Differential localization of the mRNA of the M and B isoforms of creatine kinase in myoblasts

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Creatine kinase (CK) plays an important role in buffering ATP and ADP levels in tissues which have intermittently high and fluctuating energy demands, such as skeletal muscle. This buffering function has a spatial, as well as a temporal aspect, which is dependent on the localization of different enzyme isoforms within the cell. We show here, by *in situ* hybridization, that the mRNAs for the cytoplasmic isoforms of CK are differentially localized in a mouse myoblast cell line (C_2C_{12}). The mRNA for the M form is localized at the cell periphery, while that for the B form is localized in the perinuclear region. Deletion of segments of the 3′ untranslated regions of these mRNAs or swapping of these segments between the mRNAs for the two isoforms demonstrated that localization signals lie within these regions. Localization appears to be tissue-specific, since both the M and B mRNAs were distributed uniformly over the cytoplasm in a non-muscle cell line. These results, in conjunction with other studies which have shown that mRNA localization can lead to co-localization of the encoded protein, suggest that the localization of the mRNAs for the cytoplasmic isoforms of CK may be involved in the localization of the enzymes themselves.

INTRODUCTION

Creatine kinase (CK) (EC 2.7.3.2) plays a key role in the cellular energetics of vertebrates, where it catalyses the reaction:

$$PCr^{2-} + MgADP^{-} + H^{+} \rightleftharpoons creatine + MgATP^{2-}$$

The enzyme is expressed predominantly in muscle and neural tissue, where increases in ATP demand are buffered by the breakdown of phosphocreatine (PCr). The subcellular localization of the enzyme at sites of cellular ATP production and utilization has led to the proposal that the enzyme acts as a spatial as well as a temporal energy buffer. For example, in muscle the enzyme is localized at the mitochondria and myofibrils. Diffusion of PCr and creatine between these sites is thought to act as an energy 'shuttle' [1,2], effectively enhancing the transport of ATP and ADP respectively.

The two cytosolic forms of CK, brain (B) and muscle (M), are encoded by separate genes and dimerize to form active enzyme. These show tissue-specific expression, with neural tissue containing predominantly BB, cardiac muscle MB and MM and skeletal muscle MM [3]. Myoblast cells express BB and switch to MM during differentiation [4]. Tissue-specific expression is achieved, at least in part, by differential transcriptional control through tissue-specific enhancers [5,6]. The isoforms also show tissue-specific subcellular localization. For example, the MM isoform is found associated with the M line in myofibrils, while the BB isoform is found within photoreceptors and sperm tail and is associated with synaptic vesicles [3].

There is evidence that the subcellular localization of some proteins is mediated via localization of their mRNAs. This appears to be the case particularly in spatially complex systems such as oocytes and in neural and motile cells [7–9]. One of the most detailed studies of mRNA localization in cultured cells has been that of the actin mRNAs [10–12]. The signals for subcellular localization have been found within the 3′ untranslated regions (UTRs) [13,14]. Binding of this region to the cytoskeleton is thought to be responsible for the anchorage, movement and subsequent site-specific translation of the mRNAs [11,15]. In developing muscle cells, co-localization of several cytoskeletal proteins and their cognate mRNAs have been demonstrated [16–18]. Localization of these mRNAs has been proposed to play a key role in the formation of cellular structures. Earlier work on the localization of CK in muscle indicated that localization of CK was due to an interaction with the C-terminus of the protein. Schäfer and Perriard [19] showed that it was possible to mislocalize the M isoform of CK in chicken cardiac muscle cells by exchanging the 3′ end of the coding region with that for the B form. However, the 3′ UTRs of the mRNAs had also been exchanged. We show here that the mRNAs for the M and B isoforms of CK are localized in a mouse myoblast cell line (C_2C_{12}) and that this localization is dependent on signals found within their 3′ UTRs. This suggests that localization of the enzyme in muscle might also be dependent on localization of its mRNA. We believe that this is the first report of the subcellular localization of the mRNAs of different isoforms of an enzyme in somatic cells.

EXPERIMENTAL

Materials

Tissue-culture medium was purchased from Gibco–BRL (Paisley, Renfrewshire, Scotland, U.K.) and sera from Seralab (Crawley, West Sussex, U.K.). Tissue-culture plastics were obtained from Falcon (Becton Dickenson, Oxford, U.K.). Restriction enzymes were purchased from Boehringer (Lewes, East Sussex, U.K.) or Gibco–BRL. Other enzymes were obtained from Boehringer or Sigma (Poole, Dorset, U.K.). Tandem R immunoradiometric kit was obtained from Hybritech, Nottingham, U.K. Hybond N^+ was obtained from Amersham, Slough, Berks., U.K. [α-^{32}P]UTP was obtained from Amersham. Digoxigenin detection and labelling kits were obtained from Boehringer. Plasmid pSP72 was obtained from Promega, Southampton, U.K. All other chemicals were of analytical grade.

Abbreviations used: CK, creatine kinase; BCK, brain creatine kinase; MCK, muscle creatine kinase; GAPDH, glyceraldehyde-phosphate dehydrogenase; hGH, human growth hormone; PCr, Phosphocreatine; UTR, untranslated region.

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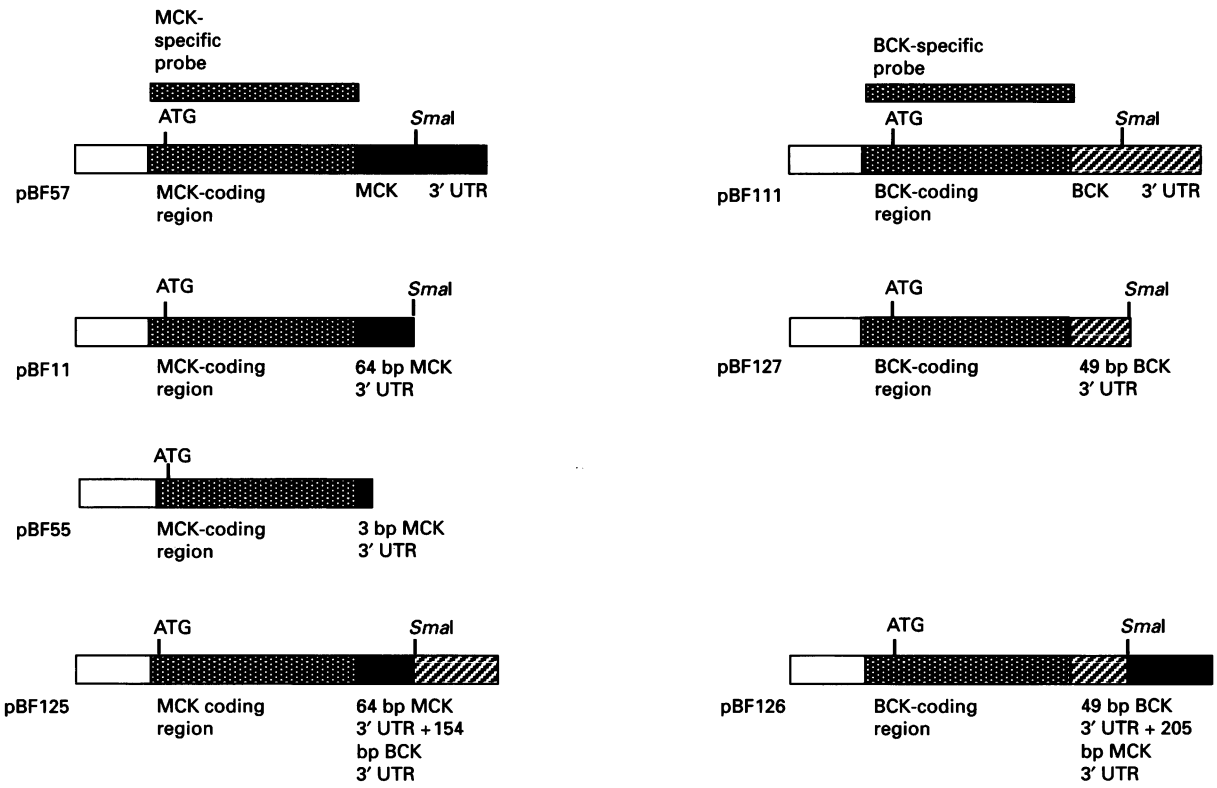

Figure 1 Expression vector inserts

The line diagrams are representations of the CK sequences inserted at the *Bgl*II site of pKV461. The numbers given to the resulting plasmids are indicated.

Plasmid constructions

The mRNAs for the CK isoforms were expressed off the human cytomegalovirus promoter in the plasmid pKV461 [20]. Rabbit muscle CK (MCK) cDNA was a gift from P. Schimmel [21] and rat brain CK (BCK) cDNA was a gift from P. Benfield [22]. A *Bam*HI oligonucleotide (5′ CCGGATCCGG 3′) (Gibco–BRL) was inserted 5 bp upstream of the ATG in the MCK cDNA sequence. The cDNA was subcloned as a *Bam*HI fragment into the *Bgl*II expression site of pKV461 to give pBF57 (Figure 1). The deleted versions of the MCK cDNA were made by inserting the *Bam*HI oligonucleotide at the *Bss*HII site 3 bp downstream of the stop codon (pBF55) or at the *Sma*I site, 64 bp into the 3′ UTR sequence (pBF11) (see Figure 1). The rat BCK cDNA was subcloned from λgt 11 [22] as a double *Eco*RI fragment, into the *Eco*RI site of pSP72. This fragment was then moved as a *Bam*HI–*Bgl*II fragment into the *Bgl*II expression site of pKV461 to give pBF111 (Figure 1). pBF125 and pBF126 were generated by swapping the *Sma*I–*Bam*HI fragments of pBF57 and pBF111, which contain part of the 3′ UTR sequences of MCK and BCK respectively (Figure 1).

Plasmid DNAs were prepared by the method of Birnboim and Doly [23].

Cell culture

C_2C_{12} cells were obtained from the American Type Culture Collection (A.T.C.C.), Rockville, MD, U.S.A. (A.T.C.C. no. CRL 1772) and COS 1 cells from the European Collection Animal Cell Cultures (E.C.A.C.C.), Porton Down, Salisbury, Wilts., U.K. (E.C.A.C.C. no. 88031701). Cells were grown in Dulbecco's modified Eagle's medium/10% heat-inactivated foetal-calf serum. Transient transfections were carried out using the DEAE-dextran method described by Foecking and Hofstetter [24], using 15 μg of CK plasmid DNA. The cells were also co-transfected with 5 μg of RSVhGH, a plasmid expressing human growth hormone (hGH) [25] from the Rous-sarcoma-virus promoter [26]. In transient transfections, CK expression levels were standardized against hGH levels, as a control for transfection efficiency. hGH assays were carried out using a tandem R immunoradiometric kit, following manufacturer's instructions. Transfection efficiencies were greater than 80%, as judged by *in situ* hybridization to the expressed mRNAs. Such a high efficiency is not unusual for this transfection protocol [27]. Stable transfections were carried out by electroporation [28] using a Bio-Rad Gene Pulser (settings: 960 μF, 0.3 V double pulse), using 15 μg of CK plasmid DNA and 5 μg of RSVneo DNA [26]/5×10^6 cells in 0.75 ml of Hepes-buffered saline, pH 7.2, plus 50 μl of foetal-calf serum. Selection was carried out for 14 days on 475 μg/ml G418 (Geneticin; Gibco–BRL). A control cell line (RSV-5) was produced, which was taken through the same transfection procedure, but using RSVhGH rather than a CK-expressing plasmid.

Probes

Digoxigenin-labelled RNA probes were generated by *in vitro* transcription in the presence of digoxigenin-labelled UTP using a digoxigenin labelling and detection kit. Sections of the coding regions of the M and BCK cDNAs (see Figure 1) were inserted into pSP72. Sense and antisense probes were made using either the SP6 or T7 promoters as appropriate.

Radioactively labelled RNA probes were generated in the same way, using [α-^{32}P]UTP in place of the digoxigenin-labelled UTP. Probes were labelled to a specific radioactivity of (1–3) $\times 10^9$ c.p.m./μg of RNA. Glyceraldehyde-phosphate dehydrogenase (GAPDH) (EC 1.2.1.12) probes were made by transcription of rat GAPDH cDNA, inserted as a *Pst*I fragment (from pGAPDH, [29]) into pSP72.

Northern-blot hybridizations

Total cellular RNA was prepared from 70% confluent cells by the method of Chomczynski and Sacchi [30]. RNAs were separated on a 1% agarose/formaldehyde gel [31] with 20 μg of RNA/lane. The RNA was transferred to Hybond N$^+$ nylon membrane by capillary blotting and fixed by exposure to UV light. Filters were prehybridized for 1 h in hybridization buffer {50% formamide/5 × SSPE [1 × SSPE is 0.15 M NaCl/10 mM sodium phosphate (pH 7.4.)/1 mM EDTA]; 5 × Denhardt's solution/0.5% (w/v) SDS/20 μg/ml denatured salmon sperm DNA} at 42 °C and then hybridized in fresh hybridization buffer, containing 4 ng/ml of freshly heat-denatured probe, for a minimum of 12 h at 42 °C. Filters were washed twice for 10 min in 2 × SSPE/0.1% SDS at room temperature, once for 15 min in 1 × SSPE/0.1% SDS at 65 °C and twice for 10 min in 0.1 × SSPE/0.1% SDS at 65 °C. Hybridization with a GAPDH probe was used to demonstrate equal loading in all lanes.

Membranes were exposed in a Phosphorimage cassette for 48 h and scanned using a Molecular Dynamics Phosphor 450 Imager.

In situ hybridization

Cells were grown on acetylated glass slides [32]. The cells were fixed by washing for 5 min in Dulbecco's PBS (Gibco–BRL), 15 min in ice-cold 4% paraformaldehyde in Dulbecco's PBS, 5 min in Dulbecco's PBS and 10 min at −20 °C in ethanol/acetic acid (19:1, v/v). The slides were left to dry and were then frozen in liquid-nitrogen vapour for 15 s and stored at −20 °C. The cells were dehydrated by washing in an ethanol series (30, 50, 75, 95 and 100%). Transiently transfected cells were treated with DNase. Slides were incubated for 15 min at 37 °C in 40 mM Tris/HCl (pH 7.9)/10 mM NaCl/ 6 mM $MgCl_2$/0.1 mM $CaCl_2$/1 unit/μl DNase (RNAase-free) [31] and then washed in diethyl pyrocarbonate-treated water at room temperature for 5 min and in 0.2% glycine in Dulbecco's PBS at room temperature for 10 min. The slides were placed in a humid chamber and pre-hybridized for 3 h at 45 °C by placing 200 μl of prehybridization buffer (5 × SSC/5 × Denhardt's solution/50% formamide/0.25 mg/ml yeast tRNA/1.25 mg/ml denatured salmon sperm DNA) on each slide and covering with a silicone-treated glass coverslip. The slides were then washed in an ethanol series (70, 90 and 100%) for 2 min in each solution at room temperature. They were then dried and hybridized overnight at 45 °C in hybridization buffer that lacked the salmon sperm DNA but which contained 10 ng/μl digoxigenin-labelled probe. Single-stranded RNA probes were made as described above. The probe was revealed by using a digoxigenin detection kit according to the manufacturer's instructions.

CK assays and gels

Total cellular protein extracts were prepared as described by Trask et al. [33]. Protein concentration was determined by the method of Bradford [34]. CK assays were carried out as described previously [35].

RESULTS

Differential localization of MCK and BCK mRNAs

Plasmids pBF57 and pBF111 (Figure 1) were used to generate transfectants of the mouse myoblast cell line C_2C_{12}. These plasmids direct overexpression of the MCK and BCK isoforms respectively. We chose to analyse further two clones, namely 57-4 and 111-7, which showed similar levels of CK overexpression (8–11 fold) (Table 1). The Northern blot in Figure 2 shows that these cell lines expressed similar levels of CK mRNA. Endogenous CK mRNAs in untransfected control cells were not detected.

Figure 3 shows the results of *in situ* hybridization of antisense MCK and BCK digoxygenin-labelled RNA probes to cell lines 57-4 and 111-7 respectively. A pronounced difference is seen in the intracellular localization of the mRNAs for the different isoforms. In the majority of cells of 57-4, MCK mRNA is found concentrated in discrete patches at the cell periphery (Figure 3a), although in a few cases (< 5%) it is found throughout the cell. However, in cell line 111-7, BCK mRNA was confined to the perinuclear region (Figure 3c). Screening of a further three clones of cells transfected with pBF111 and two clones of cells transfected with pBF57 produced similar results. A signal was detected only with probes complementary to the sense strand of MCK or

Table 1 CK activity in stably transfected C_2C_{12} cells

The cell lines were named according to the plasmid with which they were transfected (see Figure 1 and the Experimental section). The last numbers represent the clone number. Cultures were plated at a cell density of 2×10^5 cells/cm^2 and grown for 48 h. CK activity was determined in cell extracts using the assay previously described [35]. Protein content was estimated by the method of Bradford [34]. Values, which are based on assays of extracts from cells grown on *n* different dishes for each clone, are means ± S.D. (n).

Cell line	CK activity (μmol/min/g of protein)
RSV-5	48 ± 1 (20)
57-4	518 ± 8 (12)
55-2	418 ± 8 (12)
11-3	384 ± 9 (17)
111-7	376 ± 9 (11)

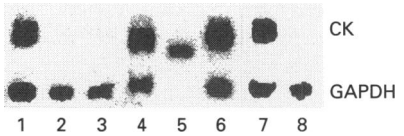

Figure 2 Comparison of expression levels of mRNA in CK-overexpressing C_2C_{12} cell lines

A 20 μg sample of total RNA from each stably transfected cell line was fractionated on a 1% agarose/formaldehyde gel, transferred to a nylon membrane and hybridized with ^{32}P-labelled RNA probes complementary to MCK, BCK and GAPDH mRNA as appropriate. Membranes were exposed in a Phosphorimage cassette for 48 h and scanned using a Molecular Dynamics Phosphor 450 Imager. GAPDH was used as an internal control for RNA loading on the gel. Lane 1, 11-2 (truncated MCK 3′UTR); lane 2, C_2C_{12}; lane 3, RSV-5 (transfection control); lane 4, 55-3 (no MCK 3′UTR); lane 5, *in vitro*-transcribed MCK RNA, which is shorter than the plasmid-directed mRNA; Lane 6, 57-4 (full-length MCK cDNA); lane 7, 111-7 (full-length BCK cDNA); lane 8, RSV-5.

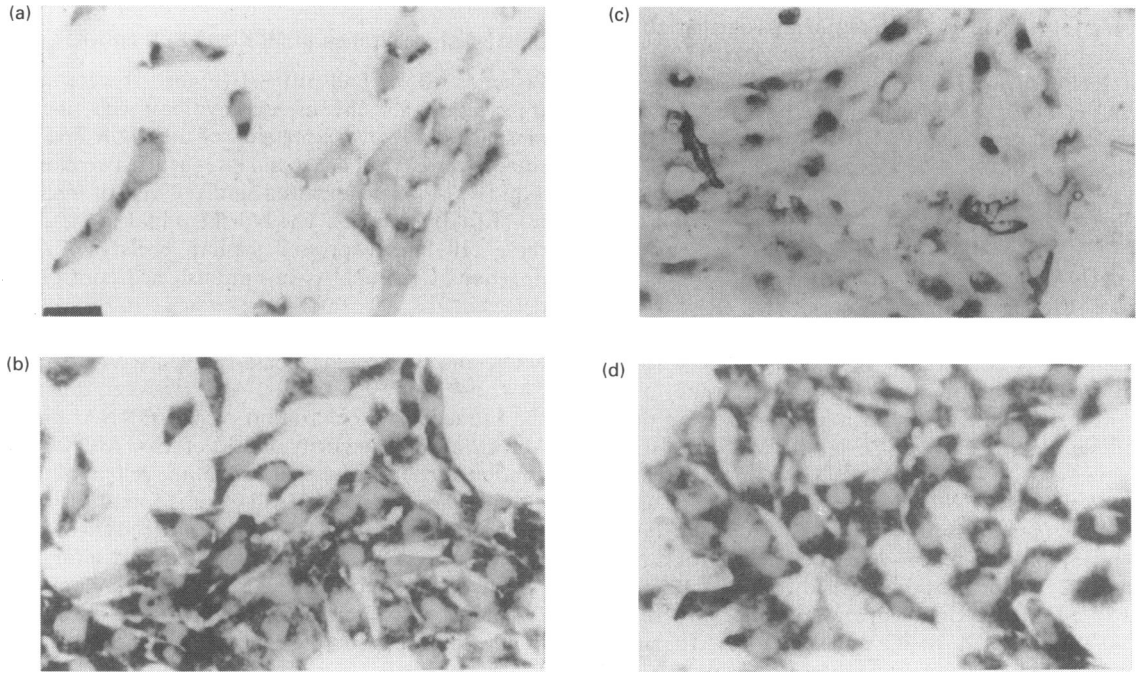

Figure 3 ***In situ*** **hybridization to stably transfected C_2C_{12} cells overexpressing CK**

In (**a**), (**b**) and (**d**), *in situ* hybridization was carried out using digoxigenin-labelled antisense MCK RNA probes. In (**c**) the probe was digoxigenin-labelled antisense BCK RNA. (**a**) MCK including the full-length 3′UTR (pBF57); (**b**) MCK minus the 3′UTR, (pBF55); (**c**) BCK including the full-length 3′UTR, (pBF111); (**d**) MCK cDNA, with a truncated 3′UTR, (pBF11). Slides probed with sense RNAs gave no signal (results not shown). The bar represents 50 μm.

BCK and not with probes which were complementary to the antisense strand (results not shown). Furthermore, no signal was obtained in untransfected cells, i.e. the endogenous CK mRNAs were not detected (results not shown).

Localization of MCK mRNA is dependent on signals within the 3′UTR

Given the importance of the 3′UTR in the localization of actin and other mRNAs [7,10,36], we investigated the role of the MCK 3′UTR in the localization of MCK mRNA. Two plasmids were constructed. In one the entire 3′UTR was deleted (pBF55), while in the other the sequence downstream of the *Sma*I site was deleted (pBF11), which left the first 64 nucleotides of the 3′UTR (Figure 1). These plasmids were used to generate stable cell lines (55-2 and 11-3) which overexpress MCK 8–9 fold (Table 1). Northern-blot analysis (Figure 2) showed that the deletion of the 3′UTR sequences did not affect the steady-state levels of expression of the MCK mRNA. *In situ* hybridization to these cell lines showed that, in both 55-2 and 11-3, the MCK mRNA was no longer localized in discrete patches close to the cell periphery, but was evenly distributed throughout the cytoplasm, although excluded from the nucleus (Figures 3b and 3d).

To expedite screening of different regions of the 3′UTR for their effectiveness in directing localization, we investigated whether the CK mRNAs showed the same distribution in transiently and stably transfected C_2C_{12} cells. Figures 4(a), 4(b), 4(c) and 4(e) show that the intracellular distribution of the mRNAs was not affected by the method of transfection. Therefore transient transfections were used subsequently to assess the effectiveness of different 3′UTR sequences in mediating mRNA localization. The CK activities achieved in transient transfections were similar for all the plasmids (Table 2).

Localization of BCK mRNA is dependent on signals within the 3′UTR

The sequence downstream of the *Sma*I site in the 3′UTR of BCK mRNA was deleted, leaving the first 49 nucleotides of the 3′UTR (plasmid pBF127; see Figure 1). *In situ* hybridization showed that removal of this sequence altered the localization of the mRNA (Figure 4f). The label was still concentrated around the nucleus, but it was more diffuse and extended into the cytoplasm.

Transfer of localization signals between BCK and MCK mRNAs

To determine whether the sequences downstream of the *Sma*I sites in the MCK and BCK 3′UTRs are sufficient to confer isoform-specific mRNA localization, these sequences were swapped between the two mRNAs. Transfer of sequences from the BCK 3′UTR to the MCK 3′UTR and vice versa gave the constructs pBF125 (M+B tail) and pBF126 (B+M tail), which are shown in Figure 1. These were used in transient transfections. *In situ* hybridization with a probe containing predominantly the M-coding region (see Figure 1) to cells transfected with pBF125 shows that the B 3′UTR sequences downstream of the *Sma*I site are sufficient to confer B-type localization on M mRNA (Figure 4d). The label was found concentrated around the nucleus, as with the B mRNA with an intact B 3′UTR (Figures 3c and 4e).

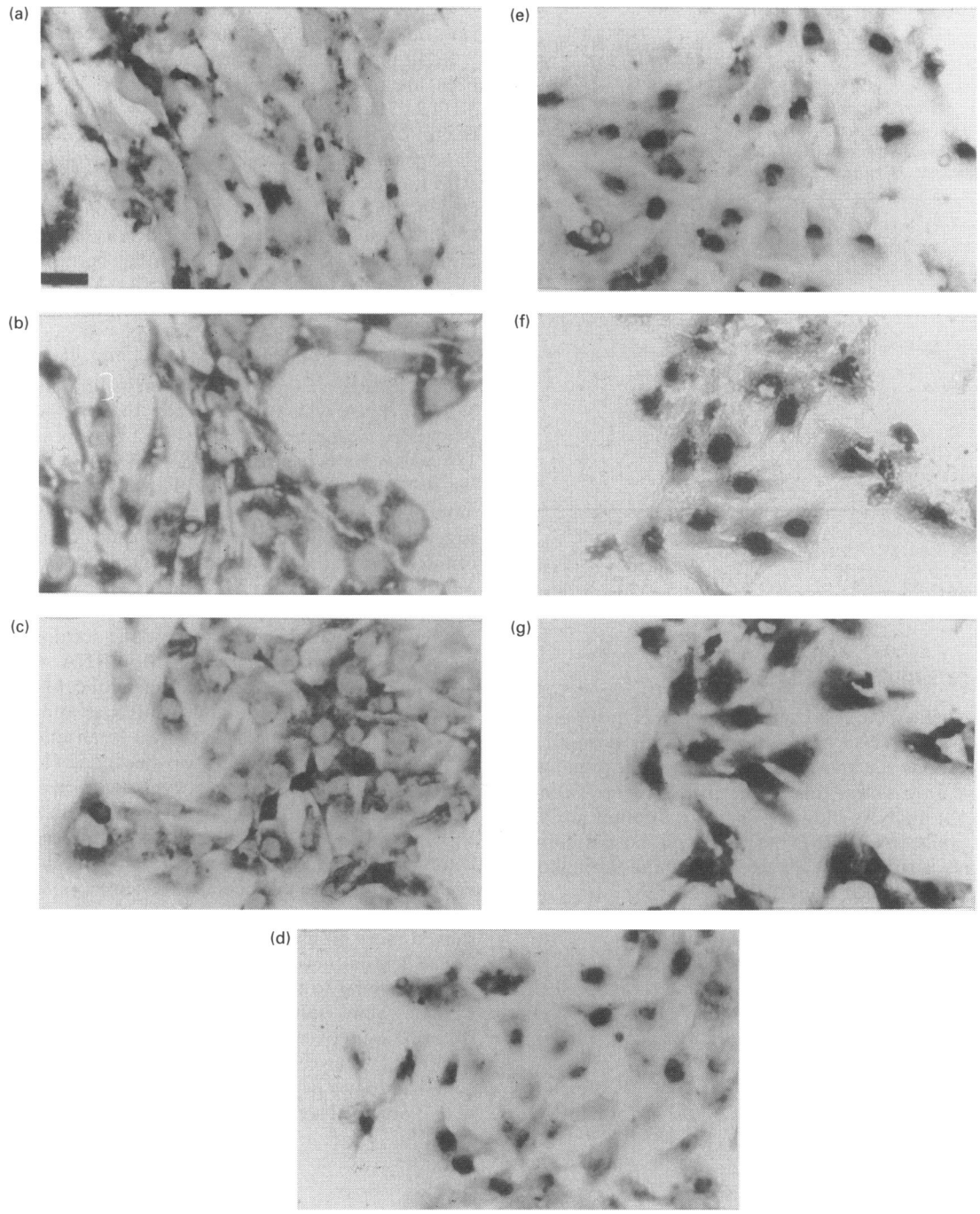

Figure 4 ***In situ*** **hybridization to transiently transfected C_2C_{12} cells overexpressing CK**

In (**a**)–(**d**) the probe was digoxigenin-labelled antisense MCK RNA, and in (**e**)–(**g**) the probe was digoxigenin-labelled antisense BCK RNA. (**a**) pBF57, full-length MCK; (**b**) pBF11, MCK with the 3′UTR truncated at the *Sma*I site; (**c**) pBF55, MCK with no 3′UTR; (**d**) pBF125, M cDNA with sequences downstream of the *Sma*I site replaced with BCK sequences (M + B); (**e**) pBF111, full-length BCK; (**f**) pBF127, BCK with the 3′UTR truncated at the *Sma*I site; (**g**) pBF126, B cDNA with sequences downstream of the *Sma*I site replaced with MCK sequences (B + M). The bar represents 50 μm.

This distribution was similar to that of pBF127 with the B 3′UTR deleted beyond the *Sma*I site (Figure 4f), except that, in this case, the label extended further into the cytoplasm. However, sequences downstream of the *Sma*I site in the MCK 3′UTR do not confer M-type localization on B mRNA. Although the pBF11 results have shown that these sequences are necessary for M-specific mRNA localization (Figures 3c and Figure 4b) they are clearly not sufficient to confer M localization on the B mRNA (Figure 4g). The B mRNA with the M 3′UTR sequence shows a similar distribution to the B mRNA with the sequence beyond the *Sma*I site deleted (pBF127). Either the signal for M-specific distribution spans the *Sma*I site or there is more than one sequence motif involved in signalling correct localization of the MCK mRNA. An alternative explanation is that the remaining

Table 2 CK activity in transiently transfected C_2C_{12} cells

C_2C_{12} cells were transiently transfected with the designated plasmid using the DEAE-dextran method and incubated for 48 h before harvesting. The transfection efficiency for each experiment was estimated by co-transfecting with a plasmid expressing hGH and measuring secreted hGH (see the Experimental section). The quoted activities have been normalized to the level of hGH in the medium. The concentration of secreted hGH was similar in all transfections (results not shown). Values, which are based on assays of extracts from cells from *n* different transfections for each plasmid, are means ± S.D. (*n*).

Plasmid transfected into the cells	CK activity (μmol/min per g of protein/ng of hGH)
pBF57	10.2 ± 0.4 (4)
pBF111	9.9 ± 0.6 (4)
pBF55	10.4 ± 0.5 (5)
pBF11	9.5 ± 0.7 (9)
pBF125	9.8 ± 0.5 (5)
pBF126	8.7 ± 0.7 (5)
pBF127	9.4 ± 0.6 (5)
pKV461	1.0 ± 0.1 (10)

B sequences override any M-specific signal downstream of the *Sma*I site.

Localization of CK mRNA in COS1 cells

To determine whether the localization of the CK mRNAs in myoblast cells was tissue-specific, we examined localization in transient transfections of a kidney cell line, COS1. The results are shown in Figure 5. It is clear that neither BCK (Figure 5a) nor MCK (Figure 5b) mRNAs show the same localization in COS cells as in C_2C_{12} cells. In COS cells the mRNAs of both isoforms showed the same distribution, with the RNA found throughout the cytoplasm, but excluded from the nucleus.

DISCUSSION

There are an increasing number of reports of subcellular localization of mRNAs. Many of the reports in somatic cells concern mRNAs for structural proteins such as actin, vimentin and tubulin [10,12,16,37]. The mRNAs for the M and the B isoforms of CK have been shown to display differential localization in cultured mouse myoblasts. The M isoform mRNA is localized in discrete patches at the cell periphery, while the B isoform mRNA has a perinuclear distribution (Figures 3 and 4).

In all previous cases where mRNA localization signals have been identified, these have been shown to reside within the 3′UTR. The data presented here show that this is also true for the mRNAs of the M and B isoforms of CK. The 3′UTRs of these genes are highly conserved. The nucleotide sequence of the 3′UTR of BCK shows greater than 90% identity among species [38,39], while the MCK sequence is also conserved, but to a lesser extent, with greater than 70% identity among species [40]. Deletion of these 3′UTR sequences has been shown to result in mislocalization of the M and B mRNAs. A sequence required for M-specific localization lies within the final 205 nucleotides of the M mRNA. Furthermore, exchange of the 205 nucleotides of the M mRNA 3′UTR downstream of the *Sma*I site for the final 154 nucleotides of the B 3′UTR results in a distribution of the M+B mRNA which is more like that of the B form. Thus B-specific localization signals must be located within this 154-nucleotide sequence of the B 3′UTR. However, the fact that the B 3′UTR truncated at the BCK *Sma*I site (pBF127) has a more diffuse pattern around the nucleus than the intact B mRNA suggests that there may be a component of the signal upstream of the BCK *Sma*I site. Additional signals upstream of the MCK *Sma*I site also appear to be required for M-specific localization, as transfer of sequences downstream of this site to a B mRNA are not sufficient to confer M-specific localization on the B mRNA. Rather the hybrid B+M mRNA shows the same distribution as before the addition of the M tail (Figure 4). Therefore both MCK and BCK mRNAs appear to require a combination of sequences for correct localization.

Ysraeli et al. [41] proposed a two-step model for the localization of Vg1 mRNA in *Xenopus* oocytes, with microtubules being important for translocation and microfilaments for anchorage of Vg1 mRNA. There is evidence from other studies that signals within the 3′UTR which specify the localization and the anchorage of mRNAs are independent (reviewed in [8,42]). This could explain why the CK mRNA species with only part of the 3′UTR show a more diffuse localization.

The distributions of the mRNAs for the M and B isoforms of CK are similar to those observed by Hill and Gunning [12] for β- and γ-actin isoforms in myoblasts. When these non-muscle isoforms were overexpressed in myoblast cells, β-actin mRNA was found at the cell periphery and around the nucleus, while γ-actin had only a perinuclear distribution, which was very similar to that observed here for BCK mRNA. Although the distribution

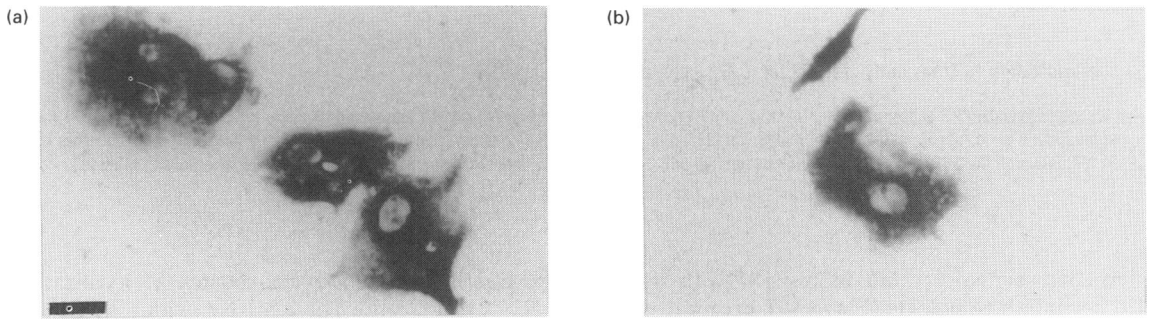

Figure 5 *In situ* hybridization to transiently transfected COS cells

(**a**) Transfected with pBF111 expressing CK from the full-length BCK cDNA, probed with BCK-specific probe. (**b**) Transfected with pBF57 expressing CK from the full-length MCK cDNA, probed with MCK-specific probe. The bar represents 50 μm.

of the mRNAs for the actin and CK isoforms were similar, no sequence similarity could be detected between their 3′UTRs. This is not unusual. For example, *nos*, *osk* and cyclin B mRNAs have similar subcellular localization in *Drosophila* oocytes, the signals residing in the 3′UTRs, but show no significant sequence similarities in these regions [43]. On the basis of these data it seems likely that the three-dimensional structure of the RNA may be more important than the actual sequence of the nucleotides in terms of signalling RNA localization.

RNA localization has been proposed to be a means of 'addressing' or defining subcellular locations other than the recognized membrane-bound compartments [9]. In somatic cells it has been suggested to be of general importance for the generation and maintenance of cell structures [17,18]. Localization of mRNAs in myoblasts could be an essential part of the differentiation of the cell. In the case of skeletal muscle, the mRNA for myosin has been shown to be localized in the myotendinous region where new myofibrils form [44], and the mRNAs for several cytoskeletal proteins have been shown to be localized and to change this localization during development [16].

CK is an important enzyme involved in the maintenance of energy homoeostasis in muscle. This function is thought to be dependent on its subcellular localization, with the M isoform localized at the M line in myofibrils and a mitochondrial isoform located in the intermembrane space of the mitochondria [3]. The B isoform, which is the most abundant species in myoblasts, is present in only very low concentration in mature muscle. Diffusion of PCr and creatine between the mitochondria and myofibrils is thought to provide a parallel diffusion pathway for ATP and ADP respectively, thus facilitating the transport of energy between the sites of production and utilization in the cell. Localization of the M isoform at the myofibrils is thought to depend on an interaction with the C-terminus of the protein. Schäfer and Perriard [19] microinjected MCK and hybrid MCK/BCK mRNAs into chicken heart cells. Immunolocalization studies showed that MCK molecules and translation products of chimaeric cDNA molecules containing the N-terminal half of the BCK- and the C-terminal half of the MCK-coding regions were localized to the M-line of the myofibrils. They concluded that the C-terminus of the MCK protein was essential for its association with the M line. However, in this experiment they had also exchanged the 3′UTRs of the mRNAs. The data presented here demonstrate that the mRNAs of BCK and MCK have different subcellular distributions in myoblast cells and that this localization is specified by their 3′UTRs. A tissue-specific protein which binds to the 3′UTR of BCK mRNA and which could be involved in mRNA localization, has been detected in osteoblasts [45]. Given the evidence from studies of other proteins that the localization of their mRNAs specifies the localization of the protein and that the 3′UTR determines this localization, these data suggest that the subcellular localization of CK mRNA could also serve to localize the cytosolic CK isoforms to specific regions of the cell. The isoform specificity of this localization in cells which normally express CK (C_2C_{12}) and the lack of localization in non-muscle cells (COS) supports this hypothesis.

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