MORPHOLOGICAL AND FUNCTIONAL STUDIES ON THE EPIDERMAL CELLS OF AMPHIOXUS (BRANCHIOSTOMA BELCHERI TSINGTAUENSE) AT DIFFERENT DEVELOPMENTAL STAGES

MAO Bing-yu(毛炳宇), SUN Xiao-yang(孙晓阳), ZHANG Hong-wei(张红卫)**

(Department of Biology, Shandong University, Jinan 250100)
ZHANG Shi-cui(张士璀), WU Xian-han(吴贤汉)
(Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071)

Received Apr. 27, 1996; revision accepted Jan. 21, 1997

Abstract

Epidermal cells of amphioxus at different developmental stages were investigated by electron microcopy and colloidal carbon tracing experiments. Amphioxus epidermal cells showed different ultrastructural characteristics at larval and adult stages. The epidermal cells at all larval stages studied (24–96 h) had numerous vesicles containing electron dense materials in their apical cytoplasm. In tracing experiments, carbon particles were found in apical vesicles and intercellular spaces. Under scanning electron microscope, many crater-like protrusions were observed on the surface of the cells. These results indicated that amphioxus larval epidermal cells may be capable of endocytosis. The epidermal cells of 3-month and adult amphioxus were obviously secretory ones characterized by well-developed peripheral filaments, a prominent Golgi apparatus and abundant apical secretory vesicles. This study also showed that adult amphioxus body surface mucus contained lectin that could agglutinate human red blood cells. The authors propose that the epidermal cells of amphioxus larva and adult may contribute to the immune defense of the animal by different means.

Key words: amphioxus, epidermal cell, ultrastructure, function

INTRODUCTION

The vertebrate epidermis is in intimate contact with the environment and reflects environmental adaptions. The epidermis of terrestrial animals is keratinized while that of amphibious and aquatic forms is related to mucus—production. The epidermis acts as a final barrier between the organism and its environment and one of its main functions is to protect the organism against external pathogens. In addition to mechanical protection of the body, the epidermis of amphibians and fishes can secret lectins and other antibacterial peptides that help in the immune defense of the animal (Suzuki and Otake, 1994; Bevins and Zasloff, 1990).

Amphioxus, a cephalochordate, is an important animal in the study of chordate phylogeny. The structure and function of amphioxus epidermis is of great importance from the view of descriptive and comparative anatomy. Olsson (1961) was the first to report the ultrastructural and histochemical characteristics of adult amphioxus epidermis. In order to study the nature of amphioxus larval tail fin-rays, Flood (1975) examined the fine structure of epidermal cells of the tail of two-week old larva. The cell junctions in adult amphioxus epidermis were also reported (Baskin, 1975; Lane et al., 1987; Welsch1983). However, there are few works on the structure of earlier stages amphioxus epidermal cells and no

* Project 3860811 supported by NSFC and study also supported by the Shandong Natural Science Foundation (Grant No. 92D1144).
** To whom correspondence should be addressed.
functional information is available. To investigate the structure and function of amphioxus epidermis in a developmental context, we examined the fine structure of epidermal cells of different development stages amphioxus and obtained evidences that amphioxus epidermis may serve in the self-defense system of the animal.

MATERIALS AND METHODS

1. Materials

Adult amphioxus (Branchiostoma belcheri tsingtauense) were collected from the vicinity of Qingdao, China. Twenty-four to ninety-six hours amphioxus larvae and 3-month young, amphioxus were obtained by artificial fertilization and culture in the laboratory of the Institute of Oceanology, Chinese Academy of Sciences.

2. Transmission electron microscopy (TEM)

The specimens were fixed in a mixture of 2% glutaraldehyde and 2% paraformaldehyde (Zhang et al., 1992) for 2 hours and postfixed in 1% osmium tetroxide for 1 hour. Following dehydration with graded ethanols, tissue blocks were embedded in Epon 812. Ultrathin sections, stained with uranyl acetate and lead citrate, were observed under a JEM-100CX electron microscope.

3. Scanning electron microscopy (SEM)

The specimens were fixed as above, dehydrated with ethanol and dried by the critical point dry method. Observations were made under a JSM-35C scanning electron microscope.

4. Tracer impregnation

Thirty-six and forty-eight hours amphioxus larvae were exposed to Chinese ink (about 2%, v/v) for 30-40 minutes before fixation and preparation for TEM observations.

5. Skin mucus collecting, hemagglutilation and inhibition test

Mucus was collected from the body surface of twenty adult amphioxus and resolved in 1 ml 0.1 mol/L Tris-HCl buffer (pH 7.4). After centrifugation, the supernatant was used as samples for hemagglutinating tests. Serial two-fold dilutions of the sample (50μl) were made in a V type multiwell microtiter plate using 0.9% saline as diluant. To each well, 50μl 2%(v/v) human erythrocytes in 0.9% saline were added and the agglutination titer (the highest dilution giving positive agglutination) was read after incubation for 1 hour at 37°C. In inhibition tests, the samples(25μl) were mixed with various concentrations of sugars (25μl) before the erythrocyte suspension was added to test the agglutinating activity. The following sugars (100 mmol/L) were used: D-galactose, L-arabinose, D-mannose, D-glucose, D-rhamnose and L-sorbose.

RESULTS

1. Ultrastructure of amphioxus larval epidermal cells

The amphioxus larval stage was considered to begin at about 24 hours after fertilization (Hirakow and Kajita, 1994) and lasts about 50 days in this species (Wu et al., 1994). At 24-hour of development, the epidermal cells were columnar with a spherical or irregular-shaped nucleus which usually located in the basal part of the cells. Abundant yolk granules could still be seen in the cytoplasm at this stage, but were much less dense than previous stages (Hirakow and Kajita, 1994). A few rough endoplasmic reticulum and mitochondria distributed sparsely in the dense cytoplasm. A cilium, short microvilli and