Placozoa

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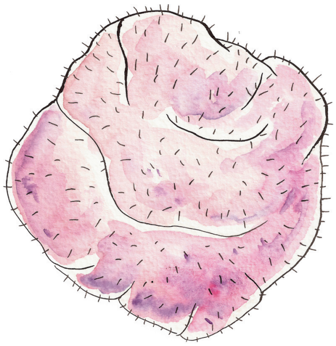

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INTRODUCTION

The most primitive metazoan animal phylum Placozoa presently harbors a single named species, the enigmatic *Trichoplax adhaerens*. In 1883, the German zoologist Franz Eilhard Schulze discovered this microscopic marine animal on the glass walls of a seawater aquarium at the University of Graz, Austria (Schulze 1883). The animal, usually measuring less than 5 mm in diameter and less than 20 µm in thickness, looked like an irregular hairy plate sticking to the glass surface (Fig. 5.1) and was thus named *Trichoplax adhaerens* (Greek for "sticky hairy plate") (see Schierwater 2005 for historical overview). Recent genetic analysis of placozoan specimens from different ocean waters around the world, including the Mediterranean Sea, revealed the presence of several cryptic species (Eitel et al. 2013), i.e., species, which are morphologically *cum grano salis* undistinguishable. The real placozoan biodiversity is estimated to include several dozen genetically, developmentally, and ecologically distinguishable species.

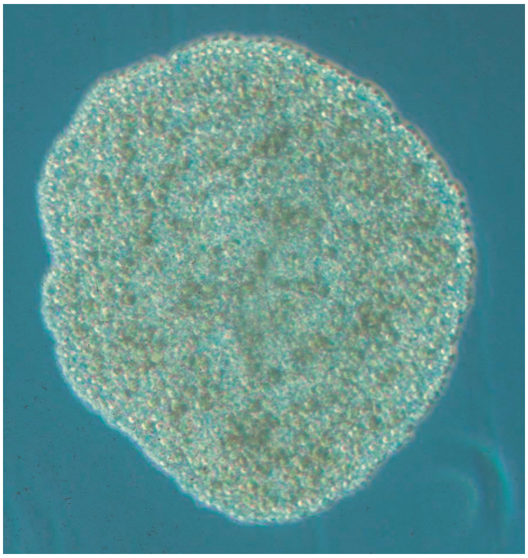

Fig. 5.1 The placozoan *Trichoplax adhaerens*, yet the only described species of the phylum Placozoa. The flat animal, which represents the most simple (not secondarily reduced) body plan of all metazoans, is found in tropical, subtropical, and certain temperate waters around the world ((Photograph by Bernd Schierwater) © Bernd Schierwater All Rights Reserved)

In contrast to the "typical" multicellular animal, *Trichoplax* does not show anything like an oral-aboral axis, nor does the animal possess any organs, nerve or muscle cells, basal lamina, or extracellular matrix (Schierwater 2013). Because of the lack of any axis, placozoans also lack any type of symmetry. The defining characteristic that separates placozoans (and any other metazoan) from protozoans is the number of somatic cell types. In contrast to protozoans, which consist of either a single cell or several cells of the same somatic cell type, Placozoa possess at least five defined somatic cell types: lower epithelia cells, upper epithelia cells, gland cells, fiber cells, and small potentially "omnipotent" cells (Jakob et al. 2004; Guidi et al. 2011). The epithelia cells are arranged in a sandwich-like manner, with the lower epithelia and gland cells at the bottom, the upper epithelia cells at the top, the fiber cells in between, and the "omnipotent" cells at the margin between the upper and lower epithelium (Fig. 5.2). Cells of the lower epithelium attach the animal to a solid substrate, enable the animal to crawl (with the aid of cilia) and allow feeding. During feeding, the animal lifts up the center region of its body to form an external digestive cavity between the substrate and lower epithelium (see Schierwater 2013 for details). Interestingly, the upper epithelium is also capable of feeding. Algae and other food particles are trapped in a slime layer coating the upper epithelium and are subsequently taken up (phagocytized) by the inner fiber cells; this unique mode of feeding is called "transepithelial cytophagy" (Wenderoth 1986). Placozoa presumably harbor endosymbiotic bacteria in the endoplasmic reticulum of the fiber cells (Grell and Benwitz 1971; Eitel et al. 2011). A possible role for these endosymbionts in feeding is not yet understood.

In general, very little is known about the biology of Placozoa, and almost all current knowledge derives from laboratory observations. Ecological data are limited to records of finding *Trichoplax* on hard substrate surfaces from tropical and subtropical marine waters around the world (Eitel and Schierwater 2010; Eitel et al. 2013).

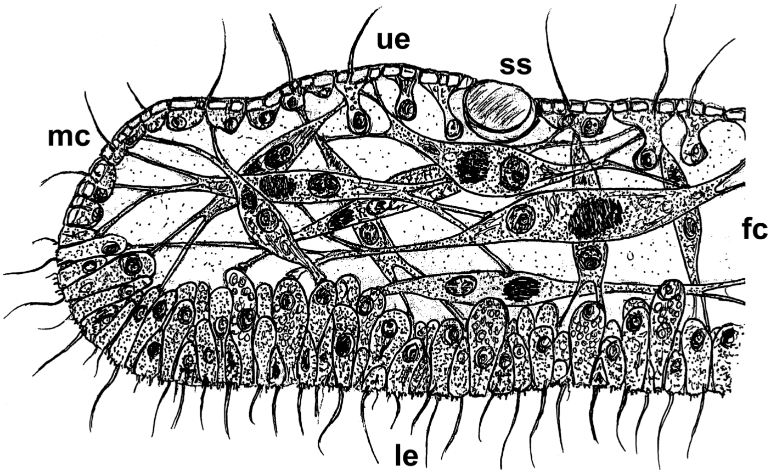

Fig. 5.2 Cross section of *Trichoplax adhaerens* showing the sandwich-like organization of the body plan: the ciliated upper epithelium, the ciliated lower epithelium, and the fiber cells in between. The interior fiber cells form a contractile, three-dimensional meshwork. The lower epithelium serves as the nutritive body region with gland cells incorporated into the epithelium. The upper epithelium shows no specializations, with the exception of the shiny spheres (originally named "Glanzkugeln"). These are lipid droplets, which are usually understood as residues of degenerated epithelial cells. A remarkable and exclusive feature of Placozoa is the lack of both an extracellular matrix and a basal lamina. *mc* marginal cells, *ue* upper epithelium, *le* lower epithelium, *fc* contractile fiber cell, *ss* shiny sphere (Modified from Eitel et al. (2011))

After its original description in 1883, *Trichoplax* attracted particular attention because it possibly mirrored the basic and ancestral state of metazoan organization. Almost a hundred years later, the German zoologist Karl Grell further highlighted this view and created the new, and monotypic, phylum Placozoa (Grell 1971). The phylum name refers to Bütschli's placula hypothesis (Bütschli 1884), which sees a placozoan-like animal as the Urmetazoon (Schierwater et al. 2010). Although a variety of molecular data support the traditional view that Placozoa are closest to the very root of metazoan origin, quantitative molecular systematics overall has created more confusion than resolution yet. We believe that eventually Haeckel's biogenetic rule (c.f. Schierwater et al. 2010) will provide the answer we are looking for.

For a comprehensive comparative analysis of the development of all non-bilaterian and some basal bilaterian phyla, however, the recent bottleneck is the Placozoa. A lot more work on the development of placozoans is needed. Given the importance of this enigmatic phylum Placozoa, any investment in developmental research seems to be justified.

SEXUAL REPRODUCTION

Despite more than half a century of research efforts, the complete life cycle of Placozoa can only be suspected. Most likely, the adult placozoon – after a series of vegetative reproductions – becomes sexually mature either as protandrous or simultaneous hermaphrodite. If the sperm is released into the open water, outcrossing might be possible, but in most cases selfing may occur. Sexual reproduction has been studied in the laboratory using different placozoan species. Experiments have identified specific environmental conditions that are required for the generation and maturation of oocytes. These include high animal density, food scarceness, and temperatures above 23 °C (see Eitel et al. 2011).

Oogenesis

Female gametocytes (oocytes) are presumably produced in the lower epithelium (Grell and Benwitz 1974), while maturation and fertilization occur somewhere in the center of the body (Fig. 5.3). During oocyte maturation, the mother

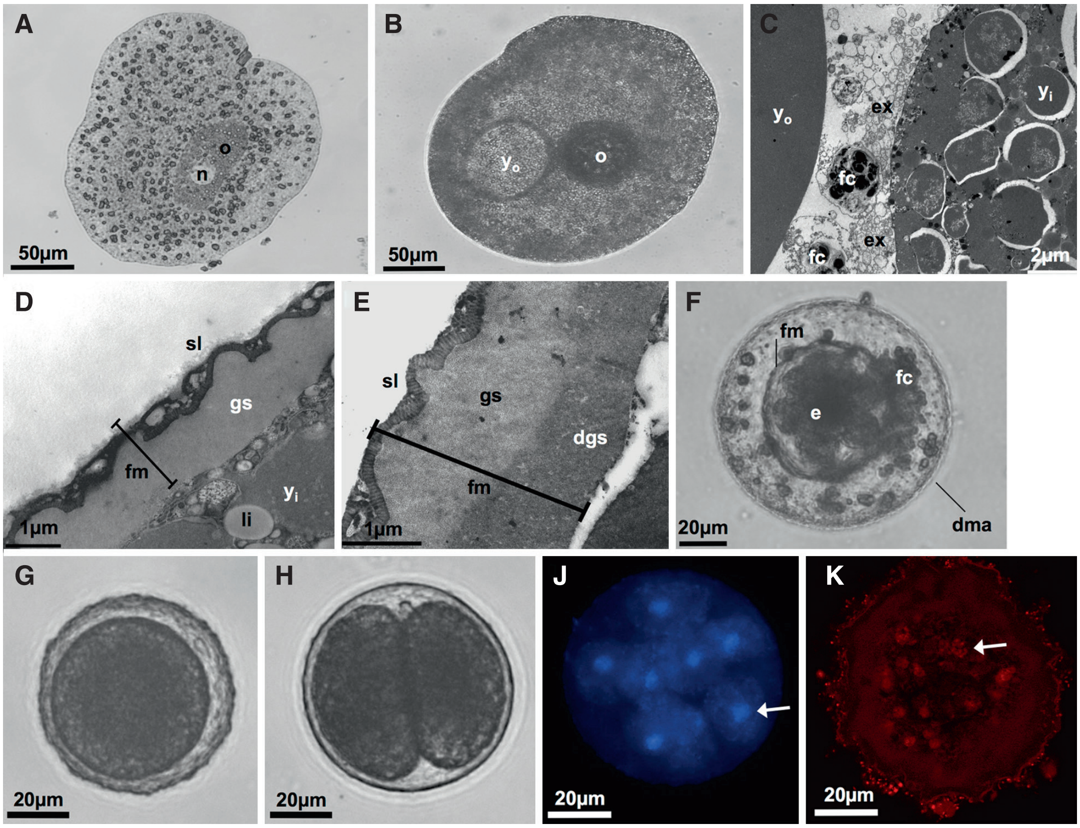

Fig. 5.3 Oogenesis and early embryonic development in the undescribed Placozoa sp. H2. Shown are light microscopy (**A**, **B**, **F–H**), transmission electron microscopy (**C–E**), and fluorescence microscopy (**J**, **K**) images of oocytes and embryos. (**A**) An oocyte with a large nucleus grows in a flat animal without any signs of degeneration. (**B**) Accompanied by the generation of yolk droplets, the animal enters the degeneration phase after 5–6 weeks. (**C**) By incorporating extensions from fiber cells through pores, the oocyte grows. (**D**) After fertilization, the "fertilization membrane" is built around the zygote. This protective egg shell drives from the fusion of cortex granules in the margin of the oocyte. It has a characteristic two-layered appearance in early stages. (**E**) Later embryo with three-layered egg shell. (**F**) By lifting the upper and condensing the lower epithelium, the mother animal rounds up and builds a "brood chamber" for the embryo. (**G–K**) The first and all later cleavages are total-equal. Fluorescence microscopy (DAPI in **J** and propidium iodide in **K**) is used to count nuclei and chromosomes in embryos (*arrows* in **J** and **K**, respectively). *n* nucleus, *o* oocyte, *yo* yolk outside oocyte, *yi* yolk inside oocyte, *fc* fiber cells, *ex* fiber cell extensions, *fm* fertilization membrane, *sl* striped layer, *gs* ground substance, *dgs* dense ground substance, *li* lipid droplet, *e* embryo, *dma* degenerating mother animal (Figure modified after Eitel et al. (2011))

animal enters the so-called D phase (degeneration phase) in which the upper epithelium lifts and the lower epithelium condenses. A key feature of this phase is the generation of yolk droplets, not only inside but also outside the oocyte. The outside yolk droplets accumulate to form a large cluster, possibly an energy source for the growing embryo while still inside the mother organism. To grow, the oocyte incorporates extensions from nursing fiber cells through pores on its surface (Grell and Benwitz 1974, 1981; Eitel et al. 2011). In the maturation process, specific granules are formed throughout the oocyte. These structures of unknown material look strikingly similar to – and likely resemble – cortical granules known from other marine invertebrates. During maturation, these granules increase in number and are transported toward the oocyte's surface. In addition to yolk droplets and cortical granules, the oocyte stores lipid droplets and glycogen granules. A fully mature oocyte reaches a variable size of 70–120 μm, depending on the size of the mother animal and the number of oocytes that are built.

From electron and fluorescence microscopy, it is known that a vast amount of bacteria are transferred into the oocyte during maturation. These are vertically transmitted from the nursing fiber cells (Grell and Benwitz 1974, 1981; Eitel et al. 2011).

Spermatogenesis

The existence of male gametocytes (sperm) was claimed based on ultrastructural observations (Grell and Benwitz 1974; Eitel et al. 2011), but their functionality has not been confirmed yet. Sperm is probably produced in the center of the animal, but the exact location and the progenitor cells are unknown. The expression of sperm-associated marker genes strongly suggests spermatogenesis and sperm maturation in placozoans. According to transcriptome analyses of three placozoan species, the potential sperm markers cover various stages of spermatogenesis, ranging from early meiosis to sperm maturation (Eitel et al. 2011). Even markers known to encode proteins for functional sperm flagella and sperm-oocyte recognition proteins used in fertilization were identified. Sperm markers were found expressed in adult, healthy growing animals that did not show any sign of degradation. This indicates production and storage of sperm before the animal experiences unfavorable conditions. It is thus likely that only oocytes are produced at that stage and that placozoans are possibly protandrous hermaphrodites.

Early Embryonic Development

Mature oocytes are fertilized internally. Subsequently, the "fertilization membrane" is built by fusion of accumulated granulae on the oocyte's surface. The "fertilization membrane" serves as a protective egg shell and resembles the cortex of other invertebrates (Grell and Benwitz 1974; Eitel et al. 2011). Early embryos grow inside the mother animal until the latter completely degenerates and releases the embryo. Despite great investigator efforts, the embryonic development was never completed under laboratory conditions. Here, embryos do not develop past the 128- to 256-cell stage. The reasons are unknown, but it must be speculated that the water chemistry (including microflora) under laboratory conditions does not meet the specific requirements for the developing embryos. What we do know from the early development is that cleavage is total and equal from the zygote to the 128-cell stage.

VEGETATIVE REPRODUCTION

Besides the sexual reproduction outlined above, two modes of vegetative reproduction are known: (1) fission (normal type of vegetative reproduction in Placozoa) and (2) swarmer formation (occasional type of vegetative reproduction). In fission, animals grow to a certain size and divide into two approximately equally sized daughter individuals, which then regrow to "normal" size. This mode of vegetative reproduction can go on *ad infinitum*, and it is conceivable that there might be placozoan species and populations out there that only reproduce vegetatively. The second mode of vegetative reproduction has only been observed in the laboratory when environmental conditions become unfavorable. Under such conditions, placozoans may develop small spherical swarmers, which are planktonic (free-floating) and thus are taken by water currents to new habitats. Several different swarmer types have been characterized among which the "hollow spheres" have been shown to open at one point to create flattened animals (Thiemann and Ruthmann 1988, 1989). These spheres are made up of an outer layer of upper epithelial cells, a fiber cell layer, and an inner layer of lower epithelial cells. After opening, the spheres will fully rebuild the normal adult habitus within a day.

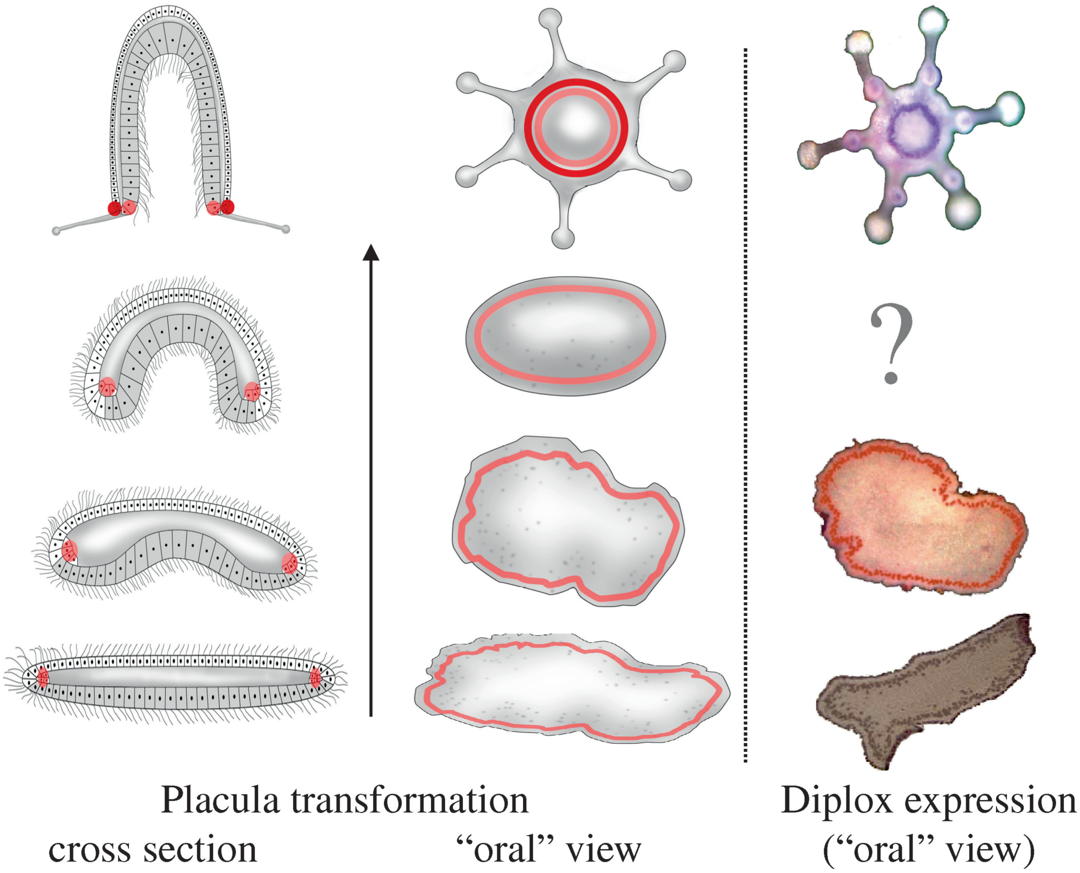

Fig. 5.4 The "new Placula hypothesis of metazoan origin" (Schierwater et al. 2009b). A nonsymmetric and axis-lacking body plan (placula) transforms into a typical symmetric metazoan body plan with a defined oral-aboral (or anterior-posterior) body axis (indicated by the *arrow*). This original idea from Bütschli (1884) has recently been complemented by expression patterns of the putative Proto-Hox/ParaHox gene, *Trox-2* (in *red*). A single regulatory gene, like *Trox-2*, can control the separation between lower and upper epithelium (*three lower rows*), i.e., create polarity as a precursor of symmetry. Once a main body axis, like oral-aboral (e.g., in cnidarians) has developed, duplication of the Proto-Hox/ParaHox gene could aid the invention and organization of new head structures originating from the ectoderm-endoderm boundary of the oral pole (*upper row*). Indeed, two putative descendants of the *Trox-2* gene, *Cnox-1*, and *Cnox-3* show these hypothesized expression patterns in the hydrozoan cnidarian *Eleutheria dichotoma* (*upper row*, for simplicity, only the ring for *Cnox-1* expression is shown) (See Schierwater et al. (2009b) for details © Bernd Schierwater. (All Rights Reserved)

DEVELOPMENTAL GENES

The study of developmental genes in placozoans is as exciting as explosive, since the interpretation of any kind of gene expression data depends on the evolutionary perspective one looks from. Those scientists believing in so-called quality data (cf. Osigus et al. 2013), which put placozoans in an ancestral position to other diploblastic animals, see a clear picture for, e.g., ancestral Hox-like genes in placozoans. Here, Hox-like genes are responsible for coordinating an ancestral symmetry pattern which is called "polarity". The putative Proto-Hox/ParaHox gene, *Trox-2*, determines the setup of polarity in *Trichoplax* (Jakob et al. 2004). Since polarity is the first step for creating symmetry, the "new placula hypothesis" (Fig. 5.4; Schierwater et al. 2009b) derives as naturally as a baby's smile. The possibly ancestral Hox-like gene fulfills a logical and predicted ancestral function, and animals, which give up symmetry and strict polarity (like sponges), do no longer need Hox-like genes. Scientists believing in one of the several contradictive evolutionary scenarios arising from so-called quantity data (Osigus et al. 2013) may have a completely different view. They may see placozoans somewhere in a derived position in the tree of life and may interpret the *Trox-2* expression patterns and Hox-like gene presence in any evolutionary and less parsimonious way they want. Nothing to blame, this is scientific freedom.

When talking about developmental genes, one automatically comes to signaling pathways. In placozoans, representatives of all important components are present for the *BMB*/*TGF* beta, *Wnt*, and *Notch* signaling pathways. In the Hedgehog pathway, however, only *Fused* was found in the genome (Srivastava et al. 2008; Schierwater et al. 2009a). We do not know anything about the expression of these pathway genes neither in the embryo nor as maternal factors in the oocyte. Given that basically all major developmental gene families are present in placozoans, it seems disappointing how few expression and functional data we have here. Clearly, much more work is needed to obtain comparative developmental genetic data from placozoans. These data will not solve but contribute to the debate of ancestral versus derived developmental patterns to be found in placozoans.

OPEN QUESTIONS

- Type of hermaphroditism
- Selfing versus outcrossing
- Completion of the life cycle
- Function and expression of developmental genes

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