ISOLATION AND PARTIAL CHARACTERIZATION OF *DROSOPHILA* MYOBLASTS FROM PRIMARY CULTURES OF EMBRYONIC CELLS

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ABSTRACT

We describe a method for preparing highly enriched cultures of *Drosophila* myoblasts from a heterogeneous cell population derived from gastrulating embryos. Enriched cultures are prepared by plating this heterogeneous population of cells in medium from which much of the free calcium is chelated by ethylene glycol-bis(β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetate (EGTA). Adhesion of myoblasts to tissue culture plastic is better than that of other cell types when plated in this medium. Data concerning cell identity, timing of S phase, and fusion kinetics document the degree of enrichment for myogenic cells and illustrate their synchronous differentiation in vitro.

KEY WORDS myoblasts · cell adhesion · EGTA · cell isolation · *Drosophila* · myogenesis

The genetic analysis of cellular differentiation can ideally be undertaken in a *Drosophila* myogenic culture system. Analysis of a much studied developmental system, such as myogenesis (2, 6) in a genetically well-characterized eucaryote, *Drosophila melanogaster* (12), offers a unique opportunity to investigate coordinate gene regulation and the assembly and functioning of the contractile apparatus. The study of *Drosophila* myogenesis would permit myogenic gene localization on polytene chromosomes using in situ nucleic acid hybridization (15) as well as efficient screening of myogenic gene mutations using genetic deficiencies (13). The avian and mammalian myogenic culture systems, which have provided considerable information to date, lack the genetic potentials of *Drosophila*.

Existing *Drosophila* cell lines and primary culture methods are not well-suited for the investigation of molecular aspects of myogenesis. Numerous cell lines are currently in existence (5, 11, 19), including some with known genetic alterations (1, 17). However, none exhibit unambiguous characteristics of myogenic cells. Primary embryonic cultures contain myoblasts but also numerous other cell types (21, 27). Furthermore, *Drosophila's* size precludes mass dissection of muscle tissue for initiating myogenic cultures. These factors have limited the investigation of in vitro *Drosophila* myogenesis to the morphological and ultrastructural levels (10, 26).

In this communication, we describe and analyze a method for the preparation of cultures enriched in myoblasts from gastrulating embryos of *Drosophila melanogaster*. The method used ethylene glycol-bis(β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetate (EGTA), a calcium chelator, to separate myoblasts from other cell types on the basis of differential cell-substrate adhesion to tissue culture plastic. Isolated myoblasts differentiate in vitro during ~24 h to form mononucleated myocytes and multinucleated myotubes, which pulsate spontaneously. We present phase and electron micrographs to illustrate that these differen-

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tiated cells possess the bipolar morphology and myofilamentous ultrastructure characteristic of *Drosophila* myogenic cells in culture (10, 26). In addition, we have assayed the cultures for myogenic cell purity and developmental synchrony in order to establish the utility of this method for the genetic investigations of myogenesis.

MATERIALS AND METHODS

Collection and Preparation of Synchronous Embryos

Drosophila melanogaster (Oregon R strain) were raised in half-pint plastic bottles and transferred to large population cages after eclosion. Flies were maintained on a light-dry 12-h, dark-moist 12-h cycle to facilitate collection of synchronous embryos. Embryos were collected at the beginning of the dark-moist phase by allowing females to oviposit on grape juice-agar plates (1,800 ml of water, 200 ml of grape juice, 100 g of agar), coated with a live yeast-water paste. The first 2-h collection was discarded to eliminate older embryos held by females. The following collection was for a period of 2 h. At the end of this collection, trays were removed and incubated at 26°C for an additional 4 h.

Preparation of embryos was like that of Friedman et al. (7). After the surface sterilization step, embryos were transferred to a sterile 35-mm tissue culture dish (Bio-Quest, BBL & Falcon Products, Cockeysville, Md.) and rinsed twice with modified Schneider's medium (24).

Homogenization of Embryos and Plating of Cells

Drosophila mass cultures and myogenic cultures were prepared according to protocols that we have previously published in an abbreviated form (4, 8). All procedures were performed under sterile conditions with modified Schneider's medium, which contained 3 mM EGTA, except where omission of the latter is indicated. Embryos and about 5 ml of medium were transferred to a 7-ml glass homogenizer having a clearance of 60 μm, and dissociated into a cellular suspension by 10–15 gentle strokes. The cell suspension was transferred to conical centrifuge tubes and spun at the lowest speed in a clinical centrifuge (Damon/IEC Div., Needham Heights, Mass.) for 2–4 min. The pellet, containing large fragments of vitellary membrane, was discarded and the supernate was spun at speed three for 5 min. The increased time and speed of this centrifugation resulted in the separation of cells (pellet) from yolk particles (supernate). This washing procedure was repeated two times with the addition of fresh medium. Before the final centrifugation, an aliquot of the cell suspension was diluted in a crystal violet staining solution and the cells were counted in a hemocytometer. Cells were plated at 1.7–2.5 $\times$ 10^5 cell/cm^2 (in Falcon 150-mm dishes) in medium which contained 3 mM EGTA and 10 mU/ml bovine insulin (Sigma Chemical Co., St. Louis, Mo.). Cultures were then incubated at 26°C in an atmosphere of humidified air for 2 h. We have designated this interval of time as the "preplating period".

Isolation of Myoblasts from Primary Cultures

After 2 h, dishes were transferred to an incubated gyratory shaker (New Brunswick Scientific Co., Inc., Edison, N. J.) and agitated at 100 rpm for 10 min to create shear force which suspended loosely adhering cells. After shaking, the EGTA-released cells were decanted, and the adhering cells were rinsed very gently three times with fresh medium (without EGTA). The adhering cells were then allowed to differentiate for 21 h in insulin-supplemented Schneider's medium lacking EGTA. Analysis of differentiated cultures demonstrates that the adhering cells are enriched for myoblasts (see Results). To plate adhering cells at a higher density, they were sheared from the bottom of the dish with a stream of medium (lacking EGTA) delivered by a Pasteur pipet. These cells were then collected by centrifugation, resuspended in medium (without EGTA), counted, and plated at the desired higher density. EGTA-released cell cultures were prepared from the decanted medium by the same procedures. EGTA-released cell cultures contain few myoblasts (see Results). The characterization of cultured cells as "EGTA released" is operational and convenient, but does not preclude that some recovered cells were never attached to the tissue culture plastic.

Assaying the Effect of EGTA on Myoblast Purity

Primary cell cultures were prepared as described, except for the alterations outlined below which were designed to minimize the exposure of cells to EGTA before preplating and also the effect of shear force. The embryos were broken without addition of EGTA. Before the final centrifugation, 2 mM EGTA was added along with the normal culture medium to help disperse cells. The pellet was resuspended in more of this medium, and cell number was determined. Immediately thereafter, 0.1 ml of cell suspension was plated at 1.7 $\times$ 10^5 cell/cm^2 in 4 ml of medium supplemented with insulin and 0, 1, 2, 3, 4, or 5 mM EGTA. After 2 h, a reduced shear force of 50 rpm for 5 min was applied. Cells from differentiated cultures were fixed, stained, and assayed for percentage of myogenic nuclei present.

Timing of Cell Culture Events

All cell culture events use the midpoint of egg collection as zero time, except for the S phase experiments which give times after initiation of egg collection. The egg collection period corresponds to the fertilization event in rapidly laid eggs. This reference point is prefer-

able to plating time because it has biological significance and allows comparison between experimental data in rapidly differentiating cells with a high degree of determined cell activity (26).

Preparation of Cells for Light Microscopy

Living cultures were observed with Zeiss inverted or standard phase optics. Fields of cells were photographed by conventional photography or time-lapse cinematography. For cell counting, medium was decanted from the cultures and the cells were rinsed three times with phosphate-buffered saline (20). Cells were rinsed in methanol, then fixed in methanol for 5 min and hydrated in 95, 75, 50, and 35% ethanol (5 min each). Cultures were stained for 6 min with Giemsa's buffered with 0.01 M phosphate, pH 6.8.

Preparation of Cells for Electron Microscopy

5–10 × 10^6 putative myoblasts were allowed to differentiate for 45 h. The medium was decanted, and the cells were rinsed three times with phosphate-buffered saline (20). After treatment with 0.1 M sodium cacodylate, pH 7.2, cells were fixed in situ for 2 h with 2.5% glutaraldehyde in the cacodylate buffer. Cells were rinsed in 10% sucrose for 10 min and postfixed with 2% OsO_4 in 0.1 M s-collidine buffer, pH 7.2, for 2 h. Cultures were stained with 2% aqueous uranyl acetate for 1 h. Cells were scraped into 50% ethanol, dehydrated through an ethanol series, and rinsed in propylene oxide for 20 min. The detached cells were pelleted after each rinse. The pellet was gently overlaid with a 1:1 mixture of Epon 812 and propylene oxide, and incubated overnight. This mixture was gently removed, and the cell pellet was embedded in Epon 812 overnight at 60°C. Sections were cut, stained with uranyl acetate and lead citrate, and observed at 60 kV in a Philips electron microscope. EGTA-released cells were prepared and observed in an identical manner.

Identification of Differentiated Cell Types

The identities of some *Drosophila* cells in culture have been determined previously. We have used the following morphological characteristics to distinguish the cell types present and to estimate myogenic cell enrichment in EGTA-treated cultures.

Myogenic cells differentiate into mononucleated myocytes, which are frequently found in pairs or nests of several cells, or fused into multinucleated myotubes. These two myogenic forms are bipolar and pulsate spontaneously (21, 26). Myocytes are ~50 μm in length, and myotubes may achieve lengths of 100 μm. The ultrastructure of myogenic cells in culture has been investigated (10) and used here as a basis of comparison. The presence of regularly packed thick and thin myofilaments is characteristic of myogenic cells.

Other cell types mentioned in this communication can be distinguished from myogenic cells. In neurogenic cells, unequal cytokinesis of the neuroblast is responsible for the formation of a ganglion-like cluster of cells from which axons extend (25). The individual cell diameter is 4–5 μm, and an axon is 0.7 μm in diameter and up to 100–200 μm in length (21). F-cells are flattened and ovoid in shape with a maximum diameter of 10–20 μm. They contain fat droplet inclusions (22). Groups of imaginal disk cells form hollow monolayer cellular vesicles. The vesicles are highly refractile, have a size range of 20–40 μm, and initially contain up to 20 cells (27).

Labeling of Cultures and Preparation for Autoradiography

Cultures were pulse-labeled with [^{3}H]thymidine (5 Ci/mmol, Amersham Corp., Arlington Heights., Ill.), for 1-h periods at 2.5 μCi/ml. Labeled medium was removed, cultures were rinsed once with fresh medium, and fresh medium was added. Some cultures were labeled continuously from the times indicated. Single embryo cultures and mass cultures were prepared as before (24, 4). At 24 h after plating, cultures were rinsed three times with phosphate-buffered saline (20). Cells were air-dried, fixed in absolute methanol for 5 min, and hydrated through an ethanol series. Some cultures were treated with 300 μg/ml DNase (Worthington Biochemical Corp., Freehold, N. J.) in 0.03 M phosphate buffer, pH 7.0 for 3 h at 37°C. Since enzyme-treated cultures showed only background grains after autoradiography, no enzyme data are presented and no corrections for grain counts are made. All dishes were treated with 5% TCA at 4°C to remove unincorporated label.

Autoradiography and Scoring of Labeled Myogenic Nuclei

Autoradiographs were prepared by coating the entire culture dish with NTB2 liquid emulsion (Eastman Kodak Co., Rochester, N. Y.). Air-dried dishes were exposed in light-tight boxes for 3 days at 5°C. Film was developed in Kodak D-19 at 18°C. Cells were stained in Giemsa's buffered with phosphate, pH 6.8, for 6 min. Immersion oil was layered directly on dry autoradiographs, and the cultures were viewed at × 1,000, to count grains over the nuclei. At least 100 myogenic nuclei were scored in each culture examined. Nuclei were considered to have undergone DNA synthesis during labeling if seven or more grains were observed over the nuclear region (23). Elongated cells containing two or more nuclei (myotubes) and bipolar mononucleate myocytes were scored separately. Since data for myocytes were similar to those generated for myotubes, they are not included.

Timing of Myocyte Fusion

To determine the kinetics of fusion in myogenic cells, adhering cells were replated at 2.5×10^5 cells/cm^2. This density was satisfactory for easily discerning myotube nuclei by light microscopy. Preliminary experiments showed that fusion was not highly density dependent (S. I. Bernstein, unpublished data). Cells were fixed at indicated intervals and the number of myotube nuclei in random fields totalling 5 mm^2 were scored. This is probably the best available method for assaying fusion, as it is not influenced by division or death of mononucleated cells (29).

RESULTS

Optimization of Myogenic Cell Purity and Yield

The myoblast enrichment achieved by our method depends upon both the EGTA concentration of the medium in which the embryonic *Drosophila* cells are preplated and the amount of shear force employed to detach loosely adhering cells. We examined the effect of EGTA upon myogenic cell purity by preplating cells in 0, 1, 2, 3, 4, and 5 mM EGTA and subsequently shaking the cultures very gently at the end of the preplating period (see Materials and Methods). The results (Fig. 1) indicate that there is a direct relationship between EGTA concentration employed during preplating and the proportion of adhering cells that are myogenic. 5 mM EGTA results in the maximum enrichment of 70% myogenic nuclei.

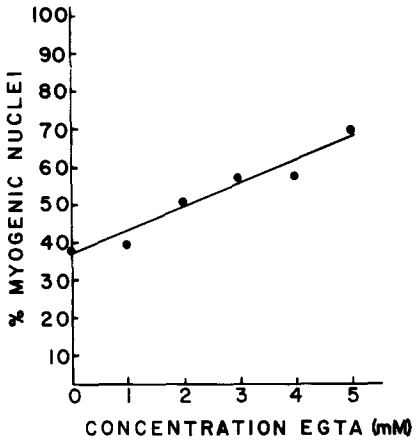


FIGURE 1 The effect of EGTA on purity of myogenic cultures. Cultures were preplated at 1.7×10^5 cells/cm^2 in medium supplemented with 0, 1, 2, 3, 4, and 5 mM EGTA. After 2 h, the cultures were shaken to loosen nonadhering cells. The medium containing these cells was removed and replaced with medium lacking EGTA. At 30 h after fertilization, the adhering cells were fixed, stained, and 1000 nuclei/dish were scored as being myogenic (myocytes and myotube nuclei) or nonmyogenic.

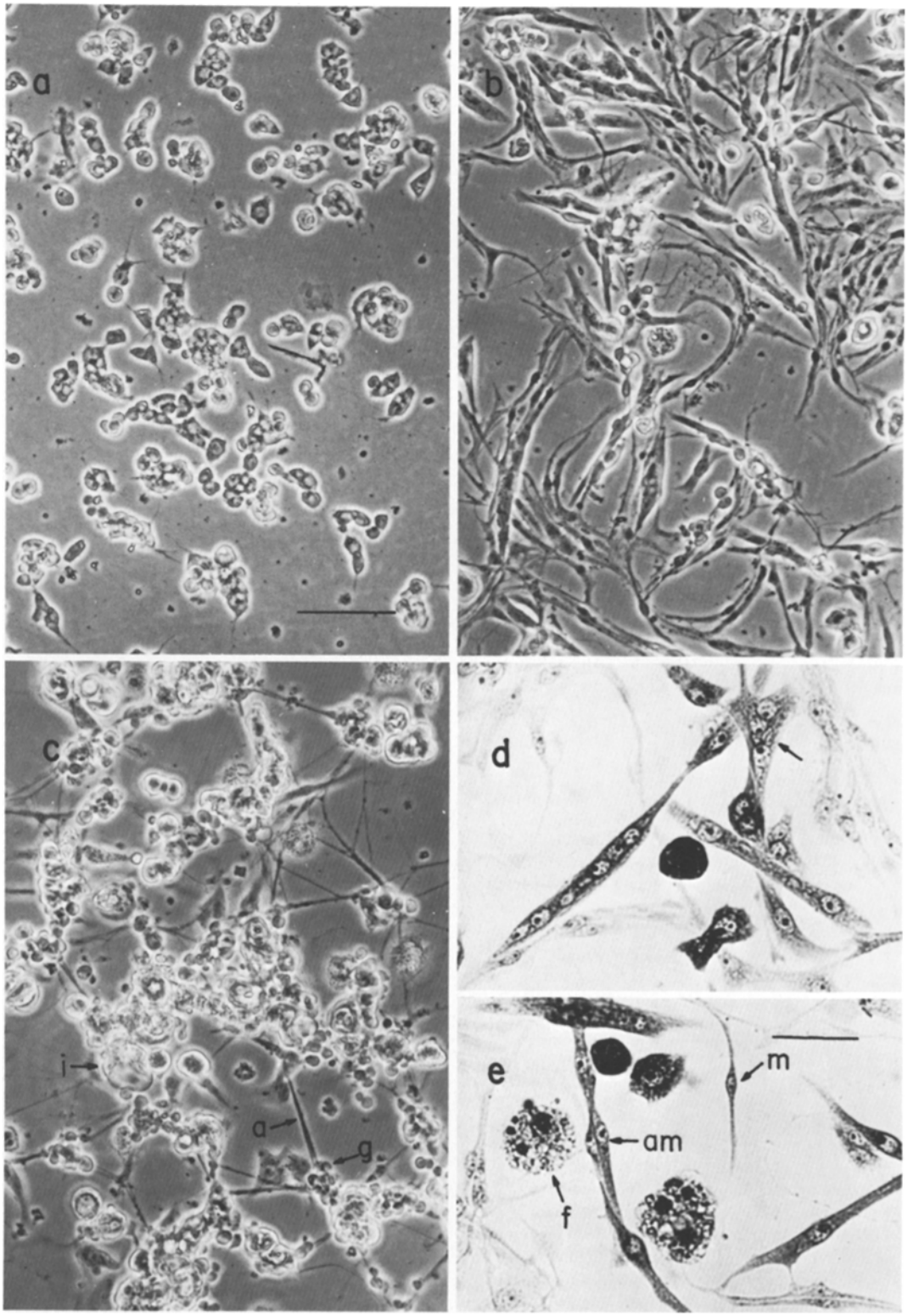
EGTA concentration and shear force affect myoblast yield in adhering cell cultures (data not presented). As EGTA concentration increases, the yield of myoblasts from a standard number of primary cells decreases. At concentrations of EGTA greater than 5 mM, myogenic cells adhere so tenuously that few myoblasts can be recovered. Increased shear force, while producing a higher proportion of myogenic cells in adhering cell cultures, also lowers the yield of myoblasts.

To optimize for both myogenic cell yield and enrichment, we have adopted a procedure that employs an intermediate concentration of EGTA and moderate shear force (see Materials and Methods). Employing this procedure allows us to obtain cultures that have almost 80% myogenic nuclei (see the following section) and up to 2×10^7 myoblasts (from 2 ml of dechorionated embryos).

Light Microscope Assay of Cell Identity

EGTA treatment of the initially heterogeneous cell population produces two classes of cells, adhering and released (Fig. 2*B* and 2*C*, respectively). We assayed cell identity in each class by phase contrast observations. As Table I indicates, cells adhering after treatment with 3 mM EGTA are primarily myogenic. At least 77.3% of the differentiated cell nuclei are myogenic: 52.4% of the total nuclei are in myotubes, 24.9% in myocytes. The proportion of myogenic cells may be greater than indicated because time-lapse cinematography demonstrates that a small fraction of cells that fuse do not display a bipolar configuration. The final morphology of multinucleated myotubes is usually straplike with bipolar attachment sites. Additional attachment sites give rise to other configurations (Fig. 2*D*). The contaminating cells (22.7%) are a mixture of all other cell types, with F-cells as the major species (Fig. 2*E*). Neurons and imaginal disk vesicles are infrequently present in adhering cell cultures.

The cells released by EGTA treatment and shear force have been cultured to determine their identity. All known cell types are represented in the differentiated products of released cells (Fig.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2*C*). The high proportion of cell clumps in released cell cultures precludes the possibility of performing accurate cell type counts. Neurons and imaginal disk cells are the predominant identifiable cell types; myogenic cells are present in low numbers.

Electron Microscopy of Presumptive Myogenic Cells

Although the cell morphology and pulsatile nature of differentiated cells in adhering cell cultures are reasonable evidence of their myogenic identity, we have chosen to substantiate this contention with ultrastructural observations. Previous electron microscope studies have indicated that mature myogenic cells of *Drosophila*, identified in culture by phase contrast microscopy, display thick and thin filament arrays (10, 26). As expected, the vast majority of cells from our adhering cell cultures contain these filaments. Fig. 3*A* shows several myogenic cells in cross section. Each cell contains tightly packed thick and thin myofilaments. High magnification cross sections, such as Fig. 3*B*, illustrate that up to 11 thin filaments orbit each thick filament resulting in a 4:1 ratio of thin to thick filaments. This is consistent with other studies of insect nonflight muscle, where ratios of 4, 5, or 6:1 are common (see reference 6 for review). We have not rigorously excluded the possibility that these sections contain double overlap of actin filaments, but contraction is not a frequent occurrence in these cells. Lattice organization may still be proceeding at 45 h since the orbit number of actin filaments per myosin is variable, most notably at the borders of the filamentous array. A micrograph of a multinucleated cell from an adhering cell culture (Fig. 3*C*) offers additional proof that these cells are myogenic, since myocytes form syncytia in vivo and in vitro (18, 26). These ultrastructural features of myogenic cells are rarely observed in EGTA released cells.

TABLE I

*Purity of Isolated Myoblast Cultures**

Trial	Myogenic nuclei‡	Nuclei in myotubes
	%	%
1	83.8	55.7
2	79.2	59.4
3	68.8	42.2
Average values	77.3	52.4

* Cells treated with 3 mM EGTA and replated at 5×10^5 cells/cm^2 were fixed, stained, and scored at 30 h after fertilization.

‡ 1,000 nuclei were scored in each trial. Myogenic nuclei include those in mononucleate differentiated myocytes and multinucleate myotubes.

Synchrony of S Phase in Myoblast Populations

Since the period of DNA synthesis (S phase) in myoblasts is highly synchronous and occurs only once in single-embryo cultures (23), we chose to assay the timing of S phase in order to determine the degree of developmental synchrony of myogenic cells derived from many embryos. We measured S phase of myoblasts in mass cultures of heterogeneous cell types rather than in adhering cell cultures. Measurement of the timing of S phase in adhering cell cultures is complicated by the fact that S phase is coincident with the preparation of these cultures. This complication makes pulse-labeling experiments virtually impossible.

We have analyzed the timing of S phase in the myoblasts of single-embryo cultures. Several such cultures were labeled with [^{3}H]thymidine for successive 1 h periods, allowed to differentiate, then

FIGURE 2 Phase contrast micrographs of living cultures (*A–C*) and bright field micrographs of Giemsa-stained cultures (*D* and *E*). (*A*) Replated cells which adhered during EGTA treatment, shown at 12.5 h after fertilization. (*B*) Same culture at 35 h after fertilization. Numerous multinucleated myotubes and nests of myocytes are present. (*C*) Cells that did not adhere during EGTA treatment, shown at 35 h after fertilization. Ganglion-like clusters of neurons (*g*), axons (*a*), and imaginal vesicles (*i*) are evident. Few myogenic cells are present. Bar for phase contrast photographs, 50 μm. (*D*) Giemsa-stained myogenic culture at 35 h after fertilization. All multinucleated myogenic cells are not tubular in morphology as evidenced by the triangular cell present in this figure (arrow). (*E*) Same condition as Fig. 2*D*. Aligned myocytes (*am*) appear ready to fuse. Small myocytes (*m*) often form nests of myogenic cells (see Fig. 2*B*). F cells (*f*) are the major contaminating cell type in myogenic cultures. Bar for bright field photographs, 25 μm.

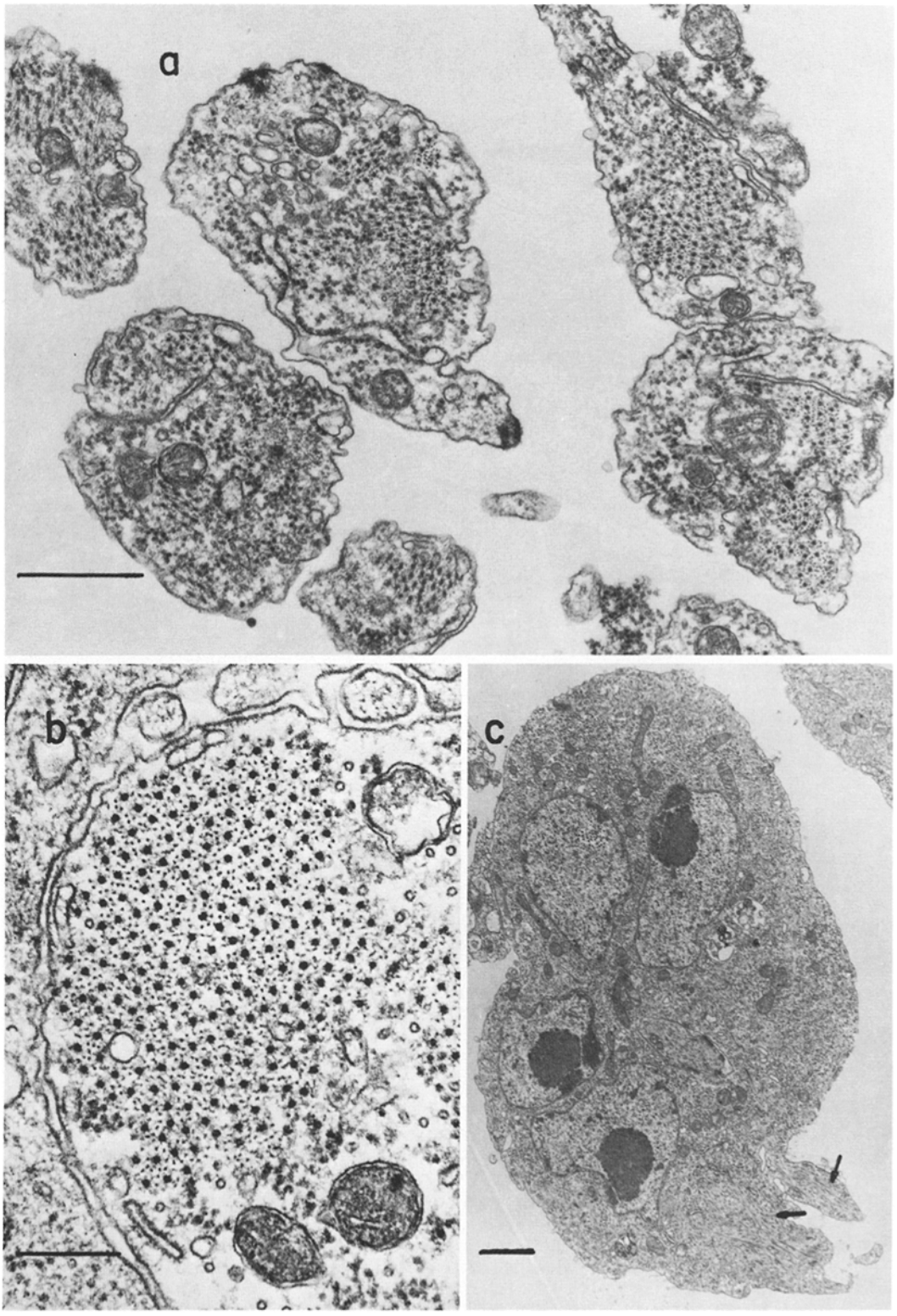

FIGURE 3 Electron micrographs of 45-h myogenic cells. (*A*) Thin section of a cell pellet. Cells display an orderly array of thick and thin filaments in cross section. Bar, 0.5 μm. (*B*) Enlarged view of a single myogenic cell in cross section. Up to 11 thin filaments circle each thick filament, and the thin to thick filament ratio is approximately 4:1. Bar, 0.25 μm. (*C*) Myotube containing four nuclei. Arrows indicate clusters of thick and thin filaments. Bar, 1 μm.

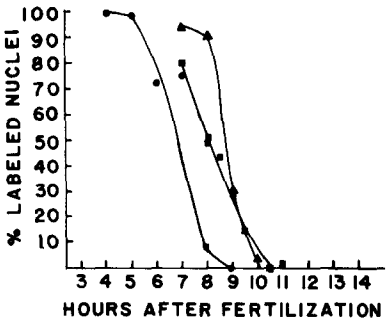

FIGURE 4 Duration of S phase in myoblasts of single-embryo and mass cultures. Cultures were pulse-labeled with 2.5 μCi/ml of [^{3}H]thymidine for 1-h periods or continuously from the times indicated. They were processed for autoradiography at 24 h after plating. At least 100 myotube nuclei were scored for each data point. ●: pulse-labeled single-embryo cultures. ■: pulse-labeled mass cultures. ▲: continuously labeled mass cultures. Times are calculated from the initiation of gastrulation (3.5 h) for single-embryo cultures and from the beginning of egg collection for mass cultures.

fixed and processed for autoradiography. Analysis of the differentiated cells revealed that significant labeling of nuclei destined to be incorporated into myotubes had occurred from the 4th to the 7th h after fertilization (Fig. 4). A 1-h pulse initiated immediately after cell plating at 4 h labels 99% of myotube nuclei. The number of myogenic nuclei labeled in subsequent 1 h pulses decreases until by 8 h after fertilization only 7% of nuclei incorporate [^{3}H]thymidine. The period of DNA synthesis of myoblasts in our single-embryo cultures agrees well with data from a previous report (23) where S phase was determined to occur 4.8–6.8 h after fertilization.

The timing of S phase of myoblasts of mass cultures, which were prepared from many embryos, is analogous to that of myoblasts of single-embryo cultures. Since the egg collection extends for 2 h, we would expect S phase for the myogenic cell population of mass cultures to continue for 2 h beyond the period observed in single-embryo cultures. As Fig. 4 shows, this is indeed the case (labeling was not performed before culture initiation, accounting for the absence of data before 7 h). At 7 h after the beginning of egg collection, 80% of the pulse-labeled nuclei and 95% of the continuously labeled nuclei destined to be incorporated into myotubes are in S phase. The decay of numbers of nuclei in S phase from pulse- and continuous labeling experiments on mass cultures indicates that only one S phase occurs and that its duration is the same as for single-embryo cultures. It is important to note that mononucleated myocytes have the same kinetics of labeling (data not presented) and therefore do not represent a separate class of myogenic cells with different DNA synthetic characteristics. These experiments demonstrate that the method of egg collection yields a highly synchronous population of embryos from which synchronously differentiating myoblasts can be obtained.

Time-Course of Myocyte Fusion

Since fusion is a significant event in myogenic cell differentiation (2), we have determined the time-course of myocyte fusion in adhering cell cultures (Fig. 5). Fusion begins at about 14 h after fertilization and is limited to a 15-h period, after which myogenic nuclei no longer enter myotubes. Included in the graph is a prefusion period during which fusion is not observed in vitro and is presumably absent in vivo (18). Phase contrast observations of cultures before 14 h after fertilization indicate that no myotubes are present, although an occasional binucleate cell is seen. These cells do not show the elongated tubular morphology of mature myotubes and are probably postmitotic cells that have not as yet undergone cytokinesis.

It is unlikely that EGTA, which is known to block myocyte fusion in vertebrate muscle cultures (16, 30), inhibits fusion during preplating of *Drosophila* myoblasts. Seecof et al. (26) have shown that myocyte aggregation, alignment, and elongation that may result in fusion occurs around 16 h,

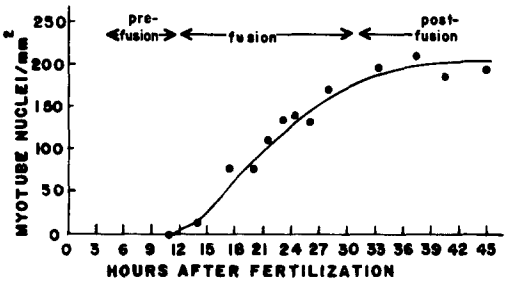

FIGURE 5 Time-course of myocyte fusion. Myoblast cultures plated at 2.5 × 10^5 cells/cm^2 were fixed and stained at times indicated. The number of myotube nuclei in a 5-mm^2 area was determined. Each point represents average results of two or three trials, normalized to the average value at 28 h to allow for myoblast purity differences between the trials. Times indicated are from the midpoint of egg collection.

which is well after EGTA is removed from our culture medium.

DISCUSSION

We have presented the first method for the isolation of a *Drosophila* cell type that subsequently differentiates in vitro. We have characterized the differentiation of these cells and have demonstrated that the vast majority possess the gross morphological and ultrastructural features of myogenic cells. These cells undergo one round of DNA synthesis, then divide once (26) and become bipolar myocytes, 70% of which fuse to form multinucleate myotubes. The synchrony of the differentiation of these myogenic cells is illustrated by the relatively short periods during which DNA synthesis and fusion occur.

The cell-substrate adhesion of cultured embryonic *Drosophila* cells is modified by the addition of EGTA to the culture medium. Embryonic *Drosophila* cells settle and attach firmly when plated in modified Schneider's medium (24). In medium containing 3–5 mM EGTA, however, only myogenic cells are able to adhere firmly. Nonmyogenic cells adhere at best tenuously, and can be suspended by a small amount of mechanical shear. Previously, Gail (9) and Shields and Pollack (28) noted that addition of EGTA to cell culture medium decreases cell-substrate adhesion. This effect may be the result of EGTA's calcium chelating ability since calcium is a required component in the complex interaction between cell surface and substrate that occurs during cell-substrate adhesion and cell spreading (3). Although other methods have employed "preplating" as a means for enriching for specific cell types (14, 31), our system is the first to incorporate EGTA into such a procedure. Employing EGTA in this manner should be applicable to other cell systems.

Utilizing the cells obtained by the myoblast enrichment procedure described here, it is feasible to investigate gene regulation, as well as other aspects, of myogenesis in *Drosophila melanogaster*. A single 2-h egg collection yields quantities of myoblasts suitable for biochemical analyses. The genetic and cytogenetic advantages of *Drosophila melanogaster* will allow investigators to dissect myogenesis at a genetic level not currently feasible in vertebrate systems.

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