Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1763 (2006) 833–843
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.elsevier.com/locate/bbamcr

The 3′-untranslated region directs ribosomal protein-encoding mRNAs to specific cytoplasmic regions

Annapina Russo [a,1], Giulia Russo [a,*,1], Monica Cuccurese [a], Corrado Garbi [b], Concetta Pietropaolo [a,*]

[a] Dipartimento di Biochimica e Biotecnologie Mediche, Università Federico II, Via Sergio Pansini 5, Napoli 80131, Italy
[b] Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università Federico II, Via Sergio Pansini 5, Napoli 80131, Italy

Received 15 December 2005; received in revised form 21 April 2006; accepted 10 May 2006
Available online 23 May 2006

Abstract

mRNA localization is a conserved post-transcriptional process crucial for a variety of systems. We have analyzed the subcellular distribution of mRNAs encoding human cytosolic and mitochondrial ribosomal proteins. Biochemical fractionation experiments showed that the transcripts for cytosolic ribosomal proteins associate preferentially with the cytoskeleton via actin microfilaments. Transfection in HeLa cells of a GFP reporter construct containing the cytosolic ribosomal protein L4 3′-UTR showed that the 3′-UTR is necessary for the association of the transcript to the cytoskeleton. Using confocal analysis we demonstrate that the chimeric transcript is specifically associated with the perinuclear cytoskeleton. We also show that mRNA for mitochondrial ribosomal protein S12 is asymmetrically distributed in the cytoplasm. In fact, this transcript was localized mainly in the proximity of mitochondria, and the localization was 3′-UTR-dependent. In summary, ribosomal protein mRNAs constitute a new class of localized transcripts that share a common localization mechanism.

Keywords: mRNA localization; Ribosomal protein; Cytoskeleton; 3′-UTR localization signal; In situ hybridization

1. Introduction

mRNA localization, which is a post-transcriptional process found in several types of organisms and cells [1], appears to exert diverse biological functions. Most mRNA localization data come from *Drosophila melanogaster*, in which the intracellular distribution of transcripts plays a critical role in cell organization and development [2]. In *Xenopus laevis*, several mRNAs are asymmetrically distributed during formation of the dorsal–ventral axis in the oocyte [3]. The specific distribution of some mRNAs at the dendritic or axonal regions of neurons results in an asymmetry that determines the highly polarized state of these cells [4]. Although observed primarily in highly polarized cells, an asymmetrical distribution of mRNAs has been observed also in other mammalian cells. For example, in fibroblasts the mRNA encoding the nuclear protein c-myc localizes at the perinuclear cytoplasm [5], whereas OXA1 mRNA localizes in the proximity of mitochondria [6]. According to the most plausible hypothesis, this subcellular localization results in efficient production of encoded proteins in the cell region where they are required, and eventually facilitates their efficient targeting [7,8].

The molecular mechanisms involved in mRNA localization include directional transport along the cytoskeleton, general degradation coupled with localized RNA stability, and random cytoplasmic diffusion and trapping [9]. Although a combination of these mechanisms may be used to localize mRNAs, in most systems correct localization of transcripts depends on the integrity of the cytoskeleton [10,11], because it occurs by way of a microtubule-dependent mechanism [12–15], or by way of actin microfilaments [1,16]. Also in non-polarized mammalian

* Corresponding authors. G. Russo is to be contacted at tel.: +39 0817463061; fax: +39 0817463074. C. Pietropaolo, tel.: +39 0817463065; fax: +39 0817463074.
E-mail addresses: russog@dbbm.unina.it (G. Russo), pietropaolo@dbbm.unina.it (C. Pietropaolo).
[1] These authors contributed equally to this work.

0167-4889/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2006.05.010

cells, transcripts such as c-myc are associated with the cytoskeleton [17]. Thus, interaction of mRNA with the cytoskeletal network appears to be a general mechanism of asymmetric accumulation of certain mRNAs at specific cytoplasmic sites.

All localized mRNAs contain cis-acting elements that are essential for their subcellular localization. Although these elements occur in coding as well as in untranslated regions, most localized mRNAs contain targeting sequences, termed zip-codes, in their 3′-UTR [1]. These sequences appear to be unique to each mRNA. They can consist of a single nucleotide sequence [18] or multiple partially redundant elements [19], which might fold in intricate stem–loop structures. Various attempts have been made to identify the proteins involved, in trans, in the diverse steps of the localization process, i.e., recognition of localization sequences, transport of RNA, and anchoring and control of its local stability. Recently, two RNA-binding proteins, ZBP1 and ZBP2, have been found to bind to a zip-code with high specificity and cooperate to localize β-actin mRNA [20].

Cytosolic and mitochondrial ribosomal proteins (r-proteins) undergo intense cell trafficking. In fact, they are encoded by the nuclear genome and are eventually localized in distinct, specific cytoplasmic regions. Cytosolic r-proteins, after being synthesized in the cytoplasm, are transported into the nucleus to assemble, in the nucleolus, with nascent rRNA. Rapid transfer into the nucleus is crucial because unassembled r-proteins are toxic to the cell [21] and are rapidly degraded [22,23]. Hovland et al. [17] observed that mRNAs encoding cytosolic r-proteins L4 and S6 are mostly associated with cytoskeleton-bound polysomes. They postulate that by binding to the cytoskeleton, mRNAs coding for nuclear proteins would be retained in the perinuclear cytoplasm and so promote efficient transport of the newly synthesized proteins to the nucleus. These observations suggest that specific localization of mRNAs for these r-proteins in the cytoplasm can serve to ensure the efficiency of protein targeting. Mitochondrial biogenesis, on the other hand, needs the expression of both nuclear and mitochondrial genomes, and mitochondrial r-proteins must be correctly addressed to the mitochondrial compartment in synchrony with biogenesis. Thus, it is conceivable that also the transcripts encoding mitochondrial r-proteins are asymmetrically distributed in the cytoplasm. Support for this hypothesis comes from the identification in yeast of >100 mRNAs that encode mitochondrial proteins associated to mitochondria-bound polysomes [24].

The aims of our study were to determine the subcellular distribution of mRNAs that encode human cytosolic and mitochondrial r-proteins (rp-mRNAs), and to identify the transcript regions containing the signal that directs the mRNA to a specific cytoplasmic region. Our data indicate that rp-mRNAs constitute a new class of transcripts distributed asymmetrically in non-polarized somatic mammalian cells, and that rp-mRNAs act via a common molecular mechanism of localization involving cis-acting elements present in the 3′-UTR.

2. Materials and methods

2.1. Drug treatment

To study the association of cytosolic rp-mRNAs with cytoskeleton, HeLa cells, cultured in Dulbecco's modified Eagle Medium supplemented with 10% fetal bovine serum, were treated for 20 min before harvesting subcellular fractions with compounds known to disrupt the integrity of distinct components of the cytoskeleton. Specifically, cells were treated with 40 μM nocodazole (Calbiochem) or 5 μM latrunculin A (Calbiochem) to depolymerize the microtubule network and actin filaments, respectively. To verify that the drugs resulted in the depolymerization of microfilaments or microtubules, cells were processed for western experiments using antibodies against actin (all isoforms, Sigma A2103) or β-tubulin (Amersham). Cells treated with latrunculin A or nocodazole were also processed for fluorescence staining experiments using rhodamine-conjugated phalloidin (Molecular Probes), or rodamine-conjugated α-tubulin antibodies (Santa Cruz).

2.2. Cellular fractionation

To analyze the subcellular distribution of cytosolic rp-mRNAs, HeLa cells were separated into S and Ck fractions [25]. Briefly, cells from two 35-mm plate (2×10^6 cells) were washed with ice cold PBS 1× and treated with 1 ml of ice cold extraction buffer (10 mM Pipes, pH 6.8, 100 mM KCl, 2.5 mM $MgCl_2$, and 0.1% Triton X-100) at 4 °C for 1 min. The material obtained under these conditions was referred to as the "S fraction". Subsequently, the cell remnants were scraped in 1 ml of ice cold cytoskeleton buffer (20 mM HEPES, pH 7.5, 0.5 M NaCl, 30 mM Mg-acetate, 0.5% deoxycholate, and 1% Tween-20) and left for 5 min on ice. This suspension was passed through a low-gauge needle and centrifuged for 5 min at 4 °C at 10,000×*g*. The recovered supernatant was referred to as the "Ck fraction". Lactate dehydrogenase activity was measured in these fractions by using a commercially available kit (Sigma-Aldrich) according to the manufacturer's instructions (Table 1a). To study the distribution of mRNA for mitochondrial rpS12, subcellular fractionation of cells was carried out using a standard protocol for mitochondrial isolation, slightly modified [26]. Essentially, cells from four 100-mm plate (2×10^7 cells) were washed once with ice cold PBS 1×; harvested in 1.3 ml of ice cold PBS 1× and centrifuged at 250×*g* for 10 min at 4 °C. The cell pellet was resuspended by gentle pipetting it in 10 volumes of ice cold 0.133 M NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 25 mM Tris–HCl, pH 7.5, and centrifuged again at 250×*g* for 10 min at 4 °C. The pellet was resuspended by pipetting up and down in 500 μl of ice-cold 10 mM NaCl, 1.5 mM $CaCl_2$, 10 mM Tris–HCl, pH 7.5, and kept on ice for 15 min.

Table 1
LDH activity in soluble and cytoskeletal fractions

a

Fraction	Control	Lat A	Noc	GFP	GFP/rpL4
Soluble	95.5 ± 0.3	93.1 ± 0.5	94.8 ± 0.5	92.4 ± 0.5	96.8 ± 0.1
Cytoskeletal	4.5 ± 0.3	6.9 ± 0.3	5.2 ± 0.5	7.6 ± 0.6	3.2 ± 0.5

b

Fraction	Control	GFP	GFP/rpS12
Cytosol	95.8 ± 1.0	96.3 ± 0.7	97.8 ± 1.0
Mitochondrial	4.2 ± 0.4	3.7 ± 0.2	2.2 ± 0.5

Aliquots from each fraction were assayed for LDH. As expected, the preferential activity of LDH resulted within the soluble fraction (a) and the cytosolic fraction (b). Data are expressed as a percent of the total activity. The heading for each column represents the different experiments performed in this study: HeLa cells untreated (control), cells treated with latrunculin A (Lat A), nocodazole (Noc), cells transfected with GFP vector and chimaeric construct containing the cytosolic rpL4 3′UTR (GFP/rpL4) (a); HeLa cells untreated (control), cells transfected with GFP vector and chimeric construct containing mitochondrial rpS12 3′UTR (GFP/rpS12) (b).

An equal volume of ice cold 0.7 M sucrose, 0.21 M mannitol, 2 mM EDTA, 20 mM Tris–HCl, pH 7.5 was added, and nuclei and cell debris were removed by two sequential centrifugations at 600×*g* for 10 min at 4 °C. The resulting supernatant was then centrifuged at 11,000×*g* for 30 min at 4 °C to separate "raw"cytosolic (supernatant) and mitochondrial (pellet) fractions. The mitochondria from four 100-mm plates were washed once with 1 ml of ice cold 0.1 M NaCl, 50 mM Tris–HCl, pH 8.5, 10 mM EDTA, and resuspended in a final volume of 300 μl of the same buffer immediately prior to nucleic acid isolation. In these fractions LDH activity was measured as described above (Table 1b).

2.3. Immunoblot analysis

Proteins were extracted from S and Ck fractions. Aliquots of samples (30 μg) were resolved by 12% SDS-gel electrophoresis and transferred into nitrocellulose filters. The membranes were blocked in PBS 1×, 0.2% Tween and 5% dry milk for 2 h, and then actin (Sigma) and tubulin (Amersham) were revealed by specific antibodies and peroxidase-conjugated antibodies (Amersham). An enhanced chemiluminescence (ECL) system (Amersham) was used for detection.

2.4. Generation of reporter constructs

The full-length cytosolic rpL4 and mitochondrial rpS12 3′-UTRs were obtained by RT-PCR using total RNA isolated from HeLa cells as template and specific primers (Table 2). The 3′-UTR fragments were subsequently inserted downstream from the reporter gene GFP in the pEGFP-C1 expression vector (Clontech) using the *Kpn*I and *Bam*HI cloning sites for cytosolic rpL4 3′-UTR and the *Hin*dIII and *Bam*HI cloning sites for the mitochondrial rpS12 3′-UTR. All fusion plasmids, prepared using QIAGEN kits, were sequenced to verify the accuracy of the constructs.

2.5. Cell transfection

Transfection was carried out using LipofectAMINE™ 2000 (Invitrogen). The cells were grown in 35-mm plates (GFP/rpL4 construct) or 100-mm plates (GFP/rpS12 construct) until 80–90% confluent and were then covered with a mixture of specific DNA and LipofectAMINE, as indicated by the manufacturer. Total RNA extracted from the cells transiently transfected with GFP/rpL4 or GFP/rpS12 constructs was analyzed by semiquantitative RT-PCR 24 h after transfection.

2.6. RNA extraction, northern blotting and semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)

All biochemical fractions were treated with 200 μg/ml proteinase K (Roche) in 0.5% SDS for 30 min at 37 °C and total RNA was purified by repeated phenol/chloroform (v/v 1:1) extractions, and ethanol precipitated. The distribution of cytosolic rp-mRNAs between the S and Ck fractions was analyzed in northern blotting experiments. Aliquots (20 μg) of total RNA for each fraction were fractionated by electrophoresis through a 1.5% denaturating formaldehyde-agarose gel, and then transferred to a positively-charged nylon membrane (Millipore) by capillary blotting. The probes for β-actin mRNA and cytosolic rpL3, rpL4, rpL7a, rpL12, rpL15 mRNAs were DNA fragments obtained by RT-PCR amplification directed by specific oligonucleotides (Table 2), using total RNA from HeLa cells as a template. Labeling of DNA fragments was performed by random priming reaction (Promega) with [α^{32}P]dCTP, according to the manufacturer's instructions. Hybridization with radiolabeled probes was performed as described by Church and Gilbert [27]. A 28S rRNA synthetic oligonucleotide was used as standard to correct any difference in loading. The relative abundance of each individual mRNA species was determined by using the PhosphorImager STORM 840 system (Amersham). The level of transcripts in the cytosolic and mitochondrial fractions was determined with RT-PCR. After DNase treatment, 1 μg of RNA was reverse-transcribed into cDNA by the random hexamers technique using 200 units of Superscript II RNAse H^- Reverse Transcriptase (Invitrogen). The reaction was carried out at 42 °C for 50 min and heated to 75 °C for 15 min to terminate it. Ten microliters of the 40 μl of this reaction were PCR-amplified in a final volume of 50 μl, using 5 μM of each specific primer (Table 2), 10 mM of each dNTP, and 0.5 units of Taq DNA polymerase (Invitrogen). Typically, 25–30 cycles of amplification were performed. In separate experiments, we ascertained that the cycle number was within the linear range of amplification. PCR products were visualized on 1% agarose gels containing the fluorescent Vistra green dye (Amersham Pharmacia Biotech) [28]. The labeling intensity of the PCR product, which is linear to the amount of DNA, was quantified using the PhosphorImager STORM 840 system (Amersham).

2.7. In situ hybridization

HeLa cells, grown on 11-mm round coverslips, 24 h after transfection with GFP/rpL4 construct were washed three times with ice-cold PBS 1× containing 5 mM $MgCl_2$; fixed for 10 min with 4% paraformaldehyde in PBS 1× on ice and permeabilized with 0.1% Triton X-100 in PBS 1× for 2 min on ice. Cells were incubated in 50% formamide, SSC 2× (1× SSC: 15 mM Na-citrate, 150 mM NaCl pH 7.4), at 55 °C for 2 h and hybridized with 500 ng of digoxigenin (DIG)-labeled GFP riboprobe in 50% formamide, 2× SSC, 10% Dextran sulphate, 10 mM $NaPHO_4$, 250 μg/ml tRNA and 100 μg/ml herring sperm DNA overnight at 55 °C in a humidified atmosphere. The GFP riboprobe was generated from the 750-bp full GFP coding sequence in pGEM-4Z vector using a DIG RNA labeling kit (Roche Molecular Biochemicals), in the sense (control) and antisense orientation by using Sp6 and T7 polymerase (Roche), respectively. After hybridization, cells were washed in SSC 2× and 50% formamide for 20 min at 55 °C twice, then in SSC 2× at 55 °C once, and at room temperature twice. Non-specifically bound probe was removed by treatment with 40 μg/ml RNase A (Sigma) in wash buffer (10 mM Tris–HCl, pH 7.5, 0.4 M NaCl and 5 mM EDTA). Labeled transcripts were detected by incubation with anti-digoxigenin rhodamine-conjugated Fab fragments (Roche Diagnostics) according to the manufacturer's instructions. The samples were analyzed at room temperature by using a laser scanning confocal microscope LSM 510 version 2.8 SP1 (Zeiss), a Plan-Apochromatic 63×/1.4 objective and a TRITC filter. Images were analyzed with LSM 510 software (Zeiss).

Table 2
Oligonucleotides sequences

	5′-Primer (5′–3′)	3′-Primer (3′–5′)
β-actin	GGCACCACCTTCTACA	CAGGAGGACAATGAT
rpL3	ATGTCTCACAGAAAGTTC	AGCTCCTTCTTCCTTTGC
rpL4	ATACGCCATCTGTTCTGC	TGTTTGGCTTGTAGTGC
rpL7a	ATATGAAGTACAGACCAGAGACA	TGTGAAGGCGACATGGT
rpL12	GGAGGCCAAGGTGCAACT	ACTGGCTGGGCATTCCACA
rpL15	AGTCACAAGCGCATGGTT	TTGAACAACCTCTACACA
rpL4 3′UTR	CCTGCTGCATAAACTCTT	CATGTTTCTCACTGCCTG
rpS12 3′UTR	CTCAGAAGAAGTGACGGT	CACAGAAGAAGTGACGGCTG
GFP	TACCGGTCGCCACCATGG	CTTGTACAGCTCGTCCAT
COXII	TTATTCCTAGAACCAGGC	GGCTCTAGAGGGGGTAGA
TOM20	TGGACAGCCACAGCAGTTAC	CCCAGAGCTGCTCAACTACC

3. Results

3.1. Asymmetric distribution of cytosolic rp-mRNAs in the soluble versus the cytoskeletal cell fraction

Interactions between mRNA and cytoskeletal elements are responsible for the spatial distribution of several transcripts in mammalian somatic cells [7,29]. We used a subcellular fractionation procedure to investigate whether mRNAs encoding cytosolic r-proteins associate with the cytoskeleton, in analogy with transcripts encoding some nuclear proteins [5,17,30], and as suggested by the association of mRNAs encoding rpL4 and rpS6 to cytoskeletal-bound polysomes [17]. Using biochemical techniques [25], we isolated from HeLa cells two distinct fractions corresponding to a detergent-soluble fraction (S), containing cytosolic components, and a detergent-insoluble fraction (Ck), enriched in cytoskeleton elements. These two fractions were assayed for lactate dehydrogenase (LDH) activity, a marker component of the soluble fraction [28]. More than 90% of LDH activity occurred in the S fraction (Table 1a); thus the detergent-insoluble fraction was free of soluble fraction contaminants. A western blot analysis demonstrated that the S fraction was free of cytoskeleton proteins (Fig. 1A). Northern blots of total RNA extracted from each fraction were probed for five mRNAs that encode the human cytosolic r-proteins L3, L4, L7a, L12 and L15 (rpL3, rpL4, rpL7a, rpL12 and rpL15). We used a β-actin mRNA [31] as a control of cytoskeleton-associated transcript. As reported by others [25], about 60% of the β-actin mRNA was recovered preferentially in the insoluble fraction Ck (Fig. 1B and C). The cytosolic rp-mRNAs were significantly more abundant in the Ck fraction (Fig. 1B and C). Specifically, a quantitative analysis revealed that about 70% of these transcripts were associated with the Ck fraction (Fig. 1C). Interestingly, the association of the cytosolic r-protein transcripts (rp-transcripts) to the cytoskeleton was more pronounced than the association of the control β-actin mRNA to the same fraction.

3.2. Cytosolic rp-mRNAs associate with actin filaments

To determine which specific class of cytoskeletal elements was required for the localization of cytosolic rp-transcripts, we used latrunculin A to disrupt selectively actin filaments, and nocodazole to disrupt microtubules. Cells treated with latrunculin A were submitted to biochemical fractionation to isolate S and Ck fractions. Treatment of cells with latrunculin A resulted in depolymerization of actin filaments as shown by fluorescence (Fig. 2A). Western blotting analysis of biochemical fractions from treated cells, using antibodies against actin and tubulin, showed depolymerization only in actin filaments. In fact, no tubulin shifting from the Ck to the S fraction was observed (Fig. 2B). Northern blot analysis of total RNA extracted from these fractions revealed that β-actin mRNA was released into the S fraction (Fig. 2C), thus confirming its association with actin filaments [31]. Latrunculin A treatment also caused a dramatic redistribution of the cytoskeleton-associated rp-mRNAs from the Ck fraction to the S fraction (Fig. 2C). Quantification of the signals obtained for each transcript showed that, after

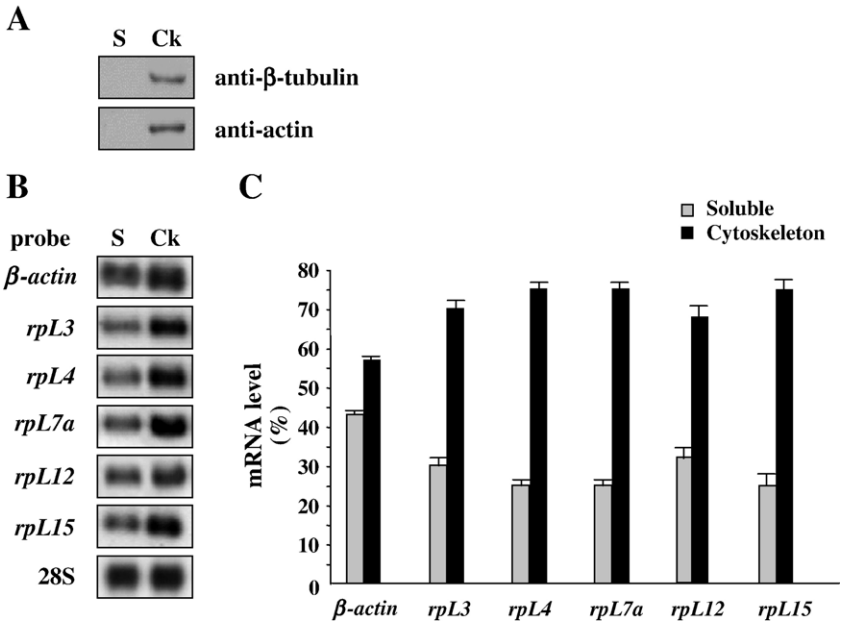

Fig. 1. Cytosolic rp-mRNAs are asymmetrically distributed between the soluble and cytoskeletal fractions. HeLa cells (2×10^6) were biochemically fractionated to separate the soluble (S) and cytoskeletal (Ck) fractions as described under Materials and methods. (A) Western blot analysis of actin and tubulin in soluble (S) and cytoskeletal (Ck) fractions. (B) 20 μg of total RNA extracted from each fraction were subjected to Northern blotting using probes for the indicated cytosolic r-proteins. A 28S rRNA probe was used as a standard. β-actin mRNA was used as a well-characterized cytoskeleton-associated transcript. (C) The hybridization signals were quantified with the PhosphorImager STORM 840 system (Amersham). For a given mRNA, the addition of both signals after normalization with *28S* rRNA signal was considered 100% and the RNA level in each fraction was expressed as a percent of the total. The results shown represent the mean ± s.d. of a minimum of three independent experiments.

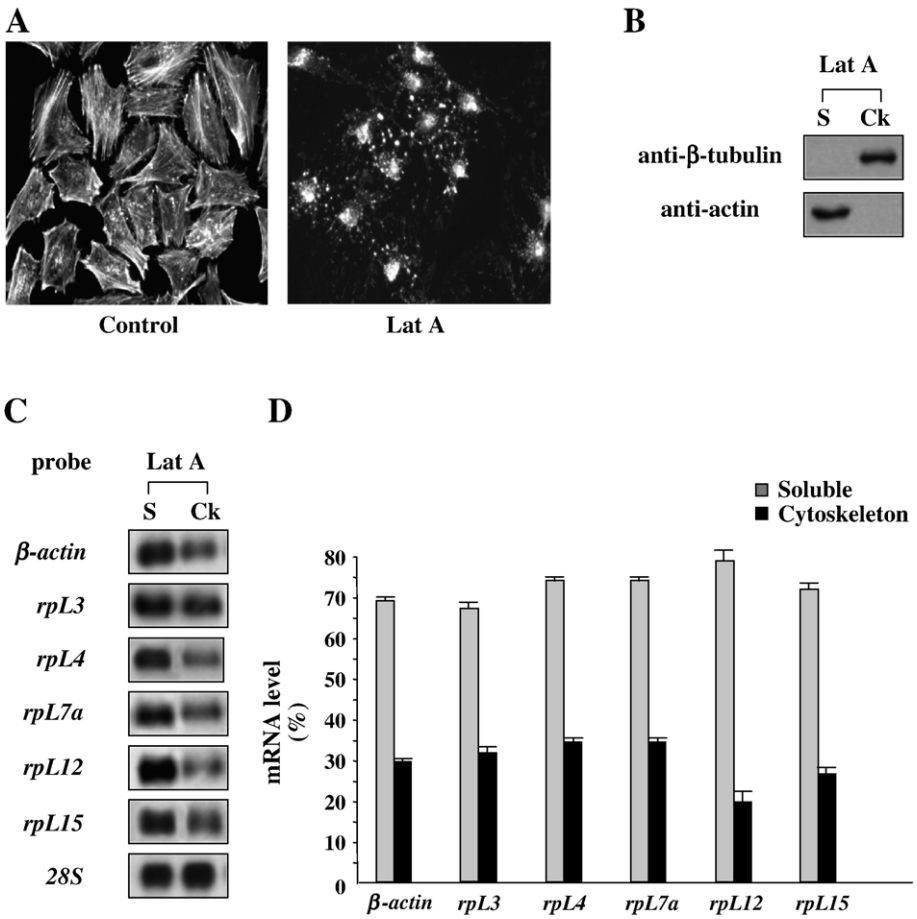

Fig. 2. Cytosolic rp-mRNAs are specifically associated with actin filaments. (A) Phalloidin-stained HeLa cells untreated (Control) and treated for 20 min with 5 μM latrunculin A (Lat A). (B) Western blot analysis of actin and tubulin in soluble (S) and cytoskeletal (Ck) fractions upon exposure to Lat A of HeLa cells. (C) HeLa cells were incubated with Lat A. Subsequently, total RNA was extracted from S and Ck fractions and subjected to northern blotting as described in the legend to Fig. 1. (D) The hybridization signals in each fraction were quantified as described in the legend to Fig. 1. The results shown represent the mean ± s.d. of a minimum of three independent experiments.

latrunculin A treatment, about 70% of the transcripts for cytosolic rpL3, rpL4, rpL7a, rpL12 and rpL15 occurred in the S fraction (Fig. 2D). These findings confirm the association of cytosolic rp-mRNAs with the cytoskeleton, and indicate that actin filaments play an essential role in mediating the association of these transcripts to the cytoskeleton. In contrast, depolymerization of microtubules, induced by nocodazole (Fig. 3A), and the consequent release of tubulin into the S fraction (Fig. 3B), did not significantly affect the distribution of mRNAs in the distinct pools (Fig. 3C, D). The minor shift (about 15%) of cytoskeleton-associated rp-mRNAs from the Ck to the S fraction was probably due to partial actin depolymerization. In fact, the western blot analysis of fractions after nocodazole treatment showed a low amount of actin in the S fraction (Fig. 3B). These results indicate that the subcellular distribution of the cytosolic rp-mRNAs does not require intact microtubules.

3.3. Subcellular distribution of mitochondrial rp-mRNAs

Studies of mRNA localization in yeast demonstrated that some mitochondrial, nuclear genome-coded transcripts are localized near the mitochondrion [8,24]. To determine whether the transcripts for mitochondrial r-proteins are localized in specific regions rather than distributed throughout the cytoplasm, we examined the subcellular distribution of the mRNA that encodes the mitochondrial r-protein S12 (rpS12). To this aim, we purified cytosolic and mitochondrial fractions from HeLa cells [26], and submitted the total RNA from both fractions to RT-PCR semi-quantitative analysis. To verify fractional efficiency, we used COXII mRNA, which encodes a mitochondrial genome-encoded cytochrome *c* oxidase II, as a marker of the mitochondrial fraction, and β-actin mRNA and cytosolic rpL4 mRNA as controls of the cytosolic fraction. As expected, the amplified COXII transcript occurred predominantly in the mitochondrial fraction (Fig. 4A). Furthermore, human TOM20, as the yeast orthologue [8], was distributed similarly in the cytosolic and the mitochondrial fraction. Consistently, densitometric analysis showed 85% of the COXII transcript in the mitochondrial fraction, and only a weak signal in the cytosolic fraction (Fig. 4B). Since β-actin and cytosolic rpL4 transcripts were recovered exclusively in the cytosolic fraction (Fig. 4A and B), the mitochondrial fraction appeared to be free of soluble fraction contaminants. We also verified the nature of the mitochondrial fraction by measuring

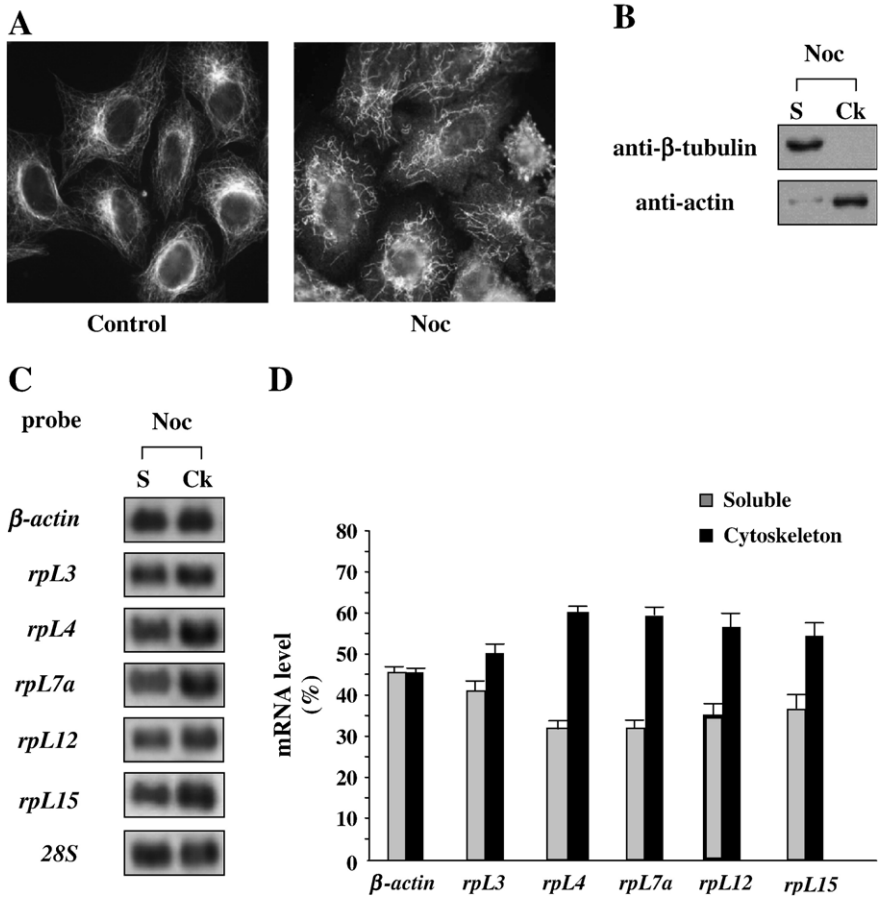

Fig. 3. Localization of cytosolic rp-mRNAs is unaffected by disorganization of microtubules. (A) Tubulin-stained HeLa cells untreated (Control) and treated with 40 μM nocodazole (Noc) for 20 min. (B) Western blot analysis of actin and tubulin in S and Ck fractions upon exposure to Noc of HeLa cells. (C) HeLa cells were incubated with nocodazole. Subsequently, total RNA was extracted from soluble (S) and cytoskeletal (Ck) fractions and subjected to northern blotting as described in the legend to Fig. 1. (D) The hybridization signals in each fraction were quantified as described in the legend to Fig. 1. The results shown represent the mean ± s.d. of a minimum of three independent experiments.

LDH activity (Table 1b). Like COXII mRNA, mRNA for mitochondrial rpS12 occurred preferentially in the mitochondrial fraction. In fact, about 80% of the total amount of the mitochondrial rpS12 transcript occurred in the mitochondrial fraction (Fig. 4B). These experiments suggest that mitochondrial rp-mRNAs are asymmetrically distributed in the cytoplasm and are associated with mitochondria.

3.4. The 3′-UTR of rp-mRNAs is competent for localization of a reporter gene

The sorting of mRNAs to specific subcellular regions is thought to depend on molecular mechanisms involving sequence signals often located in their 3′-UTR [11]. Since we demonstrated that cytosolic and mitochondrial rp-transcripts are asymmetrically distributed, we next investigated whether the 3′-UTR of these mRNAs contains signals that mediate cytoskeletal or mitochondrial targeting. To this aim we attempted to direct a reporter transcript to the cytoskeleton or to mitochondria by linking it to the cytosolic rpL4 3′-UTR, or to the mitochondrial rpS12 3′-UTR. The coding region of the GFP gene was chosen as a reporter sequence since GFP mRNA is distributed throughout the cytoplasm [32]. In these experiments we isolated the cytosolic rpL4 3′-UTR and mitochondrial rpS12 3′-UTR from HeLa cell RNA using RT-PCR, and subsequently generated GFP reporter constructs by inserting each 3′-UTR downstream from the GFP gene coding sequence (Fig. 5A). Cells were transiently transfected with GFP/rpL4, in the absence or presence of latrunculin A, or with GFP/rpS12, and then biochemically fractionated to obtain the S and Ck fractions for GFP/rpL4 transcript distribution analysis, and the cytosolic and mitochondrial fractions for GFP/rpS12 transcript localization analysis. Total RNA, extracted from each fraction, was subjected to RT-PCR. In cells transfected with control construct (GFP, Fig. 5A), the transcript was detected in the S fraction (Fig. 5B) as well as in the “raw” cytosolic fraction (Fig. 5C). This observation confirms that the transcript does not contain a localization signal. In contrast, the addition of cytosolic rpL4 3′-UTR to the reporter mRNA caused a dramatic redistribution of the corresponding transcript. In fact, this chimeric mRNA was detected exclusively in the cytoskeletal fraction which indicates that the cytosolic rpL4 3′-UTR contains information essential for the targeting of the reporter transcript to the cytoskeleton (Fig. 5B). Moreover, latrunculin A caused the release of the

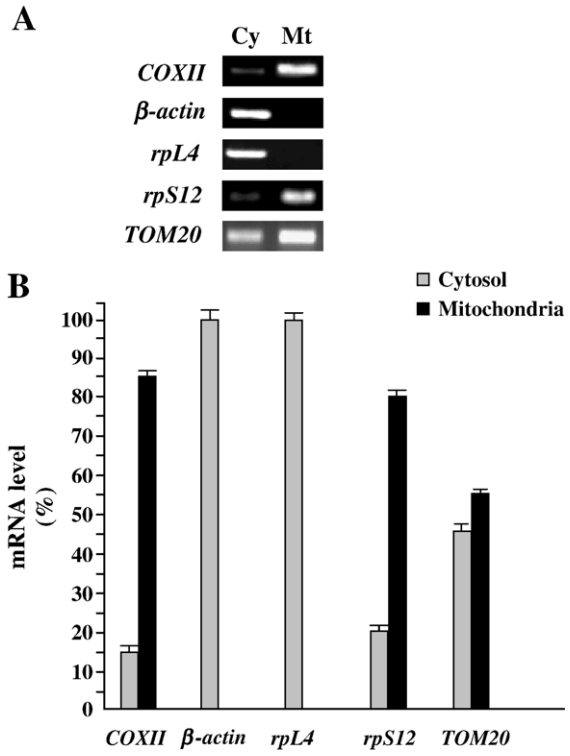

Fig. 4. Mitochondrial rp-S12 mRNA localizes to the mitochondrial fraction. (A) Examples of Vistra Green-stained agarose gels of RT-PCR products obtained from cytosol (Cy) and mitochondrial (Mt) fractions. Cross-contamination of the fractions was checked by revealing the cytosolic rpL4 and β-actin transcripts, as examples of cytosolic proteins, and COXII mRNA as a mitochondrial marker. TOM20 represents a mRNA distributed similarly between the fractions. (B) The levels of the transcripts were quantified with the PhosphorImager STORM 840 system (Amersham). The results shown represent the mean ± s.d. of a minimum of three independent experiments.

chimeric transcript from the Ck to the S fraction, thereby demonstrating that the 3′-UTR is directly involved in the association of mRNA with actin microfilaments (Fig. 5B). We next analyzed the distribution pattern of the transcript produced by the GFP/rpS12 construct, which contains the mitochondrial rpS12 3′-UTR (Fig. 5A). In cells transfected with this chimeric construct, the corresponding mRNA associated with the mitochondrial fraction, as did the *COXII* mRNA (Fig. 5C). These data indicate that also the 3′-UTR region of mitochondrial rp-mRNAs possesses cis-acting elements that guide the mRNA to the vicinity of mitochondria. Thus, an mRNA localization strategy, common to cytosolic and mitochondrial rp-mRNAs, involves directional information contained in the mRNA itself.

3.5. The cytosolic rpL4 3′-UTR is responsible for directing a reporter gene to the perinuclear cytoplasm

We conducted non-radioactive in situ hybridization to detect the spatial localization of the GFP/rpL4 transcript. Fixed HeLa cells, transiently transfected with the GFP/rpL4 or GFP construct (Fig. 5A), were hybridized with a digoxigenin-labeled riboprobe corresponding to the entire coding region of the GFP transcript, in the antisense orientation, and, as a control, in the sense orientation. The signals were visualized using digoxigenin rhodamine-conjugated antibody and confocal microscopy. Minimal, random staining was observed when cells were transfected with construct GFP/rpL4 and hybridized with the GFP sense riboprobe (Fig. 6A). Cells transfected with the GFP construct and hybridized with the GFP antisense riboprobe showed staining throughout the cytoplasm (Fig. 6B), thereby confirming our biochemical findings (Fig. 5B, C). Cells transfected with construct GFP/rpL4 and hybridized with the GFP antisense riboprobe showed redistribution of the reporter sequence from a diffuse intracellular localization to an intense perinuclear signal that formed a rim around the nucleus (Fig. 6C). Analysis of z-sections taken through the cell nucleus showed the absence of signal in the nucleus (Fig. 6D), which confirms that highly intense staining occurs specifically in the perinuclear region. These results indicate that the cytosolic rpL4 3′-UTR contains information essential for targeting a reporter transcript to the perinuclear region.

4. Discussion

Here we show that rp-mRNAs constitute a new class of transcripts that share a preferential subcellular localization. mRNA localization mechanisms have been demonstrated in a variety of systems [1], and most localized transcripts are distributed in a cytoskeleton-dependent fashion [33]. By means of a biochemical analysis of RNA distribution in the S and Ck

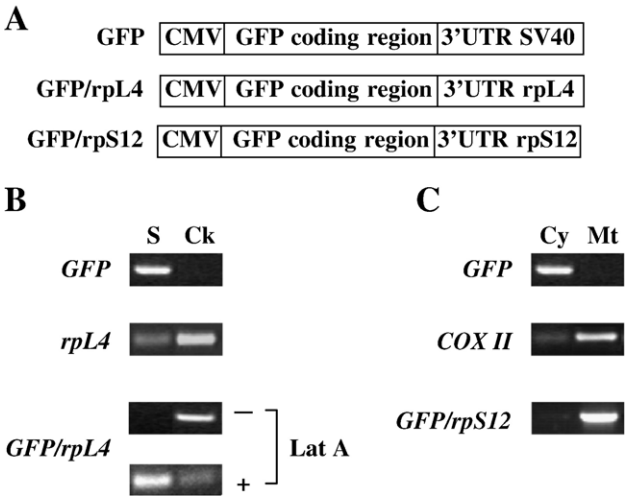

Fig. 5. The 3′-UTR of rp-mRNAs is required to target GFP mRNA to specific subcellular regions. (A) Schematic representation of the constructs tested. In all plasmids, the GFP gene is expressed under the control of CMV promoter and in the chimeric constructs the SV40 3′-UTR was replaced with the cytosolic rpL4 3′-UTR (GFP/rpL4) or mitochondrial rpS12 3′-UTR (GFP/rpS12). (B, C) HeLa cells were transiently transfected with the constructs described above. Total RNA extracted from soluble (S), cytoskeletal (Ck), cytosolic (Cy), and mitochondrial (Mt) fractions was subjected to RT-PCR analysis with the appropriate primers (Table 2). B) The distribution of GFP/rpL4 mRNA in the S and Ck fractions, with and without latrunculin A (Lat A) was analyzed by RT-PCR. C) Distribution of GFP/rpS12 in the Cy and Mt fractions analyzed by RT-PCR. Cross-contamination of the fractions was checked by revealing the endogenous cytosolic rpL4 transcript, as a cytoskeleton-associated mRNA (B), and COXII mRNA as a mitochondrial marker (C). The figure shows examples of Vistra green-stained agarose gels that correspond to the amplified products.

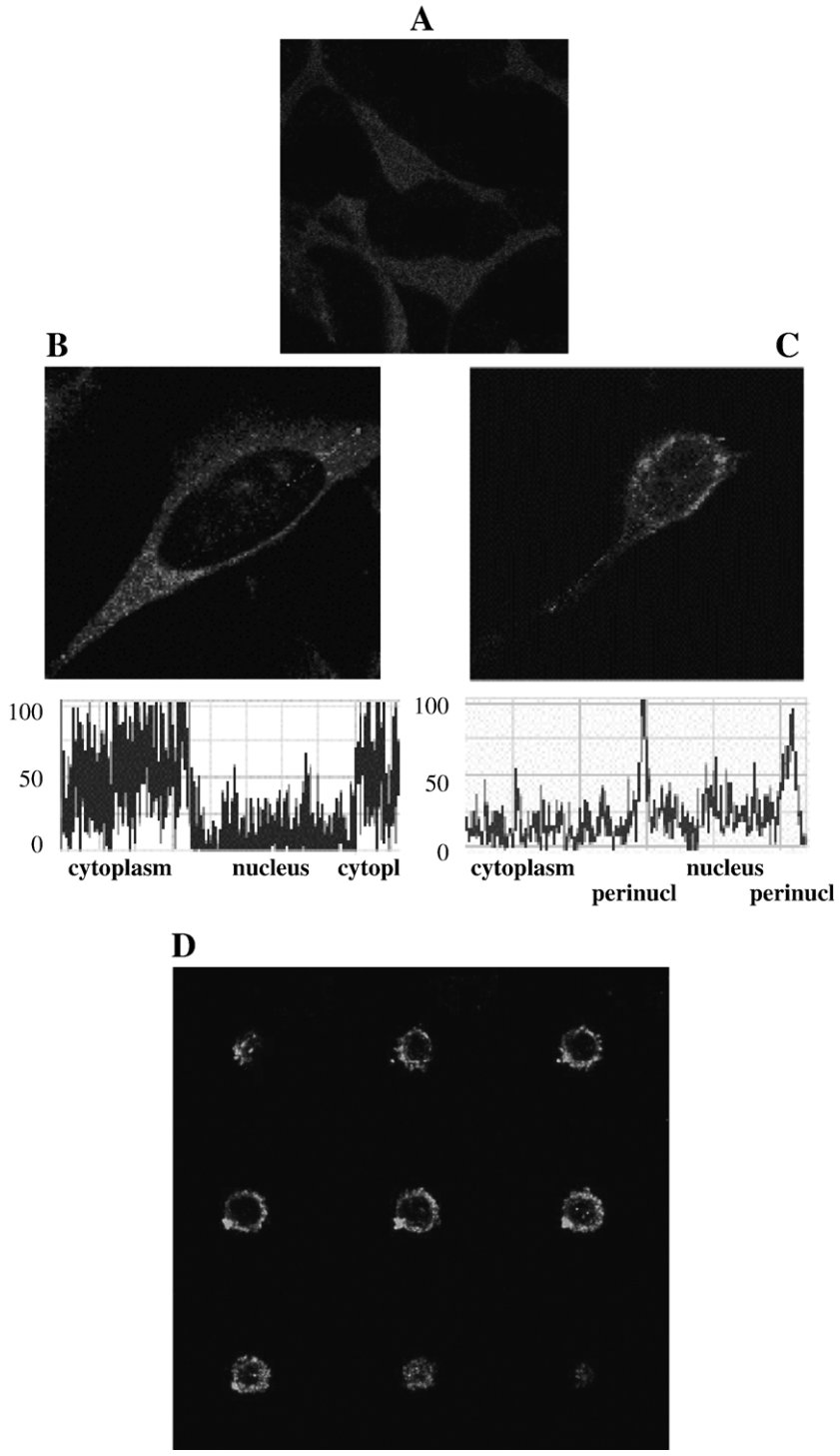

Fig. 6. Cytosolic rpL4 3′-UTR directs a reporter transcript to the perinuclear cytoplasm. HeLa cells were transiently transfected with GFP/rpL4 (A, C, D) or the GFP construct (B). The distribution of the corresponding transcript was detected by in situ hybridization using a DIG-labeled GFP riboprobe in sense (A) and antisense (B, C) orientation and rhodamine-conjugated anti-digoxigenin antibodies. (D) Z-stack gallery of 0.5-μm optical sections taken through the nuclear region from the same sample shown in (C).

fractions from HeLa cells we demonstrate that mRNAs for cytosolic rpL3, rpL4, rpL7a, rpL12 and rpL15 are asymmetrically distributed (Fig. 1B). Specifically, these mRNAs accumulate to a large degree (70% on average) in the cytoskeleton fraction (Fig. 1C). Selective depolymerization of actin microfilaments, but not of microtubules, caused a dramatic redistribution of rp-transcripts from the Ck to the S fraction (Fig. 2C, D). These data suggest that the intracellular distribution of these mRNAs requires the involvement of microfilaments. The latter could serve as a "motor" for the transport of rp-mRNAs and/or as an anchor to confine them to a specific cytoplasmic region. However, the process could be

more complex and involve such other cytoskeletal elements as the intermediate filaments, which were not considered in this study.

In yeast, a genome-wide analysis identified >100 mRNAs encoding mitochondrial proteins associated to mitochondrion-bound polysomes [24]. Our study supports the hypothesis that import of mitochondrial proteins encoded by the nuclear genome is facilitated through a co-translational process that leads to mRNA sorting in the vicinity of mitochondria [6]. We show that also mitochondrial ribosomal protein mRNAs are asymmetrically distributed in the cytoplasm. Our cell fractionation experiments demonstrate that the mRNA for mitochondrial rpS12 is distributed unequally between the cytosolic and mitochondrial fractions, and associates preferentially with mitochondria (Fig. 4A, B).

Analysis of signals that mediate mRNA localization has revealed that the 3′-UTR is the region that contains the cis-acting sequence involved in the targeting of most transcripts [34]. For example, the metallothionein-1 3′-UTR targets a reporter mRNA to the cytoskeleton [30], and in yeast the 3′-UTR of ATP2 is required to direct a reporter transcript to the mitochondria [35]. The role of the 3′-UTR in targeting mRNA to the mitochondria appears to be conserved from yeast to human cells [6]. Consequently, we asked whether the localization of the rp-mRNAs examined in our study is mediated by a sequence in the 3′-UTR. To address this issue, we produced chimeric constructs containing the cytosolic *rpL4* 3′-UTR or mitochondrial *rpS12* 3′-UTR downstream from the *GFP* gene and expressed them in HeLa cells. RT-PCR analysis of the transcripts in the various subcellular fractions showed that the addition of mitochondrial *rpS12* 3′-UTR results in translocation of *GFP* mRNA to the proximity of the mitochondria. In fact, it was associated to the mitochondrial fraction (Fig. 5A, C). On the other hand, the *rpL4* 3′-UTR is responsible for the association of the chimeric mRNA to the cytoskeleton, via actin microfilaments (Fig. 5A, B), and is sufficient to target the *GFP* mRNA to the perinuclear region (Fig. 6). These data demonstrate that the 3′-UTRs tested contain sequences able to target a transcript close to where the protein will function. The mechanism by which 3′-UTR sequences target a transcript to the perinuclear cytoskeleton or to the mitochondrion remains unclear. Very likely, these sequences fold into secondary structures recognized by specific trans-acting factors. Sequence elements relevant for the cytoskeleton association and localization of several mRNAs in the perinuclear cytoplasm have been identified; these range from segments with a defined nucleotide sequence to repeated short motifs scattered throughout the 3′-UTR. In the *c-myc* 3′-UTR, an AU-rich sequence element responsible for its localization lies within 86 nucleotides [36], and in the c-fos 3′-UTR the signal consist of a 145 nucleotide stretch [37]. The vimentin 3′-UTR contains a highly conserved region of 100 nucleotides that is able to localize a reporter transcript to the perinuclear cytoplasm [32]. This region is predicted to fold into a Y-shaped, stem–loop secondary structure that is able to bind protein complexes including HAX-1 [38]. An eleven-nucleotide motif, containing a CACC sequence repeat has been shown to be critical for the perinuclear localization of rat metallothionein-1 mRNA [39]. The authors suggest that the CACC containing motif may fold into a configuration comparable to that proposed for β-actin mRNA in which ZBP1 binds a CAC-containing motif. Recently, a bioinformatic analysis has identified CAC-rich clusters in localized transcripts in all chordates, including some human gene transcripts [40]. The authors suggest that these elements may work in a variety of mRNA localization pathways through slight variations in sequence composition, differences in sequence context or in higher order structure, or a combination of these features, thereby determining the specificity of mRNA localization.

A catalog of motifs that may regulate the stability and subcellular localization of mRNAs in yeast has recently been produced [41]. Twenty-three subcellular localization motifs were identified including some mitochondrial localization motifs. Of these, the M1 motif (TGTAHATA) has been found over-represented in mRNAs enriched at the mitochondrial surface, is highly conserved in several yeasts and authors proposed this conservation as having functional implications. A TGTA core sequence is present in the 3′-UTR of human mitochondrial rpS12; however, experimental data are needed to give a functional significance to these sequence elements.

Regarding the biological significance of the asymmetric distribution of rp-mRNAs in the cytoplasm, our data indicate that once transcribed in the nucleus, cytosolic rp-mRNAs are exported to the cytoplasm and directed to the actin cytoskeleton by localization elements contained in the mRNA 3′-UTR. The association of cytosolic rp-mRNAs to the cytoskeleton could be essential for the targeting and/or anchoring to the perinuclear region [17]. In turn, this selective localization might contribute to the rapid, efficient import of the newly synthesized r-proteins into the nucleus. In fact, the cytosolic rp-transcripts, anchored to the perinuclear cytoskeleton, could serve as templates for various translation cycles by cytoskeleton-associated polysomes [42]. Therefore, high levels of the corresponding proteins, directed by nuclear and nucleolar localization peptide signals [43], could enter in the nucleus and participate in ribosome biogenesis in the nucleolus.

The specific subcellular distribution of mitochondrial rp-mRNAs could also play a crucial role in the biogenesis of mitochondria. Previous studies led to the notion that the outer surface of mitochondria is rich in ribosomes and that translation and import are coupled processes [44,45]. The proximity of the mitochondrial rp-transcripts to the mitochondrion might contribute to the control of mitochondrial activity. Indeed, mRNA localization could promote the rapid co-translational import of the mitochondrial r-proteins into the organelle to ensure the biosynthesis of proteins encoded by the mitochondrial genome.

In conclusion, we believe that the spatial organization of rp-mRNAs serves a regulatory function in the complex mechanism of rp-gene expression. Although transcriptional control is central to this regulation, r-proteins are regulated at translational level in response to the growth status or to physiological stimulation [46,47]. Furthermore, some r-proteins post-transcriptionally regulate the expression of their own genes by

modulating the production of alternative transcripts that are targets of nonsense-mediated mRNA decay [48,49]. Based on our results, together with the fact that some mRNAs are translated only if they are correctly located [11], we propose that the asymmetric intracellular distribution of rp-mRNAs is a new post-transcriptional mode of rp-gene regulation.

Acknowledgements

This work has been supported by MIUR, Progetti di Ricerca di Interesse Nazionale (PRIN 2003, PRIN 2005) and Fondo Investimenti Ricerca di Base (FIRB 2001). We are grateful to Fabrizio Loreni for kindly providing the mitochondrial rpS12 cDNA. We are indebted to Jean Gilder for editing the text.

References

[1] R.P. Jansen, mRNA localization: message on the move, Nat. Rev., Mol. Cell. Biol. 2 (2001) 247–256.
[2] D. St Johnston, C. Nusslein-Volhard, The origin of pattern and polarity in the Drosophila embryo, Cell 68 (1992) 201–209.
[3] D.A. Melton, A. Ruiz i Altaba, J. Yisraeli, S. Sokol, Localization of mRNA and axis formation during Xenopus embryogenesis, Ciba Found. Symp. 144 (1989) 16–29 (discussion 29–36, 92–98).
[4] C. Job, J. Eberwine, Localization and translation of mRNA in dendrites and axons, Nat. Rev., Neurosci. 2 (2001) 889–898.
[5] J. Hesketh, G. Campbell, M. Piechaczyk, J.M. Blanchard, Targeting of c-myc and beta-globin coding sequences to cytoskeletal-bound polysomes by c-myc 3′ untranslated region, Biochem. J. 298 (Pt. 1) (1994) 143–148.
[6] J. Sylvestre, A. Margeot, C. Jacq, G. Dujardin, M. Corral-Debrinski, The role of the 3′ untranslated region in mRNA sorting to the vicinity of mitochondria is conserved from yeast to human cells, Mol. Biol. Cell 14 (2003) 3848–3856.
[7] M. Levadoux, C. Mahon, J.H. Beattie, H.M. Wallace, J.E. Hesketh, Nuclear import of metallothionein requires its mRNA to be associated with the perinuclear cytoskeleton, J. Biol. Chem. 274 (1999) 34961–34966.
[8] M. Corral-Debrinski, C. Blugeon, C. Jacq, In yeast, the 3′ untranslated region or the presequence of ATM1 is required for the exclusive localization of its mRNA to the vicinity of mitochondria, Mol. Cell. Biol. 20 (2000) 7881–7892.
[9] M. Kloc, N.R. Zearfoss, L.D. Etkin, Mechanisms of subcellular mRNA localization, Cell 108 (2002) 533–544.
[10] M. Lopez de Heredia, R.P. Jansen, mRNA localization and the cytoskeleton, Curr. Opin. Cell Biol. 16 (2004) 80–85.
[11] I.M. Palacios, D. St Johnston, Getting the message across: the intracellular localization of mRNAs in higher eukaryotes, Annu. Rev. Cell Dev. Biol. 17 (2001) 569–614.
[12] J.K. Yisraeli, S. Sokol, D.A. Melton, A two-step model for the localization of maternal mRNA in Xenopus oocytes: involvement of microtubules and microfilaments in the translocation and anchoring of Vg1 mRNA, Development 108 (1990) 289–298.
[13] S. Lall, H. Francis-Lang, A. Flament, A. Norvell, T. Schupbach, D. Ish-Horowicz, Squid hnRNP protein promotes apical cytoplasmic transport and localization of Drosophila pair-rule transcripts, Cell 98 (1999) 171–180.
[14] G.J. Bassell, H. Zhang, A.L. Byrd, A.M. Femino, R.H. Singer, K.L. Taneja, L.M. Lifshitz, I.M. Herman, K.S. Kosik, Sorting of beta-actin mRNA and protein to neurites and growth cones in culture, J. Neurosci. 18 (1998) 251–265.
[15] J.H. Carson, K. Worboys, K. Ainger, E. Barbarese, Translocation of myelin basic protein mRNA in oligodendrocytes requires microtubules and kinesin, Cell. Motil. Cytoskeleton 38 (1997) 318–328.
[16] P. Chartrand, R.H. Singer, R.M. Long, RNP localization and transport in yeast, Annu. Rev. Cell Dev. Biol. 17 (2001) 297–310.
[17] R. Hovland, G. Campbell, I. Pryme, J. Hesketh, The mRNAs for cyclin A, c-myc and ribosomal proteins L4 and S6 are associated with cytoskeletal-bound polysomes in HepG2 cells, Biochem. J. 310 (Pt. 1) (1995) 193–196.
[18] A.P. Chan, M. Kloc, L.D. Etkin, fatvg encodes a new localized RNA that uses a 25-nucleotide element (FVLE1) to localize to the vegetal cortex of Xenopus oocytes, Development 126 (1999) 4943–4953.
[19] E.R. Gavis, D. Curtis, R. Lehmann, Identification of cis-acting sequences that control nanos RNA localization, Dev. Biol. 176 (1996) 36–50.
[20] Y. Oleynikov, R.H. Singer, Real-time visualization of ZBP1 association with beta-actin mRNA during transcription and localization, Curr. Biol. 13 (2003) 199–207.
[21] W.H. Mager, Control of ribosomal protein gene expression, Biochim. Biophys. Acta 949 (1988) 1–15.
[22] L.H. Bowman, The synthesis of ribosomal proteins S16 and L32 is not autogenously regulated during mouse myoblast differentiation, Mol. Cell. Biol. 7 (1987) 4464–4471.
[23] P. Pierandrei-Amaldi, N. Campioni, P. Gallinari, E. Beccari, I. Bozzoni, F. Amaldi, Ribosomal-protein synthesis is not autogenously regulated at the translational level in *Xenopus laevis*, Dev. Biol. 107 (1985) 281–289.
[24] P. Marc, A. Margeot, F. Devaux, C. Blugeon, M. Corral-Debrinski, C. Jacq, Genome-wide analysis of mRNAs targeted to yeast mitochondria, EMBO Rep. 3 (2002) 159–164.
[25] M. Mallardo, S. Schleich, J. Krijnse Locker, Microtubule-dependent organization of vaccinia virus core-derived early mRNAs into distinct cytoplasmic structures, Mol. Biol. Cell 12 (2001) 3875–3891.
[26] D.P. Tapper, R.A. Van Etten, D.A. Clayton, Isolation of mammalian mitochondrial DNA and RNA and cloning of the mitochondrial genome, Methods Enzymol. 97 (1983) 426–434.
[27] G.M. Church, W. Gilbert, Genomic sequencing, Proc. Natl. Acad. Sci. U. S. A. 81 (1984) 1991–1995.
[28] A.O. Gramolini, G. Belanger, B.J. Jasmin, Distinct regions in the 3′ untranslated region are responsible for targeting and stabilizing utrophin transcripts in skeletal muscle cells, J. Cell Biol. 154 (2001) 1173–1183.
[29] J.E. Hesketh, I.F. Pryme, Interaction between mRNA, ribosomes and the cytoskeleton, Biochem. J. 277 (Pt. 1) (1991) 1–10.
[30] P. Mahon, K. Partridge, J.H. Beattie, L.A. Glover, J.E. Hesketh, The 3′ untranslated region plays a role in the targeting of metallothionein-I mRNA to the perinuclear cytoplasm and cytoskeletal-bound polysomes, Biochim. Biophys. Acta 1358 (1997) 153–162.
[31] C.L. Sundell, R.H. Singer, Requirement of microfilaments in sorting of actin messenger RNA, Science 253 (1991) 1275–1277.
[32] G. Bermano, R.K. Shepherd, Z.E. Zehner, J.E. Hesketh, Perinuclear mRNA localisation by vimentin 3′-untranslated region requires a 100 nucleotide sequence and intermediate filaments, FEBS Lett. 497 (2001) 77–81.
[33] H.D. Lipshitz, C.A. Smibert, Mechanisms of RNA localization and translational regulation, Curr. Opin. Genet. Dev. 10 (2000) 476–488.
[34] A. Bashirullah, R.L. Cooperstock, H.D. Lipshitz, RNA localization in development, Annu. Rev. Biochem. 67 (1998) 335–394.
[35] A. Margeot, C. Blugeon, J. Sylvestre, S. Vialette, C. Jacq, M. Corral-Debrinski, In *Saccharomyces cerevisiae*, ATP2 mRNA sorting to the vicinity of mitochondria is essential for respiratory function, EMBO J. 21 (2002) 6893–6904.
[36] J.L. Veyrune, G.P. Campbell, J. Wiseman, J.M. Blanchard, J.E. Hesketh, A localisation signal in the 3′ untranslated region of c-myc mRNA targets c-myc mRNA and beta-globin reporter sequences to the perinuclear cytoplasm and cytoskeletal-bound polysomes, J. Cell Sci. 109 (Pt. 6) (1996) 1185–1194.
[37] G. Dalgleish, J.L. Veyrune, J.M. Blanchard, H.J., mRNA localization by a 145-nucleotide region of the c-fos 3′-untranslated region. Links to translation but not stability, J. Biol. Chem. 276 (2001) 13593–13599.
[38] M. Al-Maghrebi, H. Brule, M. Padkina, C. Allen, W.M. Holmes, Z.E. Zehner, The 3′ untranslated region of human vimentin mRNA interacts with protein complexes containing eEF-1gamma and HAX-1, Nucleic Acids Res. 30 (2002) 5017–5028.
[39] D. Nury, H. Chabanon, M. Levadoux-Martin, J. Hesketh, An eleven nucleotide section of the 3′-untranslated region is required for perinuclear

localization of rat metallothionein. 1 mRNA, Biochem. J. 387 (2005) 419–428.
[40] J.N. Betley, M.C. Frith, J.H. Graber, S. Choo, J.O. Deshler, A ubiquitous and conserved signal for RNA localization in chordates, Curr. Biol. 12 (2002) 1756–1761.
[41] R. Shalgi, M. Lapidot, R. Shamir, Y. Pilpel, A catalog of stability-associated sequence elements in 3′ UTRs of yeast mRNAs, Genome Biol. 6 (2005) R86.
[42] R. Moss, I.F. Pryme, A. Vedeler, Free, cytoskeletal-bound and membrane-bound polysomes isolated from MPC-11 and Krebs II ascites cells differ in their complement of poly(A) binding proteins, Mol. Cell. Biochem. 131 (1994) 131–139.
[43] G. Russo, G. Ricciardelli, C. Pietropaolo, Different domains cooperate to target the human ribosomal L7a protein to the nucleus and to the nucleoli, J. Biol. Chem. 272 (1997) 5229–5235.
[44] M. Nobumoto, M. Yamada, S. Song, S. Inouye, A. Nakazawa, Mechanism of mitochondrial import of adenylate kinase isozymes, J. Biochem. (Tokyo) 123 (1998) 128–135.
[45] L. Ni, T.S. Heard, H. Weiner, In vivo mitochondrial import. A comparison of leader sequence charge and structural relationships with the in vitro model resulting in evidence for co-translational import, J. Biol. Chem. 274 (1999) 12685–12691.
[46] F. Loreni, F. Amaldi, Translational regulation of ribosomal protein synthesis in Xenopus cultured cells: mRNA relocation between polysomes and RNP during nutritional shifts, Eur. J. Biochem. 205 (1992) 1027–1032.
[47] R.L. Kaspar, T. Kakegawa, H. Cranston, D.R. Morris, M.W. White, A regulatory cis element and a specific binding factor involved in the mitogenic control of murine ribosomal protein L32 translation, J. Biol. Chem. 267 (1992) 508–514.
[48] Q.M. Mitrovich, P. Anderson, Unproductively spliced ribosomal protein mRNAs are natural targets of mRNA surveillance in *C. elegans*, Genes Dev. 14 (2000) 2173–2184.
[49] M. Cuccurese, G. Russo, A. Russo, C. Pietropaolo, Alternative splicing and nonsense-mediated mRNA decay regulate mammalian ribosomal gene expression, Nucleic Acids Res. 33 (2005) 5965–5977.