NUCLEAR ENVELOPE-ASSOCIATED RESUMPTION OF RNA SYNTHESIS IN LATE MITOSIS OF HeLa CELLS

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ABSTRACT

The restitution of RNA synthesis in cultures progressing from metaphase into interphase (G_1) has been investigated in synchronized HeLa S_3 cells by using inhibitors of macromolecular synthesis and the technique of electron microscope autoradiography. The rate of incorporation of radioactive uridine into RNA approached interphase levels in the absence of renewed protein synthesis. In contrast, maintenance of this rate in G_1 was dependent upon renewed protein synthesis. Restoration of synthesis of heterogeneous nuclear RNA occurred under conditions that inhibited production of ribosomal precursor RNA. In autoradiographs of individual cells exposed to radioactive uridine, silver grains were first detected after nuclear envelope reformation at the periphery of the chromosome mass but before chromosomal decondensation. These data are consistent with the following interpretation. Multiple RNA polymerase activities persist through mitosis and are involved in the initiation of RNA synthesis in early telophase at sites on the nuclear envelope.

INTRODUCTION

The transition from mitosis to interphase in the mammalian cell cycle involves extensive reorganization of cell structure and function. A detailed analysis of this transition conceivably could help to elucidate regulatory phenomena in mammalian cells. The morphological alterations that have been observed include the reformation of the nuclear envelope, the reappearance of nucleoli, the decondensation of chromosomes into chromatin, the depolymerization of the spindle microtubules, and the increased formation of cytoplasmic polyribosomes (1–3). Concomitant biochemical events include the reutilization of some preexisting messenger RNA (4), the restitution of protein synthesis to interphase levels (4, 5), and the reversal of the nearly complete restriction of RNA synthesis (6).

To gain an understanding of these events in the reestablishment of the interphase state, each process needs to be analyzed separately. We have examined aspects of one event, the restoration of RNA synthesis, in vivo. This type of study requires large numbers of cells that are synchronized in mitosis and that will progress into interphase. Reversal of Colcemid inhibition of Chinese hamster ovary cells was used by Fan and Penman (7) to study synthesis and processing of ribosomal precursor RNA 2 h after release. However, since such populations were well into G_1, the events surrounding the initial restoration of RNA synthesis are not clear. To examine this restoration in more detail, it appears advantageous to use populations in metaphase prepared in the absence of inhibitors of cell division. We have employed

the technique of selective detachment (8) and have studied the first 15–90 min after synchronization. During this time period, the mitotic index fell from 90% or greater to less than 10%, the cell number approximately doubled, the interphase cellular morphology was reestablished, and the interphase levels of RNA and protein synthesis were attained. For biochemical studies on the kinetics of resumption of RNA synthesis, we have employed inhibitors of both RNA and protein syntheses. In addition, the relationship between ultrastructural and biochemical events in individual cells has been explored employing the procedure of electron microscope autoradiography.

MATERIALS AND METHODS

Cell Culture and Synchronization

HeLa S_3 cells were maintained in suspension culture in Eagle's medium (9) supplemented with 7% calf serum and 2 mM glutamine. Cells were synchronized in mitosis by the technique of selective detachment (8) after a double thymidine blockade. This procedure was used as previously described (4), except that cells were arrested in S phase by two 14-h exposures to 2.0 mM thymidine (10). The yield from 32 Blake bottles was 6–8 $\times$ 10^7 cells with a metaphase index ranging between 88 and 95%. The number of interphase cells did not exceed 5% of the total. Inhibitors of RNA or protein synthesis, when used, were present in the medium at the time of collection of mitotic cells. The concentrations of inhibitors are indicated in the appropriate legends.

Determination of Protein and RNA Synthesis

2 or 4 ml of cells at a concentration of 40–50 $\times$ 10^4/ml were incubated with either 1.0 μCi of [^{14}C]-mixed amino acids (uniformly labeled mixed amino acids from New England Nuclear, Boston, Mass.) or 1.0 μCi of [^{14}C]uridine (>50 mCi/mmol, New England Nuclear) for 15 min in a prewarmed tube gassed with 5% CO_2. The tubes were shaken intermittently by hand and the pulse was stopped by the addition of ice-cold Earle's saline. Cells were collected by centrifugation at 600 *g* for 5 min at 5°C, washed twice in the cold, resuspended in 1.0 ml of RSB (1.5 $\times$ 10^{-3} M $MgCl_2$; 10^{-2} M Tris-HCl, pH 7.2; 10^{-2} M NaCl) and precipitated with an equal volume of 10% cold trichloroacetic acid (TCA) for 15 min at 5°C. Precipitates were collected by filtration onto Millipore filters (type HA) and were washed with cold 5% TCA. Filters were then pasted onto planchettes, dried, and the radioactivity was determined using a Nuclear Chicago low-background counter.

Preparation of Nucleoplasmic and Nucleolar RNA

Nuclear heterogeneous and nucleolar pre-ribosomal RNAs were prepared from HeLa cells by the procedure of Penman, using detergents (11). RNA was precipitated at –20°C for 16 h with 0.15 M sodium chloride, 0.1% of 2-mercaptoethanol, and 2.5 volumes of ethanol. This precipitate was suspended in 2.0 ml STE (5 $\times$ 10^{-2} M Tris-HCl (pH 7.4) with 10^{-3} M EDTA and 10^{-1} M NaCl) containing 1% sodium dodecyl sulfate (SDS) and extracted three times at room temperature with a phenol solution composed of 80% phenol and 20% STE. RNA was precipitated at –20° C for 16 h from the aqueous layer as above. Recovery of RNA was monitored by determining the acid-precipitable radioactivity before and after extraction.

Sedimentation Analysis of RNA

Samples of nuclear RNA to be analyzed in SDS-sucrose gradients were solubilized in 0.5 ml of PEMS (10^{-2} M sodium phosphate buffer (pH 7.4) containing 1% SDS, 0.1% of 2-mercaptoethanol, 10^{-1} M NaCl, and 2 $\times$ 10^{-3} M EDTA). RNA was sedimented in 16 ml 15–30% (wt/wt) linear sucrose gradients, consisting of 10^{-2} M Tris-HCl (pH 7.2), 0.5% SDS, and 10^{-1} M NaCl, which were centrifuged for 16 h at 20,000 rpm in a Beckman SW 27 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Absorbance at 260 nm was monitored using a Gilford Recording Spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) with a continuous flow cell. 0.75-ml fractions were collected and the trichloroacetic acid-insoluble radioactivity was determined.

Preparation of Polyribosomes

Cytoplasmic polyribosomes were prepared with Nonidet P-40 (Shell Chemical Co., New York) (12) followed by separation in linear 7.5–45% sucrose gradients which were centrifuged for 3¼ h at 24,000 rpm in an SW 25.1 rotor (13). Absorbance at 260 nm was monitored as described above.

Electron Microscope Autoradiography

A preparation of metaphase cells was exposed to [^{3}H]uridine (100 μCi/ml; 2–20 Ci/mmol, New England Nuclear) for 40 min at 37°C. Fixation was carried out at 5°C for 1 h, employing a modified fixative of Franke et al. (14) consisting of a 10^{-1} M sodium potassium phosphate buffer (pH 6.8) containing 1.5% glutaraldehyde and 1% osmium tetroxide. Cells were then washed four times in buffer before postfixation for 2 h in buffer containing

1% (wt/vol) osmium tetroxide. After several washes and a 16-h rinse in buffer, cells were dehydrated in an ethanol series and embedded in Epon. Fixation procedures of this type remove mononucleotides and precursor pools from fixed cells (15–17). Gold-to-silver sections were mounted on grids, coated with Ilford L4 emulsion (Ilford Ltd., Ilford, Essex, England) and stored for 3 wk or 10 wk in light-tight slide boxes according to the technique of Caro and van Tubergen (18).

Grids were developed for 5 min in Microdol X (33.5 g/250 ml, Eastman Kodak Co. Rochester, N.Y.) at 22°C, exposed for 30 s to 1% acetic acid and fixed for 5 min in acid fixer (Kodak Rapid Fixer and Kodak Hardener, Eastman Kodak Co.). After several rinses in deionized, glass-distilled water, grids were thoroughly dried. The gelatin was removed by immersion for 4.0 min in 10^{-1} M sodium hydroxide.

Grids were stained with aqueous uranyl acetate and lead citrate and were examined with an RCA electron microscope operating at 50 kV. Measurements were made on micrographs printed at either × 14,060 or × 24,300 magnification.

RESULTS

Restoration of RNA Synthesis in the Presence of Inhibitors of Macromolecular Synthesis

To explore the various parameters involved in the resumption of RNA synthesis, inhibitor studies have been employed. Mitotic cells were collected in the presence and absence of inhibitors of macromolecular synthesis, and the incorporation of [^{14}C]uridine into acid-precipitable radioactivity for successive 15-min intervals was determined. Untreated metaphase preparations demonstrated the expected restoration of RNA synthesis, which reached interphase levels by 60–75 min after synchronization (Fig. 1 A). When cells were collected in the presence of inhibitors of protein synthesis (puromycin or cycloheximide), the initial rate of increase of uridine incorporation was maintained for 60–75 min and was similar to that in the untreated populations. Thereafter, the

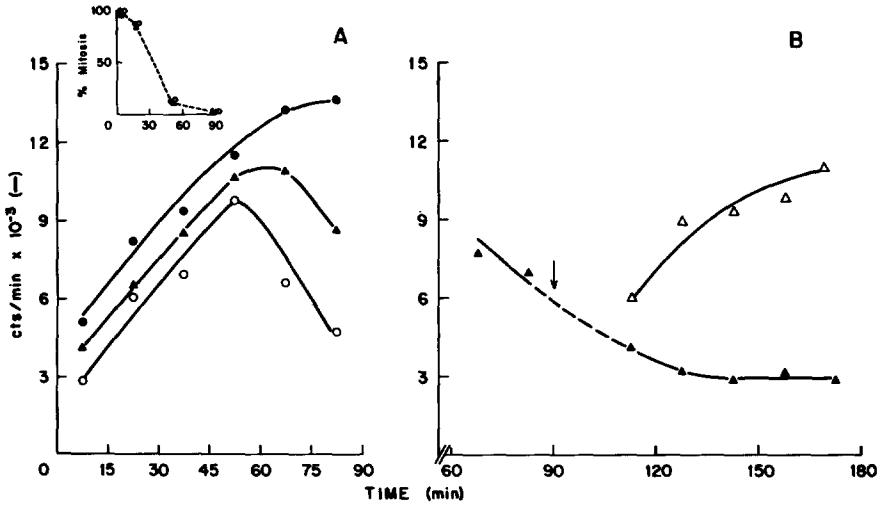


FIGURE 1 Effect of inhibition of protein synthesis on the restoration of RNA synthesis. Preparations of mitotic cells were collected in the absence or presence of inhibitors of macromolecular synthesis and maintained at 37°C as described under Materials and Methods. (A) At the indicated times 4.0-ml aliquots of each culture were incubated at 37°C for 15 min with 1.0 μCi [^{14}C]uridine and total acid-precipitable radioactivity was determined (see Materials and Methods). The data were plotted at the midpoint of each labeling period and have been normalized for an initial concentration of 43 × 10^4 cells per ml. *Insert:* The mitotic index of control and inhibitor-treated cells was ascertained at various times after synchronization by phase microscopy. Control cells (●—●); cells collected with 60 μg per ml cycloheximide (▲—▲); cells collected with 25 μg per ml puromycin (○—○). (B) Mitotic cells were collected in the presence of 100 μg per ml of cycloheximide. At 90 min after synchronization (arrow) the culture was divided into two equal parts and washed: one portion was resuspended in medium containing inhibitor; the second portion was resuspended in medium lacking inhibitor. All procedures were performed at 37°C. The incubation was extended for an additional 90 min. Aliquots were removed from each culture as indicated and the incorporation of [^{14}C]uridine was determined as described for A. Cells continuously incubated with 100 μg per ml of cycloheximide (▲—▲); cells resuspended in cycloheximide-free medium (△—△).

rate of incorporation declined. To determine the extent of this decrease, cycloheximide-treated preparations were first incubated for 60 min and the rates of uridine incorporation were measured. The rate of incorporation continued to decline until a plateau of approximately 25% of the maximum was reached 120–135 min after synchronization (Fig. 1 B). These results indicate that RNA synthesis is initiated but fails to be maintained in cultures treated with the inhibitor. However, this failure of maintenance of synthesis in G_1 cultures was reversible. Cells incubated 90 min with cycloheximide and then resuspended in cycloheximide-free medium approached interphase levels of uridine incorporation by 75–90 min after resuspension (Fig. 1 B). Concentrations of 60–100 μg of cycloheximide did not alter either the rate of initiation or the time at which the failure of maintenance of RNA synthesis was observed. These concentrations of cycloheximide are effective in inhibiting protein synthesis in randomly growing cells (19).

The concentration of inhibitors used in these experiments was sufficient to prevent the restoration of protein synthesis to interphase levels (Fig. 2 A). Uninhibited cells attained G_1 levels of protein synthesis within 60 min, whole cells treated with puromycin (25 μg/ml) or cycloheximide (60 μg/ml) showed no change in protein synthesis above that observed initially. The level of incorporation of radioactive amino acids amounted to 25–30% of the G_1 level found in untreated cultures. This is similar to the rate observed in mitotic cells, in which polyribosomes are disaggregated. Since polyribosomes reform as the cell enters interphase (3, 5), we have documented this inhibition of protein synthesis in more detail by examining the profile of polyribosomes isolated from treated and untreated cultures. Sedimentation analysis, 60 min after synchronization, revealed that polyribosomes sedimented less rapidly in the treated cultures (Fig. 2 B). The monosome to polyribosome ratio was 1.4:1 in the control cultures, as compared to 6.2:1 and 2.1:1 in the puromycin and cycloheximide-treated cultures, respectively. It appears that coincident with the blocked restoration of protein synthesis, there is also a failure of complete polyribosome reformation.

Analogous studies were carried out with the inhibitor actinomycin D. In randomly growing cells, low doses (0.04 μg/ml) preferentially inhibit nucleolar ribosomal precursor RNA synthesis while high doses (1–4 μg/ml) also inhibit extra-

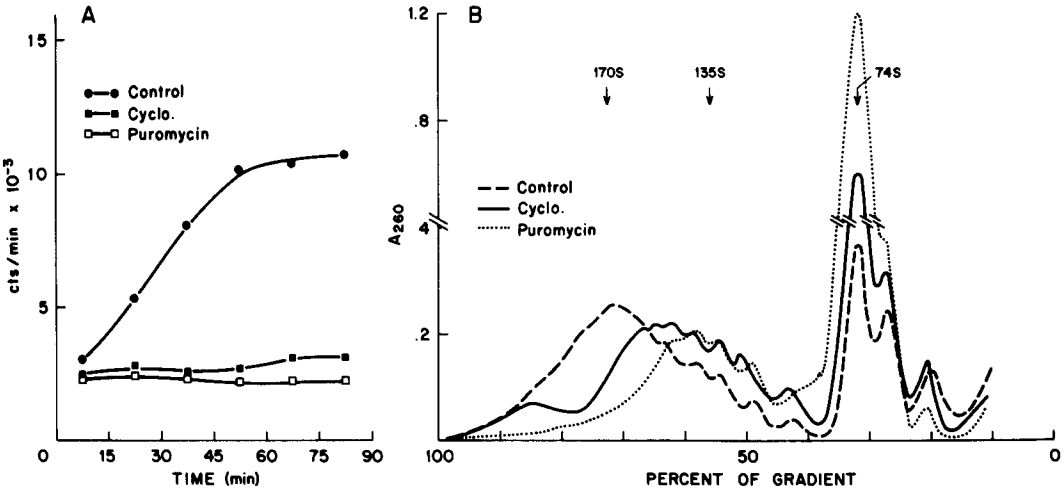


FIGURE 2 Protein synthesis and polyribosome reformation in the presence of inhibitors of protein synthesis. Mitotic cells were collected and maintained at 37°C in the presence or absence of inhibitors (see Materials and Methods). (A) At the indicated times, 2.0-ml aliquots of each culture were exposed to 1.0 μCi of [^{14}C]mixed amino acids for 15 min. Total acid-precipitable radioactivity was determined and the data are presented as described in the legend of Fig. 1 A. Control cells (●—●); cells collected with 60 μg per ml puromycin (□—□). (B) A preparation of mitotic cells was divided into three equal parts which were incubated at 60 min with or without inhibitors. Cytoplasmic polyribosomes were isolated and separated in 7.5–45% sucrose gradients (see Materials and Methods). Control cells (– –); cells incubated with 100 μg per ml cycloheximide (—); cells incubated with 25 μg per ml puromycin (· · · · ·).

nucleolar synthesis (20). As previously observed (4), in synchronous populations there was no resumption of total RNA synthesis in the presence of the high dose of inhibitor (Fig. 3). In contrast, at the low dose of inhibitor the incorporation of radioactivity became constant at 45–50% of the untreated controls by 45–60 min after synchronization. To analyze the patterns of restoration of synthesis of heterogeneous and nucleolar ribosomal precursor RNA, mitotic cells, collected in the absence or presence of inhibitors, were then exposed to radioactive uridine for a 15-min period at 60–75 min after synchronization. Nuclear RNA was fractionated, extracted with phenol, and analyzed by sedimentation in linear 15–30% SDS-sucrose gradients (Fig. 4). Thus, the effect of cycloheximide was evaluated at 75 min after synchronization, when the decreasing rate of incorporation was first noted. In comparison to the untreated G_1 population, there was reduced synthesis of both species of nuclear RNA in the cycloheximide-treated cells (Fig. 4 A and B). There was no restoration of either RNA species in cultures containing high levels of actinomycin D, as previously shown (4). In contrast to the synthesis of ribosomal precursor RNA, significant restoration of the synthesis of heterogeneous RNA was observed in the presence of the low dose of actinomycin D (Fig. 4 A and B). Although there is no obvious ribosomal precursor RNA in the nucleolar preparation, contamination of this fraction with heterogeneous nuclear RNA is apparent. Similar contamination of nucleolar preparations has been reported in randomly growing mouse L cells in which ribosomal RNA synthesis was inhibited selectively by low levels (0.08 μg/ml) of actinomycin D (21).

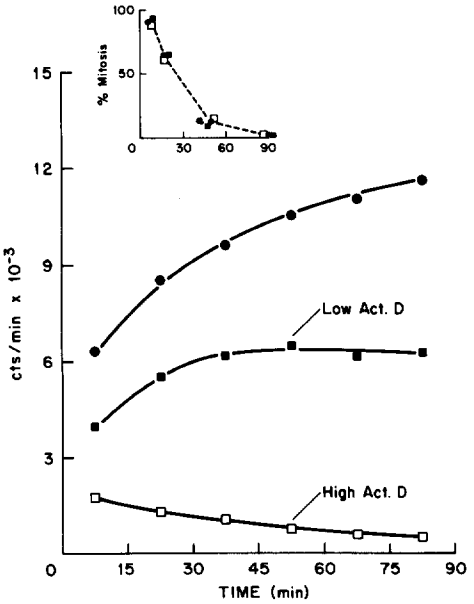


FIGURE 3 Selective restoration of RNA synthesis with actinomycin D. Preparations of mitotic cells were collected in the absence and in the presence of high and low amounts of actinomycin D (see Materials and Methods). As described in the legend of Fig. 1 A, 4.0-ml aliquots were exposed to [^{14}C]uridine and acid-precipitable radioactivity was ascertained. The data were corrected for concentration of cells and plotted at the midpoint of the labeling period. *Insert:* The mitotic index for each culture was determined as described in the legend of Fig. 1. Control cells (●—●); cells collected with 0.04 μg per ml actinomycin D (■—■); cells collected with 2.0 μg per ml actinomycin D (□—□).

Restoration of RNA Synthesis in Individual Cells

To make a correlation between changes in fine structure and the restoration of RNA synthesis in individual cells, the procedure of electron microscopic autoradiography has been employed. Cells in various stages of transition from mitosis to G_1 have been classified according to chromosome migration, nuclear envelope reformation, chromosome decondensation, nucleolar reaggregation, and formation of the intercellular bridge (2, 22). In metaphase cells chromosomes were arranged across the equator, the nuclear envelope was not evident and the cytoplasm lacked polyribosomes. In anaphase, groups of progeny chromosomes were moved apart; later in anaphase the members of each group were fused and an incomplete nuclear envelope was observed. During telophase the nuclear envelope was reformed, the process of chromosome decondensation was evident, and cytokinesis had begun. Progeny cells in early G_1 had irregularly shaped nuclei, contained nucleoli, and were joined by an intercellular bridge.

A population enriched for late anaphase, telophase, and early G_1 was needed, since analysis by light autoradiography indicated that new RNA syntheses occurred at some time between telophase and early G_1. Our data demonstrated that at 30–60 min after synchronization, when there was a rapid increase in RNA synthesis, a majority of the cells were completing mitosis (Figs. 1 and 4). Therefore, a 40-min incubation at

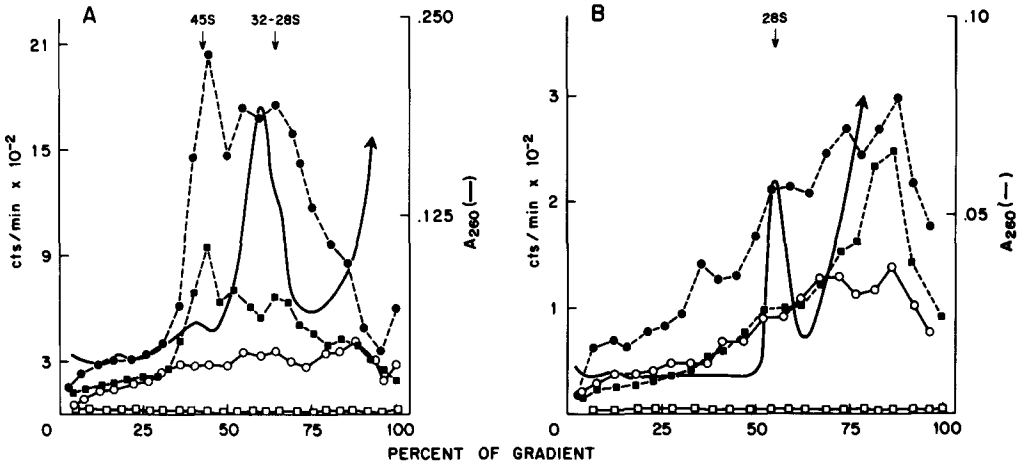
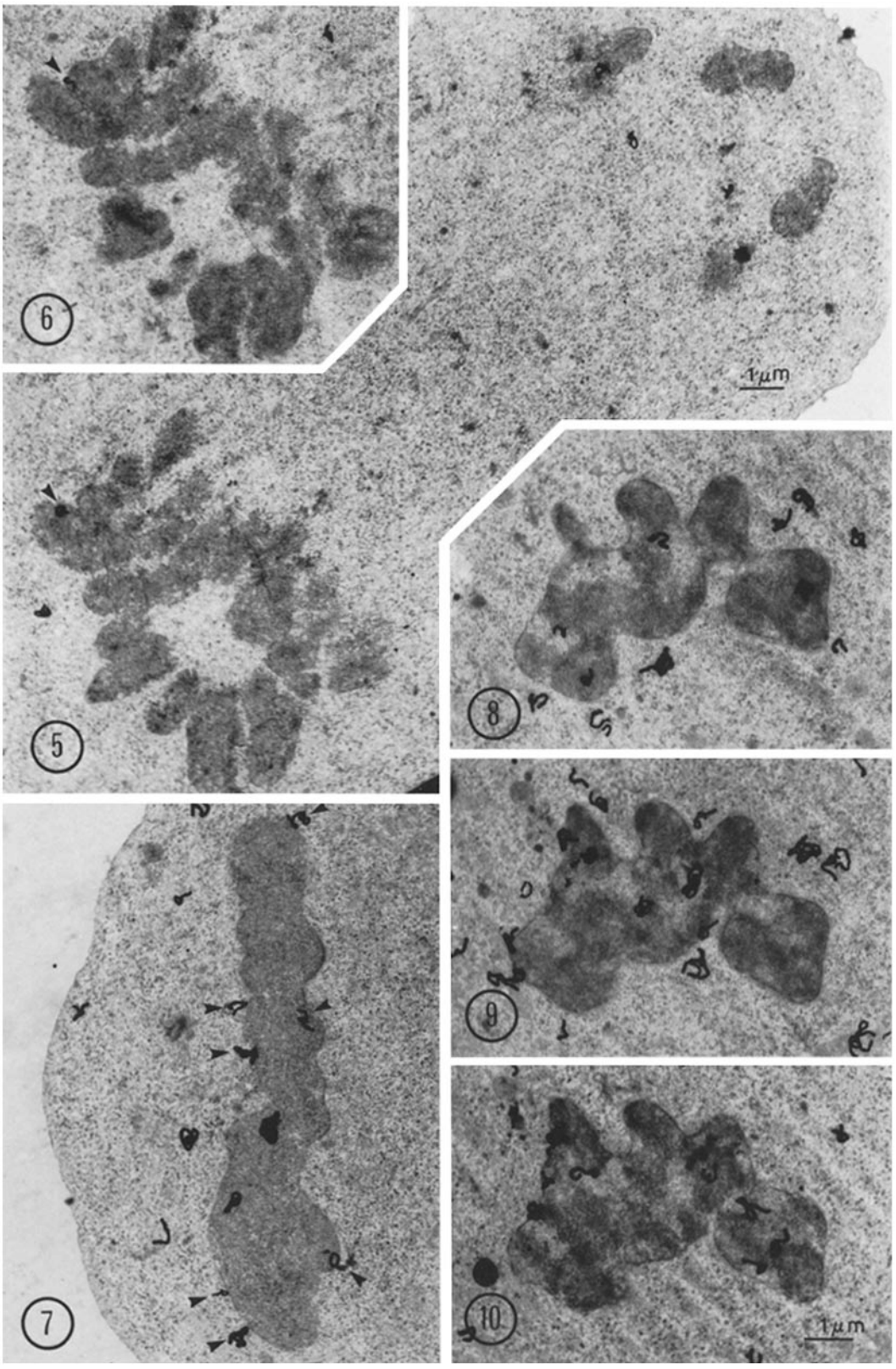


FIGURE 4 Synthesis of nucleolar pre-ribosomal RNA and nuclear heterogeneous RNA in the presence of inhibitors. Mitotic cells were collected as described in Materials and Methods. 20-ml cultures were incubated at 37°C in the presence or absence of inhibitors. At 60 min after synchronization, when the fraction of mitotic cells was less than 20%, cultures were exposed to [^{14}C]uridine for 15 min. Nucleolar pre-ribosomal RNA and heterogeneous nuclear RNA were prepared and subjected to sedimentation analysis (see Materials and Methods). The data were normalized for an initial cell concentration of 43 × 10^4 cells per ml and were corrected for recovery after phenol extraction. (A) Nucleolar pre-ribosomal RNA: control cells (●--●); cells incubated with 100 μg per ml cycloheximide (■--■); cells incubated with 0.04 μg per ml actinomycin D (○—○); and cells incubated with 4.0 μg per ml actinomycin D (□—□). (B) heterogeneous nuclear RNA: control cells (●--●); cells incubated with cycloheximide (■--■); cells incubated with low actinomycin D (○—○); and cells incubated with high actinomycin D (□—□).

37°C after synchronization was employed to maximize the probability of observing both the earliest time and location(s) of restored RNA synthesis. During this time cells were exposed to high levels of tritiated uridine. The cells were then collected by centrifugation, washed, and processed for analysis by electron microscope autoradiography. Photographs of individual cells were analyzed for the location and distribution of silver grains.

72 cells in various stages of anaphase and telophase have been examined for the appearance and distribution of grains about the reforming nucleus. Few cells in earlier stages were found. Late anaphase cells generally contained small numbers of grains, and the nuclear envelope appeared as a discontinuous structure (2, 22). A typical cell in this stage is illustrated in Figs. 5 and 6. The grains occasionally observed were peripheral to the fused chromosome mass at or near segments of observable nuclear envelope. There were insufficient numbers of grains for quantification at this stage. Numbers of grains per nucleus were found to increase significantly in early telophase cells (Fig. 7). The majority of these nuclear grains were at or near the envelope at a variety of locations around the entire chromosomal mass. Of importance, these peripherally located grains were present before decondensation of chromosomes, before nucleolar reformation, and before obvious intercellular bridge formation. In mid to late telophase, when chromosomal decondensation became marked, grains were seen both peripherally at the nuclear envelope and centrally over the chromatin (Figs. 8–13). By early G_1, chromosomal decondensation was near completion and the central distribution of grains was generalized over the interior of the nucleus (Figs. 14–16). Distinct nucleolar structures usually were not observed until this stage and showed a highly localized pattern of grains (Fig. 15). It was therefore reasoned that the grains observed in early and mid telophase nuclei represented primarily extranucleolar RNA synthesis.

These results indicate that the earliest detectable nuclear RNA synthesis in telophase is at or near the nuclear envelope. To analyze this apparent association more completely, the distribution of grains was quantified in cells in telophase and in early G_1, and was examined in contiguous


6
1μm
5
8
9
7
10
1μm

serial sections of individual cells. The comparison of the grain distribution in telophase and G_1 cells employed sections of the latter not containing nucleoli, since these structures were not seen in telophase nuclei. The distribution of grains was expressed as the relative grain density (Table I) and by histograms (Fig. 17) constructed according to the half-distance method of Salpeter et al. (23). In Table I, grain density is the number of grains per cell compartment divided by the relative area of that compartment. Comparisons were made by normalizing densities to that calculated for the nuclear envelope. For both telophase and G_1 cells, grain densities were greater at the nuclear envelope than in either the respective nuclear or cytoplasmic compartments. However, the relative grain density of the nucleus has increased approximately threefold in G_1 cells as compared to telophase cells, while the density over the cytoplasm was similar in both stages (Table I). The histograms were constructed from the grains within 10 half-distance units on either side of the nuclear envelope. This tabulation should represent over 90% of the grains originating from a defined source (23). The distribution in telophase cells is skewed toward the envelope. For example, approximately 85% of the nuclear grains were within three half-distances of this structure (Fig. 17). In the nuclear compartment of the G_1 cells, the distribution is more dispersed (Fig. 17) and approximately 48% of the grains were within three half-distances. A simple calculation demonstrates that this association with the nuclear envelope is not typical of another cellular membrane (Figs. 7, 11, 14, 18). In a total length of cell membrane of 243 μm, only 14 grains were found within 1000 Å on either side of this membrane. For a total length of nuclear envelope of 190 μm, 42 grains were within 1000 Å of this structure.

Additional evidence for the peripheral location of restored RNA synthesis is offered by autoradiography of serial sections of the same cell. Figures 18–22 represent an early to mid telophase cell with minimal chromosomal decondensation. At this stage the nucleus is convoluted and the invaginations of membrane-bound nuclear material are separated by areas of cytoplasm. On the basis of silver-to-gold interference colors, each section was estimated to be approximately 1000 Å in thickness. Consequently, the four contiguous sections represent a cell depth of approximately 0.4 μm. With this three-dimensional perspective, the majority of grains were at or near the nuclear envelope. For example, of the 45 grains about the nuclear segments, 38 appear, by inspection, to be associated with this structure. When grains are found over the chromosome mass, they generally appear in nuclei in which chromosomal decondensation has begun. Serial sections of mid to late telophase cells with increasing degrees of decondensation have revealed increasing numbers of intranuclear grains (Figs. 8–10).

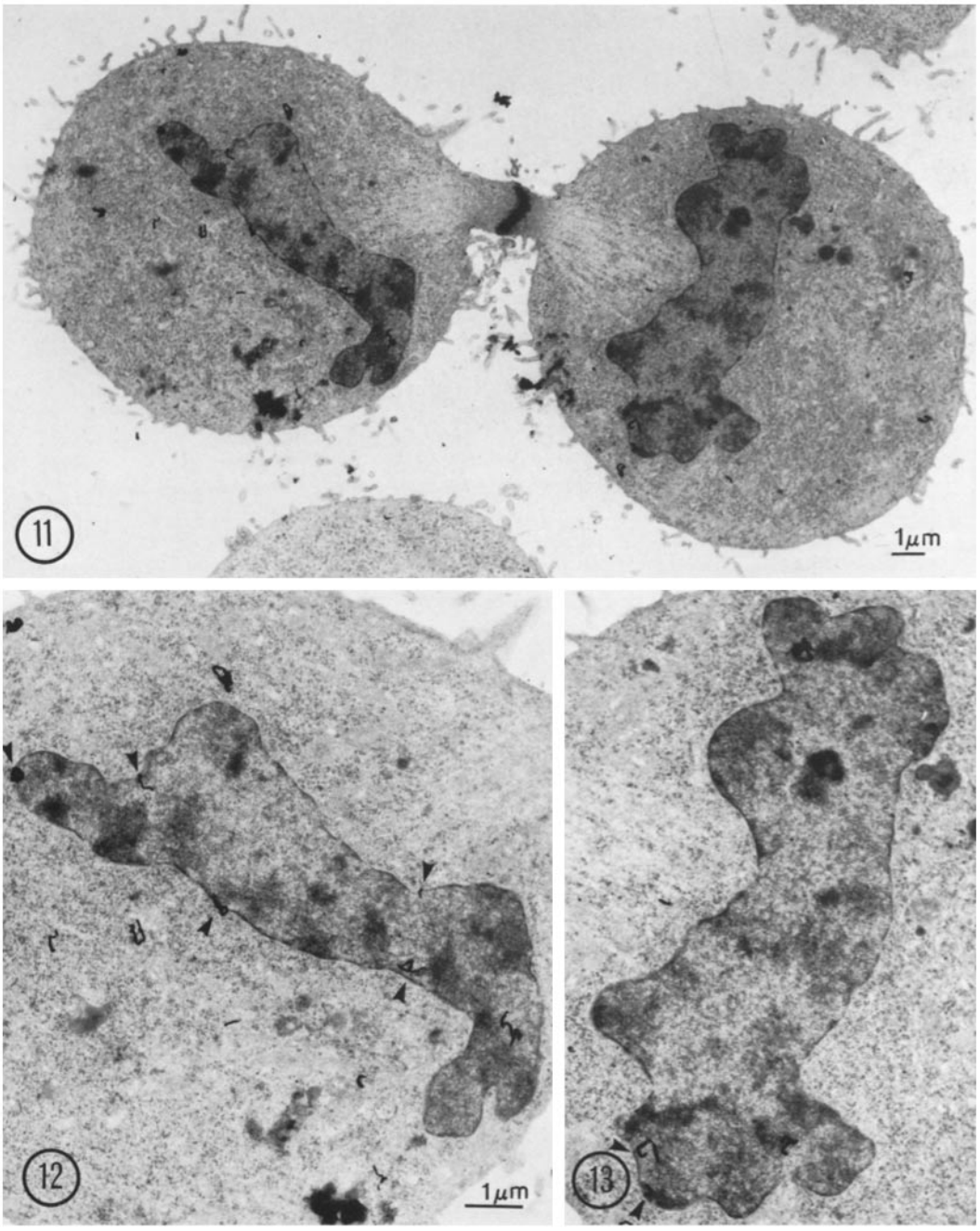
DISCUSSION

Aspects of the reinitiation of nuclear RNA synthesis during the transition from mitosis to G_1 have been investigated. An obvious approach was to determine the effect of inhibitors of macromolecular synthesis. Cautious interpretation of these studies can imply more about this process, as well as suggest further experimental approaches. In the presence of inhibitors of protein synthesis, the kinetics of the increase in RNA synthesis could have resembled those of untreated control cells, could have exhibited a slowly increasing rate, or could have shown a delay followed by an increasing rate. Any of these patterns of restoration would explain the synthesis of some precursor

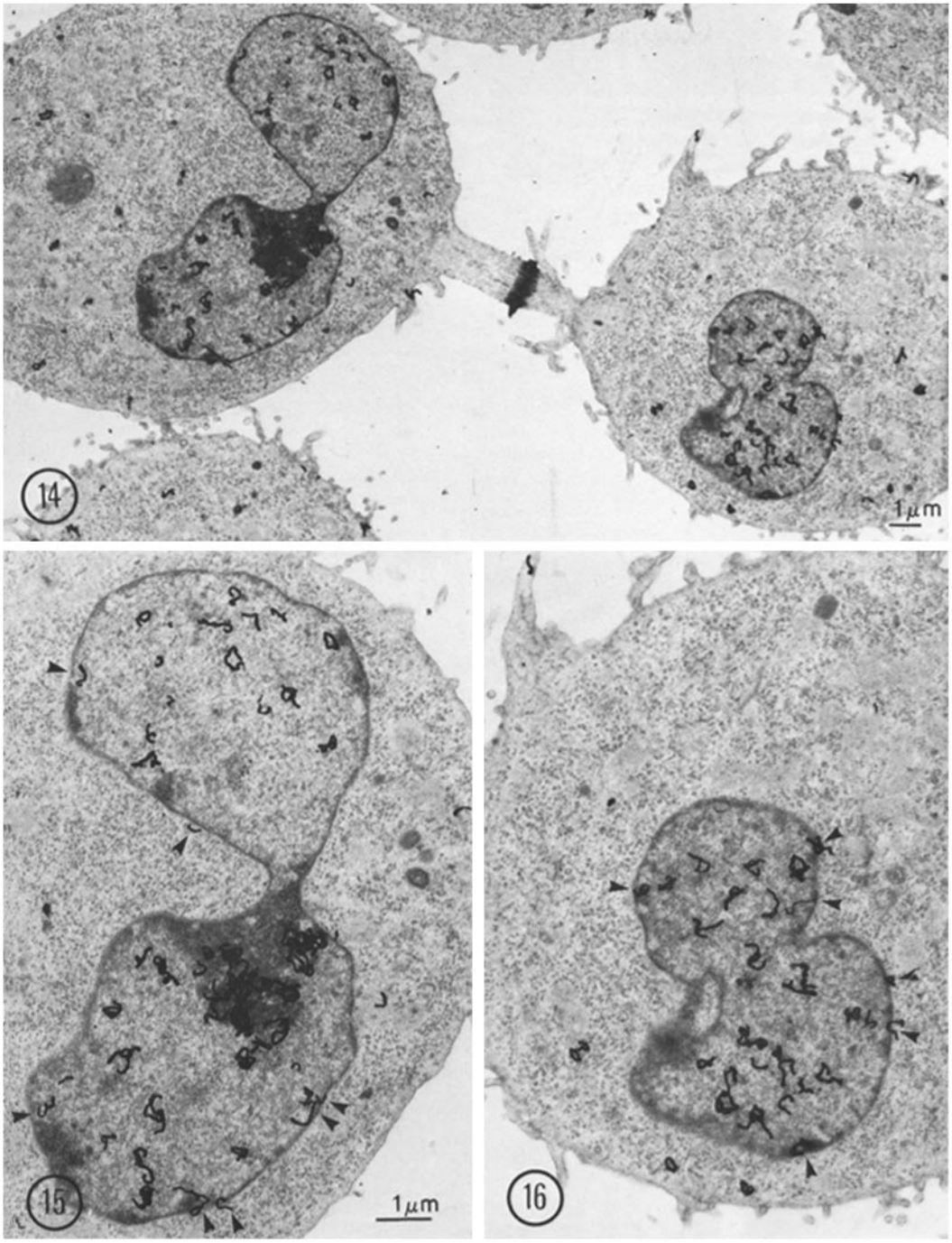
FIGURES 5 and 6 Oblique longitudinal section through a late anaphase cell. A contiguous serial section of the larger chromosome group is shown in Fig. 6. Few grains were observed over anaphase cells. Of the silver grains found over this cell, three of seven are near sites of completed nuclear envelope. Note the similarity of location in the two sections of the grains associated with an area of reforming nuclear envelope (arrowheads). × 7,000.

FIGURE 7 Early telophase nucleus. The chromatin remains condensed although at this stage individual chromosomes are not distinguishable. The reformed nuclear envelope is nearly complete. Note the peripheral, apparently random, pattern of grains (arrowheads) at or near the nuclear envelope. × 8,000.

FIGURES 8–10 Three contiguous serial sections through a mid to late telophase nucleus. The nucleus is irregularly shaped and areas of chromosome decondensation are present. Grains are found over the nuclear envelope and over the areas of chromatin. × 8,000.



FIGURES 11–13 Mid to late telophase cell. Fig. 11: Progeny cells are connected by an intercellular bridge which is relatively short compared to that in Fig. 14. × 4,400. Figs. 12 and 13: Each nucleus has a similar morphology, degree of chromosomal decondensation and a peripheral location of grains (arrowheads). × 8,000.



FIGURES 14–16 Early G_1 cells. Fig. 14: Progeny cells joined by an elongated intercellular bridge. × 4,400. Figs. 15–16: Nuclei appear more round and the chromatin seems to be decondensed. Silver grains are distributed over the entire nuclear area, and a highly localized concentration of grains is found over the nucleolus in Fig. 15. Some grains still appear at or near the nuclear envelope (arrowheads). × 8,000.

TABLE I

Relative Grain Density in Telophase and Early G_1 Cells

Stage in cell cycle	Compartment	Number of grains	Relative area	Relative grain density
			%	
Telophase	Nuclear envelope	101	2.3	1.00
	Nuclear	103	13.8	0.17
	Cytoplasmic	440	83.9	0.12
Early G_1	Nuclear envelope	85	2.9	1.00
	Nuclear	522	28.5	0.62
	Cytoplasmic	185	68.6	0.09

Measurements of cellular area and grain counts were made from autoradiographs of 32 telophase and 36 G_1 cells printed at × 14,060. The relative area of each cellular compartment was estimated with a uniformly dispersed grid of points by dividing the points per compartment by the total number of points. The relative density represents the grains per area for each compartment normalized to that of the nuclear envelope.

ribosomal RNA by Chinese hamster ovary cells 2 h after release from Colcemid arrest in the presence of cycloheximide (7). Our data indicate that the kinetics of the resumption of RNA synthesis, in the absence of the return to interphase levels of protein synthesis, closely parallel those of untreated cultures. This level of synthesis was not maintained in G_1. The initial increase in rate of synthesis implies that activity of preexisting RNA polymerase(s) persists through mitosis. The decline in the rate of synthesis in G_1 populations implies that synthesis of new enzyme(s) and/or factor(s) are necessary to maintain interphase levels of RNA synthesis. Both heterogeneous RNA and ribosomal precursor RNA were affected similarly by the inhibition of protein synthesis. In addition, by varying the concentrations of actinomycin D, it was shown that the restoration of heterogeneous RNA synthesis was independent of ribosomal precursor RNA synthesis. These results, combined with the observations with the inhibitors of protein synthesis, imply that at least preexisting DNA-dependent RNA polymerase I and II (24, 25) were involved and were functioning independently in the restitution of RNA synthesis. This contention is supported by our observations of in vitro RNA synthesis by preparations of metaphase chromosomes (26). Preparations of isolated chromosomes were used as sources of both substrate and endogenous RNA polymerase activity (or activities) in an in vitro assay measuring tritiated UTP incorporation. The radioactive product of this reaction was extractable with phenol and was sensitive to digestion by pancreatic ribonuclease. Although not previously documented, this possibility had been suggested (27).

The maximum increase in the rate of nuclear RNA synthesis occurred before a major portion of the population had reestablished an interphase morphology. This population consisted of cells in a continuum of stages from late anaphase to early G_1. According to Robbins and Gonatas (2), these stages represent a 20–25-min interval at the end of cell division. There was evidence for a small amount of synthesis in late anaphase, while significant amounts of radioactivity abruptly appeared in telophase. Restoration of RNA synthesis seemed to occur in early telophase after reformation of the nuclear envelope but before decondensation of the chromosomal mass. Light microscope autoradiographic studies of cultured mammalian cells following exposure to tritiated uridine have provided estimates of the time of resumption of RNA synthesis that varied from late telophase to early G_1 (28–32). Our use of high resolution electron microscope autoradiography, as well as a tightly synchronized population of cells, has permitted a more precise determination of the time of restoration of synthesis in relation to defined nuclear morphology. It could be argued that the RNA synthesis in telophase cells represented continuing synthesis of small RNA species during cell division. There is evidence for synthesis of near interphase levels of nonmitochondrial 4S and 5S RNA in HeLa and Chinese hamster ovary cells

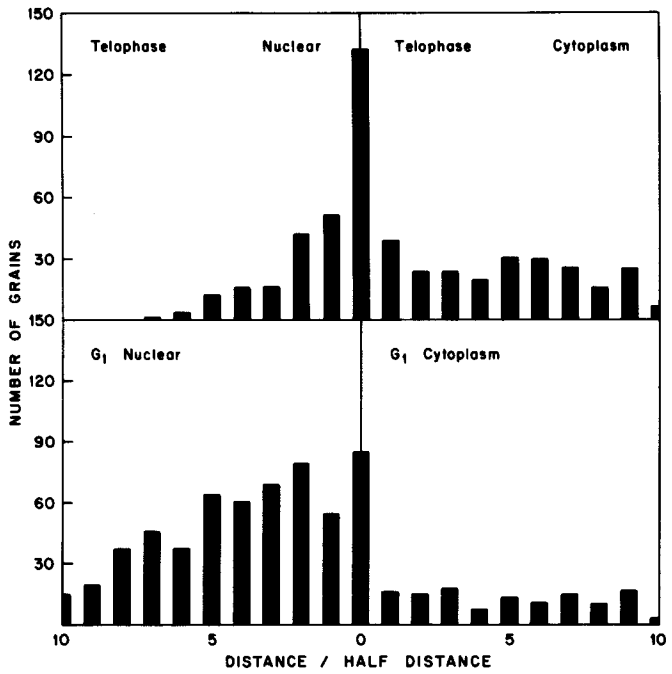
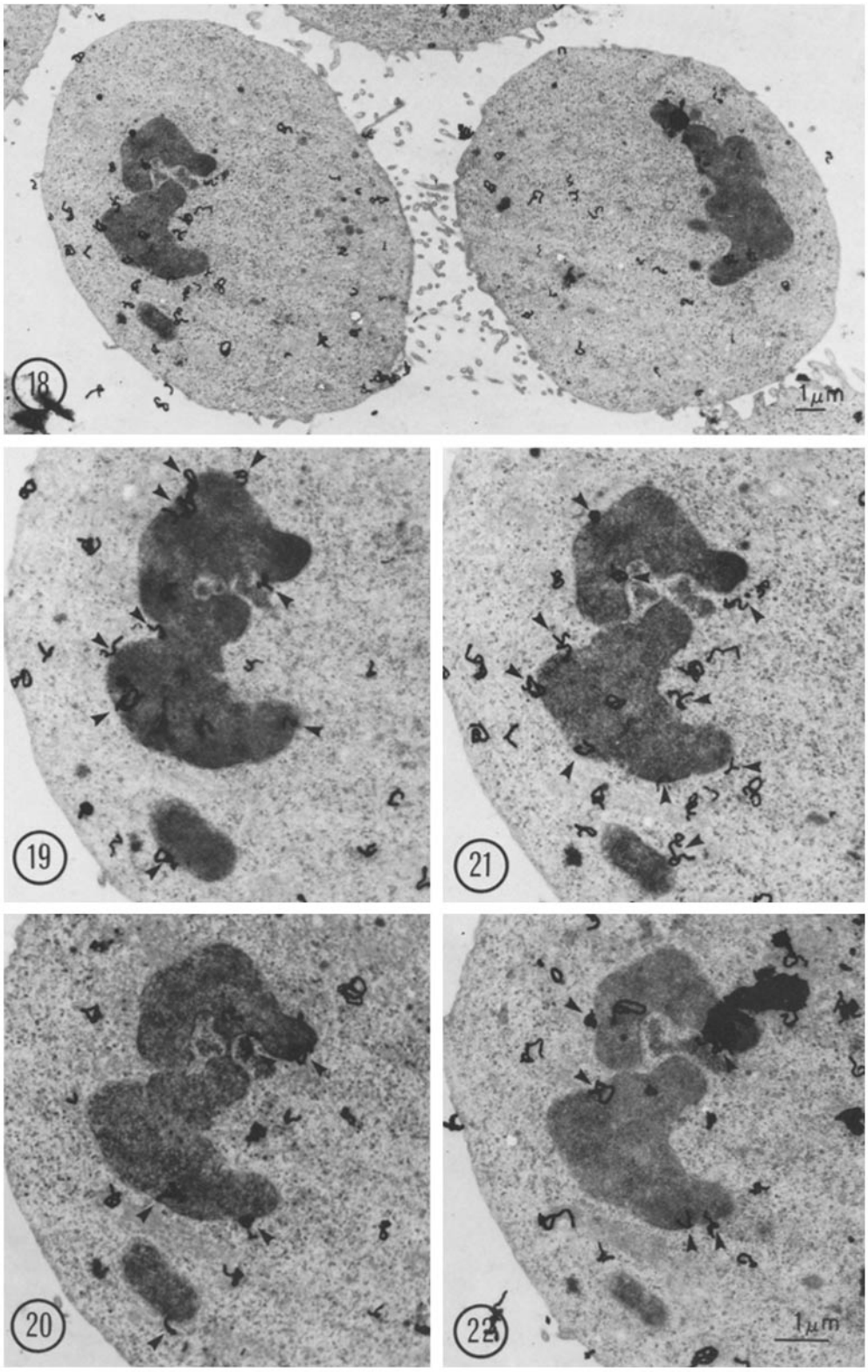


FIGURE 17 Autoradiographs at × 14,060 or × 24,300 were quantified according to the method of Salpeter et al. (23), using 1,500 Å as one half-distance (HD). The perpendicular distance (x) from the midpoint of a grain to the nuclear envelope was converted to the lowest HD. Grains within 10 HD on either side of the nuclear envelope at which x/HD = 0 were tabulated. Upper panels display the distribution of 492 grains from 49 telophase cells. The lower panels display the distribution of 590 grains from 36 early G_1 cells. Left quadrants represent nuclear areas and the right quadrants represent cytoplasmic areas.

arrested in metaphase (33). It is not clear whether these experiments, using an exposure of 4–8 h to Colcemid, are analogous to ours, in which the process of cell division progressed unimpeded. The interval of time between late anaphase and early telophase has been estimated to be 5 min (2). Synthesis of 4S and 5S RNA could have been occurring in our preparations during the 40-min interval after synchronization in metaphase. However, it is unlikely that continuing high levels of 4S and 5S RNA synthesis could be responsible for the abrupt increase in numbers of grains observed in early telophase relative to late anaphase.

Quantitative analysis of the grain distribution in telophase and G_1 cells suggested that synthesis is initiated at or near the site of the nuclear envelope. This observation is of some interest, since electron microscope autoradiography studies using randomly growing exponential cultures have not demonstrated this correlation. For example, with short pulses of radioactivity for 1 or 15 min in mouse L cells (34), or short pulses of 2.5 or 15 min in monkey kidney cells strain BSCl (35), no preferential site of labeling over the nuclear envelope was detected. Many sites of "ongoing" RNA synthesis exist in interphase nuclei and may have obscured any evidence of membrane-associated synthesis. In addition to synthesis, the accumulation, processing, and transport of RNA occurring in interphase nuclei would contribute to the generalized distribution of silver grains. The use of metaphase preparations that progress synchronously from a state of near total restriction of nuclear RNA synthesis to a state of rapidly resuming nuclear RNA synthesis, before the reestablishment of complete nuclear function, obviates the problems inherent in studies not employing synchronized cells. Although metaphase populations were exposed to radioactive uridine for 40 min, individual cells would have utilized the nucleoside for shorter periods of time; these can be correlated with the cells' respective mitotic stages. For example, the grains observed at or near the nuclear envelope in early telo-



FIGURES 18–22 Early to mid telophase cell. Fig. 18 is an oblique longitudinal section that does not include the central portion of the cell. × 4,400. Figs. 19–22 are contiguous serial sections of the left nucleus. The lobed configuration of the condensed chromatin is apparent from these serial sections. Reformation of the nuclear envelope is almost complete. Grains at or near the nuclear envelope are indicated by arrowheads. × 8,000.

phase could represent synthesis for less than 1 min. Several explanations for the peripheral location of silver grains should be considered. The straightforward interpretation of this observation is that the appearance of grains represents sites of RNA synthesis. It appears unlikely that the autoradiographic data represent the association of uridine or its derivatives with the nuclear envelope, for two reasons. The fixation and preparation of samples made use of a variety of aqueous and organic solvents, and the same association of grains with the cell membrane was not observed. It is conceivable that there has been an accumulation of previously synthesized nuclear RNA at this structure before transport to the cytoplasm. However, we have observed no evidence for earlier synthesis at other sites in the reforming nucleus. Peripheral labeling may represent sites of synthesis in dispersed subnucleolar particles (36). However, the ultrastructural evidence for such particles was not seen. Therefore, we presume that this synthesis represents extranucleolar RNA. Autoradiographic studies employing low levels of actinomycin D may provide additional evidence as to the species of RNA synthesized. It is also possible that membrane associated RNA synthesis is unique to the restoration occurring in telophase cells and cannot be generalized to synthesis during interphase. Conceivably, nuclear membrane vesicles that coalesce and fuse at the polar surface of the chromosomes are the sources of enzyme(s) and substrate(s) that persist in mitotic cells and are necessary for the initiation of RNA synthesis.

The functional significance of the nuclear envelope in eukaryotes is a matter of conjecture but several speculations concerning the role of this structure have been proposed. DNA replication in at least a portion of S phase may be associated with the nuclear envelope, possibly in the vicinity of the nuclear pores (37–40). Attachment of chromosomes to the nuclear envelope may be a mechanism for ensuring that interphase chromatin is nonrandomly arranged (41). In mitotic and interphase cells the inner and outer membranes of the nuclear envelope may serve as centers for microtubule growth (42, 43). The nuclear envelope may give rise to the synaptonemal complex (44). Transport of messenger RNA may, in part, be regulated by enzymes associated with the nuclear envelope (45). Our data permit the additional speculation that the nuclear envelope may also serve as the site of resumption of RNA synthesis in mammalian cells at early telophase.

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