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TRICHOPLAX ADHAERENS: DISCOVERED AS A MISSING LINK, FORGOTTEN AS A HYDROZOA, RE-DISCOVERED AS A KEY TO METAZOAN EVOLUTION

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ABSTRACT. - Trichoplax adhaerens is more simply organized than any living metazoan. After its original description by FE Schulze 1883, it attracted particular attention because it possibly possessed the basic and ancestral state of metazoan organization. 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. Hox genes and whole genome sequencing promise insights into the genetics underlying the origin and development of basal metazoan phyla. We here review the history of research on Trichoplax, and provide a modern interpretation of special Trichoplax features in an evolutionary context.


I. The discovery of Trichoplax adhaerens – Schulze’s original description and Bütschli’s “Placula-Hypothesis”

In 1883, the German zoologist Franz Eilhard Schulze published a short communication describing a new species, Trichoplax adhaerens (the "sticky hairy plate"; Greek trich=hair, plax=plate): a flattened, crawling marine animal of up to a few millimeters in size (Fig. 1). Schulze found these organisms settling on the glass sides of seawater aquaria at the University of Graz (Austria), recognizing their amoeba-like movements and continual shape changes. These were new features for metazoan animals.

Schulze’s histological analysis of Trichoplax, based on microtome sections and various staining procedures, revealed a three-layered sandwich organization of the animal, with morphologically different upper and lower epithelia. The epithelia enclose an inner, connective-tissue-like union of cells. A more detailed description was published by Schulze in 1891, and most of his results are still valid today (Fig. 2). The upper epithelium consists of a thin squamous layer while the lower epithelium consists of relatively large columnar cells and smaller gland cells. The lower epithelium is spe-
Fig. 1. - Photograph of living specimen of *Trichoplax adhaerens*. The animal shown is changing from an infolded (upper and right side) to its preferred flattened form (left side). The "shiny spheres" of the upper epithelium (cf. Fig. 2) are mainly visible on the left, where the body is already flattened.

Fig. 2. - Schematic cross section of *Trichoplax adhaerens* (modified after Grell & Ruthmann 1991). UE = upper epithelium, LE = lower epithelium, FC = contractile fibre cell, GC = gland cell, SS = shiny sphere, Me = mitochondrial complex, B = (endosymbiotic?) bacterium in endoplasmic reticulum. Note that the interspace between fibre cells and epithelia is free of ECM and that a basal lamina is missing.
TRICHOPLAX ADHAERENS: A KEY TO METAZOAN EVOLUTION

vermiform phyla. Consequently, he assumed that *Trichoplax* was an isolated, basal offshoot close to the root of the metazoan phylogenetic tree.

Schulze's original description of *Trichoplax* soon sparked debate on the hypothetical first metazoan ("urmetazoan" or "archimetazoan") between Haeckel, Lankester, Metschnikoff, and other zoologists (for overview see: Gruner 1993). Only one year after Schulze's original description of *Trichoplax adhaerens*, O. Büttschli (University of Heidelberg) published an improved version of Haeckel's "gastraea-hypothesis". While Haeckel's "gastraea", a hypothetical spherical, pelagic organism, invaginates from a pelagic "blastaea" at its posterior pole, Büttschli (1884) tried to derive the gastraea from a flat, benthic-vagile ancestor, the hypothetical "placula" (cf. Fig. 3). According to Büttschli, the first metazoans emerged after colonial flagellates (Protozoa) fused into a benthic, single-layered organism with ciliary locomotion. From this stage, the two-layered "placula" developed with an upper "ectoderm" and a lower "entoderm". Gradual invagination of the "entodermal" layer led to a benthic gastraealike animal. The entodermal invagination finally led to closed gastric cavities or through-guts, as was already described in Haeckel's model.

Büttschli argued that the three-layered *Trichoplax* is a comparatively derived organism, still mirroring the two-layered placula's mode of life. It is important to note that both Schulze and Büttschli agreed in interpreting the upper epithelium of *Trichoplax* as an ectoderm and the lower epithelium as an entoderm homolog. The question then arises whether the interior fibre cell complex of *Trichoplax* is a mesoderm homolog or not. Both authors hesitated to interpret the fibre cells as a mesoderm homolog because this would have implied a close affinity of *Trichoplax* to the triploblastic phyla. Büttschli (1884, p. 425) therefore saw in the fibre cell layer an analogy to mesodermal structures of triploblasts. Schulze (1883) pointed out that observations on the ontogeny of *Trichoplax* would be required to solve this question. Both researchers were aware of the principal counter-hypothesis that *Trichoplax* might be a secondarily simplified organism, as this was already proposed for some parasitic mesozoa. The latter alternative was regarded as relatively unlikely as there was no evidence for parasitism by *Trichoplax*. After the first morphological descriptions and resulting phylogenetic interpretations 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 the Placozoa.

II. The hydrozoan-interpretation -- *Trichoplax is forgotten*

In 1890, one year before Schulze's detailed *Trichoplax* description, FC Noll from the Senckenberg Museum in Frankfurt reported observations on the animals' normal mode of vegetative reproduction, which is binary fission of the entire body into two new individuals. Noll wrongly suggested the presence of otoliths in large *Trichoplax* specimens and thus a close relationship to the acoel turbellarians. This idea was supported by L von Graff (1891), an expert on Acoela. Schulze (1891) remained skeptical about this interpretation, mainly because of the arrangement of the inner contractile cells of the animal (which do not resemble a myoepithelium), and also because of the lack of any fixed axes of symmetry. At this time, most zoologists agreed with Schulze (1883) that the functional layer of contractile cells rejects a close relationship to either coelenterates or sponges. Through the end of the 19th century a close relationship of *Trichoplax* to acel turbellarians or mesozoa was discussed. After FS Monticelli (1893, 1896) described another *Trichoplax*-like animal, *Treptoplax reptans*, both forms were united as Mesenchyma, in reference to the fibre cells, and grouped within the Mesozoa (Delage & Herouard 1899), a phylum that had already become "a dumping ground for a host of multicellular but presumed nonmetazoan organisms" (Busca & Busca 1990). True metazoan phyla were seen as showing an invaginating gastrula stage during embryogenesis (e.g., Neresheimer 1912), although this definition had been intensely debated from the very beginning.

The question of the complete life cycle of *Trichoplax* initially yielded a most surprising -- and completely wrong -- answer when the German zoologist Thilo Krumbach observed these animals in a seawater aquarium that was settled by sexual medusae of the hydrozoan *Eleutheria krohni* (Krumbach 1907). As in medusae of other *Eleutheria* species, for example *E. dichotoma*, the eggs of *E. krohni* develop in a brood pouch, which eventually opens to release well-developed planula larvae (Hauenschild 1956, Schierwater 1989,

Fig. 3. -- Placula-hypothesis of metazoan evolution according to O Büttschli 1884. Flagellated protozoans unite to form a benthic-vagile, plate-like metazoan organism. The one-layered form (a) evolves to the two-layered "placula" (b). Upper cells of the placula are presumed ectoderm, while the lower cells adapt to nutritive function and thus are presumed entoderm (c). Note that there is no gradual transformation from stage a to b (modified from Gruner 1993).
Hadrys et al. 1990). Krumbach (1907) reported that he found Trichoplax individuals in exactly those positions where Eleutheria planulae had settled before. Krumbach was convinced that Trichoplax adhaerens was a deformed larva of Eleutheria krohni, although he never observed the supposed metamorphosis. Surely Krumbach’s interpretations were influenced by speculations of other authors, who thought of Trichoplax as a “paranormal” organism that was unable to complete its life cycle under culture conditions (e.g., Ehlers 1887: 497). Although refused by Schulze (1891), this interpretation persisted in zoological textbooks of that time (e.g., Lankester 1901: 158). Krumbach’s 1907 publication led to a corresponding statement on Trichoplax in the first installment of Bütscbl’s “Vorlesungen über vergleichende Anatomie” (1910) and was cited as fact in a reference book (Neresheimer 1912: 827). However, the hydrozoan interpretation was soon criticized by Schubotz (1912) and Schulze (1914). Schubotz compared the histological organization of Eleutheria krohni planulae and Trichoplax. He noted that the ectoderm of the planula already contains nematocysts, which would have to vanish during any transformation into a Trichoplax. Schulze completed Schubotz’s argumentation by mentioning some special features of the inner fibre cell layer of Trichoplax. Note that Schulze’s article of 1914 was the last publication on Trichoplax in a zoological journal for more than half a century.

How can an exciting animal like Trichoplax be pushed out of scientific research by a shaky larva-hypothesis? It is an amazing fact that the completely unsupported larva-hypothesis remained in German, French, and Anglo-American textbooks for decades. After World War One, the first German encyclopedia on animal phyla was prepared by the meritorious zoologist W. Kuekenthal, who died one year before the first volumes were published in 1923 (also, F.E. Schulze died in 1921). The editor who finished the volumes was Thilo Krumbach, a supporter of the larva-hypothesis. Through his hands, volume one of the “Handbuch der Zoologie” contained the Protozoa, Porifera, Coelenterata, and Mesozoa. Trichoplax is briefly mentioned in the chapter “Hydroida” (H Broch, Oslo) and the chapter “Mesozoa” (M Hartmann, Berlin-Dahlem). Both authors interpreted Trichoplax as a transformed planula of Eleutheria krohni. Hartmann (p. 1014) cites Krumbach’s paper from 1907 but ignores the replies of Schubotz (1912) and Schulze (1914). It was through this single, pivotal circumstance that Krumbach’s larva-hypothesis became widely accepted. In her influential “Invertebrates”, LH Hyman (1940) also cites only Krumbach’s paper (p. 247) and ignores the other data: “... Trichoplax and Treptoplax, which have the construction of planulae, were found actually to be modified planulae of Hydroidea” (p. 243). PP Grasse’s “Traité de zoologie IV” (1961: 694) mentions Trichoplax in a similar way.

III. The rediscovery of Trichoplax adhaerens and birth of the phylum “Placozoa”

It is often said that Trichoplax adhaerens was rediscovered when the German protozoologist KG Grell (University of Tübingen) found this animal in an algal sample from the Red Sea in 1969. Although it is true that the first electron-microscopical examinations by Grell were decisive for the final falsification of Krumbach’s larva-hypothesis, at this time the animal had already found its way back into science.

In July 1961, the cell biologist W. Kuhl (University of Frankfurt) found Trichoplax in a seawater aquarium containing organisms from the Mediterranean Sea. Although Kuhl’s research on Trichoplax concentrated on locomotion and regeneration, he and co-workers clearly stated that they had never observed any connection between Trichoplax and hydrozoans in the aquarium (Kuhl & Kuhl 1966: 433). At about the same time, Trichoplax was also cultured in Moscow, where it inspired Russian researchers to reinforce E. Metschnikoff’s phagocytoella-hypothesis of metazoan evolution (Ivanov 1968, cited in Ivanov 1973, 1988).

When Grell (1971b, 1972, Grell & Benwitz 1974) discovered oogenesis and cleavage processes (after mixing Trichoplax clones from different locations) it became clear that Trichoplax specimens in culture represent an adult stage. Unfortunately, the embryos regularly died after reaching the 64-cell-stage (cf. Ruthmann et al. 1981, Grell 1984), and the further development of Trichoplax remains unknown. However, Grell’s meticulous research provided sufficient support for placing Trichoplax adhaerens in a new phylum, the “Placozoa” (Grell 1971a). The new phylum was named after Bütscbl’s placula hypothesis, and consequently the Russian researchers around AV Ivanov responded by proposing a phylum “Phagocytoellozoa” for Trichoplax (Ivanov 1973). Grell’s phylum Placozoa has survived to this day and has marked the rediscovery of Trichoplax adhaerens.

German and Russian research groups focused mainly on morphological descriptions, while researchers in the US began fieldwork on placozoans (close to nothing was known about the biology of Trichoplax in its natural habitat). It quickly became clear that Trichoplax adhaerens could be found worldwide in the littoral of subtropical and tropical regions (e.g., Pearse 1989). Treptoplax reptans Monticelli 1893 (see II.) has never been found again, and its existence can be doubted.

Detailed electron-microscopical studies by the groups around KG Grell in Tübingen and
A Ruthmann in Bochum confirmed and extended Schulze's (1883, 1891) classical descriptions of *Trichoplax*. No basal lamina could be found, and the interspace between the fibre cells and epithelia was found to be free of any collagenous ECM (e.g. Grell & Benwitz 1971, 1981). For the fibre cells, a syncytial (Buchholz & Ruthmann 1995) and wide-meshed organization (instead of a compact mass; Stiasny 1903) was described. Interestingly, von Graff (1891) and Stiasny (1903) had described unicellular algae in the cell bodies of the fibre cells and interpreted them as symbiotic or commensal zooxanthelles. Wenderoth (1986) found that algae and other food particles adhere to the slime layer of the upper epithelium and are subsequently phagocytized by the inner fibre cells. Food particles must be pulled through gaps of the upper epithelium, and Wenderoth (1986) called this unique mode of feeding "transepithelial cytophagy". Thus, the incorporated algae are prey. However, there may also be endosymbionts present in *Trichoplax*, as bacteria were regularly found in the endoplasmic reticulum of the fibre cells (Grell & Benwitz 1971).

The ability for transepithelial cytophagy indicates a relatively loose arrangement of the epithelia.

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Fig. 4. – Bilaterogastraea-hypothesis of metazoan evolution according to G Jägersten 1955/59: A “benthoblastaeae”-stage gives rise to the “bilateralgastraea” with a through-gut. A-benthoblastaea showing polarity, specialized lower epithelium and gonads, B-digesting benthoblastaea, C, E-benthoblastae changes to bilaterogastraea by developing an inner gastric cavity (D: transverse section showing “oral slit”), F: a through-gut develops by partial closing of the “oral slit”. Note that the vagile benthoblastaea had already developed an A/P-axis and bilateral symmetry, inferring that a placozoan-construction without such axes of symmetry would have to be derived from an earlier stage. In the given scenario, the presumed entoderm of the benthoblastaea (orange color) would be homologous to the nutritive lower epithelium of *Trichoplax*. 
Only two types of epithelial cell-cell connections are present in Trichoplax, belt and septate desmosomes (Ruthmann et al. 1986, Ruthmann 2000). Connections between the epithelia and the fibre cells remain unknown. It seems likely that these connections get continually rearranged. Studies on isolated fibre cells (Thiemann & Ruthmann 1989) revealed their ability to build up cytoplasmatic extensions by microtubuli-assembly. Those extensions are probably mediated by an actinomyosin system (Ruthmann 2000). Since isolated fibre cells live for hours in seawater, Grell & Ruthmann (1991) suggest that the interspace between epithelia and fibre cells may not be very different from the seawater medium. The loose arrangement of the epithelia and the lack of an underlying basal lamina support this idea.

IV. Trichoplax adhaerens, the “Archimetazon”?

With the beginning of the molecular revolution in biology, molecular phylogenetic approaches have been used in an attempt to unravel the phylogenetic position of the Placozoa. Ironically some of the modern molecular analyses moved the phylogenetic view on Trichoplax back to the turn of the century, and Krumbach’s larva hypothesis almost was rejuvenated in a “phylogenetic” version. Based on DNA sequence analyses of ribosomal genes it was proposed that the phylogenetic position of Trichoplax could fall within or very close to Cnidaria, and therefore placozoans could potentially be derived from a neotenic planula (e.g. Bridge 1994, Aleshin et al. 1995, Collins 1998). Later, limitations of rRNA sequence based phylogenies were noted and so far rRNA sequences have failed to unambiguously resolve evolutionary relationships among the diploblast phyla (perhaps due to the age of these groups; cf. Adoutte et al. 2000). The use of new molecular markers, such as mtDNA and rRNA molecular morphology, however, has supported the hypothesis that Placozoans are not very closely related to Cnidaria (Ender & Schierwater 2002). For example, the secondary structure of the 16S rRNA molecule is substantially more complex in Trichoplax than what is known in any cnidarian. At present, molecular systematics has not resolved the issue of the phylogenetic position of the Placozoa. The sum of evidence available to date suggests a basal position for Trichoplax within Metazoa (e.g. Schierwater & DeSalle 2001, Syed & Schierwater 2002, Ender & Schierwater 2002). Resolving this issue is clearly of key importance to our understanding of the origin of the Metazoa.

Identification of the earliest diverging lineage of metazoans will be of decisive value for reconstructing the hypothetical urmetazoan bauplan, and thus early character evolution within Metazoa. In a previous paper we reconstructed the placozoan bauplan as derived from hypothetical “gallertoid” precursors (compare Fig. 5) and we argued in detail against the evolutionary possibility of a gradual transformation of a neotenic cnidarian planula larva into a placozoan (Syed & Schierwater 2002). Although planulae occur in a great variety of forms and creeping planulae exist (e.g. Tardent 1978), two main morphological problems contradict the larva-hypothesis. Essentially, planulae are composed of an outer ectoblastema and an inner endoblastema with both blastemas connected by a thin mesoglea. If one takes such a bilayered construction as a placozoan ancestor, one must explain how the interior contractile fibre cells of placozoans emerged. Furthermore, if one regards the “nutritive” endoblastema of a planula as a homolog to the “nutritive” lower epithelium of Trichoplax, it becomes difficult to explain why and how the interior cell layer was exposed to the outside. For these and other reasons (cf. Syed & Schierwater 2002) the “phylogenetic” larva-hypothesis seems as unrealistic as Krumbach’s (1907) “ontogenetic” precursor.

If we regard the Placozoa as a phylum of animals whose simple organization is primary, the nutritive lower epithelium and the interior fibre cells of Trichoplax require particular discussion.
TRICHOPLAX ADHAERENS: A KEY TO METAZOAN EVOLUTION

? - discoid ediacaran "problematica" (extinct)

b.1

Placozoa

b.2

b.3

b.4

Trichoplax adhaerens
The specialized lower (“nutritive”) epithelium: Precursor of the Entoderm?

As Bartolomaeus (1993) and Ax (1995) pointed out, the term “entoderm” has always been used in a toposcriptive sense, i.e. it has only been applied to inner nutritive cell layers. Nonetheless, in a cladistic analysis, Ax (1995) considered the nutritive epithelium of *Trichoplax* as entoderm, implying homology. As a consequence – if one assumes a basal position for *Trichoplax*, the possibility we are addressing here – one faces the hypothetical evolutionary scenario of a *Trichoplax*-like, benthic animal, which gradually had its lower, nutritive epithelium displaced into the interior of the body. This is the case in both the placula hypothesis (Fig. 3) and the so-called benthoblasten/bilaterogastrea hypothesis of Jägersten (1955, 1959; cf. Fig. 4). Both are modified versions of Haeckel’s blastaea-gastrea hypothesis and propose benthic vagile forms as the first metazoans. Considering the nutritive epithelium of *Trichoplax* to be an independently evolved character instead of an entoderm homolog has some explanatory advantages. Specifically, if the earliest lineages of metazoans are derived from a small pelagic metazoan, adaptation of the lower epithelium in the line leading to the recent *Trichoplax* and an infolding of inner canal systems in the line leading to the other metazoan phyla appear reasonable (cf. Fig. 5).

At present we are left with two plausible evolutionary scenarios:

1. a benthic-vagile, flattened urmetazoan with an adapted lower epithelium (=homology scenario, as it is assumed in the placula/benthoblastae/bilaterogastrea hypothesis, Fig. 4); 2. a pelagic urmetazoan of variable outer shape (depending on the arrangement of inner contractile elements): flat individuals adapt to benthic vagile life, while the remaining pelagic forms develop inner canal systems for ventilation and filtration/digestion (=analogy scenario, as it is assumed in the gallertoid hypothesis, Fig. 5).

It is noteworthy that the upper and lower epithelia of *Trichoplax* are often misleadingly called “dorsal” and “ventral” epithelia. Since these terms are reserved for bilaterian animals -- and here defined upon the possession of a mouth -- one should avoid these terms in reference to *Trichoplax*. Grell (1971) proposed the functional terms “protective” and “nutritive” for the upper and lower epithelia, respectively. Observations by Vicki Pearse and us support the term “protective” for the upper layer in a more concrete way than originally proposed by Grell. Potential predators usually shrink back when encountering placozoans, perhaps because the shiny spheres (cf. Figs 1, 2) serve as reservoirs of chemical defense substances (V Pearse, pers comm). A biochemical analysis of the shiny spheres would be desirable.

It should further be mentioned that some textbooks incorrectly describe some suprastructures of the lower epithelium of *Trichoplax* as “microvilli” (comp. Fig. 2). These suprastructures are not comparable to typical “entodermal” microvilli, since they consist of a system of fenestrated ledges and folds which likely have an important function in the animals’ ability to adhere to flat surfaces (Grell & Benwitz 1981).

In summary, it will be difficult to verify the homology or analogy hypothesis. Potentially, a biochemical characterization of digestive enzymes, which are thought to be produced by the gland cells of the nutritive epithelium, and a molecular characterization of entodermal gene expression would be informative features to investigate.

The interior fibre cells: mesenchyme, parenchyme, or mesoderm?

As discussed above (I), Schulze and Bütschi hesitated to denote the inner fibre cell layer of *Trichoplax* as mesoderm, so as not to imply homology. When the phylum Placozoa was established, the fibre cells were denoted as “mesenchyme-like” by Grell & Benwitz (1971). This more neutral term is still in use (e.g. Buchholz & Rathmann 1995) and means “connective tissue-like” here (not undifferentiated embryonal tissue, as it is often used). Connective tissue cells are normally embedded in an ECM. Because there is no ECM in placozoans, and also because the fibre cells are clearly involved in the “amoeboid” locomotion of the animal, the term “mesenchyme” appears to be inadequate. Ivanov (1973, 1988) appropriately described the fibre cell system as “differentiated multifunctional tissue” and used the term “parenchyme” for it (also v. Salvini-Plawen 1978).

Given that the diploblast-triploblast distinction may become subject to debate, the question of the putative historical relationship of the fibre cells to typical mesodermal structures of triploblastic phyla may become of interest. Referring to the models outlined above, one can discuss the following theoretical scenarios.

The gallertoid hypothesis (Bonik et al. 1976) describes an early differentiation between outer epithelial and inner mesenchymal (in the sense of connective tissue) cells. Deriving a placozoan from this stage implies reduction of ECM and basal lamina (comp. Fig. 5, see also Syed & Schierwater 2002). As a consequence, the mesenchymal cells gradually increase their function in co-ordination of movement. This scenario implies that both placozoan fibre cells and all muscular cell types of other phyla can be derived from contractile mesenchymal cells of early pelagic gallertoids. According to this model both lines diverge very early, which – together with biomechanical reasons – could explain why placozoan fibre cells are diffi-
cult to compare to other muscular cell types or mesodermal tissues, as they are known from more derived phyla.

Because 19th century models of early metazoan evolution mainly focused on the ectoderm-entoderm specialization, they offer less precise statements about connective tissue-like elements. Therefore, Haeckel’s classical blastaea-gastracea model was often criticized from a biomechanical point of view, as it is hard to imagine how gradual invagination and forming of inner canals could be stabilized in a hollow sphere (e.g., Bonik et al. 1978). Jägersten (1959: 99), whose bilateral gastraea-hypothesis was based on Haeckel’s view, addressed this problem by modifying the early benthoblastaea-stages: “It is quite conceivable, and nothing prevents it, that the entire blastocoel was filled with a mesogloea-like substance, containing also somatic cells that had immigrated from the blastoderm. In other words, it is possible that a kind of mesoderm existed even prior to the evolution of the entoderm and the intestine.” Thus, Jägersten (1959: 100) describes the term “mesoderm” as follows: “It is now obvious that the mesoderm is not a uniform germ layer in the same sense as the ectoderm and the entoderm. The very fact that it is formed exclusively mesenchymatically in several recent groups, but mainly the circumstance that this way of formation ought to be considered as the original in the metazoans, prevents its interpretation as a strict germ layer.” To some extent, this view fits modern definitions of the mesoderm, as they are given by Bartolomaeus (1993) and Ax (1995).

Moreover, it seems clear that the gallertoid-hypothesis and the bilateral gastraea-hypothesis describe nearly the same type of earliest metazoan, from which the Placozoa would have to be derived. The assumption of Jägersten (1955) and Bonik et al. (1978) that a mesogloea-like connective tissue served as precursor of the later mesoderm may find support in current molecular studies. Homologs of triploblast mesodermal transcription factors were found to be expressed in the entocodon (an interconnecting structure) of a hydrozoan (Spring et al. 2000).

V. Two models of metazoan evolution

At the time when Jägersten (1955, 1959) outlined his bilateral gastraea-model of early metazoan evolution, Trichoplax was still assumed to be an aberrant hydrozoan planula and therefore not considered as a distinct phylum. Thus, after Grell established the phylum Placozoa in 1971, the bilateral gastraea-hypothesis was modified by some authors. While Jägersten (1955) derived his bilateral gastrae from a “benthoblastaea” (comp. Fig. 4), Grell (1971) proposed a placula sensu Bütschli (Fig. 3) as precursor of the bilateral gastrae.

Today such modified versions of Jägersten’s bilateral gastraea-hypothesis occur in several textbooks (e.g. Siewing 1987, Erben 1990, Ax 1995, Ruthmann 2000). However, there are some critical points in this scenario to note. First, Bütschli’s (1884) original model of a one-layered organism that develops a second layer — resulting in precursors of the ecto- and entoderm — looks dubious, because this scenario hides a phylogenetic saltation. The two-layered stage cannot emerge in a gradual way (comp. Fig. 3). Bütschli did not mention this problem. He notes, however, that there is no plausible selective advantage for the newly developed placula.

Bütschli regarded the three-layered Trichoplax as derived from the two-layered placula. This is another critical point of the placula-hypothesis, because it is not straightforward how the interior fibre cells could have gradually emerged from a two-layered construction. It might be helpful in this respect to consider the hollow amphiblastula of some sponges, which flatten out after settlement (Grüner 1993). Also, the behavior of Trichoplax adhaerens may provide insight on this question. Starving placozoans change from their normal flattened shape to a spherical form, as the interior fibre cells degenerate and lose contact with the epithelia (personal observations). These starving forms are not able to regenerate and soon die (see also Thiemann & Ruthmann 1990). Thus, Jägersten’s (1959) improved description of the benthoblastaea (as cited in IV) and the “gallertoid” as proposed by Bonik et al. (1976) are among the preferable models of early metazoans.

When comparing the gallertoid-hypothesis with Jägersten’s benthoblastaea-bilateral gastraea scenario, there is one important difference regarding the evolution of placozoans. In Jägersten’s model, the early benthic metazoans develop an anterior-posterior polarity before transforming to the bilateral gastraea-stage (cf. Fig. 4). Since Trichoplax does not exhibit any indication of an A/P polarity, we prefer the alternative shown in Fig. 5. According to this view, Placozoa would be the oldest extant metazoan group, probably a sister group of some enigmatic discoid “Vendobionta” as they are known from about 600 million year old strata (comp. Fig. 5 and Syed & Schierwater 2002).

VI. Current Research and Conclusions

Trichoplax attracts the attention of modern multi-disciplinary research for at least three good reasons. First, it is the most simply organized metazoan animal; second, it possesses the smallest genome of all known metazoans (Ruthmann & Wenderoth 1975, Ruthmann 1977, Ruthmann et al. 1981); and third, it might be relatively basal to all recent metazoan phyla (see above). Thus understanding the genetic control of its development will
redefine the basic, and possibly also the ancestral genetic programming of metazoan organization. With excitement we await the answers to how many genes control the development of a basic metazoan bauplan, how big these genes are, how different they are compared to protists, how regulatory genes in Trichoplax have switched or gained new functions in derived animals, and how these genes interact. For example, at present it seems that Trichoplax possesses a single Hox gene only (Schierwater & Kuhn 1998), which is substantially smaller and more simply structured than other Hox genes (Schierwater & DeSalle 2001), and which in sharp contrast to those of higher animals has no function with respect to the formation of body polarity (Jakob et al. in prep; see also Schierwater & DeSalle 2001). Furthermore, the deduced amino acid sequence of the Trichoplax Hox gene, Trox-2, looks like a genetic chimera that harbors diagnostic domains from several families of Hox genes (Kuhn et al. 1999, Schierwater & DeSalle 2002). At the same time we study the genetics, and particularly the developmental genetics, we need to unravel the complete life cycle, describe the morphological changes during development and reproduction in more detail, and finally resolve the phylogenetic position of Trichoplax and the relationships among the diploblastic animals. The answer to the latter seems close, the complete mtDNA genome of Trichoplax has been sequenced (Stephen Dellaporta, Bernd Schierwater, and co-workers), and comparative mtDNA genomes from sponges, hydrozoans, scyphozoans, cubozoans, and cnidarians are being sequenced now. At present it is probable that Trichoplax is not very closely related to the cnidarians (Ender & Schierwater 2002) and that only the Placozoa or Porifera qualify as candidates for the basal position within recent diploblasts.

Some key answers to the above questions are expected soon, since expression and functional studies on regulatory genes and whole genome sequencing of the Trichoplax genome are in progress (Trichoplax Consortium 2002). Soon the no-longer hydrozoan Trichoplax adhaerens might become what the hydrozoan Hydra has been, one of the most promising diploblastic model organisms for development and evolution (Tardent 1988).

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LITERATURE