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Alterations in Cellular and Plasma Membrane Glycoproteins in Chicken Myogenesis *in Vitro**

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鷄胚의 筋細胞分化에 있어서 細胞 및 原形質膜 糖蛋白質의 變化

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요 약

본 연구는 계배근세포가 분화하는 과정에서 조절을 받는 단백질이 있는 지의 여부를 가려내기 위해서 근세포 및 그 원형질막의 당단백질의 변화양상을 표지된 Con A 염색법을 써서 검토한 것이다.

당단백질 중에는 세포에서만 발견되는 것이 8종, 원형질막에서만 발견되는 것이 4종, 그리고 공통적으로 발견되는 것이 9종이 있었다. 그중에서 분화하는 동안에 변하지 않는 것, 증가하는 것, 감소하는 것, 증가하다 감소하는 것, 그리고 감소하다 증가하는 것 등 다섯가지 종류가 있었다.

본 연구에서 fibronectin이 근관형성후에는 감소되는 사실이 판명되었는데, 이 결과는 지금까지 논란의 대상이 되어오던 상반된 결과들이 분화과정 중에서 택한 시기의 차이에 따라 나타난 결과들로 볼 수 있음을 보여주었다. 일반적으로 근세포의 융합이 진행될수록 고분자량의 당단백질은 감소하고, 대신 저분자량의 당단백질은 증가하는 경향성을 보였고, 이와같은 결과는 근원세포가 융합을 함에 따라서 당단백질의 구조적인 재편성이 일어남을 암시하는 것으로 받아들여졌다.

INTRODUCTION

One of the steps in the differentiation of skeletal myoblasts has been recognized as the fusion of the cells to form long, multinucleated myotubes. The fusion process is proceeded by recognition of or by specific adhesion of myoblasts followed by membrane-membrane interaction culminating in cell fusion. There have been numerous reports dealt with the

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fusion mechanisms (Whateley *et al.*, 1976; Kalderon *et al.*, 1977; Pauw and David, 1979; Kaur and Sanwal., 1981; Parfett *et al.*, 1981; Couch and Strittmatter., 1983; Kang *et al.*, 1983; Dubois *et al.*, 1984; Lucy, 1984). To date, however, the molecular mechanisms involved have been largely unknown, although it is reasonable to expect that alterations in the cell surface should play a vital role. In addition, changes in the cell surface proteins and lipids are presumed to be important in myogenesis, both for cell-cell interaction and for other interactions of the cells with its environment.

Under appropriate conditions, myoblast fusion is inhibited by lectins such as Concanavalin A (Con A) which binds to specific cell surface carbohydrates (Den *et al.*, 1975; Sharon and Lis, 1962). This effect might be due to a restriction of the mobility of glycoprotein receptors within the myoblast membrane. Furthermore, some of the lectin-resistant mutants are unable to fuse into myotubes (Parfett *et al.*, 1981), indicating possible lack of glycoproteins which are involved at certain stages of differentiation process leading to fusion. The observations that inhibition of protein glycosylation by the antibiotics, tunicamycin and pantomycin, inhibits myoblast fusion (Gilfix and Sanwal, 1980; Cates *et al.*, 1984) suggested that some glycoproteins are not essential for growth but are necessary for myoblast fusion. Thus, it appears that glycoproteins are important components in cell-cell recognition and in cell fusion as well.

Many attempts have been made to study whether alterations exist in glycoproteins using radioiodination and other techniques in differentiating myoblasts. Hynes *et al.* (1976) found no qualitative label difference between the surface proteins of prefusion and postfusion rat L8 cells, but observed an increased iodination of large-external-transformation sensitive (LETS) protein(fibronectin). Moss *et al.* (1978), studying chick muscle cells, found a similar increase in fibronectin levels as well as an increase in low-molecular-weight components. In contrast, Chen (1977) using radioimmunoassay, found that the level of fibronectin decreased after fusion. Likewise, using radioiodination method, Pauw and David (1979) found that a number of proteins that appeared with cell fusion and that a 66K protein which increased and subsequently decreased in relative amount. On the other hand, Walsh and Phillips (1981) found that fibronectin and a 48K protein (Con A receptor) increased with cell fusion and subsequently decreased as the myotubes differentiated. A similar result was obtained by Kang *et al.* (1983) that the level of fibronectin decreased with cell fusion and that 165K and 93K proteins appeared at the onset of fusion as well as the increase in the number of low-molecular-weight proteins.

In the present study, we aimed at finding out how the expression of surface glycoproteins vary with myogenesis and to identify developmentally regulated proteins by analyzing the surface as well as cellular glycoproteins (many of them are exposed on the cell surface) during myogenesis of chicken myoblast, The rationale for the use of Con A as a probe of glycoproteins is that the mannose moieties in plasma membrane glycoproteins (receptors for Con A) may be involved in fusion, but not sialic acid (receptors for wheat germ agglut-

inin) (Cates *et al.*, 1984). It is suggested that the glycoproteins may play an important role in the mediation of fusion process, especially in the recognition stage.

MATERIALS AND METHODS

Cell Culture

Fertile Hi-Line chicken eggs were purchased from local poultry farm. Myoblast cultures were prepared according to the method of O'Neill and Stockdale (1972). Briefly, breast muscle from 12-day-old embryos were dissected out, minced, and digested with 0.1% trypsin (Difco) for 30 min and dispersed by repeated pipetting. Cells were collected by centrifugation and suspended in RPMI 1640 medium supplemented with 10% horse serum (GIBCO), 10% chick embryo extract, and 1% antibiotics. Cells were preplated on collagen-coated dishes for 20 min to remove fibroblasts. The cell suspension was then filtered through a two-fold lens paper to remove undissociated cells. Approximately 5×10^5 cells per ml were inoculated on collagen-coated dishes. Medium was changed after 24 hr with RPMI 1640 containing 10% horse serum, 2% chick embryo extract, and 1% antibiotics. Cultures at various stages of differentiation were labelled at 32 hr, 48 hr, and 72 hr which correspond to prefusion, midfusion, and postfusion stage in our culture systems, respectively.

Preparation of Plasma Membranes

Plasma membranes were prepared as described by Schimmel *et al.* (1973). Monolayer cultures were washed three times with cold phosphate-buffered saline (PBS), and the cells were removed from the dish by scraping off with a rubber policeman and collected by centrifugation for 5 min at $1,100\times g$. The collection of cells and all the subsequent steps were done at 0-4°C.

One gram of cell pellet (wet weight) was suspended in 15 ml of 0.25 M sucrose-1 mM triethanolamine (TEA)-HCl, pH 7.4 and was broken in a Dounce homogenizer with 15 strokes. The homogenate was centrifuged for 10 min at $1,700\times g$ and the supernatant was removed and centrifuged for 60 min at $33,000\times g$. The pellet was resuspended in 1.0 ml sucrose-TEA and layered over a sucrose discontinuous gradient. The sucrose-gradient was prepared by layering 0.5 ml of 40% (w/w) sucrose, followed by 1 ml each of 32%, 27% and 20% sucrose, successively. After centrifugation at $206,000\times g$ for 90 min, the bands of turbid material at the interfaces in the gradient were removed and were designated as follows: I, 8.3~20%; II, 20~27%; III, 27~32%; IV, 32~40%; and V, the pellet. Each fraction was diluted to about 4 ml with 1 mM TEA-HCl, pH 7.4, and the proteins were then collected by centrifugation at $105,000\times g$ for 60 min.

Assay of Ouabain-sensitive Na^+,K^+-ATPase Activity

Ouabain-sensitive Na^+, K^+-ATPase activity was determined in a 400 μl reaction mixture containing 300 mM imidazole-HCl, pH 7.5, 0.11 M NaCl, 15 mM KCl, 5 mM NaN_3, 0.5

mM ethylenediamine-bis (β-aminoethylether) N, N′-tetraacetic acid (EDTA), 4 mM $MgCl_2$, 3 mM ATP, and 50 μg of protein. Na^+, K^+-ATPase activity was calculated as the difference in ATPase activity in the presence and absence of 1 mM ouabain (DePierre and Karnovsky, 1973). Reactions were carried out at 37°C for 1 hr and stopped by addition of 400 μl of 10% perchloric acid. After removal of protein by centrifugation, inorganic phosphate was assayed according to the method of Chen *et al.* (1956). Briefly, 4 ml of reagent consisting of 1 vol of 6N sulfuric acid, 2 vol of distilled water, 1 vol of 2.5% ammonium molybdate, and 1 vol of 10% ascorbic acid was added to each supernatant, capped with Parafilm, mixed, and incubated at 37°C. After 1.5 hr, absorbance at 680nm was read against the reagent blank. Potassium monohydrogen phosphate (K_2HPO_4) was used as a standard.

Gel Electrophoresis

Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. Electrophoresis was carried out using the triglycine discontinuous system of Laemmli (1970) on 7.5% acrylamide slab gel with 3% stacking gel. Samples were dissolved in SDS-sample buffer (0.0625 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5%

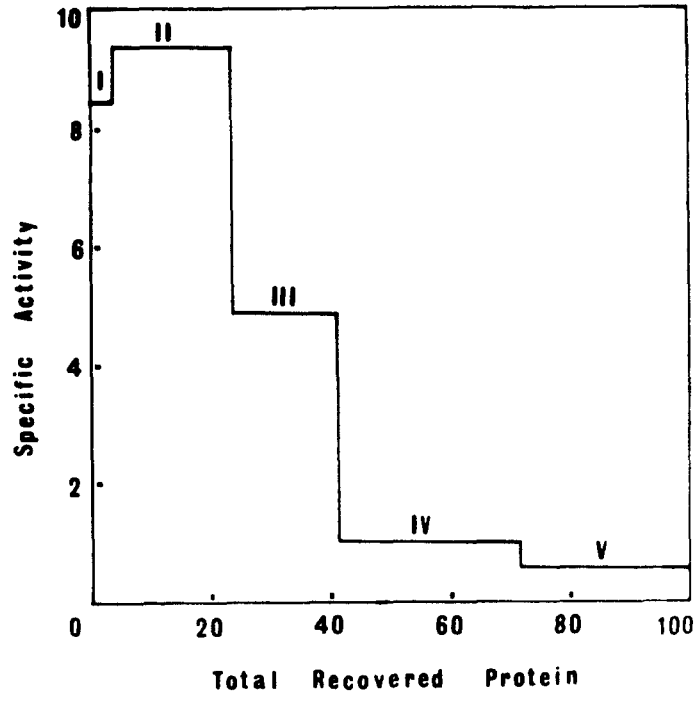

Fig. 1. Specific activities of Na^+, K^+-ATPase in membrane preparations. Cells of postfusion stage were collected by scraping, homogenized in 0.25M sucrose/1mM TEA-HCl, pH 7.4, and fractionated on a sucrose discontinuous density gradient as described in the Materials and Methods. Fractions were collected from the interfaces of the gradient and are as follows: I, 8.3~20%; II, 20~27%; III, 27~32%; IV, 32~40%; and V, the pellet. Specific activities are expressed as μmole Pi released/min/mg protein.

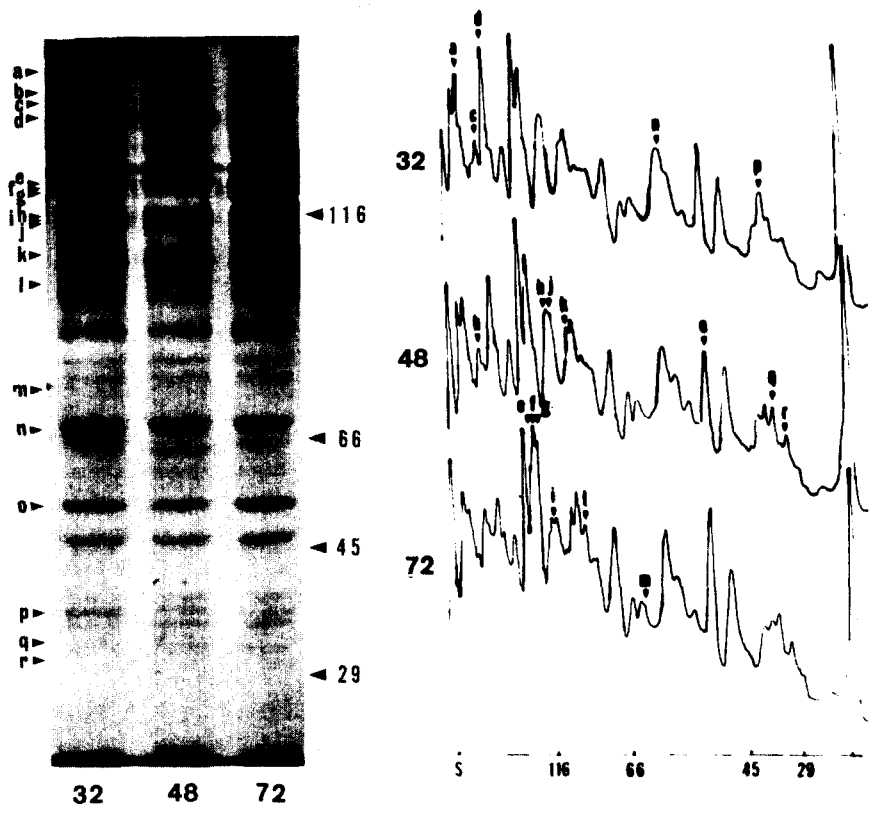

Fig. 2. Autoradiogram of cellular glycoproteins and its densitometric scan. After electrophoresis, gel was stained with ^{131}I-Con A, dried, and autoradiographed. The film was scanned at 525 nm. 32, prefusion time point; 48, midfusion time point; and 72, postfusion time point. The positions of molecular weight standards are indicated, and Mr's are shown $\times 10^{-3}$. Glycoproteins with Con A receptors are indicated with arrows.

2-mercaptoethanol, and 0.01% bromophenol blue) and were boiled for 3 min. Electrophoresis was carried out with a constant current of 20~30 mA for 5~7 hr.

Lectin Staining of Polyacrylamide Gels

Lectin staining of polyacrylamide gels was carried out essentially according to Walsh *et al.* (1981) and Mintz and Glaser (1978). Briefly, 1 mg of Con A in PBS (10 mM Na-phosphate buffer, 0.05M NaCl, pH 7.4) was labelled with 1 mCi of ^{131}I, using chloramine-T (Greenwood *et al.*, 1963). The iodinated Con A was stored at −20°C until used.

After electrophoresis and staining in Coomassie blue, polyacrylamide gels were soaked in 50% methanol for 30 min, and was transferred to 50% methanol containing 0.05%

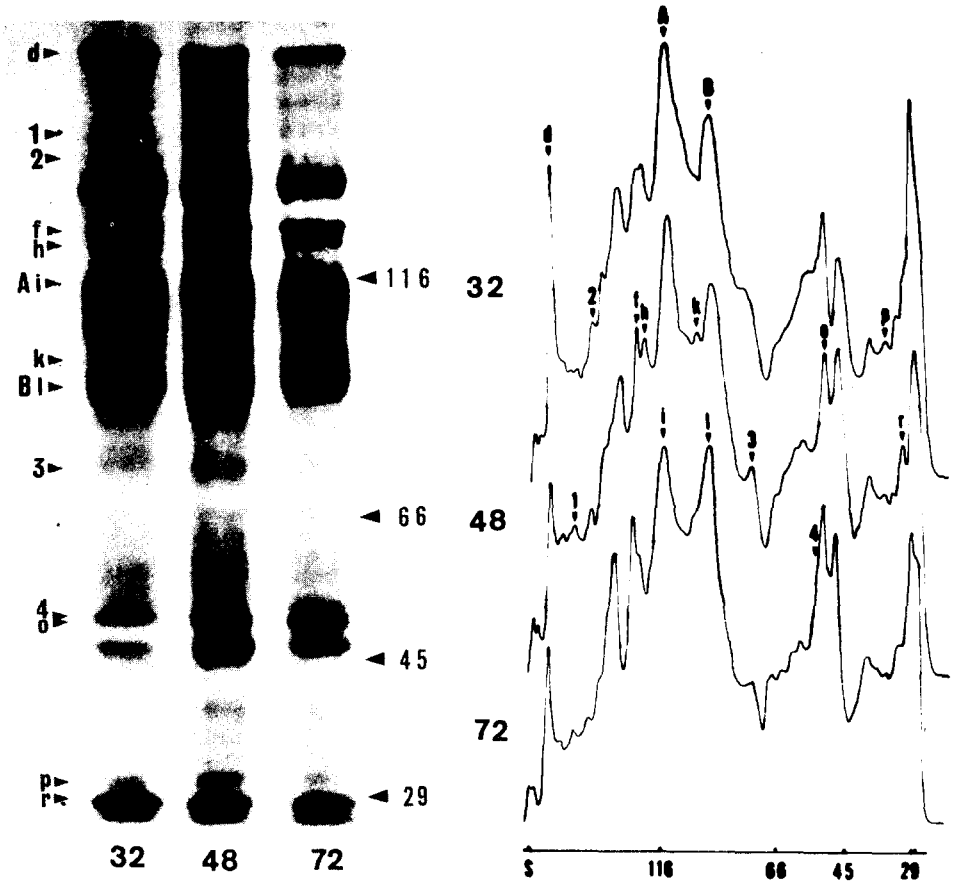

Fig. 3. Autoradiogram of plasma membrane glycoproteins and its densitometric scan. Methods were the same as those described in the legend to Fig. 2. A and B stand for the intensely stained bands which are peculiar to plasma membrane glycoproteins.

glutaraldehyde and further incubated for 90 min. The fixing solution was replaced by 0.1 M NaCl, 0.03 M Na-phosphate buffer, pH 8.0, containing $NaBH_4$ to a final concentration of 20 μg/ml. After 1 hr, it was replenished with the same fixing solution and left at 20°C for 16 hr. The gels were then soaked in PBS for 8 hr with a change of PBS. Gels were placed in a flat plastic box containing 100 ml of PBS including 0.05% NaN_3 and 1 mg hemoglobin per ml of the buffer. ^{131}I-Con A was added at a level of 10^6 cpm per ml of PBS. The gels were incubated for 48 hr at 20°C, followed by intensive washing with PBS for 72 hr. The gels were finally stained with Coomassie blue, dried, and autoradiographed. After development, X-ray film was scanned by a densitometer (Helena Lab.) at 525 nm.

Table 1. Alterations in cellular and plasma membrane glycoprotein levels during myogenesis.

Band	Apparent M.W.($\times 10^{-3}$)	Cellular glycoprotein			Membrane glycoprotein		
		32	48	72	32	48	72
a	330	+	+	−			
b	270	−	+	+			
c	250	−	−	−			
d	240	+	+	−	+	+	−
1	165				−	+	+
2	155				+	+	−
e	150	+	+	−			
f	140	−	+	+	−	+	+
g	130			+			
h	120	+	+	−	+	+	−
i	114	+	+	−	+	+	−
j	110	+	+	−			
k	97	−	−	+	−	−	+
l	93	+	−	+	+	−	+
m	80	−	−	+			
3	70				−	+	−
n	57	−	+	+			
4	52				−	+	+
o	48	−	+	+	−	+	+
p	40	+	+	−	+	+	−
q	35	−	+	+			
r	32	−	+	+	−	+	+

Glycoprotein levels are expressed as increase(+) or decrease(−) in amounts in the same band at different time points.

RESULTS

Purity of Muscle Cell Plasma Membranes

Schimmel *et al.* (1973) demonstrated that their method for the purification of plasma membrane from skeletal muscle cell cultures was applicable to myoblasts and early myotubes. The purity of membrane fractions was estimated by Na^+, K^+-ATPase activity in the membrane fraction.

The specific activities of Na^+,K^+-ATPase assayed in membrane fraction obtained from the sucrose discontinuous density gradient were reproducible and were in agreement with those originally reported by Schimmel *et al.* As shown in Fig. 1, membrane fractions between 8.3% and 20% (w/w) sucrose (fraction I) or between 20% and 27% (fraction II) had the highest ouabain-sensitive Na^+,K^+-ATPase activity.

Staining of Glycoproteins with ^{131}I-Con A

Chicken muscle cells were stained with ^{131}I-Con A at three time points during myogenesis. At the prefusion time point, 32 hr after plating, the cultures consisted of mononucleated cells only; at the midfusion time point, 48 hr after plating, the cells began to align each other; and at the postfusion time point, 72 hr after plating, the cultures consisted mainly of myotubes.

In Fig. 2 are shown the staining patterns of cellular glycoproteins along with their densitometric scans. Similarly, the staining patterns and their scans of membrane glycoproteins are shown in Fig. 3. As is evident from Figs. 2 and 3, many glycoproteins appear to change in relative amounts during myogenesis. These are glycoproteins which are common in cellular and membrane fractions and those which are specific to cellular or membrane fraction. The glycoproteins, which are stained with iodinated-Con A and show changes in levels during myogenesis can be classified into at least four types. These are the glycoproteins which increase, decrease, increase and subsequently decrease, or decrease and subsequently increase. All the glycoproteins which vary in their levels during myogenesis are collectively listed in Table 1.

DISCUSSION

Myoblast fuse only in the G1 phase of the cell cycle and fusion is preceded by entry of myoblasts into an extended G1 phase (Devlin and Konigsberg, 1981). The changes in the composition of plasma membrane of myoblasts immediately before or concomitant with fusion may therefore reflect differentiation of the membrane and are dependent upon the entry of the nucleus into the G1 phase of the cell cycle. Three concomitant events, myoblast fusion, withdrawal from the cell cycle, and initiation of muscle-specific protein synthesis, are interdependent. As has been noted earlier, the glycoprotein plays a key role in cell-cell recognition and in cell fusion. Changes in level of the plasma membrane glycoprotein, along with the altered rate of synthesis and accumulation coincident with the onset of the rapid phase of myoblast fusion, could be the developmentally-regulated process.

The characterization of the cell surface changes during myogenesis has been attempted by a number of investigators using mainly avian or murine myoblasts in culture. The methods used for cell surface characterization have been lactoperoxidase-catalyzed iodination of tyrosine residue in cell surface, labelling of cell-surface sialoglycoproteins by periodate-tritiated borohydride, and staining of polyacrylamide gels of separated cellular or plasma glycoproteins with ^{125}I-Con A or ^{125}I-wheat germ agglutinin. The results obtained so far differ from one experiment to another. For example, Hynes *et al.* (1976), using lactoperoxidase-catalyzed iodination of rat L8 cells, found no difference between the myoblast and myotube stages of differentiation, except for an increase in the level of LETS protein (fibronectin) in postfusion myotubes. Using the same technique, Moss *et al.* (1978) found

a similar change in fibronectin in chick embryo muscle cells, as well as some increases in low-molecular-weight proteins. Thus, the findings made by these study groups were that only minor cell surface changes occur during myogenesis.

In contrast, Pauw and David (1979), using the same method applied to rat L6 cells, found a number of proteins which appear during myogenesis and a 66K protein which increases in amounts after fusion and myotube formation and subsequently decreases. However, no data on the levels of fibronectin during myogenesis can be deduced from this investigation. In apparent contradiction to the studies above reporting an increase in fibronectin levels during myogenesis, Chen (1977) using radioimmunoassay technique, found that the level of fibronectin decreases after fusion.

These apparent conflicting results obtained using surface labelling and radioimmunoassay made Walsh and Phillips (1981) to conduct an extensive study on the level of fibronectin during G8-1 clonal muscle cell differentiation using four different labelling techniques. The conclusion reached by these authors was that fibronectin increases in amount with cell fusion and myotube formation, but subsequently decreases to the prefusion levels with maturation of myotubes. The time points used by them was myoblast stage and three stages in myotubes, and the decreased level of fibronectin was found in stage III myotubes.

The present study was undertaken to elucidate whether there are developmentally regulated proteins in chick myogenesis by observing cellular as well as membrane glycoproteins. The cellular glycoprotein levels are thought to give some insight into the synthesis, processing and transport of glycoproteins and their relation to the surface membrane characteristics.

At least three classes of glycoprotein can be identified in terms of their occurrence and five classes by change in their levels. As to the former category, glycoproteins which are specific to cellular fraction are found to be eight (a, b, c, e, g, j, q, n), those specific to membrane fraction are four (1, 2, 3, 4), and those in both fractions are nine (d, f, h, i, k, l, o, p, r). For the latter category, there are glycoproteins which are invariant, those which increase in level, those which decrease in level, those which increase and subsequently decrease, and those which decrease and subsequently increase. A significant finding to be noted in the present study is that fibronectin does decrease in level after myotube maturation and that such a behavior is commonly found in cellular as well as membrane fractions. The decrease of fibronectin after myotube formation is in good agreement with the report by Walsh and Phillips. The decrease of fibronectin after maturation of myotube may have a correlation with the suggestion that fibronectin promotes cell adhesion and it is no longer required after cell fusion and myotube formation in myoblast differentiation. Since fibronectin has been found in high concentration in the myoblasts, this glycoprotein appears to influence on cell adhesion (Yamada and Olden, 1978; Ruoslahti *et al.*, 1981; Hynes, 1982). Another interesting fact to be noted is that 130K glycoprotein appears to be a cellular glycoprotein specific to myotubes. The significance of this specific glycoprotein

remains to be elucidated.

In summary, glycoproteins of high-molecular-weight appear to decrease and those of low-molecular-weight to increase in their relative amount as the fusion proceeds. Thus, these results suggest that a major structural reorganization of glycoproteins seem to occur in association with myoblast fusion.

ABSTRACT

The present study has been undertaken to elucidate whether there are developmentally regulated proteins in chick myogenesis by observing cellular and plasma membrane glycoproteins by use of labelled-Con A staining.

At least three classes and five classes of glycoproteins can be identified in terms of their occurrence and their change in levels, respectively. As to the former, there are glycoproteins which are specific to cellular fraction are eight, those which are specific to plasma membrane are four, and those which occur in both fractions are nine. Likewise, as to the latter, there are glycoproteins which are invariant, those which increase in level, those which decrease, those which increase and subsequently decrease, and those which decrease and subsequently increase.

The present experiment reveals that the level of fibronectin does decrease after the fusion and that the apparent conflicting results so far reported may be due to the difference in time point setting during fusion and myotube stages. It is thought to be a general tendency that glycoproteins of high-molecular-weight decrease and those of low-molecular-weight increase in relative levels as the fusion proceeds. These results are suggestive of the fact that a major structural reorganization of glycoproteins does occur in association with myoblast fusion.

REFERENCES

Cates, G.A., H. Kaur, and B.D. Sanwal, 1984. Inhibition of fusion of skeletal myoblasts by tunicamycin and its reversal by N-acetylglucosamine. *Can. J. Biochem.* 62:28.

Chen, P.S. Jr., T.Y. Toribara, and H. Warner 1956. Microdetermination of phosphorus. *Anal. Chem.* 28:1756.

Chen, L.B., 1977. Alteration in cell surface LETS protein during myogenesis. *Cell* 10:393.

Couch, C.B. and W.J. Strittmatter, 1983. Rat myoblast fusion requires metalloendoprotease activity. *Cell* 32:257.

Den, H., D.A. Malinzak, H.J. Keating, and A. Rosenberg, 1975. Influence of Concanavalin A, wheat germ agglutinin, and soybean agglutinin on the fusion of myoblast *in vitro*. *J. Cell Biol.* 67:826.

DePierre, J.W. and M.L. Karnovsky, 1973. Plasma membranes of mammalian cells. *J. Cell Biol.* 56:275.

Devlin, B.H. and I.R. Konigsberg, 1983. Reentry into the cell cycle of differentiated skeletal myocytes.

Dev. Biol. **95**:175.

Dubois, C., B. Hauttecoeur, M-J. Coulon-Morelec, D. Montarras, C. Rampini, and M.Y. Fiszman, 1984. Changes in ganglioside metabolism during *in vitro* differentiation of quail embryo myoblasts. *Dev. Biol.* **105**:509.

Gilfix, B.M. and B.D. Sanwal, 1980. Inhibition of myoblast fusion by tunicamycin and pantomycin. *Biochem. Biophys. Res. Commun.* **96**:1184.

Greenwood, F.C., W.M. Hunter, and J.S. Glores, 1963. The preparation of ^{131}I -labelled human growth hormone of high specific radioactivity *Biochem. J.* **89**:114.

Hynes, R.O., 1982. Fibronectin and its relation to cellular structure and behavior. In "Cell Biology of Extracellular Matrix" (E.D. Hay, ed.) pp. 295-334. Plenum, New York.

Hynes, R.O., G.S. Martin, M. Shearer, D.R. Critchley, and C.J. Epstein, 1976. Viral transformation of rat myoblasts: Effects on fusion and surface properties. *Dev. Biol.* **48**:35.

Kalderon, N., M.L. Epstein, and N.B. Gilula, 1977. Cell-to-cell communication and myogenesis. *J. Cell Biol.* **75**:788.

Kang, M.S., S. Choe, and W. Song, 1983. Studies on the fusion mechanism of the cell (I). *Korean J. Zool.* **26**:235.

Kaur, H. and B.D. Sanwal, 1981. Regulation of the activity of a calcium-activated neural protease during differentiation of skeletal myoblasts. *Can. J. Biochem.* **59**:743.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**:680.

Lowry, O.H., N. G. Rosenbrough, A.L. Farr, and R.J. Randall, 1951. Protein measurement with the Folin reagent. *J. Biol. Chem.* **193**:265.

Lucy, J.A., 1984. Do hydrophobic sequences cleaved from cellular polypeptides induce membrane fusion reactions *in vitro*? *FEBS Letters* **166**:223.

Mintz, G. and L. Glaser, 1978. Specific glycoprotein changes during development of the chick neural retina. *J. Cell Biol.* **79**:132.

Moss, M., J.S. Norris, E.J. Peck, Jr., and R.J. Schwartz, 1978. Alterations in iodinated cell surface proteins during myogenesis. *Exp. Cell Res.* **113**:445.

O'Neill, M.C. and F.E. Stockdale, 1972. A kinetic analysis of myogenesis *in vitro*. *J. Cell Biol.* **52**:52.

Parfett, C.L.J., J.C. Jamieson, and J.A. Wright, 1981. A correlation between loss of fusion potential and defective formation of mannose-linked lipid intermediates in independent Concanavalin A-resistant myoblast cell lines. *Exp. Cell Res.* **136**:1.

Pauw, P.G. and J.D. David, 1979. Alterations in surface proteins during myogenesis of a rat myoblast cell line. *Dev. Biol.* **70**:27.

Ruosalhti, E., E. Engvall, and E.G. Hayman, 1981. Fibronectin: Current concepts of its structure and function. *Cell Res.* **1**:95.

Schimmel, S.D., C. Kent, R. Bischoff, and P.R. Vagelos, 1973. Plasma membranes from cultured muscle cells: Isolation procedure and separation of putative plasma-membrane marker enzymes. *Proc. Natl. Acad. Sci. U.S.A.* **70**:3195.

Sharon, N. and H. Lis, 1962. Lectins: Cell-agglutination and sugar specific proteins. *Science* **177**:949.

Walsh, F.S. and E. Phillips, 1981. Specific changes in cellular glycoproteins and surface proteins

during myogenesis in clonal muscle cells. *Dev. Biol.* **81**:229.

Walsh, F.S., S.E. Moore, and S. Dhut, 1981. Monoclonal antibody to human fibronectin: Production and characterization using human muscle cultures. *Dev. Biol.* **84**:121.

Whateley, R., S.K.C. Ng, J. Rogers, W.C. McMurray, and B.D. Sanwal, 1976. Developmental changes in gangliosides during myogenesis of a rat myoblast cell line and its drug resistant variants. *Biochem. Biophys. Res. Commun.* **70**:180.

Yamada, K.M. and K. Olden, 1978. Fibronectin-adhesive glycoproteins of cell surface and blood. *Nature* **257**:179.