PERSISTENCE OF MESSENGER RNA THROUGH MITOSIS IN HELA CELLS

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ABSTRACT

The decrease in protein synthesis which occurs in mammalian cells during cell division is associated with significant disaggregation of polyribosomes. For determining whether messenger RNA survives this disaggregation, the reformation of polyribosomes was investigated in synchronized HeLa cells as they progressed from metaphase into interphase in the presence of 2 μg/ml Actinomycin D. The persistence of messenger during cell division was evidenced by: (*1*) a progressive increase in the rate of protein synthesis in both treated and untreated cells for 45 min after metaphase; (*2*) reformation of polyribosomes, as determined by both sucrose gradients and electron microscopy, within 30 min after the addition of Actinomycin D to metaphase cells; (*3*) the persistence of approximately 50% of the rapidly labeled nonribosomal RNA which had associated with polyribosomes just before metaphase; (*4*) the resumption of synthesis, following cell division, of 6 selected peptides in Actinomycin-treated cells.

INTRODUCTION

When mammalian cells divide, the rate of protein synthesis decreases sharply to 20–40% the level in interphase cells, remains depressed throughout metaphase, and increases again as the cell reenters interphase. A 60–70% decrease in the rate of incorporation of radioactive amino acids into proteins has been observed radioautographically in mitotic cells of random cultures (1–5) and in synchronized cells in suspension culture (5). The decreased incorporation is associated with disaggregation of polyribosomes (5) and reflects an actual decrease in protein synthesis, since the amino acid pool size and rate of equilibration with the medium are the same in interphase and metaphase cells (5, 6). Since mitosis lasts for only 30–40 min, the rapid disappearance of large polyribosomes and the decrease in protein synthesis cannot be explained by the lack of RNA synthesis during metaphase (2, 7) or by the natural decay of messengers, which in HeLa cells have an average half-life of 3–4 hr (8). In this paper, it will be shown that rapidly labeled polyribosome-associated RNA made prior to mitosis persists into the subsequent G_1 period and that new RNA synthesis is not necessary for either the restitution of protein synthesis in early interphase, the reformation of polyribosomes, or for the ability to synthesize selected polypeptides in G_1.

MATERIALS AND METHODS

Maintenance and Synchronization of Cells

HeLa S3 cells were maintained in suspension culture in Eagle's medium (9) supplemented with 7% calf serum. Cells were synchronized by a modification of the technique of selective detachment (10). 700 ml of cells suspended at 25–30 × 10^4 per ml were prelabeled with 10–15 μc of uridine-^{14}C (30 mc/mmole, New England Nuclear Corp., Boston) for 12 hr and were then arrested in the DNA synthetic

phase of the cell cycle (S) with 2.0 mM thymidine (11, 12). After 20 hr of thymidine treatment, the cells were centrifuged, resuspended in complete prewarmed (37°C) medium for approximately 9 hr, and reexposed to 2.0 mM thymidine for 14 additional hr. The thymidine was again removed by centrifugation, the cells were resuspended in Eagle's monolayer medium containing 10% fetal calf serum to a concentration of 2.4×10^5 per ml, and 50 ml of the suspension were inoculated into each of 16 Blake bottles. 8½ hr later, the bottles were shaken vigorously to detach the loosely adherent cells, rinsed twice, and 20 ml of fresh complete prewarmed medium were added to each bottle. 30 min later, the mitotic cells were collected by gentle agitation of the bottles (5, 10). All operations were carried out in a warm room at 37°C. The 16 Blake bottles yielded $2–4 \times 10^7$ cells, with a metaphase index ranging between 88 and 92%. The remaining cells were in anaphase, telophase, or early G_1, and at no time did the number of nucleated cells exceed 5% of the total.

RNA synthesis was inhibited by adding 2.0 μg/ml of Actinomycin D to the cells either 30 min before or at the time of synchronization, since similar results were obtained with either procedure. 1 μg/ml Actinomycin D sufficed to reduce the incorporation of uridine to 5% that of control cultures, a level which has been shown by Hecht et al (13) and Tamaoki and Mueller (14) to reflect the incorporation of the isotope into transfer RNA.

Preparation and Analysis of Polysomes

Cells were chilled to 5°C, washed twice in cold spinner salts, and resuspended in 0.5 ml of RSB buffer (0.01 M NaCl, 1.5×10^{-3} M $MgCl_2$, and 0.01 M Tris-HCl pH 7.2). Nonidet P-40 (Shell Chemical Co., New York) (15) was added to the suspension at a final concentration of 0.5% for 10 min at 5°C. Nuclei and chromosomes were removed by centrifugation at 600 *g* for 10 min, and the supernatant solution was layered on an appropriate linear sucrose gradient and centrifuged as described in each of the legends. The gradients were analyzed for absorbance at 260 mμ with a continuous flow cell and a Gilford Spectrophotometer. Approximately 1.0 ml fractions were collected and, after the addition of carrier (15 μg each) of yeast nucleic acid (Schwartz Bio-Research Corp., Orangeburg, N. Y.) and Bovine Gamma Globulin (Pentex), the samples were adjusted to a final concentration of 5% with trichloroacetic acid (TCA). After at least 15 min at 5°C, the resulting precipitates were collected by filtration onto Millipore filters (1500, type HA white Plain) which were either pasted onto planchettes and counted in the Nuclear Chicago low background counter (Nuclear-Chicago Corp., Desplaines, Ill.), or dissolved in a modified Bray's solution (containing only naphthalene, PPO, and dioxane) and counted in a Beckman Scintillation Counter (Model No. LS 200 B). Approximately 10% of the ^{14}C radioactivity was recorded in the tritium channel, while less than 1% of the ^3H radioactivity was present in the ^{14}C channel. Appropriate corrections were made for channel overflow.

Extraction and Sedimentation Analysis of RNA

Samples which had been labeled with radioactive uridine and separated on sucrose gradients were pooled and, after the addition of sodium dodecyl sulfate (SDS) to 1% and 2-mercaptoethanol to 0.15 M, were incubated at 37°C for 15 min. NaCl was then added to final concentration of 0.15 M, and the RNA was precipitated with two volumes of ethanol at −20°C for 16 hr. The precipitate was collected by centrifugation at 100,000 *g* for 30 min, and resuspended in 0.01 M sodium phosphate buffer pH 7.4 containing 1% SDS and 0.002 M EDTA. This suspension was layered on top of a 15–30% linear sucrose gradient containing 0.5% SDS, 0.005 Tris-HCl pH 7.2, and 0.1 M sodium chloride, and centrifuged at 24,000 rpm for 16 hr at 25°C in a Spinco SW 25.3 rotor. The gradient was collected in approximately 0.75-ml fractions, and the absorption at 260 mμ was determined. The acid-precipitable radioactivity in each fraction was determined by adding an equal volume of 25% TCA, and, after addition of the globulin-nucleic acid carrier described above, the precipitates were filtered onto Millipore filters.

Determination of the Rate of Protein and RNA Synthesis

1 or 2 ml of cells at 30×10^4/ml were incubated with either 1.0 μc of ^{14}C-mixed amino acids (U.L. mixed amino acids from New England Nuclear) or 0.5 μc of uridine-^{14}C for 15 min in a prewarmed tube gased with 5% CO_2. The tubes were intermittently shaken by hand, and the pulse was stopped by the addition of Earle's saline. Cells collected by centrifugation at 600 *g* for 5 min were resuspended in 5 ml of 5% cold trichloroacetic acid (TCA) for 15 min at 5°C, and the resulting precipitate was collected on Millipore filters for determination of radioactivity.

Analysis by Electron Microscopy

Monolayers of HeLa S3 cells were grown on carbon-coated coverslips (16) and mounted in specially constructed perfusion chambers (17). Individual prophase cells were located by phase contrast microscopy, and, while in view, were exposed to Actinomycin D in Eagle's medium (2 μg/ml). The cell's progression through the remainder of mitosis was unimpeded by the treatment, and the cell upon

reaching G_1 was fixed in 5% glutaraldehyde buffered to pH 7 with Eagle's medium. Cell marking, embedding, and sectioning were carried out as previously described (16, 18).

Preparation and Analysis of Selected Peptides

Cells were suspended at a concentration of 1×10^7 cells per ml in complete medium containing $\frac{1}{20}$ the normal amount of threonine and either 40 μc of threonine-^{14}C (148 mc/mmole, New England Nuclear) or 150 μc of threonine-^{3}H (615 mc/mmole, Schwarz Bio-Research). After 1 hr at 37°, the cells were washed three times with cold Earle's saline, resuspended in 2 ml of RSB (1.5×10^{-3} M MgC_2l, 10^{-2} Tris-HCl pH 7.2, 10^{-2} M NaCl) for 10 min at 5°C, and disrupted by Dounce homogenization. The homogenate was then centrifuged at 100,000 *g* for 30 min in the No. 40 rotor in a Spinco L2 centrifuge. The supernate solution was adjusted to pH 5.1 with 0.5 M acetic acid and centrifuged at 34,000 *g*. The supernate was readjusted to pH 7.1 with 0.5 M KOH, precipitated at 50% ammonium sulfate for 12 hr, and centrifuged at 34,000 *g* for 5 min. The supernate was dialyzed against 0.1 M sodium phosphate buffer pH 7.1 at 5°C for 12 hr and concentrated to approximately 0.5 ml by dialysis against 60% sucrose. After the sucrose was removed by dialysis against 0.01 M sodium phosphate buffer pH 7.1, the protein solution was dissociated into individual polypeptide chains by making it 1% with SDS and 0.15 M with 2-mercaptoethanol for 30 min at 37°C (19). Iodoacetamide was added to a final concentration of 0.20 M and, after incubation at 37° for 20 min, the reduced and alkylated protein was dialyzed for 12 hr against 0.01 M sodium phosphate buffer pH 7.2 containing 0.1% SDS and 0.0002 M sodium azide. Reduction and alkylation in the presence of the denaturing reagent was carried out because preliminary experiments had shown that this procedure would make it possible to obtain clearly discernible peaks of radioactivity on acrylamide gels.

After removal of 0.05 ml for protein determination, the remainder of the sample was mixed with 0.05 ml of 60% sucrose and layered on top of a 20-cm 7.5% acrylamide gel containing 0.1% SDS and 0.1 M sodium phosphate buffer pH 7.2 (19). After electrophoresis for 20 hr at 95 v with a 0.1 M sodium phosphate buffer pH 7.1 containing 0.1% SDS, gels were fractionated by the Maizel Autogeldivider (19), and the samples were collected in vials for determination of radioactivity.

Trypsinization of G_2 Cells and Protein Determination

Cells were removed from Blake bottles by using three times recrystallized trypsin (Worthington Biochemical Corp., Freehold, N.J.) and lima bean trypsin inhibitor as described by Levine et al. (20). Protein was determined by a modified Lowry technique (21).

RESULTS

Restitution of Protein Synthesis

Actinomycin D (2 μg/ml) was added at the time of selective detachment to aliquots of HeLa cells, and the per cent of cells in mitosis and the rates of protein and RNA synthesis were compared with those of untreated cultures (Fig. 1). As reported by others (22), the presence of actinomycin did not prevent cell division. The rate of movement through the different stages of mitosis was also unaffected by the presence of the inhibitor (bar graph, Fig. 1). For the first 45 min after synchronization, there was a comparable increase in the rate of protein synthesis in both treated and untreated cells. Thereafter, however, the rate of protein synthesis in the Actinomycin D-treated cells began to fall, while that of the control cultures continued to rise. 4 hr after synchronization, protein synthesis in the treated cells was only 25–30% that of nontreated cells. In contrast, cells arrested in metaphase with colchicine showed no rise in the rate of protein synthesis (Fig. 1). The inhibition of RNA synthesis under these conditions is demonstrated by the lack of incorporation of uridine-^{14}C (Insert, Fig. 1). These experiments indicated that the restitution of protein synthesis which occurs in cells as they move from metaphase into interphase does not depend upon the synthesis of new RNA, and suggested that polysomes would reform under these conditions.

Polysome Reformation

As compared with G_1 cells, metaphase cells contained relatively fewer large polysomes, with a compensatory increase in single ribosomes (Fig. 2).

The most effective means of measuring the reformation of polysomes proved to be the examination of the single ribosome peak in sucrose gradients (23). In the presence of Actinomycin D, there was a significant decrease in the number of single ribosomes as the cells moved from metaphase into interphase (Fig. 3 *A*). This reformation was complete by 30 min. However, consistent with the pulse data in Fig. 1, the reformation of polyribosomes after 90 min in untreated control cells was greater than that found in cells treated

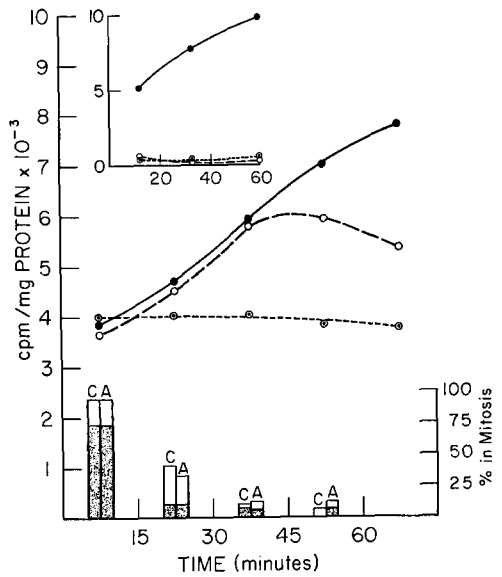

FIGURE 1 Restoration of protein synthesis during G_1. Metaphase cells were collected in medium containing $\frac{1}{20}$th the normal amount of amino acids (cf. Materials and Methods): Control cells without metabolic inhibitor (●———●); cells in medium containing 2 μg/ml of Actinomycin D (○— — —○); and cells in 0.1 μg/ml colchicine and Actinomycin D (◉---------◉). Colchicine was added 20 min before the hard shake. After adjusting the cell count in each sample to 30 × 10^4/ml, 4.0-ml samples were removed at the indicated times. Half of each sample was incubated at 37°C for 15 min with 1 μc of ^{14}C-mixed amino acids (U. L. mixture from New England Nuclear), as described in Materials and Methods, while the other half was used for determination of protein. For each pulse, the specific activity (cpm/mg protein) is plotted at the midpoint time of that pulse. Uridine incorporation in the presence and absence of Actinomycin D was carried out similarly, except that aliquots were incubated with 5 μc uridine-^{14}C (30.0 mc/mmole, New England Nuclear). The colchicine-treated culture also was treated with Actinomycin D. The bar graph at the bottom of the figure shows the per cent of cells in metaphase (scored bar) and in anaphase plus telophase (open bar) in both control (*C*) and actinomycin-treated cultures (*A*).

with Actinomycin D (Fig. 3 *B*). Reformation of polyribosomes did not involve a detectable change in the amount of 40S ribosomal subunits.

For more precise measurements, the ribosomal RNA of these cells was prelabeled prior to synchronization, and the acid-precipitable radioactivity in single ribosomes, 40S subunits, and the pellet was determined (Table I). In the absence of Actinomycin D, approximately 30% of the single ribosomes shifted from the 74S region of the sucrose gradient (Fractions 9–16) into the pelleted polyribosomes. The corresponding shift in the presence of actinomycin was 40% that of the untreated cells, and, as indicated by the optical density tracings in Fig. 3, there was no significant net loss of radioactivity from the 40S subunit area of the sucrose gradient.

Cells passing through metaphase and into interphase in the presence of Actinomycin D were examined microscopically. Light microscopic observations of such cells showed that, even when the inhibitor was added in prophase, cells divided with the formation of two daughter cells (Fig. 4 *a*). In contrast to the nuclei of the nondividing cells in Fig. 4 *a*, the nuclei of the cells which had divided in the presence of inhibitor contained a large number of what appeared to be nucleolar fragments. This provided further evidence for the effectiveness of the inhibitor, since similar fragments have been observed during interphase in various cell types after much longer exposures to doses of Actinomycin D which rapidly inhibit RNA synthesis. The reformation of polyribosomes was confirmed by electron microscopic examination of the cytoplasm of the dividing cell shown in Fig. 4 *a* (Fig. 4

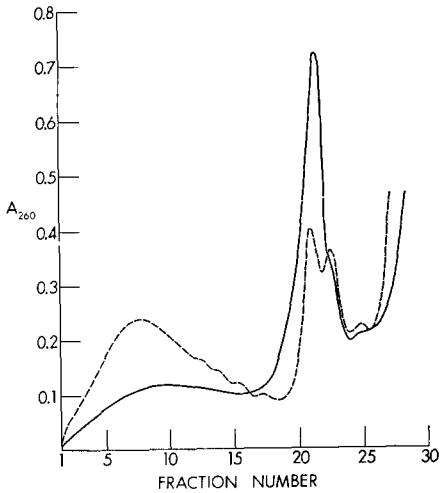

FIGURE 2 Polyribosome reformation following G_1. Mitotic cells were collected as described in Materials and Methods and immediately divided into two aliquots, one of which was chilled at 5°C and the second was incubated at 37°C for 60 min. Polysomes (cf. Methods) were separated with a 7½–45% sucrose gradient centrifuged at 24,000 rpm for 3¼ hr in a SW 25.1 Spinco Rotor. (——— = Mitotic cells; --------- = G_1 cells).

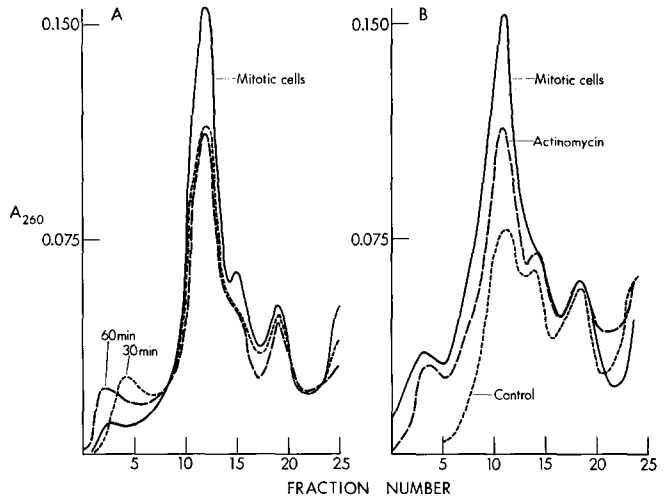

FIGURE 3 Kinetics of polysome reformation in the presence of Actinomycin D. *A*, uridine-^{14}C-prelabeled mitotic cells were collected in the presence of Actinomycin D (as described in Methods) and immediately divided into three aliquots: one was chilled at 5°C (————), the other two aliquots were incubated at 37°C with 2 μg/ml of Actinomycin D for 30 (·····) and 60 (— — — —) min, respectively. Polysomes were separated with a 15–30% sucrose gradient centrifuged at 24,000 rpm for 8½ hr in a SW 25.3 Spinco Rotor. The patterns have been normalized on the basis of total ^{14}C cpm in each of the three aliquots. *B*, Metaphase cells (————) were collected, divided into three aliquots, and processed as in Fig. 3 *A* except that the incubation at 37°C was carried out for 90 min either in the absence (·····) or presence (— — — —) of 2 μg/ml of Actinomycin D. The patterns were normalized as in Fig. 3 *A*.

TABLE I

Quantification of Polysome Reformation

	74S	40S	Pellet
	cpm	*cpm*	*cpm*
Mitotic	9735	1890	3422
G_1	6430	1789	6244
G_1(Actino. D)	8689	1750	4392

Fractions 9–16 of Fig. 2 *B* have been taken as single ribosomes (74S), and Fractions 20–24 as the 40S ribosomal subunit. The radioactivity in the pellet is considered to represent the counts in polysomes (Joklik and Becker, 1965).

b). Large numbers of small aggregates of ribosomes are noted in the cell treated with inhibitor. Few such aggregates were present in a typical metaphase cell (5, 24) because polysomes that persist are fewer in number and smaller in size.

Fate of Rapidly Labeled RNA During Mitosis

The reformation of polyribosomes in the presence of actinomycin indicated that at least some of the messenger RNA formed before mitosis survived into the subsequent G_1. This possibility was further investigated by pulse-labeling cells with uridine-^{3}H for 20–30 min before synchronization and examining polyribosome-associated radioactivity in G_2, mitotic, and G_1 cells (Fig. 5–7). The comparison of nucleated (G_2 and G_1) with nonnucleated (mitotic) cells was complicated by the appearance of unexpectedly large amounts of rapidly labeled RNA in the cytoplasm of mitotic cells. As seen in Fig. 5 *A*, some of this rapidly labeled RNA is found in the single ribosome area of the gradient. Sedimentation analysis of this rapidly labeled RNA and the RNA present in the polyribosomes (pellet) revealed that some of this rapidly labeled RNA sedimented more rapidly (Fig. 6, *I* and *II*) than the material found in G_1 cells (Fig. 6, *I'* and *II'*). The significance of this "heavy" RNA in mitotic cells is not clear since there is at present little information as to the fate of nuclear RNA upon dispersion of the nuclear membrane. The persistence of rapidly labeled RNA has, therefore, been examined by comparing G_2 and G_1 cells, both of which are nucleated. RNA made in late G_2 approximately 25 min before

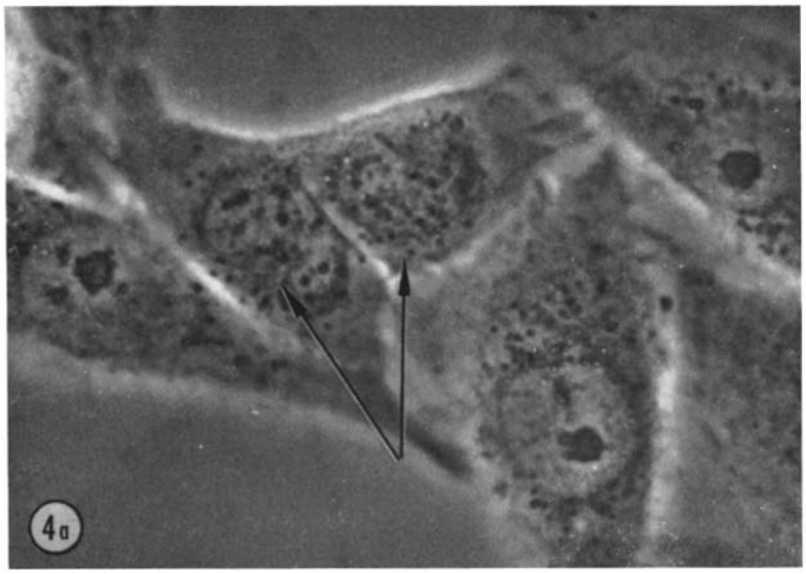

FIGURE 4 Microscopic demonstration of polysome reformation.

FIGURE 4 *a* Phase-contrast light micrograph of early G_1 cells (arrows) which had been exposed to Actinomycin D in prophase: note numerous micronucleoli (? nucleolar fragments). × 1200.

synchronization becomes associated with polyribosomes (Fig. 7 *A*), and 44% persists into G_1 (Fig. 7 *C*). The rapidly labeled RNA extracted from the heavier polyribosome area of a sucrose gradient (Fractions 1–13) is heterogeneous in its sedimentation and contains little, if any, ribosomal RNA (inserts, Fig. 7). As reported by others (25) at least 50% of this material is associated with polyribosomes since it no longer sediments as heavy material after the polyribosomes have been disrupted by EDTA (Fig. 7 *B* and 7 *D*).

Synthesis of Selected Polypeptides

All of the preceding experiments indicated that some messenger RNA persisted through mitosis, and was functional in the following G_1 phase. This question was examined in more detail by fractionating the soluble proteins of the cell (as described in Materials and Methods) until 1.5–2% of the total cell protein remained. This residual material could be resolved in acrylamide gels as six distinct polypeptides (or groups of polypeptides). For determining whether the synthesis of these polypeptide chains in G_1 required new RNA synthesis, actinomycin-treated G_1 cells were incubated with threonine-^{3}H, while control G_1 cells were labeled with threonine-^{14}C. The cultures were mixed, and extracts were prepared and analyzed on SDS-containing acrylamide gels (Fig. 8). The qualitative patterns were similar in both preparations, indicating that the messenger RNA for these selected polypeptides had not only persisted but remained functional. Similar experiments in which the cultures were analyzed separately after ^{14}C-labeling showed that the synthesis of these peptides was reduced by approximately 43% in the actinomycin-treated cultures.

DISCUSSION

The present experiments show that actinomycin does not prevent the postmitotic increase in the rate of protein synthesis, a finding indicating that at least some of the messenger RNA had persisted through mitosis. Buck et al. (26) failed to observe a similar restoration of protein synthesis when cells were treated during mitosis with 5 μg/ml of Actinomycin D. However, in their experiments even the nontreated cells failed to maintain the expected increase in the rate of protein synthesis for more than 1 hr after synchronization, suggesting that the cells may not have been collected under optimal conditions. In addition to being unable to maintain macromolecular synthesis,

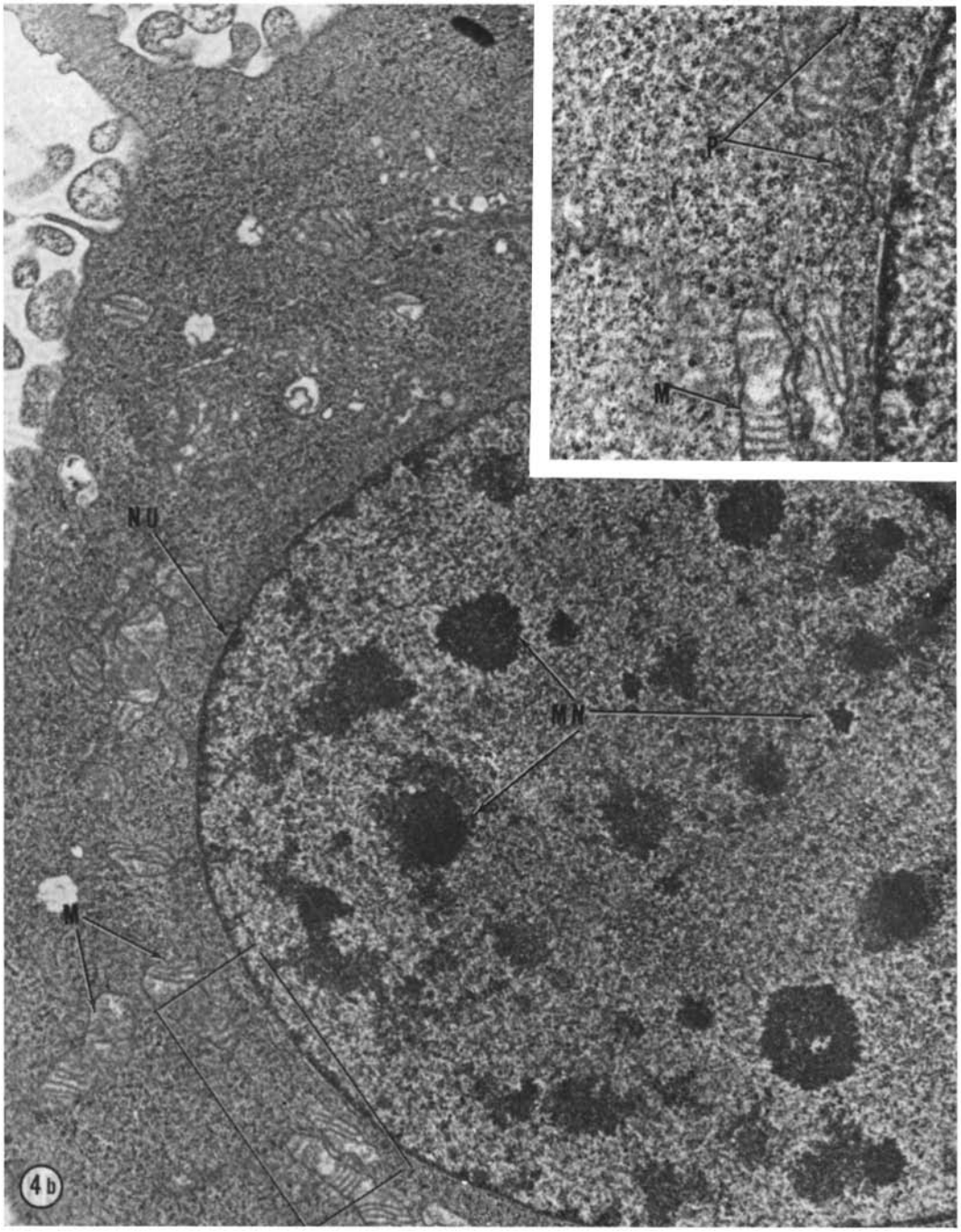

FIGURE 4 *b* Low-power electron micrograph of section through same G_1 cell shown in Fig. 4 *a*. Numerous polysomes are visible throughout the cytoplasm. The multiple (fragmented?) nucleoli seen in Fig. 4 *a* are apparent and are a constant characteristic of G_1 cells treated with Actinomycin D during mitosis. The *insert* shows a higher magnification of the area outlined by the black square. Polysome aggregates are clearly discerned. *P* = polysomes, *M* = mitochondria, *NUC* = nucleus, *MN* = micronucleoli. × 15,000; insert, × 30,000.

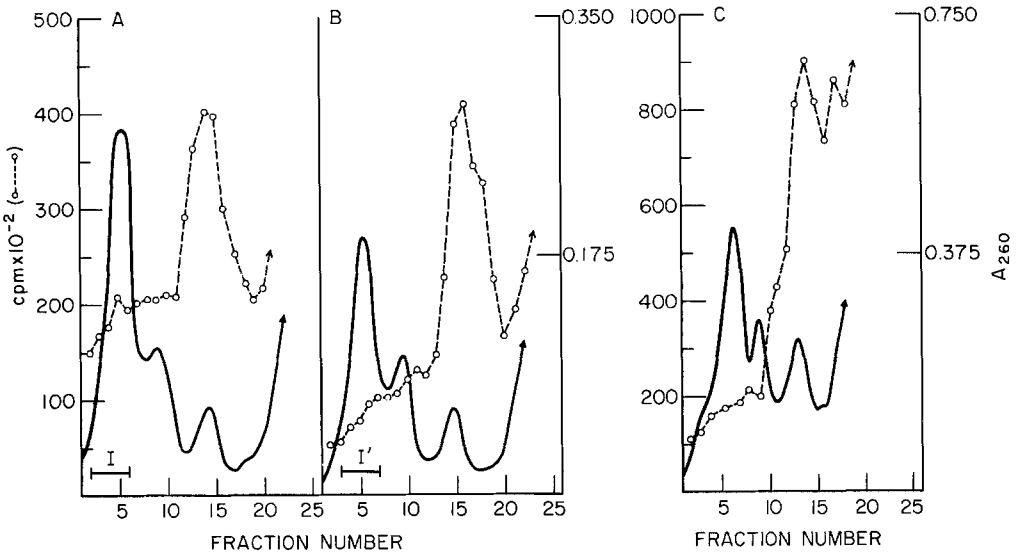

FIGURE 5 Fate of pulse-labeled RNA in mitotic cells. *A* and *B*, Cells were prelabeled with uridine-^{14}C, arrested, and pulse labeled with 90 μc uridine-^{3}H (30.0 mc/mmole) (as described in Fig. 3) before collection of mitotic cells in medium containing Actinomycin D. One-half of the sample was put immediately on ice (mitotic cells), and the second half was incubated at 37°C for 45 min (G_1 cells). Polysomes were prepared in a 15–30% linear sucrose gradient centrifuged at 24,000 rpm for 11 hr in a SW 25.3 Rotor. The patterns have been normalized as in Fig. 3 *A*. *C*, 100 ml of nonsynchronized cells at a count of 40 × 10^4/ml were incubated for 20 min with 20 μg uridine-^{3}H (20 c/mmole, sp act), and polysomes were prepared as described above. (———— = OD; o---------o = uridine-^{3}H).

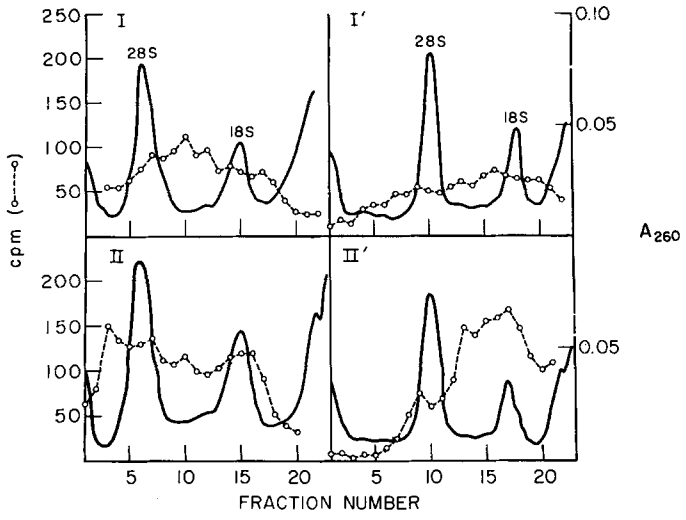

FIGURE 6 Characterization of pulse-labeled RNA in mitotic cells. 0.4-ml aliquots from each fraction of the experiment in Fig. 5 *A* and *B* were pooled as indicated in that figure: *I* = fractions 2–6 of Fig. 5 *A*, *I'* = fractions 3–7 of Fig. 5 *B*. *II* and *II'* are the pellets of gradients Fig. 5 *A* and B, respectively. The RNA of each pooled sample was extracted and analyzed in SDS gradients as described in Methods. (———— = OD; o---------o = uridine-^{3}H).

such cells usually exhibit ultrastructural abnormalities (5). Further, the use of five to ten times the concentration of actinomycin necessary to inhibit RNA synthesis may have had an effect unrelated to the primary action of the drug. In our hands, however, even 5 μg/ml actinomycin did not prevent the postmitotic restitution of protein synthesis here described.

In logarithmically growing cells, newly synthesized ribosomal subunits contribute to the forma-

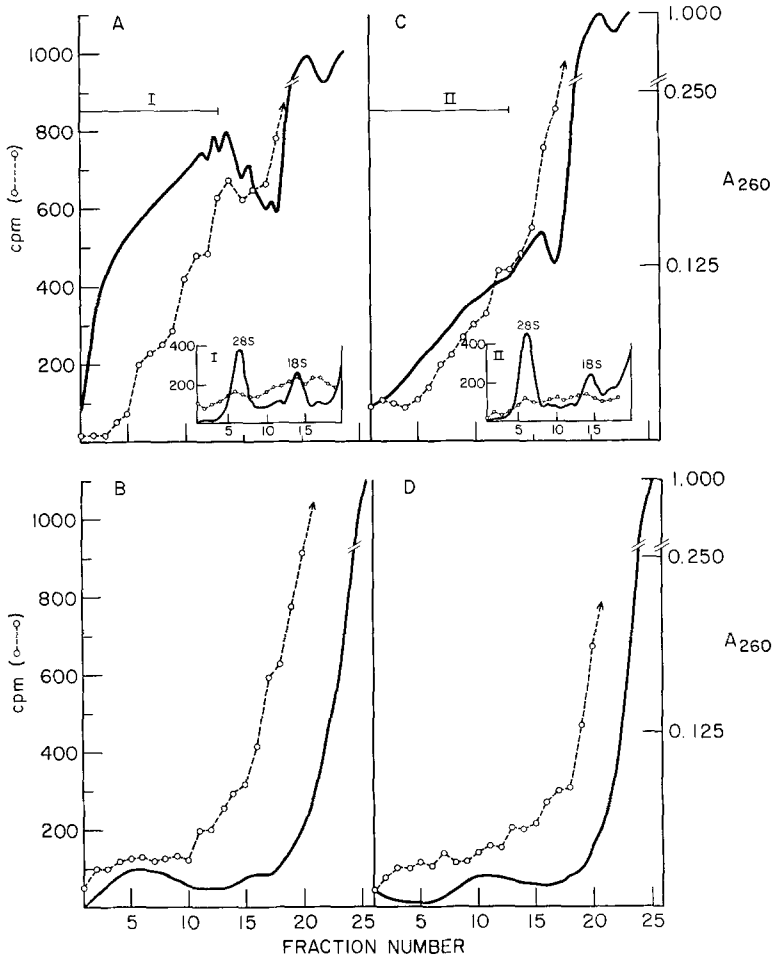

FIGURE 7 Fate of pulse-labeled RNA. 90 μc of uridine-^{3}H (20 c/mmole, sp act, Schwarz BioResearch) in 20 ml of complete medium was added to each of 16 Blake culture bottles 25 min before collection of mitotic cells. After cells were collected in the presence of Actinomycin D (2 μg/ml), they were incubated at 37°C for 1 hr (G_1 cells). At the time of collection, four other Blake bottles were placed immediately in an ice-water bath (at 5°C) for 30 min, and cells were collected (G_2 cells) by trypsinization (Levine et al., 1965). Each sample was adjusted to a cell count of 1.6×10^7, divided into two aliquots, and processed for polysomes as described in methods; one aliquot was separated with a standard 15–30% sucrose gradient and the second aliquot was made 0.01 M with respect to EDTA and separated in a 15–30% sucrose gradient containing 0.01 M EDTA. All four sucrose gradients were centrifuged at 24,000 rpm for 130 min in an SW 25.1 Spinco Rotor. *A* = G_2 cell; *B* = G_2 cell (EDTA); *C* = G_1 cell; *D* = G_1 cell (EDTA).

Inserts *A* and *C*, 0.6 aliquots from fractions 1–13 of the gradients shown in *A* and *C* were pooled separately. RNA extracted and analyzed on SDS gradients as described in Methods. (———— = OD; O--------O = uridine-^{3}H).

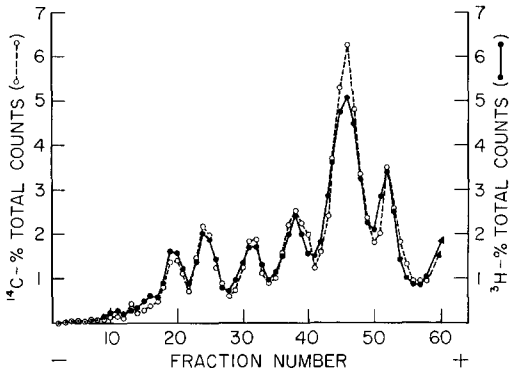

FIGURE 8 Synthesis of selected polypeptides by cells in G_1. Two preparations of metaphase cells were collected: one in the presence of Actinomycin D (2 μg/ml), and the other in the absence of inhibitor. The cell count in each was adjusted to 1.4×10^7 cells. Following a 1-hr incubation, during which the cells had passed into interphase, the inhibitor-treated cells were pulsed with 150 μc of threonine-^{3}H (615 mc/mmole, Schwarz Bioresearch), while the untreated cells were pulsed with 40 μc of threonine-^{14}C (148 mc/mmole) as described in Methods. The two cultures were mixed, extracts were prepared, reduced, alkylated, and the resulting polypeptides were separated in 7.5% acrylamide gels containing SDS as described in Methods. Based on a Lowry determination, the amount of protein applied to the gel was approximately 0.7 mg.

tion of polyribosomes (23, 27). Following mitosis, however, there is no net change in the number of 40S (and probably 60S) ribosomal subunits and reformation takes place from single ribosomes rather than the existing subunits. Similar reformation from single ribosomes occurs when puromycin-induced polyribosome disaggregation is reversed (23).

When the fate of rapidly labeled RNA made in late G_2 was examined during metaphase, unexpectedly large amounts of rapidly sedimenting material were found in the single ribosome and polyribosome (pellet) areas of the gradients. While this rapidly sedimenting heterogeneous RNA probably represents material which was in the nucleus prior to metaphase, some of that found in the single ribosome area could be messenger-single ribosome complexes resulting from polysome disaggregation. Because of the uncertainties in comparing mitotic cells with nucleated G_2 or G_1 cells, labeled heterogeneous RNA associated with polysomes was compared in G_2 and G_1. Although this polysome-associated RNA has not been characterized by either base ratio analysis or hybridization for technical reasons (28), its sedimentation properties, kinetics of labeling, and association with polyribosomes suggest that it represents messenger RNA. Our inability to reliably examine the rapidly labeled RNA on single ribosomes and polyribosomes in metaphase cells made it impossible to determine whether the rapidly labeled RNA found on G_1 polyribosomes was free during metaphase, or, as is more likely, represents that proportion of the RNA which remained attached to ribosomes during mitosis.

The functionality of this persisting messenger was demonstrated by the continuing ability of actinomycin-treated G_1 cells to synthesize six selected polypeptides (or groups of peptides). While all six polypeptides were synthesized, the possibility of selective degradation of certain messengers cannot be eliminated since the peptides examined represent less than 2% of the total cell proteins.

From all of these experiments, it has been concluded that at least 40% of the messenger RNA present in late G_2 persists through mitosis and continues to function as the cell reenters interphase. It is of interest that during the first two cleavage cycles in sea urchin development there is no disaggregation of polyribosomes, indicating that most if not all the messenger RNA persists (29). In HeLa cells, the exact mechanism of polyribosome disaggregation, the significance of the loss of some of the messenger RNA, and the functional capability of metaphase polyribosomes remain to be fully elucidated.

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