RIBONUCLEIC ACID AND PROTEIN SYNTHESIS IN MITOTIC HELA CELLS

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ABSTRACT

HeLa cells arrested in mitosis were obtained in large numbers, with only very slight interphase cell contamination, by employing the agitation method of Terasima and Tolmach, and Robbins and Marcus. Protein synthesis and RNA synthesis were almost completely suppressed in mitotic cells. Active polyribosomes were nearly absent in mitotic cells as compared with interphase cells treated in the same way. Cell-free protein synthesis and RNA polymerase activity were also greatly depressed in extracts of metaphase cells. The deoxyribonucleoprotein (DNP) of condensed chromosomes from mitotic cells was less efficient as a template for *Escherichia coli* RNA polymerase than was DNP from interphase cells, although isolated DNA from both sources was equally active as a primer. Despite very poor endogenous amino acid incorporation by extracts of metaphase cells, polyuridylate stimulated phenylalanine incorporation by a larger factor in mitotic cell extracts than it did in interphase cell extracts. These results suggest that RNA synthesis is suppressed in mitotic cells because the condensed chromosomes cannot act as a template, and that protein synthesis is depressed at least in part because messenger RNA becomes unavailable to ribosomes. This conclusion was supported by the demonstration that cells arrested in metaphase supported multiplication of normal yields of poliovirus, thereby showing that the mitotic cell is capable of considerable synthesis of RNA and protein.

INTRODUCTION

Robbins and Marcus (15) have modified the method of Terasima and Tolmach (19) for the harvesting of mitotic cells so that large numbers of metaphase cells can be obtained for biochemical studies. This method is based on the poor adhesion of mitotic cells to glass in monolayer cultures in a low-calcium medium.

Taylor (18) showed by radioautography that RNA synthesis is depressed in cells in mitosis, but he found normal incorporation of arginine into protein. More recent radioautographic evidence (12, 14) suggests that both RNA synthesis and protein synthesis are inhibited during mitosis in mammalian cells. The availability of a method for obtaining quantities of metaphase cells nearly free of interphase cell contamination made it possible to examine the synthesis of protein and nucleic acid in mitotic HeLa cells using conventional biochemical techniques. Marcus and Robbins (9) used their technique to show that mitotic cells incorporate uridine into RNA at a very low level, and that they are refractory to virus infection as compared with interphase cells.

MATERIALS AND METHODS

Cell Culture

HeLa cells were grown in monolayer cultures in 16-ounce or 32-ounce glass bottles. The medium consisted of Eagle's minimal medium with 10 per cent calf serum.

Metaphase arrest cells were obtained essentially by the method of Robbins and Marcus (15). After a period of growth in calcium-free Eagle's medium

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supplemented with 10 per cent whole calf serum and 0.05 μg/ml vinblastine (Velban, Eli Lilly and Company, Indianapolis, Indiana), cells arrested in metaphase were freed from the monolayer by gentle agitation with warm 0.15 M NaCl. The firmly adherent interphase cells did not detach from the glass even after fairly vigorous shaking. Occasionally small sheets of interphase cells were torn from the monolayer, but it was found that these small cell clumps could easily be removed by filtering the cell suspension through several layers of gauze. The mitotic cells were then collected by centrifugation.

The remaining interphase cells were collected by 0.05 per cent trypsin treatment. After the removal of the mitotic cells as described above, to assure that very few mitotic cells remained, the monolayers were shaken vigorously with warm 0.15 M NaCl solution prior to the addition of trypsin.

RNA Polymerase Assay

RNA polymerase was prepared from *Escherichia coli* and purified and assayed as described by Chamberlin and Berg (1), except that *E. Coli* K12 was used and was harvested in logarithmic phase from brain-heart infusion broth.

HeLa cell RNA polymerase activity was assayed as the deoxyribonucleoprotein complex referred to as aggregate enzyme (21). Aggregate enzyme was prepared from nuclei of interphase cells (or the 0.15 M saline-insoluble deoxyribonucleoprotein of mitotic cells), as described by Weiss (21), and assayed for polymerase activity in the high ionic strength reaction mixture of Goldberg (3).

RNA synthesis by either polymerase was assayed as the incorporation of P^{32}-uridylate into acid-insoluble material which remained after repeated alcohol and acid precipitation. The final precipitate was collected on nitrocellulose filters and counted in a Nuclear-Chicago gas-flow counter. Exact conditions for each experiment are presented in the appropriate figure or table in the Results section.

Preparation of Polymerase Substrate

UTP labeled in the α position with P^{32} was employed as radioactive substrate for RNA polymerase reaction mixtures along with unlabeled ATP, GTP, and CTP. A modification of the method of Lehman, Bessman, Simms and Kornberg (8) for deoxyribonucleotide kinases was used to prepare crude ribonucleotide kinases for the conversion of 5′-UMP^{32} to the triphosphate. The UMP^{32} was incubated with the crude kinase, phosphoenolpyruvate, pyruvate kinase (Calbiochem) and ATP; UTP^{32} was isolated by chromatography on Dowex 1 × 2 (8). UMP^{32} was chemically synthesized from 10 to 20 mc P^{32} orthophosphate and uridine by a modification of the method used by Hurwitz (5) for preparation of cytidylate, and the 5′-isomer was separated from the 2′- and 3′-isomers by chromatography on Dowex 1 × 2. Yields of 5 to 10 μmoles of UTP^{32} with specific activities above 5×10^7 CPM per μmole were usually obtained.

In vitro Amino Acid Incorporation

Protein synthesis *in vitro* by cytoplasmic extracts of HeLa cells was measured by a modification of the method of Keller and Zamecnik (7). The reaction mixtures contained in 1.0 ml: 50 μmoles of tris-HCl buffer, pH 7.7; 5 μmoles of $MgCl_2$; 60 μmoles of KCl; 250 μmoles of sucrose; 5 μmoles of mercaptoethanol; 1 μmoles of ATP; 0.1 μmole of GTP; 10 μmoles of phosphoenolpyruvate; 20 μg of pyruvate kinase; ^{14}C-amino acids as indicated; and cytoplasmic supernatant after the removal of nuclei from disrupted HeLa cells. HeLa cells were thoroughly washed with 0.15 M NaCl before disruption. The last wash was with 0.07 M KCl at 0°C, and the cells were quickly concentrated by centrifugation, and suspended in 3 to 4 volumes of 0.001 M $MgCl_2$ solution. The cells were allowed to swell for 5 minutes at 0°C and were disrupted with 5 strokes of a close-fitting Dounce homogenizer at a low speed of rotation. Radioactive amino acids employed for incorporation studies were obtained from New England Nuclear Corp., Boston: C^{14}-valine (185 mc/mmole), C^{14}-arginine (242 mc/mmole), C^{14}-lysine (223 mc/mmole), C^{14}-phenylalanine (334 mc/mmole), and C^{14} algal protein hydrolysate (1.43 mc/mg). After incubation at 37°C the proteins were repeatedly washed and precipitated with hot 5 per cent TCA (17), and 1 mg or less of the washed precipitate was collected on a nitrocellulose filter, dried, and counted in a Nuclear-Chicago gas-flow counter. Washing of the precipitate with lipid solvents had little or no effect, and was not usually performed.

Sucrose Gradient Velocity Sedimentation

RNA was analyzed by sedimentation in the Spinco SW 39 swinging bucket rotor in a 5 to 20 per cent sucrose gradient containing 0.02 M tris-HCl buffer, pH 7.5, 0.002 M EDTA; and 0.1 M KCl. Centrifugation was carried out at 39,000 RPM for 4.5 hours at 2 to 4°C. Polyribosomes were analyzed in the SW 25.1 swinging bucket rotor in a 5 to 20 per cent sucrose gradient containing RSB (0.1 M NaCl, 0.01 M tris-HCl buffer, pH 7.4, 0.015 M $MgCl_2$). Cytoplasmic supernatants were prepared as described by Penman, Scherrer, Becker and Darnell (13). Centrifugation was carried out at 27,500 RPM for 90 minutes at 2 to 4°C.

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TABLE I

*Incorporation of P^{32} Into RNA in Mitotic and Interphase HeLa Cells**

HeLa cells employed	Labeling time	P^{32} CPM in total cellular RNA¶	Per cent of labeling
	hrs.		
Experiment 1‡			
Interphase cells	2	500,000	100 (control)
Mitotic cells	2	16,700	3
Experiment 2§			
Interphase cells	2.5	790,000	100 (control)
Mitotic cells	2.5	40,000	5
Experiment 3‖			
Interphase cells (not treated with vinblastine)	2.5	667,000	100 (control)
Interphase cells (treated with vinblastine)	2.5	686,000	—
Mitotic cells	2.5	53,700	8

* P^{32} orthophosphate (0.25 mc) in Eagle's medium, minus cold phosphorus, was incubated at 37°C with 10^7 interphase cells.

‡ Cells were harvested after 20-hr. incubation with 0.05 μg/ml vinblastine.

§ Cells were harvested after 12-hr incubation with 0.05 μg/ml vinblastine.

‖ Cells were harvested after 24-hr. incubation with 0.1 μg/ml vinblastine (except for the untreated interphase cells).

¶ RNA was extracted by the hot phenol-SDS method of Scherrer and Darnell (16) and repeatedly precipitated from ethanol to free bound radioactivity.

RESULTS

RNA and Protein Synthesis by Interphase and Mitotic HeLa Cells

HeLa cells arrested in mitosis and HeLa cells in interphase were obtained by the above described method after treatment with the spindle inhibitor vinblastine as employed by Marcus and Robbins (9). Interphase cells had been exposed to vinblastine for the same period of time as were the cells arrested in mitosis by the vinblastine treatment and removed from the monolayers by agitation. Vinblastine caused no depression of the ability of interphase cells to synthesize protein or nucleic acid. It can be seen in Table I (experiment 3) and Table II (experiment 1) that even after 24-hour exposure to 0.1 μg/ml vinblastine (twice the usual concentration), interphase cells synthesized protein and RNA at the same rate as untreated cells. Furthermore, it appears that vinblastine was not selectively taken up by mitotic cells and excluded from interphase cells. Short periods of treatment of interphase cells with 1 μg/ml of vinblastine at 37°C followed by thorough washing and replacement with fresh medium led to accumulation for the next 3 to 5 hours of cells arrested in mitosis. This indicates that interphase cells take up vinblastine and maintain an intracellular concentration in the absence of vinblastine in the medium.

Table I shows that the synthesis of P^{32}-RNA by isolated mitotic HeLa cells is only a small fraction of the rate in interphase cells, in confirmation of the findings of Taylor (18), Prescott and Bender (14), Monesi (12), and Marcus and Robbins (9). Fig. 1 shows the sucrose gradient sedimentation patterns of the P^{32}-RNA synthesized by mitotic and interphase cells. It can be seen that almost no ribosomal RNA is made during mitosis, and the small amount of P^{32} incorporated into transfer RNA probably represents terminal addition of nucleotides (4), rather than synthesis.

Table II shows that incorporation of amino acids into HeLa cell protein during mitosis is also markedly reduced as compared to similarly treated interphase cells. It can be seen, however, that the uptake of C^{14}-amino acids into the intracellular pool of mitotic HeLa cells is about the same as, or slightly greater than, that occurring in interphase cells. This confirms the radioautographic

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TABLE II

*Incorporation of C^{14}-Amino Acids into Protein and into the Intracellular Pool of Mitotic and Interphase HeLa Cells**

HeLa cells employed	C^{14}-amino acid	Labeling time	C^{14} CPM in intracellular pool per mg cell protein	C^{14} CPM in protein per mg cell protein	Per cent amino acid in protein
		hrs.			
Experiment 1‡	Phenylalanine				
Interphase cells (not treated with vinblastine)		1.0	—	75,000	—
Interphase cells (vinblastine treated)		1.0	—	92,000	100
Mitotic cells		1.0	—	7,900	9
Experiment 2§	Arginine				
Interphase cells		1.0	4,900	24,500	100 (control)
Mitotic cells		1.0	5,400	2,200	9
Experiment 3‖	Valine				
Interphase cells		1.0	5,000	87,000	100 (control)
Mitotic cells		1.0	5,190	17,500	20
Experiment 4¶	Lysine				
Interphase cells		0.5	4,200	64,000	100 (control)
Mitotic cells		0.5	3,900	9,000	12

* All cells were incubated with 1 μc of C^{14} amino acid in Eagle's medium prepared without the labeled amino acid. After labeling, cells were washed and repeatedly precipitated with hot TCA (Siekevitz, reference 17).

‡ Cells were harvested after 24-hr. incubation with 0.1 μg/ml vinblastine (except for untreated interphase cells).

§ Cells were harvested after 20-hr. incubation with 0.05 μg/ml vinblastine.

‖ Cells were harvested after 3-hr. incubation with 0.05 μg/ml vinblastine.

¶ Cells were harvested after 20-hr. incubation with 0.05 μg/ml vinblastine.

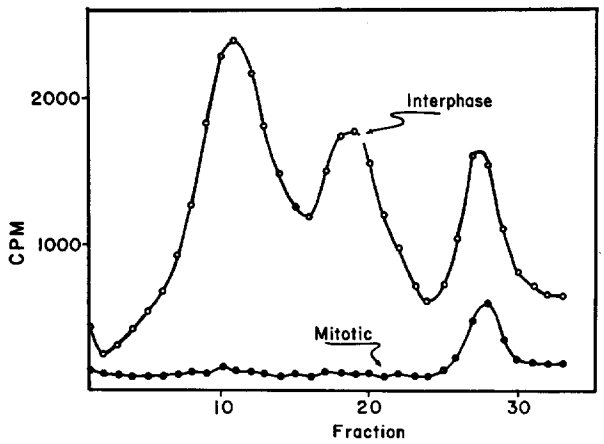

FIGURE 1 Sucrose sedimentation pattern of P^{32}-RNA from mitotic and interphase HeLa cells. Mitotic (●—●) and interphase (○—○) cells were separated by agitation after 12-hour treatment with 0.05 mg/ml vinblastine in Eagle's medium, then exposed to 0.25 mc P^{32} orthophosphate in Eagle's medium minus cold phosphate for 2.5 hours at 37°C. Sedimentation was carried out with the Spinco SW 39 rotor for 4.5 hours, 39,000 RPM, 2 to 4°C.

evidence of Prescott and Bender (14). It is not clear why Taylor (18) observed considerable C^{14}-arginine incorporation in radioautography of HeLa cells, whereas under our conditions the incorporation of arginine into acid-insoluble polypeptide is greatly depressed (Table II). Experiment 3 in Table II shows that mitotic cells exhibited reduced protein synthesis even when the

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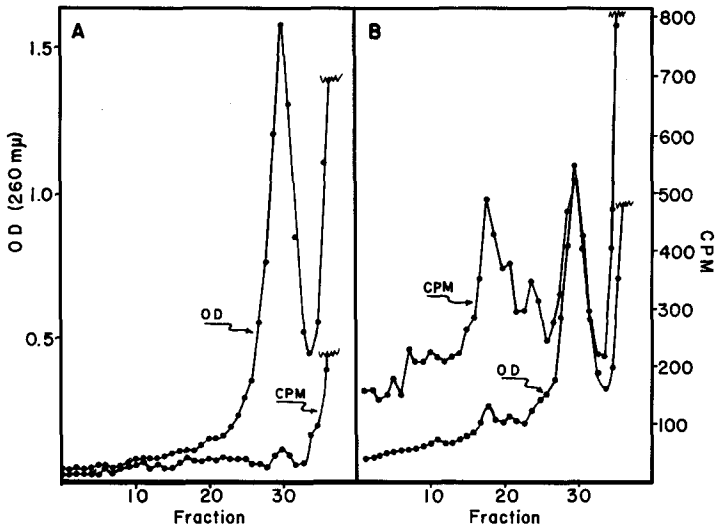

FIGURE 2 C^{14}-valine-labeled polyribosomes from (A) mitotic and (B) interphase HeLa cells. Mitotic and interphase cells were separated by agitation after 18 hours at 37°C in Eagle's medium with 0.05 μg/ml vinblastine. Aliquots of 2×10^7 cells of each type were then starved for valine for 30 minutes in Eagle's medium minus valine at 37°C, pulse-labeled for 2 minutes with 5 μc C^{14}-valine, washed 3 times, lysed in 1.0 ml RSB; the nuclei were removed by centrifugation, and the cytoplasmic supernatants were brought to 0.5 per cent final concentration of sodium deoxycholate (Penman *et al.*, reference 13). Sedimentation was carried out in 5 to 20 per cent sucrose containing RSB in the Spinco SW 25.1 rotor, 27,500 RPM, 2 to 4°C. Fractions collected from the bottom of the tube were analyzed for OD at 260 mμ, and aliquots were precipitated in 5 per cent TCA and carrier serum albumin, collected as precipitates on nitrocellulose filters, and counted.

TABLE III

*"Aggregate Enzyme" RNA Polymerase Activity of Cell-Free Extracts from Mitotic and Interphase Hela Cells**

Cell source of "aggregate enzyme"	mμ moles of P^{32}-UTP incorporated/mg "aggregate enzyme" protein	Per cent of polymerase activity
Experiment 1		
Interphase cells (not treated with vinblastine)	0.85	—
Interphase cells	0.85	100 (control)
Mitotic cells	0.09	11
Experiment 2		
Interphase cells	0.88	100 (control)
Mitotic cells	0.09	11
Experiment 3		
Interphase cells	0.60	100 (control)
Mitotic cells	0.12	20
Interphase cells plus 1 mg cytoplasmic protein from mitotic cells	0.84	140

* Mitotic and interphase cells were harvested after 20-hr. incubation with 0.05 μg/ml vinblastine. "Aggregate enzyme" RNA polymerase was prepared from interphase nuclei or 0.15 M NaCl-insoluble chromatin from mitotic cells essentially as described by Weiss (21). The reaction mixture of Goldberg (3) contained in 0.5 ml: 50 μmoles of tris-HCl buffer, (pH 8.0); 1.5 μmoles of $MnCl_2$; 0.05 ml of saturated $(NH_4)_2SO_4$ (pH 8.0); 7 μmoles of 2-mercaptoethanol; 0.4 μmoles of CTP, GTP, ATP; 0.4 μmoles of P^{32}-UTP; and "aggregate enzyme" containing 1 mg of protein. The reaction mixtures were incubated at 37°C for 20 min. The amount of DNA was about equivalent in preparations of "enzyme" from interphase and mitotic cells.

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TABLE IV

Ability of DNA and Deoxyribonucleoprotein from Mitotic and Interphase Cells to Serve as a Template for E. coli RNA Polymerase

Nature of nucleic acid template, and conditions of reaction	mμ moles of nucleotide of template DNA or DNP added	Incorporation of mμ moles of P^{32}-UTP
Experiment 1		
Interphase cell DNP, no added polymerase	160	0.1
Mitotic cell DNP, no added polymerase	200	0.1
Interphase cell DNP plus *E. coli* polymerase	160	4.6
Mitotic cell DNP plus *E. coli* polymerase	200	1.6
Interphase cell DNA plus *E. coli* polymerase	160	12.3
Mitotic cell DNA plus *E. coli* polymerase	200	10.2
Experiment 2		
Interphase cell DNP plus *E. coli* polymerase	15	0.57
Mitotic cell DNP plus *E. coli* polymerase	15	0.15
Interphase cell DNA plus *E. coli* polymerase	15	3.3
Mitotic cell DNA plus *E. coli* polymerase	15	3.4
No template, plus *E. coli* polymerase	0	0.1

Reaction mixture of Chamberlin and Berg (1), contained in 0.25 ml: 10 μmoles of tris-HCl, pH 8.0; 0.25 μmole $MnCl_2$; 1.0 μmole of $MgCl_2$; 100 μmoles each of GTP, UTP, CTP; 100 μmoles of P^{32}-ATP; 3.0 μmoles of 2-mercaptoethanol; and 200 units *E. coli* polymerase.

Enzyme was always added in excess, and DNP or DNA was added in low concentrations so that it would be the rate-limiting component. DNA or DNP added as indicated. Reaction allowed to proceed for 10 min. at 37°C, and stopped by addition of cold perchloric acid.

Chromosomal DNP from mitotic cells and nuclear DNP from interphase cells was prepared by sonication of cells suspended in 10^{-3} M $MgCl_2$ (two 3-second bursts at maximum intensity with a Branson 20 KC sonifier) followed by addition of 4 M KCl to bring the KCl concentration to 0.2 M. The insoluble DNP was sedimented, washed, and resedimented three times in 0.15 M NaCl solution, then dispensed into aliquots, some of which were extracted with phenol and 1 per cent SDS at pH 7.5 at 60°C to obtain DNA for parallel testing.

mitotic cells were collected after only 3-hour incubation in vinblastine.

Polyribosomes in Interphase and Mitotic HeLa Cells

Since the original studies of polyribosomes (20, 10, 22), evidence has accumulated that most protein synthesis takes place on a structure, referred to as polyribosomes, which is composed of messenger RNA and a number of ribosomes. We examined mitotic and interphase HeLa cells for the presence of polyribosomal complexes. It can be seen in Fig. 2 that interphase HeLa cells incorporated considerable C^{14}-amino acid into acid-insoluble polypeptide in the polyribosomal region. However, the mitotic cells showed almost no incorporation in the polyribosomal or the ribosomal area, and the optical density profiles show an increase of single 76S ribosomes with very little optical density in the polyribosomal area as compared with interphase cells. These results suggest (but do not prove) that messenger RNA is nearly absent in mitotic cells or else that it is in some way prevented from forming active polyribosomal complexes.

Synthesis of RNA by "Aggregate Enzyme" RNA Polymerase in Cell-Free Extracts of Interphase and Mitotic HeLa Cells

It was of interest to determine if the depression of RNA synthesis observed in intact mitotic cells is reflected by a depressed polymerase activity in cell extracts. Table III shows that "aggregate enzyme" RNA polymerase activity (21) is markedly reduced in cell-free extracts from mitotic cells. This indicates that RNA polymerase is not functional in mitotic HeLa cells, or else it is no longer in an active complex with DNA as it is in interphase cells, or it is more unstable. Preparation of aggregate enzyme RNA polymerase depends on the enzyme being complexed with DNA in the insoluble deoxyribonucleoprotein precipitate (21). Table III also shows that extracts of mitotic cells did not contain an inhibitor of interphase cell RNA polymerase activity, since mixture of mitotic cell cytoplasmic supernate with interphase cell aggregate enzyme did not depress the activity of the latter. It can also be seen (Table I) that vinblastine treatment has no effect on the polymerase activity of interphase cells.

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TABLE V

Effect of Poly U on Incorporation of C^{14}-Amino Acids in vitro by Cytoplasmic Extracts from Mitotic and Interphase HeLa Cells

Source of cytoplasmic extract	Cts/min C^{14}-amino acid incorporated: No added poly U	With 200 μg poly U added
Experiment 1*		
Interphase cells	987	2,556
Mitotic cells	229	1,839
Experiment 2*		
Interphase cells	1,300	9,800
Mitotic cells	380	6,400
Experiment 3§		
Interphase cells	1,400	11,000
Mitotic cells	141	11,800
Experiment 4‡		
Interphase cells	743	—
Mitotic cells	202	—

* Mitotic and interphase cells obtained after 20-hr. incubation in 0.05 μg/ml vinblastine. 0.5 μc C^{14}-phenylalanine employed.

‡ Mitotic and interphase cells obtained after 11-hr. incubation in 0.05 μg/ml vinblastine. 0.05 μc C^{14}-protein hydrolysate employed.

§ Mitotic and interphase cells obtained after 7-hr. incubation in 0.05 μg/ml vinblastine. 0.5 μc C^{14}-phenylalanine employed.

Reaction mixture contained in 1.0 ml: 5 μmoles of tris-HCl buffer, pH 7.7; 5 μmoles of $MgCl_2$; 10 μmoles phosphoenolpyruvate; 20 μg of pyruvate kinase; C^{14}-phenylalanine, plus 10^{-5} M of all the other 10 cold amino acids. Incubation was at 37°C for 30 min.

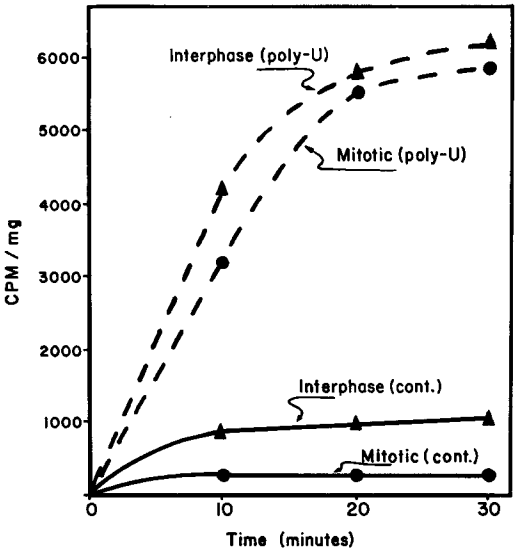

FIGURE 3 Kinetics of C^{14}-phenylalanine incorporation into polypeptide by cytoplasmic extracts of mitotic and interphase HeLa cells, with and without added poly-U template. Reaction conditions exactly as for Table V, except that the total reaction mixture contained 0.6-ml, and 0.1-ml aliquots, were withdrawn at intervals during incubation. ▲—▲, interphase cell extract with no added polynucleotide. ▲---▲, interphase cell extract with 400 μg poly-U added at 0 minutes. ●—● mitotic cell extract with no added polynucleotide. ●---● mitotic cell extract with 400 μg poly-U added.

Ability of Deoxyribonucleoprotein from Mitotic and Interphase Cells to Serve as a Template for E. coli RNA Polymerase

The above results suggest the obvious possibility that RNA synthesis might be depressed in mitotic cells because the DNA in condensed chromosomes is inaccessible to RNA polymerase as compared with the more dispersed DNA of interphase cells. Table IV demonstrates that the deoxyribonucleoprotein (DNP) of mitotic cells is less efficient as a template for purified RNA polymerase from *E. coli* than is the same amount of DNP from interphase cells.

However, DNA extracted from this DNP was equally active as a template whether it originated from mitotic or interphase cells. The template activity which mitotic cell DNP did exhibit could have resulted from partial dispersion of its DNA during preparation.

Amino Acid Incorporation by Cell-Free Extracts of Mitotic and Interphase HeLa Cells

We next examined polypeptide synthesis in cell-free cytoplasmic extracts from mitotic and interphase cells, since the results in Table I and Fig. 2 would indicate that ribosomes from mitotic cells might be relatively inactive in amino acid incorporation and free rather than complexed with messenger. We also examined the incorporation of C^{14}-phenylalanine with and without the addition of poly-U as a template. Table V shows that mitotic cell extracts were greatly depressed in their ability to incorporate amino acids into acid-insoluble polypeptide, as compared to interphase cell extracts. However, it can be seen that poly-U

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TABLE VI

Replication of Poliovirus in Mitotic and Interphase HeLa Cells

HeLa cells employed	Total cell number	Total number of infectious centers	Total virus yield	Yield of plaque-forming units per cell
Experiment 1*				
Interphase cells	7×10^5	3×10^5	3×10^8	430
Mitotic cells	7×10^5	3×10^5	3×10^8	430
Experiment 2*				
Interphase cells	1.4×10^6	5×10^5	4×10^8	290
Mitotic cells	8×10^5	3×10^5	3×10^8	370
Experiment 3‡				
Interphase cells	4×10^6	—	1.2×10^8	300
Mitotic cells	4×10^6	—	1.1×10^8	280
Mitotic cells (incubation in presence of 10^{-3} M guanidine)	4×10^6	—	4×10^5	0.1

* Monolayer cultures were allowed to adsorb 100 PFU/cell type 1 (Mahoney) poliovirus at 37°C, then were washed and incubated for 9 hrs. in Eagle's medium containing 0.05 μg/ml vinblastine and 10^{-3} M guanidine (a specific inhibitor of enterovirus replication). After 9 hrs. the mitotic and interphase cells were separated by agitation, washed free of guanidine, and incubated for another 12 hrs. in the presence of vinblastine before virus assay (or aliquots were plated under agar medium for infectious center assay).
‡ Mitotic cells and interphase cells were separated after 9-hr. incubation in 0.05 μg/ml vinblastine, infected with 100 PFU/cell type 1 poliovirus, washed free of excess virus, and incubated for an additional 12 hrs. in vinblastine medium before cell disruption and virus assay. As a control to prove that virus replication is involved, one aliquot of infected mitotic cells was incubated in the presence of 10^{-3} M guanidine to prevent virus replication.
All virus assays were performed after disruption of cells by repeated freezing and thawing. Virus assays and infectious center assays were carried out under Eagle's medium containing 0.6 per cent agar.

stimulated C^{14}-phenylalanine incorporation by a much greater factor in mitotic cell extracts than it did in interphase cell extracts. The kinetics of one experiment are shown in Fig. 3. In some experiments, the total incorporation of C^{14}-phenylalanine by poly-U–stimulated mitotic cell extracts exceeded that of interphase extracts. Since poly-U was added at saturating concentrations of 200 μg/ml, the limiting component in these experiments was probably ribosomes. These findings indicate that the enzymatic and ribosomal machinery for protein synthesis are capable of function in mitotic cells, but are lacking in messenger RNA template.

Multiplication of Poliovirus in Mitotic and Interphase HeLa Cells

All of the results presented above suggest that RNA synthesis might be depressed in mitotic cells because the condensed state of the chromosomes precludes their acting as template for RNA synthesis. Without renewal of messenger RNA, protein synthesis would become depressed for lack of template. If this were the case, then mitotic cells might be expected to synthesize RNA and protein if an appropriate nucleic acid template were substituted for the condensed chromosomal DNA. An RNA virus might be an ideal candidate.

Marcus and Robbins (9) have reported that mitotic cells are resistant to a number of animal viruses and that they are resistant to poliovirus at low multiplicities. We have carefully determined the yields of poliovirus from mitotic and interphase HeLa cells under two different conditions of high multiplicity infection. Virus was either adsorbed to cells already arrested in mitosis and then incubated in the presence of vinblastine, or it was adsorbed to interphase cells which were then kept in the presence of the specific virus inhibitor guanidine, and in the presence of vinblastine for 9 hours to allow infected cells to accumulate in mitotic arrest. After 9 hours, the mitotic cells were separated from interphase cells by agitation, and further incubation in the presence of vinblastine, but in the absence of guanidine, was provided. Regardless of which technique was employed, it was found (Table VI) that mitotic

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cells supported the replication of normal yields of poliovirus (several hundred plaque-forming units per cell). Furthermore, as many mitotic cells as interphase cells produced virus after infection, because an approximately equal number of infectious centers were found for each.

It can be concluded that mitotic cells are capable of synthesizing normal yields of poliovirus RNA and protein. This shows that mitotic cells have no general mechanism which blocks all protein and nucleic acid synthesis, nor is there a lack of metabolic energy to drive protein and nucleic acid synthesis. It is obvious that replication of poliovirus does not require an intact nucleus, since normal yields were obtained in mitotic cells in which the nuclear membrane was dissolved.

DISCUSSION

All of the above data are compatible with, but do not prove, the hypothesis that condensation of chromosomes during mitosis renders DNA unavailable as a template for RNA polymerization, and that the subsequent failure of renewal of messenger RNA leads to loss of polyribosomes and depressed protein synthesis. The near absence of polyribosomes in mitotic cells is similar to the loss of polyribosomes in HeLa cells in which RNA synthesis is blocked by actinomycin D (13). There might, in addition, be an accelerated breakdown of messenger RNA, or an inhibitor of its function in mitotic cells, but the data in Table V suggest that any such mechanism is not able to stop the function of poliovirus RNA.

We have introduced a major artifact into this study by the employment of the spindle inhibitor vinblastine to hold the cells in mitosis. We found this necessary in order to obtain very high yields of mitotic cells with a very low percentage of contamination (less than 10 per cent interphase cells). Consequently, most of the mitotic cells employed here have been in mitosis for hours longer than the normal mitotic period. This would tend to accentuate any depression of protein synthesis resulting from progressive decay of messenger RNA without synthesis.

Taylor's (18) failure to find radioautographic evidence of depressed protein synthesis may be due to the shorter period of chromosome condensation in normal mitosis. However, Prescott and Bender (14) found definite evidence for depression of protein synthesis in normal mitosis by radioautography. Also, it can be seen in Table II that mitotic cells obtained after only 3 hours of vinblastine treatment showed 84 per cent reduction in valine incorporation. Less than half of these cells could have been in mitosis for more than 2 hours.

Finally, the above results certainly do not exclude the possibility that the residual, low-level incorporation of precursors into RNA and protein of mitotic cells might represent significant and specific synthesis related to this stage of the cell's cycle. In fact, it is clear from the work of Gall and Callan (2) and of Izawa, Allfrey, and Mirsky (6) that the lateral "loops" of newt meiotic lampbrush chromosomes are quite active in RNA and protein synthesis. It is likely that cell synchronization techniques will make it possible to study with more refinement some of the intricate biochemical controls which must be involved (11) in mammalian cell division.

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