RNA IN CYTOPLASMIC AND NUCLEAR FRACTIONS OF CELLULAR SLIME MOLD AMEBAS

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ABSTRACT

A method is described for the rapid separation of cellular slime mold (*Dictyostelium discoideum*) cells into nuclear and cytoplasmic fractions. Sucrose density sedimentation profiles of radioactivity from cells that had been grown for long or short periods in the presence of uridine-^{3}H indicate very low levels of cross-contamination between the fractions. The nuclear fraction contains few, if any, ribosomes. In exponentially growing cells, at least 80% of the ribosomes were associated in polysomal complexes. No loss of counts from prelabeled rRNA was observed during 2 generations (24 hr) of logarithmic growth and, within the polysomal complexes, the distributions of the preformed material and of rRNA synthesized during the 2 generations were identical. In stationary phase cells that had entered the developmental program leading to fruiting body construction, the rRNA turned over rapidly so that by the end of development at least 75% of the ribosomes fabricated during exponential growth had disappeared and had been replaced by new ones synthesized during the morphogenetic sequence. The preformed ribosomes disappeared preferentially from the monosomal contingent; the newly synthesized ribosomes appeared exclusively in the polysomal contingent and did not appear as monosomes in appreciable numbers for at least 6 hr. The possible significance of this wholesale replacement of ribosomes is discussed.

INTRODUCTION

Recent studies of RNA metabolism in mammalian cells have been facilitated by the application of rapid and gentle methods for separation of the cell contents into nuclear and cytoplasmic components (Prescott et al., 1966; Penman, 1966; Penman et al., 1968; Soeiro et al., 1968). The present study describes a corresponding fractionation procedure suitable for cellular slime mold amebas. The level of cross-contamination between the nuclear and cytoplasmic fractions is shown to be very low. The conditions are gentle enough to preserve the polysomal associations in the latter, and these associations have been systematically examined in cells growing exponentially, cells in the stationary phase, and cells embarked on the morphogenetic program leading to the construction of fruiting bodies. Some studies of RNA distribution between the nucleus and cytoplasm of cells that had incorporated uridine-^{3}H over brief or sustained time periods are described. The results shed some light on the ontogeny and decay of ribosomes and rRNA during growth and morphogenesis.

METHODS

Organism and Growth Conditions

Dictyostelium discoideum, strain Ax-1, was grown at 22°C with shaking in 70 ml volumes of sterile liquid

medium referred to hereafter a HL-5 and contains the following: glucose (16 mg/ml); proteose peptone (14 mg/ml); yeast extract (7 mg/ml); $Na_2HPO_4 \cdot 7H_2O$ (0.95 mg/ml); KH_2PO_4 (0.5 mg/ml). Growth was exponential with a doubling time of about 12 hr and a stationary phase yield of $1-2 \times 10^7$ cells/ml (about 1–2 g dry weight per liter).

Strain Ax-1 was derived from the parent strain NC-4 (which can grow only in association with bacteria), by serial selection in a medium like the above but supplemented with liver concentrate and embryo extract (Sussman and Sussman, 1967). Sustained serial passage in liquid where fruiting body construction is impossible tends to introduce morphogenetically deficient mutants into the population. To avoid this, the stock was plated clonally at monthly intervals on nutrient agar with *A. aerogenes*. A clone that had constructed normal fruiting bodies was selected, and spores, free of bacteria, were used as inoculum in the sterile liquid HL-5 medium for the next cycle of serial passages.

Conditions of Morphogenesis

Cells were harvested from the growth medium by centrifugation at ca 500 *g* for 2–3 min, washed once in cold water, and resuspended in cold water at a density of 1×10^8 cells/ml. Aliquots of 0.5 ml were dispensed evenly on 2 in. Millipore membrane filters that rested on absorbent pads saturated with 1.4 ml of lower pad solution (LPS), a solution containing 1.5 mg/ml KCl, 0.5 mg/ml $MgCl_2$, 0.5 mg/ml streptomycin sulfate, and 0.04 M phosphate pH 6.5, inside 60 mm plastic Petri dishes. Under these conditions the cells proceed through the morphogenetic sequence synchronously and construct fruiting bodies over a 24 hr period (Sussman, 1966). Cells could be recovered quantitatively from a filter simply by immersing it in a few milliliters of cold water and placing the test tube on a Vortex mixer for a few seconds.

Separation of Nuclear and Cytoplasmic Fractions

A sample of 10^8 cells was suspended in 2 ml of cold HMK[1] solution containing 5% (w/v) sucrose and 4% (v/v) of the detergent Cemulsol NPT 12 (Societé des Produits Chimiques de Synthèse, Bezons, Seine-et-Oise, France). The suspension was agitated for 1 min on a Vortex mixer, incubated for 9 min in an ice bath, and then diluted with 2 ml of cold HMK containing 22% sucrose and 4% NPT 12. The suspension was examined briefly at this time under phase optics at 1250 × magnification to check the efficiency of cell breakage. The incidence of whole cells was routinely less than 4×10^{-4}. The nuclei were collected by centrifuging at 1000 *g* for 5–10 min and washed once in a 1:1 mixture of the 5 and 22% sucrose HMK-solutions. The supernatant from the first spin was further fractionated by centrifuging for 5–10 min at 10,000 *g* to yield a pellet called the *particulate cytoplasmic fraction* and a supernatant called the *soluble cytoplasmic fraction*.

A rapid fractionation procedure, a variant of the above method, was used in order to avoid significant breakdown of polysomes in the soluble cytoplasmic fraction. The cells were suspended in the first detergent solution, agitated on the Vortex mixer for 1 min, immediately diluted 1:1 with the second detergent solution, centrifuged at 10,000 *g* for 1.5 min, and the supernatant was immediately diluted 1:1 with HMK solution and centrifuged in a sucrose density gradient as described in the legend to Fig. 3.

[1] HMK solution contains: 0.05 M HEPES buffer (*N*-2-hydroxyethylpiperazine-*N*[1]-2-ethane sulfonic acid, Calbiochem) adjusted to pH 7.5 with NH_4OH; 0.04 M $MgCl_2$; 0.02 M KCl.

RESULTS

Distribution of Radioactivity after Incorporation of Uridine-^{3}H

Cells growing exponentially in liquid medium were exposed to uridine-^{3}H for a period of 24 hr (2 generations). Sister cells were exposed to uridine-^{3}H for 15 min. The cells were harvested, separated into nuclear and cytoplasmic fractions, treated with sodium dodecyl sulfate (SDS), and centrifuged in 13–23% linear sucrose gradients. Fig. 1 shows the profiles of optical density and trichloroacetic acid (TCA)-insoluble radioactivity for samples of 10^8 cells.

Fig. 1 (left) summarizes the data for cells labeled with uridine-^{3}H during a 24 hr period. The radioactivity profiles show great disparities in several regions of the gradients and indicate that cross-contaminations must have been relatively slight. The OD_{260} traces of both the particulate and soluble cytoplasmic fractions show the characteristic rRNA peaks with sedimentation coefficients variously calculated as 16 and 23S (Ashworth, 1966) or 17 and 25S (Ceccarini et al., 1968). The radioactivity profiles appear to coincide with the OD_{260} profiles, and the levels of radioactivity associated with the peaks are in the ratio that would be expected for the two kinds of rRNA molecules. However, the profiles observed in the nuclear fraction appear to differ from the above profiles in two respects. First, the OD_{260} profiles do not correspond precisely. The main peak in the nuclear fraction is slightly heavier than the 23–25S rRNA of the cytoplasm. This difference is considered to be significant.

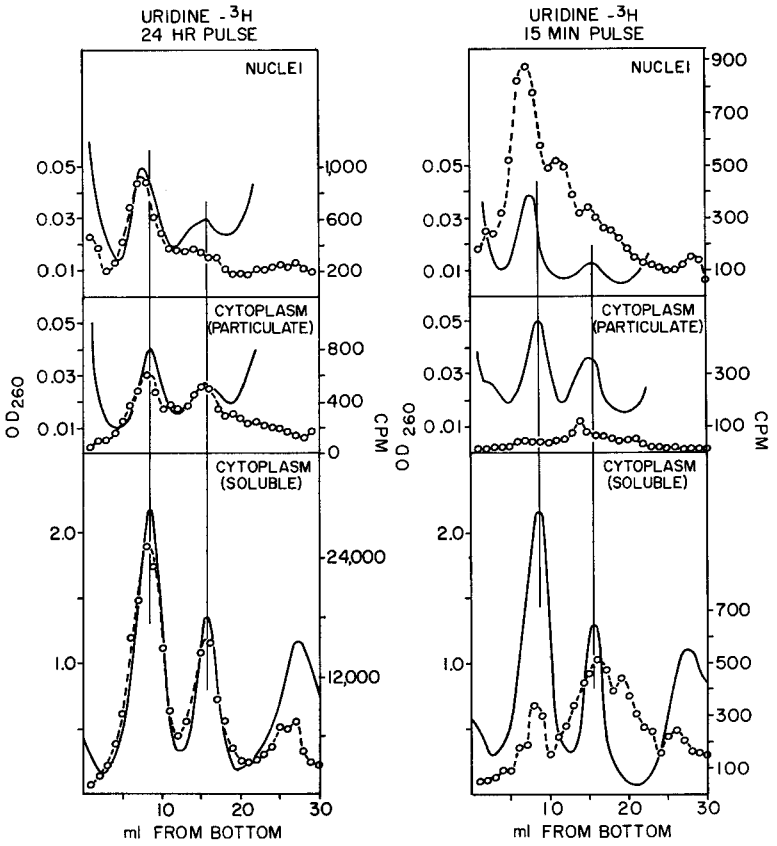

FIGURE 1 (*Left*) To a log phase culture (15 ml) of *D. discoideum* at a density of about 2×10^6 cells/ml was added 50 μCi of uniformly labeled uridine-^{3}H (27 Ci/mmole). Incubation was continued for 24 hr by which time the density of each culture was 1×10^7 cells/ml. The culture was harvested by centrifugation at 2000 *g* for 1.5 min. A sample containing 10^8 cells was fractionated as described in Methods. The nuclear and particulate cytoplasmic fractions were washed once, and each fraction was suspended in 0.9 ml of H_2O plus 0.1 ml of 10% SDS. The soluble cytoplasmic fractions were precipitated in 90% cold ethanol (to reduce the level of the alcohol-soluble detergent that has a high UV absorbance), and the pellet was suspended in 2.7 ml of H_2O plus 0.3 ml of 10% SDS. Samples of 1 ml were layered over linear gradients prepared from solutions containing 13–23% sucrose (w/w), 0.1 M NaCl, 0.01 M Tris buffer pH 7.4, 0.5% SDS and were centrifuged for 17 hr at 22500 rpm in an SW-25 rotor. The tubes were pierced and emptied from below, with a finger pump, through the flow cell of a Gilford recording spectrophotometer (Gilford Instrument Laboratories Inc., Oberlin, Ohio) 1-ml fractions were precipitated with 10% TCA, collected on membrane filters, washed, and counted in liquifluor scintillation fluid. (*Right*) A 15 ml culture of log phase cells (5×10^6 cells/ml) was exposed to uridine-^{3}H (12 Ci/mmole, 33 μCi/ml) for 15 min, harvested, and treated as described above. The solid lines represent OD_{260} traces (meaningless at the tops of the gradients because of the high absorbance of the detergent at 260 mμ). The dotted lines and circles represent TCA-insoluble radioactivity. All data including those for the cytoplasmic fractions are given as amounts per 10^8 cells.

Each set of three fractions was centrifuged in the same rotor and thus mutually serve as external references The difference was consistent in the experiments shown in Fig. 1 (and in repeat experiments not shown here), while in the replicate centrifugations of any one such fraction the positions of each of the peaks with respect to the meniscus agreed very closely. Second, there is a great disparity between the absolute levels of radioactivity associated with the rRNA peaks in the soluble cytoplasmic fraction on the one hand and the two peaks observed in the nuclear frac-

tion on the other, as well as disparate relative levels of radioactivity between the nuclear fraction peaks themselves.

The data suggest that, as in HeLa cells (Penman, 1966), the isolated *D. discoideum* nuclei are deficient in intact ribosomes and consequently in the capacity to synthesize proteins. The data shown in Table I support this conclusion. In cells exposed briefly to a mixture of ^{14}C-labeled amino acids, the TCA-insoluble radioactivity associated with the nuclei was less than 3% of the radioactivity found in the cytoplasm.

The distribution of radioactivity after a 15-min pulse of uridine-^{3}H is shown in Fig. 1 (right). About 60% of the total radioactivity is associated with the nucleus, a large proportion coincident

TABLE I

Amino acid incorporation in the nuclear and cytoplasmic fractions. 5 ml of a culture at a density of 1.5×10^7 cells/ml was incubated for 10 min with 20 μCi of a ^{14}C-amino acid mixture (New England Nuclear Corp., Boston, Mass.). The cells were harvested and fractionated, and the fractions were assayed for TCA-insoluble radioactivity.

Fraction	Total TCA-insoluble radioactivity
	cpm
Nuclei	160
Cytoplasm (particulate)	295
Cytoplasm (soluble)	6160

with the 26–27S OD_{260} peak or perhaps even slightly heavier, a lesser proportion in the 16–24S region, a shoulder at 16S, a small 4S peak, and a small amount of very heavy material. Most of the remaining 40% is associated with the soluble cytoplasmic fraction, and the distribution of this radioactivity has only limited correspondence with the OD_{260} trace. There are a small 23–25S peak, a pronounced 16–17S peak, a considerable proportion in the 4–16S region, and a small 4S peak. Most of this material must be unstable in view of the different distribution found after sustained exposure to uridine-^{3}H (Fig. 1, left). This result is in agreement with the results of corresponding experiments previously carried out with *Polysphondelium pallidum* (R. Sussman, 1967) in which total cell RNA was examined.

The Level of Polysomes during Growth and Morphogenesis

Soluble cytoplasmic fractions prepared from cells growing exponentially in liquid medium were centrifuged in sucrose gradients for examination of their polysomal profiles These profiles are shown in Fig. 2 *A* and *B*. A sharp peak of monosomes comprising about 20%[2] of the total ribosomal population was observed near the meniscus, and the remaining 80% was distributed through the polysomal region. Treatment with pancreatic RNase in the cold converted about 95% of the polysomes into monosomes. Addition of 0.5% sodium deoxycholate to the extracting medium did not alter the profile, thus eliminating the possibility that the heavy fraction was associated with membranous fragments. The profile is similar to that observed in log phase HeLa cells (Penman et al., 1963; Latham et al., 1965).

When the cells left the log phase and began the last generation of growth at a progressively decreasing rate (Fig. 2 *C*), the proportion of polysomes fell rapidly, and the proportion of monosomes rose. The total ribosomal population increased by about 20% as the cells grew slightly larger. When the cells entered the stationary phase at a density of about 2×10^7/ml, the proportion of polysomes had fallen to 40–45% (Fig. 2 *D*). Sister cells were harvested at this time and dispensed on membrane filters (see Methods) so that they could begin fruiting body construction. By 3 hr the level of polysomes had risen dramatically to approximately 65% of the total (Fig. 2 *E*), and by 6 hr the proportion was back to 80%. Meanwhile the total ribosomal population per cell had fallen back to the approximate level characteristic of log phase amebas. If the cells were not harvested but instead were left in the nutrient broth to complete the stationary phase, the polysome level fell to about 15% after which the cells began to die in significant numbers.

Fig. 3 summarizes an experiment in which the cells were harvested directly from the log phase and dispensed on membrane filters in order to begin morphogenesis. The level of polysomes did not fall but was maintained at about 80% throughout, even as late as 20 hr by which time the fruiting bodies were almost complete. Thus the fall in

[2] Relative proportions of polysomes and monosomes were determined by cutting graphs into appropriate segments and weighing them.

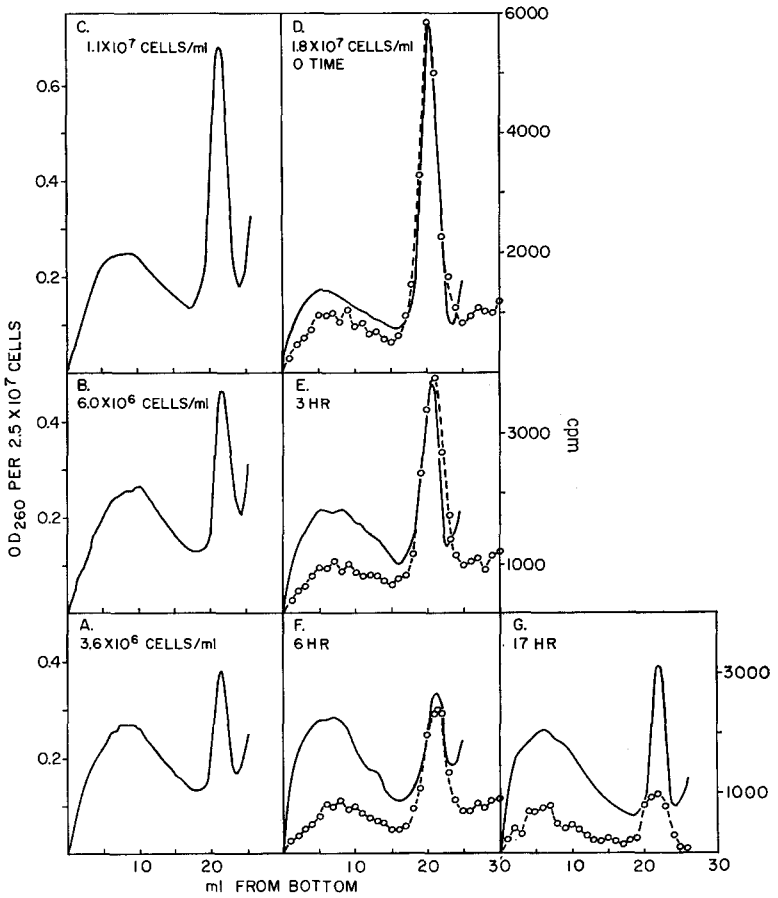

FIGURE 2 Polysomal profiles of cells in various stages of growth and morphogenesis (*A–C*). Cells were harvested from cultures that had attained the cell densities noted. Soluble cytoplasmic fractions were prepared by the rapid procedure described in the Methods section. Aliquots of 2 ml (each containing material from 2.5×10^7 cells) were layered over 28 ml of a linear 7–42% sucrose gradient (w/v) in HMK solution and centrifuged for 2 hr at 23,500 rpm in an SW-25 rotor at 3°C. The tubes were pierced and emptied from below, with a finger pump, through the flow cell of a Gilford recording spectrophotometer. (*D*) Prior to harvesting, the cells had been grown for 48 hr (4 generations) in the presence of uniformly labeled uridine-^{3}H, specific activity = 12 Ci/mmole, 1.5 μCi/ml. The cells were washed once by centrifuging for 1.5 min at 1500 *g* before preparing the soluble cytoplasmic fraction. After sucrose density centrifugation as described above, the drops issuing from the spectrophotometer flow cell were collected in 1 ml fractions and immediately precipitated with 10% TCA acid in the presence of carrier bovine serum albumin. The precipitates were collected on membrane filters, washed with TCA, dried, and counted in liquifluor scintillation fluid. (*E–F*) The cells (sisters of those employed in *D*) were washed by centrifugation, resuspended in LPS solution containing 10^{-3} M cold uridine, and aliquots of 2.5×10^7 cells were dispensed on filter papers over support pads saturated with the LPS-uridine solution (see Methods section). At 3 hr and again at 6 hr, the cells from four filters were harvested and treated as in *D*. (*G*) In a separate experiment cells were harvested from filters after 17 hr of development and treated as described above. The solid lines are the OD_{260} traces. The open circles and dotted lines represent cpm.

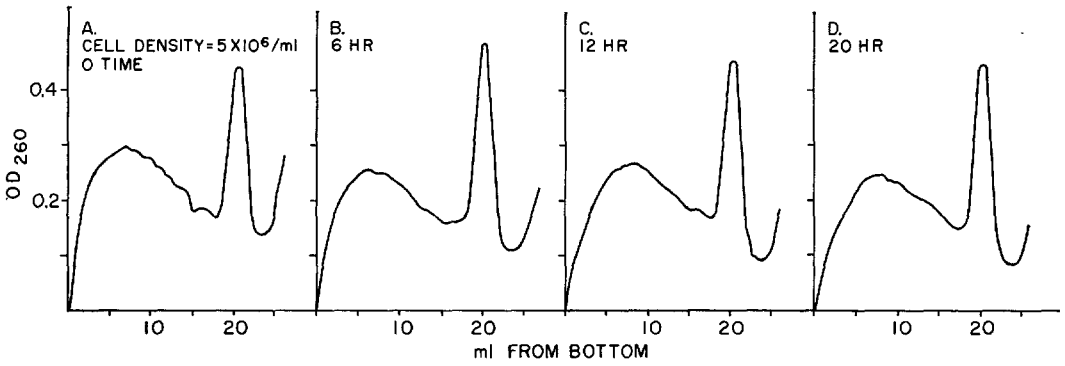

FIGURE 3 Cells were harvested from the culture medium when they had reached a density of 5×10^6 cells/ml. The soluble cytoplasmic fraction that was prepared from one sample was layered over a linear 7–42% sucrose (w/v) gradient in HMK solution and centrifuged for 2 hr at 23,500 rpm in an SW-25 rotor at 3°C; then it was collected and monitored as described in the legend to Fig. 2. The remaining cells were dispersed on filters (as described in the Methods section) in order to begin morphogenesis. Samples were harvested at the times noted and treated as above.

polysomal levels observed in Fig. 2 is not obligatory but is a specific consequence of the cells' entrance into the stationary phase under conditions that make the construction of fruiting bodies impossible.

The Turnover of Ribosomes during Morphogenesis

R. Sussman, studying axenically grown *Polysphondelium pallidum*, has shown that rRNA synthesized during vegetative growth is degraded during morphogenesis and replaced by newly synthesized rRNA having the same base composition and the identical capacity to hybridize with *P. pallidum* DNA (R. Sussman, 1967). The turnover rate was such that, over the entire morphogenetic sequence, roughly two-thirds of the old rRNA disappeared and was replaced by a quantity of new rRNA roughly equal in amount to the remaining old rRNA.

This analysis was extended by examining the RNA content of *D. discoideum* ribosomes during morphogenesis. The cells harvested upon entrance into the stationary phase (Fig. 2 *D*) had been grown for 48 hr (4 generations) in the presence of uridine-^{3}H, and the radioactivity profile is seen to coincide with the OD trace. Sister cells had been washed and dispensed on membrane filters resting on support pads saturated with LPS containing an excess of cold uridine. By 3 hr (Fig. 2 *E*) the absolute level of radioactivity in the polysome region had fallen as old ribosomes disappeared, and the specific radioactivity had decreased dramatically because new ribosomes containing unlabeled rRNA had entered the polysomal region. The absolute level of radioactivity in the monosome peak had decreased much more markedly than in the polysome region as old ribosomes were degraded, but the specific radioactivity did not decrease, i.e. no new ribosomes containing unlabeled rRNA entered this pool. By 6 hr (Fig. 2 *F*), both total and specific radioactivity had further decreased in the polysomal region due to the disappearance of old polysomes and the appearance of new ones. The total radioactivity of the monosomes had again decreased more markedly than that of the polysomes, but the specific radioactivity had fallen only slightly (although probably significantly). In a separate experiment, samples taken at 13.5 and 17 hr displayed progressive decreases to as low as 30% of the initial specific radioactivities of both polysomes and monosomes (Fig. 2 *G*). It should be noted, however, that in the later samplings the decreases in specific radioactivity of the monosomes varied considerably possibly due to increased fragility of a polysomal contingent.

In Fig. 4 the change in the specific radioactivity of the combined ribosomal population has been plotted as a function of time. It is roughly first order with a half-time constant of 12 hr. Thus by the end of fruiting body construction (24-hr), it would appear that at least 75% of the old ribosomes have disappeared and have been

replaced by new ones whose RNA was synthesized during the morphogenetic sequence. This value is minimal since it assumes that no recycling whatsoever occurred.

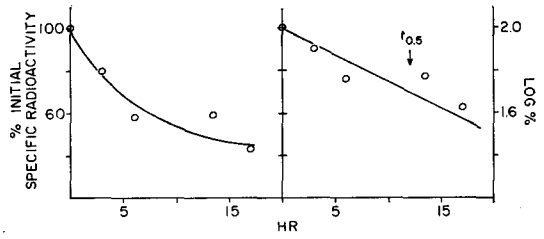

FIGURE 4 The decay of ribosomal RNA during morphogenesis. The ratios of total radioactivity: total OD_{260} were calculated from the data given in Fig. 2 *D–F* and also from data obtained from a second experiment (not shown) in which prelabeled cells were sampled at 0 time, 13.5, and 17 hr. Left curve shows a linear plot of the relative specific radioactivities (as percentages of the initial values) as a function of the time at which the cells were harvested. Right curve is a semilog plot of the same data.

Persistence of RNA (and Ribosomes) during Exponential Growth

Cells were allowed to grow for 2 generations in medium containing uridine-^{3}H in order that the RNA be uniformly labeled (see Figs. 1 and 2). They were then harvested, washed free of exogenous label, and resuspended at a density of 2×10^6 cells/ml in HL-5 medium containing excess (10^{-3} M) cold uridine, a concentration that decreases uptake of exogenous label to an insignificant level under these conditions. As seen in Fig. 5 (left), they continued to increase exponentially for more than 2 generations and then grew at a diminished rate as they approached the stationary phase. At intervals, samples of 2×10^6 cells were taken to determine TCA-insoluble radioactivity. Fig. 5 shows the cpm/ml of culture as per cent of the initial value (the latter being the value at the time the washed cells were suspended in cold medium). The cells appeared to have retained at least 90% of the label even after 2 generations, and the preincorporated label did not disappear

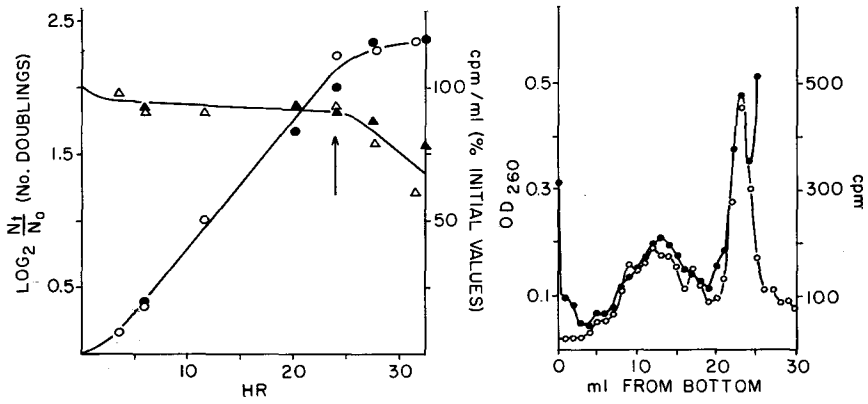

FIGURE 5 Persistence of RNA during exponential growth. (*Left*) Log phase amebas at an initial density of 5×10^5 cells/ml were the inoculum for 20 ml aliquots of HL-5 medium containing 50 μCi uridine-^{3}H (27 Ci/mmole) and were incubated for 24 hr until they reached a density of 2×10^6 cells/ml. The cells were harvested and washed in unlabeled HL-5 medium under conditions of sterility, resuspended at a density of 2×10^6 cells/ml in HL-5 medium containing 10^{-3} M cold uridine and 500 μg/ml streptomycin, and incubated further. At intervals thereafter, cell counts were made. Samples containing 2×10^6 cells were centrifuged and the pellets were examined for TCA-insoluble radioactivity. The data from two separate experiments are shown. Growth (*circles*) is plotted as Log_2Nt/No; TCA-insoluble radioactivity (*triangles*) is plotted in terms of cpm/ml of culture as a percentage of the initial value (the radioactivity was measured at the time the cells were removed from uridine-^{3}H medium and introduced into cold uridine medium). (*Right*) At 24 hr after being introduced into cold uridine medium (see arrow in the graph on the left), cells were harvested and samples were treated according to the procedure described in the legend to Fig. 2 to yield polysome profiles. (Closed circles, OD_{260}; Open circles, TCA-insoluble radioactivity.)

at an appreciable rate until the end of exponential growth. (In another experiment not shown here, the decrease in radioactivity was followed until it had dropped to about 50% of the initial level.) While the possibility exists that the preformed RNA was in fact destroyed and the labeled nucleotides were then recycled, one would not expect virtually total conservation as observed in Fig. 5, particularly since the presence of 10^{-3} M cold uridine in the medium has been shown to saturate the internal nucleotide pool and would therefore be expected to dilute out the labeled nucleotides.

Even if the preformed RNA were not destroyed by exponentially growing cells, the possibility remained that it had been discarded by them in terms of function. The data in Fig. 5 (right) argue compellingly against this. The distribution, in a polysomal profile, of RNA synthesized 2 generations before, representing 25% of the total RNA, was not detectably different from that of the remaining 75% of the RNA that had been synthesized subsequently.

DISCUSSION

The levels of polyribosomes have been examined in *D. discoideum* cells that (*a*) were growing exponentially, (*b*) had approached or entered the stationary phase, and (*c*) were embarked on the program of fruit construction. In exponentially growing cells, the level is very high and involves almost all of the available ribosomes. The level falls precipitously in the stationary phase but, if such cells are permitted to initiate the program of fruit construction, it rapidly rises to the original value. If this is taken as a direct reflection of the rate of protein synthesis, then *D. discoideum* amebas that are engaged in fruit construction may be synthesizing protein just as actively as if they were in the log phase. However, since the cells do not increase in mass during morphogenesis but in fact decrease somewhat, this high rate of protein synthesis must be more than matched by protein turnover. Wright et al. (1960) have studied protein turnover during fruit construction by examining the dilution of cell protein previously labeled with methionine-^{35}S, and they have estimated the rate as about 7% per hr. Over the 24 hr period required, this would lead to an almost quantitative replacement of preexisting protein. At least some of the replacement appears to be concerned with the preferential synthesis of specific enzymes previously absent or in low concentrations (Sussman and Sussman, 1969). The results taken together would seem to invalidate the supposition that the morphogenetic sequence is a metabolically trivial event.

The data in Figs. 2 and 4 confirm a previous finding in the sister genus *Polysphondelium* (R. Sussman, 1967). They show that in *D. discoideum* at least 75% of the RNA synthesized during the cell growth and division cycle is degraded during morphogenesis and is replaced by newly synthesized RNA. Since it is likely that the labeled nucleotides are recycled to some extent at least, this estimate of turnover is minimal. The pattern of ribosome disappearance observed in Fig. 2 indicates that newly made ribosomes go directly into the polysomal complexes and that, for at least the first 6 hr, it is the detached ribosomes (all of them old) whose RNA is preferentially destroyed. During this time the old ribosomes still in polysomal complexes disappear more slowly, presumably first entering the monosomal pool. In contrast, the label (uridine-^{3}H) in preformed rRNA persists in exponentially growing cells through at least 2 generations (24 hr) after removal of the labeled precursor from the medium and addition of excess cold uridine.

This poses an intriguing question. *D. discoideum* amoebae do not begin fruiting body construction until they have stopped growing and can complete the normal morphogenetic sequence in the absence of all exogenous nutrients. Clearly the cells would have a selective advantage if they were able to utilize old ribosomes and to avoid as much as possible the fabrication of new ones. The question is why do they not utilize old ribosomes exclusively.

It might be that, during morphogenesis, only newly made ribosomes can convey nascent mRNA to the cytoplasm and/or effect its translation. Studies of HeLa cells have indicated that mRNA transport into the cytoplasm does continue for some time after the cessation of ribosome synthesis (Darnell, 1968), but it should be noted that these cells were exponentially growing ones. In the present instance, it is necessary that transport be sustained for a period of 24 hr by stationary phase cells engaged in a morphogenetic program.

A second possibility is that the ribosomes synthesized during fruiting body construction constitute a new class of ribosomes that are functionally different from ribosomes present during the cell growth and division cycle with respect to their capacity to transport and/or to translate mRNA that is transcribed during fruiting body

construction. In this connection it is of interest to note that, in heterokaryons of HeLa cells and hen erythrocytes (whose cytoplasm had been allowed to leak out prior to cell fusion), the previously quiescent hen nuclei synthesized RNA for the first few days but no RNA was found in the cytoplasm, whereas RNA newly made in the HeLa nuclei was found there; only after nucleoli developed in the hen nuclei and ribosome synthesis began did hen RNA appear in the cytoplasm and could hen-specific proteins be detected shortly thereafter (Harris et al., 1969).

It is also noteworthy that sea urchin and amphibian blastulae utilize preformed ribosomes exclusively and only resume ribosome synthesis after they enter the gastrula stage. While this correlation may spring merely from a quantitative requirement for additional protein synthesizing capacity, it could conceivably reflect the appearance of a new class of ribosomes that match the mRNA needed for the new developmental program (Gross et al., 1964; Brown and Littna, 1966; Slater and Spiegelman, 1966; Crippa et al., 1967).

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