The Relationship between the Edge of the Chick Blastoderm and the Vitelline Membrane

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Received February 3, 1969

Summary. 1. The edge of the chick blastoderm has been filmed as it migrated over the inner surface of the vitelline membrane. Ruffled membranes are described, comparable to those reported by ABERCROMBIE and AMBROSE for other types of cells growing in tissue culture.

2. The ruffled membranes, which have been examined both by scanning and transmission electron microscopy, are visible as broad, flattened processes which adhere closely to the inner surface of the vitelline membrane.

3. The inner and outer surfaces of the vitelline membrane, and the endodermal surface of the area opaca, have also been examined by scanning electron microscopy.


2. „Ruffled membranes“ wurden im Transmissions- und auch im Raster-Elektronenmikroskop untersucht. Sie sind sichtbar als breite, abgeflachte Fortsätze, die nahe an der inneren Oberfläche des Dottermembran kleben.


Introduction

Our understanding of the processes involved in cell movement is largely derived from experiments carried out in tissue culture. Much of this recent work has been summarised by ABERCROMBIE (1966), CURTIS (1967), TRINKAUS (1965) and WEISS (1967). It is, however, often difficult to know whether we are justified in assuming that similar processes take place in the developing embryo.

The edge of the chick blastoderm is consequently of great interest, for during the first few days of incubation it has many features in common with sheets of epithelia, and even with isolated cells, growing in tissue culture. For instance, moving cells in vitro are usually in a state of contractile tension, whether they are migrating individually (ABERCROMBIE, 1966) or in sheets (VAUGHAN and TRINKAUS, 1966). Similarly the chick blastoderm is under a state of tension (NEW, 1959) and cannot expand properly if this tension is destroyed or markedly reduced (BELLAIRS et al., 1967). Most cells growing in tissue culture are subject to contact inhibition (ABERCROMBIE and HEAYSMAN, 1954) and a similar situation holds for the advancing edge of epithelial sheets in culture (VAUGHAN and TRINKAUS, 1966); in the same way, the cells at the edge of the chick blastoderm appear to be capable of contact inhibition for, if two blastoderms are grown close to one another, migration ceases when they come into contact (BELLAIRS and NEW, 1962).

* The “Stereoscan” scanning electron microscope was provided by the Science Research Council. We would like to thank Mr. R. F. MOSS and Mrs. J. HAMILTON for technical assistance, and MRS. J. ASTAFIEV for the drawings.

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Our aim has been to consider whether or not the cells at the edge of the chick blastoderm also move in the same way as cells growing in tissue culture. In the hen's egg, the blastoderm is attached by its edge cells to the inner surface of the vitelline membrane and this structure is the normal substrate over which the edge cells move. In the present investigation, therefore, we have studied the morphology both of the edge cells and of the vitelline membrane by scanning electron microscopy. In addition we have filmed the edge of the blastoderm moving over the vitelline membrane.

Material and Methods

A. Scanning and Transmission Electron Microscopy

The eggs were incubated for about 20 hours. A piece of vitelline membrane together with the attached blastoderm was then dissected from each yolk in a bath of Pannett and Compton's saline (PANNETT and COMPTON, 1924). The specimen was fixed in Karnovsky's fluid (KARNOVSKY, 1965) for three hours, washed, treated with osmium tetroxide for one hour, and subsequently re-washed in water and air dried. The vitelline membrane from each of three unincubated eggs was also prepared in a similar way.

After dehydration, all specimens were given conducting coatings of ca. 20 μm carbon and ca. 30 μm gold before the examination of their surfaces in the scanning electron microscope (SEM) Cambridge Scientific Instruments Stereoscan operated at 10 kV. Stereo-pair micrographs were recorded by tilting the specimen through 10° between exposures.

Some difficulty was experienced in removing enough of the adherent yolk without loosening the connection between the blastoderm and the vitelline membrane, so that, although 15 specimens of this region were prepared, only one proved suitable for photography.

In addition to the material examined by scanning electron microscopy, sections through the tip of the edge cells were also prepared and were examined in the Siemens Elmiskop I transmission electron microscope. The techniques used in preparation are described elsewhere (BELLAIRS, 1963).

B. Filming of the Edge Cells

Five eggs were incubated for periods of 24—36 hours, after which the yolk was emptied into a bath of saline. A large piece of vitelline membrane, together with the entire, adherent blastoderm, was carefully dissected from the yolk and mounted in a Prior chamber (ROBERTS and TREVAN, 1961). The vitelline membrane was pulled taut around the silicone-rubber O-ring and the entire chamber was filled with Pannett and Compton's saline (PANNETT and COMPTON, 1924). This chamber was maintained at 37° C on the stage of a Wild phase contrast microscope, and filming of the edge cells was carried out with a Vinten time-lapse cinecamera.

Three further embryos were each dissected from the yolk in the same way and then transferred to a plastic petri-dish, the base of which had been replaced by a thin cover-glass. The vitelline membrane was pulled taut around a glass ring and a thin layer of Pannett and Compton's saline was added. A glass lid was sealed over the petri-dish which was then transferred to the stage of a Prior phase contrast microscope enclosed in a Prior filming apparatus.

An interval of five seconds was used in filming, and magnifications on the film before projection were 900 times.

Results

The gross morphology of a chick blastoderm after 20 hours of incubation is shown diagrammatically in Fig. 1. It is attached to the vitelline membrane only by the edge cells. The cells of the greater part of the area opaca contain much intra-cellular yolk, but this is absent from the cells at the edge.

An electron micrograph of a section through an edge cell process is shown in Fig. 2. These edge processes may be as much as 500 μm long though as little as