Journal of Supramolecular Structure 7:307–322 (1977)
Cell Surface Carbohydrates and Biological Recognition 279–294

Cell Surface Changes Accompanying Myoblast Differentiation

L. T. Furcht, G. Wendelschafer-Crabb, and P. A. Woodbridge

Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota 55455 (L.T.F., G.W-C., and P.A.W.), and St. Paul Regional Red Cross Blood Center, St. Paul, Minnesota (L.T.F.)

Myoblasts are mononucleated cells and associated with differentiation undergo cell fusion and become multinucleated. The current studies have examined cell surface dynamic changes of Concanavalin A lectin receptor mobility and the role of hormones in modulating myoblast differentiation. A uniform distribution of Con-A receptors is observed in undifferentiated cells when reacted with Con-A at 37°C. Cells from differentiating cultures or fully differentiated myotubes reacted similarly at 37°C show a significant redistribution of Con-A into patches, "caps," and endocytic vesicles containing Con-A. If undifferentiated and differentiated cells are first prefixed with glutaraldehyde then reacted with Con-A continuous distribution of Con-A is seen across the cell surface. This suggests redistribution of Con-A and its receptors occurs in differentiated cells reacted with lectin at 37°C. It is further shown that insulin (10 μg/ml) significantly enhances myoblast differentiation but that this occurs after an apparent stimulation of proliferation. In contrast to insulin, dexamethasone (10 μM and 100 μM) profoundly inhibits myoblast differentiation while having different effects on proliferation; 10 μM dex stimulates cell growth while 100 μM dex suppresses cell proliferation. Lastly, an extracellular filamentous matrix which binds Con-A is observed at the ultrastructural level in high density cultures. No significant redistribution of Con-A is observed on this matrix in distinction to the redistribution observed on the cell membrane in differentiated cells.

Key words: myoblast differentiation, Con-A, extracellular filamentous matrix, insulin, dexamethasone

Recent work on cell membrane organization dynamics and composition has provided useful insights into studies of cell proliferation of various cell types. Early studies by Aub et al. (1) and Burger (2) showed differences in the agglutination of normal and transformed cells with lectins. This was followed by studies of Edidin (3) and dePetris and Raff (4) which showed the "fluid" nature of membrane receptors subsequent to binding of antibodies to histocompatibility antigens and xenoantibodies to surface immunoglobulin on lymphocytes, respectively.

Abbreviations: Con-A – Concanavalin A; CPK – creatine phosphokinase; dex – dexamethasone

Received April 5, 1977; accepted June 16, 1977.

We have been interested in cell surface changes accompanying differentiation and have used L6 rat myoblast cell line originally isolated by Yaffe (5, 6). This cell line was isolated from embryonic rat striated muscle, and in its undifferentiated state consists of mononucleated cells. Differentiation is associated with cell fusion and myotube formation, increased synthesis of myofibrillar proteins and myofilaments, increased levels of creatine phosphokinase (CPK) and other enzymes, and the development of acetylcholine receptors (5–10). The current studies have examined cell surface topography and the ability for Concanavalin A (Con-A) to undergo redistribution at various stages of myoblast differentiation. In addition, we have examined the role of insulin and dexamethasone (dex) in affecting cell proliferation and differentiation in this system.

METHODS

Clonal isolates of L6 myoblasts were kindly supplied by Dr. David Schubert, Salk Institute. Cells are typically plated at 2,500/cm^2 in 100-mm sterile Falcon plates, in Dulbecco's minimal essential media supplemented with 10% fetal calf serum (Flow, Rockville, Maryland) under conditions described for other cell types (11).

In addition to the formation of myotubes, differentiation is assessed by an increase in CPK activity (6). To assess the proliferation of the cells we have made sequential measurements of total DNA per culture dish. We have found the DNA assay more reliable than cell counts as once the cells start fusing it becomes rather difficult to get reliable data. In addition to sequential determinations of DNA to assess cell proliferation, [^{3}H]dThd incorporation was assayed. Cells are pulsed with 10 μCi of [^{3}H]dThd (New England Nuclear Corporation, Boston, Massachusetts, specific activity 40–60 μCi/M) for 2 h, harvested, and counted as described (11).

For biochemical assays cells are scraped from plates in 50 mM imidazole-phosphate buffer, pH 6.75, and homogenized at 4°C for 90–120 sec using a motorized Dounce homogenizer with a Teflon pestle at 600 rpm. Each sample is then split for determination of CPK and DNA. CPK activity is measured using a modification of the method of Oliver using a GEMSAC fast analyzer (12). DNA is extracted with 0.5 N perchloric acid and quantitated according to the method of Burton (13) by measuring the optical density at 600 nm in a Beckman 25 spectrophotometer using calf thymus DNA (Sigma Chemical Company, St. Louis, Missouri) as a standard.

Studies are also performed to examine the effects of insulin or dexamethasone on L6 proliferation and differentiation. Cells are allowed to grow up to a density of 20,000–40,000 cells/cm^2 and the medium is changed to one of the following: 1) medium + 10 μg/ml insulin (Sigma); 2) medium + 100 μM dex (Sigma); 3) medium + 10 μM dex; or 4) routine medium. Stock dex solutions are made 5×10^{-2} M in absolute ethanol with final concentrations of ethanol less than 0.2% in the dex and control medium samples.

Cells for Con-A localization are taken at 3 stages: 1) low density undifferentiated (approximately 20,000/cm^2); 2) predominantly mononucleated cells showing early signs of differentiation (cell density approximately 100,000/cm^2) and 3) advanced differentiation with preponderantly myotubes. At these various stages cells are washed 3 times in phosphate-buffered saline (PBS) (pH 7.4) and incubated with either 30 or 50 μg/ml Con-A (Sigma) for times ranging from 5 to 20 min. Samples are then washed 3 times with PBS and fixed in this buffer with 1.6% glutaraldehyde (Electron Microscopy Sciences, Fort Washington, Pennsylvania) for 20 min at 37°C. All above incubations and washings were performed at 37°C. To assess the native distribution of cell receptors for Con-A, parallel

samples are prefixed with 2.5% glutaraldehyde-PBS for 20 min at 37°C, and then reacted with Con-A and fixed again as above. Con-A is then localized by the peroxidase reaction with diaminobenzidine after the method of Bernhard and Avaremas (14). Samples are processed for ultrastructural cytochemistry as described for routine electron microscopy (see below) with the omission of tannic acid and staining with lead citrate.

For routine electron microscopy samples are washed in 0.1 M cacodylate buffer, pH 7.2, fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, at 37°C and subsequently postfixed for 1 h with 1% OsO_4 (Electron Microscopy Sciences) 0.1 M cacodylate (15). Cells are then treated with 1% tannic acid (Mallinckrodt, St. Louis, Missouri) (16), dehydrated in graded series of ethanol (30, 50, 70, 95, and 100%) and embedded in Epon 812 (17). Silver to gray sections (40–60 nm thick) are cut with a diamond knife on a LKB Ultratome III and mounted unsupported on 200-mesh copper grids. Thin sections are counterstained with Reynold's lead citrate (Baker) (18) and examined in a Philips 300 electron microscope.

To quantitate the surface distribution of Con-A, 1-μm thick epon sections are cut and examined with a Zeiss light microscope at 400 or 1,000 ×. The distribution of Con-A is denoted as either uniform showing a continuous distribution or showing redistribution, i.e., patching and/or capping evidenced by a segregation from a uniform distribution.

RESULTS

L6 myoblasts in their undifferentiated state at low density (approximately 10,000–20,000/cm^2) are mononucleated. These are very active cells having a very dilated rough endoplasmic reticulum, numerous ribosomes, and a paucity of myofilaments, though occasional microfilament bundles are observed (Fig. 1). Differentiation is dependent on medium conditioning, cell contacts, and on calcium ions among other things (5–10). At the early stages of differentiation the free ribosomes appear to be actively synthesizing the contractile proteins and most are associated with numerous thin and thick filaments (Fig. 2). Dense bodies which may correspond eventually to Z-line material are seen within these filament bundles and numerous microtubules, generally not associated with ribosomes, are observed (Fig. 2). At more advanced stages of differentiation the density of myofilaments increases (Fig. 3). At more advanced stages myofilaments organize and the relative number of microtubules appears to decrease compared to undifferentiated cells (Fig. 4).

Using this background information and biochemical estimations of differentiation (see below) we have examined Con-A receptor topography in cells at various stages of differentiation and the ability of cells to undergo receptor redistribution upon interacting with this ligand. Undifferentiated L6 myoblasts, interacted with 50 μg/ml Con-A at 37°C for 10 min, show a predominantly uniform distribution of Con-A (Fig. 5). Undifferentiated cells which are pre-fixed prior to reacting with Con-A also have a uniform distribution of Con-A at the cell surface (Fig. 6).

As cells begin to differentiate, we see an alteration of lectin distribution when compared to the uniform distribution seen in undifferentiated myoblasts. When these cells are reacted with Con-A at 37°C and then fixed, a predominantly uniform pattern of Con-A is seen at the cell surface; however, microvilli show markedly decreased staining for Con-A (Fig. 7). Interestingly, if samples from these cultures showing early differentiation are pre-fixed and then reacted with Con-A a uniform distribution is seen over the plasma membrane and the microvilli (Fig. 8). The uniform distribution of lectin receptors on pre-fixed cells suggests the native topographical array and that changes from this seen in samples reacted with Con-A prior to fixation represent redistribution (19).

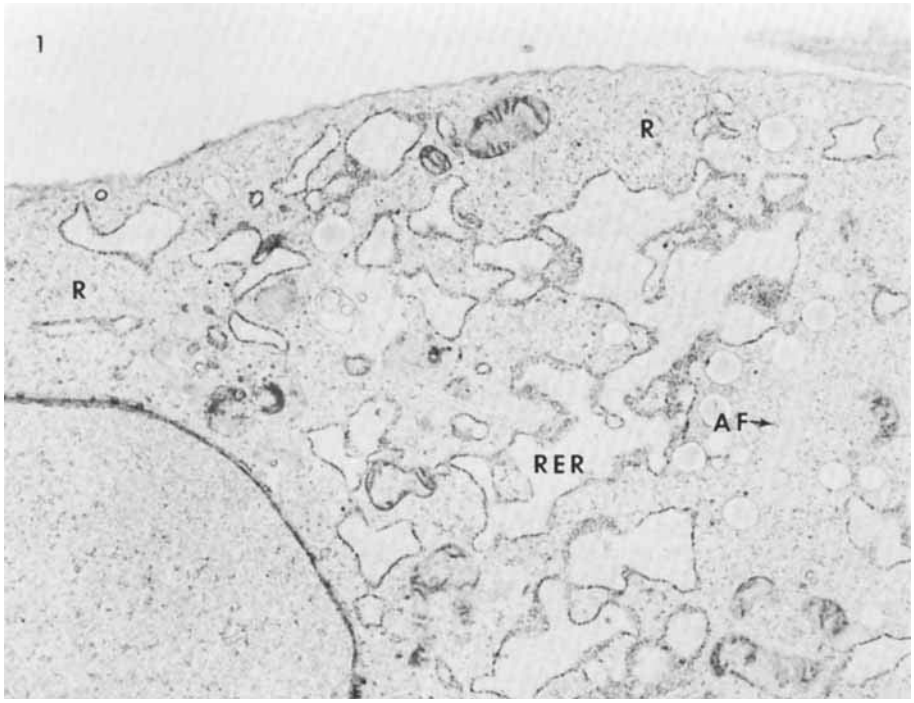

Fig. 1. Electron micrograph of undifferentiated L6 myoblast. Distended rough endoplasmic reticulum (RER) predominate in thin section of myoblast. Note great number of free ribosomes (R) and actin filaments (AF). Heavy metal counterstain (9,500 ×).

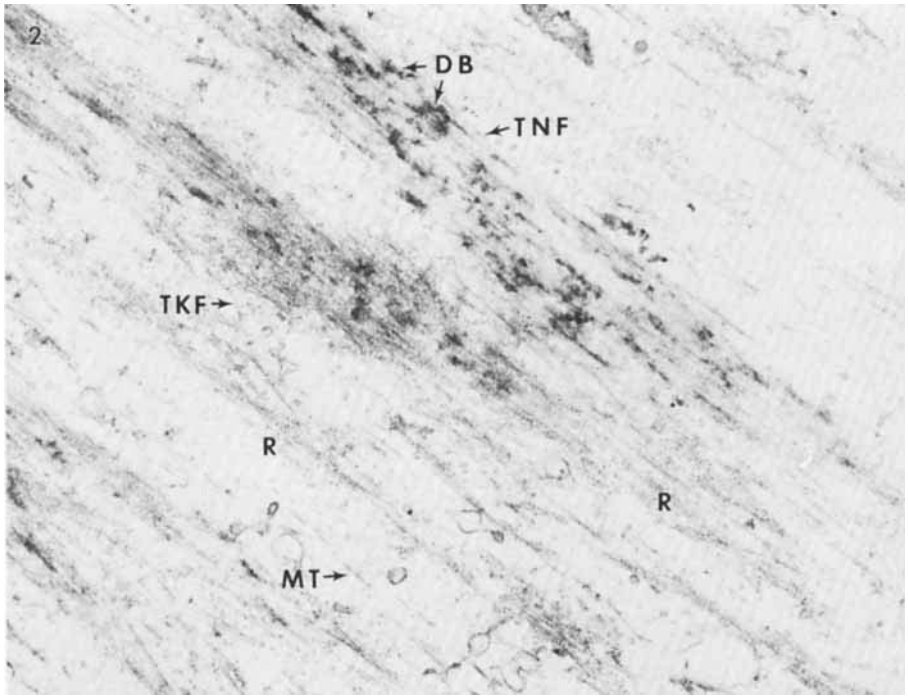

Fig. 2. Electron micrograph of differentiating L6 myoblast. Numerous ribosomes (R) are visible in close proximity to thin filaments (TNF) and thick filaments (TKF). Dense bodies (DB) are present in association with filament bundles while microtubules (MT) commonly course between filament bundles. Heavy metal counterstain (12,300 ×).

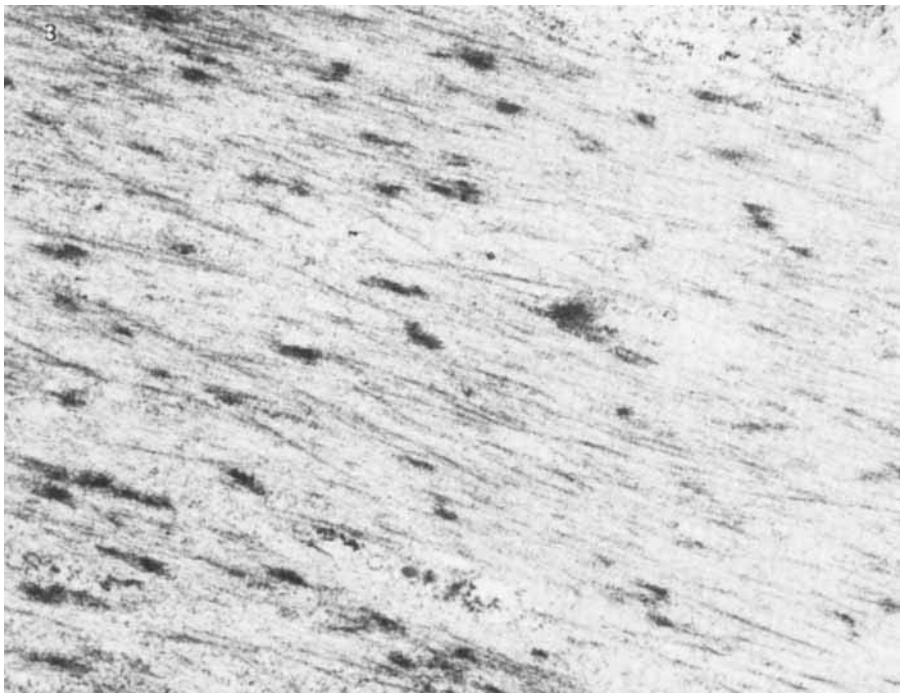

Fig. 3. Thin section micrograph of more highly differentiated L6 myotube. Increased numbers of myofilaments course in parallel through myotube. Heavy metal counterstain (17,900 X).

Fig. 4. Thin section of advanced stage of myotube differentiation. A more classic organization of myofilaments characterizes well-differentiated myotubes. Heavy metal counterstain (20,000 X).

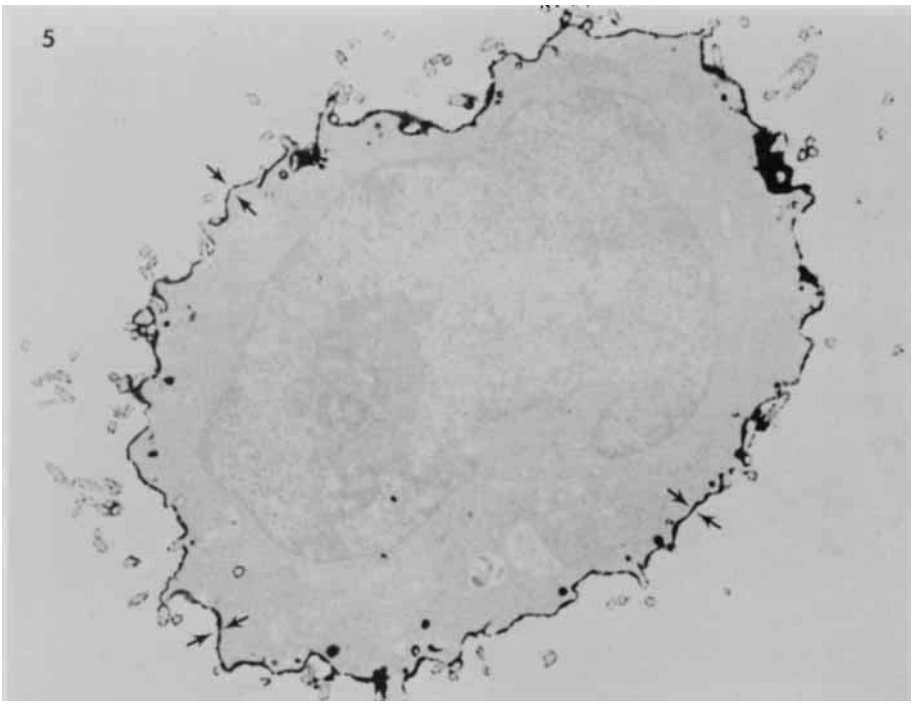

Fig. 5. Ultrastructural Con-A localization on low density L6 myoblast. Cells reacted with 50 μg/ml Con-A at 37°C for 10 min before fixation. Con-A was localized with peroxidase and diaminobenzidine as described in Methods. Con-A is uniformly distributed at the cell membrane (arrows). No heavy metal counterstain (9,600 ×).

As these cells differentiate into multinucleated myotubes they show greater redistribution of Con-A receptors. When myotubes are interacted with lectin at 37°C without prior fixation a "patching" of Con-A receptors occurs (Fig. 9), or a more pronounced redistribution occurs forming what others have termed a "cap" (Fig. 10). In addition to this redistribution the internal membrane surface of endocytic vesicles is labeled with Con-A (Fig. 10). When myotubes are first pre-fixed then reacted with Con-A none of these rearrangements (patching, capping, or endocytosis of Con-A receptors seen in unfixed samples), is observed (Figs. 11 and 12).

The binding of lectin and the reaction product show the expected specificity with the virtual complete absence of reaction in the presence of 50 mM α-methyl-D-mannoside (Fig. 13). Interestingly, in addition to the cell membrane binding of Con-A there is significant binding to an extracellular filamentous matrix in high density cultures (Fig. 11), the precise nature of which is unknown. We have failed to observe redistribution of receptors on this extracellular filamentous matrix as is observed on the plasmalemma of differentiated cells. It should be noted that the presence of the matrix is so extensive in high density cells in situ that it precludes definitive resolution of membrane receptor dynamics using fluorescein-conjugated Con-A (unpublished observation). The redistribution of receptors in differentiated cells or myotubes has been quantitated in Table I. For samples reacted with 50 μg/ml Con-A at 37°C for 20 min we observed: a uniform distribution in 99% of undifferentiated cells, redistribution into "patches" and/or caps in 37.5% of cells in a culture showing early differentiation, and a redistribution of lectin in 95% of the cells in a culture showing a high degree of differentiation and myotube formation. If parallel

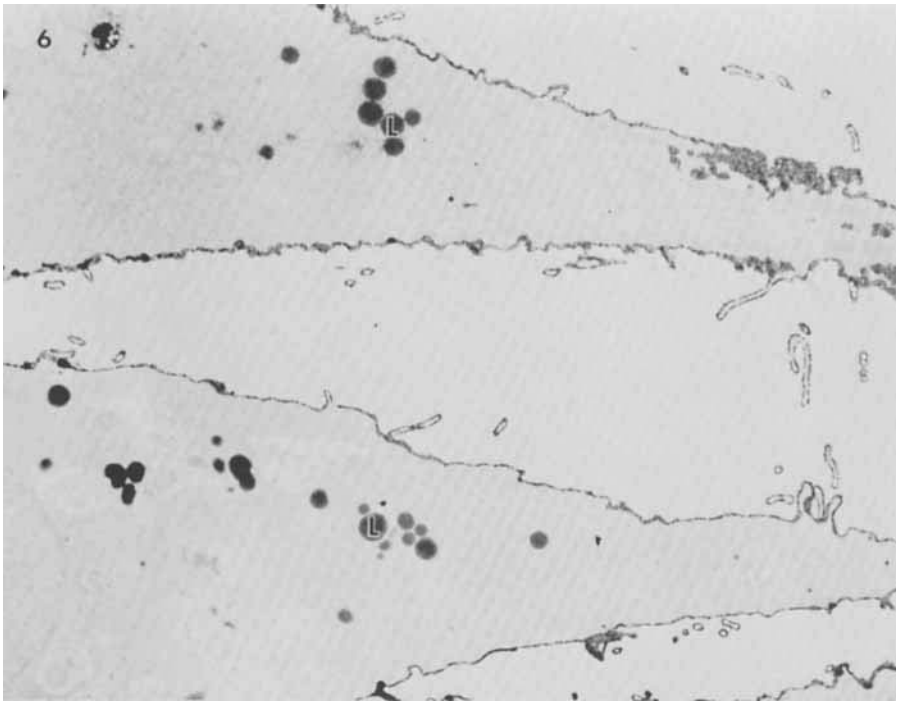

Fig. 6. Con-A localization on pre-fixed undifferentiated L6 myoblast. The uniform distribution of Con-A is interpreted as the native configuration of Con-A "receptors." Lysosomes (L) which appear dark are osmophilic. No heavy metal counterstain (10,200 ×).

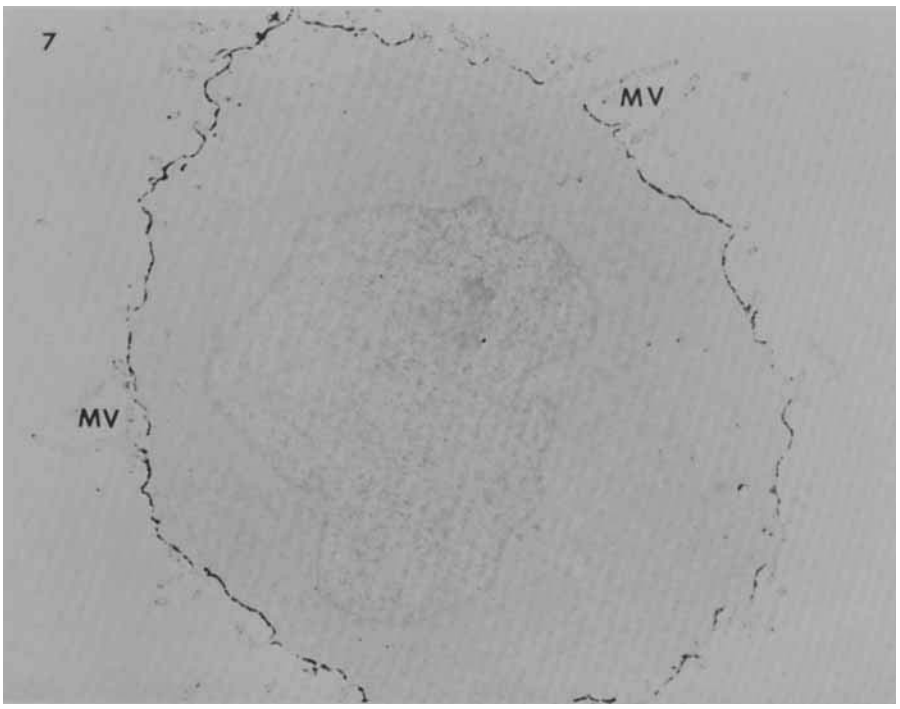

Fig. 7. Con-A localization on high density L6 culture showing early differentiation. Changes from the uniform distribution seen in Fig. 5 are observed as microvilli (MV) being less stained. No heavy metal counterstain (4,600 ×).

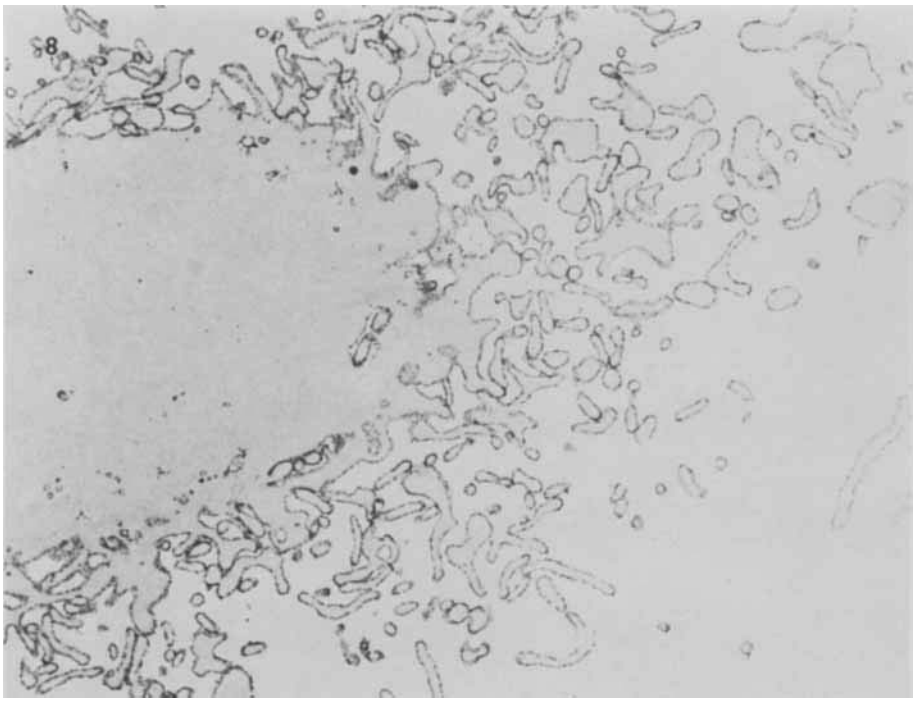

Fig. 8. Con-A localization on pre-fixed high-density L6 culture. Con-A is uniformly distributed over the entire surface of pre-fixed cells, including microvilli. No heavy metal counterstain (9,000 ×).

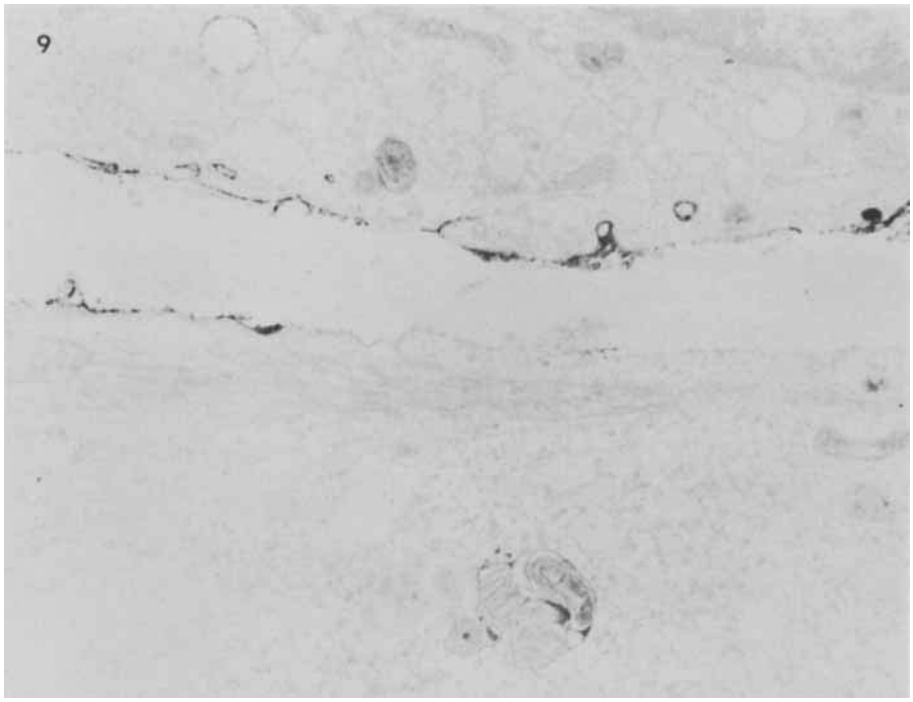

Fig. 9. Con-A localization on differentiated myotube. The Con-A reaction product is unevenly distributed into patches. No heavy metal counterstain (12,400 ×).

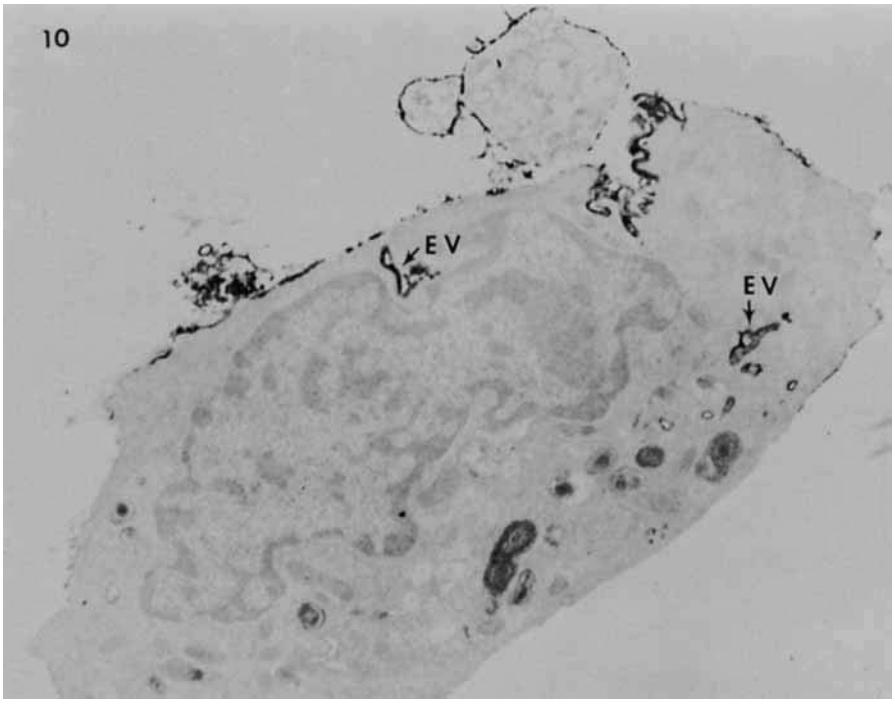

Fig. 10. Con-A localization on differentiated myotube. The Con-A reaction product is segregated (globally redistributed into a cap). Some Con-A appears in endocytic vacuoles (EV) within the cell. No heavy metal counterstain (10,300 ×).

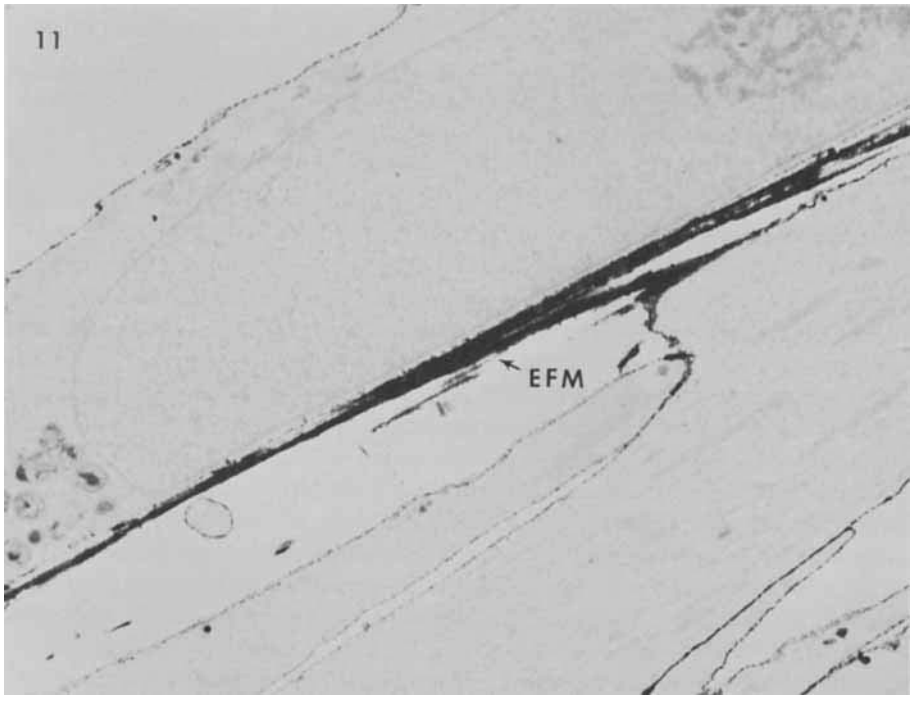

Fig. 11. Con-A localizalization on prefixed differentiated myotube. The Con-A reaction product is uniformly distrubuted. Note the extracellular filamentous matrix (EFM) which binds Con-A quite heavily. No heavy metal counterstain (8,000 ×).

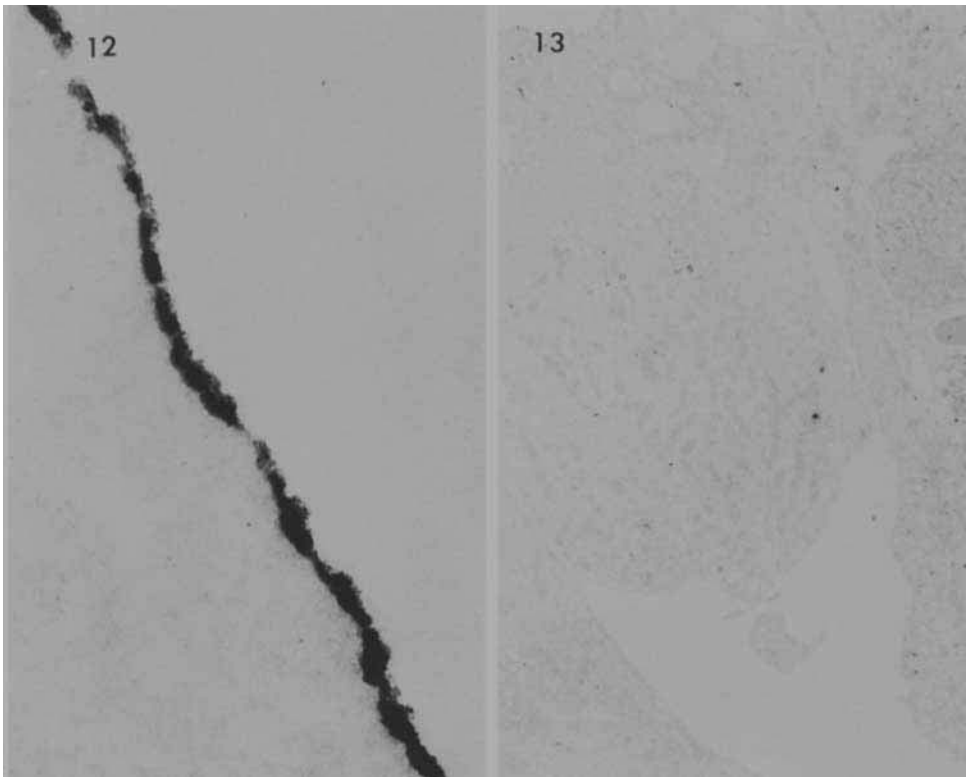

Fig. 12. High magnification micrograph of prefixed differentiated myotube. Con-A is distributed in a uniform pattern, continuously along the cell membrane. No heavy metal counterstain (93,000 ×).

Fig. 13. Micrograph of myotube reacted with Con-A in the presence of 50 mM α-methyl-D-mannoside. The mannoside competatively blocked all Con-A binding. No heavy metal counterstain (6,100 ×).

samples are first pre-fixed then reacted with Con-A a uniform distribution of membrane lectin receptor is seen in virtually all of the cells under all of the conditions (Table I).

Insulin (10 μg/ml) and dexamethasone (100 μM) have profound effects on proliferation of L6 myoblasts (Fig. 14). Even though addition of fresh medium and serum to cells moderately enhances [^{3}H]dThd incorporation, insulin produces a threefold elevation in incorporation. Following this initial burst in [^{3}H]dThd incorporation in insulin-treated samples incorporation falls to levels below 50 cpm/μg DNA at 5 days of treatment, while controls remained relatively high at approximately 200 cmp/μg DNA. This would be compatible with a larger percentage of insulin-treated cells arresting in the G_1 phase of the cell cycle. Dexamethasone (100 μM) profoundly inhibits proliferation and blocks the expected rise in [^{3}H]dThd incorporation from adding fresh medium. This blockade of proliferation by dexamethasone is completely reversible (unpublished observation).

Somewhat similar results are observed when sequential determinations of total DNA/plate are made (Fig. 15). Significant increases in DNA are observed in controls for the first 3–5 days. As would be expected from the enhancement of [^{3}H]dThd incorporation, insulin treatment produces a rapid rise in total DNA/plate which plateaus at the onset of differentiation. Dex (100 μM) produces a profound inhibition of proliferation

TABLE I. The Redistribution of Receptors in Differentiated Cells or Myotubes

Cells and conditions[a]	Con-A distribution[b]	
	% Uniform	% Nonuniform (Redistribution into patches and/or caps)
I. Low density undifferentiated L6 (N = 200)		
(A) Postfixed after reacting with Con-A	99%	1%
(B) Pre-fixed	99%	1%
II. High density confluent L6 showing (N = 110)		
(A) Postfixed	62.5%	37.5%
(B) Pre-fixed	99%	1%
III. Differentiated L6 myotubes (N = 200)		
(A) Postfixed	5%	95%
(B) Pre-fixed	99%	1%

[a]Postfixed: cells incubated with 50 μg/ml Con-A, 15 min, 37°C, then fixed with 1.6% glutaraldehyde. Pre-fixed: cells fixed with 2.5% glutaraldehyde, 15 min, 37°C, then reacted with Con-A as in postfixed specimens.

[b]These observations are made from 1-μm "thick sections" of samples used for electron microscopic localization and viewed at 1,000 ×.

N = number of cells.

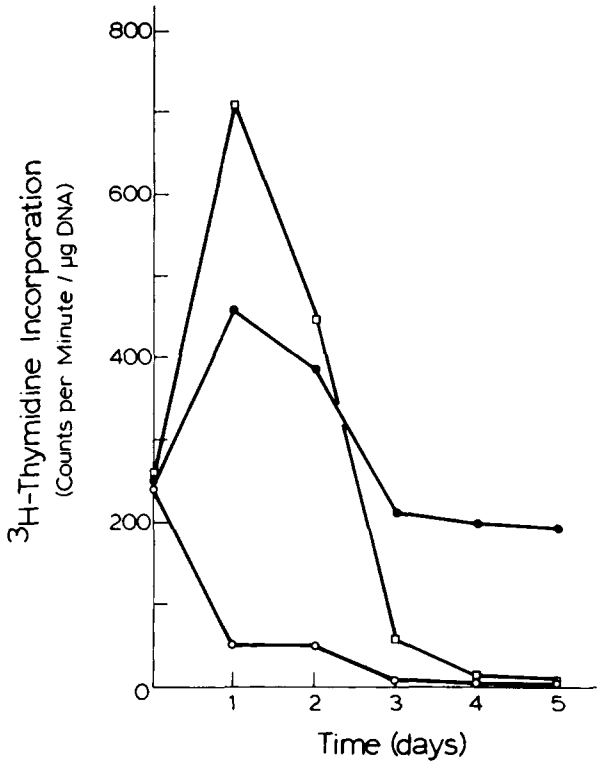

Fig. 14. [^{3}H] Thymidine incorporation (cpm/μg DNA) in L6 cells as a function of time in days. At time zero, the following are added: 1) medium (●—●); 2) medium + 10 μg/ml insulin (□—□); 3) medium + 100 μM Dexamethasone, (○—○)

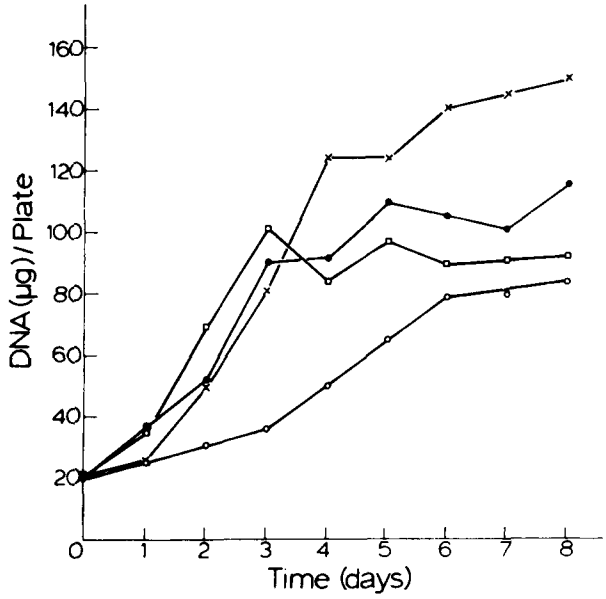

Fig. 15. Total DNA (μg) per 100 mM tissue culture plate as a function of time (in days) of culturing. Medium (•—•); or medium plus the following additions: 10 μg/ml insulin (□—□); 10 μM dex (x—x); 100 μM dex (○—○).

with only minimal increases in DNA with levels being consistently below controls. Treatment of cells with 10 μM dex has the opposite effect where an enhancement of proliferation occurs and elevated levels of DNA relative to controls are observed after 3–5 days of treatment.

L6 cells differentiate and increased levels of creatine phosphokinase are observed (Fig. 16). Significant increases of CPK/DNA are observed in controls from day 5 and later. As was shown above, insulin will stimulate DNA synthesis which then plateaus after day 3. Associated with the cessation of proliferation, insulin-treated cells show a greater rate of differentiation than controls as measured by CPK/DNA (Fig. 16). In contrast to the insulin effects both 100 μM and 10μM dex appear to inhibit differentiation with CPK levels remaining at baseline levels throughout the course of the experiment.

DISCUSSION

These studies have examined the cell surface topography for Con-A and its ability to redistribute at various stages of myoblast differentiation. A uniform distribution of Con-A receptors was observed in undifferentiated cells reacted with Con-A at 37°C, and in differentiated or undifferentiated cells pre-fixed with glutaraldehyde and then reacted with Con-A. Cells from differentiating cultures or fully differentiated myotubes reacted with Con-A at 37°C prior to fixation show a significant redistribution of Con-A into patches or caps and endocyte vesicles.

Studies of Singer and Nicholson (20) and Wallach (21) were the first to postulate the "fluid" or mobile nature of membrane components. Studies by Frye and Edidin (3) and dePetris and Raff (4) supported this hypothesis showing a redistribution of histo-

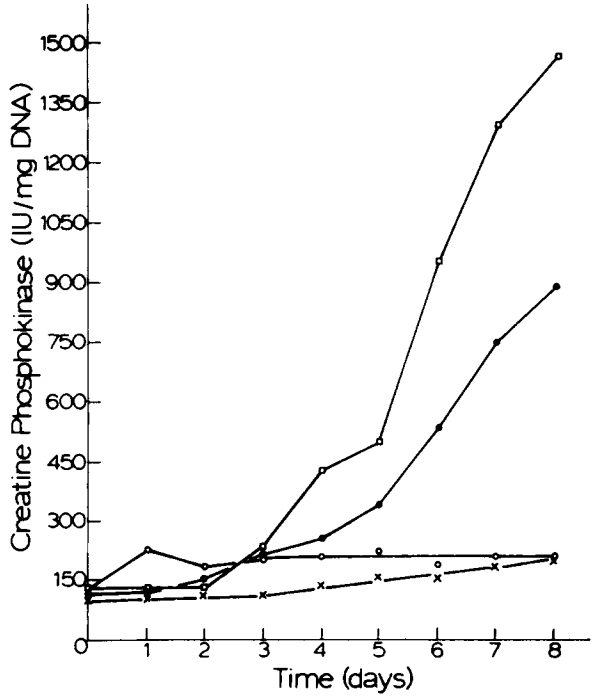

Fig. 16. Creatine phosphokinase activity in international units/mg DNA as a function of time in days of culturing. Medium (•—•); or medium plus the following additions: 10 μg/ml insulin (□—□); 10 μM dex (x—x); 100 μM dex (○—○).

compatibility antigens on heterokaryons and alloantibodies on lymphocytes. Other interesting studies have shown a difference in the topographical distribution or dynamic redistribution of lectin receptors in normal and transformed cells for Con-A and Ricinis communis agglutinin (19, 22). Both normal and transformed cells in their native state have a uniform distribution of Con-A receptors. This was determined by pre-fixing cells with glutaraldehyde then reacting with Con-A or reacting unfixed cells with lectin at 4°C without prior fixation (19, 22, 23). In both cases a uniform lectin distribution was observed in transformed cells. However, when cells are reacted with Con-A at 37°C a re-distribution occurs on transformed cells but not on normal cells (19, 22). The interpretation of these, as well as our own studies, is that numerous receptors for multivalent ligands can undergo redistribution at the cell surface (23).

The nature of the Con-A receptor mobility in terms of the Singer and Nicholson membrane model could have 2 possible explanations: alterations of membrane lipid fluidity or interactions with cytoskeletal components. Redistribution as a result of enhanced membrane lipid fluidity would be a plausible explanation for a receptor site that is loosely associated with the cell membrane, or partially embedded in the lipid bylayer, or a glycolipid component of the membrane. Studies have shown that reduced temperature, thus presumably modulating membrane lipid environment, can effect the mobility of receptors or agglutination of cells by Con-A (24).

Redistribution of Con-A receptors as a result of interactions with cytoskeletal components would require that there be interactions between Con-A and the cytoskeletal

structure via a transmembranous moiety (23, 25). However, there is no correlation between Con-A receptor sites and intramembranous particle distribution in myotubes as seen by freeze fracture (Furcht, in preparation). This does not rule out that the Con-A receptor may be a transmembranous component not represented within the intramembranous particle. Also, transformed cells which are thought to have more "fluid" or less "restricted" membranes will patch membrane receptors for Con-A and have few organized cytoskeletal components demonstrable by thin section electron microscopy or immunocytochemical localizing of contractile proteins (26–28). It is also interesting to note that Con-A receptors fail to patch or cap in lymphocytes unless microtubular disruptive drugs are utilized (29).

To account for the apparent inconsistencies we have postulated the existence of 2 operationally separable contractile systems in cells. The first is the classical cytoskeleton suggested by Porter (30) and the second is an undefined membrane-associated contractile network which may regulate cell surface dynamics (31, 32). This hypothesis is based on the ability of low concentrations of either cytochalasin B, vinblastine, or colchicine to independently modulate intrinsic membrane structure without affecting cell shape thought to be maintained by the classical cytoskeletal system (31). Until the true nature of Con-A receptors is known it would be speculation to suggest which of the 2 or perhaps other mechanisms may be occurring to produce the redistribution observed in these differentiated myoblasts or myotubes.

In this system, differentiation is associated with an increase in Con-A mobility. It could be argued that differentiation and fusion of cells leads to a more "active" or less restricted state of the cell membrane so that developing myotubes can fuse and incorporate new cells and cell membranes. A more restricted or rigid membrane in cells could inhibit cell fusion thereby inhibiting myotube formation. This hypothesis is supported by observations that agents which may enhance fusion have been suggested to have at least locally some membrane disorganizing or detergent-like effect (33). Whether this is the case or not awaits further experimentation. However, recent work using the laser bleaching of fluorescent-labeled acetylcholine receptors on myotubes suggests a fluid nature for this intrinsic membrane protein (34).

In view of the clearly different cell surface lectin receptor dynamics of the L6 myoblasts and myotubes, we are investigating the interactions of various agents that modulate differentiation and the distribution of Con-A receptors. As reported above, our preliminary studies show that insulin enhances differentiation while dexamethasone at 10 μM and 100 μM concentration inhibits differentiation.

The insulin promotion of differentiation has been reported by others (35). However, our studies suggest that this is somehow secondary to the initial enhancement of proliferation demonstrated by increased [^{3}H] thymidine uptake, DNA synthesis, and by the several-day delay in the appearance of differentiation following insulin treatment. This explanation would reconcile the observation that insulin and other mitogens are known to raise cGMP in other cell systems (36–38), whereas differentiation is normally associated with an elevation in cAMP (39–40). In contrast to insulin, dexamethasone at 10 μM and 100 μM concentrations inhibits differentiation. More interestingly, the 2 doses seem to act through mechanisms different from insulin as 10 μM dex stimulates cell growth (without differentiation) while 100 μM dex suppresses proliferation.

It is interesting to note that in undifferentiated cultures, there is an extensive network of an extracellular filamentous matrix which binds Con-A. The distinction between this network and membrane receptors for Con-A is readily seen using the peroxidase

method of localization at the ultrastructural level. The nature of this matrix is unknown and the Con-A agglutination of certain cells cultured in vitro has been suggested to be a function of an extracellular matrix (41). Myoblasts are known to synthesize collagen; thus, the extracellular network could be collagen fibers. Possibly of greater interest, at least currently, is that this extracellular filamentous matrix may represent the glycoprotein fibronectin (LETS, Z, etc.). These proteins are a class of high-molecular-weight (250,000 dalton), externally disposed, loosely attached glycoproteins shown to be present in contact inhibited fibroblasts and myoblasts (42, 43). Studies are in progress to define the biochemical nature of this extracellular filamentous matrix which binds Con-A, and its relationship to fibronectin or LETS. Studies in progress are also examining the interrelationship of various hormone treatments, dynamic alterations in membrane structure and function, and the role of this extracellular matrix in myoblast differentiation.

NOTE ADDED IN PROOF

We have recently observed at the light and ultrastructural level using immunocytochemistry that antibodies made against purified fibronectin react with the extracellular filamentous matrix and the plasma membrane. The matrix is most pronounced in high density contacted cultures and appears to decrease with myotube formation (Furcht, Mosher, Wendelschafer-Crabb – submitted).

ACKNOWLEDGMENT

We wish to thank S. Gentry and R. Scott for technical assistance, C. Furcht for preparation of this manuscript, and Ellis Benson for his advice and support. This work is supported by a Basil O'Connor Starter Research Grant from the National Foundation-March of Dimes; and Grants 1R01 CA21463-01 and 5P01 CA16228-03 from the National Cancer Institute, DHEW, and the Minnesota Leukemia Task Force to L.T.F.

REFERENCES

1. Aub JC, Sanford BH, Cote MN: Proc Natl Acad Sci USA 54:396, 1965.
2. Burger MM, Goldberg AR: Proc Natl Acad Sci USA 57:359, 1967.
3. Frye CD, Edidin M: J Cell Sci 7:313, 1970.
4. dePetris S, Raff MC: Eur J Immunol 2:523, 1972.
5. Yaffe D: Proc Natl Acad Sci USA 61:477, 1968.
6. Yaffe D: Curr Top Dev Biol 4:37, 1973.
7. O'Neill MC, Stockdale FE: Dev Biol 29:410, 1972.
8. Shainberg A, Yagil G, Yaffe D: Dev Biol 25:1, 1971.
9. Yaffe D: Exp Cell Res 66:33, 1971.
10. Fluck RA, Strohman RC: Dev Biol 33:417, 1973.
11. Furcht LT, Scott RE: Exp Cell Res 96:271, 1975.
12. Oliver IT: Biochem J 61:116, 1955.
13. Burton K: Biochem J 62:315, 1956.
14. Bernhard W, Avarameas S: Exp Cell Res 64:232, 1974.
15. Terzakis JA: J Ultrastruct Res 22:168, 1968.
16. Simonescu N, Simonescu M: J Cell Biol 70:608, 1976.
17. Luft JH: J Biophys Biochem Cytol 9:409, 1961.
18. Reynolds ES: J Cell Biol 17:208, 1963.

19. Nicholson GL: Nature (London) New Biol 243:218, 1973.
20. Singer J, Nicholson G: Science 175:720, 1972
21. Wallach DFH, Zahler HP: Proc Natl Acad Sci USA 56:1552, 1966.
22. Nicholson GL, Lacorbiere M: Proc Natl Acad Sci USA 70:1672, 1973.
23. Nicholson GL: Biochim Biophys Acta 457:59, 1976.
24. Noonan K, Burger M: J Cell Biol 59:134, 1973.
25. Rittenhouse HG, Fox CF: Biochem Biophys Res Commun 57:323, 1974.
26. McNutt NS, Culp LA, Black PH: J Cell Biol 50:691, 1971.
27. Pollack RM, Osborn M, Weber K: Proc Natl Acad Sci USA 72:994, 1975.
28. Schollmeyer JE, Furcht LT, Goll DE, Robson RM, Stromer MH: In Goldman R, Pollard T, Rosenbaum J (eds): "Cell Motility." New York: Cold Spring Harbor Press, 1976, pp 361–388.
29. Yahara I, Edelman GM: Nature (London) 246:152, 1973.
30. Porter KR: In "Locomotion in Tissue Culture Cells." Porter R, Fitzsimons DW (eds). Ciba Foundation Symposium. Amsterdam: Elsevier Publishing Company, 1973, vol 14, pp 149–170.
31. Furcht LT, Scott RE, Maerklein P: Cancer Res 36:4584, 1976.
32. Furcht LT, Scott RE: Biochem Biophys Acta 401:213, 1975.
33. Ahkong QF, Fisher D, Tampion W, Lucy JA: Nature (London) 253:194, 1975.
34. Axelrod D, Ravidin P, Koppel DE, Schlessinger J, Webb W, Elson EL, Podleski TR: Proc Natl Acad Sci USA 73:4594, 1976.
35. Mandel JL, Pearson ML: Nature (London) 25:618 (1974).
36. Goldberg ND, Haddox M, Dunham E, Lopez C, Hadden JW: In Clarkson B, Baserga R (eds): "Control of Proliferation in Animal Cells." New York: Cold Spring Harbor Press, 1974, pp 627–634.
37. Hadden JW, Hadden EM, Haddox MK, Goldberg ND: Proc Natl Acad Sci USA 69:3024, 1972.
38. Haddox MK, Furcht LT, Gentry SR, Moser ME, Stephenson JH, Goldberg ND: Nature (London) 262:146, 1976.
39. Prasad K, Vernadakis A: Exp Cell Res 70:27, 1972.
40. McMahon D: Science 185:1012, 1974.
41. Skehan P, Friedman SJ: Exp Cell Res 92:350, 1975.
42. Hynes RO, Martin GS, Shearer M, Gritchley DR, Epstein CJ: Dev Biol 48:35, 1976.
43. Yamada KM, Weston JA: Proc Natl Acad Sci USA 71:3492, 1974.