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# Control of Translation in Cultured Cells: Continued Synthesis and Accumulation of Messenger RNA in Nondividing Cultures

**(cellular growth state/ribosomal RNA synthesis/protein synthesis/stringent control)**

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**ABSTRACT Nontransformed animal fibroblasts in tissue culture regulate total protein and ribosomal RNA synthesis coordinately with changes in the cellular growth state. Here it is shown that the amounts and rates of synthesis of cytoplasmic poly(A)-containing RNA, presumed to be mRNA, do not appreciably change with alterations in growth state. In nongrowing (resting) cultures of BALB/c 3T3 cells presumptive mRNA molecules predominantly accumulate as cytoplasmic ribonucleoprotein particles; the RNA from which can be chased into the polyribosomal structure upon activation of the resting cultures with animal sera. It is suggested that the decreased protein synthetic rate in resting as compared with growing cells is in part due to a failure of preexisting mRNAs to attach to ribosomes and that addition of serum to the tissue culture medium can partially overcome this translational lesion.**

Fibroblastic cells in tissue culture exist in one of two reversible growth states: a state of rapid proliferation (growing) and a state of relative quiescence (resting)(1, 2). In nontransformed mouse fibroblast cultures, transition between these two states is predominantly regulated by growth-promoting substances in animal sera (3, 4). Transition from a growing to a resting state is accompanied by a decrease in the rate of synthesis of DNA, RNA, and protein (1, 2, 5), disaggregation of cytoplasmic polyribosomes (2), and an apparent decrease in the DNA-dependent RNA polymerase activity (5). Mechanisms to regulate the decline in the overall protein synthetic rate could operate either by controlling the amount of cytoplasmic messenger RNAs (mRNAs) synthesized (transcription and/or processing) and/or by controlling the rate at which mRNA is translated. Recently over 90% of cell mRNAs [except the histone mRNAs, (6)] have been shown to contain long stretches (100 to 200 nucleotides) of poly(adenylic acid), whereas the major stable RNA components of the cell [ribosomal (*r*) and transfer (*t*) RNAs] do not (7, 8). I have utilized this fact to measure directly both the rate of synthesis and the amount of total cellular mRNA species by hybridization either to [$^3$H]poly(uridylic acid) [poly(U)] in solution or to nonradioactive poly(U) attached to glass-fiber filters (7) for both growth states (growing and resting) of monolayer cultures of animal fibroblasts.

## MATERIALS AND METHODS

*RNA Extraction and Hybridization to* [$^3$*H*]*Poly*(*U*). Permanent cell lines (3) were seeded at 4 to 5 $\times$ $10^5$ cells per 9-cm petri dish in Dulbecco's modified Eagle's medium with 10% calf serum (Colorado Co.) except where otherwise stated. Total cellular RNA was isolated by lysing cell cultures with buffer A [50 mM Tris·HCl (pH 7.4), 0.1 M NaCl, 1 mM $Na_2$ EDTA, 0.5% sodium dodecyl sulfate (SDS) w/v] and selectively precipitating most of the DNA and some proteins with 0.5 M NaCl at 4° according to Lindstrom and Rudland (manuscript in preparation). Greater than 85% of either poly(A)-containing RNA or total cellular RNA was recovered, and the fractional amount of poly(A)-containing RNA was independent of the RNA isolation procedure. Various amounts of cell RNA were added to each annealing mixture containing 0.03 M sodium citrate, 0.3 M NaCl, and 8.5 $\mu$g of [$^3$H]poly(U) (Schwarz/Mann, specific activity 7500 cpm/$\mu$g). Reactants were incubated 12 hr at 50° and allowed to cool to 20° for 2 hr before digestion with 40 $\mu$g/ml of pancreatic RNase for 2 hr at 20°. Samples were then precipitated with 10% trichloroacetic acid (w/v), collected on glass-fiber filters, and counted in a liquid scintillation spectrometer. Maximum hybridization was attained within 2 hr and remained constant for a further 24 hr. Control experiments showed that only poly(A)-containing RNA species hybridized to [$^3$H]poly(U).

*Determination of Macromolecular Synthesis.* Either 50 $\mu$Ci/ml of [5-$^3$H]uridine at 14 $\mu$M, 0.13 $\mu$Ci/ml of L-[U-$^{14}$C]leucine, or 3 $\mu$Ci/ml of [*methyl*-$^3$H]thymidine at 3 $\mu$M were added to parallel cell cultures. Upon isolation, the cultures were rinsed twice with "Tris" (3) and lysed with 2 ml of either buffer A (RNA) or 0.5 N NaOH (DNA and protein). Cell suspensions in NaOH were incubated several hours at 37°; 0.2 ml was sampled for total radioactivity and the remainder was precipitated with trichloroacetic acid. For RNA synthesis, the [$^3$H]uridine samples were deproteinized by phenol–chloroform extraction. Then the total radioactivity incorporated into the cell and into trichloroacetic acid-precipitable material was measured. One milliliter of each sample was diluted to 5 ml with 0.3 M NaCl–0.03 M sodium citrate–0.5% SDS (w/v) and filtered under gravity through two successive poly(U) filters (7) which were subsequently counted (13% efficiency). Incorporation of [$^3$H]uridine into structural RNA (rRNA and tRNA) was calculated by deduction from the total incorporation into trichloroacetic acid-precipitable material, that incorporation due to mRNA [poly(U) filter-bound radioactivity] and to DNA (5–10% of the total trichloroacetic acid-precipitable radioactivity after extensive NaOH treatment). Analysis of radioactive RNA in the filtrate from the poly(U) filters on sucrose gradients showed that 80% was rRNA and 10% tRNA for either resting or growing cells labeled for 12 hr

Abbreviations: SDS, sodium dodecyl sulfate; RNP, ribonucleoprotein.

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with [$^{3}$H]uridine. Results were corrected for changes in the internal specific activities of the radioactively labeled RNA precursors. The relative specific activities were measured from the incorporation of $^{3}$H radioactivity into the total trichloroacetic acid-soluble fraction of the cell. Results were expressed in terms of the specific activity of the RNA precursor when the precursor pool became saturated with radioactivity.

*Isolation and Characterization of Messenger Ribonucleoprotein (mRNP) Particles.* Polysomes were prepared from monolayer cultures with 1% Nonidet P40 (NP40) in ice-cold hypotonic buffer B [10 mM Tris·HCl (pH 8.5), 5 mM NaCl, 1.5 mM $MgCl_2$] as described (9). The supernatant from the 1000 × *g* step was then layered onto an 11-ml 0–40% (w/w) exponential concave sucrose gradient (10) in buffer B and centrifuged at 200,000 × *g* for 1 hr (Beckman SW41 rotor) before 0.5-ml fractions were collected. Fractions from cells labeled with [$^{3}$H]aminoacids were precipitated with trichloroacetic acid while RNA in different fractions was extracted with phenol–chloroform. Polysomes were completely destroyed by prior exposure to RNase or EDTA. RNA fractions labeled with uridine were passed through poly(U) glass-fiber filters; those labeled with adenosine were digested with pancreatic and T1 RNase. Radioactively labeled *mRNP* particles from resting cultures of BALB/c 3T3 cells were isolated as described above in buffer C [10 mM triethanolamine·HCl (pH 8.5), 5 mM NaCl, 1.5 mM $MgCl_2$]. Fractions containing mRNA (Fig. 2) were pooled (30–70S region of the gradient). RNP complexes in growing cells were prepared by treating combined fractions from the polysomal region of the gradient (about 200–1000 S) with $Na_2$ EDTA as described by Perry and Kelley (11). Both preparations were analyzed on 15–30% sucrose gradients in buffer C containing 10 mM $Na_2$ EDTA instead of $MgCl_2$, or were fixed with formaldehyde before buoyant density analysis (11). *RNA* from the RNP particles prepared above was extracted with phenol–chloroform and purified from contaminating ribosomal RNAs by adsorption and elution from poly(U) filters. Sedimentation values were determined in either 5–20% sucrose gradients in buffer C containing 0.1 M NaCl, 0.2% SDS (w/v) or by electrophoresis through 2.9% SDS–polyacrylamide gels (w/v) (12) with ribosomal RNAs as standards. *Poly(A)* tracts were prepared by digestion of adenosine-labeled RNA with pancreatic and T1 RNases, and the residues were subjected to electrophoresis through 10% SDS–polyacrylamide gels together with *E. coli* 4S and 5S RNAs as markers. *Nucleotide composition* was estimated by exhaustive alkaline hydrolysis of $^{32}$P-labeled RNP RNA and subsequent paper electrophoresis of the products at pH 3.5. For *hybridization kinetic measurements* (13), the entire BALB/c 3T3 cell mRNA (specific activity 2 to 5 × $10^{5}$ cpm/μg) was isolated from poly(U) filters and then annealed with 3000 times the weight of cell DNA at 68° in 10 mM Tris·HCl (pH 7.4), 0.5 M NaCl, 0.1% SDS (w/v). Samples were removed at various times and digested with 40 μg of pancreatic, 40 units of T1, and 10 units of T2 RNase/ml for 1 hr at 37° before trichloroacetic acid precipitation.

## RESULTS

*mRNA Synthesis.* Initially, to estimate the amount of mRNA in either asynchronously growing or resting cell cultures we hybridized [$^{3}$H]poly(U) to the total RNA preparations isolated from various nontransformed fibroblasts. These included established cell lines from mice, BALB/c 3T3,

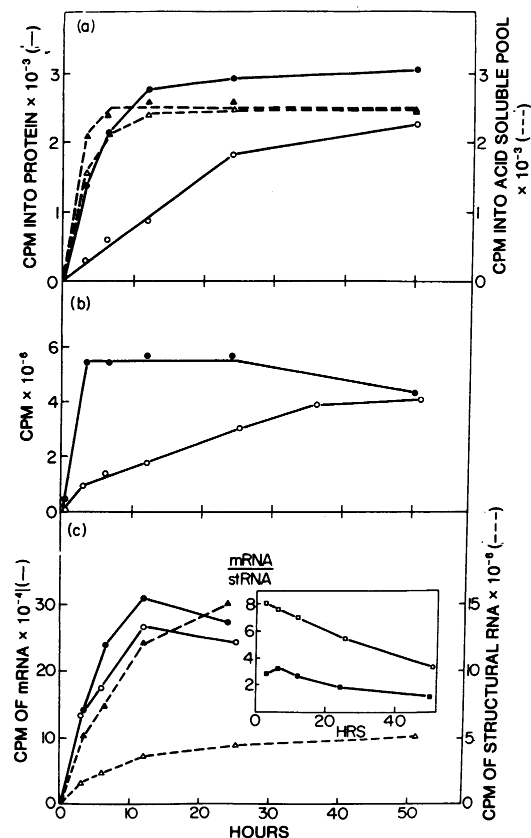


FIG. 1. RNA and protein synthesis in resting and growing cells. The relative amounts of incorporation of either (*a*) [$^{14}$C]-leucine into protein (——) and into the trichloroacetic acid-soluble fraction of the cell (– – –), or [$^{3}$H]uridine into (*b*) trichloroacetic acid-soluble fraction of the cell (——), into (*c*) mRNA (——), and into structural species (rRNA and tRNA) (– – –) was measured as a function of the labeling time for either resting (O, Δ, □) or asynchronously growing (●, ▲, ■) BALB/c 3T3 cell cultures of $10^{6}$ cells. Cell densities immediately before addition of the radioactive isotopes were 8 × $10^{5}$ cells and 2.5 × $10^{6}$ cells per 9-cm petri dish for growing and resting cultures, respectively, and about 90 and 0.3% of the nuclei from growing and resting cells, respectively, became radioactively labeled with [$^{3}$H]thymidine during the subsequent 16-hr periods. Incorporation into RNA species was corrected for the variability in the intracellular specific activity of the radioactive precursor. The relative proportions of uridine, UMP, UDP, and UTP were the same in resting and growing cultures; UTP represented 65–70% of the total pool. The ratio of incorporation into mRNA ÷ incorporation into structural RNA (*stRNA*) (%) is shown in the *insert*.

3T3-4A, monkey, BSC-1, hamster, BHK-21, and secondary cultures from mouse embryos. For comparison, two virus-transformed lines were also studied; the simian virus 40-transformed 3T3 line, SV3T3, and the polyoma virus-transformed hamster line, PyBHK. Quiescent cultures from the nontransformed cells were obtained either by allowing the cultures to form a nearly confluent cell monolayer in medium containing 10% serum or by serum deprivation with 1% or 2% serum (3, 4) (Table 1). The cells were presumed to be arrested at a point in the cell cycle before DNA synthesis rather than in mitosis since less than 2% of the cells were synthesizing DNA (Table 1, except BHK) and upon addition of fresh serum to the cultures DNA synthesis occurred before cell multiplication. Although virally transformed cell lines

TABLE 1. *Hybridization of [$^3$H]poly(U) to RNA from resting and growing cells*

| | BALB/c 3T3 2% serum | BALB/c 3T3 10% serum *DNase-treated sample | 3T3-4A | Mouse embryo | BHK-21 | BSC-1 | SV3T3 | PyBHK |
|---|---|---|---|---|---|---|---|---|
| % Labeled nuclei (resting cells)/(growing cells) | 0.5 | 0.7 | 0.4 | 0.8 | 5.6 | 2.4 | 90 | 95 |
| % Thymidine into DNA (resting cells)/(growing cells) | 0.4 | 0.5 | 0.3 | 1.8 | 6.5 | 2.2 | 74 | 70 |
| % Poly(U) hybridized per cell RNA | | | | | | | | |
| Resting cells | 0.32 ± 0.03 | 0.35 ± 0.02<br>0.34 ± 0.03* | 0.23 ± 0.02 | 0.34 ± 0.02 | 0.31 ± 0.02 | 0.20 ± 0.02 | 0.29 ± 0.04 | 0.24 ± 0.03 |
| Growing cells | 0.31 ± 0.04 | 0.32 ± 0.02<br>0.34 ± 0.05* | 0.28 ± 0.02 | 0.32 ± 0.02 | 0.36 ± 0.03 | 0.20 ± 0.02 | 0.31 ± 0.01 | 0.23 ± 0.02 |
| mRNA (resting cells)/mRNA (growing cells) | 1.0 ± 0.2 | 1.1 ± 0.1<br>1.0 ± 0.2* | 0.8 ± 0.2 | 1.1 ± 0.1 | 0.9 ± 0.1 | 1.0 ± 0.2 | 0.9 ± 0.2 | 1.0 ± 0.2 |

The fractional amounts of poly(U) hybridized per weight of total cellular RNA (%) were obtained as described in *Methods*. Average determinations of the incorporation of [$^3$H]thymidine into DNA during a 4-hr labeling period or of the fraction of cells with radioactively labeled nuclei during a 12-hr period are expressed as the ratio (%) of those values in resting (7–8 days after seeding) and in growing cultures. Quiescent cell cultures were isolated 2 or 3 days after termination of DNA synthesis. For resting cell populations of nontransformed cells, the fraction of cells with radioactively labeled nuclei was 2% or less, except for BHK-21 which was 6%. Addition of 20% serum induced at least 65% of the cell nuclei to become radioactively labeled with [$^3$H]thymidine within 30 hr. Quiescent cultures from BALB/c 3T3 cells isolated 12 days after seeding (7–8 days after termination of DNA synthesis) gave identical results. DNA content of the RNA preparations was less than 5% (w/w). Half of the BALB/c 3T3 RNA preparations from cells originally grown in 10% serum was extensively treated with DNase so that the final preparation contained less than 0.01% DNA (*).

were also studied, their confluent cultures were actively synthesizing DNA (Table 1). The amount of [$^3$H]poly(U) hybridized per $\mu$g of total cellular RNA and, therefore, the poly(A) content of the RNA was constant (within experimental error: Table 1), for a given cell culture independent of its growth state. As the mean size and the relative size distribution of the poly(A) sequences attached to mRNA and of the mRNA molecules themselves did not vary when isolated under different cellular growth states from either resting or growing BSC-1 or BALB/c 3T3 cells (unpublished results and Table 2), the fractional poly(A) content was, therefore, a direct measure of the fraction of mRNA molecules present in the total cell RNA. For BALB/c 3T3 cells, the RNA (Fig. 1) and packed cell volumes (8.6 ± 0.5 for growing and 6.6 ± 0.5 for resting $\times 10^{-3}$ ml per $10^6$ cells) both only decreased by about 20–25% when the growing cells enter the resting phase, and hence the amount and concentration of mRNA per cell were relatively independent of the cellular growth state.

Measurements of the synthesis of macromolecules in cells in different growth states based upon addition of a radioactively labeled precursor to the tissue culture medium have to be corrected for the variability in the intracellular specific activity of the radioactive precursor (14). For protein synthesis, the rate of equilibration between the intra- and intercellular leucine pools was relatively fast so that for labeling times greater than 3 hr there was very little apparent difference in the extent of saturation of the cellular leucine pool for growing and resting cultures, and the actual size of the total pool did not alter appreciably (Fig. 1*a*). Thus, the rate of incorporation of [$^3$H]leucine into trichloroacetic acid-precipitable material was a direct measure of protein synthesis; the rate for asynchronously growing cultures was approximately three times higher (1, 2, 15) than for resting cultures of BALB/c 3T3 cells (Fig. 1*a*). The incorporation rate of RNA precursors into the cell, however, markedly depends on the cellular growth state (Fig. 1; refs. 16 and 17). Assuming that a single cellular RNA precursor pool exists, the relative amounts of RNA synthesized were determined from the incorporation of [$^3$H]-uridine into RNA and the average incorporation into the trichloroacetic acid-soluble cellular pool. Fig. 1*c* shows that although the rate of synthesis of stable RNA species (rRNA and tRNA) is approximately 3- to 4-fold higher in asynchronously growing than in resting cultures, the rate of synthesis of mRNA [defined as RNA that binds to poly(U) filters (7)] is only 20–30% higher in growing than in resting cultures. Similar results were obtained with cultures labeled with adenosine rather than uridine. The ratio of tRNA to rRNA synthesis was found to be approximately constant, independent of the radioactive labeling time and the cellular growth state. Radioactive labeling of cell cultures for an extended period of time showed that the total amount of labeled mRNA in resting cells was about 80–90% of that in growing cultures (Fig. 1*c*). The ratio of mRNA to structural RNA species (rRNA, tRNA) synthesized was approximately three times higher in resting than in growing cells for at least the first 6 hr of labeling.

TABLE 2. *Properties of RNP particles*

| | Resting cells | Growing cells |
|---|---|---|
| Sedimentation value of RNP | 30–70S | 30–80S |
| Density of RNP in CsCl | 1.42 ± 0.07 | 1.44 ± 0.08 |
| Sedimentation value of RNA | 10–25S | 12–28S |
| Sedimentation value of poly(A) | 3–5S | 3–5S |
| Nucleotide composition (%) (A, C, G, U) | 35, 24, 21, 20 | 34, 23, 22, 21 |
| Hybridization kinetics ($C_0t_{1/2}$: mol × sec/liter) | 2,600 ± 300* | 2,700 ± 400* |

RNP particles were isolated directly from resting BALB/c 3T3 cultures or by $Na_2$ EDTA treatment of polysomes derived from growing cultures. The approximate range (±) of sedimentation values of material that, after phenol–chloroform extraction, bound to poly(U) filters was recorded. The range of buoyant densities (±) of formaldehyde-fixed particles other than ribosomal subunits is also shown. RNP RNA was purified by adsorption and elution from poly(U) filters. For the hybridization kinetics to cell DNA approximately 85% of the [$^3$H]mRNA annealed with 2 × $C_0t_{1/2}$ [DNA concentration (mol of nucleotide per liter) × time required for half-maximum annealing (sec)]. Structural RNA (rRNA and tRNA) from either growing or resting cells purified by three passages through 2 poly(U) filters to adsorb out all the poly(A)-containing RNA species annealed to cell DNA with a $C_0t_{1/2}$ of 2.2 mol × sec/liter.

* Total cellular mRNA.

*Localization of mRNA in Resting Cells.* Although mRNA amounts per cell were maintained when actively growing cells became quiescent, the polyribosomes disaggregated and protein synthesis was reduced (2). Fig. 2 shows a 3- to 4-fold difference in both the $A_{260}$ and cpm of [$^3$H]aminoacids incorporated into nascent protein in 2 min for the polyribosomes isolated from growing than from resting cultures. In actively growing cells approximately 75–80% of the cytoplasmic radioactively labeled mRNA was associated with polysomes and approximately 20–25% was associated with material that sedimented slower than monosomes on sucrose gradients (Fig. 2*a*) (30S–70S region of the gradient, Table 2). In resting cells approximately two-thirds of the cytoplasmic labeled mRNA was found in the 30S–70S region of a sucrose gradient (Fig. 2*b*) and only one-third in polysomes. Localization of nonradioactive poly(A)-containing RNA (mRNA) was also determined by hybridization of RNA from different cell fractions (obtained by NP40 treatment of whole cells, Fig. 2) to [$^3$H]poly(U). For growing BALB/c 3T3 cells 6% of the total occurred in the nucleus, 80% in the polysomal region, and 13% in 30S–70S region of the cell cytoplasm, while in resting cultures 10% was found in the nucleus, 35% in polysomes, and 54% in the 30S–70S region of the sucrose gradient.

The mRNA-containing particles from the 30S to 70S region of the sucrose gradient obtained from resting cell cultures were compared with the RNP particles released from polyribosomes by EDTA treatment (11) (Table 2). In particular, their buoyant densities after formaldehyde fixation were approximately the same (1.42–1.44 g/ml), intermediate between that of RNA (1.90) and protein (1.25) and distinct from the 60S (1.57) and 40S (1.49) ribosomal subunits (11). 30S–70S

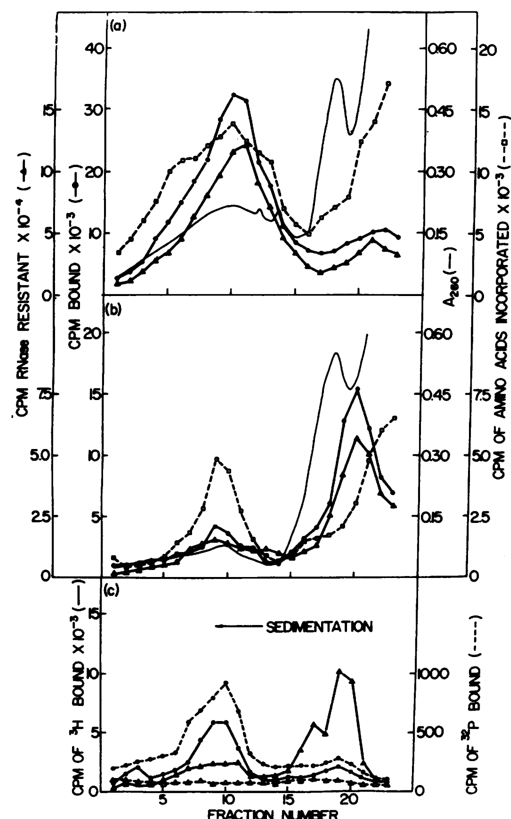


FIG. 2. Polysomal mRNA in resting and growing cells. Sucrose gradients of polysomal RNA isolated from either (*a*) growing or (*b*) resting BALB/c 3T3 cells. The cpm of RNA labeled with [$^3$H]uridine bound to poly(U) filters (●——●) or labeled with [$^3$H]adenosine which was then resistant to RNase digestion (▲——▲) are shown for each gradient fraction. Incorporation of [$^3$H]aminoacids into nascent peptides (□– – –□) and $A_{260}$ (——) are also indicated. Cell cultures in 10% nondialyzed serum grown to a cell density of 1.8 × $10^6$ (3 days after seeding) for (*a*) growing or 2.1 × $10^6$ (9 days after seeding) for (*b*) resting cultures were radioactively labeled for 6 hr with 100 μCi/ml of [$^3$H]uridine (10 μM); 100 μCi/ml of [$^3$H]adenosine (5 μM); or for 2 min with 250 μCi/ml of [$^3$H]aminoacids (Amersham) in Dulbecco's modified Eagle's medium containing one-tenth the normal concentration of amino acids. Two 9-cm petri dishes were isolated for each isotope and three for nonradioactive cultures and processed as described in *Methods*. The peak fraction (10) of the polysomal profile corresponded to 5 or 6 polyribosomes calibrated against a reticulocyte lysate. In (*c*) resting cultures were labeled with [$^3$H]uridine as for (*b*) and two combined petri dishes were isolated as above, (▲——▲), or fresh medium containing 20% serum, 5 μCi/ml of [$^{32}$P]$H_3PO_4$, and 0.5 μg/ml of actinomycin D was added and the cells from two combined cultures were isolated 3 hr later. Fractions containing polysomal RNA were filtered through poly(U) filters and the cpm bound for [$^3$H]uridine-labeled (●——●) or $^{32}$P-labeled (△——△) RNA were recorded. Two control cultures were labeled solely with [$^{32}$P]-$H_3PO_4$ for 3 hr after a change into fresh medium without actinomycin D (○– – –○). There was no appreciable alteration in the rate of incorporation of radioactive uridine or phosphate into the trichloroacetic acid-soluble precursor pool of the cell with or without actinomycin D.

particles from resting cell cultures contained about 30–40% RNA and 60–70% protein (11) and no detectable DNA. RNAs isolated from either EDTA-treated polysomes from growing cell cultures or from the 30S to 70S particles in

resting cultures were identical in size, poly(A) content, nucleotide composition, and their reannealing kinetics to cell DNA (Table 2). The functional significance of mRNP particles in resting cell cultures of BALB/c 3T3 or BSC-1 (not shown) cells was unclear. Fig. 2 shows, however, that when resting cell cultures were prelabeled with [$^3$H]uridine and fresh medium containing 20% serum (or serum alone) was added together with actinomycin D, then approximately 50% of the mRNA originally in the 30S–80S RNP complex was recovered in the polysomes after 3 hr (Fig. 2). Actinomycin D was added to prevent fresh synthesis of mRNA from degradation products of the preexisting mRNA species.

## DISCUSSION

Results in this paper demonstrate that the amounts and rate of synthesis of cytoplasmic poly(A)-containing RNA (presumed to be mRNA) did not vary greatly with changes in the cellular growth state, whereas the rate of ribosomal and tRNA synthesis were reduced approximately 3- to 4-fold when growing cultures became quiescent. The relative rates of synthesis depended upon estimating the changes in the specific activities of the total intracellular RNA precursor pools, and the values obtained for ribosomal RNA synthesis of 1.1–1.5 $\mu$g and 0.4–0.6 $\mu$g/hr per $10^6$ cells for growing and resting BALB/c 3T3 cultures, respectively, closely agree with those obtained from direct measurement of the intracellular specific activity of the radioactively labeled RNA precursor pool in chicken-skin fibroblasts (18). In addition, estimation of the amounts of mRNA either by hybridization to [$^3$H]poly(U)(Table 1), assuming that the average poly(A) content of mRNA is 10% (Table 2), or from an extended incorporation (12–24 hr) of radioactive precursors into RNA binding to poly(U) filters (Fig. 1*c*) yield consistent values of about 0.4–0.6 $\mu$g of mRNA for growing and 0.4–0.5 $\mu$g for resting cultures of $10^6$ cells.

The localization of mRNA in resting cell populations predominantly in cytoplasmic RNP particles and the ability to transfer a major proportion of this mRNA into polysomes upon activation of the resting cell cultures with animal sera suggest that the decreased protein synthetic rate observed when nontransformed fibroblasts enter the resting state is partially due to a failure of mRNA's to attach to ribosomes (initiation of translation) and not primarily due to either a reduction in the transcription of total mRNA's or a failure to process heterogeneous nuclear RNA into cytoplasmic mRNA. Similar conclusions have been drawn from the ability of quiescent cell cultures to reform polysomes and increase protein synthetic rates in the presence of antibiotic inhibitors of RNA synthesis (19). Finally, the dependence of ribosomal RNA (20) and tRNA (21) synthesis on the cellular growth state and the relatively uncoordinated synthesis of mRNA argue that the synthesis of gross cellular mRNA and stable RNA are under independent control in fibroblastic cells in culture, similar to the regulation of RNA synthesis in bacteria by external concentrations of amino acids (stringent control). Recently also several reports have suggested the occurrence of mRNP particles of various sizes (25S–160 S) in the cytoplasm of cultured cells starved for amino acids and the reassociation of mRNA in those particles with polysomes upon restoration of the amino acids to the culture medium (22). This similarity between the biochemical events involved in the regulation of RNA and protein synthesis in cell cultures starved for amino acids and those events in monolayer fibroblast cultures depleted of animal sera may not prove to be fortuitous (15).

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