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DISAPPEARANCE OF A BASIC CHROMOSOMAL PROTEIN FROM CELLS OF A MOUSE TEMPERATURE-SENSITIVE MUTANT DEFECTIVE IN HISTONE PHOSPHORYLATION

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SUMMARY

The amount of a basic nuclear protein which migrates a little slower than histone H1 in urea-acetic acid-polyacrylamide gel electrophoresis and a little faster than H1 in sodium dodecylsulfate-polyacrylamide gel electrophoresis, decreases when cells of a temperature-sensitive mutant, ts85, derived from a mouse carcinoma cell line, are incubated at the nonpermissive temperature (39°C). This protein appears again, when cells cultured at 39°C are shifted down to the permissive temperature, indifferent to the added cycloheximide. Neither in wild type nor in a revertant of ts85, the protein disappeared at 39°C. Since the ts85 cells were found to be defective in chromosome condensation and in the phosphorylation of histone H1 at 39°C (1,2), this basic protein may relate to the both events.

INTRODUCTION

We have isolated a temperature-sensitive mutant, designated as ts85, from a mouse carcinoma cell line, FM3A (1). The major part of ts85 cells cultured at 39°C (nonpermissive temperature) are arrested in the late S and G_2 phases, and incompletely condensed chromosomes are observed in these cells. The mutant cells are also defective in the phosphorylation of histone H1 at 39°C (2). From our data and previous findings (3,4), the defect in chromosome condensation of this mutant seems to be due to the lack of histone H1 phosphorylation. The preliminary experiments revealed, however, the enzyme, histone kinase itself was not temperature-sensitive, prompting us to scrutinize the real cause of defects in this mutant. We report here the disappearance of a basic chromosomal protein from ts85 cells at the nonpermissive temperature.

MATERIALS AND METHODS

Cell cultures: The ts85 strain, a temperature-sensitive mutant of mouse cells was selected from FM3A c128 cells and partially characterized as previously described (1). As the permissive and nonpermissive temperature 33°C

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and 39°C were used, repsectively. Cells were cultured in suspension in RPMI 1640 medium supplemented with 10 % calf serum.

Synchronization of cells: Logarithmically growing cells were treated with excess thymidine (2 mM) at 33°C for 18 h. After the removal of excess thymidine by washing twice with phosphate-buffered saline, the cells were incubated in fresh medium at 33°C for 8 h, then treated again with excess thymidine for 10 h. By these treatment the most of cells were accumulated at G1/S boundary. The synchronized ts85 cells were permitted to enter S phase at 33°C or 39°C in the medium containing 0.03 μg/ml colcemid (Ciba) which was added in order to prevent cells from proceeding to the next cell cycle. DNA synthetic activity was monitored by pulse labeling with [^{3}H] deoxyadenosine as described previously (2).

Extraction of chromosomal basic proteins and gel electrophoresis: Basic nuclear proteins were extracted as follows; cells (5-10 × 10^6) were suspended in 1 ml of hypotonic buffer T (10 mM Tris-HCl, pH 7.8, 2 mM $MgCl_2$, 0.5 mM EDTA, 2 mM 2-mercaptoethanol and 0.025 % Triton X-100). After standing at 4°C for 10 min, they were homogenized with a teflon pestl Potter-Elvehjem homogenizer by 15 strokes and centrifuged at 800 g for 5 min. Precipitate was washed with buffer T, buffer A (50 mM Tris-HCl, pH 7.4, 0.14M 2-mercaptoethanol and 50 mM sodium bisulfite) and with buffer A containing 0.15 M NaCl, successively. Chromatin was pelleted by centrifugation at 12,000 g for 10 min. Chromosomal basic proteins were extracted with 0.4 N sulfuric acid at 4°C. Proteins were precipitated by the addition of trichloro-acetic acid (18 %), washed with acetone-HCl (0.5 % v/v HCl) and twice with acetone. Basic proteins obtained were dissolved in sample buffer (2M urea, 0.9 N acetic acid, 10 % glycerol) and analyzed by acid-urea-polyacrylamide gel electrophoresis according to the method of Paynim and Chalkley (5). Basic proteins were electrophoresed also in SDS polyacrylamide gel (6) with a 15 % acrylamide concentration. Gels were stained with Coomassie Brilliant Blue R and were scanned with a densitometer.

Phosphorylation of histones: The ts85 cells synchronized at G1/S boundary were permitted to enter S phase at 33°C or 39°C, then cells were labeled with [^{32}P] orthophosphate (100 μC/ml, carrier free) for 2 h from 13.5 h after the release, when cells were proceeded to M phase at 33°C or arrested at 39°C. Histones were extracted and analyzed by gel electrophoresis as described above. Gels were stained, frozen and sliced into pieces of 1.2 mm in length. Each slice was put into a vial, and the radioactivity was measured.

Chemicals: Acrylamide and bis-acrylamide were purchased from Eastman. RPMI 1640 medium was purchased from Nissui Co., calf serum from Flow Laboratory, and thymidine from Sigma. Other chemicals were purchased from Wako Pure Chemica Industries, Japan.

RESULTS AND DISCUSSION

Disappearance of a basic protein at the nonpermissive temperature: Basic proteins in chromatin of ts85 cells were extracted with 0.4 N sulfuric acid and analyzed by acid-urea gel electrophoresis as described in MATERIALS AND METHODS. Five kinds of histones and several minor proteins were detected by staining (Fig. 1). A nonhistone protein which migrated a little more slowly than histone H1 was designated as A protein. When ts85 cells that were synchronized at G1/S boundary were incubated at 39°C, the amount of A protein decreased to 50 % of the initial value within 3 h and virtually to null in

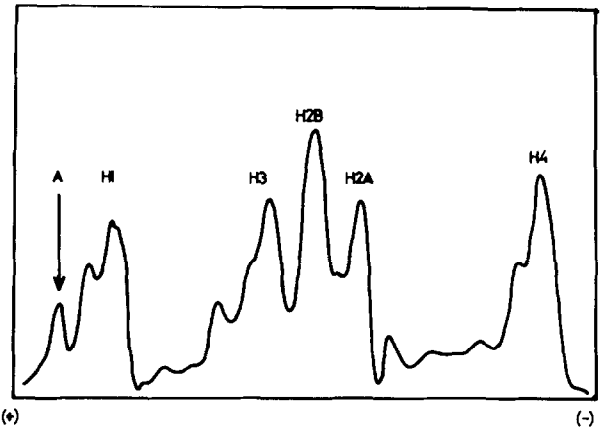

Fig. 1 The densitometric scan of acid-urea gel electrophoresis of chromosomal basic proteins. Chromosomal basic proteins of ts85 cells synchronized at G1/S boundary were extracted and analyzed by gel electrophoresis as described in MATERIALS AND METHODS. A protein was indicated by an arrow.

6 h (Fig. 2A). The amount of the A protein was reduced when ts85 cells synchronized at G1/S boundary were incubated at 39°C in the presence of added thymidine (2mM), cycloheximide (10 μg/ml), or actinomycin D (5 μg/ml). These data indicate that the disappearance of the A protein at the nonpermissive

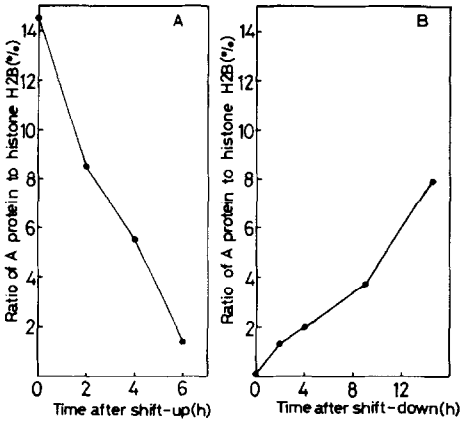

Fig. 2A Disappearance of A protein by shift-up of the temperature to the nonpermissive one. The ts85 cells synchronized at G1/S boundary by the treatment with excess thymidine were incubated at 39°C for various length of time in the presence of excess thymidine and ratio of the amount of A protein to H2B histone was calculated from the densitometric scans of gel electrophoresis stained with Coomassie Brilliant Blue R.

Fig. 2B Reappearance of A protein by shift-down of the temperature to the permissive one. The ts85 cells synchronized at G1/S boundary were released from the blockade and permitted to enter S phase at 39°C. After 8 h incubation at 39°C, the temperature was shifted down to 33°C in the presence of colcemid. The ratio of the amount of A protein to H2B histone was calculated as described in Fig. 2A.

Table 1. Ratio of phosphorylation of H1 histone to H2A histone at the nonpermissive temperature

Temperature	Incorporation of [^{32}P] phosphate into histones (cpm/2 h/ 1 × 10^7 cells)		
	H1	H2A	H1/H2A
33°C	4622	6174	0.75
39°C	1912	10440	0.18

temperature was resulted neither from the progression of cell cycle nor from the production of RNAs or proteins. In addition, the A protein did not disappear in wild type cells nor in the cells of a revertant of ts85 at both temperatures (data not shown). As a corollary, the disappearance of the A protein in ts85 cells was likely to be a temperature-dependent event.

As reported previously (1,2), ts85 cells showed abnormal chromosome condensation as well as the decrease in the phosphorylation of histone H1 at nonpermissive temperature (Table 1). The former event was not detectable until cells were incubated at 39°C for longer than 10 h, whereas the latter was evident in 3 h at 39°C. So, the disappearance of the A protein occurred earlier than the appearance of abnormal chromosome condensation and parallel to the decrease in the phosphorylation of histone H1.

Reappearance of the A protein by the shift-down of the temperature: The most of the ts85 cells synchronized at G1/S boundary are able to proceed through S phase at 39°C, when they are freed from the synchronizing agent, and finally they are arrested at the end of S or in G2 phase (1). If the temperature is shifted down to the permissive one at this time, when the A protein disappears completely, the protein appears gradually. Fig. 2B shows the time course of the reappearance of the A protein. The amount of the A protein increased to about 60 % of the initial level at 12 h after the down-shift of the temperature.

The characteristics of the A protein: Although the function of the A protein is not clear yet, our data show that it is related to the chromosome condensation and the phosphorylation of histone H1.

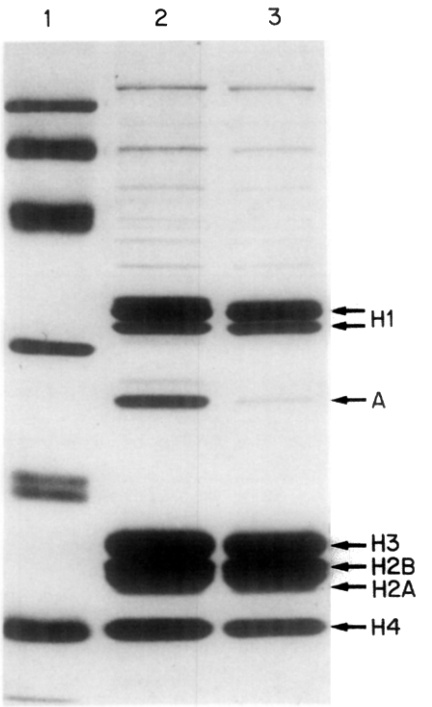

Fig. 3 SDS-polyacrylamide gel electrophoresis of chromosomal basic proteins. 1. Protein standards (molecular weight) were: phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), α lactalbumin (14,400). 2. Chromosomal basic proteins extracted from ts85 cells cultured at 33°C. 3. Chromosomal basic proteins extracted from ts85 cells cultured at 39°C for 16 h. Histones and A protein were indicated by arrows.

The A protein is not soluble in 5 % perchloric acid as histone H1. Furhtermore, the A protein is not extractable from the nuclei with 0.6 M sodium chloride. When the A protein is applied to SDS-polyacrylamide gel electrophoresis, it migrates a little faster than histone H1 (Fig. 3), in contrast to the electrophoresis in acid-urea-polyacrylamide gel. The approximate molecular weight of the protein A is 25,000 as determined by the gel electrophoresis. The amount of the A protein in the cells at G1/S boundary is about 13 % of histone H2B, as calculated from the intensity of Coomassie Brilliant Blue stainning. These data suggest that the character of the A protein quite resembles that of protein A24 reported by Goldknopf et al (7). Recently Matsui et al reported that protein A24 disappeared in mitosis and discussed its role in chromatin condensation (8). If the protein A is identi-

cal with protein A24, and if it has some roles in chromatin condensation, there may exist some structural similarity between mitotic chromatin and chromatin of ts85 cells arrested at nonpermissive condition.

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