Isoform-specific 3′-untranslated Sequences Sort α-cardiac and β-cytoplasmic Actin Messenger RNAs to Different Cytoplasmic Compartments

Edward H. Kislauskis, Zhifang Li, Robert H. Singer, and Krishan L. Taneja

Department of Cell Biology, University of Massachusetts Medical Center, Worcester, Massachusetts 01655

Abstract. We demonstrate that in differentiating myoblasts, the mRNAs encoding two actin isoforms, β-cytoplasmic, and α-cardiac, can occupy different cytoplasmic compartments within the same cytoplasm. β-actin mRNA is localized to the leading lamellae and α-actin mRNA is associated with a perinuclear compartment. This was revealed by co-hybridizing, in situ, fluorochrome-conjugated oligonucleotide probes specific for each isoform. To address the mechanism of isoform-specific mRNA localization, molecular chimeras were constructed by insertion of actin sequences between the Lac Z coding region and SV-40 3′UTR in a reporter plasmid. These constructs were transiently expressed in a mixed culture of embryonic fibroblasts, myoblasts and myotubes. β-galactosidase activity within transfectants was revealed by a brief incubation with its substrate (X-gal). Since the blue-insoluble reaction product co-localized with the specific mRNAs expressed from each construct, it was used as a bioassay for mRNA localization. Transfectants were scored as either perinuclear, peripheral or nonlocalized with respect to the distribution of the blue product. The percentage of transfectants within those categories was quantitated as a function of the various constructs. This analysis revealed that for each actin mRNA its 3′UTR is necessary and sufficient to direct reporter transcripts to its appropriate compartment; β-actin peripheral and α-actin perinuclear. In contrast, sequences from the 5′UTR through the coding region of either actin gene did not localize the blue product. Therefore, 3′UTR sequences play a key role in modulating the distribution of actin mRNAs in muscle cells. We propose that the mechanism of mRNA localization facilitates actin isoform sorting in the cytoplasm.

ACTIN is a key structural component of all eukaryotic cells. It is integral to the processes of intracellular transport, cell motility, and the maintenance of cell architecture. In higher animals, actin is encoded by a family of 6 to 10 genes (Vandekerckhove and Weber, 1978; Bergsma et al., 1985; Sawtell et al., 1988; Sadano et al., 1990) which are subject to developmental and tissue-specific regulation (Chang et al., 1984; Ruzicka and Schwartz, 1988; Sawtell and Lessard, 1989; Rubenstein, 1990; McHugh et al., 1991). The few differences in amino acid sequence which occur between actin isoforms are predominantly at the amino terminus (reviewed by Herman, 1993). However, 3′-untranslated sequences are isoform specific (Yaffe et al., 1985). It is entirely possible that differences among actin genes in the noncoding regions are functionally significant.

Immunocytochemical analyses, using isoform-specific antibodies, have suggested that actin isoforms are differentially sorted in the cytoplasm (Pardo et al., 1983; Otey et al., 1988; DeNofrio et al., 1989; reviewed in Herman, 1993). Actin polymerization at the leading edge of motile cells is involved in the extention the lamellipodia, filopodia, or pseudopods (McKenna et al., 1985; Okabe and Hirokawa, 1989;

Theriot and Mitchison, 1991). Extension of the lamellae appears to involve the β-cytoplasmic isoform (Hoock et al., 1991). During myogenesis, myoblasts fuse into syncytial myotubes and express muscle-specific actin genes. In early myotubes, nonmuscle actin isoforms segregate from the skeletal muscle isoforms which are involved in forming nascent myofibrils (Otey et al., 1988). In fusion-competent myogenic cells, derived from the quail fibrosarcoma (QT6), the α-actin isoforms are not expressed during differentiation and sarcomeres are not formed, even though other muscle-specific genes as well as nonmuscle actins are expressed (Antin and Ordahl, 1991). These data also support the functional specificity of actin isoforms.

How are actin isoforms sorted in the cytoplasm? One possibility is that mRNAs are sorted prior to translation. In motile cells, such as chicken embryonic fibroblasts (CEFs)[1] and myoblasts, β-actin mRNA localizes to the leading lamel-

---

1. *Abbreviations used in this paper:* α-actin, α-cardiac actin; β-actin, β-cytoplasmic actin; CEF, chicken embryonic fibroblast; 3′UTR, 3′-untranslated region; X-gal, 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

lae (Lawrence and Singer, 1986; Sundell and Singer, 1990). Similarly, β-actin mRNA is recruited to the extending lamellae in response to the wounding of a monolayer of endothelial cells (Hoock et al., 1991). As myoblasts differentiate to form myotubes and assemble myofilaments, β-actin gene transcription is repressed and the α-cardiac actin gene is expressed (Paterson and Eldridge, 1984; Hayward and Schwartz, 1986). The α-cardiac isoform is expressed in single cells before their fusion into myotubes and its mRNA is localized in the perinuclear cytoplasm (Lawrence et al., 1989; Taneja and Singer, 1990). Therefore, the differential compartmentalization of actin mRNAs could explain the preferential distribution of their cognate proteins.

How do actin mRNAs occupy different cytoplasmic compartments? One possibility is that localization is dependent on the cytoarchitectural changes which occur during myogenesis, concurrent with the repression of the β-actin gene and the expression of the α-cardiac gene. Alternatively, actin isoform mRNAs could be targetted by their unique sequences, independent of cell morphology or state of differentiation. A functional role has been established for the 3'UTR in the localization of maternal and zygotic transcripts in oocytes or embryos (reviewed in Kislauskis and Singer, 1992). It is reasonable that sequences which reside in actin mRNA 3'UTRs which are isoform-unique and evolutionarily conserved perform a similar function (Ponte et al., 1983; Vandekerckhove et al., 1984; Yaffe et al., 1985). Our data indicate that isoform-specific 3'UTR sequences contain the spatial information for the differential compartmentalization of actin mRNAs within the cytoplasm of single cells and myotubes. We propose that mRNA localization is a key aspect of gene expression in somatic cells, important in the organization of intracellular structure.

Materials and Methods

Probes

Oligonucleotide probes (50–55 bases) specific to α-cardiac and β-cytoplasmic actin mRNA were synthesized on a DNA synthesizer (model 396; Applied Biosystems, Foster City, CA). Five amino-modified thymidine residues (Glen Research, Sterling, VA) were incorporated approximately every 10 bases. Incorporation of fluorochromes less than every 10 bases causes quenching of the fluorescent signal (Taneja, K. L., unpublished observation). After deprotection, probes were gel purified on a 10% polyacrylamide gel, and then labeled with a specific fluorochrome. Oligonucleotide complementary to β-actin mRNA were labeled with Cy-3 (Biological Detection Lab, Pittsburgh, PA), and α-specific probes with fluorescein isothiocyanate (Molecular Probes, Eugene, OR) in 0.1 M NaHCO₃/Na₂CO₃, pH 9.0, overnight, in the dark, at room temperature, and then fractionated through Sephadex G-50. Fractions were combined, lyophilized, and further purified on 10% polyacrylamide native gels. Six probes were made to β-specific mRNA and four probes were made to α-specific 3'UTR sequences. The sequences of the six β-actin oligos KLT-4, -15, -18, -19, -20, and -23 were previously published as was one α-probe, KLT-9 (Taneja and Singer, 1990). The three additional α-actin oligos (oriented 5' to 3') were:

KLT-10
(CTCCAAGAAGCATACTGTCGTCATCCTGAGTGGGAAGTAAATGTAGGGAAAAGT) ;

KLT-11
(TGCACAAGTGTGTAAACAAACTGTACAATGACGGAGGAGTTCAGGAAGGGCTGC) ;

KLT-12
(CATTTGTAGGTTGCAAGCCCAGATCTTTATTTATTTATAAAGCAATAAATATTAGAAG-
CACAAATA) .

Cell Culture

12-d-old chicken embryonic skeletal muscle myoblasts and fibroblasts, pre-

pared using standard techniques, were cultured onto gelatin-coated coverslips as previously described (Lawrence et al., 1989; Sundell and Singer, 1990). Cells on coverslips were washed with HBSS, fixed for 15 min at room temperature in 4% paraformaldehyde in PBS/5 mM MgCl₂, washed again in PBS, and, when necessary, stored in 70% ethanol at 4°C.
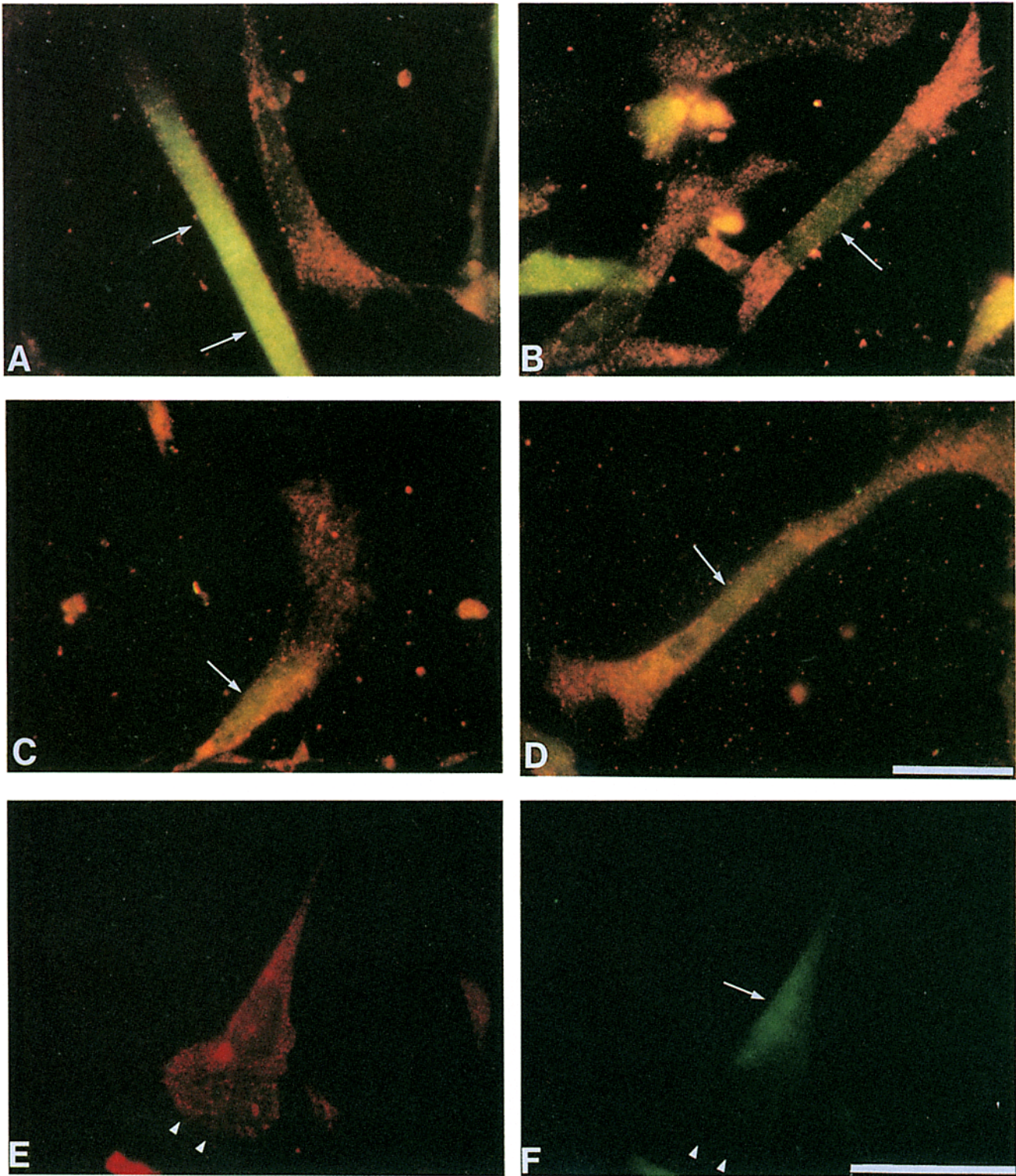
In situ Hybridization

To detect endogenous actin mRNAs, coverslips were hydrated in PBS/5 mM MgCl₂, and then hybridized for two h at 37°C with the mixture of oligonucleotide probes: α-specific (four probes, 5 ng/probe) and β-specific (six probes, 5 ng/probe) in 50% formamide containing 2× SSC, 0.2% BSA, 10% dextran sulfate, 2 mM vanadyl adenosine complex, and 1 mg/ml each of Escherichia coli tRNA and salmon sperm DNA. This mixture of oligonucleotide probes increases signal intensity (Taneja and Singer, 1990). After hybridization and washing successively for 30 min in 50% formamide/2× SSC at 37°C, 30 min in 50% formamide/1× SSC at 37°C, and 30 min in 1× SSC, coverslips were mounted on the slides using phenylene diamine (antibleaching agent) in 90% glycerol and PBS. To detect Lac Z reporter mRNAs, each coverslip was hybridized for 3 h at 37°C to 20 ng of digoxigenin-labeled, nick-translated probes generated from the entire RSVβgal plasmid. For methods where that enzyme activity and the reporter mRNA were detected simultaneously, nick-translated probes were generated using standard procedures (Lawrence and Singer, 1989). After in situ hybridization, coverslips were washed three times in 1× SSC for 15-min each, then incubated with an anti-digoxigenin mouse IgG₁ mAb (Boehringer Mannheim Biochemicals, Indianapolis, IN) at a 1:25 dilution in 1× SSC for 1 h at 37°C. For detection, a second incubation with a 1:8 dilution of the AuroProbe™ One GAM 1 nM gold-labeled goat anti-mouse IgG (H + L) (Amersham Intl., Buckinghamshire, U.K.), followed by several washes with 1× SSC, a final wash with water, and 18 min of development with equal volumes of IntenSE™ M Silver Enhancement Reagents A and B (Amersham Intl.).
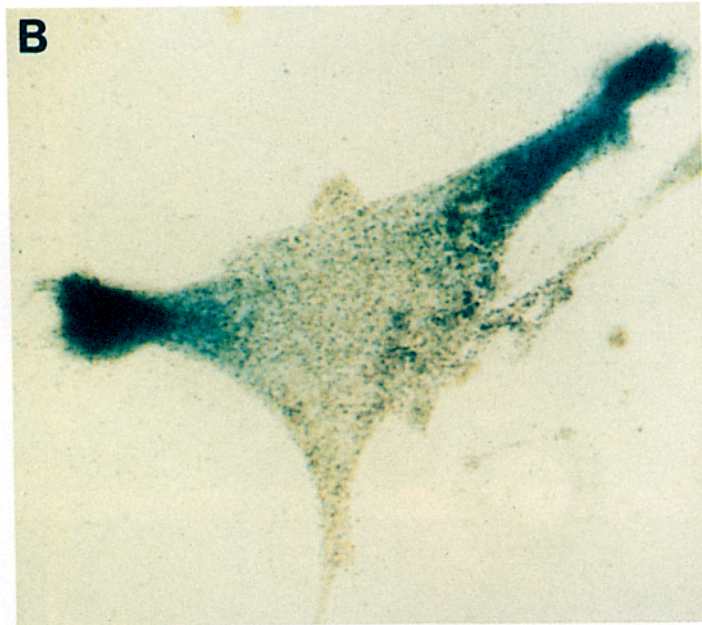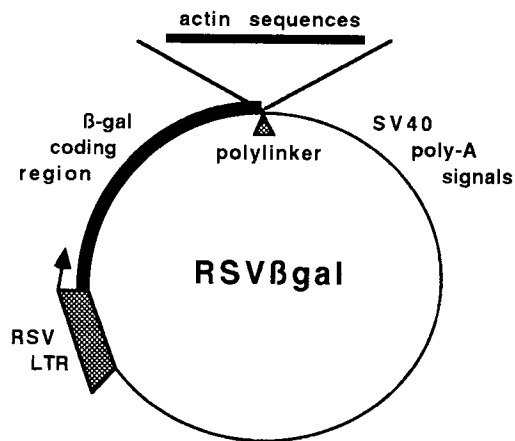
Transfection

CEFs and myoblasts were plated onto gelatin coated coverslips (22 × 22 mm) essentially as described above with the following exceptions: cells were plated at a density of 1 × 10⁶ cells/100 mm plate in OPTI-MEM (BRL-GIBCO Gaithersburg, MD) supplemented with 10% FBS and 2% chicken embryo extract. At ∼50% confluence (24–48 h), the cells were washed with HBSS and incubated for 12 h in 10 mls of OPTI-MEM containing 20 μg of cesium-purified plasmid DNA with 30 μg Lipofectin (BRL-GIBCO) at 37°C. Subsequently, the medium was replaced with 10 mls. of OPTI-MEM supplemented with 10% FBS and the cells were incubated for an additional 12 h. Posttransfection, cells were fixed for 10 min in cold 4% formaldehyde/PBS + 5 mM MgCl₂ and processed to detect β-galactosidase, as previously described (Lim and Chae, 1989). In control experiments, we have shown that the transfection conditions do not appreciably reduce the percent of cells with peripherally localized endogenous β-actin mRNA (data not shown).

Vector and Plasmid Constructions

The complete chicken α-cardiac actin cDNA (Chang et al., 1985) and the full-length chicken β-cytoplasmic (Cleveland et al., 1980) were used to construct the various chimeric genes. The original plasmid RSVβgal was modified by removing a 250 base DraI/BamHI fragment which contained a consensus polyadenylation signal upstream of the polylinker used in the constructions. The Rous Sarcoma Virus long terminal repeat (RSV LTR) drives the constitutive expression of the reporter gene (Gorman et al., 1982). The reporter gene encodes β-galactosidase from the bacterial Lac Z operon fused to SV-40 3' processing signals (An et al., 1982). In this vector, The SV-40 sequences contain two consensus polyadenylation signals downstream of the RSVβgal polylinker. Plasmid constructions were verified by restriction endonuclease analysis and dideoxy sequencing. The construct containing the 5'UTR plus coding region of the β-cardiac actin cDNA was generated by polymerase chain reaction (PCR) (upper primer: 5'-AAG-GAATCCGTGTGACGACGAGGAGACCA; lower primer: 5'-GGTCTAGAG-CGGTGGACAATGGATGGAC) and cloned into the BamHI and XbaI sites of RSVβgal. The 3'UTR of β-cardiac actin cDNA was similarly generated (upper primer 5'-AAGGATCCGCAAGTGCTTCTATAC; lower primer 5'-GTTCTAGAGTAGGTTGCAAGTCCAGATC). The 5'UTR and coding region of the β-actin cDNA corresponding to sequences 1–1192 was subcloned by modifying a AvaII site at position 1192 with BamHI linkers (New England Biolabs, Beverly, MA) and inserting it as a 1.2-kb fragment into

Figure 1. α-cardiac and β-cytoplasmic actin mRNAs occupy distinct cytoplasmic compartments in the same cell. In situ hybridization using fluorochrome-labeled, isotype-specific oligonucleotide probes specific for α-actin (*green*, arrows point to nucleus) and β-actin (*red*) mRNAs on primary cultures of chicken embryonic muscle cells. (*A*) Peripheral β-actin mRNA signal in a fibroblast, and a perinuclear α-actin signal in a myotube. (*B*) A bipolar myoblast with a predominant peripheral distribution of β-actin mRNA signal and a perinuclear α-actin signal. (*C*) Another myoblast with perinuclear α-actin mRNA signal and peripheral β-actin mRNA signal. (*D*) Small myotube (five to six nuclei) with a perinuclear α-actin mRNA signal and a peripheral β-actin signal. (*E* and *F*) Separate images showing peripheral β-actin mRNA (*red*) and perinuclear α-actin mRNA (*green*). Arrowheads indicate the periphery of the lamellipod. These images are not digitized, they are registered analog double exposures. Note there is little yellow color indicative of substantial overlap of equally strong red and green signals. Bars, 10 μm.

Figure 2. Transfection strategy using a Lac Z reporter gene for the analysis of *cis*-acting localization sequences. (*A*) Schematic of the RSVβgal plasmid and the relevant sequences, including the position of the RSV LTR, Lac Z coding region, polylinker for insertion of actin sequences, and SV-40 processing sequences. (*B*) Insertion of the entire β-actin cDNA sequence, in the correct (sense) orientation, between the Lac Z gene and SV-40 polyadenylation signals resulted in colocalization of Lac Z mRNA (*black*) and β-galactosidase activity (*blue*) to the peripheral compartment (leading lamellae).

RSVβgal. The 3′UTR of β-actin, corresponding to positions 1221–1778 was synthesized by PCR (upper primer: 5′-CCGGATCCTAAACCGGACTGTT-ACCAAC; lower primer: 5′-GGTCTAGATCAGTGTACAGGTAGCCCCT. The β-actin 3′UTR fragment excludes sequences downstream of position 1780, 14 bases before the consensus polyadenylation signal (position 1794–1880). Also excluded from this fragment was half of a previously characterized 40 nucleotide transcriptional control element in the distal β-actin 3′UTR (Deponti et al., 1988).

Statistical Analyses

Analysis of variance was performed for a two factor factorial design. In the presence of significant main or interactive effects, pairwise comparisons were made using Tukeys HSD multiple comparisons procedure. The number of individual transfectants (single cells) scored for each construct in Fig. 3 *A* ranged from 232–365. The number of transfected myotubes scored for each plasmid in Fig. 3 *B* ranged 272–656.

Results

Actin mRNAs are Differentially Sorted in Individual Cells and myotubes

It was important to determine directly whether each actin isoform mRNA occurred in unique cell populations (differentiating vs. nondifferentiating) or whether a subset of cells existed which contain both isoforms. To address this question, we synthesized two sets of amino-linked deoxyoligonucleotide probes specific to α-cardiac and β-cytoplasmic actin mRNA, and subsequently conjugated each set to a different fluorochrome. Each set of probes hybridized to its specific mRNA target in a mixed culture of chicken embryonic muscle cells. Specific hybridization of Cy-3–conjugated probes to β-actin mRNA was verified by localization of signal at the leading edge of fibroblasts. Hence, only red fluorescence was seen in fibroblasts where α-actin mRNA was not expressed

(Fig. 1 *A*). Specific hybridization of fluorescein-conjugated probes to α-cardiac actin mRNA was verified by signal in myotubes. Hence, only green fluorescence was seen in these cells where minimal β-actin mRNA was present (Fig. 1 *A*, *arrows*). In a differentiating muscle cell culture, ~20% of the cells have both signals. In that population, α-actin signal was predominantly perinuclear and the β-actin signal was predominantly peripheral whether in differentiating myoblasts (Fig. 1, *B* and *C*) or in small myotubes (Fig. 1 *D*). These compartments, as defined by their respective probes, showed minimal congruence as indicated by the lack of yellow fluorescence resulting from the overlap of red and green signals (Fig. 1 *C*). To visualize each probe, in the absence of the other, individual red and green images revealed the extent of β-actin mRNA in the periphery (Figs. 1, *E* and *F*, *arrowheads*) compared to the sequestration of α-actin mRNA around the nucleus. These are analog images; the colors are true to the fluorochrome and not enhanced or altered relative to each other. Therefore, the two actin mRNAs denote isoform-enriched regions within the same cytoplasm.

Actin mRNAs Are Sorted via Isoform-specific 3′UTRs

It was reasonable to postulate that isoform-specific sequences partition actin isoform mRNAs to different cellular compartments. Consequently, a bioassay was developed to determine whether or not isoform-specific actin cDNA sequences could function in *cis* to target heterologous reporter molecules to the appropriate compartment in the cytoplasm. Actin sequences were inserted into a polylinker between the Lac Z termination codon and SV-40 3′UTR, in the expression vector RSVβgal (Fig. 2 *A*), to create a fusion transcript but not a fusion protein. Following a period of transient expression, β-galactosidase–positive transfectants were de-

β–actin α–actin
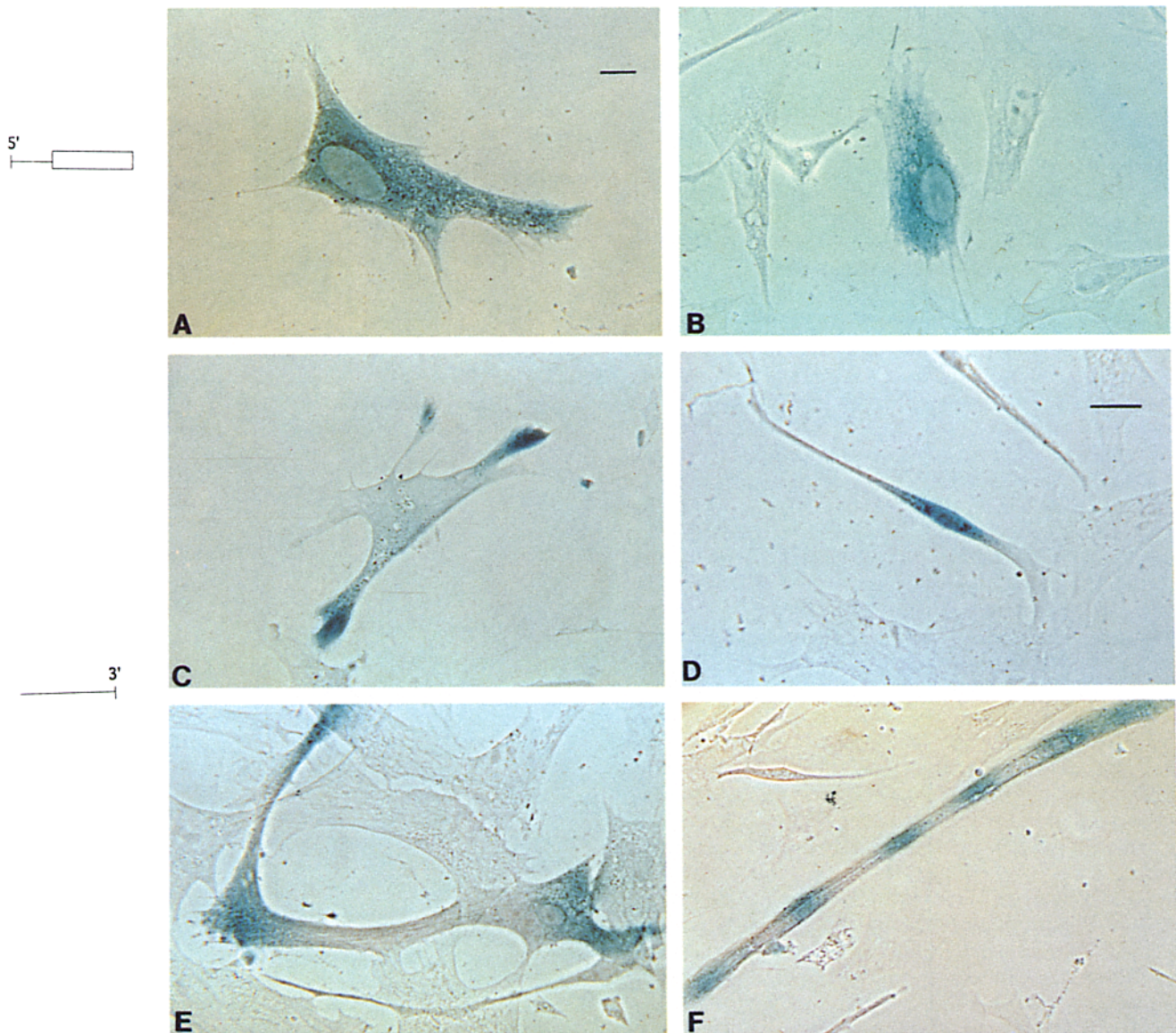
Figure 3. Distribution of β-galactosidase activity in cells following transient transfection of chimeras between Lac Z and α-cardiac or β-cyto-plasmic actin sequences. Intracellular β-galactosidase activity (*blue*) detected in single cells (fibroblasts and myoblasts, *A–D*) and myotubes (*E* and *F*). The portion of the actin isoform, either 5′UTR plus coding (*A* and *B*) region or the 3′UTR (*C–F*), fused to the reporter gene are schematically depicted. Neither β-actin (*A*) nor α-actin (*B*) 5′UTR and coding sequences localized β-galactosidase activity whereas, β-galactosidase activity localized to a peripheral compartment in single cells (*C*), and myotubes (*E*) which expressed the reporter gene fused to the β-actin 3′UTR, and to a perinuclear compartment in single cells (*D*) and myotubes (*F*) which expressed the reporter gene fused to the α-actin 3′UTR. Note the segmented pattern of β-galactosidase activity around the nuclei (*F*). Bars: (*A–C*) 10 μm; (*D–F*) 25 μm.

tected in fixed cells using the chromogenic substrate X-gal (Lim and Chae, 1989). Since β-galactosidase is functional as a homodimer or tetramer of 135-kD subunits, we predicted that it would diffuse slowly in the cytoplasm and would serve as a reasonable indicator of the distribution of its cognate mRNA. The size of a β-galactosidase tetramer, ~70 Å by 120 Å (Karlsson et al., 1964), would have restricted diffusion within the cytoplasm compared to an aqueous solution (Luby-Phelps et al., 1987).

We scored transfectants as peripheral, perinuclear or non-

localized based upon where the majority of β-galactosidase activity (blue stain) appeared in the cytoplasm. For instance, "non-localized" cells showed a homogeneous distribution of blue stain; "perinuclear" cells had most of their blue stain associated with the nucleus, and not toward the leading lamel-lae; and "peripheral" cells had blue stain separated from the nucleus and in the leading lamellae. In situ hybridization was performed using Lac Z specific probes to verify that the distribution of Lac Z mRNA correlated with the distribution of blue stain in transfectants. The distribution of in situ reaction

product (black) in a cell transfected with a reporter construct containing the entire β-actin cDNA sequence colocalized with the blue staining in the lamellae (Fig. 2 B). In general, the distribution of β-galactosidase is more diffuse than its mRNA in the cytoplasm (see also Ralston and Hall, 1992), but sufficient to be an assay for the distribution of the cognate mRNA.

Micrographs of single cells (Fig. 3, A–D) and myotubes (Fig. 3, E and F) represent examples of homogenous, perinuclear, or peripheral distributions of β-galactosidase activity. The quantitation of localized β-galactosidase activity in cells transfected with the various constructs is presented in Fig. 4 (see below). We found that β-galactosidase activity was not localized in cells transfected with the vector alone. When the Lac Z gene was fused to the 5′UTR plus the coding regions of either β-actin (Fig. 3 A) or α-actin (Fig. 3 B), the resulting cells showed no significant difference from the vector alone. In sharp contrast, a significant increase in percent of transfectants which exhibited peripheral β-galactosidase activity was seen with β-actin 3′UTR (Fig. 3, C and E). Remarkably, cells transfected with the construct containing the α-cardiac actin 3′UTR showed a significant increase in perinuclear β-galactosidase activity (Fig. 3, D and F). The increase in peripheral localization of β-galactosidase activity was not as strong as the localization of the endogenous β-actin mRNA, which can be seen in approximately 40–60% of the cells. We attribute this difference to some diffusion of the enzyme away from its site of synthesis. However, it is possible that β-galactosidase is unstable in the cytoplasm and blue stain is localized near newly synthesized and functional β-galactosidase. We have noted a reduced correlation between the distribution of β-galactosidase activity and localized reporter mRNAs when reporter gene expression was allowed to continue for longer than 18-h posttransfection. Therefore, it was imperative to assess localization soon after transfection, presumably before a steady-state could be established in each transfectant.

Statistical analyses of the percent of transfectants showing localized β-galactosidase activity indicated that isoform-specific 3′UTR sequences were necessary and sufficient to target heterologous transcripts to the appropriate cytoplasmic compartment. In fibroblasts and myoblasts (Fig. 4 A), we found that the β-actin 3′UTR insert increased the percent of transfectants with peripheral β-galactosidase activity by 6.0-fold relative to the vector alone (31.2%/5.2%), and α-actin 3′UTR sequences increased the number of cells with perinuclear β-galactosidase activity by 3.5-fold, relative to the vector alone (43.4%/12.5%). The differences were determined to be significant ($P \leqslant 0.0002$ in each case) by analysis of variance for a two factor factorial design. In myotubes (Fig. 4 B), equally significant differences between the constructs were obtained ($P \leqslant 0.0007$, in each case). Remarkably, isoform-specific fusion transcripts were localized correctly, even though the endogenous α-actin gene is not expressed in fibroblasts; likewise, the endogenous β-actin gene is not expressed in most myotubes. Therefore, the 3′UTR of each isoform was sufficient to sort β-galactosidase activity to the appropriate compartment. Because the 5′UTR plus coding region of either actin mRNA did not significantly alter the distribution of β-galactosidase, compared to the vector control ($P = 1.0$ in each case), the 3′UTR of each isoform was also necessary for compartmentalized expres-
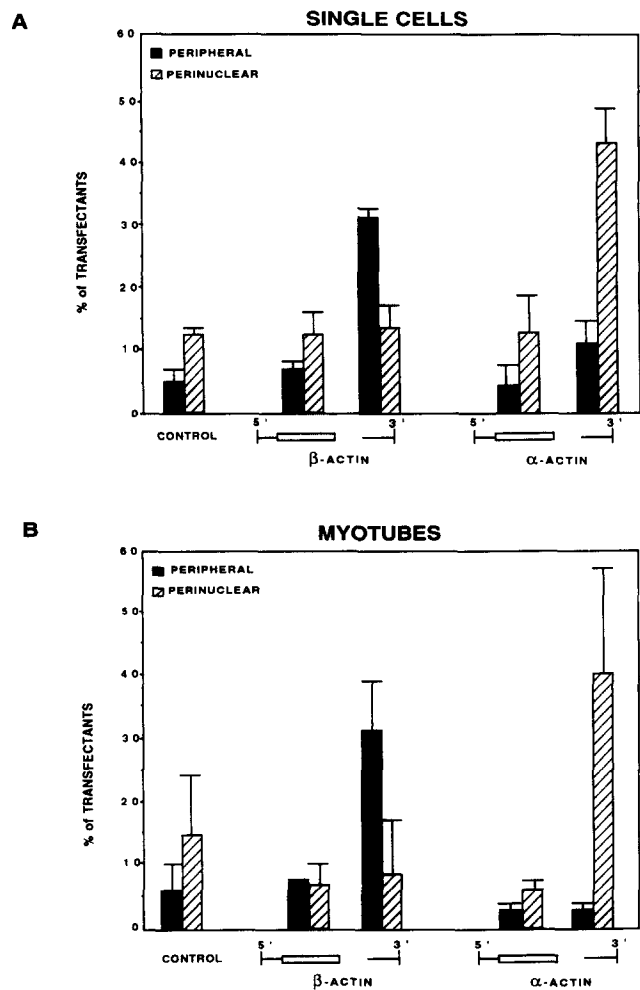
Figure 4. Effects of actin sequences on the distribution of reporter encoded β-galactosidase activity. Chimeric genes between Lac Z and portions of the β-actin or α-actin genes (as illustrated in Fig. 3) were transiently expressed in (A) single cells (fibroblasts and myoblasts) and (B) myotubes. The data was expressed as percent of transfectants with either a peripheral (■) or a perinuclear (▨) distribution of β-galactosidase activity (blue reaction product). The vector, RSVβgal, was used as the control. Standard deviation (error bars) from calculated experimental mean for each plasmid are depicted.

sion of β-galactosidase. The nonlocalized (homogeneous) distribution of blue staining in RSVβgal transfectants, imparted by SV-40 3′UTR sequences, and in transfectants expressing the reporter gene fused to either actin 5′ plus coding region, may represent the default position for nucleic acids in the cytoplasm without a localization signal. We conclude that sequences in both actin 3′UTRs, and not their 5′UTR or coding regions encode positional information for each its site of synthesis. This information is independent of the state of cell differentiation or whether the endogenous isoform is expressed. A more detailed characterization of the cis-acting localization sequences in the α-cardiac and β-actin is currently underway.

Discussion

Genetic approaches in *Drosophila* have clearly established

the functional significance of mRNA localization for embryogenesis. Prior localization of maternal RNAs encoding the anterior and posterior morphogenetic determinants, bicoid, and nanos, respectively, generate morphogenetic gradients and establish polarity in the oocyte (review by Lipshitz, 1991). A 625-nucleotide segment of the *Drosophila* bicoid gene 3'UTR was necessary and sufficient to sequester Lac Z/bicoid fusion transcripts to the anterior half of transgenic *Drosophila* oocytes as demonstrated by RNase protection (McDonald and Struhl, 1988), and later verified by in situ hybridization (Gavis and Lehmann, 1992). In *Xenopus* oocytes, a 340-nucleotide segment of the Vg-1 3'UTR was necessary for vegetal pole localization (Mowry and Melton, 1992). The nanos 3'UTR was sufficient to localize nanos mRNA to the posterior pole in *Drosophila* embryos (Gavis and Lehmann, 1992). In addition, 3'UTR sequences are responsible for directing pair–rule gene transcripts (even-skipped, hairy, and fushi tazaru) to the apical periplasm in the blastoderm (Davis and Ish-Horowicz, 1991). These examples of localization of maternal and zygotic mRNAs are reinforced by our description of the role of 3'UTRs for sorting α- and β-actin mRNAs in somatic cells.

The work presented here adds to the physiological importance of actin mRNA sorting in somatic cells and suggests a functional relevance for actin isoform sorting. The targetting of each actin isoform to its particular cytoplasmic domain may create localized concentrations of new actin monomers, each available to polymerize at different sites within the cytoplasm. Modifications which may occur posttranslationally, or binding proteins which may sequester the actin in nonpolymerizable form may be obviated at these sites. Furthermore, the distribution of actin mRNAs may coincide with other mRNAs for proteins physiologically relevant for actin regulation. For instance, tropomyosin and α-actinin mRNAs are localized at the leading edge of motile fibroblasts where actin mRNA is found (Sundell, C., and R. Singer, unpublished data). The formation of macromolecular complexes involving actin would facilitate the physiology of the relevant cellular compartment (e.g., motility, or sarcomere formation).

Evidence for mRNA localization determinants in the 3'UTR adds support to the notion that the 3'UTR has evolved to be specialized in posttranscriptional regulation of mRNA metabolism. The 3'UTR has been described as a repository for *cis*-acting sequences which regulate RNA localization, stability, cytoplasmic polyadenylation, and translation (reviewed by Jackson, 1993). Most recently, it has been suggested that specific 3'-untranslated sequences can modulate gene transcription (Deponti-Zilli et al., 1988) and muscle cell differentiation (Rastinejad and Blau, 1993). It is probable that each of these aspects of RNA metabolism is mediated by a specific *trans*-acting factor(s) which interact with RNA sequences similar to the assembly of transcription factors on DNA. Proteins are being identified which modulate RNA metabolism in the cytoplasm; proof for their direct roles will require further work (reviewed by Peltz and Jacobson, 1992; Jackson, 1993). β-galactosidase activity in the cytoplasm is a demonstration of the expression, through translation and assembly, of each reporter gene construct. Therefore, it is reasonable that the corresponding endogenous actin isoforms are likewise expressed in their respective compartments. The ability of the localization mechanism to

correctly compartmentalize heterologous mRNAs containing localization sequences indicates that the mechanism was not saturated, at least within the time frame of our bioassay. Since the α-actin 3'UTR-targetted β-galactosidase perinuclearly in fibroblasts, which do not express this isoform, and the β-actin 3'UTR-targetted β-galactosidase peripherally in myotubes where this gene is transcriptionally repressed, our data indicate that the "reading" of the peripheral and perinuclear localization signals were not affected by changes in gene expression and morphology which accompanied differentiation. In fibroblasts, the actin cytoskeleton is required for localization of actin mRNAs to the periphery (Sundell and Singer, 1991). Whether or not the actin cytoskeleton is also involved in perinuclear compartmentalization of α-actin mRNA remains to be determined.

We propose that isoform-specific 3'-UTRs effect and affect the compartmentalization of actin isoforms in the cytoplasm. This compartmentalization may be important for the assembly of intracellular structures involving actin. In accordance with this view, it has been reported that overexpression of β- or γ-actin in mouse C2 myoblasts altered cell shape and motility in an isoform-specific manner (Schevzov et al., 1992). Hill and Gunning (1993) have shown that the β-actin mRNA exists throughout the cytoplasm of mouse fibroblasts but extended into the most peripheral cytoplasm, whereas γ-actin mRNA is not detected in the outermost periphery. This reinforces the spatial restriction of isoform-specific actin mRNAs by providing information on a third isoform (γ-actin) and enhances the validity of arguments for the importance of protein isoform sorting as a physiological mechanism for determining cell morphology. In apparent contrast to this view, α-skeletal actin can be incorporated into myofibrils preferential to β-actin in a cell-free system indicating that actin isoform sorting can occur at the protein level (Peng and Fischman, 1991). However, these results may be concordant with our viewpoint since, in vivo, localized synthesis could act to concentrate and sequester the α-cardiac isoform close to developing myofilaments, and hence facilitate the process of assembly, and the probability of spatial control of sarcomere formation. Thus, the mechanisms of mRNA sorting followed by protein sorting and assembly may act synergistically.

References

An, G., K. Hidaka, and L. Siminovitch. 1982. Expression of bacterial β-galactosidase in animal cells. *Mol. Cell. Biol.* 2:1628–1632.

Antin, P. B., and C. P. Ordahl. 1991. Isolation and characterization of an avian myogenic cell line. *Dev. Biol.* 143:111–121.

Bergsma, D., K. Chang, and R. Schwartz. 1985. Novel chicken actin gene: third cytoplasmic isoform. *Mol. Cell Biol.* 210:1151–1162.

Chang, Jr., K. S., W. E. Zimmer, D. J. Bergsma, J. B. Dodgson, and R. J. Schwartz. 1984. Isolation and characterization of six different chicken actin genes. *Mol. Cell. Biol.* 4:2498–2508.

Davis, I., and D. Ish-Horowitz. 1991. Apical localization of pair-rule tran-

scripts requires 3' sequences and limits protein diffusion in the Drosophila blastoderm embryo. *Cell.* 71:927-940.

DeNofrio, D. D., T. C. Hoock, and I. M. Herman. 1989. Functional sorting of actin isoforms in microvascular pericytes. *J. Cell Biol.* 109:191-202.

Deponti-Zilli, L., A. Seiler-Tuyns, and B. Paterson. 1988. A 40-base-pair sequence in the 3' end of the β-actin gene regulates β-actin mRNA transcription during myogenesis. *Proc. Natl. Acad. Sci. USA.* 85:1389-1393.

Gavis, E., and R. Lehmann. 1992. Localization of nanos RNA controls embryonic polarity. *Cell.* 71:301-313.

Gorman, C. M., G. T. Merlino, M. C. Willingham, I. Pastan, and B. Howard. 1982. The Rous Sarcoma Virus long terminal repeat is a strong promoter when introduced into a variety of eukaryotic cells by DNA-mediated transfection. *Proc. Natl. Acad. Sci. USA.* 79:6777-6781.

Gunning, P., P. Ponte, L. Kedes, R. J. Hickey, and A. I. Skoultchi. 1984. Expression of human cardiac actin in mouse L cells: a sarcomeric actin associates with a nonmuscle cytoskeleton. *Cell.* 36:709-715.

Hayward, L. J., and R. J. Schwartz. 1986. Sequential expression of chicken actin genes during myogenesis. *J. Cell Biol.* 102:1485-1493.

Hill, M., and P. Gunning. 1993. Beta and Gamma Actin mRNAs are differentially located within myoblasts. *J. Cell Biol.* 122:825-832.

Herman, I. M. 1993. Actin isoforms. *Current Opin. Cell Biol.* 5:48-55.

Hoock, T. C., P. M. Newcomb, and I. M. Herman. 1991. β-actin and its mRNA are localized at the plasma membrane and the regions of moving cytoplasm during the cellular response to injury. *J. Cell Biol.* 112:653-664.

Jackson, R. 1993. Cytoplasmic regulation of mRNA function: the importance of the 3'-untranslated region. *Cell.* 74:9-14.

Karlsson, U., S. Koorajian, I. Zabin, F. S. Sjostrand, and A. Miller. 1964. High resolution electron microscopy on highly purified β-galactosidase from *Escherichia coli. J. Ultrstruct. Res.* 10:457-469.

Kislauskis, E. H., and R. H. Singer. 1992. Determinants of RNA localization. *Curr. Opin. Cell Biol.* 4:975-978.

Lawrence, J. B., and R. H. Singer. 1986. Intracellular localization of messenger RNAs for cytoskeletal proteins. *Cell.* 45:407-415.

Lawrence, J. B., K. L. Taneja, and R. H. Singer. 1989. Temporal resolution and sequential expression of muscle-specific genes revealed by in situ hybridization. *Dev. Biol.* 133:235-246.

Lim, K., and C.-B. Chae. 1989. A simple assay for DNA transfection by incubation of the cells in culture dishes with substrates for beta-galactosidase. *Biotechniques.* 7:576-579.

Lipshitz, H. D. 1991. Axis specification in the Drosophila embryo. *Curr. Opin. Cell Biol.* 3:966-975.

Luby-Phelps, K., P. E. Castle, D. L. Taylor, and F. Lanni. 1987. Hindered diffusion of inert tracer particles in the cytoplasm of mouse 3T3 cells. *Proc. Natl. Acad. Sci. USA.* 84:4910-4913.

Macdonald, P. M., and G. Struhl. 1988. Cis-acting sequences responsible for anterior localization of bicoid mRNA in Drosophila embryos. *Nature (Lond.).* 336: 595-598.

McHugh, K. M., K. Crawford, and J. L. Lessard. 1991. A comprehensive analysis of the developmental and tissue-specific expression of the isoactin multigene family in the rat. *Dev. Biol.* 148:442-458.

McKenna, N. M., J. B. Meigs, and Y.-L. Wang. 1985. Identical distribution of fluorescently labeled brain and muscle actins in living cardiac fibroblasts and myocytes. *J. Cell. Biol.* 100:292-296.

Mowry, K. L., and D. A. Melton. 1992. Vegetal messenger RNA localization directed by a 340-nt RNA sequence element in Xenopus oocytes. *Science (Wash. DC).* 255:991-994.

Okabe, S., and N. Hirokawa. 1989. Incorporation and turnover of biotin-labeled actin microinjected into fibroblastic cells: an immunoelectron study. *J. Cell Biol.* 109: 1581-1595.

Otey, C. A., M. H. Kalnoski, and J. C. Bulinski. 1988. Immunolocalization of muscle and non-muscle of actins in myogenic cells and adult skeletal muscle. *Cell Motil. Cytoskeleton.* 9:337-348.

Pardo, J. V., M. F. Pittenger, and S. W. Craig. 1983. Subcellular sorting of isoactins: selective association of α-actin with skeletal muscle mitochondia.

Cell. 32:1093-1103.

Paterson B. M., and J. D. Eldridge. 1984. α-cardiac actin is the major sarcomeric isoform. *Science (Wash. DC).* 224:1436-1438.

Peltz, S. W., and A. Jacobson. 1992. mRNA stability: in trans-it. *Curr. Opin. Cell Biol.* 4:979-983.

Peng, I., and D. A. Fischman. 1991. Post-translational incorporation of actin into myofibrils in vitro: evidence for isoform specificity. *Cell Motil. Cytoskeleton.* 20:158-168.

Ponte, P., P. Gunning, H. Blau, and L. Kedes. 1983. Human actin genes are single copy for alpha-skeletal and alpha-cardiac actin, but multicopy for beta- and gamma-cytoskeletal genes: 3'-untranslated regions are isotype specific, but are conserved in evolution. *Mol. Cell. Biol.* 3:1783-1791.

Ralston, E., and Z. Hall. 1992. Restricted distribution of mRNA produced from a single nucleus in hybrid myotubes. *J. Cell Biol.* 119:1063-1068.

Rastinjad, F., and H. Blau. 1993. Genetic complementation reveals a novel regulatory role for 3'-untranslated regions in growth and differentiation. *Cell.* 72:903-917.

Rubenstein, P. A. 1990. The functional importance of multiple actin isoforms. *Bioessays.* 12:309-315.

Ruzicka, D., and R. J. Schwartz. 1988. Sequential activation of α-actin genes during avian cardiogenesis: vascular smooth muscle α-actin gene transcripts mark the onset of cardiomyocyte differentiation. *J. Cell Biol.* 107:2575-2586.

Sadano, H., S. Taniguchi, and T. Baba. 1990. Newly identified type of β-actin reduces invasiveness of mouse B16-melanoma. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 271:23-27.

Sawtell, N. M., and J. L. Lessard. 1989. Cellular distribution of smooth muscle actins during mammalian embryogenesis: expression of the α-vascular but not the γ-enteric isoform in differentiating striated myocytes. *J. Cell Biol.* 109:2929-2937.

Sawtell, N., A. L. Hartman, and J. L. Lessard. 1988. Unique actin isoforms are present in the brush border of rat intestinal epithelial cells. *Cell Motil. Cytoskeleton.* 11:318-325.

Schevzov, G., C. Lloyd, and P. Gunning. 1992. High level expression of transfected β- and γ-actin genes differentially impacts on myoblast architecture. *J. Cell Biol.* 117:775-785.

Singer, R. H. 1992. The cytoskeleton and mRNA localization. *Curr. Opin. Cell Biol.* 4:15-19.

Sundell, C. L., and R. H. Singer. 1990. Actin mRNA localizes in the absence of protein synthesis. *J. Cell Biol.* 111:2397-2403.

Sundell, C. L., and R. H. Singer. 1991. Requirement of microfilaments in sorting actin messenger RNA. *Science (Wash. DC).* 253:1275-1277.

St. Johnston, D., and C. Nusslein-Volhard. 1992. The origin of pattern and polarity in the Drosophila embryo. *Cell.* 68:201-219.

Taneja, K. L., and R. H. Singer. 1987. Use of oligonucleotide probes for quantitative hybridization to actin mRNA. *Anal. Biochem.* 166:389-398.

Taneja, K. L., and R. H. Singer. 1990. Detection and localization of actin mRNA isoforms in chicken muscle cells by in situ hybridization using biotinylated oligonucleotide probes. *J. Cell. Biochem.* 44:241-252.

Taneja, K. L., L. M. Lifshitz, F. S. Fay, and R. H. Singer. 1992. Poly(A) RNA codistribution with microfilaments: Evaluation by in situ hybridization and quantitative digital imaging microscopy. *J. Cell Biol.* 119:1245-1260.

Theriot, J. A., and T. J. Mitchison. 1991. Actin microfilament dynamics in locomoting cells. *Nature (Lond.).* 352:126-131.

Vandekerckhove, J., and K. Weber. 1978. At least six different actins are expressed in a higher mammal: an analysis based on the amino acid sequence of the amino-terminal tryptic peptide. *J. Mol. Biol.* 122:783-802.

Vandekerckhove, J., and K. Weber. 1984. Chordate muscle actins differ distinctly from invertebrate muscle actins: the evolution of the different muscle actins. *J. Mol. Biol.* 179:391-413.

Yaffe, D., U. Nudel, Y. Mayer, and S. Neuman. 1985. Highly conserved sequences in the 3' untranslated region of mRNAs coding for homologous proteins in distantly related species. *Nucleic Acids Res.* 13:3723-3737.