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Development and ultrastructure of larval skin in *Scophthamus maximus*

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Abstract: The histological development and ultrastructure of larval turbot skin were studied by both light and transmission electron microscopes. The early larvae showed a complete bisymmetry of skin. The skin of 1- 25 days old larvae developed slowly and was composed of the thin epidermis of 1- 2 layers epidermal cells and undeveloped dermis before the metamorphosis. But the skin developed significantly when the metamorphosis began. Till the completion of metamorphosis (about 60 days old), the skin contained 3- 4 layers epidermal cells and very developed collagenous strata. With the process of metamorphosis, the ocular side skin and blind side skin became different in ultrastructure. Ultrastructural observation showed the epidermis of turbot contained three types of epidermal cells: filament-containing cells, mucus cells and chloride cells. The ocular side epidermis with looser structure consisted one type of filament-containing cells, but the blind side epidermis was structured densely and two types of filament-containing cells were observed in it. Melanophores, iridophores, fibroblasts and other cell types and tissues were found in the spongiosum of dermis. The distribution of melanophores depended on the larval developing stage and the pigmentation of skin. The differentiation of skin seems to be an adaptation for the change of life style of turbot larvae from pelagic living to benthonic living after the metamorphosis.

Key words: *Scophthamus maximus*; larvae; skin; development; ultrastructure
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1 Introduction

The turbot *Scophthamus maximus* (Linnaeus), belonging to the family Bothidae, the genus *Scophthamus*, lives originally in Baltic, Black and Mediterranean Seas. Since 1960's, this turbot has become an important fish for mariculture in the world[1]. It is well known that one of the commonest defects in hatchery-reared flatfish is malpigmentation, especially the albinism. Albinism is characterized with white patches or areas devoid of normal pigmentation on the surface of ocular side skin. Hatchery-reared turbot shows a very high proportion of albinism, sometimes more than 50%, and extreme albinism reduces the commercial price[2]. This abnormality is one of major problems in turbot culture. In past years, many studies on the occurrence of albinism have been carried out in relation to environmental, genetical and nutritional factors. These studies were mainly focused on the Japanese flounder *Paralichthys olivaceus* by Japanese researchers[3,4]. One important aspect for understanding the mechanism

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of albinism is to get more knowledge of development and structure of skin. In early 1970's, Robert reported the development and structure of skin of plaice *Pleuronectes platessa* from hatching larvae to 4 years old fish[5, 6]. Suzuki also described the ultrastructure of blind side skin of hatchery-reared Japanese flounder in 1994[7]. However, few reports have been available on development and ultrastructure of larval turbot skin. The present study described the histological development of turbot skin in its early life stage and the ultrastructure of its larval skin to provide some helpful information for turbot mariculture and control of its albinism.

2 Materials and Methods

2.1 Samples

Fertilized eggs of the turbot were obtained and reared in feeding tanks of Huaxin Marine Product Corporation in Weihai, Shandong, China. The salinity was 34, and the temperature was in a range of 15- 18℃. 10 individuals of 0- 60 days old larval turbot were collected respectively every five days for histological observation on the development of skin by light microscope. The turbot larvae (30.0 mm in average total length, 40 days old) at the climax metamorphosis, which showed normal, albinic and black body color, were sampled for ultrasturctural observation of skin by electron microscopic study.

2.2 Light microscopic study

The samples in every stage were dehydrated by ethanol gradually and embedded in paraffin. Continuous transverse sections of skin (4 μm in thickness) were made, stained with hematoxylin and eosin, and then examined by light microscope. The sections of middle portion of trunk of fish were chosen to observe the structure of skin in different developmental stage. Photography was taken as required.

2.3 Electron microscopic study

Two pieces of skin were removed from the same region on ocular side and blind side of live fish. The skin pieces were transected into 1- 2 mm^2 smaller ones and were prefixed with 3% glutaraldehyde in phosphate buffer of pH 7.2 at 4℃ for 12h. After rinsing, they were postfixed with 1% osmium tetroxide in phosphate-buffer at 4℃ for another 12h. The fixed tissues were dehydrated gradually with ethanol and embedded in Epon 812. Then ultrathin sections of skin (600Å) were cut, double stained in uranyl acetate and lead citrate, and then examined with H7000 Hitachi electron microscope. Photography was taken at 2000 to 15000 times.

3 Results

3.1 Development of larval turbot skin

The skin of 1 day old larvae was a single layer of epithelial cells. Many mucous cells were located regularly in the surface of epidermis. No obvious dermis was observed (Plate Ⅰ- 1). From 1 to 10 days after hatching, the skin developed very slowly. The skin of 10 days old larvae was still composed of one layer of epidermal cells. Numerous larval-type melanophores were found under the epidermis (Plate Ⅰ- 2). From 15 days after hatching, the structure of skin became complex and dense, but the thickness of skin increased slowly until 25 days. Many flask-shaped mucus cells were located in the outer layer of epidermis of 15 days old larvae (Plate Ⅰ- 3). The epidermis of 25 days old larvae was comprised of 2- 3 layers of densely arranged epidermal cells. The plasma membrane of outer epidermal cells formed many microridges which arranged regularly on the surface of skin. The collagenous strata were constructed loosely. Some large larval melanophores were located evenly

under the epidermis (Plate Ⅰ - 4). No difference in structure was observed between both sides of skin of larval body in this stage. During the process of metamorphosis, the thickness of skin increased significantly, and especially the dermis developed quickly (Plate Ⅰ - 5). Till the metamorphosis completed (about 60 days after hatching), the epidermal cells became flattened and densely arranged, the collagenous strata became so developed that they reached to 3- 4 times of the epidermis in thickness (Plate Ⅰ - 6). The changes of the skin thickness showed similar trend to changes of total length and total width of turbot larvae (Fig. 1- A, B).

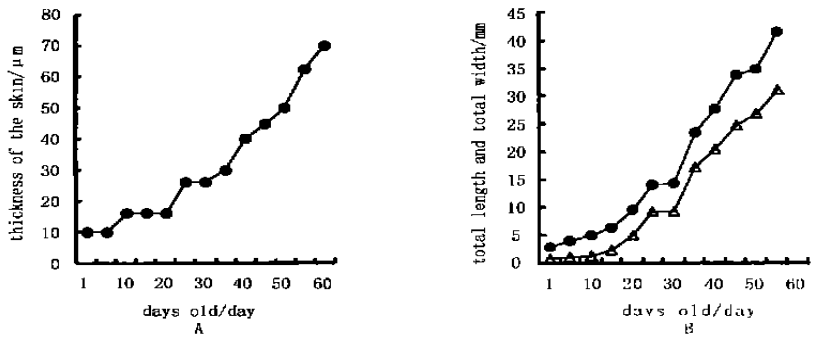

Fig. 1 A: Change of thickness of larval turbot skin;
B: Change of total length (●) and total width (△) of larval turbot

3.2 Ultrastructure of larval turbot skin

3.2.1 Epidermis

TEM observation revealed that the ultrastructure of turbot larvae skin was similar to that of other flatfish. The epidermis consisted of three types of epidermal cells: mucus cells, filament-containing cells and chloride cells. Filament-containing cells were by far the most numerous.

The ocular side skin and blind side skin became different in ultrastructure in the process of metamorphosis. As shown in Plate Ⅱ- 1, filament-containing cells in ocular side epidermis of 40 days old larvae were irregular in shape and densely stained. Large nucleus was located centrally in the cytoplasm. The plasma membrane of free surface filament-containing cells formed a series of cavities and numerous microridges which were covered by a fuzzy fibrilla on the surface of skin. Their cytoplasm contained a large number of microfilaments, numerous rough-surfaced endoplasmic reticula, free ribosome, a few mitochondria, Golgi apparatus and many vesicles. The filament-containing cells, which constructed the superficial layer of epidermis, became more regular oval-shaped. The epidermal cells were linked with neighboring cells by developed interdigitation and numerous desmosome. In the epidermis near the basal membrane, ball-shaped chloride cells were observed (Plate Ⅱ- 2). The cytoplasm of chloride cell was occupied by numerous mitochondria and a three-dimensional developed tubular system, in which the sodium and chloride ions exchange occurred. Some sediment was observed in lumens of tubular system. The nucleus was stained densely. The plasma membrane of chloride cells often formed an apical pit exposed to the external environment, and had flexural channels through the basal membrane to connect with the interior environment. But the apical pit was not observed in this section. No melanophore and blood capillary were found in the epidermis.

Blind side epidermis was mainly composed of two different types of filament- containing cells (Plate Ⅱ- 3). The filament-containing cells, which constructed the outer layer of epidermis, were quadrate-shaped and contacted with each other with powerful desmosome. A series of microridges were observed on the free surface of

these cells and covered with a thin layer of cuticle. The cytoplasm and nucleus were stained lightly. A large number of microfilaments, rough-surfaced endoplasmic reticula, a few mitochondria, free ribosome, some small vacuoles and many Golgi apparatus located centrally were found in the cytoplasm (Plate Ⅱ-4). It was noted that the number of Golgi apparatus was more than that of other epidermal cells. The number of mitochondria and vesicles in their cytoplasm was less than that of filament-containing cell in ocular side skin. The other type of filament-containing cells was located deeper in the epidermis. They were stained densely and similar to the filament-containing cells in ocular side skin in morphology and ultrastructure, but these cells arranged more orderly and densely. Their cytoplasm was denser in structure and few vesicles were found (Plate Ⅱ-3). Blind side skin was obviously denser than ocular side skin in structure.

3.2.2 Dermis

The dermis of larvae was composed of developed spongiform strata and dense collagenous strata. The subepidermal spongiform strata were full of loose colloid fibres. Melanophores, iridophores, fibroblasts, blood capillaries, myeloid bodies and other types of cells and tissues were observed in these strata. TEM observation revealed three kinds of melanosomes distribution status in normal, dark-colored and albinic ocular side skin of larvae at the climax metamorphosis (Plate Ⅲ-1, 2, 3). Numerous melanosomes distributed in the dermis of normal skin, while in the dermis of dark-colored larvae, large melanophores were observed and a great number of melanosomes in high density were found in its cytoplasm. The nucleus of melanophore was small and lightly stained. In albinic skin, a few melanosomes were observed. No melanophore was found in blind side skin of 40 days old normal larvae (Plate Ⅱ-3).

The iridophores with a flat, rod-like shape and stained densely cytoplasm were also observed in the spongiform strata of the dermis (Plate Ⅱ-5). Its cytoplasm contained piles of thin flat crystals, which were located in parallel to each other and to the planar surface of the cell. Each crystal was closely invested by a thin crystal chamber membrane, and lay in a crystal sac formed by an undulatory unit membrane. The nucleus of iridophore was located laterally. Many mitochondria, Golgi apparatus, small vesicles, endoplasmic reticula, and some microtubules were observed in the cytoplasm. A few of fibroblasts with large nucleus and poor cytoplasm surrounded the iridophores. The endothelium of blood capillaries found in the dermis was well developed. The very developed dense collagenous strata was structured with a great number of colloid fibres, which were stratified and overlapped densely in crossways. Some fibroblasts were also found among the colloid fibres (Plate Ⅱ-6).

4 Discussion

The present study showed that the skin of early larval turbot developed very slowly before the metamorphosis. There was no difference between both sides of skin of larval body in this stage. At the onset of metamorphosis, the skin began to develop quickly, including the proliferation of epidermal cells and the development of the dermis. Following the metamorphosis, the ocular side and blind side skin became different in ultrastructure. The ocular side skin was mainly composed of one type of filament-containing cells in which many vesicles existed. But in the blind side skin, two types of filament-containing cells, which had not been reported, were arranged densely and orderly. The surface of blind side skin was covered with a thin layer of cuticle. This structural difference in both side of skin was owing to the change of life style of turbot larvae from pelagic life style to benthonic life style after metamorphosis. The blind side skin might contact the sea bottom; so tough and smooth structure of skin was needed.

Typical chloride cells with numerous mitochondria and developmental tubular system were found in epidermis of turbot larvae. Chloride cell was regarded as sodium secretion cell of fish. They often were found in the gill of almost all kinds of euryhaline teleosts[8]. Skin chloride cells of flatfish were also described in some reports[7].

But TEM observation showed the skin chloride cells of turbot were similar to those gill chloride cells of salmon[8] or pupfish[9] in ultrastructure.

According to the different pigmentation status of turbot larvae, the number of melanosomes in the ocular side skin varied largely. It was well known that albinism occurred in the early developmental stage of flatfish[10], especially in the period of metamorphosis. Our previous research on the distribution of melanophores in the skin of turbot larvae also showed that the albinism began to form gradually at the onset of metamorphosis[11]. The present study revealed that albinism had formed at the climax metamorphosis, so the factors that induce albinism might be effective before the metamorphosis. Thus we might pay more attention to the living environment, nutrition or other fields of early turbot larvae before metamorphosis. Suzuki[7] had reported that the melanophores and iridophores coexisted in the dermis of Japanese flounder larvae. But in the dermis of turbot larvae, the iridophore was located in the spongiosum isolatedly; it was not accompanied with melanophores. The further research will be carried out in the future for understanding the mechanism of albinism.

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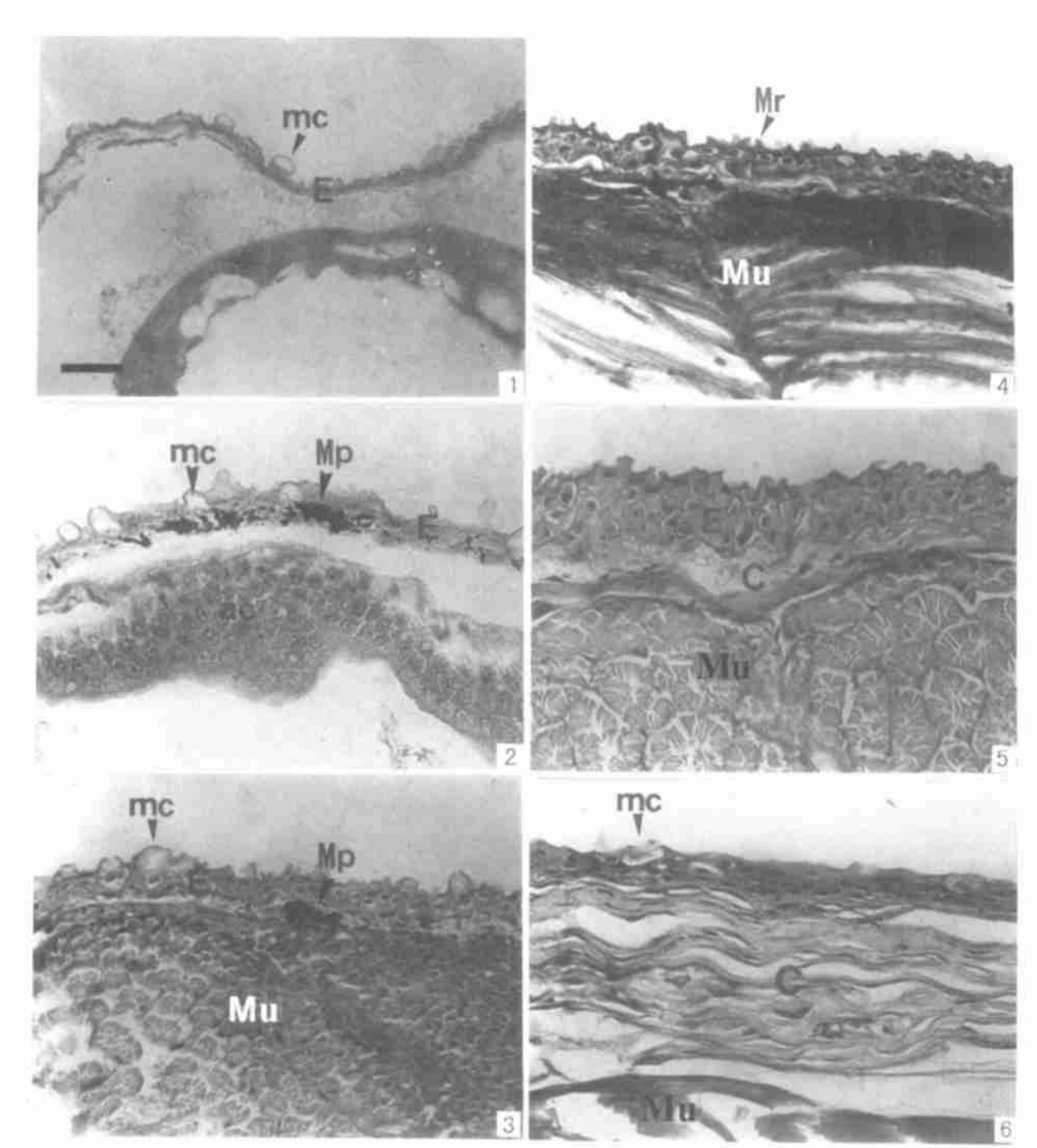

Plate Ⅰ Three kinds of melanosome distribution status in ocular side skin of turbot larvae

1. Skin of 1 d turbot larvae; 2. Skin of 10 d larvae; 3. Skin of 15 d larvae; 4. Skin of 25 d larvae; 5. Ocular side skin of 40 d larvae; 6. Ocular side skin of 60 d larvae. Scale bar= 20 μm

E: epidermis, C: subepidermal collagenous strata, Mp: melanophore, Mu: muscle, mc: mucus cell, Mr: microridge, ac: alimentary canal

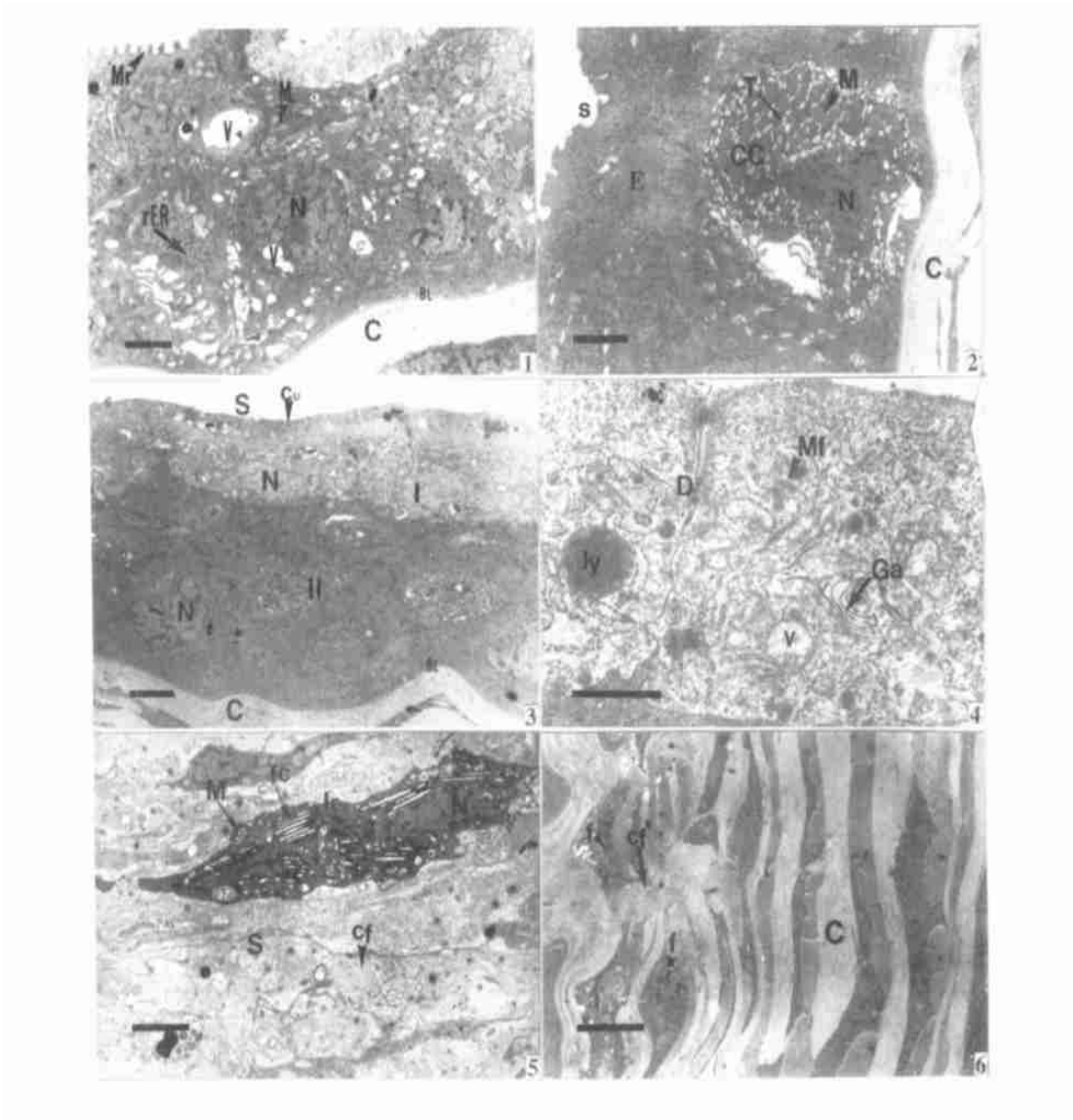

Plate Ⅱ Electron micrographs of the ocular side and blind side skin of turbot larvae

1. Electron micrograph of ocular side skin, scale bar= 2 μm; 2. Magnified view of chloride cell in the ocular side epidermis, scale bar = 2 μm; 3. Electron micrograph of the blind side skin containing two types of filament-containing cell, scale bar= 2 μm; 4. Magnified view of the filament-containing cell stained lightly in blind side epidermis, scale bar= 1 μm; 5. Iridophore in the spongiform strata of dermis, scale bar= 2 μm; 6. Developed cross stratified collagenous strata, scale bar= 5 μm

C: subepidermal collagenous strata, BL: basement lamella, N: nucleus, V: vesicle, M: mitochondria, Mr: microridge, rER: rough surfaced endoplasmic reticular, S: surface of skin, E: Epidermis, CC: chloride cell, T: tubular system, Cu: cuticle, I: type Ⅰ filament-containing cell stained lightly, II: type Ⅱ filament-containing cell stained densely, Mf: microfilament, Ga: Golgi apparatus, D: desmosome, ly: lysome mucus cell, cf: collagenous fibre, Ir: iridophore, fc: flat crystal, f: fibroblast

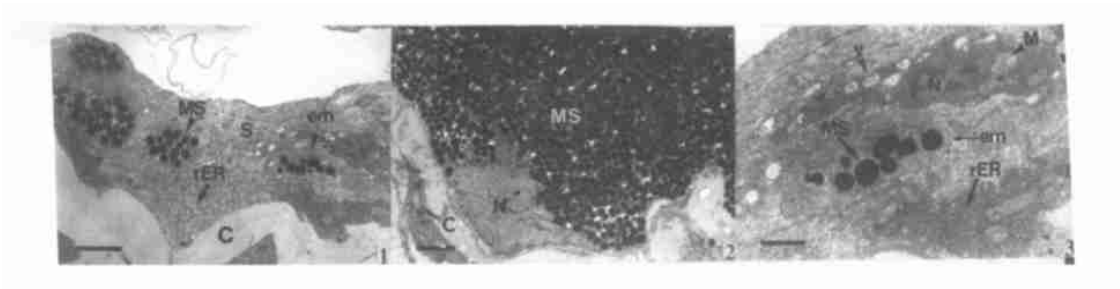

Plate Ⅲ Cross sections of skin in middle part of the trunk of normal turbot larvae in different developmental stages

1. Melanosme distributed in the normal ocular side dermis, scale bar= 2μm; 2. Melanosome distribution in the dark-colored ocular side dermis, scale bar= 2μm; 3. Melanosome distribution in albinic ocular side dermis, scale bar= 1μm.
C: collagenous strata, S: spongiform strata; MS: melanosome, em: endoplasmic membrane, M: mitochondria, N: nucleus, V: vesicle, rER: rough surfaced endoplasmic reticulum

大菱鲆仔鱼皮肤发育及超微结构

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摘要: 利用光镜和透射电镜技术对大菱鲆仔鱼皮肤的发育及超微结构进行了观察。大菱鲆早期仔鱼鱼体两侧皮肤结构完全对称。变态前仔鱼的皮肤发育缓慢, 1~25d仔鱼的皮肤由1~2层表皮细胞和不发达的真皮层组成。变态开始后, 皮肤的发育速度明显加快。至变态结束(60 d左右), 仔鱼的皮肤由3~4层表皮细胞和发达的皮下胶原层组成。随着变态发育, 大菱鲆仔鱼有眼侧皮肤与无眼侧皮肤结构上出现差异。超微结构观察显示大菱鲆的表皮包括3种表皮细胞: 含微丝细胞, 黏液细胞和氯细胞。有眼侧表皮细胞胞质内有大量空泡结构, 表皮结构较疏松, 仅有1种含微丝细胞。无眼侧表皮细胞胞质密度较高, 表皮细胞排列紧密, 具有两种结构和电子密度各异的含微丝细胞。在真皮层的疏松结缔组织层中有黑色素胞、虹彩色素胞, 纤维芽细胞和其他类型的细胞及组织分布其中, 黑色素胞中色素颗粒的密度及分布随仔鱼的发育及皮肤的着色状况而发生变化。这种伴随变态而产生的鱼体两侧皮肤结构的差异, 是大菱鲆仔鱼由浮游生活转入底栖生活的一种生态学适应。

关键词: 大菱鲆; 仔鱼; 皮肤; 发育; 超微结构
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