

Serum-induced Signal Transduction Determines the Peripheral Location of β -Actin mRNA within the Cell

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Abstract. Cell motility is dependent upon the reorganization of the cellular cytoskeleton. Actin filaments form the major component of the cytoskeleton and respond rapidly to serum growth factors. We have previously shown that myoblasts sort the two cytoskeletal β - and γ -actin isoform mRNAs to different intracellular regions and that only β -actin mRNA was associated with peripheral regions of cell motility (Hill, M. A. and P. Gunning. 1993. *J. Cell Biol.* 122: 825–832). We now show by in situ hybridization that 3T3 fibroblasts similarly sort actin isoform mRNAs and that peripheral β -actin mRNA is regulated by serum. In the absence of serum, we could not detect β -actin mRNA at the periphery. Addition of serum rapidly redistributed β -actin mRNA to the pe-

riphery. γ -actin mRNA distribution was not altered by serum addition at any time. Both proteins, as identified by immunocytochemistry with isoform-specific antibodies, were found in similar cellular structures. Serum-stimulated cell motility is mediated through the GTPase signal transduction pathway. We find that an RNA-binding protein, p62, that is part of this pathway, displays a localization pattern similar to β -actin mRNA. Our results suggest a new biological mechanism which integrates signal transduction with the supply of an architectural component required for membrane remodeling. We propose that active transport of β -actin mRNA to regions of cell motility is one possible objective of these signal transduction pathways.

LOCALIZATION of specific mRNAs to sites within cells for localized protein synthesis/function is emerging as a significant biological mechanism (for review see Singer, 1993; Wilhelm and Vale, 1993). In the embryo, localization of maternal mRNAs by their 3' untranslated region (3'UTR)¹ (Kim-Ha et al., 1993; Macdonald et al., 1993) involves the cytoskeleton (Yisraeli et al., 1990) and allows topographical protein synthesis for correct development (Nusslein-Volhard et al., 1987; Gavis and Lehmann, 1992). In mature cells, mRNA can also be localized to different intracellular regions by their 3'UTR (Kislauskis et al., 1993) using the cytoskeleton (Hesketh and Pryme, 1991; Sundell and Singer, 1991), though the functional significance of this localization is less clear.

Singer and co-workers (Lawrence and Singer, 1986; Sundell and Singer, 1991) have shown that β -actin mRNA is located at peripheral sites in the cell which are involved with motility. Hooek et al. (1991) also showed that β -actin mRNA

is recruited to the periphery of wounded endothelial cells and that this correlates with an increase in β -actin protein at these motile regions. We (Hill and Gunning, 1993) and others (Kislauskis et al., 1993) have recently shown that the peripheral location of β -actin mRNA is specific for the β isoform of actin. This peripheral actin mRNA pool may also influence the impact of transfected actin genes on myoblast morphology. We have found that transfections that increase the β -actin mRNA pool promote cell spreading whereas those which decrease the pool result in restricted spreading (Lloyd et al., 1992; Schevzov et al., 1992). This has led us to propose that translation of the peripheral β -actin mRNA pool may contribute newly synthesized actin for membrane remodeling and cell spreading (Hill and Gunning, 1993).

Recent studies have shown that cell motility in Swiss 3T3 fibroblasts can be stimulated by growth factors which regulate the actin cytoskeleton (Ridley and Hall, 1992; Ridley et al., 1992). This pathway is mediated by two of the small GTP-binding proteins: rac induces membrane ruffles and rho induces stress fiber formation (Ridley and Hall, 1992). Withdrawal of serum for 16 h leads to a loss of both stress fibers and cell motility (Ridley and Hall, 1992). If localization of β -actin mRNA is involved in membrane remodeling and motility, then this may be a target for growth factor regulation. We have tested this in the 3T3 fibroblast system and demonstrate that indeed, peripheral location of β -actin

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1. *Abbreviations used in this paper:* 3'UTR, 3' untranslated region; CLSM, laser scanning confocal microscope; GAP, GTPase-activating protein; LPA, lyophosphatidic acid.

mRNA is under growth factor regulation and correlates with changes in cell motility.

Materials and Methods

Cell Culture and Protein Analysis

The intracellular distribution of γ - and β -actin mRNA and protein was analyzed in Swiss 3T3 fibroblasts. Cells were grown on collagen-coated (calfskin collagen; Calbiochem, Sydney, Australia) glass multiwell slides (Nunc Inc., Naperville, IL) in MEM plus 10% FCS (Cytosystems, Sydney, Australia). Serum-starved cells were washed and grown for at least 16 h in MEM alone. For serum readdition, MEM plus 10% FCS was added and cells fixed at appropriate time points. Specific growth factors were added to final concentrations as previously described for biological activity (Ridley and Hall, 1992); 200 ng/ml lyophosphatidic acid (LPA) and 10 ng/ml PDGF (both Sigma-Aldrich, Sydney, Australia). Cells were fixed in 4% paraformaldehyde then in cold methanol for 20 min.

For protein studies cells were fixed and incubated for 1 h at 37°C with primary antibody, washed three times with PBS, and identified with a fluorescently conjugated secondary antibody (Jackson ImmunoResearch Lab. Inc., West Grove, PA). Confocal microscope analysis on double-labeled cells was carried out as described for in situ analysis. The β -actin monoclonal antibody was kindly provided by Dr. Langbeheim (Biomakor, Rehovot, Israel; now available through Sigma Chem. Co., St. Louis, MO; #A5441/#A5316). The γ -actin rabbit antisera was provided by Dr. J. C. Bulinski (Columbia University, New York) and has been previously characterized (Otey et al., 1986). The GTPase-activating protein (GAP)-associated p62 antisera was provided by Santa Cruz Biotechnology Inc. (Santa Cruz, CA).

RNA Probes and In situ Hybridization

Probes were as previously described (Hill and Gunning, 1993). Briefly, isoform-specific 3'UTR probes were derived from the mouse genes with complementary strands used as the controls. The β -actin probe was a 198-bp fragment from 1468–1665. The γ -actin probe was a 264-bp fragment from 2228–2494. (These data are available from EMBL/Genbank/DBJ under accession numbers X03672 and X13055, respectively.) Fragments were cloned into pGEM-3Z (Promega Corp., Madison, WI) and orientation determined by Northern and slot blot analysis (data not shown). Plasmids were linearized before RNA synthesis. RNA was labeled by incorporating either digoxigenin-UTP or fluorescein-conjugated UTP using appropriate labeling kits (Boehringer Mannheim, Sydney, Australia). RNA was quantified by comparison with known standards on slot blot analysis.

Hybridization procedures were as previously described (Hill and Gunning, 1993). All chemicals were of RNA grade, solutions were diethylpyrocarbonate treated and/or autoclaved, and sterile plasticware, glassware, and instruments were baked overnight at 200°C. Prehybridization was in 500 μ l of hybridization solution without probe, 45°C for 1 h. The solution was removed and probe or control RNA added in 100 μ l, then hybridized at 45°C overnight. Slides were washed with 2 \times SSC at 45°C and then 1 \times SSC/0.1% SDS at 65°C. High stringency wash conditions were determined by comparison with controls, usually several washes of 0.1 \times SSC at 65°C. Slides were then equilibrated in PBS. Conjugated antibodies (Boehringer Mannheim) were diluted as directed by manufacturer, reacted with blocked slides (10% inactivated FCS in PBS) for 1–2 h at 37°C, and then washed in PBS at pH 7.5 or 8 to reduce nonspecific binding.

Northern Blot Quantitation of β -Actin mRNA

Northern blots and analyses were exactly as described by Lloyd et al. (1992). β -actin mRNA levels (corrected for loading by densitometric comparison of 18S ribosomal RNAs) were compared using the level from cells growing in serum as 100%.

Laser Scanning Confocal Microscope (CLSM) Analysis

A CLSM (Wild Leitz Instruments, Heidelberg, Germany) with a 40 \times objective (1.3NA) allowing a minimum slice thickness of 0.5 μ m was used to analyze label distribution within cells. Cells with a fully spread morphology, previously determined by time-lapse video analysis (data not shown), were analyzed for β - and γ -actin mRNA distribution. Cells were optically

sectioned in the xy and xz planes (parallel and perpendicular to substratum) with multiple scan averaging and all images stored on optical disc. Extended focus is an algorithm which compiles individual sections of the whole cell (Wilson and Carlini, 1988). In double labeling experiments identical confocal slices were taken for both probes allowing direct comparison of both mRNAs within each cell.

Labeling intensity was initially analyzed directly on the CLSM with supplied software. Analysis of intracellular labeling was carried out on images transferred to Macintosh computers and examined with NIH Image 1.43. Quantitation of labeling was as shown in Fig. 2. Lowest optical CLSM slice of cells (in contact with substratum) were collected (as in Fig. 1) under identical conditions. Fluorescent intensity was then measured in three separate 4- μ m squares in each region (nuclear, perinuclear, cytoplasmic, and peripheral) representing areas of highest label intensity as well as in regions outside the cell (background). All cell averages were then pooled.

All cell treatments in this current study were also analyzed by time-lapse video (Leitz inverted microscope; Minitub heated stage [Tiefenbach, Germany]; Sony DXC101P camera; Panasonic AG6720 Time-Lapse Video).

Results

Effect of Serum on β -Actin mRNA Localization

The serum starvation of Swiss 3T3 fibroblasts has been shown to dramatically decrease cell motility and alter the actin cytoskeleton (Ridley and Hall, 1992). Serum starved Swiss 3T3 fibroblasts do not have peripherally located β -actin mRNA but do have this message concentrated around the nucleus (perinuclear) (Fig. 1 *a*). The overall size of cells was not altered by this treatment, but motility (evaluated by time-lapse video microscopy) was decreased (data not shown).

Cells growing in serum continuously (Fig. 1 *b*) had two separate pools of β -actin mRNA: peripheral and perinuclear. The perinuclear pool was approximately the same as that seen for serum-starved cells (Fig. 1 *a*). The peripheral pool varied from cell to cell; with both even and concentrated pockets of peripheral labeling seen (Fig. 1 *b*, arrows). This variable distribution may reflect differences occurring between individual cells in terms of cytoskeletal reorganization or motility. Intranuclear hotspots (Fig. 1 *b*, nucleus yellow speckles) were also seen in these cells, but absent in the serum-starved cells. Interestingly, specific sites of poly(A)⁺ RNA processing in the nucleus have a similar hotspot pattern of labeling (Carter et al., 1991, 1993). Our decrease in nuclear staining following serum starvation could represent a decrease in transcription/transcript accumulation/processing, as β -actin is upregulated in the presence of serum (Thomas and Thomas, 1986; Lamal and Ziff, 1990).

Readdition of serum to starved cells shifts β -actin mRNA away from the perinuclear region toward the periphery (Fig. 1, *c* and *d*). After 2 min of serum addition some cells have identifiable peripheral labeling (Fig. 1 *c*, arrows) and after 10 min (Fig. 1 *d*) have labeling indistinguishable from cells growing in serum (Fig. 1 *b*). Lower serum concentrations gave identical redistributions (data not shown), 10% serum add-back was used to allow direct comparison with cells growing in serum.

Quantitation of Localized Intracellular β -Actin mRNA

An explanation of how quantitation was carried out is shown in Fig. 2 and allowed the direct comparison of different intracellular regions as well as treatments. Analysis was performed on a single 1- μ m slice through the bottom of the cell so that intensity differences were due to concentration differences in mRNA and not due to differences in cell thickness.

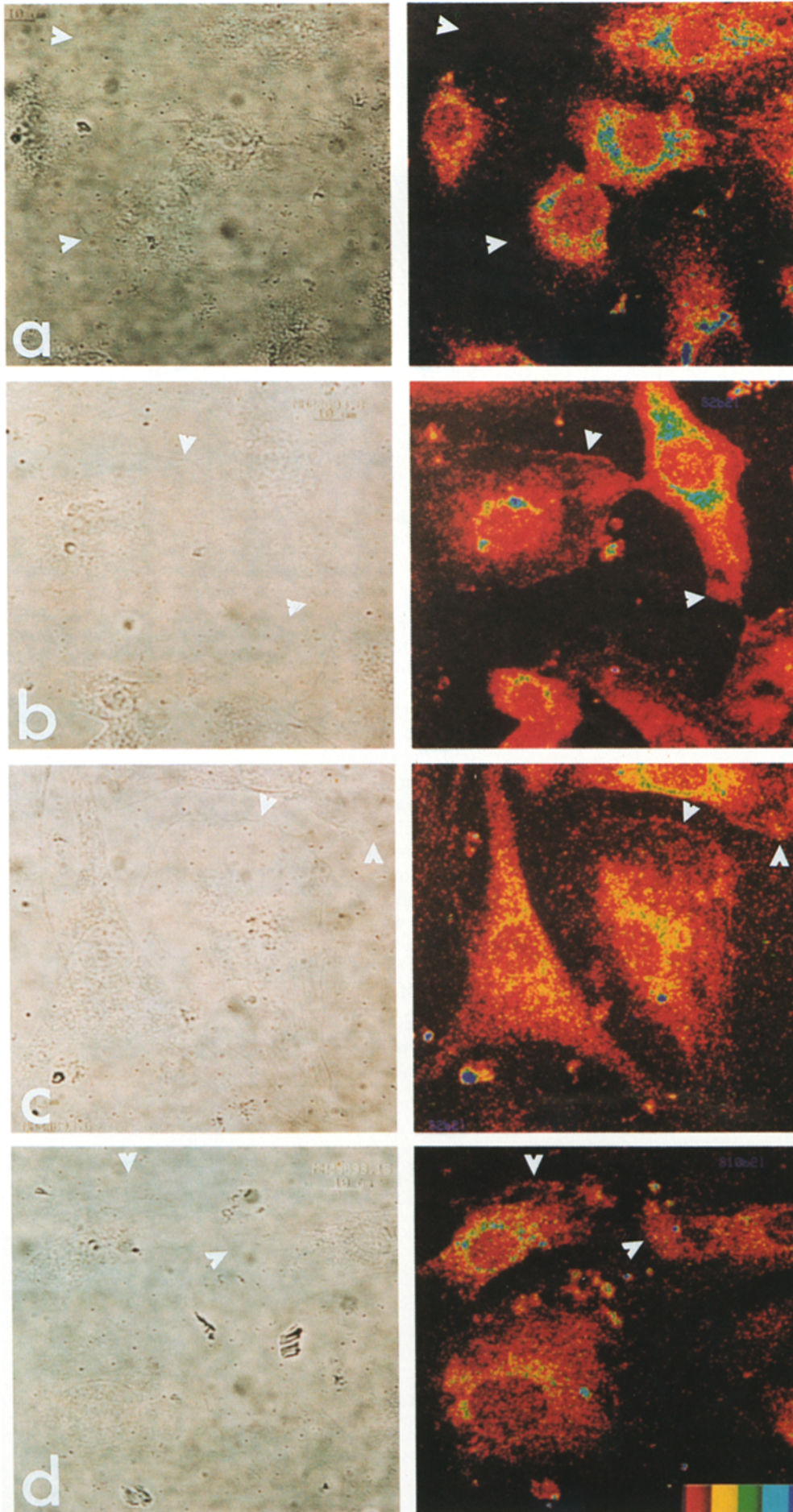


Figure 1. β -actin mRNA localization in Swiss 3T3 fibroblasts is regulated by presence or absence of serum. Cells growing in serum-free conditions (a) have no detectable β -actin mRNA at their periphery (arrows). The concentration of β -actin mRNA is highest (blue areas) directly around the nucleus. Cells growing in serum (b) locate β -actin mRNA both around the nucleus and at the cell periphery (arrows). In fully spread cells (cell center left) the perinuclear concentration decreases with distance into the cytoplasm and then increases at the periphery. After addition of serum to serum-starved cells for 2 (c) and 10 min (d) mRNA redistributes toward the cell periphery (arrows). This distribution was also found at 15-, 30-, and 60-min serum readdition time-points (data not shown). Bright field (left column) and in situ hybridization (right column) are matched for a-d. Color bar in (d) shows increasing label intensity from red to blue and is 40 μ m in length.

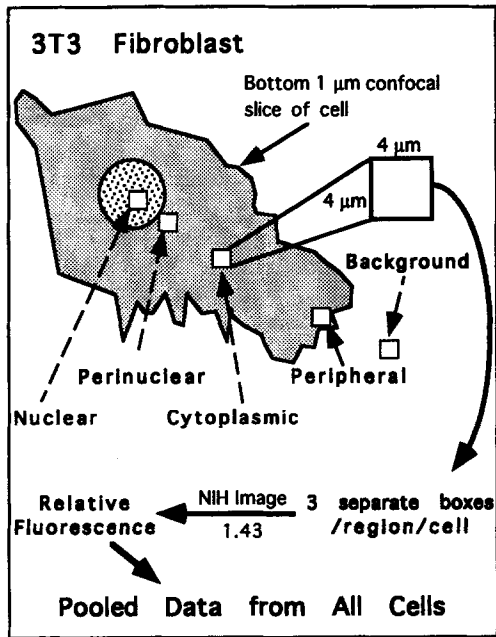


Figure 2. Sample method of quantifying mRNA within specific intracellular regions. Lowest optical 1- μm CLSM slice of cells (in contact with substratum) was collected for each cell under identical scanning conditions. The image was then transferred to a Macintosh computer and using NIH Image 1.43 a 4- μm square was placed in the three brightest areas of each region (nuclear, perinuclear, cytoplasmic, and peripheral). In addition a region outside the cell (background) was also measured. The fluorescent intensity values for all cells were pooled and averaged. The results of this analysis are shown in Fig. 3.

Quantitation of β mRNA localization (Fig. 3) in all cells analyzed supported the above qualitative finding both regionally and in terms of serum supplementation.

Cells serum starved for 16 h had high perinuclear labeling, and decreased both cytoplasmic and peripheral labeling (Fig. 3, *filled circles*). The level of labeling at the periphery in these cells approached that of nonspecific background. Cells growing in serum (Fig. 3, *open circles*) had lower perinuclear, similar cytoplasmic, but substantially higher peripheral labeling compared to serum-starved cells (Fig. 3, *filled circles*). The decrease in perinuclear labeling may indicate that it is this pool that is drawn on for peripheral β mRNA localization.

After serum addition for 2 min (Fig. 3, *open triangles*) cells shifted the perinuclear pool towards the cytoplasm and periphery. At this time point the perinuclear level dropped marginally and cytoplasmic labeling doubled to even higher levels than that found for cells growing in serum. Labeling at the periphery was also doubled but still intermediate between serum supplemented and starvation values.

After serum addition for 10 min (Fig. 3, *filled triangles*) cells had both high perinuclear and peripheral pools. Perinuclear labeling equaled that found in serum-starved cells, cytoplasmic labeling equaled the 2 min time point, and peripheral labeling equaled that of cells growing in serum. The peripheral pool is therefore reestablished within this period, at specific sites, to levels found in serum supplemented cells, and was maintained at 30 and 60 min of se-

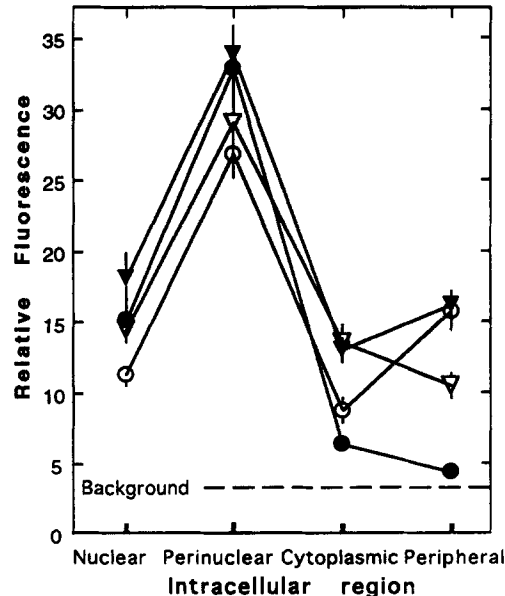


Figure 3. Quantitative analysis of intracellular distribution of β -actin mRNA in Swiss 3T3 fibroblasts in presence and absence of serum. The β -actin mRNA distribution in discrete intracellular regions in the bottom 1 μm of cells was quantified as described in Fig. 2. The nuclear, perinuclear, cytoplasmic, and peripheral regions (\pm SE) was scored for the total number of cells studied by CLSM: +serum (*open circles*) 28 cells, serum free (*closed circles*) 51 cells, +serum 2 min (*open triangles*) 44 cells, and +serum 10 min (*closed triangles*) 37 cells. The background level (*hatched line*) was established from areas outside the cell.

rum addition (data not shown). The rapid reestablishment of the peripheral pool suggested that synthesis of new protein was not required for this process (Sundell and Singer, 1990). Our own experiments with protein synthesis inhibitors, puromycin and cyclohexamide, also support this finding (data not shown). This suggested to us that β -actin mRNA localization was closely linked to the actual signal transduction pathway/timecourse.

Signaling Mechanisms for Peripheral β -Actin mRNA

There are several characterized intracellular signaling pathways that have been described for serum-derived growth factors. We have initially looked at only the two factors, LPA and PDGF, that have been described to alter the actin

Table 1. Changes in Peripheral β -Actin mRNA Level in Serum-starved Swiss 3T3 Cells Induced by Growth Factors

Addition	Peripheral β -actin mRNA level	
	5 Min	30 Min
	%	
LPA (200 ng/ml)	108	59
PDGF (10 ng/ml)	48	105

A total of 84 cells were analyzed from three separate experiments for their increase in peripherally located β -actin mRNA. Quantitation was as described in Fig. 2. To compare values from all three experiments we calculated with reference to serum-starved cells as 0% and cells grown in the presence of serum as 100% for each experiment, numbers are the mean percentage.

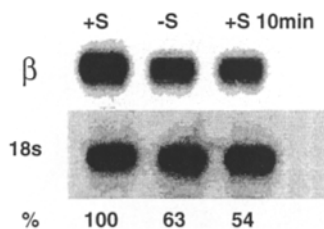


Figure 4. Northern blot analysis of β -actin mRNA in Swiss 3T3 fibroblasts. Cells growing in serum (+S) have the highest expression of β -actin mRNA, serum starvation (-S) decreased β -actin mRNA levels. After 10 min of serum readdition (+S 10 min) β -actin mRNA levels had slightly decreased still more. The bottom panels show the 18S ribosomal RNA autoradiogram used to correct for loading differences and values beneath this are the corrected quantified percentages relative to cells grown in serum.

cytoskeleton within the times described in this current study (Ridley and Hall, 1992; Ridley et al., 1992). Both LPA and PDGF increased peripheral β -actin mRNA levels/localization in serum-starved cells to levels found in serum sup-

plemented cells (Table I). Interestingly, each factor appeared to have different timecourses: LPA was initially maximal and decreased with time, while PDGF initially increased but was maximal at a longer time of stimulation (Table I). These timecourses matched those seen with these same two factors for effects on the actin cytoskeleton (see Table 1 in Ridley and Hall, 1992). Though it is still not clear whether our serum readdition result (of peripheral β -actin mRNA) reflects a single or a combination of factors acting on the cell, it does suggest that multiple mechanisms/pathways can result in the same redistribution.

Quantitation of Intracellular β - and γ -Actin mRNA

Northern blot analysis showed that the total level of β -actin mRNA falls with serum starvation to 63% of control (Fig. 4). After 10 min of serum addition the β -actin mRNA was slightly lower again at 54% of serum value, even though the β -actin gene responds rapidly to serum addition (Thomas

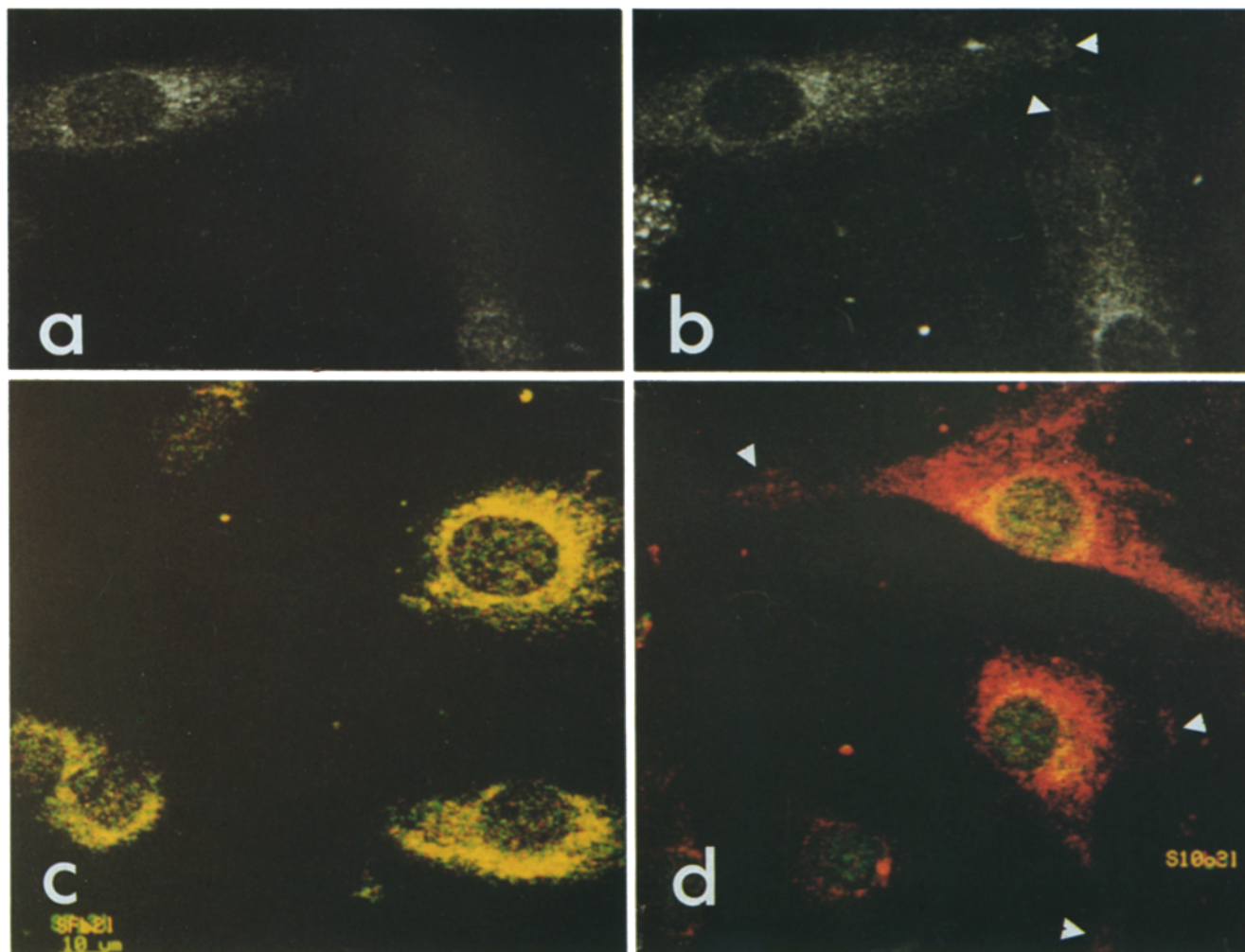


Figure 5. Swiss 3T3 fibroblasts show a perinuclear distribution of γ -actin mRNA which is not changed by serum addition to cells growing without serum. Cells growing in serum (a and b) have perinuclear located γ -actin mRNA (a) and both perinuclear and peripherally located β -actin mRNA (b, arrows). Serum-starved 3T3 fibroblasts (c) locate both mRNAs only in the perinuclear region (double label, yellow). Readdition of serum for 10 min (d) did not alter γ -actin mRNA distribution (green) but β -actin mRNA (red) is redistributed to the periphery of the cells (arrows). Confocal slices of cells from double in situ hybridization for both β - and γ -actin mRNA (a-d). In (a) β and (b) γ images of the same cells have been separated. In (c and d) the β -actin mRNA is shown in red, the γ -actin mRNA is shown in green and colocalization is shown in yellow.

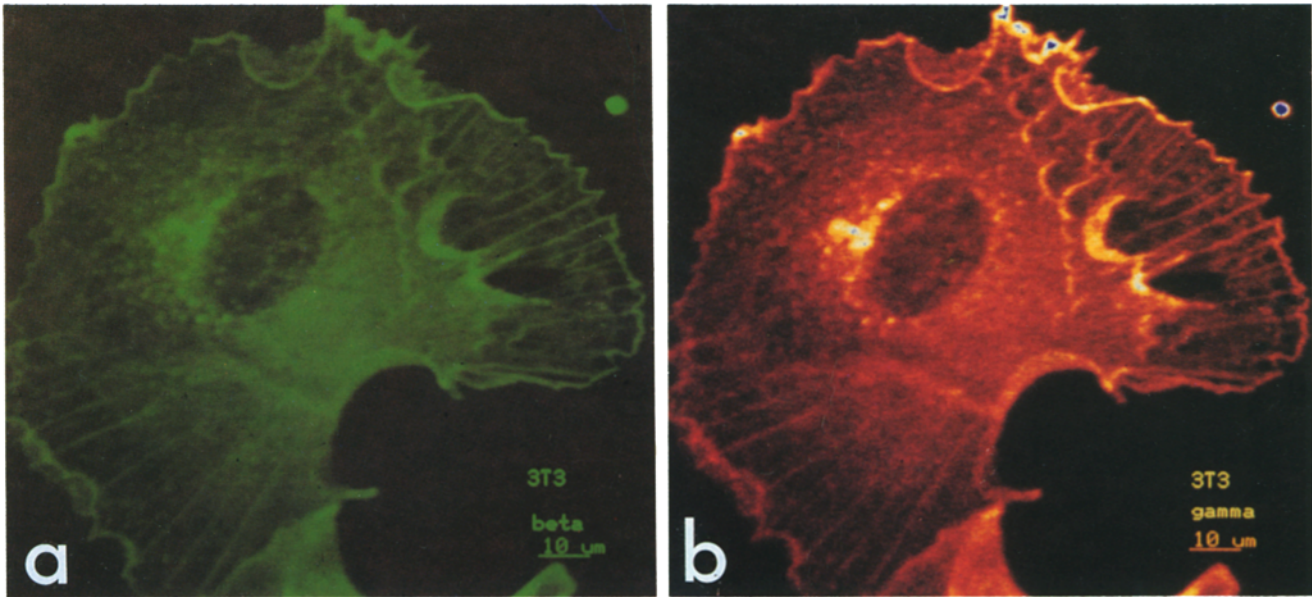


Figure 6. Both actin protein isoforms show overall similar intracellular distributions. Labeling of β - (a) and γ -actin (b) in 3T3 fibroblast after readdition of serum for 10 min. CLSM extended focus visualization show similar staining patterns near the nucleus, in stress fibers, in punctate patches, and at the periphery of the cell. The dynamic redistribution of β -actin mRNA did not result in new β -actin specific filament bundles within the existing structures at this or later timepoints. Subtle differences in protein concentration may be masked in the overall protein distribution shown by these antibodies. The antibodies identify both filamentous and monomeric actin.

and Thomas, 1986; Lamal and Ziff, 1990). Our data suggests that there is no significant increase in the total β -actin mRNA pool during the time of mRNA redistribution. This in turn suggests that peripheral β -actin mRNA observed after serum readdition is essentially a rapid redistribution of the existing perinuclear pool.

Intracellular β - vs. γ -Actin mRNA Localization

It has been shown previously that myoblasts sort actin isoform mRNA to different intracellular regions (Hill and Gunning, 1993; Kislauskis et al., 1993). Double in situ hybridization labeling for both β - and γ -actin mRNA show that 3T3

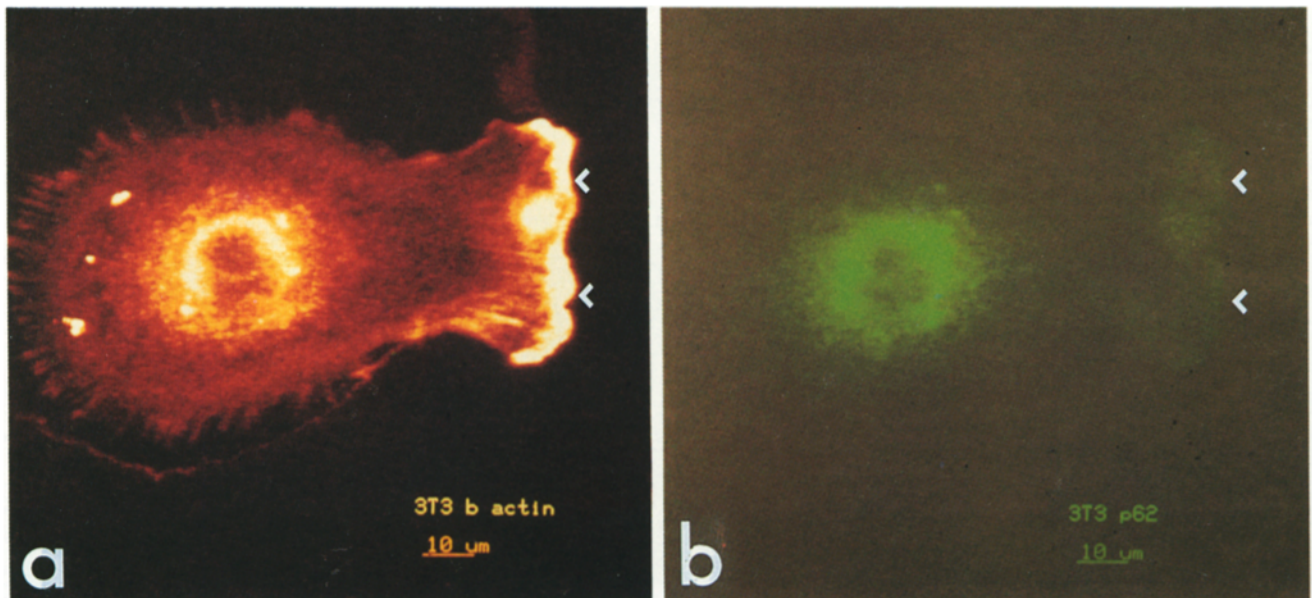


Figure 7. A representative 3T3 fibroblast growing in serum showing β -actin and the RNA-binding GAP-associated p62 colocalized to the motile edge. The cell morphology indicates either a large extending region or overall cell movement on the substrate to the right where both β -actin (a) and p62 (b, arrows) are found at the very edge of this extension. All cells where peripheral colocalization was observed had a similar morphology. The majority of p62 was always found located within the cell nucleus. The peripheral p62 may reflect localized intracellular growth factor signal transduction and correlates with our previous localization of peripheral β -actin mRNA (See Figs. 1, 3, and 5).

fibroblasts growing in serum similarly sort actin isoform mRNAs (Fig. 5, *a* and *b*). γ -actin mRNA (Fig. 5 *a*) was found concentrated around the nucleus with some speckly intranuclear labeling. β -actin mRNA (Fig. 5 *b*) was found both around the nucleus and at the cell periphery (Fig. 5 *b*, arrows). 3T3 fibroblasts therefore sort actin isoforms to the same intracellular regions as described for myoblasts (Hill and Gunning, 1993).

Serum starvation (Fig. 5 *c*) removed the peripheral β -actin mRNA pool; both β - and γ -actin mRNA now colocalized around the nucleus. Readdition of serum for 10 min (Fig. 5 *d*) shifted the β -actin mRNA back to the cell periphery, but did not alter γ -actin mRNA distribution. If any γ -actin mRNA redistribution did occur it was below the limits of our detection techniques. The difference between the intracellular location of β - and γ -actin mRNA therefore is under regulation by serum and presumably involves a signal transduction pathway.

Intracellular β - and γ -Actin Protein Localization

Serum addition to 3T3 fibroblasts can rapidly increase protein levels of all actin isoforms (Thomas and Thomas, 1986). Within individual cells 10 min after serum readdition we were unable to discriminate between the overall distribution of β - (Fig. 6 *a*) and γ -actin (Fig. 6 *b*) protein as detected by isoform specific antibodies. The antibodies are able to detect both filamentous and monomeric actin. Both isoforms were identified in filaments, at the cell periphery, and in broadly distributed punctate patches within the cell. These small punctate patches have been shown in other cells to contain mainly monomeric actin (Cao et al., 1993), and at higher magnification (data not shown) we could also identify the same peripheral labeling behind lamellipodia. Previous studies in other cell types (Otey et al., 1986; Hill and Gunning, 1993) have also found γ -actin in all structures where β -actin is located; although other studies (DeNofrio et al., 1989; Hoock et al., 1991) suggest β -actin enrichment at the cell periphery. These findings may reflect differences between the different cell types. Alternatively, the total quantity of actin protein within the cell may swamp any subtle isoform distribution differences since dynamic processes of the cell cannot be easily seen in the overall steady-state distribution of the proteins.

p62 Localization within 3T3 Fibroblasts

Peripheral localization of β -actin mRNA could be tied directly to rac/rho signal transduction. It has recently been suggested that the ras GTPase-activating protein may be an effector that couples tyrosine kinases and ras-related proteins such as rac/rho to control of the cytoskeleton (McGlade et al., 1993). Since GAP is known to interact with the RNA-binding tyrosine phosphoprotein p62 (Ellis, et al., 1990; Wong et al., 1992), we considered that p62 may be an ideal candidate to mediate transport and/or anchorage of β -actin mRNA in a serum-dependent manner. We therefore carried out immunolocalization of p62 within 3T3 fibroblasts. The majority of p62 was located within the cell nucleus (Fig. 7 *b*), however, some cytoplasmic p62 was also detected at the motile edges (Fig. 7 *a*) of 3T3 fibroblasts growing in serum. This motile region corresponded to the area where we had previously located β -actin mRNA (Figs. 1, 3, and 5). p62 is therefore a potential candidate molecule which could link

signal transduction to actin mRNA localization and regulation of the cytoskeleton.

Discussion

Distribution of β - and γ -Actin mRNA and Protein in 3T3 Fibroblasts

There is still controversy as to the functional significance of having two cytoskeletal actin isoforms that only differ by four conservative amino acids at the NH₂ terminus (Vanderkerckhove and Weber, 1978). While the proteins themselves may be different, we have shown different regulatory feedback responses (Lloyd et al., 1992), cell morphologies (Schevzov et al., 1992), and mRNA localizations (Hill and Gunning, 1993). Sorting of actin isoform mRNAs occurs in different cells (Hill and Gunning, 1993; Kislauskis et al., 1993) and there are at least two different and separable distributions of the mRNAs for different actin isoforms: γ and α perinuclear, β both perinuclear and at the cell periphery. This sorting of actin isoforms strongly suggests that cells can functionally discriminate between signals in the mRNA for each actin isoform. The signals for localization for all protein mRNAs so far identified exist in their 3'UTR sequences. A recent study (Kislauskis et al., 1993) has further identified specific subsequences within the β -actin 3'UTR that determine peripheral localization. This concept of isoform sorting is further supported by the differences between each isoform's 3'UTR, but evolutionary conservation of 3'UTR sequences for each isoform between species (Ng et al., 1985; Erba et al., 1986).

Does this translate into differences in actin isoform protein distribution? While cell to cell differences were seen in the distribution of β - and γ -actin, we did not see a consistent difference in the steady-state distribution of the proteins within cells. The antibodies used in this study identify both filamentous and globular actin, and newly synthesized and old actin alike. At this stage, we cannot exclude that subtle differences in isoform distribution do exist and are functionally significant. The problem is seeing these differences in two of the most abundant protein components within the cell. Furthermore, the nondirected, random motility seen under time-lapse video (data not shown) of these cells compared with directed motility seen in cells repairing a wound (Hoock et al., 1991; Martin and Lewis, 1992) may be a significant factor in showing differences in actin isoform distribution.

Redistribution of β -Actin mRNA

Cells growing in the presence of serum have peripherally located β -actin mRNA, serum starvation leads to the loss of this peripheral pool of mRNA. Serum starvation leads to many changes in cell metabolism, chiefly as a result of growth factor withdrawal. The loss of peripheral β -actin mRNA following serum withdrawal implies that serum-related cellular events are required to maintain its distribution. A two-stage mechanism for mRNA localization (*[a]* transportation and *[b]* site fixation) has been demonstrated in the embryo (Yisraeli et al., 1990) and may result from separate 3'UTR signals (Kim-Ha, 1993). Time-lapse studies of microinjected mRNA shows that additional intermediate stages may also occur during the transport/translation of mRNA (Ainger et al., 1993). In the case of β -actin mRNA, it has been shown that microfilaments are required for its pe-

ripheral translocation (Sundell and Singer, 1991) though no specific factor for its site fixation has yet been described. The finding that the RNA-binding protein p62 is associated with motile regions provides a possible candidate for involvement in anchoring β -actin mRNA by a growth factor-dependent mechanism. At this stage we cannot determine whether one or more steps are being altered with serum starvation, though serum starvation does deplete microfilament bundles (Ridley and Hall, 1992) and this may compromise transport.

Signaling Pathway for Redistribution of β -Actin mRNA

The peripheral β -actin mRNA is both serum dependent and is redistributed within minutes of serum readdition. Similar results have been obtained by Latham et al. (1994). This rapid peripheral redistribution with serum addition occurs within the timeframe of signal transduction mechanisms that generate changes in the actin cytoskeleton (Ridley and Hall, 1992; Ridley et al., 1992; Peppelenbosch et al., 1993). Both blockage of protein synthesis (Sundell and Singer, 1990) and the speed of redistribution suggests that newly synthesized proteins are not involved in this process. This in turn suggests that the components of this pathway are regulated in the same manner as other signal transduction pathway molecules, that is, activation/inactivation by some event such as phosphorylation/dephosphorylation. An alternative explanation is that the redistribution occurs as a secondary event related to changes in the actin cytoskeleton itself (Ridley and Hall, 1992; Ridley et al., 1992), the two signaling mechanisms described for this pathway, LPA and PDGF, individually stimulate β -actin mRNA redistribution to the cell periphery. There is also a difference in the timecourse of LPA and PDGF effects: LPA is more rapid and falls with time, PDGF is initially smaller and increases with time. This is the same timecourse for structural changes seen in the cytoskeleton stimulated by these individual factors (see Table 1 in Ridley and Hall, 1992). The finding that PDGF has multiple pathways of action (Olivera and Spiegel, 1993) may also contribute to the differences in timecourse between it and LPA. This does however suggest that there may also be more than one pathway of signaling which can control the distribution of β -actin mRNA. The recent linking of the Ras and cyclic AMP pathways (Cook and McCormick, 1993; Wu et al., 1993) also means that disparate signaling pathways may generate similar (or different) cellular events dependent upon their intracellular background and this may impact on how mRNA distribution is regulated within the cell. Our current results suggest that extracellular signals can be rapidly converted into intracellular localization cues for β -actin mRNA.

Function of Peripheral β -Actin mRNA

In microvascular epithelial cells (DeNofrio et al., 1989; Hooek et al., 1991) and in the embryo (Martin and Lewis, 1992) wound repair is associated with peripheral actin protein localization/concentration for cytoskeleton remodeling associated with cell motility. β -actin mRNA is located in the same intracellular region, at the leading edges of migrating cells. Myoblasts and fibroblasts also locate β -actin mRNA to regions of cell motility (Hill and Gunning 1993; Kislauskis et al., 1993). One possible function of peripheral

β -actin mRNA is for positional translation in situ of new actin required for cytoskeleton remodeling driving/responding to cell motility and shape change. Thus newly translated actin monomer could provide a pool of actin to locally polymerize for the formation of new actin filaments. This may occur by direct incorporation of new monomer into actin filaments or indirectly by replenishing the pool of actin bound to actin monomer binding proteins (Pollard and Cooper, 1986) which may have also responded to serum-stimulated signal transduction by releasing bound actin monomer. The continued cytoskeletal response in the presence of protein synthesis inhibitors and the rapidity of new actin structures appearing within the cell (Ridley and Hall, 1992) would support the idea of replenishing a monomer pool. The model being: growth factor signal \rightarrow mRNA localization \rightarrow positional translation \rightarrow new actin monomer \rightarrow monomer-binding proteins and/or new filaments \rightarrow cytoskeleton changes \rightarrow motility/shape change. Positional translation is further supported by time-lapse studies of new actin monomer location in spreading cells: in continuously forming discrete pools concentrated at the cell periphery directly behind lamellipodia (Cao et al., 1993). Localized translation may be yet further complicated by the recent description of a requirement for a cytosolic actin chaperonin, t-complex polypeptide 1 complex (Sternlicht et al., 1993), for actin folding to occur. This chaperonin is itself associated with cell membranes (Willison et al., 1989).

Alternatively, the function of β -actin mRNA localization may involve the distribution of either the ribosomal structures themselves or other proteins bound to this abundant mRNA. The fact that β -actin mRNA is so abundant and stable makes it an ideal candidate for such a role. For example, in the rac/rho signal pathway p62 is involved in growth factor signal transduction and it also binds RNA (Wong et al., 1992). p62 was found in the cell cytoplasm only in regions where β -actin mRNA was located at the cell periphery. p62 may be transported/located at the periphery by being bound to this mRNA or ribosomal RNA. Interestingly, other proteins in these signal transduction pathways that complex with p62-GAP are themselves bound and localized by the cytoskeleton (Bar-Sagi et al., 1993). Alternatively, p62 could anchor β -actin mRNA at the periphery at sites of growth factor signal transduction. At this stage motor molecules for mRNA transport along microfilament/microtubule systems are implied but not identified (Wilhelm and Vale, 1993). These putative motor molecules may have additional functions at the cell periphery, for example acting either locally, or once the mRNA has been anchored, available for molecular retrograde transport to the nucleus. Finally, the biological function of actin mRNA localization may also be different in different cell types.

From our current results signaling through growth factors (or even extracellular matrix) for directed cell motility in chemotaxis could be explained by positional translation of new actin cytoskeleton. For example, in wound healing hormone/growth factor levels may increase at the wound site which would in turn promote signal transduction and movement of β -actin mRNA to the adjacent membrane region (Hooek et al., 1991). In this way, supply of new actin would promote growth of the appropriate cell membrane region to allow wound closure (see also Martin and Lewis, 1992). This also opens up the concept of how extracellular signals

are translated into long-term cellular changes, particularly with so many membrane receptors linked to the actin cytoskeleton. At this stage we cannot discount the possibility of a multifunctional cellular requirement for localization of β -actin mRNA. Nevertheless, the temporal correlation between mRNA redistribution and motility (Ridley and Hall, 1992) is consistent with signal transduction and mRNA localization as linked processes.

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