The presence of mRNA coding for creatine kinase M (M-CK) and creatine kinase B (B-CK) in RNA from myogenic and fibrogenic cell cultures, embryonic muscle, and embryonic brain tissue was demonstrated by “in vitro” translation in a heterologous cell-free protein-synthesizing system from rabbit reticulocytes. The products were isolated by sensitive immunochemical methods and their identity with isolated M-CK and B-CK was shown by the following criteria: (a) the in vitro synthesized creatine kinases react with the specific antibody against these antigens; (b) the labeled peptides co-migrate with purified creatine kinase on sodium dodecyl sulfate gels in single bands; (c) the labeled peptides form homo- and heterodimers with isolated enzymatically active creatine kinase, thus behaving like authentic creatine kinases. The assay was shown to be reproducible and gave a linear response with increasing amounts of RNA, allowing relative quantitation of mRNA in polysomal RNA for the creatine kinases M and B.

mRNA for M-CK was detected in polysomal RNA and total cellular RNA from myogenic cells. It is also present in polysomal RNA from embryonic muscle and the fraction binding to oligo(dT)-cellulose. mRNA for B-CK could be found in RNA extracted from young myogenic cultures and the fraction of polysomal embryonic brain RNA binding to oligo(dT)-cellulose.

In the course of differentiation of myogenic tissue from the chicken embryo (1) as well as of myogenic cells, many structural and biochemical changes take place (2, 3). Most of the studies of the regulation of the biosynthesis of cell-specific proteins have dealt with the major proteins of the contractile apparatus, the outstanding feature of differentiated muscle cells. Myosin heavy chains and light chains as well as the different forms of actin have been studied at the level of the biosynthesis of these proteins and at the level of their mRNAs (4–16). These studies were facilitated by the rather unique size of myosin heavy chain or the relative abundance of actin and light chains of myosin. For some of these proteins, however, polymorphic forms have been found not all of which are specific for myogenic cells and muscle tissue (7, 17–20) and some seem to be ubiquitously distributed (see Ref. 21). Hence the advantage from the quantitative considerations cannot be fully exploited and probably will require reinterpretation of earlier results.

Other changes that have been observed comprise the accumulation of muscle-specific enzymes, which could easily be measured with the available specific assays for enzymatic activity (for review, see Refs. 2 and 3), but usually only total activity was accounted for. In the case of creatine kinase, an isoenzyme transition was observed to take place simultaneously with the differentiation of both the embryonic tissue (22) and the cultured myogenic cells (23–25). Mononucleated cells from presumptive avian muscle and the undifferentiated tissue contain only the B form of creatine kinase while M-CK becomes the predominant form in the differentiated myotubes or tissue (25, 26). The M-CK is characteristic for differentiated muscle and is only found in significant concentrations in striated muscle (22, 27). This isoenzyme transition provides a good model for one of the processes involving differential gene expression associated with myogenic terminal differentiation.

The two polypeptides are very probably the products of at least two different genes. Although the peptides have identical or very similar molecular weights of about 40,000, their amino acid composition and their tryptic peptide maps are clearly different (28). Furthermore, the antibodies against the two homomorphic enzymes do not cross-react with the heterologous antigen (28, 29) and can be used to distinguish between the two polypeptides.

Studies that concern differential gene expression in myogenic cells must necessarily also concentrate on the identification and the metabolism of the mRNA of such molecules. Since these mRNAs have no distinct size or structural features which can be used to separate them and furthermore, since they are expected to occur only in small concentrations in myogenic tissue or cells, we characterized these mRNAs by their property to be translated in a heterologous cell-free “in vitro” protein-synthesizing system. This allowed not only the demonstration of the distribution of the mRNA for M-CK and B-CK but also their relative quantitation, providing the basis for further studies of the levels of regulation of the creatine kinase isoenzymes.

**EXPERIMENTAL PROCEDURES**

**Animals**—Fertilized white leghorn eggs were obtained from Geflügel Wolff, Volketswil, Switzerland. Rabbits (Swiss Hair) weighing 2

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1 M. Caravatti and J. C. Perriard (1978) submitted for publication.

2 J. C. Perriard, M. Caravatti, E. R. Perriard, and H. M. Eppenberger (1978) Arch. Biochem. Biophys., in press.

3 The abbreviations used are: B-CK, M-CK, subunit of brain or muscle type creatine kinases; SDS, sodium dodecyl sulfate; BB-CK, MM-CK, homomeric creatine kinases of brain type or muscle type, respectively; MB-CK, heterodimeric creatine kinase; EGTA, ethylene glycol bis(β-aminoethyl ether)N,N'-tetraacetic acid.
to 3 kg were from the Institut für Biologisch-Medizinische Forschung, Fullsindorf, Switzerland.

**Chemicals**—Acrylamide and bisacrylamide were from Serva, rabbit muscle creatine kinase was from Boehringer, pancreatic ribonuclease and creatine phosphate were from Calbiochem, chicken MM-CK and BB-CK were from laboratory stocks.  
[4] [5]methylion (specific radioactivity 100 Ci/mmol) was dissolved in ethanol and injected intramuscularly to Wistar rats. Coon's Buffered Standard Salt Solution (BSB, 140 mM NaCl, 1.5 mM MgCl₂, and 5 mM KCl) to which a few drops of a heparin solution (1 mg/ml) were added to prevent clotting. The cells were washed three times by repeated suspension in RSB buffer and centrifugation in a Sorvall GSA rotor at 8,000 x g, 4°C, for 10 min, the buffy coat was removed by suction and the cells were resuspended in the buffer by an equal volume of fresh BSB buffer. Membranes and other cellular debris were removed by centrifugation in a Sorvall SS-34 rotor at 23,500 x g at 0-2°C for 20 min and the resulting lysate supernatant was immediately frozen and stored in liquid nitrogen in 1-ml portions.

**Cell-free Translation**—The lysates were incubated according to the method of Pelham and Jackson (37) to yield a mRNA-dependent protein-synthesizing system. One milliliter of freshly thawed lysate was made 0.00002 M hemin, 200 μg/ml of creatine kinase, 0.013 M creatine phosphate, 0.0008 M CaCl₂, 0.100 M KCl, 0.001 M MgCl₂, 65 μg/ml of the 19 common amino acids except methionine, in a final volume of 1.21 ml.

This mixture was equilibrated at exactly 20°C for 5 min; then 10 μl of poly(A) containing nuclease (1 mg/ml) were added and well mixed. The lysate was digested for 10 min at 20°C and the reaction was stopped by the addition of 10 μl of 0.25 M neutralized EGTA and immediately transferred to ice. To this lysate, radioactive methionine was added to a final concentration of 50 to 100 μc/ml and aliquots of 20 μl were supplemented with 5 μl of water for controls or RNA up to a total volume of 20 μl for assays of RNA. Incubation was usually for 60 min at 30°C. Protein synthesis was stopped by addition of 25 μl of 10 mM unlabeled methionine and placing the sample in ice. To determine total incorporation an aliquot, usually 5 μl, was spotted onto a Whatman No. 3MM filter and the filters were washed in hot trichloroacetic acid according to the method of Roberts and Paterson (38). The filters were subsequently equilibrated for 12 h in a toluene-based scintillation mixture containing 4 g/liter of Omnifluor and 3% (v/v) protosol. Radioactivity was determined in a Packard 2650 liquid scintillation spectrometer. Calibration was made by the external standard ratio method and the resulting counting efficiency using 14C settings for methionine was about 85%.

**Analysis of Products**—The remainder of the assay, typically 45 μl, was spotted to scintillation plates and 8 μg/ml of RNase and incubated for 10 min at 37°C. The mixture was diluted by the addition of 2 volumes of phosphate/NaCl buffer, pH 7.5 (10 mM NaPO₄, 0.14 mM NaCl), including 20 μg/ml unlabeled methionine and centrifuged for 15 min (27,000 x g). An aliquot of the supernatant was removed to display the synthesized peptides on a 10% discontinuous SDS-polyacrylamide gel. The remaining supernatant was used for analysis by specific immunoprecipitation.

Immunoprecipitation was carried out in two steps. A first immunoprecipitation was made with an antibody not related to muscle proteins to remove nonspecifically precipitating peptides and in a second step, both B-CK and M-CK were precipitated quantitatively. For the first immunoprecipitation, the lysate was mixed with 7 μl of rabbit anti-guinea pig serum diluted 10-fold, 15 μl of rabbit anti-guinea pig serum, antisera made 500 μl in volume by the addition of phosphate buffer and incubated for 30 min at 30°C and subsequently at least 4 h at 4°C. The incubation was underlayered with 200 μl of 1 M sucrose in phosphate buffer containing 1% Triton X-100 and the immunoprecipitate was collected by centrifugation at 9,500 x g for 15 min in a Beckman SW 40 rotor at 4°C. The supernatant including 50% of the sucrose pad was removed, transferred to a new tube, and mixed with 0.5 to 1 μg of each purified BB-CK and MM-CK and the corresponding antibody preparation in 5 to 4-fold excess. The second precipitation was carried out as above (but 300 μl of 1 M sucrose in phosphate buffer was used). After centrifugation, the supernatant was removed by suction as well as antibody and the sucrose pad was washed with the solution for the walls of the tube were rinsed with phosphate buffer which was finally removed by suction including the remainder of the sucrose pad. The precipitates were dissolved in 30 μl of SDS sample buffer and the two species could be separated on a 10% SDS-polyacrylamide gel. After staining (40), the bands representing M-CK and B-CK were cut from the gel and extracted in an SDS-soluble mixture containing protease, and the radioactivity was determined. Alternatively, gels were prepared for fluorography according to established methods.
Hybridization of "in Vitro" Synthesized CK with Purified Enzymes—The cell-free assays were scaled up 90-fold for the synthesis of B-CK and M-CK and immunoprecipitates were obtained as described before, except that only one of the antibody species was used for the final precipitation of either B-CK or M-CK. The pellets were directly dissolved in 200 μl of 3 M KCl, 0.05 M Tris-HCl, pH 8.0, and split into two equal portions. To one-half 10 μg of purified, homologous, homomeric creatine kinase was added (in the case of the pellet containing M-CK, MM-CK was added; if on the other hand the pellets contained B-CK, BB-CK was added). To the second portion, a mixture of 5 μg of MM-CK and 10 μg of BB-CK (or 10 μg of MM-CK and 5 μg of BB-CK for the portion containing synthesized M-CK) were added. The enzymes were incubated for 30 min at 30°C to dissociate the added enzymes and the immunoprecipitate into their subunits. Reassociation of dimeric enzymes was achieved by dialysis against 0.050 M Tris-HCl, 0.001 M EDTA, 0.01 M f-mercaptoethanol, 10% glycerol (v/v) pH 8.0 for 4 h and then the samples were concentrated by dialysis against the same buffer containing 50% (v/v) glycerol. The resulting enzymes were subjected to polyacrylamide gel electrophoresis under nondenaturing conditions using a 7.5% 2-mm thick gel slab prepared according to Maurer (42). Enzymatic staining with an agar overlay gel was performed as described by Turner et al. (23) and the acrylamide gel was stained for protein and processed for autoradiography as described before.

RESULTS

Cell-free In Vitro Translation of M-CK and B-CK—The total cellular or polysomal RNA obtained from myogenic cells or embryonic tissue by the CeCl/lauryl sarcosinate centrifugation method (33) did not show any signs of degradation as judged by SDS/sucrose density gradient centrifugation or agarose-polyacrylamide gel electrophoresis (data not shown). These RNA preparations were effective in priming the synthesis of peptides in a mRNA-dependent in vitro protein-synthesizing system derived from rabbit reticulocytes (37) and the synthesis of M-CK and B-CK could be demonstrated. Aliquots taken from different in vitro incubations were analyzed on 10% SDS-polyacrylamide slab gels (39) and the radioactively labeled peptides synthesized were revealed by fluorography. In Fig. 1, the data are compiled for the comparison of the different steps. The part labeled a displays the gels stained for protein, while Part b displays the fluorograph of the same gel. Lane 1 displays a sample of a lysate treated with micrococcal nuclease but without added RNA and demonstrates that the digestion procedure effectively removes the background of peptides synthesized due to the presence of endogenous mRNA, leaving only two faintly labeled bands, already described by Pelham and Jackson (37). If, however, polysomal RNA from myogenic cell cultures was added, the system yields a host of radioactively labeled, different products, presumably reflecting all different kinds of mRNA species. The size of these products extends well above a M, of 40,000 (Fig. 1, Lane 3). We have made sure that no creatine kinase is precipitated in these immunoprecipitates by immunoreplica of SDS gels (44), by autoradiography as described before. The autoradiograph of the resulting immunoprecipitate analyzed on a SDS gel demonstrates the unspecific precipitation of a variety of proteins, some of them migrating in the critical region of the creatine kinase peptides, around the M, of 40,000 (Fig. 1, Lane 3). We have made sure that no creatine kinase is precipitated in these immunoprecipitates by immunoreplica of SDS gels (44). There is not even a trace of radioactively labeled M-CK or B-CK detectable as demonstrated by the autoradiographs of such immunoreplicas (not shown). The in vitro synthesized M-CK and B-CK peptides could be isolated by subsequent specific immunoprecipitation using the specific antibodies and the purified enzymes as carrier. The autoradiograph of the SDS gels shows that both species of creatine kinase were synthesized and could be specifically precipitated (Fig. 1, Lane 4).

If the products were precipitated with only one kind of creatine kinase antibody only one kind of creatine kinase was precipitated (Fig. 1, Lanes 5 and 6). If, however, in all the above mentioned specific immunoprecipitation steps, immune serum or antiguenius pig serum were substituted for the specific anti-creatine kinase antibody preparation, no precipitation of creatine kinase peptides was observed (Fig. 1, Lanes 8 and 9).

After the removal of the in vitro synthesized creatine kinase, new carrier protein and the corresponding anti-creatine kinase antibodies were added and this second immunoprecipitate was analyzed for the presence of either creatine kinase peptides. As shown in Fig. 1, Lane 10, no radioactivity is detectable by autoradiography. We conclude that all in vitro synthesized creatine kinase was precipitated in the specific immunoprecipitation step.

In Vitro Synthesized Creatine Kinase Peptides Are Genuine Creatine Kinase—As demonstrated before, peptides with some properties of creatine kinases are synthesized in a cell-free system. There are, however, some examples, especially of secretory proteins, synthesized in vitro in cell-free systems with properties slightly different from those of the authentic molecules isolated from the respective tissue as discussed in Palmiter et al. (45). We have already demonstrated that the

![Fig. 1. 10% SDS-polyacrylamide of samples from cell-free protein synthesis assays and immunoprecipitates stained for protein (a) and the corresponding fluorograph (b).](http://www.jbc.org/)
in vitro synthesized creatine kinase react with the specific antibodies and co-migrate on SDS gels with the molecules purified from adult chicken tissues. In the following experiment, the evidence is given that the in vitro synthesized creatine kinase are capable of interacting with purified creatine kinase in the same way as the subunits of the active enzymes do.

Radioactively labeled B-CK and M-CK were prepared and isolated in the manner described above and the immunoprecipitates containing only B-CK and M-CK were treated with high ionic strength buffer to dissociate both the enzyme dimers and the immunocomplexes. The resulting solution was divided into two parts of equal size. To one part, a 10-fold excess of homologous, enzymatically active creatine kinase was added together with twice as much of the heterologous creatine kinase species which was not present as radioactive creatine kinase. The other half of each preparation was supplemented with a 20-fold excess of the homologous enzyme present in the immunoprecipitate as the radioactive protein. The dissociated enzymes were allowed to reassociate and regain enzymatic activity by dialysis against low ionic strength buffer containing β-mercaptoethanol. All four samples were subsequently analyzed on polyacrylamide slab gels under non-denaturing conditions, allowing the separation of all three native, enzymatically active creatine kinase species, MM-CK, MB-CK, and BB-CK. The enzymatic activity of the samples was revealed by placing a detector gel on top of the polyacrylamide gel; after this incubation, it was stained for protein and autoradiographed. The enzyme activity stain is shown in the MB-CK, and BB-CK. The enzymatic activity of the samples native, enzymatically active creatine kinase species, MM-CK, was revealed by placing a detector gel on top of the polyacrylamide slab gels under non-denaturing conditions, allowing the separation of all three native, enzymatically active creatine kinase species, MM-CK, MB-CK, and BB-CK. The enzymatic activity of the samples was revealed by placing a detector gel on top of the polyacrylamide gel; after this incubation, it was stained for protein and autoradiographed. The enzyme activity stain is shown in the right portion of Fig. 2. In Lanes 1 and 3, the enzymatically active bands of BB-CK and MM-CK coincide with the autoradiograph of the same samples and the protein staining pattern (left portion of Fig. 2), indicating that the in vitro synthesized creatine kinase molecules took part in the formation of the homologous creatine kinase species. If, however, in vitro synthesized molecules were dissociated and reassociated in the presence of both kinds of homomeric creatine kinase, the formation of all three creatine kinase species could be observed as shown in Lanes 2 and 4. Due to the interaction of the radioactively labeled peptides with the heterologous enzyme subunit, the radioactivity appeared also in the position of the heteromeric enzyme MB-CK, but never in the position of the heterologous dimeric enzyme. This experiment further indicates that the in vitro synthesized creatine kinase peptides behave like the enzymatically active, purified enzymes and take part in the interaction of the subunits. This adds additional evidence that the observed peptides are identical with genuine creatine kinase subunits.

**Reproducibility of Assays for mRNA Coding for Creatine Kinase and Their Quantitation.—**Since one of the goals of the present study was to establish methods to determine relative amounts of mRNA present in complex mixtures of RNA such as total cellular RNA or polysomal RNA, it was necessary to test the reproducibility of the assays. After the isolation of the synthesized creatine kinase peptides on SDS gels, the corresponding bands were cut out, solubilized in a liquid scintillation mixture, and analyzed for radioactivity in a liquid scintillation spectrometer. Table I shows the result of a series of assays primed by similar RNA preparations at two concentrations. The results demonstrate that even at this low level of incorporation into creatine kinase peptides (0.1 to 0.4% of total), the observed radioactivity measurements were within a reasonable range of the average value. Again, reprecsipitation of the supernatant with anti-B-CK and anti-M-CK did not yield any radioactivity in the bands of the gels representing M-CK and B-CK as was shown in the autoradiograph of a similar precipitate (Fig. 1, Lane 10).

In the next experiment, duplicate assays were run with increasing amounts of RNA per standard reticulocyte lysate

![Fig. 2](https://example.com/fig2.png)

**Fig. 2.** Electrophoresis of creatine kinase isoenzymes on 7.5% non-denaturing acrylamide gel of in vitro synthesized, radioactively labeled, and enzymatically active purified creatine kinase after the dissociation reassociation procedure as described under “Experimental Procedures.” Left, stained for protein; center, autoradiography; right, stained for enzymatic activity. 1, in vitro synthesized B-CK plus BB-CK; 2, in vitro synthesized B-CK plus both BB-CK and MM-CK; 3, in vitro synthesized M-CK plus purified MM-CK; 4, in vitro synthesized M-CK plus both MM-CK and BB-CK; 5, marker enzymes MM-CK and BB-CK; purified from chicken; breast muscle and heart, respectively.

### Table 1

| Assay number | dpm in M-CK | dpm in B-CK |
|--------------|-------------|-------------|
| 1            | 410         | 265         |
| 2            | 429         | 256         |
| 3            | 485         | 285         |
| 4            | 408         | 299         |
| Mean ± S.D.  | 425 ± 21.8  | 284 ± 15.2  |
| 5            | 232         | 187         |
| 6            | 278         | 171         |
| 7            | 290         | 195         |
| 8            | 277         | 167         |
| Mean ± S.D.  | 269 ± 25.5  | 180 ± 13.2  |

**Assay for creatine kinase mRNAs in reticulocyte lysate cell-free protein-synthesizing assays primed by polysomal RNA from myogenic cells.**

For each RNA preparation, four assays were run in order to demonstrate the reproducibility of the procedure. Assays 1 to 4 were primed by 1.8 μg of polysomal RNA from 4-day-old cultures. Assays 5 to 8 by 1.15 μg of a similar preparation. The assays were run on different days, but with the same lysate and identical label concentration.

![Downloaded from http://www.jbc.org/ by guest on March 21, 2020](https://example.com/table1.png)
assay and the M-CK and B-CK produced were isolated and their radioactivity was measured as described above. The RNA, derived from 4-day-old myogenic cell cultures obviously contained both kinds of mRNA for creatine kinase as shown in Fig. 3. The curves show a linearly increasing incorporation with increasing amount of RNA assayed and saturation of incorporation into creatine kinase around 120 μg of RNA/ml. The procedure allows, therefore, not only the detection of mRNA but also the measurements of relative amounts of mRNA species and thus will be useful in studying the regulation of gene expression in differentiating myogenic cells.

Occurrence of mRNA for M-CK and B-CK—As already shown in Fig. 1 (Lane 5) and Fig. 3 as well as in Table I, the occurrence of mRNA for both kinds of creatine kinase has been established for RNA from 4-day-old myogenic cell cultures. We wanted to test further different RNA preparations from a variety of sources for the occurrence of mRNA for the creatine kinase peptides. Total polysomal RNA was extracted from the breast muscle and brain of 14-day-old chicken embryos and a crude poly(A)-containing RNA fraction was prepared by chromatography on oligo(dT)-cellulose. The resulting RNA binding to the adsorbent was assayed for creatine kinase mRNA in reticulocyte lysates as described above. In Fig. 4, the fluorographs of these assays are compiled and show the mRNA for M-CK to occur in myogenic cells and embryonic muscle tissue but not in brain tissue polysomal RNA. Furthermore, the experiment established that both kinds of mRNA have a poly(A) tract as judged by the activity of the RNA retained on oligo(dT)-cellulose to code for both M- and B-CK peptides (Fig. 4, Lanes 4 and 5). Brain RNA contains only mRNA for B-CK as expected from the accumulation patterns of this tissue (27); embryonic muscle, however, contains mRNA for the M-CK and only a trace of B-CK mRNA activity is left in the total polysomal RNA from this tissue, indicating that the isoenzyme transition at the level of the mRNA is already quite advanced.

In a second experiment, we tested for the disappearance of the mRNA for M-CK after subculturing fibrogenic cells derived from fibrogenic tissue of 11-day-old chicken embryonic breast muscle. Total cellular RNA was extracted from primary fibrogenic cultures and from cells that were subcultured two...
times. Earlier studies had shown that MM-CK and MB-CK activity was present in primary cultures of fibrogenic cells but that the M-CK subunit-containing enzymes were removed upon subculture of these cells (23) due to the selection against the myogenic cells in the subculturing process. The autoradiographs of the corresponding SDS gels show the presence of both kinds of mRNA in confluent primary fibrogenic cultures (Fig. 4, Lane 2) but the absence of the mRNA for M-CK in subcultures of the same cells (Fig. 4, Lane 3). Thus, we conclude that we are able to pick up the mRNA from contaminating cells, due to remaining myogenic cells in such fibrogenic primary cultures. Quantitative analysis demonstrated also the removal of mRNA for M-CK in the process of the subculture of fibroblast-like cells from myogenic tissue as shown in Fig. 5. Thus, the procedure is sensitive enough to pick up the mRNA derived from some contaminating myogenic cells, comprising 5 to 10% of the nuclei in myotubes, (Fig. 5a) in the primary fibroblast cultures and their removal after subculturing the cells twice (Fig. 5b).

**DISCUSSION**

The occurrence of isoenzyme transitions concomitant with other differentiative events has been observed in a variety of tissues and organisms (see Ref. 46) and is not restricted to myogenic cells undergoing differentiation. It is, however, interesting to note that myogenic cell cultures display a variety of isoprotein transitions. Not only enzymes like creatine kinase and aldolase (23, 47) go through such transitions but also the different forms of actin (7, 19) and myosin proteins display similar features (18). Since it is rather difficult to distinguish between the different forms of some of these molecules and their mRNAs, the advantages conferred by the relatively high concentration of these species are not easy to exploit. In the case of creatine kinase however, the isoprotein molecules can be identified relatively easily, in spite of their low abundance, on the basis of their immunological and biochemical properties.

Another feature of the creatine kinases of cultured myogenic cells is that B-CK, the form of creatine kinase in young embryonic muscle, never completely disappears in culture (25) and therefore, this molecule represents an internal control for basic cell function and allows experimental manipulation of the cells. The levels of creatine kinases in nonmyogenic tissues are much lower; for example, fibroblast cultures synthesize only about 0.001% of total cellular protein compared to 0.5% devoted to total creatine kinase synthesis of differentiated myogenic cells and therefore do not represent a major source of mRNA for B-CK, while M-CK was almost undetectable.

The approach to the study of these mRNAs in the present communication was to use the translatable part of the mRNA coding for the creatine kinase proteins, as a criterion for their occurrence. Of course, if these molecules were accumulated in some untranslatable form, we would be unable to detect these and molecular hybridization methods would be required. The purification of the mRNA coding for these enzymes and the subsequent synthesis of a cDNA probe seems within the reach of modern technology, but still represents a major effort for mRNA available in such small concentrations. The fact that the mRNA can be assayed for in purified, but unfractonated RNA allows us to test the RNA in larger numbers of samples without the need to purify the RNA further, thus avoiding problems of recovery, poly(A) content and the completeness of retention on affinity adsortents like oligo(dT)-cellulose or poly(U)-Sepharose and the purity of the eluted material. These considerations may be important since recent studies (48) have indicated that β-actin mRNA fails to bind to oligo(dT)-cellulose, the mRNA for the isopolypeptides γ-actin and α-actin, however, are retained. Considering such heterogeneity, we thought of using unfractonated cellular or polysomal RNA in our experiments, since it is not clear that such differences are restricted to the mRNA for actin.

The criteria for the authenticity of the in vitro synthesized peptides have been described under "Experimental Procedures" and "Results," but some features of the demonstration of authenticity deserve further attention. Since the relatively abundant actins almost co-migrate with B-CK on SDS gels, we had been worried about possible contamination of the immunoprecipitates with such molecules. Pretreatment of incubated lysates with unrelated antibody and its antigen removed the contaminating molecules without precipitating any creatine kinase peptides. The fact that the two creatine kinase peptides could be resolved on 10% SDS-polyacrylamide slab gels is astonishing since earlier studies on cylindrical gels did not show a clear separation. Further details will be discussed elsewhere.

Of course, the usefulness of an assay for mRNA based on in vitro translatability is limited if the assay is either very complicated to carry out or if it is not reproducible and thus limits the application only to qualitative investigations. With the present study, we hope to show clearly that even for a mRNA species present at low concentrations, it is possible to find suitable assay conditions to allow at least a relative quantitation of the two mRNA species involved. Lodish (49) already pointed out that α- and β-globin mRNA were translated with different efficiencies in cell-free protein-synthesizing systems. We assume that this could be the case in our system; therefore, the quantitative determinations must be considered as relative, but reproducible, if determinations are considered within one experiment measured under identical conditions. It will depend on the availability of a purified mRNA preparation for both kinds of creatine kinase to allow the standardization of these assays, in order to calibrate the assays and give the results absolute values.

We want to apply these procedures to the study of gene expression at the level of production and accumulation of mRNA for B-CK and muscle-specific M-CK. The methods described above will provide the basis for characterizing more carefully the mRNA and the isopolypeomes of the creatine kinase isoproteins, a prerequisite of their enrichment and future purification.

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