

Translational Control of mRNA Expression during the Early Mitogenic Response in Swiss Mouse 3T3 Cells: Identification of Specific Proteins

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Abstract. Addition of serum or epidermal growth factor to quiescent Swiss mouse 3T3 cells in culture leads to a number of specific changes in the pattern of protein synthesis. Earlier experiments with actinomycin D suggested that the altered expression of these proteins was controlled at either the pretranslational or translational level. Here we have identified and further characterized the regulation of mRNA expression for ten of these proteins, including protein synthesis elongation factor eEF-1 α , poly A binding protein, vimentin, the multiple forms of the actin protein family, and α - and β -tubulin. Using an *in vitro* translation system, we determined the change in the level of mRNA en-

coding for each of these proteins after serum stimulation. The results showed that the amount of mRNA coding for eEF-1 α , poly A binding protein, vimentin, and α - and β -tubulin remains unchanged during this time, whereas that of the actin family increases. Thus, with the exception of the actin family, the results argue that the expression of all the proteins identified is regulated at the translational level. The importance of this latter group of proteins in cell growth and the abundance of their cognate mRNAs should prove them useful tools in elucidating the mechanisms involved in the activation of translationally repressed mRNA during the mitogenic response.

ACTIVATION of growth has been shown in numerous biological systems to be intimately associated with a large increase in the rate of protein synthesis (10, 39, 50, 52, 53). When quiescent animal cells in culture are stimulated to proliferate by serum they exhibit a two- to threefold increase in the rate of protein synthesis within 60 min (41, 43). This increase, which appears to be controlled at the level of initiation (37, 41, 43), is essential for the activation of cell growth. In part, this large and rapid response in the rate of protein synthesis is accounted for by the recruitment of both newly transcribed mRNA and a large pool of stored nonpolysomal mRNA into actively translating polyosomes (2, 38). Indeed, this pool of stored mRNA accounts for \sim 80% of the mRNA in polyosomes during the initial 6 h of the mitogenic response (38). Although the importance of stored mRNA in the activation of cell growth is clearly established, little is known regarding the mechanism(s) regulating its expression. Such studies would be greatly facilitated by the identification of specific proteins whose cognate mRNAs could be assigned to this population.

Earlier we used [35 S]methionine pulse-labeling of Swiss mouse 3T3 cells together with two-dimensional nonequilibrium pH gradient polyacrylamide gel electrophoresis (NEPHGE)¹ to ask whether the alterations in mRNA expression described above also lead to changes in the pattern of translation (45, 47). The results showed that for nearly all

of the 300–400 proteins detected by this method, the proportion of radioactivity incorporated into each protein remained constant after activation by serum (45). However, \sim 20 marked qualitative or quantitative changes were observed in the pattern of translation. Furthermore, experiments with actinomycin D suggested that at least half of these changes were controlled at the translational level, with the remaining half controlled at some unknown pretranslational step.

Here we have used a number of methods including both NEPHGE and two-dimensional isoelectric focusing (IEF), coelectrophoresis with known proteins, immunoblotting, and peptide mapping to identify 10 of these proteins, all of which play an important role in cell growth. In addition, to ascertain whether the earlier use of actinomycin D was a valid method for determining whether the changes in expression of individual proteins was under translational or pretranslational control, total mRNA from quiescent and serum-stimulated cells was isolated and translated in the rabbit reticulocyte lysate system. The results show that most of the identified proteins belong to the translationally controlled population. Furthermore, because these proteins can be assigned to the moderately abundant class of cellular mRNAs (300–1,000 copies per cell [13]) they should prove to be useful molecular and biochemical tools for studying how the expression of translationally controlled mRNA is regulated during the mitogenic response.

1. *Abbreviations used in this paper:* NEPHGE, non-equilibrium pH gradient polyacrylamide gel electrophoresis; PABP, poly A binding protein.

Materials and Methods

Cell Cultures

Swiss mouse 3T3 cells were cultured and maintained as previously described (23, 42). No mitoses were observed 7–8 d after seeding. At this time cells were judged to be quiescent.

Radioactive Labeling of Cells

Pulse-labeling was performed on cells grown in 35-mm tissue culture plates (Falcon Labware, Oxnard, CA) as previously described (45). [³⁵S]Methionine and [³H]isoleucine were purchased from New England Nuclear (Boston, MA).

Extraction of Cellular Proteins

All operations during the extraction were carried out at 0–2°C. At the times indicated in the text cells were lysed with detergents (42), the extracts were then made 1% in SDS (Serva Feinbiochemica GmbH & Co., Heidelberg, FRG), vortexed for 5 s, and sonicated for 15 s with a Sonifer B-12 (Branson Sonic Power Co., Danbury, CT) using a microprobe set at 40 W. Each extract was then incubated with 50 µg RNase A (Boehringer Mannheim GmbH, Mannheim, FRG) for 5 min at 37°C, frozen in liquid N₂, and stored at –70°C.

Two-Dimensional PAGE

NEPHGE gel electrophoresis was carried out as described earlier (45). Two-dimensional isoelectric pH 4–6 electrophoresis (IEF pH 4–6) was performed as described by O'Farrell and O'Farrell (32) except that the acetone-precipitated protein pellets were first resuspended in 20 µl of 1% SDS and boiled for 2 min. This solution was then mixed with an equal volume of 2× sample buffer (32) which was adjusted to 9.2 M urea. Nonidet P-40 (Shell, Richmond, CA) was then added to the sample to give an eightfold excess over SDS. Electrophoresis in the first dimension was carried out as

described by O'Farrell and O'Farrell (32), except that 400 µl of pH 4–6 and 100 µl of pH 2–11 Servalytes (Serva Feinbiochemica GmbH & Co.) were used. Separation in the second dimension was performed in an SDS slab polyacrylamide gel (45). After electrophoresis the gels were soaked for 1 h in EN³HANCE (New England Nuclear) containing 1% glycerol, washed twice for 15 min in a solution containing 20% methanol and 1% glycerol, dried, and analyzed by fluorography (45).

V8 Protease Peptide Mapping by One-dimensional PAGE

Proteins to be compared were excised from Coomassie Blue (R250)-stained two-dimensional polyacrylamide gels, and then analyzed according to Cleveland et al. (11). Each gel piece was equilibrated for 30 min at 22°C in 1 ml of a solution containing 0.125 M Tris-HCl pH 6.8, 0.1% (wt/vol) SDS, and 1 mM EDTA for 30 min at 22°C. After equilibration, the gel piece was placed at the bottom of a sample well of a one-dimensional SDS polyacrylamide slab gel containing 15% (wt/vol) acrylamide (Serva Feinbiochemica GmbH & Co.) and 0.4% (wt/vol) bis-acrylamide (Serva Feinbiochemica GmbH & Co.) (11). The gel pieces were then covered with 15 µl of the equilibrium buffer containing 20% (wt/vol) glycerol and 0.1% bromophenol blue (Serva Feinbiochemica GmbH & Co.) and overlaid with 15 µl of the same solution containing 10% glycerol and 0.1 µg per ml *S. aureus* V8 protease (Miles Laboratories Inc., Naperville, IL). Electrophoresis was carried out at 40 V until the bromophenol blue reached the stacking gel-separating gel interface, then the voltage was increased to 150 V for 4 h. The gels were analyzed by silver staining (8).

Protein Blotting

Electrophoretic transfer of protein from SDS polyacrylamide slab gels to nitrocellulose paper and subsequent staining of the immunoblot were performed according to the method of Towbin et al. (49), as modified by Nielsen et al. (31). The vimentin antiserum was a gift from Dr. R. Goldman, Northwestern Medical School, Chicago. The anti-tubulin antiserum was a gift from Dr. R. Franklin, Biozentrum, University of Basel. Peroxidase-coupled antiserum were purchased from DAKO (Copenhagen).

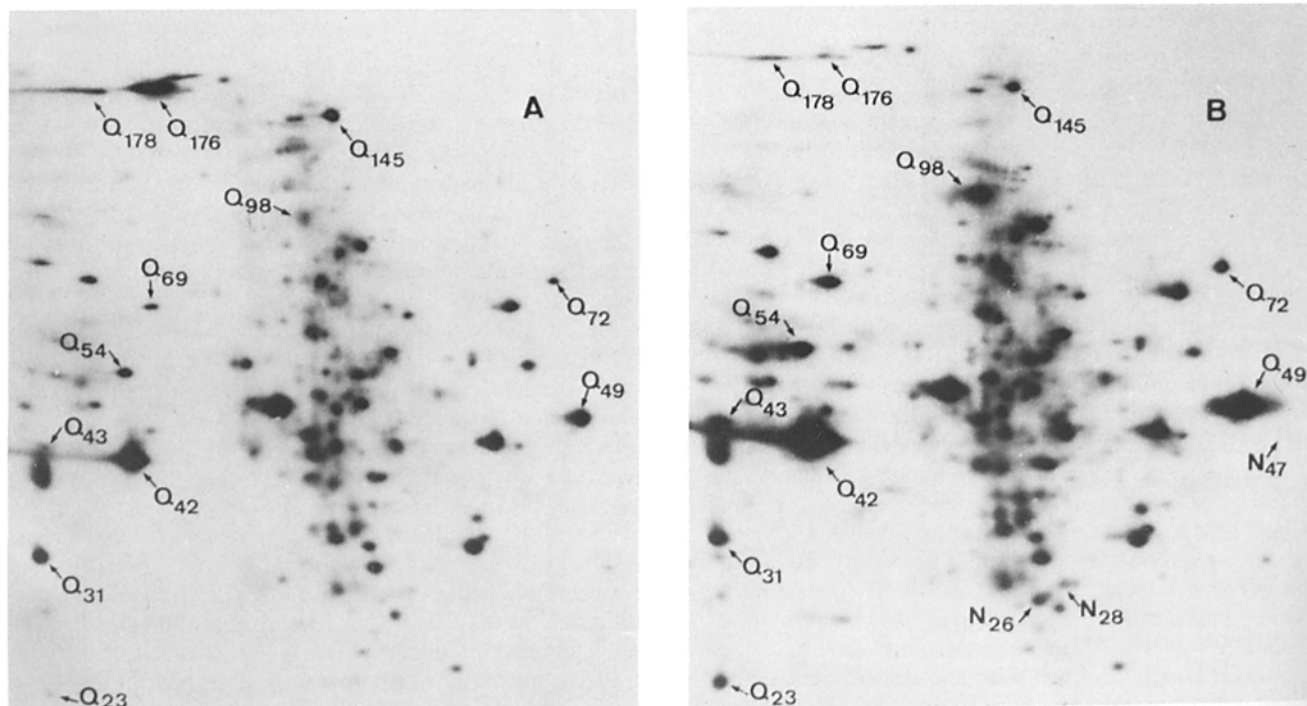


Figure 1. Fluorograms of two-dimensional NEPHGE gels depicting [³⁵S]methionine-labeled cytoplasmic proteins from (A) quiescent cells and (B) 60-min serum-stimulated cells. Parallel cultures of quiescent and 60-min serum-stimulated cultures were pulse-labeled with 400 µCi of [³⁵S]methionine. Quiescent cells were pulse-labeled for 1 h and 60-min serum-stimulated cells were pulse-labeled for a 20-min period between 50 and 70 min after the addition of serum. 1×10^6 cpm of protein, corresponding to equal amounts of total protein, were applied to each gel. Q, a protein detectable in quiescent cells; N, a protein not detectable in quiescent cells. Numerical subscript represents apparent molecular weight.

RNA Extraction

Cell lysates (45) were adjusted to 100 mM Tris-HCl pH 8.0, 100 mM NaCl, 10 mM EDTA, and 1% SDS and then extracted with phenol/chloroform/isoamylalcohol (24:24:1). Total RNA was precipitated with 2.5 vol of ethanol for 12 h at -20°C . The RNA pellets were then washed twice with 3 M sodium acetate followed by two washes with 70% ethanol precooled to -20°C . The dried RNA pellet was resuspended in 200 μl H_2O . About 50 μg of total cytoplasmic RNA was recovered from each 150-mm culture plate (6×10^6 cells).

In Vitro Translation

RNA was translated in a rabbit reticulocyte lysate as described by Pelham and Jackson (35).

Results

Protein Synthesis Elongation Factor-1 α and Poly A Binding Protein

60 min after the addition of serum to quiescent 3T3 cells the relative amount of [^{35}S]methionine incorporated into most cellular proteins remains constant when compared with qui-

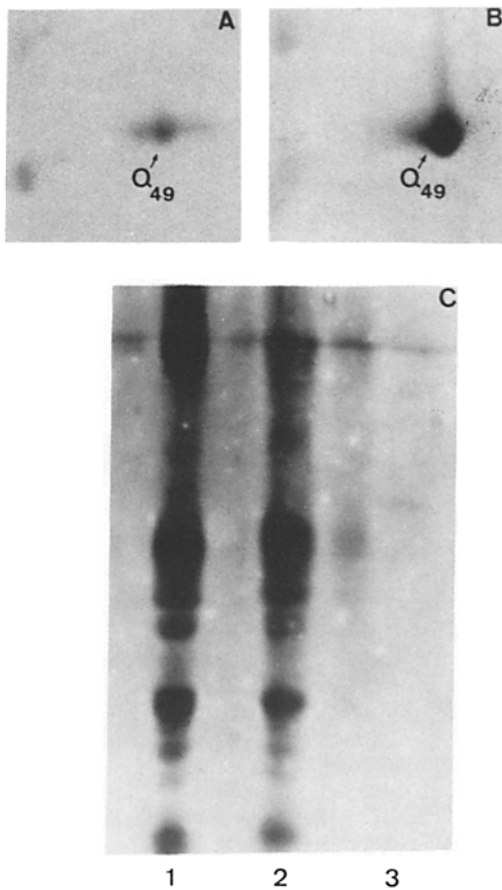


Figure 2. Identification of Q_{49} as eEF-1 α . Parallel 200 μg samples of cytoplasmic cell lysate were separated on NEPHGE two-dimensional polyacrylamide gels and stained with Coomassie Blue R250 (45) in the absence (A) and presence (B) of 2 μg of purified eEF-1 α (a gift from Dr. L. Slobin, University of Mississippi Medical School). In C, either Q_{49} (lane 1) which had been excised from the two-dimensional polyacrylamide gel, or 1 μg of eEF-1 α (lane 2) was digested with V8 protease (Materials and Methods), separated on a one-dimensional SDS polyacrylamide slab gel and analyzed by silver staining. Lane 3 contains the V8 protease alone.

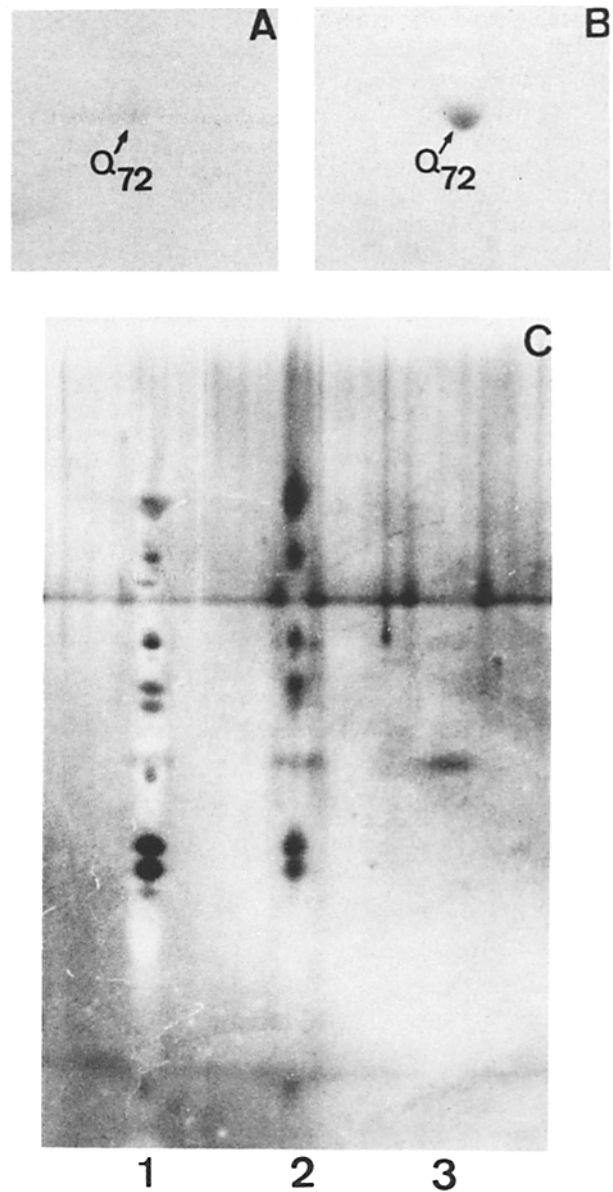


Figure 3. Identification of Q_{72} as the PABP. 200- μg samples of cytoplasmic cell lysate were separated on NEPHGE two-dimensional polyacrylamide gels and stained with Coomassie Blue R250 (45) in the absence (A) and presence (B) of 2 μg of purified PABP (a gift from Dr. A. Vincent, Institute de Recherches, Scientifiques Sur le Cancer, Villejuif). In C, either Q_{72} (lane 1), which had been excised from the two-dimensional polyacrylamide gel, or 1 μg of PABP (lane 2) were treated with V8 protease (Materials and Methods), separated on a one-dimensional SDS polyacrylamide slab gel, and analyzed by silver staining. Lane 3 contains V8 protease alone.

escent cells (Fig. 1 and references 45 and 47). However, there are a number of proteins in which the relative amount of incorporated [^{35}S]methionine changes dramatically (Fig. 1). Of these proteins, the only one previously identified was actin, Q_{42} (45). While carrying out in vitro translation studies it became evident that the most basic of these proteins, Q_{49} and Q_{72} , co-migrated on two-dimensional NEPHGE gels with two major proteins present in the rabbit reticulocyte lysate. Previously, it had been shown that a protein with similar physical properties to Q_{49} could substitute for pro-

tein synthesis elongation factor eEF-1 α in a cell free translation system (33) and that a protein with mobility on two-dimensional gels similar to Q₇₂ (51) specifically binds to the poly A tail of mRNA (4, 29, 51). To determine whether these proteins were identical to Q₄₉ and Q₇₂, a cell extract was resolved on two-dimensional NEPHGE gels either alone or in the presence of 2 μ g of eEF-1 α or 2 μ g of poly A binding protein (PABP). The results show that eEF-1 α and PABP migrate in the same position as Q₄₉ and Q₇₂, respectively (compare Figs. 2, *A* and *B* and 3, *A* and *B*). To further test this identity, the four proteins were subjected to one-dimensional V8 protease peptide analysis. The results show that the peptides generated from Q₄₉ and Q₇₂ are essentially identical to those of eEF-1 α and PABP, respectively (Figs. 2 *C* and 3 *C*). Thus based on co-migration on two-dimensional polyacrylamide gels and peptide maps, Q₄₉ is identical to eEF-1 α and Q₇₂ is identical to PABP.

Vimentin

Based on the observations of others (6), the migration of protein Q₅₄ in relation to Q₄₂ (actin) suggested that it could be equivalent to vimentin, the major intermediate filament protein of 3T3 cells. To examine this possibility, a portion of a 3T3 cell lysate, which had been labeled with [³⁵S]-methionine, was mixed with an excess of unlabeled 3T3 cell

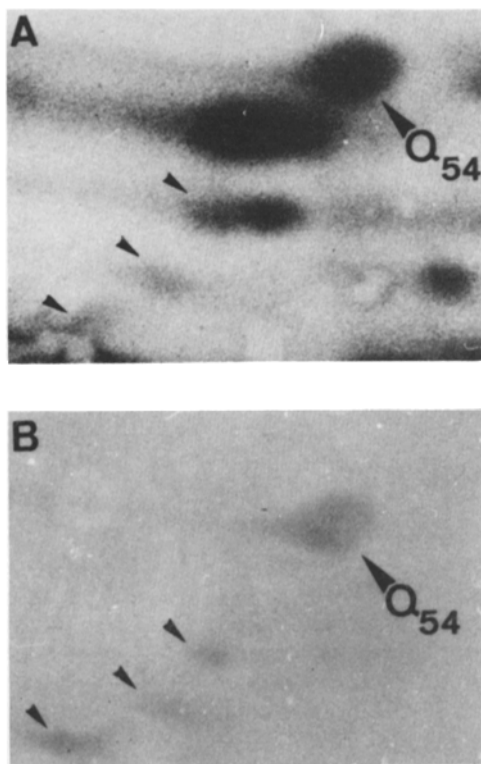


Figure 4. Identification of Q₅₄ as vimentin by Western immunoblotting. An aliquot containing 500 μ g of protein from a quiescent cell lysate was mixed with 1×10^6 cpm of [³⁵S]methionine-labeled cytoplasmic lysate, separated by NEPHGE two-dimensional PAGE, and transferred to nitrocellulose paper (Materials and Methods). The nitrocellulose blot was first subjected to autoradiography (*A*), then incubated with a rabbit anti-vimentin antiserum (a gift from Dr. R. Goldman, Northwestern University Medical School) and stained by the immunoperoxidase method (*B*). *Arrowheads*, proteolytic fragments of vimentin (see reference 30).

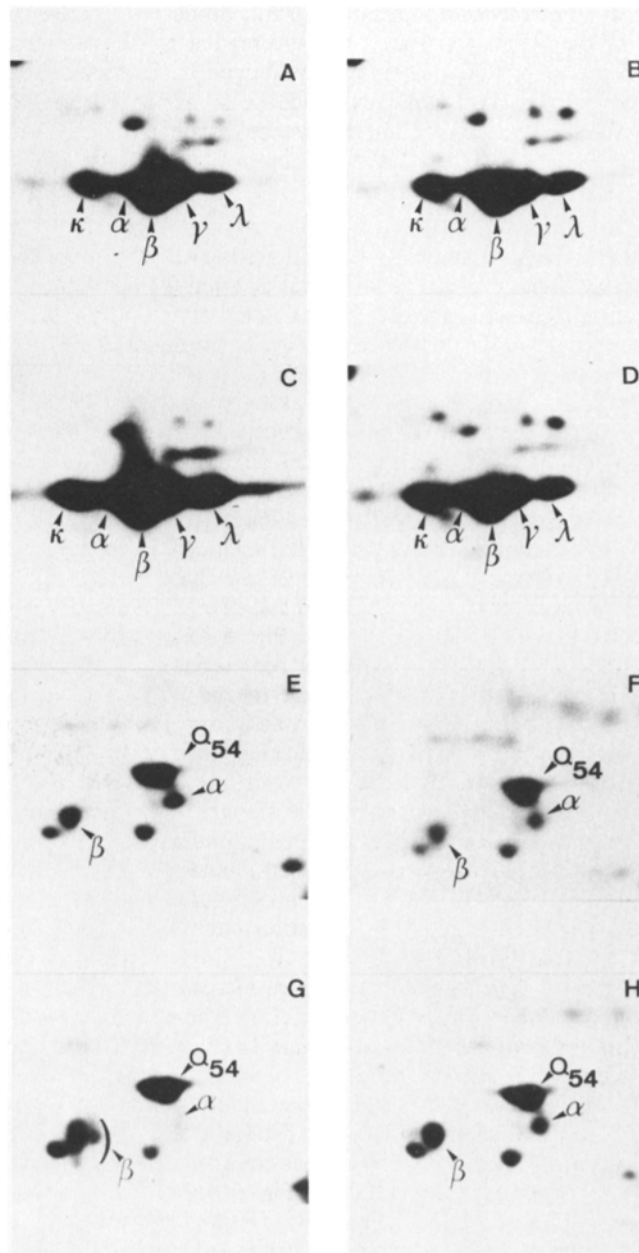


Figure 5. Fluorograms of two-dimensional IEF pH 4-6 polyacrylamide gels depicting either the actins (*A-D*) or the tubulins (*E-H*). Either parallel cultures of quiescent cells (*A, E, B, and F*) or 60-min serum-stimulated cells (*C, D, G, and H*) in the absence (*A, C, E, and G*) or presence (*B, D, F, and H*) of 2 μ g/ml actinomycin D were pulse-labeled with [³⁵S]methionine as described in Fig. 1. 1×10^6 cpm of [³⁵S]methionine-labeled extract from each sample was mixed with 1×10^6 cpm of [³H]isoleucine-labeled extract prepared from cells as previously described (45) and the proteins were analyzed by IEF pH 4-6 two-dimensional PAGE. The results show a blow-up of that area of the gel showing either the actins or tubulins.

lysate and the proteins were separated on two-dimensional NEPHGE gels. The proteins were then transferred to nitrocellulose paper, autoradiographed (Fig. 4 *A*), and localized with an anti-vimentin antibody (Fig. 4 *B*). The immunoblots show that the anti-vimentin antibody bound to protein Q₅₄, and three additional minor proteins that appear as a staircase (Fig. 4 *B*). These latter proteins most likely represent the

calcium-activated protease digestion products of native vimentin, which are thought to play a regulatory role in vimentin function (30). Therefore, as judged by its mobility on two-dimensional polyacrylamide gels, antibody staining, and the appearance of the documented staircase derivatives, protein Q₅₄ is equivalent to vimentin.

Actin and Tubulin

Like vimentin, actin and tubulin are the major components of two distinct cytoskeletal networks, microfilaments and microtubules, respectively (7). As noted above, it has previously been shown that Q₄₂ (Fig. 1) co-migrates on two-dimensional NEPHGE gels with actin (45). However, the different forms of actin, as well as the tubulins (which in Fig. 1 should migrate just to the acidic or left side of vimentin, Q₅₄) are not well resolved in this gel system (6). To distinguish the actins and tubulins we analyzed total ³⁵S-labeled cytoplasmic proteins by IEF pH 4–6 electrophoresis (Fig. 5). The fluorogram shows that in the area of the gel depicting the actin family, the actins migrate as a characteristic cluster comprised of α -, β - and γ -actin and two additional proteins that are labeled κ - and λ -actin (Fig. 5 A). The existence of such multiple forms of actin has been reported by others (17, 22). That all five proteins are of the actin family was confirmed by subjecting each protein to cyanogen bromide treatment and demonstrating that all five forms generated the same peptide pattern (data not shown). As can be seen in Fig. 1, there is a large increase in the amount of [³⁵S]methionine incorporated into actin after serum stimulation. The results show that the amount of [³⁵S]methionine incorporated into all five species increased when compared with quiescent cells (Fig. 5, A and C). Quantitation of these changes by double radioactive [³H]leucine and [³⁵S]methionine labeling (Table I) indicated that the largest increase was for κ -actin (3.1) and the smallest for α -actin (1.3). These increases in actin were inhibited by actinomycin D (Fig. 5 D), consistent with earlier studies (45).

In contrast to the actins, the tubulins are represented by two distinct proteins, α - and β -tubulin (Fig. 5 E). Identification of these two proteins was confirmed by showing that after transfer to nitrocellulose (Fig. 6 A) both react with an anti-tubulin antibody (Fig. 6 B). Unlike the actins only the amount of [³⁵S]methionine incorporated into β -tubulin increases (2.8-fold, Table II) after serum stimulation (Fig. 5, E and G). Indeed, no α -tubulin synthesis was detectable 60 min after the addition of serum (Fig. 5 G). The increase in

Table I. Changes in the Amount of [³⁵S]Methionine Incorporated into Specific Actin Species

Protein	Ratio of [³⁵ S]methionine to [³ H]isoleucine		
	Quiescent	Serum-stimulated	Change
α -Actin	1.0	1.2	1.3
β -Actin	1.3	2.3	1.8
γ -Actin	0.8	2.4	3.0
κ -Actin	1.1	3.4	3.1
λ -Actin	1.7	3.9	2.3

The actins were located on the IEF pH 4–6 two-dimensional polyacrylamide gel shown in Fig. 7 by fluorography. Each protein was then cut out of the dried gel, eluted, and the amount [³⁵S]methionine and [³H]isoleucine incorporated into each species was determined.

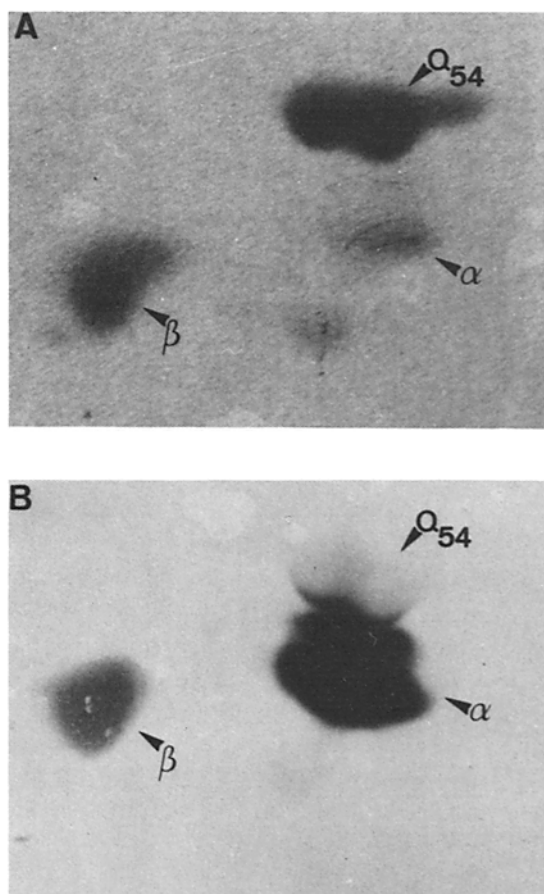


Figure 6. Identification of α - and β -tubulin by Western immunoblotting. An aliquot containing 500 μ g of protein from a quiescent cell lysate was mixed with 1×10^6 cpm of [³⁵S]methionine-labeled cytoplasmic lysate, separated by IEF pH 4–6 two-dimensional PAGE, and transferred to nitrocellulose paper (Materials and Methods). The nitrocellulose blot was first subjected to autoradiography (A), then incubated with a rabbit anti-tubulin antiserum (a gift from Dr. R. Franklin, Bizenrum, University of Basel), and stained by the immunoperoxidase method (B).

β -tubulin synthesis was marked by the appearance of a number of β -tubulin isoforms. That these isoforms were modified forms of β -tubulin was confirmed by antibody staining (data not shown). Though the increase in β -tubulin synthesis was not affected by actinomycin D, the appearance of the β -tubulin isoforms was blocked under these conditions (Fig. 5 H). Similarly, the loss of α -tubulin synthesis did not occur in the presence of actinomycin D (Fig. 5 H). Thus the increases in actin synthesis, the modifications of β -tubulin and

Table II. Changes in the Amount of [³⁵S]Methionine Incorporated into α - and β -Tubulin

Protein	Ratio of [³⁵ S]methionine to [³ H]isoleucine		
	Quiescent	Serum-stimulated	Change
α -Tubulin	0.5	ND	—
β -Tubulin	0.4	1.1	2.8

α - and β -Tubulin were located on gels as described in Table I and the amount of [³⁵S]methionine and [³H]isoleucine incorporated into each protein was determined. ND, not detectable.

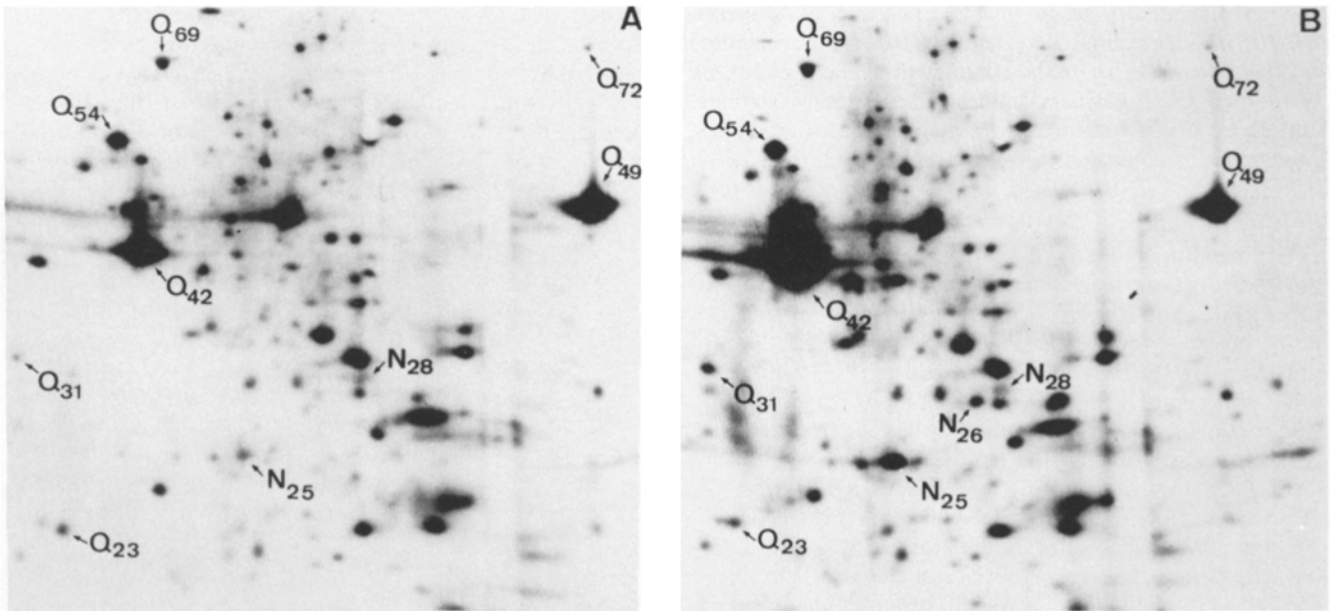


Figure 7. In vitro translation of total cellular mRNA. 30 ng of total mRNA from (A) quiescent and (B) 60-min serum-stimulated cultures (Materials and Methods) was translated in a reticulocyte lysate in the presence of [³⁵S]methionine (35). 1×10^6 cpm from each reaction mix was applied to a NEPHGE two-dimensional polyacrylamide gel and analyzed by fluorography. Protein spots are labeled as described in Fig. 1.

the disappearance of α -tubulin synthesis after serum stimulation all appear to be regulated at the pretranslational level, whereas the increase in β -tubulin synthesis appears to be regulated at the translational level.

In Vitro Translation of mRNA

Of the approximately 20 mitogen-induced changes in the pattern of translation seen in Fig. 1 half are unaffected by the presence of actinomycin D, whereas the others are completely inhibited by the drug. The former group includes eEF-1 α (Q₄₉), vimentin (Q₅₄), and β -tubulin (Fig. 5 E). The latter group includes the actin family (Fig. 5 B), α -tubulin (Fig. 5 H), the modified forms of β -tubulin (Fig. 5 H), and PABP (Q₇₂). The results suggest the synthesis of the first group is controlled at the translational level and the second group is regulated at some pretranslational step. To test these conclusions, an equivalent amount of mRNA from quiescent and 60-min serum-stimulated cells was translated in a nuclease-treated rabbit reticulocyte lysate (35). The assay was conducted under nonlimiting mRNA conditions such that unique mRNA molecules present in two distinct populations should be translated with the same efficiency, assuming they have the same primary structure (25). The results show that there is no measurable change in the amount of mRNA encoding for Q₄₉ (eEF-1 α), Q₅₄ (vimentin), and α - and β -tubulin (Figs. 7 and 8), as would be expected for translationally controlled mRNAs. Surprisingly, the amount of PABP also remains constant, arguing that the synthesis of this protein is also controlled at the translational level, rather than the pretranslational level as thought earlier. In contrast, the amount of translatable actin mRNA sharply increases after serum stimulation (Figs. 7 and 8). However, only the α -, β -, and γ -forms are detectable suggesting, as with the multiple isoforms of β -tubulin and similar to what has been reported in other cell lines for actin (22), that

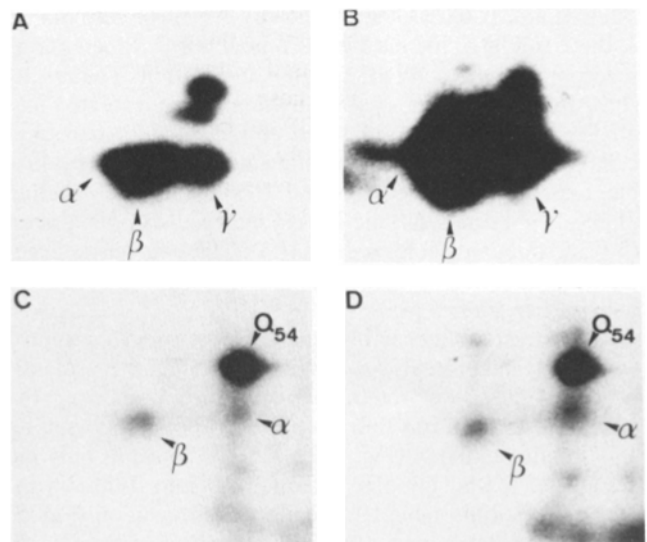


Figure 8. Analysis of in vitro translation products on IEF pH 4–6 two-dimensional polyacrylamide gels. Translations were carried out as described in Fig. 7. Blow-ups of that area of the fluorogram depicting either the actins from (A) quiescent cells and (B) 60-min serum-stimulated cells or the tubulins from (C) quiescent cells or (D) 60-min serum-stimulated cells are shown.

κ - and λ -actin are posttranslational modifications of one of the other forms of actin. Thus with the exception of α -tubulin and PABP, the conclusions drawn from the in vitro translation experiments are consistent with those taken from the actinomycin D data (45).

Discussion

The addition of either serum (Fig. 1 and reference 45) or epidermal growth factor (47) to quiescent cells in culture in-

duces a number of changes in the amount of [³⁵S]methionine incorporated into specific proteins. It was shown earlier (45) that if cells were preincubated with actinomycin D, an inhibitor of de novo mRNA synthesis, the increases observed in proteins N₂₆, Q₃₁, Q₄₂, N₄₇, and Q₇₂ were blocked (Fig. 1). Of this group of proteins we have identified Q₄₂ as representing the actin family, comprising five major actin species, and Q₇₂ as PABP. Because actinomycin D blocked the increased expression of actin and PABP, as well as N₂₆, Q₃₁ and N₄₇, it was argued that their increased synthesis *in vivo* was due to a rise in the cytoplasmic levels of their cognate mRNAs. Except for PABP (see below), these results are consistent with the fact that the amount of mRNA, as measured by *in vitro* translation for all the proteins listed above, increases after serum stimulation (Figs. 7 and 8). These higher mRNA levels could be explained by increases in either (a) transcription rates, (b) transport of heteronuclear mRNA into the cytoplasm, (c) the half-life of mRNA, or (d) activation of translationally inactive mRNA (for a review, see reference 12). Based on previous studies in 3T6 cells the most likely possibility would be increased transport from the nucleus to the cytoplasm (1, 28). In preliminary experiments with a β -actin cDNA probe we have found that the amount of hybridizable actin mRNA in the cytoplasm increases sharply after serum stimulation, as has been reported by a number of other laboratories (3, 9, 18). Cell fractionation studies in combination with this cDNA probe should distinguish between these alternative regulatory pathways.

The only protein whose increased synthesis *in vivo* was inhibited by actinomycin D and whose level of mRNA did not increase was Q₇₂, or PABP. PABP has been shown to be exclusively associated with the poly A tracks of mRNA (4). The role of poly A in mRNA expression has not been established, even though a number of experiments suggest it may effect the transport of mRNA from the nucleus to the cytoplasm (24), half-life of mRNA (27), and its translational efficiency (34). In pulse-labeling experiments it was found that newly synthesized PABP associates with the poly A tracks of newly transported mRNA (40). Treatment of cell cultures with cordycepin (3-deoxyadenosine), which prevents poly A adenylation and transport of mRNA, also blocked the synthesis of PABP in the cytoplasm (40). Actinomycin D is also known to affect transport of mRNA (14). Thus, the apparent anomaly may be explained by the fact that inhibition of general mRNA appearance in the cytoplasm by actinomycin D leads to the activation of some control mechanism that blocks the expression of PABP in the cytoplasm. Possibly, the accumulated excess PABP in the cytoplasm binds to its own mRNA and prevents its expression, as has been described for other mRNAs (16, 20, 26).

In contrast to the proteins described above the increased incorporation of [³⁵S]methionine into proteins Q₂₃, N₂₈, Q₄₃, Q₄₉ (eEF-1 α), Q₅₄ (vimentin), and Q₆₉ was not inhibited by actinomycin D (45). The results of *in vitro* translation of mRNA from quiescent and stimulated 3T3 cells shows that the level of these mRNAs remains constant after serum stimulation (Fig. 7), as would be expected for translationally controlled mRNA. In the case of eEF-1 α , we have recently used an oligonucleotide probe (a gift from L. Slobin, University of Mississippi), complementary to the first 57 coding bases of murine eEF-1 α , to show that the amount of this message remains constant after serum stimulation (data not

shown). The finding that the level of these mRNAs remains constant is also consistent with previous results of others showing that there is a large pool of stored mRNA in quiescent cells which shifts into polysomes after serum stimulation (2, 38). Such an increase at the translational level could also be explained by an increase in the mean number of polysomes attached to a single mRNA species. Preliminary results suggest this may in part explain the large increase in eEF-1 α synthesis after serum stimulation.

Like the proteins above, it was also found that both α - and β -tubulin were under translational control. Interestingly in the case of α -tubulin the control appears to be negative, since no synthesis of α -tubulin can be detected at 150 min postinduction even though the mRNA is present in the cell. cDNA probes to α -tubulin have given similar results after fertilization in Spisalaocytes. Rosenthal et al. (36) reported that in this system the α -tubulin message moves from the polysome to the nonpolysome fraction after fertilization and that this change temporally correlates with depolyadenylation of the message. Whether a similar correlation exists in 3T3 cells has not yet been examined.

The proteins identified here are ubiquitous components of all cell types and are involved in either translation (eEF-1 α), mRNA processing (PABP), or structural organization of the cell (actins, tubulin, and vimentin). Because these proteins are involved in central cellular functions it is not unexpected that their levels of synthesis are dramatically altered when quiescent cells are induced to enter the proliferative state. However it is unusual that the expression of their cognate mRNAs, with the exception of the actin family, are all under translational control. Regulation of gene expression at this level has always been an attractive possibility, even though few specific examples exist (21). In part this seemingly overabundant representation is explained by the fact that in quiescent cells there is a large pool of stored mRNA that shifts into polysomes after mitogenic stimulation (2, 38). It should also be noted that this shift is temporally paralleled by the multiple phosphorylation of a 40S ribosomal protein, S6 (44, 46). Those ribosomes containing the most highly phosphorylated derivatives of S6 have a selective advantage in entering polysomes (15, 46) and because S6 has been localized in the mRNA binding site of the ribosome (5, 48), it has led to the proposal that the phosphorylation of S6 may facilitate the initiation process by altering the affinity of the ribosome for mRNA. Since all the proteins identified above are readily visible on two-dimensional polyacrylamide gels they fall into the moderately prevalent class of cellular mRNA (19, 54). The abundance of these mRNAs, upwards of a thousand copies per cell, should make them useful models for testing the importance of proposed translational control mechanisms, such as S6 phosphorylation, in the expression of specific mRNAs during the mitogenic response.

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