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A Mouse Temperature-Sensitive Mutant Defective in H1 Histone Phosphorylation Is Defective in Deoxyribonucleic Acid Synthesis and Chromosome Condensation†

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ABSTRACT: By means of a temperature-sensitive mutant (ts85 strain), we have studied the effect of the decrease in H1 histone phosphorylation on DNA synthesis and chromosome condensation. When ts85 cells were incubated at 39 °C (nonpermissive temperature), the rate of H1 histone phosphorylation was decreased gradually and reached half that at 33 °C (permissive temperature) by 6-h incubation. Wild-type cells, growth-revertant ts85 cells (ts85R-MN3), and other ts mutants which were arrested mainly at the G2 phase at 39 °C had no defects in H1 histone phosphorylation. When ts85 cells were synchronized at the G1/S boundary at 33 °C and released from the blockade at 39 °C, ~70% of cells passed through the S phase and stopped at the G2 phase. The rest were distributed in G1/S to the S phase and mitotic cells were not detected at all. When ts85 cells, synchronized at the G1/S boundary, were further incubated for 8 h at 39 °C with a synchronizing agent, the rate of phosphorylation of H1 histone was decreased and the cells were not able to complete DNA synthesis after release from the blockade. Cytofluorometric analysis revealed that the cells had DNA contents of the S phase. Taken together with our earlier data [Matsumoto, Y., Yasuda, H., Mita, S., Marunouchi, T., & Yamada, M. (1980) *Nature (London) 184*, 181–183], both events, incomplete DNA replication and a defect in chromosome condensation, were thought to be ascribed to the decrease in H1 histone phosphorylation.

When mammalian cells grow, the structural changes of chromatin necessarily take place. The phosphorylation of histone is thought to be important for the changes in chromatin structure. In proliferating cells, the phosphorylation occurs significantly in H1, H2A, and H3 histones (Marks et al., 1973; Gurley et al., 1974a). A lot of studies with cultured cells revealed that the phosphorylation of H1 histone changes most dramatically throughout a cell cycle of that of other histones (Balhorn et al., 1972; Lake & Salzman, 1972; Lake et al., 1973; Gurley et al., 1974b). Since H1 histone phosphorylation begins at the late G1 phase and continues from the S to M phase, this phosphorylation is thought to be related to DNA synthesis and/or chromosome condensation at the M phase. To date, much evidence supporting the relationship between H1 histone phosphorylation and chromosome condensation has been reported (Bradbury et al., 1974a,b; Gurley et al., 1978). On the contrary, only a few data which suggested the role of H1 histone phosphorylation in structural changes of chromatin during the interphase have been reported. H1 histone phos-

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phorylation began at the late G1 phase and was not affected by the inhibitors of DNA synthesis which inhibited concominantly histone synthesis (Marks et al., 1973). Therefore, H1 histone phosphorylation at the late G1 phase was thought to occur in old synthesized histone, relating to the initiation of the S phase. Another report which supports the involvement of H1 histone phosphorylation in chromatin organization at the interphase showed that the inaccessibility of chromatin to heparin was increased in accordance with the increase in H1 histone phosphorylation at the late G1 phase to the S phase (Hildebrand & Tobey, 1975).

In our laboratory, a temperature-sensitive mutant (ts85 strain) was selected from mouse FM3A cells and was arrested mainly at the G2 phase and partly at the S phase by the incubation at the nonpermissive temperature (Mita et al., 1980). We have also reported briefly the defect in H1 histone phosphorylation at the arrested G2 phase at the nonpermissive temperature, and as a corollary, the involvement of H1 histone phosphorylation in chromosome condensation was elucidated (Matsumoto et al., 1980).

By use of the ts85 cells, our data presented here provide further support for the hypothesis that H1 histone phosphorylation regulates the structural change of chromatin which is necessary for not only chromosome condensation at the M phase but also DNA synthesis at the S phase.

Experimental Procedures

Cells, Cell Culture, and Synchronization. The ts85 cells and ts18 cells were obtained from FM3A (cl28), a cell line established from mouse mammary carcinoma, by the treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) as described previously (Mita et al., 1980). The growth-revertant ts85 cells (ts85R-MN3) were obtained from ts85 cells by MNNG treatment. The fused cells between ts85 and 8-azaquanine resistant FM3A cells, kindly supplied by Dr. H. Koyama, Cancer Institute, Japan, were obtained by the poly(ethylene glycol) method (Davidson & Gerald, 1976) and selected with HAT medium. 11C3 cells were obtained from CHO-K1 cells by the treatment with MNNG as described previously (Marunouchi & Nakano, 1980). All the cells except 11C3 cells were cultivated in a suspension in RPMI 1640 medium containing 10% calf serum. 11C3 cells were cultivated as a monolayer in F12 medium containing 10% fetal bovine serum. The permissive and the nonpermissive temperatures, 33 and 39 °C, respectively, were used.

Cell synchronization at G1/S boundary was performed as reported elsewhere by the treatment with excess thymidine (2 mM) (Matsumoto et al., 1980). Logarithmically growing cells were treated with excess thymidine at 33 °C for 18 h. After removal of excess thymidine by washing the cells, they were incubated at 33 °C for 8 h in normal medium; then the second treatment with excess thymidine was done for 10 h at 33 °C. In some experiments, the cells at the G1/S boundary were incubated further for 8 h without washing the excess thymidine at the permissive or the nonpermissive temperature. After the incubation, excess thymidine was washed out and the cells were permitted to enter the S phase at each temperature, respectively. When indicated, Colcemid (0.05 μg/mL in FM3A cells; 0.03 μg/mL in CHO-K1 cells) was added to the culture for inhibiting the cells to enter the next G1 phase.

Histone Extraction, Histone Phosphorylation, and Gel Electrophoresis. Labeling of cells with [^{32}P]orthophosphate and gel electrophoresis of histone were performed as reported elsewhere (Matsumoto et al., 1980). Cells were pulse labeled for 2 h with [^{32}P]orthophosphate (100 μCi/mL; carrier free) in phosphate-free Eagle's MEM (1:100 phosphate contents of normal medium) containing nonessential amino acid and 10% dialyzed calf serum. Labeled cells frozen at −20 °C were thawed and suspended in 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 for 10 min at 4 °C, they were homogenized for 15 strokes with a Teflon pestle glass Potter Elvehjem homogenizer and centrifuged at 800*g* for 5 min. The precipitate was washed successively with buffer T, buffer A (50 mM Tris-HCl, pH 7.4, 0.14 M 2-mercaptoethanol, and 50 mM sodium bisulfite), and buffer A containing 0.15 M NaCl with stirring. Nuclei were pelleted at 12000*g* for 10 min. Histones were then extracted by 0.4 N sulfuric acid for 1 h with stirring at 4 °C. Histones obtained were pelleted by 18% trichloroacetic acid and were washed with acetone–HCl (HCl, 0.5% v/v) and twice with acetone. In some experiments, H1 histone was extracted by 0.75 N perchloric acid before sulfuric acid extraction. Histones were analyzed by acid–urea–15% polyacrylamide gel electrophoresis (0.9 N acetic acid–2 M urea; 15- or 25-cm gel) according to the method of Panyim & Chalkley (Panyim & Chalkley, 1969). After electrophoresis, the gels were stained with Coomassie brilliant blue R and were scanned with a densitometer. When the radioactivities in gel were counted, gels were cut into 1.2-mm slices, each slice was solubilized with Soluene 350 (Packard), and the radioactivity was measured by liquid scintillation spectrometer.

Flow Cytofluorometric Analysis. After being fixed with ethanol, cells were treated with 0.5 mg/mL ribonuclease A at 37 °C for 1 h and 0.1 mg/mL pepsin in 0.5% HCl at 37 °C for 15 min. The cells were washed with phosphate-buffered saline, stained with ethidium bromide (20 μg/mL), and analyzed with a cytofluorograph (Model FC 200; Ortho Instruments).

Results

Decrease in H1 Histone Phosphorylation at the Nonpermissive Temperature. We reported previously that when the ts85 cells were incubated at the nonpermissive temperature (39 °C) for 16 h, they ceased to cycle mainly at the G2 phase and partly at the S phase (Mita et al., 1980). Furthermore, at the arrested state at 39 °C, the rate of the phosphorylation of H1 histone was reduced, as compared with that at the permissive temperature (33 °C) (Matsumoto et al., 1980).

In order to know how long incubation at 39 °C was necessary for the rate of phosphorylation of H1 histone to be reduced, we synchronized the ts85 cells at the permissive temperature at the G1/S boundary and permitted them to enter the S phase at the permissive or the nonpermissive temperature, as described under Experimental Procedures. Then the rate of phosphorylation of H1 histone was measured. Since the rate of phosphorylation of H2A histone was constant throughout a cell cycle of this type (data not shown), the ratio of the rate of phosphorylation of H1 histone to that of H2A histone at 33 or 39 °C was plotted against incubation period (Figure 1). The rate of the phosphorylation of H1 histone of ts85 cells was decreased gradually at 39 °C, but that at 33 °C was not decreased at all. The rate of phosphorylation of H1 histone was ~50% at the S/G2 phase (at 6 h after G1/S). The highly phosphorylated form of H1 histone of ts85 cells, which migrated more slowly than non- or a little phosphorylated one, was detected at 33 °C, whereas it was not detected at 39 °C (Figure 2). The number of phosphate residues of H1 histone of the ts85 cells at the arrested state at 39 °C was less than that of the cells logarithmically growing at 33 °C (Figure 2). In this experiment, 0.05 μg/mL Colcemid was added to the culture when the cells were released from the

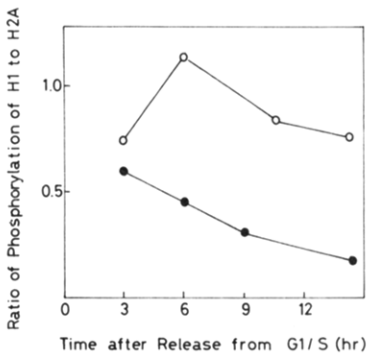

FIGURE 1: Decrease in H1 histone phosphorylation of ts85 cells at the nonpermissive temperature. The ts85 cells were synchronized at the G1/S boundary by the treatment with excess thymidine, and at 0 h, they were permitted to enter the S phase at the permissive or the nonpermissive temperature with Colcemid. Every 3 h after release from the blockade, the rate of phosphorylation of histones was measured as described under Experimental Procedures. In this figure, the S phase was ~0–6 h and the G2/M phase was 6–15 h, because Colcemid was added to the culture. At the nonpermissive temperature, mitotic cells were not detected at all. Values were indicated by the ratio of phosphorylation of H1 histone to H2A histone. (○) At the permissive temperature; (●) at the nonpermissive temperature.

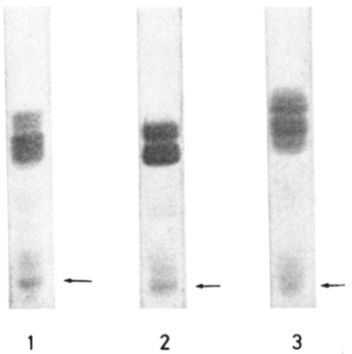

FIGURE 2: Highly phosphorylated form of H1 histone was not detected in the ts85 cells at the nonpermissive temperature. H1 histones of G2-arrested ts85 cells at 39 °C, logarithmically growing ts85 cells at 33 °C, and the ts85 cells at the M phase at 33 °C were analyzed by acid–urea–polyacrylamide gel electrophoresis (25 cm long) as described under Experimental Procedures. The electrophoresis was run for 48 h. The arrows showed the H1 histones of calf thymus as markers which had been run 4 h before the samples were done. Samples (H1 histones) were extracted from nuclei by 0.75 N perchloric acid. (1) Logarithmically growing ts85 cells at 33 °C; (2) G2-arrested ts85 cells at 39 °C; (3) ts85 cells at the M phase at 33 °C.

blockade for inhibiting the cells to enter next the G1 phase, but this dose of Colcemid showed no effects on H1 histone phosphorylation.

The H1 histone phosphorylation of growth-revertant ts85 cells (ts85R-MN3), which were obtained by MNNG treatment of ts85 cells, was investigated. The ts85R-MN3 cells grew as rapidly as wild-type cells at either temperature. The rate of phosphorylation of H1 histone was not decreased at 39 °C where that of ts85 cells was (Table I). The highly phosphorylated form of H1 histone at the G2/M phase was also detected at either temperature of the ts85R-MN3 cells. The hybrid cells between ts85 cells and wild-type cells (8AG-resistant cell) could also grow at either temperature, and the highly phosphorylated form of H1 histone was also detected at either temperature, indicating that the phenotype of the ts85 cells was recessive. The results described above indicated that the reduction in the rate of the phosphorylation of H1 histone of ts85 cells at 39 °C was really due to the temperature sensitivity of the cells and not the consequence of the cessation of the cell cycle at the G2 phase. The other

Table I: H1 Histone Phosphorylation of Various Cell Types at 33 or 39 °C[a]

cell type	^{32}P in H1/^{32}P in H2A		
	33 °C	39 °C	39 °C/33 °C
ts85	0.964	0.119	0.123
ts85R-MN3	0.875	0.618	0.706
ts18	1.496	1.061	0.709
11C3	2.109	1.667	0.790

[a] All the cells except for the ts85R-MN3 were labeled for 2 h at the arrested point (G2 phase) at 39 °C or at the M phase at 33 °C in the presence of Colcemid. The ts85R-MN3 cells were labeled for 2 h at the M phase at either temperature.

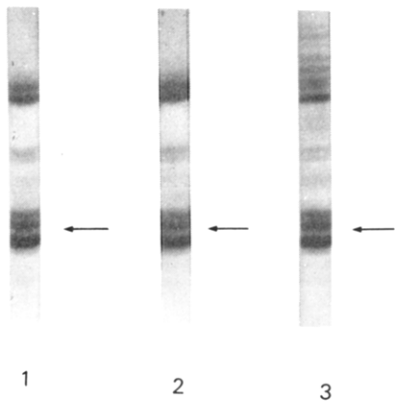

FIGURE 3: H1 histone phosphorylation of 11C3 cells. H1 histones of G2-arrested 11C3 cells at 39 °C, logarithmically growing 11C3 cells at 33 °C, and 11C3 cells at the M phase at 33 °C were analyzed as described in Figure 2. Mitotic cells were obtained as follows. After being released from the G1/S boundary, cultures were treated with 0.03 μg/mL Colcemid at the late S to G2 phase and mitotic cells were obtained by the shake-off method. The mitotic index was only 67%, for 11C3 cells were very sensitive to Colcemid (Marunouchi & Nakano, 1980). G2-arrested 11C3 cells were obtained by the incubation for 46 h at 39 °C of logarithmically growing 11C3 cells at 33 °C. (1) Logarithmically growing 11C3 cells at 33 °C; (2) G2-arrested 11C3 cells at 39 °C; (3) 11C3 cells at the M phase at 33 °C. The arrows showed the H1 histones of calf thymus as markers.

temperature-sensitive mutants, ts18 of FM3A and 11C3 of CHO-K1 (Marunouchi & Nakano, 1980), which were also arrested mainly at the G2 phase at the nonpermissive temperature, were examined in the rate of phosphorylation of H1 histone at either temperature (Table I). The rate of phosphorylation of H1 histone was not decreased at 39 °C in both temperature-sensitive mutants. Furthermore, as indicated in Figure 3, the highly phosphorylated form of H1 histone was not detected in 11C3 cells at the arrested state at 39 °C, which was detected in mitotic cells. These suggested that 11C3 cells were arrested at a point in G2 prior to the point in the cell cycle where the highly phosphorylated form of H1 histone occurred.

The rate of phosphorylation of histones was reported to be decreased by the reduction of histone synthesis (Slevely & Stocken, 1968; Ord & Stocken, 1969; Gurley et al., 1973). The possibility that the decrease in H1 histone phosphorylation of ts85 cells at 39 °C was due to the reduction of the synthesis of H1 histone was denied by the experiment as follows. The ts85 cells were synchronized at the S phase at 33 °C by the treatment with excess thymidine, and then they were released from the blockade with the medium containing [^{3}H]lysine (0.35 μCi/mL, 40 Ci/mL) and incubated for 8 h at 33 °C. By the second treatment with excess thymidine for 10 h, the cells were accumulated at the G1/S boundary and then they were divided into two groups. A group was released from the blockade and incubated at 33 °C, and the other was done at

Table II: Synthesis of Histones of ts85 Cells at 33 or 39 °C[a]

histones	[^{14}C]Lys/[^{3}H]Lys		
	33 °C	39 °C	39 °C/33 °C
H1	1.36	0.96	0.71
H2A, H2B, H3	1.32	0.99	0.75
H4	1.20	0.95	0.79

[a] The radioactivities of each histone band in polyacrylamide gel were counted as described under Experimental Procedures.

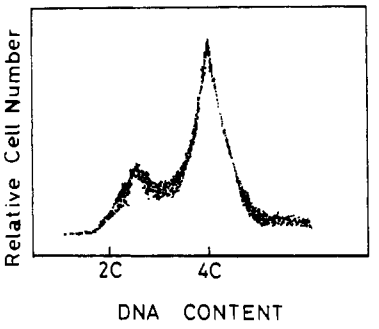

FIGURE 4: The ts85 cells were arrested mainly at the G2 phase at 39 °C after release from the blockade at the G1/S boundary. The ts85 cells synchronized at the G1/S boundary at 33 °C were permitted to proceed to the cell cycles at 39 °C and incubated for u2 h at 39 °C; then they were analyzed with the cytofluorometer. The 2C was the G1 DNA content and 4C was the G2 DNA content.

39 °C. Both cultures were labeled with [^{14}C]lysine (0.1 μCi/mL, 342 mCi/mmol) to examine the histone synthesis, when they were released from the blockade. Labeled cells were harvested at 12 h after release from the blockade in the presence of Colcemid. The ratio of the radioactivity of ^{14}C to ^{3}H was shown in Table II. The synthesis of all histones at 39 °C was ~75% at 33 °C, but the synthesis of H1 histone was not shown to be specifically inhibited at 39 °C.

DNA Synthesis and Chromosome Condensation at the Nonpermissive Temperature. When the ts85 cells synchronized at the G1/S boundary were permitted to enter the S phase at the permissive or the nonpermissive temperature, the ts85 cells cycle at the permissive temperature, passing through the mitotic phase, but at the nonpermissive temperature, they did not reach the mitotic phase at all. The study with cytofluorometry indicated that the ts85 cells at 39 °C were arrested mainly at the G2 phase and partly at the S phase (Figure 4). The inability to enter the mitotic phase at 39 °C, the defect in chromosome condensation at 39 °C, must result from the reduction in the phosphorylation of H1 histone (Matsumoto et al., 1980).

If the phosphate residues of H1 histone which may be necessary for DNA synthesis are reduced at the nonpermissive temperature, the reduction in DNA synthesis must be observed, and as a result, the ts85 cells could be arrested mainly at the S phase at the nonpermissive temperature. As shown in Figure 4, that is not the case. Figure 1 shows that the decrease in the phosphorylation of H1 histone was gradual, so that the phosphate residues available for DNA synthesis were barley decreased in the S phase, and the cells were arrested mainly at the G2 phase at 39 °C. If so, the number of phosphate residues of H1 histone that would be required for the transition from the G2 to M phase is more than that for the progression in the S phase.

In order to ascertain whether the phosphorylation of H1 histone is necessary for the progression of the S phase or not, we carried out further experiments.

DNA Synthesis after the Reduction in H1 Histone Phosphorylation at the Nonpermissive Temperature. The ts85 cells

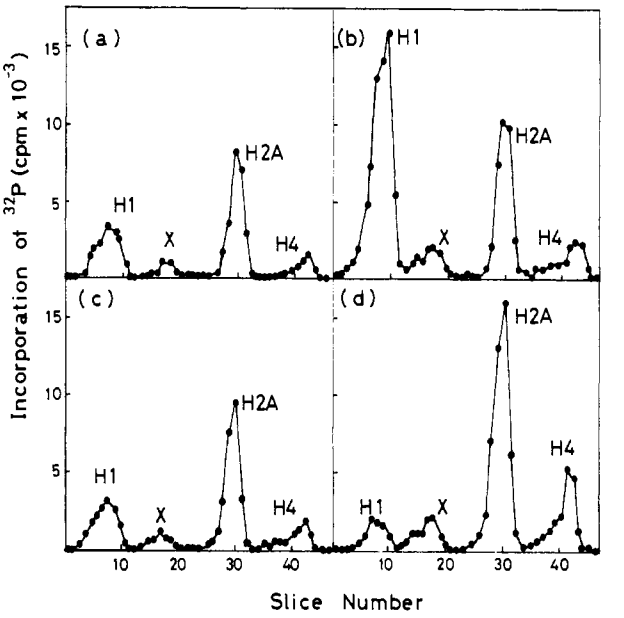

FIGURE 5: Effect of temperature shift up at the G1/S boundary on the phosphorylation of histones. The ts85 cells and wild-type cells were synchronized at the G1/S boundary at 33 °C, and then half of them of each cell type was shifted up the temperature to 39 °C. The incubation at each temperature was carried out for 8 h, and the cells were labeled with [^{32}P]orthophosphate for the last 2 h (6–8 h). The incorporation of ^{32}P in histones was analyzed as described under Experimental Procedures. H1, H2A, and H4 histones were phosphorylated. X in the figure was an unknown protein which was phosphorylated at either temperature in both cell types. (a) Wild-type cells at 33 °C; (b) wild-type cells at 39 °C; (c) ts85 cells at 33 °C; (d) ts85 cells at 39 °C.

or wild-type cells synchronized at the G1/S boundary by the treatment with excess thymidine were incubated at the permissive or the nonpermissive temperature for another 8 h in the presence of excess thymidine, and the rate of the phosphorylation of H1 histone was examined (Figure 5). In wild-type cells, by the 8-h incubation at the permissive or the nonpermissive temperature at the G1/S boundary, H1 histone was not decreased at all. The rate of phosphorylation was rather higher at the nonpermissive temperature (39 °C) than that at the permissive temperature (33 °C). On the contrary, in ts85 cells, by the 8-h incubation at the permissive temperature at the G1/S boundary, the rate of H1 histone phosphorylation was not decreased at all as seen in wild-type cells, but at the nonpermissive temperature, it was decreased.

For investigation of the relationship of H1 histone phosphorylation to DNA synthesis, the ability of DNA synthesis was examined with the cells having a low level in H1 histone phosphorylation at the G1/S boundary as described above. After the ts85 cells or wild-type cells synchronized at the G1/S boundary were incubated further at the permissive or the nonpermissive temperature for 8 h, they were allowed to be released from the blockade at the respective temperature, and the amount of DNA synthesized was measured by the flow cytofluorometer as described under Experimental Procedures.

If DNA replication was performed completely, the DNA content becomes 4C in the prescence of Colcemid. Figure 6a–d shows that in wild-type cells, DNA replication was completed, even if the cells were preincubated for 8 h at 33 or 39 °C at the G1/S boundary. DNA replication was also completed in ts85 cells which were preincubated for 8 h at 33 °C at the G1/S boundary. On the contrary, when the ts85 cells were preincubated for 8 h at 39 °C at the G1/S boundary and were permitted to proceed in their cell cycles at 39 °C, DNA replication was performed incompletely. Almost all the cells were

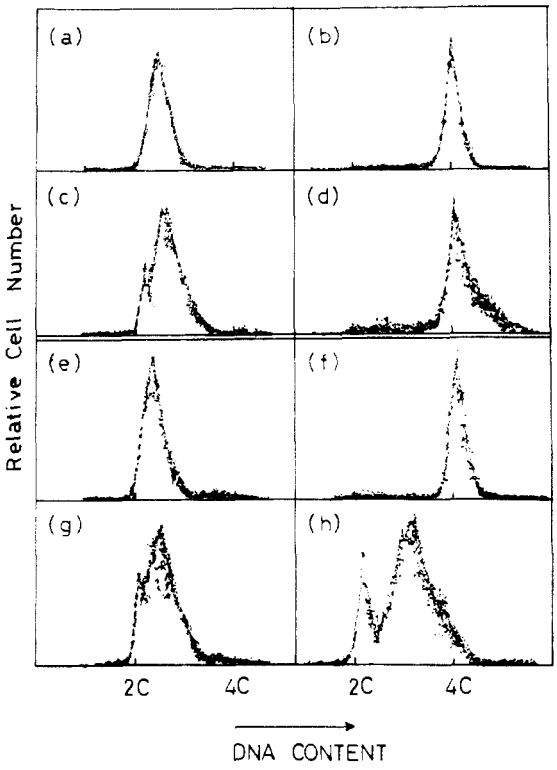

FIGURE 6: Effect of the temperature shift up at the G1/S boundary on progression in the S phase of wild-type cells or ts85 cells. The ts85 cells and wild-type cells were synchronized at the G1/S boundary at 33 °C, and half of them of each cell type was carried out for 8 h, and then the cells were released from the blockade and incubated at either temperature. At 0 and 14.5 h after release from the blockade, the cytofluorometric analyses were carried out. (a) Wild-type cells at 33 °C, 0 H; (b) wild-type cells at 33 °C, 14.5 h; (c) wild-type cells at 39 °C, 0 h; (d) wild-type cells at 39 °C, 14.5 h; (e) ts85 cells at 33 °C, 0 h; (f) ts85 cells at 33 °C, 14.5 h; (g) ts85 cells at 39 °C, 0 h; (h) ts85 cells at 39 °C, 14.5 h. When the complete DNA replication was performed, the cells had 4C contents of DNA, because the cultures were incubated with Colcemid.

arrested in the S phase and a part of the cells remained at the G1/S boundary (Figure 6e–h). This cytofluorometric pattern is quite different from that in Figure 4.

With the above experiments, we have shown that the defect in DNA replication at the nonpermissive is caused by the decrease in H1 histone phosphorylation.

Discussion

When ts85 cells were synchronized at the G1/S boundary by the double treatment with excess thymidine, were released from the blockade, and entered in the S phase, the rate of phosphorylation of H1 histone was decreased rapidly with a half-life of 6 h. On the contrary, in wild-type cells, the rate of phosphorylation of H1 histone was almost constant throughout the S to M phase. Therefore, when a mutant cell strain was arrested at the nonpermissive temperature from the S to M phase, the rate of phosphorylation of H1 histone was thought not to be decreased at the arrested point but to be constant at that point, if the phosphorylation of H1 histone was not temperature sensitive in this mutant strain. Indeed, in two G2 mutants (ts18 and 11C3 cells), the rates of the phosphorylation of H1 histone were constant at the arrested point at the nonpermissive temperature (Table I).

Furthermore, as indicated in Figure 5, when ts85 cells were incubated at the nonpermissive temperature at the G1/S boundary in the presence of excess thymidine, the rate of phosphorylation of H1 histone was decreased apparently.

The results described above agreed that the decrease in H1 histone phosphorylation of ts85 cells at the nonpermissive temperature was not the consequence of the arrest of the cell cycle but was due to the temperature sensitivity of ts85 cells.

By using this mouse temperature-sensitive mutant defective in H1 histone phosphorylation, we have reported here that the decrease in H1 histone phosphorylation causes the imcomplete DNA synthesis as well as a defect in chromosome condensation.

It was reported in CHO cells (Gurley et al., 1975; Hohmann et al., 1976; Gurley et al., 1978) that the phosphorylation of H1 histone occurs at G1/S and G2/M phases. It is now clear that the phosphorylation of H1 histone of the M phase is different from that during interphase, because at the M phase, the N-terminal region of histone which was not phosphorylated at the interphase was also phosphorylated (Hohmann et al., 1976). The phosphorylated sites at the interphase was contained among those at the M phase. Since the highly phosphorylated form of H1 histone was observed parallel to chromosome condensation in synchronous culture, it was proposed that H1 histone phosphorylation at the M phase may be related to chromosome condensation (Bradbury et al., 1974a,b; Gurley et al., 1978). Our data presented here and reported elsewhere (Matsumoto et al., 1980) supported the hypothesis that H1 histone phosphorylation at the G2 phase is one of the factors necessary for the chromosome condensation at the M phase.

As for the phosphorylation of H1 histone during interphase, there was no evidence for its role, but a possibility was formerly proposed as follows: the phosphorylation of H1 histone would be related to DNA synthesis or chromatin organization, because the phosphorylation preceded DNA synthesis (Shepherd et al., 1971; Gurley et al., 1975, 1978; Hohmann et al., 1976) and because the inaccessibility of histones to heparin occurred as cells traversed the early part of the interphase (Hildebrand & Tobey, 1975). Our results presented here suggest that H1 histone phosphorylation during the interphase may be necessary for DNA replication, because the cells which were decreased in H1 histone phosphorylation did not perform DNA replication completely. The mechanism of regulation in DNA synthesis by H1 histone phosphorylation is unclear, but the phosphorylation at the G1 phase may induce the structural changes in chromatin which may be necessary for the initiation of chromatin replication and in the S phase may play an important role for DNA synthesis at the elongation step.

The activity of histone kinase was assayed in the extract of ts85 cells by the use of H1 histone as the substrate. Cyclic AMP dependent and cAMP independent protein kinase in a cytoplasm and cAMP independent kinase in nuclei of ts85 cells were not temperature sensitive, though the purified enzyme was not tested for temperature sensitivity.

Therefore, the cause of the decrease in H1 histone phosphorylation of ts85 cells was unclear, but the decrease in H1 histone phosphorylation was not due to the decrease in histone synthesis (Table II).

As for the temperature sensitivity of the phosphorylation of H1 histone, we already reported that chromatin basic protein disappeared in ts85 cells at the nonpermissive temperature (Marunouchi et al., 1980). Recently we proved that A protein was identical with chromatin A24 protein (H. Yasuda et al., unpublished experiments).

The relationship between the phosphorylation of H1 histone and this protein was under investigation.

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Double-Resonance Experiments at 500 MHz on Gene-5 Protein and Its Complex with Octadeoxyriboadenylic Acid†

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ABSTRACT: In this paper, a detailed description is presented of the aromatic part of the 500-MHz ^1H nuclear magnetic resonance (NMR) spectrum of the helix-destabilizing gene-5 protein (GVP) encoded by the coliphage M13. As a result of the resolution obtained at 500 MHz, it was possible to perform selective decoupling and time-resolved selective Overhauser experiments. The magnitudes of the observed Overhauser effects compare favorably with magnitudes expected on the basis of theoretical calculations. These experiments in conjunction with selective decoupling experiments allowed a detailed interpretation of the aromatic part of the protein spectrum. The spectrum of the aromatic part of the GVP–$d(A)_8$ complex could be interpreted in a similar fashion. The ring protons of one phenylalanyl residue and of two tyrosyl residues show rather large shifts upon complex formation. This indicates that these residues are involved in the interaction with the DNA molecule in accordance with earlier observations. Direct evidence for the proximity of these aromatic rings and the DNA fragment in the complex was obtained by additional Overhauser experiments. It turns out that the H_3', H_4', and/or the H_5' sugar protons of the oligonucleotide are situated near the ring protons of (most likely) two or all three of the aromatic residues of which the resonances undergo large shifts upon complex formation.

Gene-5 protein (GVP)[1] is a DNA helix-destabilizing protein, encoded by the filamentous *Escherichia coli* phages M13 fl and fd. It is synthesized in great amounts in the infected *E. coli* cell and plays an essential role in the replication process of the phage, in which it induces a changeover from replicative form (RF) replication to single-stranded viral DNA synthesis (Alberts et al., 1972; Oey & Knippers, 1972; Ray, 1977).

Information about the structure of the protein and the protein–DNA complex has been obtained from various physicochemical experiments. The protein has a molecular weight of 9690 and exists in solution predominantly as a dimer (Pretorius et al., 1975; Cavalieri et al., 1976). The amino acid sequence has been determined (Cuyper et al., 1974; Nakashima et al., 1974a,b). Recently, the X-ray structure of the protein has been determined to 2.3-Å resolution (McPherson et al., 1979, 1980b). A 30-Å long groove in the protein structure is presumed to be the DNA binding region. The elucidation of the X-ray structure of a protein–oligonucleotide complex is in progress (McPherson et al., 1980a). Both the in vivo and the in vitro complexes of GVP with the viral DNA

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[1] Abbreviations used: DSS, 4,4-dimethyl-4-silapentane-1-sulfonate; FID, free induction decay; GVP, gene-5 protein; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; RF, replicative form.

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