The Comparative Embryology of Sponges

Alexander V. Ereskovsky

The Comparative Embryology of Sponges

Springer

Alexander V. Ereskovsky
Department of Embryology
Biological Faculty
Saint-Petersburg State University
Saint-Petersburg
Russia
aereskovsky@mail.ru

Originally published in Russian by Saint-Petersburg University Press

ISBN 978-90-481-8574-0 e-ISBN 978-90-481-8575-7
DOI 10.1007/978-90-481-8575-7
Springer Dordrecht Heidelberg London New York

Library of Congress Control Number: 2010922450

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

It is generally assumed that sponges (phylum Porifera) are the most basal metazoans (Kobayashi et al. 1993; Li et al. 1998; Mehl et al. 1998; Kim et al. 1999; Philippe et al. 2009). In this connection sponges are of a great interest for EvoDevo biologists. None of the problems of early evolution of multicellular animals and reconstruction of a natural system of their main phylogenetic clades can be discussed without considering the sponges. These animals possess the extremely low level of tissues organization, and demonstrate extremely low level of processes of gametogenesis, embryogenesis, and metamorphosis. They show also various ways of advancement of these basic mechanisms that allow us to understand processes of establishment of the latter in the early Metazoan evolution.

The position of Porifera within the animal kingdom has been problematic since the last decades of the nineteenth century. Due to the limited number of morphological/cytological characteristics, no much conclusive or plausible decision about the phylogenetic position of the sponges could be made. In the literature there were two opposite points of view on position of sponges in the system of Eukaryotes. Some researchers, since Balfour (1879) considered sponges as an independent direction in the evolution of Metazoans, which has arisen irrespective of others (Sollas 1884; Delage 1892; Minchin 1900; Livanov 1955; Hadži 1963; Fedotov 1966; Schulman 1974; Salvini-Plaven 1978; Zhuravleva and Miagkova 1987; Seravin 1997). The opposite point of view belongs to the authors considering the sponges and the true Metazoa as descendants of a common ancestor (Haeckel 1874; Lévi 1956; Beklemishev 1964; Brien 1967c; Brien 1973a; Ivanov 1968, 1971; Tuzet 1970; Hooper et al. 2002; Nielsen 2008).

Only the introduction of molecular phylogeny techniques rapidly increased our understanding of evolutionary processes, and definitively confirmed monophyly of Porifera and other Metazoa (Kobayashi et al. 1993; Müller 1997b, 1998; Kim et al. 1999; Lang et al. 2002; Lavrov et al. 2008; Philippe et al. 2009). At the same time, contradictory and often poorly supported trees have been proposed, leaving major issues such as the phylogenetic status of sponges – monophyletic (Müller 2001; Lavrov et al. 2008; Philippe et al. 2009) or paraphyletic unresolved (Cavalier-Smith et al. 1996; Zrzavy et al. 1998; Collins 1998; Adams et al. 1999; Medina et al. 2001; Borchiellini et al. 2001, 2004a; Peterson and Eernisse 2001; Manuel et al. 2003; Sperling et al. 2007; Robertson et al. 2009).

Attempts to understand the reason of an originality of the organization and biology of sponges were repeatedly done. It has been shown that specificity of different stages of sexual development of sponges, as the gametogenesis, along with the embryogenesis, larval development, and the metamorphosis are closely connected with a low level of tissues organization and their multifunctionality, with a high adaptive capability (Maas 1894; Delage 1892; Minchin 1900; Rasmont 1979; Korotkova 1981a, 1988a; Simpson 1984; Seravin 1986, 1992; Malakhov 1990; Gaino et al. 1995; Efremova 1997; Ereskovsky and Korotkova 1997, 1999; Ereskovsky 1999, 2005; Maldonado 2004; Leys and Ereskovsky 2006).

A lack of uniformity and completeness in the available data on ontogenesis of the representatives of various groups of Porifera complicates the typization of sponge development, and understanding the roots of their originality and ways of their evolution (Brien 1943, 1967a, 1972; Borojevic 1970; Korotkova 1981a, b, 1988a; Efremova 1997; Ereskovsky and Korotkova 1997, 1999; Ivanova-Kazas 1997; Ereskovsky 1999, 2004, 2007b; Leys and Ereskovsky 2006).

In one work, the evolution of sponges' ontogenesis has been represented in the form of a gradual complication of embryogenesis, correlating with complication of their aquiferous systems (Brien 1967c, 1972; Ivanova-Kazas 1997). In other works, the attention has been drawn to the balance of flagellated and amoeboid cells lines during morphogeneses in sponges (Borojevic 1970). In the third, the evolution of ontogenesis has been considered as a process of interdependent somatic and reproductive morphogeneses in evolution of life cycles of sponges (Korotkova 1981b, 1988a).

It is probable that one of the deepest causes, which affected the variety of points of view, is traditional consideration of the sponges as a homogeneous group. The results of the study of a representative of one clade (Hexactinellida, Calcarea, Demospongiae, or Homoscleromorpha) were extrapolated on all Porifera. It was not important, what kind of research has been conducted: morphological, embryological, cytological, or molecular.

Considering such a great value of Porifera for understanding the origin, evolution, and phylogeny of Metazoa, it becomes obvious an indispensability of extensive comparative investigations of the ontogenesis in these basal metazoans.

Despite having more than 150-year-old history of studies of sponges' development, their comparative embryology is not yet well developed. However, attempts of its development have been undertaken repeatedly as soon as new facts and ideas are accumulated (Delage 1892, 1899; Brien 1943; Lévi 1956; Ivanova-Kazas 1975; Korotkova 1981a; Ereskovsky 2004, Leys and Ereskovsky 2006). Accordingly, the main aim of the present book is to promote the advancements for comparative embryology of sponges at a new step of the study of this important animal group using the state-of-the-art information on their development and evolution.

The main goals here are

- To collect the up-to-date available information on development of sponges, its classification, and position according to current taxonomical structure of Porifera
- To show the heterogeneity of the morphogeneses and other peculiarities of ontogenesis in various taxonomical groups of Porifera (at a rank of order or higher),

as well as their correlation with the organization of both the adult sponges and the larvae
- To show not only the homology of the morphogeneses in Porifera and Eumetazoa, significant for understanding the general evolutionary roots of multicellular animals, but also peculiar characters of morphogeneses and ontogenesis of Porifera in general

The book consists of two parts: the special one and the theoretical one. In the 'Introduction' the general morphological characteristic of sponges is presented. A special attention is given to the anatomical–histological organization, as well as ultrastructural characteristics. Chapters 1–4 describe gametogenesis, embryonic development, larvae, metamorphosis, and asexual reproduction of representatives of various groups of Porifera. The special attention is given to the morphogeneses accompanying embryonic development and metamorphosis. Unequal volume of chapters reflects the different degree of knowledge of the sponges groups. The second part of the book is devoted to theoretical aspects of sponges' embryology.

The basis for the writing of the present book were my own research on development of sponges of various groups lead at the Department of Embryology of Saint-Petersburg State University (Russia) and in the Centre d'Océanologie de Marseille, Station Marine d'Endoume Marseille (France). Reading the course of 'Comparative Embryology of Invertebrates' for many years at Biological Faculty of Saint-Petersburg State University (Russia), gave me further insights on similarity and distinctions of morphogeneses during the development of Porifera and Eumetazoa.

I consider as a pleasant duty to express my deepest gratitude to my teacher and the first scientific supervisor to the professor of Saint-Petersburg State University, Galina P. Korotkova, who rendered a strong effect on development of my scientific interests. My comparative-embryological conceptions in many respects have been certain by impression of unforgettable lectures of the professor of Saint-Petersburg State University, Olga M. Ivanova-Kazas. I am also deeply indebted to my professors: Vladimir M. Koltun and Alexander N. Golikov from the Zoological Institute of Russian Academy of Sciences, Lev N. Seravin, Sophia M. Efremova, Archil K. Dondua, and Diana G. Polteva from Saint-Petersburg State University. I am grateful to my colleagues and friends Dr. Elizaveta L. Gonobobleva, Alexander S. Plotkin, Dr. Raisa P. Anakina, Dr. Ljudmila V. Ivanova, Dr. Ivan A. Tikhomirov, Prof. Andrey I. Granovich, and Prof. Andrey N. Ostrovsky from Saint-Petersburg State University, Prof. Vladimir V. Malakhov, Prof. Nikolay N. Marfenin, Prof. Lev V. Beloussov, and Dr. Yu. Kraus from Moskow State University, Dr. Nicole Boury-Esnault, Dr. Jean Vacelet, Dr. Carole Borchiellini, and Dr. Thierry Perez from Centre d'Oceanologie de Marseille, Station Marine d'Endoume Marseille, Dr. Philippe Willenz (Royal Belgian Institute of Natural Sciences, Bruxells) and Prof. Michael Manuel from University Paris VI for numerous discussions on a handful of problems of embryology, morphology, zoology, and developmental biology, which promoted deeper comprehension of many evolutionary problems of biology.

In preparing the book for print I have been assisted by a number of persons to whom I would like to express my gratitude on this occasion. In the first place I wish to thank Natalia Lentsman for translating my manuscript from Russia to English.

Also many thanks to all the authors and publisher who have kindly agreed to the reproduction of figures used for illustrating this book, as well as colleagues in many countries who by sending me reprints or PDF of their publications have facilitated the arduous task of keeping track of current literature. I would like to thank Elizaveta L. Gonobobleva for her help in preparing most of the drawings for the book.

I thank all my students, past and present, for their friendship and for the intellectual stimulation they have given me.

This work was supported in part by the grant INTAS 97-854, by grants from the French 'Ministère de l'Education nationale et de la Recherche', from Federal Scientific Policy of Belgium, intended to promote S&T collaboration with eastern and central Europe, by the programs 'Universities of Russia' no. 07.01.017 and no. 3248, by the grants of Russian Foundation for Basic Research (RFBR) no. 03-04-49773; 04-04-62000, 06-04-48660; 06-04-5857, 09-04-00337 and by European Marie Curie Mobility program (fellowship MIF1-CT-2006-040065).

Neither my scientific work, nor the writing of this book was possible without constant support and understanding from my wife Daria B. Tokina.

Saint-Petersburg State University, Russia Alexander V. Ereskovsky

Contents

Part II Theoretical Aspects of Sponge Embryology

Introduction

In this introduction we will discuss only those aspects of poriferan organization that have a direct bearing on the topic of the present book. Physiology, biochemistry, ecology and palaeontology are left outside the scope of our consideration. Moreover, some of the relevant molecular biological and genetic data will only be mentioned in passing. Up to now the molecular biological methods were seldom applied to the study of sponge embryonic development and gametogenesis, and the data available are insufficient for generalizations of any kind.

1 Organization and Taxonomic Structure of Sponges

Sponges (Porifera) are aquatic, mostly marine, sedentary multicellular animals, with filtration feeding and respiration. The degree of their organization is low: there is no distinct gut, no special digestive parenchyma or cell population specialized in digestion, no nervous and muscular system and no gonads. The body shape of sponges is very diverse; they may be film-like, encrusting, lumpy or spherical, tubular, branching, flabellate, etc. (Colour plates I-XIII). Correspondingly, it is difficult to speak about their polarity, except the apical-basal one. In monooscular sponges, however, regardless of the aquiferous system type, the apical-basal axis also remains the radial symmetry axis (Manuel 2009). The body size of sponges varies as much as their body shapes: from 3–10 mm to 1.5–2 m.

The rigidity of the sponge body is ensured by the collagen fibrils of the mesohyl, by the spongin fibers (in some Demospongiae orders) and by the inorganic skeleton consisting of various mineral compounds on the basis of either calcium carbonate ($CaCO_3$) (Calcarea) or silicon (SiO_2) (Hexactinellida, Demospongiae, Homoscleromorpha). Inorganic skeleton may be represented by separate spicules, connected or fused spicules, or monolithic mineral skeleton (Fig. 1). The organic, as well as the inorganic, skeleton is secreted or assembled by special cells.

Sponges are characterized by a high polymorphism. The shape, size and colour of the body are often depending directly on environmental conditions, mostly hydrodynamics. A high plasticity also characterizes the life cycles and the reproductive strategies of different populations of the same species.

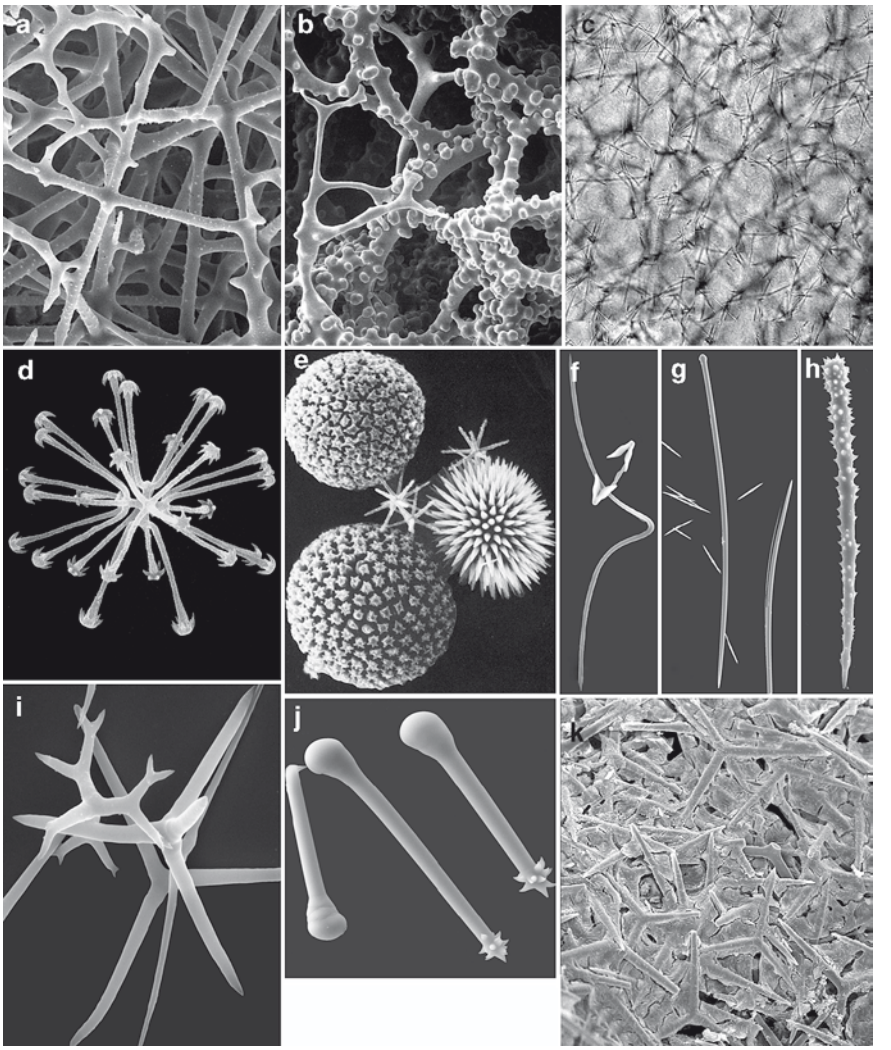

Fig. 1 Inorganic skeleton of sponges. (**a**) Scanning electron microscopy (SEM) of a skeleton of *Oopsacas minuta* (Hexactinellida). (**b**) SEM of a skeleton of *Neophrisspongia nolitangere* (Demospongiae, 'Lithistida'). (**c**) Light microscopy of a skeleton of *Plakortis* sp. (Homoscleromorpha). (**d**) SEM of the discohexaster of *O. minuta* (Hexactinellida). (**e**) SEM of the asters of *Geodia neptuni* (Demospongiae, Astrophorida). (**f**) SEM of anchorate isochela and a forceps of *Artemisina arcigera* (Demospongiae, Poecilosclerida). (**g**) SEM of the tylostyle, oxae and microrhabds of *Suberites domuncula* (Demospongiae, Hadromerida). (**h**) SEM of the acanthotylostyle of *A. arcigera* (Demospongiae, Poecilosclerida). (**i**) SEM of the diode, triode and calthrop of *Plakina* sp. (Homoscleromorpha). (**j**) SEM of echinating acanthostyles of *Hymeraphia stellifera* (Demospongiae, Poecilosclerida). (**k**) SEM of the skeleton of *Clathrina contorta* (Calcispongiae, Calcinea). (**a**, **b**, **d**, **e** – Courtesy of J. Vacelet)

Traditional systematics puts sponges in the phylum Porifera. About 8,000 sponge species are currently known, separated into four clades of the class level: Hexactinellida, Calcarea, Demospongiae and Homoscleromorpha. There is also a class of extinct sponges, Archaeocyatha, showing a close affinity with the Demospongiae. The extinct 'classes' Sphinctozoa and Stromatoporoidea, as well as Sclerospongiae are not separate taxa (Vacelet 1985; Wood 1991), but simply types of the body organization. The representatives of each clade differ in the time of origin (judging by the fossils), the skeleton structure and the spicule shape, as well as in developmental types, which are quite strikingly dissimilar.

According to the paleontological records, Porifera are the most ancient multicellular animals. The most archaic of the extant sponges are the Hexactinellida, whose fossil records date back as far as the early Proterozoic (Steiner et al. 1993; Brasier et al. 1997). Demosponges are known from the late Proterozoic (about 750 million years ago) (Reitner and Wörheide 2002). Interestingly, the first findings of the keratose, i.e. nonspicular demosponges date back to the same time (Reitner and Wörheide 2002). The calcareous sponges (Calcarea) originated somewhat later than the other Porifera, in the lower Cambrian (Reitner 1992). Judging by the paleontological data, the Homoscleromorpha are the youngest sponge group, appearing in the Early Carboniferous (Mehl-Janussen 1999).

2 Organization of 'Cellular' Sponges

The body of sponges with the cellular organization (include Demospongiae, Calcarea and Homoscleromorpha) consists of two epithelial cell layers: the pinacoderm and the choanoderm. The pinacoderm is represented by the flattened cells, the pinacocytes, which form the external cover and line the aquiferous system canals and some internal cavities. The choanoderm is formed by the flagellated collar cells, the choanocytes, lining the choanocyte chambers. The space between the external layer of pinacocytes and the aquiferous system is filled with the mesohyl. As indicated by Müller (1997a), sponge mesohyl should not be considered as an inert scaffold but as a dynamic and complex network of molecules that regulates the behaviour of cells. The mesohyl contains over ten types of highly mobile cells, as well as skeletal elements and microbial symbionts (Korotkova 1981a; Simpson 1984; Harrison and De Vos 1991).

2.1 Aquiferous System

The circulatory aquiferous system is the most characteristic feature of the poriferan organization. It comprises the following elements (Fig. 2): ostia, inhalant canals, apopyle, choanocyte chambers, prosopyle, exhalant canals and osculum.

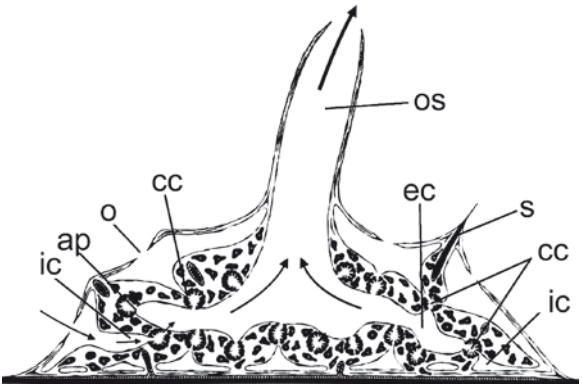

Fig. 2 Diagram of young sponge with leuconoid aquiferous system. *ap* apopyle, *cc* choanocyte chamber, *ec* exhalant canal, *ic* inhalant canal, *o* ostium, *os* osculum, *s* spicule (From Weissenfels 1975, reproduced by permission of Springer)

Water drawn into the inhalant canals via small pores called *ostia* moves to *choanocyte chambers* and then, via the system of the exhalant canals, to the large excurrent *osculum*. The unidirectional flow of water is ensured by the coordinated beating of the choanocytes' flagella. In the sponges with cellular organization, food particles and oxygen are captured from water by various cells, including choanocytes. The cells that are not included into the epithelia participate in the transport of the food particles and oxygen inside the sponge body. The aquiferous system is a modular, easily rearranged system (Gaino et al. 1995; Plotkin et al. 1999; Ereskovsky 2003). Its main physiological functions are transport and excretion of food particles, respiration and the release of the gametes and the larvae. At the same time, some representatives of the families Cladorhizidae and Esperiopsidae (Poecilosclerida, Demospongiae) lack all the elements of the aquiferous system (Vacelet 2006, 2007; Ereskovsky and Willenz 2007).

There are four main type of aquiferous system: (1) *asconoid* – the internal cavities are completely lined with choanocytes (Fig. 3a); (2); *syconoid* – the elongated choanocyte chambers pass through the whole sponge body from the cortex to the atrium (Fig. 3b); (3) *sylleibid* – the elongated choanocyte chambers are arranged radially around an invagination of the atrium cavity (Fig. 3c); (4) *leuconoid* – choanocytes are arranged into separate choanocyte chambers scattered in the mesohyl (Fig. 3d).

Choanocyte chambers are the basic elements of the aquiferous system. These chambers can be considered to be organ-like assemblies. The function of the choanocyte chambers as organs or organ-like assemblies is to orient the water flow unidirectionally from the incurrent to the excurrent to allow the extrusion of the water from the body through the exhalant oscule(s). They are divided into three types: aphodal, diplodal and eurypilous (Fig. 4). Aphodal chamber is connected directly with the inhalant canals through prosopyles and with the exhalant canal through an apopyle extended by an aphodus (Fig. 4a). Only one chamber opens into

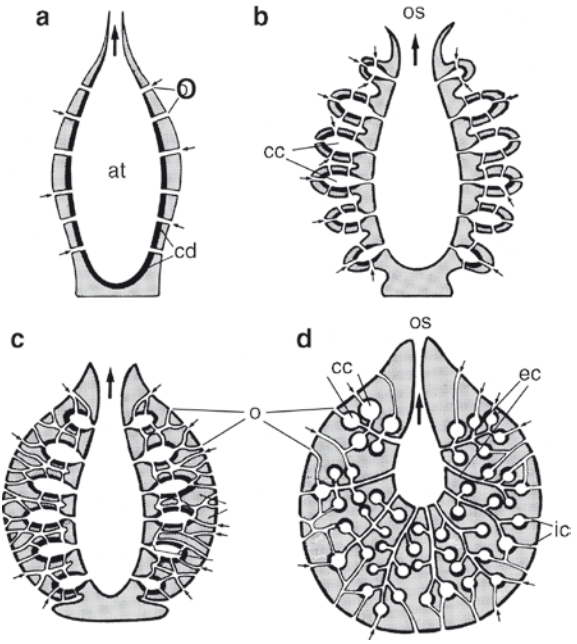

Fig. 3 Diagrams of different types of aquiferous systems in sponges. (**a**) asconoid; (**b**) syconoid; (**c**) sylleibid; (**d**) leuconoid. *at* atrium, *cc* choanocyte chamber, *cd* choanoderm, *ec* exhalant canal, *ic* inhalant canal, *o* ostium, *os* osculum (After Hyman 1940)

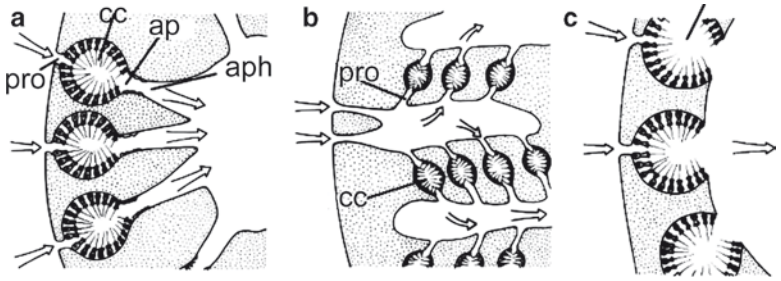

Fig. 4 Diagrams of different types of choanocyte chambers: (**a**) aphodal; (**b**) diplodal; (**c**) eurypilous. *ap* apopyle; *aph* aphodus; *cc* choanocyte chamber, *pro* prosopyle (From Boury-Esnault and Rützler 1997, reproduced by permission of Smithsonian Institution Scholarly Press)

one aphodus. Diplodal chamber connects with the inhalant canals through small canal (prosodus) and with the excurrent canal through an apopyle extended by an aphodus (Fig. 4b). Eurypylous is the type of chamber that connects directly with the inhalant canals through prosopyles and with the excurrent canal through an apopyle (Fig. 4c).

2.2 Tissue Organization

The interpretation of the sponge tissue organization is one of the most debated problems of their organization and biology (Bergquist 1978; Korotkova 1981a, 1997; Simpson 1984; Harrison and De Vos 1991; Seravin 1992; Efremova 1997; Ereskovsky 2005; Ereskovsky and Dondua 2006; Leys 2007).

Tissues of the multicellular animals are divided into two categories: the epithelial ones and the parenchymal ones. In histology, the tissue is understood as a historically formed system of elements (cells and the intercellular structures formed by them) united by a common function and structural-chemical organization (Fawcett 1994). There are four main types of tissues: (1) bordering (epithelial) tissues, (2) the tissues of the internal environment (blood, interstitial tissues, and skeletal tissues), (3) nervous system tissues and (4) muscular tissues (Fawcett 1994). Since sponges lack the latter two types, we will analyse their bordering tissues and the tissues of the internal environment.

The epithelial organization is an important characteristic of the multicellular animals, which preserve the covering epithelium throughout the life cycle (Tyler 2003). An embryonic cell layer can be considered an epithelium if their apical surface is free and non-adhesive and the baso-lateral surface contacts the embryonic cells (see Hay 1968).

An epithelium is defined as a sheet of polarized cells. The cells are joined by belt-like junctions around their apical margins, and extracellular matrix (ECM) is typically present only apically and basally due to the close apposition of cells within the epithelium (Tyler 2003). Metazoan epithelial tissues have two primary characteristics of system organization: the structural unification of the epithelial cells into uninterrupted layers or cords, functioning as integral systems, and the polarity, resulting from their bordering position (Fawcett 1994).

The tissues of the internal environment are the complex of tissues forming the internal environment of an organism and maintaining its stability (Fawcett 1994). There are three kinds of such tissues: (1) loose connective tissues (parenchyma, mesoglea and mesohyl), (2) skeletal and supportive tissues and (3) protective tissues (blood, lymph). Their main functions are the trophic function, the structural function, the maintenance of chemical and osmotic composition and the protective (immune) function.

In sponges, the bordering tissues and the tissues of the internal environment are simpler, both structurally and functionally, than in the other Metazoa. In particular, sponge tissues are always more multifunctional than their counterparts in advanced animals. Besides, the cells of sponge tissues possess a very high capacity of transdifferentiation into cells of other types (Korotkova 1981a, 1997; Gaino et al. 1995). At the same time, Porifera lacks a single category of totipotent cells, the representatives of each clad possessing its own system of stem cells: archaeocytes in the Demospongiae, choanocytes in the Calcarea, pinacocytes in the Homoscleromorpha and, apparently, archaeocytes in the Hexactinellida.

2.2.1 The Epitheliums

Pinacoderm is represented by the exo-, baso- and endopinacoderm. *Exopinacoderm* forms the external cover of the sponge. *Basopinacoderm* develops at the sponge base, attaching it to the substrate. *Endopinacoderm* forms the walls of the subdermal cavities and the aquiferous system canals. There are, correspondingly, several types of pinacocytes.

Exopinacocytes are the covering cells of the sponge. They may be spindle-shaped or T-shaped at cross section (Fig. 5). Spindle-shaped exopinacocytes are described in many Demospongiae from the orders Spongillidae and Poecilosclerida (Bagby 1970; Weissenfels 1989), in all the Homoscleromorpha (Muricy et al. 1996, 1999) and in the Calcarea (Borojevic 1969; Eerkes-Medrano and Leys 2006). T-shaped exopinacocytes are described in many Demospongiae and in the Calcarea (see Boury-Esnault 1973; Willenz and Hartman 1989).

The surface part of an exopinacocyte is polygonal in shape and covered with a mucous layer of self-secreted glycocalyx. Owing to the latter, a food particle to be phagocyted is first glued to the cell surface. Exopinacocytes lack specialized cell junctions such as desmosomes or *macula adhaerens*, but are united with a well-developed adhesive system (Blumbach et al. 1998; Müller 1982; Schütze et al. 2001). In *Hippospongia communis*, *Ephydatia fluviatilis*, *Sycon coactum, Oscarella lobularis* and *O. tuberculata* at the site of exopinacocytes' contacts there are electron-dense thickenings of the membranes resembling *zonula adhaerens* (Fig. 6) (Pavans de Ceccatty et al. 1970; Pottu-Boumendil 1975; Eerkes-Medrano and Leys, 2006; Ereskovsky and Tokina, 2007). Exopinacocytes can secrete components of the extracellular matrix and synthesize collagen (Garrone 1978; Simpson 1984; Boute et al. 1996). Exopinacocytes of the Homoscleromorpha are closely associated with the underlining dense fibrillar layer, the basal membrane, comprising collagen IV, laminin and tenascin. This basal membrane is identical to the *lamina reticulat*ain the basal lamina of the epithelia of vertebrates (Fig. 7) (Humbert-David and Garrone 1993; Boute et al. 1996).

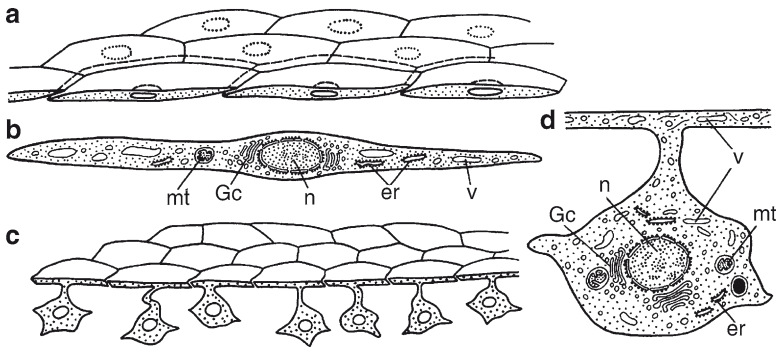

Fig. 5 Diagram of sponge's pinacoderm and pinacocytes. (**a**) pinacoderm, formed by spindle-shaped pinacocytes (**b**), (**c**) pinacoderm, formed by T-shaped pinacocytes (**d**). *er* endoplasmic reticulum, *Gc* Golgi complex, *mt* mitochondria, *n* nucleus, *v* vacuole

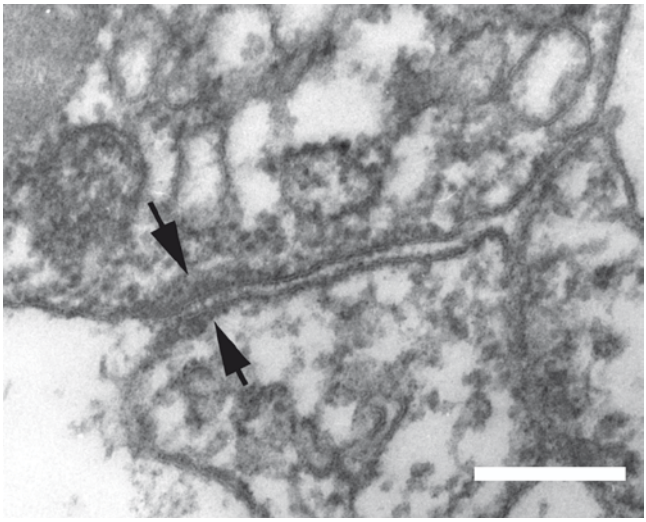

Fig. 6 Transmission electron microscopy (TEM) of cell junction (*arrows*) between the endopinacocytes of *Oscarella lobularis* (Homoscleromorpha). Scale bar 3 μm

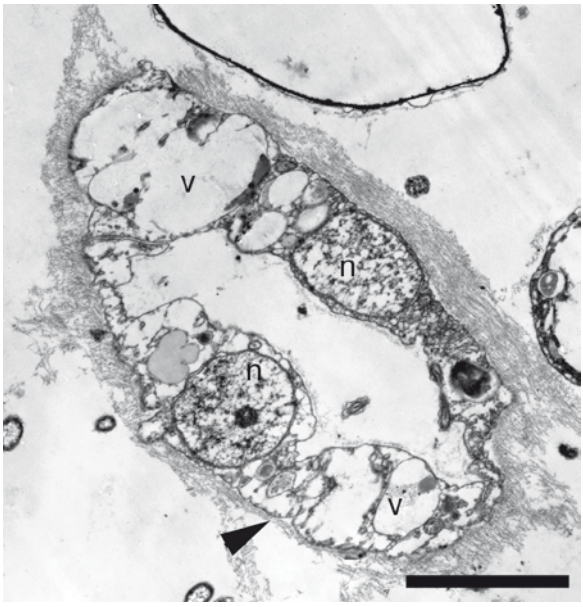

Fig. 7 TEM of basal membrane (*arrowhead*) under the endopinacocytes of *Oscarella tuberculata* (Homoscleromorpha). *n* nucleus, *v* vacuole. Scale bar 3 μm

Unique characteristics of the homoscleromorph exopinacocytes are the flagellum and the ability to synthesize spicules (Donadey 1979; Muricy et al. 1996; Ereskovsky and Tokina 2007; Maldonado and Riesgo 2007).

Exopinacoderm contains *ostia* – numerous microscopic structures which are 4-100 μm in diameter – through which the water is drawn into the aquiferous system of the sponge. In most Demospongiae and in all Homoscleromorpha ostia are intercellular (Fig. 8a). In the Calcarea, the ostia are formed inside special cylindrical tubular cells, *porocytes* (Fig. 8b) (Jones 1966; Borojevic 1969; Eerkes-Medrano and Leys 2006). In *S. coactum*, porocytes can contract in response to the mechanical stimulation and treatment with anaesthetics (Eerkes-Medrano and Leys 2006). Porocytes of some Demospongiae from order Haplosclerida are flattened cells with a central or peripheral opening, which can open and close like a sphincter (Fig. 8c) (Harrison 1972a; Weissenfels 1980; Willenz and Van de Vyver 1982; Harrison et al. 1990; Langenbruch and Scalera-Liaci 1986).

Exopinacoderm exhibits many functions characteristic of the typical eumetazoan epithelia, such as absorption, secretion, transport, excretion and protection (see Simpson 1984; Harrison and De Vos 1991; Meyer et al. 2006).

Basopinacocytes are flattened cells at the basal surface of the sponge whose main function is attachment of the sponge to the substrate. Synthesizing basal spongin and fibronectin, basopinacocytes function as spongocytes (see below) (Garrone and Pottu 1973; Garrone and Rosenfeld 1981; Labat-Robert et al. 1981). In the course of sponge growth, marginal basopinacocytes actively secrete proteins that make up spongin (Garrone 1978).

In the coralline demosponge *Acanthochaetetes wellsi* (order Hadromerida), basopinacocytes take part in the formation of the massive basal calcareous skeleton (Reitner and Gautret 1996). Basopinacocytes of other demosponges with a massive calcareous skeleton, *Ceratoporella nicholsoni* and *Stromatospongia norae* (Agelasida), also participate in its synthesis (Willenz and Hartman 1989).

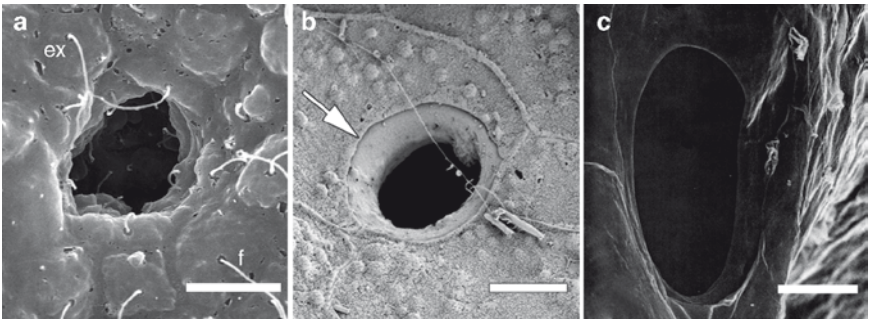

Fig. 8 Sponge's ostia. (**a**) SEM of intercellular ostium of *Oscarella lobularis* (Homoscleromorpha); (**b**) SEM of ostia formed inside of porocytes in *Sycon coactum* (Calcarea) (From Eerkes-Medrano and Leys 2006, reproduced by permission of Wiley). (**c**) SEM of porocyte of *Ephydatia fluviatilis* (Demospongiae, Haplosclerida). (From Willenz and Van de Vyver 1982, reproduced by permission of Elsevier, Ltd). *ex* exopinacocytes, *f* flagellum. Scale bars (**a**) 10 μm, (**b**) 0.5 μm, (**c**) 5 μm

As shown on the freshwater demosponges *Ephydatia muelleri* and *Spongilla lacustris*, basopinacocytes have a well-organized cytoskeleton (Pavans de Ceccatty 1986; Wachtmann et al. 1990; Wachtmann and Stockem 1992a, b; Kirfel and Stockem 1997). Actin is located in the cortical layer (directly below the plasma membrane) and in the fibrils in the cytoplasmic matrix. Microtubules radiate from the perinuclear zone, finishing at the cell periphery. At the same time, intermediate filaments have not been described (Fig. 9). In *E. muelleri*, basopinacocytes were shown to have desmosome-like junctions (Pavans de Ceccatty 1986).

Endopinacocytes are flattened, polygonal cells, spindle-shaped at cross section (Fig. 10). Endopinacocytes are divided into *prosopinacocytes*, lining the inhalant canals, and *apopinacocytes*, lining the exhalant canals. In all Homoscleromorpha (Fig. 10a, b) (Boury-Esnault et al. 1984; Vacelet et al. 1989) and in some Demospongiae endopinacocytes bear flagella. In particular, this is the case of *Tethya lyncurium* (Hadromerida) (Pavans de Ceccatty 1966) and of most studied representatives of the orders Dictyoceratida and Dendroceratida (Thiney 1972; Donadey 1982; Vacelet et al. 1989; Boury-Esnault et al. 1990). The presence of flagella appears to be associated with the involvement of endopinacocytes in the water circulation in the aquiferous system. In the sclerosponges *C. nicholsoni* and *S. norae* (Agelasida), endopinacocytes form membrane partitions perpendicular to the canals (Willenz and Hartman 1989), which are also supposed to be involved in regulation of the water passage in the sponge.

In most Demospongiae and Homoscleromorpha studied, the external surface of the endopinacocytes is covered with a glycocalyx layer (Harrison and De Vos 1991;

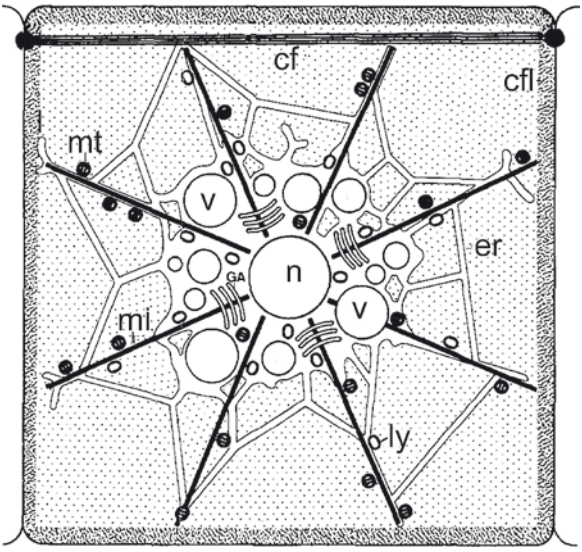

Fig. 9 Schematic drawing of the cytoplasm organization of *Spongilla lacustris* basopinacocyte (Demospongiae, Haplosclerida). *cfl* cortical filament layer, *l* lipid droplets, *ly* endosomes and lysosomes, *mi* microtubules, *mt* mitochondria, *n* nucleus, *v* vacuoles of the osmoregulating system (From Wachtmann and Stockem 1992a, reproduced by permission of Springer)

Boury-Esnault et al. 1984; Simpson 1984; Vacelet et al. 1989). The basal surface often forms numerous projections (pseudopodia) for anchoring in the extracellular matrix (Fig. 10b).

Generally, endopinacocytes contact each other by simple fitting. However, in the oscular tubes of freshwater sponges the cells of the endopinacoderm are united by desmosome-like junctions (Masuda et al. 1998). Endopinacocytes of *Oscarella* (Homoscleromorpha) are joined by *zonula adhaerens* junctions (Ereskovsky and Tokina 2007; Ereskovsky et al. 2009a).

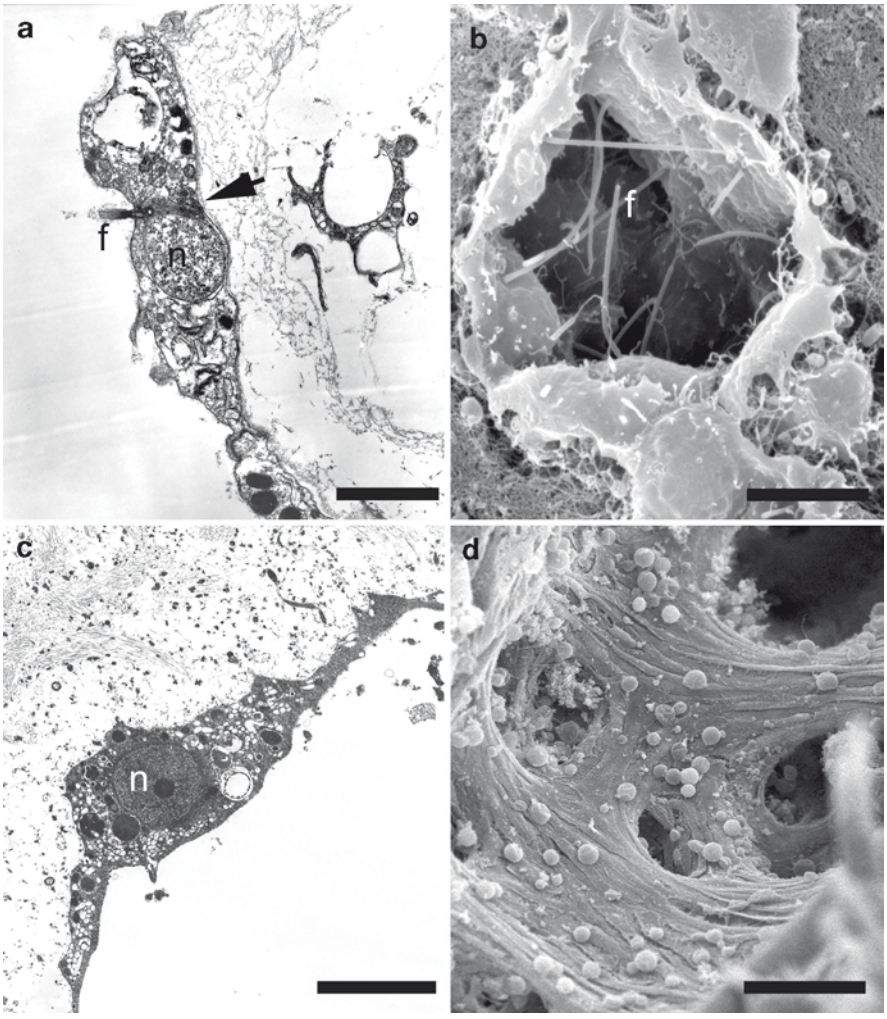

Fig. 10 Endopinacocytes. (**a**) TEM of an endopinacocyte of *Oscarella* sp. (**b**) SEM of an endopinacocytes of *Oscarella malakhovi.* (**a**, **b**) Homoscleromorpha. (**c**) TEM of an endopinacocyte of *Halisarca caerulea* (Demospongiae, Halisarcida) (Courtesy of J. Vacelet). (**d**) SEM of an endopinacoderm of *Hippospongia communis* (Demospongiae, Dictyoceratida). *Arrow* basal membrane, *f* flagellum, *n* nucleus. Scale bars (**a**, **c**) 3 μm, (**b**) 5 μm, (**d**) 50 μm

During growth of *Oscarella* (Homoscleromorpha), exopinacocytes differentiate into endopinacocytes (Gaino et al. 1987a). The latter, in their turn, can differentiate into the vacuolar cells of the mesohyl (Gaino et al. 1986a).

Endopinacocytes and choanocytes of the demosponges are thought to be functionally and ontogenetically interrelated. This is supported, in particular, by observations on *Suberites massa* (Hadromerida): its choanocytes can differentiate into endopinacocytes by reduction of the flagellum and the microvilli and the subsequent flattening of the cell (Diaz 1974).

In some Demospongiae, endopinacoderm contains special contractile cells, the *myocytes*. These long spindle-shape cells are inbuilt into the oscular tube wall of *Microciona prolifera,* the oscular sphincter of *Tedania ignis* (Poecilosclerida) and in *Aplysina cavernicola* (Verongida) (Bagby 1966; Vacelet 1966). The myocytes of *M. prolifera* contain two types of filaments: fine filaments, 50-70 nm in diameter, that form clusters around the larger ones, 150-250 nm in diameter. The myocytes of *T. ignis* have only one type of filament, 100 to 200-300 nm (Bagby 1966). Epithelial myocytes are supposed to differentiate from pinacocytes (Bagby 1970). In freshwater sponges, contractile actin filaments are present not in myocytes but in the endopinacocytes of the oscular tubes (Masuda et al. 1998).

***

Only the Homoscleromorpha have epithelium of the eumetazoan type, i.e. with a basal membrane containing collagen IV, tenascin and laminin (Humbert-David and Garrone 1993; Boute et al. 1996). The other sponges with cellular organization (Demospongiae and Calcarea) do not have a basal membrane. At the same time, the spatial organization of the cytoskeleton in the basopinacoderm of freshwater demosponges is characterized by a high degree of regularity and coordination of reaction, which are the attributes of the eumetazoan epithelia (Pavans de Ceccatty 1986; Wachtman et al. 1990). This integrated cytoskeletal organization of the basal epithelium was called the *hystoskeleton* (Pavans de Ceccatty 1986).

It is considered that typical epithelia either occupy a relatively stable surface position or line various cavities. Contrary to the Eumetazoa, the pinacocytes of sponge epithelia are contractile; they can also become amoeboid and move. For instance, the basopinacocytes of *Corvomeyenia carolinensis* become amoeboid before flattening at the new site (Harrison 1972b). It was shown that some exo- and basopinacocytes of *E. fluviatilis* can migrate (Weissenfels 1978). This unusual ability of sponge epithelia appears to be associated with the lack of true desmosomes and the presence of a well-developed network of cytoskeletal actin microfilaments associated with a system of microtubules (Pavans de Ceccatty 1986).

The pinacocytes of all types (exo-, baso, and endopinacocytes) can phagocyte. This was proved both by experiments revealing the presence of phagolysosomal acid phosphatase and by direct observations (Willenz and Van de Vyver 1984; Pottu-Blumendil 1975; Diaz 1979; Simpson 1963; Harrison 1972a). The pinacocytes are also involved in excretion.

Choanoderm consists only of choanocytes that formed the choanocyte chambers (or choanoderm in asconoid sponges) (Fig. 11). Contrary to pinacoderm, choanoderm is a cubic or palisade epithelium. Choanocytes can be cylindrical, cubic, trapezoid or slightly flattened. These cells bear a flagellum surrounded with a collar of cytoplasmic microvilli connected by glycocalyx bridges.

Choanocytes may be different both in different species and in one and the same sponge, their morphology depending on environmental conditions, physiological state or ontogenetic stage. These cells, however, do have some common characters. The nucleus occupies the apical, central or basal position, the chromatin is very condensed, the nucleolus may be present in the choanocytes of some demosponge groups (Figs. 11c, 12d) (Boury-Esnault et al. 1984; Vacelet et al. 1989), in choanoblasts

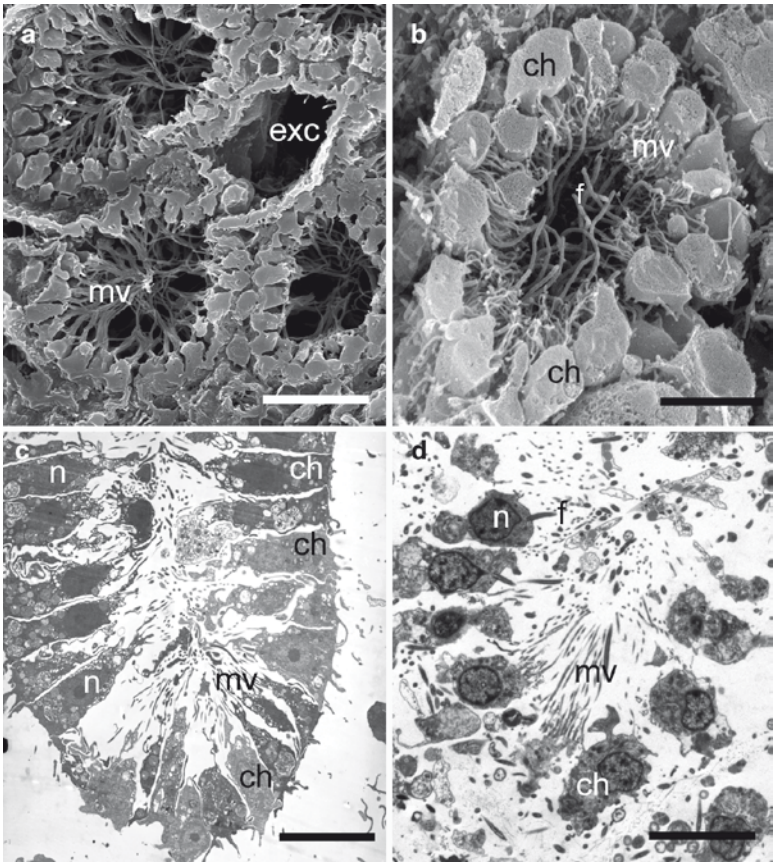

Fig. 11 Choanocyte chambers of (**a**) *Oscarella malakhovi* (Homoscleromorpha) SEM, (**b**) *Hymedesmia irregularis* (Demospongiae, Poecilosclerida) SEM, (**c**) *Halisarca dujardini* (Demospongiae, Halisarcida) TEM and (**d**) *Hymedesmia irregularis* (Demospongiae, Poecilosclerida) TEM. *ch* choanocyte, *exc* exhalant canal, *f* flagellum, *mv* microvilli, *n* nucleus. Scale bars (**a**) 15 μm, (**b**) 5 μm, (**c**) 20 μm, (**d**) 5 μm

differentiating into choanocytes in the course of asexual (Jetton et al. 1987) or sexual (Ereskovsky et al. 2007a) development and in choanocytes transforming into 'blastogenic archaeocytes' during gemmulogenesis of *Suberites domuncula* (Hadromerida) (Connes et al. 1974).

The flagellum starts from a small knob or circular depression at the apical cell surface (Fig. 12a, c-f). In some demosponges from the orders Hadromerida and Halisarcida the base of the flagellum is surrounded with a high cytoplasmic cuff (Figs. 11c, 12a, b) (Connes et al. 1971; Diaz 1979; Vacelet et al. 1989; De Vos et al. 1991; Boury-Esnault et al. 1994).

The apical cell surface within the microvilli collar, the basal part of the flagellum and the internal surface of the microvilli are covered with a thin layer of glycocalyx. In sponges from various groups the choanocyte may bear a pair of flat lateral wing-like structures (Feige 1969; Mehl and Reiswig 1991). The structure of the axoneme is typical of the Eukaryota. The basal apparatus comprises two centrioles oriented perpendicular or, as in the Halisarcida, at an angle to each other. The Golgi complex lies between the centrioles and the nucleus.

The choanocyte collar comprises 20–55 microvilli, the number correlating with the taxonomic position of the sponge (De Vos et al. 1991). The microvilli may be connected to each other by thin glycocalyx cords (Fig. 12b, e) (Boury-Esnault et al. 1984; De Vos et al. 1991; Eerkes-Medrano and Leys 2006) or cytoplasmic projections (Brill 1973; De Vos 1977; Watanabe 1978a), forming a fine honeycombed network. The microvilli are often fused at the basis (Fig. 12e). The choanocytes have a well-developed cytoskeleton, which includes F-actin and myosin; the latter are especially apparent in the dissociated cells of *Clathrina cerebrum* (Calcinea) (Burlando and Gaino 1984; Gaino and Magnino 1998).

The choanocyte chambers of the Demospongiae are usually underlined with a more or less loose layer of the extracellular matrix containing collagen, and those of the Homoscleromorpha, with a true basal membrane containing collagen IV (Humbert-David and Garrone 1993; Boute et al. 1996; Muricy et al. 1996; Ereskovsky 2006). In the basal part of the choanocytes of many demosponges, cytoplasmic projections are formed, which not only anchor the cells in the extracellular matrix, but also intertwine to lend the mechanical support to the choanoderm. Such structures are especially characteristic of the Halisarcida (Fig. 11c). The choanocytes contact by simple fitting, either in the basal or in the middle part (Boury-Esnault et al. 1984, 1990; Vacelet et al. 1990). In Homoscleromorpha (*O. tuberculata* and *O. lobularis* (Ereskovsky and Tokina 2007) and in the calcareous sponge *S. coactum* (Eerkes-Medrano and Leys 2006) the choanocytes form specialized intercellular desmosome-like junctions.

Apopylar cells are a special cell type. Intermediate between the apopinacocytes and choanocytes, these flagellated cells form the border between the exhalant canal and the choanocyte chamber. Apopylar cells are triangular at cross section; the edge facing the chamber bears a comb of microvilli (Fig. 13).

Apopylar cells are characteristic of all the Homoscleromorpha (Fig. 13a, b), Halisarcida, Dictyoceratida and Dendroceratida (Boury-Esnault et al. 1984; Vacelet et al. 1990; De Vos et al. 1990; Muricy et al. 1996; Ereskovsky 2006; Ereskovsky

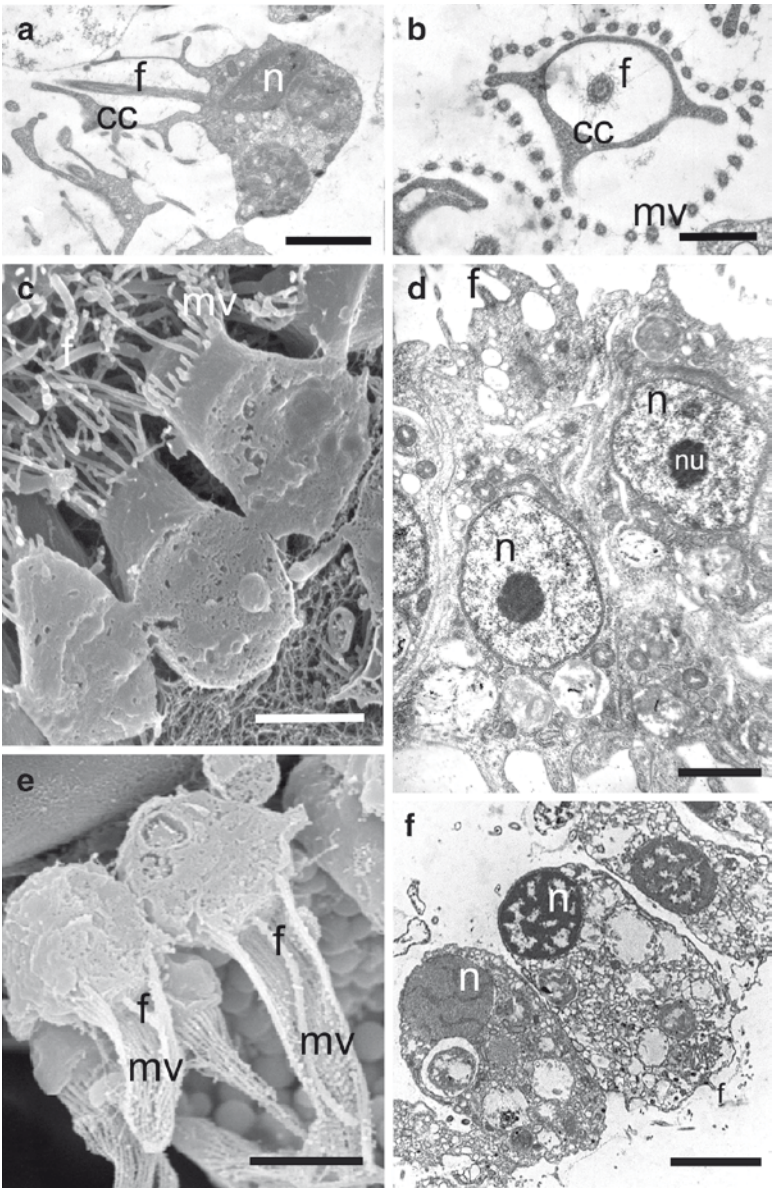

Fig. 12 Choanocytes. (**a**, **b**) Choanocytes with cytoplasm cuff (*cc*) of *Acanthochaetetes wellsi* (Demospongiae, Hadromerida) TEM longitudinal section of cell (**a**) and transversal section of the cuff (**b**), flagellum (*f*) and microvilli (*mv*). (**c**) SEM of longitudinal section of the choanocytes of *Oscarella malakhovi* (Homoscleromorpha). (**d**) TEM of longitudinal section of choanocytes of *Halisarca dujardini* (Demospongiae, Halisarcida). (**e**) SEM of choanocytes of *Clathrina cerebrum* (Calcarea, Calcinea) with the microvilli fused at the basis. (**f**) TEM of longitudinal section of choanocytes of *Clathrina clathrus* (Calcarea, Calcinea). *n* nucleus, *nu* nucleolus. (**a**, **b** – Courtesy of J. Vacelet). Scale bars (**a**, **d**, **e**) 5 μm, (**b**, **c**, **f**) 2 μm

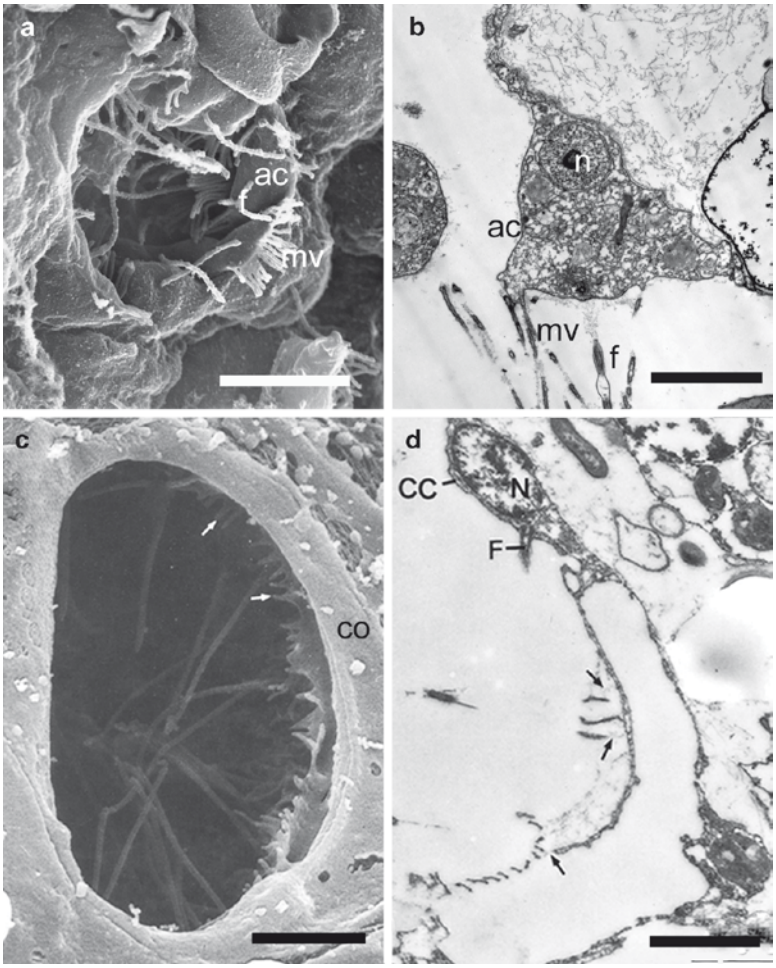

Fig. 13 Apopylar cells. (**a**) SEM of *Oscarella lobularis* (Homoscleromorpha), (**b**) TEM of *Oscarella viridis* (Homoscleromorpha), (**c**) SEM of a cone cells (*co*) of *Niphates digitalis* (Demospongiae, Haplosclerida) and (**d**) TEM of a cone cell of *Reniera sarai* (Demospongiae, Haplosclerida). *ac* apopylar cell, *cc* cone cell, *f* flagellum, *mv* microvilli, *n* nucleus. (**c**, **d** – After Langenbruch 1988, reproduced by permission of Springer). Scale bars (**a**, **b**) 4 μm, (**c**) 5 μm, (**d**) 2 μm

and Tokina 2007; Ereskovsky et al. 2009). They were also described, under the name of cone cells, in some demosponges from the orders Haplosclerida (Fig. 13c, d) (Weissenfels 1981; Langenbruch et al. 1985; Langenbruch and Scalera-Liaci 1986; Langenbruch and Weissenfels 1987; Langenbruch 1988). It is supposed that the main role of the apopylar cells is regulation of the hydrodynamics in the sponge.

Central cells are another type of cells in the choanocyte chambers. First discovered by Sollas (1888), they were repeatedly recorded at the light microscopic level (see Reiswig and Brown 1977), though only ultrastructural studies clarified their structure and function (Connes et al. 1972; Diaz 1979; Langenbruch and

Scalera-Liaci 1986; Langenbruch and Jones 1989; Sciscioli et al. 1997). In *S. massa*, the central cells have an irregular, branched shape with numerous projections and holes. The cell is perforated with a vast canal into which enter the choanocytes' flagella (Diaz 1979) (Fig. 14a). Ultrastructurally, the central cells are identical to the choanocytes. In *Pellina fistulosa* and *P. semitubulosa* (Haplosclerida), the central cells look like a perforated membrane situated at the

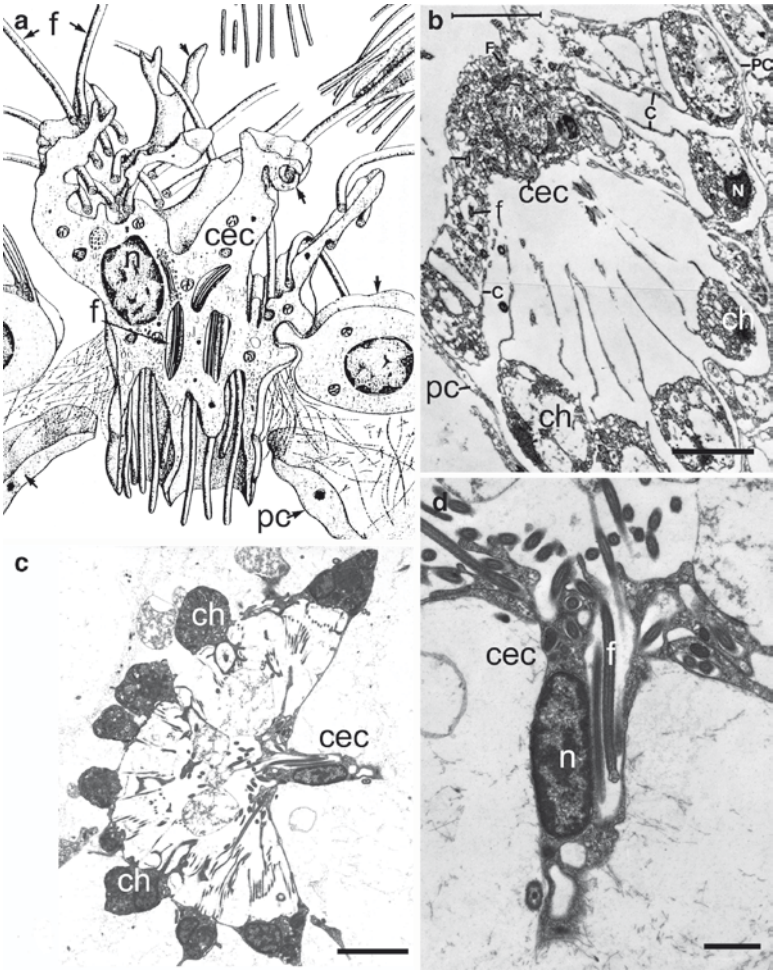

Fig. 14 Central cells of some demosponges. (**a**) Diagram of the central cell of *Suberites massa* (Demospongiae, Hadromerida). (**b**) TEM of a central cell of *Haliclona elegans* (Demospongiae, Haplosclerida) extending three processes into choanocyte *(ch)* collars (c), choanocyte flagella (*f*) penetrate the central cell it is a pinacocyte cover (*pc*) at the outer chamber surface. (**c**, **d**) TEM of a central cell of *Acanthochaetetes wellsi* (Demospongiae, Hadromerida) and its detail (**d**). *cec* central cell, *n* nucleus (**a** – Courtesy of J. Diaz; **b** – From Langenbruch and Scalera-Liaci 1986, reproduced by permission of Springer; **c**, **d** – Courtesy of J. Vacelet). Scale bars (**b**) 3 μm, (**c**) 10 μm, (**d**) 3 μm

level of the distal surface of the choanocytes' collars in each chamber (Fig. 14b) (Langenbruch and Jones 1989; Sciscioli et al. 1997). Interestingly, the central cells are present not in all the choanocyte chambers of a sponge. For instance, in *Haliclona elegans* (Haplosclerida) they are present only in 15% of the chambers (Langenbruch and Scalera-Liaci 1986).

The origin of the central cells is not quite clear: they were supposed to originate both from choanocytes (Reiswig and Brown 1977) and from endopinacocytes (Langenbruch and Scalera-Liaci 1986). The central cells participate in the regulation of beating of the choanocyte flagella within a chamber.

As noted above, sponges lack the gut epithelium and the digestive parenchyma. Food particles may be captured by almost all the cells of the covering and the internal tissues (Diaz 1979; Willenz and Van de Vyver 1982, 1984; Hahn-Keser and Stockem 1997). Therefore, neither the choanoderm nor the aquiferous system with its canals should be considered as endoderm derivatives (Ereskovsky and Dondua 2006).

2.2.2 Tissues of the Internal Environment

Taken together, the tissues of the internal environment of the sponges with cellular organization make up the *mesohyl*. There the cells, symbiotic bacteria and skeletal elements are loosely embedded in the ground matrix of mesohyl, composed primarily of collagen, galectin and glycoconjugates. Drawing an analogy with other animals, we can delimit the mesohyl of these sponges, according to the functional specialization of certain cell groups, the *supporting-connective* and the *protective-secretory* tissue (Korotkova 1981a). A characteristic feature of the sponges with cellular organization is that all the tissues of the internal environment are populations of free, mobile cells capable of transdifferentiation.

Supportive-Connective Tissue

The main function of this tissue is formation of the organic and the mineral skeleton, the supporting structures and the extracellular matrix of the mesohyl.

Collencytes, a variety of fibroblasts, are mobile cells secreting collagen. They were first described by Sollas (1888). According to the first definition, collencytes are stellate or spindle-shaped cells with branching pseudopodia and an ovoid anucleolate nucleus; their cytoplasm contains some non-specific inclusions (Borojevic 1966). It is possible, however, that the cells referred to as collencytes are actually a heterogeneous population (Simpson 1984; Harrison and De Vos 1991). Experiments on tissue transplantation demonstrate their involvement in immune reactions (Van de Vyver and Barbieux 1983; Buscema and Van de Vyver 1984a, b; Custodio et al. 2004). Interestingly, the level of the collencyte population remains rather high in allogenic pairs but drops significantly in the control. Most often, the name of collencytes has been applied to lophocytes.