Zoology 161 (2023) 126129

Contents lists available at ScienceDirect

Zoology

journal homepage: www.elsevier.com/locate/zool

Ontogenetic dynamics of the nudibranch epithelium in *Onchidoris muricata* (O.F. Müller, 1776)

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ARTICLE INFO

Keywords:
Spicule
Sclerocyte
Doridina
Ultrastructure

ABSTRACT

The integumentary system is the set of organs forming the outermost layer of an animal's body. It comprises the epithelium, muscles, and elements of connective and nerve tissue. The integument acts as a physical barrier between the external environment and the internal environment that serves to protect and maintain the body of the animal. The body of nudibranch mollusks undergo significant changes during ontogenesis, with the subepidermal space changing as the mollusk grows. As the extracellular subepidermal matrix is modified, the number of collagen fibers increases, muscles and nerves develop, and calcite spicules appear and grow. Yet, specific knowledge pertaining to the transformation of the epithelium is absent. In the present work, the ontogenetic dynamics of the surface epithelium of nudibranch mollusks are traced for the first time using *Onchidoris muricata* (O. F. Müller, 1776) during the postlarval stages of development. Ontogenetic changes in the epithelium of *O. muricata* were studied using a complex set of morphological methods. According to our data, the degree of modification to the epithelium in ontogenesis depends on individual body parts and is not consistent throughout. First x-cells were recognized as the probable precursors to sclerocytes.

1. Introduction

Doridina is a unique group of nudibranch mollusks whose notum contains subepidermal calcite spicules (Thompson, 1961; Penney et al., 2018; Nikitenko et al., 2021). The notum of mollusks is significantly transformed at the postlarval stages of development (Thompson, 1961; Goddard, 2005; Nikitenko et al., 2021), where spicules and a collagen matrix are developed in the subepidermal space (Nikitenko et al., 2021). However, there is no information on how the epithelium is transformed. This work describes the reorganization of the surface epithelium in *Onchidoris muricata* (O. F. Müller, 1776) during post-larval stages of development.

The development of doridins occurs with the veliger larval stage (Bickell and Chia, 1979; Chia and Koss, 1978; Goddard, 2005; Thompson, 1961). The body of the dorid veliger is protected by a shell, which is lost during metamorphosis (Thompson, 1961; Chia and Koss, 1978; Goddard, 2005). Shell loss may be a signal for the beginning of integument transformation. The integument plays an important role towards survival in metazoans by separating and protecting them from hostile environments. Its function ranges from protection against injury and infection, participation in the regulation of body temperature and water balance, respiratory activity, monitoring of the environment, and production of signals related to behavior (Bereiter-Hahn et al., 2012). The integument in dorids is located directly in contact with the external environment in all post larval stages of development.

At present, the epithelium in adult nudibranchs has only been partially described in a few species of dorids (Skidmore and Rivera, 1982; Thompson, 1983; Wägele, 1997/98). The basis of the

Abbreviations: Ag, alveolar gland; Aj, adherens junction; Asg, apocrine secret gland; Bl, basal lamina; C, cilia; Chs, chitin spindle; Chv, chitin vacuole; Ci, concentric inclusion; Cr, striated roots.; Ct, ctenidia; E, eye; Ecm, extracellular matrix; Eg, epithelial gland; Ep, epithelia; Epr, endoplasmic reticulum; F, foot; Ga, Golgi apparatus; Gl, gland; Gr, secretory granule; Gs, gland secret; Ic, intercellular cell; Icm, intracellular collagen matrix; Inv, invagination; Lv, large vacuole; M, muscle; Mcc, multicellular cell; Mg, mucous gland; Msc, the membrane of sclerocyte; Mv, microvilli; Mvl, microvillar layer; Mvs, the membrane of sclerocyte vacuole; N, nuclei; Nv, nerve; Pr, protrusion of x-cell; R, rhinophore; Rl, rhinophore lamellae; Rp, rhinophore pocket; S, secrete; Sc, sclerocyte; Sg, subepidermal gland; Sj, septate junction; Sp, spicule; Sr, striated roots; T, tubercle; Tf, tonofilament; Tz, transition zone from notum to tubercle; V, vacuole; Vcf, x-cells vacuole with fine content; Vs, vesicle.

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https://doi.org/10.1016/j.zool.2023.126129
Received 3 May 2023; Received in revised form 24 August 2023; Accepted 19 October 2023
Available online 4 November 2023

1967; Kocot et al., 2017), Polyplacophora (Leise, 1984), Bivalvia (Harper et al., 2006)) are formed by cells of the integumentary epithelium, it is most likely that doridid sclerocytes also are ectodermal. Numerous protrusions of x-cells into the subepidermal space may indirectly confirm the participation of these cells in spiculogenes. We suggest that x-cells are sclerocytes in the early stages of formation in the epithelium. However, to test this hypothesis, further studies using a set of methods are required. Thus, the study of epithelia at different stages of development is also relevant for understanding spiculogenesis.

5. Conclusion

A detailed study of the epithelia in *O. muricata* during postlarval stages of development made it possible for the first time to note the ontogenetic variability in the morphology of epithelia. The degree of modification of the epithelium in ontogeny depends on the part of the body. However, they are most pronounced in that the notum, which in larvae, was protected by the protoconch.

The epithelial glands were classified for dorids. Two fundamental types of gland structure have been identified. There are glands with a large vacuole, and those with numerous granules inside.

Numerous vacuoles are present in the epithelium of the rhinophores and increase in number during ontogeny. It was noted that vacuoles with amorphous contents formed ordered rows, there are fewer vacuoles in the depth of the leaves and more vacuoles at the distal edge. For the first time, a positive calcoflour white color was shown, which may indicate that the content is unpolymerized polysaccharide, could be chitin. However, our first ultrastructural data on vacuoles with amorphous contents of dorids are extremely similar to the ultrastructure of single bacteria. Thus, the chemical nature of the content has not been determined.

In the present work, unique x-cells are described in detail. These cells form protrusions into the subepidermal space and look like sclerocytes in their early stages of formation. Numerous protrusions were found in individuals whose spicules were at the stage of formation. This confirms the above assumption that x-cells belong to sclerocytes.

Thus, questions about the chemical nature of the contents of vacuoles in the epithelium of dorids rhinophores remain open. More x-cell studies are needed to test for their involvement in spiculogenesis.

Funding

This work was supported by the Russian Science Foundation, Grants no. 21-14-00042.

Declaration of Competing Interest

The authors report no conflict of interest.

Data Availability

Data will be made available on request.

Acknowledgements

We are grateful to Dr. B.C. Gonzalez for the invaluable help with the English. We would like to thank our colleagues of the Pertsov White Sea Biological Station and the Zoology Department of Lomonosov Moscow State University. We thank A.A. Semenov, D. Ozerov, G.D. Kolbasova, A. L. Mikhlina, and A.A. Prudkovsky for helping with the sampling. We want to thank the unknown reviewers for their most helpful comments on the typescript and any fruitful discussion. We are very thankful to I.A. Kosevich, A.I. Lavrov, E.N. Temereva, F.V. Bolshakov, and I.A. Ekimova for their assistance with the morphological methods and experimental protocols. Sincere appreciation goes to G. Davidovich, A. Bogdanov, and the Electron Microscopy Laboratory of the Shared Facilities Center of Lomonosov Moscow State University, sponsored by the RF Ministry of Education, Science and Research. The authors would also like to thank S. Metelev, G. Bykov, and I.D. Papanin from the Institute for the Biology of Inland Waters, Russian Academy of Sciences for their help with the electron microscopy. Light microscopy was made possible at the Center of microscopy, WSBS, MSU.

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