DIVERGENT SYNTHESES OF ACTIN AND MYOSIN IN FUSED AND UNFUSED MUSCLE CELLS

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Summary: Myosin and actin were isolated from cultured rat muscle cells after isotope labelling. The specific activity (S.A.) of myosin from unfused cells treated with lysolecithin medium, was comparable to myosin from fused untreated cells. Extensive cell fusion was therefor not a requirement for acceleration of myosin presumably synthesized on free polysomes. Actin, presumably synthesized in association with lysolecithin-affected membranes, was however inhibited and had a comparatively low S.A.

Fusion of committed myoblasts to form multinucleated straps is one of the key phases in differentiation of muscle. The fusion phenomenon is preparatory to a burst of syntheses for specific proteins such as actin, myosin, creatine kinase, myoglobin and phosphorylase among others (1). Many of these proteins also appear to be present in small quantities in all eukaryotic cells, myoblasts not excepted (2).

Rat muscle cells suitably treated with lysolecithin exhibit viable but unfused "myoblasts" for extended periods of *in vitro* culture (3). This system was used to explore two questions concerning myogenesis. One was to determine if unfused cells could be shown to precociously increase the synthesis of specific myogenic proteins. Fortuitously, the system also suggests an answer as to how *nascent* actin could be separated within cells from interacting with myosin.

Methods: Primary monolayer cultures of rat leg muscle cells were as before and replicate plates treated with lysolecithin (LL)

as indicated by Reporter and Norris (3). The methods for cell sampling, extraction of actin and myosin and their analyses were carried out as previously indicated (4-6).

Results: To examine the question concerning precocious syntheses of specific proteins, 9-day cultures of muscle cells were used. At this period of culture, cells grown in regular medium (R) indicated extensive fusion and the resulting muscle straps were well differentiated. The replicate plates treated with LL were confluent with cells but the majority of myogenic cells had not fused (2).

Probable communication between myogenic cells from the LL-treated plates was indicated by two criteria. The first observation was that intracellular cyclic AMP concentrations in these LL-cells showed a decrease after confluency in a manner similar to cells grown in regular medium (8). The second observation was that isolated plasma membrane fractions (PM) of LL-cells often exhibited interconnections (tight junctions) between the large stretches of these membranes when their respective pellets were examined in cross section. This is indicated in Figure 1.

Myosin synthesis was compared in 9-day cells grown in R- and in LL-medium after introducing [^{14}C]-arginine. This is shown in Table I. The incorporation of [^{14}C]-arginine into bulk protein is also shown in the table to indicate that the general utilization of this charged amino acid was not hindered in the treated cells.

Both actin and myosin syntheses were compared in a second experiment using an 11-day culture and [^{3}H]-methionine. The results from this experiment are shown in Table II. This was comparison between well-differentiated R-cells and LL-cells which were in a more crowded confluent monolayer. The majority of myogenic cells (>70%) in these 11-day LL-cultures were still not fused.

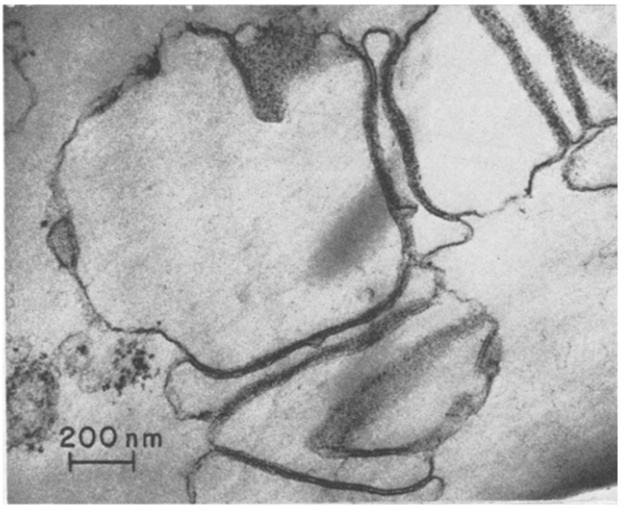

Figure 1. Electron micrograph of isolated plasma membrane fraction from lysolecithin-treated cells. Note the isolated intact junctions between extensive vesciculated pieces from different cells. (See Ref. 8)

Table I. Myosin and Bulk Protein Synthesis of Lysolecithin-treated (LL) and Control Cells (R) from a 9-day Rat Muscle Culture.

	Bulk Protein	Myosin			
	S.A. x 10^4	Final µg Isolated	S.A.* x 10^4	UMe_2Arg % Total	cpm
R-Medium	5.16	396	4.73	14.2	
LL-Medium	5.80	190	3.27	10.8	

(^{*14}C-guanidino) Arginine (S.A. x 45.7 mCi/mM) was introduced into the culture plates at 0.4 µ Ci/ml. Cells were sampled after 180 mins. Specific activity (S.A.) of bulk proteins was obtained after introducing 10% trichloroacetic acid in replicate plates and using the entire residual protein after hot ethanol, ethanol-ether and hot acid treatment. S.A. expressed as cpm.mg^{-1} isolated protein.

The specific activities of myosin from LL-cells was ca. 70% of R-cells in the 9-day culture (Table I) and 83% in the 11-day culture (Table II). The LL-cells, after 11 days in culture,

Table II. Myosin and Actin Syntheses in Lysolecithin-treated and Control Cells from an 11-day Rat Muscle Culture.

	Actin			Myosin					
						Methylation Ratio			
	Final µg isolated	S.A. x10^4	Methlation Ratio (3MeHis)	Final µg isolated	S.A. x10^4	3MeHis	UMe$_2$Arg	MeLys	Me$_3$Lys
R-medium	364	3.71	3.6	944	6.0	1.5	4.5	5.9	11.0
LL-Medium	74	1.54	2.4	196	5.0	4.4	8.0	6.6	13.2

(3H-Methyl)methionine (S.A.6·3 Ci/mM-Amersham/Searle) was added to culture media at a final concentration of 1 µCi/ml. Plates were sampled after 240 min. (Methionine equilibrium in cell protein is usally attained after 8 hrs see Ref 4) S.A. expressed as cpm.mg^{-1}.

yielded myosin with nearly normal specific activity. The myosin was collected to give constant specific activity and losses were not estimated. However the fewer cells contained in the LL-treated plates (see Ref. 1) would be expected to yield lower amounts of myosin.

The specific activity of actin (cpm.mg protein^{-1}) from the 11-day cultures of LL-cells was ca. 41% of R-cells. This lowered specific activity of actin isolated from LL-cells indicated possible interference with actin translation.

The determination of methylated bases was also examined in both experiments. In the experiment of Table I, the extent of methylation is expressed in terms of percentage of radioactivity separated as unsymmetric dimethylarginine radioactivity. Thus, the arginine residues in myosin synthesized in 9-day treated cells were methylated to a similar extent as myosin from untreated cell. In the experiment of Table II, [^{3}H]-methionine was used to label actin and myosin. In this instance methylation was expressed as a methylation ratio for each of the selected methylated residues* viz. $\left(\frac{\text{cpm in methylated residue}}{\text{cpm in methionine}}\right)$ 100. The methylation of both actin and myosin from treated cells was noted. Myosin methylation ratios were increased for histidine and arginine in comparison with untreated cells while lysine methylation was similar. Actin methylation ratio for histidine was comparatively inhibited in the treated cells.

Discussion: Single plates of confluent LL-cell cultures usually contained 50 to 55% of DNA and protein of R-cell cultures (1). Thus, in the experiment of Table I, the amount of myosin isolated to constant specific activity from 9-day LL-cells was

* 3MeHis = 3-methylhistidine; UMe_2Arg = unsymmetric dimethyl-arginine; MeLys = monomethyl-lysine and Me_3Lys = trimethyl-lysine.

49% of the myosin from R-cells. Therefore establishment of intercellular communication resulted in proportionate increase of at least one specific cell protein (myosin) in myogenic cells. An extensive amount of cell fusion was not necessary. The isolation of myosin of constant specific activity from 11-day LL-cells in the experiment of Table II was 22% of myosin from R-cells. The S.A. of myosin recovered from LL-cells was however 80% of untreated cell myosin. Turnover studies on bulk proteins as well as specific proteins like myosin are needed to explain discrepancy in the low recovery of LL-cell myosin. It is possible to have nearly normal rate of myosin synthesis but accelerated rate of myosin breakdown when concomitant intracellular actin synthesis is absent. It is likely that LL-treatment did not affect fidelity of myosin synthesized because methylation of myosin was unaffected. It also follows that methylases for myosin may not be associated with membranes since myosin methylation was unaffected.

Methylated actin of low specific activity was isolated from LL-cells suggesting that the actin synthesized was normal in form. While LL-treated cells exhibited major defects in plasma membranes, intracellur membranes were also affected (8). Actin synthesis would be perturbed if this synthesis was associated with endoplasmic reticulum (ER). It is likely that G-actin is segregated by ER after being synthesized on bound ribosomes (2). Transfer of the vescicles containing G-actin and conversion to F-actin would then occur near the plasma membrane. This would explain the repeated findings of thin filaments (diam. 50 to 60 A°) near cell-membranes during investigation of contractile proteins with the aid of the electron microscope (2,7).

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