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Regulation of Actin mRNA Levels and Translation Responds to Changes in Cell Configuration

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The role of cell configuration in regulating cell metabolism has been studied, using a system in which cell shape and surface contact can easily be manipulated. The suspension of anchorage-dependent mouse fibroblasts in Methocel results in a coordinate decrease of DNA, RNA, and protein synthesis. These processes are restored upon reattachment of cells to a solid surface. This recovery process has two or more components: a rapid recovery of protein synthesis requiring only surface contact, and a slower restoration of nuclear events which is dependent upon extensive cell spreading (A. Ben-Ze'ev, S. R. Farmer, and S. Penman, Cell 21:365–372, 1980). In the present study, we examined 3T3 cells while in suspension culture and after attachment to a tissue culture dish surface to study cell configuration-dependent expression of specific cytoskeleton protein genes. The 3T3 line of fibroblasts used here shows these responses much more dramatically compared with 3T6 cells previously studied. We demonstrate that whereas total protein synthesis was strongly inhibited upon suspension, actin synthesis was preferentially inhibited, decreasing from 12% of total protein synthesis in control cells to 6% in suspended cells. This occurred apparently at the level of translation of actin mRNA, since the amount of actin mRNA sequences in the cytoplasm was unchanged. Reattachment initiated the rapid recovery of overall protein synthesis which was accompanied by a dramatic, preferential increase in actin synthesis reaching peak values of 20 to 25% of total protein synthesis 4 to 6 h later, but then declining to control values by 24 h. Translation in vitro and hybridization of mRNA to a cloned actin cDNA probe revealed that the induction of actin synthesis was due to increased levels of translatable mRNA sequences in the cytoplasm. These results imply a close relationship among cell cytoarchitecture, expression of a specific cytoskeletal protein gene, and growth control. The expression of the actin gene appears to be regulated at both the level of translation (during suspension) and mRNA production (during recovery).

Folkman and co-workers (10, 11) first demonstrated that cell shape is a fundamental regulator of cell growth. These researchers varied cell configuration in culture from flat to nearly round by growing endothelial cells on poly(2-hydroxyethyl methacrylate) [poly(HEMA)] surfaces with different adhesiveness. A direct correlation between the degree of cell spreading and growth rate as measured by DNA synthesis was observed.

Our investigations have extended Folkman's pioneering studies to show that most of the macromolecular metabolism in the cell (i.e., DNA, RNA, and protein synthesis) is responsive to cell configuration. Anchorage-dependent fibroblasts immediately cease growth when placed in suspension culture; DNA, RNA, and protein synthesis are all inhibited as the result of the initiation of a program of shutdown mechanisms (1, 2, 9, 24). These effects result from both the change in cell shape and the loss of surface contact. After the reattachment of cells to a culture dish surface, protein synthesis recovers rapidly within a few hours. This recovery process requires only cell contact with an external surface and is shape insensitive. In contrast, the recovery of nuclear metabolism is much slower. The complete restoration of rRNA, DNA synthesis, and mRNA production requires about 18 h of culture after reattachment. This recovery process is dependent on extensive cell spreading and thus appears to be shape dependent (4).

Otsuka and Moskowitz (18) demonstrated that suspension of 3T3 cells arrests growth in the G1 phase of the cell cycle and that reattachment to a

surface reinitiates progress through the cycle, reaching S phase 14 to 20 h later. Pardee and coworkers (20, 21) have attempted to characterize and assign specific protein markers to different phases of the cell cycle. Recently, they noted the induction of actin synthesis accompanying the rescue of serum-arrested 3T3 cells and suggested that this may be a specific marker for passage from G_0 through G_1 into S. We had observed a similar increase in actin synthesis after the reattachment of suspended 3T6 cells (1, 9).

Recent studies aimed at understanding cell configuration-related growth control have been directed towards investigating the regulation of genes coding for the major cytoskeletal proteins (3, 22). The possibility that the expression of such genes is controlled by the morphological state of the corresponding cytoskeletal elements has recently been proposed (3, 8). In the present study, we have focused on the regulation of actin synthesis during suspension of 3T3 cells and after reattachment of suspended 3T3 cells. We intend initially to define the level of regulation of this gene and eventually be able to relate changes in gene expression to alterations in cell configuration.

The results presented here, using a cloned actin cDNA as a hybridization probe, indicate that the selective inhibition of actin synthesis during suspension occurs despite a constant level of actin message. In contrast, the induction of actin synthesis during reattachment is due to increased actin mRNA levels in the cytoplasm.

MATERIALS AND METHODS

Materials. [^{35}S]methionine, [α-^{32}P]dCTP, and [^{3}H]polyuridylate [poly(U)] were from New England Nuclear Corp. Methylcellulose (Methocel) was obtained from Dow Chemical Co., and Ampholines were obtained from LKB Instruments, Inc.

Cell cultures. 3T3 mouse fibroblasts were maintained either as subconfluent monolayers or in suspension in Methocel as outlined in detail in previous studies (1). For reattachment studies, cells were harvested from suspension by a 10-fold dilution of the Methocel medium with regular medium and centrifugation for 10 minutes at 2,000 × *g*. Cells were then suspended in fresh medium and plated onto tissue culture dishes.

Determination of protein synthesis rates. The rate of protein synthesis was measured by incubating cells with 25 μCi of [^{35}S]methionine per ml for 30 to 45 min in medium containing 10% of the normal concentration of methionine, followed by measurement of trichloroacetic acid-precipitable radioactivity. To express data relative to equal numbers of cells in each sample, the number of cells was most easily measured by counting the number of nuclei after detergent extraction. A convenient check for equal numbers of cells, particularly when experimenting with few cells, was to quantitate the intensity of silver staining of proteins after gel electrophoresis.

Cell fractionation and RNA extraction. Cytoplasmic extracts were prepared with a low-ionic-strength buffer RSB (10 mM NaCl, 1.5 mM $MgCl_2$, 10 mM Tris, pH 8.3) containing 1% Nonidet P-40 and 15 μg of phenylmethylsulfonylfluoride per ml for protein analysis or the ribonuclease inhibitor vanadyl adenosine at 20 mM for RNA extraction. Suspended cells were harvested by centrifugation in the cold after diluting the Methocel fivefold with ice-cold phosphate-buffered saline. The cell pellet, after being washed with phosphate-buffered saline, was suspended in RSB buffer and extracted. Monolayer cells (both control and reattached) were harvested simply by washing the tissue culture plates carefully with phosphate-buffered saline and then adding RSB buffer followed by scraping. Cells in each case were disrupted by vigorous vortexing, and cytoplasmic extracts were obtained by centrifuging out the nuclei. To obtain maximum amounts of cytoplasmic material, the nuclei were further washed with the double detergent 1% Tween–0.5% deoxycholate in RSB. The nuclei were again removed by centrifugation, and the supernatants were combined. Total cytoplasmic RNA was isolated, using the modified phenol-chloroform procedure described recently (15).

Protein gel electrophoresis, staining, and fluorography. One-dimensional sodium dodecyl sulfate slab gels were performed with 10% polyacrylamide according to the method of Laemmli (13). Two-dimensional gels were run according to the method of O'Farrell (17), using a mixture of ampholytes of 60% 3 to 10, 30% 5 to 7, and 10% 4 to 6. Proteins were routinely stained with Coomassie blue and destained with a solution containing 40% methanol and 7.5% acetic acid. On occasions when very small amounts of protein were being analyzed and it was important to stain the proteins to confirm cell counts, the slab gels were stained by the extremely sensitive silver staining procedure (Bio-Rad Laboratories) as developed by Merril et al. (16). Gels were fluorographed according to the method of Bonner and Laskey (6). After drying, gels were exposed to prefogged Kodak XR-2 film at −80°C (14).

RNA quantitation and cell-free protein synthesis. Total cytoplasmic RNA was quantitated by absorbance at 260 nm. The amount of polyadenylated [poly(A)$^+$] mRNA present in each sample was quantitated by hybridization to [^{3}H]poly(U) as described previously (5). Translation of mRNA was performed with the micrococcal nuclease-treated reticulocyte lysate system (19). Poly(A)$^+$ RNA (200 ng) was translated in 25-μl lysate volumes as outlined previously (9).

RNA gel electrophoresis and filter hybridization. Total cytoplasmic RNA [equivalent to equal quantities of poly(A)$^+$ RNA, i.e., 200 ng] was electrophoresed on a 1% agarose gel containing 6% formaldehyde by a procedure modified from that of Seed and Goldberg (unpublished data). A 1% agarose gel was prepared by melting agarose in 1.2× gel buffer (1× gel buffer is 20 mM morpholinepropanesulfonic acid–50 mM sodium acetate–1 mM EDTA, pH 7.0). The agarose solution was cooled to 65°C, then one-sixth volume of 37% formaldehyde (Mallinckrodt, Inc.) was added to give a final concentration of 6% formaldehyde. The gel running buffer was 1× gel buffer without formaldehyde. RNA samples were incubated at 55°C for 15 min in 50% deionized formamide–6% formaldehyde–1× gel buffer containing bromophenol blue. Electrophoresis

was carried out overnight at constant voltage (35 V). After electrophoresis, the gel was washed in transfer buffer (10× SSC [= 1.5 M NaCl plus 150 mM sodium citrate]) for 30 min. It was then stained with 1 μg of ethidium bromide per ml for 20 min and viewed under UV light after washing. The RNA was transferred to nitrocellulose (Schleicher & Schuell Co.) as described previously (23). After completion of the transfer (usually overnight), the filter was washed in 5× SSC, blotted dry between 3MM paper (Whatman, Inc.), and baked at 80°C for 3 to 4 h. Hybridization with 5×10^6 to 10×10^6 cpm of ^{32}P-labeled, nick-translated actin cDNA clone was performed in a plastic Seal-a-Meal bag as described recently (8).

Radiolabeling of cDNA. Radiolabeling of cDNA was performed as described previously (22).

RESULTS

Response of actin and total protein synthesis to changes in cell configuration. Our previous studies addressing the question of regulation of macromolecular metabolism by changes in cell configuration were performed with 3T6 mouse fibroblasts. A more recent study (24) demonstrated that 3T3 cells respond more rapidly and extensively to suspension culture than do 3T6 cells, reflecting their more stringent growth regulation. Furthermore, 3T3 cells were originally used by Otsuka and Moskowitz (18) to demonstrate that suspension in Methocel arrests growth in the G_1 phase of the cell cycle.

The regulation of actin synthesis is studied here with the 3T3 cell line and appears to be an aspect of the regulation of gene expression by the configuration of cell architecture. The changes in total protein synthesis in 3T3 cells during the suspension-reattachment manipulations are illustrated in Fig. 1. Control monolayer cells growing at 50 to 75% confluency were suspended for 3 days in Methocel, then allowed to reattach to tissue culture surfaces for increasing time periods. Forty-five minutes before harvest, cells were radiolabeled with [^{35}S]methionine, and cytoplasmic fractions were obtained. Figure 1A shows a sodium dodecyl sulfate-polyacrylamide gel analysis of the cell extracts. The proteins from equal numbers of cells were applied to each lane. It is clear that suspension caused a dramatic inhibition of all protein synthesis. It is difficult in the reproduction to observe the selective inhibition of structural proteins during suspension since so few counts were loaded into the S track. After reattachment, there was a rapid increase in the rate of protein synthesis, reaching a plateau value of 80 to 90% by 4 to 6 h. The synthesis of most of the cellular proteins recovered to these values, as judged by two-dimensional gel analysis (see Fig. 3), with only a few exceptions. Complete return to control values (100%) did not occur until 17 to 24 h later, coincident with DNA synthesis. The

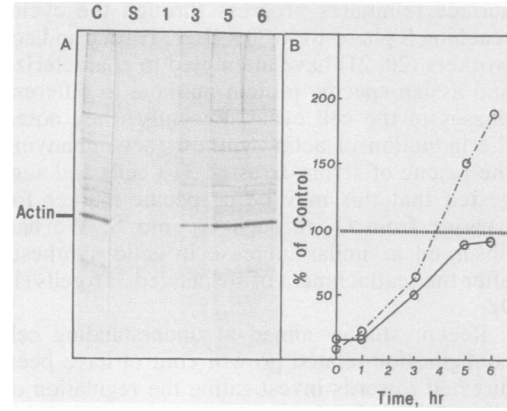

FIG. 1. Absolute rates of actin and total protein synthesis during the reattachment of suspended 3T3 cells. 3T3 fibroblasts were cultivated under the various cell culture conditions and were pulse-labeled for 45 min with 25 μCi of [^{35}S]methionine per ml as described in the text. The cytoplasmic proteins (equivalent to equal numbers of cells) were analyzed on 10% sodium dodecyl sulfate-polyacrylamide slab gels as described previously (13, 14). (A) Fluorograph of the gel analysis. C, Control cells (57,000 cpm); S, suspension cells (8,000 cpm); 1, 3, 5, 6, reattaching cells as follows: 1, 1 h after reattachment (10,000 cpm); 3, 3 h after reattachment (25,200 cpm); 5, 5 h after reattachment (42,500 cpm); and 6, 6 h after reattachment (50,000 cpm). (B) Absolute protein synthesis was quantitated by integrating the area under the representative scans for each gel track. Total protein synthesis (———) is proportional to total area and actin synthesis (–·–·–·–) to the area under the actin peak. The data are expressed as a percentage of the corresponding values obtained in control cells. 0, Time cells were suspended. Time, hr refers to time cells were allowed to reattach after suspension culture.

data plotted in Fig. 1B were obtained by scanning the fluorograph of each gel track with a Zeineh soft laser densitometer equipped with an automatic peak integrator. The areas under the total scan and under the actin peak (proportional to absolute total protein synthesis and actin synthesis, respectively) were determined. The data are expressed as percentages of the corresponding protein synthesis rate obtained in control cells. To confirm the determination of cell numbers, we stained the protein gels by the silver staining method, which can detect very small quantities of protein. Intensity of staining for different cell extracts was quantitated to correct for the differences in cell numbers. The most striking observation was the marked increase in the rate of actin synthesis, which reached values almost twofold greater than those observed for control cells.

A more accurate measure of actin synthesis relative to other proteins is afforded by the data

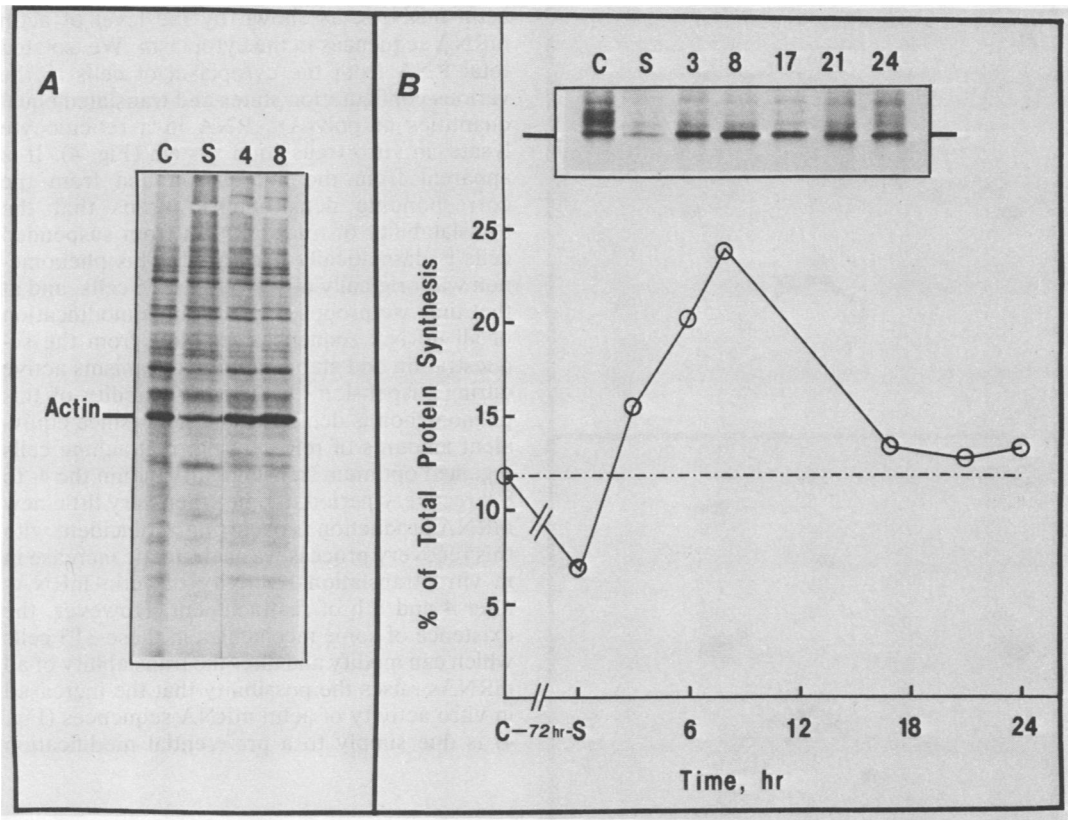

FIG. 2. Rate of actin synthesis relative to total cytoplasmic protein synthesis during suspension and reattachment of 3T3 cells. 3T3 fibroblasts suspended in Methocel for 3 days were allowed to reattach to surfaces for the times shown and were pulsed with [^{35}S]methionine for 45 min. Cytoplasmic proteins (equivalent to equal amounts of trichloroacetic acid-precipitable radioactivity, 10^5 cpm) were analyzed as described in the legend to Fig. 1. (A) Fluorograph of gel analysis demonstrates changes in actin synthesis relative to other cytoplasmic proteins. (B) Synthesis of actin at different times after reattachment. Gels similar to those in part A corresponding to the different time points were scanned, and the relative rate of actin synthesis was quantitated as described in the text and expressed as a percentage of total protein synthesis. The gel insert is the actin region of the gel used to derive this data. C, Control cells; S, suspension cells.

in Fig. 2, in which equal amounts of radioactivity were applied to each lane. Figure 2A shows the relative inhibition of actin synthesis during suspension and its early recovery, and Fig. 2B follows the complete actin synthesis time-course. The data plotted in Fig. 2B were derived by scanning individual gel tracks and integrating the area under the actin peak relative to the area of the total scan. It is apparent from the gels in Fig. 2A that the relative rate of actin synthesis decreased significantly during the suspension of 3T3 cells. It also appears that the synthesis of several other proteins of molecular weights of 50,000 to 70,000 was similarly affected. When cells reattached to surfaces, there was a rapid and extensive induction of actin synthesis. The data expressed in Fig. 2B reveal that the value of actin synthesis in control cells is 11 to 12% of total protein synthesis and agree with values previously obtained (22). By 3 days in suspension, total protein synthesis was reduced to 10 to 20% of control values (Fig. 1B), and actin synthesis fell to 6 to 7% of this reduced value. After replating, total protein synthesis recovered rapidly to control values, accompanied by a dramatic induction of actin synthesis which reached a peak of 20 to 25% of restored total protein synthesis by 6 to 8 h after reattachment. Actin synthesis then declined to the control value by 17 to 24 h. This profile is similar to that observed for the induction of actin synthesis accompanying passage from G_0 into S during the rescue of serum-arrested cells (20).

To confirm that this induced protein was indeed actin, we analyzed samples of [^{35}S]methionine-labeled extracts from control and 8-h

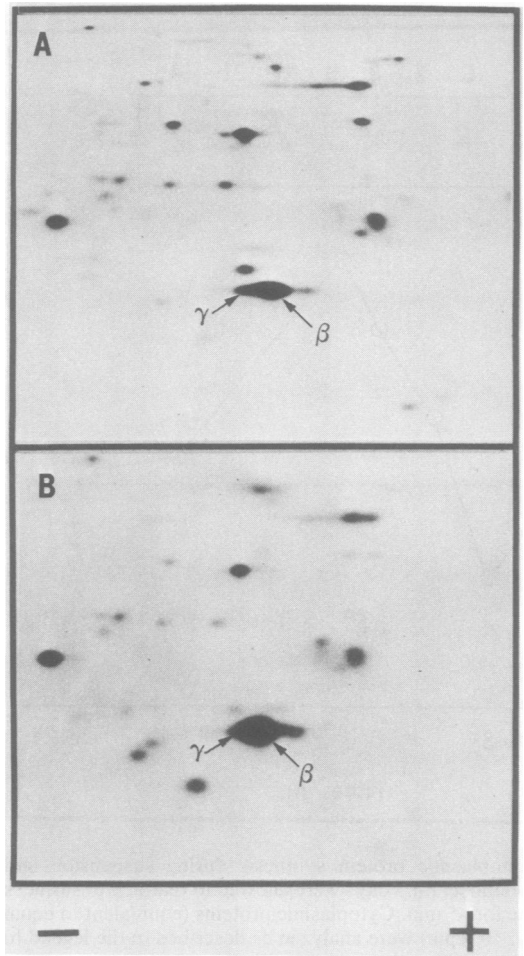

FIG. 3. Two-dimensional gel electrophoretic analysis of 3T3 cytoplasmic proteins. Cytoplasmic extracts (equal amounts of radioactivity, 10^5 cpm) from [^{35}S]methionine-labeled cells maintained either as a control culture (A) or 8 h after reattachment (B) were analyzed on two-dimensional gels as described in the text. β and γ indicate the migration of β- and γ-actins, respectively.

reattaching cells by two-dimensional gel electrophoresis (Fig. 3). It is clear that the radioactive actin spot is more intense in the gel corresponding to the reattaching cell extract. It appears that both β- and γ-actin species are induced, but a more definitive assay employing high-resolution, one-dimensional isoelectric focusing is necessary to quantitate the individual species.

Mechanisms regulating actin synthesis. The rapid recovery of total protein synthesis after reattachment has been shown previously to be primarily at the level of translational control (1, 9, 4). However, the enhanced synthesis of actin is not simply due to a preferential translation of actin mRNAs, as shown by the level of actin mRNA sequences in the cytoplasm. We isolated total RNA from the cytoplasm of cells in the various configuration states and translated equal quantities of poly(A)$^+$ RNA in a reticulocyte lysate, in vitro translation system (Fig. 4). It is apparent from the fluorograph and from the corresponding densitometric scans that the translatability of total mRNAs from suspended cells is dramatically decreased. This phenomenon was originally observed for 3T6 cells, and at that time we proposed a reversible modification of all mRNA sequences resulting from the sequestration and stabilization mechanisms active during suspension (9). The reversibility of this phenomenon is demonstrated here, since equivalent amounts of mRNA from reattaching cells regained optimum translatability within the 4- to 8-h recovery period, a time when very little new mRNA production is occurring. Coincident with this recovery process was a dramatic increase in in vitro translational activity of actin mRNAs after 4 and 8 h of reattachment. However, the existence of some mechanism in these 3T3 cells which can modify and alter the translability of all mRNAs raises the possibility that the increased in vitro activity of actin mRNA sequences (Fig. 4) is due simply to a preferential modification

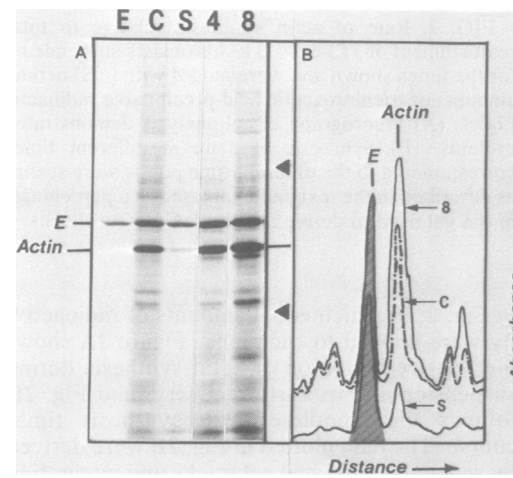

FIG. 4. Electrophoretic analysis of in vitro translation products directed by mRNA from 3T3 fibroblasts. Samples [equivalent to the same amount of input poly(A)$^+$ RNA] were taken from the in vitro translation system and analyzed on 10% sodium dodecyl sulfate-polyacrylamide gels (A) as described in the text. E, Endogenous background, i.e., no input mRNA (6,100 cpm); C, control (63,082 cpm); S, suspension (24,760 cpm); 4, 4 h after reattachment (49,480 cpm); and 8, 8 h after reattachment (74,584 cpm). (B) Densitometric scan of gel tracks C, S, and 8 between the arrowheads shown in part A.

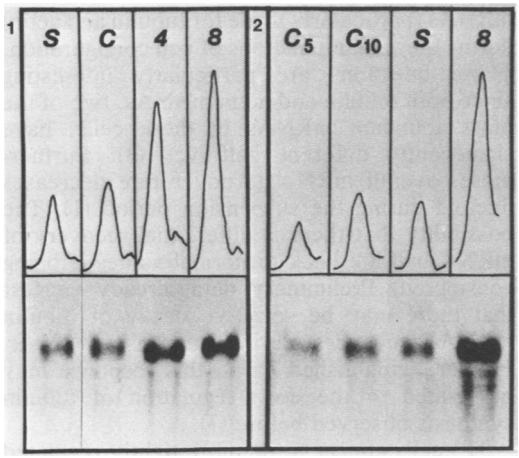

FIG. 5. Measurement of actin mRNA levels in 3T3 cells by Northern blot hybridization. 3T3 cells were manipulated in culture as described in the text. RNA was isolated and electrophoresed on 1% agarose gels containing 6% formaldehyde as described in the text. Total cytoplasmic RNA containing equal amounts of poly(A)$^+$ sequences [determined by [^{3}H]poly(U) hybridization] were loaded onto each track. There was very good correlation between the amount of total RNA estimated by the absorbance at 260 nm and the quantity of poly(A)$^+$ present. In all RNA samples, poly(A)$^+$ RNA was 2% of the total RNA (i.e., 200 ng of poly(A)$^+$ RNA per 10 μg of total RNA). The amount of RNA loaded was confirmed after electrophoresis by ethidium bromide staining. The RNA was then transferred to nitrocellulose by the method of Thomas (23). Filters were hybridized to linearized plasmid DNA containing chicken β-actin sequences, labeled with ^{32}P by nick translation as described in the text. 1 and 2 correspond to two separate experiments. C_5 and C_{10}, 5 and 10 μg of control RNA; S, suspension; 4, 4 h after reattachment; and 8; 8 h after reattachment.

and not to an increase in the amount of these sequences. To determine which of these situations is correct, we determined the actual concentration of mRNA by Northern blot hybridization, using a cloned actin cDNA. Cytoplasmic RNAs containing equal quantities of poly(A)$^+$ RNA [determined by [^{3}H]poly(U)hybridization] were electrophoresed on formaldehyde-agarose gels and transferred to nitrocellulose. Filters were hybridized to plasmid [^{32}P]DNA containing cDNA sequences complementary to chicken β-actin (7). Previous studies have shown that this clone hybridizes to both β- and γ-actin sequences present in mouse 3T3 cells (22). We observed a single discrete band of hybridization with a mobility of slightly slower than 18S rRNA (ca. 2,000 base pairs) (Fig. 5). Therefore, it appears that we have not separated the individual β- and γ-actin mRNAs, and only the sum of their signals is observed. In both of the experiments presented in Fig. 5, we observed a significant increase in β- and γ-actin mRNA levels in the RNA isolated from reattaching cells. To estimate the extent of this increase, we scanned the autoradiographs, and the corresponding scans are shown above the blots. The degree of hybridization was shown to be linear from 0 to 15 μg of total RNA. Experiment 2 presented in Fig. 5 demonstrates the doubling of signal intensity when the quantity of control RNA was increased from 5 to 10 μg. The level of actin mRNAs has been determined here in total RNA rather than in poly(A)$^+$-enriched preparations, since previous work has shown that a significant quantity of actin mRNAs are poly(A) deficient (12). Table 1 summarizes the data derived from the in vivo labeling experiments, the in vitro translations, and the hybridization analysis and clearly demonstrates that the increase in actin protein synthesis is accompanied by and due to a corresponding increase in the level of translatable actin mRNAs in the cytoplasm of reattaching cells.

In contrast to the coordinate rise in both actin synthesis and mRNA content after replating, the marked decrease in actin synthesis in suspended cells was not accompanied by a significant change in the amount of actin sequences. Integration of the areas under the densitometric scans shows that the level of actin sequences in RNA of suspended cells decreased only slightly,

TABLE 1. Regulation of actin gene expression in suspended-reattached 3T3 cells[a]

Cells	In vivo actin synthesis (%)[b]	In vitro translation of actin mRNA (%)[c]	Actin mRNA levels by hybridization (%)[d]	
			Expt 1	Expt 2
Control	100	100	100	100
Suspension	6.5	13.2	80	75
4-h reattachment	144	151	206	ND[e]
8-h reattachment	205	203	228	257

[a] Data are expressed as absolute values derived from equal numbers of cells; i.e., the amount of poly(A) RNA per cell remains constant throughout these manipulations.

[b] Data obtained from Fig. 1. Absolute rate of actin synthesis determined from area under actin peak for equal numbers of cells.

[c] Data obtained from Fig. 4. In vitro rate of actin synthesis determined from area under actin peak for equal amounts of poly(A)$^+$ RNA.

[d] Data obtained from Fig. 5. mRNA levels proportional to area under peak [equal amounts of poly(A)$^+$ RNA].

[e] ND, Not determined.

to 80% of control values. The blots also show that these sequences were intact, migrating as a discrete band of 2,000 base pairs. This observation implies that the significantly reduced translatability of suspended cell mRNA (Fig. 4) is not due to a selective decay or degradation of abundant mRNAs in this RNA preparation. This, therefore, confirms our initial reports that many of the suspended cell mRNAs are rendered untranslatable by some unknown modification (9). Furthermore, the total amount of poly(A)$^+$ RNA remained constant after 3 days in suspension, even though mRNA production was markedly reduced (legend to Fig. 5). This phenomenon was observed previously in suspended 3T6 cells and was shown to result from the cessation of mRNA turnover (1). The rapid recovery of protein synthesis after reattachment of 3T6 cells, observed previously, is apparently due to a translational control mechanism, involving the mobilization of untranslatable, quasistable mRNA onto polysomes. From the data presented here (Fig. 5; Table 1), it appears that a similar translational control is operating in 3T3 cells, both for total protein synthesis and actin synthesis (i.e., compare column 1 with columns 3 and 4 in Table 1; there is a low level of actin synthesis in vivo but very little decrease in the amount of actin mRNA sequences in the cytoplasm).

DISCUSSION

Anchorage-dependent fibroblasts offer a particularly simple system for examining the response of cellular synthesis systems to changes in cell configuration. In particular, placing such fibroblasts in suspension culture leads to the shutdown of the major macromolecular processes, whereas reattachment results in an apparently well-defined program of recovery. In this report, we show that the synthesis of actin shows unique regulatory responses to cell suspension and reattachment.

Suspension of 3T3 cells caused an overall reduction in protein synthesis, but an even greater inhibition of synthesis of actin and a few other proteins. Upon reattachment, protein synthesis recovered rapidly to 80 to 90% of control values, whereas actin synthesis was induced twofold above control levels. This induction appears to be due to an increased level of actin mRNA in the cytoplasm, as assayed by in vitro translation and by hybridization of cloned cDNA to cell mRNA. Several mechanisms could be operating to effect such a response; for instance, enhanced transcription, altered processing events, or decreased turnover rates. The data presented here do not distinguish among these possibilities. Studies are in progress to determine the turnover rates of abundant mRNAs (particularly those for tubulin and actin) during these manipulations of cell configuration. These questions are particularly interesting since both tubulin and actin mRNAs, two of the most abundant mRNAs in these cells, have significantly different half-lives (3). Furthermore, overall mRNA turnover rate decreases fivefold during the suspension period (1). The possibility that there is differential recovery of mRNA half-life back to normal values is being considered. Preliminary data already suggest that there may be selective decay of tubulin mRNA sequences during suspension (S. R. Farmer, unpublished data); this response may be related to the down-regulation of tubulin synthesis observed before (3).

3T3 cells appear to be more tightly regulated than 3T6 cells (24). They are more responsive to contact inhibition, grow to lower densities at confluence, and have a more normal spread fibroblast morphology. A recent study has shown that the metabolic responses (DNA, RNA, and protein synthesis) to shape alteration affected by suspension culture change profoundly as cells lose growth regulation and display a more transformed phenotype (24). In this regard, it is interesting that we also observed a difference between 3T3 and 3T6 cells in the response of actin synthesis to cell suspension. It appears that in the tightly controlled, less-transformed 3T3 cell, there is a significant regulation of actin gene expression. This implies a correlation between the extent of regulation of actin and other structural protein synthesis and the transformed cell phenotype. It is possible that this is related to the change in overall cell organization and morphology resulting from transformation.

The enhanced rate of actin synthesis was maximal 4 to 8 h after reattachment of suspended 3T3 cells and returned to control values by 17 to 24 h. This response is very similar to that observed previously by Pardee and co-workers (20, 21) for the passage from G_0 into S in the rescue of serum-arrested 3T3 cells. These authors proposed that actin induction may serve as a marker for this transition period. The enhanced actin synthesis after reattachment might be interpreted as signalling the G_0-to-G_1 transition, implying that suspended cells exist in a G_0 phase of the cell cycle. However, it is also possible that this response reflects solely the changes in cell architecture and that the actin induction observed during the rescue of serum-deprived cells is a similar response to changes in cell cytoskeleton configuration.

There are, as yet, few examples of systems demonstrating regulation of the structural protein genes in nonmuscle cells. Thus, little is known about their regulatory mechanisms and

the signals such mechanisms respond to. The most relevant data to date reveal that tubulin synthesis in several cell types decreases in response to increasing levels of depolymerized tubulin subunits (3, 8). It is conceivable that a similar mechanism may exist for the other cytoskeletal protein genes. In this regard, the dramatic changes in cell configuration during suspension may trigger subtle mechanisms involving a reorganization of microfilaments and consequently cause an inhibition of actin synthesis. Reattachment may then initiate processes to restore the normal cytoskeleton and therefore require an enhanced actin protein production.

A close relationship between the morphological state of a cell and the regulation of cytoskeletal protein gene expression has recently been demonstrated. Spiegelman and Farmer (22) observed a down-regulation of actin, tubulin, and vimentin synthesis before the dramatic morphological changes accompanying 3T3 preadipocyte differentiation. It is possible, therefore, that regulation of cytoskeletal protein genes plays an important role during both growth and differentiation of many nonmuscle cells.

The studies outlined above establish a system for future studies designed to expand our present knowledge of the relationship between cell configuration and growth control. We have recently demonstrated that cell surface contacts and cell shape give rise to distinctly different regulatory responses (4). In those studies, suspended cells were replated on dishes precoated with poly(HEMA) to control cell shape, and we demonstrated that protein synthesis requires only cell surface contact, whereas nuclear events are profoundly affected by cell shape. In future studies, we will analyze these different cell configuration regulatory mechanisms for specific gene products (actin and tubulin), using cDNA hybridization techniques. Furthermore, we hope that we can correlate specific cell configuration requirements for continued passage through the cell cycle.

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