Vol. 86, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 14, 1979 Pages 514-521

A ROLE FOR LIPID IN MYOBLAST FUSION

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Received December 13, 1978

SUMMARY: Alteration of the membrane fatty acyl composition modulates the fusion of myoblasts into multinucleate myotubes. The rate of fusion after addition of calcium to 50-52 hour cultures of chick pectoral myoblasts is markedly inhibited in cells possessing acyl chains enriched in elaidate and is enhanced in those enriched in oleate. The modulations appear to occur after the cells have recognized one another and adhered strongly but before the membranes have united. These observations lead to a hypothesis for membrane union (fusion) in which the lipids participate directly perhaps by a mechanism analogous to that proposed for the fusion of lipid vesicles.

The formation of multinucleate myotubes from myoblasts is one of many examples of biological phenomena in which fusion is central (1). Potential insight into the myoblast as well as other fusion mechanisms derive from the recent observations that lipid bilayer vesicles fuse under appropriate conditions (2-5). Such model studies would be more meaningful if their relevance to the in vivo mechanism could be established. The effect of systematic perturbations of the myoblast lipid composition on fusion could provide a direct indication of such a lipid involvement. In this communication we extend our previous observations (6), and using synchronously fusing myoblast cultures, we demonstrate that altered fatty acyl compositions have a marked influence on the rate

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Abbreviations: Ara C - cytosine arabinoside
EGTA - ethyleneglycol-bis (β-aminoethyl ether)-N_1N_1, $N_1^1N_1^1$-tetraacetate

0006-291X/79/030514-08$01.00/0

of myoblast fusion. We interpret these observations as implicating a central role for lipid in fusion.

METHODS: Pectoral muscle was explanted from 10-11 day chick embryos, dissociated into single cells, and plated at a density of 0.5 x 10^6 cells/ml onto 35 mm collagen coated tissue culture dishes (6, 7). The growth medium (2 mls/dish consisted of calcium-free Dulbecco's Modified Eagle's medium supplemented with 13% lipid depleted fetal calf serum, 200 μM EGTA, 2 μg/ml avidin, and one of the following: 1.25 μg/ml biotin, 21 μg/ml oleate, or 21 μg/ml elaidate (6). Fusion was assayed using 51-52 hour cultures. The assay was initiated by adding $CaCl_2$ to a final concentration of 1.8 mM (9). [Ara C (1 μg/ml was also added at this time to kill proliferating cells (8).] The kinetics of fusion was followed by fixing and staining the cultures after the designated time intervals (6). The plates were scored using phase contrast microscopy for fusion - the fraction of nuclei in myotubes (cells containing two or more nuclei in a cluster or a line within a single cytoplasma), or interacting cells - the fraction of nuclei in cells that are in close association such that their boundaries are not clearly distinguishable. These procedures are presented in detail elsewhere (6). The large apparent error arises primarily from the variability in the data derived from experiments run on different days. It does not reflect the variability within a single experiment which is relatively small. The data presented are the mean with standard deviation of 2-7 separate determinations.

RESULTS AND DISCUSSION: The influence of altered fatty acyl compositions on myoblast fusion are shown in Figs. 1 and 2. The fatty acyl chains of chick pectoral myoblasts (grown in a low calcium medium to prevent fusion) were enriched in oleate or elaidate by growth in a lipid-depleted-medium supplemented with biotin (control), oleate, or elaidate (the trans isomer of oleate which produces a relatively less fluid membrane). These procedures alter the percent of unsaturated fatty acyl chains in the cellular and plasma membrane lipids and do not appear accompanied by large secondary alterations in the composition of other lipid classes (6). Detailed analyses of the membrane lipids from the supplemented cultures are presented elsewhere (6). Upon addition of calcium (1.8 mM) to the myoblasts at 37°C, the oleate, elaidate, and biotin (control) supplemented myoblast cultures rapidly form interacting

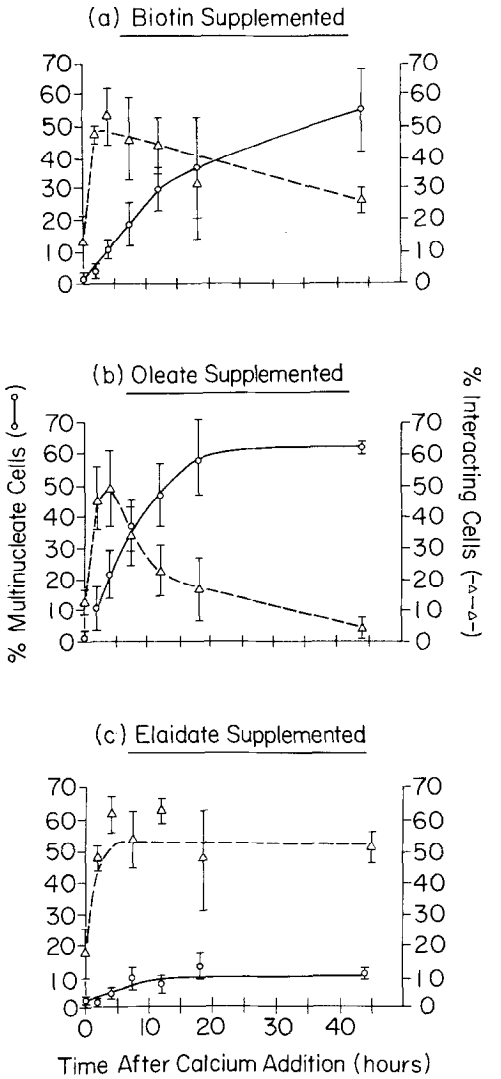

Figure 1. Fusion of myoblasts possessing altered fatty acyl compositions. Fusion was assayed using 51-52 hour cultures. The assay was initiated by adding $CaCl_2$ to a final concentration of 1.8 mM. The kinetics of fusion was followed by fixing and staining the cultures after the designated time intervals. The plates were scored using phase contrast microscopy for fusion (o) - the fraction of nuclei in myotubes,or interacting cells (Δ) - the fraction of nuclei in cells that are in close association such that their boundaries are not clearly distinguishable. The data presented are the mean with standard deviation of 2-7 separate determinations. (a) biotin supplemented, (b) oleate supplemented, and (c) elaidate supplemented cultures.

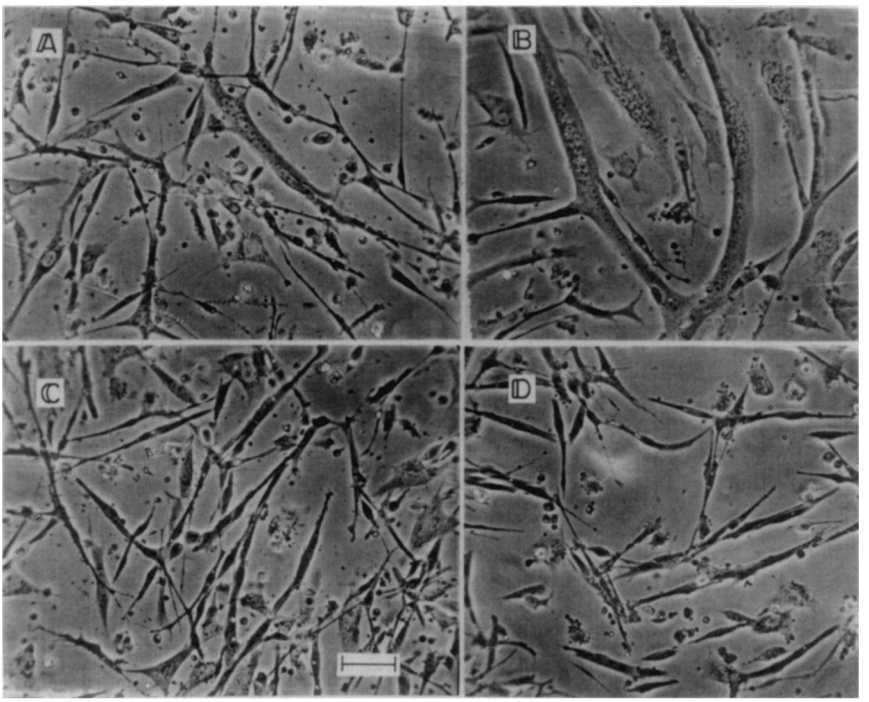

Figure 2. Phase contrast micrographs of myoblast cultures possessing altered fatty acyl compositions. Chick pectoral myoblasts were cultured and the fusion assay initiated as described in the Methods Section with the exception that the cells were plated at a density of 1 x 10^6 cells/ml. At the stated times after calcium addition, pictures of living cultures were taken using a phase contrast microscope. The bar represents 50 μm. (a) Oleate enriched cultures 5 hours after calcium addition; (b) 12 hours after calcium addition; (c) Elaidate enriched cultures 5 hours after calcium addition; (d) 12 hours after calcium addition.

structures (Figs. 1 and 2). With time, the fraction of interacting cells decreases in parallel with an increase in multinucleate myotubes in the oleate and biotin-supplemented cultures but not in the elaidate supplemented ones. When the assay was performed at 26°C, none of the cultures fused appreciably; a high level of interacting cells was present however. At 41°C, the fusion rate of oleate and biotin-supplemented cultures increased with little, if any, reversal of the elaidate inhibition. The enhanced fusion

rate of oleate supplemented cultures compared to that of the biotin controls was seen consistently in all experiments.

Two important conclusions follow from these observations: 1) the rate of multinucleate cell formation is modulated by the lipid composition, i.e. oleate enrichment enhances the rate while elaidate enrichment inhibits it, and 2) the rate limiting process occurs after migration and adhesive interactions have occurred. Before discussing the implications of these conclusions for the fusion mechanism, two points need clarification. First, the lipid alterations do not modulate fusion indirectly via a toxic metabolic perturbation. This follows from the observation that elaidate enrichment does not appreciably inhibit the migration or interaction of the myoblasts. These phenomena involve complex cellular processes including energy production and utilization. The second point is that the modulations of fusion occur at a time just prior to membrane union. In a recent publication we dissected the fusion process into operationally defined stages using suspension aggregation assays. These stages include an initial recognition, followed by a stronger adhesive interaction which, in turn, is followed by membrane union (10). The observation that the modulation of fusion occurs after the cells have interacted implies that the modulated, rate limiting step occurs after myoblast recognition. Using the suspension assay, we have determined that elaidate enrichment inhibits myoblast fusion before membrane union but after the cells have recognized each other and adhered strongly[1] (11).

Thus, it seems likely that the lipid alterations influence membrane union via a structural perturbation of the membrane

[1] The presence of interacting cells when fusion is assayed at 26°C implies that a stage occurring after recognition is temperature sensitive. In addition to this stage, we reported previously that the rate of calcium-mediated myoblast aggregation in suspension, i.e. recognition, is also temperature sensitive.

lipids. There are several possible mechanisms by which altered lipid compositions could influence the union of myoblast membranes. One mechanism is by inhibiting the movement of proteins. While we cannot exclude this possibility, we consider it unlikely. First, protein movements are implicated in recognition and adhesion processes which are not significantly perturbed in the lipid altered cells (12). Furthermore, direct estimates of the lateral diffusion rates of a lipid probe or α-bungarotoxin-acetylchloline receptor complexes are similar in oleate and elaidate enriched cells (13). Another more attractive possibility is that membrane union occurs by a mechanism analogous to that for the fusion of lipid bilayer vesicles. The rate of fusion of these vesicles is reported to be optimal when regions of solid and liquid lipid domains coexist (2-5, 14). In this view, the oleate or elaidate enrichments would modulate fusion by perturbing the membrane phase equilibria.

Hence, a reasonable hypothesis for membrane union is that it requires a direct lipid involvement and proceeds by a mechanism analogous to that of model lipid vesicles. It is quite likely that this aspect of fusion is general and extends to systems other than fusing myoblasts. In contrast, aspects of the recognition and adhesion phenomena, though required for myoblast fusion (10, 11), may be different in other systems and, in some, may not be required at all. When recognition and adhesion are present, in addition to providing the obvious requirement for specificity, they may also function to produce areas depleted of membrane protein and a close apposition of regions of membrane lipid so that fusion can occur.

It has been reported recently that a general increase in membrane fluidity parallels the onset of fusion competence in myoblast cell cultures (15, 16). Our observations on the modulating effect

of altered lipid compositions on the fusion rate open the possibility that these fluidity changes may in fact provide a regulatory contribution to the fusion rate. The physiologic significance, however, remains to be established.

ACKNOWLEDGEMENTS: These studies were supported by NIH grant GM 23244, benefitted from facilities made available from NIH grants GM 20138 and HL-18708, and were performed during the tenure (to AFH) of the W.D. Stroud Established Investigatorship of the American Heart Association. We thank Howard Goldfine for comments on the manuscripts.

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