western Blotting Detection System (GE Healthcare). Images of chemiluminescence were created with Image Quant LAS-4000 mini (GE Healthcare). Histone H4 was visualized as a loading control using histone H4 antibody (Sigma-Aldrich, 1:5,000).

2.3. Survival assay.

S. pombe cells were grown in YEA medium until OD_{600} of the culture reached 1.0. Cell density was determined by a Z1 particle counter (Beckman Coulter), and adjusted to 1.0×10^7 cells/mL with

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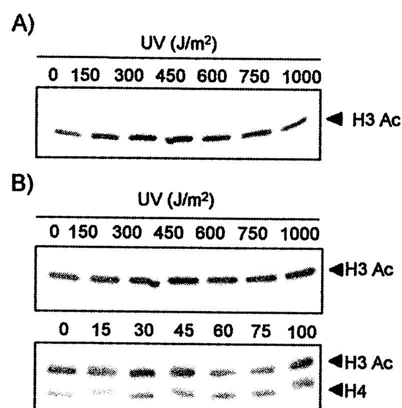

Fig. 1. DNA damage-induced H3Ac in yeast cells. (A) *S. cerevisiae* BY4724 cells were cultured in YPD medium until OD_{600} of the culture reached 1.0. The cells were washed with PBS, and resuspended with PBS at a density of 2.0×10^7 cells/mL. Ten mL of cell suspension was transferred to a Petri dish with a diameter of 83 mm, and irradiated with 254-nm UV light at various doses. The cells were collected, resuspended in 2 mL of YPD, and incubated at 28°C for 1 h with shaking. The histone proteins were extracted and acetyl-histone H3 was detected as described in the Materials and Methods. (B) Acetyl-histone H3 in *S. pombe* ED0665 cells was detected in the same way described above, except that YEA medium was used for cell culture. In the case of a low UV dose (bottom panel), histone H4 was used as the loading control.

sterilized water. The cells were diluted 10-fold serially from 10^{-1} to 10^{-3} in sterilized water. The dilutents (3 μL) containing 3×10^1 to 3×10^4 cells were spotted onto YEA medium, and irradiated with 254-nm UV light using a UV crosslinker (FS-1500; Funakoshi Inc.) equipped with an internal photodetector for measuring the UV dose. Photographs of colonies were taken after 3 days of growth at 28°C.

3. Results and Discussion

3.1. DNA damage-induced H3Ac in *S. pombe* cells.

In *S. cerevisiae*, acetylation of histone H3 was observed following UV irradiation in the overall genome as well as at the regulatory region of a specific gene (Teng *et al.*, 2008). To examine DNA damage-induced H3Ac in *S. pombe* cells, we first developed an immunological detection system for acetyl-histone H3 using an alkaline extract from UV-irradiated *S. cerevisiae* cells (Fig. 1A). The amount of acetyl-histone H3 in the whole genome was maximal at UV-doses of 300 to 600 J/m^2,

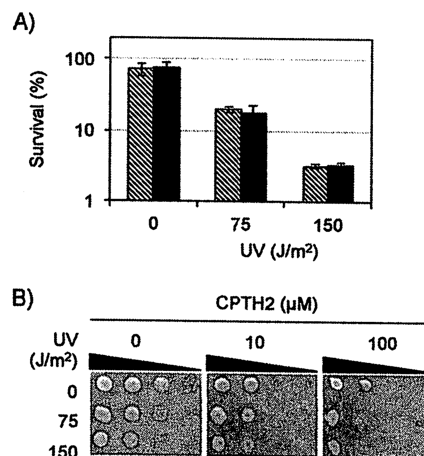

Fig. 2. Effect of a HAT inhibitor CRTH2 on the repair of UV-damaged DNA. (A) Acute exposure of CRTH2. *S. pombe* ED0665 cells were cultured and irradiated with UV light in the same way described in Fig. 1. The cells were collected, resuspended with 2 mL of YPD in the presence (closed bar) or absence (hatched bar) of 100 μM CPTH2, and incubated at 28°C for 4 h with shaking. The cells were diluted 10-fold serially in sterilized water, and the dilutents containing 1×10^5 to 1×10^3 cells were spread onto YEA medium. After culturing for 3 days cell viability was calculated from the number of colonies. Results are the mean ± SD for three experiments. (B) Chronic exposure of CRTH2. *S. pombe* cells were cultured and irradiated with UV light in the same way described above. After measuring cell density with a particle counter, 3×10^1 to 3×10^4 cells were spotted onto YEA medium containing various concentrations of CPTH2. Photographs of colonies were taken after 3 days of growth at 28°C.

indicating that UV-induced H3Ac in *S. cerevisiae* could be confirmed using our detection system described in the Materials and Methods. On the other hand, we detected acetyl-histone H3 in the *S. pombe* whole genome, but no significant change in acetylation levels was observed by varying the UV dose (Fig. 1B). Alterations in DNA repair time after UV damage did not affect the acetylation levels.

3.2. Effect of HAT inhibitors on the repair of UV-damaged DNA.

In *S. cerevisiae*, *GCN5* is essential for efficient NER at some yeast genes and UV-induced H3Ac occurs via Gcn5p HAT activity (Waters *et al.*, 2012). CPTH2 (cyclopentylidene-[4-(4'-chlorophenyl) thiazol-2-yl]hydrazine) and MB-3 ((2S,3R)-4-methylidene-5-oxo-2-propyloxolane-3-carboxylic acid) are well-known small-molecular HAT inhibitors that modulate the Gcn5p network *in vivo*

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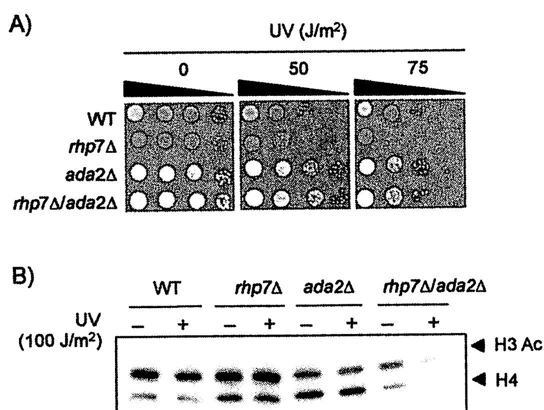

Fig. 3. Genetic interaction of *rhp7* and *ada2* during repair of UV-damaged DNA. (A) WT cells and various mutants were grown overnight in YEA. Survival assays for UV irradiation were carried out as described in the Materials and Methods. (B) After irradiation of UV light (100 J/m^2), acetylated histone H3 in the whole genome of WT cells and various mutants was detected in the same way described in Fig. 1. Histone H4 was used as the loading control.

and *in vitro* (Mai *et al.*, 2006; Chimenti *et al.*, 2009). The effect of these HAT inhibitors on the repair of UV-damaged DNA in *S. pombe* was examined. After UV irradiation, the cells were subjected to acute or chronic exposure to HAT inhibitors, and then cell survival rate was measured (Fig. 2). CPTH2 treatment under acute and chronic exposure did not affect the survival rate of UV-damaged cells. Chronic exposure to MB-3 also exhibited no effect (data not shown).

3.3. Genetic interaction of *rhp7* and *ada2* during repair of UV-damaged DNA.

Ada2p, one of the main components of the SAGA histone acetylation complex, affects HAT activity of the complex (Koutelou *et al.*, 2010). Genetic interaction of *ada2* and GG-NER gene *rhp7* during repair of UV-damaged DNA was examined by a survival assay (Fig. 3A). As expected, deletion of *rhp7* was more sensitive to UV irradiation than WT cells. However, *ada2Δ* cells exhibited slightly greater UV resistance than WT. Moreover, the *rhp7Δ/ada2Δ* double mutant showed the same UV sensitivity as the *ada2Δ* single mutant, indicating no genetic interaction between *rhp7* and *ada2* during GG-NER of UV photolesions. H3Ac levels of the whole genome in *ada2Δ* and *rhp7Δ/ada2Δ* cells were significantly lower than those of WT and *rhp7* cells, indicating that the SAGA complex is primarily responsible for H3Ac of the overall genome (Fig. 3B). However, UV-dependent H3Ac was not observed in any cells tested.

In conclusion, we examined the role of chromatin remodeling in GG-NER of *S. pombe* cells. Unlike in *S. cerevisiase*, UV-induced H3Ac was not observed during early steps of GG-NER of *S. pombe* under our experimental conditions. HAT inhibitors CPTH2 and MB-3 did not affect the repair activity of UV damage. No apparent involvement of *ada2* in GG-NER and UV-dependent H3Ac was observed. These data imply differences in the initial step of NER between both yeast species, *S. cerevisiase* and *S. pombe*. Further studies are needed to elucidate the precise role of chromatin remodeling during *S. pombe* NER.

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