MICROSCOPIC ANATOMY OF DEVELOPMENTAL STAGES OF *BRANCHIOSTOMA LANCEOLATUM* (CEPHALOCHORDATA, CHORDATA)

by

Thomas Stach

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INTRODUCTION

General remarks

In 1948 *Branchiostoma lanceolatum* was considered to be "l'animal le mieux connu après l'homme" (Drach 1948, p. 932), the best-known animal after man. In his account for the "Handbuch der Zoologie" Pietschmann (1962) stated that every single organ system of this species had been studied several times. Since then the number of publications concerned with studies of lancelets continuously increased to nearly 3000 (Gans 1996). It is mainly the prominent phylogenetic position of Cephalochordata, as the probable sister-taxon of the craniates (Maisey 1986, Jeffries 1986, Schaeffer 1987, Nielsen 1995, Salvini-Plawen 1998) that attracted the interest of both, invertebrate and vertebrate zoologists. Despite this seemingly exhaustive amount of literature on the subject, the last decade witnessed a renewed scientific interest in the evolution of lancelet development. This "return of the amphioxus" (Gee 1994) is inspired by the application of new methods, especially molecular genetics. However, even "standard" techniques such as electron or light microscopy yield new relevant data (Fritzsch 1996, Gilmour 1996, Lacalli 1996, Lacalli et al. 1999, Ruppert 1996, 1997a, b, Stokes & Holland 1995a) and still some fundamental anatomical characteristics remain controversial.

One such problematic issue in the anatomy of cephalochordates is the arrangement of the mesoderm and the coelomic cavities. The existence of extensive coelomic cavities in posterior mesodermal segments of embryos of *Branchiostoma lanceolatum* for example, as stated by Hatschek (1881) was doubted by Cerfontaine (1906) and Conklin (1932). Franz (1927) described virtual cavities ("virtuelle Hohlräume") in order to circumvent these difficulties. Despite such discrepancies the results of the classical light microscopic studies had been highly schematized in textbooks (e.g., Drach 1948, Romer & Parsons 1991), mostly following the theoretical account of Prenant (1936). Even recent publications demonstrated the uncertainty in regards to coelomic cavities. Most authors described myocoelic and sclerocoelic cavities in the myomeres of adult lancelets (Holland & Holland 1990, Welsch 1995, Holland, L. Z. 1996, Ruppert 1997b), whereas Ruppert (1991, p. 9) stated that "adult myomeres lack a cavity". Yet, mesoderm development and the arrangement of coelomic cavities have played a fundamental role in discussions concerning the evolution of chordates, and deuterostomes in general (Hyman 1959, Maisey 1986, Welsch 1995).

Most of the morphological studies of cephalochordate development were accomplished before the use of electron microscopy became widespread and had necessarily been carried out with light microscopic techniques. Some of the uncertainties may be due to the limited power of resolution of the light microscope. Relevant features of the early embryonic stages of cephalochordates are close or below the limitation given by the power of resolution of the light microscope.

Concerning the mesodermal segments, for example, Conklin (1932, p. 104), states: "The boundaries of these somites are especially difficult to see (...), and I feel much less secure in representing them than in the case of most of the other structures figured." It is thus not surprising that details of the embryogenesis, knowledge about organogenesis, and especially mesoderm development remained fairly schematic.

In order to clarify some of the ambiguities mentioned above and to provide a refined knowledge of the early organogenesis of *Branchiostoma lanceolatum* the present study combined light, scanning, and transmission electron microscopy of serially sectioned developmental stages of *B. lanceolatum*. A low power transmission electron microscopy was chosen. The advantage of this approach is that it connects the more detailed findings of the transmission electron microscope directly with the presentations of classical light microscopical studies (Kowalevsky 1867, Hatschek 1881, Cerfontaine 1906, Franz 1927, Conklin 1932). It is not aimed to provide an exhaustive account of all subcellular details of the development of different tissues. Thus, high power magnification of the transmission electron microscope is only occasionally applied. Also, while the technique of serially sectioning animals for a combined light and transmission electron microscopic examination allows for a complete three-dimensional reconstruction of the studied stages, the resolution along the antero-posterior axis makes an identification of single cells in consecutive sections in more complicated tissues difficult. The focus of this embryological study is on the formation and differentiation of the mesoderm and the coelom but organogenesis of all organ systems is covered. *Branchiostoma lanceolatum* is chosen, because it represents a species for which transmission electron microscopic studies of developmental stages are scarce but for which the light microscopic data on development are numerous.

Thus, the purpose of this study is to provide a detailed morphological description based on electron microscopy of complete serial sections of developmental stages of cephalochordates. The results of this study will be discussed in a phylogenetic framework in order to formulate hypotheses concerning the evolutionary changes in structure and function of the organ systems in developmental stages of cephalochordates.

The result-section consists of two parts. In the first part the external morphology of each of the stages is described, whereas internal features are only briefly covered. In the second part the development of each organ system is described in detail. Although this arrangement is likely to produce some overlap, it provides consistent information in each of the two parts, even when read separately. A short survey of the literature concerned with different aspects of the early development of cephalochordates and a summary of what is known about the biology of the early phases of the life cycle of lancelets supplements this information.

Literature survey on lancelet ontogeny

The subphylum Cephalochordata (= Acrania; Phylum: Chordata), according to a recent revision based on meristic variation, comprises two genera and 29 species (Poss & Boschung 1996). *Branchiostoma* includes 22, the second genus *Epigonichthys* (the former *Asymmetron*) 7 species. The ontogeny of only a few species of the genus *Branchiostoma* has been investigated, whereas data on the development of *Epigonichthys* are very scarce (but see Wickstead & Bone 1959, Wickstead 1964b). Investigations on different aspects of development are available for *Branchiostoma belcheri*, *B. californiense*, *B. floridae*, *B. lanceolatum*, *B. senegalense*, and *B. virginiae*. Amongst these are ecological studies concerned with developmental stages of *B. belcheri* (Wickstead & Bone 1959), *B. floridae* (Stokes & Holland 1995b), *B. lanceolatum* (Bone 1958), *B. nigeriense* (Webb 1956, 1958), and *B. senegalense* (Gosselck & Kuehner 1973). The outer morphology of early ontogenetic stages is described in detail by Stokes and Holland (1995b) in a study of *B. floridae*, using scanning electron microscopy. Scanning electron microscopic and transmission electron microscopic data are available for *B. belcheri*. Especially the early stages up to the neurula stage of this species from the Indian ocean were studied to some extent (Hirakow & Kajita 1990, 1991, 1994). Transmission electron microscopic studies of special developmental aspects, such as the ontogeny of Hatschek's Nephridium or the nervous system, exist also for *B. virginiae* (Ruppert 1996, 1997b and B. floridae (Lacalli & Kelly 1999, Lacalli et al 1999). On a light microscopical level the ontogeny of *B. lanceolatum* is the best studied of all species (see e. g., Kowalevsky 1867, Hatschek 1881, Cerfontaine 1906, Franz 1927, Conklin 1932, Drach 1948). Transmission electron microscopic studies have also been published recently for this species (Stach 1996, 1998, 1999; Stach & Eisler 1998).

The most recent attempt in the investigation of the ontogeny of cephalochordates is the application of biochemical and molecular-genetical techniques. The species studied under this aspect was mainly *B. floridae*, but *B. belcheri*, *B. californiense*, and *B. lanceolatum* were also investigated. A review of the research in this area was given by Holland, P. W. H. (1996); more recent publications on this subject are: Kusakabe et al. (1997), Shimeld (1997), Terazawa & Satoh (1997), Zhang et al. (1997), Naylor & Brown (1998), Langeland et al. (1998), Kozmik et al. (1999).

Biological of lancelet development (with SEM-observations of gametes)

"Considering the attention the lancelets received from morphologists and the interest this animal has aroused as a chordate of comparatively simple if not primitive form, it is surprising how little is known with certainty of its life history, ecology and behaviour." Although this statement of Webb was published in 1958 (p. 335) and considerable progress has been achieved since then, it is in general still valid.

Branchiostoma lanceolatum individuals become sexually mature during the autumn months of their second year (Courtney 1975). The mean length at this age is about 23 mm (Courtney 1975) which corresponds well with the observation of sexual maturation in *Branchiostoma floridae* at about the same length (Stokes & Holland 1996). Individuals of *B. lanceolatum* spawn for the first time in the late spring or early summer months of their third year. Animals of this latter species seem to reproduce every year reaching an age of up to six years. The exact date of spawning varies over the range of the geographic distribution. It may start as early as at the beginning of April in the Mediterranean near Naples (Hatschek 1881) and occurs around the end of May (personal observation) or later (June/ July: Courtney 1975) off Heligoland in the North Sea. A more detailed study of *B. floridae* reveals that several spawning events take place during most of the summer months within one single population (Stokes & Holland 1996). On such occasions up to 90% of the animals of a population release their gametes after sunset. The exact day time of spawning was never determined by direct observation in the field, but accounts from backcalculation of developmental stages from plankton hauls as well as observations under laboratory conditions indicate that spawning takes place shortly after sunset (Hatschek 1881, Willey 1893, Bone 1958, Wickstead 1975, Stokes & Holland 1996, personal observation).

Ontogeny is a continuous process including all alterations of a single living organism beginning with the oocyte and ending with its death (e. g., Kryzanowsky 1939, Zeller 1989, Maier 1993, 1999, Britz 1995). In order to establish a framework of orientation, this continuous developmental process is divided into a succession of phases. These specific phases are easily recognizable and defined by some gross morphological features. Fig.1 gives an overview of the timing of development, and of the different phases and stages before metamorphosis.

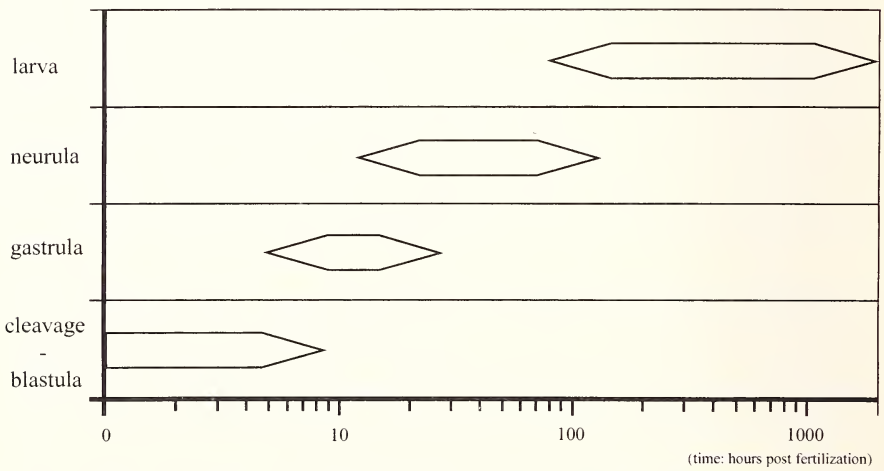


Fig.1: Stages and phases of the development of *Branchiostoma lanceolatum* and their timing at 18°C (data from Drach 1948 and personal observation).

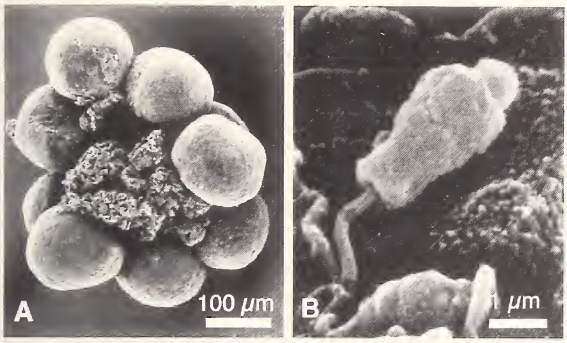


Fig.2: Scanning electron micrographs of gametes of *Branchiostoma lanceolatum*. **A** - External aspect of late oocytes, dissected from ovary. **B** - Spermatozoon. Note the three regions of the cell body corresponding to the acrosome, the nucleus, and the mitochondria (from apex to base).

In *Branchiostoma lanceolatum* the small eggs (~ 120 µm in diameter) appear to be without a micropyle (Fig.2; Holland & Holland 1989). The mature male gamete is a "primitive" aquasperm (sensu Franzén 1956), consisting of an apical acrosome, a prominent, roundish nucleus, few mitochondria, and a long motile flagellum, typical for marine invertebrates with external fertilization (Fig.2; Baccetti et al. 1972). The zygote forms a fertilization membrane (details of these early events are given by Holland & Holland 1992) and sinks to the bottom, where early development takes place. Secured in this fertilization membrane development proceeds through radial holoblastic cleavage to the blastula stage and further on through gastrulation to the early neurula stages. The late gastrula stages begin to rotate within the fertilization membrane; when a neurula stage with two to three myotomes is reached, the embryos break free. Depending on the temperature this takes place 15-18 hours post-fertilization (see Drach 1948). Locomotion in these early stages is exclusively powered by their long ectodermal cilia. During forward swimming these stages rotate alternatively clockwise or counter-clockwise. Bone (1958) noted that rotation in a counter-clockwise direction (viewed from behind) is more common. Contrary to other reports (e. g., Bone 1958, Roschmann 1975) it seems most likely that development thereafter proceeds entirely in the plankton (Courtney 1975, Stokes & Holland 1995b, personal observation). The neurula flattens laterally and elongates anteroposteriorly, in addition, it acquires the ability to move by lateral undulatory flexions of the body. While this general ability of muscular propulsion is gained relatively early (after ~ 27 hours post-fertilization - Bone 1958; ~ 30 hours - personal observation), ciliary propulsion seems to be the preferential mode of locomotion during the entire planktonic period. The muscular twitching occurs mainly in conjunction with avoidance of adverse stimuli (Stokes & Holland 1995a, Stokes 1997, personal observation).

The neurula changes into a larva when the mouth, the first gill slit, and shortly thereafter the anus are formed. Immediately after these anatomical changes the larvae begin to feed. The reports on the behavior and mode of life of normal

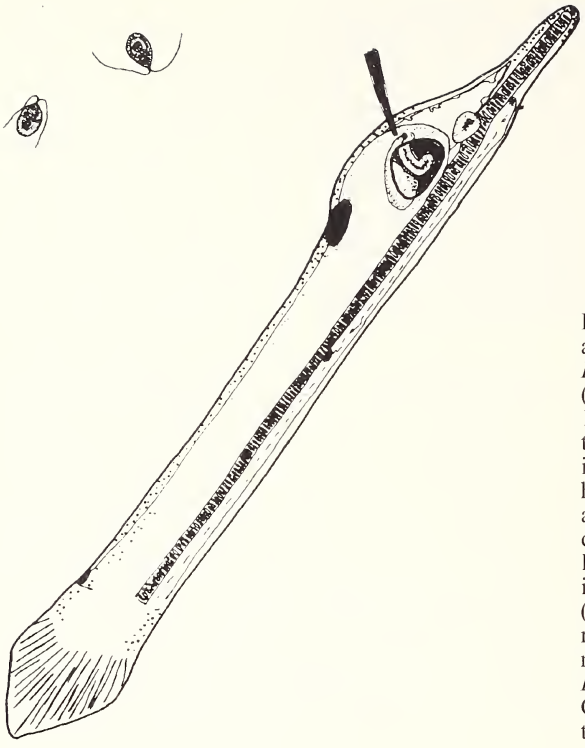


Fig.3: Line drawing of a lateral aspect of an early larva *of Branchiostoma lanceolatum* (ca. 110h post-fertilization, 18°C). Normal feeding position: the long axis of the body is at an angle of about 60 from horizontal and the anterior end and the ventral surface are directed towards the surface. In the upper left corner two individuals of *Dunaliella* (Chlorophycaea, Polyblepharidaceae), a regular food organism in laboratory cultures of *B. lanceolatum*, are depicted. Ontogenetic stage comparable to one shown in Fig.7.

planktonic larvae in the literature were often confusing or contradictory (see below). The picture emerging from more recent studies suggests that the larvae feed in an angular position with their heads directed upward (see Fig.3). Locomotion in this peculiar position by the epithelial cilia was termed "hovering" by Stokes & Holland (1995a). Although these authors confirmed the findings of Willey (1891) it may be noted that others suggest a benthonic feeding period of the larvae (e. g., van Wijhe 1927, Bone 1958, Webb 1969). A feeding current created by a ciliar band in the oesophagus, the cilia of the gill slit(s) and the general intestinal ciliation drives the food into the large asymmetrically left-sided mouth. The food is entangled in a mucus strand emerging from the larval endostyle and perhaps the club-shaped gland (Olsson 1983, Gilmour 1996).

Regarding the prey organisms of cephalochordate larvae, the claim of Webb (1969) that larvae of *Branchiostoma lanceolatum* feed on benthonic diatoms, and in addition may be capable of swallowing bigger food items such as copepods or chain diatoms, was refuted by Gosselck & Kuehner (1973) on the basis of a much larger survey of the gut contents of *Branchiostoma senegalense*. These authors convincingly showed that larvae of this latter species feed preferentially on rela-

tively small planktonic diatoms of the genus *Thalassiosira* (*T. gravida* being the most abundant prey item), ranging from cysts only 24 µm in length to species 65 µm in length. The three observations of crustaceans (an isopod, a cyclopoid, and a harpacticoid) among 3300 investigated larvae were interpreted as anomalies.

Wickstead & Bone (1959) found a diurnal vertical migration pattern in larvae of *Branchiostoma belcheri*. From plankton tows these authors concluded that larvae live near the sea bottom during the day, migrate to the top layers after sunset, and remain dispersed throughout the water column during darkness. On the other hand Hartmann & John (1971) could not detect any vertical migration pattern in a study concerned with *B. lanceolatum* larvae in the North Sea. This controversy has not yet been settled.

During the larval feeding stage of all species observed, the animals grow and primary gill slits are added consecutively. This usually continues until there are about 15 primary gill slits and the animals reach a length of approximately 3.5 - 4 mm (e. g., Franz 1927, Wickstead 1964, Stokes & Holland 1995b). However, it seems that metamorphosis can be delayed considerably when the larvae do not encounter a suitable habitat, especially if they are carried into the epipelagic realm of the open ocean by adverse currents. In this case the animals presumably preserve their planktonic mode of life and keep on adding gill slits up to about 30 and grow until they reach a length of about 8-10 mm (Bone 1957, Wickstead 1964). Some of these planktonic forms may even gain rudimentary gonads (Wickstead 1975). Originally these "giant" planktonic forms were considered to belong to a different cephalochordate genus ("*Amphioxides*", Goldschmidt 1905) but now the notion presented here prevails, although it has to be stated that these so called amphioxides larvae were never observed to metamorphose into juveniles of a known species.

The "normal" planktonic period may last from a little over a month up to several months (Webb 1975, Wickstead 1975, Stokes & Holland 1995b; Fig.1). After this time metamorphosis commences, ending the larval period.

MATERIALS AND METHODS

The goal of this study is to render a reliable detailed morphological description of developmental stages of *Branchiostoma lanceolatum*, as a basis to formulate functional and evolutionary hypotheses. Therefore, different light microscopic and electron microscopic methods have been applied.

Sexually mature specimens of *Branchiostoma lanceolatum* were provided by the Biologische Anstalt Helgoland and kept constantly at 10°C. Spawning was induced by raising the temperature to 18°C. Developmental stages were observed alive by means of light microscopy (Zeiss, Axioplan) and recorded on videotape (Sony, KSP - 60, U - matic). The entire fixation procedure was performed at 0°C.

Nine specimens of different developmental stages between 22 and 110 hours post

fertilization have been serially sectioned in a combined light microscopic / transmission electron microscopic technique. Table 1 gives an overview of the stages examined by this method.

The younger embryonic stages were fixed in 2.5% glutardialdehyde - cacodylate (0.2 M) solution at pH 7.4 for 40 minutes. Rinsing in cacodylate buffer was followed by postfixation with OsO_4 (2%) for 3 hours and 30 minutes. The embedding material was Epon 812. Sections of these stages were stained with uranyl acetate (5 minutes), lead citrate (1 minute), and with toluidine blue for light microscopy.

Table 1: Secimens sectioned

number of specimens	length [µm]	approx.age [h] at 18°C	transverse serial section	horizontal serial section	sagittal serial section	remarks
2	280	22	+	+	–	two complete series
1	~420	32	+	–	–	complete series
2	580	35	+	+	–	two complete series
1	~600	37	–	–	+	complete series[1]
1	~700	42	+	–	–	complete series[2]
2	1100	110	+	+	–	two complete series

[1] The animal was infested with bacteria

[2] The anterior third of the animal was sectioned only for light microscopy

The larvae with one primary gill slit were fixed for 30 minutes in a glutardialdehyde (8%) - sea water mixture (1:2). Rinsing in seawater was followed by postfixation for 3 hours in an OsO_4 (4%) - seawater mixture (1:1). The animals were stained with uranyl acetate "en bloc" prior to embedding in Epon 812. Sections were stained with lead citrate (1 minute) for TEM and with toluidine blue for light microscopy.

Serial sections of different developmental stages for light microscopy and transmissionelectron microscopy (TEM) were prepared on a LKB Ultratome 1 as follows: a survey series of about 20 semi-thin-sections 0.5 µm thick was made, followed by a series of about 30 ultra-thin-sections 0.05 µm in thickness. This pattern was repeated for the length (transverse sections), height (horizontal longitudinal sections), and breadth (sagittal longitudinal sections) respectively of the entire animals; see Table 1. Transmission electron micrographs were documented with a Siemens Elmiskop 102.

Scanning electron microscopy (SEM) preparation was as follows: animals were fixed in glutardialdehyde in seawater (4%), some of the embryonic stages were treated with OsO_4 (2%) as a postfixative. Fixation was followed by dehydration in a graded ethanol series. Preparation to reveal internal structures in scanning electron microscopic aspect was accomplished by applying a gummed tape on the

critical point dried and mounted embryos, then pulling the tape and thereby fracturing the embryo. Fractured and unfractured specimens were coated with gold and viewed in a Cambridge Stereoscan 250 MK II.

General technical remarks – Cell differentiation, cell proliferation, organogenesis, and cell movements take place simultaneously during the ontogeny of any bilaterian animal. On the molecular level these changes are accompanied by changes in gene activity or enzymatic equipment of single cellular units and their surrounding. This variable biochemical environment may be a reason for difficulties in tissue fixation in fast developing animals in general and of developmental stages of cephalochordates in particular (Hirakow & Kajita 1994). The same technique may result in different qualities of the final sections prepared for electron microscopy.

Other reasons that may explain the lack of ultrastructural anatomical data of developmental stages of cephalochordates, despite the great interest of developmental biologists in them, are also of a technical nature. Like other deuterostome larvae, developmental stages of cephalochordates are hard to rear under laboratory conditions. Therefore it was a major breakthrough accomplished by Stokes & Holland (1995b), when they reported the rearing of cephalochordates from the egg through metamorphosis to the adult stage for the first time under laboratory conditions.

Developing stages of cephalochordates occupy a size range unpleasantly small for conventional light microscopy and unwieldy large for a serial section of embryos or larvae at an electron microscopical level. About 20 000 semi-thin and ultra-thin sections have been analyzed to study all specimens indicated in Table 1. In addition, to be able to link the detailed electron microscopic information with the results gained by light microscopy 10 - 25 electron micrographs had to be mounted to produce plates of complete cross sections.

RESULTS

General description of ontogenetic stages

The 9-segments stage

Animals of this stage develop in the open water column and exhibit a typical mode of locomotion. Propelled by their external cilia the animals swim with their anterior end leading, thereby rotating preferentially around their longitudinal body axis in a clockwise direction when viewed from behind. Occasionally the direction of this rotation may be reversed.

General morphology

The earliest neurula stage studied in serial transmission electron microscopic sections was 22 hours old post-fertilization (18°C). It clearly displays all symmetry

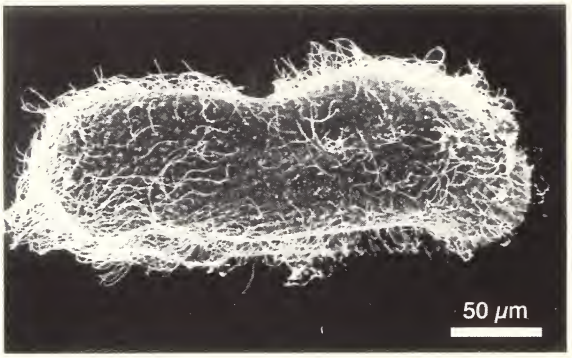


Fig.4: SEM micrograph of a neurula stage with nine mesodermal segments (22 h pf, 18°C). Anterior is to the left. Ventral aspect.

axes found in the adult animal. The neurulae are laterally compressed. The neuropore is situated at the dorsal anterior tip of the animal and cilia extend from this opening. The animals are small measuring about 280 µm in length, 75 µm in width, and approximately 100 µm in height. Scanning electron microscopic observation demonstrates a monociliated epidermis (Fig.4) and the cell boundaries of the epidermal cells are visible. The cells are of an irregular polygonal shape when viewed from above and the single cilium measures about 15 µm in length (after preparation for scanning electron microscopy). There are however a few cells that possess cilia, which are surrounded by microvilli and may represent sensory cells (see also Stokes & Holland 1995b). In general the outer cell surface has many small roundish protrusions (Fig.8; see also Bereiter-Hahn 1984, Stach 1994).

General internal organization (Figs. 8, 10A, 14A, 16A; Plates 1 & 2)

Some of the organ systems typical for all chordate taxa are already recognizable in this early stage. The outer epidermis is a monolayer of monociliated cells, which establish the contact to the surrounding seawater (Fig.8). Separated by a narrow gap from the dorsal epidermis is the neuroectoderm which is only present as a neural plate in this stage (Plates 1 & 2). The neural plate is connected at the posterior end of the animal with the endodermal archenteron via the rudiment of the canalis neurentericus. The archenteron is closed anteriorly; there is neither a mouth opening nor a gill slit developed at this stage. From the dorsal roof of the archenteron the cells of the prospective notochord, the anlage of the Chorda dorsalis, are in the process of growing out of the endodermal tissue (Plates 1 & 2). Thus the notochord is visibly distinct from the archenteron, but not separated by an extracellular matrix from it. The dorsolateral mesoderm, which constitutes the main part of the mesoderm and gives rise to the later myotomes, consists of 9 pairs of segmentally arranged units. These are separated from the archenteron only in the anterior region; there they are formed by epithelial cells surrounding a coelomic cavity. The segments of both sides are symmetrically arranged. The association of the somitic mesoderm with the archenteron in the posterior part of the animal demonstrates the ontogeny of the mesoderm from the endoderm.

The 10-segments stage

When the animals are approximately 32 h post-fertilization (18°C) the rotatory movement is slower compared to earlier ontogenetic stages, although the animals are not capable of muscular twitches yet. The neurulae are planktonic and are propelled by metachronal waves of their epidermal cilia.

General morphology

The neurulae of this stage look similar to those of the stage described above; they are of a nearly cylindrical shape but clearly laterally compressed. The proportions are slightly changed to about 400 µm in length, 50 µm in width, and 80 µm in height. The general shape does not provide definite hints regarding the orientation of the body of the neurulae, but the neuropore marks the dorsal side at the anterior part of the animals. Long cilia project from the cells of the anterior nervous system to the exterior through the neuropore. The entire epidermis is ciliated, each cell possessing a single apical cilium of about 15 µm length. Cell boundaries seen in preparations for scanning electron microscopy reveal a polygonal arrangement of the epidermal cells. Scanning electron microscopy demonstrates apical protrusions on the cell surfaces as a feature of these epidermal cells.

General internal organization (Fig. 11A; Plates 3 - 5)

The differentiation of the organ systems present in the earlier stage described above, proceeded, but no additional organ developed. The epidermis consists of a single layer of polar, monociliated cells. The nervous system is now represented by a neural tube with a layer of prismatic, ciliated cells that surround a central canal. Some of these cells seem to possess axons projecting from the base of the cells especially at the ventrolateral border of the neural tube. The neuropore connects the central canal with the exterior and the canalis neurentericus at the posterior end of the neural tube with the lumen of the archenteron. The notochord is a distinct organ and is separated from the roof of the archenteron throughout the most part of the neurula. Only the posteriormost part is still in continuity with the archenteron. The mesoderm is present in form of ten paired segments. The first pair displays a spacious coelomic cavity (Plate 4), whereas the more posterior ones show two different cell types with only a narrow coelom between them (Plate 5). In the trunk region the mesoderm extends ventrally below the archenteron where the mesodermal compartments from both sides of the body meet. This rudimentary ventral mesoderm seems to be segmented (Fig.13B; Hatschek 1881). Thus, a narrow mesodermal sheet around the intestine is formed. The archenteron has neither mouth, nor anus, nor gill slits. However, the anterior end of the archenteron is forked into the short left and right diverticula of Hatschek (Plate 3).

The 11-segments stage

The development of neurula stages still proceeds in open water, as the animals are planktonic. When the animals are about 35 h post-fertilization (18°C), they are still propelled by their epidermal cilia. Individuals swim with the anterior end leading. The rotation along the body axis is less pronounced and the swimming path is more straightened compared to previous stages. Simple muscular twitches can be observed around this age when the neurulae encounter adverse stimuli.

General morphology (Fig.5)

The later neurula stages are considerably longer with about 580 µm in length. During the process of elongation the body is even more flattened laterally, measuring 35 µm which is less than half of the width observed in the first stage described above. The height also decreased further to 60 µm; therefore elongation is connected with a process of proportion change. The anterior end is now easily distinguished as the rostrum is more pointed than the rounded posterior end of the animal. The neuropore still opens at the dorsal anterior side and cilia extend from the neural canal through the neuropore anteriorly to the outside. The ciliation of the epidermis is still regular with cilia about 15 µm long. Apical protrusions of the polygonal epidermal cells are also still visible.

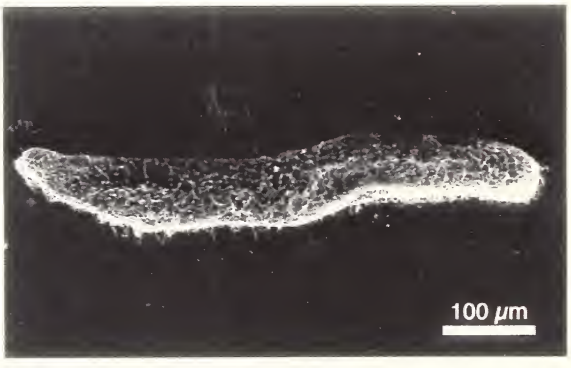


Fig.5: SEM micrograph of a neurula stage with eleven mesodermal segments (35 h pf, 18°C). Anterior to the left recognizable by the rostrum. Lateral aspect.

General internal organization (Figs.10B, 13A, 14B; Plates 6 - 8)

Although the organ systems are more differentiated than in the previous stages, no additional system is present in the 11-segments stage. The epidermis is constituted of a monolayer of monociliated cells. The nervous system represents a tube surrounding a central canal. Connections of the neural tube with the outside via the neuropore at the anterodorsal part and with the archenteron via the canalis neurentericus at the posterior part of the animal remain present. The notochord is separated from the archenteron throughout most of the body of this neurula stage;

only the most posterior part is in continuous connection with the endodermal archenteron. Eleven mesodermal segments are established and separated from the archenteron. At the posterior part of the second segment of the left side, rudiments of excretory cells are seen (Stach 1994, Stach & Eisler 1998). The trunk segments contain two different cell types in their dorsolateral main portion (Plate 7). These are medial myogenic cells and lateral coelothelic cells with a narrow slit-like myocoel separating the two groups of cells. The mesodermal segments of the left and right side of the body are out of phase from this stage on, the left segments being half a segment anterior to the right ones. Ventrally the mesoderm surrounds the archenteron. The archenteron itself has not acquired a secondary anterior opening to the exterior yet. The anterior tip of the archenteron is forked with the two anterior branches pointing antero-dorsally (Plate 6).

Late neurula / early larva

The beginning of the larval period is defined here functionally by the onset of planktotrophic feeding, following the usage in ichthyological literature (Balon 1975). Naturally this phase is not demarcated by a sharp boundary, but begins with several consecutive anatomical changes, the most prominent and earliest of which is the acquisition of the asymmetrical left-sided mouth opening. This is followed by the opening of the first gill slit and the anus slightly thereafter. These changes occur over a period of approximately 20 hours between 40 and 60 h pf (18°C).

General morphology

At an age of about 42 h post-fertilization (18°C) the late neurulae is at the so called "knife-shaped" stage (Hirakow & Kajita 1994). The rostrum of these larvae is clearly pointed and the buccal region is markedly swollen, where the mouth opening and the gill slit are to be situated (Fig.6). The length increased only slightly and measures about 600 µm. A tailfin is not developed yet, neither is the anus. Muscular undulations occur regularly when adverse stimuli are encountered by the animals.

General internal organization (Fig.6, 13B; Plate 9)

It is obvious that the cells constituting the different organs of this stage are further differentiated than in the previous stage. The narrow epidermal cells are entirely depleted of their content in yolk granules, but all other organs still possess such granules, although their number decreased. The neural tube consists of specialized cells with complex arrays of intracellular vacuoles (Plate 9). The neuropore is still open, but it could not be determined whether the same holds true for the canalis neurentericus. The notochordal cells show spacious intracellular vacuoles and thick transversal myofilaments within their cytoplasm. Light microscopical observations suggest that Hatschek's nephridium as well as the preoral pit and the

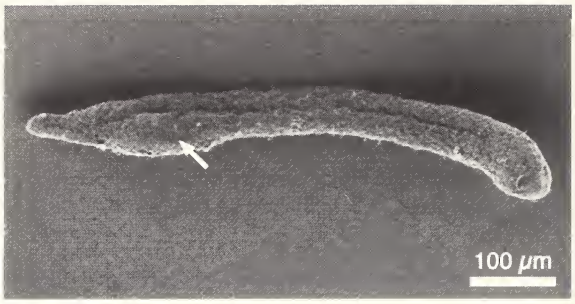


Fig.6: Scanning electron micrograph of a late neurula stage (42 h pf, 18°C). Anterior is to the left. Lateral aspect. Note the thickened area where the mouth is just beginning to open (arrow).

oral papilla are present. In the trunk region the elaborate differentiation of the medial mesodermal cells into regular myocytes can be observed. Lateral mesodermal cells border the regularly occurring myocoel. The ventral mesoderm has acquired a narrow slit in the ventral midline (Plate 9) indicating that the presumably segmental arrangement of the ventral mesoderm of slightly younger stages (see above, Fig.13B) does not prevail. The intestine is now in open contact with the surrounding seawater by the narrow left-sided mouth opening. However, the gill slit is only recognizable as a thickening of the endodermal epithelium, and the anus is not recognizable in this stage.

1 primary gill slit stage

The larval stage examined in detail here is approximately 1100 µm in length, and is about 110 h post-fertilization at a temperature of about 18°C. A feeding current is produced by the epidermal cilia, and the cilia of the intestinal system, especially those surrounding the mouth and the gill slit, and a ciliar band in the pharynx. Mucous secretions of the endostyle, the club-shaped gland, and probably sensory mechanisms are involved in the feeding mechanism (Lacalli et al. 1999, personal observation). The normal feeding position of the larvae is called hovering (see Fig.1); and the animals remain most of the time in this position by the action of their outer ciliature. Nevertheless, they are capable of complex muscular sinusoidal movements. Muscular locomotion already resembles that of the adults, but is of shorter duration, and mainly displayed when confronted with an adverse stimulus.

General morphology (Fig.7)

The larva is of the well known asymmetrical appearance, with the relatively large mouth opening on the left side of the animal. Light microscopy of living specimens reveals stiff cilia surrounding the mouth opening. Behind the mouth the first primary gill slit opens nearly in the ventral midline slightly shifted to the right side. The anus is seen at the base of the caudal fin at the ventral side, slightly to the left. A caudal fin is developed and supported by "fin-rays"(see Discussion).

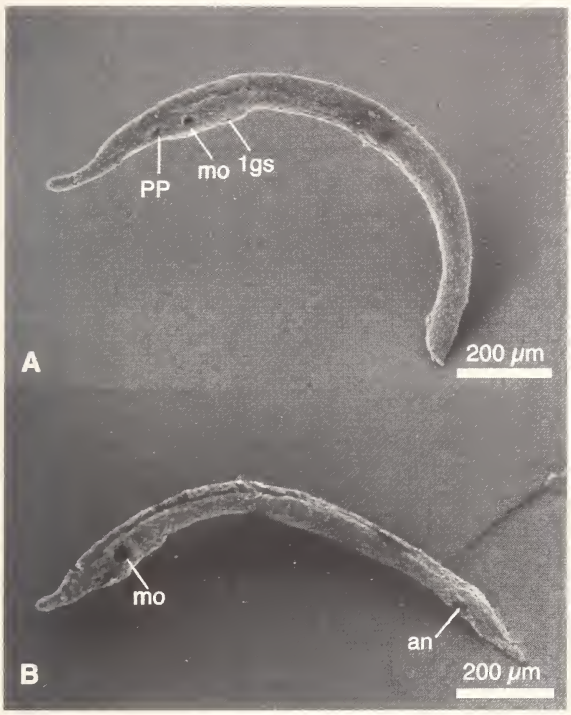


Fig.7: SEM micrograph of two larvae (**A** & **B**) with one primary gill slit (110 h pf, 18°C). Anterior is to the left. Note the left sided mouth openings, the ventral anus (B), and the tail fin. **an** - anus, **mo** - mouth, **PP** - preoral pit, **1gs** - first primary gill slit.

The rostrum is elongated anteriorly and a slight dorsal elevation marks the site of the cerebral vesicle. The opening of the neuropore is still visible with cilia emerging from it. The epidermis is supplied with cilia but the ciliature is less dense at the dorsal side. Additional ciliary specializations can be seen from the outside. These are the preoral pit at the dorsal anterior rim of the mouth opening, and the oral papilla, that consists of especially long stiff cilia situated at the ventral anterior rim of the mouth opening. Close to the oral papilla a small opening, the external opening of the club-shaped gland, can be detected.

General internal organization (Figs.9, 10C, 11B, 12, 14C, 15, 16C, 17-20; Plates 10-18)

Drastic changes accompany the development of larval features, which are to a great extent related to the newly acquired feeding capacity. Only the epidermis remains more or less unchanged and consists of a monolayer of monociliated cells. The nervous system displays a complex arrangement of cells around a central canal. Most obviously in the middle region of the body the first pigment spot is apparent. Many cells of the nervous system possess complex vacuole systems and lamellar membrane complexes. The neuropore at the dorsal anterior end of the

cerebral vesicle is still open, as is the canalis neurentericus. The notochord is now an entirely separate organ and functions as a stiffening rod by muscular contraction of its central cells. The mesoderm is differentiated considerably in the various body regions. Hatschek's diverticula are pinched off from the archenteron; the diverticulum of the right side into the spacious rostral coelom, whereas the one of the left side remains small and contacts the exterior as the preoral pit (Fig.17; Plates 10 & 11A). The more posteriorly situated segments constitute the main body musculature and are situated dorsolaterally. The second muscular segment of the left series is specialized as it contains the first larval excretory organ in its posterior part: Hatschek's nephridium (Fig.15; Plate 11B & C). This excretory organ opens to the exterior at the dorsal rim of the mouth. The mouth and the primary gill slit are surrounded by muscles (Fig.18; Plates 11 - 14; Lacalli et al. 1999). Behind the pharyngeal regions the ventral mesoderm that surrounds the intestine borders a conspicuous coelomic cavity, that ends blindly before the anus (Figs.12B & C; Plates 13-17). Additional specializations of the intestine are the primordium of the endostyle and the club-shaped gland. The endostyle is present as a cellular thickening of the right side of the buccal epithelium opposite the mouth opening. Immediately behind it the club-shaped gland is seen. This organ possesses an exterior opening at the ventral border of the mouth and an interior opening in the dorsolateral part of the intestine on the right side just behind the endostyle. As a further important change the primordia of a major blood vessel, the anterior aorta, can be detected in the extracellular matrix below the notochord (Fig.15A; see also Plates 15-17).

Description of the ultrastructure of the development of individual organ systems

Epidermis (Fig. 8)

The cells of the epidermis are equipped for their different functional needs early on during ontogeny. From the onset of gastrulation, cilia begin to grow at the apical surface of the epidermal cells. Immediately thereafter these cilia propel the early ontogenetic stages: first inside the fertilization membrane, and after neurulation in the open water column. The anchoring complex of the epidermal cilia consists of two perpendicular centrioles (Fig.8A). The proximal centriole is anchored by a rootlet fibre within the epidermis cell; this fibre shows a repetitive pattern of cross striation with a periodicity of about 70 nm. The cilium itself shows the common 9*2+2 - arrangement of its microtubules and is a clear sign of the polar organization of the epidermal cells. Other signs of polarity are the fact that the cells rest on a fibrous meshwork, the extracellular matrix, which shows an electron-dense basal lamina. In addition, individual epidermal cells are connected with each other by way of septate junctions apically. In these septate junctions about 10 bridge-like narrow intercellular structures approximately 20 nm apart

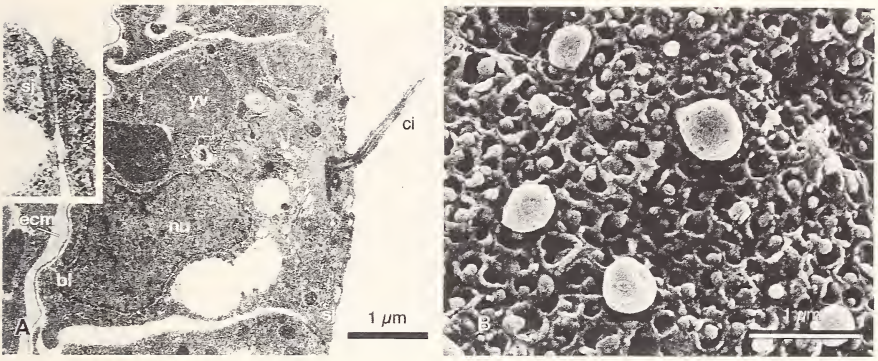


Fig.8: Electron micrographs of epidermal cells of early neurula stages of *Branchiostoma lanceolatum*. **A** - TEM-aspect. Inset: septate junction. **B** - SEM-aspect of apical surface of a deciliated epidermal cell. Note the numerous protrusions bulging to the exterior. **bl** - basal lamina, **ci** - cilium, **ecm** - extracellular matrix, **nu** - nucleus, **sj** - septate junction, **yv** - yolk granules.

span the narrow intercellular space between neighboring cells (Fig.8A, inset). Other features of the epidermal cells include a large nucleus situated in the basal part of the cells and yolk granules. Profiles of rough endoplasmic reticulum as well as a prominent Golgi complex and numerous vacuoles of different size and content indicate a high metabolic activity. The vacuoles seen just below the apical plasma membrane may correspond to the protuberances observed on the apical surface of the cells with the scanning electron microscope (Fig.8B). Among the ontogenetic stages examined in detail for this study, the main changes of the epidermal tissue is seen in the decrease of yolk granules and the flattening of the cells. Therefore several anatomical properties of the integument of adult cephalochordates as summarized by Bereiter-Hahn (1984) are acquired later in ontogeny. Such features include, e. g., the lateral interdigitation of neighboring cells or the thick layer of regularly arranged collagen fibres in the extracellular matrix below the epidermis.

Besides the locomotory function and the protection of the embryo, the epidermis probably has sensory functions as well. Single sensory cells are probably present in the epidermis of developing *Branchiostoma lanceolatum*, but could not be detected by the methods applied (but see Stokes & Holland 1995a).

Oral papilla (Fig.9; Plate 11)

A special structure of the epidermis of unknown function is the oral papilla. This structure develops as an epidermal thickening at the same time as the mouth opening pierces through and is present throughout the larval stage. The cells that comprise the oral papilla are elongated and possess a long apical cilium that appears stiffer than the rest of the epidermal cilia when viewed with a light microscope in a living specimen. Numerous mitochondria are seen in the basal part of the cells;

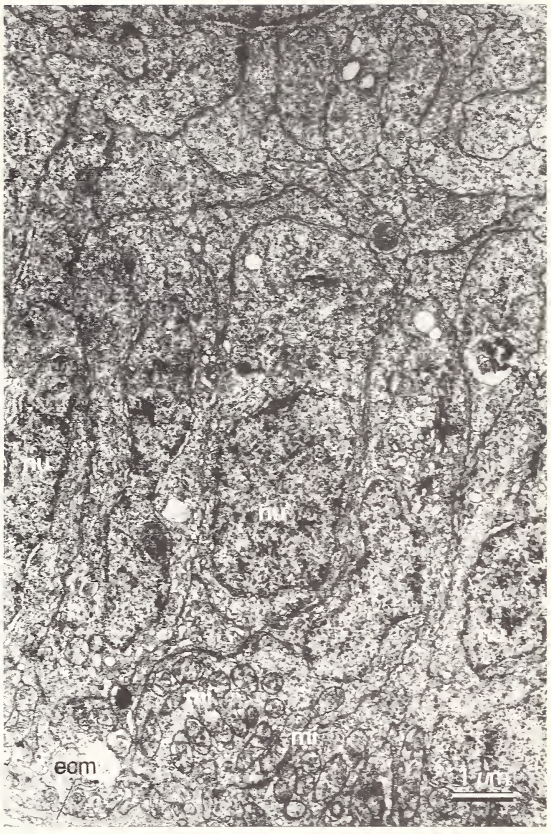


Fig.9: TEM-aspect of a cross section of the oral papilla of early larval stages of *Branchiostoma lanceolatum*. **ecm** - extracellular matrix. **mi** - mitochondrium. **nu** - nucleus.

the nucleus is situated above this basal area. Apical to the nucleus a Golgi complex is well developed and accompanied by several vesicles. No axons could be detected at the base of the papilla, suggesting that it may not be a sensory structure. Its ultrastructure (Fig.9) offers little help in attributing a definite function to this enigmatic, purely larval, organ but suggests a secretory (Golgi complex + vesicles) or a sensory function (stiff, long cilia).

Nervous system (Plates 1-18)

The differentiation of the nervous system is accompanied by the development of an entire set of numerous, highly specialized cellular units with a complex three-dimensional arrangement. The detailed description of all the cell types found in the larval stages of *Branchiostoma lanceolatum* would require a higher resolution along the anterior-posterior axis than achieved with the light microscopic / electron microscopic approach of this study, and would exceed by far the scope of this

contribution. Therefore, only general statements regarding the central nervous system of larvae are furnished. Detailed descriptions of the microanatomy based on transmission electron microscopy of parts of the nervous system of older amphioxus larvae (*Branchiostoma floridae*) are available in the papers of Lacalli (1996a, b), Lacalli et al. (1994, 1999), Lacalli & West (1993), and Lacalli & Kelly (1999).

The neuroectoderm of late gastrula stages consists of prismatic cells on the dorsal side of the animals. During neurulation the neuroectoderm is overgrown by the epidermal ectoderm from posteriorly and laterally. These processes result in a neural plate in early neurula stages with an intercellular space, the rudiment of the neural canal, separating the neural plate from the dorsal epidermis (Plates 1 & 2). The cells of the neural plate are, according to their ectodermal derivation, polar in organization. They have an elongated shape with an apical cilium, which extends into the space between dorsal epidermis and neural plate, the primordium of the neural canal. An extracellular matrix separates the neural plate from the mesoderm and the prospective notochord, but not from the dorsolateral epidermis. Cross sections of smaller diameter (around 0.5 μm) in slightly more advanced neurulae may indicate that some of the cells possess axons already (Plates 4 & 5).

In later neurula stages, with eleven segments developed, the neural plate changed into the neural tube around the neural canal. At the anterior tip the cerebral vesicle is distinguished already as a thickening of the neural tube (Plate 6). The diameter of the cerebral vesicle is, with about 18 μm, higher than in the posterior part of the central nervous system, where the diameter is about 10 μm. The height of the central nervous system in the anterior region also shows a difference in diameter: in the cerebral vesicle it measures 10 μm; in more posterior parts it measures 4.5 μm. The length of the cerebral vesicle is approximately 20 μm; and the neuropore opens at its anterior dorsal tip. The cells of the entire central nervous system are still fairly undifferentiated and resemble those of the neural plate with apical cilia surrounded by microvilli around the neuropore. Nevertheless, the arrangement of the cells around the canal is irregular, and axons can be seen more frequently at the ventral side of the neural tube (Plates 6 & 7).

The neural canal is still in open communication with the exterior and the intestine via the neuropore and the canalis neurentericus, respectively. In the neural canal sections through cilia are still numerous, but Reissner's fibre, which is present in adults of this species (e. g., Olsson 1993), was not detected. The lamellar body, which is a conspicuous structure at the posterior part of the cerebral vesicle in older larvae (Lacalli 1996b), was not seen in the 110 hours old larvae. To add to these negative findings a pigment spot at the anterior end of the cerebral vesicle is also lacking. The first pigment spot that develops during ontogeny is situated at the ventral wall of the central nervous system at the height of the fifth mesodermal segment. Action of cilia in the neural canal seems to create a flow into the central nervous system.

Notochord (Figs.10 & 11; Plates 1-18)

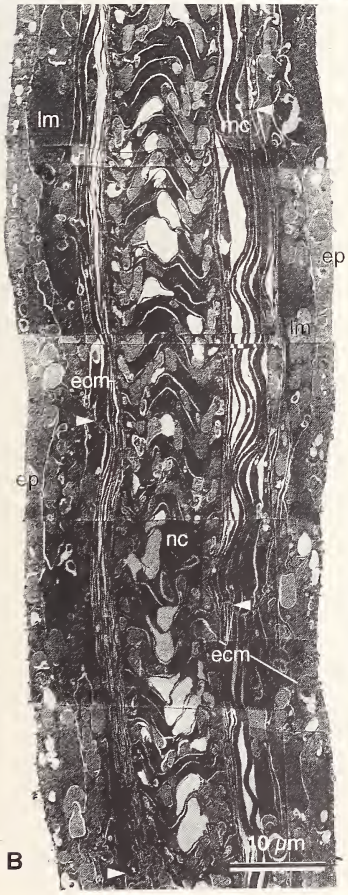
The anlage of the notochord of the early neurula stages is not separated from the epithelium of the archenteron by any extracellular matrix (Plate 1). The prospective notochordal tissue is thus continuous with the endoderm of the archenteron. Nevertheless, the horizontal longitudinal sections reveal that the prospective notochordal cells are already typically arranged in single file, a pattern known as a"stack of coins" (Fig.10A), where each cell is situated behind another and spans the entire width of the future notochord. Approximately seven prospective notochordal cells border the side of a single pair of myotomes which stretch over a length of about 15 µm. The myotomes from both sides of the body are still bilaterally symmetrical. A gradient along the antero-posterior axis of the animals is recognizable. In the posterior part the outgrowth of the prospective notochord in the dorsal medial line of the archenteron is less advanced than in the anterior part (Plate 2).

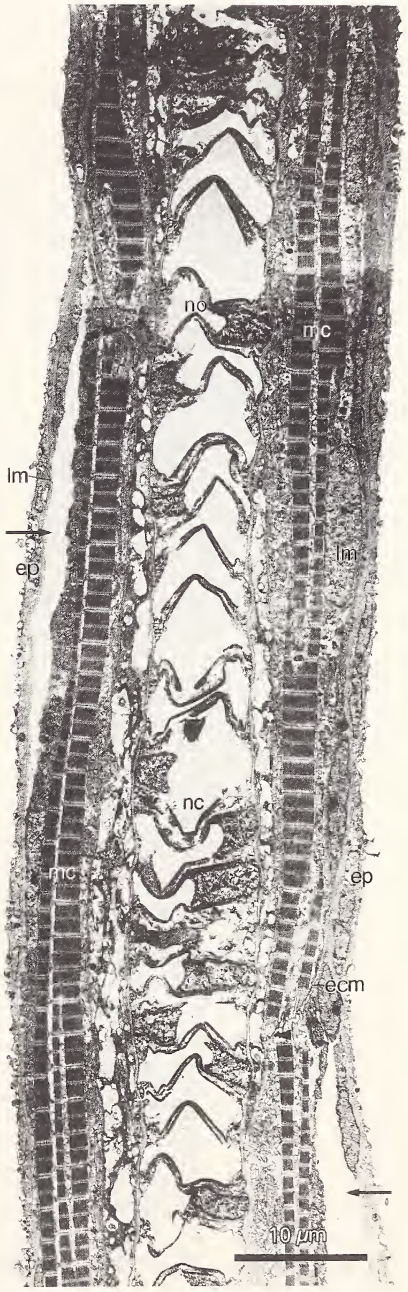
The prospective notochordal cells of this early developmental stage are fairly undifferentiated and resemble the cells of the archenteron at this age (Plates 1 & 2). There are no spacious intracellular vacuoles other than the numerous yolk granules. Profiles of rough endoplasmic reticulum are frequently encountered. No myofilaments are visible.

The notochord of later neurula stages is entirely separated by an extracellular matrix from the epithelium of the archenteron for the most part of the animal (Plates 3-7). Only posteriorly is the notochordal tissue still continuous with the endodermal archenteron (Plate 8). Like in the early neurula stage with nine mesodermal segments described above, the horizontal section reveals that the cells are arranged in single file, spanning the entire width of the notochord (Fig.10B). There are now approximately 15 central notochordal cells per myotome, considerably more than in the previous stage. The myotomes from either side of the body are asymmetrically arranged and span over approximately 50 µm.

On the cellular level several changes are recognizable (Figs.11A, 10B; Plates 6 - 8). Yolk granules are less numerous, whereas profiles of rough endoplasmic reticulum are frequently encountered indicating a high transcriptional activity; this coincides with a high number of mitochondria seen in these cells. The central notochordal cells develop a prominent intracellular vacuole. The larger central vacuoles seem to be formed by the confluence of numerous smaller vesicles (Stach 1999). Small extracellular vacuole-like spaces develop on this stage between adjacent central notochordal cells (Fig.11A). Sometimes two neighbouring

Fig.10 (page 25): Transmission electron micrographs of horizontal longitudinal sections through different developmental stages of *Branchiostoma lanceolatum*, anterior is to the top, left of Fig.is left in animals. **A** - Early neurula (22h pf, 18°C). **B** - Later neurula (35h pf, 18°C). **C** - Early larva (110h pf, 18°C). **ecm** - extracellular matrix, **ep** - epidermis, **mc** - myocytes, **ms** - mesodermal cell, **nc** - notochord, **ncA** - anlage of the notochord, arrows - myocoel, arrowheads - myoseptum.





central cells can be seen to be interconnected by cell junctions, which are possibly desmosomes of the adherens type (Fig.11A, inset).

The sagittal sections (Fig.11A) clearly show that the central notochordal cells, which are arranged as a "stack of coins" are bordered dorsally and ventrally by distinctively different cells. These dorsal and ventral cells (dorsal and ventral Müller cells of Flood 1975) are well equipped with rough endoplasmic reticulum, mitochondria, and yolk granules. They seem not to produce intracellular vacuoles like the central notochordal cells of this ontogenetic stage. On the other hand they seem to be a preferential site for the formation of vacuole-like spaces between neighbouring cells (Fig.11A). Like the central notochordal cells the dorsal and ventral cells are interconnected by desmosomes mostly basally, close to the extracellular matrix. Rarely interconnections of the same type between central and dorsal or ventral notochordal cells are found.

Later neurula stages with eleven segments are considerably longer and the rostrum at the anterior tip is markedly pointed (Fig.5). The notochord stretches from the anteriormost tip of the rostrum where it is covered by a thin epidermis to the posterior end of the archenteron. Again the notochord is separated from the intestinal archenteron throughout the greater part of the embryonic body, leaving only the most posterior part of the notochord in continuity with the archenteron (Plate 8). The number of notochordal cells per myotome (about 25) is again higher than in the neurula stage described above. The myotomes now stretch over a length of about 80-90 μm.

The overall shape of the central notochordal cells differs considerably from that of the stage with ten mesodermal segments. Although the notochordal cells are still arranged behind each other in single file, the cells as a whole have a sinusoidal appearance in longitudinal sections which may be due to shrinkage during the histological processing (Fig.10B). The central notochordal cells are otherwise comparable in ultrastructure to the ones described from the previous stage. Two changes have to be noted: First, the empty appearing vacuoles within the cells are larger in size. Second, some of the cells show small amounts of myofibres at this stage (Stach 1994, 1999).

The notochord of early larval stages is now entirely separated from the intestine throughout the larval body (Plates 9-18). The central notochordal cells are still sinusoidal in their outline on horizontal sections (Fig.10C). These central notochordal cells are now of a very specialized ultrastructural appearance. The apparently empty vacuoles occupy the major part of the cell bodies. The nucleus is situated lateral to this prominent vacuole in the medial part of the notochord and bulges into the vacuole (Stach 1999). Anterior and posterior to the central vacuole a sheath of myofilaments is found within each central notochordal cell (Figs. 10C, 11B). Thick and thin myofilaments are present; the thick ones measuring approximately 20 nm in diameter. A pattern of cross striation is not clearly marked in these larvae. Judging from horizontal sections mainly intracellular vacuo-

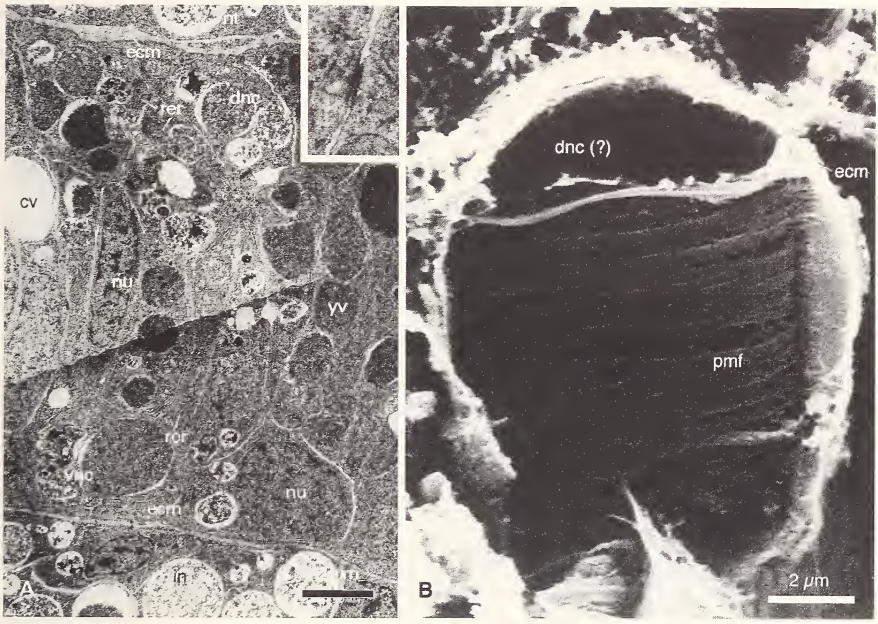


Fig.11: Electron micrographs of notochordal cells. **A** - sagittal TEM aspect of a neurula stage (30h pf, 18°C). Inset: cellular junction (adherens type) between two central notochordal cells. **B** - SEM micrograph of a fractured larval stage (110h pf, 18°C). **cv** - central vacuole, **dnc** - dorsal notochordal cell, **ecm** - extracellular matrix, **in** - intestine, **nt** - neural tube, **nu** - nucleus, **pmf** - paramyosin filaments, **rer** - rough endoplasmic reticulum, **vnc** - ventral notochordal cell, **yv** - yolk granule.

les are present among the central notochordal cells. Dorsal and ventral to the central notochordal cells nuclei of the dorsal and ventral notochordal cells can be found on cross sections (Plates 9-17). The ventral notochordal cells have also acquired a spacious, intracellular, empty vacuole (Fig.11B; Stach 1999). Although the entire notochord is now clearly separated from the intestine, there is still a gradient of ontogenetic differentiation within the notochord along the antero-posterior axis of the animals. The central cells in the most posterior part of the notochord possess smaller vacuoles and fewer myofilaments. Few yolk granules are still present in these posterior notochordal cells (Plate 18). The differentiated notochordal sheath of the adult with its distinct layers and collagen fibres (Flood 1975) is established later during ontogeny as is the peculiar innervation via two paramedian rows of pits (Stach 1999).

Mesoderm (Figs.10, 12-19; Plates 1-18)

General

The mesoderm is a so-called "endomesoderm" as it is derived entirely from the epithelium of the archenteron. This process of enterocoely can be seen in early

neurula stages and, due to a gradient of differentiation along the antero-posterior axis, the ontogenetically less differentiated "earlier" state prevails in the posterior part of the animals in later stages. The asymmetrical state of the mesodermal segments in adult amphioxus is well known: the left series of the mesodermal segments is situated half a segment anterior to the corresponding segment of the right series. Early in ontogeny, however, the arrangement of the segments is bilaterally symmetrical as seen in the neurula with nine mesodermal segments (Fig.10A). The asymmetrical situation found in the adults is established already in neurulae with eleven mesodermal segments (Fig.10B). As the number of mesodermal cells

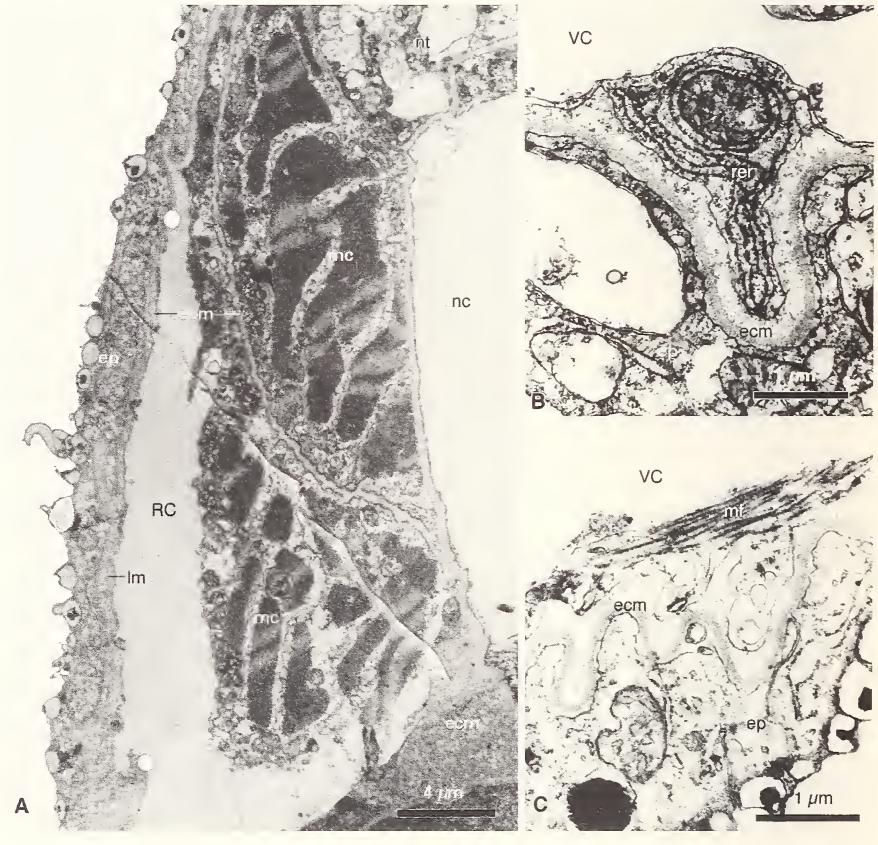


Fig.12: Transmission electron micrographs of mesodermal cells of a larva (110h pf, 18°C); compare with Fig.15. B + C - ventral mesoderm. **A** - Muscle cells building the dorsal border of the rostral coelomic cavity (left dorsal quadrant of Plate 16A). **B** - Cell with extensive profiles of rough endoplasmic reticulum around the nucleus. **C** - Mesothelial cell equipped with myofilaments. **ecm** - extracellular matrix, **ep** - epidermis, **lm** - lateral mesoderm, **mc** - myocyte, **mf** - myofilaments, **nc** - notochord, **nt** - neural tube, **nu** - nucleus, **rer** - rough endoplasmic reticulum, **RC** - rostral coelom, **VC** - ventral coelom.

recognizable in cross sections remains fairly constant whereas the shape of the segments changes considerably, cellular movement may be the main source of this rearrangement of the mesodermal segments.

The mesoderm is folded off from the archenteron dorsolaterally on both sides of the animal; and the mesodermal pouches are formed in the beginning by epithelial cells with apical cilia at least in the two anteriormost segments (Plate 1). The cells of these segments are all cuboidal in form and rest on an extracellular matrix. The cells are characterized by their prominent nucleus and numerous yolk granules. Apical cell contacts are present in the form of desmosomes (Ruppert 1996 depicts a similar apical junction in mesodermal cells of Hatschek's nephridium in older larval stages and states that this is "an adhering junction, probably a zonula adherens", p.168). In the segments posterior to the first two segments the dorsolaterally situated mesoderm consists of two cell types clearly distinguished by their shape (Stach 1994). These are the medial cells which are elongated in cross sections and stretch over the length of an entire segment antero-posteriorly (Figs. 10B, 14). The lateral cells on the other hand lie immediately below the extracellular matrix of the lateral epidermis and have a nearly cuboidal shape. A small slit is always present between these two groups of cells. At the posterior end the prospective mesoderm is nothing more than a dorsolateral elevation of the epithelium of the archenteron, the cells being entirely endodermal in nature (Plate 2).

In the first segment of slightly older neurulae with ten mesodermal segments, a spacious coelomic cavity is surrounded again by two different cell types (Plate 4). On the medial wall next to the notochord prospective muscle cells have a club-shaped appearance in transverse sections with their narrow ends adjacent to the neural tube. The lateral and ventral walls of the mesodermal segments are lined by narrow sheet like cells. The more posterior segments show the same arrangement of two different cell types, but lack the spacious coelom between them (Plate 5). Instead only a narrow slit like intercellular space is present in the more posterior segments between the lateral and the medial group of cells.

In older neurulae with eleven mesodermal segments, the differences between the two anteriormost and the nine posterior segments is still evident although the cells are more or less alike regarding their state of differentiation. The first segment is characterized by the possession of a central coelomic space (Plate 6). A small coelomic cavity is also present in the second segment (at least of the left series) and it contains rudiments of excretory cells (see below, Hatschek's nephridium; Stach & Eisler 1998). In the posterior segments intercellular spaces are small, but may be enlarged artificially during histological processing (Stach 1994). In all segments two distinct cell types can be recognized. The medial cells, club-shaped in transverse sections, span an entire segment, which measures now about 50 μm in length (Fig.10B; Plate 7). Thick myofilaments of approximately 15 nm width are seen in the cytoplasm. They are irregularly arranged sometimes perpendicular to their future orientation, but areas of a more regular arrangement may be encoun-

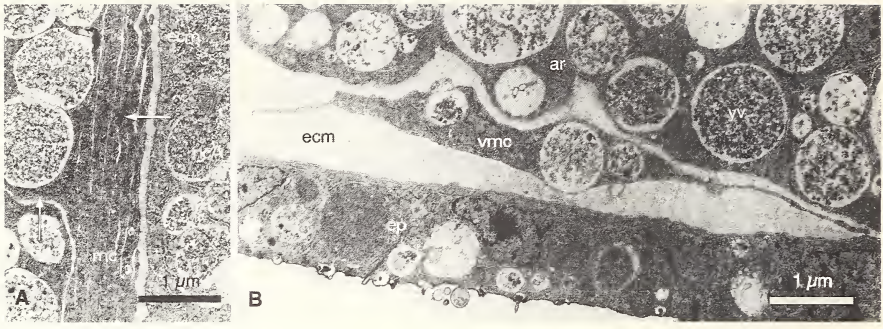


Fig.13: Transmission electron micrographs of mesodermal cells. **A** - Horizontal longitudinal section of a neurula stage (35h pf, 18°C). The ecm separates the left dorsolateral mesoderm (left in figure) from the anlage of the notochord; anterior is at the top . Note the irregular arrangement of the myofilaments (arrows). **B** - Sagittal section along the mid- ventral line from the trunk region of a neurula stage (37h pf, 18°C). Note the single mesodermal cell ventral of the archenteron. **ar** - archenteron, **ecm** - extracellular matrix, **ep** - epidermis, **mc** - early myocyte, **ncA** - anlage of the notochord, **vmc** - ventral mesodermal cell, **yv** - yolk granule.

tered (Fig.13A). The lateral cells became very narrow and often have a thickness of only 0.2 µm in transverse sections (Plate 7). In addition, mesodermal cells surround the archenteron ventrally, thus connecting the mesodermal segments of both sides. Sagittal sections suggest that this ventral mesoderm is also segmentally arranged in the beginning (Fig.13B; Hatschek 1881). The cells of this ventral mesoderm resemble those of the lateral cell-groups. In addition to the eleven mesodermal segments just described, the intestine is forked at its anterior tip, where two more compartments are in the process of becoming pinched off from the archenteron: these are the diverticula of Hatschek (see below; Plate 6).

In the late neurula / early larva stage, the main dorsolateral mesodermal segments of the trunk consist of two different cell types (Plate 9). The medial cells are differentiated into muscle cells resembling the deep lamellae of the adult in the nomenclature of Flood (1968). These are relatively poor in glycogen and mitochondria content and were therefore compared with the fast fibres of craniates by Flood (1968). The lateral cells are sheet like, extremely narrow cells bordering the segment laterally and are separated from the muscle cells by a narrow myocoel. The mesoderm that surrounds the intestine is two-layered over the main part of its length. In the midline this ventral mesoderm surrounds a well-defined ventral coelomic canal. The development of the mesoderm in the anterior part of the animal is more difficult to understand. The differentiation of the second, left segment into Hatschek's nephridium and the further development of Hatschek's diverticula into the rostral coelom and the preoral pit will be dealt with below. The origin of the muscles around the mouth opening and the first primary gill slit (Fig.18) could not definitely be clarified. The association of the posterior muscle of the mouth ope-

ning with the segment of Hatschek's nephridium (see below; Stach & Eisler 1998) suggests that this muscle originates in the ventral mesoderm of this segment.

Ontogeny of the so-called "muscle tails" (Fig.14)

The medial group of mesodermal cells that borders the notochord is distinguishable from early larval stages on. Although the cellular specialization is obviously comparable to that of the lateral mesodermal cell, which underlie the lateral epidermis, the shape of the cells in cross sections and horizontal sections differs remarkably. Even in the neurula stages with ten mesodermal segments the medial cells show a club-shaped appearance in cross sectional aspect, whereas the lateral cells appear to be rectangular (Fig.14A; Stach 1994). The more narrow dorsal end of the elongated medial cells is already situated at the lateral ventral border of the neural plate (Fig.14A). Rather than an outgrowth of processes from the prospective myocytes, which contact the central nervous system, the contact of the myocytes is established early on during the process of mesoderm formation and retained during further development. The situation found in the adult with a clearly confined narrow area of contact with the central nervous system in the so-called central motor end plate (Flood 1968) is a result of the changes in overall cell shape of the medial mesodermal cells during myogenesis (Fig.14).

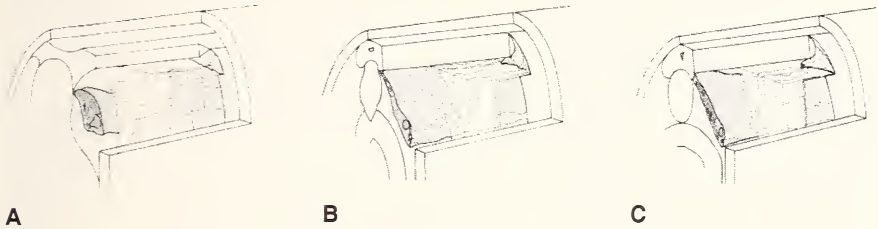


Fig.14: Schematic line drawings of the trunk mesoderm of developmental stages of *Branchiostoma lanceolatum*; not to same scale. Two different cell types are represented: a medial group of (prospective) myocytes and a lateral group of (prospective) mesothelial cells. One cell of the medial group is depicted in greater detail to demonstrate the development of myofilaments and the early association with the nervous system. **A** - Early neurula stage (22h pf, 18°C). **B** - Neurula stage (35h pf, 18°C). **C** - Larval stage (110h pf, 18°C).

Coelomic cavities (Fig. 15)

There are three spacious coelomic cavities lined by a mesodermal epithelium present in larval stages of *Branchiostoma lanceolatum*. These are (1) the rostral coelom, (2) the lumen inside of Hatschek's nephridium, and (3) the ventral coelomic canal (Fig. 15). In addition a small coelomic cleft, the myocoel, separates the medial muscle cells from the lateral coelothel in each mesodermal segment. The ontogenetic origin of Hatschek's nephridium is discussed separately below.

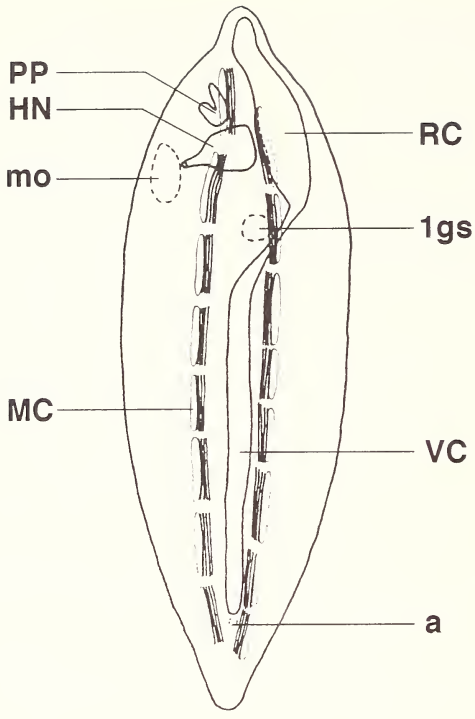


Fig.15: Diagrammatic dorsal view of coelomic cavities of a 1 gill slit larva of *Branchiostoma lanceolatum*. **a** - anus, **HN** – Hatschek's nephridium, **mo** - mouth opening, **MC** - myocoel, **PP** - preoral pit, **RC** - rostral coelom, **VC** - Ventral coelom, **1gs** - first primary gill slit. (Number of segmental myotomes arbitrary.)

The rostral coelom arises from the right diverticulum of Hatschek that pinches off from the intestine at about the neurula stage with eleven segments (Plates 3 & 6). The epithelium of this pouch is prismatic in the earlier stages, but develops into a thin layer of typical coelothelic cells in the larval stage (Plates 10-16A). In the larvae the rostral coelom is very spacious reaching from the anterior tip of the animal, where it is situated just beneath the notochord, posterior to the region behind the first primary gill slit, thus spanning over a length of approximately 300 µm nearly a third of the animal's entire length. The rostral coelom divides into two narrow canals, where the gill slit opens, passing the gill slit dorsally and with a narrower canal ventrally (Plates 13 & 14). The rostral coelom is in continuity with the ventral coelom. Just in front of the anterior rim of the mouth opening the dorsal cells of the rostral coelom are differentiated as muscle cells (Fig.12A; Plate 11B). Such a differentiation of the dorsal cells into muscle cells occurs again behind the first primary gill slit at the posterior end of the rostral coelom (Plates 13B & 16). Throughout its course the rostral coelom comes in close contact with the endostyle, the club-shaped gland, and the preoral pit.

The ventral coelomic canal of the larva extends from the region of the first primary gill slit where it consists of very narrow lacunae, which are in contact with the rostral coelom, to the region several µm anterior to the anus (Plates 13-17).

The ventral coelom is bordered by narrow epithelial cells, which may occasionally show high transcriptional activity (Fig.12B) or possess myoepithelial features (Fig.12C). The system is contractile in these stages (Stach 1998).

The mesoderm of the neurulae with nine segments is restricted to the dorsolateral parts of the animals (Plate 1: Stach 1994). Later the mesoderm grows out ventrally (Plates 5, 7, 8). This is seen in the neurulae with ten and eleven segments. In these stages, the ventral mesodermal cells surround the archenteron. The cells are narrow and may be present in one or two sheets, but always make up a solid tissue. In the earliest larval stages small intercellular spaces are present in the ventral mesoderm (Plate 9).

Hatschek's nephridium (Figs.15-17; Plate 11, B & C)

Hatschek's nephridium develops ontogenetically in the second mesodermal segment of the left side (counted without the diverticulum of Hatschek). The neurula

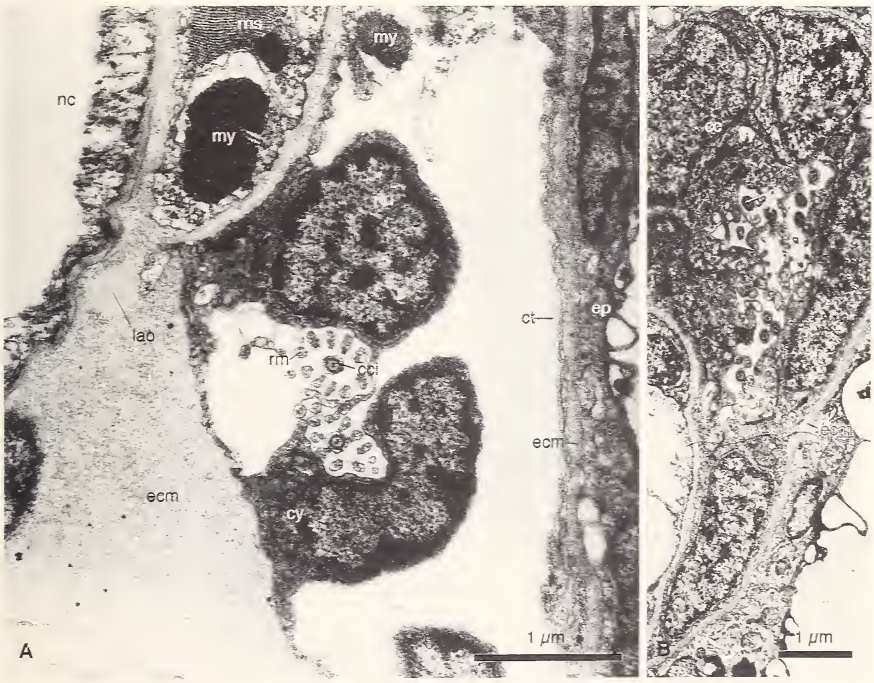


Fig.16: Transmission electron micrographs of Hatschek's nephridium of a larval stage of *Branchiostoma lanceolatum* (110h pf, 18°C). **A** - Cyrtopodocytes on the enlarged ecm where the rudiment of the aorta is formed. **B** - Cross section through the posterior canal of Hatschek's nephridium. **cc** - canal cell, **cci** - central cilium, **ci** - cilium, **ct** - coelothel, **cy** - cyrtopodocyte, **ecm** - extracellular matrix, **ep** - epidermis, **lao** - anlage of the left anterior aorta, **ms** - mesoderm of the first segment, **my** - myocyte, **nc** - notochord, **nu** - nucleus, **rm** - rod-like microvillus.

stages of *Branchiostoma lanceolatum* with nine segments do not show any cellular specializations of excretory function. The anterior mesodermal segments are restricted to the dorsolateral sides of the animal; the mesodermal cells of this region are fairly unspecialized but of a clear polar organization and surround a relatively spacious coelomic cavity. Embryos with eleven segments show organelles resembling those of excretory cells of the adults, with a central cilium surrounded by ten rod-like microvilli (Stach 1994, Stach & Eisler 1998). Only a single excretory cell could be detected in serial sections, situated in the second mesodermal segment of the left series. On its visceral side the cell rests on the extracellular matrix covering the notochord. No podocytic extensions of this cell could be seen. A coelomic space lies lateral to this cyrtocytic cell. Again the mesodermal cells of this segment, including the cyrtocyte, are definitely polar in organization. In larvae where the mouth and first gill slit are open, Hatschek's nephridium is fully developed. It is situated immediately behind the preoral pit just above the left-sided mouth opening (Fig.15, 16; Plate 11B & C). The nephridium stretches over a length of approximately 40 µm and measures some 15 µm at its broadest diameter. It is situated lateral to the caudal end of the first segment of the left series in the second segment and comprises of five different cell types (Fig.16):

– Dorsally next to the neural tube a small number of myocytes (about 5) can be seen. They possess many mitochondria. Myofilaments are arranged in the typical striated manner of skeletal muscle cells.

– The somatic side of the nephridium consists of extremely narrow epithelial cells separated from the ectodermal epithelium by a common extracellular matrix.

– The extracellular matrix ventral to the notochord is thickened. A lighter, finer-granulated region probably represents a rudimentary subchordal blood vessel (Fig. 16A; Stach 1998). Cyrtopodocytes are resting on this extracellular matrix with their foot-like processes. The cyrtopodocytes possess a prominent nucleus projecting into the coelomic space. In addition, the central cilium surrounded by ten rod-like microvilli is seen. A fibrillar extracellular material interconnects these microvilli.

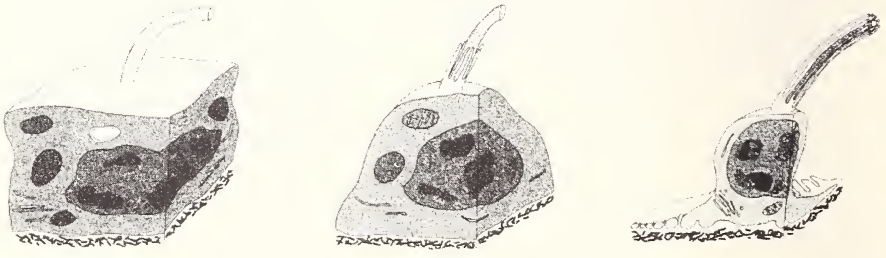


Fig.17: Schematic line drawings of the development of the nephridial cells of *Branchiostoma lanceolatum*. **A** - Epithelially organized mesodermal cell of a neurula stage (22h pf, 18°C). **B** - Hypothetical intermediate stage. **C** - Fully functional cyrtopodocyte of a early larval stage (110h pf, 18°C).

– In the anterior part of the nephridium the ventral cells are differentiated into muscle cells which clasp the mouth opening (Fig.19A).

– The posterior part of the organ consists of a narrow canal (Fig.16B). About five cells make up this short channel, which opens into the oral cavity. The cells bear prominent nuclei and possess mitochondria. Especially in the apical parts, numerous vesicles (either empty or with light, granulated contents) can be seen. The canal cells are interconnected by septate junctions and bear a single apical cilium.

Preoral pit (Figs. 15 & 18; Plates 10 & 11, A)

The preoral pit is ontogenetically derived from the left diverticulum of Hatschek which is seen in the neurulae with ten and eleven segments (Plates 3 & 6). This diverticulum is sealed off from the anterior archenteron and retains, in contrast to its right counterpart, tall cells. The cells are in the beginning not distinguishable from the endodermal, polar, epithelial cells of the archenteron. The left diverticulum comes into contact with the epidermis and opens to the exterior as the preoral pit. An intermediary stage, where the process of migration could be documented, was not observed. In the larval stage the preoral pit is an epithelially organized structure lying dorsal to the oral papilla just in front of the relatively large left-sided mouth opening (Fig.15; Plates 10 & 11A). It is a bean-like organ situa-

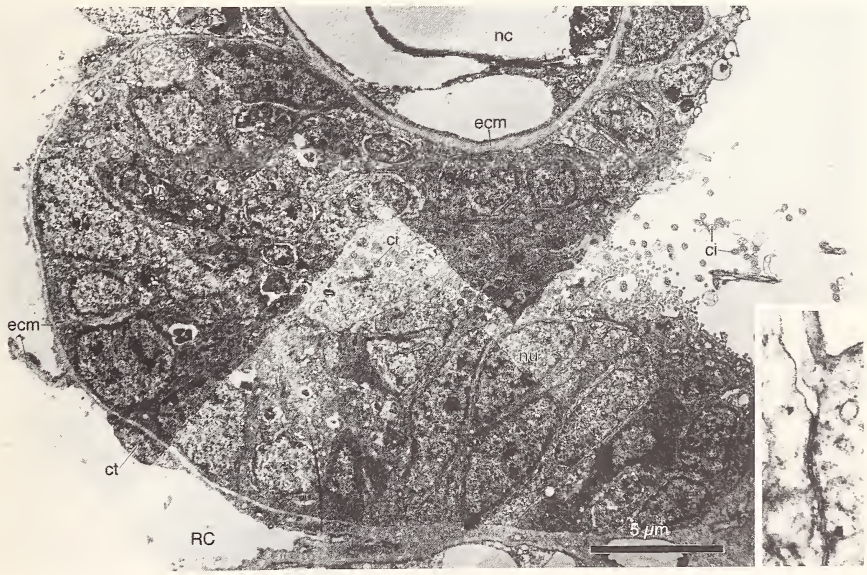


Fig.18: Transmission electron micrograph of the preoral pit of a larva of *Branchiostoma lanceolatum* (110h pf, 18°C). Note the rostral coelom at the left corner of the micrograph. Inset: septate junction connecting two preoral pit cells apically. **ci** - cilium, **ct** - coelothel, **ecm** - extracellular matrix, **nc** - notochord, **nu** - nucleus, **RC** - rostral coelom.

ted just beneath the notochord and is continuous with the ectodermal epidermis. The preoral pit consists of a single layer of elongated cells surrounding a short antero-posterior oblique groove (Figs.15 & 18). The organ extends internally to the right side of the larva, where it is situated next to the rostral coelom. The cells show a large, mainly basal nucleus of a spherical or elongated shape with numerous patches of scattered heterochromatin. Smooth and rough endoplasmic reticulum is rarely seen around the nuclei, but free ribosomes are distributed over the entire cytoplasm. Many small (about 0.1 μm in diameter) vesicles and some bigger (about 0.5 μm in diameter) electron-dense, membrane-bound vesicles are seen in the apical region of the cells. These latter vesicles resemble lysosomes. Mitochondria are found throughout the cytoplasm but are especially numerous in the apical parts of the cells. Occasionally a Golgi apparatus is seen among them. A few empty-appearing, membrane-bound apical protuberances in some cells resemble those abundant in the epidermis of neurula stages. The apical surface of each of the preoral pit cells possesses a single cilium surrounded by 15-20 microvilli. The cilia have the regular pattern of 9*2+2 microtubules and two long rootlet fibres originate from their basal bodies. Along the lateral membranes in the apical region septate junctions could be identified (Fig.18, inset). A second group of cells similar in their ultrastructural appearance to the ones described above but with a generally denser and darker, cytoplasm occurs among the ones described above (Stach 1996).

Endoderm (Plates 1-18)

The archenteron is generated by invagination of the vegetal half of the blastula during the process of gastrulation. The cells of the archenteron become tall and prismatic and acquire an apical cilium. The blastopore is closed by the ectodermal epithelium during neurulation; thus the archenteron is not in direct contact with the exterior until the mouth breaks through at the onset of the larval stage. As mentioned above, an indirect connection with the exterior persists after the closure of the blastopore via the canalis neurentericus, the neural canal, and the neuropore. In the neurula stages with nine segments, the archenteron has a straight central lumen, which is bordered by the endodermal epithelium. This epithelium is continuous with the primordium of the notochord and in the posterior part with the rudiment of the mesoderm (Plates 1 & 2). In general the endodermal cells contain the highest amount of yolk granules of all tissues. The cells of the prospective intestine are prismatic in appearance and of a polar organization. This cellular polarity becomes especially visible with the apical cilium and the apical lateral junctional complexes of these cells. In the neurula with ten mesodermal segments the notochord is separated from the archenteron while the cells remain unchanged in their ultrastructural appearance with only a slight decrease in the number of yolk granules. This decrease in the content of yolk continues up to the neurula with eleven segments where occasionally irregular microvilli around the apical

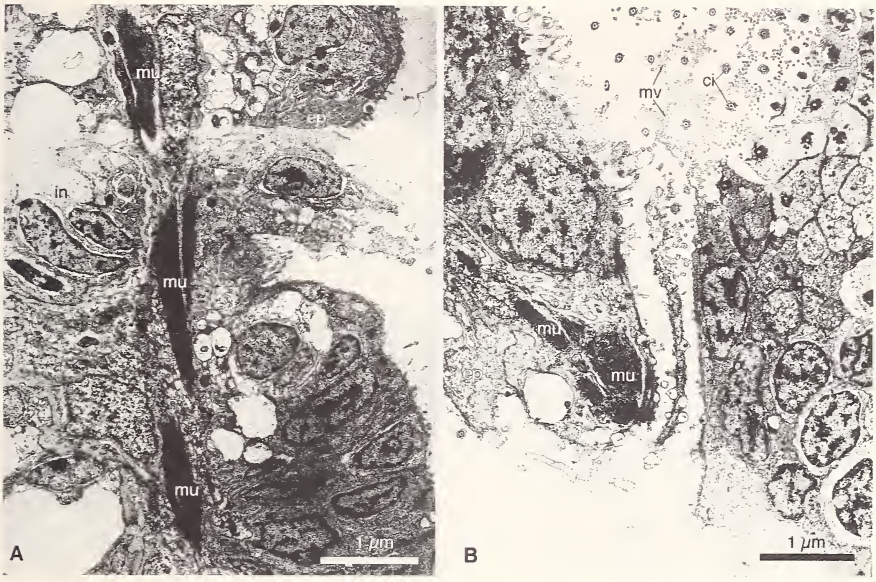


Fig.19: Transmission electron micrographs of cross sections of a larva of *Branchiostoma lanceolatum* (110h pf, 18°C). See Plates 12 & 13 for approximate planes of section. **A** - Section just behind the mouth opening shows the muscle that controls the mouth opening and the thickened epithelium of the mouth. **B** - Section through the gill slit demonstrating also muscular differentiations and regular microvilli around the intestinal cilia. **ci** - cilia, **ep** - epidermis, **in** - intestine, **mu** - musculature, **mv** - microvilli, **nu** - nucleus.

cilium are observed. The major change that can be recognized in this neurula stage is seen at the anterior tip of the archenteron (Plates 3 & 6). At its anterior tip the archenteron is forked, resulting in two short branches about 20-30 µm long. The cells are of the same type as in the remainder of the archenteron but appear less regularly arranged, a trait which may be attributed to the oblique plane of section, as the two branches are directed anterodorsally. The two branches pinch off from the archenteron during the subsequent ontogenetic events and become the left and right diverticula of Hatschek. The development of the right diverticulum into the rostral coelom and of the left diverticulum into the preoral pit is described above.

At an age between about 42 to 80 h (at 18°C) the mouth opening and the first primary gill slit break through at the anterior third of the animal. The mouth opens at the left side, whereas the gill slit pierces posterior to the mouth slightly to the right of the ventral midline. Only a short time later the anus breaks through in the ventral midline, slightly shifted to the left in front of the tail fin, that developed in the latest neurula / early larval stage (Fig.7; Plate 18). The epithelial intestinal cells of the pharyngeal region are less prismatic than in the rest of the intestine. The epithelium that builds up the border of the mouth and the gill region is specialized in a way that its cells are higher and equipped with long cilia. Muscles of probably

ventral mesodermal origin clasp both the mouth and the gill opening (Fig.19). No such muscular specializations could be detected around the anus (Plate 18). In the region anterior to the mouth, where the intestine projects rostrally for a short distance the cells of this intestinal projection possess a prominent empty-appearing vacuole (visible also in Plate 12). The intestinal cells are prismatic throughout the remainder of the endoderm and numerous microvilli are arranged in a circle around the apical cilium from this stage on (Plates 11-18). In early larval stages (Plate 9) few yolk granules are still visible in the intestine; whereas in the larva of 110 h (at 18°C) no yolk granules are found. In the pharyngeal region of the larval stage two prominent organs are situated at the right wall: these are the endostyle and the club-shaped gland.

Endostyle (Fig. 20, Plates 11 & 12)

The endostyle originates in the endoderm, opposite the mouth opening in the early larval stages. In the larval stage of 110 h (at 18°C) the endostyle stretches over a length of roughly 40 µm on the right side of the pharynx opposite of the anterior part of the mouth. It is an epithelial thickening, situated immediately anterior to the club-shaped gland, and appears as a figure "7" shaped structure, in which the dorsal branch stretches laterally over approximately 20 µm. The ultrastructure and histochemistry of the endostyle in older larvae with 4-10 gill slits and a hypothesis of the later development of this organ was presented by Olsson (1983) and Fredriksson et al. (1985). The endostyle of the larva is easily recognized on electron micrographs as the remaining endodermal cells in this area possess large intracellular vacuoles, a feature entirely missing in the cells of the endostyle (Plate 12). Different stripes of cells as seen in the later larval stages (zones 2-6 in the nomenclature of Barrington 1958 and Fredriksson et al. 1985 whose terminology is followed here) are already clearly distinguishable in these early larval stages (Fig.20).

Zone 2 is characterized by cells with a basally situated nucleus. Large amounts of rough endoplasmic reticulum can be seen and the Golgi complexes are well developed. The apical region contains numerous small vesicles (about 0.3 µm in diameter).

Zone 3 cells possess only little amounts of rough endoplasmic reticulum. The nuclei are not restricted to the basal parts of the cells only. Apically these cells show slightly bigger (about 0.4 µm) vesicles, which are not as frequent as in the cells of zone 2. Each cell bears an apical cilium and microvilli.

Zone 4 cells are easily distinguished by the combination of the following characteristics: The most prominent feature is the parallel arrangement of the cisternae of the rough endoplasmic reticulum around the basal nuclei. The cytoplasm is electron-denser than in the rest of the endostyle cells. Apically these cells contain characteristic vesicles (0.2-0.4 µm) with an electron dense content which is sepa-

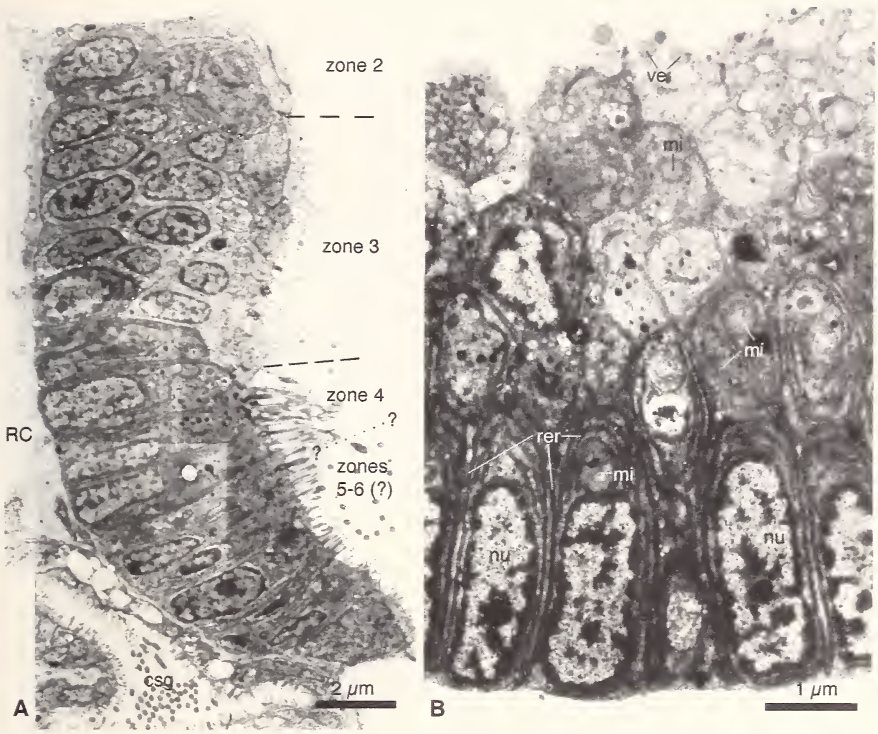


Fig.20: Transmission electron micrograph of the endostyle of a larva of *Branchiostoma lanceolatum* (110h pf, 18°C). **A** - Low power magnification of the entire organ in cross sectional aspect. Note the clear zonation and the close proximity of the rostral coelomic cavity. **B** - Higher magnification of zone 4-cells. Note the extensive profiles of endoplasmic reticulum around the nuclei. **csg** - club-shaped gland, **mi** - mitochondria, **nu** - nucleus, **RC** - rostral coelom, **rer** - rough endoplasmic reticulum, **ve** - apical vesicles; labelling of zones according to BARRINGTON (1958).

rated from its limiting membrane by an electron lucent rim (compare to Fredriksson et al. 1985). The zone 4 cells bear an apical cilium and numerous long microvilli.

Zones 5-6 are not clearly distinguishable in the electron micrographs but cells similar in ultrastructure to zone 4 cells but with a slightly lighter cytoplasm are situated in the position where zone 5 and 6 are situated in later developmental stages (Fredriksson et al. 1985).

Club-shaped gland (Fig. 21; Plates 11 & 12)

The club-shaped gland, like the endostyle, originates in the endoderm opposite of the mouth opening in the early larval stages. According to light microscopical evidence it is formed as a fold in the epithelium of the intestine and retains a dorsal

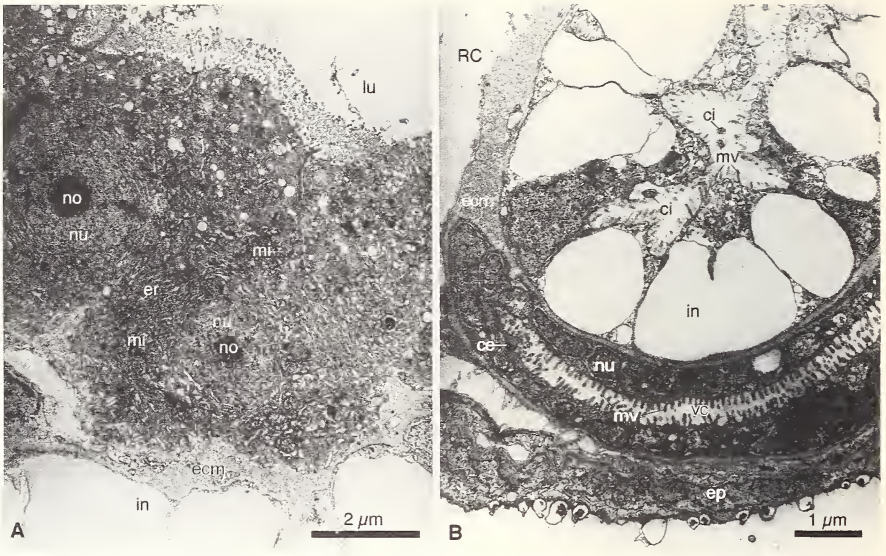


Fig.21: Transmission electron micrographs of the club-shaped gland of a larva of *Branchiostoma lanceolatum* (110h pf, 18°C). **A** - Section through the dorsal glandular area of the organ. Note the prominent nucleolus of the cells. **B** - Oblique section through the ventral canal region of the organ below the intestine. Note the regular apical microvilli of the canal cells. **ci** - cilium, **ecm** - extracellular matrix, **ep** - epidermis, **in** - intestine (note the large empty appearing intracellular spaces characterizing the epithelial cells of the buccal cavity), **mi** - mitochondrium, **mv** - mikrovilli, **no** - nucleolus, **nu** - nucleus, **RC** - rostral coelom, **rer** - rough endoplasmic reticulum, **vc** - ventral canal of the club-shaped gland.

internal opening and acquires a ventral external opening on the left side. The study of Olsson (1983) provides electron microscopical and histochemical data of this organ at later larval stages. In the larval stage of 110 h (at 18°C) the club-shaped gland stretches over a length of nearly 75 µm on the right side of the buccal wall. It consists of a glandular dorsal part (Fig.21A; Plate 11D) and a ventral duct (Fig. 21B; Plate 11C & 12) leading to the external pore and is situated immediately posterior to the endostyle. The lightmicroscopical regions observed in the club-shaped gland are clearly recognizable in the electron micrographs. The cells that build up the ventral canal are flat, squamous, typical epithelial cells. Besides the prominent nucleus, the canal cells are well equipped with mitochondria; each of the cells possesses numerous microvilli apically (Fig.21B). Although cilia are rarely seen, they seem to be present, as apical centrioles are regularly found in the canal cells (Fig.21B). Some of the cells in the canal region display an intracellular, empty-appearing vacuole. The dorsal glandular part consists of a single layer of gland cells obviously of a single type (Fig.21A). The cells are cylindrical and show basally a spherical nucleus with a conspicuous, dense nucleolus. The nucleus is surrounded by flattened cisternae of rough endoplasmic reticulum in

parallel arrangement. In the central region of the cells numerous mitochondria can be seen; apically, a prominent Golgi complex is frequently encountered. Above the Golgi apparatus a large number of vesicles of various sizes is evident, with contents of varying electron density, some even empty in appearance. Only few cilia are seen in the central canal of the glandular part of the club-shaped gland, indicating that the cells are rarely ciliated. Microvilli on the other hand are numerous on the apical surface of the gland cells.

Circulatory system (Fig. 15A; Plates 15 - 17)

The circulatory system of adult *Branchiostoma lanceolatum* is difficult to study. It consists of vessels, which are not equipped with an endothelium, which makes it extremely difficult to follow the fine branches.Using refined injection techniques together with electron microscopy, Rähr (1979, 1981) provided a detailed picture of the circulatory system of adults. No data exist about the ontogeny of the circulatory system and observations of blood vessels in the course of this study remained sporadic and ambiguous. One main blood vessel of the adult seems to be the first to appear during ontogeny. This is the anterior aorta seen in the proximity of Hatschek's nephridium. In the place where this blood vessel is found in the adult, i. e., ventral to the notochord the extracellular matrix is considerably enlarged and an area of finer granulation within this extracellular matrix is observed (Fig.15A). In addition, the extracellular matrices of the corresponding right side ventral to the notochord, where in the adult the right notochordal artery is situated, is considerably enlarged. Also in the mid ventral line below the intestine, where in the adult the endostylar artery is situated, the extracellular matrix is enlarged; no finer granulation in the extracellular material could be observed in these latter areas (Plates 15-17; terminology following Rähr 1979).

DISCUSSION

Phylogenetic systematics

Most recent researchers concerned with the phylogenetic position of cephalochordates favor the hypothesis that these animals comprise the sister-taxon of the craniates. This hypothesis is substantiated by morphological and molecular evidence (e.g., Maisey 1986, Jeffries 1986, Schaeffer 1987, Turbeville et al. 1994, Wada & Satoh 1994, Nielsen 1995, Salvini-Plawen 1998). The monophyletic taxon consisting of Cephalochordata and Craniata was named Notochordata by Nielsen (1995), whose consistent nomenclature will be followed here. However, recently Ruppert (1997a) proposed a closer relationship between the Tunicata and the Cephalochordata – a hypothesis called Atriozoa hypothesis in older publications (see Pietschmann 1962 for discussion). Ruppert based his phylogenetic interpretation on similarities in the development of the notochord (but see Stach

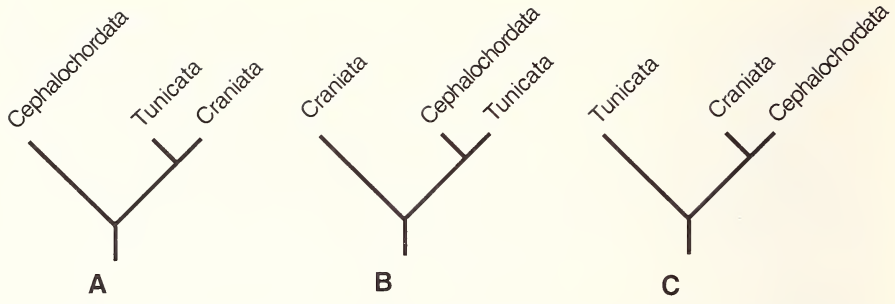


Fig.22: Logically possible phylogenetic interrelationship of the three presumably monophyletic higher taxa of the Chordata. **A** - No author favoured this possibility, although Salvini-Plawen (1998) claims that light sensitive organs of tunicate larvae are homologous to eyes of craniates, but not to eye structures of cephalochordates (but see Lacalli 1996). **B** - Atriozoa-Hypothesis (see text). **C** - Currently favoured phylogeny of the chordates (see Fig.23 and text).

1999 for a conflicting interpretation). With the assumed monophyly of the Chordata and of the three subgroups within the Chordata, i. e., the Tunicata, the Cephalochordata, and the Craniata, only three possibilities for the interrelationships between these taxa exist (Fig.22).

Since a phylogenetic framework is crucial for the interpretation of evolutionary changes, I provide a short summary of the argumentation supporting the currently favored hypothesis (Fig.23) based primarily on morphological data. It is obvious that all conflicting hypotheses have to account for the shared similarities discussed below between the Cephalochordata and Craniata.

Although Hennig (1984) is not an original publication on the subject of chordate phylogeny it is cited because of its phylogenetic perspective on previously described characters and its consistent phylogenetic interpretation of these characters.

- The most obvious and superficially visible features common to cephalochordates and craniates are the segmentally arranged muscle blocks of the trunk. It has been debated whether the muscular band of miniature muscle cells in the tail of tunicates represents a segmental arrangement comparable to that found in the Notochordata (e. g., Berril 1955, Jollie 1973). In any case, repeated lateral myomeres separated by connective tissue with a dorsal anterior angle ("chevron shape") are unique to the Notochordata and can, in combination with the other evidence presented here, be interpreted as a synapomorphy of the Cephalochordata and the Craniata.
- Another macroscopic feature unique to the Notochordata is a ventral outgrowth of the alimentary canal, the hepatic caecum that functions as a storage organ for lipids and as a site for the production of digestive enzymes (Welsch 1975). The similarity regarding the relative position of this organ is especially evident if one compares the hepatic caecum of lancelets with the ontogenetic rudiment of the liver in *Lampetra fluviatilis* (Damas 1944). Interestingly, the capillaries

around the liver and the hepatic caecum are both connected through a hepatic vein with the capillaries of the intestine. Thus this vessel fulfills the definition of a portal vein.

- In addition other blood vessels such as the paired aortae, the single caudal aorta, and the Ductus Cuvieri are comparable in their overall course (Rähr 1979). Thus the anatomy of the circulatory system can be regarded as another synapomorphy of Notochordata. Whereas the overall pattern of the circulatory system is regarded as apomorphic, the lack of an endothelium in the circulatory system of cephalochordates is seen as a plesiomorphic trait.
- The rudiment of the excretory system in both groups of Notochordata is mesodermal in origin (Nakao 1965, Stach & Eisler 1998). It is situated between the dorsal muscular mesoderm and a ventral portion of the mesoderm, and is at least in its embryonic origin segmentally arranged (Goodrich 1934). These similarities are also regarded as synapomorphies of the Notochordata, and the acquisition of a cyrtocytic part to the plesiomorphic podocyte in the cephalochordates is interpreted as an autapomorphy of the Cephalochordata.
- Hennig (1984) mentions another morphological character, which he assumes to be synapomorphic for the Notochordata: this is the preoral cavity ("Präoralhöhle") which is situated anterior to the velum.
- The velum is considered to be another synapomorphy of the Craniata and Cephalochordata by Hennig (1984), who remarked that there is no hint of reduction of this character in the third clade of the Chordata, i. e., the Tunicata.
- A unique feature again common to craniates and cephalochordates is seen in the phosphagens which provide muscular energy supply. Whereas in all invertebrate groups investigated phosphoarginin or phosphoarginin and phosphocreatin provide the energy for muscular contractions, phosphoarginin is entirely reduced in the Notochordata. Thus phosphocreatin seems to be the only phosphagen source in this clade (Watts 1975, Hennig 1984).
- The original suggestion of molecular biologists that the Notochordata are distinguished from other deuterostomes by the duplication of the HOX gene cluster which duplicated again in the line that led to the craniates (Pendleton et al. 1993) was shown to be erroneous (Garcia-Fernàndez & Holland 1994). Nevertheless P.W.H. Holland (1996, pp. 261-262) claims that "although a single set of HOX genes is also seen in all other invertebrates studied in detail to date, only lancelets show the remarkable 1:1 correspondence to mammalian paralogy groups."
- Recently Lacalli (1996a, b) and Lacalli et al. (1994) found evidence for the hypothesis that certain cellular arrangements in the frontal brain of larval amphioxus resemble the paired eyes of craniates. If this is substantiated further this anterior, light-sensitive organ could constitute another synapomorphy of the Notochordata, although the Tunicata should be investigated in this regard as well.

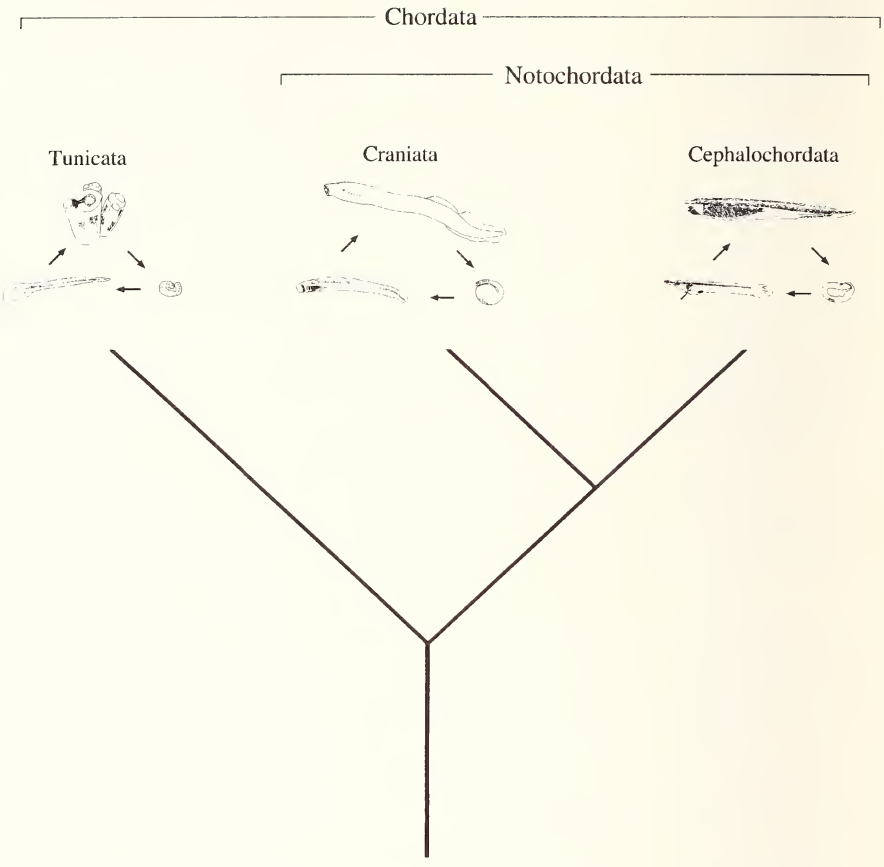


Fig.23: Assumed phylogenetic systematics of the Chordata. Discussion see text.

The phylogenetic hypothesis which results from this evidence is used as a framework to discuss the evolution of ontogenetic characters. The corresponding cladogram is depicted in Fig.23.

The fossil record

Following Hennig (1950), fossils are the only source of evidence regarding the absolute age of any monophyletic taxon. Whenever a fossil is attributable to a monophyletic taxon, the age of this taxon **and** its sister-taxon is at least as old as the fossil in question. If the phylogenetic interpretation presented above is used as a framework to assign fossils to a monophyletic taxon, several fossils become crucial.

Pikaia gracilens from the Middle Cambrian Burgess Shale is often considered to be a cephalochordate (Conway Morris & Whittington 1979, Conway Morris et al.

1982, Conway Morris 1993, Briggs & Kearr 1993). According to these authors *Pikaia gracilens* possesses a notochord and a zig zag arrangement of segmentally repeated lines which are interpreted as the myocommata of serial muscle blocks (Conway Morris & Whittington 1979). However, a detailed description supplemented by adequate figures of this interesting fossil is still lacking. The picture reproduced in the article of Conway Morris & Whittington (1979) depicts only straight segmental lines, without the special chevron shape of the dorsal part of the myomere, considered to be a synapomorphy of the Notochordata here. There are other problems with the interpretation of *P. gracilens*. Butterfield (1990) argued that *P. gracilens* possessed a decay resistant cuticle, which is not found in any of the extant cephalochordate, but characterizes e. g. many protostome taxa. In addition it is usually reported, that *P. gracilens* possessed paired antennae, also common in protostomes (Conway Morris & Whittington 1979, Conway Morris et al. 1982, Conway Morris 1993, Briggs & Kearr 1993). *P. gracilens* is therefore not easy to be attributed to any of the chordate taxa and a relation to the annelids can not entirely be excluded although it lacks parapodial chetae. A detailed study of this fossil is needed.

Appendicularia are soft, small, planktonic animals which hardly fossilize. Nevertheless *Oesia disjuncta* from the celebrated Burgess Shale fauna probably has to be interpreted as an appendicularian (Lohmann 1956). The evolutionary division of the Chordata into Tunicata and Notochordata thus had already taken place by the time the Burgess Shale animals were buried. Another fossil convincingly interpreted as the remains of an appendicularian has been described from the Lower Cambrian of southern China (Zhang 1987).

Aldridge et al. (1993) convincingly showed from well-preserved carboniferous fossil material that conodonts are nested within the craniates. If the older fossil material such as *Hertzina* from the upper Middle Cambrian (Clark & Miller 1969), or the oldest euconodont *Cambropustula* from the lower Upper Cambrian (Mueller & Hinz-Schallreuter 1998) belongs to the same conodont taxon, the splitting of the Notochordata into Cephalochordata and Craniata occurred in the Middle Cambrian at the latest.

There have been several claims for the oldest fossil chordate, among which *Yunnanozoon* from the Chinese Cambrian Chengjiang fauna recently attracted some attention (Chen et al. 1995, Dzik 1995, Shu et al. 1996). These fossils are (at least from the published material) hard to interpret; and given the evidence presented above, would not necessitate an earlier splitting event of the Chordata into Tunicata and Notochordata or into Tunicata, Cephalochordata, and Craniata than inferred from the fossil appendicularians and conodonts.

The calcichordate hypothesis of Jefferies (e.g., 1986, 1996, 1997) which heavily relies on the interpretation of Middle Cambrian and younger fossil material, is not reiterated here. Like *Yunnanozoon* the calcichordate hypothesis would not infer an earlier separation of the extant chordate lineages than deduced from the fossil evi-

dence mentioned in the paragraphs above (*Oesia*, *Cambropustula*). The fossils in question, the "calcichordates" or "carpoids", are interpreted as chordates with gill slits and notochord, amongst other chordate features, by Jefferies (ibid.). However, Philip (1979) and Jollie (1982) concluded in their review articles that the "carpoids" are best interpreted as primitive echinoderms, as they possess, e.g., a calcitic stereom-like endoskeleton, which is a synapomorphic character of extant echinoderms. Gee (1996) seems to arrive at a similar, albeit more ambiguous conclusion, when he states: "For the moment, I suggest that [...] carpoids are echinoderms with chordate (or hemichordate) affinities..." (Gee 1996: 292). Moreover Gee points out a crucial point of reservations about the calcichordate hypothesis: "Jefferies' detailed reconstructions of the insides of carpoids are thus intriguing, but impossible to test" (Gee 1996: 292). Peterson (1995) chooses a different approach and rejects the calcichordate hypothesis due to its failure to challenge the traditional phylogenetic hypotheses derived from computer-based analyses of extant phyla. This claim was refuted by Jefferies (1997).

Ontogeny and phylogeny

Ontogeny is understood here as the life history of an individual starting with fertilization and ending with its death (Kryzanowsky 1939, Zeller 1989, Maier 1993, 1999, Britz 1995). It has to be noted that this point of view contrasts with the position held by Haeckel (e. g., 1891) that ontogenetic stages are opposed to a mature adult stage. Contrary to the definition of Haeckel, the former definition of ontogeny leads to the conclusion that the ontogenetic sequence seen in the individul's development does not necessarily recapitulate any phylogenetic sequence. "For phylogenetic systematics this means that the transformation stages that led to a character condition during phylogeny, as it is realized in the adults of a particular species, cannot be read off with certainty from the ontogeny of the species" (Hennig 1979, p. 96). For the purpose of this paper, it suffices to point out that all ontogenetic characters have to be analyzed in the same way, i. e., primarily by comparison with the proposed sister-taxon and, if necessary, with the probable sister-taxon of the resulting higher clade (= outgroup), in order to reconstruct evolutionary changes.

In the discussions of chordate evolution, heterochrony has played a prominent role since the publications of Garstang (1928) and Berrill (1955). Elaboration of the ideas of these authors led to the suggestion that the Notochordata developed from an ancestral stage with an ascidian-like tadpole larva by ways of neoteny (Romer 1972, Berrill 1987a, b, Lacalli 1994, 1996c, Salvini-Plawen 1998). Recently Ruppert (1997a) pointed out that the number of cell divisions until gastrulation is only slightly smaller in cephalochordates (9-10, resulting in ~780 cells) than that found in echinoderms (*Echinus*: ~9 / 808 cells; *Lytechinus*: ~10 / ~1000 cells). Craniates show a remarkable retardation regarding gastrulation which occurs after 11 cycles of cell division (2200 cells) in *Petromyzon* and 14 divisions (16000

cells) in *Triturus* (Ruppert 1997a). Compared to echinoderms as an outgroup, for which data show the same order of magnitude in the number of cell division / cell number at a comparable ontogenetic stage in cephalochordates, tunicates appear to be distinguished by acceleration. This is especially pronounced in appendicularians: Gastrulation occurs after 6 -7 cell cleavages (76 cells) in *Styela* and 5-6 cell cycles (38 cells) in *Oikopleura* (Ruppert 1997a). Clearly this is a somewhat different view from the scenarios mentioned in the beginning of this paragraph. A major hindrance for more detailed discussions of the origin of the Chordata and the Notochordata is the fact that hypotheses concerning the morphology of the last common ancestor of the tunicates are not well-established.

Evolution of anatomical features of cephalochordate ontogeny and its bearings for the reconstruction of the probable Grundplan of the Notochordata

Preoral pit

Hatschek's pit in the roof of the preoral cavity of cephalochordates is thought to be homologous with the craniate adenohypophysis (e. g. Goodrich 1917, Welsch & Welsch 1978, Sahlin & Olsson 1986, Ruppert 1990, 1997b, Stach 1996). Evidence to support this hypothesis is based on studies of structure and function that revealed similarities. Sahlin & Olsson (1986) demonstrated that Hatschek's pit contains two types of cells with secretory specialization with type I cells being probably endocrine. Several studies were concerned with the presence of craniate adenohypophyseal substances in Hatschek's pit (see Table 2). Though most researchers claimed the presence of such substances, the results remain controversial (Sahlin 1988); a reinvestigation seems desirable. Despite these uncertainties the effect of gonadotropins on the maturation of lancelet gonads (Fang & Lin 1993) supports the studies reporting the presence of gonadotropins in Hatschek's pit. Moreover this result demonstrates the functional similarities of these hormones in the two taxa.

If the hypothesis of the homology between Hatschek's pit and the adenohypophysis is accepted, it is only logically consequent to consider the preoral pit of the larval amphioxus homologous to Rathke's pouch of craniate embryos (Stach 1996, Ruppert 1997b). The general similar position in front of the larval mouth is in favor of this hypothesis; but the preoral pit is regarded to be of mesodermal origin whereas Rathke's pouch originates entirely in the ectoderm (Starck 1975, Chester-Jones et al. 1987). However, the affiliation to a definite germ layer is highly variable during the ontogeny of the preoral pit (see above; Stach 1996) and the correlation with the anteriormost mesoderm may be plesiomorphic for the Notochordata (see below). The function of the preoral pit in larval amphioxus remains unknown (see Stokes & Holland 1995a, Stach 1996) but secretory and sensory functions have been suggested. In vertebrate embryos Rathke's pouch seems to fulfill endocrinological functions already in early stages of their development (Woods et al. 1994, Sasaki & Nishioka 1998).

Table 2. Hormones and peptides reported to be present in Hatschek's pit and groove in adult lancelets (references indicated) and the localization of their counterparts in craniates (three lower lines; Gorbman et al. 1983, Schmidt 1992).

	luteinizing hormon (LH)	chorionic gonadotropin	substance P	met-enkephalin	thyrotropin releasing hormone (TrH)	cholecystokinin (CCK)	gastrin
Hatschek's pit and groove	Chang et al. 1982, 1985 Nozaki & Gorbmann 1992	Chang et al. 1982, 1985	Nozaki & Gorbmann 1992	Nozaki & Gorbmann 1992	Chang et al. 1982, 1985	Sahlin 1988	Sahlin 1988
Adenohypophysis	+	+	(+)	(+)	–	–	–
Hypothalamus	–	–	(+)	(+)	+	+	+
Gastrointestinal tract	–	–	+	+	–	+	+

\+ present
– not present
(+) present in the "hypothalamus-hypophyseal area"

Note: The presence of these substances in Hatschek's pit and groove were described by the authors indicated. Other authors were sometimes unable to confirm the results of their predecessors. E. g., Sahlin (1988) could not detect any immunoreactivity against any of the substances listed in the table but CCK and gastrin (see Ruppert 1997b for discussion).

Questions regarding homologous structures in invertebrate deuterostomes are even harder to answer than those regarding homologs in craniates. In tunicates a homologous structure seems to be missing (see Ruppert 1990; endocrinologists, however, regard the subneural gland of ascidians as a homolog: Chester-Jones et al. 1987). This lack would hardly be surprising if one considers the almost entire reduction of mesoderm in this taxon. The developmental origin from the anteriormost mesodermal compartment and its connection with the exterior prompted several authors to suggest the homology of the preoral pit with the proboscis pores of enteropneusts and echinoderms (Goodrich 1917, Ruppert 1990, Salvini-Plawen 1998). Another structure of mesodermal origin just behind the preoral pit, but not from the anteriormost mesodermal compartment, acquires a connection with the exterior. This is Hatschek's nephridium, which in addition has a similar excretory function as the protocoels in enteropneusts and echinoderms (see p. 73). Welsch & Welsch (1978) proposed yet another hypothesis. The similar position and the similar appearance of a "präorale Wimpergrube" (= preoral ciliar pit) in the enteropneust *Saccoglossus horsti* prompted these authors to propose the homology of this structure with the preoral pit of amphioxus.

Conclusion: Despite the somewhat ambiguous evidence, the present state of knowledge may justify the notion held by most recent researchers that Hatschek's pit in cephalochordates and the adenohypophysis of craniates and hence their ontogenetic precursors preoral pit and Rathke's pouch, respectively, are homolo-

gous structures. The last common ancestor of the Notochordata may have had an endocrine function for the groove-like structure, with an eminent role in reproduction. In the ancestors of the craniates, this function became more and more dominant. In addition, its mode of development shifted, and Rathke's pouch became entirely ectodermal.

Endostyle

The endostyle of cephalochordates is homologous with the endostyle of tunicates and ammocoete larvae (Olsson 1963). Like the two latter structures it is situated in the ventral midline of the pharynx and is the main site for the production of the mucus net for filter feeding. Moreover, the endostyles of all three taxa have been shown by autoradiography to be a site of iodination. It is this latter fact and the fact that the endostyle of the ammocoete larva metamorphoses into the thyroid gland of the adult lamprey, that support the homology of the endostyles with the thyroidea of craniates.

The endostyle of adult cephalochordates consists of 6 different cell zones, arranged longitudinally in stripes symmetrically along the ventral midline of the pharynx (Barrington 1958, Welsch & Storch 1969a, Ruppert 1997). Zones 2-6 are discernible in older larval stages (Fredriksson et al. 1985) and functional in the production of mucus and iodination (Olsson 1983, Fredriksson et al. 1985, Gilmour 1996). In these and in younger stages the endostyle is asymmetrically situated in the right wall of the pharynx (Olsson 1983; see above). Most of the cell zones described by Barrington (1958) and Fredriksson et al. (1985) are already present in the earliest larval stages (see above). This is hardly surprising as larval feeding commences immediately with the formation of the left-sided mouth (Gilmour 1996, personal observation). The endostyle must be capable of mucus secretion at this stage. For this purpose, the cells of zone 2 and 4 are ultrastructurally specialized as active secretory cells. Whether iodination occurs at this early larval stage remains to be tested.

Iodothyroines and a thyroglobulin-like iodoprotein are reported to be present in the endostyle of adult cephalochordates (Salvatore 1969, Monaco et al. 1981). There seems to be no study on the effects of thyroid hormones on the physiology of lancelets, although it would be interesting to know whether these hormones play a role during metamorphosis.

The endostyle of chordates does not seem to have a corresponding structure in enteropneusts (Benito & Pardos 1997) although classical studies suggested that the hypobranchial ridge of spengelid enteropneusts may be a homologon (e. g. van der Horst 1939). Recently Ruppert et al. (1999), based on light microscopical observations, proposed a challenging hypothesis by emphasizing similarities between the dorsal epibranchial ridge of *Schizocardium brasiliense* and the endostyle of chordates. This report as well as the classical investigations of enteropneusts

should be re-investigated and substantiated by electron microscopic and autoradiograpic studies.

Conclusion: The endostyle probably represents a synapomorphy of the Chordata. In the grundplan of the Chordata it may have been a ventral-medial trough in the pharynx, consisting of different longitudinal stripes of cells, and had at least a double function in mucus production and iodination. This arrangement was most probably retained in the last common ancestor of the notochordates, as it is still present in adult cephalochordates. The asymmetrical arrangement of the endostyle in larval lancelets is interpreted as an autapomorphic character of this clade and arose probably in association with the larval feeding strategy. In Craniata the endostyle became more and more independent of the pharynx. The function of mucus production is only seen in ammocoete larvae, whereas in the gnathostomes the thyroidea is the only remaining homologous structure of the endostyle.

Club-shaped gland

So far there seems to be little evidence suggesting the homology of the club-shaped gland of larval cephalochordates to structures in craniates or tunicates. The supposition that the club-shaped gland could represent a differentiated gill slit (van Wijhe 1927, Goodrich 1931) is substantiated by its position in the lateral wall of the pharynx only. It is not associated with any of the structures associated with gill slits such as muscle cells (see above) or nephridia (Goodrich 1934). This "rätselhafte Organ" (= enigmatic organ, Schultze 1851) seems to vanish with metamorphosis (Goodrich 1931, Wickstead 1975, Olsson 1983) and its function is by no means clearly established. The hypothesis of earlier studies that it could secrete an adhesive substance which attaches the larvae temporarily to the sea bottom (Orton 1914, Barrington 1979) is implausible given the evidence of Olsson (1983) that the internal pore is the site of secretory release. Moreover the larvae are thought to be planktonic until metamorphosis commences (Stokes 1997, personal observation). According to Olsson (1983, p. 12), the position of the internal opening club-shaped gland "indicates that the discharged material does not participate in the food-trapping mechanism, since food particles have already been packe in mucus and are en route to the intestine when the gland material is added". As the dorsal part is definitely secretory (see above), the hypotheses of van Wijhe (1914) that it produces a gastric juice and of Wickstead (1975) that it may secrete a hormone should be tested.

Conclusion: The club-shaped gland, consisting of a secretory dorsal part and a narrow ventral canal, is a purely larval structure unique to cephalochordates, and is thus interpreted as an autapomorphy of this clade. Its precise function is not known, but according to Olsson (1983) it seems to secrete its substances into the pharynx by way of its dorsal internal opening.

Central nervous system

The neural tube dorsal of the notochord is clearly homologous throughout the chordates (see Nielsen 1995, pp. 396-398 for discussion). This is substantiated by the positional similarity as well as by similarities of anatomical structures (e. g., neuropore, canalis neurentericus, infundibular organ, Reissner's fibre, Rohan-Beard cells) and molecular evidence (reviewed by Fritzsch 1996, P.W.H. Holland 1996). As already mentioned, the resolution along the antero-posterior axis achieved in this study makes a reconstruction of details of the highly complicated neural system difficult. The peculiar mode of innervation of the somatic trunk musculature by so called "muscle tails" is discussed below. In order to complete this discussion section and stimulate further research, two important recent findings have to be mentioned.

In a series of papers Lacalli and co-authors (Lacalli & West 1993, Lacalli et al. 1994, Lacalli 1996 a, b) described the detailed anatomy of the rostral part of the central nervous system of a larva (12.5 days old) of *Branchiostoma floridae*. These authors concluded that this part of the central nervous system is similar to the visual system of craniates and described it as a "frontal eye" consisting of a pigment cup, two transverse rows of receptor cells, and a closely associated cluster of neurons. Moreover, this "frontal eye" was reported to project into a dorsal, tectum-like structure of the larval brain. More research is needed to corroborate the suggested homologies and the intriguing implications.

The neural crest and its derivatives account for numerous apomorphic characters of the craniates (Gans & Northcutt 1985, Northcutt 1996). Because of the developmental potential of this tissue, unique for craniates, the neural crest was thought to represent a "key invention" in the evolution of the craniates. Recently Langeland et al. (1998) showed that a homolog gene of the *snail* gene, which is expressed in the neural crest of developing craniate embryos, is also expressed in cells situated in the ventrolateral part of the neural tube of larval amphioxus (*Branchiostoma floridae*). These cells could therefore be homologous to the craniate neural crest cells. The cytological properties and identity of the corresponding cells as well as their function in the central nervous system of cephalochordates are yet to be determined.

Notochord

The notochord is the homologous structure after which the taxon Chordata is named. It is accepted by most authors that the notochord of tunicates, cephalochordates, and craniates is derived from a common ancestor (e. g. Maisey 1986, Nielsen 1995, Stach 1999), although the detailed structure of notochords varies immensely among different species (see Welsch & Storch 1969b, Ruppert 1997a). This concept of homology is soundly based on the relative position of the notochord ventral to the neural column and dorsal to the endodermal intestine. The

development of the notochord as a dorsal outgrowth of the archenteron is strikingly similar in all three taxa. Moreover molecular studies revealed conserved genes and gene expression in the notochords of the three chordate taxa (Holland, P.W. et al. 1995, Terazawa & Satoh 1995, 1997, Zhang et al. 1997). The notochord is only caudally developed in tunicates, stretches over the main part of the trunk in the two taxa of the Notochordata, and reaches the anterior tip of the body in cephalochordates only. In all three taxa a "Geldrollenstadium", the arrangement of the notochordal cells in single file, exists during early ontogeny (Stach 1999, but see Ruppert 1997a).

The main function of the notochord is thought to be mechanical, as a stiffening rod acting against the lateral body musculature. The notochord of cephalochordates is unique in containing striated muscle cells under neural control. The paramyosin fibres are seen already in late neurula stages and in early larval stages they are clearly contractile (Stach 1999).

Concerning the stomochord of hemichordates, Ruppert (1997a, p. 8) has recently stated that this structure "of hemichordates may not be a homologue of the chordate notochord, as nearly all modern authors contend." This notion would not contradict the view that the notochord may have evolved in the caudal part of the body (see below). Also, a molecular study of the expression pattern of the *Brachyury* gene in developing *Ptychodera flava* could not detect the expression of this gene at any time (Peterson et al. 1999). The *Brachyury* gene is expressed in the notochord of developing chordates (ibid.).

Conclusion: The notochord of the common ancestor of the Notochordata spanned (at least) over the main part of the trunk into the post-anal tail. It developed ontogenetically through a "Geldrollenstadium" and served as a stiffening rod to counteract against the muscular contractions through undulatory swimming. The latter function is present in tunicates as well and is thus interpreted as a plesiomorphy for the Notochordata. Whether the limitation of the notochord to the tail in tunicates is a primitive feature can not be established with certainty, as outgroup comparison fails to elucidate this question. Based on the cladogram suggested on other evidence (Fig.23) this caudal restriction may well have characterized the last common ancestor of the Chordata. The cranial extension of the notochord in the Notochordata would then turn out to be another synapomorphy of this clade. The notochord of cephalochordates is autapomorphic in its content of striated muscle filaments (Ruppert 1997a). Other autapomorphic traits of the cephalochordate notochord are its extension to the rostral tip and its direct innervation from the neural tube.

Mesoderm

The mesoderm is the germ layer, which gives rise to a variety of tissues and organ systems, especially in craniates. In cephalochordates the bulk of mesodermal tis-

sue is epithelially organized and does not undergo an epithelial-mesenchymal transition (Ruppert 1997b). Nevertheless the mesoderm of the two taxa can be homologized, as this germ layer shares ontogenetic similarities. In both groups the mesoderm is derived from a somewhat crescent-like region between the vegetal and animal pole of the mature egg (see Nielsen 1995). In both taxa the mesoderm develops from dorsolateral strands of the archenteron ("enterocoely" see below) and the main part develops into the segmentally arranged body musculature. The results of recent molecular studies support this hypothesis of general homology of the mesoderm in the two groups. In a study on the expression pattern of homologous genes of the murine *Brachyury (T)* gene Holland, P.W. et al. (1995, p. 4283) found that "the spatial and temporal distribution of *Brachyury* transcripts during amphioxus development is remarkably similar to vertebrate *Brachyury* in presumptive mesoderm, posterior mesoderm and the notochord". In addition to this similarity between cephalochordates and craniates, a *Brachyury* homolog is also expressed in the notochord of ascidian larvae.

A fate map of the tunicate egg reveals a similar distribution of the mesodermal portion as in the notochordate taxa (Conklin 1905, Nielsen 1995), and, shows also a developmental correlation with the endoderm (Nishida 1987). The region of the presumptive mesoderm in enteropneusts is less well established (Colwin & Colwin 1951); but the origin of the mesoderm from the archenteron seems to be well documented in several species (Bateson 1884, Dawydoff 1948).

Conclusion: In general, the mesoderm of cephalochordates and craniates is homologous. The derivation of the mesoderm from the archenteron is a plesiomorphic character for the common ancestor of the Notochordata. The segmentation of the mesoderm seems to be a synapomorphy for the Cephalochordata and Craniata, although the lack of knowledge concerning the last common ancestor of the Tunicata hampers a definitive answer to this question.

Coelom

The term "coelom" as understood in this study was defined by Rieger & Lombardi (1987) as a "body cavity lined by tissues of mesodermal origin" (ibid., p. 192) and an "epithelium" as a "single layer of cells with an apical belt-shaped junctional complex, with mostly parallel cell polarities and the extracellular matrix deposited on apical (cuticles) or basal (basal matrix) surfaces" (ibid. p. 192-193). The development of the coelom has played an eminent role in discussions of the phylogenetic relations between anatomically drastically different taxa (see Hyman 1959, Willmer 1990 for reviews). Our earliest understanding of mesoderm development in the cephalochordates comes from studies of the last century (Kowalevsky 1867, Hatschek 1881). Because the extracellular matrix separates germ layers during ontogeny, it is one of the important features to distinguish germ layers. The extracellular matrix in cephalochordate embryos, has, with sometimes only 0.2 μm in transverse sections, a thickness close to the limit of

resolution in light microscopy (e. g., Figs.8, 10, 11). In addition the delicate embryos of cephalochordates have a tendency to show artificial widening of intercellular spaces not unfamiliar to former authors (Goodrich 1931, Stach 1994). This makes it extremely difficult to distinguish between coelomic spaces and artificial gaps in histological preparations. Nevertheless, highly schematic drawings of the development of the mesoderm and the coelom, depending on older light microscopical studies persist in modern textbooks and publications (Fig.24; Franz 1927, Prenant 1936, L.TZ. Holland et al. 1995).

Three spacious coelomic cavities lined by a mesodermal epithelium are present in larval *Branchiostoma lanceolatum* (Fig.15; also Cerfontaine 1906, Conklin 1932, Hirakow & Kajita 1994 for *B. belcheri*): 1) the prominent rostral coelom on the right anterior side of the larva; 2) the coelomic space ventral to the intestine, just below the anlage of the ventral aorta (Figs.2C & 3B); and 3) the coelomic space of the first larval excretory organ - Hatschek's nephridium (Fig.16; Ruppert 1996, Stach & Eisler 1998). In addition the segmental narrow myocoels are present lateral to the myotomal musculature.

The preoral pit has been discussed above (see also Stach 1996). Hatschek's nephridium is covered below and elsewhere (Stach & Eisler 1998).

The rostral coelom is peculiar in several aspects. The rostral coelom arises from the right diverticulum of Hatschek. This anterior extension of the archenteron on the right side becomes separated from the endoderm and develops into a thin layer of typical coelothelic cells in the larval stage. The rostral coelom is very spacious and is bordered by two (perhaps three) groups of dorsal myocyte in larval stages (Fig.12a; Plates 11B, 13B, 16). Thus the rostral coelom resembles an enlarged and anteriorly stretched myocoel and it may be that it originated by fusion of the right diverticulum of Hatschek with one or even two anterior mesodermal segments. This fusion-hypothesis would agree with the disappearance of the two segments with conspicuous enterocoelic spaces seen in the neurula stages (Plates 1, 4, 6; see Stach 1994 for a diagrammatic presentation of earlier neurula stages) and the presence of two muscular portions in the dorsal part of the rostral coelom. A higher resolution along the antero-posterior axis and the examination of intermediate developmental stages should clarify some of these issues. Throughout its course the rostral coelom comes in close contact with the endostyle, the club-shaped gland, and the preoral pit. It should be tested, whether the rostral coelom has a role in the storage of products of these glandular or endocrine structures. In addition it is in continuity with the ventral coelom (Plates 13 & 14) for which a function in the distribution of substances was recently hypothesized (Stach 1998).

On the other hand the schemes mentioned above represent various coelomic cavities in the mesodermal segments of the trunk (Fig.24). In the trunk region of neurula stages, I could not detect any spacious coelom in the mesodermal segments by means of electron microscopy. There are no traces of a perivisceral coelom or sclerocoel (Plates 6-8). According to established textbook knowledge these com-

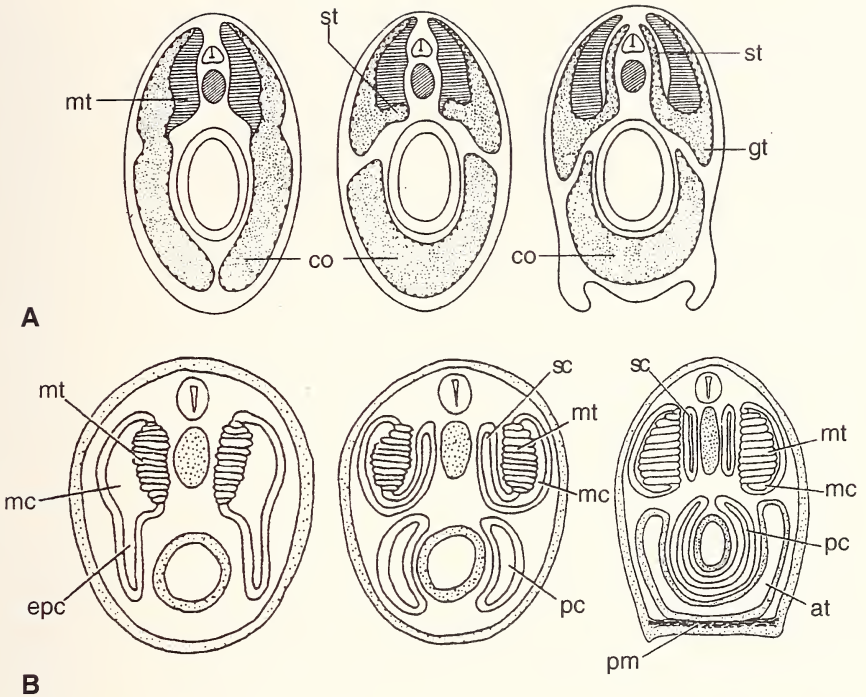


Fig.24: Schematic line drawings showing the ontogenetic origin of different coelomic spaces. **A** - *Branchiostoma lanceolatum*, after Prenant (1936); **B** - *Branchiostoma floridae*, after Holland et al. (1995). at - atrial cavity, co - coelom, epc - evaginating perivisceral coelom, gt - gonotome, mc - myocoel, mt - myotome, pc - perivisceral coelom, pm - pterygial muscle, sc - sclerocoel, st - sclerotome.

partments should become pinched off from the dorsal mesodermal segments during the first two days of development (Fig.24). The ventral part of the mesoderm surrounds the intestine and its cells constitute a solid mesoderm for most of the early development (Fig.13, Plates 5 & 7). In the larval stages examined in this study, a coelomic gap is seen in the posterior third of the larvae in this ventral region (Plates 9, 15-17, Figs.12 & 15). This space should become the perivisceral coelom of the adult (Prenant 1936, Holland, L.Z. 1996).

A sclerocoel is never present in the developmental stages examined in this study. Recent anatomical studies are ambiguous regarding the presence of a sclerocoel in adult cephalochordates. A coelomic cavity with the epithelial sclerothel is described by Holland & Holland (1990) and Ruppert (1997b). However, Ruppert (1991, p. 9) states that "adult myomeres lack a cavity". This issue is in need of reinvestigation.

The myocoel could be detected as a narrow intercellular space at the lateral side of most of the mesodermal segments (e. g., Fig.10, Plates 5, 9, 14-18). Although

in some sections the myocoel is not wider than other intercellular spaces such as those between muscle cells (Plate 7), it is encountered regularly even in material which does not seem to show artificially widened spaces. Thus earlier studies (e. g., Hatschek 1881, Cerfontaine 1906, Franz 1927, Conklin 1932) seem to be correct in noting that there is a lateral sheet of cells constituting a coelothel on the lateral side of each segment and a medial sheet of cells which differentiates into the numerous mononuclear myocytes. These sheets are separated by a myocoel.

As far as it is known all mesodermal cells are derived ontogenetically from the archenteron (Cerfontaine 1906, Conklin 1932, personal observation). Spacious coelomic cavities directly derived from the cavity of the archenteron are restricted to the first two (perhaps three) pairs of segments (Plates 1, 3, 4, 6). The posterior segments are more or less solid. Nevertheless they seem to gain a narrow myocoel during ontogeny (Plates 5, 7, 8, 17, Conklin 1932). Although this process was termed schizocoely by some authors (Conklin 1932, Willmer 1990, Hirakow & Kajita 1994, Holland, L.Z. 1996), this term can not be applied in a phylogenetic sense. A similarity between members of a single taxon in the lineage of the deuterostomes with the schizocoely of protostome taxa has to be interpreted as an example of convergent evolution. Moreover, this is confirmed by the fact that in cephalochordates the mesoderm is derived from the epithelium of the archenteron (Cerfontaine 1906, Conklin 1932, personal observation), whereas in the protostomes the mesoderm is proliferated by the so-called mesoteloblast (or the 4d cell; Kaestner 1980). I see no argument why the ontogenetic origin of the mesoderm in the cephalochordates should not be called enterocoely as in other deuterostome taxa.

The different arrangement of mesodermal compartments during ontogeny in the various taxa of the deuterostomes has gained much attention from zoologists. A widely accepted theory states that a condition with an unpaired protocoel and two pairs of subsequent coeloms, the mesocoel and metacoel, is primitive for the deuterostomes (Hennig 1984, Willmer 1990). These coelomic compartments are of enterocoelic origin. Comparison with the development of *Lampetra fluviatilis* (Damas 1944) shows that the last common ancestor of cephalochordates and vertebrates probably had paired mesodermal segments along its entire body. Tunicates seem to have reduced most of their coelom during evolution (Nishida 1987), a fact that at the present state of knowledge has to be interpreted as an autapomorphy of this clade. Whether the protocoel of enteropneusts in its primitive condition was paired or unpaired awaits a phylogenetic revision of this group. A wide range of processes leading to an unpaired condition is known (classical review by Stiasny 1914).

Pterobranchia are reported to possess an unpaired protocoel as larvae. However, although the paired mesocoel and metacoel is separated from the mesenchyme by an extracellular matrix, this is not the case for the protocoel (Lester 1988). The coelomic character of this intercellular space consequently remains dubious.

Echinoderms show a wide range of variation in mesoderm formation. *Asterias rubens* (Gemmill 1914), a sea star, *Strongylocentrotus lividus* (von Ubisch 1913), a sea urchin, and others, possess paired protocoelic compartments in early stages. The protocoel of *Echinus esculentus* (MacBride 1903) on the other hand is unpaired from its ontogenetic origin. One common feature of the protocoels of all these taxa in addition to their similar position may allow their homologization. All of them gain a connection to the outside via a canal and an exterior pore.

In *Branchiostoma lanceolatum* the most anterior mesodermal compartment of the left series opens to the outside and becomes the larval preoral pit (Hatschek 1881, Conklin 1932, Stach 1996). To complicate the picture, one additional mesodermal compartment of *B. lanceolatum* is connected with the outside: the second of the left series of myotomes opens as Hatschek's nephridium through a canal to the outside (see above; Stach & Eisler 1998). The protocoels of larval and adult enteropneusts and echinoderms play a role in excretion as well (Ruppert & Balser 1986, Balser et al. 1993). Therefore, homology is not easy to establish.

To complete this short survey, the studies concerned with tentaculates, the probable sister group of the deuterostomes (Hennig 1984), have to be mentioned. The phoronids develop an unpaired protocoel from proliferating amoeboid cells (Zimmer 1980). At least one species of brachiopods (*Crania anomala*) has four pairs of mesodermal sacs as larvae (Nielsen 1991). Finally, concerning bryozoans zoologists state that "there are no body cavities in the larvae of bryozoans" (Reed & Cloney 1982, p. 128).

This short review demonstrates that the dual character state of the protocoel (paired versus unpaired) probably changed several times during evolution. It also reveals the lack and necessity of modern morphological studies with a phylogenetic framework in smaller taxa.

Excretory cells

Differences of the excretory cells of amphioxus compared to vertebrates and superficial similarities with protonephridia have puzzled researchers over a long period (Boveri 1892, Goodrich 1902, 1934, Legros 1909, Brandenburg & Kümmel 1961, Nakao 1965, Ruppert 1994, 1996).

Among craniates, a podocyte with an apical cilium as the excretory cell is widespread (Bargmann 1978, Kluge & Fischer 1990, 1991). In Tunicata, comparable excretory organs seem to be missing, but Enteropneusta, Pterobranchia, and Echinodermata possess similar podocytes. In the latter two taxa, the podocytes bear apical cilia (Dilly et al. 1986; Ruppert 1994). It is therefore reasonable to suggest a podocyte with a cilium as the excretory cell of the last common ancestor of the Deuterostomia. The structure of the excretory cells of *Branchiostoma*, known as cyrtopodocytes, has to be interpreted as an autapomorphy of this taxon. Based on light microscopic evidence the situation in adults of the genus *Epigonichthys* is essentially the same as in *Branchiostoma* (Goodrich 1933).

In an electron microscopical study of the gill region of the enteropneust *Glossobalanus minutus,* Pardos & Benito (1988) described podocytes associated with coelomic spaces in the gill bars. It thus seems that there are two excretory complexes present in enteropneusts: 1) the protocoelic glomerulus and 2) the podocytes associated with coelomic spaces in the gill bars. It may be speculated that the anteriorly situated unpaired Hatschek's nephridium in cephalochordates may be homologous to the glomerulus in enteropneusts and the paired nephridia associated with the gills in cephalochordates may be homologous to the excretory structures in the branchial sacs of enteropneusts described by Pardos & Benito (1988). Studies of the detailed structure of the excretory system in the branchial apparatus of enteropneusts are necessary to substantiate such a hypothesis.

Branchiostoma and craniates exhibit similarities in their excretory organs in addition to their cells possessing podocytic extensions and an apical cilium. The position of Hatschek's nephridium in the mesoderm just ventral to a group of myocytes in *Branchiostoma* is comparable to the developing nephrotome of a craniate embryo in the mesoderm ventral to the somite. Moreover, in the case of the paired nephridia, the repeated arrangement is similar to the segmental arrangement of the tubules of the pronephros of craniates (Hatschek 1884, Legros 1910, Stach & Eisler 1998). Molecular studies of the expression of homologous genes in Hatschek's nephridium and the craniate pronephros strengthen the hypothesis that these structures are homologous (Kozmik et al. 1999).

Conclusion: The hypothesis that a separation into an anterior unpaired excretory system and paired excretory organs associated with the pharynx is homologous in Enteropneusta and Cephalochordata remains speculative until more is known about the excretory structures in the branchial sacs of enteropneusts (see above). The last common ancestor of the Notochordata possessed segmental excretory organs derived from the mesoderm. These excretory organs were situated ventral to segmental muscular compartments. The cyrtopodocyte itself, although with rod-like microvilli that resemble protonephridia, is an autapomorphy of the cephalochordates. The similarities to the pronephros of craniates (e.g., in regard to its germ layer affiliation) suggests that the nephridial structure in *Branchiostoma* is a secondarily modified metanephridium (see also Stach & Eisler 1998).

"Muscle tails"

Schneider (1879) and Flood (1966, 1968) found that the muscle cells of adult cephalochordates were not innervated by spinal nerves but by "muscle tails". It was shown in this present study that the close association of the muscle cells and the neural tube, which forms the basis for the pattern of innervation of the somatic musculature in cephalochordates, is formed very early during the ontogeny of the animals. The mesodermal myoblasts are from the beginning of their formation

in a position close to the dorsal neural tube. Later in development the differentiating myoblasts elongate, leaving a narrow dorsal part of themselves at the neural tube. Comparable muscular innervation via extensions of the muscle cells was described for nematodes (Debell 1965) and echinoderms (Cobb & Laverack 1967). The phylogenetic positions of these animals indicate that this similarity is not based on common ancestry.

In the last common ancestor of the craniates a different mode of innervation of the muscle cells was present. The somatic musculature of the trunk is innervated by the ventral roots of the spinal nerves. Tannenbaum & Rosenbluth (1972) demonstrated that in a larval ascidian (*Amaroucium constellatum*, Polyclinidae, Enterogona) the innervation of the tail muscle cells is comparable to that of the muscle cells of cephalochordates. The lack of detailed studies of tunicate anatomy makes it difficult to estimate if this condition existed in the last common ancestor of the Tunicata.

Therefore, whether the situation found in cephalochordates is an autapomorphy of this taxon or a plesiomorphic condition present in the grundplan of the Chordata and inherited by the Notochordata can not be resolved at present. It could even support the "Atriozoa-hypothesis" which suggests that the Tunicata + Cephalochordata comprise a monophyletic taxon (see Pietschmann 1962, Ruppert 1997a).

Dermatome, Sclerotome

"Amphioxus somites, unlike those of vertebrates, form no dermatome, and no cells migrate away from the myotome to found muscles elsewhere." (Holland, L.Z. 1996, p. 238). In the present study a cell type different from the developing myocytes from early neurula stages on could be distinguished: the cells of this type lie on the lateral side of each segment, forming the lateral wall (Plates 4-7, 9-17). Early in the ontogeny they are cuboidal. Later they differentiate into very thin sheet-like cells (Fig.14). They resemble cells, which build up coelomic linings elsewhere in the larval body. As they are separated from the myocytes in all stages by a regularly appearing intercellular space, they seem to constitute a coelothel. Their position in the segment on the lateral side of the muscle cells is comparable to the position of the dermatome of craniates (Boyd 1960, Keynes & Stern 1988). As the homology of the muscular part of the segments and the myotome of craniates seems to be well established (Maisey 1986, Ruppert 1997b), it is reasonable to hypothesize the homology between the lateral coelothel of cephalochordates and the dermatome of craniates. In contrast to the dermatome cells in craniates, the coelothel cells do not undergo an epithelial-mesenchymal transition. But they may be involved in the formation of the more elaborate connective tissue between coelothel and epidermis in the adult. It would also be of interest to know whether the fibroblasts reported by Welsch (1968) are in a generic correlation to the coelothelial cells or their precursors.

The myoblasts and myocytes fill the central and the medial part of the mesodermal segments. On the medial side next to the notochord no other cell type can be found. In craniates a medial part of the early ontogenetic somite becomes mesenchymal. Its cells migrate around the Notochord and build up skeletal material; these cells constitute the sclerotome. It is lacking entirely in the developing cephalochordates. This corresponds to the lack of hard skeletal material.

Functional aspects

In adult cephalochordates there are two clearly different types of muscle lamellae present (Flood 1968, 1977, Grocki 1982, Ruppert 1997). A third intermediate type may exist but it could also represent a developmental stage of the deep lamellae (Flood 1968). Indirect structural evidence, and comparison with various fish groups, led to the hypothesis that this dual fibre pattern is of similar function as the dual muscle cell types in craniates (Flood 1968, Bone 1989). The superficial lamellae are richer in mitochondria and glycogen content as well as in the amount of sarcoplasmatic vesicles. Compared with the deep lamellae they constitute a small part of the segmental trunk muscle cells. Only about 1% of the myocytes are of the superficial type (Bone 1989). The superficial lamellae may be comparable to the slow muscle fibres in craniates whereas the deep lamellae may represent the fast type (Flood 1968). In the early larval stage examined in this study, only myocytes similar to the deep lamellae of the adults are present. It is important in this context to consider that the principal way of locomotion in these stages is not by muscular body undulations. Most of the time neurulae and larvae hover in the water column by their pronounced body ciliation (Bone 1958, Stokes and Holland 1995a). Nevertheless they are capable of rapid wriggling movements very early when adverse stimuli are encountered. It is possible that the dual fibre pattern is correlated with the burrowing adult hemi-sessile mode of life, which is totally dependent on muscular propulsion.

Post pharyngeal intestine

In larval amphioxus the post pharyngeal intestine consists of a straight tube made up of monociliated cells, which is derived from the endodermal archenteron. During early development the rich intracellular yolk resources are depleted and the cells acquire numerous microvilli apically. In living specimens the rotation of food particles (e. g. *Dunaliella*) entangled in a mucous thread can be observed. Together with a narrowing of the intestinal tube, this indicates the presence of the iliocolonic ring. However, a differentiation into oesophagus, stomach, iliocolonic ring, and hindgut as easily visible in adults (Ruppert 1997b) could not be detected. More absent features are the caecum and the sphincter muscle around the anus. Nevertheless, the intestine is clearly functional in food transport, uptake, and processing.

As in cephalochordates, the food is transported by ciliated cells in all regions of the post-pharyngeal tract of adult ascidians (Burighel & Cloney 1997). The same holds true for the adult appendicularian (personal observation); and both tunicate taxa possess a curved U-shaped alimentary canal. The presence of a straight, ciliated, post-pharyngeal tract (although with the rudiment of a spiral fold) in the larval ammocoete (Fontaine 1958) leads to the conclusion that a comparable situation was present at least in the development of the last common ancestor of the Notochordata.

Tail fin

A "distinct somatic tail region extending behind (the) visceral cavity, with lateral muscle bands derived from caudal mesoderm" is best interpreted as a synapomorphy of the three major chordate taxa ascidians, cephalochordates, and craniates (Maisey 1986, p. 203). Swalla & Jeffery (1996) demonstrated that this anatomical complex might be easily lost in larval tunicates during evolution through loss or down regulation of a single zinc finger *Manx* gene. The fin rays of a larval amphioxus are easily visible with a light microscope (Tenbaum 1955; personal observation). Flood (1975) demonstrated that these rays are most probably intracellular rootlet fibres of the epithelial epidermis. He concluded from the ultrastructural appearance and the birefringency of the fin rays that they consist of a protein, which was probably not collagen. In contrast, the fin rays of craniates develop in the mesodermal mesenchyme and consist mainly of collagen or elastoidin (Starck 1979). Burighel & Cloney (1997, p. 302-303) demonstrated that the tailfin of certain ascidian larvae is supported by "birefringent bundles of filaments (...) in the outer compartment of the tunic". As an ultrastructural analysis of these filaments is lacking, it is not clear whether they consist of rootlet fibres. Thus, at present, it may be concluded that birefringent fin rays built by epidermal cells have been present in developmental stages of the last common ancestor of the Chordata.

ABSTRACT

Developmental stages of *Branchiostoma lanceolatum* (subphylum: Cephalochordata) were studied from complete serial sections in a combined light microscopic / transmission electron microscopic technique for the first time. Developmental stages examined included early neurula stages, with nine mesodermal segments developed (22 hours post fertilization at 18°C) until early larval stages, with the mouth opening, first primary gill slit, and the anus developed (110 hours post fertilization at 18°C). As the Cephalochordata are probably the sistergroup of the Craniata, knowledge about the anatomy of the lancelets is of utmost importance for the reconstruction of the evolution of the Chordata. The combined light microscopic / transmission electron microscopic - approach allowed three-

dimensional reconstruction of the entire animals in great detail. The frequent and regular transmission electron micrographs made it possible to detect early primordia of organs and draw conclusions regarding their probable functions. These anatomical and functional results were discussed in an evolutionary context within the theory of phylogenetic systematics.

Special attention was paid to the development of the mesoderm and several interesting findings have been reported. Three spacious coelomic cavities were described besides the myomeric, narrow myocoels (see Fig.15). These are 1) the rostral coelom, 2) the ventral coelom, and 3) the space around Hatschek's nephridium. The hypothesis that the spacious rostral coelom develops by fusion of the right diverticulum of Hatschek with adjacent myocoelic cavities is presented. The ventral coelom is connected with the rostral coelom and has probably a role in substance distribution when the circulatory system is rudimentary. A comparison with coelomic cavities of other deuterostome taxa was accomplished in the discussion. The derivation of the first excretory organ during development, Hatschek's nephridium, from the mesoderm was demonstrated. It was concluded that the nephridia in cephalochordates are homologous to the pronephros of craniates. The first blood vessel that could be recognized in transmission electron microscopic aspect was the left anterior aorta, which was correlated with Hatschek's nephridium. The nematode-like innervation of the trunk muscles is established very early during development and may be a plesiomorphic trait in cephalochordates, already present in the last common ancestor of the Chordata. Only deep lamellae, which had been compared to fast fibres in chordates (Flood 1968), were encountered in larvae. It was speculated that the superficial fibres originate (and may thus be functionally linked) with the post metamorphic burrowing behavior. Because of similar position and structural difference from the medial myocytes the lateral coelothelic cells were tentatively homologized with the craniate dermatome cells. No homologous structure to the craniate sclerotome could be detected.

Differences to earlier descriptions based on light microscopical examination of the ontogeny of *B. lanceolatum* were minor and mainly due to the improved microscopic techniques. The notochord was found to grow out of the archenteron rather than be folded off. The ventral mesoderm was seen to grow around the archenteron very early and in single sheet. Other organ systems, which were covered by the transmission electron microscopy-based descriptions and the discussion, were: the preoral pit, the endostyle, and the club shaped gland. To a lesser extend this was accomplished for the epidermis, the oral papilla, the central nervous system, and the tail fin.

Despite remarkable differences between cephalochordate and craniate structures, no evidence to challenge the suggested monophyly of the Notochordata (sensu Nielsen 1995) was found.

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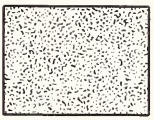
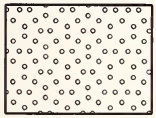
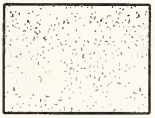
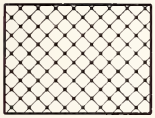
Explanation of plates


epidermis


endoderm


neural plate/tube

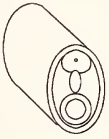

notochord

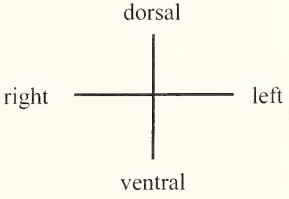

mesoderm

fine lines: cell borders
bold lines: extracellular matrix separating tissues / organs

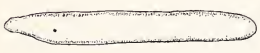
Special features are labeled in the plates

Note: All cross sections are presented in the same orientation:





The small line drawings of the animals at the top of each plate that show the plane of the section are also oriented in the same way:



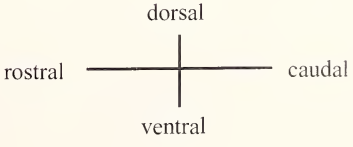


Plate 1
Neurula
22 h pf, 18°C

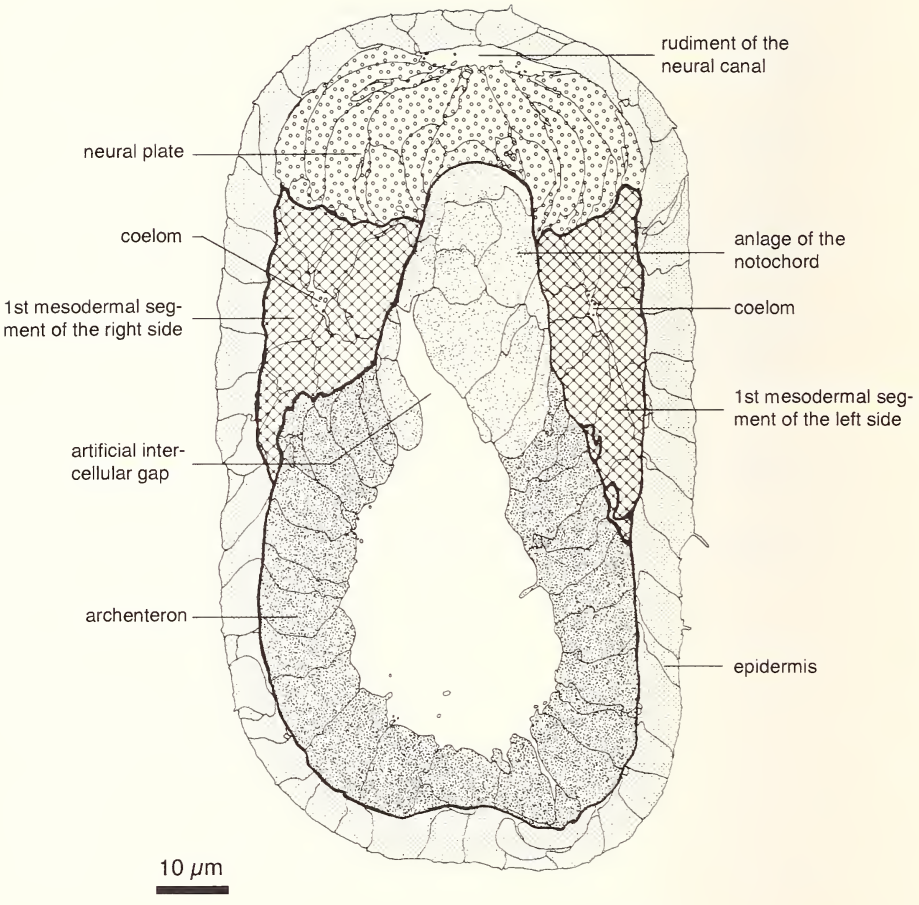


Plate 1: Several chordate features are visible in this cross section of an early neurula stage (22h pf, 18°C). The dorsolateral mesoderm in this stage is epithelially organized. Narrow coelomic spaces can be recognized in both sides of this cross section through the first pair of mesodermal segments. The anlage of the notochord is seen in the process of growing out of the dorsal part of the archenteron and is continuous with the epithelium of the archenteron, i. e. the anlage of the notochord is not separated by an extracellular matrix from the archenteron. The dorsal neural plate is overgrown by the dorsal epidermis and is in continuity with the epidermis (from Stach 1994, modified).

Plate 2
Neurula
22 h pf, 18°C

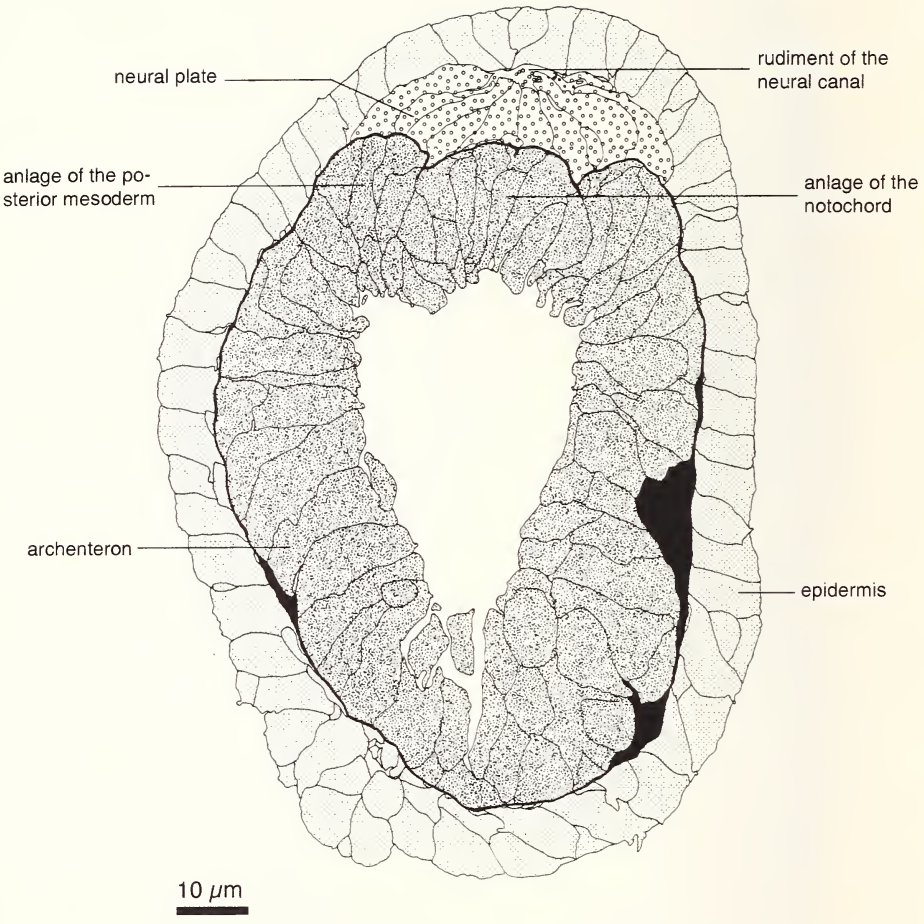


Plate 2: A developmental gradient exists along the antero-posterior axis of all developmental stages examined in this study. The posterior parts are less differentiated than the anterior ones. This is clearly recognizable in this cross section of an early neurula (22h pf. 18°C). In the posterior parts of this stage the anlage of the dorsolateral mesoderm is continuous with the archenteron and forms merely two shallow bulges at the dorsolateral archenteron. Also the anlage of the notochord is only discernible as a shallow bulge at the dorsomedial archenteron (from Stach 1994, modified).

Plate 3
Neurula
32h pf, 18°C

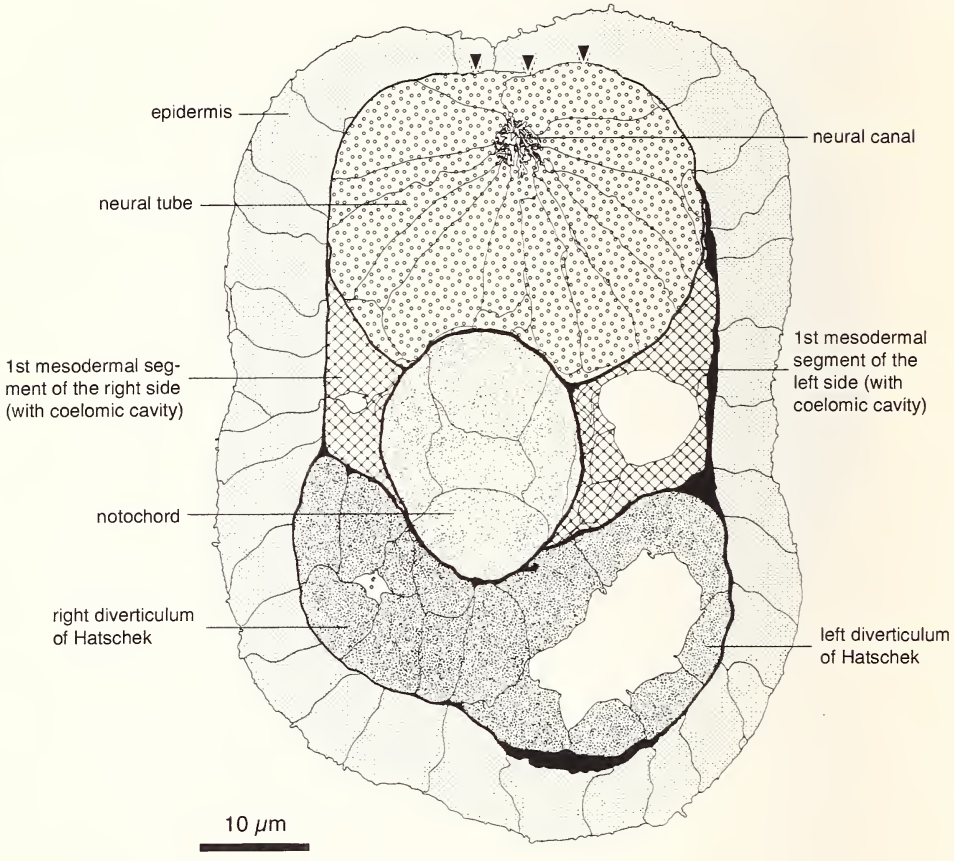


Plate 3: In this cross section of a neurula (32h pf, 18°C) coelomic cavities are clearly recognizeable in the first mesodermal segments of both sides. The archenteron is forked at its anterior tip: both branches, the diverticula of Hatschek, are seen in this cross section. The notochord is entirely separated by an extracellular matrix from the archenteron. The neural plate rolled up to form a neural tube around a neural canal. This neural tube is also almost entirely separated from the adjacent tissue, the epidermis by a layer of extracellular matrix. Only the most dorsal part of the neural tube and the epidermis are still in direct contact (arrowheads).

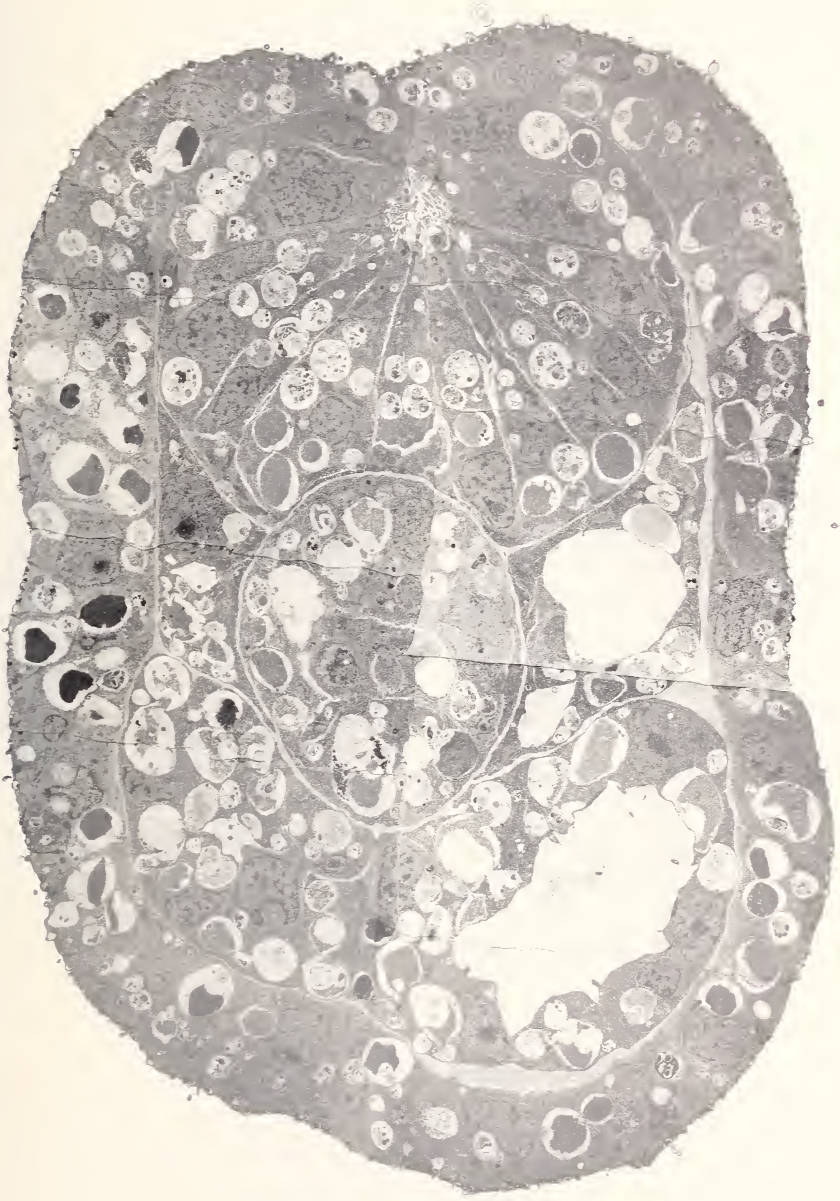


Plate 4
Neurula
32h pf, 18°C

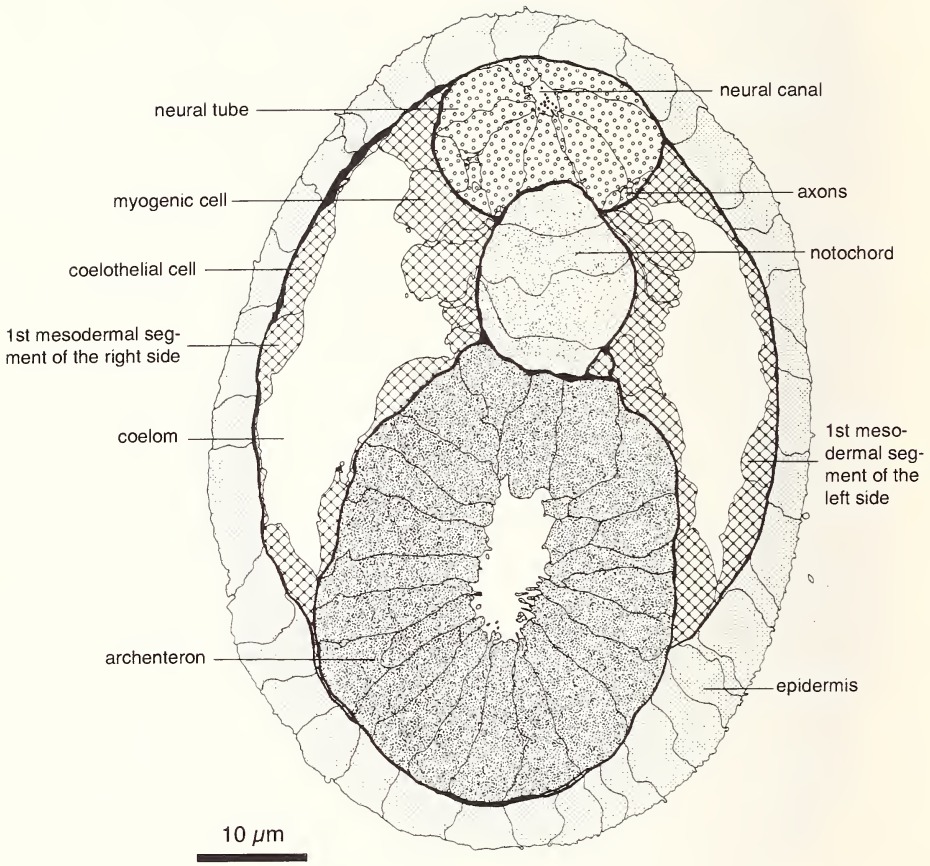


Plate 4: The extend of the coelomic cavities in the first mesodermal segments is seen in this cross section of a neurula stage (32h pf, 18°C). Moreover the tendency of the mesoderm to grow ventrally around the archenteron is visible. Note also the different aspects of the mesodermal cell types. The medial, myogenic cells have a somewhat club shaped appearance in cross sections, with their narrow ends pointing towards the neural tube. The lateral cells display a flatter, epithelial character. As they border the extensive coelomic cavity laterally they are termed coelothelial cells. The ventrolateral area of the neural tube shows cross sections of axons.

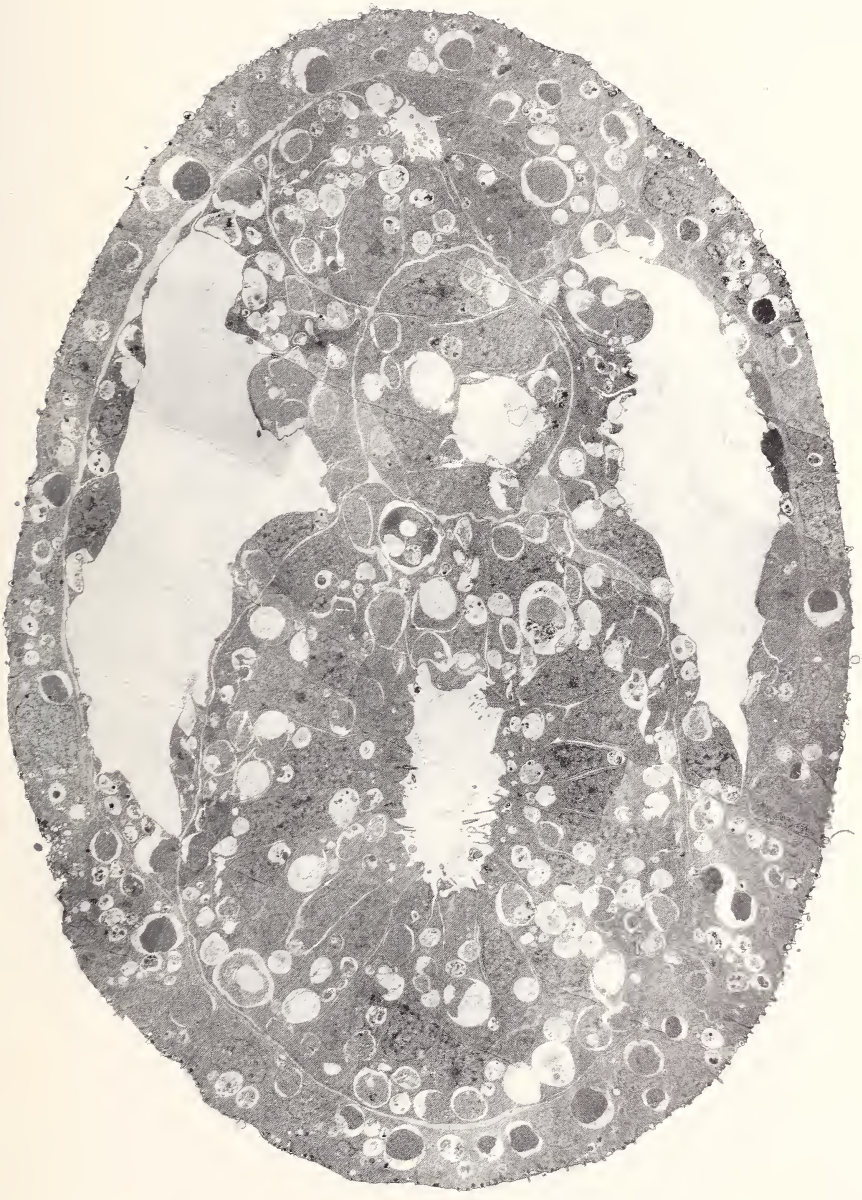


Plate 5
Neurula
32h pf, 18°C

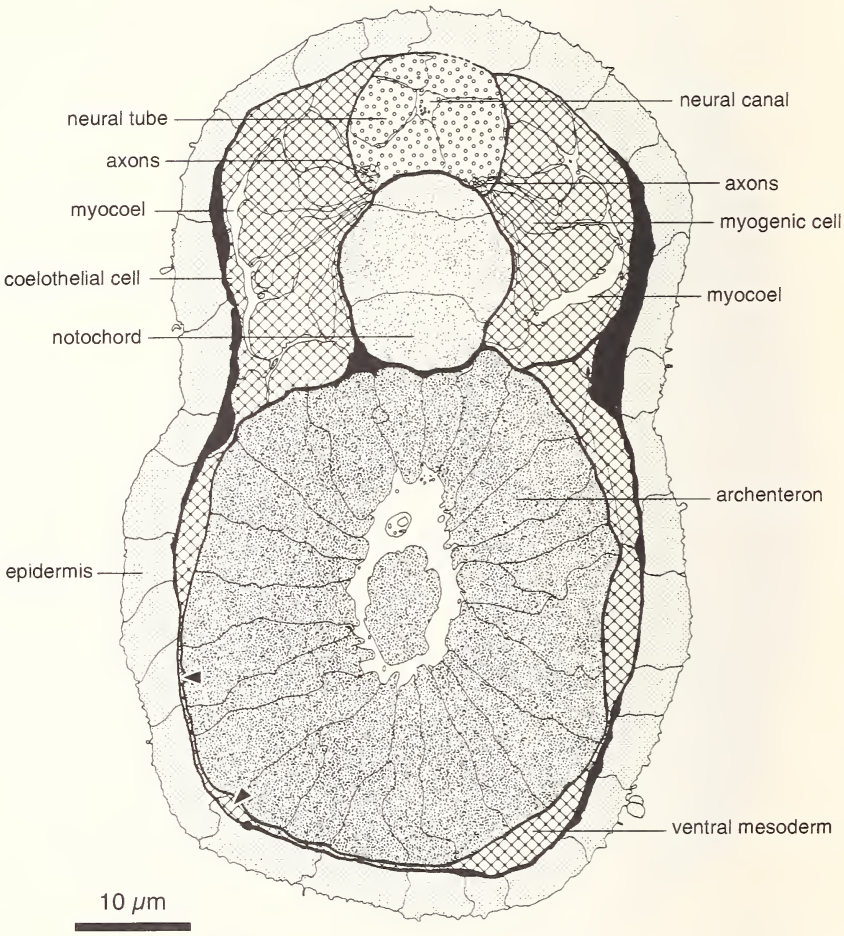


Plate 5: In the dorsolateral mesoderm of this neurula (32h pf, 18°C) two cell types can be observed, medial myogenic cells and lateral coelothelial cell. These are separated from each other by a narrow coelomic cavity, the myocoel. Notice that the mesoderm surrounds the archenteron in a very narrow sheet (arrowheads). The contact between myogenic cells and the putative axons running along the ventrolateral neural tube is well established. A cellular material is seen in the lumen of the archenteron.

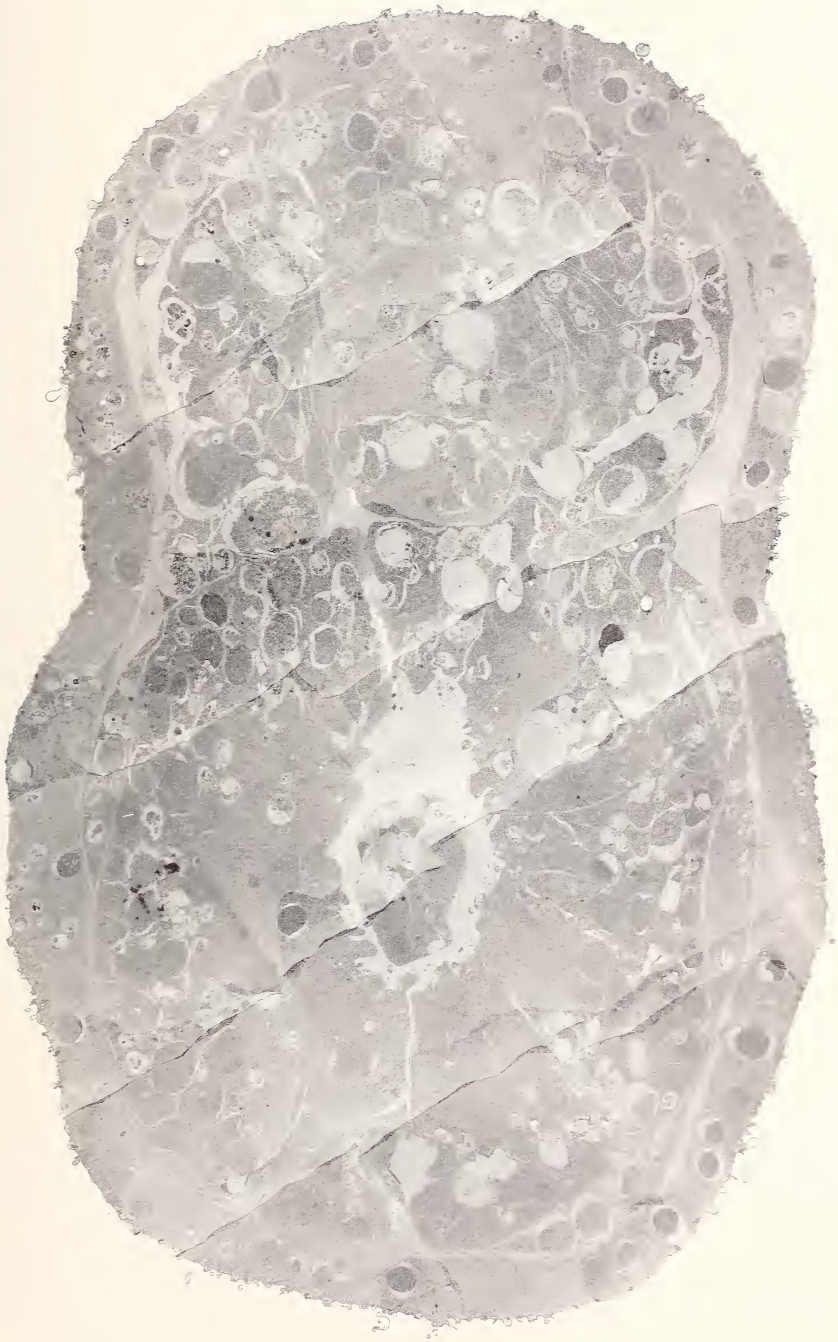
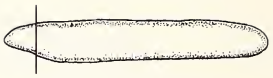


Plate 6
Neurula
35h pf, 18°C



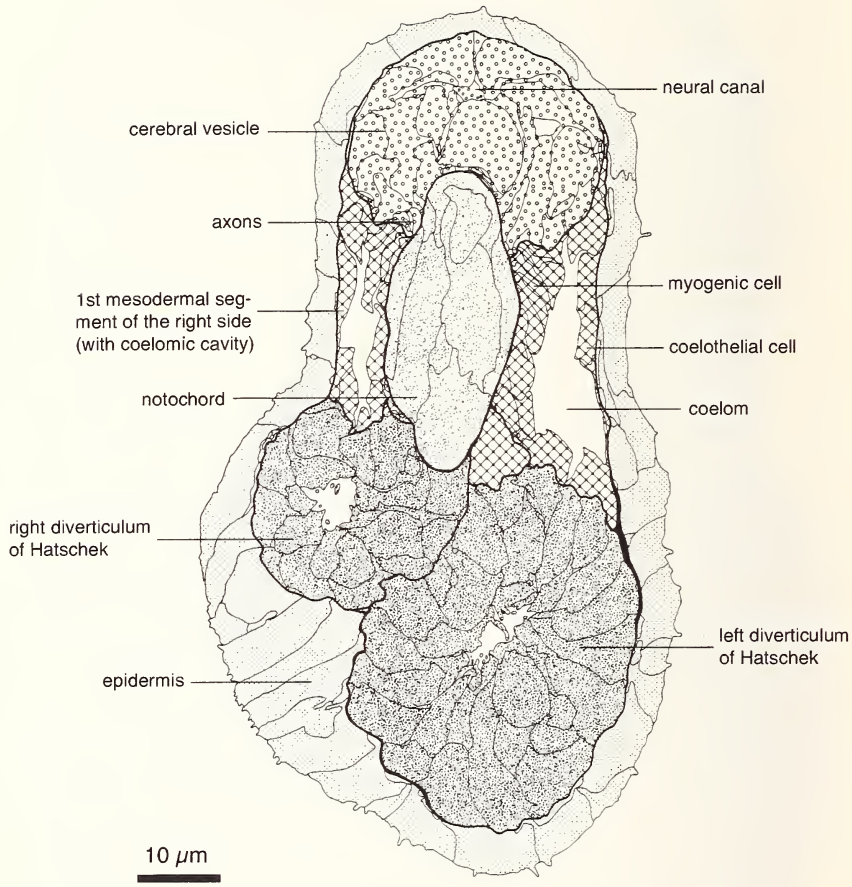
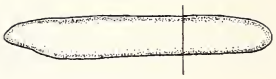


Plate 6: This cross section of a later neurula stage (35h pf, 18°C) reveals that the coelomic cavities of the first mesodermal segment are still comparatively spacious. Medial myogenic and lateral coelothelial mesoderm cells are easily distinguished. The medial cells differentiate into myocytes displaying irregularly arranged myofilaments at this stage already. The two diverticula of Hatschek are seen on this cross section. They are still continuous with the archenteron. Note also the relatively voluminous neural tube forming the cerebral vesicle in this anterior part of the animal (from Stach 1994, modified)

Plate 7
Neurula
35h pf, 18°C



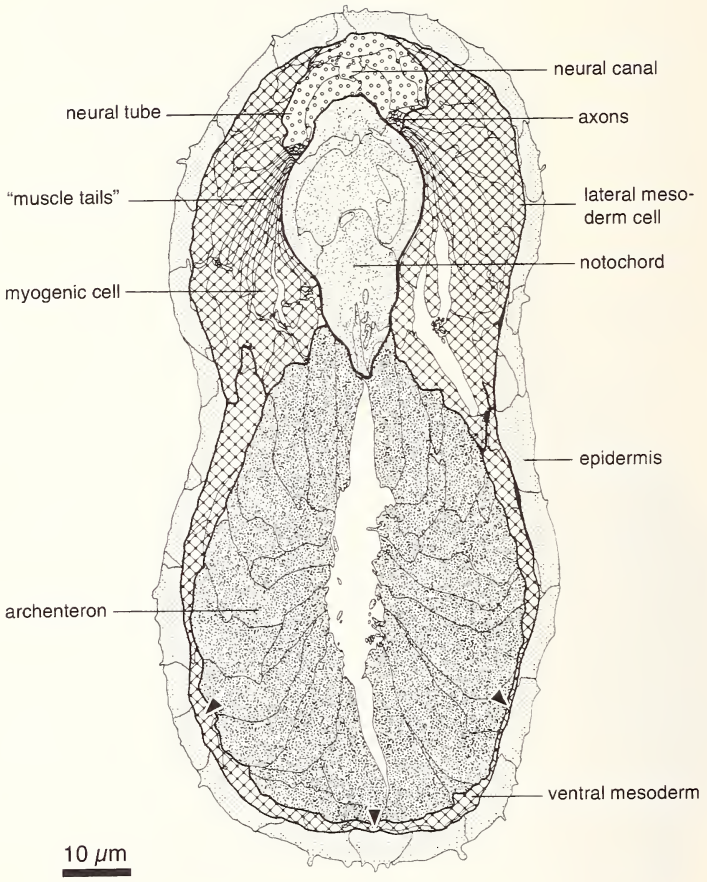


Plate 7: A 'typical' cross section through the trunk region of this late neurula stage (35h pf, 18°C) shows the dorsolateral mesoderm consisting mainly of myogenic cells. These cells possess narrow extensions ("muscle tails") pointing towards the ventrolateral neural tube where axons can be seen. Higher magnification would demonstrate myofilaments in these myogenic medial cells. The ventral mesoderm surrounds the archenteron (arrowheads). (Intercellular spaces seem to be slightly widened artificially in this specimen) (from Stach 1994, modified).

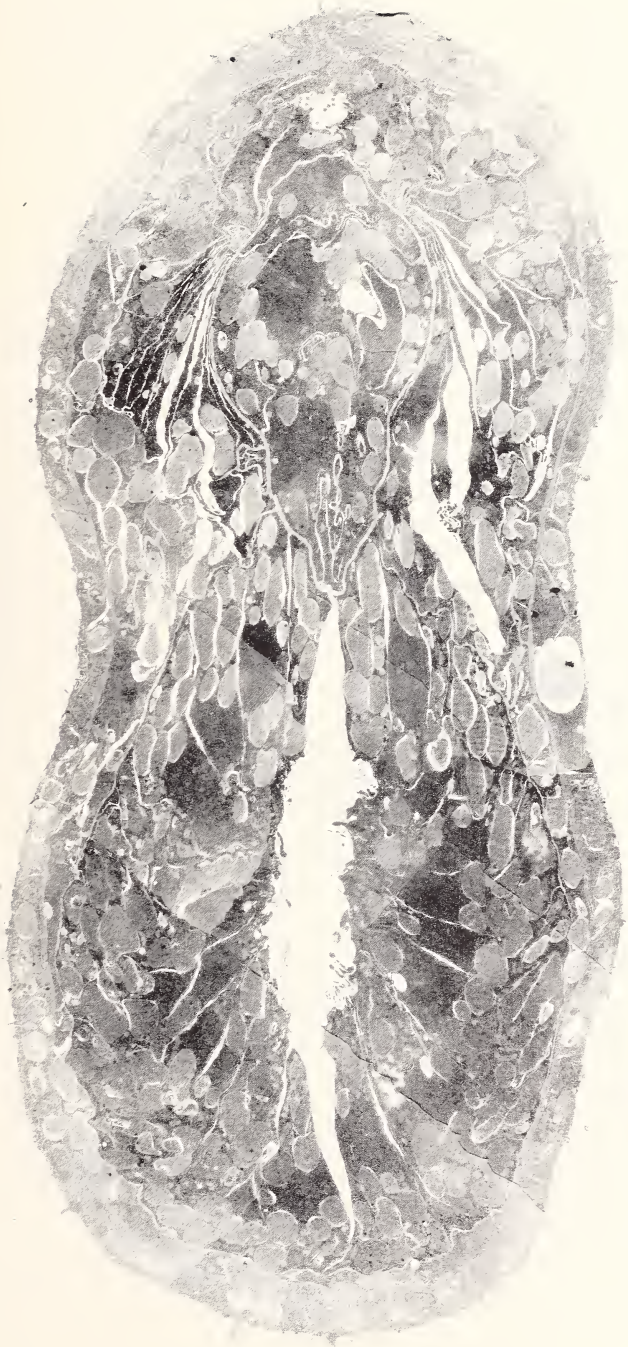
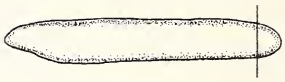


Plate 8
Neurula
35h pf, 18°C



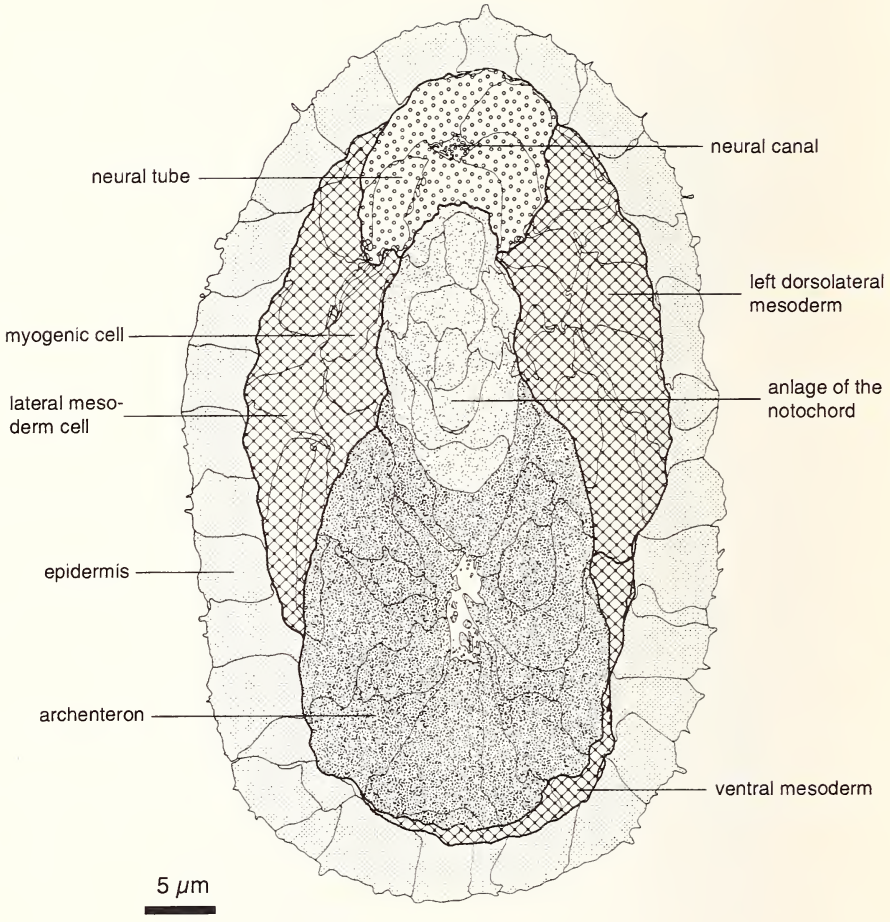


Plate 8: The cross section of the posterior part of this neurula stage (35h pf, 18°C) again reveals a developmental gradient. Especially the cells of the endoderm and its derivatives, the mesoderm and the notochord, are less differentiated compared to the more anterior regions of the animal. Most notably the anlage of the notochord is not separated from the archenteron. In the dorsolateral mesoderm the medial and lateral cells are hardly differentiated (from Stach 1994, modified).

PLATE 9
Larva
42h pf, 18°C

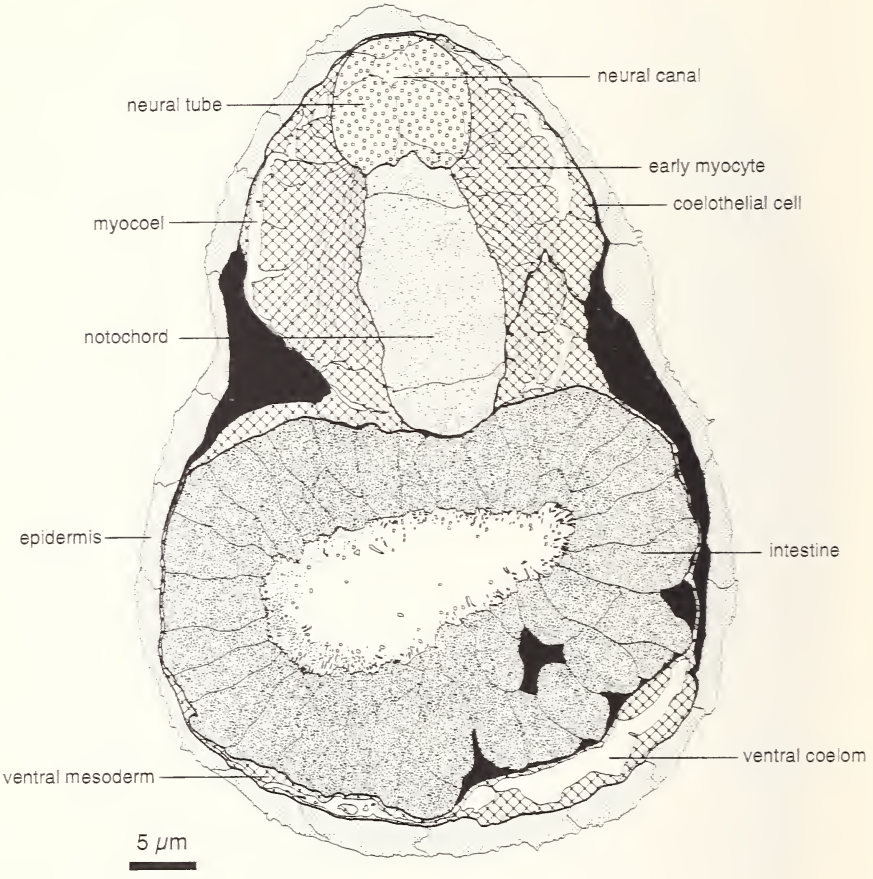


Plate 9: The cross section of the trunk region of this early larval stage (42h pf. 18°C) clearly shows the different aspects of the medial and lateral cells in the dorsolateral mesoderm. A myocoel separates these two cell types on both sides. The ventral mesoderm displays a coelomic slit, the ventral coelom. Notice the extensive intracellular vacuoles of the notochordal cells. The endodermal cells possess numerous irregular microvilli around their single apical cilium. The extracellular matrix (black) of certain areas is enlarged. The significance of this expanded extracellular matrix remains unknown.

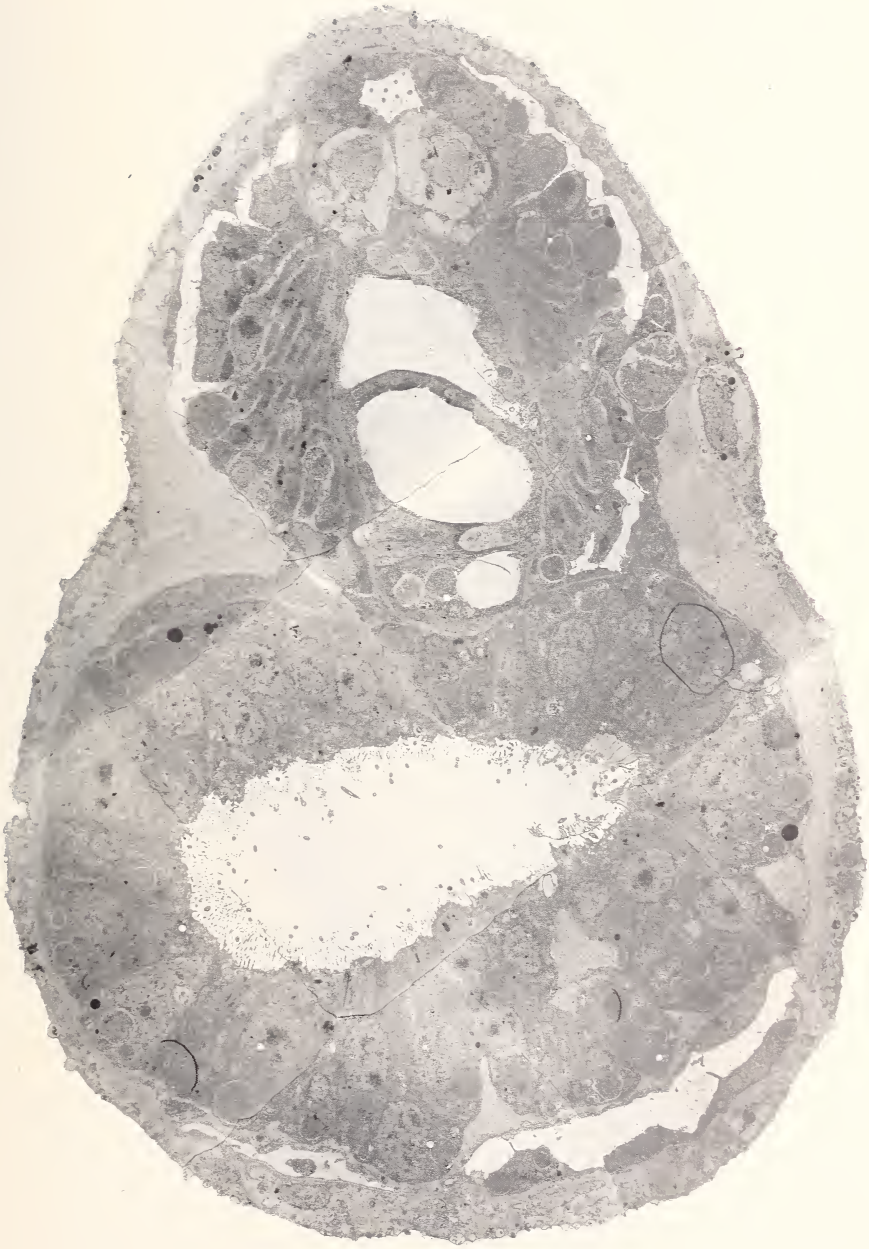


Plate 10
Larva
110h pf, 18°C

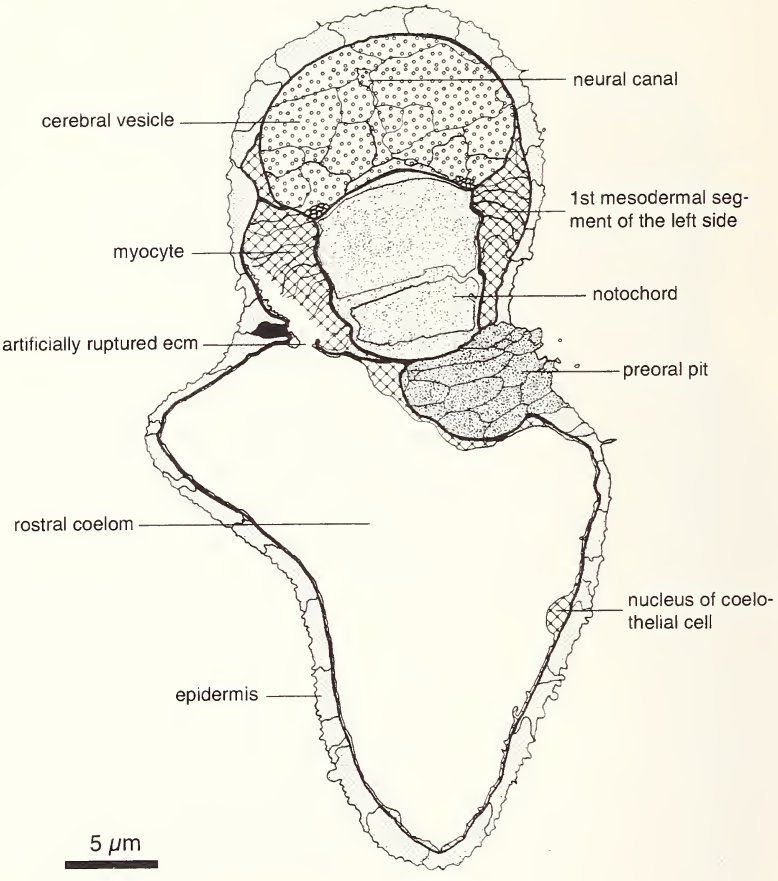


Plate 10: The cross section of this larva (110h pf, 18°C) demonstrates that cell differentiation and organogenesis considerably proceeded. Most dorsolateral mesodermal cells differentiated into fully developed myocytes. The rostral coelom, derived from the right diverticulum of Hatschek, is very spacious and contains some coagulated material. Notice the preoral pit, which developed from the left diverticulum of Hatschek. The notochordal cells possess a huge intracellular vacuole and contractile filaments. The cells of the cerebral vesicle show irregular patterns of intracellular vacuoles. ecm - extracellular matrix.

Plate 11: This series of line drawings of cross sections of a larval stage (110h pf, 18°C) demonstrates:

– the extension of the preoral pit (A)
– the position of the oral papilla in front of the mouth opening (A)
– the blind ending intestine rostral to the mouth opening (A)
– the connection of the rostral coelom with the myocoel of the first myomere of the right side. The dorsomedial border of the rostral coelom is lined by myocytes (B). (Compare also with Plates 13, 15; Fig.12A.)
– the position of Hatschek's nephridium (B + C)
– the different aspects of the endostyle (B + C)
– the different aspects of the club shaped gland (B – D)
– the extension of the mouth opening (C + D)
– the course of the rostral coelom (A – D)

One can follow the course of the entire rostral coelom from Plate 10 to Plate 16. It seems also to be continuous with the midventral ventral coelom; see Plates 13 to 15. The coelomic spaces of this larval stage are represented in a schematic line drawing in Fig.15.

Plate 11
Larva
110h pf, 18°C

A

notochord
preoral pit
rostral coelom
buccal epithelium
oral papilla

B

neural tube
myocytes lining the rostral coelom dorsally
Hatschek's nephridium
rostral coelom
buccal epithelium
endostyle
club shaped gland

C

notochord
endostyle
canal of Hatschek's nephridium
rostral coelom
endostyle
mouth opening
club shaped gland

D

neural tube
dorsolateral mesoderm
club shaped gland
mouth opening
rostral coelom

10 µm

Plate 12
Larva
110h pf, 18°C

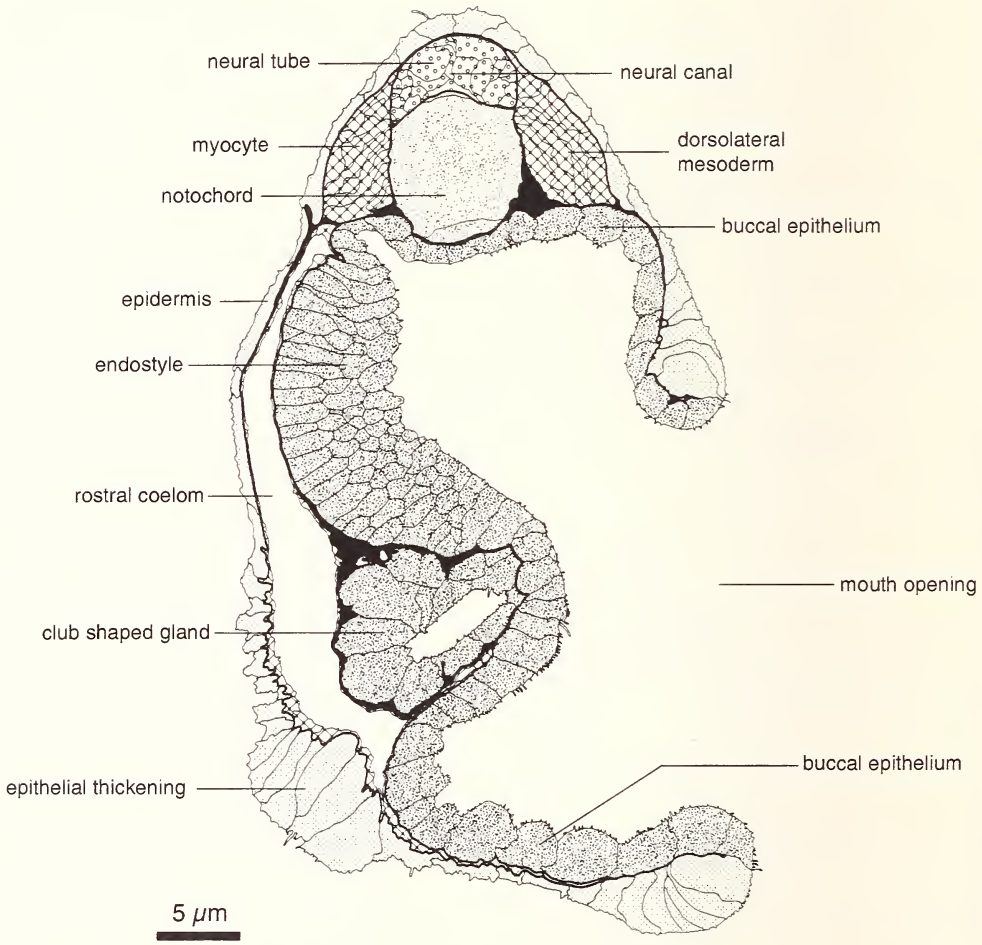


Plate 12: This cross section through the mouth opening of a larva (110h pf, 18°C) demonstrates the relative positions of the posterior part of the endostyle, the medial part of the club shaped gland, and the rostral coelom. Note the conspicuously empty appearing vacuoles of the buccal epithelium. An epidermal thickening of unknown function at the right ventrolateral side is seen.

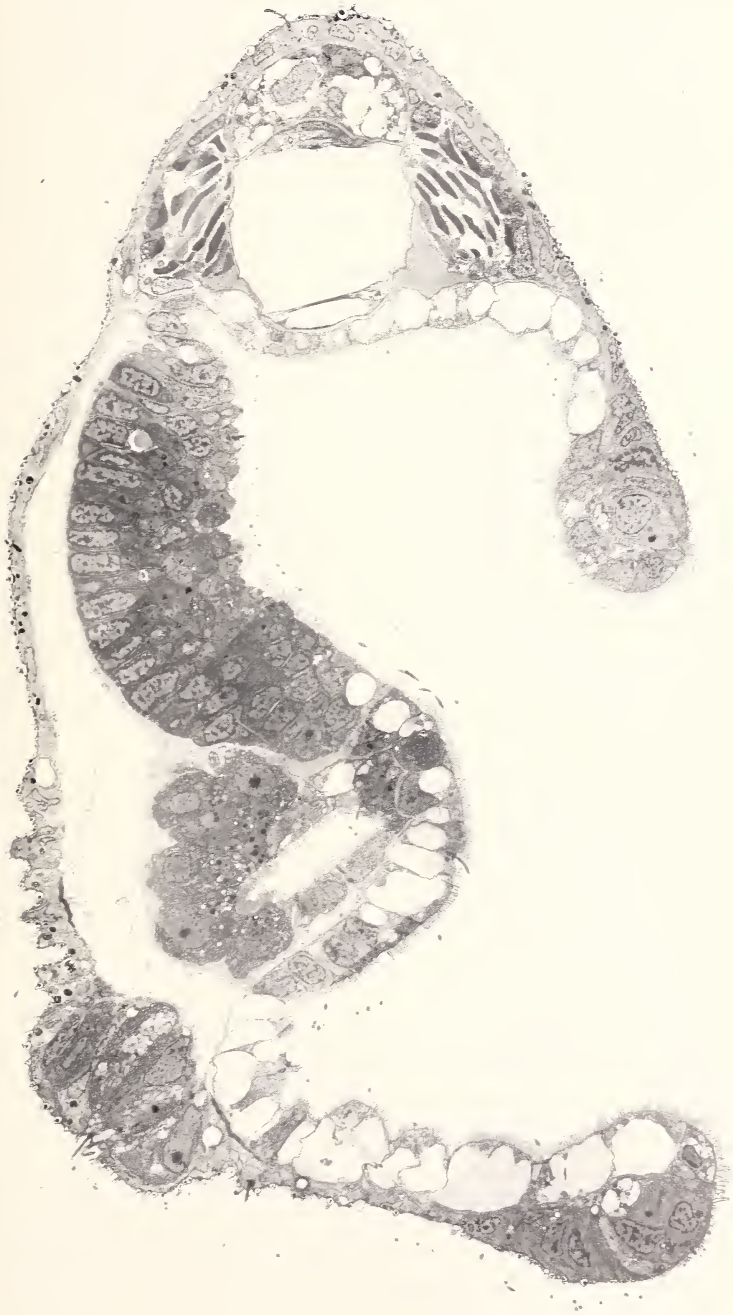
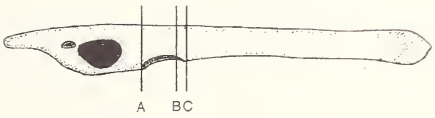


Plate 13: This series of line drawings of cross sections of a larval stage (110h pf, 18°C) demonstrates:

– the posterior end of the club shaped gland (A)
– the position of the first primary gill slit (B)
– a group of myocytes lining the dorsal border of the rostral coelom (B). (Compare with Plates 11B, 15; Fig. 12A.)
– the position of the rostral coelom in relation to this gill slit and the probable connection of the rostral coelom with the ventral coelom (A – C)
– the anterior extension of the ventral coelom (B + C).

Plate 13
Larva
110h pf, 18°C



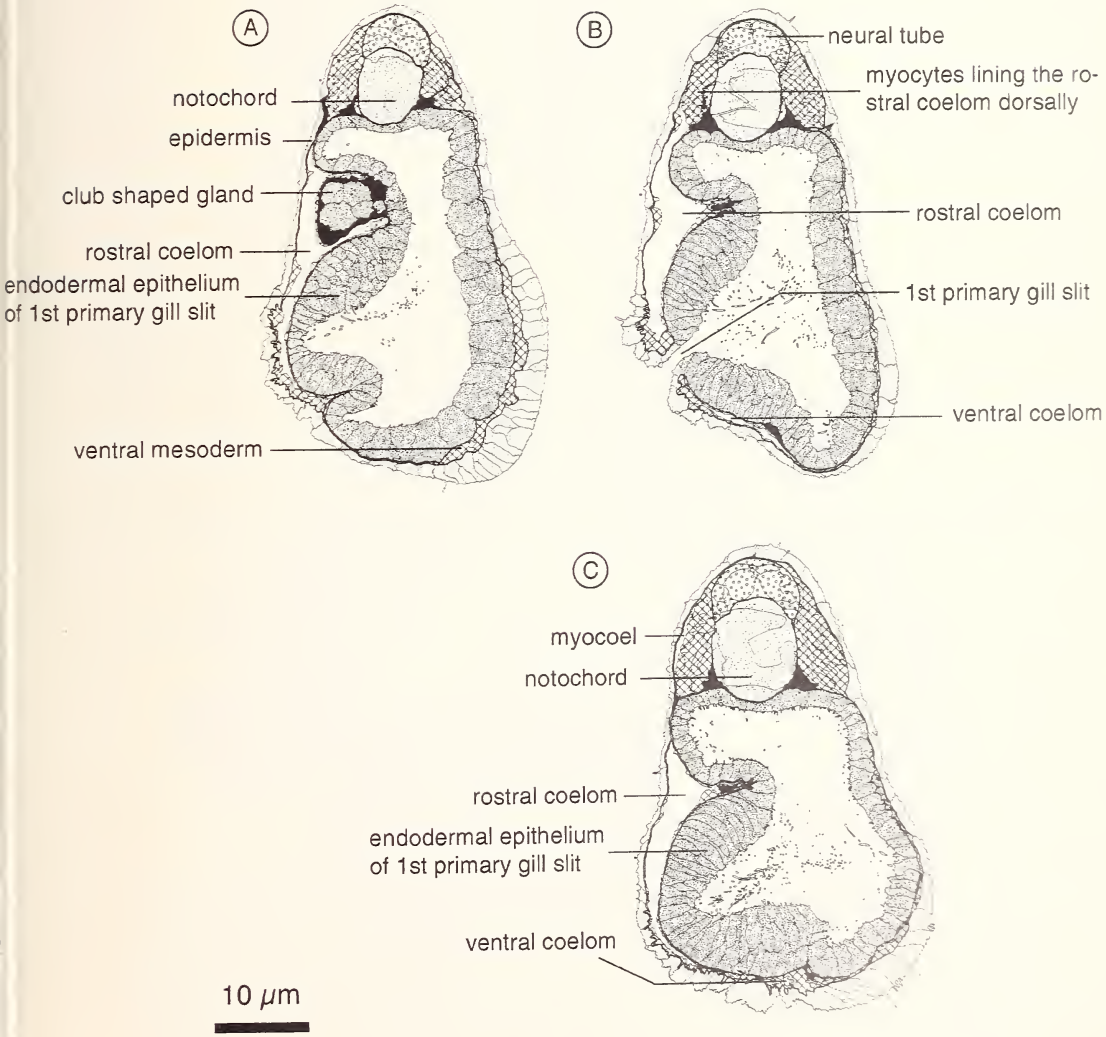


Plate 14
Larva
110h pf, 18°C

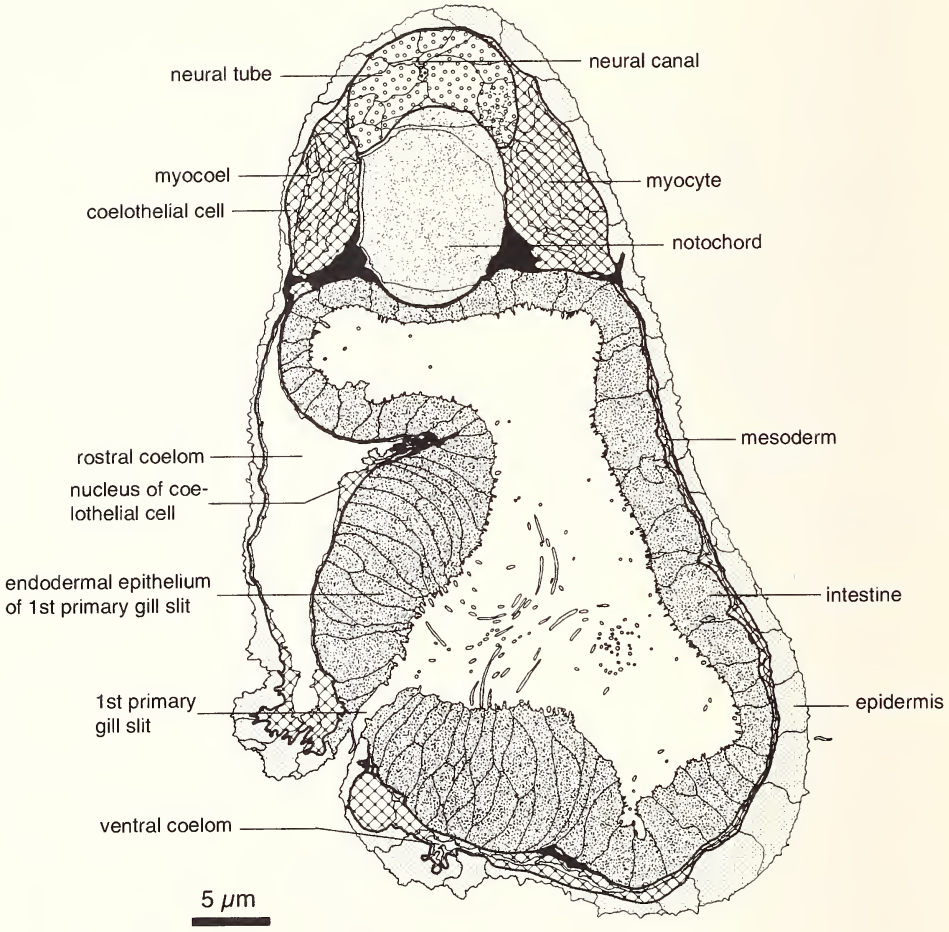


Plate 14: This cross section of a larva (110h pf, 18°C) shows the first primary gill slit and associated structures in more detail. Note the position of the rostral and ventral coelom and the thickened endodermal epithelium surrounding the gill slit. The cilia of this specialized epithelium seem to be considerably longer than the cilia of the intestinal cells. Fig.19B depicts a higher magnification of a similar plane of section, in which the muscles around the gill slit are discernible.

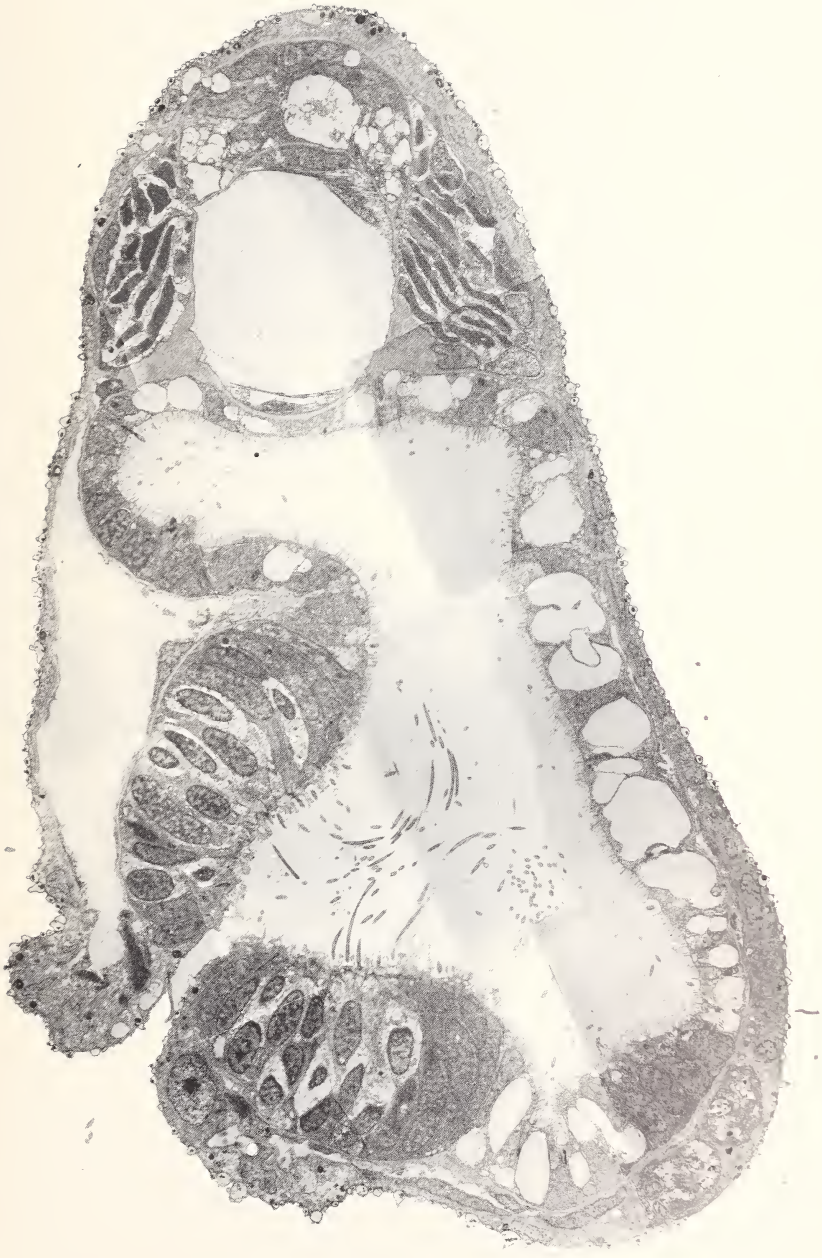


Plate 15
Larva
110h pf, 18°C

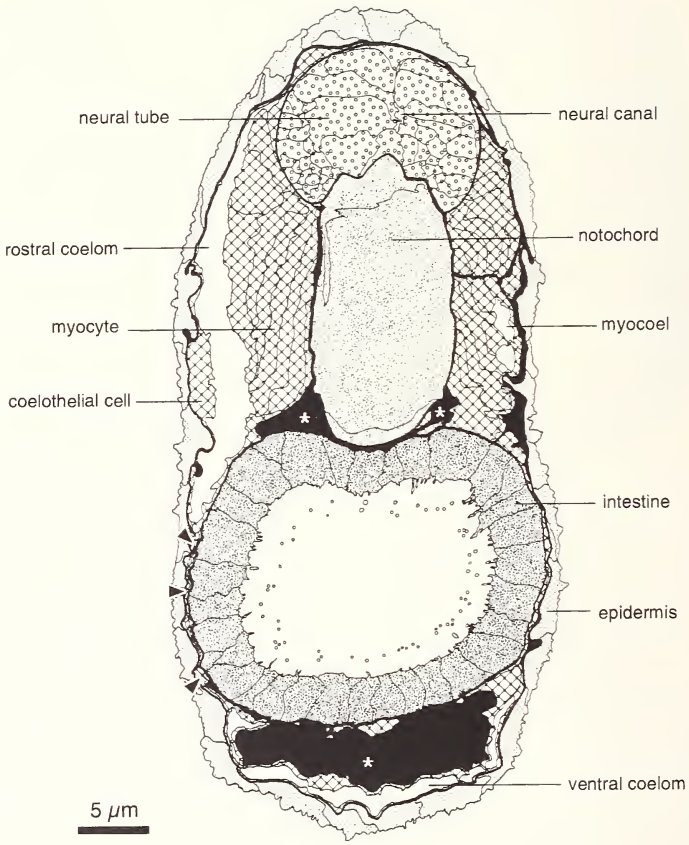
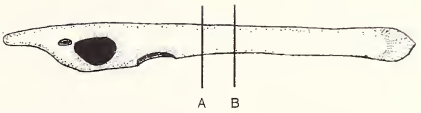


Plate 15: Even posterior to the first primary gill slit the rostral coelom on the right side of the larva (110h pf. 18°C) is spacious in this cross section. Note that the rostral coelom is bordered by myocytes on its dorsomedial border (compare to Plates 11B, 13; Fig.12A). The ventral cells of the rostral coelom seem to be in continuity with the coelomocytes of the ventral coelom. The mesoderm cells of the rostral and ventral coelom do not seem to be separated by an extracellular matrix. The ventral coelom in this region is more spacious compared to the area just behind the gill slit. Enlarged areas of extracellular matrix are conspicuous dorsal of the ventral coelom and at the ventrolateral borders of the notochord (asterisks). These are areas where major blood vessels are situated in adult specimens.

Plate 16: The two line drawings of cross sections through the trunk region of a larval stage (110h pf, 18°C) show the posterior end of the rostral coelom. (A) is easily compared to Plate 15, whereas in (B) the major difference is the disappearance of the rostral coelom. (Asterisks as in Plate 15).

Plate 16
Larva
110h pf, 18°C



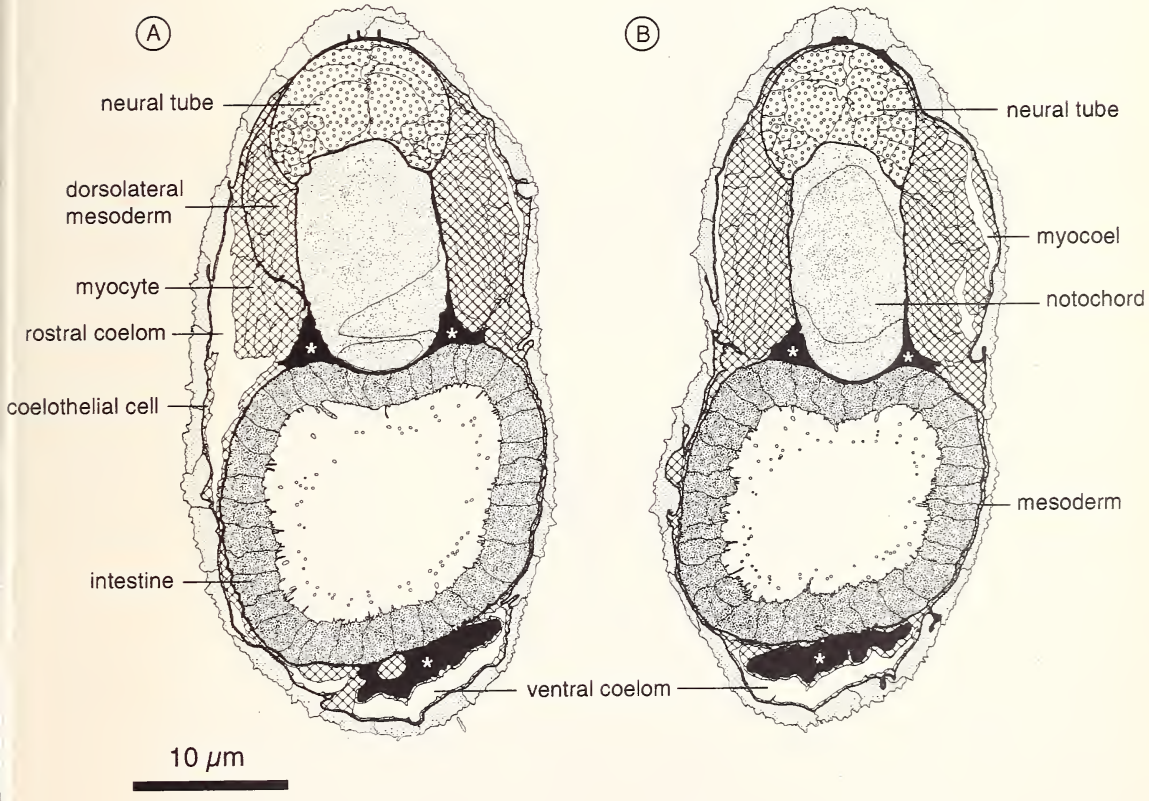


Plate 17
Larva
110h pf, 18°C

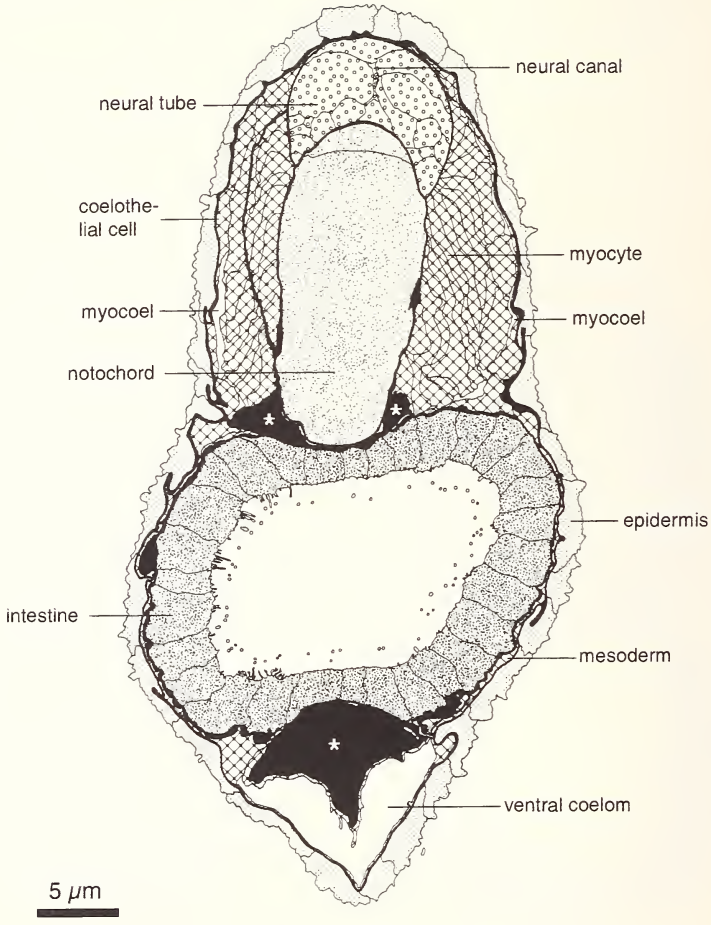


Plate 17: A "typical" cross section through the trunk region of a larval stage (110h pf, 18°C). Two cell types are seen in the dorsolateral mesoderm – medial myocytes and lateral coelothelial cells separated by a myocoel. The ventral mesoderm surrounds the intestine and borders the ventral coelom in the midventral line. Note again the enlarged areas of extracellular matrix dorsal of the ventral coelom and at the ventrolateral borders of the notochord (asterisks). These are areas where major blood vessels are situated in adult specimens.

Plate 18
Larva
110h pf, 18°C

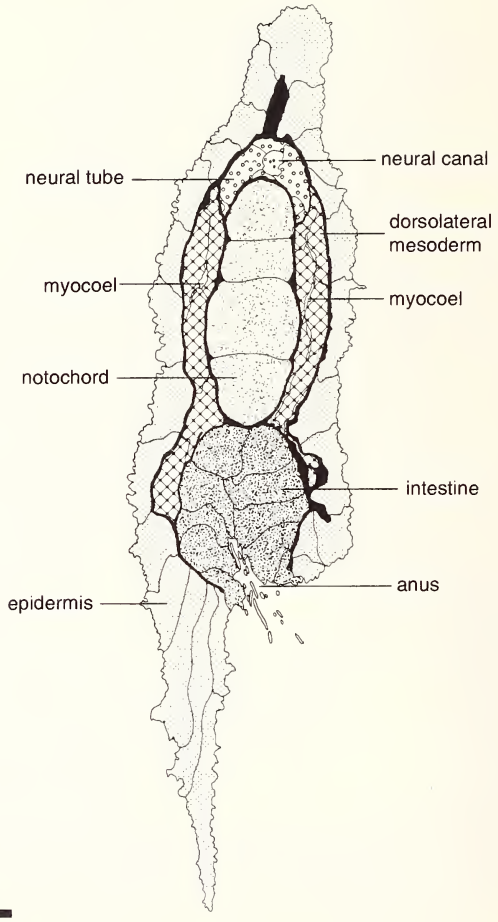


Plate 18: Cross section through the posterior part of a larva (110h pf, 18°C). Notochordal cells are less differentiated compared to anterior body regions. This is seen in the minor extension of the intracellular vacuoles and myofilaments. Moreover some of the notochordal cells still possess yolk granules. The mesoderm cells are not clearly divided into two distinct cell types – medial and lateral cells are nearly identical in regards of their ultrastructure. The anus is seen on the left side of the animal and the beginning of the tail fin just right to it.

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