

Chiton Myogenesis: Perspectives for the Development and Evolution of Larval and Adult Muscle Systems in Molluscs

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ABSTRACT We investigated muscle development in two chiton species, *Mopalia muscosa* and *Chiton olivaceus*, from embryo hatching until 10 days after metamorphosis. The anlagen of the dorsal longitudinal rectus muscle and a larval prototroch muscle ring are the first detectable muscle structures in the early trochophore-like larva. Slightly later, a ventrolaterally situated pair of longitudinal muscles appears, which persists through metamorphosis. In addition, the anlagen of the putative dorsoventral shell musculature and the first fibers of a muscular grid, which is restricted to the pretrochal region and consists of outer ring and inner diagonal muscle fibers, are generated. Subsequently, transversal muscle fibers form underneath each future shell plate and the ventrolateral enrolling muscle is established. At metamorphic competence, the dorsoventral shell musculature consists of numerous serially repeated, intercrossing muscle fibers. Their concentration into seven (and later eight) functional

shell plate muscle bundles starts after the completion of metamorphosis. The larval prototroch ring and the pretrochal muscle grid are lost at metamorphosis. The structure of the apical grid and its atrophy during metamorphosis suggests ontogenetic repetition of (parts of) the original body-wall musculature of a proposed worm-shaped molluscan ancestor. Moreover, our data show that the “segmented” character of the polyplacophoran shell musculature is a secondary condition, thus contradicting earlier theories that regarded the Polyplacophora (and thus the entire phylum Mollusca) as primarily eumetameric (annelid-like). Instead, we propose an unsegmented trochozoan ancestor at the base of molluscan evolution. *J. Morphol.* 251:103–113, 2002.

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Adult polyplacophorans show a complicated system of eight sets of paired dorsoventral shell muscles that correspond to the eight distinct shell plates in the adult animal. In addition, a ventrolaterally positioned circular enrolling muscle, an unpaired dorsal longitudinal “rectus” muscle, the buccal apparatus, and transversal and oblique muscles underneath each shell plate are present (see, e.g., Sampson, 1895; Plate, 1897; Henrici, 1913; Wingstrand, 1985). Despite numerous detailed studies on the anatomy of the adult polyplacophoran musculature, no data on its ontogenetic development exist until today. Several recent articles (Page, 1995, 1997a,b, 1998; Degnan et al., 1997; Wanninger et al., 1999a,b) as well as earlier studies (e.g., Meisenheimer, 1901; Smith, 1935; Crofts, 1937, 1955; Cole, 1938; Anderson, 1965; Smith, 1967; Cragg, 1985; Cragg and Crisp, 1991) showed that specific larval retractor systems do exist in several gastropod and bivalve clades. These data raise the question whether the existence of independent larval retractor(s) may either be (syn)apomorphic for the entire phylum Mollusca, solely for the Conchifera, or evolved independently within the several molluscan taxa. In order to answer this question, knowledge of the polyplacophoran condition is crucial.

The Polyplacophora have retained numerous characters that are considered plesiomorphic for the Mollusca, e.g., a chitinous cuticle with calcareous spicules, lack of jaws, bipectinate ctenidia, and a cord-like tetra-neuran nervous system with a supra-rectal commissure and serial pedal commissures. Therefore, they are phylogenetically regarded as either generally primitive (Scheltema, 1996) or as linking the aplacophoran clades Solenogastres and Caudofoveata to the Conchifera (Monoplacophora, Gastropoda, Cephalopoda, Bivalvia, Scaphopoda) (Boettger, 1955; Salvini-Plawen, 1980; Salvini-Plawen and Steiner, 1996). However, the prominent feature of seriality of shell plates, muscles, and ctenidia has often been and still is used to argue in favor of a primary segmented molluscan ancestor (Götting, 1980; Ghiselin, 1988; Lake, 1990; Nielsen, 1995; but see Russell-Hunter, 1988).

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In order to solve the question of an independent larval musculature and to provide new data for the discussion of the "segmentation problem" in the Mollusca, we analyzed the ontogeny of the shell plate musculature in two chiton species, *Chiton olivaceus* and *Mopalia muscosa*, by means of fluorescence staining of F-actin as well as by scanning and transmission electron microscopy.

MATERIALS AND METHODS

Animal Cultures

Adult specimens of *Chiton olivaceus* Spengler, 1797 were collected on the rocky shore near the STARESO marine station in Calvi/Corsica. Individuals of both sexes spawned during the evening after collection. The eggs were rinsed in seawater and fertilized immediately. Embryos and larvae were kept in glass dishes at 24–27°C.

Breeding of the mossy chiton *Mopalia muscosa* Gould, 1846 was carried out at the Friday Harbor Laboratories, WA, USA. Adult individuals were found near Argyle Creek, San Juan Island, and transported to the laboratory, where some of them immediately released gametes. After insemination the embryos and larvae were maintained in Millipore-filtered seawater (MFSW) in small custard dishes within a temperature range of 10–12°C. To avoid bacterial or fungal infection, 60 mg penicillin and 50 mg streptomycin were added per liter MFSW.

Metamorphosis was induced by adding either small rocks covered with encrusting coralline red algae or stones from which adult specimens had been removed to the culture dishes with metamorphic-competent larvae. Thus, most animals induced at the age of 215 h postfertilization (hpf) or older settled at the bottom of the culture dish within a few hours after the rocks had been added and the first metamorphosed animals were found at 24–48 h after induction (cf. Leise, 1986; Strathmann and Eernisse, 1987). Juveniles were cultured until 10 days after metamorphosis, bearing seven well-developed shell plates but still lacking the eighth plate.

F-Actin Staining

Animals were relaxed by adding drops of 7% MgCl₂ to the MFSW and fixed overnight at 4°C in 4% paraformaldehyde in 0.1 M PBS with 10% sucrose. Late larval and juvenile stages were decalcified in 2% EDTA for 2 h prior to staining. Staining of filamentous F-actin was performed with Oregon Green 488 phalloidin (Molecular Probes, Eugene, OR) and followed the detailed description of Wanninger et al. (1999a). Analyses were done using confocal laser scanning microscopy (CLSM) on a Leica DM IRBE microscope with Leica TCS NT software.

Scanning and Transmission Electron Microscopy

Relaxation (see above), fixations, and all further preparations and analyses exactly followed the procedures described by Wanninger et al. (1999a).

RESULTS

General Remarks

Myogenesis followed the same chronological patterns in *Chiton olivaceus* and *Mopalia muscosa*. However, due to lower rearing temperatures, the timing of development was more synchronous and could be followed more easily in *Mopalia muscosa*. Thus, the data presented herein were obtained from *Mopalia* cultures under the conditions mentioned above, if not stated otherwise.

Please note that herein the term "trochophore" is used in the broad sense as proposed by Rouse (1999), which characterizes all spiralian larval types that bear a prototroch and thus defines the taxon Trochozoa.

Myogenesis

In *Mopalia muscosa*, hatching of the embryos starts at around 21 hpf at 10–12°C. The first myocytes are formed at 74 hpf (Figs. 1A, 2A). Dorsally, myogenesis starts with the anlagen of the prototroch muscle ring and the first two myocytes of the putative rectus muscle, which arise along the median body axis underneath the prototroch and ventrally cross the prototroch muscle ring (Fig. 2A, left). A yet delicate, paired longitudinal muscle appears ventrolaterally on both sides of the larva and starts to extend posttrochally (Fig. 2A, right). Relative to the rectus muscle, the myocytes of the prototroch ring are situated more dorsally. During subsequent development, the fibers of the prototroch muscle ring and the ventrolateral longitudinal muscles gain strength and the two myofibrils of the rectus muscle grow both towards the anterior and the posterior pole of the larva. Ventrally, the anlage of the dorso-ventral musculature becomes visible and the fibers of the ventrolateral longitudinal muscle pair start to expand into the pretrochal region. At this stage, the first ring muscles of the pretrochal muscle grid become visible on the dorsal and ventral side (Fig. 2B).

Fig. 1. SEM of the larval development of *Mopalia muscosa*. **A:** Early trochophore-like larva at the beginning of myogenesis with well-defined prototroch (pt) and apical tuft (at), lateral view. Age: 74.25 hpf. **B:** Late trochophore, dorsolateral view. Note the pretrochally extending anlage of the first shell plate (I) and the posttrochally transversal dorsal depressions of the subsequent shell fields (arrowheads). The foot (ft) and mantle fold (mf) start to form. Age: 142 hpf. **C:** Late trochophore during metamorphosis, lateral view. Note the partially shed prototroch (pt). Age: 240 hpf. **D:** Early juvenile, approximately 2 days after metamorphosis with seven well-developed shell plates (I–VII), dorsal view.

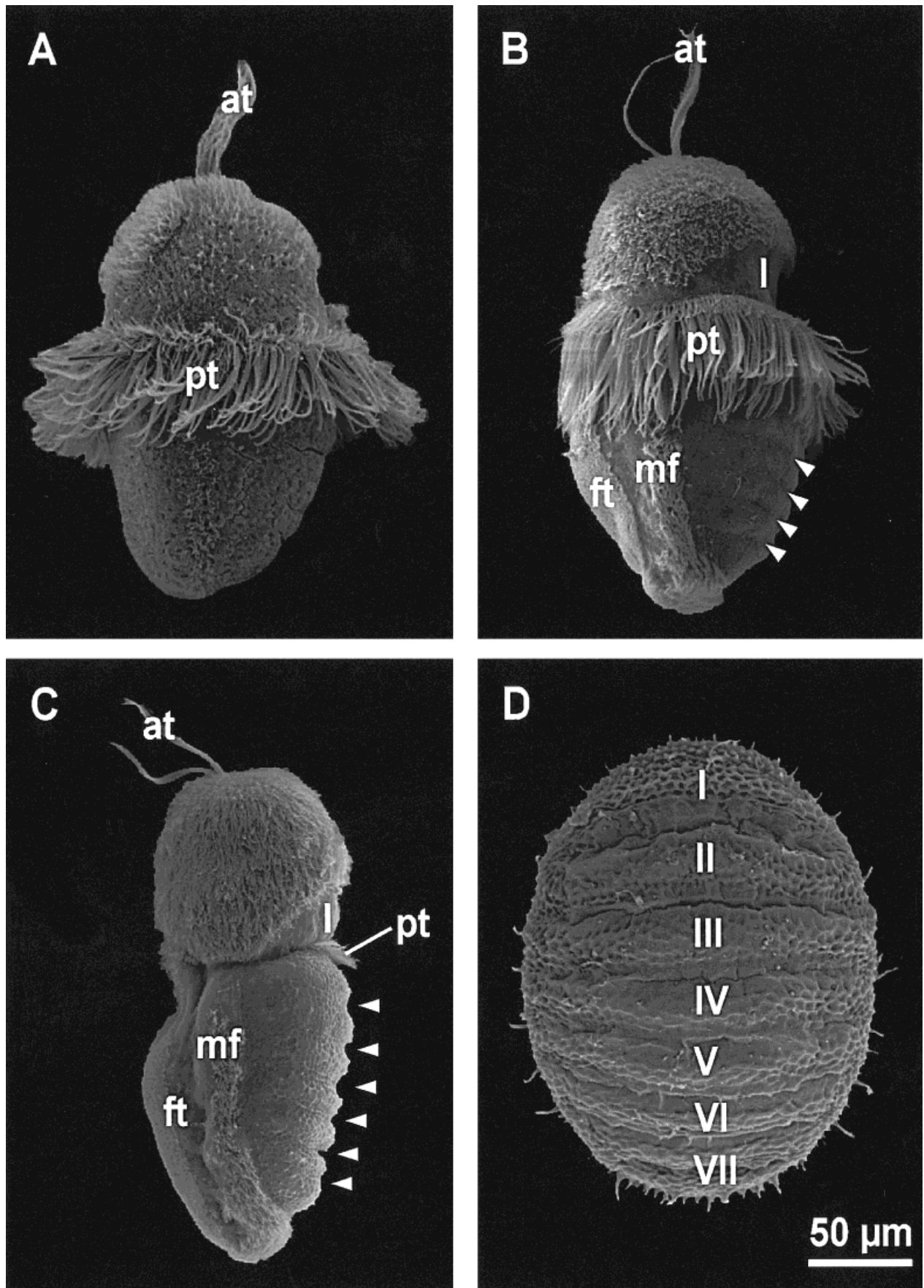


Figure 1

These muscle systems grow subsequently. New myocytes of the rectus muscle are formed laterally on both sides, resulting in a bilaterally symmetrical, prominent muscular system. However, these newly formed fibrils strongly diverge towards the anterior pole of the larva with only the two earliest formed fibers marking a strict anterior–posterior axis through the animal by running parallel to each other along the middle of the larval body. In addition, ring muscles extend throughout the whole pretrochal region and form a muscular meshwork around the fibers of the rectus muscle (Figs. 2C,D, 3A,B, 4A). This “apical grid” is engulfed laterally by a still weak, circular muscle that later becomes the ventral enrolling muscle. In the posttrochal body region, transversal muscle fibers are formed that are situated immediately underneath the epithelium of each putative shell plate, i.e., dorsal of the fibers of the rectus muscle (Figs. 2C,D, 3A,B, 4B).

As larval development proceeds, proportions of the larval body plan change, resulting in an elongated posttrochal area relative to the pretrochal region at metamorphic competence (cf. Figs. 1A–C, 2, 3A,B). The anlagen of the putative first seven shell plates are already present in the late trochophore larva. In both species, *Mopalia muscosa* (Fig. 1B,C) and *Chiton olivaceus* (not shown), the anlage of the first plate (head valve) extends into the pretrochal region.

At around 129 hpf, the various muscle systems have reached an intermediate stage of differentiation: the rectus muscle forms a predominant, dorsal, longitudinal unit and extends anterolaterally, while the apical grid surrounds the pretrochal body part as a three-dimensional muscular net, consisting of distinct outer ring and inner diagonal muscle fibers. This network encircles the fibers of the rectus muscle and some of them bifurcate at their anterior end. The prototroch ring is a solid band of muscle fibers located directly underneath the prototroch. In addition, a layer of posttrochal transversal myofibrils is found under each of the seven shell plates, which have already started to calcify. Laterally, the enrolling muscle encircles all other muscle systems, forming a border against the outer mantle. The ventrolateral longitudinal muscle pair lies more ventral and medial to the latter muscle and consists of two distinct muscle strands that do not form anterior contact. This ventrolateral longitudinal muscle interconnects on both sides with the dorsoventral musculature via numerous short muscle fibers (Figs. 2D, 3A). The dorsoventral musculature appears as a multiple repetition of minute myofibrils that intercross in the pedal region (Figs. 2D, 3A,B).

During subsequent larval (i.e., premetamorphic) development from approximately 145 hpf until metamorphic competence at around 210–215 hpf, the only major changes regarding myogenesis are the growing number of myofibrils and the increasing thickness of the muscle bundles of the respective muscle systems (Fig. 3A,B). At metamorphic compe-

tence (Figs. 1C, 3B), all muscles show a bright fluorescent signal, indicating that no muscular atrophy has taken place so far (cf. Fig. 3A,B).

During metamorphosis, the larval prototroch muscle ring and the apical muscle grid degenerate (Fig. 3C,D). The buccal musculature arises immediately after metamorphosis and consists of numerous fibers that insert symmetrically on the first shell plate. The former distinct, delicate dorsoventral muscle fibers start to concentrate (Fig. 3C) and 10 days after metamorphosis the paired shell muscle bundles are already differentiated under each shell plate. Additionally, the radula retractor muscles are formed. They insert on the second shell plate and are situated on both sides of the rectus muscle (Fig. 3D). The paired ventral longitudinal muscle persists in the juvenile animal, although it has not yet been described for any adult polyplacophoran species (see Discussion). The circular enrolling muscle is already functional in early juvenile animals (i.e., at 1 day after metamorphosis, see Fig. 3C), enabling the animal to protect its soft body parts on the ventral side if separated from the substratum.

The myofibrils of the dorsal rectus muscle undergo considerable rearrangement during larval life and especially at metamorphosis: their strong anterior divergence ceases (cf. Figs. 2C,D, 3A,B) and after metamorphosis all fibers follow the longitudinal anterior–posterior orientation of the first two myocytes, which are still situated on the mediadorsal line of the animal (cf. Figs. 2A,B, 3C).

Ultrastructure of Muscle Systems

Nearly all larval and adult muscle systems in *Mopalia muscosa* and *Chiton olivaceus* are smooth (Fig. 4) except for the obliquely striated buccal musculature. Tendon cells, which form the shell attachment junctions of various gastropod shell muscles (see Page, 1995, 1998; Wanninger et al., 1999a) and contain a high density of F-actin fibers, were not found in the larvae of the two polyplacophoran species investigated. The outer ring muscles of the apical grid and the posttrochal transversal muscles under the shell plates lie dorsad of the rectus muscle (Fig. 4A,B).

DISCUSSION

General Notes on Polyplacophoran Larval Development

As in many animal taxa with a biphasic life cycle, the transition from a free-swimming larval to a benthic juvenile stage involves dramatic changes of gross morphology. In the Polyplacophora, the dorsoventral axis flattens considerably and the postmetamorphic juvenile chiton becomes typically oval-shaped. At the same time, the animal sheds its prototroch cells and parts of the pretrochal area are lost (Fig. 1).

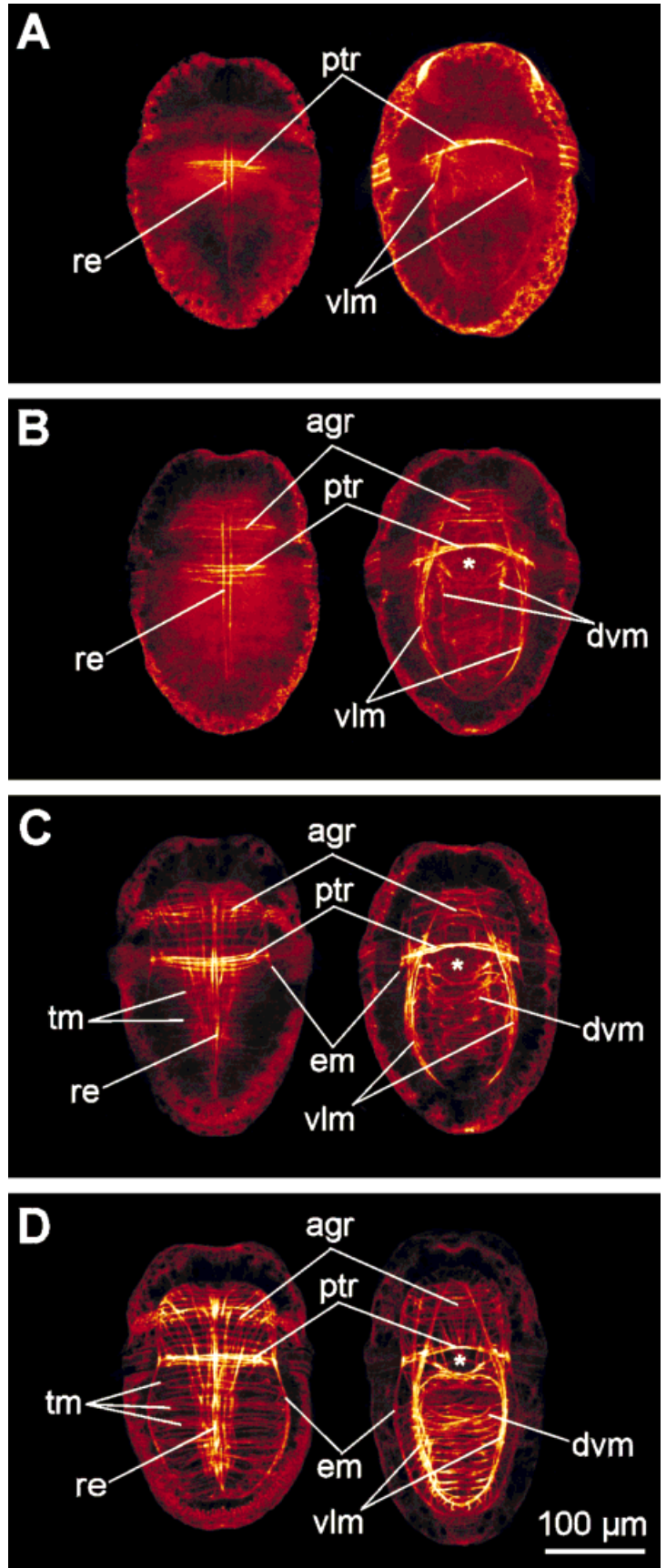


Fig. 2. Myogenesis in *Mopalia muscosa*, CLSM, early larval stages. Each pair of fluorescence images shows a dorsal (left) and a ventral (right) view of the respective developmental stage. Ages are given in hours postfertilization (hpf) at 10–12°C. Asterisks mark the mouth opening. **A:** Early trochophore stage, showing the first two fibers of the dorsal rectus muscle (re), fine myofibrils of the prototroch ring (ptr), and the paired ventrolateral longitudinal muscle (vlm). Age: 74.25 hpf (left), 82.25 hpf (right). **B:** The fibers of the rectus muscle (re) and ventrolateral longitudinal muscle (vlm) elongate and the first anlagen of the apical grid (agr) and the dorsoventral (shell) musculature (dvm) are formed. Age: 86.25 hpf (left), 93 hpf (right). **C,D:** Further differentiation of all muscle systems; the enrolling muscle (em) and transversal myofibrils (tm) in the region of the putative shell plates start to form. Age: 108 hpf (C, left), 96 hpf (C, right), 129 hpf (D, left), 142 hpf (D, right).

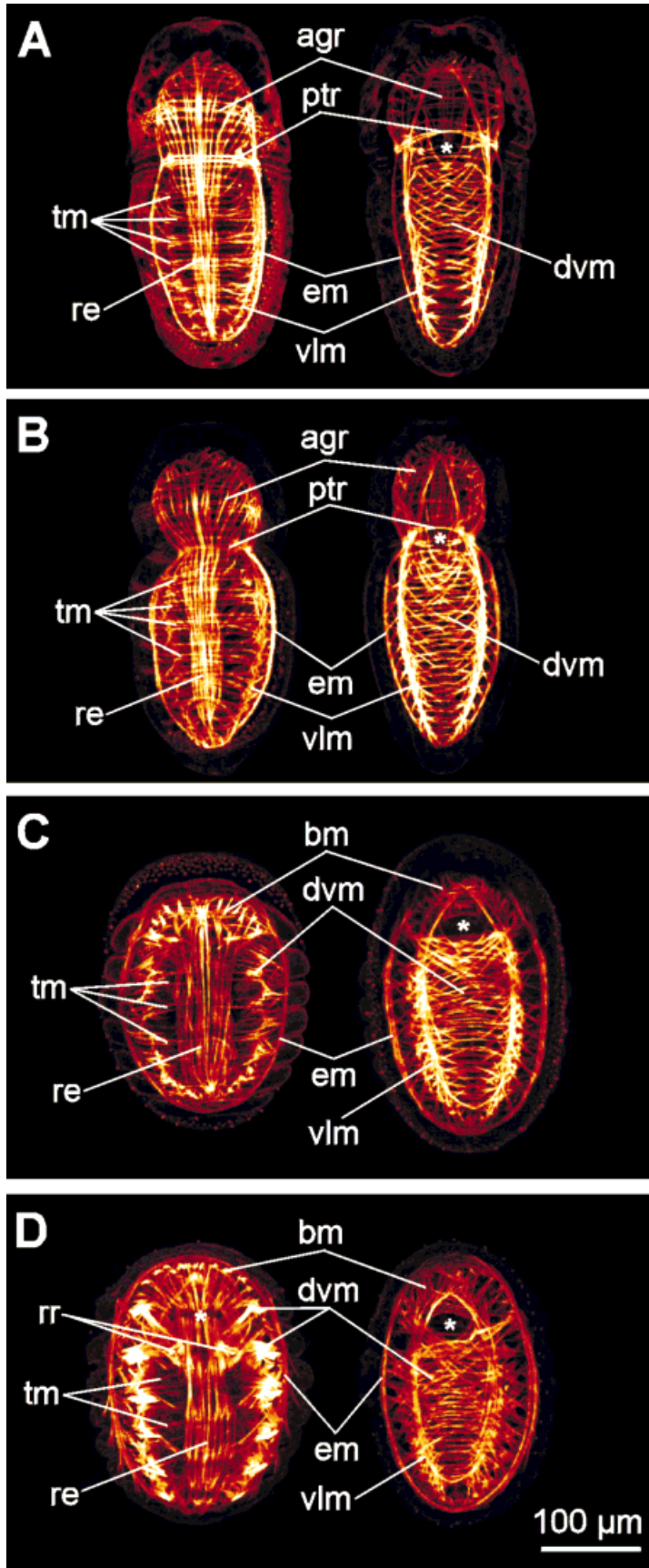


Fig. 3. Myogenesis in *Mopalia muscosa*, CLSM, late larval and early juvenile stages. Each pair of fluorescence images shows a dorsal (left) and a ventral (right) view of the respective developmental stage. Ages are given in hours postfertilization (hpf) or days postmetamorphosis (dpm) at 10–12°C. Asterisks mark the mouth opening. **A,B**: Muscle development in late premetamorphic stages until metamorphic competence. The transversal musculature (tm) under each shell plate, the putative enrolling muscle (em), the prototroch muscle ring (ptr), as well as the rectus muscle (re) and the dorsoventral (shell plate) musculature (dvm) are all heavily stained. Note also the three-dimensional apical grid (agr) in the pretrochal area and the prominent ventrolateral longitudinal muscles (vlm). Age: 161.15 hpf (**A**, left and right), 239.75 hpf (**B**, left and right). **C,D**: Postmetamorphic juvenile stages at one dpm (**C**) and ten dpm (**D**). The buccal musculature (bm) forms soon after metamorphosis and attaches at the first shell plate. The rearrangement of the dorsoventral shell muscles (dvm) into paired functional units has started in **C** (cf. their relative position to the weakly stained rims of the first seven shell plates), but is fully achieved only in later stages (**D**). The radula retractors (rr) are the last muscles to be formed. Note the still prominent staining of the ventral longitudinal muscles (vlm).

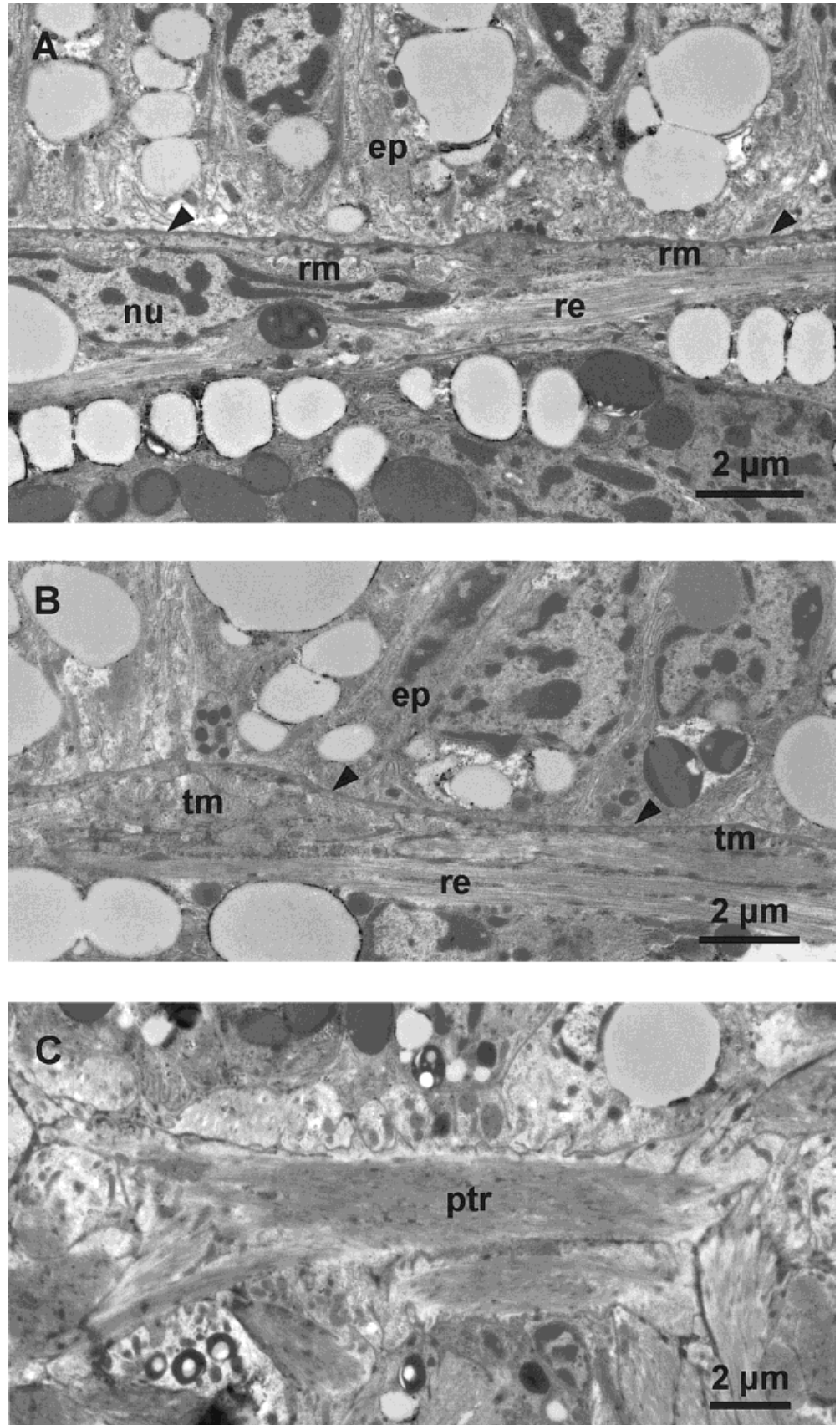


Fig. 4. Ultrastructure of several smooth muscle systems in larvae of *Mopalia muscosa*. Dorsal side faces upwards in **A** and **B** and to the right in **C**. **A**: Longitudinal section of the apical area of a late trochophore stage. The myocytes of the ring musculature (rm, with its adjacent nucleus (nu)) of the apical muscle grid lie directly underneath the basal membrane (arrowheads) of the dorsal epidermis (ep), thus encircling the fibers of the rectus muscle (re). **B**: Longitudinal section of the posttrochal region of the same specimen as in **A**. The transversal muscle fibers (tm), which underlie each shell plate, are ventrally bordered by the rectus muscle (re), while the basal membrane (arrowheads) of the dorsal epidermis (ep) lies on their dorsal side. **C**: Longitudinal section of the smooth larval prototroch muscle ring (ptr).

As shown on SEM micrographs of earlier studies on *Lepidochitona thomasi* (Eernisse, 1988: fig. 7C; Eernisse and Reynolds, 1994: fig. 5A) and confirmed by our observations on *Mopalia muscosa* and *Chiton olivaceus* (see above), the first shell plate extends pretrochally, thus contradicting former statements on the sole posttrochal origin of all shell plates in the Polyplacophora (Kniprath, 1980; Eernisse and Reynolds, 1994). This raises doubts about the homology of the polyplacophoran shell plates and the conchiferan shell, since the latter is entirely of posttrochal origin and position (Kniprath, 1981) and because shell (plate) secretion is different in conchiferan and polyplacophoran larvae (Haas, 1981). Moreover, shell plate ontogeny in the Polyplacophora does not show a stage of shell field invagination, as found in the Conchifera (Kniprath, 1981). The very gradual and, compared to gastropods and bivalves, slow establishment of the eventual juvenile body plan seems to be a general feature in polyplacophoran ontogeny. This is indicated by the fact that organs like gills, aesthetes, and the final shell plate are usually formed weeks after metamorphosis. On the other hand, several larval structures such as protonephridia and larval eyes are carried over into the postmetamorphic stage (Heath, 1904; Grave, 1932; Creese, 1986; Strathmann and Eernisse, 1987: p. 213).

Recent studies on the myogenesis in the Gastropoda (Page, 1995, 1997a,b, 1998; Degnan et al., 1997; Wanninger et al., 1999a,b) as well as earlier works on several bivalves (Meisenheimer, 1901; Smith, 1935; Crofts, 1937, 1955; Cole, 1938; Anderson, 1965; Smith, 1967; Cragg, 1985; Cragg and Crisp, 1991) and the data presented herein allow a comparison of the various muscle systems and the mechanisms involved in molluscan myogenesis.

Prototroch Muscle Ring

As in the basal gastropod *Patella* (Wanninger et al., 1999a), both polyplacophoran species investigated show a smooth muscular ring (see Fig. 4C) that is situated directly underneath the ciliated prototroch cells and that is lost during metamorphosis. These positional, structural, and ontogenetic similarities in both groups suggest supraspecific homology of this larval muscle system for the Gastropoda and the Polyplacophora. No similar structure has yet been described for either higher planktotrophic gastropod larvae with a much more complicated velum or any bivalve. Thus, it may be a molluscan plesiomorphy that is conserved only in some of the basal lecithotrophic molluscan larvae that possess a "simple" prototroch rather than a highly specialized and complicated velum.

Polyplacophoran vs. Aplacophoran Enrolling Muscles

The data presented herein raise doubts about the homology of the enrolling muscles of chitons and

aplacophoran molluscs as proposed by Salvini-Plawen (1972). The enrolling muscle in the Polyplacophora clearly represents a single circular muscle system, while it is longitudinally paired in adult Caudofoveata and Solenogastres (Salvini-Plawen, 1972). In addition, the enrolling muscle is a strengthened part of the longitudinal body-wall musculature in the aplacophoran taxa, but an independent system in chitons. However, data on the myogenesis in aplacophorans are necessary to finally solve this problem.

The fate and function of the paired ventral longitudinal muscle in *Mopalia muscosa* and *Chiton olivaceus*, which is retained in the juvenile animal (see Fig. 3D), remains enigmatic. Since it has not been found in any of the numerous detailed anatomical studies of adult chitons, it is very likely that this muscle disappears during subsequent development. Functionally, it may support the still relatively weak enrolling muscle, although its early ontogenetic appearance seems to contradict this hypothesis.

Larval and Adult Shell (Plate) Muscles

Larval velar and mantle retractor muscles that disappear through or shortly after metamorphosis are common throughout the Gastropoda (e.g., Smith, 1935; Smith, 1967; Fretter, 1972; Bonar and Hadfield, 1974; Page, 1995, 1997a, 1998; Degnan et al., 1997; Wanninger et al., 1999a,b) and are also found in several bivalves (Meisenheimer, 1901; Cragg, 1985; Cragg and Crisp, 1991). The absence of such larval shell muscles in the Polyplacophora indicates that they are probably not a part of the ancestral molluscan bauplan, although a secondary loss at the base of the polyplacophoran line cannot be completely ruled out. The restriction of larval retractor systems to those molluscan taxa that possess a protective shell in the early larval stages suggests coevolution of larval retractors and a functional larval or heterochronically shifted adult shell. Thus, the presence of specific larval retractor system(s) seems to be characteristic neither for the entire Mollusca nor for the Testaria (Polyplacophora + Conchifera), but may be so for the Conchifera. However, preliminary data on the myogenesis in Scaphopoda (pers. obs.) makes independent evolution of larval retractors in gastropods and bivalves equally possible.

Compared to the conditions found in gastropods and bivalves (see Meisenheimer, 1901; Cragg, 1985; Cragg and Crisp, 1991; Page, 1995, 1997a,b, 1998; Wanninger et al., 1999a,b), the formation of the adult shell musculature in the Polyplacophora shows striking differences. In free-swimming larvae of several gastropod and bivalve taxa, the adult shell muscles arise after the functional establishment of the larval retractor systems. In these groups, the adult shell musculature is formed very fast (in basal gastropods after the completion of torsion) and, with the exception of steady growth, does not undergo

major morphological rearrangement during its ontogeny. In *Mopalia* and *Chiton*, however, their generation and ultimate functional arrangement appears as a much more gradual process starting in the early trochophore-like larva, with continuous elaboration until considerably after metamorphosis (see Fig. 3).

Dorsoventral Musculature and the "Segmentation Problem"

The polyplacophoran dorsoventral musculature, inserting on the shell plates in postmetamorphic animals, starts to form as numerous distinct, serially repeated muscle fibers along the whole posttrochal larval body. The adult morphological and functional arrangement in seven (and later eight) sets of paired shell muscles is clearly a secondary condition that starts after the completion of metamorphosis. The latter condition is thus not indicative for a proposed segmented bauplan in chitons, as previously proposed (e.g., Götting, 1980; Lake, 1990). Instead, these findings argue in favor of recapitulation, as proposed by Salvini-Plawen (1969, 1981), who regarded the shell (plate) musculature as having evolved from serially arranged dorsoventral muscle fibers as found in adult Solenogastres. Accordingly, the testarian shell muscles evolved by subsequent concentration of such fibers, a condition which can still be traced ontogenetically in the recent Polyplacophora (see above).

Recently, gene expression pattern analyses of the homeobox gene *engrailed*, which is involved in arthropod segment formation, showed that this gene plays an important role in embryonic shell morphogenesis in gastropods (Moshel et al., 1998), bivalves (Jacobs et al., 2000), and scaphopods (Wanninger, pers. obs.), as well as in shell plate and spicule formation in polyplacophorans (Jacobs et al., 2000). Thus, the serial expression of *engrailed* in seven transversal stripes in the dorsal ectoderm of late chiton larvae reflects the function of "exoskeleton" formation of this gene in molluscs rather than proving their annelid-like "segmented" character.

Microanatomical and ontogenetic studies on the partly pedomorphic monoplacophoran *Micropilina arntzi* (Haszprunar and Schaefer, 1997) also suggest a nonsegmented body plan for the Monoplacophora, mainly because the ontogenetic formation of several organ systems such as ctenidia and gonads occurs from posterior to anterior, not vice versa as in the Annelida. The fundamental differences regarding the coelomic conditions in the Annelida and the Mollusca support this hypothesis: the coelomic cavities in the Mollusca are restricted to two sacs, one around the heart (pericardial cavity) and one enclosing the gonad, while they appear as multiple paired sacs along the anterior-posterior axis of the Annelida, which defines true segmentation or eumetamerism. Comparative analyses of the ontogeny of these epithelially

lined cavities even suggest their diphyletic origin between molluscs and the eucoelomate taxa, thus making the possibility of secondary loss of segmentation within the Mollusca very improbable (see Salvini-Plawen and Bartolomaeus, 1995). In addition, most authors nowadays (e.g., Salvini-Plawen and Steiner, 1996) consider the aplacophoran taxa (i.e., Solenogastres and Caudofoveata) as most basal clades of the Mollusca, and neither their adult body plan nor ontogenetic data on the Solenogastre *Neomenia carinata* (Thompson, 1960) show any trace of eumetamerism in these groups.

Ancestral Condition: From Worm to Mollusc

The adult dorsoventral musculature of the Mollusca, which intercrosses just dorsal of the foot sole, is phylogenetically distinct from that of all other phyla. Thus, the molluscan dorsoventral musculature can be regarded as apomorphic for the phylum (e.g., Salvini-Plawen, 1980; Haszprunar, 1988; Haszprunar and Wanninger, 2000). Platyhelminthes, however, also express dorsoventral muscles different from that of molluscs and distinct from the typical worm-like body-wall musculature (Tyler and Rieger, 1999). The body-wall musculature of worm-shaped groups like annelids, platyhelminths, or nemerteans mainly consists of three layers of ring, diagonal, and longitudinal muscles (e.g., Rieger et al., 1994; Reiter et al., 1996; Hooge and Tyler, 1999). The Solenogastres and Caudofoveata are the only major molluscan taxa which express in their adult body plan a three-layered body-wall musculature similar to the phyla mentioned above (Salvini-Plawen, 1972, 1981; Scheltema et al., 1994; Haszprunar and Wanninger, 2000). Our results suggest that the fibers of the apical muscle grid in the chiton larva may be vestiges of such body-wall muscles of a proposed worm-shaped molluscan ancestor. Due to the evolution of protective larval and adult shells in the Conchifera, the original body-wall muscles were completely reduced in this clade. Instead, the conchiferans elaborated the dorsoventral musculature as the main adult shell muscle system (note that gastropods and bivalves possess distinct larval retractors which are independent of the adult shell muscles; see above). Indeed, all gastropods with a planktonic veliger stage investigated so far show a larval shell and larval retractor systems early in development, but no "worm-like" body-wall muscles are present. In cases of shell reduction, however, a secondary "worm body" is found (e.g., nudibranchs, slugs, ship-worms). The Polyplacophora, which are phylogenetically situated at the interface of the primary worm-shaped aplacophorans and the Conchifera, lack a distinct larval shell and the adult shell plates are not protective before metamorphosis, but relics of such ancestral body-wall muscles (i.e., the apical grid) are present. However, in both chiton species investigated, *Mopalia muscosa* and *Chiton*

olivaceus, longitudinal muscles were not observed in the apical grid. Thus, it seems that the longitudinal fibers are replaced by the diverging rectus muscle fibers in the chiton larva, which is indicated by the fact that after metamorphosis (i.e., after the loss of the apical grid) the rectus muscle appears as a solid median band of parallel longitudinal myocytes. However, the question whether this is a result of myofibrillar rearrangement and/or cell death of these fibers remains open. Accordingly, two evolutionary pathways appear equally possible: 1) assuming recapitulation of an ancestral body-wall musculature, the longitudinal fibers of the apical grid are completely lost in the Polyplacophora, or 2) the original longitudinal muscles are modified and contribute to (parts of) the rectus muscle. The latter assumption infers that at least parts of the rectus muscle are homologous to the longitudinal body-wall muscles of aplacophoran molluscs (see Salvini-Plawen, 1972). For further insights, ontogenetic data on the myogenesis of aplacophoran molluscs and the cytological mechanisms on the myofibrillar rearrangement of the rectus muscle fibers during chiton metamorphosis are crucial.

The transversal muscle fibers under the shell plates are most likely a polyplacophoran apomorphy which co-evolved with the shell plates. The strictly transversal character throughout their ontogenetic development makes a derivation from the body-wall ring muscles of a molluscan ancestor unlikely.

CONCLUSIONS

Myogenesis in the Polyplacophora involves several mechanisms in the transition from the larval planktonic to the juvenile benthic lifestyle: 1) degeneration of larval muscle systems (prototroch muscle ring, apical grid, and probably the paired ventral longitudinal muscle); 2) de novo generation of the buccal musculature, including the paired radula retractors after metamorphosis; and 3) gradual morphological rearrangement of the dorsoventral shell musculature and the dorsal rectus muscle. The cytological mechanisms and epigenetic background of these muscular dynamics, however, remain unknown but are highly promising for future studies.

Our study supports the concept that the "segmented" character of the adult polyplacophoran shell musculature is a secondary condition, contradicting previous attempts to derive the Polyplacophora (and the entire phylum Mollusca) from a primarily segmented stem species. The data currently available suggest their descent from an unsegmented, non-eucoelomate trochozoan ancestor (cf. Haszprunar, 1996).

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