Plasma Membranes from Cultured Muscle Cells: Isolation Procedure and Separation of Putative Plasma-Membrane Marker Enzymes

(Na⁺, K⁺-ATPase/5'-nucleotidase/ α -bungarotoxin)

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ABSTRACT Partially purified plasma membranes were obtained from chick-embryo muscle cells grown in tissue culture. The purification procedure involved homogenization in buffered isotonic sucrose followed by differential and sucrose density gradient centrifugations. The activities of five plasma-membrane markers, as well as microsomal and mitochondrial markers, were followed throughout the purification. When cultures were labeled with [¹²⁵I] α -bungarotoxin, which binds to the surface of cultured muscle cells, the distributions of bound α -bungarotoxin and Na⁺,K⁺-ATPase (EC 3.6.1.3) activity were nearly identical. The activities of these two plasma-membrane markers were maximal in the upper two fractions of the sucrose density gradient and were purified 5- to 7-fold with respect to total particulate protein. These fractions contained 20-30% of the Na⁺,K⁺-ATPase activity and bound α -bungarotoxin, 4% of the microsomal marker TPNH-dependent cytochrome c reductase, 0.2% of the mitochondrial marker succinate-dependent cytochrome c reductase, 2.7% of the cellular RNA, and 0.02% of the DNA. The activity of the commonly used plasma-membrane marker, 5'-nucleotidase (EC 3.1.3.5), was low in the upper two sucrose gradient fractions and was maximal in a more dense fraction. The distributions of the other two plasma-membrane markers, leucyl β -naphthylamidase and phosphodiesterase I, were intermediate between Na⁺,K⁺-ATPase and 5'-nucleotidase. The distributions of all markers were similar in preparations from cultures containing mononucleated myogenic cells, multinucleated myotubes, fibroblasts, or all three cell types. Modification of the procedure to include homogenization in the absence of sucrose resulted in a 3.4-fold purification of the membranes containing 5'-nucleotidase, which were shifted to a lower density.

Mononucleated muscle cells grown in tissue culture fuse to form multinucleated myotubes which elaborate muscle-specific proteins and eventually contract spontaneously (1). Under appropriate conditions *in vitro* more than 80% of the cells in culture may fuse to form myotubes (2). (For a recent review, see ref. 3.) However, the mechanism by which fusion occurs is unknown, although it is reasonable to expect that alterations in the cell surface must play a vital role. In order to elucidate such fusion-related surface alterations, we have undertaken a study of the plasma membranes of chick-embryo muscle cells grown in monolayer culture. In this paper we report the isolation of plasma membranes from cultured muscle cells at various stages in their development and the separation of two widely accepted plasma-membrane

"marker" enzymes, 5'-nucleotidase (EC 3.1.3.5) and ouabain-sensitive Na⁺- plus K⁺-stimulated adenosine triphosphatase (Na⁺,K⁺-ATPase, EC 3.6.1.3). (For a review of plasma-membrane isolation and characterization techniques, see ref. 4.) To examine further the significance of the separate distributions of these plasma-membrane markers, we labeled muscle cultures with radioactive α -bungarotoxin, which blocks acetylcholine receptors of skeletal muscle (5) and binds to the surface of myotubes in culture (6, 7). In subcellular fractions of cells labeled with [¹²⁵I] α -bungarotoxin, the distribution of bound toxin was nearly identical to the distribution of Na⁺,K⁺-ATPase but not of 5'-nucleotidase.

MATERIALS AND METHODS

Culture Conditions. Fertilized chicken eggs were obtained from Ken-Roy Hatcheries, Bergen, Mo. Cultures of muscle cells from 12-day chick embryos were prepared essentially as described (8) except that cells were preplated for 10 min at 37° to remove some of the fibroblastic cells which adhere quickly to the dish (9). The cells were diluted to 0.35-0.5 $\times$ 10⁶ cells per ml in culture medium [82.4% Eagle's minimum essential medium, 8.3% horse serum (Grand Island Biological Co., Grand Island, N.Y.), 8.3% chick-embryo extract (high-speed supernate of quickly frozen 50% extract of 11-day embryos in Earle's balanced salt solution) and 1% antibiotic-antimycotic solution (GIBCO)], and 20 ml were added to each 150-mm collagen-coated dish. The cultures were incubated at 37° in a humidified atmosphere at 5% CO₂-95% air, and 10 ml of culture medium was replaced daily.

To obtain cultures of nonfused myogenic cells, we used 5 μ g of 5-bromodeoxyuridine (BrdU; Nutritional Biochemicals Co., Cleveland, Ohio) per ml of culture medium (10, 11). For cultures of myotubes, 5-fluorodeoxyuridine (FdU; Calbiochem, La Jolla, Calif.), at a final concentration of 10 μ M, was added to cultures 36-48 hr after they were plated (12). After an additional 24-48 hr, nearly all dividing mononucleated cells had detached from the dish and nondividing myoblasts had fused into myotubes. Fibroblasts were obtained from subcutaneous connective tissue in the groin region of 12-day chick embryos, plated at 0.25 $\times$ 10⁶ cells per ml and grown for 3 days. The cells were then subcultured at the same density and harvested after 2 days.

Isolation of Plasma Membranes. Monolayer cultures of muscle cells or fibroblasts were washed four times with cold Earle's salt solution; then 10 ml of Earle's salt solution was

Abbreviations: BrdU, 5-bromodeoxyuridine; FdU, 5-fluorodeoxyuridine; TEA, triethanolamine.

TABLE 1. Distribution and activities of protein and subcellular markers in the fractionation of 3-day muscle cultures

Fraction	Protein	[¹²⁵ I]α-Bungarotoxin*	Na ⁺ ,K ⁺ -ATPase	5'-Nucleotidase	Phosphodiesterase I	Leucyl β-naphthylamidase	TPNH-cytochrome c reductase	Succinate-cytochrome c reductase
Homogenate, particulate	(100)	13.1(100)	17.8(100)	5.11(100)	6.08(100)	3.09(100)	9.00(100)	14.3(100)
	8.99†	118,000‡	161§	46.0§	54.6§	27.8§	80.8§	129§
1700 × g Supernate	(32.8)	28.1(70.5)	30.2(55.5)	7.26(46.3)	9.59(51.9)	4.88(51.8)	13.5 (49.5)	9.43(21.5)
1700 × g Pellet	(80.6)	5.1(31.3)	10.6(47.7)	2.54(62.8)	4.07(53.3)	1.74(45.3)	5.74(52.0)	17.3 (97.6)
28,000 × g Supernate	(6.0)	11.0 (5.1)	9.0 (3.1)	1.80 (2.1)	3.13 (3.1)	2.10 (4.1)	2.80 (1.9)	0.76 (0.3)
28,000 × g Pellet	(21.5)	33.0(54.6)	38.8(46.6)	8.73(36.8)	11.3 (40.0)	5.24(36.3)	15.4 (36.8)	6.88(10.3)
I + II	(4.3)	93.6(29.8)	94.1(22.8)	4.35 (4.3)	17.9 (12.7)	8.08(12.9)	8.99 (4.4)	0.58 (0.2)
Total gradient	(20.3)	(52.0)	(45.3)	(32.4)	(36.8)	(42.1)	(27.8)	(19.2)

3-Day muscle cultures were prepared and fractionated as described in *Methods*. Enzyme activities are expressed as nmol min⁻¹ mg of protein⁻¹. Numbers in parentheses are the percent recovery of the initial activity in the particulate homogenate.

* cpm/μg of protein.

† mg of total protein in the particulate homogenate.

‡ Total cpm.

§ Total activities, expressed as nmol per min.

added to each 150-mm dish. The cells were removed from the dish by mopping with a small sheet of perforated cellophane (Microbiological Associates, Bethesda, Md.) and collected by centrifugation for 5 min at 1100 × *g*. (All centrifugal forces refer to *r*_{av}.) This and all subsequent steps were done at 0–4°. The cell pellet was suspended in 15 ml of 0.25 M sucrose–1 mM triethanolamine (TEA)·HCl (pH 7.4) (sucrose–TEA) per g of cells (wet weight). The cells were broken in a Dounce homogenizer (Kontes Glass Co., Vineland, N.J.) with the tight (B) pestle. Fifteen up-and-down strokes were sufficient for complete breakage, as judged by phase contrast microscopy. The homogenate was centrifuged for 10 min at 1700 × *g*; the supernate was removed and centrifuged for 60 min at either 27,000 or 33,000 × *g*. A slightly better yield of plasma membranes was obtained with 33,000 × *g*, and that centrifugal force is now used routinely. The resulting

pellet was suspended in 1.0 ml sucrose–TEA and layered over a sucrose discontinuous gradient of the following composition: 0.5 ml of 55% (w/w) sucrose, then 2.5 ml each of 40%, 32%, 27%, and 20% sucrose.

After centrifugation in an SW 41 rotor at 41,000 rpm (206,000 × *g*) for 1.5 hr, the bands of turbid material at the interfaces in the gradient were removed and designated as follows: 8.3–20%, I; 20–27%, II; 27–32%, III; 32–40%, IV; and 40–55%, V. Each fraction was diluted to about 9 ml with 1 mM TEA·HCl (pH 7.4), then collected by centrifugation for 60 min at 105,000 × *g*. The pellets were suspended in TEA·HCl and assayed for protein and enzyme activities. Samples were also taken at each step of preparation before the gradient step, diluted with TEA·HCl, and centrifuged for 60 min at 105,000 × *g*, so that only particulate protein was assayed for enzyme activity. At least 30 min before dilution and centrifugation, beef-pancreas deoxyribonuclease I (Sigma Chemical Co., St. Louis, Mo.), at a final concentration of 50 μg/ml, was added to the samples of homogenate and 1700 × *g* pellet to facilitate later resuspension for assay. All conditions were kept the same for preparations homogenized in TEA·HCl instead of TEA–sucrose, except that a 15,000 × *g* centrifugation step was substituted for the 33,000 × *g* centrifugation step.

FIG. 1. Relative distribution of marker activities on the sucrose discontinuous density gradient. 3-Day muscle cultures were labeled with [¹²⁵I]α-bungarotoxin, homogenized in sucrose–TEA, and fractionated. Fractions were collected at each interface as follows: I (8.3–20% sucrose); II (20–27%); III (27–32%); IV (32–40%); and V (40–55%). This is the same experiment as shown in Table 1. Relative specific activity was calculated as the percent in each fraction of the total activity recovered from the gradient divided by the percent in that fraction of the total protein recovered from the gradient.

[¹²⁵I]α-Bungarotoxin. Pure α-bungarotoxin was a gift from Dr. Regis B. Kelly. It was labeled with ¹²⁵I by the procedure of Berg *et al.* (5), with 0.5 mg of toxin and 5 mCi of carrier-free Na¹²⁵I (Industrial Nuclear Co., St. Louis, Mo.). To label cultures with radioactive toxin, the cells in a 150-mm dish were washed twice with Earle's salt solution, then incubated for 10 min at 37° in 10 ml of culture medium containing 30 pmol (8.5 × 10⁶ cpm) of [¹²⁵I]α-bungarotoxin. The toxin-containing medium was removed and the cells on the dish were washed four times in Earle's salt solution containing 2 mg/ml of bovine-serum albumin, then twice with Earle's salt solution before they were harvested. Addition of 1 mM carbamylcholine to the incubation mixture resulted in a 90–95% decrease in binding of labeled toxin. ¹²⁵I-Containing samples were counted on a Packard model 3001 Auto-Gamma Spectrometer.

Biochemical Determinations. 5'-Nucleotidase was assayed in a 0.1-ml incubation mixture containing 50 mM glycine-HCl (pH 8.7), 0.2 mM EDTA, 0.2 μ g of sodium dodecyl sulfate per μ g of protein, 6 mM adenosine-5'-monophosphate, and 5–20 μ g of protein. The reaction was carried out for 30–60 min at 37° and terminated by addition of 0.1 ml of 10% perchloric acid. The precipitated protein was removed by centrifugation and the inorganic phosphate content of the supernate was determined (13). Ouabain-sensitive Na^+ , K^+ -ATPase was determined in a 50- μ l reaction mixture containing 30 mM imidazole-HCl (pH 7.5), 0.11 M NaCl, 15 mM KCl, 5 mM NaN_3 , 0.5 mM ethyleneglycol-bis(β -aminoethyl ether)*N,N'*-tetraacetic acid (EGTA), 4 mM MgCl_2 , 3 mM ATP, and 2–30 μ g of protein. Na^+ , K^+ -ATPase activity was calculated as the difference in ATPase activity in the presence and absence of 1 mM ouabain. Reactions were carried out at 37° for 45 min and stopped by addition of 50 μ l of 10% perchloric acid. After removal of protein by centrifugation the inorganic phosphate content was determined (13). In muscle-cell homogenates, 35–50% of the total ATPase assayed in the presence of Na^+ and K^+ was ouabain sensitive. For purified plasma-membrane fractions (I and II) from muscle cells, the inhibition was 60–85%.

The spectrophotometric assays for TPNH-dependent and succinate-dependent cytochrome *c* reductases were essentially those described by Sottocasa *et al.* (14). Phosphodiesterase I was determined as described by Touster *et al.* (15), except that the reaction was terminated by addition of 0.4 M NaOH and the absorbance at 400 nm was measured directly. Leucyl β -naphthylamidase (leucine aminopeptidase) was assayed as described by Hubscher *et al.* (16). Protein was determined by the method of Lowry *et al.* (17) with bovine-serum albumin (Pentex; Miles Laboratories, Inc., Kankakee, Ill.) as standard. The distribution of DNA or RNA in subcellular fractions was determined in preparations from cultures grown for 3 days in the presence of 3 μ M [*methyl*- ^3H]thymidine (0.5 μ Ci/ml; New England Nuclear Corp., Boston, Mass.) or 3 μ M [^3H]uridine (0.5 μ Ci/ml; Schwarz/Mann, Orangeburg, N.Y.).

EXPERIMENTAL RESULTS

Purification of Fractions Enriched in Na^+ , K^+ -ATPase. 3-Day-old muscle cultures were labeled with [^{125}I] α -bungarotoxin, harvested, homogenized in sucrose-TEA buffer, and fractionated by differential and sucrose discontinuous density gradient centrifugations. The results of a typical purification procedure are shown in Table 1. Recoveries of protein, radioactivity, and all measured enzymatic activities were close to 100% in each step of the purification. In the 1700 $\times g$ centrifugation step, essentially all of the nuclei (98.8% of the total DNA) and about 80% of the mitochondrial marker, succinate-dependent cytochrome *c* reductase, were found in the pellet. In addition, 63% of the 5'-nucleotidase and 48% of the Na^+ , K^+ -ATPase were found in the pellet fraction, apparently associated with the large particles. More vigorous homogenization with a motor-driven pestle did not alter the result. After the 28,000 $\times g$ centrifugation, all but 6% of the remaining particulate protein was found in the pellet.

The distribution of the 28,000 $\times g$ pellet material on a discontinuous sucrose gradient is shown in Fig. 1. In the gradient shown in Fig. 1, [^{125}I] α -bungarotoxin radioactivity and Na^+ , K^+ -ATPase were distributed similarly with maximum specific activities in fractions I and II. 5'-Nucleotidase,

on the other hand, was low in those fractions and maximal in fraction IV. The distributions of leucyl β -naphthylamidase and phosphodiesterase I, which have also been reported to be plasma-membrane marker enzymes (4, 15), were nearly identical and were intermediate between those of Na^+ , K^+ -ATPase and 5'-nucleotidase.

The overall purification of Na^+ , K^+ -ATPase in fractions I and II combined was 5.3-fold (with respect to particulate protein), with a 23% yield of enzyme activity. In this same preparation, specific radioactivity of [^{125}I] α -bungarotoxin increased 7.2-fold with a 30% yield of radioactivity. The purified membrane fractions (I and II) contained 4.4% of the initial TPNH-dependent cytochrome *c* reductase, an enzyme commonly used as a microsomal marker (18), 0.2% of the mitochondrial marker succinate-dependent cytochrome *c* reductase, 0.02% of the total cellular DNA, and 2.7% (183 μ g/mg of protein) of the cellular RNA. The microsomal marker, TPNH-dependent cytochrome *c* reductase, was maximal in fraction IV but differed from 5'-nucleotidase in its distribution in fractions IV and V. Under other conditions (see below) these two markers differed even more distinctly. The mitochondrial marker, succinate-dependent cytochrome *c* reductase, was found in fractions IV and V.

Purification of a Fraction Enriched in 5'-Nucleotidase. For further study of the membranes containing the separated marker activities, Na^+ , K^+ -ATPase and 5'-nucleotidase, it was necessary to obtain fractions that were enriched for one marker but that contained only low levels of the other. This condition was satisfied for Na^+ , K^+ -ATPase in fractions I and II of the membrane preparation described above. In this same preparation, however, there was no overall purification and only a 4% yield of 5'-nucleotidase activity in fraction IV, where this activity was maximal.

When cells were homogenized in sucrose-TEA, about 63% of the 5'-nucleotidase activity was found in the pellet after centrifugation at 1700 $\times g$ for 10 min. If, however, a portion of the same cells was suspended and homogenized in 1 mM TEA-HCl (pH 7.4), without sucrose, only 20–40% of the 5'-nucleotidase was recovered in the 1700 $\times g$ pellet, and the distribution of the remaining 5'-nucleotidase was maximal in fraction III rather than fraction IV (Fig. 2). Fraction III in the TEA preparation contained 36.2% of the total 5'-nucleotidase recovered from the gradient, corresponding to a yield of 15.5% of the total homogenate activity and a purification of 3.4-fold.

Fig. 2. Relative distribution of marker activities from 3-day muscle cultures after homogenization in 1 mM TEA-HCl (pH 7.4). Sucrose density gradient conditions were the same as described for Fig. 1. Total protein and activities recovered from the gradient were as follows: protein, 2.02 mg; Na^+ , K^+ -ATPase, 68.3 nmol/min; 5'-nucleotidase, 19.7 nmol/min; TPNH-dependent-cytochrome *c* reductase, 15.0 nmol/min.

FIG. 3. Distribution of Na^+, K^+ -ATPase and 5'-nucleotidase from BrdU- and FdU-grown cultures, 3-day cultures, and fibroblasts. This is the same experiment as shown in Table 2. Sucrose density gradient conditions were the same as described for Fig. 1. Hatched area, 5'-nucleotidase activity.

In contrast to the large change in 5'-nucleotidase distribution in the preliminary fractions and on the sucrose gradient, there was little change in the distributions of α -bungarotoxin radioactivity, Na^+, K^+ -ATPase, leucyl β -naphthylamidase, phosphodiesterase I, or TPNH-dependent cytochrome *c* reductase in cells homogenized in TEA. The succinate-dependent cytochrome *c* reductase, however, was shifted in relative distribution between fractions IV and V on the gradient.

Membrane Preparations from Mononucleated Cells and Myotubes. 3-Day-old cultures contain myotubes, nonfused myogenic cells, and fibroblasts. In order to determine if the separation of Na^+, K^+ -ATPase and 5'-nucleotidase activities was a result of the heterogeneous cell population, it was necessary to determine the distribution of markers from more homogeneous cultures. Accordingly, we prepared membrane fractions from cultures of each of the three cell types. Mononucleated myogenic cells were grown in the presence of BrdU to reversibly inhibit formation of myotubes (10, 11). FdU was used to obtain cultures with large numbers of myotubes and few mononucleated cells (12). Fibroblasts were cultured as described in *Methods*. All three cell types were homogenized in sucrose-TEA and were fractionated as described above. In each case, the distributions of all marker activities were similar to those shown in Fig. 1 for 3-day cultures. The distributions of 5'-nucleotidase and Na^+, K^+ -ATPase were nearly identical for the different cell types and for 3-day cultures (Fig. 3). Each showed the same clear separation of these two markers. In addition, the purification and yield of Na^+, K^+ -ATPase in gradient fractions I plus II was very similar for each preparation (Table 2). When FdU- and BrdU-grown cells were labeled with [^{125}I] α -bungarotoxin before harvest, the distribution of radioactivity on the gradient closely followed that of Na^+, K^+ -ATPase. (For BrdU-grown cells, specific binding of the toxin was less than 7% of that found with 3-day cultures).

DISCUSSION

In this paper we report the isolation and partial purification of plasma membranes from muscle cells grown in tissue culture. The purification procedure involves differential and sucrose discontinuous density gradient centrifugations. One result of this work is the unusually clear separation of two enzymes commonly used as markers for plasma membrane, Na^+, K^+ -ATPase and 5'-nucleotidase. This separation occurs in membrane preparations from both muscle cells and fibroblasts. To our knowledge, the subcellular location of these

enzymatic markers in cultured muscle cells has not previously been established. In Ehrlich ascites tumor cells, 5'-nucleotidase was detected by histochemical techniques on the nuclear membrane but not on the plasma membrane (19). It is possible that in cultured muscle cells 5'-nucleotidase likewise may be absent from the plasma membrane. Perdue and Snider (20) purified plasma membranes from chick-embryo fibroblasts and obtained similar purifications of 5'-nucleotidase and Na^+, K^+ -ATPase activities in their membrane fraction. Because the distributions and recoveries of these two enzymes were not reported, however, comparison with the present findings is difficult. There have been reports of the separation of Na^+, K^+ -ATPase and 5'-nucleotidase activities in membrane preparations from liver (21-24). Evans (22) found that the 5'-nucleotidase from mouse liver was present in a fraction of small vesicles while Na^+, K^+ -ATPase was present in a denser fraction of large membranous sheets. The Na^+, K^+ -ATPase was associated with the large junctional complexes which are characteristic of liver cell surfaces. In this report we have demonstrated that α -bungarotoxin, which binds to nicotinic acetylcholine receptors on the surface of cultured muscle cells (6, 7), was present in membrane fractions that also contain Na^+, K^+ -ATPase, indicating that this ATPase is actually on the surface of muscle cells. (In the experiment shown in Table 1, a larger percentage of α -bungarotoxin than Na^+, K^+ -ATPase remained in the supernate after centrifugation at $1700 \times g$; however, in all other preparations the same percentage of each remained in the supernate.) The 5'-nucleotidase from the muscle cells, on the other hand, was located predominantly in a more dense fraction that contained little toxin or Na^+, K^+ -ATPase.

The specific activities of Na^+, K^+ -ATPase and α -bungarotoxin in fractions I plus II were 5- to 7-times higher than those in the particulate homogenate. Only the particulate protein was assayed for marker-enzyme activities to prevent inter-

TABLE 2. Specific activities of marker enzymes from BrdU- and FdU-grown cultures, 3-day cultures, and fibroblasts

Cell type	Na^+, K^+ -ATPase	5'-Nucleotidase	TPNH-cytochrome <i>c</i> reductase
Muscle, BrdU			
Homogenate	12.7	6.56	5.44
I & II	63.5 (5.0 $\times$)	9.55	6.00
Yield	23.3	6.9	5.3
Muscle, FdU			
Homogenate	16.6	4.29	n.d.*
I + II	110 (6.6 $\times$)	5.10	11.3
Yield	25.4	5.0	n.d.*
Muscle, 3-day			
Homogenate	17.8	5.11	9.00
I & II	94.1 (5.3 $\times$)	5.13	8.99
Yield	22.8	4.3	4.4
Fibroblasts			
Homogenate	6.86	6.61	2.40
I & II	34.8 (5.2 $\times$)	11.1	5.62
Yield	17.6	5.7	8.2

Enzyme activities are expressed as $\text{nmol min}^{-1} \text{mg of protein}^{-1}$. Numbers in parentheses refer to purification of Na^+, K^+ -ATPase in fractions I plus II combined with respect to the particulate homogenate.

* Not determined.

ference from soluble enzymes such as nonspecific phosphatases. About 25% of the total protein in the mononucleated muscle cells and myotubes was soluble, so that the purification with respect to total cell protein was 7- to 10-fold.

When the cells were homogenized in sucrose-TEA, 5'-nucleotidase and TPNH-dependent cytochrome *c* reductase had similar distributions on the sucrose gradient. When TEA was used for the homogenization, however, the particles containing 5'-nucleotidase were less dense than the ones containing TPNH-dependent cytochrome *c* reductase, demonstrating that the 5'-nucleotidase is probably not microsomal.

Phosphodiesterase I and leucyl β -naphthylamidase had identical distributions both in preliminary centrifugations and on the sucrose gradient, which suggests that they are present in the same particle or particles. The density distribution of these particles was intermediate between particles containing Na^+ , K^+ -ATPase and 5'-nucleotidase. This observation suggests either that the phosphodiesterase I and leucyl β -naphthylamidase are present on yet a third type of particle or that they are present in both the particles containing Na^+ , K^+ -ATPase and those containing 5'-nucleotidase.

The separation of plasma-membrane markers from liver has been explained on the basis of plurality of cell types within the tissue and the heterogeneity of surfaces on a single cell type. In the present study, the identical separation (Fig. 3) of the markers in preparations from relatively homogeneous cultures of mononucleated cells and myotubes rules out an explanation based on heterogeneity of the cell population. We cannot say, however, if the separation of markers is the result of heterogeneity of the cell surface or the absence of 5'-nucleotidase from this surface.

The demonstrated feasibility of preparing similar membrane fractions from cells at different stages in their development and the availability of methods to inhibit that development make the isolated plasma membranes of cultured muscle cells an excellent system for the study of cell fusion, cell-cell interaction, and the topology of the cell surface.

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