Studies on the Efficiency of Translation and on the Stability of Actin Messenger Ribonucleic Acid in Mouse Sarcoma Ascites Cells†

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ABSTRACT: The actin mRNA of mouse sarcoma ascites cells differs from the other abundant mRNA species with respect to its poly(A) content. It is enriched in chains with short poly(A) segments, and a substantial portion may not have any poly(A) at all. In order to determine whether this unusual feature of the actin mRNA influences its physiological behavior, we studied actin synthesis in cells incubated under various conditions. Two treatments of the cells that reduce their capacity for polypeptide chain initiation, incubation in high salt and starvation, did not affect actin synthesis in any unique fashion. The synthesis of actin was relatively resistant to these treatments, as was the synthesis of some other abundant polypeptides. Different patterns of inhibition of polypeptide synthesis were obtained with the two treatments. The actin mRNA was also translated with relatively high efficiency in the reticulocyte cell-free system supplemented with polysomal RNA from ascites cells. Actin was by far the major polypeptide produced in this system, and its synthesis was not inhibited in the presence of excess mRNA. In contrast, actin mRNA translation in wheat germ extracts is highly sensitive to inhibition by excess mRNA. Prolonged incubation of the ascites cells in a culture medium caused a gradual decay in their capacity for actin synthesis. This was accompanied by corresponding losses in translatable actin mRNA. The poly(A)-containing and poly(A)-deficient components were affected to about the same extent. Other major mRNA species were not affected by this treatment. Addition of actinomycin D to the incubation medium prevented the loss of actin mRNA. It appears that this drug causes a stabilization of this RNA species. The changes in actin mRNA do not appear to be linked to the proliferative state, since rapidly growing and contact-inhibited mouse 3T3 cells showed the same relative rate of actin synthesis.

Actin is synthesized in large amounts in mouse sarcoma ascites cells (Geoghegan et al., 1978). It is also the major polypeptide produced in wheat germ extracts under the direction of polysomal RNA from these cells. Recent studies in this laboratory have revealed an unusual feature of the mRNA for this protein (Geoghegan et al., 1978; Sonenshein et al., 1976). A substantial portion of the actin mRNA chains occurs either without poly(A)[1] at their 3′ terminus or with very short segments unable to bind to oligo(dT)–cellulose. Of the poly(A)-containing actin mRNA chains, the major portion has segments shorter than about 50 nucleotides. The other abundant mRNA species of the ascites cells, on the other hand, bind nearly quantitatively to oligo(dT)–cellulose, and most of these chains have poly(A) segments greater than 50 nucleotides in length.

In the present study, we have examined the possibility that the unusual poly(A) content of the actin mRNA may have a bearing on the function of this RNA species in the cells. We chose to compare the behavior of the actin mRNA in intact cells to that of other abundant species with a normal poly(A) complement. First we examined the relative efficiency of actin mRNA translation in cells subjected to treatments that reduce their capacity to initiate polypeptide synthesis. It has been shown that the translation of mRNAs with a low initiation efficiency is preferentially inhibited under such conditions (Lodish, 1971; Sonenshein & Brawerman, 1976; Nuss & Koch, 1976). The two inhibitory treatments used here, incubation of the cells in high salt concentration and incubation in the absence of nutrients, affected actin synthesis to different extents. In neither case, however, did the synthesis of this protein show any unique response.

Experiments were also designed to see whether the actin mRNA might differ from the other mRNA species with respect to stability, owing to its unusual poly(A) content. We observed that incubation of the ascites cells in a culture medium leads to a progressive reduction in their capacity to synthesize actin. The cells also showed corresponding decreases in translatable actin mRNA content. Cells incubated in the presence of actinomycin D did not show these changes, as if this drug were causing a stabilization of the mRNA.

Materials and Methods

Cell Incubation and Labeling. Sarcoma-180 cells were maintained by weekly transfers into the peritoneal cavity of albino mice. Cells harvested from the infected animals were washed first in a solution of 0.42% NaCl, 0.8% sodium citrate, and 2% glucose buffered at pH 6.1 and then in Krebs bicarbonate buffer (Lee et al., 1971). They were resuspended and incubated at 37 °C in Krebs bicarbonate buffer suplemented with 1% glucose, 10% dialyzed calf serum, and amino acids as described previously (Lee et al., 1971) but containing one-tenth the usual concentration of methionine. For the starvation experiments, the cells were incubated in Krebs bicarbonate buffer modified to contain only 0.05% bicarbonate, in the absence of glucose, amino acids, and serum (Sonenshein & Brawerman, 1977). For incubations in complete medium for periods longer than 2 h, phenol red was included, and the pH was maintained around neutrality by periodic additions of sodium bicarbonate.

Small aliquots of cell suspensions, containing approximately 2.5×10^6 cells, were incubated with [^{35}S]methionine (500

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‡ Silvia Cereghini is a fellow from the Consejo Nacional de Investigaciones Cientificas y Tecnicas de la Republica Argentina.

[1] Abbreviations used: poly(A), poly(adenylic acid); oligo(dT), oligo(deoxythymidylic acid); DNase, deoxyribonuclease I (EC 3.1.4.5.); Tris-HCl, 2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride; $NaDodSO_4$, sodium dodecyl sulfate.

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Ci/mol; New England Nuclear). After labeling for the periods of time indicated, we chilled the cells quickly and washed them twice with phosphate-buffered saline (3 mM KCl, 140 mM NaCl, 0.5 mM $MgCl_2$, and 10 mM phosphate buffer, pH 7.5) and twice with a hypotonic buffer consisting of 10 mM Tris-HCl, pH 7.6, 10 mM KCl, and 1 mM $MgCl_2$. The swollen cells were lyzed by the addition of 0.2 mL of the same buffer containing 0.1% Triton X-100, 0.2% sodium deoxycholate, and 7 mM 2-mercaptoethanol. The lysate was centrifuged at 1000*g* for 5 min and the supernatant (cytoplasmic extract) saved. The crude nuclear pellet was resuspended in a solution of 10 mM Tris-HCl, pH 7.6, and 3 mM $MgCl_2$, and pancreatic DNase (Boehringer Manheim) was added to a final concentration of 15 μg/mL. After 30 min of incubation at 37 °C, $NaDodSO_4$ was added to a final concentration of 0.5%. The nuclear and cytoplasmic extracts were combined and subjected to polyacrylamide gel electrophoresis as described below. Samples were removed for determination of hot trichloroacetic acid insoluble radioactivity prior to electrophoresis.

Swiss mouse 3T3 cells, kindly supplied by Peter Rosow of the Sidney Farber Cancer Research Institute, were grown in Dulbecco's modified Eagle's medium with 10% dialyzed calf serum (Gibco). They were labeled for 1 h at 37 °C in methionine-deficient medium in the presence of 60 μCi/mL of [^{35}S]methionine. After labeling, the medium was quickly withdrawn by us, and we washed with phosphate-buffered saline and harvested them by scraping and subsequent centrifugation. Cytoplasmic and nuclear extracts were prepared as described for the ascites cells.

RNA Preparations. Polysomes and small mRNA–protein complexes were obtained by magnesium precipitation from the cytoplasmic fraction of cells lyzed with Triton X-100 (Mendecki et al., 1972), and RNA was prepared by the phenol extraction procedure at alkaline pH (Brawerman et al., 1972), modified as described previously (Geoghegan et al., 1978). For the preparation of poly(A)-deficient RNA fractions, the RNA was mixed at room temperature with oligo(dT)–cellulose (Collaborative Research; type T-3) in the presence of 500 mM NaCl, 5 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.6, and 0.1% $NaDodSO_4$. After 30 min of gentle stirring, we poured the mixture through a column and washed it with an equal volume of the same buffer. The combined unadsorbed material, representing poly(A)-deficient RNA, was precipitated by addition of 2.5 volumes of ethanol, kept overnight at 4 °C, collected by centrifugation, and washed with a 2:1 mixture of ethanol and 0.1 M NaCl. Poly(A)-containing RNA was eluted with 0.1% $NaDodSO_4$ and precipitated by addition of NaCl to 0.1 M and of 2.5 volumes of ethanol. Excess ethanol was removed with ether after dissolving the precipitate in distilled water. Residual ether was removed with a stream of air.

Protein Synthesis in Wheat Germ Extracts. The reaction mixtures (25 μL) contained 5 μL of wheat germ extract (Roberts & Paterson, 1973), 24 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), pH 7.2, 0.8 mM spermidine, 1 mM magnesium acetate, 2 mM dithiothreitol, 1.2 mM 2-mercaptoethanol, 1 mM ATP, 0.2 mM GTP, 8 mM creatine phosphate, 116 mM potassium acetate, 24 mM KCl, 50 μg/mL creatine phosphokinase, 30 μM each of the common amino acids except methionine, 10 μCi of [^{35}S]methione, and the indicated amounts of exogenous RNA. After incubation at 22 °C for 2 h, pancreatic ribonuclease A (Sigma) was added to a final concentration of 200 μg/mL, and the reaction mixture was digested at 37 °C for 30 min. Samples were removed for measurement of hot trichloroacetic acid insoluble radioactivity.

Protein Synthesis in Rabbit Reticulocyte Lysates. The reaction mixtures (28 μL) contained 18 μL of rabbit reticulocyte lysate digested with micrococcal nuclease (Pelham & Jackson, 1976), 0.5 μg of hemin, 0.6 μg of creatine phosphokinase, 5 mM creatine phosphate, 0.3 mM $MgCl_2$, 80 mM KCl, 135 μM each of the common amino acids except leucine, 5 μCi of [^{3}H]leucine (115 Ci/mM; New England Nuclear), and the indicated amounts of RNA. After incubation at 30 °C for 45 min, pancreatic ribonuclease A was added to a final concentration of 100 μg/mL, and the reaction mixtures were digested at 37 °C for 30 min. Samples were removed for measurement of hot trichloracetic acid insoluble radioactivity, after treatment with hydrogen peroxide to avoid color quenching.

Polyacrylamide Gel Electrophoresis and Autoradiography. Extracts of labeled cells and in vitro reaction mixtures were applied to the gel after adjusting to 50 mM Tris-HCl, pH 6.8, 2% $NaDodSO_4$, 35 mM 2-mercaptoethanol, and 10% glycerol. The samples were subjected to electrophoresis on 13% polyacrylamide slab gels (0.8 mm thick) containing 0.1% $NaDodSO_4$ and 5.5 M urea, with a 5% polyacrylamide stacking region containing 2.7 M urea (Storti & Rich, 1976). Electrophoresis was carried out for 16 h at 80 V for the labeled cell extracts and at 65 V for the cell-free incubation mixtures. The gels were stained with coomassie blue, destained, and dried onto paper in vacuo. Autoradiograms were obtained by exposure to Kodak no-screen X-ray film. The gels containing tritium-labeled material were processed for fluorography (Bonner & Laskey, 1974) and exposed to RP Royal X-Omat film at −70 °C.

Quantitative data were obtained by scanning the autoradiograms and fluorograms at 540 nm with a Beckman Model DU spectrophotometer adapted with a Gilford linear transport. The polypeptides other than actin were identified by their approximate molecular weights, as determined by their mobility relative to that of known standards. Gel scan peaks were integrated manually.

Results

Measurement of Actin Synthesis. Actin migrates as a broad band when cell extracts are subjected to one-dimensional $NaDodSO_4$–polyacrylamide gel electrophoresis (Figures 1 and 2). This is not due to poorly resolved contaminating polypeptides, since material purified by binding to DNase–Sepharose or by polymerization–depolymerization cycles shows the same behavior (Geoghegan et al., 1978).

We observed that a substantial portion of the radioactive actin sediments with the nuclear pellet after lysis of the cells, even after short labeling periods. Thus, it was important to include in the samples used for gel electrophoresis the protein present in the rapidly sedimenting fraction, in order to obtain quantitative measurements of actin synthesis. This was achieved by first treating the nuclear pellet with DNase and then dissolving the residue in $NaDodSO_4$ (see Materials and Methods).

The extent of synthesis of actin, and of other polypeptides, was determined by making densitometry tracings of autoradiograms and measuring the area under the peaks. In order to compare changes in the rates of synthesis of individual polypeptides as a result of the various treatments, we determined the *relative synthesis* of each polypeptide (extent of synthesis relative to total amino acid incorporation into polypeptides). The changes in relative synthesis are expressed as percent of values in control cells, setting the control value

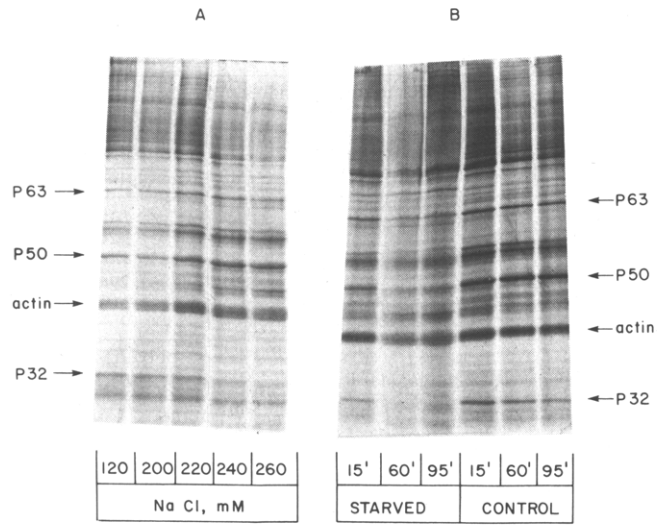

FIGURE 1: Polypeptide synthesis in Sarcoma-180 ascites cells incubated in high salt concentration and in starvation medium. (A) Cells incubated for 30 min in complete medium containing the normal (120 mM) NaCl concentration and higher concentrations as indicated. Cells were labeled for the last 15 min of incubation. For other details, see Table I. (B) Cells incubated in complete medium or in starvation medium for 15, 60, and 95 min and labeled for the last 15 min of incubation. For other details see Table II. Polypeptides are identified by their approximate molecular weights; for instance, P50 stands for a 50 000-dalton polypeptide.

Table I: Effect of Incubation in High Salt Concentration on Synthesis of Individual Polypeptides in Ascites Cells[a]

excess NaCl in medium (mM)	changes in polypeptide synthesis: rel synthesis of individual polypeptides[c] (% of control) actin	P32	P46	P50	P63	total polypeptide[b] (% of control)
80	116	93	122	88	99	65
100	121	71	126	115	112	54
120	152	68	174	149	120	40
140	153	66	284	163	93	24

[a] Ascites cells were incubated in complete medium for 15 min prior to the addition of excess NaCl. After an additional 15 min of incubation, [^{35}S] methionine was added to a concentration of 40 μCi/mL, and the incubation was continued for 15 min. Extracts were prepared as described under Materials and Methods. [b] Total polypeptide synthesis represents the hot trichloroacetic acid insoluble radioactivity measured on separate aliquots of cell extracts. The control value was 61 000 cpm. [c] Relative synthesis is defined in the text.

for each polypeptide as 100%.

Actin Synthesis in Cells Incubated in High Salt Concentration. Incubation of intact cells in the presence of excess NaCl causes inhibition of polypeptide chain initiation (Nuss & Koch, 1976; Saborio et al., 1974). The capacity for protein synthesis in the ascites cells is reduced progressively as the NaCl concentration in the medium is increased. The results of a typical experiment are described in Figure 1 and Table I. As can be seen in Figure 1, individual polypeptides are affected to different extents in the inhibited cells. Table I shows the changes in the relative synthesis of several polypeptides, expressed as percent of values in control cells. It can be seen that actin synthesis is relatively resistant to the salt treatment. The relative synthesis of this polypeptide increased by about 50% when total protein synthesis was inhibited 60–76%. The synthesis of another abundant polypeptide, P50, was affected in the same manner as actin synthesis. The

Table II: Effect of Starvation on Synthesis of Individual Polypeptides in Ascites Cells[a]

incubation conditions	changes in polypeptide synthesis: rel synthesis of individual polypeptides (% of control) actin	P32	P50	P63	total polypeptides (cpm)
complete medium 15 min	100	100	100	100	4370
starvation 15 min	82	59	46	52	3370
60 min	45	25	15	23	1190

[a] Cells were incubated in complete or starvation media for the indicated time periods and labeled with 40 μCi/mL of [^{35}S] methionine for the last 15 min of incubation. For other details, see Table I and Figure 1.

mRNA for P50 has been shown to have a normal poly(A) complement (Geoghegan et al., 1978). Some polypeptides, like P32, were inhibited to a greater extent than the bulk of the protein. One partially resolved band, P46, showed a particularly high resistance to the salt treatment, with a threefold increase in relative synthesis when total protein synthesis was inhibited about 75%. This would correspond to a rate of synthesis nearly equal to that in the control cells.

Effect of Starvation on Actin Synthesis. Incubation of cells in a medium lacking amino acids and glucose provides another means to inhibit polypeptide chain initiation. Very little capacity for protein synthesis remains after incubation of S-180 ascites cells in such a medium for 60 min, as judged by polysome profiles from cell lysates (Sonenshein & Brawerman, 1977; Lee et al., 1971). The data on amino acid incorporation in the cells subjected to this treatment (Table II), however, are not an accurate measure of the extent of inhibition, because of the possibility of changes in amino acid pools. The patterns of labeling of individual polypeptides during starvation were quite different from those observed in cells subjected to excess NaCl (Figure 1). Actin and the other abundant polypeptides tended to be affected preferentially in the starving cells (Table II). P50 synthesis was far more sensitive to this treatment than was actin synthesis. P32 and P63 were affected to about the same extent, although P32 was more sensitive than P63 to the salt treatment.

The above changes can be attributed to the starvation treatment and not simply to incubation of the ascites cells outside the animal. This is indicated in Figure 1, where the labeling patterns of cells incubated in complete medium for similar time periods are displayed. As is shown below, however, prolonged incubation in complete medium causes marked changes in the synthesis of some polypeptides.

Efficiency of Actin Synthesis in Cell-Free Systems. The above studies indicate that the actin mRNA is translated with relatively high efficiency in intact cells which show a reduced capacity for polypeptide chain initiation. Our previous studies of actin mRNA translation in wheat germ extracts, however, had shown that this RNA species seems particularly ineffective when incubated in the presence of excess S-180 mRNA (Geoghegan et al., 1978). The apparent discrepancy between in vitro and in vivo results is particularly striking when the behavior of the actin mRNA is compared to that for P50. The latter mRNA species seemed more effective than the actin mRNA in vitro but is less effective in the intact cells. The in vitro characteristics, however, were determined with the use of a nonmammalian cell-free system, and it is possible that the translation apparatus of wheat germ differs from that of

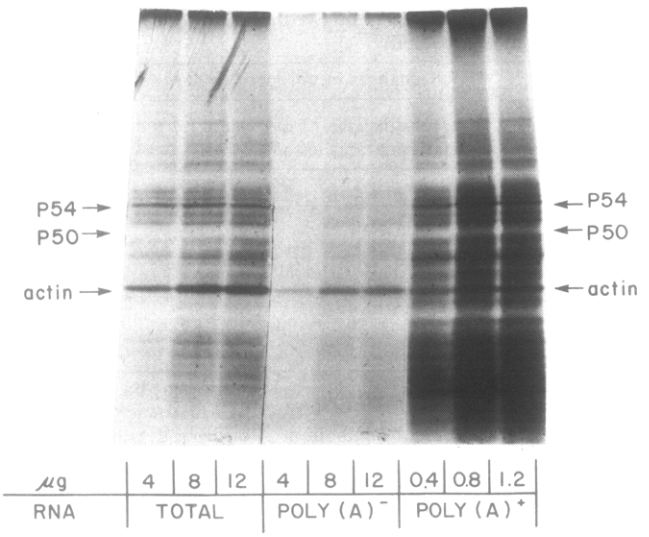

FIGURE 2: Translation of ascites cell polysomal RNA fractions in the rabbit reticulocyte cell-free system. The indicated amounts of total polysomal RNA, poly(A)-deficient RNA, or poly(A)-containing RNA were used to direct translation in a 28-μL incubation mixture containing 180 μCi/mL of [^{3}H]leucine. Products of the reaction were subjected to $NaDodSO_4$ gel electrophoresis and fluorography as described under Materials and Methods.

mammals with respect to mRNA recognition specificity. In order to verify this possibility, we examined the translation characteristics of S-180 mRNA in a rabbit reticulocyte cell-free system. The efficiency of translation of actin mRNA appears to be somewhat greater in the latter system, as judged by the high proportion of actin among the translation products (Figure 2). Moreover, actin synthesis is not affected by the presence of saturating amounts of mRNA as it is in the wheat germ system. The poly(A)-deficient RNA fraction is quite active in promoting actin synthesis in the reticulocyte lysate.

There were pronounced differences in the translation of some abundant mRNA species in the two cell-free systems. P50, which is quite prominent among the translation products in wheat germ extracts, seems not to be synthesized in the reticulocyte lysate. On the other hand, an abundant product in the latter system, P54, is not synthesized in wheat germ extracts (compare Figures 2 and 4).

Decay of Actin mRNA in Ascites Cells Incubated in Culture Medium. The S-180 ascites cells used in these studies are not adapted to grow in culture, but they remain highly active in protein synthesis for at least 6 h (Table III). After the first 2 h of incubation, changes in the capacity to synthesize individual polypeptides became apparent (Figure 3). Most prominent was the loss in actin synthesis. The relative synthesis of this protein was reduced to about 30% of the control level after 6 h of incubation (Table III). The capacity to synthesize P50 and P63 remained essentially unchanged during this time period. Some other polypeptides, such as P32, were also produced in reduced amounts.

In order to determine whether the changes in actin synthesis might be due to loss of the mRNA for this protein, we extracted cytoplasmic RNA from the cells after various times of incubation and translated it in the wheat germ cell-free system. The capacity to promote the synthesis of individual polypeptides, expressed as arbitrary units per constant amount of RNA, varied in parallel with the changes observed in the intact cells (Figure 4 and Table III). The template activity for actin synthesis decreased to 26% of the control value after 6 h of incubation. There was no significant change in the activity for P50 synthesis, and the activity for P63 synthesis

Table III: Changes in Polypeptide Synthesis and in Translatable mRNA in Ascites Cells Incubated in Culture Medium[a]

incubation time (h)	changes in polypeptide synthesis[b] rel synthesis (% of control) actin	P50	P32	P63	total polypeptide (cpm)
0	100	100	100	100	28 750
2	92	100	77	105	56 520
4	48	95	54	87	52 440
6	30	97	47	91	51 720

incubation time (h)	changes in template act.[c] total polysomal RNA actin (%)	P59 (%)	P34 (%)	P63 (%)	total (cpm)	poly(A)-deficient RNA actin (%)	total (cpm)
0	100	100	100	100	37 000	100	17 300
2	95	103	105	128	33 800	75	17 700
4	67	98	89	128	35 900	32	21 100
6	26	70	54	122	35 400	14	14 800

[a] Cells were incubated in complete medium, and samples of cell suspension were withdrawn at specified time periods. At each time point, small samples (about 2.5×10^6 cells) were supplemented with 30 μCi/mL of [^{35}S] methionine and labeled for 30 min, for analysis of polypeptide synthesis in intact cells. Large samples (10^8 cells) were used for RNA extraction. [b] Polypeptides synthesized during a 30-min labeling period. See Table I for details. [c] Polypeptides synthesized in the wheat germ cell-free system supplemented with RNA prepared from cell samples. Total incorporation into polypeptides was measured on small aliquots of reaction mixtures; endogenous incorporation was 3700 cpm. Template activity for the synthesis of individual polypeptides was determined by measuring the amount of polypeptide synthesized in reaction mixtures supplemented with 4 μg of total RNA or 8 μg of poly(A)-deficient RNA. Values for control cells were set at 100%, and changes in mRNA activity in incubated cells are expressed as percent of values obtained with control RNA.

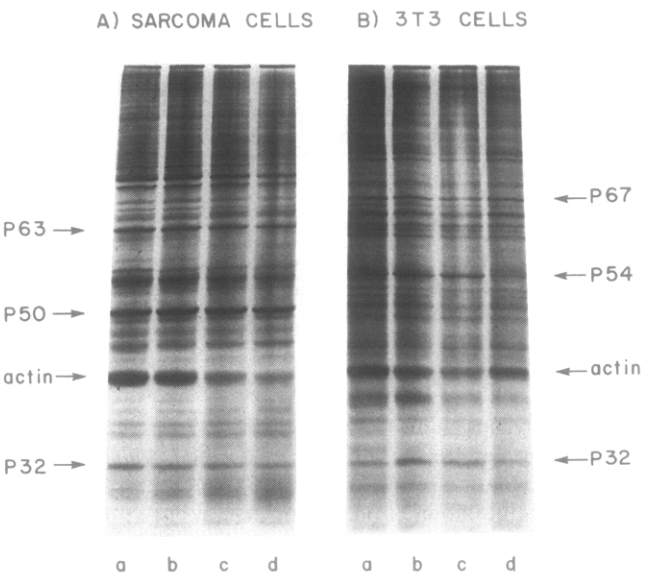

FIGURE 3: Polypeptide synthesis in Sarcoma-180 ascites cells incubated in culture medium and in 3T3 cells deprived of serum. (A) Ascites cells incubated in complete medium and samples pulse-labeled for 30 min at zero time (a), 2 h (b), 4 h (c), and 6 h (d). For other details see Table III. (B) Cultures of 3T3 cells pulse-labeled for 1 h as described under Materials and Methods. Cells grown to confluency (a); confluent cultures deprived of serum for 6 (b) and 16 h (c); cell cultures deprived of serum for 16 h incubated in complete medium for 24 h (d). For other details see Table VI.

also remained essentially unchanged. Another major polypeptide, P34, was synthesized in reduced amounts in the cell-free system. This may correspond to the P32 synthesized

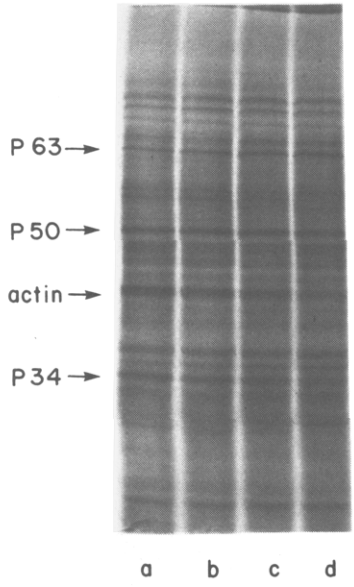

FIGURE 4: Polypeptides synthesized in wheat germ cell-free system supplemented with RNA from ascites cells incubated in culture medium. Total polysomal RNA preparations from cells incubated in full medium for zero time (a), 2 h (b), 4 h (c), and 6 h (d) were used for incorporation.

in intact cells, which is affected in the same manner.

There was no significant loss in total RNA from the cells incubated in the culture medium, as judged from the recoveries after deproteinization. Also, the overall template activity showed little change (Tables III and IV). It appears from these results that incubation of the ascites cells outside the animals leads to selective inactivation of some mRNA species, with the actin mRNA being particularly sensitive to this treatment.

A portion of the actin mRNA chains, about 25%, have either very short poly(A) segments or no poly(A) at all (Geoghegan et al., 1978). The fate of this component was examined by comparing the activity for actin synthesis of the RNA unable to bind to oligo(dT)–cellulose. As can be seen in Table III, the latter activity also decreased, to about the same extent as the activity for actin in total RNA. No consistent changes in the overall template activity of the poly(A)-deficient RNA were observed.

Effect of Actinomycin D on Actin Synthesis in Cells Incubated in Culture Medium. In order to study the decay of individual mRNAs in the absence of further RNA synthesis, we added actinomycin D to the cell suspensions. To our surprise, this drug prevented the changes in polypeptide synthesis that were taking place in the cells incubated in the culture medium. The changes in patterns of polypeptide synthesis in cells incubated for 5 h in the presence and absence of actinomycin are illustrated in Figure 5 and in Table IV. The relative synthesis of actin was consistently somewhat higher in the actinomycin-treated cells as compared to the control cells (cells incubated for 30 min only). The corresponding value for P50 was increased by more than 50%. There was no significant effect on P63 synthesis.

The actinomycin treatment also affected the levels of translatable RNA in the cells. The activity for actin synthesis tended to be considerably higher than in the RNA from control cells (Table IV). Increases ranging from 10 to 100% were observed in different experiments. The template activity for P50 synthesis was increased to a lesser extent (only up to 30%), in contrast to the large increases in P50 synthesis in the intact cells.

Table IV: Effect of Actinomycin D on Polypeptide Synthesis and on Levels of Translatable mRNA in Ascites Cells Incubated in Culture Medium[a]

incubation conditions	changes in polypeptide synthesis: rel synthesis (% of control) actin	P50	P32	P63	total polypeptide (cpm)
control	100	100	100	100	52 300
5 h	42	111	51	70	110 400
5 h + Act. D	116	151	108	76	121 200

incubation conditions	changes in template act.: total polysomal RNA actin (%)	P50 (%)	P34 (%)	P63 (%)	total (cpm)	poly(A)-deficient RNA actin (%)	total (cpm)
control	100	100	100	100	44 800	100	27 100
5 h	45	80	36	130	44 100	45	11 000
5 h + Act. D	110	90	114	113	55 100	175	25 800

[a] Ascites cell suspensions were incubated in complete medium for 10 min, and samples were taken for pulse labeling and for RNA extraction as described in Table III. Incubations continued for 5 h in the presence or absence of actinomycin D (Act. D) (4 μg/ml), and samples were taken for analysis. For other details, see Table III.

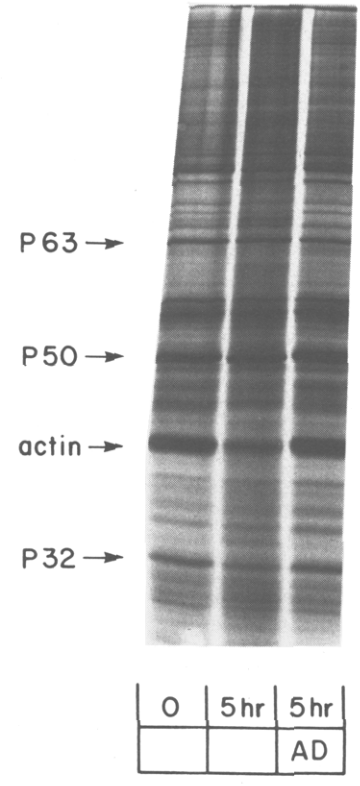

FIGURE 5: Polypeptide synthesis in Sarcoma-180 cells treated with actinomycin D. Ascites cells were incubated in complete medium for 10 min (zero time), 5 h, and 5 h in the presence of 4 μg/mL actinomycin D. Aliquots of 2.5×10^6 cells were supplemented with 30 μCi/mL of [^{35}S]methionine, labeled for 30 min, and processed as described under Materials and Methods.

The drug treatment tended to have a preferential effect on the activity of the poly(A)-deficient actin mRNA. In some experiments, the increase in activity for actin synthesis was considerably greater in the RNA fraction unable to bind to oligo(dT)–cellulose than in the total cytoplasmic RNA. This apparent accumulation of poly(A)-deficient actin mRNA,

Table V: Effect of Delayed Addition of Actinomycin D to Ascites Cells Incubated in Culture Medium[a]

incubation conditions	changes in rel synthesis (% of control)			
	actin	P50	P32	P63
4 h	69	127	28	79
6 h	23	68	25	68
4 h, then 2 h ± Act. D	52	131	36	84

[a] Ascites cells were incubated as described in Tables III and IV. Samples were taken for pulse labeling for 30 min after incubation for 4 h and for 6 h and after 4-h incubation followed by 2 h in presence of actinomycin D (Act. D).

Table VI: Effect of Serum Deprivation on Synthesis of Individual Polypeptides in 3T3 Cells[a]

incubation conditions	changes in polypeptide synthesis				
	rel synthesis (% of control)				total polypeptide (cpm)
	actin	P32	P54	P67	
control	100	100	100	100	48 950
without serum, 6 h	79	183	190	75	23 030
without serum, 16 h	55	133	215	58	12 850
recovery, 24 h	78	59	75	94	53 640

[a] Cells grown to confluency served as the control. Confluent cells were incubated in serum-free medium for 6 and 16 h, and then incubation continued in complete medium for 24 h. Cells were pulse-labeled as indicated in Figure 3.

however, was not observed in all experiments. This variability may have been due to technical difficulties in the quantitative recovery of functional RNA after oligo(dT)–cellulose fractionation or in the translation of the poly(A)-deficient RNA in wheat germ extracts.

Actinomycin was also effective in arresting the decay in polypeptide synthesis, when added to the cell suspensions after 4 h of incubation (Table V).

Actin Synthesis in Mouse 3T3 Cells. The above results indicated that certain conditions of incubation can lead to preferential inactivation of the mRNA for actin. In an effort to define the physiological state that affects actin mRNA in this manner, we used mouse 3T3 cells grown under a variety of conditions. These cells also make large amounts of actin (Figure 3). Their overall pattern of synthesis of abundant polypeptides, however, differs markedly from that of the S-180 cells. Rapidly growing and contact-inhibited cells showed the same extent of relative actin synthesis (data not shown). Also, incubation in the presence of hydroxyurea, an inhibitor of DNA synthesis, failed to affect this parameter. A differential effect on actin synthesis was observed in cells incubated in the absence of serum (Figure 3 and Table VI), but this effect was not as pronounced as it was in the case of the S-180 cells. Other polypeptides, such as P67, were equally sensitive. It is also not known whether the observed changes in the 3T3 cells are due to loss of mRNA or to selective alterations in translation efficiency.

Discussion

The present study was undertaken with the expectation that actin synthesis in the S-180 cells might show a unique pattern of regulation, owing to the unusual characteristics of the mRNA for this protein. A substantial portion of this mRNA appears to be deficient in poly(A), and much of the rest has segments shorter than those of the bulk of the S-180 mRNA (Geoghegan et al., 1978). It is possible that the poly(A) sequence in the actin mRNA chains is subject to relatively rapid decay and that these RNA chains can persist in the cytoplasm after loss of this sequence. While there is as yet not a clear understanding of the physiological role of the poly(A) segment, it is likely that this sequence is involved in the control of mRNA stability (Marbaix et al., 1975). The significance of mRNA species that normally lack this segment is also unclear. The mRNAs for histones, which are deficient in poly(A), are stable during the S phase of the cell cycle but are rapidly inactivated upon cessation of DNA synthesis (Robbins & Borun, 1967). If it is the lack of poly(A) that is the determining factor in this unique behavior, then it could be expected that the actin mRNA, or at least its poly(A)-deficient component, would be subject to some special inactivation process as well. Also, the apparent rapid decay of poly(A) in actin mRNA could mean that this RNA has a relatively short half-life.

We did observe that the mRNA for actin is particularly sensitive to inactivation when the ascites cells are maintained outside the animal. It is not known, however, what physiological process is affected under these conditions of incubation. The cells are not adapted for growth in culture, but they remain active in protein synthesis and do not suffer any significant loss of total mRNA or of rRNA. The selective inactivation of the actin mRNA is probably not due to a block in DNA synthesis. This is indicated by the fact that the relative synthesis of actin in mouse 3T3 cells is not affected by an inhibitor of DNA synthesis and is also not altered when actively growing cells become contact inhibited. Thus, the nature of the metabolic process responsible for the selective inactivation of the actin mRNA remains to be identified.

Actinomycin D had an unexpected effect on the ascites cells incubated in culture medium. Instead of accelerating the loss of mRNA through inhibition of transcription, it promoted the accumulation of the mRNA. This drug is known to prevent the decay of induced protein synthesis that normally takes place after removal of the inducer (Tomkins et al., 1972). This "superinduction" by actinomycin seems similar to the effect on actin synthesis observed in the present study. Thus, it is possible that the synthesis of this protein may be controlled by an "induction" process that becomes inactive when the cells are maintained outside the animal. The actin mRNA and the mRNAs for induced proteins would then share the property of being subject to selective degradation and of becoming stabilized when the cells are treated with actinomycin. An enhancing effect of actinomycin on levels of translatable mRNA has also been reported in the case of rat myoblast cultures (Kessler-Icekson et al., 1978).

The results of the present study did not show any obvious correlation between the behavior of the actin mRNA in the ascites cells and its unusual poly(A) content. Both the poly(A)-containing and the poly(A)-deficient components were subject to inactivation, to about the same extent. Both components were also stabilized by the actinomycin treatment, although it is possible that the poly(A)-deficient component accumulates to a greater extent under these conditions. The possibility remains that the selective inactivation of the actin mRNA may be related to the apparent rapid decay of its poly(A) segment. It must be noted, however, that the mRNA for P34, which has a normal poly(A) complement, was also subject to inactivation in the cells incubated outside the animal.

The studies of translational inhibition in intact cells did not reveal any unusual behavior of the actin mRNA. This RNA species is translated with a greater efficiency than the bulk of the mRNA in cells subjected to the salt treatment. It appears to be as efficient under these conditions as the mRNA

for another polypeptide, P50, which has a normal poly(A) complement. Actin mRNA translation was inhibited preferentially in cells subjected to starvation but to a lesser extent than was P50 mRNA translation. Both treatments cause a reduction in the capacity of the cells to initiate polypeptide synthesis and lead to the accumulation of the mRNA as nucleoprotein particles. Under these conditions, the actin mRNA seems quite effective in competing with other mRNA species.

The synthesis of some of the polypeptides investigated in this study showed differences in the response to the two inhibitory treatments. Actin synthesis and P50 synthesis were both relatively resistant in the cells exposed to high salt concentration but were preferentially inhibited in the cells subjected to starvation. Moreover, the P50 mRNA seemed as effective as the actin mRNA in the salt-treated cells but was far less effective than actin mRNA in the starved cells. There were also differences in the response of other mRNAs. Since a large number of factors are involved in the initiation process (Revel & Groner, 1978), it is possible that the two cell treatments affect different components of this process. Thus, the differential response of individual mRNAs to these treatments could be due to differences in their requirements for particular initiation factors. It should be interesting to determine whether these characteristics can play a role in the regulation of synthesis of individual polypeptides.

There were also pronounced differences in the behavior of some mRNA components in the two cell-free systems used in this study. Actin mRNA is translated with high efficiency in both systems, but it is highly sensitive to inhibition in the presence of saturating amounts of mRNA only in the wheat germ system. The basis for this inhibition is difficult to understand. The actin mRNA is also translated with high efficiency in the intact cells. The mRNA for P50, which is highly effective in wheat germ extracts, seems not to be translated in the reticulocyte lysate. It is a major product in the intact ascites cells, but it is translated in these cells with poor efficiency, as judged by the distribution of this RNA species in polysomes and in free particles (unpublished experiments). Thus, neither cell-free system reflects in this case the situation in intact cells. Another polypeptide, P54, is produced in large amounts in the reticulocyte lysate but is not detected as a major translation product in the wheat germ system. These results indicate that cell-free systems can exert a high degree of selectivity in their interaction with individual mRNA species. It is also clear that the translation characteristics of isolated mRNA components do not necessarily provide an accurate reflection of their behavior in intact cells.

Acknowledgments

The authors thank Edwin Richards and Sherrie Rozenzwog for their excellent technical assistance.

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