Expression of Histone Genes in a G_1-Specific Temperature-Sensitive Mutant of the Cell Cycle†

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ABSTRACT: The expression of genes coding for the four core histones (H2A, H2B, H3, and H4) was studied in tsAF8 cells. These baby hamster kidney-derived cells are a temperature-sensitive (ts) mutant of the cell cycle that arrest in G_1 at the restrictive temperature. When serum-deprived tsAF8 cells are stimulated with serum, they enter the S phase at the permissive temperature of 34 °C, but are blocked in G_1 at the nonpermissive temperature of 39.6 °C. Northern blot analysis using cloned human histone DNA probes detected only very low levels of histone RNA either in quiescent tsAF8 cells or in cells serum stimulated at the nonpermissive temperature for 24 h. Cellular levels of histone RNA were markedly increased in cells serum stimulated at 34 °C for 24 h. Temperature shift-up experiments after serum stimulation of quiescent populations showed that the amount of histone RNA was related to the number of cells that entered the S phase. Those cells that synthesized histone RNA and entered the S phase were capable of dividing. This is the first demonstration in a mammalian G_1-specific ts mutant that the expression of H2A, H2B, H3, and H4 histone genes depends on the entry of cells into the S phase of the cell cycle.

Since the report by Robbins & Borun (1967) that the synthesis of histones was limited to the S phase of the cell cycle, a great number of reports, sometimes contradictory, have appeared in which histone synthesis and levels of histone RNA have been determined in the various phases of the cell cycle. The situation has been further complicated by the existence of minor histone variants, some of which are synthesized even in quiescent cells (Pehrson & Cole, 1980; Wu & Bonner, 1981; Chiu & Marzluff, 1982; Lenox & Cohen, 1983), and by the fact that, among the five major histones, H1 seems to behave somewhat differently from the other four—H2A, H2B, H3, and H4. Thus, it seems that H1 protein is synthesized to a notable extent even in quiescent cells (Tarnowka et al., 1978; Wu & Bonner, 1981). As to the other four major histones, despite some contradictory results (Groppi & Coffino, 1980; Sheinin & Lewis, 1980; Waithe et al., 1983), the majority of the evidence favors synthesis restricted to the S phase (Borun et al., 1967; Robbins & Borun, 1967; Spalding et al., 1966; Gallwitz & Mueller, 1969; Gurley et al., 1972; Moll & Wintersberger, 1976; Delegeane & Lee, 1982; Marashi et al., 1982). When cellular levels of histone RNA are measured by hybridization to histone probes, the majority of the evidence indicates that the RNA of the five histones H1, H2A, H2B, H3, and H4 is detectable in appreciable amounts only in the S phase (Stein et al., 1978; Detke et al., 1979; Hereford et al., 1981; Rickles et al., 1982; Plumb et al., 1983, 1984) although some contradictory reports have appeared (Melli et al., 1977; Sittman et al., 1983).

Temperature-sensitive (ts) mutants of the cell cycle, especially ts mutants that block in G_1 at the restrictive temperature, are ideal tools for studying the cell cycle dependence of gene expression in general, and histone gene expression in particular. The tight ts block reduces and almost eliminates the background due to cells that, despite the synchronization procedures, are not in the desired phase of the cell cycle. Pochron & Baserga (1979) used one of these G_1-specific ts mutants, tsAF8 cells, to show that histone synthesis, but not histone phosphorylation, was suppressed when the cells were serum stimulated at the nonpermissive temperature. The effect of a G_1-specific ts block on histone synthesis was recently confirmed in K12 cells, another ts mutant of the cell cycle (Delegeane & Lee, 1982).

In the present experiments, the levels of histone RNA in tsAF8 cells serum stimulated at either the permissive or the nonpermissive temperature were studied. Genomic human DNA clones of H2A, H2B, H3, and H4 histones were used as probes.

Materials and Methods

Cell Lines and Culture Conditions. tsAF8 cells are G_1-specific, temperature-sensitive mutants originally isolated from BHK cells (Meiss & Basilico, 1972). These cells have been extensively characterized (Rossini & Baserga, 1978; Ashihara et al., 1978; Talavera & Basilico, 1978). Monolayer cultures were routinely maintained in Dulbecco's modified Eagle's medium containing 10% (v/v) donor calf serum at the permissive temperature of 34 °C. To obtain quiescent (G_0) cultures, tsAF8 cells were plated at 1×10^6 per 100-mm plate, grown to near-confluence at 34 °C (about 4 days), rinsed twice in prewarmed Hanks solution, and then refed with medium containing 0.5% (v/v) donor calf serum. After an additional 48-h incubation at 34 °C, the cells were stimulated with fresh medium containing 10% (v/v) donor calf serum and incubated either at the permissive temperature of 34 °C or at the nonpermissive temperature of 39.6–40 °C. Cells used for shift-up experiments were stimulated at the permissive temperature and then shifted to the nonpermissive temperature at 6, 16, or 20 h after serum stimulation. All cells were harvested for RNA isolation 24 h after serum addition.

The effect of temperature shift-up on the ability of tsAF8 cells to enter the S phase and divide was determined by cell density and autoradiography (see below). A quiescent population was trypsinized and replated at one-fifth its original cell density in medium containing 10% (v/v) donor calf serum. Control cultures were kept at 34 and 39.6 °C. Other cells were

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shifted up from 34 to 39.6 °C at 16, 20, or 26 h after stimulation. The experiment was terminated 41 h after stimulation, and cell density was determined in triplicate by trypsinization and counting.

HeLa cells were maintained and synchronized as previously described (Stein & Borun, 1972). Synchrony was monitored by the incorporation of [^{3}H]thymidine as described below.

Analysis of Entry into the S Phase. tsAF8 cells were plated as described above except that the plates contained 22 × 22 mm glass coverslips. Cells were labeled with [^{3}H]thymidine (6.7 Ci/mmol, New England Nuclear) in growth medium at a final concentration of 0.5 μCi/mL. Quiescent populations were labeled for at least 24 h in low serum at the permissive or nonpermissive temperature. Label was added to all stimulated populations at the time of serum addition and allowed to incorporate for 24 or 41 h at the permissive and nonpermissive temperatures as indicated in each experiment. Cells were fixed in −20 °C methanol, autoradiographed, and scored as described by Baserga & Malamud (1969).

RNA Isolation. Cells were harvested at the indicated times by scraping into phosphate-buffered saline (PBS). After a low-speed centrifugation, the cell pellet was resuspended and rinsed in PBS. After another low-speed centrifugation, the cell pellet was fast-frozen in a dry ice–ethanol bath and stored frozen until extracted.

Cell lysis was performed in 10 volumes of 1.3 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 7.4), 0.7 mM ethylenediaminetetraacetic acid (EDTA), 1.3 μg/mL polyvinylsulfuric acid (PVS), 2.4% (w/v) sodium dodecyl sulfate (SDS), and 0.9 mg/mL proteinase K for 15 min at ambient temperature. NaCl (5 M) was added to the cell lysate to a final concentration of 0.25 M before organic extractions—twice with 2 volumes of buffered phenol–chloroform–isoamyl alcohol (25:24:1 v/v). The aqueous phase was adjusted to 0.25 M NaOAc by using a 5 M stock solution at pH 5.0, and total nucleic acids were precipitated with 3 volumes of ethanol at −20 °C.

Nucleic acids were recovered by centrifugation at 10 000 rpm (12000*g*) at 4 °C for 30 min, resuspended in 10 mM Tris-HCl (pH 7.4), 10 mM $CaCl_2$, and 10 mM $MgCl_2$, and incubated at 37 °C for 20 min in the presence of 0.1 mg/mL DNase I (Sigma, electrophoretically pure) which had been pretreated for 2 h with proteinase K, as described by Tullis & Rubin (1980). After incubation, 0.05 volume of 5 M NaOAc, pH 5.0, and 0.25 volume of 10% (w/v) SDS were added, and total cellular RNA was recovered following organic extractions and ethanol precipitation as described above.

For isolation of total cytoplasmic RNA, cell pellets were resuspended in 10 volumes of RSB (10 mM Tris-HCl, pH 7.8, 10 mM NaCl, and 1.5 mM $MgCl_2$) containing 0.65% NP-40. Cells were lysed in a Dounce homogenizer, and the nuclei were pelleted at 1000*g* for 10 min. The supernatant was extracted with phenol and chloroform as described above, and the RNAs were recovered by ethanol precipitation at −20 °C.

Northern Blots. RNA (50 μg) was fractionated electrophoretically in a denaturing agarose–formaldehyde gel and transferred to nitrocellulose filters. The subsequent hybridization to ^{32}P-labeled histone gene probes was performed as described by Plumb et al. (1983).

Probes. The *Eco*RI–*Eco*RI or *Eco*RI–*Hin*dIII restriction fragments of λ-Charon 4A recombinant phage containing histone genes (Sierra et al., 1982) were subcloned into pBR322. The recombinant plasmids were amplified in *Escherichia coli* strain HB101 and isolated by the alkaline lysis procedure of Ish-Horowicz & Burke (1981) followed by CsCl–ethidium bromide buoyant density gradient centrifugation as described by Clewell & Helinski (1970).

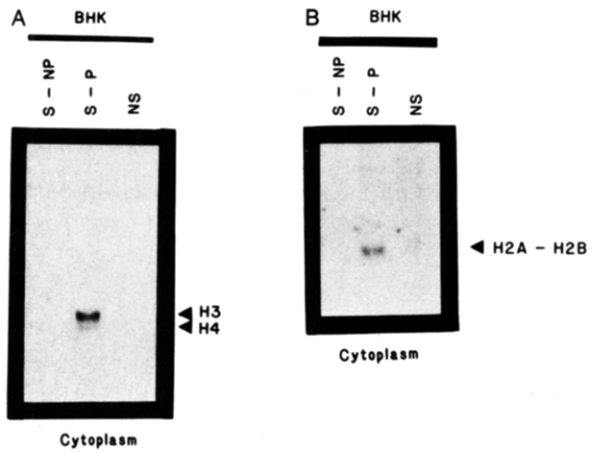

FIGURE 1: Northern blot analysis of total cytoplasmic RNA fractionated electrophoretically in a 1.5% agarose–6% formaldehyde gel. The blot was hybridized to (A) nick-translated plasmid DNAs containing H3 (pST519) and H4 (pF0108A) human histone gene sequences and (B) ^{32}P-labeled plasmid pFF435-B containing H2A and H2B human histone gene sequences. Abbreviations: S, serum stimulated for 24 h; NS, nonstimulated; P, cells maintained at the permissive temperature (34 °C); NP, cells maintained at the nonpermissive temperature (39.6 °C).

For preparation of ^{32}P-labeled histone gene probes, appropriate plasmids (Plumb et al., 1983) were nick translated with [α-^{32}P]dCTP as described by Maniatis et al. (1975).

Results

tsAF8 cells are a ts mutant of BHK cells that arrest in G_1 at the nonpermissive temperature (Meiss & Basilico, 1972). These cells grow normally at 34 °C; if shifted to the restrictive temperature of 39.6 °C, they are capable of completing S phase, G_2, and mitosis, but arrest in G_1, the arrest being reversible at least for 4–5 days after shift-up (Burstin et al., 1974). If made quiescent by serum deprivation, subsequent serum stimulation at 34 °C causes them to enter DNA synthesis, but at 39.6–40 °C, virtually no cells are capable of entering S phase after serum stimulation. At 34 °C, the cells begin to enter S phase at about 20 h and reach a peak at 24–28 h (Ashihara et al., 1978). If the cells are stimulated at the permissive temperature and then shifted up, the fraction of cells entering S phase is proportional to the time the stimulated cells were left at the permissive temperature. Thus, if the cells are shifted up 6 h after stimulation, the percentage of cells entering S phase is virtually the same as when the cells are shifted up at the time of serum stimulation. If one delays 24 h after serum stimulation before shift-up, the fraction of cells entering DNA synthesis is close to that obtained with stimulation at the permissive temperature. Intermediate shift-up times give intermediate values. All these kinetic data were reported in detail in previous papers from one of our laboratories (Rossini & Baserga, 1978; Ashihara et al., 1978) and are therefore omitted from this paper. We will simply give, when necessary, the percentage of cells labeled by incorporation of [^{3}H]thymidine, followed by autoradiography. The percentage of cycling cells in the quiescent population labeled over a 24-h period was 3%. Stimulated cells were labeled from 0 to 41 h and gave a percentage of labeled cells of 60% at 34 °C and 2% at 40 °C. Cytoplasmic RNA from these three populations was isolated 24 h after serum stimulation and fractionated by agarose–formaldehyde gel electrophoresis. Following electrophoresis, the RNA was transferred from the gel to nitrocellulose paper and hybridized with human histone

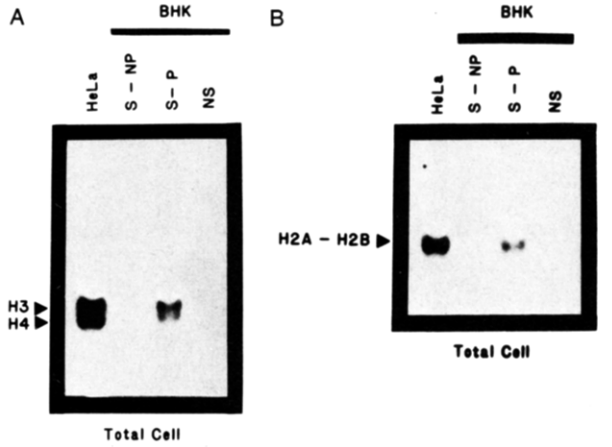

FIGURE 2: Northern blot analysis of total cellular RNA fractionated electrophoretically in agarose–formaldehyde gels. The human histone DNA probes [(A) H3 and H4, (B) H2A and H2B] used for hybridization and the abbreviations for the different lanes are the same as those described in the legend of Figure 1.

gene probes. The results are shown in Figure 1. In panel A, the probes used were H3 and H4; in panel B, the probes were H2A and H2B. In all cases, there were strong signals with cells stimulated at 34 °C and only very low signals with unstimulated cells or with cells stimulated at 40 °C. H2A and H2B RNAs do not resolve into separate species under the conditions of this electrophoresis.

These experiments were repeated with total cellular RNA instead of cytoplasmic RNA to determine if any precursor molecules were synthesized. The results for H3 and H4 are shown in Figure 2A where total RNA isolated from growing HeLa cells has been added for comparison. Again, there is a strong signal with cells stimulated at the permissive temperature and only a very low signal with unstimulated cells or with cells stimulated at the nonpermissive temperature. The amount of hybridization though is much stronger with HeLa cells than with tsAF8 cells, which may reflect the amount of histone mRNA in these different cell lines or, alternatively, the use of a heterologous hybridization probe. Similar results are shown for H2A and H2B in Figure 2B.

In one of the experiments, the quiescent population of tsAF8 cells contained more cycling cells than expected (30% of the cells were labeled by [^{3}H]thymidine over a 24-h period). A Northern analysis of total RNA isolated from this "quiescent" population and cells stimulated from this population is shown in Figure 3. These results are included to emphasize the importance of the true physiological state of the cell populations in this type of analysis. Notice in Figure 3A that when the RNA is hybridized with H2A and H2B probes a signal is detectable in the stimulated and nonstimulated (very faint band) populations at permissive temperatures although it is still nondetectable in cells at the nonpermissive temperatures whether stimulated or not. Figure 3B is the same Northern blot but with a longer exposure. Preventing the cells from entering DNA synthesis by arresting them in G_1 at the nonpermissive temperature clearly prevents the synthesis of H2A- and H2B-specific RNA. A background level of cycling cells in a so-called quiescent population allows a low level of synthesis of H2A- and H2B-specific RNA that is clearly prevented by the temperature shift.

Histone RNA Levels in tsAF8 Cells Shifted to the Nonpermissive Temperature at Different Times after Serum Stimulation. The proportion of tsAF8 cells entering DNA synthesis increases if one delays the shift-up to the nonpermissive temperature after serum stimulation. The appearance

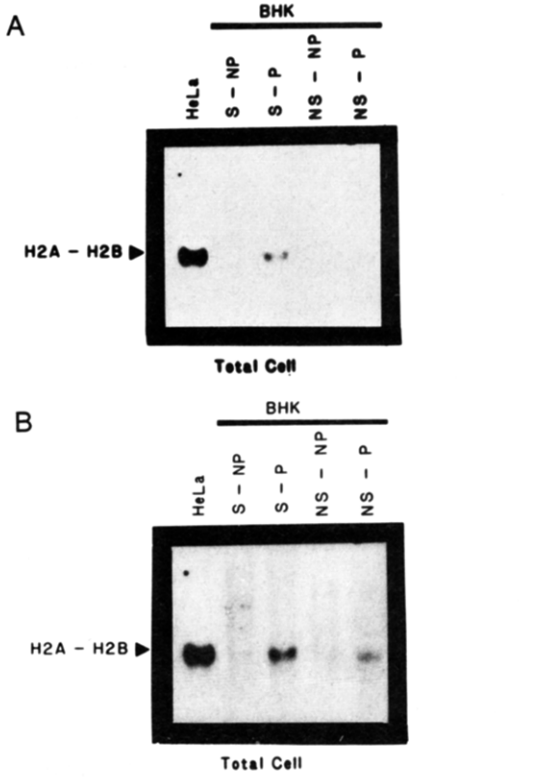

FIGURE 3: Northern blot analysis of total cellular RNA isolated from temperature-sensitive BHK cells. Abbreviations: S, serum stimulated for 24 h; NS, unstimulated quiescent cells incubated for 24 h (P) the permissive temperature or (NP) the nonpermissive temperature. The hybridization probe was pFF435B (H2A + H2B). Panel B is a longer exposure of the Northern blot shown in panel A.

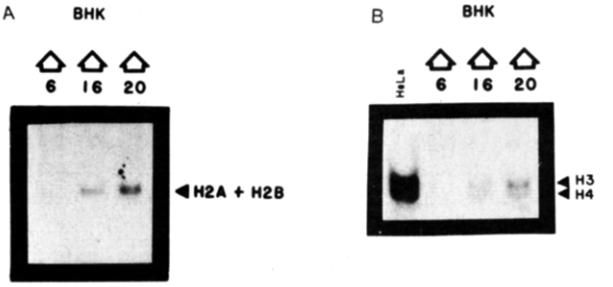

FIGURE 4: Accumulation of histone RNAs in tsAF8 cells shifted to the nonpermissive temperature at various times following serum stimulation. Cells were serum stimulated at time zero and then were shifted to the nonpermissive temperatures at either 6, 16, or 20 h. Total cellular RNA was extracted after 24 h of incubation following serum stimulation as described under Materials and Methods. Cellular levels of histone mRNAs were determined by Northern blot analysis using ^{32}P-labeled pFF435B (H2A and H2B) or pST519 (H3) and pF0108A (H4) as hybridization probes, panels A and B, respectively.

of histone RNA (total cellular) in tsAF8 cells that were shifted to the nonpermissive temperature at 6, 16, and 20 h after serum stimulation was investigated. The percentage of cells in DNA synthesis (labeled from 0 to 41 h) was respectively 5, 16, and 27%, roughly similar to the results usually obtained in previous experiments, as already reported in detail (Rossini & Baserga, 1978; Ashihara et al., 1978). The results of the Northern blots for histone probes H2A and H2B are shown in Figure 4A. The intensity of the hybridization signals is proportional to the fraction of cells entering DNA synthesis. Similar results are seen with H3 and H4 probes in Figure 4B.

DNA Synthesis and Cell Growth in tsAF8 Cells Shifted to the Nonpermissive Temperature at Different Times after Serum Stimulation. The ability of tsAF8 cells to enter DNA synthesis and subsequently divide when cells were shifted to

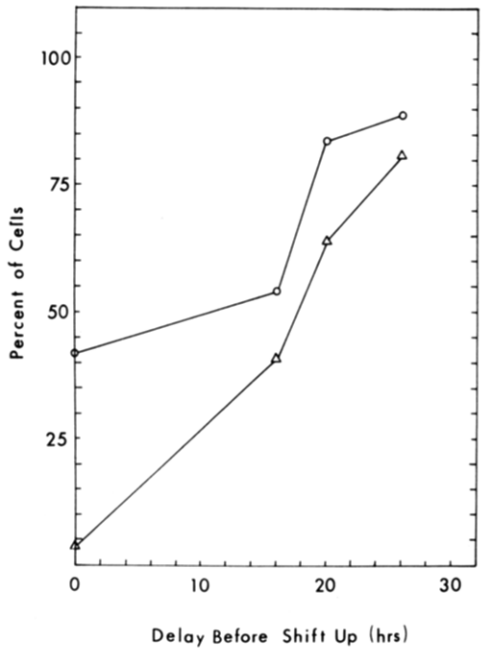

FIGURE 5: DNA synthesis and cell growth in tsAF8 cells shifted to the nonpermissive temperature at different times after serum stimulation. Quiescent tsAF8 cells were trypsinized and replated at one-fifth their density in medium containing 10% serum, at the permissive temperature. Cultures were immediately shifted up to the nonpermissive temperature, or the shift-up was delayed for 16, 20, or 26 h. Percent of cells in culture relative to 34 °C (○); percent of cells incorporating [^{3}H]thymidine (△).

the nonpermissive temperature at different times after serum stimulation was investigated. Cells were made quiescent in low serum and then trypsinized and replated at one-fifth the density in high serum. Cells were shifted to the nonpermissive temperature immediately, or the shift-up was delayed for 16, 20, or 26 h. After a total of 41 h from the time of plating, the cell density was determined and correlated with the ability of these cells to enter DNA synthesis. As can be seen in Figure 5, cells immediately shifted to the nonpermissive temperature have less than half (43%) the number of cells as compared to control cultures which remained at 34 °C for the same time period. However, only 2% of the cells could enter DNA synthesis compared to 87% of the cells at the permissive temperature. When the shift was delayed for 16 h, 41% of the cells entered DNA synthesis with a corresponding increase in cells to 54% of the control. Similar results were seen when the shift was delayed 20 and 26 h. A direct correlation was seen between the ability to enter DNA synthesis and the ability to divide.

Levels of Aspecific mRNAs. A cDNA clone was synthesized from poly(A+) mRNA isolated from exponentially growing HeLa cells (data not shown). The expression of one of these clones has been shown to be independent of the cell cycle. Figure 6 (panel A) shows a Northern blot analysis of RNA from HeLa cells in different phases of the cell cycle, which was hybridized to the non cell cycle specific cloned DNA. Figure 6 (panel B) shows a Northern blot on tsAF8 RNA isolated 24 h after serum addition, at both permissive and nonpermissive temperatures, using the same cDNA probe. The extent of hybridization is the same in all phases of the HeLa cell cycle and in tsAF8 at either temperature. This suggests that the different levels of expression exhibited with the histone probes are not a generalized phenomenon and do not result from a specific toxicity of the nonpermissive temperature, but result from the specific block imposed by the higher temperature.

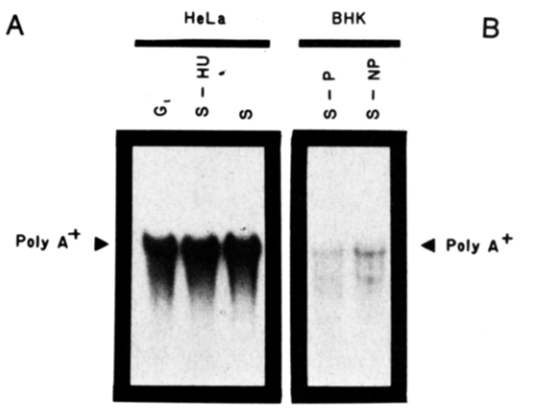

FIGURE 6: Northern blot analysis of total cellular RNAs using a ^{32}P-labeled cDNA clone (pHG 404) as probe: (A) HeLa cells; (B) temperature-sensitive tsAF8 cells. (A) HeLa cell RNAs from G_1 phase, S phase, and S phase cells treated with hydroxyurea for 1.5 h (S-Hu). (B) Serum-stimulated (S) tsAF8 BHK cells at permissive (P) or nonpermissive (NP) temperatures, for 24 h.

Discussion

The advantage of using ts mutants of the cell cycle to study cell cycle dependent gene expression has been vividly illustrated by several studies on cdc (cell division cycle) mutants of yeast (Hartwell, 1971, 1973; Hereford & Hartwell, 1974; Hereford et al., 1982; Beach et al., 1982; Arendes et al., 1983). In mammalian cells, the use of ts mutants allows reduction of or altogether eliminates the background due to cells that are not in the selected phase of the cell cycle. This is especially true of BHK mutants (like tsAF8 cells), because the parent cell line grows vigorously, even at 41 °C (Rossini et al., 1980). This will allow the placement of ts mutants at temperatures of 39.6–40 °C, where the ts block is very tight, as demonstrated by numerous reports (Rossini & Baserga, 1978; Ashihara et al., 1978; Rossini et al., 1980; this paper). Experiments with ts mutants of the cell cycle can be correctly interpreted only if the cells at the restrictive temperature are viable and not undergoing general damage. tsAF8 cells remain viable at the restrictive temperature for 4–5 days (Burstin et al., 1974) and protein synthesis remains at the same levels as in cells at 34 °C for at least 24–30 h after shift-up to the restrictive temperature (Rossini et al., 1980).

This is the first reported group of experiments in which histone RNA levels were determined in mammalian cells whose entry into S was prevented by a temperature-sensitive mutation. Detectable levels of H2A, H2B, H3, and H4 histone RNAs were only found in tsAF8 cells stimulated for 24 h at the permissive temperature and not in quiescent cells or cells stimulated at the nonpermissive temperature. This is confirmed by the experiment shown in Figure 3, where quiescent cells that contained a high percentage of cycling cells at the permissive temperature contain readily detectable levels of histone RNA. This experiment also points out how an undesirable background can affect the interpretation of the results. The data obtained with cells shifted to the nonpermissive temperature at various times after stimulation show that the amount of histone RNA reflects not only the percentage of cells entering the S phase but also the ability to divide. The cDNA clone whose expression is not cell cycle dependent confirms that our cells at the restrictive temperature were not damaged.

In conclusion, these experiments show that when cells are blocked in G_1 by a ts block, histone RNA (H2A, H2B, H3, and H4) does not accumulate, supporting previous findings that the accumulation of the specific histone RNAs is largely restricted to the S phase.

References

Arendes, J., Kim, K. C., & Sugino, A. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 673–677.

Ashihara, T., Chang, S. D., & Baserga, R. (1978) *J. Cell. Physiol. 96*, 15–22.

Baserga, R., & Malamud, D. (1969) *Autoradiography: Techniques and Applications*, Harper and Row, New York.

Beach, D., Durkacz, B., & Nurse, P. (1982) *Nature (London) 300*, 706–709.

Borun, T. W., Scharff, M. D., & Robbins, E. (1967) *Proc. Natl. Acad. Sci. U.S.A. 58*, 1977–1983.

Burstin, S. J., Meiss, H. K., & Basilico, C. (1974) *J. Cell. Physiol. 92*, 457–468.

Chiu, L.-M., & Marzluff, M. F. (1982) *Biochim. Biophys. Acta 699*, 173–183.

Clewell, D. B., & Helinski, D. R. (1970) *Biochemistry 9*, 4428–4440.

Delegeane, A. M., & Lee, A. S. (1982) *Science (Washington, D.C.) 215*, 79–81.

Detke, S., Lichtler, A., Phillips, I., Stein, J., & Stein, G. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4995–4999.

Gallwitz, D., & Mueller, G. C. (1969) *J. Biol. Chem. 244*, 5947–5952.

Groppi, V. E., & Coffino, P. (1980) *Cell (Cambridge, Mass.) 21*, 195–204.

Gurley, L. R., Walter, R. A., & Tobey, R. A. (1972) *Arch. Biochem. Biophys. 148*, 633–641.

Hartwell, L. H. (1971) *J. Mol. Biol. 59*, 183–194.

Hartwell, L. H. (1973) *J. Bacteriol. 115*, 966–974.

Hereford, L. M., & Hartwell, L. H. (1974) *J. Mol. Biol. 84*, 445–461.

Hereford, L. M., Osley, M. A., Ludwig, J. R., & McLaughlin, C. S. (1981) *Cell (Cambridge, Mass.) 24*, 367–375.

Hereford, L., Bromley, S., & Osley, M. A. (1982) *Cell (Cambridge, Mass.) 30*, 305–310.

Ish-Horowicz, D., & Burke, J. F. (1981) *Nucleic Acids Res. 9*, 2989–2998.

Lenox, R. W., & Cohen, L. H. (1983) *J. Biol. Chem. 258*, 262–268.

Maniatis, T., Jeffrey, A., & Kleid, D. G. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1184–1188.

Marashi, F., Baumbach, L., Rickles, R., Sierra, F., Stein, J. L., & Stein, G. S. (1982) *Science (Washington, D.C.) 215*, 683–685.

Meiss, H. K., & Basilico, C. (1972) *Nature (London), New Biol. 239*, 66–68.

Melli, M., Spinelli, G., & Arnold, E. (1977) *Cell (Cambridge, Mass.) 12*, 167–174.

Moll, R., & Wintersberger, E. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1863–1867.

Pehrson, J., & Cole, R. D. (1980) *Nature (London) 285*, 43–44.

Plumb, M., Stein, J., & Stein, G. (1983) *Nucleic Acids Res. 11*, 2391–2410.

Plumb, M., Marashi, F., Green, L., Zimmerman, A., Zimmerman, S., Stein, J., & Stein, G. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 434–438.

Pochron, S. F., & Baserga, R. (1979) *J. Biol. Chem. 254*, 6356.

Rickles, R., Marashi, F., Sierra, F., Wells, J., Stein, J., & Stein, G. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 749–753.

Robbins, E., & Borun, T. W. (1967) *Proc. Natl. Acad. Sci. U.S.A. 57*, 409–416.

Rossini, M., & Baserga, R. (1978) *Biochemistry 17*, 858–863.

Rossini, M., Baserga, S., Huang, C. H., Ingles, C. J., & Baserga, R. (1980) *J. Cell. Physiol. 103*, 97–103.

Sheinin, R., & Lewis, P. N. (1980) *Somatic Cell Genet. 6*, 225–239.

Sierra, F., Lichtler, A., Marashi, F., Rickles, R., Van Dyke, T., Clark, S., Wells, J., Stein, G., & Stein, J. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 1795–1799.

Sittman, D. B., Graves, R. A., & Marzluff, W. F. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1849–1853.

Spalding, J., Kajiwara, K., & Mueller, G. C. (1966) *Proc. Natl. Acad. Sci. U.S.A. 56*, 1535–1542.

Stein, G. S., & Borun, T. W. (1972) *J. Cell Biol. 52*, 292–307.

Stein, G. S., Stein, J. L., Park, W. D., Detke, S., Lichtler, A. C., Sheppard, E. A., Jansing, R. L., & Phillips, I. R. (1978) *Cold Spring Harbor Symp. Quant. Biol. 42*, 1107–1120.

Talavera, A., & Basilico, C. (1978) *J. Cell. Physiol. 97*, 429–440.

Tarnowka, M. A., Baglioni, C., & Basilico, C. (1978) *Cell (Cambridge, Mass.) 15*, 163–171.

Tullis, R. H., & Rubin, H. (1980) *Anal. Biochem. 107*, 260–264.

Waithe, W. I., Renaud, J., Nadeau, P., & Pallotta, D. (1983) *Biochemistry 22*, 1778–1783.

Wu, R. S., & Bonner, W. M. (1981) *Cell (Cambridge, Mass.) 27*, 321–330.