Japanese Journal of Ichthyology
Vol. 28, No. 4 1982

魚類学雑誌
28巻4号 1982年

Periblast in the Egg of the Eel, *Anguilla japonica*

Kiichiro Yamamoto

(Received July 3, 1981)

Abstract In the egg of the eel, *Anguilla japonica*, the peripheral periblast may be recognized as early as the one cell stage, while the subgerminal periblast is discernible for the first time at the blastula stage. The periblast nucleus seems to be derived from the nuclei of both the marginal cell and bottom cell of the blastoderm. The nuclei of the periblast increase in number by mitotic division until the end of the blastula stage, thereafter they multiply by amitosis. Thus, the periblast becomes a syncytial layer enclosing the whole yolk sphere until the late stage of gastrula. The periblast does not take part in the formation of a permanent embryonic body, but it supposedly takes on some important role in the consumption of yolk.

The periblast is a characteristic structure present in the developing eggs of fishes and birds. It is a thin syncytial layer enveloping the vitelline sphere. The existence of the periblast in the teleost egg was first discovered as long ago as 1854 by Lereboullet. Since then, many workers have investigated its origin, function and fate in various kinds of teleost eggs. Thus, a great deal of knowledge on the subject has been accumulated, but some controversial problems still remain unsolved.

The present study deals with the periblast in the egg of the eel, *Anguilla japonica* Temminck et Schlegel, and the results obtained will be presented in the following sections.

Material and methods

The material used in the present study and the methods for the preparation of sections were the same as those reported in a preceding paper by the present author (Yamamoto, 1981).

Observations

In the eel, the peripheral periblast, though very thin, may be clearly recognized in an egg at the one cell stage, while the subgerminal periblast becomes discernible for the first time in the egg at the blastula stage. Until the egg arrives at the blastula stage, however, no nucleus has ever been detected, even in the peripheral periblast.

Figure 1A shows the blastoderm at the later stage of morula. The blastomeres of the blastoderm are almost spherical in shape, their dimensions being 70 to 60 μ in diameter. They contain a spherical nucleus about 12 μ in diameter. A marginal cell embedded in the yolk is shown in Fig. 1B as having a clear boundary, but one portion of the cell is continuous with the peripheral periblast. This suggests that the marginal cell is destined to be transformed into the peripheral periblast and that the nucleus of the cell becomes the precursor of the periblast nucleus.

On the other hand, the nucleus in the subgerminal periblast in this species is supposed to be derived from the bottom cells of the blastoderm. Figure 1C shows an egg at the time when the bottom blastomere takes part in the formation of the subgerminal periblast. In this egg, a clear segmentation cavity is not yet found between the blastoderm and yolk sphere. However, the upper surface of the yolk sphere becomes flattened and a slit appears between the yolk sphere and the lowest layer of the blastomeres. The blastomere in Fig. 1D shows no distinct boundary and has almost completely fused with the subgerminal periblast. The nucleus of the blastomere still retains the same spherical shape and size (about 12.5 μ in diameter) as those of other blastomeres.

In an egg at the blastula stage, both the peripheral and subgerminal periblasts are clearly revealed and the nuclei found in the layers show active multiplication. Figure 1E and F show mitotic figures seen in the subgerminal periblast. Fine asters and spindles are recognized in the dividing nuclei. Besides these dividing nuclei,

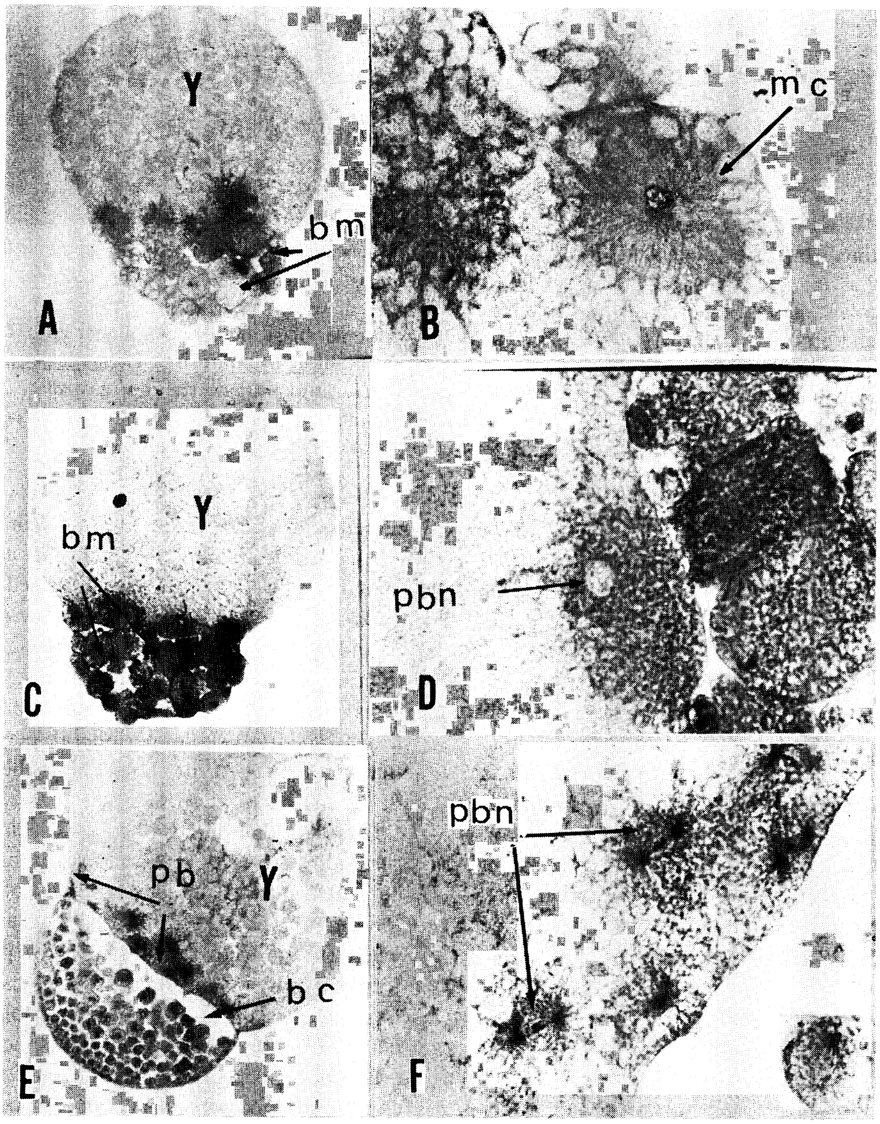

Fig. 1. Periblast nucleus and its division in the egg of the eel. A: A vertical section of morula stage egg. B: A marginal cell participating in the formation of the peripheral periblast, from the same egg as above. C: A vertical section of morula stage egg. D: A bottom cell almost fused with the sub-germinal periblast, from the same egg as above. E: Periblast in the egg at the blastula stage. F: Nuclei showing mitotic figures, from the same egg as above. bc, blastocoel; bm, blastomere; mc, marginal cell; pb, periblast; pbn, periblast nucleus; y, yolk.

there are resting nuclei situated in the periblast. They have a spherical form and a diameter of about 15 μ.

The gastrulation of eel eggs begins to take place about 10 hours after fertilization at a water temperature of 23°C. In the egg at the gastrula stage, no mitotic figures have been discovered in the periblast. However, the nuclei distributed in the periblast increase markedly in number, and they are observed not only in the sub-germinal periblast, but also in the peripheral periblast (Fig. 2G), Therefore, it is probable that they have multiplied by amitotic division.

In Fig. 2H, nuclei distributed in the sub-germinal periblast of the egg at the early stage of gastrula are shown. There are two large

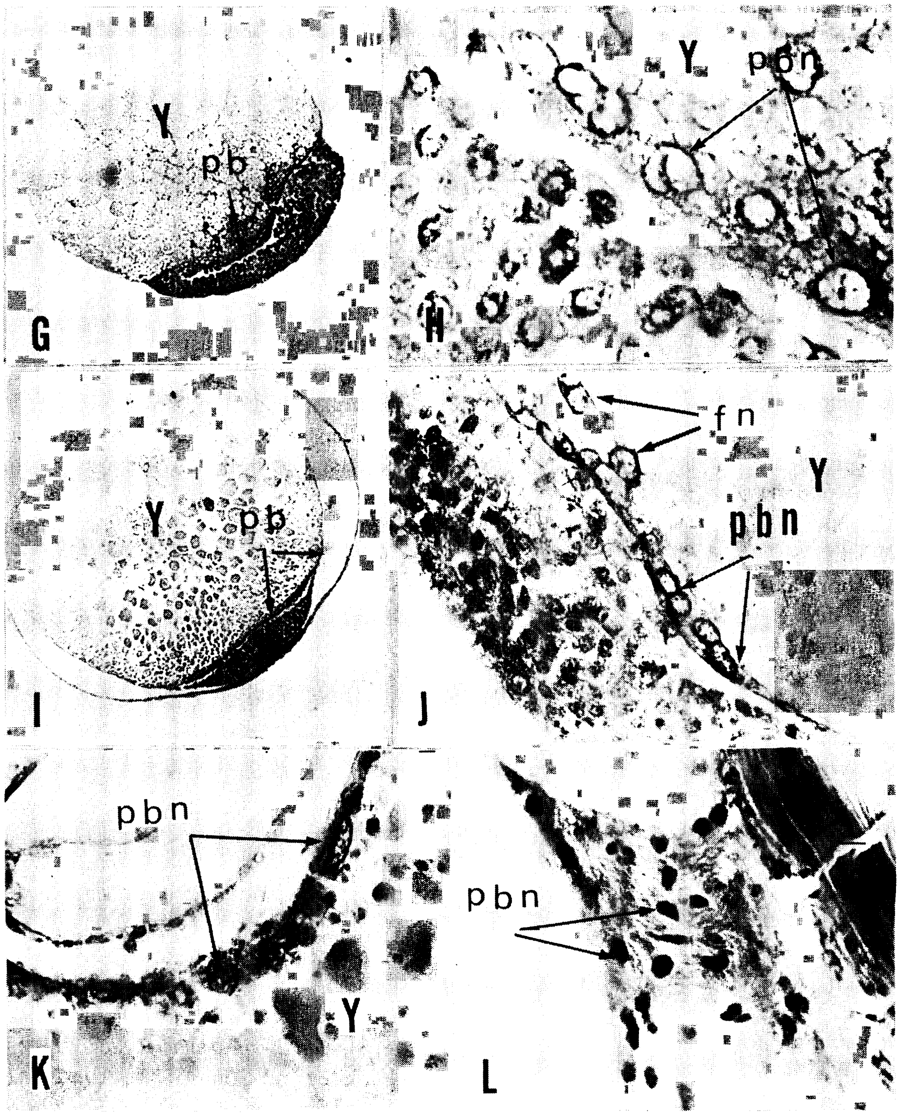

Fig. 2. Syncytial periblast and its nuclei. G: Subgerminal periblast present in the egg at the early stage of gastrula. H: Nuclei showing amitotic division, from the same egg as above. I: Periblast found in an embryo at the blastopore closing stage. J: Nuclei lying among yolk granules, from the same egg as above. K: Periblast nuclei in an egg just before hatching. L: Clusters of periblast nuclei found in the yolk sac syncytial layer (Periblast) of a larva one day after hatching. fn, free nucleus; other abbreviations as in Fig. 1.

nuclei elliptical in shape, the long axis of which being about 23 μ. A wall is present at the middle part of the nuclei, which seems to separate the nuclei into two parts. In addition to the elliptical nuclei, there are many spherical nuclei having diameters from 12 to 15 μ. Some of them are observed very close together, as if they had resulted from a division of the elliptical nuclei. At this stage, the nuclei are not yet broadly distributed in the peripheral periblast and are found limited to a narrow region near the margin of the blastoderm. They are elliptical in shape, being 20 to 15 μ in long axis and 10 μ in short axis.

As the development of the egg proceeds further, the nuclei are widely dispersed into the peripheral periblast. In eggs at the blastopore closing stage, the nuclei are found all through the periblast (Fig. 2I). The size and shape of the nuclei are about the same as those described above. As shown in Fig. 2J, there are frequently some nuclei situated outside the periblast, embedded among yolk globules.

Along with the progression of development, the boundary between the periblast and yolk sphere becomes more and more obscure. In Fig. 2K, the periblast in the egg is shown just before hatching. The figure shows that the periblast is uneven in contour and many cytoplasmic processes extend from the periblast into the masses of small yolk granules. The periblast nuclei are comparatively large in size, some elliptical ones being 20 μ in long axis and 10 μ in short axis, while spherical ones are about 12 μ in diameter.

Hatched larvae absorb the yolk quickly. Therefore, the yolk sacs of larvae become smaller in size rapidly. Simultaneously, the periblast enclosing the yolk sphere rapidly decreases in area and this results in a denser distribution of nuclei in the periblast. The contour of the periblast becomes more uneven and many cytoplasmic processes extend from the periblast into the masses of small yolk granules. The nuclei of the periblast are almost elliptical in shape and measure about 15 μ in long axis and 7.5 μ in short axis.

Besides these nuclei, there are many nuclei of various sizes and shapes which stain deeply with haematoxylin and are distributed in the periblast, forming many small clusters (Fig. 2L).

Discussion

Agassiz and Whitman (1884) proposed the term periblast to represent the thinner portion of the protoplasmic mantle of teleostean ova, which envelops the yolk sphere and is continuous with the blastodisc. Before that time, the periblast had been called "feullet muquex" by Lereboullet (1854, 1861), "dotterhaut" by Oellacher (1872), "intermediate layer" by Van Beneden (1878), "parablast" by Klein (1876) and Hoffmann (1881), and "yolk hypoblast" by Ryder (1882). The "keimwall" described by His (1876) may correspond to the periblast, although free cells instead of free nuclei are found embedded in the layer.

Since the paper of Agassiz and Whitman was published, however, the term periblast has been commonly employed for the representation of the protoplasmic layer. After the segmentation cavity has been formed, a thin protoplasmic layer appears on the bottom of the cavity, which is also designated as periblast. To distinguish the mantle portion from the portion under the blastoderm, the former is called a "peripheral portion of periblast" and the latter a "subgerminal portion of periblast" (Agassiz and Whitman, 1884).

When the egg reaches the early stage of the blastula, the peripheral portion of the periblast is separated from the blastodisc and becomes connected to the subgerminal periblast. Thus, the periblast becomes a thin protoplasmic mantle enclosing the whole yolk sphere.

In all teleosts hitherto studied, the peripheral portion of the periblast has been observed in the fertilized egg at the one cell stage. This is also true in the egg of the eel, as confirmed in the present study. On the other hand, the subgerminal periblast may be revealed after the segmentation cavity appears. In some gadoid fish (Van Beneden, 1878), *Ctnolabrus* (Aggasiz and Whitman, 1884) and *Serranus* (Wilson, 1891), the segmentation cavity appears as early as the 16 cell stage. Therefore, in these kinds of fishes the subgerminal periblast may be already found in the egg at the 16 cell stage, while in many kinds of fishes such as *Esox* (Kupffer, 1868), *Gadus morrhua* (Ryder, 1882), *Merlucius* (Kingsley and Conn, 1883), *Belone* (Wenckebach, 1886), *Salmo fario* and *Salmo irideus* (Samassa, 1896), *Salmo fario* (Kopsch, 1911), the Japanese killifish *Oryzias latipes* (Kamito, 1928) and the dog-salmon *Oncorhynchus keta* (Saito, 1950; Mahon and Hoar, 1956; Nishida, 1958), the subgerminal periblast first makes an appearance in the egg at the blastula stage.

As mentioned above, the egg of the eel belongs to the latter type and the subgerminal periblast is first revealed in the egg at the blastula stage.

When the eggs of fishes develop into the blastula stage, many free nuclei are observed in the periblast, thus the layer is transformed into a syncytium.

As for the origin of the periblast nucleus, five theories have been offered. The first one was asserted by Kupffer (1868). According to him, in the egg of *Gasterosteus* and *Spinachus* the periblast nucleus originates in situ, independent of the cell of the blastoderm. Klein (1876) who studied the development of *Salmo fario* and Van Kowalewskii (1886) who studied goldfish agreed with Kupffer's opinion.

The second theory was proposed by Hoffmann (1881). According to him, the first cleavage of *Scorpaena* and *Juli* is horizontal and divides the blastodisc into two cells of unequal size. The upper small 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 large cell shows only nuclear division and no cytoplasmic one. Thus, a layer of syncytium, designated as periblast, results. Ryder (1882) who studied eggs of a cod, *Gadus morrhua*, gave the same opinion as that of Hoffmann.

This theory was criticized severely by Agassiz and Whitman (1884). Hoffmann accepted these criticisms and again studied this subject using salmon eggs as material (1888). As a result, he arrived at the conclusion that horizontal cleavage in the salmon egg occurs at the 8-cell stage, and that the upper cell layer thus formed takes part in the formation of the blastoderm, while the lower cell layer participates in the formation of the periblast.

The third theory was offered by Agassiz and Whitman (1884). They maintained that the periblast nucleus merely originates in the marginal cells of the balstoderm. List (1887) who studied *labriden* fish, Wilson (1891) who studied *Serranus*, Ziegler (1896) who studied *Labrax* and Kamito (1928) who studied the Japanese killifish *Oryzias latipes* all arrived at the same conclusion.

Meanwhile, Wenckebach (1886) who studied *Belone acus*, Samassa (1896) who studied *Salmo fario* and *Salmo irideus*, Kopsch (1911) who studied *Salmo fario*, Kanoh (1949) who studied *Tribolodon* and Gamo and Araki (1961) who studied *Oryzias latipes* presented a fourth theory that both the marginal and bottom cells of the blastoderm take part in the formation of the periblast nucleus.

Further, Gamo (1961b) stated a fifth theory that, in *Oryzias latipes,* some nuclei of the embryonic cells in contact with the periblast undergo transformation into the periblast nucleus during the gastrula, blood circulation and tail-bud stages.

As mentioned above, in the egg of the eel, the periblast nucleus seems to be derived from the nuclei of both marginal and bottom cells of the blastoderm. The phenomenon that some of the embryonic cells are transformed into periblast nuclei during the gastrula, blood circulation and tail-bud stages has not been recognized in the present study.

Until the end of the blastula stage, the periblast nucleus increases actively in number by mitotic division as revealed by Agassiz and Whitman (1884), Wilson (1891), Kamito (1928), Mahon and Hoar (1956) and Gamo and Araki (1961). After that time, the multiplication of the nucleus is supposed to be performed by amitotic division (Wenckebach, 1886; Wilson, 1891; Samassa, 1896; Kanoh, 1949; Gamo and Araki, 1961; Long, 1980). The behavior of the periblast nucleus at the time of amitotic division, however, has not yet been described by these investigators.

The multiplication of the periblast nucleus in the eel egg is the same as that reported by the above investigators, namely, the nuclei increase in number by mitosis until the end of the blastula stage, and after that time they seem to multiply by amitotic division due to the formation of a wall in the middle of the nucleus.

As for the role of the periblast, some authors in the past such as Kupffer (1868), Cunningham (1875), Van Beneden (1878), Ryder (1882), Brook (1885) and Reinhard (1898) maintained that the periblast takes part in the formation of the blood, circulatory system and alimentary canal. However, many authors since, for instance, Agassiz and Whitman (1884), Wenckebach (1886), Wilson (1891), Samassa (1896), Ziegler (1896), Kamito (1928), Yamada (1959a, b), and Gamo (1961a, c) asserted that the periblast does not share in formation of any portion of a permanent embryonic body, but that it takes on some important role in breaking down the yolk and makes it available for the growing embryo.

Recentry this opinion has been supported by evidence obtained through electron microscopy (Yamamoto, 1967; Walzer and Schönenberger, 1979a, b; Shimizu and Yamada, 1980), and histochemistry (Walzer and Schönenberger, 1979a, b). The presence of cell organelles related to active cell metabolism, such as markedly elongated cisternae of the endoplasmic reticulae, developed Golgi complexes and many mitochondria, and the location of several enzymes necessary for the consumption of yolk have been demonstrated in the periblast by the above studies.

Further, Trinkaus (1951, 1971) emphasized that at the time of epiboly, the periblast aids the spreading of blastoderm by exerting pull on the margin of the blastoderm.

From the observations of the present study it seems certain that the periblast in the egg of the eel does not take part in the formation of a permanent embryonic body, but that it takes on some important role in the absorption of the yolk. The role of the periblast during epiboly, however, has not been investigated in the present study.

Many clusters of transformed nuclei distributed in the yolk sac syncytial layer have already been reported by Wenckebach (1886) who studied the larvae of *Belone* and by Yamada (1959a, b) who studied the larvae of the pond smelt, the dog-salmon and the rainbow trout. Wenckebach gave the opinion that this transformation of the nuclei may be a sign of degeneration. The present author could not follow the fate of the periblast nuclei until their final moments, but it seems highly probable that the supposition of Wenckebach (1886) is also applicable in the case of the eel egg.

Acknowledgments

The author would like to express his cordial thanks to Professor Hiroya Takahashi, Associate Professor Kazunori Takano and Dr. Kohei Yamauchi at the Laboratory of Freshwater Fish Culture, Faculty of Fisheries, Hokkaido University for their valuable assistance during this study. His thanks should also be extended to Mrs. Janet A. M. Kramer for improvement of the munuscript, and Professor Tamotsu Iwai of Kyoto University for his help in collecting literature.

This study was supported by a grant from the Hokkaido Prefecture Goverment.

Literature cited

Agassiz, A. and C. O. Whitman. 1884. On the development of some pelagic fish eggs. Preliminary notes. Proc. Amer. Acad. Arts. Sci., 20 (1): 23～74, figs. 1～6.

Brook, G. 1885. On the origin of the hypoblast in pelagic teleostean ova. Quart. J. Microscop. Sci., 25: 29～35, pl. 1.

Cunningham, J. T. 1875. On the relations of the yolk to the gastrula in teleosteans, and in other vertebrate types. Quart. J. Microscop. Sci., 26 (1): 1～38, pls. 1～4.

Gamo, H. 1961a. On nutrient transmission through periblast into embryo of a teleost, the medaka, *Oryzias latipes*. Bull. Aichi Gakugei Univ., 10: 73～82, figs. 1～12.

Gamo, H. 1961b. On later addition of periblast nucleus in embryo of the Medaka, *Oryzias latipes*. Bull. Japan. Soc. Sci. Fish., 27 (3): 232～235, figs. 1～5.

Gamo, H. 1961c. Further report on nutrient transmission through periblast in the Medaka, *Oryzias* latipes. Bull. Japan. Soc. Sci. Fish., 27 (10): 893～896, figs, 1～10.

Gamo, H. and F. Araki. 1961. An in vivo observation of mitotic multiplication of periblast nuclei in embryo of the medaka, *Oryzias latipes*. Bull. Japan. Soc. Sci. Fish., 27 (10): 897～902, figs. 1～10.

His, W. 1876. Untersuchungen über die Entwicklung von Knochenfischen, besonders über die jenige des Salmons. Zeit. Anat. Entwick., 1 (1): 1～40, figs. 1～14, pls. 1～2.

Hoffmann, C. K. 1881. Zur Ontogenie der Knochenfische. Naturk. Verh. der Konik. Akademie Westens. Amsterdam, 21 (1): 1～168, pls. 1～7.

Hoffmann, C. K. 1888. Über den Ursprung und die Bedeutung der sogenannten "freien" Kerne in dem Nahrungsdotter bei den Knochenfischen. Zeit. Wiss. Zool., 46: 517～548, pl. 15.

Kamito, A. 1928. Early development of Japanese killifish (*Oryzias latipes*), with notes on its habitats. J. Coll. Agr. Tokyo Imp. Univ., 10 (1): 21～38, figs. 1～8.

Kanoh, Y. 1949. On the early development of egg of the dace, *Tribolodon hakuensis hakuensis* (Günther), with special reference to the periblast. Zool. Mag. (Tokyo), 58 (6): 91. (In Japanese).

Kingsley, J. S. and H. W. Conn. 1883. Some obsevations on the embryology of the teleosts. Mem. Boston Soc. Nat. Hist. Boston, 3: 183～212, pls. 14～16.

Klein, E. 1876. Observations on the early development of the common trout (*Salmo fario*). Quart. J. Microscop. Sci., 16: 113～131, pl. 4.

Kopsch, Fr. 1911. Die Entstehung des Dottersackentblast und die Furchung bei der Forelle (*Salmo fario*). Arch. Mikro. Anat., 78: 618∼659, figs. 1∼16.

Kupffer, C. 1868. Beobachtung über die Entwicklung der Knochenfishce. Arch. Mikro. Anat., 4: 209∼272, pls. 16∼18.

Lereboullet, M. 1854. Travail d'embryologie comparée sur le dévéloppement du Brochet, de la Perche et de l'Ecrevisse. Ann. Sci. Nat., 4 serie, 1: 237∼289.

Lereboullet, M. 1861. Recherches d'embryologie comparée sur le dévéloppement de la Truite, du Lezard et du Limnee. Ann. Sci. Nat., 4 serie, 16: 113∼192, pls. 2∼3.

List, J. H. 1887. Zur Entwicklungsgeschichte der Knochenfische (Labriden) I. Morphologische Ergebnisse. Zeit. Wiss. Zool., 45: 595∼645, figs. 1∼9, pls. 31∼33.

Long, W. L. 1980. Proliferation, growth, and migration of nuclei in yolk syncytium of *Salmo* and *Catostomus*. J. Exp. Zool., 214: 333∼342, figs. 1∼12.

Mahon, E. F. and W. S. Hoar. 1956. The early development of the chum salmon, *Oncorhynchus keta* (Walbaum). J. Morph., 98 (1): 1∼47, pls. 1∼8.

Nishida, H. 1958. The periblast nucleus in the dog-salmon embryo, *Oncorhynchus keta* (Walbaum). Sci. Rep. Hokkaido salmon Hatchery, 12: 37∼44, figs. 1∼11. (In Japanese with English summary).

Oellacher, J. 1872. Beiträge zur Entwicklungsgeschechte der Knochenfische, nach Beobachtungen am Bachforelleneie. Zeit. Wiss. Zool., 22: 373∼421, pls. 32∼33.

Reinhard, W. 1898. Die Bedeutung des Periblastes und Kupfferschen Blase in der Entwicklung der Knochenfische. Arch. Mikro. Anat., 52: 793∼820, pls. 35∼36.

Ryder, J. A. 1882. A contribution to the embryology of osseous fishes, with special reference of the development of the cod (*Gadus morrhua*). Rep. U.S. Fish. Comm., 455∼605, pls. 1∼12.

Saito, S. 1950. The embryological study of fishes. 1. General observation on the early development of the dog salmon, *Oncorhynchus keta* Walbaum. J. Fac. Agri. Hokkaido Univ., 48 (3): 267∼289, pls. 1∼8.

Samassa, P. 1896. Studien über Einfluss des Dotters auf die Gastrulation und die Bildung der Primaren Keim-blätter der Wirbeltiere. III. Teleostier. Arch. Ent.-Mech., 3: 191∼218, pls. 12∼13.

Shimizu, M. and J. Yamada. 1980. Ultrastructural aspect of yolk absorption in the vitelline syncytium of the embryonic rockfish, *Sebastes schlegeli*. Japan. J. Ichthyol., 27 (1): 56∼63, figs. 1∼13.

Trinkaus, J. P. 1951. A study of the mechanism of epiboly in the egg of *Fundulus heteroclitus*. J. Exp. Zool., 118: 269∼319, figs. 1∼7.

Trinkaus, J. P. 1971. The role of the periblast in *Fundulus* epiboly. Ontogenesis, 2: 401∼405. (In Russian with English summary).

Van Beneden, E. 1878. A contribution to the history of the embryonic development of the teleostean. Quart. J. Microscop. Sci., 18: 41∼57, pl. 4.

Van Kowalewskii, M. 1886. Über die ersten Entwicklungsprocesse der Knochenfische. Zeit. Wiss. Zool., 43: 434∼483, pl. 18.

Walzer, C. and N. Schönenberger. 1979a. Ultrastructure and cytochemistry study of the yolk syncytial layer in the alevin of trout (*Salmo fario trutta* L.) after hatching. I. The vitellolysis zone. Cell Tissue Res., 196: 59∼73, figs. 1∼16.

Walzer, C. and N. Schönenberger. 1979b. Ultrastructure and cytochemistry of the yolk syncytial layer in the alevin of trout (*Salmo fario trutta* L. and *Salmo gairdneri* R.) after hatching. II. The cytoplasmic zone. Cell Tissue Res., 196: 75∼93, figs. 1∼4.

Wenckebach, K. F. 1886. Beiträge zur Entwicklungsgeschichte der Knochenfische. Arch. Mikro. Anat., 28: 225∼250, pls. 16∼17.

Wilson, H. V. 1891. The embryology of the sea bass (*Serranus atrarius*). Bull. U. S. Fish Comm., 9: 209∼278, figs. 1∼12, pls. 88∼106.

Yamada, J. 1959a. The structure of the yolk sac and the absorption of the yolk in the pond smelt fry. Bull. Fac. Fish. Hokkaido Univ., 10: 196∼203, pls. 1∼3.

Yamada, J. 1959b. On the vitelline syncytium and the absorption of the yolk in the fry of two salmonids. Bull. Fac. Fish. Hokkaido Univ., 10: 205∼210, pls. 1∼2.

Yamamoto, M. 1967. Electron microscopy of fish development V. The fine structure of the periblast in *Oryzias latipes*. J. Fac. Sci. Univ. Tokyo, ser. 4, 10: 483∼490, pls. 23∼28.

Yamamoto, K. 1981. Cleavage pattern and formation of the blastocoel in the egg of the eel, *Anguilla japonica*. Japan. J. Ichthyol., 27 (4): 301∼308, figs. 1∼2.

Ziegler, H. F. 1896. Die Entstehung des Periblastes bei den Knochenfischen. Anat. Anz., 12: 353∼370, figs. 1∼12.

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

ウナギの卵の周縁質

山本喜一郎

ウナギの卵では周辺部の周縁質は分割前の受精卵で既に認められるが，胚下周縁質は胞胚期の卵で初めて形成される．周縁質の核は胚盤の周辺および底部の細胞から由来される．それらの核は胞胚期の間は有糸分裂により活発に増殖するが，原腸胚に達すると無糸分裂により増殖を続け，終局的に周縁質は卵黄全体を包む多核質構造の薄い細胞質層となる．周縁質は胚体の形成に直接関与しないが，胚が卵黄を消費する際重要な役割を演ずるものと考えられる．

(041　函館市港町 3-1-1　北海道大学水産学部)