

My favorite animal, *Trichoplax adhaerens*

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Summary

Trichoplax adhaerens is more simply organized than any other living metazoan. This tiny marine animal looks like a irregular “hairy plate” (“tricho plax”) with a simple upper and lower epithelium and some loose cells in between. After its original description by F.E. Schulze 1883, it attracted particular attention as a potential candidate representing the basic and ancestral state of metazoan organization. The lack of any kind of symmetry, organs, nerve cells, muscle cells, basal lamina and extracellular matrix originally left little doubt about the basal position of *T. adhaerens*. Nevertheless, the interest of zoologists and evolutionary biologists suddenly vanished for more than half a century when *Trichoplax* was claimed to be an aberrant hydrozoan planula larva. Recently, *Trichoplax* has been rediscovered as a key species for unraveling early metazoan evolution. For example, research on regulatory genes and whole genome sequencing promise insights into the genetics underlying the origin and development of basal metazoan phyla. *Trichoplax* offers unique potential for understanding the minimal requirements of metazoan animal organization. *BioEssays* 27:1294–1302, 2005. © 2005 Wiley Periodicals, Inc.

How I became interested in Placozoa

On Tuesday night, around 6 p.m. on 11 December 1979, my most respected teacher, Carl Hauenschild, lists several reasons for Placozoa possibly being a basal metazoan phylum. Arguments range from smallest metazoan genome, to the lack of a basal lamina, to overall morphological simplicity. The black and white pictures thrown from the solid 30 year old Leitz slide projector onto the well-aged screen are not very impressive and yet they are somehow fascinating and breathtaking. This animal looks more like an amoeba than a metazoan (Fig. 1A). If it wasn't for the fact that *Trichoplax* possesses four different somatic cell types, it could be

classified as a multicellular or colonial protist and nobody would argue. As a rather inexperienced undergraduate student, I see only one interpretation: the screen shows the mother of metazoan life. *Trichoplax adhaerens*, the only species of the phylum Placozoa, looks like living proof of (or possibly just the inspiring model for) Bütschli's placula hypothesis and his hypothetical “urmetazoan”. According to Bütschli the latter was a uniform two-cell layered disk adapted to a benthic life style (similar to living *Trichoplax*).

When Carl Hauenschild discussed alternative interpretations regarding the phylogenetic position of Placozoa, he could not convince me of a derived position of Placozoa. In the latter case, *Trichoplax* would represent a secondarily reduced, simplified bauplan. If, for example, Placozoa were derived from Cnidaria, the following question would be hard to answer. How could a cnidarian give up its cnidae, l-cells, gastric cavity, entoderm, nerve cells, epitheliomuscular cells, basal lamina, extracellular matrix and other favorable inventions without dropping into an ecological and evolutionary no-man's-land? In other Hauenschild lectures, I have seen examples of dramatic secondary reduction of bauplans, the most extreme ones a result of adopting a parasitic life style or reducing the life-cycle to neoteny. No example, however, would be as dramatic as a secondarily reduced *Trichoplax* scenario. Placozoans possess neither a basal lamina nor an extracellular matrix (ECM), and losing both these features would have substantial cytological, physiological and morphological consequences. Even for a very creative zoologist, it would be hard to come up with a scenario in which the loss of these features becomes advantageous to selection.⁽¹⁾

It is still Tuesday, 11 December 1979, and almost 7 p.m. now, and the cross-section of *Trichoplax* (Fig. 2A) shown on the screen is the last slide for today's lecture. Already I know, when I become a researcher I want to study *Trichoplax adhaerens* and test Bütschli's placula hypothesis.

My first contact with *Trichoplax*

In 1989, the Department of Biology at Yale University, and in particular the laboratory of my postdoctoral adviser, Leo Buss, was a very exciting place for me as a postdoc to explore new things. Alongside my research on cnidarians, I started a *Trichoplax* culture with three animals that I had collected from an aquarium in Philadelphia. It was exciting just to watch this small, flat and unstructured disc of cells that forms an irregular body change its shape all the time (Fig. 1C). While

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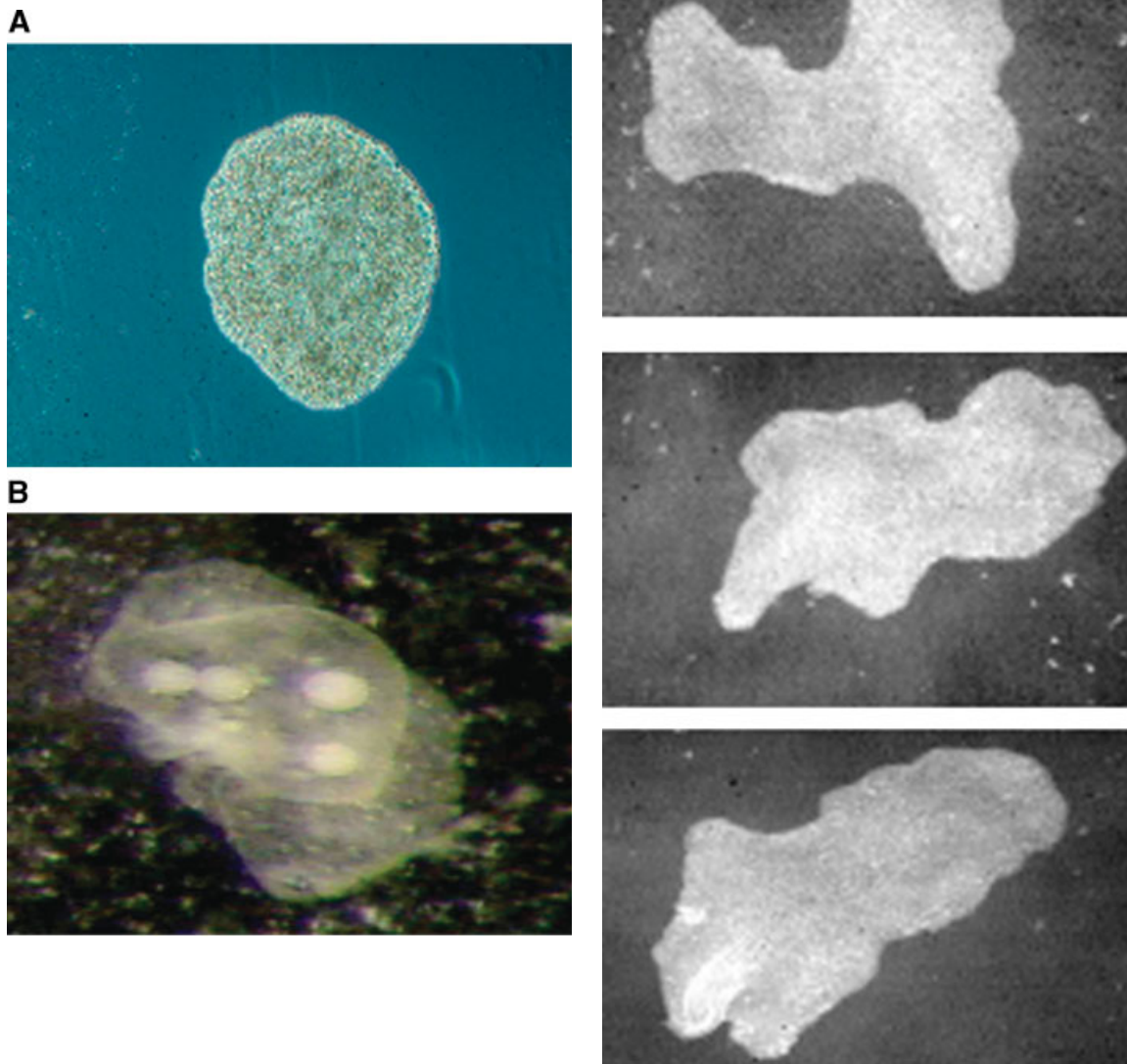


Figure 1. Photographs of *Trichoplax adhaerens*. **A,B:** These specimens belong to the so-called “Grell clone” and are about 2 mm in diameter. **B:** Eggs are growing in the interspace between the lower and upper epithelium (photograph by Sven Sagasser, Hannover). **C:** Changes of body form. Fig. 1A from Ender A, Schierwater B. 2003 *Mol Biol Evol* 20:130–134 with permission from Springer. Fig. 1C from Syed T, Schierwater B. 2002 *Senckenbergiana lethaea* 82:315–324 with permission from Springer.

moving—and movement is noticeable only when the animal is watched closely for several seconds—it leaves behind a grazing track of digested and phagocytised algae at the bottom of the Boveri dish (see Schierwater & Kuhn⁽²⁾) for feeding conditions). I enjoyed watching individuals grow bigger and divide into two. This vegetative mode of reproduction is quite effective: soon I had a few hundred individuals growing in my

Boveri dishes and I had to move them into larger dessert bowls. I had enough animals to perform phototaxis experiments and get ready for extracting DNA and RNA for isolating Hox genes, just before I had to travel to Stanford (California) for a week. When I came back, all *Trichoplax* cultures had died. This happened a second and a third time, and I had to wait until 1993 to establish a new culture in Frankfurt. Two recognized

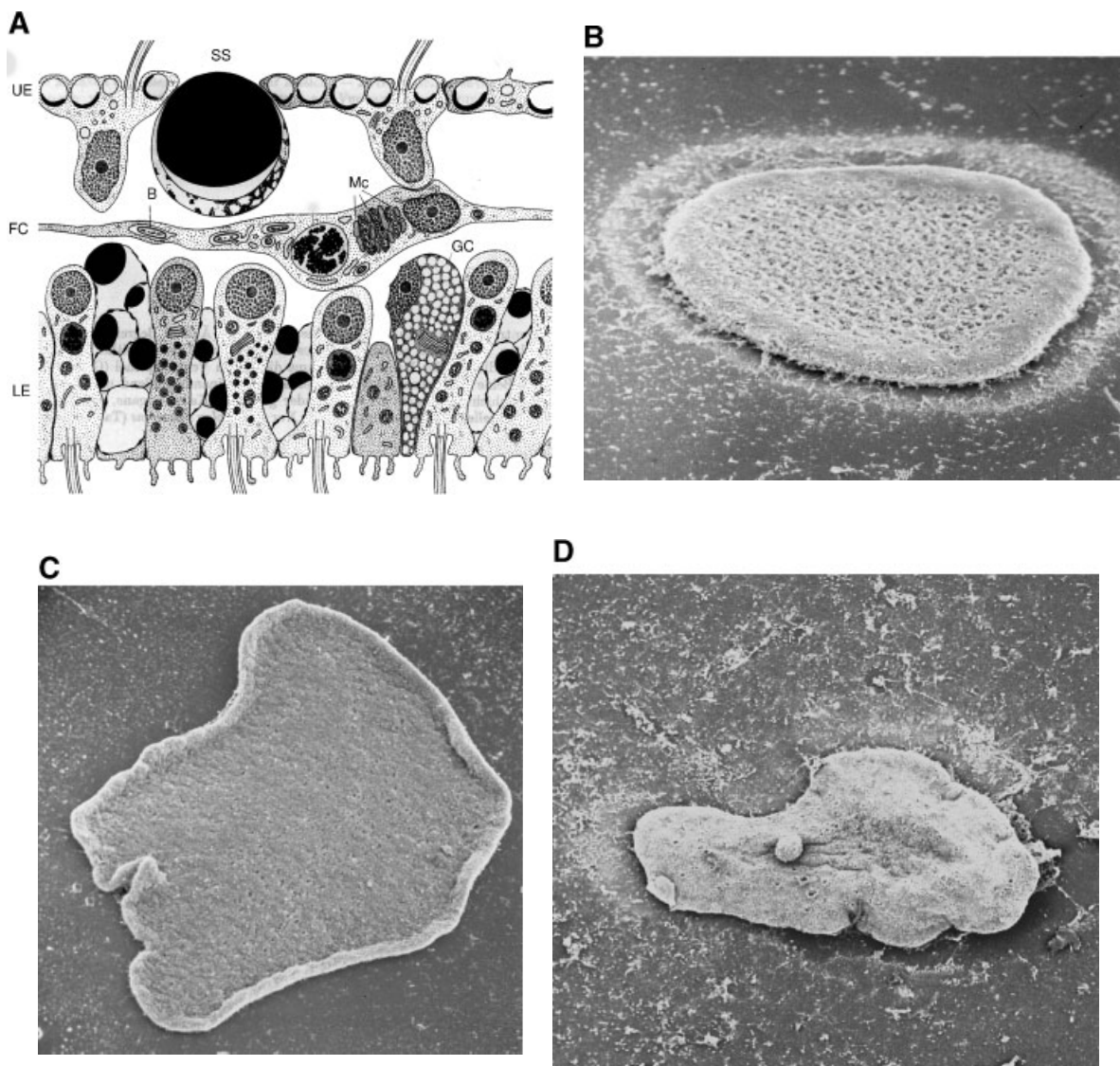


Figure 2. Placozoan morphology. **A:** Cross section of *Trichoplax adhaerens*; modified after Grell & Ruthmann,⁽⁵⁴⁾ see also Syed and Schierwater.⁽²³⁾ UE, upper epithelium; LE, lower epithelium; FC, contractile fiber cell; GC, gland cell; SS, shiny sphere; Mc, mitochondrial complex; B, (endosymbiotic?) bacterium in endoplasmic reticulum. Note that the interspace between fiber cells and epithelia is free of ECM and that a basal lamina is missing. **B–D:** Low-magnification scanning electron micrographs of diverse placozoans; the differences seen are not necessarily characteristic of the various lineages (electron micrographs by Christiane Pech, Hannover). **B:** Indo-Pac lineage. **C:** Panama #2 lineage. **D:** Grell clone individual with a “swarmer” being budded off from the upper epithelium. (See also Thiemann & Ruthmann.⁽⁵⁵⁾)

Trichoplax researchers, Drs. Ruthmann and Wenderoth, who had been culturing the original Grell clone^(3–6) in Bochum for more than two decades, provided us with animals. In 1999 both colleagues, Heinz Wenderoth and August Ruthmann, retired from university work and handed over the *Trichoplax* cultures to our lab. The “Grell” clone was found by the German protozoologist K.G. Grell, University of Tübingen, in an algal sample from the Red Sea in 1969 and is now the DNA

source that we provided for the *Trichoplax* Genome Project (*Trichoplax* Genome Consortium 2005; www.jgi.doe.gov/sequencing/why/CSP2005/trichoplax.html).

The poorly known biology of *Trichoplax adhaerens*

If whole genome sequencing had been possible at the start of the 20th century, *Trichoplax* would probably have been one of



Figure 3. Placula hypothesis of metazoan evolution. Flagellated protozoans unite to form a benthic-vagile, plate-like metazoan organism. The one-layered protist form (a) evolves to the two-layered “placula” (b,c). Cells of the upper layer form the ectoderm, while cells of the lower layer (orange) adopt a nutritive function and later invaginate to form the entoderm (d–g); modified from O. Bütschli,⁽²⁴⁾ and Syed & Schierwater.⁽²³⁾

the very first animals to have its genome sequenced because of its unique, highly primitive morphology. The unique bauplan is based on a simple, irregular sandwich organization. An upper and a lower epithelium surround a loose network (not an epithelium) of so-called fiber cells (Fig. 3a). Traditionally only four cell types have been described in *Trichoplax*, upper and lower epithelial cells, gland cells within the lower, feeding epithelium, and fiber cells sandwiched between the epithelia.^(7–11) No organs or specialized nerve or muscular cells are present. A basal lamina and extracellular matrix are likewise lacking. All these simple bauplan characteristics make placozoans more similar to protozoans than to any other existing metazoans. Body shape is irregular and changes constantly (Fig. 1C). No symmetry of any kind is seen, and nothing like an oral–aboral or even a dorsoventral polarity exists. The only polarity present results from the fact that the lower (=feeding) epithelium faces the substrate while the upper epithelium faces the open water (in laboratory cultures *Trichoplax* sometimes also floats upside-down at the water surface).

In the laboratory, we commonly see *Trichoplax* undergoing binary fission. Animals grow and then pull apart into two daughter individuals of similar size.⁽¹²⁾ Another mode of vegetative reproduction has also been seen, the budding off of small spherical swimmers (Fig. 2D) which are planktonic. The latter most likely are dispersal stages, which may float in the open water for up to a week.⁽¹³⁾ Sexual reproduction remains enigmatic. Most likely *Trichoplax* can reproduce bisexually, i.e. by producing female and male gametes. The latter have not been observed, the former are comparatively huge (some 100 μm in diameter) and appear in small numbers in individual placozoans in the laboratory (Fig. 1B). Beyond aberrant early cleavage stages, no embryonic development has been observed.^(6,12) We know nothing about sexual reproduction in the field. So far, field specimens of *Trichoplax* have shown no signs of sexual reproduction (Vicki Pearse and Allen Collins, personal communications).

Trichoplax occurs in the littoral of all warm oceans and is distributed globally in tropical and subtropical waters.^(14,15) Finding *Trichoplax* in the field, however, can be tough. In three locations of the Great Barrier Reef, I could not find a single

animal within 4 weeks, while in another location I found 10 animals on a single microscope slide, our standard traps for collecting *Trichoplax* in the field. Vicki Pearse has been collecting *Trichoplax* for almost 2 decades at tropical and subtropical latitudes throughout the Pacific as well as in the Caribbean and has observed clear seasonal changes in abundance (cf also Maruyama⁽¹⁶⁾). It has been extracted from algae, pieces of coral and smooth stones. This is about all we know of the life of placozoans in the field. From laboratory cultures, we know that, in addition to the normal mode of feeding (pinocytosis in the lower epithelium), *Trichoplax* can also feed by means of the upper epithelium, a unique mode called “transepithelial cytophagy”.⁽¹⁷⁾ Nothing is known about the relative importance of these feeding modes or about the role of potentially symbiotic bacteria, regularly found in the endoplasmic reticulum of the fiber cells.⁽¹⁸⁾

Is *Trichoplax adhaerens* the mother of all metazoans?

When the German zoologist Franz Eilhard Schulze published the description of a new marine animal that looked like a “sticky hairy plate”, he called it *Trichoplax adhaerens* (Greek *trich* = hair, *plax* = plate, Latin *adhaerere* = to stick). Schulze’s histological analysis of *Trichoplax*, based on microtome sections and various staining procedures, revealed the three-layered sandwich organization⁽¹⁹⁾ outlined above. Schulze^(20–22) noted features peculiar to *Trichoplax*: the slow gliding movement and fluid body shape, the presence of only four somatic cell types, the lack of typical metazoan histology (no basal lamina or extracellular matrix); and the lack of any kind of symmetry. These features indicated to him that *Trichoplax* did not fit the patterns of sponges, cnidarians, ctenophores, or any of the vermiform phyla. Consequently, he suggested that *Trichoplax* represents a primitive bauplan close to the root of the metazoan phylogenetic tree. Schulze’s observations soon sparked debate on the evolutionary origin of Metazoa and the hypothetical “urmetazoan” (“archimeta-zoa”) between Haeckel, Lankester, Metschnikoff and other zoologists (for overview and refs. see Syed & Schierwater⁽²³⁾).

The idea that Placozoa is basal within Metazoa is reflected in at least one of the different hypotheses. Otto Bütschli

(University of Heidelberg) modified Haeckel's "gastrea hypothesis" into the "placula hypothesis", in which the gastrea derived from a flat, benthic-vagile ancestor, the hypothetical "placula" (Fig. 3).^(24,25) Later, *Trichoplax adhaerens* was placed in the new phylum "Placozoa",⁽³⁾ named after Bütschli's placula. According to Bütschli, the first metazoans evolved from colonial flagellates (Protozoa), which formed a benthic, single-layered organism with ciliary locomotion. Subsequently a two-layered "placula" developed with an upper "ectoderm" and a lower "entoderm", and invagination of the "entodermal" layer led to a benthic gastrea-like animal (Fig. 3). The evolutionary scenario of a *Trichoplax*-like, benthic animal, which gradually had its lower, nutritive epithelium displaced into the interior of the body, is also seen in the so-called benthoblastea–bilaterogastraea hypothesis of Jägersten,^(26,27) yet another modified version of Haeckel's blastaea–gastrea hypothesis.⁽²⁸⁾ It was expected that elucidating the ontogeny and the life cycle of *Trichoplax adhaerens* would be the next crucial step in resolving the phylogenetic position of Placozoa—a step that remains to be achieved. Instead modern developmental genetics is contributing to the "urmetazoon" discussion and a first attempt to incorporate regulatory gene data into the placula hypothesis has been made.⁽²⁹⁾

A number of researchers have tried to resolve the phylogenetic position of Placozoa through molecular studies, so far without complete success. Analyses of ribosomal RNA sequences have created several conflicting hypotheses (e.g. Collins,⁽³⁰⁾ Ender & Schierwater⁽³¹⁾ for references). So far, the only comparatively safe conclusion seems to be based on mtDNA genome structure, which indicates that Placozoa are not derived cnidarians but leaves open the question of a basal position relative to Porifera.⁽³¹⁾ Recent cladistic analyses of all mitochondrial genes and mtDNA composition suggest that Placozoa possess the largest and most ancestral mtDNA of Metazoa.⁽³²⁾ Taken together, analysis of morphology,

several nuclear genes and 16S rRNA structure also point towards a basal position of Placozoa.⁽²⁹⁾

Current and future research on

Trichoplax adhaerens

The description of Placozoa in an evolutionary context represented the first wave of research on *Trichoplax*. It lasted for about three decades around the turn of the 20th century. Schulze's article of 1914,⁽²²⁾ in which he rejected the weird and unsupported hypothesis that *Trichoplax* was a cnidarian larva, was the last publication on *Trichoplax* in a zoological journal for more than half a century. In the 1970s and 1980s a second wave of organismal data was initiated when the cell biologist W. Kuhl (University of Frankfurt) found *Trichoplax* in a seawater aquarium containing organisms from the Mediterranean Sea⁽³³⁾ (note that most people say *Trichoplax adhaerens* was rediscovered by K.G. Grell in 1969). The third wave of placozoan research has just recently started and includes studies at all levels of biological organization.

Trichoplax attracts the attention of modern multidisciplinary research for at least three good reasons: (1) it is the most simply organized metazoan animal, (2) it possesses the smallest genome of all known metazoans,^(35–37) and (3) it is currently undergoing whole-genome sequencing (see also Ref. 34). Thus, understanding the genetic control of its development will clarify basic principles of metazoan organization. The success of this research will also depend on progress at the organismal level, particularly with respect to documenting the life cycle.

Biodiversity

Placozoan specimens collected from diverse tropical and subtropical waters around the world appear to be highly similar morphologically and all fit the description of *Trichoplax adhaerens*. Molecular analyses of ITS1-2 and three rRNA

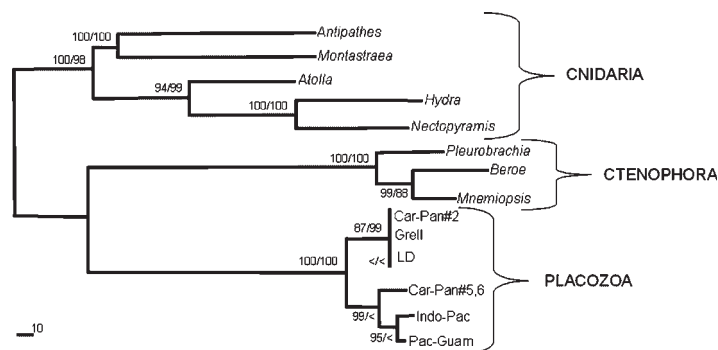


Figure 4. Unexpected diversity has been found in the phylum Placozoa, formerly assumed to be monotypic. Shown is a MP phylogram of placozoans based on combined SSU and LSU data. The sampling has been very limited so far and in order to unravel biodiversity of this unique phylum, more and extended studies are urgently needed. See Voigt et al.,⁽³⁸⁾ and Tomasetti et al.⁽⁵⁶⁾

genes, however, reveal substantial genetic variation and deep phylogenetic separation between strains (Fig. 4).^(38–40) According to these data, the phylum Placozoa is not monotypic (note that the existence of a formerly described second species, *Treptoplax reptans*,⁽⁴¹⁾ has never been confirmed). It has become clear that Placozoa is a group harboring a larger—although yet unknown—number of species that likely will fall into different taxonomic units at the genus, family, or even higher taxonomic level. Unfortunately, the absence of documented morphological and ecological differences and the lack of life-cycle information hinder the assignment of new species and higher taxonomic units. Aleshin et al.⁽⁴²⁾ found no substantial differences between the “Grell” clone and a clone possibly originating from the Sea of Japan. It seems that a placozoan species can have world-wide distribution and that different lineages may occur sympatrically.^(38,39) Yet sampling has been rather limited and more intensive world-wide collecting is required in order to unravel the biodiversity of Placozoa. Currently, the paucity of morphological characters and ecological data hamper the description of new species and the creation of a taxonomic system for Placozoa. As a first step, we have recently begun an endeavor to barcode⁽⁴³⁾ all the different placozoan lineages. Placozoa would be the first metazoan phylum barcoded as a whole. Barcoding aims to identify DNA sequences as unique taxon barcodes for each single animal species. Ideally only a few genes, e.g. the mitochondrial COI and some ribosomal genes, will harbor sufficient information to identify all animal species by individual DNA barcodes. For the phylum Placozoa, we expect barcode markers to also be informative for resolving placozoan systematics, i.e. assigning taxonomic levels to genealogical branching patterns.

In collaboration with Maria Balsano (Urbino, Italy), we have begun electron microscopical studies on the morphology of different phylogenetic lineages in order to identify morphological differences between deeply separated clades and thus facilitate the description of new species. In collaboration with John and Vicki Pearse (University of California, Santa Cruz), Allen Collins (National Marine Fisheries Service, Washington DC), Stephen Dellaporta (Yale University, New Haven), Paolo Tomasetti (Central Institute for Marine Research, Rome, Italy) and Rob DeSalle (American Museum of Natural History, New York City), we also seek to gain more information from the biology of Placozoa in the field. Efforts from more research groups are urgently needed in order to resolve the biodiversity of Placozoa.

General Biology

Understanding the complete life-cycle of placozoans is an urgent priority. Although sexual reproduction was suspected in the early 1970s, (Grell and Benwitz^(4,6,44) reported oogenesis and cleavage processes), observations on embryonic development have never gone beyond the 64-cell stage.^(12,37) Since no researcher has yet been able to follow development beyond early stages of embryogenesis, we do not even know if *Trichoplax* develops directly or possesses a larval stage. At present it appears difficult to sustain either full sexual or embryonic development under laboratory conditions. Whether these stages will be found in the field also seems uncertain. It is conceivable that placozoans in the field reproduce vegetatively only or that sexual reproduction is a rare event occurring only under certain environmental conditions. Molecular data

Table 1. Developmental regulatory genes reported from Placozoa

Developmental gene	Expression	Effect of Inhibition	Reference
<i>Trox-2</i>	Body margin between ecto- and endoderm.	Cease of vegetative growth and reproduction.	Jakob et al., 2004
<i>Not</i>	Body folds of intact animals.	—	Martinelli & Spring, 2004
<i>PaxB</i>	“Distinct cell patches along a ring region close to the outer edge of the animal body.”	—	Hadrys et al., 2005
T-box			
1. <i>Brachyury</i>	“In a few cells or groups of (unknown) cells, marginal to the edge of potential outgrowth zones of larger animals.”	—	Martinelli & Spring, 2003
2. <i>Tbx2/3</i>	“Body margin in the upper and lower epithelium.”		
<i>Hmx</i> (NK5)	Very low to not detectable in adult <i>Trichoplax</i> .	—	Monteiro et al., 2005, Unpublished data
<i>Dlx</i> (distal-less)	Spatially restricted expression around the periphery.	—	Monteiro et al., 2005, Unpublished data
<i>Mnx</i> (NK5)	Spatially restricted expression around the periphery.	—	Monteiro et al., 2005, Unpublished data

All genes somehow show spatial expression patterns near the outer margin of the animal. This observation might be related to the suggestion that, in this—in addition to expression studies—gene inhibition studies have been successfully performed; it seems to be involved in polarity determination.⁽⁴⁷⁾ I would like to note that the listed *Not* expression could not be reproduced in our lab. Instead, we regularly see high background signal in “body folds of intact animals” of *Trichoplax*.

My favorite animal

on allelic variation of single-locus genes support neither the regular occurrence of sex nor the complete lack of sex in the life cycle.⁽³⁸⁾ Probably the best means towards progress in unraveling the life cycle is to culture diverse phylogenetic lineages in the laboratory under different and changing conditions. *Trichoplax* has been cultured in several labora-

tories (in Germany, Italy, Russia, and the US) but no progress beyond observation of cleavage stages has been reported. In our laboratory, so far we have found sexual specimens only in a Panama clone (Fig. 2C).

Likewise, very little is known about the ecology of Placozoa.^(34,45) Perhaps the *Trichoplax* Genome Project

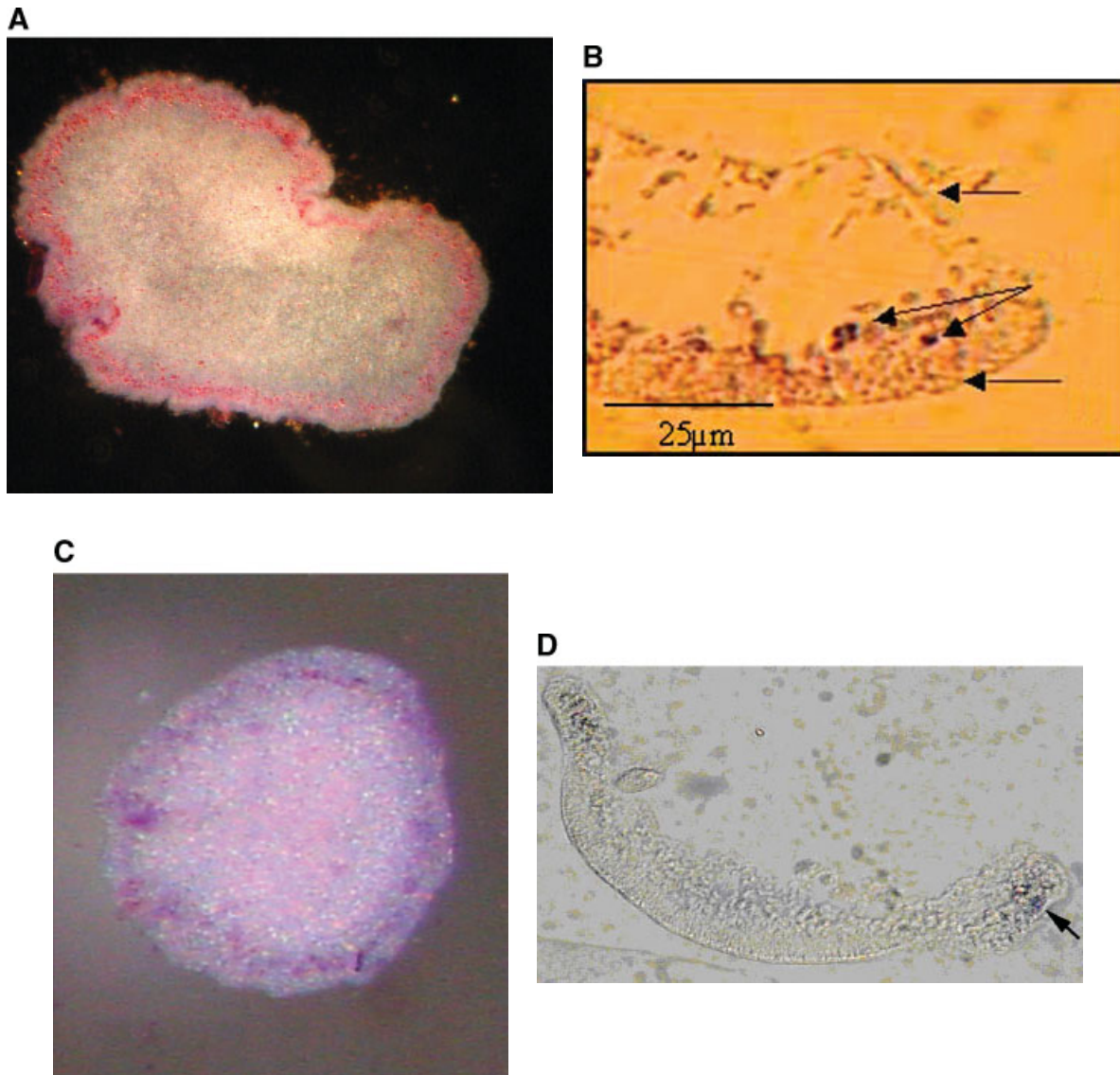


Figure 5. The study of regulatory genes reveals insights into basic and basal mechanisms of metazoan development and evolution. Most regulatory genes studied so far are expressed within or close to a small region of potentially undifferentiated cells embedded between the lower and the upper epithelium. **A,B:** *Trichoplax* whole mount in situ hybridization for the putative ProtoHox/ParaHox gene, *Trox-2*; note the strong and homogenous expression close to the body margin; arrows in **B** point to small undifferentiated—yet undescribed—cells between the lower and upper epithelium; from Jakob W, Sagasser S, Dellaporta S, Holland P, Kuhn K, Schierwater B. 2004 *Dev Genes Evol* 214:170–175 with permission from Springer. **C,D:** *Trichoplax* whole mount in situ hybridization for the putative ProtoPax gene, *TriPaxB*; note the more spotted expression along the body margin; the arrow in **D** points to a small *TriPaxB*-expressing cell that is similar to cells expressing the *Trox-2* gene (**B**); from Hadrys T, DeSalle R, Sagasser S, Fischer N, Schierwater B. 2005 *Mol Biol Evol* 22:1–10 with permission of Springer.

(see below) will highlight the importance of Placozoa and also fuel more ecological research and field work. The success of the latter will obviously also depend on progress with respect to biodiversity and systematic issues of Placozoa (see above).

EvoDevo research

In 2001 the Human Frontier Science Program awarded a Research Grant to the Antp Superclass Gene Consortium in order to clarify the early evolution of *Antp*-type genes in basal metazoans and to develop *Trichoplax adhaerens* as a model system for research in development and evolution. Together with Peter Holland (Oxford) and Stephen Dellaporta (Yale), we explored *Trichoplax adhaerens* from different perspectives. Several new *Antp*-type genes were isolated, the existence of a single Hox/ParaHox gene was verified, comparative functional characters for several Antp superclass genes were analyzed, and the mitochondrial genome was sequenced. This research has yielded some surprising results. For example, the putative Proto-Hox gene *Trox-2*^(2,46) and the Pax B gene are expressed in a region where the upper and lower epithelia meet, and where yet undescribed pluripotent cells are suspected.^(47–49) The Not and T-box gene also seem to be expressed in the same area (Table 1).^(50,51) Ongoing research promises insights into the evolutionary origin of a nervous system, a head (or oral pole) and a body axis (e.g. oral–aboral) from an animal that lacks any kind of nerve cells, symmetry or main body axis.^(47,48,52,53)

Current efforts also aim to produce cell cultures of different cell lineages, including the yet-undescribed potentially “pluri-potent” cells connecting the lower and the upper epithelia (Fig. 5).⁽⁴⁷⁾ It has been known that isolated fiber cells live for hours in seawater,⁽⁵⁴⁾ and our own experiments indicate proliferation, in cell culture, of small cells that do not belong to any of the four standard cell types. Unraveling the function of these cells will likely add to our understanding of placozoan development.

Whole genome sequencing

As a logical consequence of the work of the Antp Superclass Gene Consortium, a *Trichoplax* Genome Consortium was founded aiming to sequence and describe the complete genome. The DOE Joint Genome Institute (Walnut Creek, California) is completing this project in 2005. Once the genome sequence is released, I expect the number of research groups working on *Trichoplax adhaerens* to substantially increase. Together with anticipated genome sequences from other diploblasts (those of the anthozoan *Nematostella*, the hydrozoan *Hydra*, and the sponge *Reniera* are imminent), these data will be of crucial importance for reconstructing the evolution of metazoan genomes, bauplans and development. I expect the *Trichoplax* genome to become the standard basal genome for the comparative analysis of animal genomes,

genes and biological processes (<http://www.jgi.doe.gov/sequencing/why/CSP2005/trichoplax.html>).

At present, it is easier to sequence the whole genome than to describe the basic life cycle of *Trichoplax*. Maybe this is another reason why *Trichoplax* is my favorite animal and challenge.

Acknowledgments

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