Cleavage Pattern and Formation of the Blastocoel in the Egg of the Eel, *Anguilla japonica*

Kiichiro Yamamoto

(Received August 19, 1980)

Abstract The cleavage of the Japanese eel eggs is discoidal. The furrows of the cleavage from the first to the fourth are meridional and the resultant blastomeres are arranged in a single layer. After the 32-cell stage, some blastomeres are divided vertically while others horizontally. They increase in number to form a morula composed of three or four cell layers. Between the blastomeres of the morula many intercellular spaces are present, but no cavity is discernible between the blastodisc and the yolk. When the egg reaches the blastula stage, segmented blastomeres gradually come together under the epidermic stratum to form the blastoderm. Together with this change, the yolk under the blastodisc begins to flatten and develop into the periblast. As a result, a cavity appears between the blastoderm and periblast. This is the blastocoel of an early stage. As the egg develops further, the blastocoel becomes larger due to the close arrangement of the blastodermal cells and the sinking of the yolk surface.

Materials and methods

During September and October, 1973, forty silver females and eight silver males of *Anguilla japonica* were caught in the Mabuchi and Takase Rivers in Aomori Prefecture, Japan. They were transferred to Hakodate and raised in recirculating purified tanks with a capacity of 2.5 tons with water temperature maintained at about 17°C during the experiment. The eight silver and twenty cultivated males were injected with synahorin, a mixture of pituitary gonadotrophin and chorionic gonadotrophin (Teikoku Zoki Co.), and the silver females were injected with a saline suspension of chum salmon pituitary as previously reported (Yamamoto et al. 1974). During December, 1973 and January, 1974, more than twenty males and five females became fully sexually mature following these treatments. Eggs were stripped from the female into glass dishes and fertilized by the usual dry method. The fertilized eggs were kept in glass vessels containing normal sea water and held in water baths at approximately 23°C. The developing eggs were observed in the living state and photographed before they were fixed every hour by immersing into Bouin’s solution for five hours or Gilson’s solution for one hour.
Fig. 1. Early development of *Anguilla japonica*. A: 1-cell stage (lateral view), one hr. after fertilization. B: 2-cell stage (lateral view), 1 hr. 30 min. C: 2-cell stage (surface view), 1 hr. 30 min. D: 4-cell stage (lateral view), 2 hrs. E: 4-cell stage (surface view), 2 hrs. F: 8-cell stage (lateral view), 2 hrs. 30 min. G: 8-cell stage (surface view), 2 hrs. 30 min. H: 16-cell stage (surface view), 2 hrs. 50 min. I: 62-cell stage (surface view), 3 hrs. 20 min. J: Morula stage (lateral view), 4 hrs. K: Early blastula stage (lateral view), 6 hrs. L: Late blastula stage (lateral view), 8 hrs.

Embedding, the chorionic membranes of the eggs were removed with a fine needle under a dissecting microscope. The naked eggs were dehydrated and embedded in paraffin by the usual method. Sections, 8 to 10 μ in thickness, were prepared and stained with Delafield’s haematoxylin-eosin or Heidenhain’s iron-haematoxylin light green. Mallory’s triple stain was also employed for some sections.

**Observations**

**Cleavage pattern.** The fully matured fertilized eggs are pelagic in nature, spherical in form, transparent and almost colourless in the living state, measuring 1.0 mm in diameter. The chorionic membrane of the egg is very thin and smooth, having no appendages. The radial striation revealed commonly in the egg membrane of fishes is obscure in this egg.
even in thin sections. The yolk of the egg is composed of many globules of various sizes and the globules are found embedded in the mesh of the cytoplasm which is continuous with the cortical layer surrounding the whole egg proper. A few oil drops of large size are situated in the yolk near the vegetative pole. The cortical alveoli are discernible along the surface of the cortical layer, the diameter of the alveoli being about 5–10 μ. After fertilization, drastic changes occur. The cortical alveoli disappear in a progressive wave spreading out from the micropyle, followed by the elevation of the chorionic membrane and formation of a large perivitelline space. Consequently, the diameter of the whole egg becomes larger, measuring about 1.3 mm. Along with these changes, the cytoplasm of the egg begins to flow gradually towards the animal pole which is situated on the lower side of the floating egg. About an hour after fertilization, there appears a distinct blastodisc of lens-like shape, which measures about 0.6 mm in diameter and is continuous with the cortical layer at its margin (Fig. 1A).

The segmentation of the egg is discoidal and it starts about one and half hours after...
fertilization at a water temperature of about 23°C.

The first cleavage is meridional and the furrow runs through the centre of the blastodisc, resulting in the formation of two blastomeres of equal size (Fig. 1B, C). The sections of the egg in this stage show that the cleavage furrow is deep but it does not reach the basal surface of the blastodisc; further, that no membrane separating the blastodisc from the underlying yolk is discernible and that the blastodisc is connected intimately with the yolk by cytoplasmic processes extending from its basal part (Fig. 2A). The second cleavage plane, also meridional, appears about two hours after fertilization. It cuts through the centre of the blastodisc perpendicularly to the first and divides the blastodisc into four cells similar in shape and size (Fig. 1D, E). The resultant blastomeres are still connected intimately with the yolk as seen in the last stage (Fig. 2B).

The third division consists of two meridional furrows at right angles to the second and parallel to the first. They appear about two and half hours after fertilization and divide the blastodisc into eight cells which are arranged symmetrically in two rows of four cells each. The blastodisc in this stage becomes elliptical in shape due to the elongation of the axis in the direction of the second cleavage plane (Fig. 1G). As shown in Fig. 2C, the blastomeres are still connected tightly to the underlying yolk by their basal cytoplasmic processes.

About three hours after fertilization, the fourth cleavage takes place. Two meridional furrows parallel to the second make an appearance and divide the blastodisc into sixteen blastomeres arranged in four rows of four cells each. Of these blastomeres, four cells occupying the central part are somewhat smaller than those along the periphery. The blastodisc takes on a circular shape again (Fig. 1H).

In eggs later than this stage, cleavage does not take place simultaneously and regularly in all blastomeres. Generally speaking, however, the blastomeres in the peripheral region are divided meridionally and those of the central area horizontally in the beginning (Fig. 1I). As a result, the peripheral region of the blastodisc is composed of a single layer of cells while the central area is made up of a double layer of cells. From sections of the blastodisc it may be demonstrated that the upper blastomeres in the double layer region become free from the lower ones and a narrow space appears between the two layers. No cavity, however, is discernible between the blastomeres of the lower layer and the underlying yolk (Fig. 2D). Hereafter, the division of blastomeres continues on steadily and the cells of the blastodisc increase in number and become small in size. About four hours after fertilization, the blastodisc resembles a mulberry in appearance. The embryo in this stage is called a morula (Fig. 1J). In Fig. 2E and F, microphotographs taken from the sections of the embryos are presented. The blastodisc of this stage consists of three or four cell layers. The blastomeres are spherical or polygonal in shape and are almost similar in size. They are arranged loosely and irregularly, having many small spaces between the cells.

It is worthy to note that the cells situated at the most basal part are still connected tightly to the underlying yolk by their extended cytoplasmic processes and no cavity is found between the blastodisc and yolk.

Formation of the blastocoel. With successive cleavage, there is a progressive increase in cell number and a reduction in cell size. About five hours after fertilization, it becomes difficult to distinguish the individual blastomeres under the dissecting microscope. Although the blastodisc itself is similar in shape and size to the last stage, a narrow cavity can be seen between the blastodisc and the yolk (Fig. 1K). Sections of the egg in this stage demonstrate that the cells of the outermost part become small in size and flat in shape, and they are arranged regularly and tightly to form the so-called epidermic stratum. The blastomeres in the inner part are spherical or polygonal and are greater in size than the cells of the epidermic stratum. They gather gradually under the epidermic stratum to form a kind of germinal layer called the blastoderm. The surface of the yolk under the blastodisc becomes flat and smooth,
though still somewhat wavy. This region is covered with a thin layer of syncytium designated as the periblast. Between the blastoderm and the periblast is a distinct, narrow cavity. The embryo in this stage is called blastula and the cavity revealed in the embryo is called the blastocoel (Fig. 2G).

As the development of the egg proceeds, the blastocoel becomes large and may be seen more clearly even in the living state (Fig. 1L). The preparations made from the egg illustrate that the enlargement of the blastocoel depends upon the sinking of the yolk surface and the close arrangement of the blastodermal cells (Fig. 2H, I).

Discussion

Concerning the developing egg of the eel, two known papers have been published to date. One is Fish’s paper (1927) which deals with American eel eggs collected at a station 10 miles southwest of Bermuda from a depth of 227 m. The other is Ebceenko’s (1974), which is concerned with European eel eggs sampled at stations from 65 to 720 m in depth in the North Sea off George Bank. The American eel eggs are bathypelagic in nature, have no oil drops and measure 3.3 mm in diameter, while the European eel eggs are also bathypelagic in nature, measure 2.3 to 2.9 mm in diameter, and contain quite developed embryos with 115 to 119 somites. These eggs are very different from Japanese eel eggs as demonstrated by the present study. Moreover, the mature unfertilized eggs of European eel described by Fontaine et al. (1964) are about the same as those of Japanese eel, being pelagic, with oil drops and 0.93–1.4 mm in diameter. Therefore, it is doubtful that the fish eggs reported by Fish (1927) and Ebceenko (1974) are just corresponding to eel eggs.

All papers hitherto published agree that the eggs of teleosts show discoidal cleavage. The eggs of the eel are included in the former group and they show meridional cleavage during the early stage of development. As for the formation of the blastocoel in fish embryo, two theories have been offered. One was formulated by Oellacher (1873), Goette (1873), Ryder (1882), Henneguay (1888) and Mahon and Hoar (1956). According to these investigators, the blastocoel of fish eggs is formed by the elevation of the blastoderm on one side. The blastoderm consists of blastomeres intimately connected to each other and it lies close to the flattened upper part of the yolk until the early stage of blastula. No cavity can be recognized between the blastoderm and yolk. When the eggs arrive at the latter stage of blastula, a clear difference in thickness appears in the blastoderm, with one side being thicker than the other side. Then, the blastoderm of the

and Child, 1965), Platypoecilus (Hooper, 1943), the Atlantic salmon (Pellet, 1944; Battle, 1944), a dace (Kanoh, 1950), the carp (Hikita, 1956; Neudecker, 1976), a stickleback (Swarup, 1958), the goldfish (Kazishima, 1960), the channel catfish (Saksena et al. 1961), the Japanese killifish (Gamo and Terajima, 1963), the dog-salmon (Kume, 1965), the rainbow trout (Vernier, 1969; Ballard, 1973), etc., the cleavage of the egg is meridional during the early stage ranging from the first division to the fourth one. As a result, the blastodisc is divided in turn into two, four, eight and 16 blastomeres arranged in a single layer. In these fishes, the horizontal cleavage usually takes place in the central four blastomeres of 16-cell stage eggs and the double layer of cells first makes an appearance in the egg of the 32-cell stage.

On the contrary, one striking exception has been reported by Hoffman (1881). According to him, the first cleavage of Scorpaena porus is horizontal and divides the blastodisc into a double layer of cells. The upper layer cell thus formed goes on to display the same segmentation as seen in other fish eggs and develops into a normal blastoderm, while the lower layer cell shows only nuclear division with no cytoplasmic one. Thus, a layer of syncytium, designated as periblast, results.

As revealed in the present paper, the eggs of the eel are included in the former group and they show meridional cleavage during the early stage of development. For the formation of the blastocoel in fish embryo, two theories have been offered. One was formulated by Oellacher (1873), Goette (1873), Ryder (1882), Henneguay (1888) and Mahon and Hoar (1956). According to these investigators, the blastocoel of fish eggs is formed by the elevation of the blastoderm on one side. The blastoderm consists of blastomeres intimately connected to each other and it lies close to the flattened upper part of the yolk until the early stage of blastula. No cavity can be recognized between the blastoderm and yolk. When the eggs arrive at the latter stage of blastula, a clear difference in thickness appears in the blastoderm, with one side being thicker than the other side. Then, the blastoderm of the
thinner side is elevated freely from the yolk. In such a way, a cavity is formed between the blastoderm and the yolk, which is regarded as an anlage of the blastocoel.

The other theory concerning the formation of blastocoel is based on the opinion that the blastocoel may be originated from the segmentation cavity.

The stage when the segmentation cavity appears, however, is different in different species. In Ctenolabrus (Agassiz and Whitman, 1884) and Serranus (Wilson, 1891) the cavity is formed in the early stage of development. In the eggs at the 16-cell stage, the four central cells become free from the underlying yolk and a narrow cavity appears between the blastodisc and yolk, which is called the segmentation cavity and is surmised to develop into blastocoel.

On the other hand, in fishes such as the goldfish (Von Kowalewski, 1886), a salmon (Kopsch, 1911), the Japanese killifish (Kamito, 1928) and the dog-salmon (Saito, 1950), the segmentation cavity appears at a much more advanced stage of development, i.e., the early stage of blastula. In the embryos at this stage, the blastomeres come together under the epidermic stratum to form the blastoderm, and the yolk surface under the blastodisc develops into periblast. Thus, a narrow cavity is formed between the blastoderm and the periblast, which is called the segmentation cavity or blastocoel.

As described in the preceding section, it is clear that the formation of blastocoel in the eggs of the Japanese eel belongs to the latter group.

Some authors such as Klein (1876), Wilson (1891), Riddle (1917), Kamito (1928), Price (1934), Yusa (1954), Mahon and Hoar (1956) called the cavity between the blastoderm and the periblast a subgerminal cavity. The term "subgerminal cavity," however, had originally been used to indicate the cavity between the hypoblast and yolk in the embryo of birds. In birds, the blastoderm of the blastula stage is differentiated into two layers, the superficial epiblast and the inner hypoblast. The cavity between the epiblast and hypoblast is called the hypoblast while the cavity between the hypoblast and the yolk is designated as the subgerminal cavity (Balinsky, 1965; Torrey and Feduccia, 1979).

On the contrary, the blastoderm of fish blastula consists of only one layer and no one has ever proved the presence of two differentiated layers. Therefore, it seems unsuitable to use the term "subgerminal cavity" for indicating the cavity between the blastoderm and yolk in the egg of fish.

Acknowledgments

The author would like to express his cordial thanks to Professor Hiroya Takahashi and Associate Professor Kazunori Takano at the Laboratory of Fresh-water Fish Culture, Faculty of Fisheries, Hokkaido University, for their valuable assistance during this study. He is also indebted to Drs. Osamu Hiroi and Kohei Yamauchi and many students of the Laboratory of Fresh-water Fish Culture for their kind help in the course of the present study. His thanks should be extended to Professor Tamotsu Iwai of Kyoto University, Drs. Yoshitaka Nagahama, Yoshiaki Matsuda, Tatsuro Ikeuchi and Akira Taniguchi for their help in collecting literature, and also Mrs. Janet A.M. Kramer for improvement of the manuscript.

This study was supported by a grant from the Hokusui Kyokai.

Literature cited


Yamamoto: Anguilla Cleavage Pattern


(Faculty of Fisheries, Hokkaido University, 3-1-1 Minato-cho, Hakodate 041, Japan)

ウナギ卵の分割様式と胞胚腔の形成

山本喜一郎

ウナギの卵分割は盤割で、第一から第四分割までは卵割である。これにより生じた卵割球は常に1層に並び卵黄と密に接した胚盤を形成する。第五分割以降では卵割により分割時期に差が見られるばかりでなく、卵割の外に水平分割を行うものも認められる。分割がさらに進むと3層から4層の細胞からなる実質期の胚となる。この時期の卵割球は円形または多角形、すべてほぼ同じ大きさで、互いにやや干渉しあう間隔があるが、胚盤と卵黄の間には分裂腔は存在しない。発生がさらに進み胞胚腔に達すると、胚盤の表面の細胞は扁平となり密に並んで表皮層を作り、また他の大部分の卵割球は表皮層の下側に集まり次の第に胚盤巢の形態をとる。一方胚盤の下の卵黄表面は平坦な周縁質で覆われる様になり、これと胚盤腔の間に狭い腔があらわれる。これが初期の胞胚腔である。発生がさらに進むと胚盤巢は密に薄い層となり、周縁質の部分の卵黄は凹むため、胞胚腔は大きく発達し外観からもよく認められるようになる。

(O41 函館市港町 3-1-1 北海道大学水産学部)