REPOPULATION OF THE POSTMITOTIC NUCLEOLUS BY PREFORMED RNA

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ABSTRACT

This study is concerned with the fate of the nucleolar contents, particularly nucleolar RNA, during mitosis Mitotic cells harvested from monolayer cultures of Chinese hamster embryonal cells, KB6 (human) cells, or L929 (mouse) cells were allowed to proceed into interphase in the presence or absence (control) of 0.04–0 08 μg/ml of actinomycin D, a concentration which preferentially inhibits nucleolar (ribosomal) RNA synthesis 3 hr after mitosis, control cells had large, irregularly shaped nucleoli which stained intensely for RNA with azure B and for protein with fast green. In cells which had returned to interphase in the presence of actinomycin D, nucleoli were segregated into two components easily resolvable in the light microscope, and one of these components stained intensely for RNA with azure B. Both nucleolar components stained for protein with fast green In parallel experiments, cultures were incubated with 0.04–0 08 μg/ml actinomycin D for 3 hr before harvesting of mitotic cells, then mitotic cells were washed and allowed to return to interphase in the absence of actinomycin D. 3 hr after mitosis, nuclei of such cells were devoid of large RNA-containing structures, though small, refractile nucleolus-like bodies were observed by phase-contrast microscopy or in material stained for total protein. These experiments indicate that nucleolar RNA made several hours before mitosis persists in the mitotic cell and repopulates nucleoli when they reform after mitosis

Nucleoli, the most obvious and distinctive structures seen within interphase nuclei, appear to disintegrate at each mitotic prophase. From late prophase until telophase, discrete morphological entities equivalent to interphase nucleoli are not cytologically detectable in most cell types. What becomes of the high concentrations of RNA (Caspersson and Schultz, 1940) and protein (see Gates, 1942) which characterize interphase nucleoli is not clear. Recently, it was shown that nucleolar RNA's do persist in mitotic cells (Fan and Penman, 1971) Though these nucleolar RNA's are biochemically distinctive (Penman et al., 1966), their location within the mitotic cell and their fate after mitosis have not been worked out

It also seems likely that some nucleolar proteins might be stable through mitosis. At telophase, amorphous material appears to be sloughed from along the chromosomes and to be incorporated into the forming nucleoli (Heitz, 1931; Derman, 1933, Jacob, 1940). Thus, though the nucleoli are known to be organized in association with specific chromosomal loci (McClintock, 1934) which contain the cistrons for ribosomal RNA (Brown and Gurdon, 1964; Ritossa and Spiegelman, 1965), their formation at telophase may not be (exclusively) dependent upon *de novo* synthesis at those loci.

The present study was undertaken to investigate the fate of the nucleolar contents during mitosis. It is particularly concerned with the problem of whether nucleoli are synthesized *de novo* at each telophase or whether they are reassembled from preformed components which exist in a stable form during mitosis.

MATERIALS AND METHODS

Experiments were performed using early passages of fibroblastic cell lines derived from macerated Chinese hamster fetuses, KB6 (human oral carcinoma) cells obtained from the laboratory of Dr. Maurice Green (St. Louis University Medical School), and L929 cells (American Type Culture Collection). Cells were maintained in monolayer culture in Joklik's modified minimal essential medium (MEM) with 10% fetal calf serum and were subcultured by trypsinization every 4–6 days. Experiments were done on nonconfluent cells fed fresh medium 16.5 hr previously, as it had been determined that this resulted in improved yields of mitotic cells. Preconditioned medium was always used for medium changes during the course of an experiment.

Mitotic cells were harvested from Blake bottles by selective detachment (Terasima and Tolmach, 1963). Mitotic cells were then pelleted by centrifugation, resuspended in 0.3 ml of preconditioned medium, and carefully pipetted onto a glass cover slip inside a plastic Petri dish so that the medium did not run over the edge of the cover slip. This procedure made it possible to work with the small numbers of mitotic cells which could be obtained from unsynchronized cultures. The cover slip cultures were then incubated for 3 hr in a humidified atmosphere containing 5% CO_2. The percentage of mitotic cells obtained was monitored each time the mitotic cells were collected by removing a small portion of cells and fixing it with 3 parts methanol:1 part acetic acid at the time of harvesting, air drying the cells on a slide, staining them with Giemsa's, and scoring the per cent mitotic cells. Only experiments in which the mitotic index was greater than 50% were considered in evaluation of the results. Though cells used to initiate the cover slip cultures were not all in mitosis, the postmitotic cells could be identified since at the time they were fixed they still were distributed as pairs of morphologically similar (mirror image) sister cells, usually with a point of contact between them. 3 hr after plating of mitotic cells, cover slip cultures were fixed for 0.5 hr with 3 parts methanol:1 part acetic acid and stained for RNA and DNA with azure B (Flax and Himes, 1952). In some cases, fixed cultures were treated with DNase (RNase-free, Worthington Biochemical Corp., Freehold, N. J.), 0.2 mg/ml, or RNase (Worthington Biochemical Corp., pretreated for 10 min at 100°C to remove DNase activity), 0.2 mg/ml, for 2 hr at 37°C before staining. Some cultures were stained for total protein with 0.5% fast green, pH 2.0. Actinomycin D was used at 0.04 μg/ml for Chinese hamster and KB cells and at 0.08 μg/ml for L929 cells. These concentrations were found by quantitative radioautography in our laboratory to inhibit nucleolar RNA synthesis without significantly reducing extranucleolar nuclear RNA synthesis. In cases where cultures were treated with actinomycin D before mitotic cells were collected, removal of the actinomycin D was accomplished by pelleting the mitotic cells, resuspending them in 10 ml of Hanks' balanced salt solution, repelleting them, and then resuspending them in conditioned medium for plating out on cover slips. This washing reduced the concentration of actinomycin D by more than 100-fold.

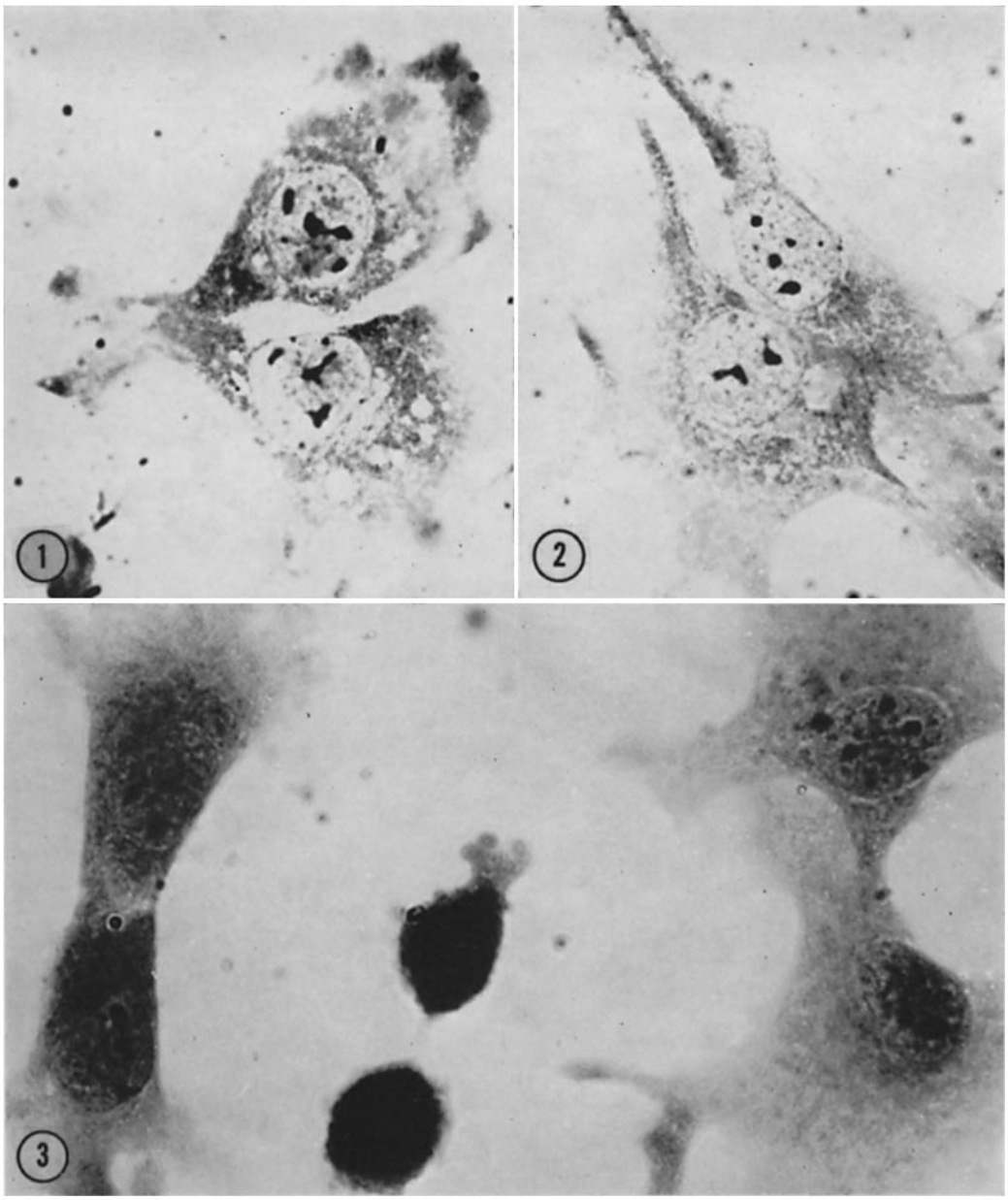
RESULTS

Chinese hamster cell strains were the original cells which were used for these experiments, and similar results were obtained in more than five trials with Chinese hamster cells. Similar, but less extensive, experiments were performed on KB6 or L929 cells, and the results were similar to those obtained with Chinese hamster cells, except in one instance. Thus, all results will be discussed together and readers may assume that they hold for all three cell types unless it is otherwise stated.

The great majority of mitotic cells collected by agitation of monolayer cultures were in stages from metaphase through very early G_1 (sister cells still connected by a midbody). Very few prophases were obtained by this method. Nevertheless, when cultures were fixed 3 hr later, a degree of asynchrony among pairs of postmitotic cells was already apparent. Some sister cells (identifiable as adjacent cells of similar morphology with a point of contact between them) were well spread out and had numerous cytoplasmic processes and large, round, lightly staining nuclei with discrete nucleoli. Other cell pairs were still in late telophase, and the sister cells were roundish and had compact, irregularly shaped, deeply staining nuclei in which nucleoli were difficult to distinguish among the clumps and strands of condensed chromatin. In some experiments, it appeared that the process of agitating the cultures, centrifuging the mitotic cells into a pellet, and resuspending them by pipetting caused a lag in the recovery of the cells from mitosis, as some cells still remained in mitosis when fixed 3 hr later. However, most of the cells in these cultures had attached to the glass cover slip and proceeded into interphase.

Controls: Reformation of Nucleoli

Variable numbers of nucleoli (Phillips and Phillips, 1969) were clearly visible in nuclei of sister cells which had entered G_1 in the 3 hr since harvesting. In preparations stained for nucleic acids with azure B, these nucleoli appeared as irregu-



FIGURES 1 and 2 Chinese hamster cells 3 hr after mitosis. Azure B. Sister cells occur in pairs. Large, irregularly shaped nucleoli stain intensely for RNA with azure B. × 1500.

FIGURE 3 Chinese hamster cells 3 hr after mitosis stained with fast green, pH 2. Deeply stained nucleoli are visible against less deeply stained nucleoplasm. × 1500.

larly shaped, deeply stained, large nuclear bodies (Figs. 1, 2, and 4) RNase pretreatment abolished all visible staining, and DNase pretreatment did not noticeably affect staining. (The nuclei of interphase tissue culture cells are generally large and the chromatin is too diffuse to stain much with azure B.) It was difficult to distinguish nucleoli from clumps of chromatin in those cells which

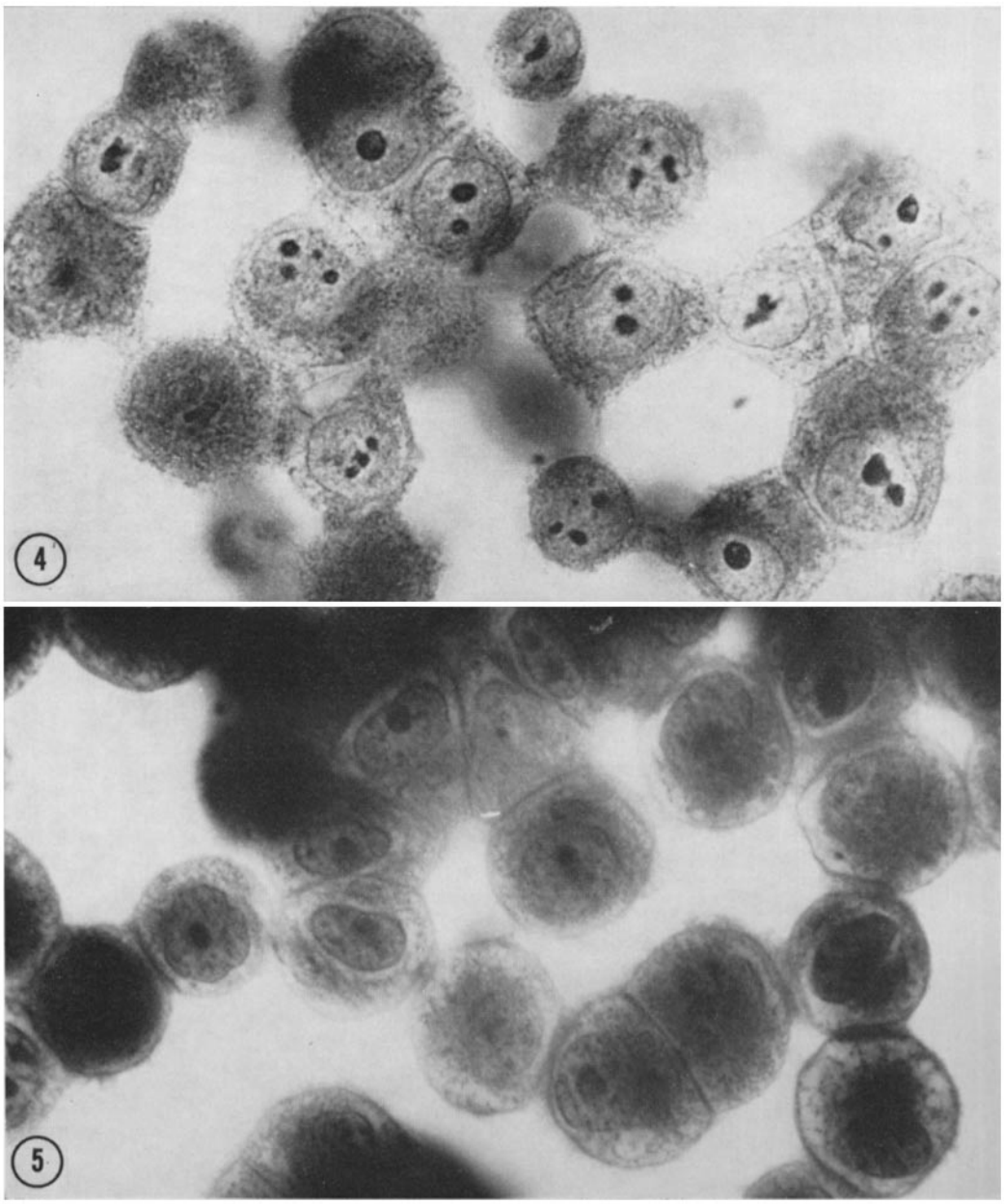


FIGURE 4 KB cells 3 hr after mitosis. Large nucleoli are deeply stained for RNA with azure B. × 1500.

FIGURE 5 KB cells 3 hr after mitosis stained with fast green. × 1500.

were still in late telophase, since the nuclei were still small and dense and the chromosomal strands appeared to be associated with much RNA, which stained purple with azure B. In addition, the condensed chromosomes stained deep blue-green, the color characteristic of DNA under these staining conditions. RNase pretreatment abolished all staining in telophase cells except the blue-green of the chromosomes. DNase pretreatment removed the blue-green staining, but nucleoli were still dif-

ficult to identify in late telophase cells because of RNA apparently associated with clumped chromatin

In preparations stained for total protein with fast green at pH 2, nucleoli were deeply stained and stood out against the more lightly stained nucleoplasm of G_1 cells (Figs 3 and 5). However, in late telophase cells, nucleoli were difficult to distinguish from the decondensing chromosomes which also stained deeply, giving a reticular or splotchy appearance to the nucleus.

Inhibition of Nucleolar RNA Synthesis during Interphase

Before discussing the effects of actinomycin D on mitotic cells, we would like to mention briefly how actinomycin D affected nucleolar morphology in interphase cells. When interphase Chinese hamster or KB6 cells were treated for 30 min or more with 0 04 μg/ml of actinomycin D, nucleoli became small and spherical A crescent-shaped nucleolar "cap" which stained for RNA with azure B appeared to be segregated from a refractile central area which stained little or not at all with azure B Less frequently, the stainable region was centrally located, with spherical unstained regions to either side The segregated nucleoli stained uniformly with fast green. They persisted in this segregated form for at least 6 hr in the presence of a low level of actinomycin D. (In the presence of 5.0 μg/ml actinomycin D, nucleoli of Chinese hamster cells tended to disaggregate and disappear after about 3 hr [Phillips and Phillips, 1971 *b*] Thus, nucleolar integrity may be dependent upon the synthesis of nonnucleolar RNA, perhaps the mRNA for nucleolar proteins) When cultures were washed free of actinomycin D and incubated in medium lacking the inhibitor, the segregated appearance of nucleoli persisted for at least 4 hr, even when the initial exposure to actinomycin D was as short as 1 hr.

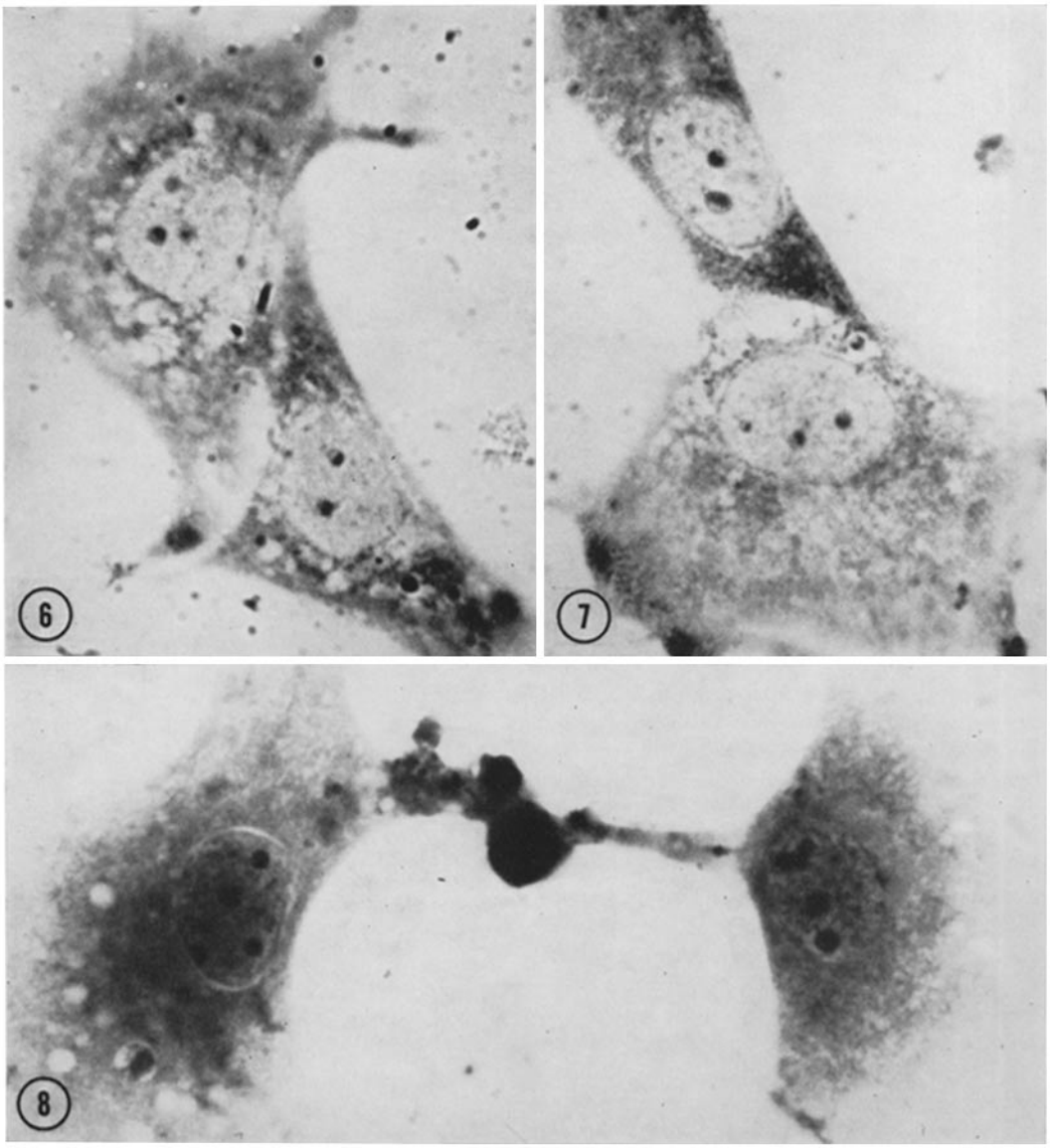
Inhibition of Postmitotic Nucleolar RNA Synthesis: Reformation of Nucleoli

In experiments dealing with the fate of nucleolar RNA during cell division, mitotic cells were collected mechanically and actinomycin D was immediately added at a concentration which preferentially inhibited nucleolar RNA synthesis The mitotic cells were plated onto cover slips in the presence of actinomycin D, incubated at 37°C for 3 hr, fixed, and stained There was no apparent lag in the progression of cells into G_1 due to the presence of actinomycin D. In the case of Chinese hamster and L929 cultures, pairs of G_1 cells were found to possess varying numbers of small, roundish nucleoli. These nucleoli, which had reformed postmitotically in the presence of actinomycin D, generally appeared to have a crescent-shaped cap which stained intensely for RNA with azure B The central portions of these segregated nucleoli were highly refractile, but they stained very little, if at all, with azure B (Figs 6 and 7). In KB and L cells, the cap occupied proportionately more of the nucleolar volume than in Chinese hamster cells (Phillips and Phillips, 1971 *a* and *b*). In preparations stained with fast green, the small roundish nucleoli appeared deeply and uniformly stained; the RNA-containing cap was not apparent (Fig. 8) This indicates that both major regions of the segregated nucleoli contained protein, but only the cap contained significant amounts of RNA. The cap corresponds to a particulate component seen in electron micrographs, whereas the central, principally proteinaceous region appears fibrillar in the electron microscope (Phillips and Phillips, 1971 *a* and *b*) Generally, these results suggest that RNA made before mitosis repopulates nucleoli after mitosis in the absence of further nucleolar RNA synthesis

KB6 cells reacted somewhat differently from Chinese hamster and L929 cells in this experiment When mitotic KB6 cells were collected and allowed to return to interphase in the presence of actinomycin D, the nuclei of some G_1 cells displayed small, segregated nucleoli, but in most cells the nuclei contained only tiny, irregular flecks which were stainable with azure B These flecks may have represented remnants of nucleoli which formed and then disintegrated, or possibly they were RNA which had been associated with the telophase chromosomes

Inhibition of Nucleolar RNA Synthesis before Mitosis

It was felt that if nucleolar RNA synthesized before mitosis indeed repopulated nucleoli after mitosis, it should be possible to interfere with this process by incubating cells for a time before mitosis with actinomycin D at a level just sufficient to inhibit nucleolar RNA synthesis This was accomplished by adding actinomycin D to unsynchro-



FIGURES 6 and 7 Chinese hamster cells which had been harvested at mitosis and then incubated for 3 hr in the presence of 0.04 μg/ml actinomycin D. Stained with azure B. Small, round, segregated nucleoli are seen. The caps stain intensely for RNA. × 1500.

FIGURE 8 Chinese hamster cells which had been harvested at mitosis and then incubated for 3 hr in the presence of 0.04 μg/ml actinomycin D. Stained with fast green. Though the roundish nucleoli appeared segregated when stained for RNA (Figs. 6 and 7), the segregation is not apparent in this preparation stained for total protein. × 1500.

nized cultures for 1, 2, or 3 hr before harvesting mitotic cells. The mitotic cells were washed and plated onto cover slips in medium lacking actinomycin D. The cover slip cultures were fixed after 3 hr.

The results of this experiment were very striking. In cultures which had been treated with actinomycin D for the last 3 hr before mitosis, postmitotic cell pairs had no large bodies in the nucleus which were stainable for RNA with azure

B (Figs. 9, 10, 13–15). This was true in repeated experiments with Chinese hamster cells, KB6 cells, and L cells. Nuclei of such cells frequently contained a number of very small, refractile (i.e., visible by phase-contrast microscopy) round bodies rimmed by condensed chromatin. These tiny bodies stained with fast green for total protein (Figs. 11 and 12). This experiment suggests that a major portion of the complement of RNA normally observed in postmitotic (early G_1) nucleoli is synthesized in nucleoli during the last 3 hr before mitosis.

Cells treated with actinomycin D for less than 3 hr before mitosis displayed this phenomenon (absence of RNA from nucleolar bodies) to a lesser degree. For instance, when cultures were exposed to actinomycin D for the last 2 hr before mitosis, most postmitotic cells appeared to lack nuclear structures which stained for RNA with azure B, but some cells had nucleoli, occasionally with a segregated appearance, which stained lightly for RNA. (It should be recalled that it took longer than 3 hr for nucleoli of interphase cells to lose their segregated appearance after exposure to actinomycin D.) The nucleoli of cells treated before mitosis with actinomycin D were generally rimmed by more than the normal amounts of nucleolus-associated heterochromatin, which stained blue-green with azure B. In G_1 cells which had been exposed to actinomycin D for only the last hour before mitosis, nuclei resembled controls except that, again, nucleoli seemed to have abnormal amounts of associated heterochromatin. Thus, it appeared under these conditions that the RNA which repopulated postmitotic nucleoli was synthesized over a time period extending back at least 2 hr before mitosis. (This estimate of premitotic synthesis time of postmitotic nucleolar RNA may be too long. The normal interphase transit time for ribosomal RNA precursor is approximately 1 hr, but treatment with actinomycin D lengthens this time [Weinberg et al., 1967].)

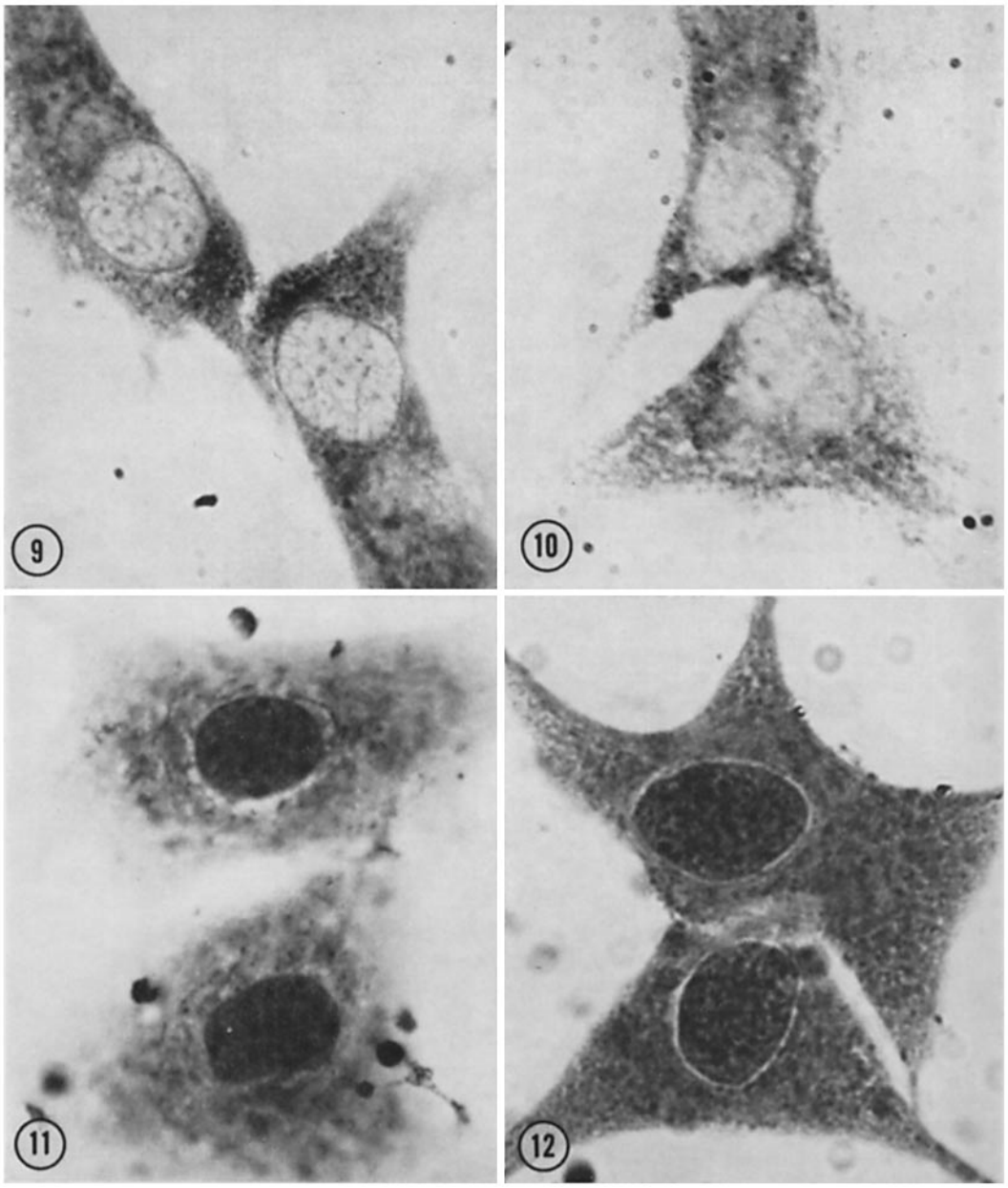
In some experiments with L929 cells, the cells were incubated in actinomycin D for 3 hr after mitosis as well as for various times before mitosis. In the case of cells incubated 1 or 2 hr before mitosis in actinomycin D, collected, then plated onto cover slips still in the presence of actinomycin D, very small nucleoli appeared to form in association with an unusually large amount of reticular-appearing, clumped chromatin. Cells grown 3 hr before mitosis in actinomycin D and 3 hr after mitosis in actinomycin D lacked any structures stainable with azure B which resembled nucleoli.

DISCUSSION

We have considered the problem of whether the nucleolus is created *de novo* after each mitosis or whether it is reassembled from preformed elements. Our observations may help to resolve the discrepancies between the classical picture of the postmitotic nucleolus being "organized" from matrix material sloughed from the telophase chromosomes (Heitz, 1931; McClintock, 1934) and the modern concept of a dynamic nucleolus composed of molecules of ribosomal precursor RNA in various stages of processing (Weinberg et al., 1967; Perry, 1967) brought into association at the site of the nucleolus with proteins previously synthesized on cytoplasmic polysomes (Heady and McConkey, 1970) before being returned to the cytoplasm in the form of the complex RNP particles called ribosomes (Warner and Soeiro, 1967; Liau and Perry, 1969).

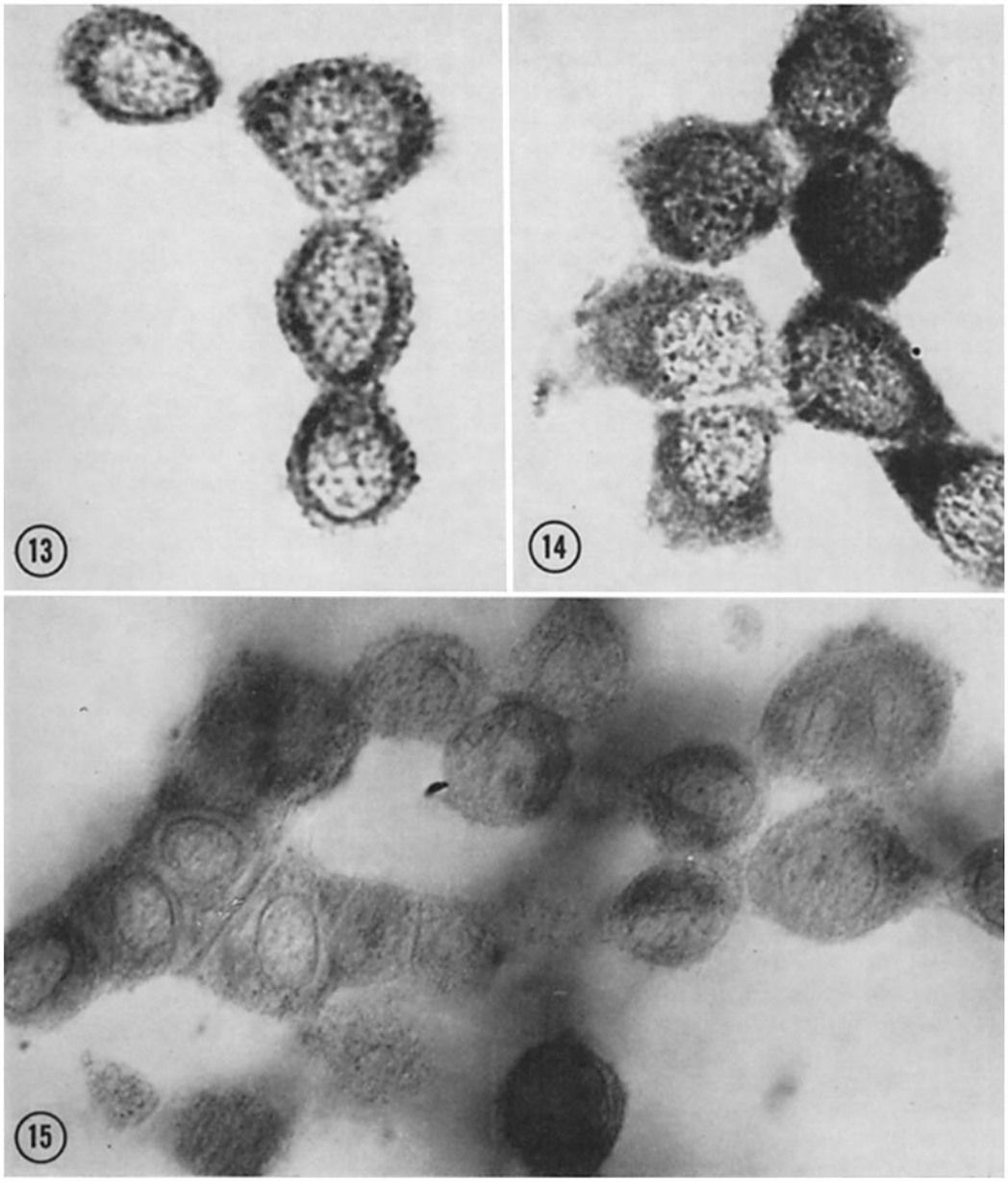
The dynamic concept of the nucleolus as a concentrated collection of sequentially turning over RNA and protein molecules applies only to the interphase cell. Nuclear RNA synthesis comes to a halt during prophase and is not detectable again until telophase (Taylor, 1960; Feinendegen et al., 1960, Prescott and Bender, 1962). Thus, during mitosis there can be no new input of ribosomal precursor RNA's into the pool of molecules subject to processing. Cytoplasmic protein synthesis is sharply depressed during mitosis (Prescott and Bender, 1962; Konrad, 1963, Salb and Marcus, 1965), and presumably the synthesis of proteins destined for the nucleolus is likewise depressed at this time as it accounts for a large fraction of cellular protein synthesis (Heady and McConkey, 1970); in any case, it seems that ribosomal proteins synthesized in the absence of ribosomal RNA synthesis are not usable for later ribosome assembly (Craig and Perry, 1971). Thus, the entry of newly synthesized proteins into the pool of nucleolar proteins is probably also disrupted during mitosis. Concurrent with the halt in RNA synthesis and the decreased rate of protein synthesis are the physical disintegration of nucleoli and their disappearance as discrete morphological entities for the duration of mitosis (Prescott and Bender, 1962).

Ribosomal RNA in mammalian cells is originally transcribed in the nucleolus as a 45S (Scherrer et al., 1963) or possibly a 47S (Tiollais



FIGURES 9 and 10 Chinese hamster cells incubated for the last 3 hr before mitosis in the presence of 0.04 μg/ml actinomycin D, collected, washed, and incubated for 3 hr in the absence of actinomycin D. Stained with azure B. The nuclei are devoid of large, obvious RNA-containing bodies. × 1500.

FIGURES 11 and 12 Same as above, but stained with fast green for total protein. Small nucleolus-like bodies appear to be present, but these are barely detectable against the background of stained chromatin. × 1500.



FIGURES 13 and 14 L cells incubated for the last 3 hr before mitosis with 0 08 μg/ml actinomycin D, washed, and incubated 3 hr in the absence of the inhibitor. Stained with azure B. No large RNA-containing bodies are seen in the nuclei. Staining in the nucleus is due to chromosomes which are not yet completely decondensed. × 1500

FIGURE 15 KB cells incubated for the last 3 hr before mitosis with 0.04 μg/ml actinomycin D. harvested, washed, and incubated for 3 hr in the absence of actinomycin D. Stained with azure B. No large RNA-containing bodies are seen in the nuclei. × 1500.

et al., 1971) molecule. This large precursor molecule is cleaved about 15 min after synthesis, giving rise to a series of intermediates of lower sedimentation constant (Weinberg et al, 1967). The major intermediate found in nucleoli of mammalian cells is a 32S molecule which has a lifetime of about 45 min. The entire process from the synthesis of a 45S RNA molecule in the nucleolus to the appearance of its derivative 18 and 28S RNA's in the cytoplasmic ribosomes normally takes about an hour (Weinberg et al, 1967). During mitosis, however, processing of ribosomal RNA precursor molecules is apparently stopped, and 45S and 32S intermediates are stable in cells arrested in c-metaphase for long periods by Colcemid (Fan and Penman, 1971).

We have investigated the fate of nucleolar RNA, presumably consisting mostly of ribosomal RNA precursor molecules, during mitosis by analyzing the reformation of nucleoli in cells in which postmitotic synthesis of nucleolar RNA was suppressed We find that when mitotic cells are plated out into medium containing sufficient actinomycin D to block nucleolar (ribosomal precursor) RNA synthesis (Perry, 1962), they proceed into G_1 and form discrete RNA-containing nucleoli These nucleoli formed in the presence of actinomycin D have the segregated appearance characteristic of nucleoli of cells treated with actinomycin D or other inhibitors of RNA synthesis (Schoefl, 1964; Reynolds et al., 1964). Stevens and Prescott (1971) similarly observed that ameba reform RNA-containing nucleoli after mitosis when RNA synthesis is blocked by actinomycin D. These nucleoli likewise display a segregated appearance. From these experiments, it is apparent that postmitotic ribosomal RNA synthesis is not necessary for the formation of RNA-containing nucleoli after mitosis This suggests that the RNA found in nucleoli of early G_1 cells is synthesized before mitosis.

Fan and Penman (1971) reported that nucleoli of CHO cells (an established Chinese hamster line) which were allowed to recover from mitotic-arrest in the presence of actinomycin D contained mostly 32S RNA by 2 hr after mitosis. The 45S RNA, which apparently was stabilized in mitotic cells, had broken down when cells returned to interphase. If we can extrapolate these results to our system, then it is likely that the stainable RNA of the cap we see in postmitotic segregated nucleoli contains mostly 32S RNA derived from 45S RNA synthesized before mitosis. This stainable cap corresponds to a region of the segregated nucleoli which appears granular in the electron microscope (Phillips and Phillips, 1971 *a* and *b*). The work of Das et al. (1970) on *Urechis* eggs also suggests that a major cleavage product of the original, large, rRNA transcription unit occupied the cap region of segregated nucleoli in actinomycin D–treated cells.

In a parallel series of experiments, we find that blocking premitotic ribosomal RNA synthesis results in G_1 cells devoid of RNA-containing nucleolus-like bodies, even when these cells have been washed free of actinomycin D at the time of mitosis. This experiment suggests that at least the bulk of the RNA which normally repopulates posttelophase nucleoli is ribosomal precursor RNA formed in the nucleolus before mitosis.

The localization of the ribosomal precursor RNA's within the mitotic cell has not been worked out. Certain experiments are, however, suggestive. It appears that the stabilized 45S and 32S RNA's of mitotic cells cosediment with the condensed chromosomes (Fan and Penman, 1971) Numerous cytochemical studies report that mitotic chromosomes appear to have a coating of RNA (Jacobson and Webb, 1952, Kleinfeld and von Haam, 1959; Mazia, 1961; Feinendegen and Bond, 1963). In addition, thioacetamide injections, which cause a dramatic increase in the amount of stainable RNA in liver nucleoli, also induce the appearance of greatly increased amounts of stainable RNA on metaphase chromosomes (Kleinfeld and von Haam, 1959). Thus, one might suspect that stable ribosomal RNA precursors reside on the chromosomes during mitosis.

Radioautographic investigations generally have not demonstrated the existence of RNA on metaphase chromosomes In fact, when cells are labeled before mitosis with radioactive RNA precursors and then radioautographed, the mitotic chromosomes appear to be unlabeled (at least relative to other structures) (Freedman et al., 1967; Neyfakh and Kostomarova, 1971; Neyfakh et al., 1971). However, in short-term labeling experiments with RNA precursors, most of the incorporation of isotope occurs in nuclear heterodisperse (chromosomal) RNA which turns over rapidly within the nucleus (Scherrer et al., 1966). Several experiments, however, make it

appear that such rapidly labeled HnRNA is shed from the chromosomes during prophase, resides in the cytoplasm during mitosis, and is returned to the nucleus after mitosis (Rao and Prescott, 1967; Neyfakh and Kostomarova, 1971; Neyfakh et al., 1971) In our laboratory we have observed radioautographically that, in cells in which ribosomal RNA synthesis has been suppressed, rapidly labeled RNA leaves the chromosomes and appears in the cytoplasm late in prophase (unpublished). This rapidly labeling RNA obscures the fate of the ribosomal precursor RNA in radioautographs.

There are many reports, both classical and modern, that at telophase "matrix" material is sloughed from many places on the chromosomes and that this material aggregates into a limited number of bodies localized at nucleolar organizer sites on the chromosomes. These bodies develop into the definitive interphase nucleoli (Heitz, 1931; Derman, 1933; McClintock, 1934; Lafontaine, 1958; Stevens, 1965). It seems likely that the bulk of the material seen on the chromosomes may represent nucleolar proteins In our experiments, when nucleolar RNA synthesis was inhibited before mitosis so that no RNA was left to populate postmitotic nucleoli, refractile proteinaceous nucleolus-like bodies often formed in the nuclei. These proteinaceous bodies may have been aggregates of nucleolar proteins, synthesized before mitosis, disaggregated during mitosis, and reorganized into nucleolus-like bodies after mitosis. In early embryogenesis in some animals, it has been reported that proteinaceous nucleolus-like bodies are organized in nuclei before the onset of ribosomal RNA synthesis (Elsdale et al., 1958, Karasaki, 1968; Hillman and Tasca, 1969). In onion and beans, which normally have only 2 nucleoli per diploid cell, one can induce the formation of micronuclei by X-irradiation which breaks chromosomes and causes chromosomal fragments to be left out of the spindle It appears that if such micronuclei lack a nucleolar organizer region, proteinaceous nucleolus-like bodies form in them, nevertheless, but these are incapable of RNA synthesis (Das, 1962). Such proteinaceous bodies, apparently formed from proteinaceous material on the chromosomes, may be analogous to the bodies we observe when premitotic nucleolar RNA synthesis has been inhibited.

From our experiments and those of others, it appears to us that the most likely explanation of the fate of nucleolar components at mitosis is as follows. The dynamic processes which occur in nucleoli during interphase appear to be abruptly interrupted by the onset of mitosis Both RNA and protein components of the nucleolus seem to be conserved during mitosis, perhaps in association with the mitotic chromosomes, and these elements appear to assemble again into functional association in the form of morphologically discrete nucleoli at the end of telophase.

I would like to thank two students, Ruth Imershein and Philip Jen, who helped keep the lab functioning during the course of these experiments.

I would also like to thank the National Science Foundation which subsidized this work under Research Grant GB-29214. The work was initiated under an American Cancer Society Institutional Grant No. IN 36 K.

Received for publication 3 November 1971, and in revised form 7 February 1972.

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