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DNA and Histone Synthesis in Mouse Cells which Exhibit Temperature-Sensitive DNA Synthesis

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Abstract—*It is demonstrated that temperature inactivation of histone synthesis is coupled to inhibition of DNA replication in ts AlS9 and ts Cl mouse L-cells, which are temperature-sensitive (ts) in an S-phase function. In contrast, uncoupling of histone and DNA synthesis occurs in BalB/C-3T3 ts 2 cells which are ts in a function of the pre-DNA-synthetic phase. Termination of histone synthesis in ts AlS9 and ts Cl cells is 16–18 h after onset of temperature inactivation of DNA replication and appears to be associated with general cessation of chromatin replication triggered by the earlier event. Synthesis of histone and other chromosomal proteins proceeds in ts 2 cells under conditions in which DNA synthesis undergoes temperature inactivation. It is suggested that the terminal phenotype of coupled temperature inactivation of DNA and histone synthesis may be diagnostic of cells ts in an S-phase function and may therefore be a useful secondary screen in designation of cell cycle mutants.*

INTRODUCTION

Mammalian cells which are temperature-sensitive (*ts*) in a protein of DNA replication (i.e., *dna*^{ts}) have proved difficult to isolate and identify (1,2). A possible biochemical marker for such S-phase mutants is suggested by an earlier study which showed that DNA and histone synthesis are coupled in *ts* AlS9 cells at the permissive and nonpermissive temperatures (3). This was not a surprising observation in view of the wealth of evidence linking histone synthesis to DNA replication in the duplication cycle of eukaryotic cells (4,5). Such linkage reflects highly regulated, coordinate replication of the nucleosomal subunit constituents and other chromatin components which proceeds normally only during S phase (cf. 6, 7).

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It was therefore of interest to discover that temperature-inactivated CH-K12 hamster cells, which are *ts* in a G_1 function (8), continue to make large amounts of histone long after inhibition of DNA synthesis has occurred (9). In light of the evidence with embryos of amphibians (10) and sea urchins (11), which indicate that an extended G_1 period is associated with histone synthesis in the absence of DNA synthesis, we postulated that true *dna*^{ts} mutants should exhibit coupled and coordinated inactivation of DNA and histone synthesis at the nonpermissive temperature (npt); whereas mutants *ts* in a G_1 function should not.

To test this hypothesis we have studied chromatin DNA and protein synthesis in three mouse cell mutants which exhibit *ts* DNA synthesis. The *ts* AlS9 mouse L cells are known to be *ts* in the conversion of newly replicated, single-stranded DNA of $\not> 5 \times 10^6$ molecular weight to mature chromosomal DNA (12). By definition this cell is *dna*^{ts} and therefore an S-phase mutant. The *ts* Cl mouse L-cell derivative has also been shown to be *dna*^{ts} (13), although the *ts* function remains unidentified. The BalB/C-3T3 *ts* 2 mouse fibroblast (14) is now thought to be *ts* in a protein which functions in a pre-DNA-synthetic phase (Sheinin et al., manuscript in preparation). The results obtained indicate that cells which are *dna*^{ts} display coordinate and coupled inhibition of DNA replication, histone synthesis, and synthesis of other chromosomal proteins. Such is not necessarily the case with non-S-phase mutants.

MATERIALS AND METHODS

Cells and Their Cultivation. Wild-type (WT-4), *ts* AlS9, and *ts* Cl mouse L cells were grown in suspension culture as described elsewhere (3, 12). Wild-type BalB/C-3T3 mouse fibroblasts (15) and the *ts* 2 derivative were cultivated on a glass surface using routine methods, in which cells were grown to confluence and then subcultured after brief trypsin treatment for release (16). Unless otherwise noted the medium was α-MEM (17) supplemented with 7.5% (v/v) fetal calf serum. The permissive temperature (pt) was 34°C, the nonpermissive (npt), 38.5°C.

General Experimental Regimen with L Cells. WT-4, *ts* AlS9, and *ts* Cl cells were grown at 34°C from 3–5 $\times 10^4$/ml to midlogarithmic phase ($\sim 3 \times 10^5$ cells/ml), and then either maintained at this pt or upshifted to 38.5°C, in a test situation. In experiments examining DNA replication, cells were grown at 34°C in the presence of added [1-^{14}C]dThd [0.01 μCi/ml, approximate specific activity 55 mCi/mmol, approximate concentration (0.2 μM)]. Such prelabeled cells were collected by centrifugation, resuspended in fresh, prewarmed medium, and incubated for the interval designated in each experiment. To label newly made DNA [*methyl*-^{3}H]dThd was added to

10–100 μCi/ml (approximate specific activity 20 Ci/mmol; approximate concentration 40–400 μM). For analysis the radiolabeled cells were harvested by centrifugation (2°C, 800*g*, 10 min), washed three times with cold α-MEM containing nonradioactive dThd (41 μM), and processed further.

In experiments studying protein synthesis, cells were grown as above but they were prelabeled in medium supplemented with [U−^{14}C]L-arginine plus [U−^{14}C]L-lysine or [U−^{14}C]L-leucine plus [U−^{14}C]L-valine, all at 0.01 μCi/ml (approximate specific activity 280–350 mCi/mmol). To label protein newly made under test conditions [3(N)−^{3}H]L-arginine plus [G−^{3}H]L-lysine or [4,5−^{3}H]L-leucine [G−^{3}H]L-valine were added to the medium (approximate specific activity of 20, 2, 60, 1 Ci/mmol, respectively). These amino acids are designated throughout as arg, lys, leu, and val.

General Experimental Regimen with 3T3 and ts *2 Cells.* 3T3 and *ts* 2 cells were cultivated at 34°C from an inoculum of 1–3 × 10^4 cells/cm^2 of glass surface to allow for 4–5 doublings before confluence was attained. In experiments which required that cells be prelabeled in their DNA or protein, cells were plated in 32-oz Brockway bottles in medium containing [^{14}C]dThd or [^{14}C]amino acids as described above for L cells. They were grown just to confluence and then subcultured in the usual way in 32-oz Brockway bottles in nonradioactive medium. The cells were incubated at 34°C for at least 28 h to permit full recovery from the trypsin treatment (18) prior to further experimental manipulation. Recovery of cells was estimated on the basis of the preformed DNA and/or protein. For postlabeling, the growth medium (usually 40 ml/culture) was reduced to 20 ml and supplemented with [^{3}H] dThd or [^{3}H]amino acids as noted above.

To analyze cellular components, the medium was decanted; the cells were then washed three times and released from the growing surface by brief trypsinization, harvested by centrifugation, and processed further. Cell numbers were measured in an electronic cell counter.

Incorporation of dThd or Amino Acids. The rate of DNA and protein synthesis was measured in terms of the incorporation of radioactive dThd or amino acids during a 1-hr interval into macromolecules precipitated by 5% (w/v) of trichloroacetic acid (TCA)(3). In experiments with mouse L cells ~4 × 10^6 cells were treated with [^{3}H]dThd or [^{3}H]amino acid (10 μCi/ml) for 1 hr. Triplicate samples of $\triangleq 10^6$ cells were then collected by centrifugation, washed, and processed. Replicate cultures of 3T3 and *ts* 2 cells grown in 2-oz Brockway bottles were incubated with 5 ml medium containing [^{3}H]dThd or [^{3}H]amino acid. Triplicate cultures were frozen and thawed three times to release cells from the glass, for measurement of radioactively labeled macromolecules. Additional cultures were used to measure cell number. Rates of macromolecule synthesis were calculated for each time point as dpm/10^6 cells. They were normalized to the activity of the zero time sample for each temperature of incubation.

Chromatin Isolation. Chromatin from 1–5 × 10^8 cells was released from partially purified nuclei and isolated by isopycnic centrifugation in sucrose (glucose) density gradients by the procedure described and validated elsewhere (3, 19). In experiments in which the chromatin was processed further, 1/10 volume of each 1-ml gradient fraction collected was precipitated for analysis of radioactively labeled material. Fractions of buoyant density >1.32 g/cm^3 (e.g., fractions 26–36 of Fig. 3a) were combined and centrifuged at 15,000 *g* at 2°C for 15 min to obtain the chromatin pellet.

Histone Analysis. Chromatin pellets were diluted with water containing 1 mM phenylmethane sulfonyl fluoride and then precipitated by the addition of $CaCl_2$ to 1 mM. The collected precipitate (10,000*g*, 10 min) was used for extraction of histones with 0.2 N H_2SO_4 as described elsewhere (20). The final histone precipitate was vacuum dried, and dissolved in 10 mM HCl for measurement of protein content (21). The resulting histone solutions were made 20% (w/v) in glycerol and samples containing 50 and 100 μg of protein were analyzed by electrophoresis on 15% polyacrylamide gels, using the method of Paynim and Chalkley (22). The more heavily loaded gels were stained for about 1 min in Coomassie blue G-PCA (23) to identify the histone bands which were excised with a razor blade for measurement of radiolabeled histones (3). The other gels were stained with Amido black and, after destaining, were scanned at 540–600 nm.

RESULTS

DNA Synthesis in Mouse Cells Incubated at 34° C and 38.5° C. Figure 1 presents a kinetic study of DNA synthesis in wild-type BalB/C-3T3 mouse fibroblasts and in mutant *ts* 2, *ts* AlS9, and *ts* Cl cells incubated at 34°C and 38.5°C. As shown in Fig. 1a, incorporation of [^{3}H]dThd into acid-insoluble material proceeds at a high rate in BalB/C-3T3 cells incubated at 34°C and 38.5°C, an expected pattern already established for WT-4 mouse L cells (3, 24). The same is true for the *ts* cells grown at 34°C, as illustrated in Fig. 1b) with *ts* 2 cells. Figure 1b also shows the somewhat different kinetics of temperature inactivation of [^{3}H]dThd incorporation in *ts* AlS9, *ts* Cl, and *ts* 2 cells. In the case of the *ts* mouse L cells, it has been established that such incorporation accurately reflects DNA synthesis and not precursor transport to sites of utilization (13, 25). This also appears to be the case for *ts* 2 cells.

Protein Synthesis in Wild-Type Mouse Cells and Those which Exhibit ts DNA Replication. As an initial step in the study of coupled DNA and histone synthesis in *ts* mouse cells, we examined the kinetics of protein synthesis, using [^{3}H]leu (or val or isoleucine) as general marker for protein synthesis, and [^{3}H]lys and/or [^{3}H]arg to focus more on the formation of the basic proteins, of which the histones form a major fraction (26).

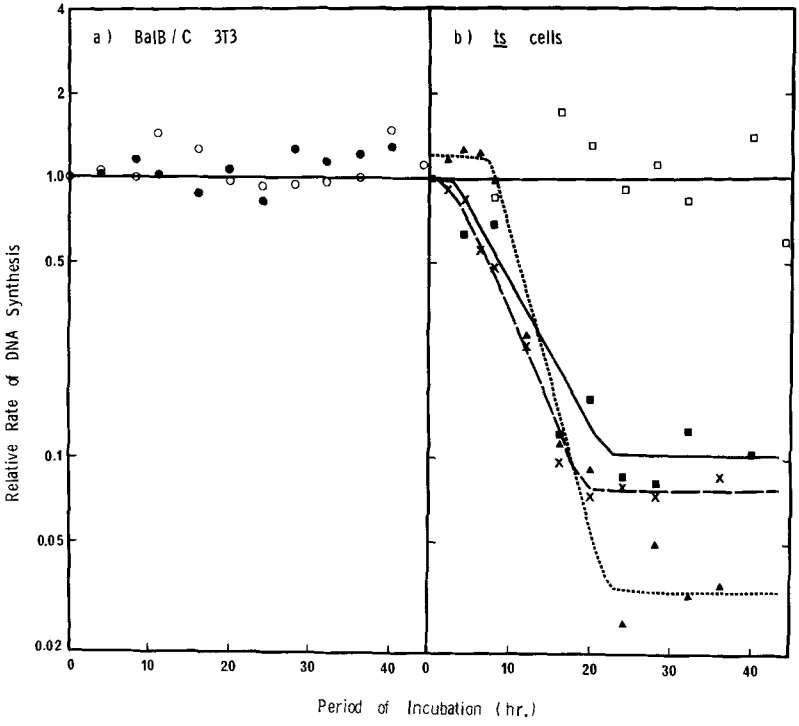

Fig. 1. Kinetics of [^{3}H]dThd incorporation in wild-type mouse cells and those which exhibit temperature-sensitive DNA synthesis. Wild-type BalB/C-3T3 mouse fibroblasts, *ts* A1S9, *ts* Cl, and *ts* 2 cells, incubated at 34°C or 38.5°C from early logarithmic phase, were treated with [^{3}H]dThd (10 μCi/ml) for 1 hr and processed for measurement of incorporation into TCA-insoluble material. O——O , ●——●, Balb/C-3T3 incubated at 34°C and 38.5°C; □——□, ■——■, 3T3-*ts* 2 incubated at 34°C and 38.5°C; ▲---▲, X—X, *ts* A1S9 and *ts* Cl incubated at 38.5°C.

As shown in Fig. 2a and b, incorporation of leu and lys into macromolecular form proceeds at a constant rate in wild-type BalB/C-3T3 cells growing logarithmically at 34°C or at 38.5°C for at least 72 hr. Similar observations were made for leu incorporation by *ts* 2 cells (Fig. 2c), by *ts* Cl cells (13), and by *ts* AlS9 cells (3).

Comparative studies of protein synthesis from [^{3}H]lys for the *ts* cells incubated at low and high temperature are shown in Fig. 2d–f. (Similar results, not shown here, were obtained with arg as precursor.) The data show that the rate of incorporation of basic amino acid remained essentially constant in all *ts* cells growing logarithmically at 34°C. When *ts* AlS9 cells were upshifted to 38.5°C such incorporation proceeded at control rate for ~16 h, after which it declined, reaching a plateau of ~22% of this rate ~20–24 h posttemperature shift (pts). In the case of *ts* Cl cells incubated at the npt, lys

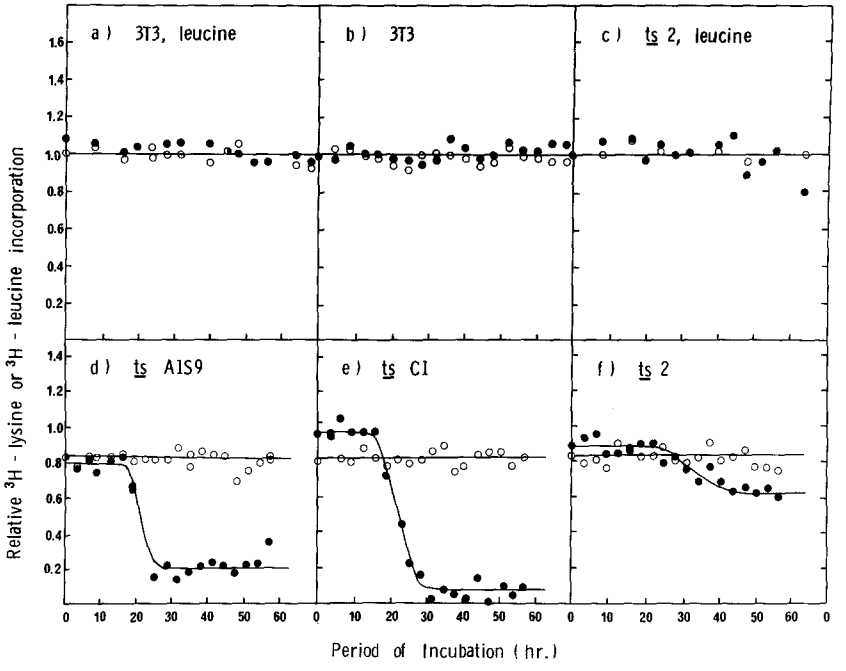

Fig. 2. Incorporation of amino acids by mouse cells incubated at 34°C and 38.5°C; 3T3, *ts* 2, *ts* A1S9 and *ts* Cl cells were grown to midlogarithmic phase at 34°C. Replicate sets of cultures were incubated further at 34°C (O——O) or at 38.5°C (●——●). At the intervals noted they were labeled with [^{3}H]leucine (panels a and c) or [^{3}H]lysine (all other panels) to measure incorporation into protein.

(arg) incorporation was maintained for ~16 h; it then fell to ~9% of the control rate ~24–28 h pts. In contrast, in *ts* 2 cells at 38.5°C, protein synthesis from basic amino acid continued actively for ~24–28 h, after which it decreased to ~80% of the control rate ~40 h pts.

Chromatin Formation in Normal and ts Mouse Cells. The central problem was pursued by examining the formation of chromatin-bound DNA and histone. As a basis for these studies we first determined that incubation of the cells of interest for up to 40 h at 38.5°C had little or no effect on the chromatin. This is exemplified by data like that shown in Fig. 3. They demonstrated that in all cases the chromatin is recovered in fractions (e.g., 28–37 in Fig. 3a) with a peak buoyant density of ≙1.384 g/cm^3, in accord with earlier work with L-cells (3) and mouse cells generally (19, 27).

We went on to examine the synthesis of chromatin-bound DNA in wild-type and *ts* cells incubated at 34°C and 38.5°C for 16–24 h to permit full expression of the *ts* defect. The details of the experiment with WT-4 and *ts* A1S9 cells are set out in Fig. 3. It is clear from a comparison of the data shown in Fig. 3a and b and in Fig. 3c and d, that essentially full recovery of the

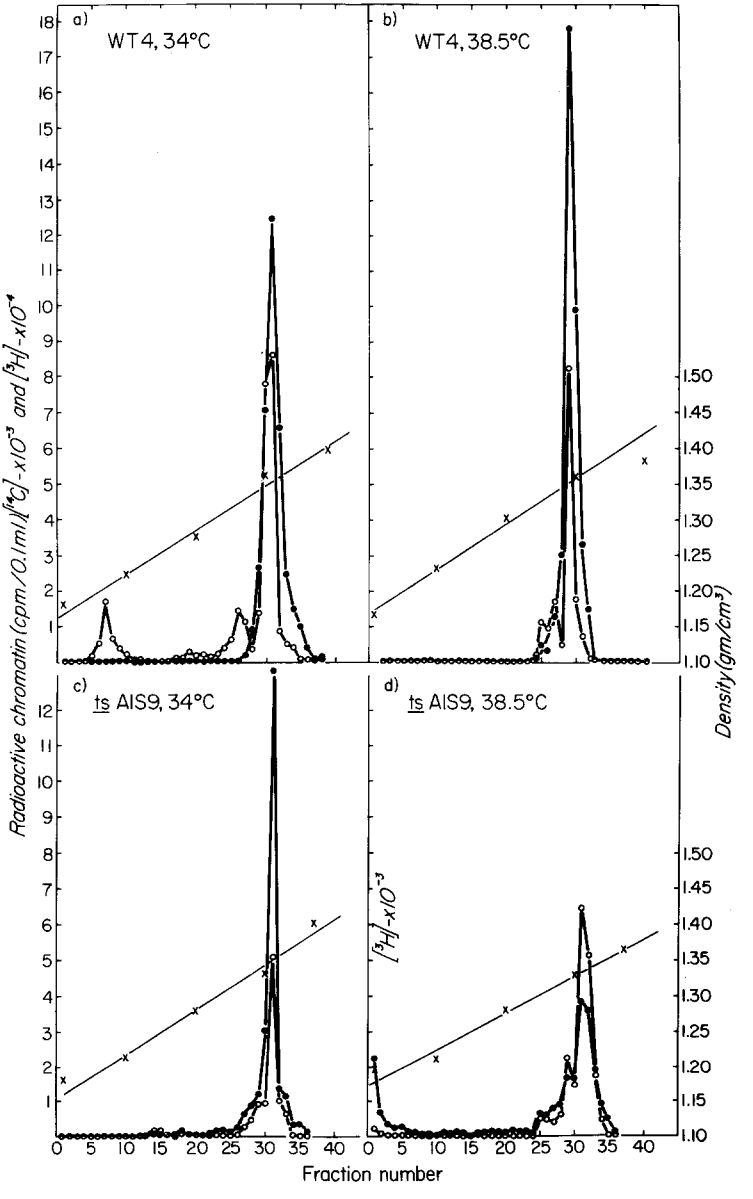

Fig. 3. Isopycnic centrifugation of chromatin from mouse L cells radiolabeled with dThd. WT-4 and *ts* A1S9 cells were grown at 34°C from 2–4 × 10^4/ml to 3 × 10^5/ml in medium supplemented with [^{14}C]dThd (0.01 μCi/ml). The cells were harvested and resuspended in prewarmed, nonradioactive medium and incubated either at 34°C or at 38.5°C. Twenty-four hours later they received [^{3}H]dThd (100 μCi/ml) for 10 min. Approximately 5 × 10^8 cells were processed for isolation of chromatin by isopycnic centrifugation. The resulting gradients were collected manually in ~1-ml fractions, which were analyzed for [^{14}C]dThd incorporated during the prelabeling interval at 34°C (O——O); [^{3}H]dThd incorporated during subsequent labeling at 34°C or 38.5°C (●—●); and density (X——X).

Table 1. Recovery of Prelabeled Nuclear DNA and Protein in Mouse Cells Incubated at 38.5°C

Cells	Prelabel at 34°C	% prelabel dpm recovered[a] at 38.5°C. in		
		Nuclei	Chromatin	Histones
WT-4	dThd	104.6 ± 15.2 (4)[c]	86.3 (1)	—
WT-4	Amino acids[b]	101 ± 12.7 (6)	120 ± 28.8 (5)	105 ± 20.3 (3)
ts A1S9	dThd	104.8 (4)	89.0 (1)	—
ts A1S9	Amino acids	107.7 ± 15.9 (6)	116 ± 11.5 (7)	97.1 ± 17.9 (3)
ts C1	dThd	97.3 (2)	101 (2)	—
ts C1	Amino acids	103.8 ± 19.8 (6)	91.6 ± 17.4 (6)	105 ± 31.4 (3)
3T3	dThd	95.4 (1)	90.0 (1)	—
3T3	Amino acids	107 (2)	104 (2)	108 (1)
ts 2	dThd	87.4 (1)	82.1 (1)	—
ts 2	Amino acids	112 ± 23.2 (6)	90.4 ± 13.7 (6)	91.1 ± 14.6 (3)

[a]Recovery was calculated in terms of the TCA-precipitable material in the designated fraction, taking as 100% that observed with cells incubated throughout at 34°C.
[b]Amino acid pairs used were *lys-arg* or *leu-val*.
[c]( ) = Number of expeirments used to calculate average and standard error where indicated by ± value.

[^{14}C]DNA preformed at 34°C is obtained in WT-4 and in *ts* AlS9 cells subsequently incubated at pt or at npt. The quantitative aspects of such studies are presented in Table 1. The results also indicate high and comparable rates of formation of chromatin-associated [^{3}H]DNA in WT-4 cells cultivated at both temperatures, and in *ts* AlS9 cells grown at 34°C. Such DNA synthesis is severely depressed in *ts* AlS9 cells incubated at 38.5°C. Calculations from such banding profiles show quantitative agreement with in vivo incorporation data like those shown in Fig. 1. Entirely analogous data (not shown) were obtained with wild-type BalB/C-3T3 cells on the one hand, and with *ts* Cl and *ts* 2 cells on the other.

The legend of Fig. 4 describes experiments designed to examine synthesis of lys- and arg-labeled chromatin proteins in wild-type and *ts* cells incubated at 34°C or at 38.5°C during the 16–40 h interval, i.e., after full temperature inactivation of DNA synthesis. The data obtained with wild-type 3T3 cells (Fig. 4a and b) compare favorably with those observed with WT-4 cells (3). The gradient profiles for the [^{14}C]proteins preformed at 34°C and the ^{3}H-labeled proteins made under the test conditions were similar. The material banding at the top of the gradient is nonchromatin nuclear protein; that banding with average peak buoyant density at = or ~ 1.34 g/cm^3 is the chromatin-bound protein (e.g., Fig. 4a and b, fractions 18–26 and 22–25, respectively).

The data shown in Fig. 4a and b and in Table 1, indicate that the ^{14}C-prelabeled chromatin-bound protein is fully recovered from cells incubated for 40 h at 38.5°C. They also reveal the comparable extent of synthesis

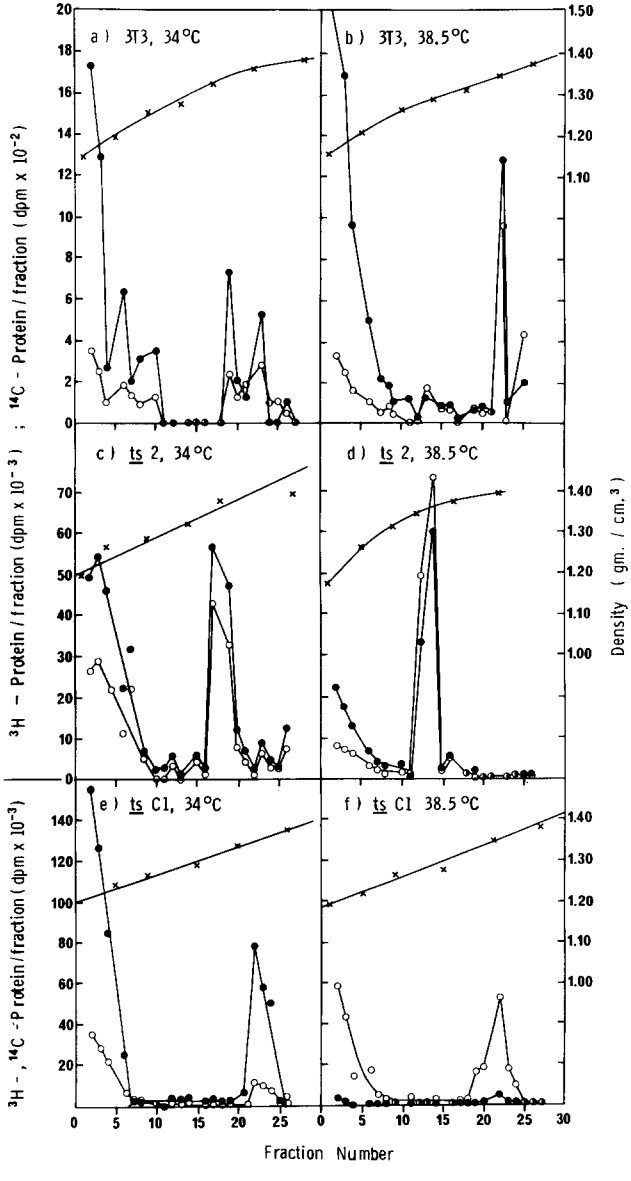

Fig. 4. Isopycnic centrifugation of chromatin from mouse cells labeled with lysine and arginine. 3T3, *ts* 2, and *ts* Cl cells were grown at 34°C to midlogarithmic phase in medium containing [^{14}C]lys and [^{14}C] arg (0.01 μCi/ml). This was replaced with fresh prewarmed medium, and half of each culture set was incubated at 34°C, the other at 38°C. After 16 h, the cells were incubated in the presence of [^{3}H]lys and [^{3}H]arg (10 μCi/ml). After 24 h at the appropriate temperature 1–5 × 10^8 cells were processed for chromatin isolation and analysis as noted in the legend of Fig. 3. O——O, ^{14}C-labeled protein; ●—●, ^{3}H-labeled protein; X——X, density.

of [^{3}H]lys–arg-labeled chromatin protein in wild-type cells grown at 34°C and at 38.5°C.

Results from analogous studies with *ts* 2 cells are presented in Fig. 4c and d. They suggest little or no perturbation in the synthesis of lys–arg-containing chromatin proteins in cells incubated at 38.5°C as compared with those grown at 34°C. This finding is in marked contrast to those made with ts Cl cells (Fig. 4e and f) and with *ts* AlS9 cells (3). Thus [^{3}H]lys–arg incorporation into chromatin proteins was minimal in such cells incubated at 38.5°C, under conditions in which essentially full recovery of ^{14}C-prelabeled chromatin proteins was observed. Entirely analogous results were obtained with cells labeled with [^{14}C]leu–val and [^{3}H]leu–val to pre- and post-label proteins, respectively.

Histone Recovery from and Synthesis in Normal and ts Mouse Cells. The foregoing studies suggested that temperature inactivation of DNA replication in *ts* AlS9 and *ts* Cl cells is associated with significant inhibition of synthesis of basic chromatin proteins (indeed of all chromatin proteins) and that such inhibition may be minimal in *ts* 2 cells. We therefore proceeded to study the recovery and synthesis of histone which is normally coupled to DNA synthesis in mammalian cells. We first asked whether preformed histones were altered in wild-type or *ts* cells incubated at 38.5°C. Cells were grown to midlogarithmic phase at 34°C, after which half the cultures were

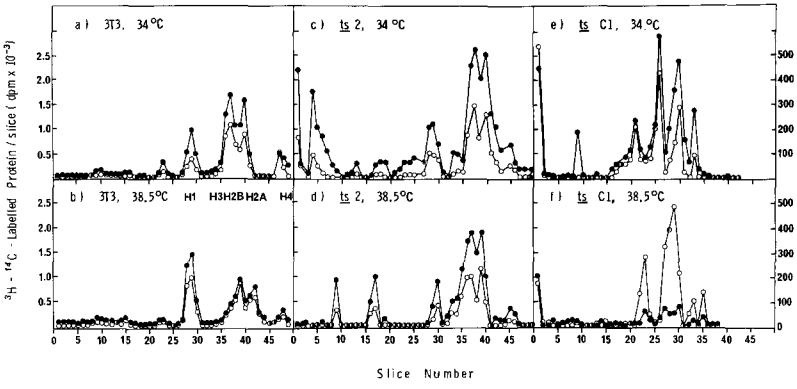

Fig. 5. Electropherograms of radiolabeled histones isolated from chromatin of mouse cells incubated at 34°C and 38.5°C. 3T3, *ts* 2, and *ts* Cl cells were prelabeled at 34°C through 4–5 generations with 0.1 μCi/ml [^{14}C]lys and [^{14}C]arg. They were then incubated in isotope-free medium at 34°C or 38.5°C for 16 h at which time the medium was replaced with prewarmed medium containing 1/4 the usual amount of lysine and arginine, and supplemented with [^{3}H]lys and [^{3}H]arg (10 μCi/ml). After 24 h, 1–5 × 10^8 cells were processed for isolation of chromatin as described in the legend of Fig. 3. The histones were extracted from the purified chromatin and separated by polyacrylamide gel electrophoresis. Gel slices were counted for ^{3}H (●——●) and ^{14}C-labeled (O——O) material. (The right *Y* axis applies to ^{14}C-labeled protein in panels e and f only.)

incubated for 40 h at 34°C, half at 38.5°C. The chromatin from each cell lot was isolated by isopycnic centrifugation in sucrose–glucose density gradients. The chromatin fractions were collected and used to extract the histones which were separated by polyacrylamide gel electrophoresis (Fig. 5).

A quantitative assessment of the histone distribution was made on material stained with Amido black. As demonstrated elsewhere (3) this indicated full recovery of preformed histones in wild-type and *ts* cells incubated for up to 40 h at 34°C or at 38.5°C. A similar conclusion derives from an analysis of the [^{14}C] histones resolved in electropherograms like those shown in Fig. 5 for 3T3 cells (Fig. 5a and b), *ts* 2 cells (Fig. 5c and d), and *ts* Cl cells (Fig. 5e and f). The quantitative aspects of such studies are seen in Table 1.

Of particular interest in the present context are the electropherograms of the ^{3}H-labeled histones shown in Fig. 5. In general they are coincident with those of the marker [^{14}C]histones. Such electropherograms indicate extensive histone synthesis in wild-type 3T3 cells (Fig. 5a and b), and in the *ts* 2 cells (Fig. 5c and d) incubated at 34°C or at 38.5°C for at least 40 h. These results are in sharp contrast with those obtained with *ts* Cl cells (Fig. 5e and f) and with *ts* AlS9 cells (3), in which little or no de novo synthesis of chromatin histones was detected at the npt.

Table 2 shows the quantitative relationship between the synthesis of DNA, basic proteins of the whole cell, and the chromatin, and of histones by cells incubated first at 34°C and then at 38.5°C for 16–40 h. These parameters are presented as the ratio of activity at 38.5°C to that at 34°C. The results show the expected high ratio of synthesis of DNA, histone, total basic protein, and chromatin-bound, basic protein in wild-type WT-4 and 3T3

Table 2. Synthesis of DNA and Lysine–Arginine-Containing Proteins by Mouse Cells

	^{3}H precursor incorporated (dpm ratio 38.5°C/34°C)			
		Basic proteins		
Cells	DNA[a]	Total[b]	Chromatin-bound[c]	Histone[d]
WT-4	1.00	1.00	1.12 ± 0.23 (5)[e]	0.98 ± 0.17 (4)
ts A1S9	0.034	0.22	0.147 ± 0.14 (5)	0.18 ± 0.04 (4)
ts Cl	0.078	0.11	0.19 ± 0.03 (3)	0.092 ± 0.14 (3)
3T3	1.00	1.00	1.36 (1)	1.08 (1)
ts 2	0.102	0.67	1.01 ± 0.20 (5)	1.04 ± 0.18 (3)

[a] Calculated from plateau values like those shown in Fig. 1b.
[b] Calculated from palteau values like those shown in Fig. 2.
[c] Calculated from chromatin gradient analyses like those of Fig. 3.
[d] Calculated from electropherograms of Amido black stained histone gels (3).
[e] ( ) = Number of experiments used to calculate average and standard error where indicated by ± value.

cells. In the case of *ts* AlS9 and *ts* Cl cells these ratios are greatly decreased and indicate coupled temperature inactivation of synthesis of DNA, histone, and basic protein. Synthesis of DNA is uncoupled from synthesis of histones in *ts* 2 cells incubated at the npt. Thus under conditions in which DNA synthesis is reduced to ~10% of control activity, the formation of histones proceeds almost at the rate observed at 34°C and formation of basic proteins generally occurs at ~70% of the control rate.

DISCUSSION

The present study demonstrates that termination of formation of chromatin-bound histones follows temperature inactivation of DNA replication in *ts* AlS9 and *ts* Cl cells, which are known to be *ts* in an S-phase function (3, 13). In marked contrast, histone synthesis was little affected under conditions which severely restricted DNA synthesis in the non-S-phase *ts* 2 mutant of BalB/C-3T3 fibroblasts (Sheinin et al., manuscript in preparation). Coupled inhibition of histone formation in *ts* AlS9 and *ts* Cl cells is reflected in reduced synthesis of total basic proteins of the cell and of the chromatin. It is associated with a general reduction in synthesis of chromatin proteins, not observed with temperature-inactivated *ts* 2 cells. An examination of total lys–arg incorporation in *ts* 2 cells revealed no effect at 38.5°C until 28–30 h pts. Thereafter there was a slow decline over the next 30 h to a plateau level of 70–80% of the control rate. This finding is reminiscent of that observed with the *ts* mutant of Chinese hamster Wg AI cells designated as CH-K12 (8). At the npt these cells arrest late in G_1 approximately 1.6 h before S-phase entry (28). Rieber and Bacalao (9) demonstrated that under conditions in which DNA synthesis was temperature-inactivated, the CH-K12 cells continued to make histone at 60–70% of the control rate for many hours. This imbalance in formation of chromatin components was accompanied by enhanced synthesis of at least three other proteins (9, 29).

One possible explanation for the absence of formation of chromatin-bound histones in temperature-inactivated *ts* AlS9 and *ts* Cl cells is that newly made protein is not deposited on the preformed chromatin. Preliminary studies suggest that this is not the case. Together these findings suggest that temperature inactivation of DNA replication in *ts* AlS9 and ts Cl cells is followed by cessation of de novo synthesis of histones.

Coordinate inhibition of DNA and histone synthesis in the dna^{ts} *ts* AlS9 and *ts* Cl cells is perhaps not an unexpected finding in view of evidence which points to obligatory coupling of synthesis of the nucleosomal constituents during replication of the genome of somatic cells (cf. 4, 7). Little is known about the mechanism of the linked stimulation of histone and DNA replication at the beginning of S phase. Although histone mRNA may be present

throughout the cell cycle (30), its synthesis is greatly enhanced and its translation is set in motion in association with initiation of DNA replication at the G_1/S traverse (4, 30, 31). The molecular trigger for these events remains unidentified (32).

Information concerning the mechanism of the conjoint termination of DNA and histone synthesis as chromatin replication nears completion is equally sparse (32). However, it is clear that cessation of DNA replication, whether it occurs normally during S phase or is induced by viral or chemical inhibitors of DNA synthesis (4,33–35), is followed by coupled inhibition of histone synthesis. It is suggested that the latter occurs as a result of dissociation of polysomes carrying histone mRNA and degradation of at least some of this mRNA (33, 36, 37). Once again, the molecular trigger for this series of events remains unrecognized. It is likely to act prior to termination of DNA replication since the evidence indicates little or no pool of free histones in or out of S phase; nor is there an accumulation of excess histone on the chromatin (4, 5).

In this context, the kinetics of incorporation of lys and arg into proteins of the *ts* cells (Fig. 2) acquires added interest, because this parameter serves as a simple, if not absolute index of the formation of basic proteins of the chromatin, including histones. In *ts* AlS9 cells inhibition of such synthesis begins ~7–9 h after onset of temperature inactivation of DNA replication is first detected; i.e., ~16 h after upshift to the npt. In *ts* Cl cells the delay is ~16–18h. Once initiated, inhibition of synthesis of histones and other basic proteins continues until a plateau is attained 20–24 h after upshift. Continued lys–arg incorporation at the npt is clearly due to synthesis of nonchromosomal proteins, since our evidence indicates coupled temperature inactivation of the replication of chromatin DNA and protein.

These studies suggest that termination of histone synthesis in *ts* AlS9 and *ts* Cl cells is not directly triggered by inhibition of DNA replication but may be mediated by another kind of signal normally expressed late in S phase (cf. 2). Thus the onset of such inhibition in both *ts* AlS9 and *ts* Cl cells is related to duration of S phase at the npt (3, 13). It may be linked to the survival of histone mRNA, once made, throughout S phase (36).

The studies with *ts* 2 cells described here, and those with CH-K12 cells (9), suggest that histone synthesis may be switched on late during G_1 by a regulatory process which can be disassociated from initiation of DNA replication (cf. 2). They are thus in accord with a growing body of evidence which indicates that significant histone synthesis can occur in the absence of DNA replication, e.g., in developing oocytes of sea urchins (10) and amphibians (11), which go through an extended G_1 period during early embryogenesis; in Friend leukemia cells forced into a prolonged G_1 state during induction of differentiation by *n*-butyrate or dimethyl sulfoxide (38); and in BHK-21 cells brought into G_1-arrest by isoleucine starvation. (39).

On the basis of the experiments discussed here, it is postulated that mutants of mammalian cells which are truly *ts* in DNA replication should exhibit coordinate termination of histone synthesis upon expression of the *ts* defect. Such may not be the case with cells which are *ts* in a late G_1 function, or in non-S-phase functions normally affecting turnover and modification of specific histones (cf. 40–42). Clearly this is a tentative hypothesis, because the number of *dna*^{ts} mammalian cells available for study has been limited.

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