Annelida

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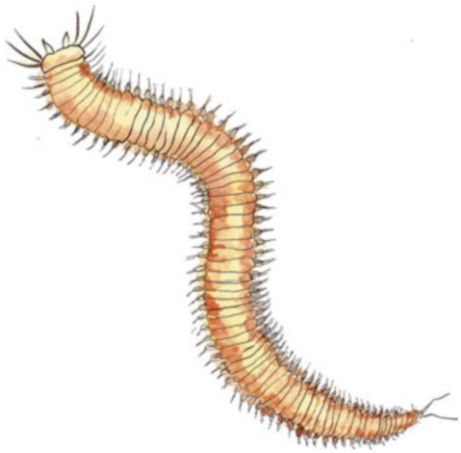

Chapter vignette artwork by Brigitte Baldrian.

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INTRODUCTION

Annelids are a taxon of protostomes comprising more than 17,000 worldwide-distributed species, which can be found in marine, limnic, and terrestrial habitats (Zhang 2011). Their phylogeny was under discussion for a long time, but recent phylogenomic analyses resulted in a solid backbone of this group (Struck et al. 2011; Weigert et al. 2014). According to these analyses, most of the annelid diversity is part of Errantia or Sedentaria, which both form reciprocally monophyletic sister groups (Fig. 9.1) and are now known as Pleistoannelida (Struck 2011). The Sedentaria also include the Clitellata, Echiura, and Pogonophora (Siboglinidae) as derived annelid taxa. Outside Sedentaria and Errantia, several groups can be found in the basal part of the annelid tree, namely, Sipuncula, Amphinomida, Chaetopteridae, Magelonidae, and Oweniidae. The latter two taxa together represent the sister taxon of all other annelids. Given this hypothesis, it has to be assumed that the early diversification of extant annelids took place at least in the Lower Cambrian (520 Ma ago) (Weigert et al. 2014). The phylogenetic position of Myzostomida, a group of commensals or parasites of echinoderms (and, rarely, cnidarians), remains still uncertain. Whereas there is strong support for an annelid ancestry, its exact position awaits to be determined (Bleidorn et al. 2014). Likewise, the phylogenetic position of several interstitial taxa is still under debate (Westheide 1987; Worsaae and Kristensen 2005; Worsaae et al. 2005; Struck 2006). A position of Diurodrilidae outside Annelida, as suggested by Worsaae and Rouse (2008), was rejected by molecular data (Golombek et al. 2013), and the position of the enigmatic *Lobatocerebrum* and *Jennaria* remains unresolved (Rieger 1980, 1991). Likewise, the position of Annelida within Protostomia is still uncertain. However, recent phylogenomic analyses recover a clade uniting annelids with Mollusca, Nemertea, Brachiopoda, and Phoronida, but without strong support for any sister group relationship (Edgecombe et al. 2011).

Annelids show a huge diversity of body plans, and it is difficult to describe a consistent anatomy matching most of this variety (Fig. 9.2). Most annelids are coelomate organisms, possessing multiple segments which occur repetitively along the anterior-posterior body axis (Purschke 2002). If segmentation is present, the annelid body is divided into a prostomium, an either homonomously (i.e., identical segments) or heteronomously (i.e., segments differ from each other) segmented trunk and a pygidium (Fauchald and Rouse 1997). In many annelid taxa, the prostomium contains the brain; however, in Clitellata the brain may be found in the following segments (Bullock 1965). The head of the annelids may bear appendages, as palps or antennae, but these are lacking in a number of taxa. The mouth can be found in the first segment which is termed peristomium. Several members of the Errantia as well as Amphinomida bear sclerotized mandibular structures, which may be replaced by a mechanism resembling molting (Paxton 2005). The segments of many annelids contain a pair of nephridia (usually metanephridia), coelomic cavities, ganglia, and ventral and dorsal groups of chitinous chaetae which might be organized in parapodia (Purschke 2002; Bartolomaeus et al. 2005). Segments are generated by a posterior growth zone which is located in front of the pygidium (Nielsen 2004). The pygidium contains the anus, which is usually either dorsally or terminally located and is often equipped with pairs of cirri.

Annelids show a wide variety in the organization of their nervous system (Bullock 1965; Orrhage and Müller 2005; Müller 2006). Müller (2006) proposed a nervous system with paired circumesophageal connectives, four cerebral commissures, five connectives, and numerous commissures in the ventral nerve cord as a hypothetical ground pattern. However, many variations of this pattern exist, and many taxa have not been investigated at all. Accordingly, alternative hypotheses suggest that a strict rope-ladder-like nervous system with segmental

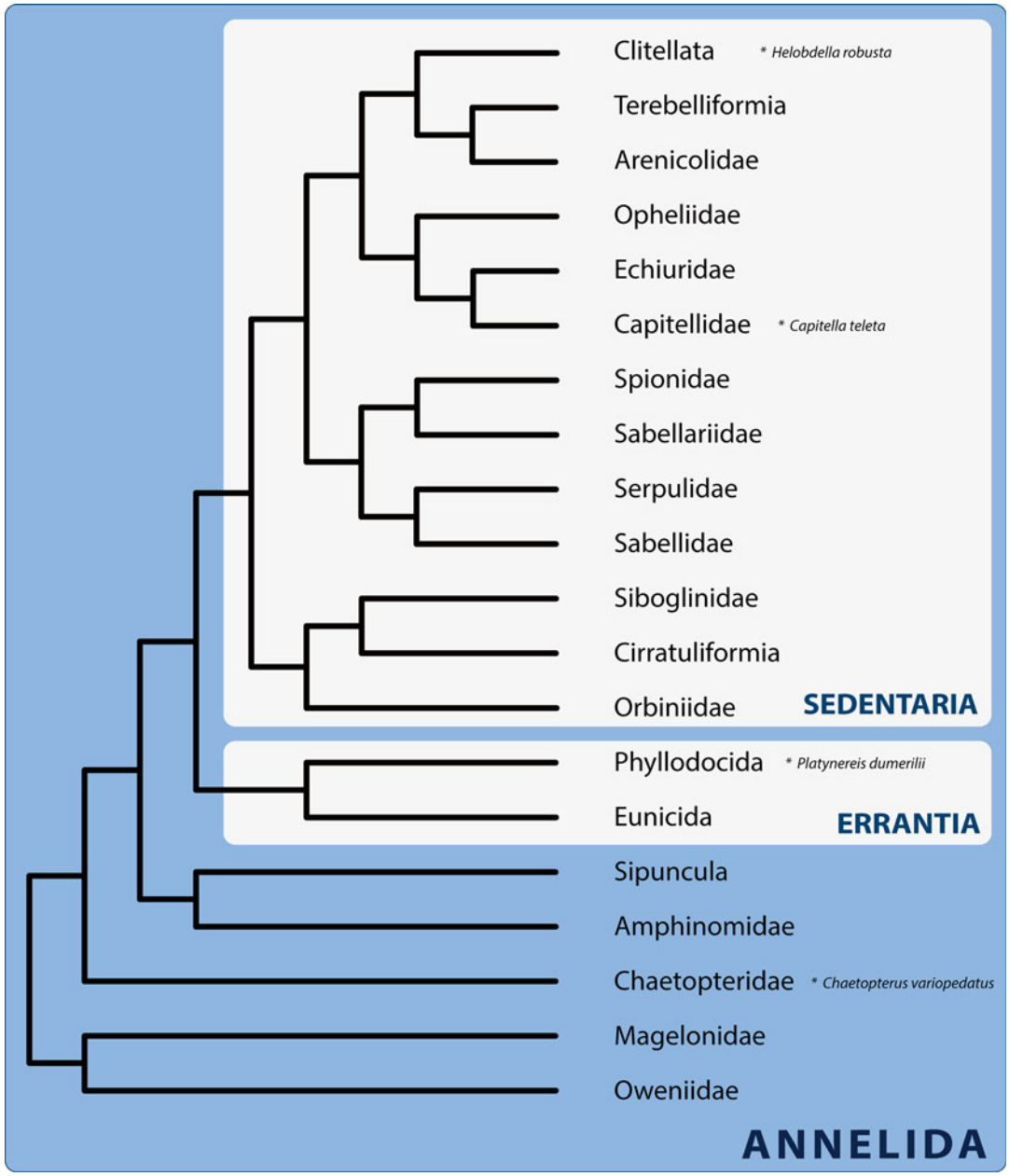


Fig. 9.1 Phylogeny of Annelida based on Weigert et al. (2014). Placement of well-investigated model annelids indicated with *asterisks*

ganglia interconnected by a pair of connectives and commissures was not present in the last common ancestor of annelids (Purschke et al. 2014). Instead, an orthogonal arrangement of the peripheral nervous system and the presence of additional longitudinal nerves might constitute the ground pattern of Annelida (Lehmacher et al. 2014).

Annelids show varying grades of brain complexity which may comprise a number of ganglia.

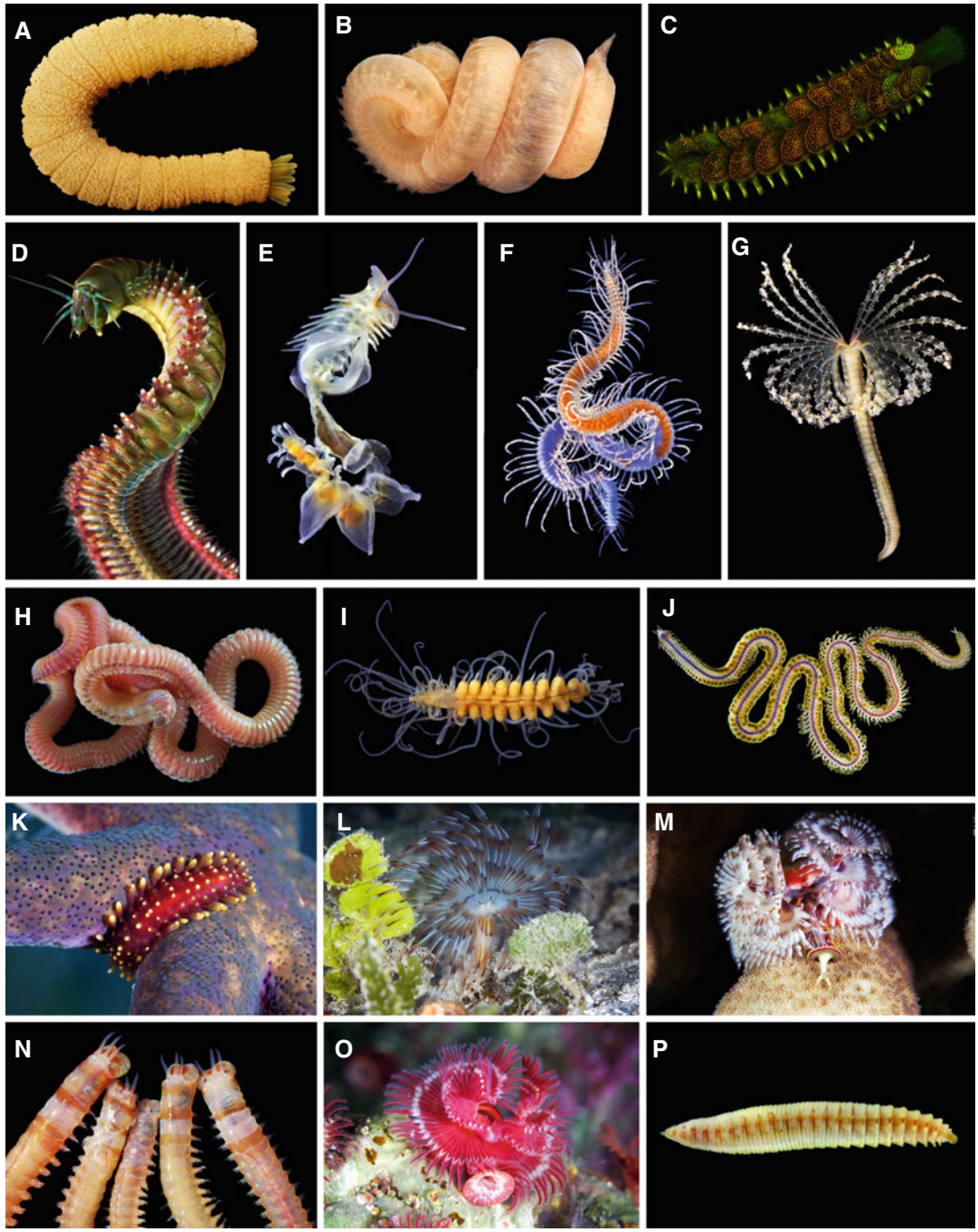


Fig. 9.2 The diversity of marine Annelida. (**A**) *Brada villosa*, Flabelligeridae. (**B**) *Glycera capitata*, Glyceridae. (**C**) *Lepidonotus squamatus*, Polynoidae. (**D**) *Nereis pelagia*, Nereididae. (**E**) *Chaetopterus* sp., Chaetopteridae. (**F**) Syllinae indet., Syllidae. (**G**) *Branchiomma arctica*, Sabellidae. (**H**) *Lumbrineris* sp., Lumbrineridae. (**I**) *Amblyosyllis* sp., Syllidae. (**J**) *Phyllodoce* sp., Phyllodocidae. (**K**) Polynoidae indet. (**L**) Sabellidae indet., Sabellidae. (**M**) *Spirobranchus giganteus*, Serpulidae. (**N**) *Lysidice* sp., Eunicidae. (**O**) *Serpula* sp., Serpulidae. (**P**) *Travisia* sp., *Travisia* (All images provided by Alexander Semenov (www.clione.ru).

Mushroom bodies have been reported for several taxa of the Phyllodocida (Heuer et al. 2010). As for the nervous system, many different variations can be found in the muscular system. The presence of an outer layer of circular muscle fibers and an inner layer of four longitudinal bands of muscle fibers is often regarded as a possible ground pattern (Tzetlin and Filippova 2005; Lehmacher et al. 2014), but circular muscles are missing in several annelid taxa and may thus not constitute a basal annelid feature. Additionally, other muscular fiber bundles referred to as oblique, diagonal, bracing, or dorso-ventral fibers might be present and are often compensating missing circular musculature (Purschke and Müller 2006).

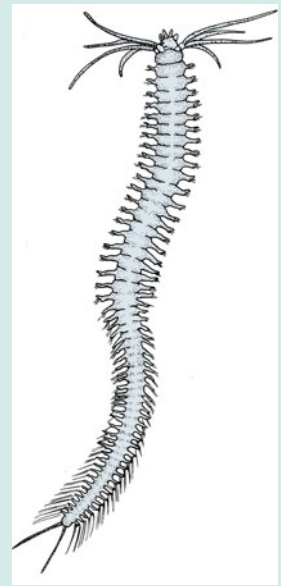
However, many taxa, such as myzostomids, sipunculids, or echiurids, are clearly deviating from the pattern described above in various aspects, and all of them seem to have lost segmentation convergently. Interestingly, all these examples still show some traces hinting to a secondary loss of segmentation (Purschke et al. 2000; Hessling 2003; Kristof et al. 2008; Helm et al. 2014). Other taxa such as clitellates and many other sedentarians lost their parapodia. Siboglinidae (Pogonophora + Vestimentifera) show many reductions as adaptation to their lifestyle in close association with bacterial endosymbionts (Schulze and Halanych 2003). Loss of key characters in Annelida is well-documented and is regarded as one of the problems to converge to a well-accepted phylogeny of the whole group (Purschke et al. 2000; Bleidorn 2007; Miyamoto et al. 2013).

Several systems of sensory organs are described for annelids, including a type of chemosensory organ called "nuchal organ." This type of sensory organ can be found in the posterior part of the prostomium and usually consists of ciliated supporting cells, sensory cells, and retractor muscles (Purschke 1997). Clitellates as well as several other annelids such as the basal branching Oweniidae and Magelonidae lack nuchal organs completely. Many annelids possess some kind of light receptive photoreceptors which show great structural diversity (Purschke et al. 2006). Generally, rhabdomeric, ciliary, and phaosomous photoreceptor cell types are distinguished, and they might represent either larval or adult eyes (Purschke et al. 2006; Arendt et al. 2009). Larval eyes are simple organized, and the eye spots of the trochophore of *Platynereis dumerilii* consist of a rhabdomeric photoreceptor cell and a pigment cell which provide a direct coupling of light-sensing ciliary locomotory control (Jekely et al. 2008). Eyes of adult annelids might be present on the head, palps, segments (usually laterally), or even the pygidium (Purschke et al. 2006).

Annelids show a variety of reproductive strategies, and sexual as well as asexual reproduction is well-documented for many taxa (Wilson 1991; Bely 2006). For sexual reproduction, different types of free spawning, brooding, and encapsulation of embryos in cocoons can be distinguished, and all types involve either planktotrophic or lecithotrophic developmental stages (Thorson 1950; Wilson 1991). Multiple modes of development (poecilogeny) are reported for some annelid species, with the spionid *Streblospio benedicti* as the best-investigated example (Levin 1984; Zakas and Wares 2012). By far the most spectacular diversity of reproductive modes can be found across syllids, including swarming and external fertilization, internal fertilization, viviparity, and parthogenesis, as well as different forms of hermaphroditism (Franke 1999). Several annelid taxa show a pronounced sexual dimorphism resulting in dwarf male forms as found, for example, in some echiurids, siboglinids, or antonbruunids (Spengel 1879; Hartman and Boss 1965; Worsaae and Rouse 2010). Not surprisingly, annelids are also a prime example for the investigation of heterochrony, and several putative paedomorphic taxa have been hypothesized (Westheide 1987; Struck 2006; Bleidorn 2007; Osborn et al. 2007).

Platynereis dumerilii as a Model for Evolutionary Developmental Biology

Platynereis dumerilii is a marine annelid belonging to the errant family Nereididae, which emerged as a thoroughly investigated model species. The life cycle of this indirect developing gonochoric species with planktotrophic larvae is well established and controllable in the lab. Immature, atokous worms live in self-constructed tubes. Sexually mature, epitokous individuals, which appear morphologically different to atokous individuals, leave the tube and begin swimming to find partners for spawning during the night. The day of swarming is controlled by an endogenous lunar cycle which can be triggered artificially in the lab. Cultures were established in the lab in 1953 and are bred since then without interruption. Experimental techniques such as cell ablation, whole-mount in situ hybridization, RNA interference, and Morpholino knockdowns are routinely applicable. First transgenic lineages have been created, and a project sequencing the genome is underway for this species (http://4dx.embl.de/platy/). Comparative genomic studies suggest that the genome of *P. dumerilii* retains a more ancestral organization compared to other protostomian model organisms such as *Drosophila melanogaster* or *Caenorhabditis elegans*. Important insights into the evolution of segmentation, vision, and the nervous system in Bilateria were provided by evolutionary developmental studies on *P. dumerilii*, and since a number of labs now use this animal as a model, important results with considerable relevance for our understanding of animal evolution and development are likely to keep emerging in the near future.



Atokous juvenile of *Platynereis dumerilii* (After Fischer and Dorresteijn (2004))

EARLY DEVELOPMENT

Egg Structure and Fertilization

Annelids with planktotrophic development usually have small, non-yolky eggs, whereas species with lecithotrophic development bear larger and yolk-rich eggs (Irvine and Seaver 2006). Ultrastructural studies of annelid eggs are scarce given the immense diversity of this group. One of the best-investigated examples is the egg of parchment worms of the genus *Chaetopterus*. Different regions are distinguished based on staining properties, divided into a cortical ectoplasm, endoplasm, and hyaloplasm (Lillie 1906, 1909), the latter two regions forming the cytoplasm. The ectoplasm contains large membrane-bound spherules, nuage (a germ line-specific organelle containing several proteins), and intracellular membrane systems. The endoplasm contains yolk and lipid, interspersed with mitochondria, granular bodies, and endoplasmatic reticulum. In contrast, the hyaloplasm (or teloplasm) is characterized by the absence of granular bodies (Eckberg 1981; Jeffery 1985). Eggs of many annelid species show a clear polarity with an accumulation of developmental factors in the cortex of future polar regions (Dorresteijn 2005). The spatial distribution of

maternal mRNA in the ectoplasm has been described for *Chaetopterus* (Jeffery and Wilson 1983; Jeffery 1985). After fertilization and before initiation of the first cleavage, a reorganization of the yolk-free hyaloplasm (teloplasm) has been observed in several annelids (Dorresteijn 1990; Weisblat and Huang 2001). It has been shown for *Platynereis dumerilii* that after attachment of the sperm to the egg surface, cortical granules released by exocytosis from the ectoplasm start forming an egg jelly on the outside, which removes supernumerary sperm from its surface (Dorresteijn 1990). Ultrastructural investigations of the ectoplasm of eggs of *P. dumerilii* and the clitellate *Theromyzon rude* reveal an extensive framework of actin filaments that are involved in remodeling the egg surface after fertilization (Fernandez et al. 1987; Kluge et al. 1995).

Following fertilization a reorganization of the endoplasm can be observed. The distribution of the two cytoplasmic domains can be categorized into different types, which seem to be restricted to certain annelid taxa (Shimizu 1999). Most investigated non-clitellate annelids (e.g., chaetopterids, nereidids, and onuphids) show a stratification of the endoplasm into two domains, and, in most cases, the clear hyaloplasm (teloplasm) is localized at the animal pole (Wilson 1892; Huebner and Anderson 1976; Jeffery and Wilson 1983). In contrast, three domains can be distinguished in the clitellate endoplasm, with teloplasm localized at both the animal and vegetal poles of the egg (Shimizu 1999; Weisblat and Huang 2001). These cytoplasmatic movements are coordinated by complex cytoskeletal mechanisms which even seem to vary among taxa. Whereas in the leech *Helobdella triserialis*, microtubules are shown to play an important role, movement of the teloplasm in the oligochaete *Tubifex* is orchestrated by an actin network (Astrow et al. 1989; Shimizu 1995).

Cleavage

Annelids develop by spiral cleavage which is characterized by cleavage furrows which are oblique to the egg axis due to an inclination of the mitotic spindle (see Chapter 7). This cleavage starts with two orthogonal cell divisions which generate four blastomeres, called A, B, C, and D (Costello and Henley 1976). Correlating with the differing developmental modes in annelids, blastomeres usually exhibit the same size (equal cleavage) in species with indirect development and planktotrophic larvae, whereas direct developers show pronounced differences in blastomere size (unequal cleavage) (Anderson 1966; Arenas-Mena 2007). However, exceptions to this trend exist. For example, *Platynereis dumerilii* and *Platynereis massiliensis* both show unequal spiral cleavage patterns, even though the former species develops indirectly and the latter directly (Schneider et al. 1992). In most cases unequal cleavage is achieved due to positioning of the mitotic spindle. However, in some annelids this cleavage pattern is facilitated due to the presence of membrane-bound polar lobes (Freeman and Lundelius 1992). Such a polar lobe is also reported for the myzostomid *Myzostoma cirriferum* (Eeckhaut and Jangoux 1993). The different modes of spiral cleavage across annelids have been thoroughly reviewed in Dorresteijn (2005). The future axis of the developing embryo is already determined, with A and C corresponding respectively to the left and right side of the embryo and blastomeres B to D defining the antero-ventral to postero-dorsal axis (Nielsen 2004). Due to uneven cleavage, starting from the third cell division, a shifting in the angle of the mitotic spindles, which alternates during subsequent divisions, becomes obvious in almost all annelids. These shifts are either dextral (clockwise) or sinistral (anti-clockwise) and lead to the name-giving spiral arrangement pattern of blastomeres. By oblique divisions animal and vegetal daughter cells are generated, referred to as micromere quartets and macromere quartets. Some annelids such as the opheliid *Armandia brevis* show equal cleavage also in the third cleavage, generating micro- and macromeres of equal size (Hermans 1964). In the oweniid *Owenia collaris* and some leeches, micromeres are larger than macromeres in the eight-cell stage, a pattern which is also known from several nemerteans

(Dohle 1999; Smart and Von Dassow 2009). Several authors introduced the idea that a specific, phylogenetically conserved pattern of blastomeres can be seen at this stage, termed the "annelid cross." This pattern is regarded as typical for most annelids (including echiurans) but cannot be found in sipunculids, which show the so-called molluscan cross. However, a continuum of different variants between these patterns is demonstrated, and consequently, these concepts have been neglected for phylogenetic purposes (see also Chapter 6; Maslakova et al. 2004a; Nielsen 2004). The cell fate of individual blastomeres is conserved across annelids, and a specific nomenclature is used to trace the fate of blastomeres throughout development, using capital letters for macromeres and small letters for micromeres (Conklin 1897; Costello and Henley 1976; Nielsen 2004). A number is used as prefix to designate the quartet of which the macromeres or micromeres originated from. The micromeres continue to divide, and daughter cells inherit the name of their mother cell, with modification, to trace its origin (animal vs. vegetal) (Fig. 9.3). An alternative cell nomenclatural system is in use for leeches (Dohle 1999). Many clitellates show a cleavage pattern that nearly obscures the original spiral mode of cleavage. In these cases where yolk content and egg size are reduced, the embryo is nourished by the surrounding fluid within the cocoon (Dohle 1999). Several siboglinids show elongated eggs with a high yolk content leading to an aberrant pattern of spiral cleavage (Southward 1999). In most annelids, the cleavage pattern shifts from spiral to bilaterally symmetric after the formation of the fourth quartet of micromeres (Meyer and Seaver 2010). In *P. dumerilii* cell fates of sister blastomeres along the animal-vegetal axis are specified by levels of beta-catenin. High levels specify vegetal sister cell fates, while lower levels specify animal sister cell fates. Interestingly, no beta-catenin asymmetry is observed after the first bilaterally symmetrical and transverse cell divisions (Schneider and Bowerman 2007).

Descendants of the first micromere quartet (1a–1d) form larval head structures including the apical organ, larval eyes, and the head ectoderm as well as the primary trochoblasts (Nielsen 2004). The trochoblasts received their name as they will give rise to the prototroch, and this has been demonstrated for several annelids,

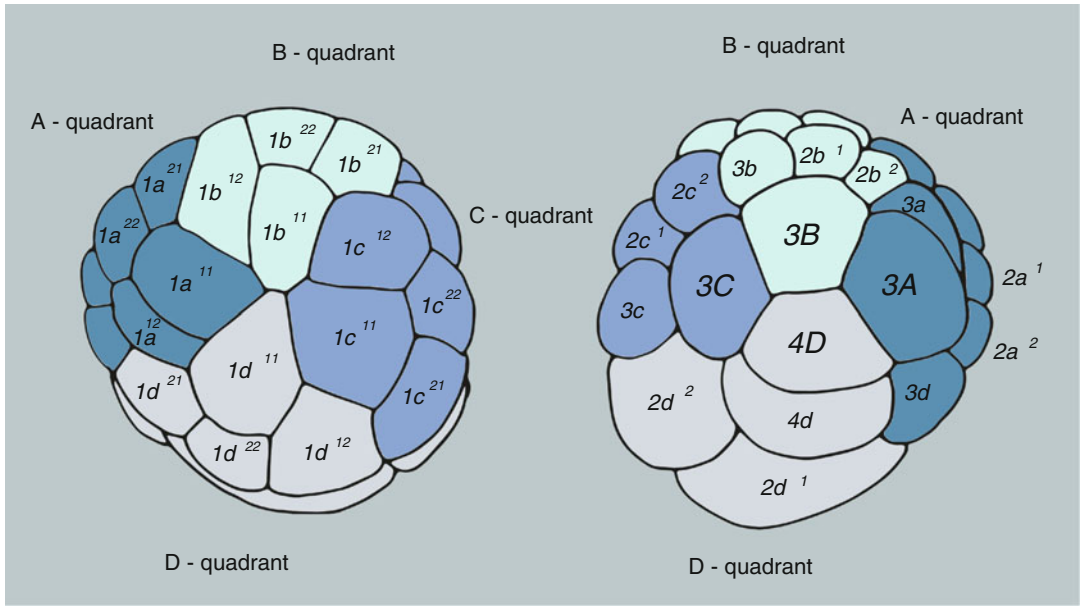


Fig. 9.3 Diagram illustrating the spiral cleavage cell nomenclature in the 33-cell stage of an unequally cleaving embryo of *Arenicola cristata* (Child 1900). The four quadrants (*A*–*D*) are indicated by colors

e.g., capitellids, dinophilids, and nereidids (Wilson 1892; Eisig 1898; Nelson 1904). Trochophore larvae of Myzostomida lack or show a reduced prototroch (Rouse 1999), but as it has been shown for the nemertean *Carinoma tremaphorus*, this need not be reflected in the formation of trochoblasts (Maslakova et al. 2004b). Three sets of trochoblast cells are involved in prototroch formation, a pattern which is highly conserved across Spiralia (Henry et al. 2007). Besides the primary and accessory trochoblasts, which are derived from the first micromere quartet, this includes secondary trochoblasts formed by some descendants of the second micromere quartet (2a–2c). Some annelids deviate from this pattern, e.g., the terebellid *Amphitrite ornata*, who lacks the accessory trochoblasts (Damen and Dictus 1994). Other descendants of the second micromere quartet generally develop into the foregut (stomodaeum) as well as part of the ectoderm (Nielsen 2004). The 2d cell is the somatoblast, developing into the major part of the body ectoderm posterior of the prototroch (Meyer and Seaver 2010). Using cell ablation studies, it has been shown for *Capitella teleta* that the 2d cell is responsible for organizing activity during early embryonic development, as well as bilateral symmetry and dorso-ventral axis organization of the head, and formation of neural, foregut, and mesoderm tissue (Amiel et al. 2013). In clitellates, four pairs of ectoteloblasts (called N, O, P, Q) are descendants of the 2d cell and give rise to four germbands including smaller cells (Dohle 1999; Goto et al. 1999). Cells derived from the third micromere quartet (3a–3d) form the foregut and ectomesoderm and might be the origin of protonephridia (Nielsen 2004; Ackermann et al. 2005). Interestingly, in *C. teleta*, mesodermal bands are generated by 3c and 3d (Meyer et al. 2010).

Usually the mesoderm and endoderm are formed by cells of the fourth micromere quartet (4a–4d), as characteristic for spiral cleavage in Lophotrochozoa in general (Chapter 7; Gline et al. 2011). Of special interest is the fate of the 4d cell, which has been called "mesenteloblast" or "primary mesoblast" (Wilson 1898). This cell gives rise to the adult mesoderm in most spiralians including mollusks or entoprocts (Chapters 6 and 7). In Clitellata, a fifth teloblast (M) is derived from the 4d cell, specifying a mesodermal germband (Goto et al. 1999). Progenitors of 4d form bilaterally symmetrical mesodermal anlagen in *Platynereis dumerilii* (Ackermann et al. 2005; Fischer and Arendt 2013). Prior to gastrulation, four secondary mesoblast cells bud from descendants of the 4d cell and show the morphology and gene expression signature of primary germ cells (Rebscher et al. 2012). These primary germ cells stay in mitotic arrest until individuals enter gametogenesis (Lidke et al. 2014). In *C. teleta* the 4d cell generates few muscle cells, primordial germ cells, and the anus (Meyer et al. 2010). It has been suggested that mesoteloblast-like mesodermal stem cells forming continuous mesodermal bands are part of the Pleistoannelida ground pattern (Fischer and Arendt 2013).

Gastrulation

The process of gastrulation in annelids has been reviewed in detail by several authors (Okada 1957; Anderson 1973; Weisblat and Huang 2001; Irvine and Seaver 2006), and the following descriptions provide a generalized pattern found in clitellate and non-clitellate annelids.

Gastrulation of embryos with less yolk starts with the invagination (embolic gastrulation) of putative midgut cells, and epithelia derived from the micromere cap grow toward the ventral side. Mesoteloblasts can be found in a posterior position in the blastocoel, whereas ectoteloblasts are located adjacent to them, below the larval ectoderm. The fate of the blastopore differs across annelid taxa, and protostomy, where the blastopore becomes the mouth, is found in most annelid taxa. Notably, deuterostomy, where the blastopore becomes the anus, has been demonstrated for eunicids (Åkesson 1967). The concept of amphistomy, in which both the mouth and the anus are derived from the corresponding ends of the blastopore, which was claimed to be present in *Polygordius* (Arendt and Nübler-Jung 1997), might not occur in any organism at all (Hejnol and Martindale 2009). Some differences apply for the gastrulation of yolk-rich annelid embryos.

Here, the process is rather described as epiboly, where the micromere cap grows over putative midgut cells and teloblasts (Irvine and Seaver 2006). In clitellates, the blastopore is found in the point where the germinal bands coalesce to form the germinal plate.

LATE DEVELOPMENT

Larval Ciliary Bands

Larval morphological characters vary across different annelid families (Fig. 9.4). A trochophore has distinct larval ciliary regions forming prominent bands or tufts, and the presence of a prototroch is regarded as defining (Bhaud and Cazaux 1987; Rouse 1999). However, detailed investigations concerning the homology of the respective ciliated regions within the different annelid families are lacking. At the anterior end of the episphere, the apical tuft marks the position of the larval apical organ, a feature well known for most invertebrate taxa with ciliated larvae (Marlow et al. 2014). Appearing early in development, the apical tuft forms a sensory region that is located in the direction of larval movement but often disappears in early larval stages (see Chapter 7 for details on apical tuft morphology). Although an apical tuft is widespread within annelids, larvae without an apical tuft are known for most cirratulids, histriobdellids, lopadorhynchids, orbiniids, sabellids, and tomopterids (Rouse 1999).

The prominent prototroch is represented by an equatorial ring consisting of usually compound cilia formed by a group of specific trochoblasts (Damen and Dictus 1994). Situated anterior to the mouth opening, a prototroch is known for most annelids, mollusks, and entoprocts (Nielsen 2012). Dividing the larval body in an anterior episphere and a posterior hyposphere (see Chapter 7), the prototroch is present mainly in planktotrophic annelid larvae and some lecithotrophic stages but absent in direct developing taxa such as clitellates, aelosomatids, and histriobdellids (Rouse 1999). In some annelid taxa, the cilia of the prototroch may cover almost the whole episphere in early developmental stages (e.g., in *Chaetopterus*; see Fig. 9.4L). The prototroch may be formed by equatorially arranged ciliary tufts (*Myzostoma cirriferum*; see Fig. 9.4G), or the whole larva may be covered by cilia, and a defined prototroch is hardly distinguishable, e.g., in early larvae of the eunicid *Marphysa* (Fig. 9.4E). An epispheral ciliated band is represented by the meniscotroch, which is only known for Phyllodocida (Bhaud and Cazaux 1982; Rouse 1999). Forming a tuft of short cilia, the meniscotroch is located in a ventral position within the episphere. Posterior to the latter structure, some annelids possess a ciliated band situated anterior to the prototroch – the akrotroch (Häcker 1896). Forming a complete ring separated from the apical tuft and the prototroch, an akrotroch can be found in syllids, orbiniids (e.g., in *Scoloplos armiger*, see Fig. 9.5), onuphids, cirratulids, and several Eunicida (Rouse 1999).

Situated posteriorly to the prototroch, the metatroch is represented by a ciliated ring that often beats opposed to the latter one and lies in a pre-segmental (= peristomial) position (Strathmann 1993; Nielsen 2012). Being present in most annelid families, a metatroch seems to be absent in Echiura (Fig. 9.4J) and Opheliidae. For Capitellidae, Siboglinidae, and Syllidae, the presence of a metatroch is still discussed (Rouse 2000a). Planktotrophic larvae of several polynoid scale worms possess another bundle of long cilia, the oral brush, which seems also to be involved in feeding mechanisms (Phillips and Pernet 1996).

A prominent ventral ciliary band is represented by the neurotroch, which is known at least in some annelid families including many sedentarian taxa, e.g., Orbiniidae (Fig. 9.5), Sabellidae (Fig. 9.6), and Maldanidae. In both planktotrophic and lecithotrophic developmental stages, the neurotroch forms a distinct ventral ciliated area interconnecting proto- and telotroch, which often appears later in larval development (Rouse 1999). The telotroch, defined as a posterior ring of cilia used for locomotion (Strathmann 1993), also appears later in development and is known for both planktotrophic and lecithotrophic developmental stages (Strathmann 1993). The telotroch marks the

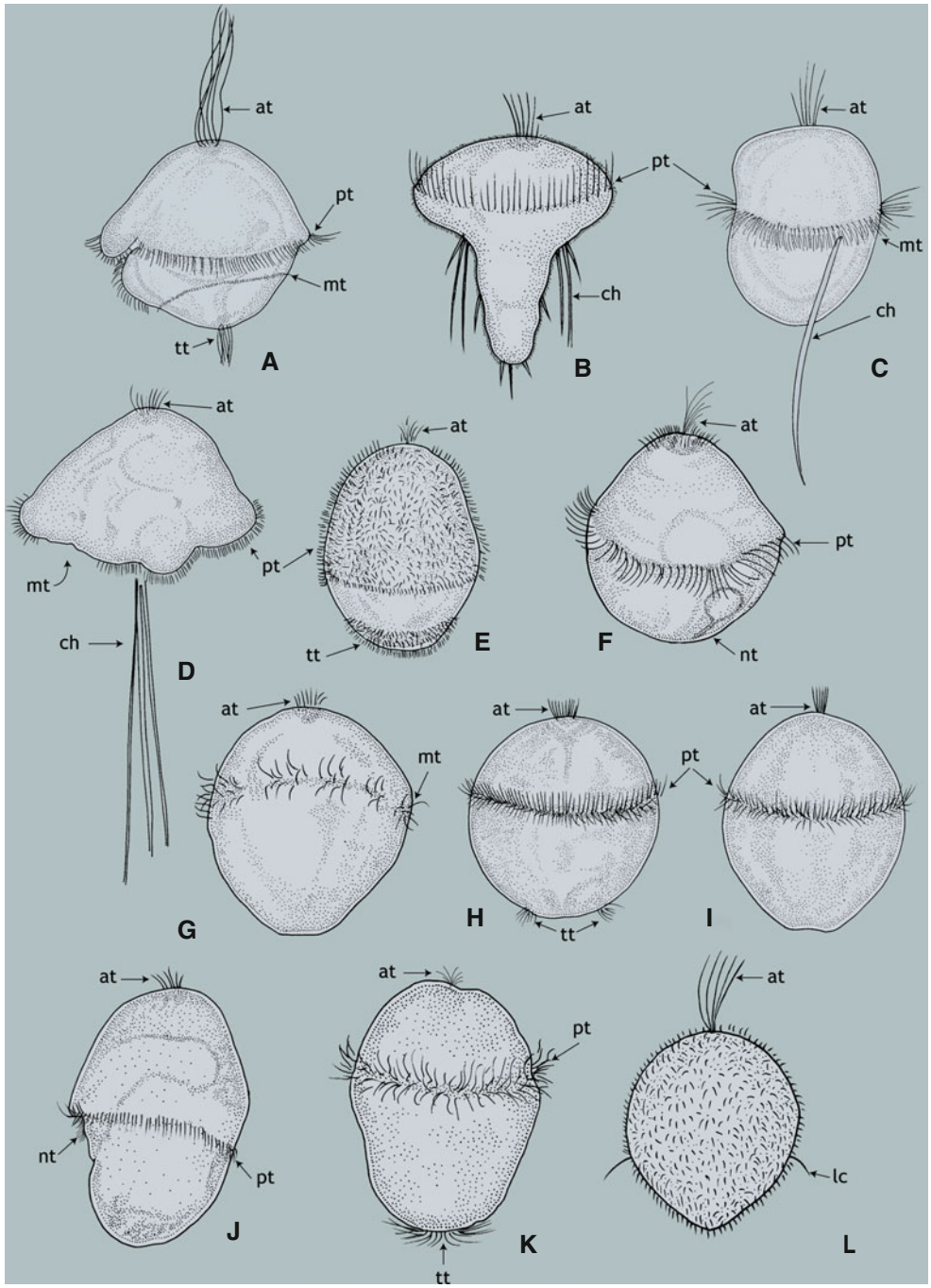


Fig. 9.4 Diversity of annelid trochophore larvae. Anterior (apical) is up in all aspects. (**A**) *Polygordius* sp. (Polygordiidae) after Woltereck (1904). (**B**) *Magelona filiformis* (Magelonidae) after Wilson (1982). (**C**) *Eurythoe complanata* (Amphinomidae) after Kudenov (1974). (**D**) *Owenia collaris* (Oweniidae) after Smart and Von Dassow (2009). (**E**) *Marphysa sanguinea* (Eunicidae) after Prevedelli et al. (2007). (**F**) *Phyllodoce maculata* (Phyllodocidae) after Voronezhskaya et al. (2003). (**G**) *Myzostoma cirriferum* (Myzostomida) after Eeckhaut and Jangoux (1993). (**H**) *Platynereis dumerilii* (Nereididae) after Fischer and Dorresteijn (2004). (**I**) *Phascolosoma perlucens* (Sipuncula) after Jaeckle and Rice (2002). (**J**) *Urechis caupo* (Echiura) after Pilger (2002). (**K**) *Osedax* sp. (Siboglinidae) after Rouse et al. (2009). (**L**) *Chaetopterus variopedatus* (Chaetopteridae) after Henry (1986). Abbreviations: *at* apical tuft, *ch* chaetae, *lc* lateral cilia, *mt* metatroch, *nt* neurotroch, *pt* prototroch, *tt* telotroch

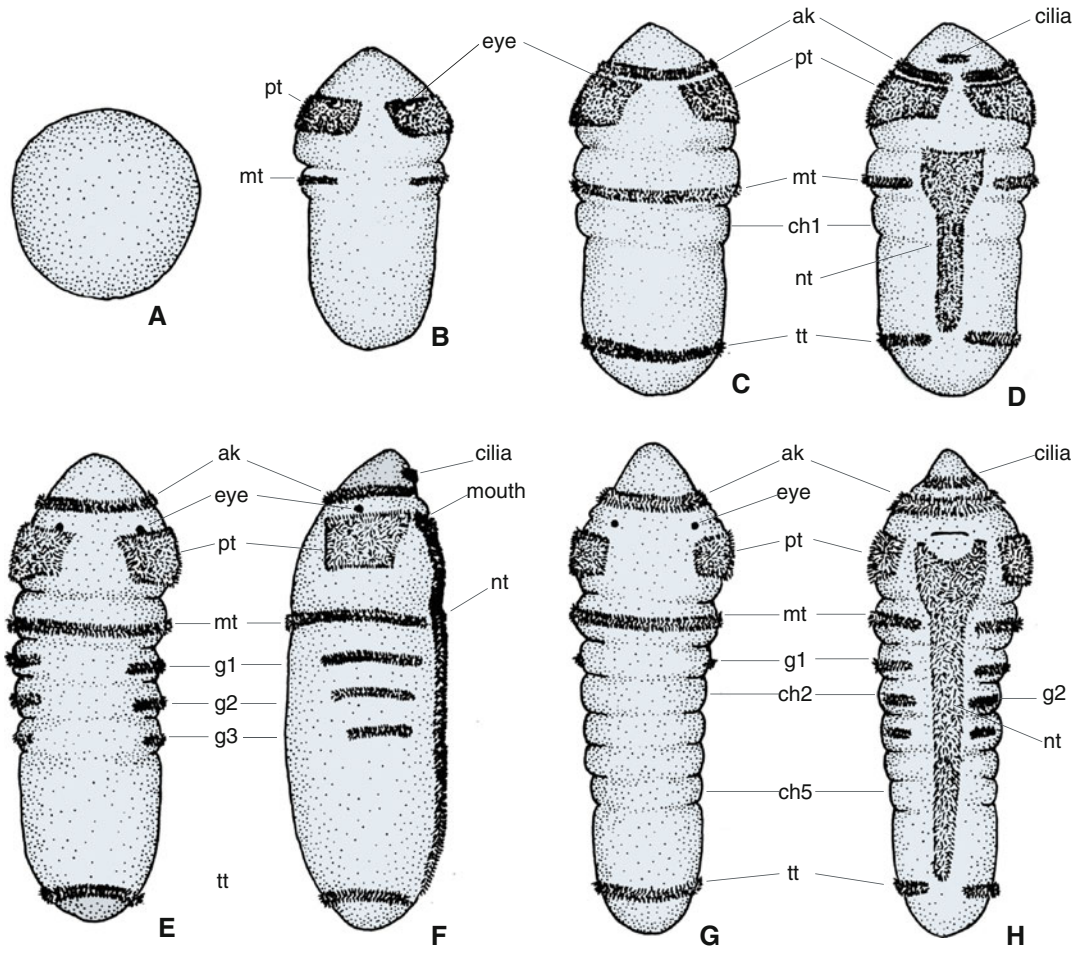


Fig. 9.5 Development of *Scoloplos armiger* (intertidal clade) after Anderson (1959). Anterior (apical) is up in all aspects. (**A**) Unfertilized egg. (**B**) Early 4-day embryo, dorsal view. (**C**) Late 5-day embryo, dorsal view. (**D**) Late 5-day embryo, ventral view. (**E**) Late 6-day embryo, dorsal view. (**F**) Late 6-day embryo, lateral view. (**G**) Early 7-day embryo, dorsal view. (**H**) early 7-day embryo, ventral view. Abbreviations: *ak* akrotroch; *ch 1, ch 2, ch 5* chaetiger 1, 2, 5; *g 1, g 2, g 3* gastrotrochs of chaetigers; *mt* metatroch; *nt* neurotroch; *pt* prototroch; *tt* telotroch

position of the posterior growth zone (Nielsen 2012). Further ciliated bands that may occur in several families are the gastro- and nototroch (= segmentally arranged ventral and dorsal ciliary bands), which are sometimes referred to as paratrochs (Bhaud and Cazaux 1982).

Post-trochophore Development and Larval Forms

Within annelid ontogeny, the metatrochophoral stage usually follows the prototrochophore/trochophore (Fig. 9.7). In this developmental stage, the first signs of segmentation are visible, e.g., the formation of the first parapodia and chaetae. In accordance with individual development, several subdivisions of the metatrochophoral stage are possible (Fischer et al. 2010). In some terebellids and pectinariids, the metatrochophore builds a tube and is called aulophore (Bhaud and Cazaux 1982).

In Oweniidae a special type of trochophore occurs, the so-called mitraria (Fig. 9.4D). The mitraria larva exhibits prominent proto- and metatrochal bands, as well as an apical tuft.

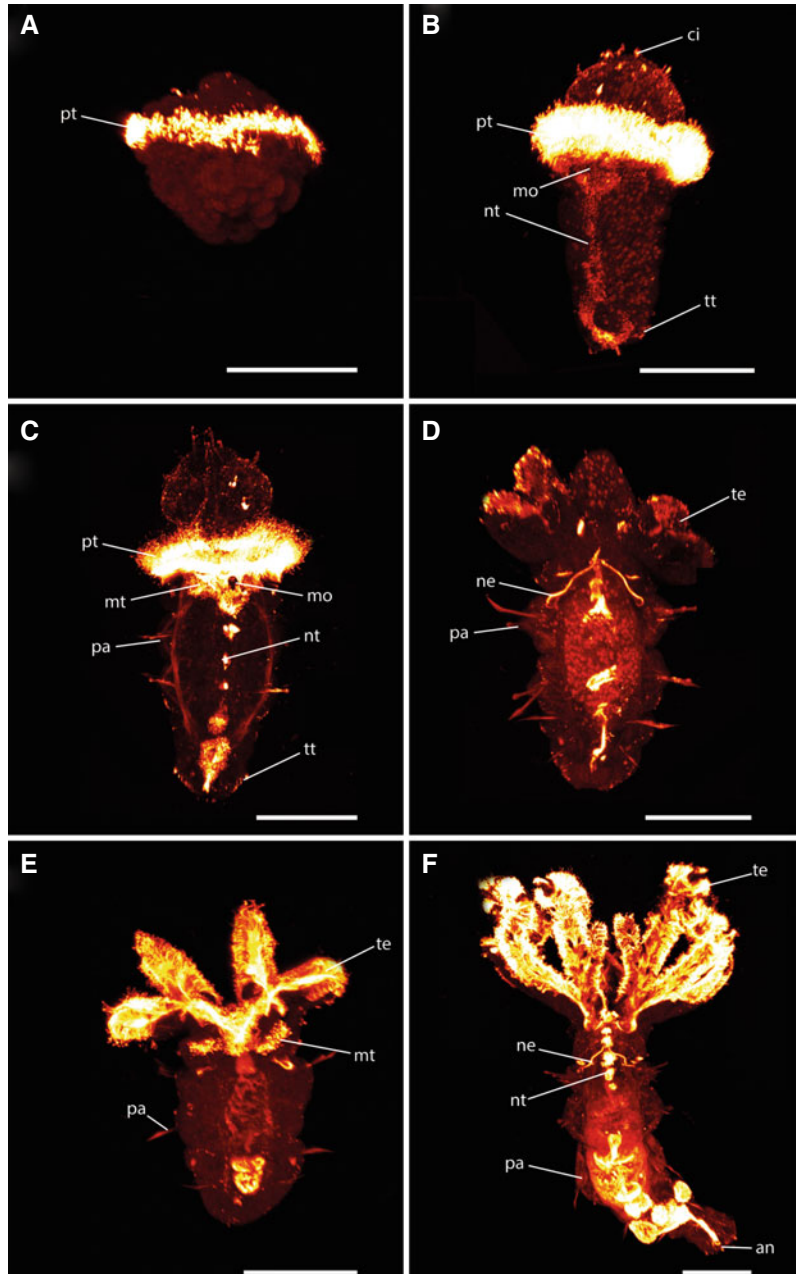


Fig. 9.6 Development of the lecithotrophic developmental stages of *Megalomma vesiculosum* revealed by anti-tubulin staining. All images are in ventral view except of (**D**) which is in dorsal view. Anterior is up. Confocal maximum projections. (**A**) The early, nonfeeding trochophore exhibits a prominent prototroch (*pt*). An apical tuft is lacking. (**B**) The later trochophore gains a well-developed prototroch (*pt*) and cilia (*ci*) at the anterior pole. Furthermore, the ventral neurotroch (*nt*) and the posterior telotroch (*tt*) develop at this stage. (**C**) The early metatrochophore exhibits three pairs of parapodia (*pa*) and a metatroch (*mt*). Neurotroch (*nt*), prototroch (*pt*), and telotroch (*tt*) are still present in this free-swimming but nonfeeding stage. (**D**) Shortly before metamorphosis, the late nectochaete has lost the main ciliary bands and starts development of the adult tentacles (*te*). (**E**) After metamorphosis the juveniles settle within a tube and start feeding. The parapodia (*pa*), the tentacles (*te*), and remnants of the metatroch (*mt*) are exhibited. (**F**) Late juvenile worms show an adult-like morphology. The tentacles (*te*) are well-developed, and the animals start to elongate by posterior segment addition. *an* anus, *ci* cilia, *mo* mouth opening, *mt* metatroch, *ne* nephridia, *nt* neurotroch, *pa* parapodia, *pt* prototroch, *tt* telotroch. Scale bars = 100 μm

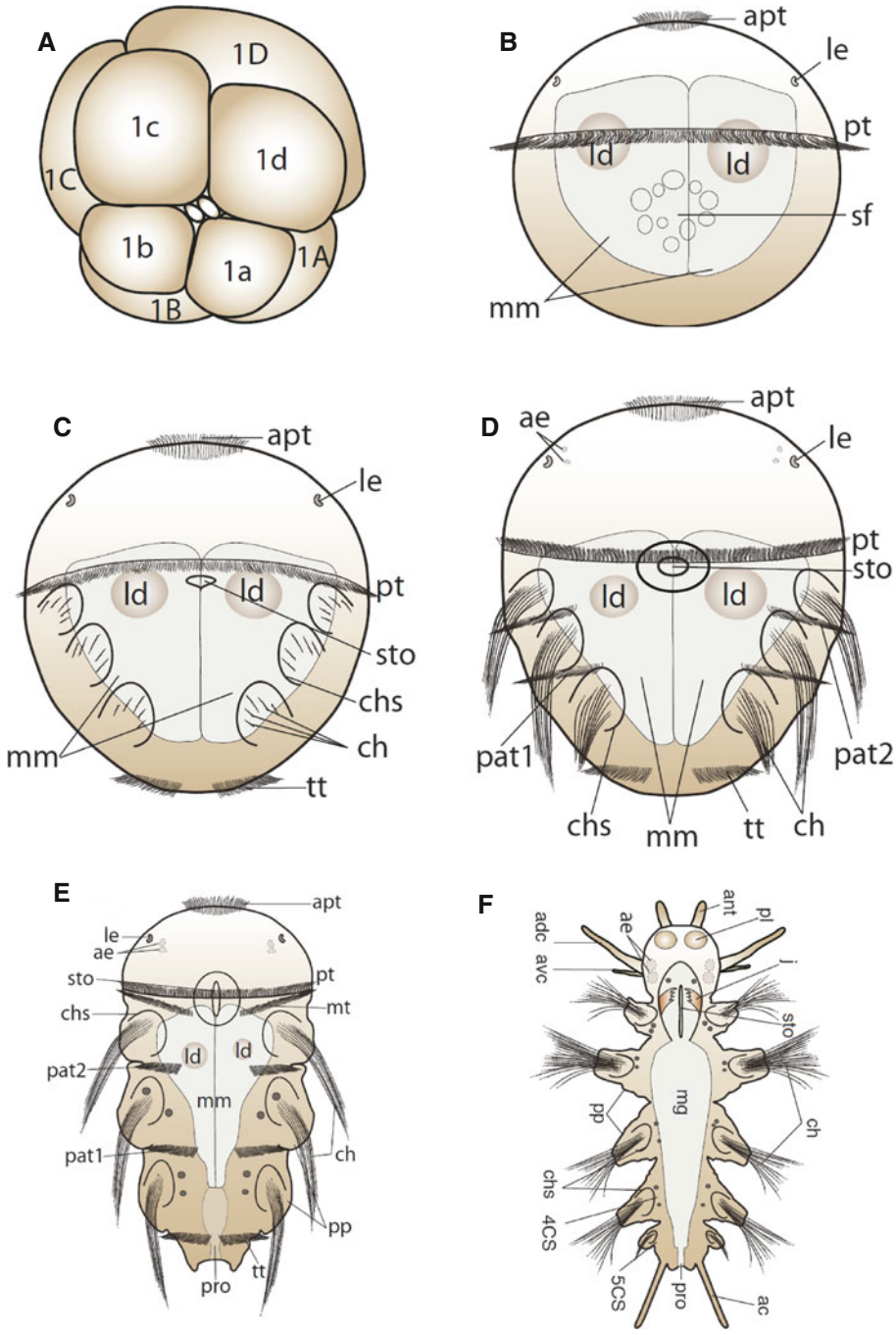


Fig. 9.7 Development of *Platynereis dumerilii*. (**A**) Cleaving embryo, where the third cleavage forms micromeres and macromeres. (**B**) Early trochophore, with prototroch and apical tuft. (**C**) Late trochophore, with simultaneous appearance of the first three larval segments. (**D**) Mid metatrochophore, with developing chaetae reaching over the body wall. (**E**) Early nectochaete, with formation of the metatroch and elongation of the trunk. (**F**) Juvenile, with rapidly growing jaws and further addition of body segments. Combined after Fischer et al. (2010). Abbreviations: *ac* anal cirrus, *ae* adult eyes, *adc* anterior dorsal cirrus, *ant* antenna, *apt* apical tuft, *avc* anterior ventral cirrus, *ch* chaetae, *chs* chaetal sac, *j* jaws, *ld* lipid droplet, *le* larval eye, *mg*, midgut, *mm* macromere, *mt* metatroch, *pat 1*, first paratroch, *pat2* second paratroch, *pl* palps, *pp* parapodia, *pro* proctodeum, *pt* prototroch, *sf* stomodeal field, *sto* stomodeum, *tt* telotroch, *4CS* 4th chaetigerous segment, *5CS* 5th chaetigerous segment

Notably, all ciliary bands are monociliated, an unusual feature for annelid larvae. The hyposphere of the mitraria is strongly reduced, and the juvenile segmental body develops within the larval body (Wilson 1932; Smart and Von Dassow 2009). Another unusual larval type is represented by the rostraria in Amphinomidae and Euphrosinidae (Mileikovsky 1960, 1961). After a trochophore stage with a proto- and metatroch and an apical tuft (Fig. 9.4C), the episphere of the metatrochophore elongates, and tentacles are formed for feeding (Jägersten 1972). Remarkably elongated metatrochophore stages can be found within siboglonids (Southward 1999). The metatrochophore of investigated vestimentiferan siboglinids is sessile and bears a prostomium, a peristomium, and two chaetigers. A prototroch, a neurotroch, and an apical organ are present as well as juvenile/adult organs such as tentacles and pyriform glands (Bright et al. 2013).

The end of the metatrochophore stage is usually marked by the point when the parapodia are fully developed. If present, the next larval stage is represented by the nectochaete (Fig. 9.7E), which is characterized by the presence of functional parapodia which are used mainly for swimming. In *Poecilochaetus* (Spionidae) this stage is sometimes called nectosoma; in other spionids, it refers to the chaetosphaera stage (Bhaud and Cazaux 1982). In this stage or the previous one, most larvae start body elongation and segment formation through the posterior growth zone (Irvine and Seaver 2006). A unique swimming larval form called pelagosphaera is known for Sipuncula (Rice 1976). The body of this larval type can be divided into three body regions: head, mid region including metatroch, and a large trunk. These larvae can be either lecithotrophic or planktotrophic forms, whereas the latter can live up to 6 months in the plankton (Jaeckle and Rice 2002).

After the nectochaetal (or pelagosphaera) stage, metamorphosis occurs. During metamorphosis, the free-swimming larvae change behavioral characteristics and start an adult-like lifestyle as juveniles. This metamorphic step can differ drastically between various species and is markedly pronounced in larvae of *Polygordius*, which rupture the larval body that is later either eaten or discarded (Rouse 2006). Such a transition of lifestyles seems to be less distinct or missing in annelids with lecithotrophic developing stages, where ciliary bands are absorbed or the chaetal morphology changes (Figs. 9.5 and 9.6).

Although late development and subsequent metamorphosis may differ between several taxa, in almost all annelid larvae, the larval episphere becomes the adult prostomium, and the posterior hyposphere becomes the pygidium and the posterior growth zone (Fig. 9.8). The remaining hyposphere forms the peristomium, which lacks chaetae in adult annelids (Nielsen 2004). The segmented body between the peristomium and the pygidium develops by segment formation from the posterior growth zone (Irvine and Seaver 2006).

Larval Feeding Modes

Several types of larval feeding behaviors and developmental modes occur in different annelid families, mostly divided into either feeding and free-swimming planktotrophic larvae with "indirect" development or nonfeeding and mostly less motile embryonic and juvenile forms with "direct" development. The latter rely on maternal sources of nutrition in the form of yolk stored in the egg during oogenesis, feeding on yolk-rich nurse eggs, or translocation of nutrition directly from the parent (Qian and Dahms 2006). An overview of larval feeding in annelids is summarized by Rouse (2006). As direct development occurs without a metamorphosis, developing stages are usually referred to as "embryonic" or "juveniles," avoiding the term "larvae" (Nielsen 2009; Winchell et al. 2010). However, debates remain about the definition of the term "larva," and alternative terminologies exist (McEdward and Janies 1993; Pechenik 1999).

Many authors regard a biphasic life cycle including a planktotrophic trochophore larva as representing the plesiomorphic condition of annelid development (Heimler 1988; Nielsen 2012). Nevertheless, this idea is doubted by some authors (Haszprunar et al. 1995; Rouse 2000a, b). Based on cladistic analyses and ancestral state

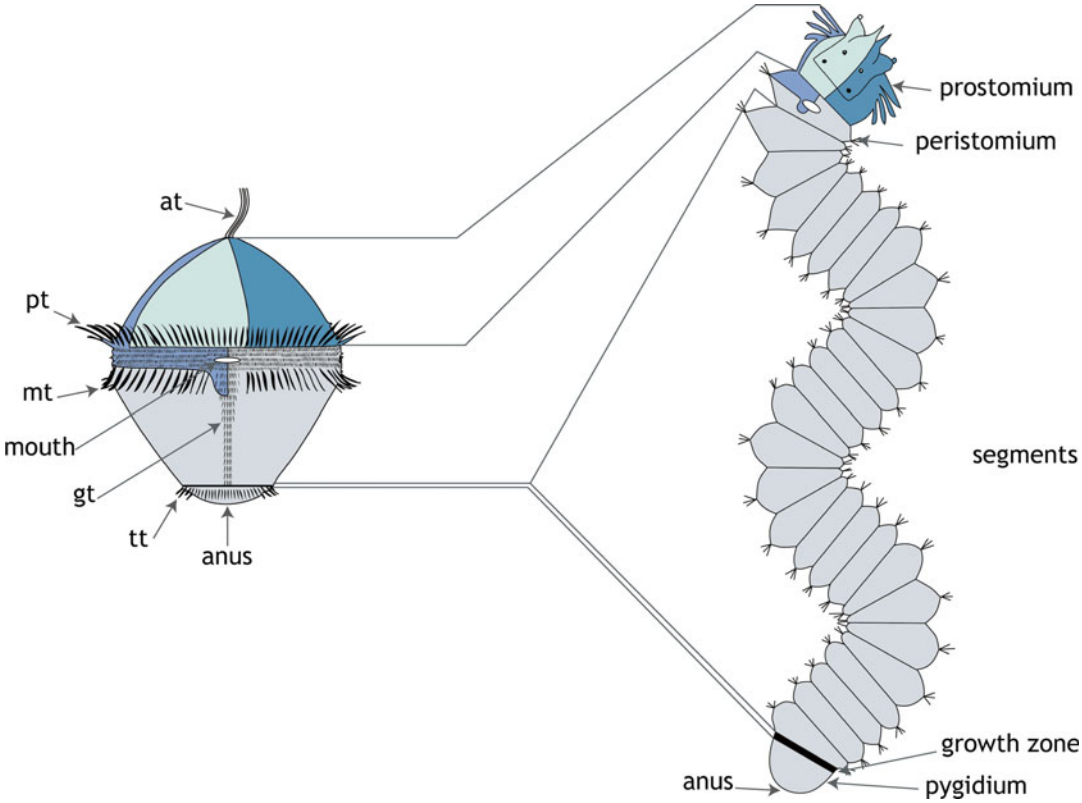


Fig. 9.8 Development of annelids indicating the contribution from the four quadrants (A–D), after Nielsen (2004). Note that the whole segmented body and the pygidium develop from descendants of the (D) quadrant. Abbreviations: *at* apical tuft, *pt* prototroch, *mt* metatroch, *tt* telotroch, *nt* neurotroch, *gt* gastrotroch

reconstruction, multiple events in the evolution of feeding larvae from lecithotrophic ancestors seem more parsimonious to assume. According to this hypothesis, the prototroch had a primary function for locomotion and became independently associated with feeding in several lineages (Rouse 2000a). By studying larval forms of sabellids, Pernet (2003) was able to demonstrate the persistence of reduced ciliary structures for food uptake in nonfeeding larvae. Consequently, it is suggested that the direction of evolution goes from planktotrophy to lecithotrophy in this case. Further on, a functional relation between egg size and gut development has been hypothesized. Non-feeding stages usually bear larger eggs, and due to the increased cell size, gut development might be hindered, resulting in nonfunctional guts in nonfeeding stages (Pernet 2003). As this scenario may be generalized for all annelids, planktotrophy seems to be the likely ancestral state in annelids.

Most planktotrophic larvae exhibit a prominent proto- and metatroch, used for locomotion and food uptake by "downstream feeding" (Rouse 2000a). This is the case for larvae of Amphinomidae, Chrysopetalidae, Glyceridae, Nephtyidae, Oweniidae, Pectinariidae, Polynoidae, and Sabellaridae. However, other annelid families with planktotrophic larval development use different modes of feeding behavior. Taxa with nonfeeding larvae possessing maternally derived nutrition can be found all over the annelid tree within various families, and lecithotrophy may have evolved secondarily (Rouse 2000a). A special case of lecithotrophic development is represented by adelphophagy, where larvae develop by uptake of nutrients from nurse eggs, which are only produced as nutrition reserve (Fig. 9.9), as in some

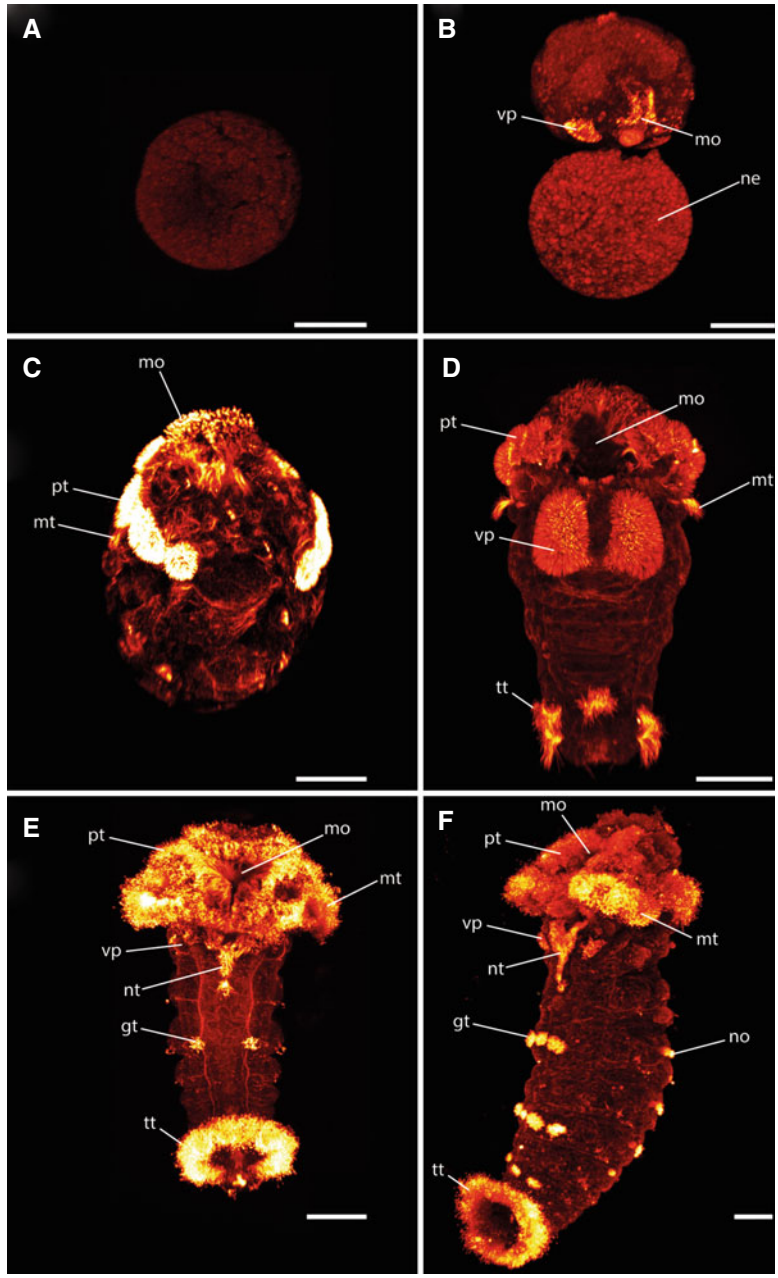


Fig. 9.9 Development of ciliation in larvae of the adelphophagous spionid *Boccardia* cf. *polybranchia* revealed by anti-tubulin staining. All images are in ventral view; anterior is up. Confocal maximum projections. (**A**) Early larvae lack ciliary regions and are full of yolk. (**B**) The trochophoral stage attaches to a nurse egg (*ne*) and starts to digest its nutrients. Ciliation is only exhibited within the mouth opening (*mo*) and in the region of the ventral ciliated patches (*vp*), which are used for attachment. (**C**) The late trochophore develops a distinct prototroch (*pt*) arranged of ciliated patches and a less-prominent metatroch (*mt*). (**D**) In the metatrochophoral stage, the prototroch (*pt*), metatroch (*mt*), the ventral ciliated patches (*vp*), and a telotroch (*tt*) are present. First signs of segmentation are visible in this stage. (**E**) In the late metatrochophore the ventral ciliated patches (*vp*) are reduced. Instead, a distinct neurotroch (*nt*) and several gastrotrochs (*gt*) form. (**F**) In the nectochaetal stage, shortly before leaving the egg capsule, three bands of gastrotrochal ciliary bands (*gt*) are developed, and notopodial cilia (*no*) are exhibited. The remaining ciliated bands are still present. *gt* gastrotroch, *mo* mouth opening, *mt* metatroch, *ne* nurse egg, *no* nototroch, *nt* neurotroch, *pt* prototroch, *tt* telotroch, *vp* ventral ciliated patch. Scale bars = 50 μm

spionid taxa (Gibson and Carver 2013). Moreover, poecilogeny, showing different types of development in one species, seems to be common in some spionid genera (Blake and Kudenov 1981; Levin 1984; Chia et al. 1996).

Segmentation

Segmented annelids generate their first segments, usually simultaneously, as larvae, and later segments are sequentially added from a posterior growth zone (Irvine and Seaver 2006). Consequently, some authors differentiate between primary and secondary segments, an idea that goes back to Iwanoff (1928) who postulated a distinct ontogenetic origin for both sets of segments. Segment formation on the cellular level is well understood for clitellate embryos, which are all direct developers and often show huge and therefore experimentally manipulable eggs. Segmentation in leeches is strongly correlated with cell division patterns of teloblast cells and their descending blast cells (Fig. 9.10). All teloblasts originate from descendants of the D quadrant, with a pair of mesoteloblasts (M) being derived from 4d and four pairs of ectodermal teloblasts (N, O, P, Q) from 2d descendants. Mechanisms of the specification of the ectoteloblast lineage are different between the hirudinean *Helobdella* and the oligochaete *Tubifex*. In *Helobdella* the O/P teloblasts constitute an equivalence group, as they are both pluripotent and may subsequently follow either the O or the P fate (Weisblat and Blair 1984). On the contrary, in *Tubifex*, the fate of the P teloblast is determined by birth, whereas the O teloblast is initially pluripotent and is restricted to the fate of the O lineage due to signaling from the P lineage (Arai et al. 2001). Each teloblast undergoes repeated series of unequal division, producing bandlets of the so-called blast cells. The N and Q lineages produce two different types of blast cells, which appear in alternation. The four ectodermal bandlets of each side of the bilateral embryo join and form together with the mesodermal band, the germinal band, which lies at the surface of the embryo. During gastrulation, the germinal bands from both sides of the embryo coalesce into the germinal plate, the origin of segments. The stereotyped cell divisions of all blast cells contribute to the forming segments (Shimizu and Nakamoto 2001; Weisblat and Huang 2001; Irvine and Seaver 2006). Each ectodermal band contributes neural and epidermal progeny, but two thirds of the neurons are derived from the N teloblast (Weisblat and Huang 2001). Ectodermal segmentation can be divided into two steps. In the first step, distinct cell clusters are generated autonomously by each bandlet. These separate clusters are aligned with the cell cluster derived from the mesodermal bandlet in a second step (Shimizu and Nakamoto 2001). It has been shown experimentally for *Helobdella* that hemilateral ablation of mesodermal precursor cells results in the loss of ectodermal segmental organization. In contrast, mesodermal boundaries are determined autonomously, without positional cues from ectodermal tissue (Blair 1982).

The cellular basis of segment generation in non-clitellate annelids is less well studied. One reason seems to be that they are more difficult to handle experimentally due to the smaller size of their embryos and larvae (Irvine and Seaver 2006). An obvious difference is the absence of large visible teloblasts in non-clitellate annelids (Seaver et al. 2005). However, using semiautomated cell tracking, mesoteloblast-like stem cells were revealed for *Platynereis dumerilii* which also form mesodermal bands of daughter cells (Fischer and Arendt 2013). For this species, two distinct sets of stem cells could be described for the posterior growth zone, where many rounds of division of small populations of teloblast-like stem cells generate new segments (Gazave et al. 2013). In contrast, Seaver et al. (2005) could not find evidence of a teloblastic growth zone in *Capitella teleta* and the serpulid *Hydroides elegans* using incorporation of BrdU, therewith confirming older studies (Wilson 1890; Shearer 1911). Instead, segments in larvae arise from a field of mitotically active cells located in lateral body regions. However, the authors could not

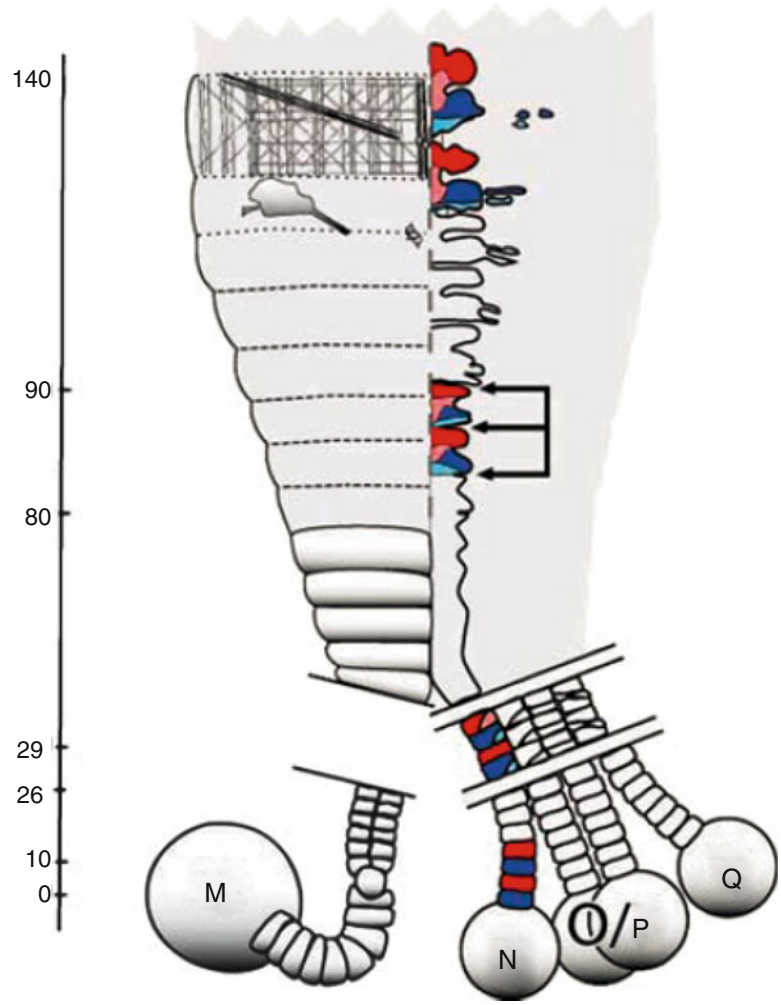


Fig. 9.10 Schematic representation of the stem cell-mediated, lineage-dependent segmentation in leeches and other clitellate annelids. Anterior is to the *top*. Pairs of *diagonal lines* indicate discontinuities in the depicted structures. One bilateral pair of mesodermal stem cells (*M* teloblasts) and four bilateral pairs of ectodermal stem cells (*N*, *O/P*, *O/P*, and *Q* teloblasts) constitute the posterior growth zone. Two types of blast cells are contributed by the N lineage, designated ns (*red*) and nf (*blue*), which arise in alternation. The numbers on the *left* side indicate the progressing time during segment formation given in hours of clonal age. *Arrows* indicate the delimitation of two ganglionic primordia (From Rivera and Weisblat 2009, with permission from the publisher)

rule out if inconspicuous teloblast-like cells might be present. For *Chaetopterus*, it has been suggested that at least the first 15 segments are formed by subdivision of existing anlagen and not by a posterior growth zone (Irvine et al. 1999). Similarly, formation of repetitive structures in myzostomids differs from an addition governed by a posterior growth zone. As such, during development the third pair of parapodial structures appears first, followed by the fourth pair, second and fifth pair (simultaneously), and first pair (Jägersten 1940). Future studies of especially non-clitellate annelids are necessary to further assess the existing variability in the process of segment formation throughout Annelida.

Muscular System

Annelids show a huge variety of muscular organization, with longitudinal musculature organized in separate bands or massive plates and circular musculature that can be fully developed, incomplete, or even completely missing (Tzetlin and Filippova 2005). Therefore, it comes without surprise that differences in the development of the muscular system have been found across the investigated taxa. Phalloidin labeling coupled with confocal microscopy revealed an origin of muscular development posterior of the apical organ in the phyllodocid *Phyllodoce groenlandica*, with distinct transversal muscles starting to

grow posteriorly. Subsequently, several longitudinal muscle fibers start to develop and grow in posterior direction. Simultaneously, outer circular muscle fibers begin to appear in a progression from anterior to posterior. The longitudinal muscle fibers reach the anal region approximately 7 days after hatching, and additional circular muscle fibers forming distinct rings develop from anterior to posterior. Additional longitudinal muscle fibers develop in the dorsal region, forming a continuous layer. Musculature of the digestive system is hardly recognizable in early stages. Notably, the organization of the body wall musculature starts before the formation of the first segments (Helm et al. 2013). Similarities to this kind of musculature development have been found in several other annelids. As such, an anterior origin in either lecithotrophic embryos or planktotrophic larvae is also reported for, e.g., capitellids, clitellates, nereidids, and sabellariids (Hill 2001; Bergter and Paululat 2007; Hunnekuhl et al. 2009; Brinkmann and Wanninger 2010a; Fischer et al. 2010). No circular musculature could be detected in developing stages of the maldanid *Axiothella rubrocincta*, even though they are present in adults (Brinkmann and Wanninger 2010b).

In contrast to the description for *Phyllodoce*, musculature of the digestive system develops before the body wall musculature in planktotrophic larvae of serpulids and sabellariids (McDougall et al. 2006; Brinkmann and Wanninger 2010a). Temporal shifts in the developmental onset of several muscle groups, a phenomenon described as heterochrony, are a common theme when comparing myogenesis between different and even closely related annelid species and are even more pronounced in the comparison of planktotrophic with lecithotrophic developing species (McDougall et al. 2006; Brinkmann and Wanninger 2010a; Helm et al. 2013). As in *Phyllodoce*, many annelid species show a successive appearance of circular musculature from anterior to posterior, which has been also described, e.g., for the tubificid *Limnodrilus* and the serpulid *Filograna implexa* (Bergter et al. 2007; Wanninger 2009). In contrast, in sipunculids, *Platynereis massiliensis* and the leech *Erpobdella octoculata*, anterior circular muscles are formed synchronously (Wanninger et al. 2005; Bergter et al. 2007; Kristof et al. 2011; Helm et al. in press). Muscular development in the non-segmented sipunculids as analyzed for *Phascolion strombus*, *Phascolosoma agassizii*, *Thysanocardia nigra*, and *Themiste pyroides* shows that the first anlagen of circular body wall musculature appear simultaneously. Rudiments of four longitudinal retractor muscles appear at the same time, with longitudinal muscle fibers forming a pattern of densely arranged fibers around the retractor muscles (Kristof et al. 2011).

Neurogenesis

Annelids show a huge variety of adult nervous system organization, and until today the ancestral ground pattern remains under discussion (Bullock 1965; Orrhage and Müller 2005). The development of the nervous system, however, has been investigated in a surprisingly small number of taxa. Several transmission electron microscopy (TEM)-based studies on the larval nervous system of phyllodocids and serpulids were published by Lacalli (1981, 1984, 1986). Some detailed comparative studies were conducted concerning the anatomy of the larval apical organ. Immunocytochemical studies revealed an almost universal occurrence of an apical organ with flask-shaped cells in larvae of Annelida, Mollusca, Entoprocta, and Platyhelminthes, exhibiting FMRFamide- and serotonin-like immunoreactivity (e.g., Hay-Schmidt 2000; Wanninger 2009). Usually, the apical organ in annelid trochophores is simple, containing a few flask-shaped cells which have slender necks, dense cytoplasm, and a single projecting cilium (Lacalli 1981). Whereas these cells are missing in echiurans and many other annelids, sipunculans show a more complex apical organ with up to ten flask-shaped cells (Wanninger 2008). Marlow et al. (2014) analyzed the molecular fingerprint of apical organ cells in *Platynereis dumerilii*. They found that orthologs of *six3* and *foxq2* are involved in the formation of the apical

plate, whereas the apical tuft is formed in a central *six3*-free area of the apical plate.

Besides this, only few comprehensive studies for developmental sequences of annelids combining immunocytochemical staining coupled with confocal laser scanning microscopy exist (Hessling 2002; Hessling and Westheide 2002; Voronezhskaya et al. 2003; McDougall et al. 2006; Brinkmann and Wanninger 2008; Kristof et al. 2008; Fischer et al. 2010; Winchell et al. 2010; Helm et al. 2013, in press). Main targets for these studies were serotonin, a biogenic amine involved in neuronal signaling, and the neuropeptide FMRFamide. Labeling of tubulin is additionally used to stain neurotubules. In summary, these studies show that neurogenesis in annelids is variable, following different developmental pathways. Planktotrophic larvae typically bear a serotonergic nerve ring underlying the prototroch and an apical organ that bears serotonergic and FMRFamidergic cells. The development of the larval nervous system usually starts from two subsystems (Fig. 9.11). FMRFamidergic immunoreactivity increases from anterior toward posterior during nervous system development. In *Phyllodoce* and some other annelids, a single serotonergic neuron located at the posterior pole of the larva is present (Fig. 9.11A). From here, anteriorly projecting nerve fibers start to grow, outlining the future ventral nerve cords (Voronezhskaya et al. 2003). Such a posterior origin of serotonin-like immunoreactivity was also detected in another phyllodocid, in syllids, nereidids, and orbiniids (Orrhage and Müller 2005; McDougall et al. 2006; Fischer et al. 2010; Helm et al. 2013). In contrast, the investigated sabellariids, spirorbids, and sipunculans show no evidence for a posterior serotonergic cell (Brinkmann and Wanninger 2008; Kristof et al. 2008; Brinkmann and Wanninger 2009). Later, the adult nervous system starts to develop along pathways established by the earliest peripheral neurons of the larva. However, other authors propose a separate development of the larval and adult nervous system (Lacalli 1984) or find that the larval nervous system is integrated in the adult one (Hay-Schmidt 1995). The relative timing of events during neurogenesis shows major shifts between compared species and is regarded as cases of heterochrony (McDougall et al. 2006; Brinkmann and Wanninger 2008; Helm et al. 2013).

A different picture is found in direct developing lecithotrophic species as investigated for nereidids. In *Nereis arenaceodentata*, the nervous system has developed already much of the complexity of the adult at hatching. This includes a large brain and the presence of circumesophageal connectives, nerve cords, and segmental nerves. Within 1 week after hatching, cephalic sensory structures and brain substructures are differentiated, and the nervous system architecture resembles that of adults (Winchell et al. 2010). A similar developmental pattern of the nervous system has been described for *Platynereis massiliensis* (Helm et al. in press).

Analyses of the development of the nervous system of the non-segmented Echiura and Sipuncula gained major interest, as they provided direct ontogenetic evidence for the indirectly inferred loss of segmentation in these taxa as suggested by molecular phylogenies. Using immunocytochemistry, a metameric organization of the nervous system has been demonstrated for two echiuran species: *Urechis caupo*, which has planktotrophic larvae, and *Bonellia viridis* with directly developing lecithotrophic stages (Hessling 2002; Hessling and Westheide 2002). The development of the nervous system in *Bonellia viridis* proceeds from anterior to posterior. This is obvious in early larvae, which show a full set of serotonergic perikarya in the anterior region, while this pattern is incomplete in the posterior area. This pattern suggests the presence of a posterior growth zone (Hessling and Westheide 2002). Similarly, in larval stages of *Urechis caupo*, a serial repetitive distribution of serotonin-containing neurons and their corresponding pairs of peripheral nerves, both formed in an anterior-posterior gradient, imply a segmental pattern. Moreover, larvae show a paired origin of the ventral nerve cord (Hessling 2002). Another case of "ontogeny recapitulating phylogeny" has been demonstrated for sipunculans, where neurogenesis of *Phascolosoma agassizii* follows a segmental pattern. During

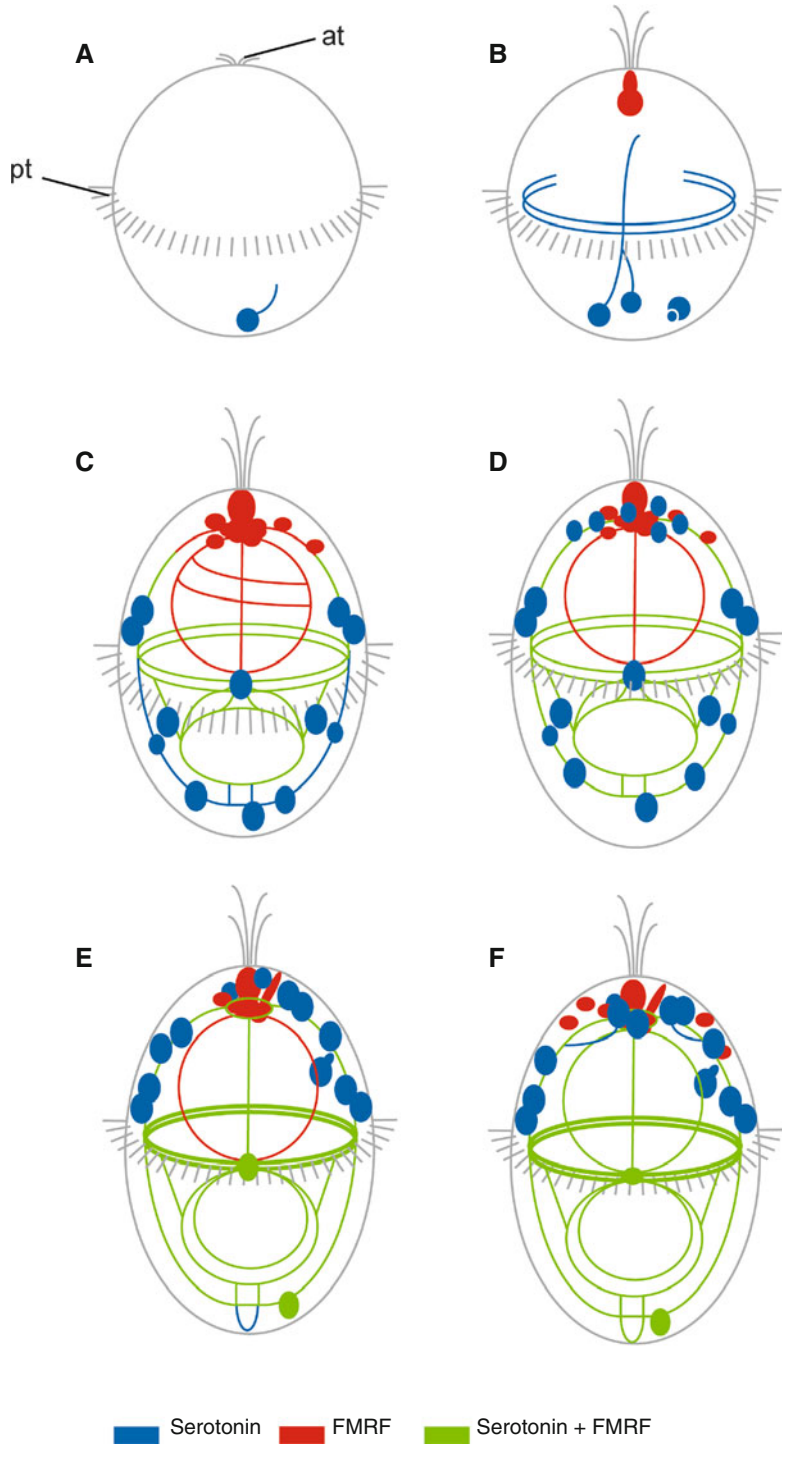


Fig. 9.11 Schematic representation of neuronal development in larval stages of *Phyllodoce groenlandica* exhibiting formation from two subsystems (Modified from Helm et al. (2013)) Diagrams are in ventral view; anterior is up. Major types of neuronal structures are color coded. (**A**) 0.5 days past hatching (*dph*). First serotonergic immunoreactivity appears at the posterior pole. (**B**) 2 dph. Nervous system originates from posterior (serotonin) and anterior (FMRFamide). (**C**) 7 dph. Serotonergic and FMRFamidergic immunoreactivities start to overlap to the greatest extent. (**D**) 11 dph. Numerous serotonergic cells are detectable within the epi- and the hyposphere. (**E**) 20 dph. Serotonergic and FMRFamidergic nerve cells are limited mainly to anterior regions. (**F**) 34 dph. The larval nervous system is fully developed. *at* apical tuft, *pt* prototroch

development of this species, a pair of FMRFamidergic and serotonergic axons gains four pairs of associated serotonergic perikarya and interconnecting commissures in an anterior-posterior progression. During later larval stages, the commissures disappear and the two serotonergic axons fuse, forming a single ventral nerve cord, and after cell migration, a nonmetameric central nervous system can be found in adults (Kristof et al. 2008). Interestingly, neurogenesis of the sipunculan *Phascolion strombus* lacks any signs of a segmented origin, and serotonergic structures are missing completely in their larvae, which may be the result of the abbreviated larval phase in this species (Wanninger et al. 2005).

Nephridia and Coelomogenesis

Most adult annelid taxa possess metanephridia as excretion organs; however, in the development they are usually preceded by protonephridia, which can be found in larvae and sometimes also in developing juveniles (Bartolomaeus and Quast 2005). These larval excretory organs were termed "head kidneys" by Hatschek (1886) and are located anteriorly in trochophore stages, closely behind the larval eyes (Bartolomaeus and Quast 2005). Later, homologous organs were also described from lecithotrophic developmental stages, as shown for the direct developing species *Scoloplos armiger* (intertidal clade), which has no free-swimming larval stage (Bartolomaeus 1998). Head kidneys are present in some echiurans but are missing in sipunculid developmental stages. It is hypothesized that the ancestral state of larval annelid protonephridia was an organ composed of three cells: a terminal cell, a nephropore cell, and a duct cell (Bartolomaeus and Quast 2005). This simple construction has been modified in adaptation to different developmental modes in several annelid lineages and especially in planktotrophic larvae, where the nephropore cell is often missing (Kato et al. 2011, 2012).

Adult segmental nephridia differentiate from a single anlage, consisting of few cells which line a small lumen filled with microvilli. This duct becomes ciliated, and the most proximal cells are separated during coelomogenesis. During coelomic cavity growth, the proximal part of the anlage is passively opened, forming the metanephridial funnel. A truncation of this process due to suppression of the separation of duct cells leads to a differentiation into a protonephridium, as, for example, observed in several taxa of the Phyllodocida (Bartolomaeus 1999). The development of metanephridia in the non-segmented sipunculids as investigated for *Golfingia minuta* seems to be similar as in segmented annelids. An overview of annelid nephridial organs is given by Bartolomaeus and Quast (2005).

Segmented annelids show a heteronomous coelomogenesis, and the coelomic lining is of mesodermal origin. Prior to metamorphosis (if present), a pair of unsegmented coelomic cavities stretches out over the first larval segments. This process has been studied in detail for the serpulid *Spirorbis spirorbis*, where two caudally located mesodermal cell clusters proliferate cells, which merge to surround the gut ventrally close to the anus. Fluid starts to accumulate between spaces of desmosomes, and at the same time myofibrils appear, and due to growth and separation processes, this myoepithelium develops into the coelomic lining. After migration, the two coelomic cavities meet dorsally, completely surrounding the gut. Postmetamorphic stages develop strictly segmental coelomic cavities during segment formation. Coelomic cavities are highly reduced or completely missing in leeches and several meiofauna annelids with a presumed progenetic origin (Koch et al. 2014).

GENE EXPRESSION

Only few model annelids are well characterized concerning gene expression patterns. Prime candidate taxa are leeches of the genera *Helobdella* and *Hirudo*, the sedentary annelid *Capitella teleta*, and the errant annelid *Platynereis dumerilii*. Besides this, some studies exist for the chaetopterid *Chaetopterus*, some sipunculid worms, and a few serpulids as well as for additional nereidid and clitellate species. These

studies mainly used candidate gene approaches, where orthologs were chosen based on studies in arthropods and vertebrates. Main points of interest are the genomic basis of segmentation, Hox gene expression, and nervous system development as well as gastrulation and gut development.

Segmentation

Metameric segmentation can be found in vertebrates, arthropods, and annelids, and the distant phylogenetic position of these taxa gave rise to the question of how often this feature evolved in animals (Seaver 2003). Traditionally well investigated is the molecular background of segmentation in vertebrates and arthropods, which show profound differences (Tautz 2004). Given the fact that a close relationship between arthropods and annelids was suspected as formulated in the Articulata hypothesis (Scholtz 2002), a possible common ancestry of segmentation in these taxa became a focus of many evolutionary developmental studies of annelids. Genes or gene families identified to play a vital role in segment formation in arthropods were used as candidates in several studies (Wedeen and Weisblat 1991; Prud'homme et al. 2003; Seaver and Kaneshige 2006; Saudemont et al. 2008; Dray et al. 2010; Steinmetz et al. 2011).

Best investigated is the molecular background of segmentation in *Platynereis dumerilii* and *Capitella teleta*. For *Drosophila* it has been demonstrated that para-segmental borders are generated by an interaction between the segment polarity genes *wingless* and *engrailed* (Tautz 2004). The gene *wingless* (or *Wnt1*) is part of the Wnt gene family, and *engrailed* is a homeodomain-bearing transcription factor. As in arthropods, a role in segment formation is suggested for this pair of genes in *P. dumerilii* (Prud'homme et al. 2003), where an expression of continuous ectodermal stripes is observed for these genes at the border of the segments during their formation. However, investigation of other annelid taxa questions the conserved nature for *engrailed* as a "segmentation gene" in annelids. In *Chaetopterus*, *engrailed* is expressed during all larval stages in different structures or organs, and no signs of a putative segment polarity pattern of expression are obvious (Seaver et al. 2001). Congruently, no conserved segment polarity pattern was found investigating the expression of this gene in developing *C. teleta* or *Hydroides elegans* individuals (Seaver and Kaneshige 2006). Finally, ablation of individual cells expressing *engrailed* in the leech *Helobdella* did not hinder remaining segmental clones in their normal development (Seaver and Shankland 2001). Consequently, the establishment of segment polarity in the leech (and possibly many other annelid taxa) seems to be independent of cell interactions across the anterior-posterior axis as known for arthropods (Seaver and Shankland 2001).

A set of pair rule genes is expressed in *Drosophila* and many other arthropods to pattern the embryo across the anterior-posterior axis, including *eve*, *hairy*, and *runt* (Damen 2007). All or some of these genes were investigated in detail for *C. teleta* and *Helobdella robusta*. In contrast to *Drosophila*, where it is expressed in stripes in the growth zone, the *Capitella* ortholog of *hairy* (*Cap-hes1*) shows an expression limited to a small band of cells in each larval segment. In juveniles its expression is limited to a small mesodermal domain of the posterior growth zone (Thamm and Seaver 2008). In vertebrates and some arthropods, the expression of hairy is controlled by the Notch pathway (Stollewerk et al. 2003). In *Capitella*, *Notch* and *hairy* do not show a broadly overlapping expression, with a *Notch* localization in already formed segments, anterior to the *hairy* signal (Thamm and Seaver 2008). In *Helobdella*, *hairy* is expressed in teloblasts and primary blast cells. The expression peak correlates with the production of blast cells by the teloblasts. However, no striped pattern suggesting a pair rule function was found (Rivera et al. 2005). Similarly, *Notch* is also expressed in teloblasts and blast cells, and functional studies revealed that the disruption of the *Notch*/*hairy* signaling results in a disruption of segmentation (Song et al. 2004; Rivera and Weisblat 2009). For *Platynereis dumerilii*, 15 *hairy* paralogs could be

identified, which are expressed in mesodermal tissue, forming segments, and during neurogenesis, where it may be involved in the patterning of the nervous system (Gazave et al. 2014). However, these authors also found no overlap with the expression of *Notch*.

The expression patterns of the arthropod pair rule genes *eve* and *runt* do not suggest a similar role in *Capitella*. Instead, the expression of *runt* can be found in the brain and ventral nerve cord, as well as the fore- and hindgut. Two *eve* paralogs were characterized for *Capitella*, both showing a complex expression pattern, which does not correspond to segmental stripes as expected by results from *Drosophila* (Seaver et al. 2012). Likewise, de Rosa et al. (2005) did find such a pattern of *eve* expression in developing *Platynereis dumerilii*. However, these authors speculate about a role of this gene in the posterior addition of segments. A detailed functional study for *eve* has been conducted for *Helobdella* (Song et al. 2002). Segments arise sequentially from five pairs of teloblasts in leeches (see above), and *eve* is expressed in these teloblasts and their primary blast cells in *Helobdella*. Later embryos express *eve* in cells of the ventral nerve cord which stem from the N teloblast. Morpholino knockdowns suggest a role of *eve* in early cell division through early segmentation in *Helobdella*. However, no pair rule pattern is found for this gene in the leech.

The zinc finger transcription factor *hunchback* plays the role of a gap gene in *Drosophila*, which defines expression domains of pair rule and Hox genes (see Vol. 5, Chapter 1). Moreover, *hunchback* is involved in mesoderm development and neurogenesis. In *Platynereis dumerilii*, hunchback expression is detected in mesodermal cells belonging to the posterior growth zone of juvenile worms. Additionally, expression in the precursors of the somatic segmented mesoderm, formed during larval development, could also be confirmed, a striking similarity with arthropods (Kerner et al. 2006). However, an expression of *hunchback* could not be detected in segmental precursor cells of the posterior growth zone in *Capitella* and *Helobdella*, and a role in the patterning of the anterior-posterior axis was rejected for these species (Iwasa et al. 2000; Werbrock et al. 2001).

NKL genes are a family of homeodomain transcription regulators that are involved in the patterning of mesodermal derivatives in *Drosophila* (Holland 2001; Jagla et al. 2001). The expression of seven genes of this cluster has been investigated in developing *Platynereis dumerilii*, and all are involved in the specification of mesodermal derivatives including muscular precursors (Saudemont et al. 2008). Notably, five of the investigated genes (*NK4*, *Lbx*, *Msx*, *Tlx*, and *NK1*) show an expression in complementary stripes in the mesoderm and/or ectoderm of developing segments. Moreover, genes of the Hedgehog signaling pathway show a similar striped pattern of expression, and segment formation in *P. dumerilii* is disrupted when treated with molecules antagonistic to this signaling (Dray et al. 2010).

Wnt genes regulate a wide range of developmental processes, including axis elongation and segmentation (Cadigan and Nusse 1997). This gene family ancestrally includes 13 paralog groups, of which several metazoan lineages lost some of the genes (Janssen et al. 2010). In *Platynereis dumerilii* and *Capitella teleta*, all paralog groups besides *Wnt3* could be discovered. In the leech *Helobdella robusta*, only nine paralog groups are present, with additionally *Wnta*, *Wnt8*, and *Wnt9* missing. Most Wnt genes in *P. dumerilii* are expressed in ectodermal segmental stripes and/or in the area around the pygidium (Janssen et al. 2010). Expression analyses in *H. robusta* and *C. teleta* led to comparable results (Cho et al. 2010). Due to similarities with arthropods, a role of Wnt genes in segment formation in both annelids and arthropods is suggested by some authors (Janssen et al. 2010).

In summary, the candidate gene approach led to the discovery of many similarities as well as differences between annelids and arthropods in gene expression patterns during the formation of segments. The expression of some genes at segmental boundaries in *Platynereis dumerilii* shows a remarkable similarity to arthropods. However, for other annelid taxa investigated for these candidate genes (as, e.g., for *engrailed* or

hunchback), the picture becomes less clear, and future studies covering segment formation in more annelid taxa are clearly wanted. Moreover, fewer similarities are found compared with arthropods when investigating pair rule genes. In the discussion of a putative common ancestry of segmentation in annelids and arthropods, different authors come to different conclusions using basically the same set of evidence (de Rosa et al. 2005; Thamm and Seaver 2008). However, homology of genes expressed during segment formation must not imply a homology of a segmented body plan itself. At present, available developmental, paleontological, and phylogenetic evidence supports a convergent evolution of segmentation in arthropods and annelids (Couso 2009; Chipman 2010). Given this hypothesis, co-option of the same set of genes into the process of segment formation leading to a convergent pattern of gene expression can explain the similarities found between annelids and arthropods (Chipman 2010; Ferrier 2012).

Hox and ParaHox Genes

Hox genes comprise a family of transcription factors bearing a DNA-binding homeodomain (Gellon and McGinnis 1998). Hox genes are usually found as linked chromosomal clusters and show spatial and temporal collinearity (Garcia-Fernandez 2004). This means that genes from the 5′-end of the cluster are usually expressed more anteriorly than the ones from the 3′-end. In similar fashion we also see a temporarily earlier onset of genes from the 5′-end compared to those from the 3′-end. In bilaterian animals Hox genes are mainly involved in the patterning of body regions; however, several examples of co-option into other areas of expression are described (Wagner et al. 2003). All these characteristics made this set of genes a prime target for evolutionary developmental biologists to understand major transitions in animal body plan evolution (Akam 1998).

For annelids, the genomic organization of the Hox cluster is only fully described for *Capitella teleta* and *Helobdella robusta* (Fröbius et al. 2008; Simakov et al. 2013). In *Capitella*, assembled whole genome shotgun data found Hox genes distributed on three scaffolds, with one scaffold containing the *Post1* genes clearly separated from the others. In contrast, the leech *Helobdella* shows an extensive fragmentation of the Hox cluster (Simakov et al. 2013). For *Capitella*, 11 Hox genes (*lab*, *pb*, *Hox3*, *Dfd*, *Scr*, *lox5*, *Antp*, *lox4*, *lox2*, *Post2*, *post1*) corresponding to 11 different paralog groups were detected, and the presence of these genes are regarded as ancestral for lophotrochozoans in general (Fröbius et al. 2008; Simakov et al. 2013). Interestingly, *Helobdella* also shows a derived pattern here, with the duplication of two paralog groups (five copies of *Scr* and two copies of *Post2*) and the loss of orthologs of *pb* and *Post1* (Simakov et al. 2013). For many other annelids, information about the Hox gene complement are available through PCR and cloning studies; however, genomic organization and absence of genes cannot be derived from this approach (Dick and Buss 1994; Snow and Buss 1994; Irvine et al. 1997; Cho et al. 2003, 2006; Kulakova et al. 2007; Bleidorn et al. 2009).

The expression of Hox genes during development has been only investigated for a few annelid taxa, *Capitella teleta*, *Chaetopterus variopedatus*, *Alitta* (*Nereis*) *virens*, *Platynereis dumerilii*, *Hirudo medicinalis*, and two *Helobdella* species (Irvine and Martindale 2000; Peterson et al. 2000; Kulakova et al. 2007; Fröbius et al. 2008; Gharbaran and Aisemberg 2013). The most inclusive study deals with *C. teleta*, where for the first time spatial and temporal collinearity for Hox genes could be demonstrated for a lophotrochozoan taxon (Fröbius et al. 2008). *Capitella* Hox genes, except for *Post1*, are all expressed in ectodermal domains during larval development, with a spatial correlation of anterior expression borders and location of genes in the Hox cluster. Anterior class Hox genes (*lab*, *pb*, *Hox3*) are the first genes expressed, occurring before the appearance of segments. The expression of *Dfd* and *Scr* can be detected after appearance of the first segments, followed by the expression of *lox5*, *Antp*, and *lox4*. The expression of *lox2* and *Post2* appears last. Interestingly, all Hox genes in

C. teleta show their highest expression level at a unique stage during the course of development, reflecting the order of activation for each gene. A unique Hox gene expression boundary can be detected for all nine thoracic segments, and the posterior-most located Hox genes (*lox2* and *Post2*) are only expressed in the abdomen (Fig. 9.12). Whereas no expression of Hox genes could be detected in the pygidium of *C. teleta*, expression of *Post2* was detected in the pygidium of nereidids (Kulakova et al. 2007). Expression of *Post1*, the gene which seems to be separated from the rest of the Hox cluster, could not be detected in any investigated stages of *C. teleta*, besides some signals in chaetal sacs (Fig. 9.12; Fröbius et al. 2008). This result is congruent with analyses of expression of this gene in nereidids (Kulakova et al. 2007).

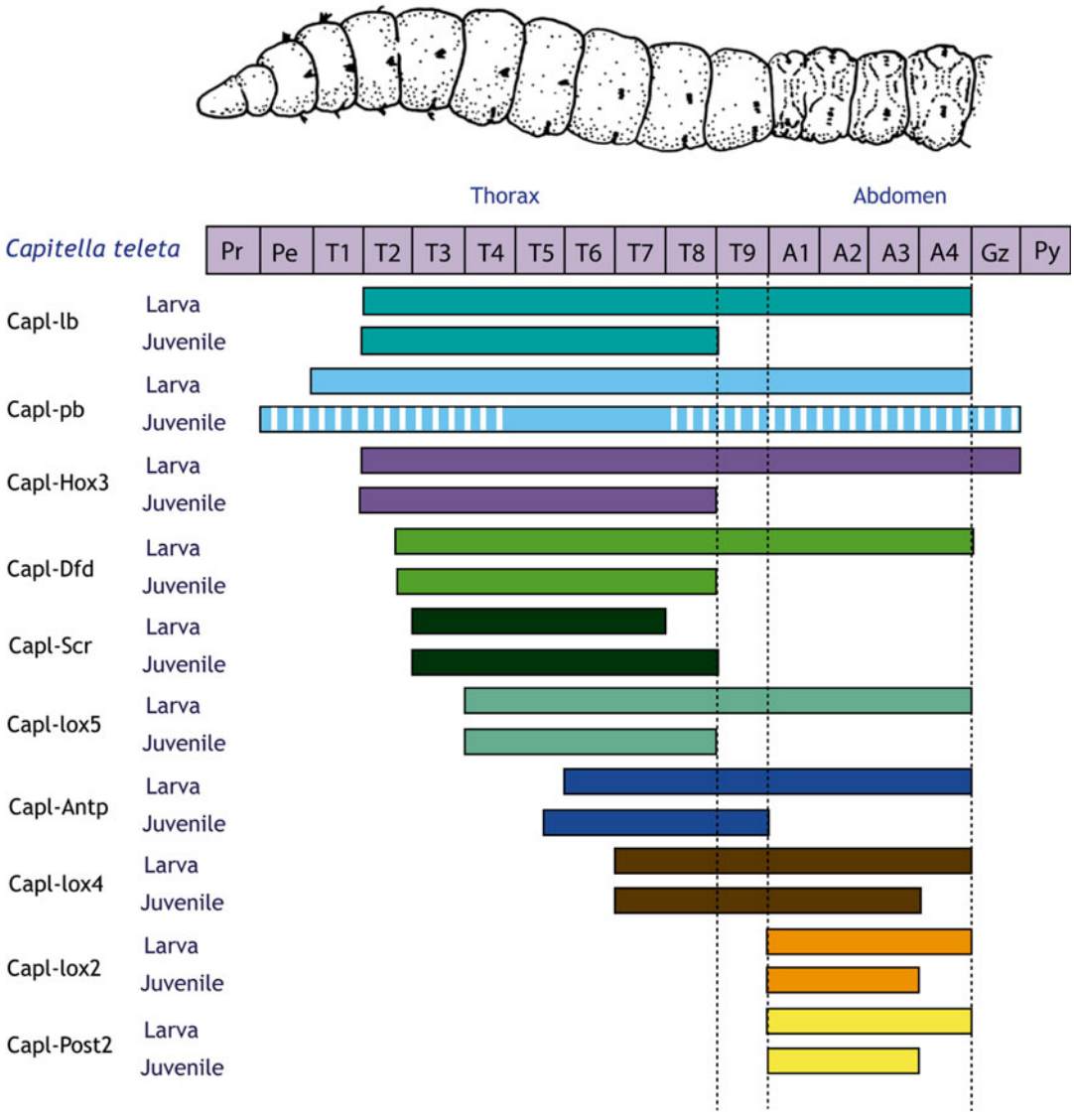


Fig. 9.12 Hox gene expression profile in larvae and juveniles of *Capitella teleta* after Fröbius et al. (2008). *Solid bars* indicate strong expression; *dashed bars* indicated weaker expression. Abbreviations: *A1–4* abdominal segments 1–4, *Gz* growth zone, *Pe* peristomium, *Pr* prostomium, *Py* pygidium, *T1–T9* thoracic segments 1–9

A staggered expression of five Hox genes generally in line with spatial and temporal collinearity can also be found in *Chaetopterus*, even though the genomic organization of the Hox cluster remains unknown in this species (Irvine and Martindale 2000). No strict temporal collinearity was found in expression studies for nereidid worms and *Helobdella* (Kourakis et al. 1997; Kulakova et al. 2007). However, all studies suggest an involvement of Hox genes in body patterning along the anterior-posterior axis, a function that seems to be the ancestral role of Hox genes in bilaterian animals (Kulakova et al. 2007; Butts et al. 2008). Notably, annelids show a predominant expression of Hox genes in neurogenic structures such as the ganglia and the ventral nerve cord. This is especially obvious in *Chaetopterus* and leeches (Shankland et al. 1991; Aisemberg and Macagno 1994; Wong et al. 1995; Kourakis et al. 1997; Irvine and Martindale 2000; Gharbaran and Aisemberg 2013).

The ParaHox cluster is a paralog of the Hox gene cluster, containing three genes (*Gsx*, *Xlox*, and *Cdx*) (Brooke et al. 1998). As for Hox genes, temporal collinearity has been likewise demonstrated for ParaHox genes in many instances. However, the ParaHox cluster seems to be lost in several investigated ecdysozoan taxa which show a breakup of the cluster and missing genes (Ferrier and Minguillon 2003). All three ParaHox genes seem to be present in annelids (Ferrier and Holland 2001; Fröbius and Seaver 2006; Park et al. 2006). The genomic organization of ParaHox genes has been studied in detail for *Platynereis dumerilii* (Hui et al. 2009). In this species, a head-to-head location of *Gsx* and *Xlox* could be demonstrated, with *Cdx* located in a separate position on the same chromosome. Expression analyses of these genes in *Alitta* (*Nereis*) *virens* suggest a role in anterior-posterior patterning of the digestive system and in the specification of neuroectodermal cell domains (Kulakova et al. 2008). Especially *Gsx* seems to be involved in the development of the brain in all investigated annelids (*P. dumerilii*, *A.* (*N.*) *virens*, *C. teleta*), a function which is regarded as ancestral for these genes for bilaterians in general (Fröbius and Seaver 2006; Kulakova et al. 2008).

Genes Involved in Neurogenesis

The development of the central nervous system has been deeply studied for *Platynereis dumerilii*. Neural progenitor cells are located close to the ventral midline and express *axin*, a negative regulator of the Wnt/β-catenin pathway, which controls the transition between these proliferating cells and differentiating neurons (Demilly et al. 2013). Wnt-controlled proliferation of neural progenitors is also well-documented for vertebrates and arthropods, especially *Drosophila* (Bielen and Houart 2014). Using a candidate gene approach, genes with a conserved expression in developing vertebrate and arthropod brains were chosen as major targets in studies on annelids. The developing head of annelid larvae is demarcated by the expression of *six3* and *otx* homeobox genes (Fig. 9.13), a patterning system that might be universal to bilaterian animals (Steinmetz et al. 2010). MicroRNAs are short noncoding RNAs that posttranscriptionally regulate gene expression (Ambros 2004). The expression of several microRNAs is highly tissue specific and conserved across animals (Christodoulou et al. 2010). In *P. dumerilii* and *Capitella teleta*, the microRNAs *mir-7*, *mir-137*, and *mir-153* show a localized expression in distinct neurosecretory brain tissue, a pattern which was also found in zebra fish (Tessmar-Raible et al. 2007; Christodoulou et al. 2010). The expression of the complementary pair *mir-9* and *mir-9*/mir-131* is restricted to two sets of differentiated neurons in the developing annelid brain, with the most apical cells located at the base of the antennae (Christodoulou et al. 2010). The three transcription factors *rx*, *otp*, and *nk2.1* are all expressed in the developing forebrain of *P. dumerilii*. All cells expressing these genes, as well as *mir-7*, are vasotocinergic extraocular photoreceptors. The expression pattern matches those known from the same cell type in zebra fish (Tessmar-Raible et al. 2007). Gene networks controlling the pattern along the anterior-posterior axis of the central nervous system are conserved across bilaterians, and the involved genes are mainly Hox genes (Ferrier 2012). The patterning of the dorso-ventral axis in *P. dumerilii* is

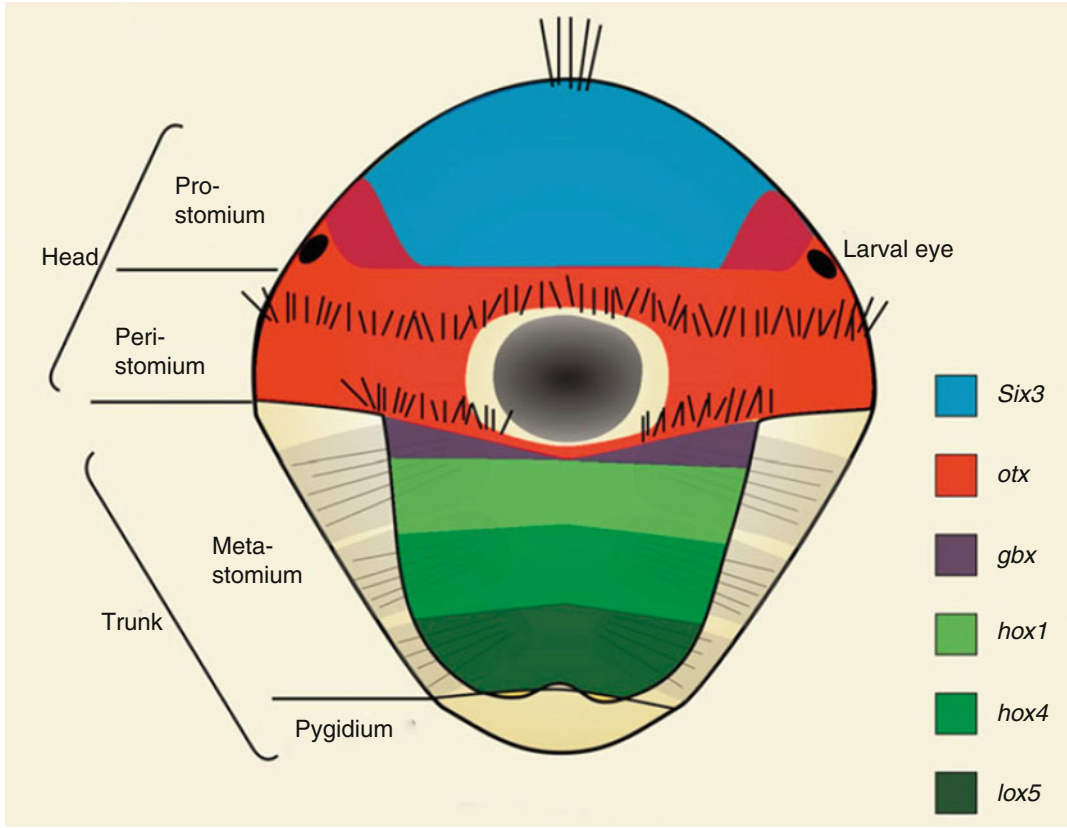


Fig. 9.13 Expression of *six3*, *otx*, *gbx*, and Hox genes in neuroectodermal regions of *Platynereis dumerilii* larvae. The *six3* and *otx* expressing regions cover the developing prostomium and the peristomium, from which the cerebral ganglia and eyes develop. *Dark gray* region marks the mouth (From Steinmetz et al. (2010))

controlled by a gene network including *nk2.2*, *nk6*, *Pax2/5/8*, *Pax6*, *Pax3/7*, *dlx*, *msx*, *gsx*, *sim*, and *dbx* (Denes et al. 2007; Ferrier 2012). A similar patterning of the neuroepithelium is obvious in vertebrates (Denes et al. 2007).

Besides the detailed investigations summarized for *Platynereis dumerilii*, some gene expression studies dealing with the development of the nervous system in leeches and *Capitella teleta* are published. As shown for *P. dumerilii*, *otx* shows a largely head-specific expression in *Helobdella* (Bruce and Shankland 1998). The expression of *Lox10*, a putative *nk2.1* ortholog, was detected in the developing brain of *Helobdella*, congruent with the results for *P. dumerilii* (Nardelli-Haefliger and Shankland 1993). In *Hirudo medicinalis*, the central class Hox gene *Lox1* controls the differentiation of the so-called "rostral penile evertor neurons" that innervate the male penis (Gharbaran and Aisemberg 2013). For the same species, expression of the axon migration guiding protein *netrin* is shown to be involved in forming interganglionic neuronal tracts and in defining ventrodorsal boundaries of peripheral innervation (Gan et al. 1999). Another protein family investigated in *H. medicinalis* is the innexins, where several cloned members show a restricted expression in neurons (Dykes and Macagno 2006). In *C. teleta*, *Delta* and *Notch* expression was detected during brain development in larvae, as well as in the forming ganglia of the ventral nerve cord of juveniles, suggesting a role of Notch signaling in neurogenesis (Thamm and Seaver 2008).

In summary, development of the central nervous system and expression patterns of genes localized in the brain show strong similarities between vertebrates and annelids. Based on

these results (and further studies involving other taxa), the presence of a centralized nervous system in the last common ancestor of protostomes and deuterostomes seems plausible for several authors (Arendt et al. 2008; Holland et al. 2013).

Genes Acting in the Development of the Digestive Tract

The gut of annelids consists of a foregut (stomodeum) and a hindgut (proctodeum), both originating from ectoderm, as well as of the midgut which is of endodermal origin. All three parts can usually be subdivided into different functional regions (Tzetlin and Purschke 2005). Several genes involved in bilaterian foregut and hindgut patterning have been investigated *for Platynereis dumerilii* (Arendt et al. 2001). As such, the T-box transcription factor *brachyury* is expressed in the ventral part of the developing foregut as well as in the hindgut of late trochophore larvae, resembling the pattern known from larvae of basal branching deuterostomes. The homeobox gene *goosecoid* is first expressed in a small number of cells at the anterior blastopore margin which develops into the foregut. Expression can be additionally detected in adjacent cells which will contribute to the development of the foregut nervous system. As the expression patterns of the investigated genes seem to be conserved in protostomes and deuterostomes, a single origin of the tripartite bilaterian through gut has been hypothesized (Arendt et al. 2001). This idea has been later challenged based on expression studies of the same set of genes in acoels, which are lacking a through gut (Hejnol and Martindale 2008).

Genes involved in the patterning of ectodermal and endodermal parts of the gut have been studied for *Capitella teleta*, *Chaetopterus variopedatus*, and the sipunculid *Themiste lageniformis*. In *C. teleta*, the transcription factor *FoxA* and genes of the GATA family are expressed across the entire developing gut (Boyle and Seaver 2008). Different genes of the GATA family are exclusively expressed in the developing midgut, with a prominent expression of *gataB1* at its boundaries. In contrast, *FoxA* expression can be detected surrounding the blastopore during development as well as in the foregut and hindgut during organogenesis. Partly similar expression patterns are reported for *Themiste* and *Chaetopterus* and might reflect the differences in the gut architecture of these species with different feeding mechanisms (Boyle and Seaver 2010). Moreover, expression of the ParaHox gene *Cdx* is also reported for anterior and posterior regions of the gut in *C. teleta* (Fröbius and Seaver 2006). In nereidids, the ParaHox gene *Gsx* is expressed during the formation of the foregut and the midgut. The expression of the ParaHox gene *Xlox* has been detected in all investigated annelids, including *Helobdella*, *H. medicinalis*, *C. teleta*, and nereidids (Wysocka-Diller et al. 1995; de Rosa et al. 2005; Fröbius and Seaver 2006; Kulakova et al. 2008; Hui et al. 2009).

Bilaterian animals are divided into deuterostomes, ecdysozoans, and lophotrochozoans (Edgecombe et al. 2011). Whereas research on several well-established model organisms in the former two groups (e.g., *Drosophila*, *Caenorhabditis*, *Danio*, *Mus*) has provided detailed insights into molecular mechanisms of the development, lophotrochozoans have traditionally been chronically understudied in this regard (Tessmar-Raible and Arendt 2003). The rise of *Platynereis dumerilii* (and other annelids like *Capitella* and *Helobdella*) as EvoDevo models has provided major insights into the evolution of the nervous system and segment formation in annelids, a key lophotrochozoan phylum (see above). Interestingly, the genomic architecture of *Platynereis* seems to be little derived from a hypothetical bilaterian ground pattern, enabling many insights into comparative developmental genomics (Raible et al. 2005; Ferrier 2012). Future studies focusing on additional annelid lineages, such as the basal branching oweniids or the non-segmented sipunculans, will certainly improve our understanding of the evolution of bilateria in general.

OPEN QUESTIONS

- Segment formation in non-clitellate annelids
- Myogenesis in Echiurida
- Development of the nervous system in basal branching annelids
- Homology of ciliary bands including the various trochi in different annelid and lophotrochozoan larvae
- Genetic background of annelids showing putative deuterostomy
- Comparative expression studies of Hox, ParaHox, and other key developmental genes across the various annelid subtaxa, especially lesser-known groups such as Myzostomida, Sipuncula, and Echiurida
- Gene expression studies of "segmentation genes" in non-segmented annelids

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