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Regulation of mRNA Utilization in Mouse Erythroleukemia Cells Induced to Differentiate by Exposure to Dimethyl Sulfoxide

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Mouse erythroleukemia cells contain several abundant mRNA species that occur to a considerable extent as untranslated molecules. For two of these species, which code for polypeptides P40 and P21, the proportion of molecules engaged in translation decreases rapidly after exposure of the cells to dimethyl sulfoxide. The extent of utilization of a third species, the P36 mRNA, is not altered. The rate of production of the P40 mRNA does not appear to be affected in the dimethyl sulfoxide-treated cells. The P21 mRNA appears to be produced in increasing amounts, leading to a large accumulation of untranslated molecules in the cytoplasm. The mRNA for actin remains nearly fully utilized during this process, but its intracellular concentration decreases, thus resulting in a reduction in the amounts present in polysomes. The results indicate that some mRNA species in mouse tumor cells are subject to a translational repression process that can serve to regulate selectively the extent of expression of the corresponding genes.

There is little knowledge of the extent to which translational control processes affect the expression of individual genes in mammalian cells. In most cases, the changes in patterns of protein synthesis during developmental processes are accompanied by corresponding changes in mRNA populations in the cytoplasm. During the early stages of embryogenesis, however, mRNA components stored in an untranslated form in the oocytes become activated (7). Such changes in mRNA utilization after fertilization have been observed in a wide range of species (3, 4, 11, 16). Cases of control of polypeptide synthesis via regulation of mRNA utilization have also been described in somatic cells. The preferential synthesis of heat-shock proteins in *Drosophila melanogaster* has been shown to be due in part to cytoplasmic factors that discriminate in favor of the corresponding mRNAs (12). In rat livers, the increase in synthesis of ferritin upon administration of iron to the animals appears to be due at first to mobilization of untranslated ferritin mRNA molecules (22). Chicken myoblasts induced to differentiate have been reported to be more efficient than the uninduced cells in the translation of myosin mRNA (10).

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Control of mRNA utilization through storage in the form of ribonucleoprotein (RNP) particles may be of widespread occurrence in mammalian cells. A substantial portion of the cellular mRNA is usually present as small particles not associated with ribosomes. The untranslated fraction of several types of cells has been shown to be enriched in a limited population of mRNA species (5, 8, 14). Some relatively abundant mRNA species have been identified in the untranslated fraction of mouse sarcoma-180 ascites cells (8). They represent functional molecules apparently maintained in an inactive state through interaction with protein components (2). Cloned cDNA probes for four of these species, coding for polypeptides of M_r 65,000, 40,000, 36,000, and 21,000, have been used to show that a portion of the corresponding mRNA molecules are engaged in translation (20) and that the untranslated molecules are somehow not accessible to the protein-synthesizing machinery (21). We have suggested that the cells might be able to regulate the synthesis of the polypeptides coded by these mRNAs by adjusting the proportion of RNA molecules engaged in translation and that the untranslated molecules could be mobilized when changes in the physiological state of the cells would call for increased amounts of these polypeptides (2).

In the present study, we have examined the behavior of several mRNA species in mouse erythroleukemia (MEL) cells exposed to dimethyl sulfoxide (DMSO). This agent induces erythroid differentiation in the MEL cells and also causes a transient inhibition of DNA synthesis and cell proliferation that lasts for a period of about 24 h (15). The untranslated mRNA species described in the mouse sarcoma ascites cells are also present in the MEL cells. We observed that, for two of these species, the amounts of molecules engaged in translation decreased sharply during the early stages of the induction process. This change was accompanied by the accumulation of the mRNA molecules in small particles. The amount of actin mRNA engaged in translation also decreased considerably, but in this case the change was apparently due to loss of mRNA from the cells. Our results indicate that control of mRNA utilization may play an important role in the regulation of synthesis of some polypeptides during erythroid differentiation.

MATERIALS AND METHODS

Preparation and fractionation of cell extracts. The MEL cell line C7D (19), kindly provided by Stuart Levy, Tufts University, was grown in Eagle basal medium (GIBCO Laboratories) supplemented with 10% fetal serum and gentamicin. The cell suspensions were chilled rapidly before harvesting to avoid ribosome runoff from polysomes. The cells, collected by centrifugation, were washed twice with ice-cold 0.9% NaCl. The packed cells were suspended in 6 volumes of 10 mM Tris-hydrochloride (pH 7.6)–10 mM KCl–1 mM $MgCl_2$ and allowed to swell for 5 min in this hypotonic medium. The suspensions were next supplemented with one-fifth volume of 32.5 mM mercaptoethanol–0.65% Triton X-100–65% sucrose (5× lysing buffer) and kept on ice for 10 min. Nuclei and cell debris were removed by centrifugation at 1,000 × *g* for 10 min and washed with 1 volume of 1× lysing buffer. The combined supernatants were fractionated by zone centrifugation, and RNA was isolated from pooled fractions by phenol extraction as described previously (9).

Hybridization and translation assays. mRNA levels in the RNA preparations were determined by dot hybridization. The RNA samples, fixed as dots on cellulose nitrate (Millipore Corp.) sheets, were hybridized with ^{32}P-labeled recombinant plasmids bearing the appropriate cDNA inserts (20). Amounts of RNA used were 4 μg for the polysomal samples and 1.2 μg for the samples from small RNP particles. These amounts from corresponding fractions of mouse sarcoma ascites cells were found to lie within the linear range of dose response (20, 21). The hybridization assays yielded very similar values for the levels of the mRNA species under study in the two types of cells. Measurements of polyadenylic acid [poly(A)] were carried out by annealing with radioactive polyuridylic acid (8).

The translation assays were carried out in the wheat germ cell-free system, and the translation products were separated by one-dimensional polyacrylamide gel electophoresis and visualized by autoradiography as described previously (9).

RESULTS

Untranslated mRNA species in MEL cells. The populations of mRNA molecules not engaged in translation in mouse sarcoma-180 ascites cells and in MEL cells were compared by incubating RNA preparations from free messenger RNP (mRNP) particles from these two cell types in the wheat germ extract. The patterns of translation products obtained by one-dimensional polyacrylamide gel electrophoresis are displayed in Fig. 1. It can be seen that the major translatable mRNA species in the RNP particles of sarcoma-180 ascites cells also occur in the RNPs of MEL cells and are highly enriched in the RNP particles of both types of cells. Although the amounts of RNA used for the translations were well below saturation (9), the dose-response curve was not verified for each mRNA species. The translation results could not be used for quantitative estimates of mRNA distribution in RNPs and polysomes mainly because of poor resolution of the translation products from polysomal RNA. The high levels of P40 and P36 mRNAs in the RNP fractions are not a true reflection of their distribution between the active and untranslated compartments, since the total mRNP

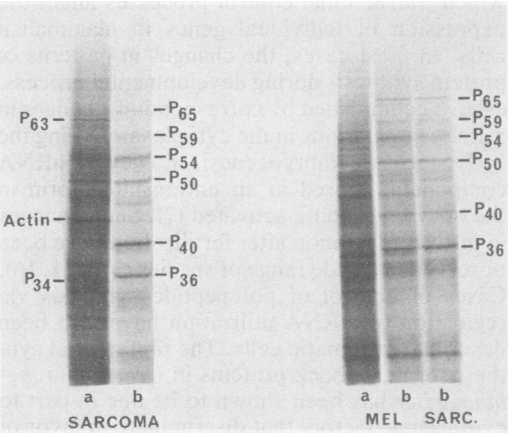

FIG. 1. Comparison of translation products of RNA preparations from mouse sarcoma-180 ascites cells and from MEL cells. RNA samples of 4 and 2 μg from polysomes (a) and from small mRNP particles (b), respectively, were translated in the wheat germ cell-free system. The sarcoma preparations were obtained as described previously (8). Translation products other than actin are designated by their approximate molecular weights. The translation product of P21 mRNA, one of the species analyzed by hybridization to cDNA, had migrated off the gel in this experiment.

RNA represents a relatively small portion of the cytoplasmic RNA (see Fig. 2).

Cloned cDNA probes, derived from the untranslated mRNA of sarcoma-180 cells (20), were used to measure the distribution of some of these species in polysomes and small RNP particles of the MEL cells. The distribution of actin mRNA, which is a major species in the polysomes of both cell types (Fig. 1), was also measured, using a cloned cDNA probe derived from chicken α-actin mRNA (17). The latter probe has been shown to be adequate for the quantitation of actin mRNA levels in the sarcoma ascites cells (20). Cytoplasmic extracts obtained from MEL cell cultures were fractionated by zone centrifugation. A typical polysome profile obtained from rapidly growing cells is shown in Fig. 2. This profile indicates that there was considerably less UV-absorbing material in the ribosomal monomer peak than in the polysomes. It also shows that most of the ribosomes engaged in translation were in relatively large polysomes. These characteristics indicate that the rate of polypeptide initiation relative to that of elongation was high in these cells. Fractions were pooled to obtain "large" polysomes, "small" polysomes (consisting primarily of monomers and dimers), and particles smaller than the ribosomal monomers (see arrows in Fig. 2). The levels of individual mRNA species in the three fractions were determined by the dot hybridization assay with the appropriate recombinant plasmid DNAs labeled by nick-translation. The data in Table 1 show that about 50 to 70% of the RNA molecules belonging to the P21, P36, and P40 mRNA species were present in untranslated particles, whereas nearly all the actin mRNA was in polysomes. In all cases, only a small proportion of the mRNA molecules was associated with monomers and dimers. As discussed elsewhere (21), this type of distribution indicates that the high proportion of molecules in the untranslated state is unlikely to be due simply to inefficient initiation on these molecules.

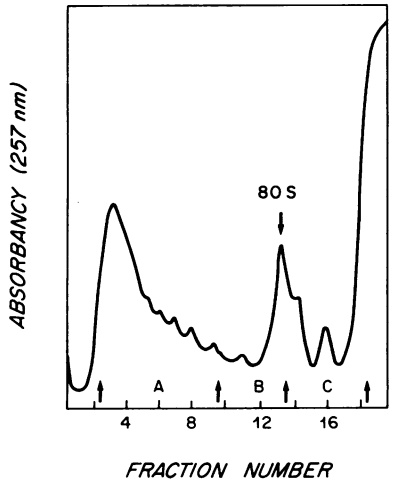

FIG. 2. Sedimentation profile of UV-absorbing material in cytoplasmic extract of MEL cells. Arrows indicate fractions pooled to represent large polysomes (A), small polysomes (consisting primarily of monomers and dimers) (B), and mRNP particles not engaged in translation (C). Direction of sedimentation is from right to left. Amounts of RNA recovered from these fractions were 133, 59, and 68 μg, respectively.

TABLE 1. Distribution of individual mRNA species in the cytoplasm of MEL cells[a]

mRNA species	Polysomes		RNP particles
	Large	Small	
Actin	0.77	0.08	0.15
P40	0.41	0.12	0.47
P36	0.22	0.08	0.70
P21	0.27	0.11	0.62

[a] MEL cells were processed as described in the text, and cytoplasmic extracts were fractionated as shown in Fig. 2. RNA samples from each fraction were used for analysis of mRNA levels by dot hybridization assays (see text). Data represent fraction of mRNA molecules of individual species present in each of the two polysomal fractions and in untranslated particles.

The analysis described in Table 1 was carried out on six different cultures of MEL cells. The range of values for the proportions of molecules of each species associated with polysomes (including dimers and monomers) is shown in Table 2. These data are compared with those derived from the analysis of sarcoma ascites cells, either taken directly from the animals or incubated in culture medium (21). It can be seen that the P21, P36, and actin mRNAs are utilized to about the same extent in the two types of cells. The data for P40 mRNA, however, show a considerably greater extent of utilization of this mRNA species in the MEL cells.

The cell cultures used for the above measurements ranged in density from 2×10^5 cells per ml (rapidly growing cells) to 2×10^6 cells per ml (cells approaching the stationary phase of growth). There was no obvious correlation between cell density and extent of utilization of any of the mRNA species. The scatter in values for mRNA utilization shown in Table 2 is probably due in part to lack in precision of the method of analysis. Variations in the recovery of fractions from the sucrose gradients and in the recovery of deproteinized RNA from the pooled fractions would affect the quantitative data on mRNA distribution. Thus, small changes in mRNA utilization could not be detected by this procedure.

TABLE 2. Extent of utilization of individual mRNA species in mouse sarcoma-180 and MEL cells[a]

mRNA species	Sarcoma-180 cells		MEL cells	
	Ascites	Cells in culture medium	Range	Avg for all cultures
Actin	0.84	0.82	0.83–0.93	0.87
P65	0.62	0.49	0.75	0.75
P40	0.39	0.26	0.50–0.71	0.59
P36	0.34	0.31	0.25–0.45	0.37
P21	0.46	0.45	0.38–0.52	0.46

[a] Cells from six MEL cultures with cell densities ranging from 2×10^5 to 2×10^6 cells per ml were analyzed as indicated in Table 1. Data on mRNA content of small and large polysomes were combined to estimate the fraction of total mRNA molecules of each species engaged in translation. Numbers represent range of values obtained with different MEL cultures. Values for the mouse sarcoma ascites cells harvested directly from the animals and from the cells incubated in culture medium are derived from data of Yenofsky et al. (21). The mRNA for P65 was analyzed in the preparations from one culture only.

The distribution of the untranslated species and of actin mRNA was also examined in mouse livers. These RNA species were present in very low amounts in this tissue. The level of P21 mRNA was about one sixth that of the corresponding level in the two types of tumor cells. About 40% of the P21 mRNA molecules were in small particles in the livers (data not shown).

Changes in mRNA utilization in cells exposed to DMSO. Exposure of the MEL cells to DMSO caused an arrest in DNA synthesis (measured as described in reference 13) and an inhibition of cell proliferation during the first 24-h period (data not shown). This was followed by a period of rapid cell proliferation. The transient effects of DMSO and of other inducers on cell cycle events have been described by other investigators (15). rRNA production was also arrested during the first day of exposure to DMSO. This was followed by a threefold increase over the next 2-day period. The progress of erythroid differentiation in the treated cultures was evidenced by the appearance of red color in the cells at the 48-h stage and by a 6.5-fold increase in globin mRNA concentration at this stage, as measured by dot hybridization of polysomal RNA with a β-globin cDNA probe (6) kindly supplied by Stuart Levy.

The polysome profiles in extracts of the differentiating cells were similar to the one shown in Fig. 2. The proportion of monomers increased somewhat during the early stages of induction. In the experiment described in Table 3, the distribution of monomers, expressed as percentage of total ribosomes in each extract, was 23, 37, 32, 30, and 29% for cells incubated for 0, 4, 24, 48, and 72 h, respectively. These data were obtained by analysis of sedimentation profiles of UV-absorbing material in the cytoplasmic extracts.

The distribution of individual mRNA species in polysomes and small RNP particles in the differentiating cells is shown in Table 3. The total mRNA, as determined by measurements of poly(A) levels, was present primarily in polysomes. There was little change in the extent of its utilization throughout the 3 days of exposure to DMSO. The actin mRNA, which was almost exclusively in polysomes, remained nearly fully utilized. The utilization of the P36 mRNA species, which was predominantly in untranslated particles, also remained unchanged. The other two species subject to translational repression, on the other hand, underwent large decreases in the proportion of their molecules engaged in translation. These decreases in utilization occurred within 1 day of exposure to the inducing agent.

The changes in levels of mRNA in the cytoplasm were also determined in these experiments. The data were obtained by summation of

TABLE 3. Changes in utilization of mRNA components in MEL cells exposed to DMSO[a]

Exposure to DMSO (h)	Total mRNA					% mRNA in polysomes				
	Poly(A)	Actin	P40	P36	P21	Poly(A)	Actin	P40	P36	P21
0	437	322	213	178	439	79	90	71	25	51
4	332	238	215	180	434	78	83	60	31	47
24	327	181	207	172	524	72	82	31	25	23
48	429	169	194	202	676	70	86	30	22	19
72	389	137	159	195	576	83	92	46	29	32

[a] Cell culture density of 10^6 cells per ml was supplemented with 1.5% DMSO. Samples of 200 ml were harvested at 0 and 4 h. At this time, samples were diluted twofold, fourfold, and eightfold with DMSO-containing medium to final volumes of 200 ml and were harvested after incubations of 20, 44, and 68 h, respectively. Cell samples were subjected to analysis of mRNA distributions in cytoplasmic fractions as in Table 2. Distributions are expressed as percentage of total mRNA of each species present in polysomes (small and large polysomes combined). Values for total mRNA are expressed as total amounts (in arbitrary units) recovered from the cytoplasmic fractions per unit amount of bulk RNA (rRNA) recovered.

the amounts of mRNA present in polysomes and in small particles and are expressed as amount of material per unit amount of total RNA recovered from the gradient fractions. The concentration of poly(A) decreased rapidly upon exposure of the cells to DMSO and returned to near normal levels after 2 days of treatment (Table 3). The level of actin mRNA decreased rapidly at first and then more gradually throughout the rest of the incubation period. No such decreases in mRNA concentration were observed in the case of the three other mRNA species. The levels of P36 and P40 mRNA remained essentially unchanged, whereas the level of P21 mRNA increased considerably.

Estimates of the levels of the various mRNA components in polysomes and in small particles were made, using the data in Table 3. The values obtained, expressed as amounts (in arbitrary units) relative to total RNA recovered from the gradient fractions, are shown in Fig. 3. The polysomal levels of the mRNAs for actin, P40, and P21 decreased to about one half the original value during the first day of exposure to DMSO. The amount of untranslated actin mRNA did not change during this period. In the case of the P40 mRNA, the decrease in polysomes was accompanied by a corresponding increase in the untranslated fraction. The P21 mRNA accumulated to a much larger extent as untranslated molecules. The levels of P36 mRNA, both in the active and inactive fractions, showed little change throughout the 3 days of treatment. The induction process was accompanied by a transient decrease in total polysomal mRNA, as indicated by the changes in the poly(A) content of the polysomes.

Distribution of translatable mRNA species. The changes in mRNA distribution upon induction by DMSO were also examined by the translation assay. Samples of polysomal and RNP RNA were incubated in the wheat germ cell-free system, and the translation products were resolved by one-dimensional polyacrylamide gel electrophoresis. After autoradiography, the patterns of bands were analyzed by densitometry tracing (Fig. 4). Major bands with mobilities corresponding to those of actin, P40, P36, and P21 could be seen. The patterns of translation products revealed changes in the distribution of mRNA species that parallel those determined by the hybridization assays. Changes in the distribution of other major species were also evident. Quantitative estimates of translatable mRNA distribution could not be made because of the poor resolution of most of the radioactive bands. The activity for actin in the polysomal RNA samples was determined by measuring the area under the peak and correcting for differences in the width of the lanes in the gel. These measurements indicated that the translatable actin mRNA decreased to 93, 74, and 48% of the original value in the samples from cells exposed to DMSO for 4, 24, and 48 h, respectively. These decreases are not as abrupt as those indicated by the hybridization assays. The polypeptide P40 produced by translation of the polysomal RNA samples was partially resolved in this experiment. A sharp reduction in the activity for this polypeptide could be seen in the polysomal samples from cells treated with DMSO for 24 and 48 h. This was accompanied by considerable increases in activity in the RNA samples from RNP particles. The activity for P21 showed similar changes. The levels of P36 mRNA seemed to remain unchanged in the RNP particles, although the appearance of a neighboring band among the translation products of the RNA samples from induced cells made it difficult to estimate the intensity of the P36 band in these samples. It can also be seen that the intensity of a band with a mobility slightly higher than that of

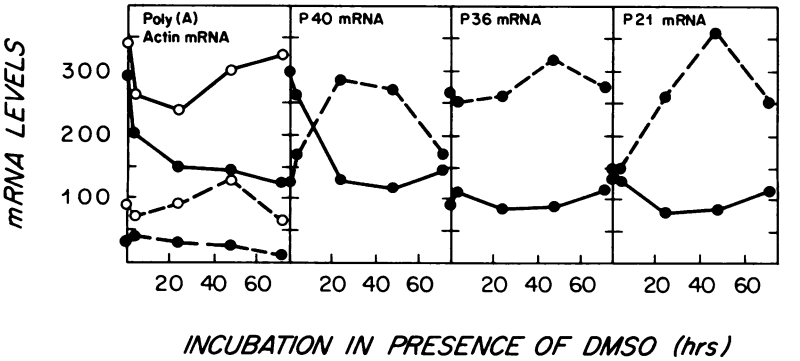

FIG. 3. Changes in mRNA levels in polysomes and in small RNP particles of MEL cells exposed to DMSO. Data, derived from values in Table 3, are expressed as activity (in arbitrary units) per 40 μg of total RNA recovered from the gradients. Symbols: ○, poly(A) content; ●, individual mRNAs; ———, levels in polysomes; and – – – –, levels in untranslated particles.

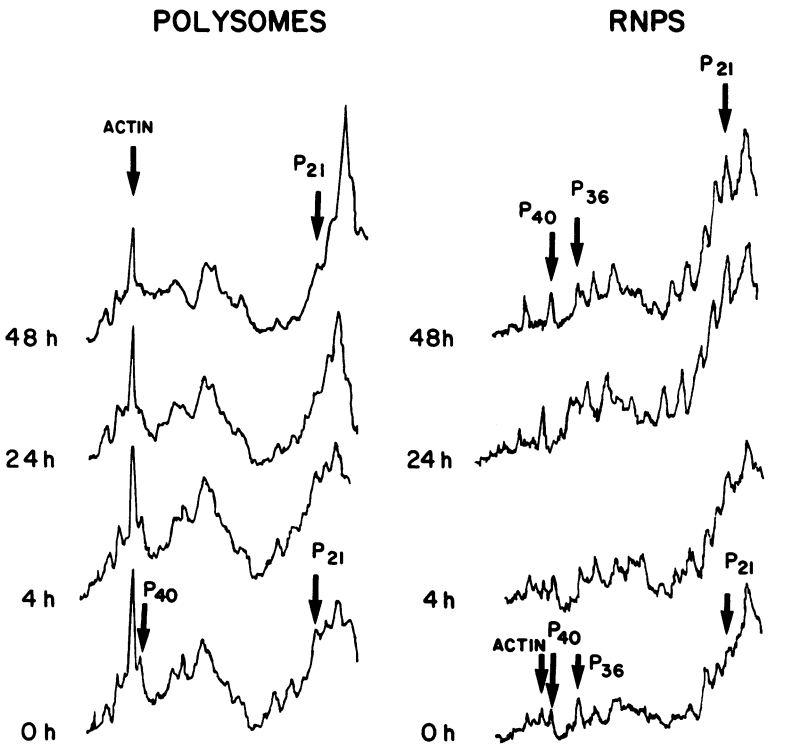

FIG. 4. Changes in the distribution of translatable mRNAs in MEL cells incubated in the presence of DMSO. RNA preparations described in Fig. 3 and in Table 3 were used in the wheat germ cell-free system as described in the text. Amounts of RNA used for translations were 4 and 3 μg for the polysomal and RNP samples, respectively. Figure shows densitometry tracings of autoradiograms.

P21 increased sharply in the polysomal samples from induced cells.

DISCUSSION

The present study showed that exposure of MEL cells to DMSO leads to a relatively rapid decrease in the amounts of several mRNA species in polysomes. Presumably, these changes provide a measure of alterations in the rates of synthesis of the corresponding polypeptides. The reduced polysomal mRNA levels persisted for up to three days. The total polysomal mRNA, as determined by poly(A) measurements, also decreased in the DMSO-treated cells but returned to nearly normal levels after 2 days of treatment. The levels of individual mRNA species in polysomes, as well as the proportions of mRNA in untranslated particles, showed some tendency to shift back to the original values in the cells exposed to DMSO for 3 days. These latter changes could be explained by the presence of cells that had not become induced and that were growing more rapidly than the induced cells. It is not known, however, whether a significant portion of the cells had remained uninduced in the present experiments. The changes described in this study represent early responses to the inducing agent, preceding the onset of globin mRNA accumulation. It remains to be determined whether these changes constitute a specific event in erythroid differentiation or whether they are the result of the transient interference with cell proliferation caused by the drug. The actin mRNA, however, did not return to the original level as cell growth resumed. It showed instead a continued tendency to decrease in amount as differentiation proceeded. The loss of actin mRNA from the polysomes of MEL cells soon after exposure to DMSO suggests that changes in the cytoskeleton may be part of the early events associated with the induction of differentiation in these cells. Large decreases in actin and tubulin synthesis have been shown to occur during the early stages of differentiation of 3T3 adipocytes (18). A rapid decrease in the synthesis of actin has also been reported to take place after fertilization of mouse blastocysts (1).

The changes in distribution of the mRNAs for P21 and P40 provide evidence for the existence of a process for the regulation of gene expression that operates in the cytoplasm. The cells responded to the inducing stimulus by reducing the extent of utilization of these two species.

The nuclear events leading to the production of P40 mRNA were apparently not affected, since the intracellular level of this RNA species remained essentially unchanged. In the case of the P21 mRNA, the rate of production appeared to increase, leading to a large accumulation of untranslated molecules of this species. The P21 and P40 mRNAs have been shown to be subject to "translational repression" in mouse sarcoma-180 ascites cells (21). They also appear to be susceptible to this kind of repression in the MEL cells. The changes upon exposure to DMSO indicate that this process can serve to regulate the rates of synthesis of the corresponding polypeptides. The P36, which is also subject to translational repression, was not affected in terms of extent of utilization for translation in the DMSO-treated cells. This indicates that the cytoplasmic repression process is selective.

The physiological significance of the translational repression process remains to be determined. The three mRNA species subject to this process that were investigated in the present study are relatively abundant in mouse sarcoma cells and MEL cells but are present in much smaller amounts in mouse livers. The P36 mRNA may correspond to a species coding for a polypeptide of M_r 35,000 that is untranslated in mouse oocytes and becomes active after fertilization (3, 4). The above features suggest a possible relationship between some of the untranslated species and the proliferative capacity of the cells. It could be, for instance, that the untranslated mRNAs function only during particular phases of the cell cycle. We have not been able, however, to detect any significant differences in mRNA utilization that could be related to the rate of proliferation of uninduced MEL cells. It is possible that relatively small shifts in asynchronous cell populations may not be detectable by the present method of analysis and that measurements on synchronized cell populations would provide more definitive information on this subject.

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