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[SHORT COMMUNICATION]

A Monoclonal Antibody Raised Against an 86-kD Subunit of Human DNA Helicase II

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ABSTRACT—Here we produced a monoclonal antibody (termed as mH2) which specifically recognized an 86-kD subunit of Human DNA helicase II (p86) in HeLa cells. Immunohistochemical analysis of p86 throughout the cell cycle showed that its localization was changed from the nuclei to the cytoplasm, when the cells entered into prophase, and p86 localized diffusely in the cytoplasm during mitosis. Double staining of interphase HeLa cells with mH2 and an antibody, which recognized a component of nuclear pore complex, showed that p86 did not localize at the nuclear envelop in interphase cells.

INTRODUCTION

We generated several strains of monoclonal antibodies against mitotic HeLa cells for studying the molecular identity of substances involved in mitosis (Okubo *et al.*, 1999). Among these, one monoclonal antibody (designated as mH2) revealed the dynamic change of subcellular localization of its antigen between interphase and mitotic HeLa cells. The mH2 specifically recognized a single 86-kD component in HeLa cell extract and its antigen was identified as an 86-kD subunit of human DNA helicase II (p86).

Human DNA helicase II (HDH2) is ATP-dependent DNA unwinding enzyme purified from HeLa cells (Tuteja *et al.*, 1990). HDH2 was first described as Ku autoantigen, to which antibodies are produced in patients with scleroderma polymyositis overlap syndrome (Mimori *et al.*, 1981), and later found in the sera of patients with other rheumatic diseases (Mimori *et al.*, 1986). HDH2 is composed of two subunits, which were called p70 and p86, respectively (Yaneva *et al.*, 1985; Reeves, 1985; Mimori *et al.*, 1986). Both subunits have a leucine zipper in their primary sequences, which explains their ability to associate with each other and to bind DNA (Reeves and Sthoeger, 1989). In the presence of double strand DNA, HDH2 associates with a 350-kD catalytic subunit to form the DNA-dependent serine/threonine protein kinase, which phosphorylates a number of transcription factors (Lees-Miller and Anderson, 1991; Dvir *et al.*, 1993; Gottlieb and Jackson, 1993; Suwa *et al.*, 1994).

Using this mH2, we present here the dynamic change of p86 localization throughout the cell cycle in HeLa cells.

MATERIALS AND METHODS

Materials

Peroxidase-conjugated sheep anti-mouse IgG, rhodamin-conjugated goat anti-mouse IgG and fluorescein-conjugated goat anti-rabbit Ig were purchased from Cappel (Turnhout, Belgium). A rabbit anti-p62 antiserum was produced previously (Saito *et al.*, 1995). Leupeptin was from Peptide institute Inc. (Osaka, Japan).

Cell Culture and Preparation of Mitotic Cells

HeLa cells (RCB0007; just like ATCC CCL2, HeLa) and SP2/0-Ag14 myeloma cells (RCB0209) were obtained from Riken Cell Bank (Tsukuba, Japan) and were grown as described previously (Hosoya *et al.*, 1997). HeLa cells grown to a subconfluent level on large culture dishes (245×245 mm; Sumitomo Bakelite Co., Tokyo, Japan) were treated with nocodazole at the final concentration of 0.25 μg/ml for 16 hr. Mitotic cells were detached by moving the media across the dishes with a pipette, collected by low-speed centrifugation and washed with PBS.

Production of monoclonal antibodies against the mitotic HeLa cells

Monoclonal antibodies were raised against the mitotic HeLa cells as described previously (Okubo *et al.*, 1999). An 8-weeks-old female BALB/c mouse was immunized with 5×10^7 cells/ml of mitotic HeLa

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cells. After immunization, the spleen cells were fused with myeloma cells. Hybridoma cells were screened using the solid-phase ELISA with mitotic HeLa cells and 14 strains of them were selected for their high reactivities (over three times the absorbance of the control). Among these, one monoclonal antibody showed the dynamic changes of its staining pattern of HeLa cells throughout cell cycle and was designated as mH2. For immunofluorescence staining and immunoblotting, hybridoma culture supernatant was used. Production of ascities and purification of IgG fraction from the ascities were described previously (Okubo *et al.*, 1999).

Indirect Immunofluorescence

Indirect immunofluorescence staining was performed as described previously (Okubo *et al.*, 1999). Stained cells were examined with a Nikon Optifluorescence microscope. Photographs were taken with Fuji film, ISO400.

Preparation of HeLa cell extract

$5\times10^{7-8}$ HeLa cells were harvested and mixed with F buffer containing 20 mM Tris-HCl (pH 7.5), 2 mM $MgCl_2$, 1 mM EGTA, 200 mM NaCl, 1 mM PMSF, 10 μg/ml leupeptin and homogenized on ice. The homogenate was centrifuged at 50,000 g for 30 min and the supernatant was collected.

Amino acid sequence analysis of mH2 antigen

The monoclonal antibody mH2 purified from mouse ascites was coupled to protein A Sepharose column (Ampure PA kit) according to manufacturer's instruction. After coupling, the column was washed with the binding buffer. The antigen-containing fractions were obtained by a gel filtration using Sephacryl S-300 column and applied to mH2 antibody-protein A Sepharose column. The column was washed with the binding buffer and then the mH2 antigen was eluted with the elution buffer (Ampure PA kit). To neutralize, 1 ml of 1 M Tris-HCl, pH 8.8 was added into the eluted fraction. The procedures for digestion of mH2 antigen with V8 protease and the determination of the amino acid sequence of the digested fragments were described previously (Okubo *et al.*, 1999). Amino acid sequences were analyzed with an Applied Biosystems amino acid sequencer model 477A (Perkin Elmer Corp., Foster City, CA).

Other procedures

SDS-PAGE and immunoblotting were carried out in the same manner as described previously (Murata-Hori *et al.*, 1998).

RESULTS AND DISCUSSION

Immunofluorescence observation using mH2 revealed that the mH2 antigen was localized at the nuclei in HeLa cells of interphase (Fig. 1, arrowheads) and diffusely in the cytoplasm of mitotic HeLa cells (Fig. 1, arrows). Immunoblot analysis of the cell extract with mH2 showed that it reacted with a single 86-kD band in both interphase and mitotic HeLa cell extracts (Fig. 2). To clarify the molecular aspects of the mH2 antigen, we determined the N-terminus amino acid sequence of the partial V8 protease digests of mH2 antigen (Fig. 3). We initially fractionated the HeLa cell extract using a gel filtration column, collected the fractions containing mH2 antigen and then purified mH2 antigen using protein A column coupled with mH2. The obtained mH2 antigen was digested with V8 protease and electrophoresed on SDS-PAGE. The transferred fragments onto PVDF membranes were subjected to amino acids sequencing. Each of four informative peptide sequences was highly homologous to that of an 86-kD subunit of human DNA helicase II (p86) (Fig. 3).

To analyze the specificity of mH2, HeLa cells in the various stages of the cell cycle were stained with mH2 (Fig. 4). p86 was localized at the nuclei in interphase cells (Fig. 4, a–c, arrowheads). When the cells entered into prophase, p86 was diffusely distributed throughout the cytoplasm except for

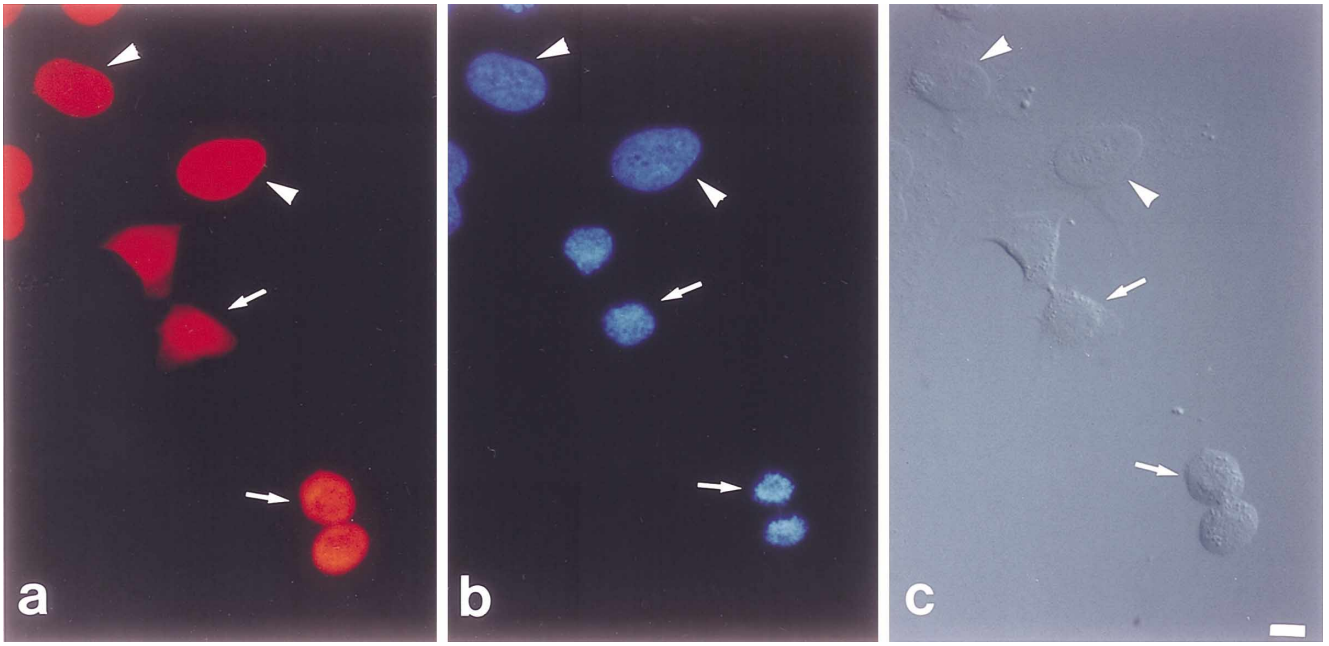

Fig. 1. Comparison of the subcellualr localization of mH2 antigen between interphase and mitotic HeLa cells. These panels are same fields. (a) Immunostaining with mH2. (b) DNA staining with 4',6-diamidino-2-phenylindole (DAPI). (c) Nomarski differential-interference micrographs. Arrows indicate mitotic cells. Arrowheads show nuclei in interphase cells. Bar 5 μm.

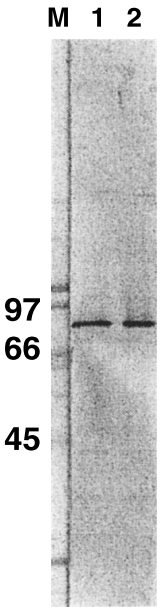

Fig. 2. Immunoblot analysis of the mH2 antigen. Total cell extracts prepared from interphase (lane 1) and mitotic (lane 2) HeLa cells were separated on SDS-PAGE, transferred to a PVDF membrane, and immunoblotted with mH2. A 86-kD band was detected in both interphase and mitotic cell extracts. Molecular weight markers are shown with molecular mass in kD (M).

chromosomes, mitotic spindles and constricted area of dividing cells (Fig. 4, d–f). And then, p86 was continuously diffused throughout the cytoplasm during mitotic phase (Fig. 4, g–r). Thus, the distribution of p86 dynamically changed from interphase to mitotic phase, but its localization was not changed during mitosis. These observations well coincided with those shown by Koike *et al.* who used the anti-Ku80 polyclonal antibody (1999).

Furthermore, we double-stained the interphase HeLa cells with mH2 and an antibody against GlcNAc-nucleoporin (p62) which was a component of nuclear pore complex and localized at the nuclear envelope (Davis and Blobel, 1986; Saito *et al.*, 1995) (Fig. 5). Using the laser scanning confocal microscopy, we did not observe colocalization of p86 with p62 at the nuclear envelope. Also we did not observe that p86 was localized with any intracellular structures and nuclear periphery. This result corresponded to that of the biochemical aspect by Vishwanatha *et al.* (1995). For conclusion, a monoclonal antibody, which specifically recognizes an 86-kD subunit of human DNA helicase II, was produced.

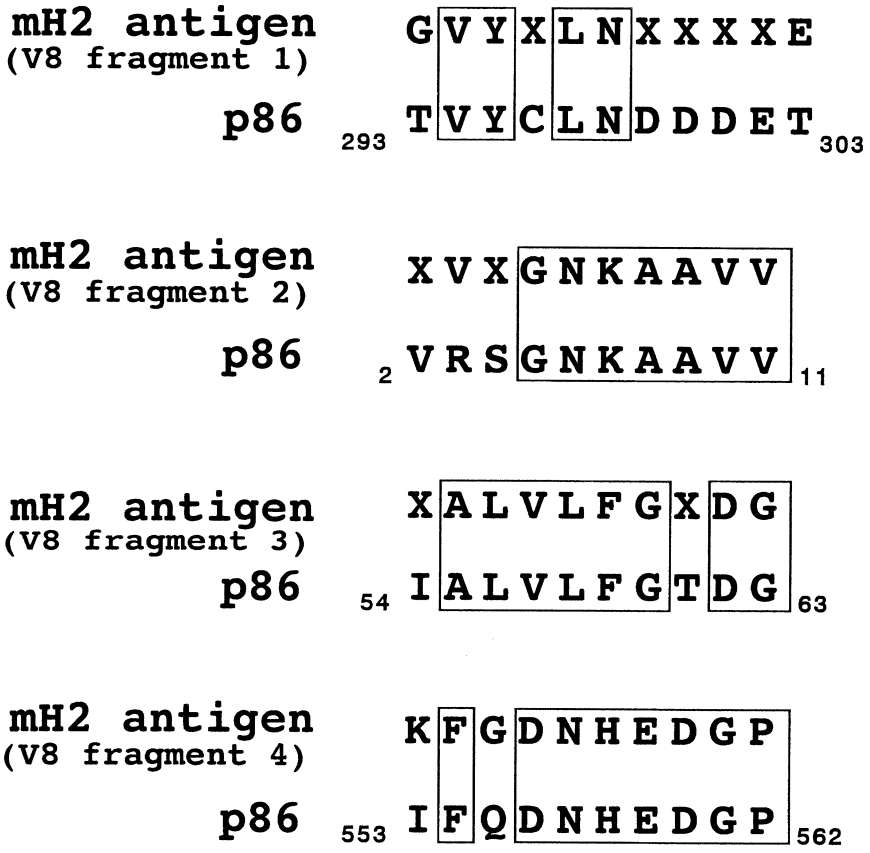

Fig. 3. Comparison of partial amino acid sequences between the mH2 antigen and p86 subunit of HDH2. Partial amino acid sequences were determined on the four fragments generated by digesting the mH2 antigen with V8 protease. X represents undetermined amino acids. The starting and the ending amino acid position in p86 subunit of HDH2 are indicated at the left and the right, respectively. Amino acid identities are shown by boxes.

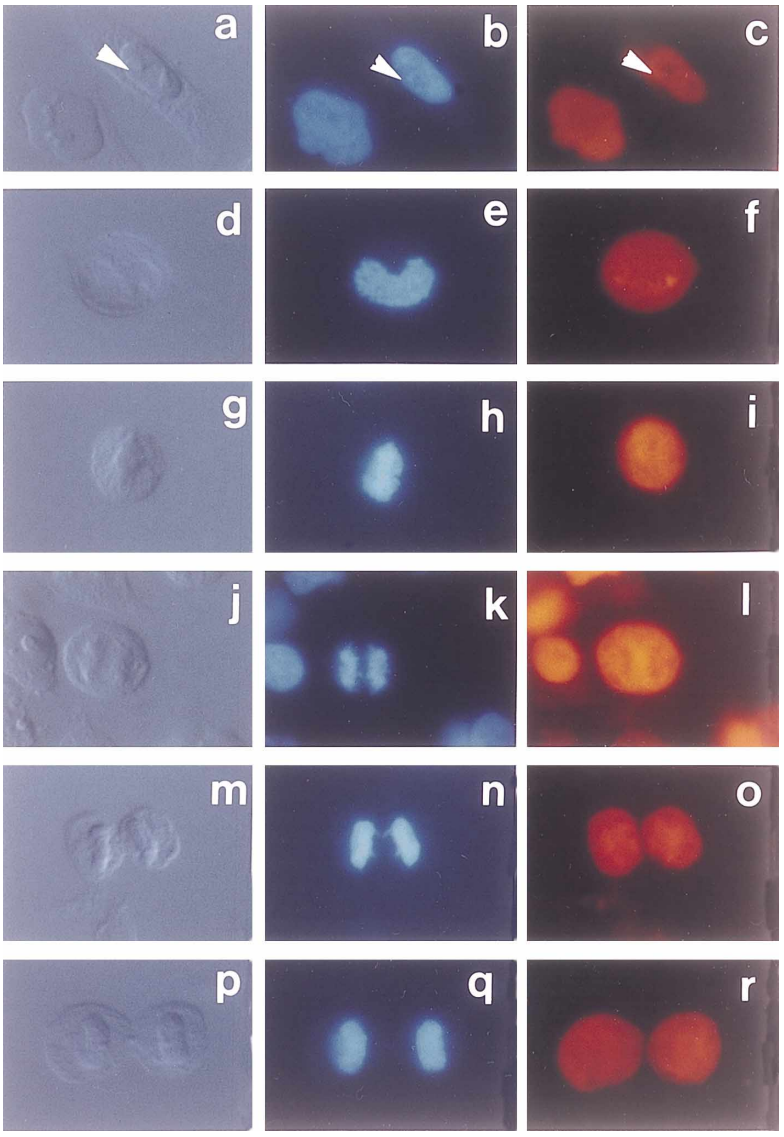

Fig. 4. Subcellular localization of p86 subunit of HDH2 throughout the cell cycle in HeLa cells. The cells were interphase (a–c), prophase (d–f), metaphase (g–i), anaphase (j–l) and telophase (m–r), respectively. (a, d, g, j, m and p) Nomarski differential-interference micrographs. (b, e, h, k, n and q) DNA labeled with DAPI. (c, f, i, l, o and r) Immunofluorescence staining with mH2. The mH2 antigen was localized diffusely throughout the cytoplasm during mitosis.

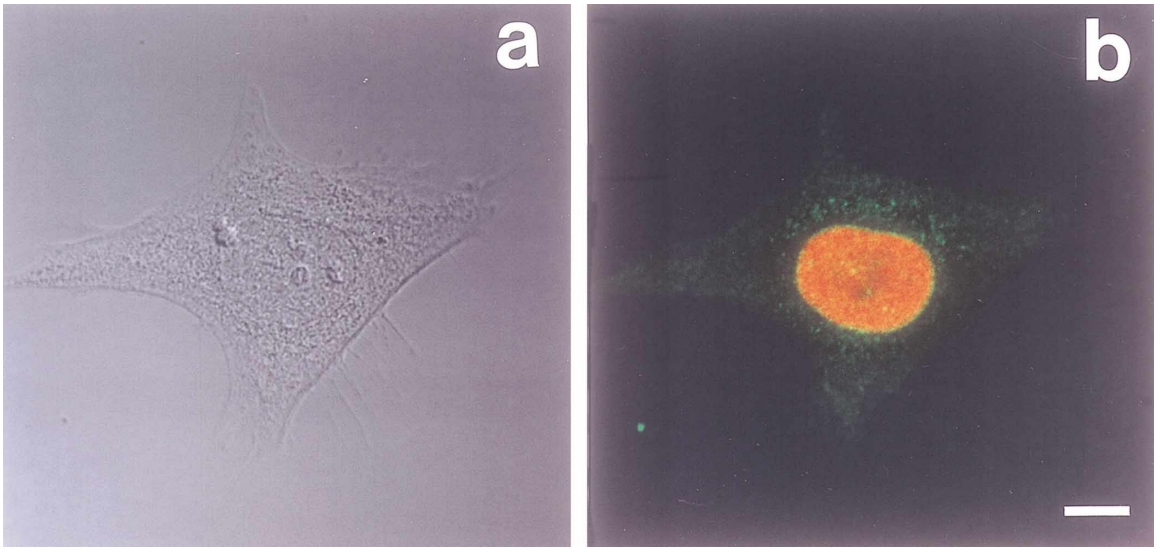

Fig. 5. Double immunofluorescence of p86 subunit of HDH2 and nuclear pore complex by confocal microscopy. (a) Nomarski differential-interference micrographs. (b) Double labeling with mH2 (red) and anti-p62 antibody (green). Merged images are shown in yellow. Bar, 5 µm

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REFERENCES

Davis L I, Blobel G (1986) Identification and characterization of a nuclear pore complex protein. Cell 45: 699–709

Dvir A, Stein L Y, Calore B L, Dynan W S (1993) Purification and characterization of a template-associated protein kinase that phosphorylated RNA polymerase II. J Biol Chem, 268: 10440–10447

Gottlieb T M, Jackson S P (1993) The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. Cell 72: 131–142

Hosoya H, Murata-Hori M, Takahashi F, Arai T, Hosoya N, Yazaki K, Nonomura Y (1997) Effects of wortmannin on the cytoskeletal structure of HeLa cells. Biomed Res 18: 321–324

Koike M, Awaji T, Kataoka M, Tsujimoto G, Kartasova T, Koike A, Shiomi T (1999) Differential subcellular localization of DNA-dependent protein kinase components Ku and DNA-PKcs during mitosis. J Cell Sci 112: 4031–4039

Lees-Miller S P, Anderson C W (1991) The DNA-activated protein kinase, DNA-PK: a potential coordinator of nuclear events. Cancer Cells 3: 341–346

Mimori T, Akizuki M, Yamagata H, Inada S, Yoshida S, Homma M (1981) Characterization of a high molecular weight acidic nuclear protein recognized by autoantibodies in sera from patients with polymyositis-scleroderma overlap. J Clin Invest 68: 611–620

Mimori T, Hardin J A, Steitz, J A (1986) Characterization of the DNA-binding protein antigen Ku recognized by autoantibodies from patients with rheumatic disorders. J Biol Chem, 261: 2274–2278

Murata-Hori M, Murai N, Komatsu S, Uji Y, Hosoya H (1998) Concentration of singly phosphorylated myosin II regulatory light chain along the cleavage furrow of dividing HeLa cells. Biomed Res 19: 111–115

Okubo M.-A, Chiba S, Nishikata T, Matsuno A, Hosoya H (1999) Generation and characterization of a monoclonal antibody, mH1, raised against mitotic HeLa cells. Develop Growth Differ 41: 381–389

Reeves W H (1985) Use of monoclonal antibodies for the characterization of novel DNA-binding proteins recognized by human autoimmune sera. J Exp Med, 161: 18–39

Reeves W H, Sthoeger Z M (1989) Molecular cloning of cDNA encoding the p70 (Ku) lupus. J Biol Chem, 264: 5047–5052

Saito M, Kita K, Sekiya K, Omata S, Horigome T (1995) Purification and molecular shape of a 144 kDa protein bearing N-acetylglucosamine residues from rat liver nuclear envelopes. J Biochem (Tokyo) 117: 47–53

Suwa A, Hirakata M, Takeda Y, Jesch S A, Mimori T, Hardin J A (1994) DNA-dependent protein kinase (Ku protein -p350 complex) assembles on double-strand DNA. Proc Natl Acad Sci USA, 91: 6904–6908

Tuteja N, Tuteja R, Rahman K, Kang L Y, Falaschi A (1990) A DNA helicase from human cells. Nucleic Acids Res 18: 6785–6792

Vishwanatha J K, Tauer T J, Rhode III S L (1995)Characterization of the HeLa cell single-stranded DNA-dependent ATPase/DNA helicase II. Mol Cell Biochem 146: 121–126

Yaneva M, Ochs R, McRorie D K, Zweig S, Busch H (1985) Purification of an 86-70 kDa nuclear DNA-associated protein complex. Biochim Biophys Acta 841: 22–29

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