CONTINUED SYNTHESIS OF NON-HISTONE CHROMOSOMAL PROTEINS DURING MITOSIS*

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SUMMARY. The synthesis of non-histone chromosomal proteins in synchronized HeLa S_3 cells was studied in 3 phases of the cell cycle: S, G_2 and mitosis. A 70-90% decrease in the rate of synthesis of total cellular protein and of chromatin-associated protein fractions extractable with low salt concentrations or with dilute acid was observed in cells in mitosis. On the contrary, the residual non-histone chromosomal proteins were synthesized during mitosis at a rate which did not differ significantly from that of S and G_2 phases. Thus, at variance with the other cellular proteins, acidic chromosomal proteins are synthesized at an undiminished rate even during mitosis, when there is complete cessation of RNA synthesis.

INTRODUCTION

Teng and Hamilton (1) have recently suggested that acidic nuclear proteins may be involved in the response of the uterus to estrogen stimulation, and Stein and Baserga (2) have demonstrated that synthesis of acidic nuclear proteins is markedly increased in salivary glands stimulated to synthesize DNA by isoproterenol. Similar findings have been reported by Rovera and Baserga (3) in contact-inhibited human diploid fibroblasts stimulated by a change of medium. These studies suggest that non-histone chromosomal proteins may have a role in the control of DNA synthesis and cell division, as well as of gene expression in general (4).

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In 1960, Taylor (5) demonstrated that mammalian cells synthesize RNA in all phases of the cell cycle except during mitosis, when there is cessation of RNA synthesis. These findings, indicating an interruption of gene expression, have been confirmed by several investigators (6,7,8), and it is generally accepted now that mammalian cells do not synthesize RNA during metaphase and anaphase (9). It was therefore thought interesting to compare the synthesis of non-histone chromosomal proteins during mitosis and during other phases of the cell cycle in synchronized HeLa S_3 cells.

METHODS. HeLa S_3 cells were maintained in suspension culture in Eagle's Minimum Essential Medium for suspension culture (10) supplemented with 3.5% each of calf and fetal calf serum. Synchronization of cells was achieved by a 24-hour, 2 mM thymidine block (11,12) followed by selective detachment of mitotic cells (13,14). The entire procedure was carried out in a warm room maintained at 37°C.

Chromatin was prepared by the method of Marushige and Bonner (15). The cells were homogenized in 0.075 M NaCl, 0.024 M Na EDTA, pH 8.0 with a Dounce homogenizer and centrifuged at 1500 • g for 15 minutes. The pellet was washed with the same solution and centrifuged again at 1500 • g for 15 minutes. It was next washed with 0.05 M Tris pH 8.0 and centrifuged at 1500 • g for 15 minutes. This was followed by homogenization in 0.05 M Tris, pH 8.0 with a Dounce homogenizer and centrifugation at 10,000 • g for 15 minutes. The last step was repeated. The pellet was homogenized in 5 ml of 0.05 M Tris, pH 8.0 and layered over 25 ml of 1.7 M sucrose in a 1"x3" centrifuge tube. The upper two-thirds of the tube were gently mixed and then centrifuged at 50,000 • g for 3 hours in a Spinco SW 25 rotor.

The pelleted chromatin was extracted twice with 0.15 M NaCl and twice with 0.35 M NaCl. This was followed by two extractions with 0.25 N H_2SO_4, yielding a crude histone fraction in the supernatant and a pellet containing residual proteins and nucleic acids. This pellet was extracted with hot 5% trichloracetic acid to remove nucleic acids. The pellet containing

the residual chromatin proteins was solubilized in 1 N NaOH. Perchloric acid was added to the nucleic acid extract to a final concentration of 0.5 N.

DNA was determined by Burton's modification of the diphenylamine reaction (16). RNA was determined by the orcinol reaction (17) and protein was assayed by the method of Lowry et al. (18).

The rates of DNA and protein synthesis at various times following release of HeLa S_3 cells from a 2 mM thymidine block were determined as described in Fig. 1 and Table 1. For autoradiography, aliquots of the HeLa cells pulse-labeled with thymidine-^{3}H were made hypotonic with water, smeared on glass slides, fixed in Carnoy's fluid and autoradiographed as described in detail by Baserga (19). Eastman Kodak NTB emulsion was used and exposure time was 7 days. The mitotic index (metaphase, anaphase and telophase cells per 1000 cells) and the percentage of labeled cells were determined on 1000 cells per preparation.

MATERIALS. Eagle's Minimum Essential Medium for suspension culture and Eagle's Minimum Essential Medium with Earle's Balanced Salts were purchased from Microbiological Associates, Bethesda, Maryland. Spinner Salts Solution (Eagle's) was purchased from Grand Island Biological Company, Grand Island, New York. Calf serum and fetal calf serum were purchased from Flow Laboratories, Rockville, Maryland. Thymidine was purchased from Sigma Chemical Company, St. Louis, Missouri. L-leucine-^{3}H (58 Ci/mM) and thymidine-methyl-^{3}H (6.7 Ci/mM) were purchased from New England Nuclear Corporation, Boston, Mass. The HeLa S_3 cells were a gift from Dr. Matthew Scharff of the Department of Cell Biology, Albert Einstein College of Medicine, New York, and were free of mycoplasma contamination.

RESULTS. The rate of incorporation of thymidine-^{3}H into DNA, the percentage of cells in DNA synthesis and the mitotic index were determined every hour following release of the thymidine block (Fig. 1). The selection of S and G_2 cells, as well as the time for selective detachment of mitotic

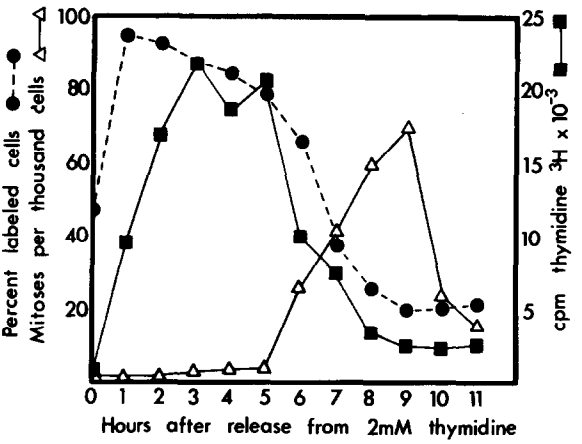

Figure 1. Incorporation of thymidine-^3H into DNA, percentage of cells in DNA synthesis and mitotic index at various times following release of HeLa S_3 cells from a 2 mM thymidine block. Cells were pulsed with 5 μCi/ml of thymidine-^3H for 15 minutes. The rate of DNA synthesis was determined by the amount of radioactivity incorporated into 5% trichloroacetic-acid precipitable material. The percentage of cells in DNA synthesis and the mitotic index were determined from the autoradiographic preparations (see Methods).

figures was based on these results. S and G_2 cells were harvested at 3-1/2 and 7-1/2 hours respectively after release of the thymidine block, and mitotic cells were selectively detached at 9 hours. Ninety five per cent of the selectively detached cells were in mitosis, as determined by light microscopy.

The rate of synthesis of cellular proteins during S, G_2 and mitosis is shown in Table 1. The 0.15 M and 0.35 M NaCl fractions from chromatin show a decreased rate of synthesis during mitosis in comparison to S and G_2, and this marked decrease in the rate of protein synthesis is also evident in the total cellular proteins. As would be anticipated, the incorporation of leucine-^3H into the 0.25 N H_2SO_4 fraction, which contains histones, is drastically reduced below the S phase level during mitosis. At variance with the other fractions examined, the synthesis of non-histone chromosomal proteins (residual acidic proteins) continues during mitosis at approximately the same rate as during G_2 and S. This experi-

TABLE 1

Synthesis of Total Cellular and Chromatin Proteins During S, G_2 and Mitosis

Protein Fraction	Phase of Cell Cycle		
	S	G_2	M
Total Cell Protein	7919	7258	2881
0.15 M NaCl	61	50	21
0.35 M NaCl	38	31	11
0.25 N H_2SO_4	940	127	110
Residual Chromatin Fraction	1372	1316	1240

Protein synthesis was assayed by harvesting 300 ml of cells and resuspending them in 10 ml of leucine-free suspension medium supplemented with 2% fetal calf serum and 150 µCi of leucine-^{3}H. After incubation at 37°C for 15 minutes the cells were washed 3 times in cold Spinner Salts Solution. Total cell protein synthesis was based on the 20% TCA precipitable leucine-^{3}H counts of the initial cellular homogenate. The other protein fractions were all obtained from chromatin preparations as described in the text. The incorporation of leucine-^{3}H into the various protein fractions is expressed as cpm/µg DNA.

TABLE 2

Amount of Protein in Each Fraction of the Chromatin Preparation

Fractions	Protein/DNA
.15 M NaCl	0.04
.35 M NaCl	0.07
.25 N H_2SO_4 (crude histones)	0.80
Residual Proteins	0.91

Chromatin was prepared by the method described in the text and the proteins were fractionated as described; however, the hot TCA extraction of nucleic acids was omitted. The amount of protein in each fraction was determined by the method of Lowry et al. (18) and the amount of DNA in the whole chromatin preparation was determined on a separate aliquot.

ment was repeated twice and, although the absolute values varied somewhat from one experiment to the next one, the difference between non-histone chromosomal proteins and the other protein fractions during mitosis was quantitatively constant.

The chromatin isolated from HeLa log phase S_3 cells had an RNA/DNA ratio of 0.095 and a protein/DNA ratio of 1.8. The amount of each protein fraction in the chromatin preparation, expressed as protein/DNA ratio, is given in Table 2. The two NaCl-soluble fractions constitute a small part of the chromatin-associated proteins and probably represent cytoplasmic contamination (20) while it is evident that histones and residual proteins which are acidic (Table 3) account for the bulk of the chromatin proteins. The latter two fractions are present in approximately equal amounts.

DISCUSSION. The decreased rate of total cellular protein synthesis during mitosis is in agreement with previous findings reported by Baserga (21), Prescott and Bender (7), Konrad (22), and Robbins et al. (23). This is paralleled by a similar decrease in the synthesis of the 0.15 M and 0.35 M NaCl fractions extractable from chromatin. Robbins and Scharff (23) have

TABLE 3

Amino Acid Composition of Residual Chromosomal Proteins

	Moles per 100 Moles Recovered		
alanine	6.01	phenylalanine	4.74
valine	5.12	aspartic acid	9.66
glycine	6.98	glutamic acid	16.07
isoleucine	4.33	tyrosine	3.90
leucine	9.52	lysine	8.19
proline	4.50	histidine	1.60
threonine	4.54	arginine	6.81
serine	5.60	half cystine	0.38
methionine	2.08		

$$\frac{\text{aspartic acid + glutamic acid}}{\text{arginine + lysine + histidine}} \quad 1.55$$

The protein was hydrolysed in 6 N HCl in a vacuum at 145°C prior to amino acid analysis by gas-liquid chromatography.

determined the size of the amino acid pools throughout the cell cycle and found that there are only minor fluctuations; thus, incorporation of leucine-^{3}H ought to reflect synthesis and should not be secondary to changes in the precursor pool. This is also confirmed by the differential decrease in specific activity of the various chromatin fractions. Since the per cent of leucine in the residual chromatin protein fraction (Table 3) is similar to that found in total cellular proteins (24,25), it is unlikely that the continued high rate of incorporation of leucine-^{3}H into non-histone chromosomal proteins during mitosis may represent the synthesis of a particularly leucine-rich protein. It is of considerable interest that the non-histone chromosomal proteins (residual acidic proteins) continue to be synthesized during mitosis at a rate that differs little from that observed during S phase and G_2. The fact that these proteins are actively synthesized at a time when RNA synthesis is completely shut down is a further indication that they may be involved in the regulation of gene expression as already suggested by Gilmour and Paul (26) and by Stellwagen and Cole (4).

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