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H1.X with different properties from other linker histones is required for mitotic progression

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Abstract **We report here the characterization of H1.X, a human histone H1 subtype. We demonstrate that H1.X accumulates in the nucleolus during interphase and is distributed at the chromosome periphery during mitosis. In addition, the results of fluorescence recovery after photobleaching indicate that the exchange of H1.X on and off chromatin is faster than that of the other H1 subtypes. Furthermore, RNA interference experiments reveal that H1.X is required for chromosome alignment and segregation. Our results suggest that H1.X has important functions in mitotic progression, which are different from those of the other H1 subtypes.**

Keywords: Histone H1; Histone H1.X; Chromosome; RNAi; FRAP; Mitosis

1. Introduction

Histone H1 stabilizes chromatin structure by binding close to the entry–exit sites of linker DNA [1] and is thought to be important for chromatin condensation and the chromatin higher structure. In fact, depletion of histone H1 leads to structural and functional defects of chromosomes [2,3] and affects proper embryonic development [4]. In mammals, 10 H1 subtypes have been reported to date, i.e., H1.1–H1.5 [5,6], H1° [7], H1.t [8], H1Foo [9], HILS1 [10] and H1.X [11].

Our previous studies of human metaphase chromosomes indicated that H1.X is a structural component of chromosomes [12,13]. H1.X expression is commonly detected throughout human tissues [11]. Its amino acid sequence does not show high similarities with the other somatic histone H1 subtypes (~30%), although it does possess some of the characteristic features of linker histones [14]. In addition, H1.X is partially associated with nucleosomes and enriched in micrococcal nuclease-resistant chromatin [15].

In the present study, we analyzed the function of H1.X in mitosis, since the biological functions of H1.X have not yet been elucidated. We demonstrate by fluorescence recovery after photobleaching (FRAP) and RNA interference (RNAi) analyses that H1.X has distinct properties from the other H1 subtypes that are essential for mitotic progression.

2. Materials and methods

2.1. Cells and transfection

HeLa cells were grown in Dulbecco's modified Eagle's medium (GIBCO BRL) supplemented with 10% fetal bovine serum (Equitech-Bio Inc.). For construction of a cell line stably expressing GFP-H1.X, a cDNA of H1.X was inserted into a pEGFP-C1 vector (Clontech), and the resulting plasmid encoding H1.X-GFP was transfected into HeLa cells using the FuGene6 reagent (Roche). A GFP-tagged expression vector for H1.2 visualization was constructed by integration of a human H1.2 cDNA into pEGFP-C1.

2.2. Antibodies

The primary antibodies used were: anti-H1.X rabbit polyclonal (Abcam), 1:200 dilution; anti-nucleolin mouse monoclonal (Upstate Biotechnology), 1:50 dilution; anti-α-tubulin mouse monoclonal (Calbiochem), 1:100 dilution; anti-γ-tubulin rabbit polyclonal (Sigma), 1:2000 dilution; anti-Bub1 mouse monoclonal (MBL), 1:20 dilution; anti-BubR1 mouse monoclonal (BD Transduction Laboratories), 1:1000 dilution; anti-MAD2 rabbit polyclonal (Covance), 1:100 dilution; and CREST (Cortex Biochem), 1:1000 dilution.

2.3. RNAi

A small interfering RNA (siRNA) duplex for H1.X (5′-CCAAGAAGGUUCCGUGGUUTT-3′) was chemically synthesized and HeLa cells were transfected with 120 nM of the siRNA duplex using Lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions. A control siRNA duplex (5′-UUCUCCGAACGU GUCACGUTT-3′; Qiagen) was used for control transfections. Cells were collected at 48 h after transfection and used for further analysis.

2.4. Immunofluorescence microscopy

HeLa cells grown on coverslips were fixed with 4% paraformaldehyde in PBS (pH 7.4) for 15 min at 37 °C, and then permeabilized with 0.2% Triton X-100 in PBS for 10 min at room temperature. After incubation with 1% BSA-PBS for 15 min, the cells were incubated with a primary antibody for 1 h at room temperature, followed by incubation with an appropriate secondary antibody for 1 h. The secondary antibodies (labeled with Alexa 488 or TRITC) were used at a dilution of 1:200. After washing, the cells were stained with 1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) and observed under an Axioplan II imaging fluorescence microscope (Carl Zeiss). Deconvoluted images were captured with a cooled CCD camera (CH 350/L; Roper Scientific) using an IX-70 microscope (Olympus) and analyzed using the Delta vision software (Applied Precision).

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Abbreviations: RNAi, RNA interference; siRNA, small interfering RNA; GFP, green fluorescent protein; FRAP, fluorescence recovery after photobleaching; DAPI, 4′, 6-diamidino-2-phenylindole; NEBD, nuclear envelope breakdown

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Metaphase chromosome spreads were prepared as described previously [12].

2.5. FRAP analysis

FRAP analysis was performed using an IX-71 inverted microscope (Olympus) equipped with an FV300 scanning unit. First, a single image was captured by the 488 nm-line of a 10 mW-argon laser (set to 1%) and then a selected area ($2.3 \times 2.3\ \mu m^2$) was bleached by the 488 nm-line of the argon laser (set to 40%). After 3 s of photobleaching, images were captured using the same scanning settings as above at intervals of 1.1 s (GFP-H1.X) or 20 s (GFP-H1.2). The relative intensities were calculated as follows:

$$\text{Relative intensity} = (I_t/W_t)/(I_0/W_0)$$

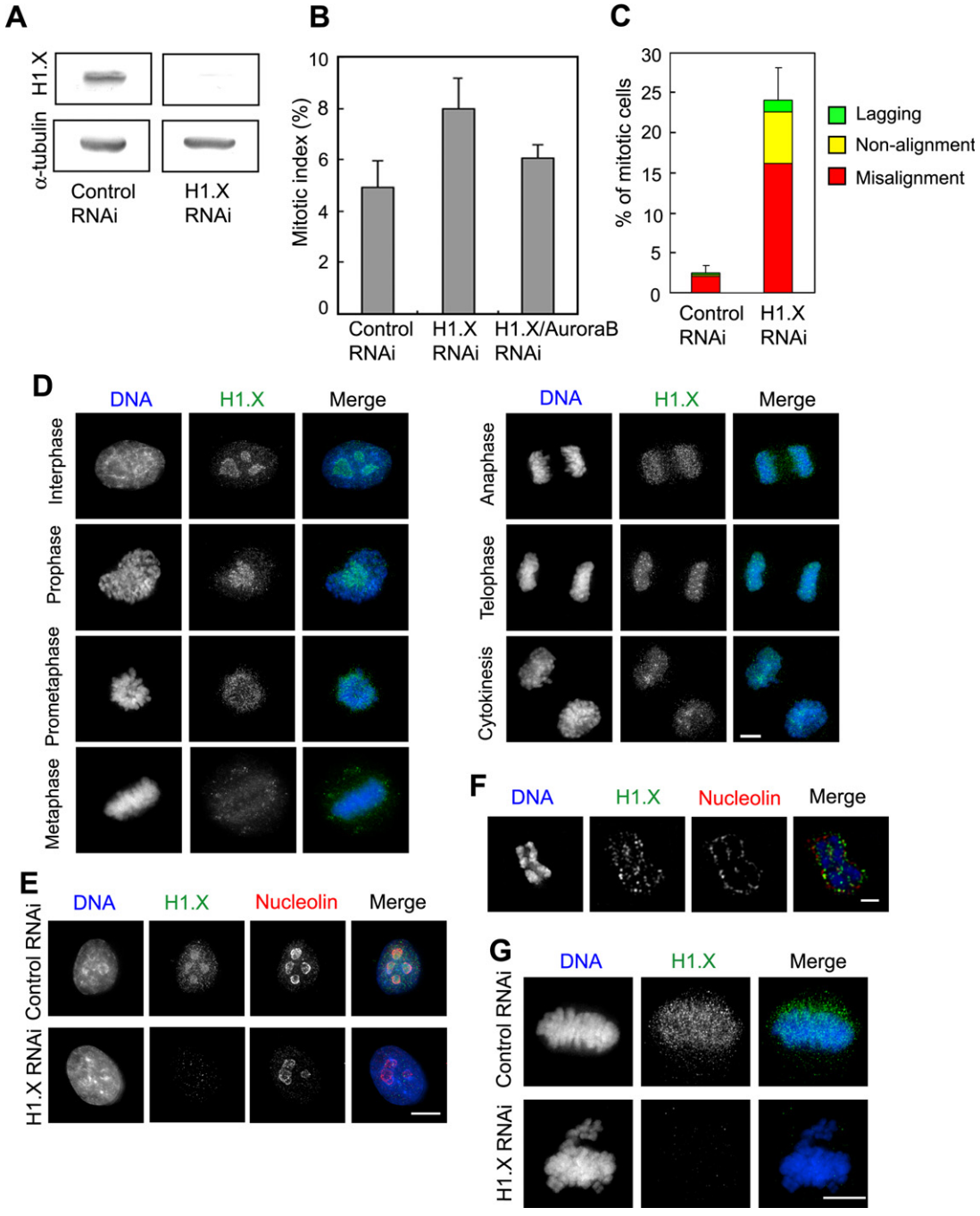

Fig. 1. Mitotic defects in H1.X-depleted cells and localization of H1.X during the cell cycle. (A) Efficient repression of H1.X after RNAi treatment was confirmed by immunoblotting. The expression of α-tubulin was used as a control. (B) The mitotic indexes in control and H1.X RNAi cells were calculated after immunostaining with an anti-α-tubulin antibody. The mitotic index in H1.X and Aurora B double-knockdown cells was also calculated. $n = 5$; >1000 cells were counted. (C) Chromosome aberrations were categorized into three groups: misalignment, non-alignment and lagging chromosomes. (D) HeLa cells were fixed with 4% paraformaldehyde and stained with an anti-H1.X antibody (green) at different mitotic phases. DNA was stained with DAPI (blue). Bar, 5 μm. (E) The nucleolar localization of H1.X (green) was confirmed by counterstaining for nucleolin (red). Bar, 5 μm. (F) Localization of H1.X to the chromosome periphery. Metaphase chromosome spreads were stained with anti-H1.X (green) and anti-nucleolin (red) antibodies. Bar, 1 μm. (G) Typical metaphase chromosome images in control and H1.X RNAi mitotic cells stained with DAPI (blue) and an anti-H1.X antibody (green). Bar, 5 μm.