Vol. 92, No. 2, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 29, 1980 Pages 517-523

THE TRANSLATION OF CHICK SKELETAL MUSCLE POLY(A)-CONTAINING mRNA IN PRIMARY HEART MUSCLE CELLS IN CULTURE

Chris Barnard, Glenda A Durrheim, Honor M Robinson and André J Bester

M R C Research Unit for Molecular and Cellular Cardiology, P O Box 63, Tygerberg 7505, Republic of South Africa

Received December 3, 1979

SUMMARY: Chick skeletal muscle mRNA (40 µg/0.8 mℓ) in phosphate-buffered saline was added to 60 mm petri dishes containing a monolayer of primary heart muscle cells. After 30 minutes absorption the cultures were supplemented with complete medium and the incubation continued in a humidified CO_2-incubator. Sucrose density gradient analysis of the absorbed RNA showed no degradation. This skeletal muscle mRNA was translated in primary heart muscle cells in culture into functional proteins as indicated by the linear increase in the accumulation of acetylcholine receptors as well as a similar increase in creatine kinase activity. In addition, the synthesis of the three unique light chains of skeletal muscle myosin (SLC_1, SLC_2, SLC_3) in these primary heart muscle cells was also demonstrable.

INTRODUCTION

Several important questions in the developmental biology of specialised cells can be studied by the introduction of protein factors which are involved in the control of translation and transcription, as well as messenger RNA and cytoplasmic RNP particles, from one kind of highly differentiated cell into another differentiated cell-type. A prerequisite for such an approach should be that the recipient cells be highly differentiated and that the introduced macromolecules be accepted by the recipient cell in a normal and functional way. In addition, it is necessary that the recipient cells continue to grow and differentiate following the introduction of such exogenous macromolecules or cytoplasmic particles.

Muscle cells possess many features which make them one of the most suitable cel systems for the study of differentiation and its control. For instance, terminal differentiation is characterised by the formation of multinucleated myotubes which arise from the fusion of single myoblasts (1) and cell fusion is paralleled by an increased production of many muscle-specific proteins (2 - 4). Although many studies have indicated that muscle mRNAs are synthesised at one stage of development (myoblast) and that their translation occurs at another stage (myotubes) (3, 5 - 7), very little is known about the *in vivo* processing of such mRNAs. Beating heart muscle cells obtained from the hearts of 1 - 2 day old neonatal hamsters (terminally differentiated cells) offer a unique *in vivo* system to study the translation or processing of mRNAs isolated from skeletal or breast muscle cells which are not at a terminally differentiated stage of development (single myoblasts). In

0006-291X/80/020517-07$01.00/0

the following, we report that heart muscle cells were able to translate the introduced skeletal muscle mRNA into functional proteins.

METHODS

Primary heart cell cultures. Primary heart cell cultures were prepared and established from the hearts of 1 - 2 day old neonatal hamsters; the cells harvested, counted and homogenized as previously described (8).

Primary chick skeletal muscle cultures. Primary chick skeletal muscle cultures were prepared from thigh muscle of 11-day pathogen-free chick embryos and grown as described previously (9).

Myofibrillar ATPase activity. The Ca^{2+}-stimulated myofibrillar ATPase activity of heart muscle cells was measured as described (8), using [γ-^{32}P] ATP.

Determination of creatine kinase activity. Creatine kinase activity was measured as described (5). The rate of ATP formation from ADP and creatine phosphate was determined by coupling the reaction to hexokinase and glucose-6-phosphate hydrogenase (10).

α-Bungarotoxin binding assay. Iodination of α-bungarotoxin was carried out using the chloramine-T method (11) and the cultures assayed essentially as described (12).

Isolation of unlabelled myosin. Myosin was isolated from both skeletal muscle and heart muscle tissue according to the method of Coetzee *et al.* (13) and subsequently purified by gel filtration on Sepharose 4B as described by Pollard *et al.* (14).

Isolation of labelled myosin. After 12 hours in culture heart muscle cultures were labelled for 24 hours in 5 mℓ of complete medium containing 25 μCi of [^{35}S] methionine (217 mCi/mmol) per mℓ. At the end of the labelling period, myosin was extracted in the cold as described (15).

Isolation of labelled total cytoplasmic RNA. After 12 hours in culture primary chick skeletal muscle cultures were labelled for 48 hours in 5 mℓ complete medium containing 25 μCi of [^{3}H] Uridine (40 Ci/mmol) per mℓ. After labelling, the cells were harvested and lysed as described (16). Total RNA was extracted and ethanol precipitated without oligo (dT)-cellulose chromatography (17).

Isolation of unlabelled poly(A)-containing RNA. The post-mitochondrial supernatant from 14-day embryonic chick leg muscle (18) was centrifuged for 5 hours at 200 000 x g to pellet the polysomes together with the mRNP particles. The obtained polysomes and mRNP particles were subsequently used for preparation of poly(A)-containing RNA (17).

The absorption of [^{3}H]-labelled skeletal muscle RNA by heart muscle cells. Primary heart muscle cultures were allowed to absorb radioactive chick skeletal muscle RNA for different time periods. This was done to estimate the maximum absorption of the exogenous RNA by the heart cell cultures as well as to ensure optimum growth properties of the cells in culture. After the initial 12 hours in culture, the complete medium was decanted and the cells washed once with phosphate-buffered saline (PBS). [^{3}H]-labelled RNA was dissolved in PBS and 0.8 mℓ (55 x 10^{6} cpm) was added to each 60 mm petri dish (this volume was enough to just cover the monolayer of heart cells) and the cultures incubated for 30, 45 and 60 min at 37 °C in a humidified CO_2-incubator. Complete medium (4.2 mℓ) was added to the cultures after the respective absorption periods, and the cultures maintained for the indicated time periods. Immediately after the respective absorption time periods and on day 1 through to day 5, the cells were harvested by trypsinization (8) and the Ca^{2+}-stimulated myofibrillar ATPase activity measured. Some of the cultures were used to re-extract labelled RNA which was subsequently analysed on sucrose density gradients.

The translation of unlabelled poly(A)-containing skeletal muscle RNA in primary heart cell cultures. After 12 hours in culture, the medium was removed and the cells washed once with PBS; 40 μg of poly(A)-containing RNA in 0.8 mℓ PBS was added to each 60 mm petri dish. An absorption period of 30 min was allowed, after which the cells were supplemented with 4.2 mℓ complete medium and maintained in a humidified CO_2-incubator for the indicated times (see results). Subsequently creatine kinase activity was determined; the appearance of acetylcholine receptors

monitored using the α-bungarotoxin binding assay; and the synthesis of the light chains of skeletal muscle shown by autoradiography. In the latter case, heart muscle cells were allowed to absorb the RNA for 30 min, followed by the addition of 25 μCi of [^{35}S]-methionine (217 mCi/mmol) per mℓ complete medium and the cells allowed to grow for a further 24 hours.

RESULTS AND DISCUSSION

Very little is known concerning the introduction of the mRNA of highly differentiated cells (i.e. skeletal muscle cells) into another differentiated cell type (i.e. heart muscle cells) in such a way that the introduced mRNA is accepted in a normal and functional way by the recipient cells. Thus, experiments involving the introduction of mRNA into differentiating cells fall into two categories. Firstly, it is necessary to establish whether the RNA absorbed by the recipient cells is still intact, and secondly, that the cells do not degenerate due to possible deleterious effects of the introduced RNA.

After 30 minutes of absorption, the labelled RNA was extracted and analysed by sucrose density gradient centrifugation. As can be seen in figure 1, the RNA sedi-

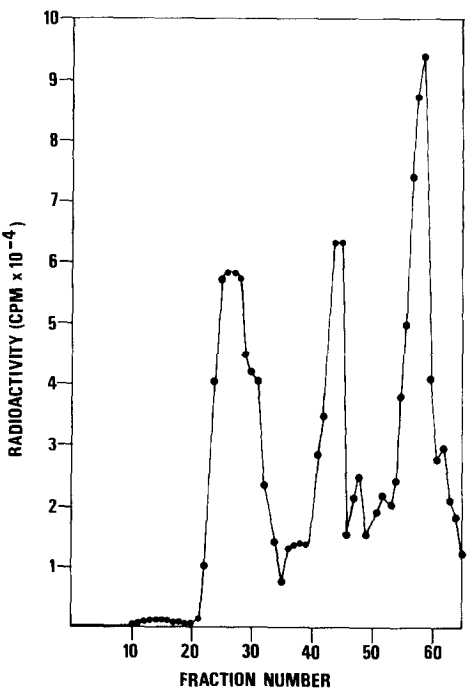


Figure 1 Sucrose density gradient analysis of skeletal muscle [^{3}H]-labelled RNA extracted from heart muscle cells in culture. Labelled skeletal muscle RNA was absorbed by heart muscle cells for 30 min. and the cells cultured for a further 24 hours before re-extraction of the labelled RNA. The 10 - 30 % (w/w) sucrose gradients in 0.05M Tris HCℓ (pH 7.4), 0.005M EDTA, 0.5 % (W/v) sodium dodecylsulphate were centrifuged for 10 hours at 40 000 r.p.m. at 4 °C in an I.E.C. 283 rotor. Each 10.5 mℓ gradient was layered with the RNA sample in the above buffer. Fractions (10 drops per fraction) were collected from the bottom of the tubes. Radioactivity was determined by mixing each fraction with 10 mℓ of Packard Insta Gel and counted in a Packard Tricarb Scintillation counter.

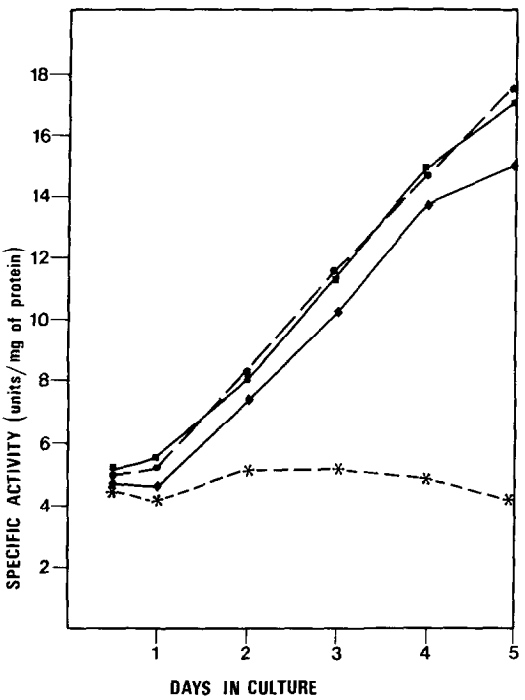


Figure 2 Ca^2 -dependent ATPase activities of heart cells grown in culture following the introduction of skeletal muscle RNA. RNA was added to the heart muscle cells after the initial 12 hours in culture, different absorption periods allowed and then the cells maintained in culture as described in methods. Untreated cells (●——●), cells allowed to absorbed RNA for 30 min (■——■), for 45 min (◆——◆) and for 60 min (*----*). Activities were determined and expressed as a function of total cellular protein.

ments from approximately 4S to 28S, exhibiting characteristic sedimentation properties of undegraded muscle RNA. To test viability of the heart muscle cells following the introduction of the skeletal muscle RNA, different absorbing times were allowed and the Ca^2 -stimulated myofibrillar ATPase activity, the enzyme generally considered to be especially prevalent in muscle cells, determined from day 1 to day 5 (Figure 2). Small differences are observed between the untreated cultures and those treated with the exogenous RNA for 30 min and 45 min. However, the cultures treated with the RNA for 60 min did not recover and displayed low ATPase activities

To evaluate the efficiency of such translation, the synthesis of the small subunits of skeletal muscle myosin, a unique set of proteins characteristic of each muscle type, together with creatine kinase activity and the appearance of acetylcholine receptors were studied. Primary heart muscle cells were allowed to absorb chick skeletal muscle mRNA for 30 min after which complete medium was added and the incubation continued. Cells were harvested at the indicated times (Figure 4) and assayed for the synthesis of creatine kinase and acetylcholine receptors. While

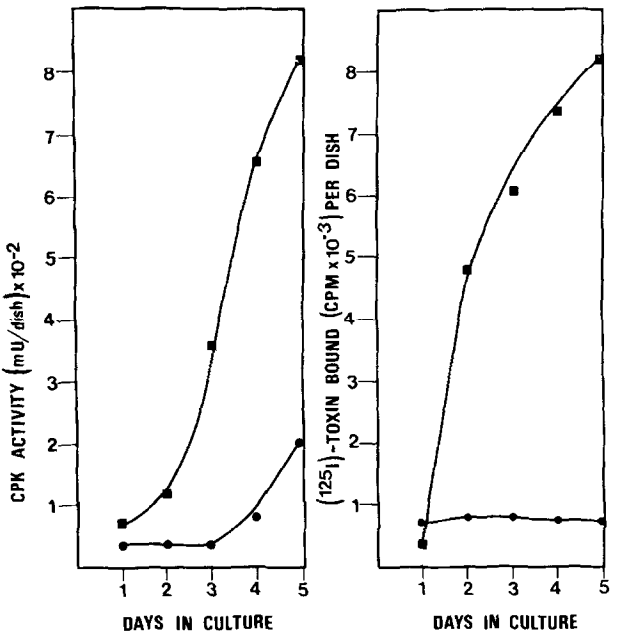
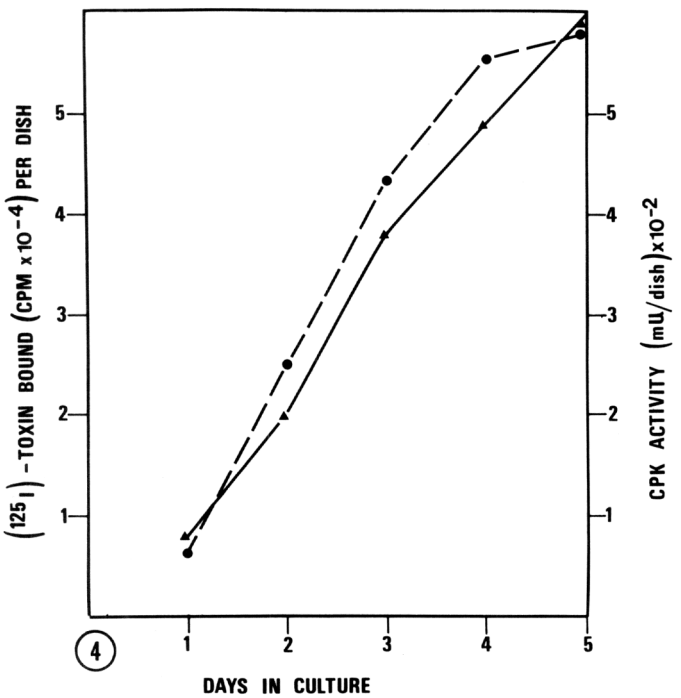


Figure 3 Creatine kinase activity (left panel) and the appearance of acetylcholine receptors (right panel) in untreated primary heart muscle (●——●) and skeletal muscle cultures (■——■).

primary heart muscle cells in culture showed very low endogenous activity of these two proteins (Figure 3), an almost linear increase in creatine kinase activity and appearance of acetylcholine receptors was observed following the introduction of poly(A)-containing skeletal muscle mRNA into heart muscle cells (Figure 4). To demonstrate the synthesis of the skeletal muscle specific myosin light chains in heart muscle cells, [^{35}S] methionine was added to the primary muscle cultures after the introduction of the skeletal muscle mRNA, and allowed to incubate for a further 24 hours. Myosin was subsequently isolated from the heart muscle cells, the light and heavy chains separated by gel electrophoresis and the radioactive bands visualised by autoradiography (Figure 5). It is apparent from figure 5 that not only the two light chains (HLC_1 and HLC_2 with molecular weights of 26 000 and 20 000 dalton respectively) of heart muscle myosin have been synthesized, but in addition, the appearance of the three light chains of skeletal muscle myosin, the SLC_1, SLC_2 and SLC_3 with molecular weights of 22 000, 17 000 and 15 000 dalton respectively, were also observed.

It is apparent from the results of the present study, that during the process of heart muscle cell development and differentiation, these cells were still able to translate functional proteins from exogenous mRNA.

Several RNAs which are possibly involved in the control of translation have emerged during recent years (19 - 21), but their role in normal cellular processes



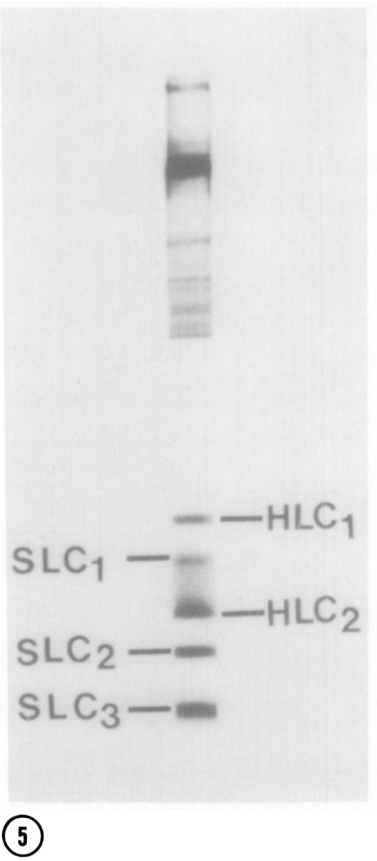


Figure 4 The effect of the absorbed chick skeletal muscle poly(A)-containing mRNA on the creatine kinase activity (●----●) and accumulation of acetylcholine receptors (▲——▲) in primary heart muscle cells in culture.

Figure 5 Autoradiography of myosin extracted from primary heart muscle cultures after the introduction of poly(A)-containing chick skeletal muscle mRNA. The separation was carried out on a 10 - 20 % SDS polyacrylamide gradient slab gel and the autoradiograph developed as previously described (15). The migration of purified unlabelled markers are indicated alongside the autoradiograph: HLC_1 HLC_2 light chains of heart muscle myosin; SLC_1, SLC_2, SLC_3: light chains of chick skeletal muscle myosin.

is still undefined. Similarly, although substantial information about protein factors involved in translational control mechanisms were described in cell-free systems (22 - 24), very little is known about their actual role in living cells. In addition, many reports have dealt with inactive or stored cytoplasmic messages as RNP particles (25 - 27), but their activation or processing during cell development in unclear. Therefore the above approach of introducing exogenous mRNA from one cell-type (e.g. chicken myoblasts) into another differentiated cell-type (e.g. primary heart muscle cells) by tissue culture techniques, in sufficient amounts

and in such a way that they function normally, may be useful in studying protein factors and small molecular weight RNAs involved in mRNA translation and also the processing of stored cytoplasmic mRNP particles.

REFERENCES

1. Yaffe, D. (1969) *Current Topics Dev. Biol.* 4, 37 - 77.
2. Paterson, B.M., Strohman, R. (1972) *Dev. Biol.* 29, 113 - 138.
3. Yaffe, D., Dym, H. (1972) *Cold Spring Harbor Symp. Quant. Biol.* 37, 543 - 547.
4. Emerson, C.P., Beckner, S.K. (1975) *J. Mol. Biol.* 93, 431 - 447.
5. Shainberg, A., Yagil, G., Yaffe, D. (1971) *Dev. Biol.* 25, 1 - 29.
6. Buckingham, M.E., Cohen, A., Gros, F. (1976) *J. Mol. Biol.* 103, 611 - 626.
7. Yaffe, D., Yablonka, Z., Kessler, G., Dym, H. (1975) mRNA and protein synthesis in differentiating muscle cultures. *Proc. 10th FEBS Meetings* 38, 313 - 323.
8. Coetzee, G.A., Van der Westhuizen, D.R., Gevers, W. (1977) *Biochem. J.* 164, 635 - 643.
9. Tepperman, K., Morris, G., Essien, F., Heywood, S.M. (1975) *J. Cell. Physiol.* 86, 561 - 565.
10. Eppenberg, H.M., Dawson, D.M., Kaplan, N.O. (1967) *J. Biol. Chem.* 242, 204 - 209.
11. Greenwood, F.C., Hunter, W.H., Glover, J.S. (1963) *Biochem. J.* 89, 114 - 123.
12. Vogel, Z., Sytkowski, A.J., Nirenberg, M.W. (1972) *Proc. Natl. Acad. Sci. U.S.A.* 69, 3180 - 3184.
13. Coetzee, G.A., Gevers, W. (1978) *Dev. Biol.* 63, 128 - 138.
14. Pollard, T.D., Thomas, S.M., Wiederman, R. (1974) *Anal. Biochem.* 60, 258 - 266.
15. Yablonka, Z., Yaffe, D. (1977) *Differentiation* 8, 133 - 143.
16. Robbins, J., Heywood, S.M. (1978) *Eur. J. Biochem.* 82, 601 - 608.
17. Aviv, H., Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A.* 69, 1408 - 1412.
18. Heywood, S.M., Rich, A. (1968) *Proc. Natl. Acad. Sci. U.S.A.* 59, 590 - 597.
19. Heywood, S.M., Kennedy, D.S. (1976) *Biochemistry* 15, 3314 - 3319.
20. Bester, A.J., Kennedy, D.S., Heywood, S.M. (1975) *Proc. Natl. Acad. Sci. U.S.A.* 72, 1523 - 1527.
21. Lee-Huang, S., Sierra, J.M., Naranjo, R., Filipowicz, W., Ochoa, S. (1977) *Arch. Biochem. Biophys.* 180, 276 - 287.
22. Heywood, S.M., Kennedy, D.S., Bester, A.J. (1974) *Proc. Natl. Acad. Sci. U.S.A.* 71, 2428 - 2431.
23. Golini, F., Thack, S.S., Birge, C.H., Safer, B., Merrick, W.C., Thach, R.E. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 73, 3040 - 3044.
24. Trachsel, H., Erni, B., Schreier, M.H., Staehelin, T. (1977) *J. Mol. Biol.* 116, 755 - 767.
25. Bag, J., Sarkar, S. (1976) *J. Biol. Chem.* 251, 7600 - 7609.
26. Bag, J., Sarkar, S. (1975) *Biochemistry* 14, 3800 - 3807.
27. Heywood, S.M., Kennedy, D.S., Bester, A.J. (1975) *FEBS Letters* 53, 69 - 72.