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Studies on the Fusion Mechanism of the Cell (1)

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細胞의 融合機作에 관한 硏究(1)

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摘 要

배양한 계배 근원세포의 융합기작을 밝혀보기 위해서 분화과정에 있는 근세포를 lactoperoxidase를 촉매로 써서 막표면단백질을 iodination하여 본 결과, 융합의 과정에서 막표면단백질의 정성적 및 정량적 변화를 볼 수 있었다. 융합전의 세포에서 12개의 주요단백질을 검출할 수 있었는데, 융합시의 세포에서는 165K와 93K의 단백질이 새롭게 나타났으며 동시에 245K 단백질의 감소와 저분자 단백질의 증가를 볼 수 있었다.

이 고분자 단백질의 감소는 세포주기와 관계가 있는 것 같이 생각되었으며, 저분자 단백질의 증가는 고분자 단백질의 가수분해의 결과 나타나는 것으로 생각되었다.

한편, 세포의 융합과 관계가 있는 것으로 생각되고 있는 세포내 cAMP 수준은 융합에 앞서서 현저한 일시적인 증가를 보였는데, 이와같은 결과는 cAMP의 증가가 세포의 융합의 유발과 관계가 있음을 보여 주는 것이었으며, 분화하는 근원세포에서는 고도의 동조성이 보였다.

아울러, 근세포의 융합과정에서 적어도 4 가지의 iodination된 저분자 단백질을 배양액에서 발견할 수 있었는데, 이들은 막단백질의 가수분해산물로 생각되었고, 역시 세포의 융합과정과 유관할 것으로 추정되었다.

세포막 표면단백질의 변화, 분화과정 중에 배양액 속으로 방출되는 단백질, 융합과 유관한 cAMP의 증가 및 융합과 관련되는 외적 요인의 가능성에 관해서 논의하였다.

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INTRODUCTION

During the differentiation of skeletal muscle, mononucleated myoblast fuse to form multinucleated syncytia or myotube. Mononucleated myoblast obtained from chick embryonic breast muscle, when cultured *in vitro*, proliferate and subsequently fuse to produce myotube with the high degree of temporal specificity.

The synthesis of cellular components also varies dramatically during myogenesis, and includes such enzymes as creatine phosphokinase (Turner *et al.*, 1974), acetylcholine receptor proteins (Paterson *et al.*, 1973), and contractile proteins (Devlin *et al.*, 1978). The relative amount of these proteins increases after cell fusion.

To bring the problems of cell fusion in focus, it can be considered into two, possibly interdependent, separate segments, morphological and biochemical changes. The question to be made along this line is what is primarily responsible for the induction of these changes. Proteins exposed at the surface of cells are likely to be involved in intercellular communication. The present study was made on this assumption.

The first attempt has been made to characterize surface proteins that might be involved in cell fusion. Characterization of surface proteins have been usually made by lactoperoxidase-catalyzed iodination. Hynes *et al.* (1976) found no qualitative labelling differences between prefusion and postfusion in rat L8 cells, but observed an increased iodination of large-external-transformation-sensitive (LETS) protein (fibronectin). Moss *et al.* (1978), studying with chick embryonic muscle, found a similar increase in fibronectin levels and an increase in low molecular-weight proteins. In addition, Pauw *et al.* (1979) found a number of proteins that appeared with cell fusion and, in particular, a 66K protein increased and subsequently decreased. But these findings are not sufficient to conclude that those proteins are directly engaged in cell fusion.

The second attempt has been made to find diffusible fusion-promoting molecules that are present in culture media. This idea was based on the finding that the initiation of fusion was delayed by continuous replacement of the culture media with fresh ones (Konigsberg, 1971). Schubert *et al.* (1973) have characterized at least 12 soluble proteins that were secreted from both myoblast and myotube. Although there was a quantitative change in the rate of appearance of five of these proteins, but no qualitative change in the profile of the secreted proteins was detected.

In relation with the diffusible fusion-promoting molecules, examination of components of the cAMP system has revealed that both the intracellular cAMP level and the adenylate cyclase activity increase ten- to fifteen-fold for approximately two hours before the initiation of cell fusion (Zalin *et al.*, 1974). This result suggests that the increase in adenylate cyclase activity is followed by the rapid increase in intracellular cAMP and the onset of fusion. The possibility that membrane-bound adenylate cyclase is activated by external

signal and the intracellular cAMP level controls the genetically-programmed expression of cell fusion process might be suggested.

In the related findings, Rutishauser *et al.* (1976) has suggested the mechanism of cell-cell adhesion among cells, from the iodination profile and immunodiffusion analysis of surface proteins of neural tissue of chick embryo. According to their result, 140K and 110K proteins were released into culture media by proteolytic cleavage of surface precursor with a molecular weight of 240K and 150K, respectively. They finally suggested that cell-cell contact invokes proteolytic activation and modualtion of cell surface ligands.

On the basis of all these findings, we could hypothesize of fusion mechanism of chick myoblast that cultured *in vitro* proliferate fairly synthronously and make contact with each other to activate proteolytic cleavage of high molecular-weight proteins. Degraded protein fragment(s) diffuse into culture media and activate surface exposed adenylate cyclase. Successively, the rapid and transient increase in intracellular cAMP level results in a series of regulation on intracellular enzymes that are engaged in membrane fusion process.

In this paper, we presented first the observation of surface protein changes during myogenesis, considering from the iodination profile. The result of intracellular cAMP level during myogenesis was then presented. Lastly, we presented the preliminary data on fibroblast growth factor (FGF) activity. Purification of FGF was performed in order to cultivate cells in a biochemically-defined medium, which contains chemicals supplemented with FGF in place of serum and embryo extract. Fibroblast growth factor was isolated in recent years from bovine brain (Gospodarowicz *et al.*, 1978). Although the data from the reports on the nature of FGF are contradictory (Westall *et al.*, 1978; Thomas *et al.*, 1980), FGF prepared according to Gospodarowicz *et al.* (1978) was the major component of the biochemically-defined medium developed by Dollenmeier *et al.* (1981). The development of biochemically-defined medium will make it more feasible to identify diffusible fusion-promoting molecule(s).

MATERIALS AND METHODS

Cell Culture

Fertile chicken eggs were purchased from Purina Korea chick farm. Cultures were prepared essentially according to the method of O'Neill *et al.* (1972). Breast muscle from 12-day old embryos was dissected and the clear, minced tissue was digested with 0.2% trypsin for 30 minutes and dispersed by repeated pipetting. Cells were collected by centrifugation and resuspended in RPMI 1640 supplemented with 10% horse serum, 10% chick embryo extract, and 1% antibiotics. Cells were preplated on collagen-coated dishes for 15 minutes to remove fibroblast. Approximately 0.5 to 1.0×10^6 cells per milliliter were inoculated on collagen-coated dishes. Medium was changed after 24 hours and at 2-day intervals thereafter with RPMI 1640 containing 10% hourse serum, 2% chick embryo

extract and 1% antibiotics, unless otherwise noted.

Labelling of Surface Proteins with ^{131}I

Lactoperoxidase (LPO) used in the present study was purified according to the method of Thorell *et al.* (1971) with minor modifications. $Na^{131}I$ was obtained from the Korea Energy Research Institute.

Surface protein labelling with ^{131}I was essentially based on the method of Hubbard *et al.* (1972). Culture media were removed by aspiration and dishes were washed three times with 10 mM phosphate-buffered saline(PBS). Cells were scraped with a rubber policeman and collected by centrifugation. Cell pellet was added by 140 μl of LPO (880μg/ml) and 300 μCi $Na^{131}I$ (1 mCi/ml). Iodination reaction was initiated by the addition of 20μl of 90 mM H_2O_2 and another 20 μl of 90 mM H_2O_2 was added 10 minutes later. After 10 minutes of incubation, reaction was stopped by the addition of 10 ml PBS containing 0.5 mM $Na_2S_2O_3$ and 2 mM phenylmethylsulfonyl fluoride(PMSF). Cells collected by centrifugation were suspended in 0.1 M Tris-HCl, pH 6.8, and were sonicated at 80 to 100 watts for half a minute three times. Homogenate was stored in a refrigerator until required for analysis.

Gel Electrophoresis

The discontinuous SDS-polyacrylamide gel electrophoresis was performed essentially according to Laemmli (1970). Protein content of each homogenate was measured by Lowry method (1951) and equal amounts of radioactivity were applied to separate slots of 7.5% slab gel. Samples were dissolved in 0.65 M Tris-HCl, pH 6.8, containing 2% SDS, 5% mercaptoethanol, and 0.01% bromophenol blue. Samples were boiled for three minutes. Electrophoresis was carried out with a constant current of 50 to 70 mA for 4~6 hours. Gels were stained in 0.2% Coomassie brilliant blue in 40% methanol and 10% acetic acid for 3 hours, and destained in 35% methanol and 7% acetic acid until the background became clear.

Destained gels were either dried under gentle vacuum for autoradiography. Autoradiograms were prepared by exposing the dried gels on X-ray film for a few days. Developed X-ray films were scanned in a Transidyne densitometer.

Radioimmunoassay of cAMP

A method described by Brown *et al.* (1971) was used to examine cAMP level in cell homogenate. An appropriate amount of 30% (w/v) trichloroacetic acid (TCA) was added to the cell homogenate prepared as described above. The protein precipitate formed after standing for one hour at 0~2°C was removed by centrifugation. The pH of the supernatant was adjusted to neutrality by repeated washing with water-saturated ethylether and finally by the addition of 1N NaOH. Samples were freeze-dried overnight and resuspended in Tris-HCl, pH 7.5, containing 4 mM EDTA. Samples were added by 0.9 *p*mol of ^3H-cAMP and binding protein. After 2 hours of incubation at 0~2°C, charcoal suspension was added to remove unbound free cAMP by 2 minutes centrifugation at 12,000 rpm (Eppendorf microcentrifuge). Aliquots of the supernatant were counted for radioactivity.

An aliquot of cell homogenate was taken before TCA precipitation to measure the DNA content, using a modification of Kissane *et al.* (1958). Samples were incubated for 45 minutes at 60°C with 100 μl of 40% (w/v) diaminobenzoic acid. Diaminobenzoic acid solution was freshly prepared just before use and decolorized with Norit A charcoal to reduce the background fluorescence. The reaction was stopped by the addition of 3 ml of 1N HCl. Fluorescence was then measured at 520 nm by the excitation at 405 nm using 10 nm slits. Calf thymus DNA was used as a standard.

FGF Purification

The partial purification of FGF from bovine brain was essentially based on the method of Gospodarowicz *et al.* (1978). Bovine brain obtained from a local slaughter-hourse was homogenized in a waring blender with two volume of water containing 0.15 M $(NH_4)_2SO_4$. The crude homogenate was adjusted to pH 4.5 with HCl and stirred for 2 hours. The homogenate was then centrifuged at 23,000xG for 45 minutes to remove cell debris and heavy organelles. The supernatant was adjusted to pH 6.8 with 1N NaOH. The supernatant was then added by 250 g of $(NH_4)_2SO_4$ per liter and the suspension was centrifuged at 23,000xG for 20 minutes after standing for one hour. After repeating the same procedure, the precipitate was dissolved in water and desalted by Sephadex G-25 column. The eluate was then mixed with CM-Sephadex C-50 that was activated previously. The gel was equilibrated with 0.1M sodium phosphate, pH 6.0 and eluted with 0.1M sodium phosphate, pH 6.0, containing stepwise gradient of NaCl. The protein content of each fraction was measured at 280 nm and mitogenic activity of the fraction was then tested.

Cell Growth Measurement

Mitogenicity of FGF F_3 fraction was tested by comparing the rate of DNA synthesis for the cells cultured in FGF added medium and those in standard medium. The rate of DNA synthesis was evaluated by double labelling technique. Two ml of 12 day-old chick embryo myoblast at a cell density of $3x10^5$/ml was seeded in a Falcon culture dish (35mmx10mm). The culture medium contained 0.01 μCi/ml of methyl-^{14}C-thymidine in plain RPMI 1640. After 24 hours the cells were washed three times with plain medium and were transferred to new medium which contained various amounts of FGF F_3 along with methyl-^{3}H-thymidine, 5μg/ml of insulin and 10μg/ml of transferrin in place of horse serum and chick embryo extract. After 30-hour incubation the cells were washed with sodium chloride-sodium sulfate solution three times and were harvested using a rubber policeman. Following recovery by centrifugation the cells were added by 4% perchloric acid and the reaction was completed in 30 minutes. The cell suspension thus obtained was dehydrated by a series of 70~100% ethanol on a GF/A filter. The radioactivity on the filter was counted differentially in a Packard Liquid Scintillation Spectrometer.

Labelled Proteins Released into the Medium

Thirty hours following myoblast plating, the cells were washed three times with PBS and to each culture dish were added 100 μCi/ml of $Na^{131}I$, 20 μl of 90 mM H_2O_2 and 150 μl

of LPO (8 unit/mg). After 20 minutes of reaction at room temperature the reaction was terminated by adding 10 ml of phosphate buffered sodium iodide solution which contained 0.5 mM $Na_2S_2O_3$ and 2 mM PMSF. Then the cells were washed twice with PBS and were routinely cultured. Forty hours later, the culture media were pooled and centrifuged to remove dead cells and cell debris at 20,000 x G for 30 minutes. The supernatant thus obtained was added TCA at final concentration of 10%. The reaction mixture was kept at 0~4°C for 30 minutes and centrifuged at 20,000 x G for 1 hour. The pellet was dissolved in 1N NaOH for electrophoretic analysis.

RESULTS

Cell Surface Protein Changes during Fusion

Fig. 1 represents the fusion process of chick myoblast cultured *in vitro*. As shown in the figure, myoblast dramatically changes in the morphology during a relatively short period of time. Under the culture conditions of chick myoblast adopted in the present study, fusion began at about 48 hours of culture and then proceeded at a linear rate, yielding 70% of the total nuclei in myotubes 70 hours after plating. It could be observed under a microscope that myoblast underwent cell division fairly synchronously for about five to six hours just before the onset of fusion. At about three hours after the termination of mitosis, cells entered S phase (Data not shown). Cells at G_1 phase were labelled for the analysis of prefusion surface components.

Autoradiograms of ^{131}I-labelled surface proteins of chick myoblast at before, during and after fusions are shown in Fig. 2. The densitometric scan of the autoradiogram shows more clearly the labelled protein profile and is shown in Fig. 3. Twelve major bands were identified from the prefusion sample. There seems to be some qualitative change in bands during the course of fusion as is evident from 48-hour sample which showed two extra bands a and b. The densitometric scanning patterns also show clearly the difference between 32 and 48 hours samples. The band c might be the increase of bands 7 and 8 to make up a dense band. Thus, it is ascribed due to the quantitative increase instead of a qualitative change.

It is very suggestive that protein bands might be grouped into two distinct ones; the protein with high molecular-weight decreased and those with low molecular weight increased in relative amount. The significance of this finding is discussed in the next section. As a whole, these data suggest that a major structural reorganization of surface proteins does occur in association with fusion.

Change in cAMP Level during Fusion

In all cultures studied, the intracellular cAMP levels, expressed in relative amount per DNA content, varied with time. The result from a typical experiment is shown in Fig. 4. The result shows a transient large peak of cAMP, with a short time span of about

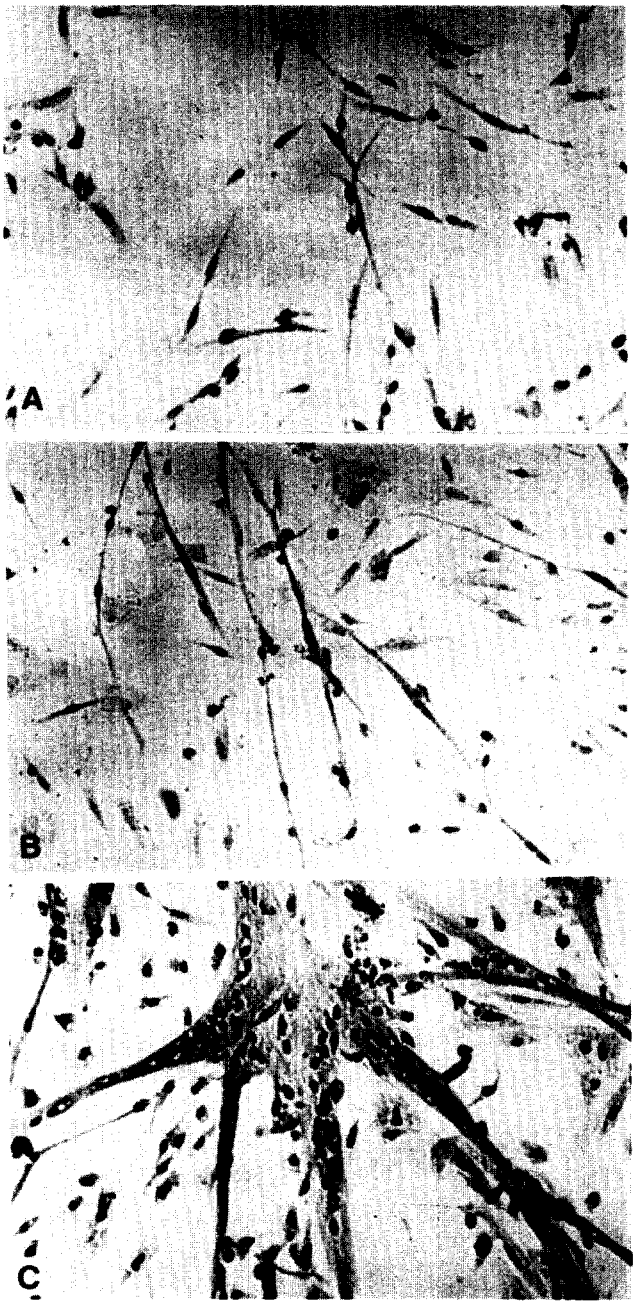

Fig. 1. Morphology and the process of fusion of chick myoblast cultured in vitro. A, 32 hr; B, 48 hr; C, 72 hr after plating. Initial seeding density was 5×10^5/ml.

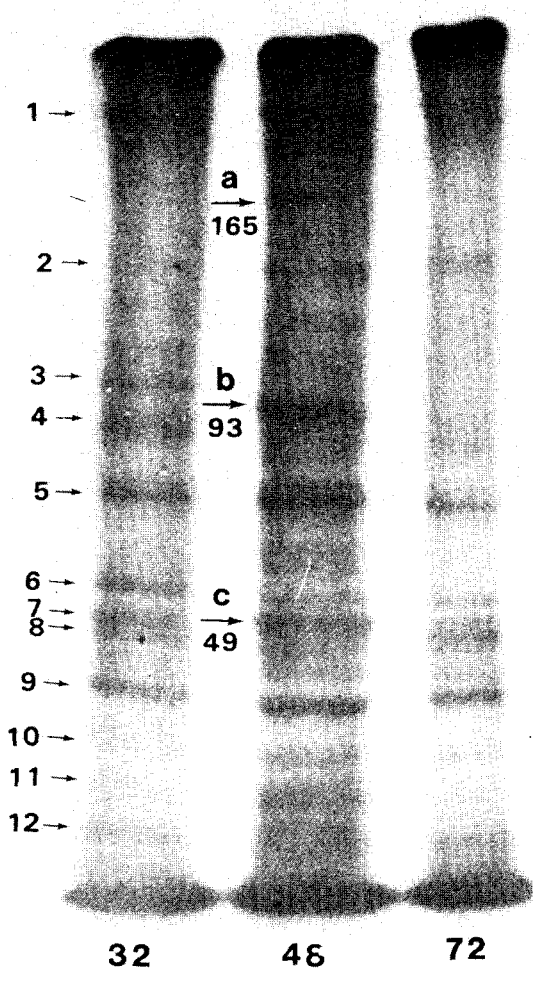

Fig. 2. Autoradiograms of cell surface proteins isolated by SDS-gel electrophoresis before, during, and after fusion. Numerials represent the time after cell seeding.

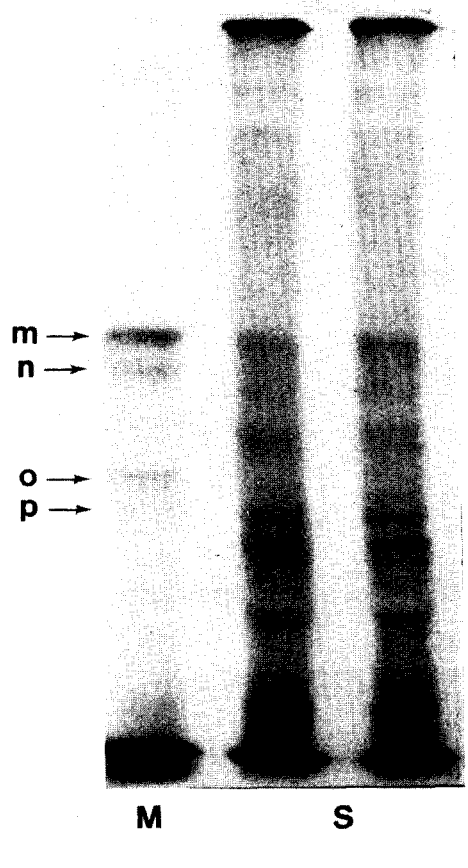

Fig. 6. Autoradiograms of labelled cell surface proteins and those recovered from the culture medium which might be released from the labelled cell surface proteins during the course of myoblast fusion. S, cell surface proteins; M, proteins released into culture medium.

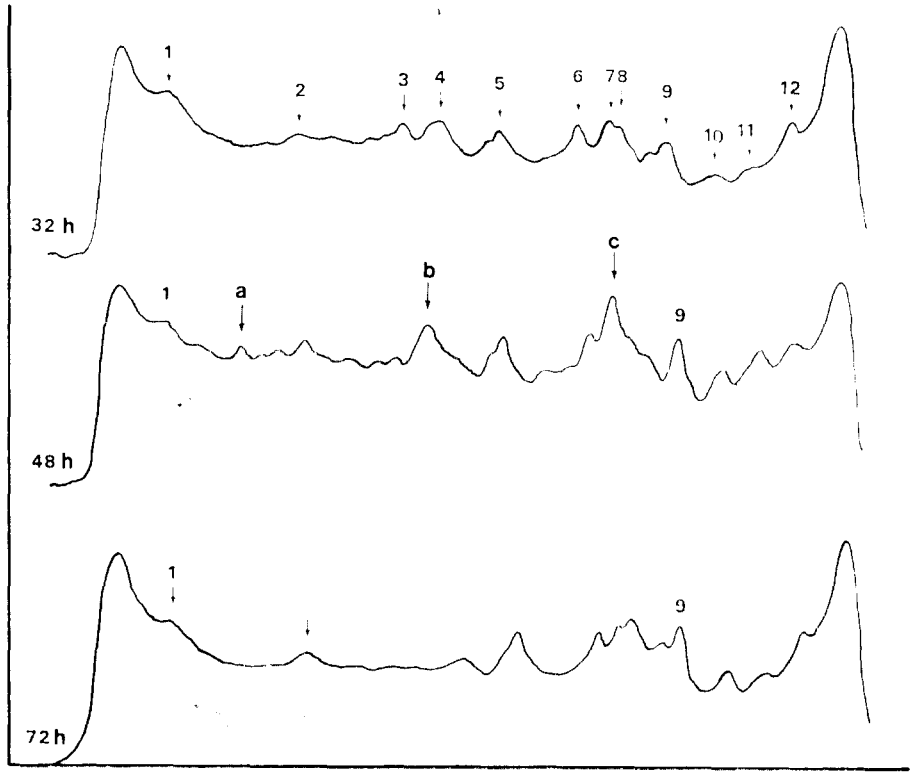

Fig. 3. Densitometric scanning patterns of the autoradiogram of cell surface proteins before, during, and after the fusion. The hours represent the time after cell seeding.

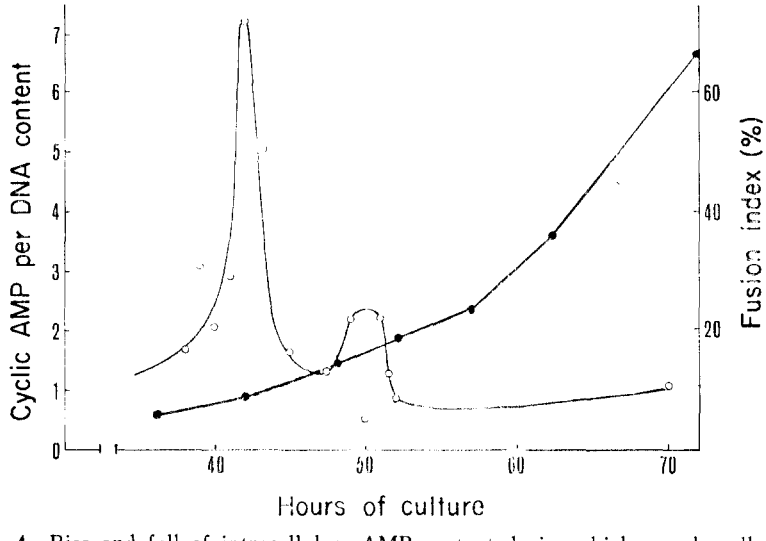

Fig. 4. Rise and fall of intracellular cAMP content during chick muscle cell differentiation. Solid circled line denotes the fusion index as estimated by a direct microscopic observation of ethanol-fixed and Giemsa-stained dishes.

three hours. It increased seven- to eight-fold at maximum as compared to basal level. In the case, the cAMP increase occurred at 42 hours and fusion began seven hours later, at 49 hours. During this time, cells underwent mitosis, most actively at 47 hours.

Since the analyses were concerned with the initial phase of the differentiation process, involving the cessation of DNA synthesis and the initiation of cell fusion, there may be another subsequent change in intracellular cAMP levels during development, before it is stabilized to those values characteristic of mature muscle. The significance of the rapid increase and/or rapid decrease of cAMP level is discussed below.

Biochemically-defined Culture Medium

In almost all of the published studies with primary cultures of chick muscle, cells have been cultivated in a complex medium containing serum and/or embryo extracts as unidentified components. Although cultivation in the complex media is satisfactory for many purposes, the use of completely defined medium will make it more feasible to isolate and characterize materials secreted or released from the cells during the culture. In addition, as the studies of cell surface changes during differentiation is somewhat complicated by the binding of media-derived serum or embryo extract components, working with a biochemically defined medium is in some respects advantageous. Fig. 5 represents the elution profile of FGF from CM-Sephadex C-50 column. The major growth factor activity was retained in fraction 3 (F_3). Table 1 shows the mitogenecity of F_3 which is expressed in terms of the rate of DNA synthesis using double isotopic labelling method. The highest mitogenicity of F_3 was found at 8 μg/ml which approached 69% of the control. The

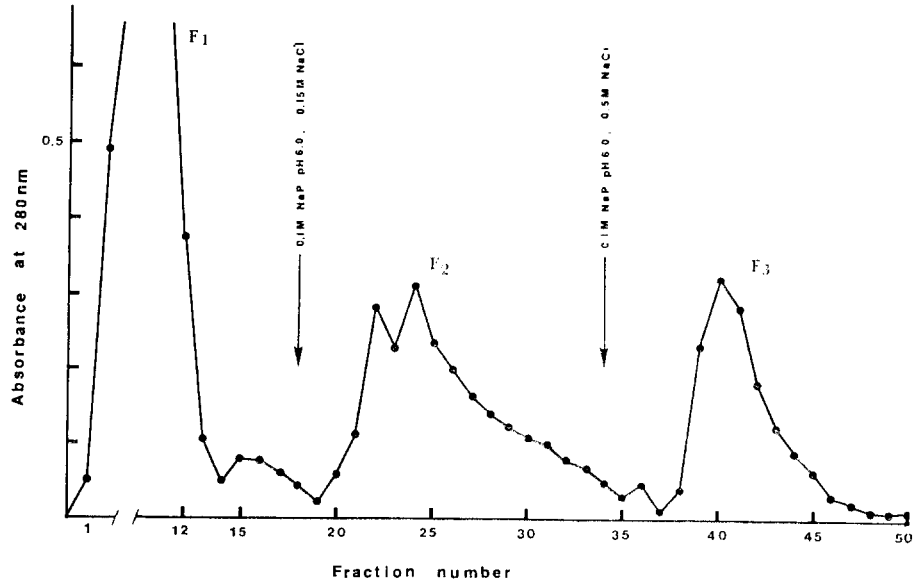

Fig. 5. Elution pattern of fibroblast growth factor on CM-Sephadex C-50 column. Most of the mitogenic activity was found in F_3 fraction.

Table 1. Rate of DNA synthesis in the cells cultured in the medium containing FGF F_3 as evaluated by double labelling.

FGF F_3 (μg/ml)	methyl-^{3}H-thymidine* / methyl-^{14}C-thymidine	Rate of DNA synthesis (per cent of control)
1	5.23	33 %
2	7.35	47 %
4	8.43	54 %
6	10.62	68 %
8	10.76	69 %
15	10.27	66 %
20	9.45	60 %
25	9.20	59 %

* The ratio for the cells in standard medium was 15.65.

data, however, are not sufficient to conclude that the addition of FGF in place of serum and embryo extract allows myoblast to grow substantially and to fuse. It thus remains to be demonstrated.

Proteins Released into the Medium

In Fig. 6 is shown the labelled proteins released into the medium during myoblast fusion along with labelled cell surface proteins for comparison. As is evident from the autoradiograms, at least four proteins (m,n,o, and p) were identifiable in the medium. These are thought to be membrane origin and not cytoplasmic in view of the fact that they do not fit with any of the major membrane proteins (now shown). Thus, at least these four proteins could be ascribed to be released from the membrane by proteolytic cleavage of surface proteins in the course of myoblast fusion.

DISCUSSION

Involvement of Surface Protein(s) in Fusion

No direct function is yet attributable to the LETS glycoprotein that seems to migrate as band 1. It could, however, have some other functions in differentiation, since its synthesis is reduced at mitosis and accelerated when cells are arrested in G_1 (Hynes *et al.*, 1974; Y. Kim, unpublished data). The fact that exposure of this protein can be switched up or down in response to growth stimuli in normal rat myoblast transformed by Rous sarcoma virus after temperature shifts (Hynes *et al.*, 1976) suggests that it could play an important role in growth controls which are aborted by transformation. It could fulfill the role either as a receptor for external signals from serum factors or from neighboring cells, or in a structural role affecting the activity of membrane proteins. There are numerous reports that surface-specific lectins in embryonic chick muscle are engaged in cell fusion (MacBride *et al.*, 1980; Nowak *et al.*, 1976; Parfett *et al.*, 1981; Mir-Lechaire

et al., 1978).

The possibility that specific cell surface glycoproteins may be an important factor in myoblast fusion is indirectly supported by the result of Den *et al.* (1975) that concanavalin A was shown to inhibit myoblast fusion effectively at a concentration of 15 μg/ml. Cell-cell recognition and intercellular communication during the fusion is, thus, believed to be mediated by specific macromolecular glycoprotein components located on the cell surface and between cells. High molecular-weight components of myoblast which decrease in amount at postfusion also seem to be cell cycle-dependent, as appears to be the case for cAMP levels during fusion.

Rutishauser *et al.* (1976), studying with the chick neural tissue, have suggested that 140K and 110K protein fragments were released from the surface components with a molecular weight of 240K and 150K by proteolytic cleavage. This proteolytic alteration of major surface components results in the appearance of determinants required for the initial formation of cell-cell adhesion. Therefore, the observed increase of bands a and b along with bands 9~12 the present study may be suggestive of an increase in proteolytic activity associated with myoblast fusion. This possibility associated with fusion remains to be conclusively demonstrated.

Involvement of cAMP in Fusion

Recently, purified plasma membrane preparation from BALB/3T3 cells have been shown to contain two protein substrates which undergo phosphorylation catalyzed by cAMP-dependent protein kinase (Scott *et al.*, 1981). These plasma membrane substrates were not phosphorylated in the presence of cAMP when cells were arrested in G_1 phase. These data suggest that cAMP modulates the phosphorylation of membrane protein in association with cell cycle. It is generally accepted that normal cells show a continuing rise in their cAMP level mediated by phosphodiesterase and membrane-associated adenylate cyclase when cell growth was contact-inhibited (Anderson *et al.*, 1973).

In the case of chick myoblast, phosphorylated protein(s) that are significantly engaged in fusion process were not detected (Ha, D. B., personal communication). This result, with the findings described above, strongly suggests that the rapid decrease of cAMP level to its base level seven hours before the onset of fusion may have a role in fusion initiation, rather the rapid increase of cAMP level may do. This idea is further supported by the findings that cAMP and phosphodiesterase inhibitors were found to delay markedly the time of myoblast fusion but have no observable effect upon the increase of creatine phosphokinase activity and cell proliferation (Zalin, 1973).

The sharpness of the cAMP peak obtained in the present study suggests a high degree of synchrony in the differentiating myoblast. One possible cause of synchrony is that an interaction exists between the myoblast and their immediate environment during differentiation.

Involvement of Cell Cycle and External Factor(s) in Fusion

It is generally believed that myoblast fusion does not occur in S, G_2, or M, but in early G_1. The position of the cell in the division cycle appears to result in the surface alterations required for fusion (Bischoff *et al.*, 1969). From the microscopic observation, the time of mitosis corresponds to the period of rapid decrease in cAMP level (Fig. 4). It is, thus, conceivable that the mitosis may condition myoblast for an abrupt shift to the synthetic activity required for fusion that occurs in the subsequent G_1. Whatever the precise mechanism of the cell fusion related with cell cycle, cell proliferation and differentiation are somewhat considered to be antithetical process (Blau *et al.*, 1979).

Myoblast fusion was completely inhibited when the culture medium was replaced with Eagle's MEM at 18 hours after plating. But the fusion was inhibited to some extent when the medium was replaced at 24 hours (B. Yoo, personal communication). The effect of DMSO on fusion inhibition was also to be the case. These observations support the idea that myoblasts are already conditioned or committed during this period to differentiate by external fusion-promoting factor(s) or by the genetically-programmed regulation, or both.

There are numerous data on the influence of external diffusible factors on myoblast differentiation (Delain *et al.*, 1981; Konigsberg, 1971; Yeoh *et al.*, 1977; Doering *et al.*, 1977; Schubert *et al.*, 1973). It has been suggested that the fusion stimulation in muscle-conditioned medium (M-CM) is solely the result of a depletion of replication-stimulating factors in the medium, and that the retardation of myoblast replication is the normal stimulus for fusion (Konigsberg, 1971). It was concluded, however, by Doering *et al.* (1977) that myoblast replication is a necessary, but not sufficient prerequisite for the onset of fusion and subsequent muscle differentiation. They also found that the fusion-promoting activity of M-CM is derived from a high molecular weight protein(s) with a molecular weight at least over 10K daltons which was secreted by muscle cell populations at the mono- or multi-nucleated stage of differentiation rather than at myotube stage. In the present study, in the culture media in which iodinated cells were grown for 30 hours were found at least four proteins. These are thought to be released from the iodinated surface proteins by proteolytic cleavage and were different from the maior surface proteins. The significance of these released proteins deserve further investigation.

On the mechanism of fusion-promoting action of M-CM, one might consider the possibility that the mitogen(s) derived from serum or embryo extract competes with this fusion-promoting macromolecule(s) for the identical site for action at the cell surface. This possibility remains to be investigated.

As cited above by many investigators, there are ample evidences that chick myoblast fusion is modulated by environmental factors such as Ca^{2+} (Ha *et al.*, 1979), depleted nutrients (Konigsberg, 1971), cAMP (Zalin *et al.*, 1974), some lectins (Nowak *et al.*, 1976), surface glycoproteins (Walsh *et al.*, 1981), cell-secreting components (Doering *et al.*, 1977), cell density (Yeoh *et al.*, 1977), cell-dependent surface alterations (Bischoff

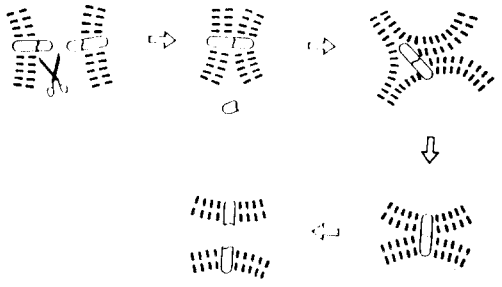

Fig. 7. A suggested mechanism for the role of high molecular weight protein which was observed to decrease at post-fusion stage as a mediator of cell-cell binding and membrane fusion process. Scissors denote media-derived protease. Proteolytic degradation of high molecular weight protein at its protease-sensitive site would suffice to induce a structural change which is required for binding. Successively, structural reorganization and dissociation would result in membrane fusion and immediate intracellular changes that may possibly mediated by cAMP and Ca^{2+}.

et al., 1969), neural influence (Bonner *et al.*, 1982), and even by the mitochondrial gene expression (Brunk, 1981). As the differentiation process is, at least in some sense, an intrinsic gene expression under very intricate regulation, it is difficult to speculate which one is the first trigger of a variety of successive changes engaged in the regulation of myoblast fusion. It is tempting to postulate that the expression of surface alterations required for fusion invokes the subsequent intracellular changes for membrane fusion reaction, which is cell cycle-dependent. This conditioning effect of surface alterations for cells to differentiate would be mediated by cAMP and Ca^{2+}, as described in Fig. 7.

ABSTRACT

Several approaches have been made to access the mechanism of fusion in chick myoblast in vitro.

Lactoperoxidase-catalyzed iodination was applied to labell cell surface proteins during myogenesis. Quantitative as well as qualitative changes were observed in ^{131}I surface components of prefusion and postfusion cells. Two proteins with a molecular weight of 165K and 93K daltons were observed to appear at the onset of fusion as compared to prefusion stage. At the same time, 245K dalton protein decreased whereas the low molecular weight proteins increased consistently. The decrease of high molecular weight proteins appears to be associated with the cell cycle of myoblast during differentiation. The increased appearance of low molecular weight proteins might be due to the proteolytic cleavage of the high molecular weight proteins.

Examination of intracellulr cAMP levels during fusion has revealed that a large but transient increase in cAMP occurs before the onset of fusion. This result suggests a causal relationship between the increase of cAMP and the onset of fusion, and further, that differentiating myoblasts are synchronized to a high degree.

During the course of myoblast differentiation, at least four low molecular weight proteins, which different from major surface proteins iodinated, were identifiable in the culture medium. These proteins could be ascribed to be released from the membrane by

proteolytic cleavage of surface proteins in the course of myoblast fusion.

The significance of cell surface alterations and the released proteins during the fusion, the involvement of cAMP in the onset of fusion and the possibility that fusion is promoted by external factor(s) are discussed.

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