# Histone Proteins in HeLa $S_3$ Cells Are Synthesized in a Cell Cycle Stage Specific Manner

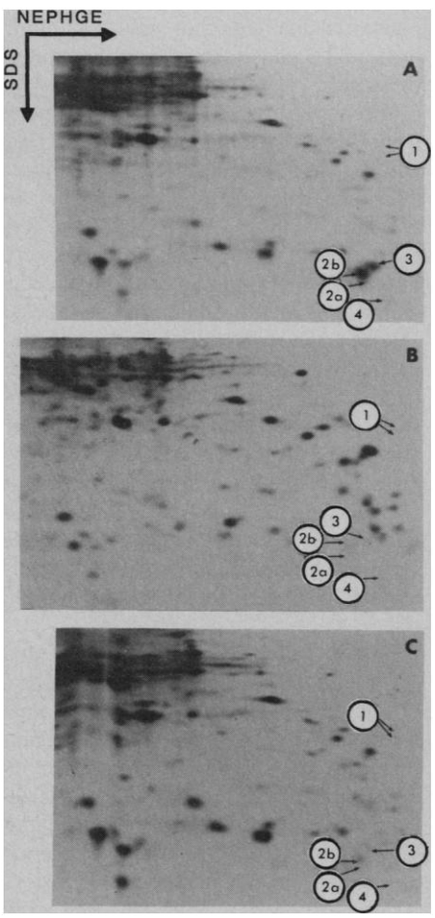
Abstract. *The synthesis of histone proteins in $G_1$ and S phase HeLa $S_3$ cells was examined by two-dimensional electrophoretic fractionation of nuclear and total cellular proteins. Newly synthesized histones were detected only in S phase cells. Histone messenger RNA sequences, as detected by hybridization with cloned human histone genes, were present in the cytoplasm of S phase but not $G_1$ cells.*

To understand the level at which the regulation of histone gene expression occurs, it is essential to establish whether or not histone proteins and histone messenger RNA are synthesized in a cell cycle stage specific manner. Equally important is an understanding of the relation between histone protein synthesis, histone gene transcription, and the representation of histone messenger RNA sequences in the cell nucleus and cytoplasm.

Our previous results and those of others suggest that histone gene expression and DNA replication are tightly coupled biological processes in HeLa cells and also in several normal and transformed mammalian cells (*1–12*). The synthesis of histones, with the possible exception of H1 under certain circumstances, has been reported to be restricted to the S phase of the cell cycle in continuously dividing cells and after quiescent cells are stimulated to proliferate (*1–11*). Inhibition of DNA replication by drugs such as cytosine arabinoside or hydroxyurea results in a rapid inhibition of histone synthesis (*1–11*). Also, results from in vitro translation (*9–11*) and nucleic acid hybridization studies (*12–18*) suggest that histone messenger RNA sequences are present in the nucleus and cytoplasm only during the S phase of the cell cycle.

In contrast with these observations, Groppi and Coffino recently reported (*19*) that histone proteins are synthesized at a constant rate throughout the cell cycle in cultured S49 mouse lymphoma cells and CHO cells, and they postulate that the newly synthesized histones become associated with DNA only during S phase. They proposed that the inability of other investigators to detect histone synthesis outside of S phase is that previously only nuclear rather than total cellular proteins were analyzed for the presence of newly synthesized histones.

Because of the growing body of information regarding expression and regulation of histone genes in HeLa cells, we have reevaluated the question of when histones are synthesized during the cell cycle in these cells, and report that, using a two-dimensional electrophoretic fractionation procedure (*19*), we were unable to detect newly synthesized histones among the nuclear or total cellular proteins of $G_1$ HeLa cells. Additional evidence for the absence of histone synthesis in $G_1$ HeLa cells is that we did not, in our analysis, find significant amounts of histone messenger RNA sequences in



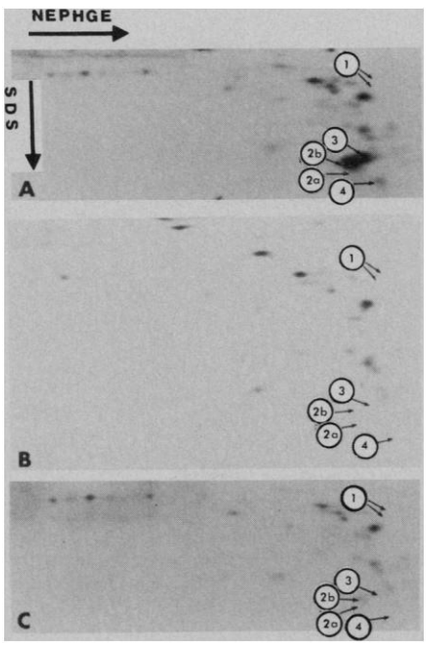




Fig. 1 (left). Autoradiograms of two-dimensional electrophoretic analysis of [$^{35}$S]methionine-labeled total cellular proteins. (A) S phase; (B) $G_1$ phase; and (C) cytosine arabinoside–treated S phase. HeLa cells in suspension culture were synchronized by two successive treatments with excess thymidine (2 m*M*) (*3*). After release from the second treatment, cells were maintained at a density of $5 \times 10^5$ per milliliter for 1 hour. The S phase cells were incubated further for 15 minutes at 37°C either in the presence or absence of the DNA synthesis inhibitor cytosine arabinose (40 μg per milliliter). Ten milliliters of S phase cells treated or not treated with cytosine arabinoside were centrifuged, washed in Earle's balanced salt solution containing 5 μ*M* methionine (37°C), and resuspended in 2.8 ml of low (5 μ*M*) methionine medium (containing cytosine arabinoside when appropriate). [$^{35}$S]Methionine (140 μCi) was added and cells were labeled for 45 minutes at 37°C. The $G_1$ phase HeLa cells were obtained 2 hours after mitotic selective detachment (*3, 23*) and labeled as above for S phase cells. Cells were harvested by centrifugation at 1500*g* for 5 minutes, rinsed with ether, and dried before solubilizing in 9.5*M* urea, 2 percent (weight to volume) NP-40, 2 percent Ampholine (weight to volume), 5 percent mercaptoethanol (weight to volume), 0.3*M* NaCl, and protamine sulfate (1 mg/ml) (*24*). $^{35}$S-Labeled peptides were fractionated in a two-dimensional electrophoretic system in which the first dimension was NEPHGE (*24*) and the second dimension was an SDS-containing, 15 percent acrylamide gel (*25*). The numbers in circles indicate the histones H1, H2A, H2B, H3, and H4. Fig. 2 (center). Autoradiograms of two-dimensional NEPHGE/SDS electrophoresis of acid-extracted nuclear proteins of (A) S phase, (B) $G_1$, and (C) cytosine arabinoside–treated S phase cells. Treatment with cytosine arabinoside was as described in legend of Fig. 1. [$^{35}$S]Methionine-labeled cells were lysed in a buffer of 10 m*M* KCl, 10 m*M* Tris, and 1.5 m*M* $MgCl_2$ (*p*H 7.4) containing 0.65 percent Triton X-100, and nuclei were centrifuged at 800*g*. The pellets were extracted with 0.4*M* $H_2SO_4$ for 30 minutes and centrifuged; the acid-soluble nuclear proteins in the supernatant were precipitated by the addition of three volumes of 95 percent ethanol and held at −20°C overnight. These precipitated proteins were rinsed with ether and dried before solubilizing in a mixture of 10*M* urea, 0.2 percent SDS, 30 m*M* lysine, and 2.5 m*M* $ZnCl_2$ (*p*H 4.8). Protein concentrations were adjusted to 2mg/ml and NaCl was added to a final concentration of 0.4*M*. Samples were digested with Sl nuclease (Sigma) (*24*) to eliminate the nucleic acids in the nuclear samples. Two-dimensional electrophoretic analysis of $^{35}$S-labeled peptides was as described in the legend to Fig. 1.


0036-8075/82/0205-0683$01.00/0

the cytoplasm of such cells by hybridization of electrophoretically fractionated $G_1$ RNA with cloned human histone sequences.

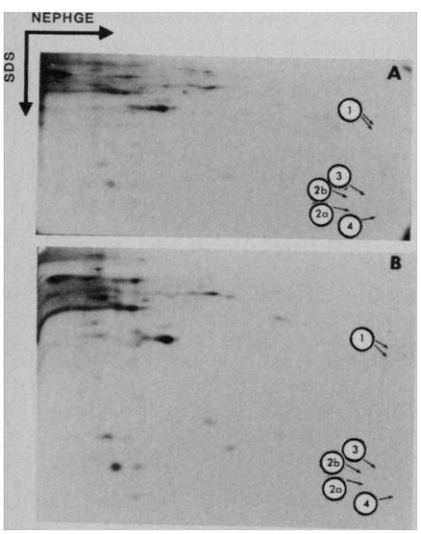
To compare the synthesis of histone proteins during the cell cycle in HeLa $S_3$ cells, $G_1$ and S phase cells were labeled for 45 minutes with [$^{35}$S]methionine and the total cellular proteins were separated electrophoretically according to charge and molecular weight by a two-dimensional [nonequilibrium *p*H gradient gel electrophoresis (NEPHGE)–sodium dodecyl sulfate (SDS) polyacrylamide electrophoresis] gel system (*19*). The $G_1$ HeLa cells were obtained by mitotic selective detachment, which yields a $G_1$ cell population containing less than 0.5 percent S phase cells. Although [$^{35}$S]methionine is incorporated into only the histones H3 and H2B mainly, and H4 to a much lesser extent, the high specific activity of the labeled histones thus obtained facilitates detection of newly synthesized histone polypeptides. Newly synthesized histones are apparent in electrophoretically fractionated total cellular proteins from S phase but not $G_1$ phase HeLa cells (Fig. 1, A and B). Identification of labeled histone polypeptides was by coelectrophoresis of unlabeled HeLa cell histone standards, the locations of which were detected by staining the gels with Coomassie blue prior to autoradiography. Confirmation of the identity of the labeled polypeptides designated H3, H4, and H2B as histones was based on the sensitivity of their synthesis to treatment with the DNA synthesis inhibitor cytosine arabinoside (Fig. 1C). The residual labeling of histones (such as H2B) was due to the treatment of S phase cells with the DNA synthesis inhibitor for only 15 minutes before the labeling; under these conditions [$^{14}$C]thymidine incorporation is inhibited by about 85 percent compared with untreated S phase cells.

If H3, H4, and H2B histones were synthesized to a significant extent during $G_1$ [especially at the same rate as during S phase, as reported (*19*) for S49 cells], their presence should have been detected in the autoradiograms of $^{35}$S-labeled total cellular $G_1$ proteins (Fig. 1B) since newly synthesized histones were readily detected after similar analysis of such preparations from S phase cells (Fig. 1A). It is unlikely that newly synthesized H3, H4, and H2B histones from $G_1$ cells are selectively degraded during sample preparations because the cells were lysed in detergent immediately after the labeling. Because histones were not acid-extracted from the total cellular proteins of $G_1$ or S phase cells, an argument for newly synthesized $G_1$ histone being acid-labile cannot be invoked. Moreover, it is reasonable to assume that those labeled polypeptides visible in the autoradiograms (Fig. 1, A and B) are an accurate reflection of all methionine-containing proteins synthesized during $G_1$ and S phase. Within this context the similar intensities of the predominant nonhistone polypeptides of $G_1$ and S phase cells indicate that similar amounts of $G_1$ and S phase samples are present in these gels.

When sulfuric acid extracts of nuclei from [$^{35}$S]methionine-labeled S (Fig. 2A) and $G_1$ (Fig. 2B) phase HeLa cells were fractionated electrophoretically in the two-dimensional NEPHGE-SDS system, newly synthesized histones were observed only in the S phase. Again, the identity of the [$^{35}$S]methionine-labeled H3, H4, and H2B histones was confirmed by staining with Coomassie blue and by the sensitivity of histone synthesis to cytosine arabinoside (Fig. 2C). The absence of detectable amounts of labeled histones in the cytoplasm of S phase or $G_1$ cells (Fig. 3, A and B) is not surprising and is probably attributable to the rapid transfer of histones to the nucleus immediately after completion of synthesis.

In a parallel series of experiments, [$^{35}$S]methionine-labeled histones from $G_1$ and S phase HeLa cells were analyzed autoradiographically after separation in a two-dimensional system consisting of acetic acid–urea polyacrylamide gel electrophoresis in the first dimension and SDS polyacrylamide gel electrophoresis in the second dimension. Again histone synthesis was observed only in the total cellular and nuclear samples from the S phase cells (data not shown).

One of the strongest arguments against



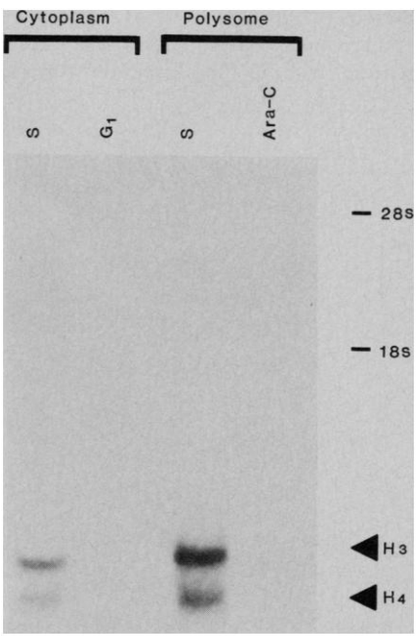




Fig. 3 (left). Autoradiography of two-dimensional NEPHGE/SDS analysis of [$^{35}$S]methionine-labeled cytoplasmic peptides. The S phase (A) and $G_1$ phase (B) cells were lysed as described in legend of Fig. 2, except that Triton was omitted. Cytoplasmic polypeptides were precipitated from the 800*g* supernatant (cooled for 30 minutes on ice) by the addition of cold 100 percent trichloroacetic acid to a final concentration of 20 percent. The precipitated proteins were washed with 70 percent ethanol (−20°C) and analyzed electrophoretically as described in the legend of Fig. 1. Fig. 4 (right). Hybridization analysis of histone messenger RNA's. Samples (100 μg) of $G_1$ and S phase total cytoplasmic and S phase and cytosine arabinoside (*Ara-C*)–treated S phase polysomal RNA's were incubated in 50 m*M* methylmercury hydroxide and fractionated on a 2 percent agarose gel containing 5 m*M* methylmercury (*26*). The gel was stained and neutralized in a solution containing 8.5 m*M* β-mercaptoethanol and ethidium bromide (1 μg/ml) and photographed. The gel was prepared for transfer by incubation in 10 m*M* iodoacetic acid for 30 minutes and then in transfer buffer (50 m*M* $KH_2PO_4$, *p*H 5.0) for 30 minutes. Diazobenzyloxymethyl (DBM) paper was prepared (*20*), and the transfer was performed with an E-C electroblot (E-C Apparatus, St. Petersburg, Fla.) apparatus (*27*) for 4 hours at 3.6 V/cm and chilled transfer buffer. Solutions for the steps in hybridization were as described (*20*), except that 5× Denhardt solution (without bovine serum albumin), 0.1 percent SDS, and 0.7 mg of carrier RNA per milliliter were used, and the hybridization was conducted at 50°C. Lambda HHG 55, a recombinant phage containing H3 and H4 genomic human histone sequences, was nick-translated in a reaction containing [α-$^{32}$P]-deoxycytidine triphosphate to a specific activity of $4 \times 10^7$ cpm/μg. The DBM paper was washed with decreasing concentrations of SSC (0.15*M* NaCl, 15 m*M* sodium citrate, *p*H 7.5) at 68°C to a final concentration of 0.1× SSC in the presence of 0.1 percent SDS. Autoradiography was performed with Kodak XAR 5 film and a Cronex Lightening Plus intensifying screen. The exposure shown was for 5 hours at −70°C.

synthesis of histone proteins in $G_1$ HeLa cells is the inability to detect histone messenger RNA sequences in the cytoplasm during $G_1$. When cytoplasmic RNA of $G_1$ and S phase cells was fractionated electrophoretically in methyl-mercury-agarose gels, transferred electrophoretically to diazotized cellulose (*20*), and analyzed by hybridization with $^{32}P$-labeled cloned human H3 and H4 histone DNA sequences (*21*, *22*), annealing with the S phase but not the $G_1$ RNA's was observed (Fig. 4). The validity of using cytosine arabinoside as an inhibitor of HeLa cell histone and DNA synthesis is substantiated by the obvious loss of more than 95 percent of the histone messenger RNA sequences from S phase polysomes after drug treatment for 1 hour (Fig. 4). We observed a similar absence of H1 and H2B histone messenger RNA sequences from $G_1$ Hela cell cytoplasmic RNA and a loss of H1 and H2B histone RNA sequences from polysomes after treatment of S phase cells with cytosine arabinoside (data not shown).

Taken together, results from our analyses of newly synthesized histone polypeptides and of the representation of cytoplasmic histone messenger RNA sequences in $G_1$ and S phase HeLa cells are consistent with cell cycle stage specific synthesis of the histone proteins. Thus, while it appears from our results that the control of histone gene expression during the cell cycle in HeLa cells is different from that in mouse lymphoma cells (*19*), such an observation would not be unprecedented. For example, it has been well documented that histone proteins are synthesized in the absence of DNA replication during oocyte maturation. The emerging picture may be one in which the level at which regulation of histone gene expression is mediated can vary depending on the cell type and the biological circumstance.

F. MARASHI, L. BAUMBACH
R. RICKLES, F. SIERRA
J. L. STEIN, G. S. STEIN

*Departments of Biochemistry and Molecular Biology* and *Immunology and Medical Microbiology, University of Florida College of Medicine, Gainesville 32610*

### References and Notes

1. J. Spalding, K. Kajiwara, G. Mueller, *Proc. Natl. Acad. Sci. U.S.A.* **56**, 1535 (1966).
2. E. Robbins and T. W. Borun, *ibid.* **57**, 409 (1967).
3. G. S. Stein and T. W. Borun, *J. Cell Biol.* **52**, 292 (1972).
4. G. S. Stein and C. L. Thrall, *FEBS Lett.* **34**, 35 (1973).
5. D. Gallwitz and G. C. Mueller, *J. Biol. Chem.* **244**, 5947 (1969).
6. T. W. Borun, M. D. Scharff, F. Robbins, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 1977 (1967).
7. W. B. Butler and G. C. Mueller, *Biochim. Biophys. Acta* **294**, 481 (1973).
8. M. Breindl and D. Gallwitz, *Eur. J. Biochem.* **45**, 91 (1974).
9. T. W. Borun, F. Gabrielli, K. Ajira, A. Zweidler, C. Baglioni, *Cell* 4, 59 (1975).
10. M. Jacobs-Lorena, C. Baglioni, T. W. Borun, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2095 (1972).
11. D. Gallwitz and M. Breindl, *Biochem. Biophys. Res. Commun.* **47**, 1106 (1972).
12. J. L. Stein, C. L. Thrall, W. D. Park, R. J. Mans, G. S. Stein, *Science* **183**, 557 (1975).
13. S. Detke *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4995 (1979).
14. I. Parker and W. Fitschen, *Cell Differ.* **9**, 23 (1980).
15. L. M. Hereford, M. A. Osley, J. R. Ludwig, C. S. McLaughlin, *Cell* **24**, 367 (1981).
16. G. S. Stein, W. D. Park, C. L. Thrall, R. J. Mans, J. L. Stein, *Nature (London)* **257**, 764 (1975).
17. R. L. Jansing, J. L. Stein, G. S. Stein, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 173 (1976).
18. R. Rickles, F. Marashi, F. Sierra, S. Clarke, J. Wells, J. L. Stein, G. S. Stein, *ibid.*, in press.
19. V. E. Groppi and P. Coffino, *Cell* **21**, 195 (1980).
20. J. C. Alwine, D. J. Kemp, G. R. Stark, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5350 (1977).
21. G. S. Stein, J. L. Stein, F. Marashi, M. I. Parker F. Sierra, *Cell Biophys.* **2**, 291 (1980).
22. F. Sierra *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
23. T. Terasima and L. J. Tolmach, *Exp. Cell Res.* **30**, 344 (1963).
24. M. M. Sanders, V. E. Groppi, E. T. Browning, *Anal. Biochem.* **103**, 157 (1980).
25. P. H. O'Farrell, *J. Biol. Chem.* **250**, 4007 (1975).
26. J. M. Bailey and N. Davidson, *Anal. Biochem.* **70**, 75 (1976).
27. E. J. Stellwag and A. E. Dahlberg, *Nucleic Acids Res.* **8**, 299 (1980).
28. Supported by NSF grant PCM-8018075 and March of Dimes Birth Defects Foundation grant 5-217.

26 August 1981; revised 29 October 1981

## Uninvolved Skin from Psoriatic Patients Develops Signs of Involved Psoriatic Skin After Being Grafted onto Nude Mice

Abstract. *Clinically involved psoriatic epidermis maintains its histological appearance, increased labeling index, and increased level of plasminogen activator after being grafted onto athymic nude mice. Uninvolved psoriatic epidermis develops increases in plasminogen activator activity after being grafted onto athymic nude mice; this is accompanied by an increased labeling index. Thus, psoriatic skin can develop markers of psoriasis independent of the host.*

Psoriasis vulgaris, a common skin disease, is characterized by circumscribed scaly plaques. Epidermal cells in psoriatic lesions are hyperproliferative (*1*), and psoriatic epidermis is thickened and papillomatous (*2*). The transit time of cells through the epidermis is markedly shortened (*3*), and the epidermis does not attain differentiated characteristics. The cause of epidermal hyperproliferation is unknown. Numerous hypotheses have been suggested, including intrinsic dysfunction of epidermal keratinocytes, alterations in cyclic nucleotide and prostaglandin metabolism, aberrations of beta-adrenergic receptor function (*4*), and abnormalities of the immune system (*5*). A major difficulty in investigating this disease in vitro is that psoriatic epidermis is not distinctive in tissue or cell culture. However, it was recently shown that clinically involved psoriatic skin maintains its morphological characteristics and accelerated mitotic rate when transplanted onto athymic nude mice (*6*).

Psoriatic scales (*7*) and psoriatic epidermis (*8*) have greater levels of plasminogen activator than normal epidermis. The activity of this enzyme increases in numerous situations in which cell transformation or activation occurs (*9*). We report here that clinically involved epidermis from psoriatic patients maintains its high plasminogen activator level when grafted onto nude mice. We also report that plasminogen activator activity in clinically uninvolved psoriatic epidermis increases significantly after the tissue is grafted onto nude mice; this is associated with an increased labeling index. These findings suggest that psoriatic epidermis can manifest markers of psoriasis independent of the host.

Specimens were removed from psoriatic plaques and from uninvolved (normal-appearing) skin on the hip and proximal thigh in nine male patients with psoriasis. Three normal males provided control skin (informed consent was obtained from all the donors). The tissue was removed with a Castroviejo dermatome to a depth of 0.4 mm for uninvolved skin and 0.6 to 0.7 mm for lesions. Half of each specimen was immediately grafted onto athymic nude mice. Each animal received two grafts 6 mm in diameter (10 to 18 animals per specimen). The remaining half of each specimen was incubated in 2*M* KBr for 30 minutes at 37°C, permitting mechanical separation of epidermis from the dermis. The harvested epidermis was washed in sterile saline, mixed into 2*M* KCl and 0.01*M* $NaH_2PO_4$ and $Na_2HPO_4$ buffer (*p*H 7.0), and frozen at −70°C. The brief potassium bromide treatment did not affect the level of plasminogen activator or of the lysosomal proteinase cathepsin D in separated epidermis.

The transplanted skin was harvested from the nude mice 6 weeks after grafting. One hour before the mice were killed, [$^3H$] thymidine (Schwarz/Mann; specific activity, 7 to 10 mCi/mole) was


0036-8075/82/0205-0685$01.00/0  