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Histone H2A mobility is regulated by its tails and acetylation of core histone tails

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Abstract

Histone tail domains play important roles in cellular processes, such as replication, transcription, and chromosome condensation. Histone H2A has one central and two tail domains, and their functions have mainly been studied from a biochemical perspective. In addition, analyses based on visualization have been employed for functional analysis of some chromatin proteins. In this study, we analyzed histone H2A mobility *in vivo* by two-photon FRAP, and elucidated that the histone H2A N- and C-terminal tails regulate its mobility. We found that histone H2A mobility was increased following treatment of host cells with a histone deacetylase inhibitor. Our results support a model in which core histone tails directly regulate transcription by interacting with nucleosome DNA via electrostatic interactions.

Keywords: Histone H2A; FRAP; Two-photon excitation; Histone tail; Acetylation

A nucleosome consists of a histone octamer and 147 bp of DNA that wraps around it [1]. The nucleosome structure should be dynamic, since some types of proteins, such as polymerases, require access to the DNA packed into nucleosomes during the cell cycle [2]. FRAP is an indispensable technique for investigating molecular dynamics in nuclei [3]. Dynamic binding of histone H1 to chromatin was revealed by FRAP [4,5]. Many factors affect histone H1 mobility, such as chromatin condensation [4] and post-translational modifications of linker and core histones [2,6]. In the case of core histones, the nuclear kinetics [7] and cell cycle-dependent mobility change [2] of histone H2A have been revealed. The mobility of histones is closely related to chromatin and/or nucleosome stability. Unlike other core histones, histone H2A has three domains: an N-terminal tail, a central domain, and a C-terminal tail. The N-terminal tail is highly basic and often post-translationally modified. Histone tail modification has important roles in cellular processes, such as replication, transcription, and chromosome condensation. In the unmodified state, the N-terminal tail of histone H2A is unstructured and thought to interact with nucleosomal DNA in a charge-dependent manner. Acetylation of histone tails decreases their positive charge, and diminishes the strength of their interaction with DNA [8].

To elucidate the physiological roles of histone tails, enzymatic removal of the tails of all four core histones has previously been employed [9,10]. Although this method

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is useful for analyzing the overall function of histone tails, we cannot obtain any information about the role of individual histone tails using this approach. On the other hand, analysis using the GFP-fusion protein method is useful and has been employed to elucidate the effects of individual domains or amino acid residues on histone H1 function, especially regarding its localization and mobility [6,11–13].

A two-photon microscope equipped with a femtosecond laser has become a powerful tool for investigating biological phenomena. The advantages offered by the two-photon microscope for observation of samples include the following: background-free signals, deep penetration property, and reduced photon-induced damage [14]. Two-photon microscopy has been applied to localization analysis of proteins [15], FRAP [16], photodisruption of organelles [17,18], and photoconversion of fluorescent protein [19] *in vivo*.

In this study, we first analyzed GFP-H1 mobility by two-photon FRAP, and concluded that two-photon FRAP was valuable for mobility analyses of chromatin proteins. To elucidate whether the N- and C-terminal tails affect histone H2A mobility, we analyzed the mobility of tailless histone H2A. In addition, treatment with a histone deacetylase (HDAC) inhibitor, trichostatin A (TSA), caused an increase in histone H2A mobility. Our results showed that acetylation of core histone tails affected the mobility of histone H2A.

Materials and methods

Cell culture. Human carcinoma cell line HeLa cells were maintained as previously described [20,21].

Vector construction and transfection. A cell line expressing GFP-histone H1 was constructed as previously described [22]. The histone H2A gene was a sincere gift from Dr. Shimabara, Japan Advanced Institute for Science and Technology. Histone H2A deletion mutants were produced by PCR using the following primers 5′-GCTAGCATGAAGACTCGGTCTTCTC GTGC-3′ and 5′-ACCGGTAGCTTTCCCTTGGCCTTATGATGG-3′ for N-terminal tail deletion; and 5′-AGATCTATGTCTGGTCGCGGCA AACAAG-3′ and 5′-GAATTCTCACGCCTGAATATTAGGCAAAAC-3′ for C-terminal tail deletion. H2AX and H2A.Z genes were purchased from Funakoshi. GFP-tagged protein expression vectors were constructed by integration of the target genes into appropriate restriction enzyme sites of pEGFP-C1 (Clontech).

HeLa cells were transfected with the constructed vectors using Lipofectamine (Invitrogen) or FuGENE6 (Roche) following the corresponding manufacturer's protocols. Transfectants were selected with 800 μg/mL of G418 (Sigma) and cloned by the limiting dilution method.

Indirect immunofluorescence. HeLa cells inoculated onto coverslips (Matsunami) were fixed with 4% paraformaldehyde in 0.1 M Hepes (pH 7.2), permeabilized with 0.05% Triton X-100 in PBS, and incubated with 1% BSA in PBS for 30 min at room temperature. The samples were then incubated with an anti-acetyl histone H2A polyclonal antibody (Upstate) for 1 h at room temperature and washed with PBS for 20 min. The cells were incubated with anti-rabbit IgG-Cy2 (Molecular Probes) for 1 h at room temperature and washed with PBS for 20 min. DNA was counterstained with Hoechst 33342 (Sigma). The samples were observed with a fluorescence microscope (AxioPlan2; Zeiss) equipped with a 40× (PlanNeo, NA = 0.75; Zeiss) or 63× (PlanApo, NA = 1.40; Zeiss) objective lens.

Immunoblotting. Immunoblotting was performed as previously described [20,23] except that the membrane was blocked with 4% BSA in PBS, and an anti-acetyl histone H2A antibody (07–376; Upstate) and anti-rabbit IgG-AP (Vector Laboratories) were used as the primary and secondary antibodies, respectively.

Live cell imaging and single-photon FRAP. HeLa cells were inoculated onto 35-mm glass-bottomed dishes (Matsunami), maintained in DMEM without phenol-red (Invitrogen) with 10 mM Hepes (pH 7.2) in a humidified atmosphere on a heated stage, and imaged using an inverted fluorescence microscope (IX-71; Olympus) with a 60× objective lens (PlanApo, NA = 1.2; Olympus). Single-photon FRAP was performed using an FV300 confocal microscopic system (10 mW Ar-ion laser; wavelength 488 nm; Olympus).

To carry out single-photon FRAP, the entire image was taken by the Ar-ion laser at a mean power of 0.1 mW (5 μW at the sample) before the microscope, and then a nuclear region of $2.3 \times 2.3\ \mu m^2$ was bleached by scanning galvanometer mirrors by increasing the power of the Ar-ion laser up to 2–4 mW (110–220 μW). After 20 s, subsequent single-photon fluorescence images were captured with the original setting.

Two-photon FRAP. Two-photon FRAP with 150 femtosecond laser pulses was performed using a mode-locked Ti:sapphire laser oscillator with a wavelength of 928 nm and a repetition rate of 76 MHz. The laser pulses passed through a series of SF10 prisms to compensate for the dispersion of the optical components in the light path and the microscope. The entire imaging was performed using two-photon excitation at a wavelength of 928 nm at a mean power of 20–30 mW (2–3 mW). A nuclear region of $2.3 \times 2.3\ \mu m^2$ was bleached at a power of 70–80 mW (7–8 mW) by scanning galvanometer mirrors. After the bleaching, subsequent images were captured with the original setting.

Results and discussion

Two-photon FRAP of GFP-H1 and GFP-H2A

To check the usefulness of two-photon FRAP for mobility analysis of chromatin proteins, we constructed a two-photon excitation system (Supplementary Figure 1) and analyzed the mobilities of GFP-histone H1 and GFP-histone H2A (Supplementary Figure 2 and Supplementary Table 1). In the case of two-photon excitation, the bleaching region was limited to the vicinity of the focus (Supplementary Figures 2A and 2B) for nonlinear optical effects [24].

The apparent recovery time by two-photon FRAP was 2- to 4-fold faster than that by single-photon FRAP (Supplementary Table 1), while the maximum recovery rate was not affected by the excitation manner (Supplementary Figures 2C and 2D). These results suggest that histone H1 and H2A show diffusion-coupled behaviors [25,26], since the volume/surface area ratio mainly affects the recovery time in the case of diffusion-coupled behaviors. Differences in the $t_{1/2two}/t_{1/2single}$ ratios between histone H1 and H2A were observed. These differences are probably caused by differences in the binding statuses of the linker and core histones on chromatin, since the chromatin binding statuses are known to differ among histone types [2,7].

The difference in the laser power between single- and two-photon FRAP was caused by the difference in the absorption efficiencies of GFP between single- and two-photon excitation [27]. Two-photon excitation causes less photo-damage to living cells than single-photon excitation [19], since a near-infrared laser is employed for two-photon excitation. This reduction in photo-damage is one of the key factors for observing living cells over a long period of time [28]. Moreover, the three-dimensional diffusion rate cannot be accurately measured by single-photon FRAP [29].