Targeting of c-*myc* and β-globin coding sequences to cytoskeletal-bound polysomes by c-*myc* 3′ untranslated region

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The influence of the 3′ untranslated region on mRNA localization was investigated by measuring the distribution of *myc*, β-globin and hybrid *myc*–globin mRNAs between free, cytoskeletal-bound and membrane-bound polysomes in cells transfected with either control or chimeric gene constructs. c-*myc* sequences and β-globin-coding sequences linked to the *myc* 3′ untranslated region were present at greatest enrichment in cytoskeletal-bound polysomes. β-Globin mRNA and *myc*-coding sequences linked to the β-globin 3′ untranslated region were recovered largely in the free polysomes. *In situ* hybridization confirmed that replacement of the c-*myc* 3′ untranslated region by that of globin caused a relocalization of the mRNA. The results suggest that mRNA localization in differentiated eukaryotic cells depends on a mechanism that involves directional information in the 3′ untranslated region of mRNAs.

INTRODUCTION

The complex organization of eukaryotic cells requires transport of newly synthesized proteins to precise and particular cell compartments. This is partly achieved by signals within the polypeptide chains (Silver, 1991), and in the case of membrane and secreted proteins the first step in targeting involves direction of mRNA–ribosome complexes to the endoplasmic reticulum and synthesis of the proteins on a specific class of membrane-bound polyribosomes (polysomes) (Blobel and Dobberstein, 1975). In addition, the asymmetric distribution of non-membrane protein mRNAs has been shown to occur in cultured cells (Lawrence and Singer, 1986; Sundell and Singer, 1990), in nerves and muscle (Garner et al., 1988; Russell and Dix, 1992) and in the oocytes of *Drosophila* and *Xenopus* (Weeks and Melton, 1987; MacDonald and Struhl, 1988). It thus appears that specific location of mRNAs is a general phenomenon which influences protein targeting. In addition, there is increasing evidence for an association of mRNAs and polysomes with the cytoskeleton (Hesketh and Pryme, 1991; Vedeler et al., 1991), and the observation that such cytoskeletal-bound polysomes contain specific mRNAs (Bird and Sells, 1986; Pondel and King, 1988; Hesketh et al., 1991) suggests that this may provide a mechanism by which at least some of the mRNAs for non-membrane proteins are sorted before translation (Hesketh and Pryme, 1991).

In fibroblasts, both actin and c-*myc* mRNAs have been reported to be present in cytoskeletal-bound polysomes (Bird and Sells, 1986; Hesketh et al., 1991), and, in the case of the actin mRNA, its translocation to the cell periphery is not dependent on nascent polypeptide chains or on association with ribosomes (Sundell and Singer, 1990). Thus, at least in the case of the actin mRNA, it appears that the information required for direction to the cytoskeletal compartment is present in the mRNA itself. This also appears to be the case in *Xenopus* oocytes where microinjection of exogenous mRNA results in correct localization (Yisraeli and Melton, 1988), suggesting that the asymmetric distribution of mRNAs depends on directional information in the mRNA. Furthermore, in both *Drosophila* and *Xenopus* oocytes there is evidence that such directional information is present in the 3′ untranslated region of the mRNAs concerned (MacDonald and Struhl, 1988; Yisraeli and Melton, 1988; Davis and Ish-Horowicz, 1991). The aim of the present work was to investigate whether such a mechanism of mRNA localization also occurs in differentiated eukaryotic cells. The experimental approach taken was to investigate, using L929 fibroblasts transfected with chimeric gene constructs, the ability of the 3′ untranslated region of the c-*myc* mRNA to direct c-*myc* and β-globin-coding sequences to cytoskeletal-bound polysomes.

MATERIALS AND METHODS

Cell lines and cell fractionation

Four lines of stable transfectants, all derived from an L TK$^-$ fibroblast line, were studied. All four lines were produced by co-transfection of a pSV_2 plasmid carrying neomycin resistance together with gene constructs under the control of the simian virus 40 (SV40) early promoter, and have been previously characterized (Bonnieu et al., 1988, 1990): one line (pSV-*myc*) was transfected with two exons and the 3′ untranslated region of the murine c-*myc* gene, a second (pSV-globin) with a construct in which the c-*myc* fragment was deleted from pSV-c-*myc* by *Xba*I–*Hin*dIII restriction and replaced by the *Xba*I–*Hin*dIII fragment of the rabbit β-globin gene under the control of the same promoter (Land et al., 1983); the third line was transfected with a chimeric construct in which the three exons of the globin gene were linked to the c-*myc* 3′ untranslated region by deletion of an *Xba*I–*Xho*I *myc* fragment from pSV-*myc* and replacement by an *Xba*I–*Xho*I globin subfragment; the last cell line was transfected with a complementary gene construct in which exons 2 and 3 of the c-*myc* gene were linked to the 3′ untranslated region of β-globin by substitution of the *Xba*I–*Xho*I region of pSV-globin with that of pSV-*myc*. The four constructs are shown diagrammatically in Figure 1.

Abbreviation used: 1 × SSC, 0.15 M NaCl + 0.015 M sodium citrate.
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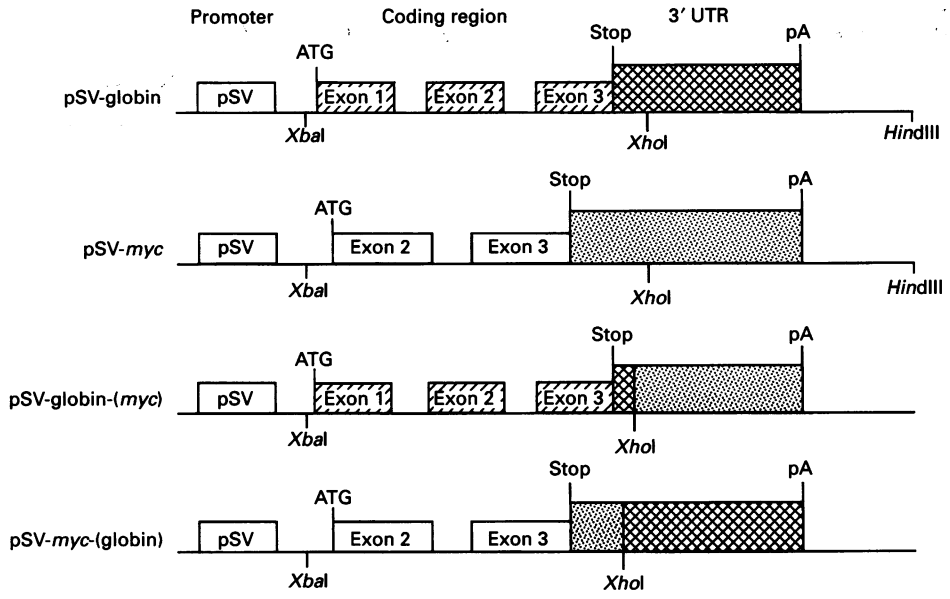

Figure 1 Chimeric *myc*–globin gene constructs used to investigate the role of 3′ untranslated sequences

3′ UTR, 3′ untranslated region; pA, polyadenylation signal; pSV, SV40 early promoter; ▨, β-globin-coding region; □, c-*myc*-coding region; ⊠, β-globin 3′ untranslated region; ▩, c-*myc* 3′ untranslated region.

Cells were grown in 90 mm Petri dishes in Dulbecco's minimal Eagle's medium supplemented with 10% fetal calf serum and in an atmosphere of 5% CO_2. They were treated with emetine (100 ng/ml of medium) for 30 min before extraction of polysomes in order to maintain mRNA–ribosome interactions. Polysome fractions were isolated as described previously (Hesketh et al., 1991). After being rinsed three times in PBS, cells from 20 dishes were harvested into 2 ml of lysis buffer (10 mM Tris/HCl, pH 7.6, containing 0.25 M sucrose, 25 mM KCl, 5 mM $MgCl_2$, 0.5 mM $CaCl_2$ and 0.05% Nonidet P40), and after 10 min at 4 °C the suspension was centrifuged at 200 *g* for 5 min. The supernatant (free polysome fraction) was removed and stored on ice. The pellet was washed once in the same buffer and then resuspended in 2 ml of a similar buffer containing 130 mM KCl. After incubation for 10 min at 4 °C, the suspension was centrifuged at 800 *g* for 10 min and the supernatant (cytoskeletal-bound polysome fraction) removed and stored on ice. Membrane-bound polysomes were solubilized by incubation of the pellet in 2 ml of lysis buffer containing 0.5% Nonidet P40 and 0.5% deoxycholate for 10 min and collected by centrifugation at 1850 *g* for 10 min. Polysomes were prepared from the above fractions by centrifugation at 32000 *g* for 16 h through a 15 ml cushion of 40% sucrose. All buffers and cushion solutions were autoclaved where appropriate and contained 100 μg/ml heparin to inhibit RNAase activity.

RNA extraction and hybridization

Total RNA was extracted by the acid/guanidinium/phenol/chloroform method of Chomczynski and Sacchi (1987), and the preparations were assessed by the A_{260}/A_{280} absorbance ratio. RNA species were then separated by electrophoresis through a denaturing 2.2 M formaldehyde/1.2% agarose gel (Sambrook et al., 1989) and transferred to Nylon membrane (Genescreen from NEN Dupont Ltd.) by capillary blotting. RNA was fixed to the membrane by exposure to u.v. light and the membranes were stored dry until required. Membranes were prehybridized overnight at 42 °C with a solution containing 0.1 mg/ml denatured salmon sperm DNA in 50% formamide, 10% dextran sulphate, 0.2% BSA, 0.2% poly(vinylpyrrolidone), 0.2% Ficoll, 0.1% sodium pyrophosphate, 1% SDS and 50 mM Tris/HCl, pH 7.5.

The c-*myc* probe, a gift from Dr. M. Cole, Princeton University, NJ, U.S.A., was the cDNA of the three exons of the mouse c-*myc* gene. The 2.3 kbp cDNA was cloned into pT7 and a 1.8 kbp *Hin*dIII fragment used in hybridization studies after separation on 0.6% low-melting-point agarose gels. The β-globin probe corresponded to the *Xba*I–*Xho*I fragment which was used in the construction of the chimeric pSV-globin-*myc* (Figure 1) and which contains the three exons of the β-globin-coding region. DNA probes (50–100 ng) were labelled with [^{32}P]dCTP by random priming (Multiprime kit from Amersham International), and the labelled DNA then separated from free nucleotides by gel filtration on Sephadex G-50; probe-specific radioactivities were approximately 10^9 c.p.m./μg of DNA. The labelled probes were added to the prehybridization mix and hybridized at 42 °C for 24 h. The membranes were washed to remove non-specific hybridizations: twice in 2 × SSC at room temperature for 5 min, followed by 0.5 × SSC/1% SDS at 65 °C for 1 h (twice). Specific hybridization was then detected by autoradiography using Hyperfilm-MP (Amersham International) at −70 °C. After autoradiography, membranes were washed in 0.1% SDS for 5–7 min at 95 °C before rehybridization to other probes. Quantification of the RNA loaded in each lane was by hybridization with a 1.4 kb probe for 18S rRNA (Erikson et al., 1981).

The absorbance of the bands on the film was quantified using a QUIPS image-processing work station (Torch Computers, Cambridge, U.K.) operating with VCS image-processing software (Vision Dynamics, Hemel Hempstead, Herts., U.K.). The amounts of specific mRNAs recovered in each fraction were calculated in arbitrary units by correcting the absorbance of the band on the autoradiograph for background absorbance of the

film. Alternatively, in certain experiments hybridization was quantified by direct measurement of specifically-bound radioactive probe using a Packard Instant Imager.

In situ hybridization

Cells were grown on glass chamber slides and washed three times with PBS before fixation for 10 min with 4% paraformaldehyde in PBS. The cells were partially dehydrated in 70% ethanol for 30 min and then fixed for a further 10 min in 4% paraformaldehyde/0.2% Triton in PBS. Controls were treated with 500 μg/ml RNAase A in 2 × SSC for 30 min at 37 °C and washed twice in PBS before all cells were given a further brief fixation for 5 min to destroy any remaining RNAase activity. After two further washes in PBS, cells were incubated in 50% formamide/2 × SSC at room temperature before being hybridized overnight at 55 °C with 200 ng of digoxigenin-labelled antisense riboprobe. The probe was generated from the 2.3 kbp c-*myc* cDNA using a T7 RNA-labelling kit (Boehringer-Mannheim) and subjected to alkaline hydrolysis. After hybridization, cells were washed in 5 × SSC for 30 min and then 50% formamide/2 × SSC, both at 55 °C. Non-specifically bound probe was removed by treatment with 20 μg/ml RNAase, and, after two further washes in 2 × SSC, the bound probe was detected by incubation with alkaline phosphate-linked anti-digoxigenin (Boehringer-Mannheim) and incubation with 4-Nitro Blue Tetrazolium for 2–3 h, all following the manufacturer's instructions. The formazon produced by the alkaline phosphatase activity was quantified by microdensitometry (Campbell et al., 1986; Farquharson et al., 1993) using a Vickers M85A microdensitometer. Absorbance readings were taken at 585 nm with a × 100 oil-immersion lens and a size-A1 circular mask, 2 μm effective diameter. Measurements were taken in both perinuclear and adjacent peripheral cytoplasm of 40 individual cells of each cell line and corrected for slide blanks measured in cell-free areas.

RESULTS AND DISCUSSION

The distribution of normal and chimeric c-*myc* and β-globin mRNAs was studied in four transfected cell lines in which β-globin, c-*myc* and globin–*myc* hybrid genes were under the control of a strong viral promoter. The constructs introduced (Bonnieu et al., 1988, 1990) are shown in Figure 1: in pSV-globin and pSV-*myc*, both coding and non-coding regions were introduced into an L cell line by transfection; in pSV-globin-(*myc*), cells were transfected with a chimeric gene construct in which the globin 3′ untranslated region was replaced by the complete 3′ untranslated region of c-*myc*; in pSV-*myc* (globin), cells were transfected with a complementary construct in which the 3′ untranslated region of the c-*myc* gene was replaced by the 3′ untranslated region of the β-globin gene. All four cell lines were extracted sequentially with non-ionic detergent, 130 mM KCl and deoxycholate which, as has been described previously for 3T3 fibroblasts, MPC11 cells and Krebs II ascites cells (Hesketh and Pryme, 1991), allows separation of free, cytoskeletal-bound and membrane-bound polysomes. Polysomes were recovered from each fraction and, as judged by RNA content, the distribution of polysomes between the three fractions was similar in all cell lines; thus 12–23% of total recovered polysomal RNA was in the polysomes released by non-ionic detergent at 25 mM KCl (free polysomes), 15–32% in the polysomes released by 130 mM KCl (cytoskeletal-bound) and 47–67% in polysomes solubilized by deoxycholate (membrane-bound polysomes).

The distribution of c-*myc*- and β-globin-coding sequences produced from the transfected gene constructs was studied using Northern-blot hybridization. The high expression achieved with the SV early promoter ensured that analysis of the mRNAs present in these cell lines reflected the distribution of mRNAs transcribed from the transfected constructs rather than those from the endogenous c-*myc* gene. Results from cells transfected with the whole globin gene showed that the free polysomes released by non-ionic detergent were enriched in the globin mRNA (Figure 2). In a series of further experiments the extent of hybridization was quantified and the ratio of globin-coding sequences in cytoskeletal-bound polysomes to those in free polysomes was found to be approximately 0.7 (Table 1).

In cells transfected with the complete c-*myc* gene, the c-*myc* mRNA was present at highest abundance in the polysomes released by 130 mM salt (Figure 2), showing that, as in 3T3 fibroblasts (Hesketh et al., 1991), the cytoskeletal-bound polysomes in such transfected cells were enriched in c-*myc* mRNA. Quantification of the hybridization showed that the abundance of the *myc*-coding sequences was approximately 2-fold higher in the cytoskeletal-bound polysomes than in free polysomes (Table 2); the similar difference in the abundance per unit of 18S rRNA showed that the enrichment in cytoskeletal-bound polysomes was not due to an artifact of RNA loading. The data indicate that both the enrichment of c-*myc* mRNA in cytoskeletal-bound polysomes and the proportion of total c-*myc* mRNA recovered

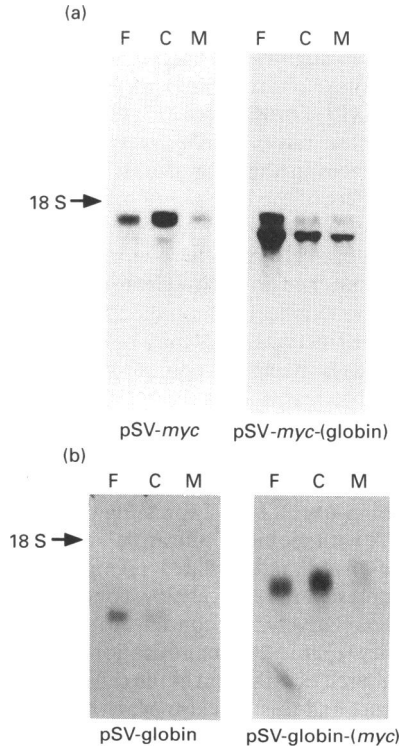

Figure 2 Northern-blot hybridization of total RNA from polysomes isolated from cells transfected with either chimeric *myc*–globin constructs or control transcripts

All lanes were loaded with 20 μg of total RNA and filters were hybridized successively with probes to the coding regions of the c-*myc* (**a**) and β-globin (**b**) genes. Probes were labelled with [^{32}P]dCTP by random priming. After removal of non-specifically bound probe, mRNAs were detected by autoradiography. Hyperfilm β-max was exposed for 3 days (**a**) or 7 days (**b**) with intensifying screens. F, free polysomes; C, cytoskeletal-bound polysomes; M, membrane-bound polysomes.

Table 1 Quantification of the distribution of β-globin-coding sequences in cells transfected with control and chimeric gene constructs

Results are expressed in arbitrary units obtained from densitometry of autoradiographs and direct radioactivity imaging. FP, free polysomes; CBP, cytoskeletal-bound polysomes; MP, membrane-bound polysomes. Sequence abundance values were normalized taking the abundance in FP of pSV-globin cells as 100 for each experiment. Results are means ± S.E.M. ($n = 4$). Groups were compared using Student's t test; $^*P < 0.001$.

	Sequence abundance			Sequence abundance per unit of 18S rRNA			Sequence abundance in CBP/sequence abundance in FP	18S rRNA abundance in CBP/18S rRNA abundance in FP
Cell line	FP	CBP	MP	FP	CBP	MP		
pSV-globin	100	72 ± 9	33 ± 22	0.27 ± 0.02	0.23 ± 0.05	0.10 ± 0.06	0.72 ± 0.09*	1.05 ± 0.14
pSV-globin-(*myc*)	128 ± 65	171 ± 82	76 ± 28	0.79 ± 0.26	1.08 ± 0.32	0.31 ± 0.17	1.63 ± 0.14*	1.21 ± 0.34

Table 2 Quantification of the distribution of c-*myc*-coding sequences in cells transfected with control and chimeric gene constructs

Results are expressed in arbitrary units obtained from densitometry of autoradiographs and direct radioactivity imaging. FP, free polysomes; CBP, cytoskeletal-bound polysomes; MP, membrane-bound polysomes. Sequence abundance values were normalized taking the abundance in FP of pSV-myc cells as 100 for each experiment. Results are means ± S.E.M. ($n = 5$). Groups were compared using Student's t test; $^*P < 0.05$.

	Sequence abundance			Sequence abundance per unit of 18S rRNA			Sequence abundance in CBP/sequence abundance in FP	18S rRNA abundance in CBP/18S rRNA abundance in FP
Cell line	FP	CBP	MP	FP	CBP	MP		
pSV-*myc*	100	224 ± 22	107 ± 12	0.48 ± 0.10	0.79 ± 0.10	0.52 ± 0.06	1.95 ± 0.35*	1.09 ± 0.15
pSV-*myc*-(globin)	210 ± 41	167 ± 55	123 ± 29	0.52 ± 0.09	0.53 ± 0.17	0.23 ± 0.14	0.99 ± 0.22*	0.96 ± 0.10

in that fraction are slightly less in the L TK$^-$ cells used in the present experiments (52% recovered in cytoskeletal-bound polysomes) than in 3T3 fibroblasts (70%). The reasons for this difference are unclear but may reflect variations in fractionation caused by differences in the detergent/salt susceptibility of the cell lines or the effect of the high c-*myc* mRNA abundance when the gene is driven by the viral promoter. Although in the pSV-*myc* and pSV-globin cell lines the c-*myc*- and β-globin-coding sequences were enriched in cytoskeletal-bound and free polysomes respectively, significant amounts of the mRNAs were also recovered in other fractions. This may be due to either mRNA–polysome complexes being in equilibrium between free and cytoskeletal compartments or to incomplete extraction of free polysomes with non-ionic detergent/25 mM KCl and incomplete release of cytoskeletal-bound polysomes by salt treatment.

In contrast with cells transfected with the whole globin gene, in the pSV-globin-(*myc*) cells expressing large amounts of a chimeric mRNA with globin 5′ untranslated and coding regions linked to the c-*myc* 3′ untranslated region, the globin-coding sequences were present in relatively low concentration in the free polysomes and in relatively high concentration in the cytoskeletal-bound polysomes (Figure 2). Quantification of the hybridization confirmed the altered distribution in the cells transfected with the chimeric construct and this was also evident when the data were expressed per unit of ribosomal RNA (Table 1). The absolute levels of expression of the transfected genes, as judged by mRNA levels, were different between the cell lines and therefore the distribution of coding sequences between free and cytoskeletal-bound polysomes was expressed as a ratio. As shown in Table 1, the ratio of globin-coding sequences in the cytoskeletal-bound polysomes to those in free polysomes was significantly different between cells transfected with the whole globin gene and those transfected with a chimeric construct in which the globin-coding sequences were linked to the c-*myc* 3′ untranslated region (0.72 ± 0.09 compared with 1.63 ± 0.14, $P < 0.001$). There was no significant difference in the 18S rRNA ratio between the fractions, thus precluding effects due to different amounts of polysomal RNA, and the observed difference in distribution of globin-coding sequences between the two cell lines cannot therefore simply be due to differences in polysomal RNA, and thus total mRNA, loaded. Thus replacement of the globin 3′ untranslated region by that of c-*myc* causes a redistribution of the β-globin-coding region of the mRNA and indicates that there are features within the c-*myc* untranslated region that are capable of directing coding sequences to the cytoskeletal-bound polysomes.

Analysis of polysome fractions from pSV-*myc*-(globin) cells showed that mRNA containing the c-*myc*-coding region linked to the globin 3′ untranslated region was present largely in the free polysomes with a higher concentration of the *myc*-coding regions recovered in the free polysomes compared with the cytoskeletal-bound fraction (Figure 2). This contrasts with the enrichment of the cytoskeletal-bound polysomes with c-*myc* mRNA in cells transfected with the full c-*myc* transcript, and the difference in distribution between the two cell lines was also evident from data in which mRNA abundances were quantified and expressed either per unit of 18S rRNA or as a ratio of mRNA abundance in the free polysomes compared with that in the cytoskeletal-bound polysomes (Table 2). There was a statistically significant difference between the cytoskeletal-bound polysome/free polysome ratio of c-*myc* coding sequences in cells containing the chimeric *myc*–globin construct compared with the pSV-*myc* cells (0.99 ± 0.22 compared with 1.95 ± 0.35, $P < 0.05$). The results thus indicate that replacement of the c-*myc* 3′ untranslated region by that of β-globin causes a redistribution of the chimeric *myc* mRNA such that there is a loss of the chimeric mRNA from cytoskeletal-bound polysomes; this supports the results from the cells transfected with the pSV-globin-(*myc*) construct in indicating that the 3′ untranslated region of c-*myc* contains sequences that are essential for the c-*myc* mRNA to be directed to be translated in the cytoskeletal-bound polysomes.

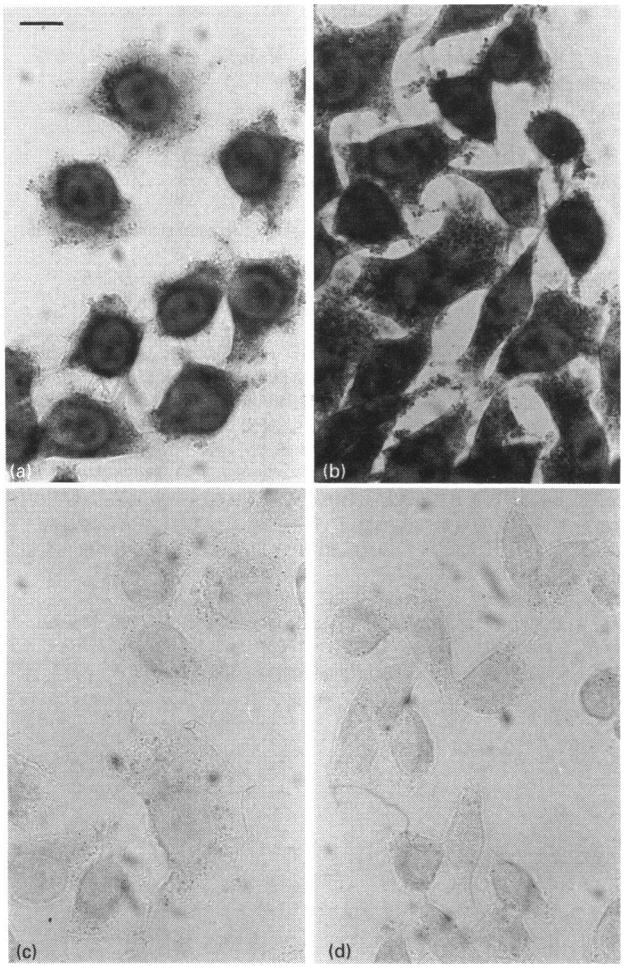

Figure 3 *In situ* hybridization showing the distribution of transcripts containing the c-*myc*-coding sequences in cells transfected with either control c-*myc* or hybrid *myc*–globin constructs

Cells transfected with either pSV-*myc* (**a**,**c**) or pSV-*myc*-(globin (**b**,**d**) constructs were fixed with 4% paraformaldehyde and hybridized with a digoxigenin-labelled antisense riboprobe specific for the c-*myc*-coding sequences. Specific labelling was detected using an alkaline phosphatase-linked anti-digoxigenin antibody and 4-Nitro Blue Tetrazolium as substrate; note the strong perinuclear staining in the pSV-*myc* cells and the staining throughout the cytoplasm, even the peripheral areas, in the pSV-*myc*-(globin) cells. Controls pretreated with RNAase (**c**,**d**) showed little or no labelling. The magnification is the same for all parts of the Figure and the scale bar = 10 μm.

Cells transfected with either the c-*myc*-coding region attached to the β-globin 3′ untranslated region [pSV-*myc*-(globin)] or the control c-*myc* construct were analysed by *in situ* hybridization. As shown in Figure 3, the control transcripts were found to be localized primarily in the perinuclear region of the cytoplasm, whereas in cells containing the chimeric gene the transcripts were distributed throughout the cytoplasm with significant amounts found in the peripheral cytoplasm. The relative amounts of specific mRNA present in perinuclear and peripheral cytoplasm were assessed by quantification of the product of the alkaline phosphatase bound to the digoxigenin-labelled riboprobe (Table 3). In the pSV-*myc* cells, the concentration of *myc* transcripts was 2–3-fold higher in the perinuclear region than in the peripheral cytoplasm. The overall extent of hybridization was greater in the pSV-*myc*-(globin) cells, as also found in the Northern-blot hybridization studies (e.g. Figure 1), and this may be due to the greater stability of the chimeric mRNA than the messenger derived from the pSV-*myc* construct (Bonnieu et al., 1988). However, the increase in hybridization observed in the pSV-*myc*-(globin) cells was not evenly distributed throughout the cytoplasm but was largely due to an almost 2-fold greater concentration of the chimeric mRNA in the peripheral cytoplasm, there being only a 20% increase in the perinuclear region; as a result, the amount of c-*myc* probe bound in the perinuclear region was only approx. 50% higher than that in the peripheral cytoplasm. Quantification of the residual phosphatase activity present after RNAase treatment showed that the difference in the ratio of perinuclear/peripheral phosphatase activity was not due to differences in background alkaline phosphatase activity. Furthermore after correction for residual activity in the RNAase-treated controls the ratio of perinuclear to peripheral activity was markedly different in the pSV-*myc* and pSV-*myc*-(globin) cells: the perinuclear/peripheral activity ratio was 4.4 ± 0.8 in the pSV-*myc* cells and 1.7 ± 0.1 in the pSV-*myc*-(globin) cells ($P < 0.001$). Thus microdensitometry of the *in situ* hybridization reaction product confirmed that there is an altered distribution of *myc*–globin chimeric mRNA compared with the control mRNA with the c-*myc* 3′ untranslated region. The *in situ* hybridization data provide further evidence that the c-*myc* 3′ untranslated region is required for the correct targeting and localization of this mRNA.

In conclusion, these studies with chimeric gene constructs in which c-*myc* and β-globin 3′ untranslated region have been exchanged show that the ability of sequences in the 3′ untranslated region of certain mRNAs to direct those mRNAs to specific subcellular locations is not restricted to *Drosophila* and *Xenopus* oocytes (Yisraeli and Melton, 1988; Davis and Ish-Horowicz, 1991; Mowry and Melton, 1992), but also occurs in differentiated mammalian cells. The results indicate that, at least in the case of c-*myc*, the 3′ untranslated region has a role in the sorting and directing of mRNAs and this provides the first

Table 3 Microdensitometry of *in situ* hybridization showing quantification of transcripts containing c-*myc*-coding sequences in perinuclear and peripheral cytoplasm

Values shown are mean absorbances ± S.E.M. with the number of cells analysed in parentheses. Cells transfected with either pSV-*myc* or pSV-*myc*-(globin) constructs were fixed and hybridized with a digoxigenin-labelled c-*myc* riboprobe specific to the c-*myc*-coding region. Hybridization was detected using an anti-digoxigenin antibody linked to alkaline phosphatase and the reaction product quantified by microdensitometry. Controls which were treated with RNAase (500 μg/ml, 30 min, 37 °C) before hybridization showed very little residual activity. Groups were compared using a two-tailed Student's *t* test; *$P < 0.001$ compared with the parallel measurement in the pSV-*myc* cells.

	c-*myc* probe bound (A_{585})		Residual activity after RNAase (A_{585})	
Cell line	Perinuclear	Peripheral	Perinuclear	Peripheral
pSV-*myc*	26.9 ± 0.7 (40)	11.8 ± 0.7 (40)	5.0 ± 0.3 (20)	4.6 ± 0.3 (20)
pSV-*myc*-(globin)	32.2 ± 0.9* (40)	21.1 ± 0.9* (40)	2.8 ± 0.4 (20)	2.4 ± 0.5 (20)

evidence that mRNA localization in differentiated eukaryotic cells depends on a mechanism that involves directional information within the 3′ untranslated region of mRNAs. The occurrence of such a mechanism in both oocytes and mammalian cells suggests that the mechanism is widespread and possibly universal. Furthermore, the results support the hypothesis that mRNA targeting involves the 3′ untranslated region in direction of specific mRNAs to cytoskeletal-bound polysomes (Hesketh and Pryme, 1991). Such mechanisms presumably involve consensus sequences and/or secondary features in the RNA which interact with specific proteins associated with the cytoskeleton and may be additional or related to the role of the 3′ untranslated region in regulation of mRNA stability (Bonnieu et al., 1988; Cole and Mango, 1990).

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REFERENCES

Bird, R. and Sells, B. (1986) Biochim. Biophys. Acta **868**, 215–225
Blobel, G. and Dobberstein, B. (1975) J. Cell Biol. **67**, 835–851
Bonnieu, A., Piechaczyk, M., Marty, L., Cuny, M., Blanchard, J.-M., Fort, P. and Jeanteur, P. (1988) Oncogene Res. **3**, 155–166
Bonnieu, A., Roux, P., Marty, L., Jeanteur, P. and Piechaczyk, M. (1990) Oncogene **5**, 1585–1588
Campbell, D. A., Du Bois, R. M., Butcher, R. G. and Poulter, L. W. (1986) Clin. Exp. Immunol. **65**, 165–171
Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159
Cole, M. D. and Mango, S. E. (1990) Enzyme **44**, 167–180
Davis, I. and Ish-Horowicz, D. (1991) Cell **67**, 927–940
Erickson, J. M., Rushford, C. L., Dorney D. J., Wilson, G. N. and Schmickel, R. D. (1981) Gene **16**, 1–9
Farquharson, C., Milne, J. and Loveridge, N. (1993) Bone Miner. **22**, 105–115
Garner, C. C., Tucker, R. P. and Matus, A. (1988) Nature (London) **330**, 477–479
Hesketh, J. E. and Pryme, I. F. (1991) Biochem. J. **277**, 1–10
Hesketh, J. E., Campbell, G. P. and Whitelaw, P. F. (1991) Biochem. J. **274**, 607–609
Land, H., Parada, L. and Weinberg, R. (1983) Nature (London) **304**, 596–602
Lawrence, J. B. and Singer, R. H. (1986) Cell **45**, 407–415
MacDonald, P. M. and Struhl, G. (1988) Nature (London) **336**, 595–598
Mowry, K. L. and Melton, D. A. (1992) Science **255**, 991–994
Pondel, M. D. and King, M. L. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 7612–7616
Russell, B. and Dix, D. J. (1992) Am. J. Physiol. **262**, C1–8
Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
Silver, P. A. (1991) Cell **64**, 489–497
Sundell, C. L. and Singer, R. H. (1990) J. Cell Biol. **111**, 2397–2403
Vedeler, A., Pryme, I. F. and Hesketh, J. E. (1991) Mol. Cell Biochem. **100**, 183–193
Weeks, D. L. and Melton, D. (1987) Cell **51**, 861–867
Yisraeli, J. K. and Melton, D. A. (1988) Nature (London) **336**, 592–595

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