Ultrastructural Evaluation of the Myogenic Cell Fusion in Chick Embryo Using the Goniometer Stage of Electron Microscopy

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Ultrastructural Evaluation of the Myogenic Cell Fusion in Chick Embryo Using the Goniometer Stage of Electron Microscopy

Tateki KIKUCHI

Department of Animal Science, Faculty of Agriculture, Tohoku University, Sendai, Japan

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Summary

In order to study in stereographic analysis of the closed plasma membrane attachment between neighboring myogenic cells, the muscle tissues (*M. complexus*) of chick embryo at 12 days of incubation were sampled and observed by a JEM-100B electron microscope equipped with JEOL's Universal Goniometer.

It may be concluded that the focal discontinuity of the apposed membranes and the cytoplasmic interdigitations found between successive generation myotube and primary myotube are not due to lateral fusion but artefacts resulting from the tangential plane of sectioning.

It is the commonly accepted view today that during development the multinuclear myotubes increase their number of nuclei by cell fusion with neighboring mononuclear myoblasts (1, 2). The myotubes are divided into two groups, the primary and successive generation myotubes. The plasma membranes of neighboring myotubes close proximity to one another, showing how they follow a tortuous course, are, in some areas, discontinuous, as if they were caught in the act of cell fusion. It cannot be determined, however, whether the two adjacent myotubes have undergone local fusion or whether the discontinuities in definition of plasma membranes are the result of the oblique plane of sectioning through the membrane (3).

The purpose of this work was to study the ultrastructural aspects of these specialized membrane attachments by using a Goniometr stage which is indispensable for the study of high resolution stereography.

Materials and Methods

Skeletal muscle (*M. complexus*) were obtained from chick embryos at 12 days of incubation. The tissues were fixed in 2.5% glutaraldehyde in a phosphate buffer (pH 7.4) for 1 hr at 4°C and postfixed with 1% OsO_4 in the same buffer for

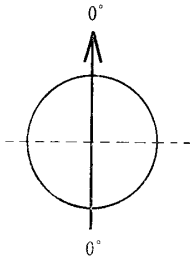


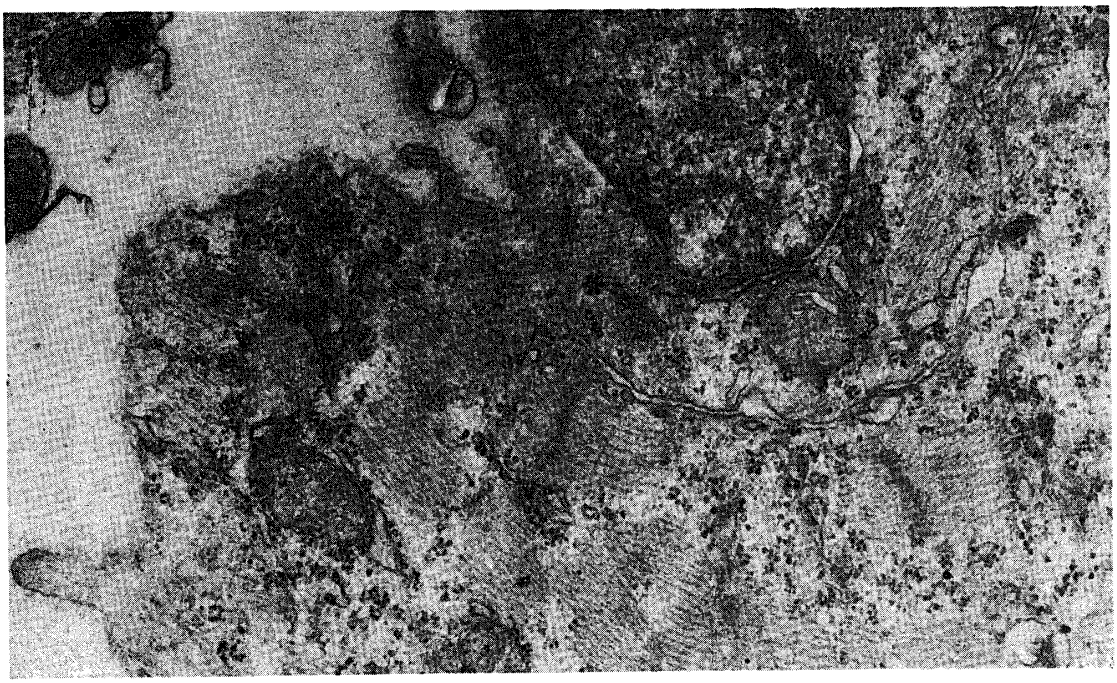
FIG. 1. Cross section of M. complexus at 12 days of incubation. 0° represents the specimen positioned horizontally. The successive generation myotube (sMT) is in close proximity to the primary myotube (pMT) by the limiting plasma membranes. The opposing limiting membrane seem to disappear for a short stretch (*), as if they are caught in the act of cell fusion. ×24,000

1.5 hr at 4°C. Other tissues were fixed in 1% OsO_4 in a Veronal acetate buffer (pH 7.2) for 1.5 hr at 4°C. Both tissue samples were dehydrated in graded alcohols, passed through propylene oxide and embedded in Epon 812. The section showing silver to light gold interference color were cut with a Porter-Blum MT-2 ultramicrotome using glass knives, mounted on carbon coated copper grids, and stained with uranyl acetate and lead citrate (4). The sections were examined with a JEOL's Universal Goniometer, an attachment for the JEM-100B electron microscope.

Results and Discussion

The primary myotube, myoblasts and successive generation myotubes consist of a group of myogenic cells which are enveloped by the same basement membrane outside of the cell group. They were focally connected to each other by close junctions.

Fischman has given a detailed description of these myogenic cell clusters in leg muscle of chick embryos and suggested that all successive generation myotubes were destined to fuse lateraly with primary myotube, thus forming one large multinucleated myotube (5, 6). As the basis of his opinion, he gave, as a proof,



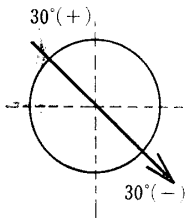
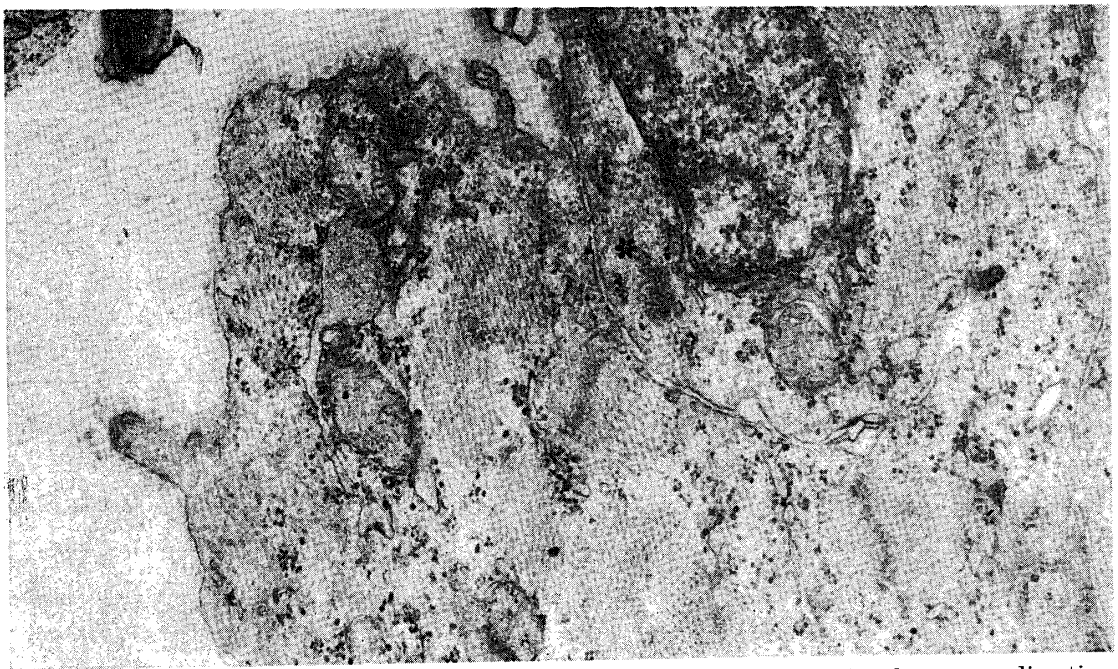


FIG. 2. The specimen of Fig. 1 was tilted 30° in the arrow direction. There are no changes in the area of fusion-like process (*). ×24,000



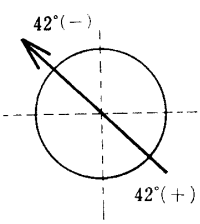
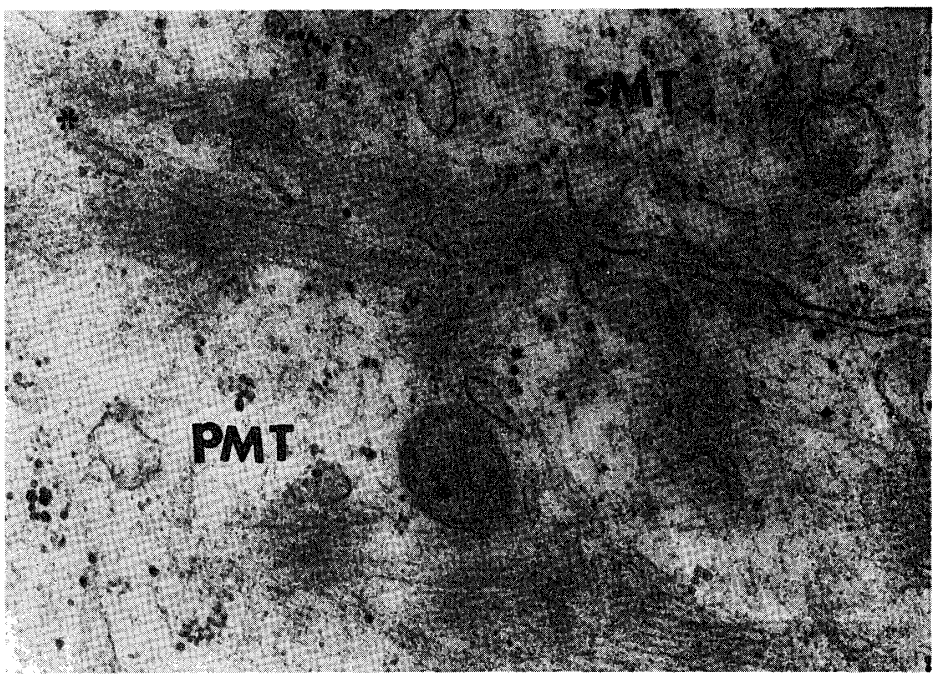


FIG. 3. The specimen of Fig. 1 was tilted 42° in the arrow direction. There are remarkable changes in the area of fusion-like process (*). Prominent parallel electron opaque membranes are seen and separated by an intercellular space. ×24,000



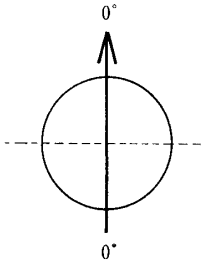
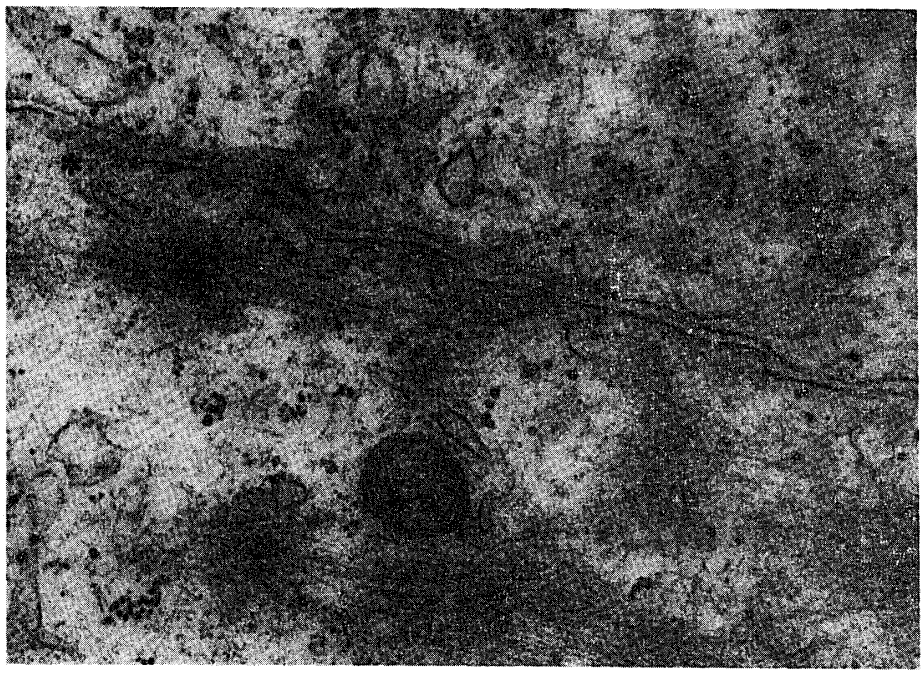


FIG. 4. Longitudinal section of M. complexus at 12 days of incubation. 0° represents the specimen positioned horizontally. The successive generation myotube (sMT) is bordered on the primary myotube (pMT) by the limiting membranes which are discontinuous and indistinct in defined area (*). ×36,000

the close apposition of these myotubes with cytoplasmic interdigitation and the discontinuity of the plasma membranes. However, little attention has been paid to the question of whether these appearance results from lateral cell fusion or are due to the tangential plane of sectioning through the membrane.

The main features of these specialized membrane attachments between neighboring myotubes has also been observed by the present author. However, he suggested that the possibility that such membrane changes are associated with obliquely cut cell membranes cannot be ruled out (7). The present report was carried by using a Goniometer stage to determine whether the indistinct membrane changes mentioned above actually point out a cell fusion process or not. Figure 1 shows a cross section of M. complexus when it was positioned horizontally. It can be seen that a successive generation myotube when traced back, appeared to fuse or join with a part of the peripheral sarcoplasm of a primary myotube (Fig. 1). When the section was tilted 30° in the arrow direction, there is little change in the fusion process of these membranes just stated (Fig. 2). Being tilted 42° in the opposite arrow direction, however, the area of fusion is observed to be parrallel showing two plasma membranes clearly separated by an intercellular space (Fig. 3).



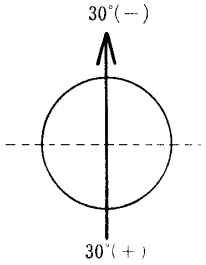


FIG. 5. The specimen of Fig. 4. was tilted 30° in the arrow direction. The two parallel electron opaque membranes appeared in this area. ×36,000

In the longitudinal sections of the same tissues, the limiting membranes between immature successive generation myotube, with many polysomes and free myofilaments, and the primary myotube follow a tortuous course and are indistinct in some areas (Fig. 4). But being tilted 30° in the arrow direction, the membrane structures appear clearly (Fig. 5).

On the basis of these results, it seemed reasonable to assume that the focal discontinuity of the limiting membranes and the cytoplasmic interdigitations found between successive generation myotube and primary myotube are not due to a lateral fusion process but to the oblique plane of sectioning. The cell fusion process observed between the myoblast and myotube was characterized by the presence of numerous small vesicles and the dissolution of the closed membrane attachment (8, 9, 10). The preliminary studies of the present author show that tilting the specimen in various directions reveals no remarkable changes in the photos of this cell fusion process. This suggested that true cell fusion occurs between myotubes and myoblasts. Further studies must be done to reveal the membranous changes between neighboring myogenic cells by using a Goniometer stage.

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References

1) Lash, J.W., H. Holtzer and H. Swift., *Anat. Res.*, **128**, 679 (1957)
2) Bischoff, R. and H. Holtzer., *J. Cell Biol.*, **36**, 111 (1968)
3) Kelly, A.M. and S.I. Zacks., *J. Cell Biol.*, **42**, 135 (1969)
4) Reynolds, E.S., *J. Cell Biol.*, **17**, 208 (1963)
5) Fischman, D.A., *J. Cell Biol.*, **32**, 557 (1967)
6) Fischman, D.A., "*Current topics in developmental biology*" *ed. by A. Moscona and A. Monroy, Academic Press Inc. New York, Vol.* **4**, p 235 (1969)
7) Kikuchi, T., *Tohoku J. Agr. Res.*, **22**, 1 (1971)
8) Hay, E.D., *Z. Zellforsch.*, **59**, 6 (1963)
9) Shafiq. S.A., *J. Cell Biol.*, **17**, 363 (1963)
10) Przbylski, R.J. and J.E. Blumberg., *Lab. Invest.*, **15**, 836 (1966)