Decrease in uH2A (protein A24) of a mouse temperature-sensitive mutant

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ts85 cell is a temperature-sensitive mutant of cell cycle, and chromosomal protein uH2A of this mutant disappears at the non-permissive temperature. uH2A in nucleosomes is thought to be synthesized or degradated as follows. H2A + Ubiquitin ⇄ uH2A. Up to date, the degradation of uH2A was shown to be catalyzed by uH2A lyase, however no enzymes (factors) concerning its synthesis have been elucidated. Here, we show that ATP is prerequisite for the synthesis of uH2A, and that the disappearance of uH2A at the non-permissive temperature may be due to a reduction in the rate of synthesis rather than an increase in the rate of its degradation.

uH2A *Temperature-sensitive mutant* *ATP* *Mouse* *Ubiquitin*

1. INTRODUCTION

Protein A24 (hereafter uH2A) is a conjugate of histone H2A and ubiquitin [1,2], a polypeptide occurring universally in nature [3]. Both of these peptides are bound by an iso-peptide linkage from a C-terminal glycine of ubiquitin to the ϵ-amino group of lysine 119 of histone H2A [2,4]. Thus, uH2A is considered to be involved in the core of nucleosomes as a modified form of histone H2A [5]. uH2A constitutes about 2% of the total amount of core histones [1], and the change in the quantity of this protein is influenced by the particular physiological state of an organism, such as partial hepatectomy [6], and by treatment with thioacetamide [7]. Also it has been reported that the quantity of uH2A changes during the cell cycle progression of a culture cell [8,9]. The role of uH2A in chromatin, however, still remains unsolved. We have reported that a basic protein of chromatin, designated as protein A, disappeared at a non-permissive temperature in a temperature-sensitive (ts) mutant of a culture cell, ts85 [10], which is defective in the phosphorylation of histone H1 [11,12]. Amino acid analysis of purified protein A shows that protein A is identical to uH2A (unpublished). Here, we show that the decrease in the amount of uH2A at the non-permissive temperature may be caused by a reduction in the rate of its ATP-dependent synthesis rather than an increase in the rate of its degradation.

2. MATERIALS AND METHODS

The ts85 cell was grown as in [13]. The whole cell homogenates and 0.4 N sulfuric acid soluble chromosomal protein was prepared as in [10]. It was separated by SDS–polyacrylamide gel system [14] or two-dimensional acid urea–SDS polyacrylamide gel system [15]. The cellular ATP-level was measured as in [16].

3. RESULTS AND DISCUSSION

We examined the cause of the decrease in the amount of uH2A in the ts85 cells at 39°C. There are 3 possibilities:

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(i) Nucleosomal uH2A and histone H2A are exchanged at an increased rate (uH2A ⇄ H2A);
(ii) Although uH2A is formed, it remains free in either cytoplasm or nucleoplasm;
(iii) The steady state equilibrium between the synthesis and the degradation of uH2A is inclined toward the following mode of degradation uH2A ⇄ H2A + ubiquitin.

(A specific enzyme, uH2A lyase, which catalyzes the split of uH2A into H2A and ubiquitin has been reported [17]).

In regard to possibilities (i) and (ii), we compared the amount of uH2A in whole cell homogenate at 33°C and 39°C and found that there was a decrease at 39°C (fig.1). Thus, these two possibilities may be excluded.

In regard to possibility (iii) (i.e., whether the change in the amount of uH2A is due to an increase in the rate of degradation or decrease in the rate of synthesis) we examined the effect of depletion of ATP on the formation of uH2A. Although no enzyme catalyzing the synthesis of uH2A has been found yet, in the course of the study on energy-dependent proteolysis reactions in reticulocytes, it has been shown that ubiquitin forms conjugates with multiple forms of polypeptide via ATP-dependent reactions [18,19].

We measured the amount of ATP and uH2A in the cells after the incubation with or without drugs depleting cellular ATP. The amount of cellular ATP was summarized in table 1. By the addition of

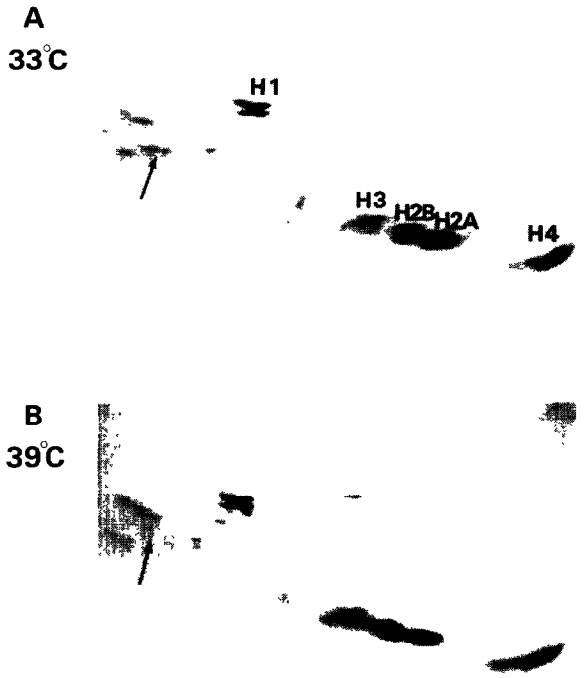

Fig.1. Two-dimensional gel electrophoresis of acid extracts of ts85 cells. The ts85 cells of logarithmically growing phase (A) or incubated at 39°C for 6 h (B) were harvested and 0.4 N sulfuric acid soluble proteins were extracted from whole cell homogenates and analyzed by two-dimensional gel electrophoresis as described in section 2. The arrow indicates the position of uH2A.

Table 1

Cellular ATP-level in ts85 cells at 33°C or 39°C in the presence or absence of DNP and DgG

Sample no.	Temp. (°C)	Time (h)	Addition of drugs	Amount of ATP (nmol/10^5 cells)	%
1	33	0	−	1.01	100
2	33	3	−	1.08	106
3	33	3	+	0.044	4.4
4	33	6	−	1.23	122
5	33	6	+	0.026	2.6
6	39	3	−	0.984	97
7	39	3	+	0.038	3.8
8	39	6	−	1.351	134
9	39	6	+	0.039	3.9

The ts85 cells (log phase) were incubated at 33°C for 18 h in the presence of excess thymidine (2.5 mM). Then 2,4-dinitrophenol (DNP, 0.5 mM) and 2-deoxy-*d*-glucose (DdG, 80 mM) were added to the culture medium, and incubated for an indicated period at 33°C or 39°C. The viability of cells (tested by the method of dye exclusion) was about 80%, in the presence or absence of drugs at either temperature

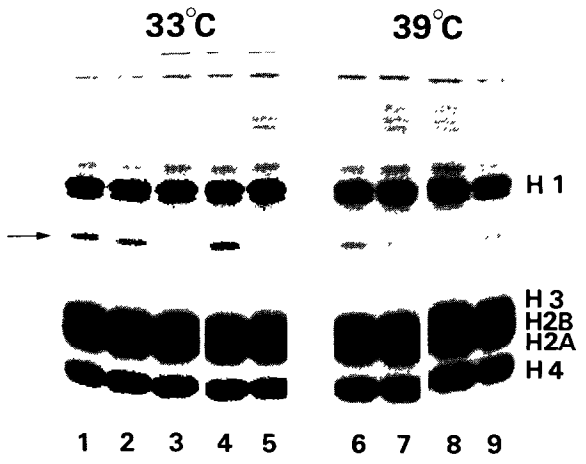

Fig.2. The effect of depletion of cellular ATP on the content of uH2A in ts85 cells incubated at 33°C or 39°C. The ts85 cells were prepared as in table 1. Lanes 1–9 corresponds to samples 1–9 in table 1. The arrow indicates the position of uH2A.

both of 2,4-dinitrophenol and 2-deoxy-*d*-glucose, cellular ATP-level was distinctly diminished at either 33°C or 39°C, while in the absence of drugs, it remained nearly the same at either temperature.

Exhaustion of cellular ATP results in a remarkable decrease in the amount of uH2A at 33°C (fig.2, lane 3 *vs* 2 or 5 *vs* 4). In an in vitro homogenate system, we also observed that the addition of ATP inhibited the disappearance of uH2A at 33°C (not shown). Thus, ATP seems to be necessary for the formation of uH2A, and this result makes possible the detection of differences in the degradation rate of uH2A under conditions which suppress the formation of uH2A. Therefore, the degradation rates of uH2A at both temperatures were compared. In cells deprived of ATP, the amount of uH2A at 39°C, was somewhat more than the amount at 33°C (fig.2, lane 7 *vs* 3 or 9 *vs* 5), indicating that the degradation rate was not stimulated at a higher temperature. Accordingly, we may conclude that the decrease in the amount of uH2A in ts85 cells at 39°C is due to a reduction in the rate of synthesis and not to an increase in the rate of degradation. Furthermore, at 39°C, the amount of uH2A of drug-treated cells, in which ATP-level was exhausted, was almost the same as that of untreated cells (fig.1, lane 8,9). This result suggests that the temperature-sensitive factor(s) of this mutant may closely participate in the formation of uH2A.

Considering the above, and the biological role of uH2A, it is of interest to consider whether the putative enzyme system which catalyzes the conjugation of H2A and ubiquitin is temperature-sensitive in this mutant and whether the enzyme has any common properties with that functioning in the ATP-dependent proteolysis in reticulocytes.

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