The coelom and the origin of the annelid body plan

Reinhard M. Rieger[1],* & Günter Purschke[2]
[1]*Abteilung Ultrastrukturforschung und Evolutionsbiologie, Institut für Zoologie und Limnologie, Universität Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria*
[2]*Spezielle Zoologie, Fachbereich Biologie/Chemie, Universität Osnabrück, D-49069 Osnabrück, Germany*
*(*Author for correspondence: E-mail: reinhard.rieger@uibk.ac.at)*

Abstract

The biphasic life cycle in annelids is characterized by two completely different types of organisation, i.e. the acoelomate/pseudocoelomate larva and the coelomate adult. Based on this observation the recent literature on the different assumptions on the organisation of the bilaterian stem species with special emphasis on the evolution of the annelid body plan is reviewed. The structure of the coelomic lining ranges between a simple myoepithelium composed of epithelio-muscle cells and a non-muscular peritoneum that covers the body wall muscles. The direction of the evolution of these linings is discussed with respect to coelomogenesis. As the coelom originates from mesodermal cell bands, different assumption on the acoelomate condition in Bilateria can be substantiated. The origin of segmentation in annelids is explained by current hypothesis. Although no final decision can be made concerning the origin of the annelid body plan and the organisation of the bilaterian stem species, this paper elaborates those questions that need to be resolved to unravel the relation between the different body plans.

Introduction

The different basic designs of bilaterian body cavities, that is acoelomate/pseudocoelomate vs. coelomate constructions (Fig. 1A–E), have been employed to define body plans in several ways (see Rieger, 1985). Recently the usefulness of this concept in phylogeny has been seriously questioned both by molecular phylogenetesists (e.g., Adoutte et al., 1999, 2000) and some comparative morphologists (Willmer, 1995). This paper cannot be the place to respond in full length to this critique, it warrants, however, a general comment: irrespective of the question as to whether the different kinds of body cavities may be homologous (see Minelli, 1995 for a thorough discussion of this point), which type of body cavity may be more primitive (e.g., Rieger, 1986) or whether they may have multiple origins (e.g., Salvini-Plawen & Bartolomaeus, 1995), there is no doubt that the

distinction between the a- and/or pseudocoelomate body cavity, as derivatives of the primary body cavity, and the coelomate condition as the secondary body cavity will remain a central question in discussions of the origins of the bilaterian body plans. Since Hyman's (1951) original proposal, acoelomate, pseudocoelomate and coelomate conditions have been defined further through ultrastructural work (e.g., see Rieger, 1985, 1986; Rieger & Lombardi, 1987; Fransen, 1980, 1988; Willmer, 1991; Bartolomaeus, 1994; Salvini-Plawen & Bartolomaeus, 1995; Westheide & Rieger 1996; Ax, 1996). Here it is shown for the coelomate organization of the Annelida that the concept of the three body cavity designs appears vague primarily due to the lack of sufficient ultrastructural & molecular information and that it is not simply 'imprecise and often unhelpful' (Willmer, 1995, p. 23). Until such investigations have been carried out it would seem premature to abandon the
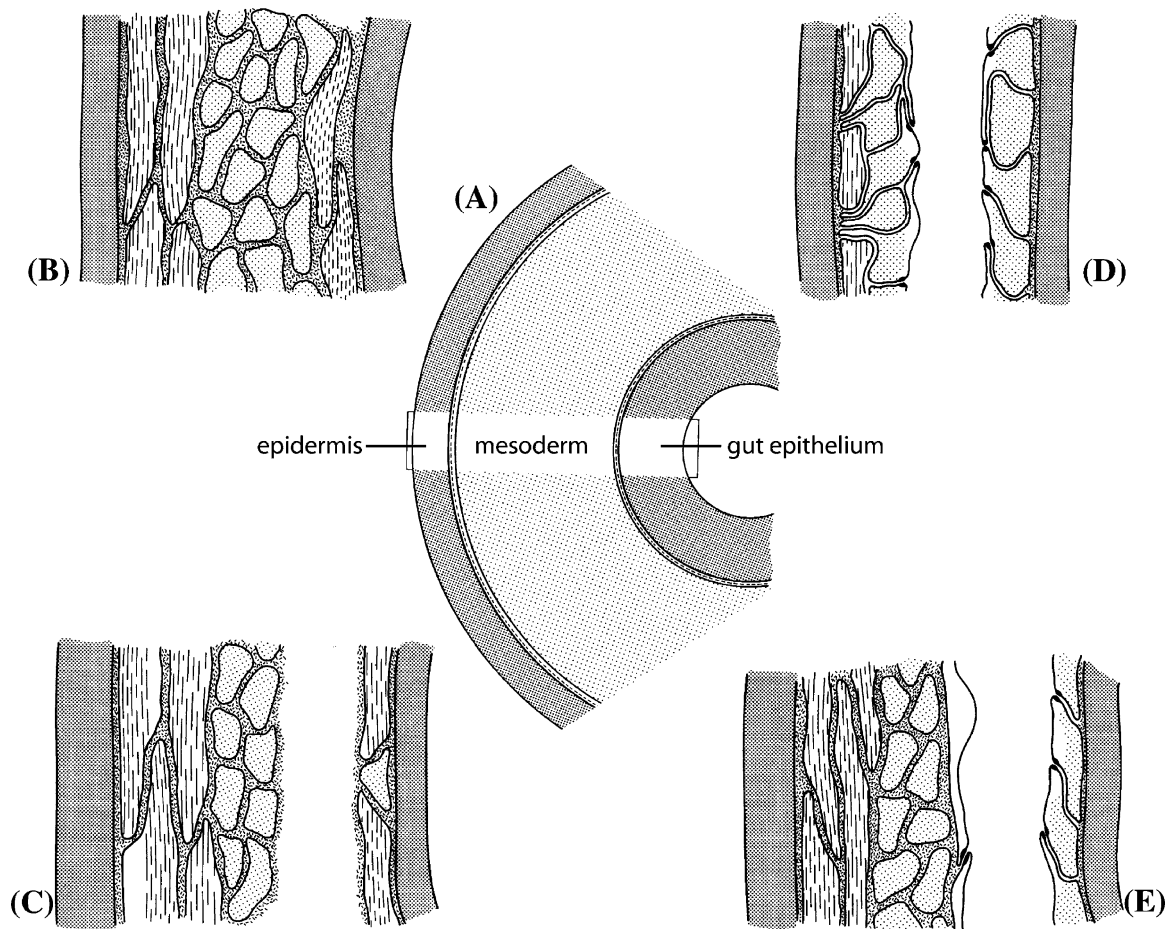
Figure 1. Diversity of mesoderm differentiation in Bilateria. (A) Mesoderm as defined as a median tissue layer situated between epidermis and gut epithelium, and primarily derived from the entoderm. (B) Acoelomate organization. (B) Pseudocoelomate organization (primary body cavity); body cavity lined by ECM only. (D–E) Coelomate organzation (secondary body cavity) as usually found in many Annelida. (D) Body cavity lined by a myoepithelium which also constitutes the muscular system of the animal. (E) Body cavity lined by a peritoneum on the somatopleure and by a visceral myoepithelium. Modified from Bartolomaeus (1994).

concept of the three body cavity designs in discussions of bilaterian phylogeny.

Adult coelomate annelids develop from pseudocoelomate larvae

If, as is widely accepted (see Nielsen, 2001), the annelid stem species exhibited a biphasic life cycle with a microscopic larva and a larger vermiform adult, it is evident that two sequential, extreme forms of body cavity design characterize the ancestral annelid body plan. It is acoelomate or pseudocoelomate in the larva and it is coelomate in adult animals (Fig. 2A, A′; Rieger, 1986, 1994). From this point of view it is not entirely correct to specify that annelids are coelomates.

As has been illustrated by Westheide (1987) progenesis is a widespread phenomenon in the evolution of interstitial annelids as well as in the interstitial fauna in general. This fact has led to the proposition that acoelomates may be secondarily derived from coelomates with schizocoelous coelom formation, without reduction of the coelom (Fig. 2B, B′; Rieger, 1986, 1991a, b; Smith et al., 1986). Among the Spiralia (but see also Tyler, 2001 for deuterostomes) the annelid example could serve as a model for the origin of acoelomate taxa
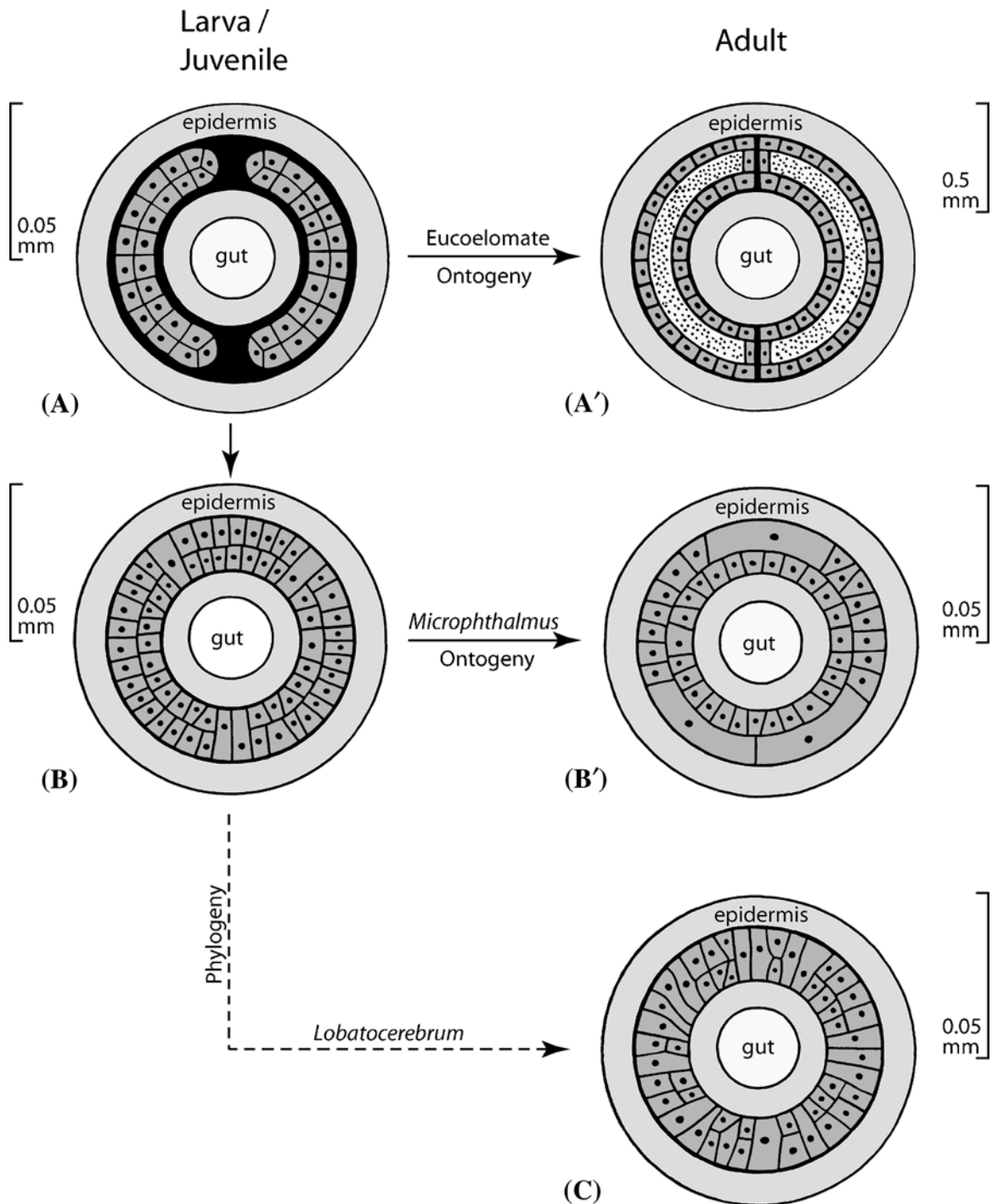
Figure 2. Body cavity designs as occurring in ontogeny and phylogeny in Annelida. (A-A′) Proposed ancestral body plan with pseudocoelomate or acoelomate larva and coelomate adult. (B–B′) Progenetic evolved interstitial species with acoelomate larva/juvenile and acoelomate adult. (C) Direct development of acoelomate adult with loss of larval stages. Original J. Lombardi, P. R. Smith & R. M. Rieger.
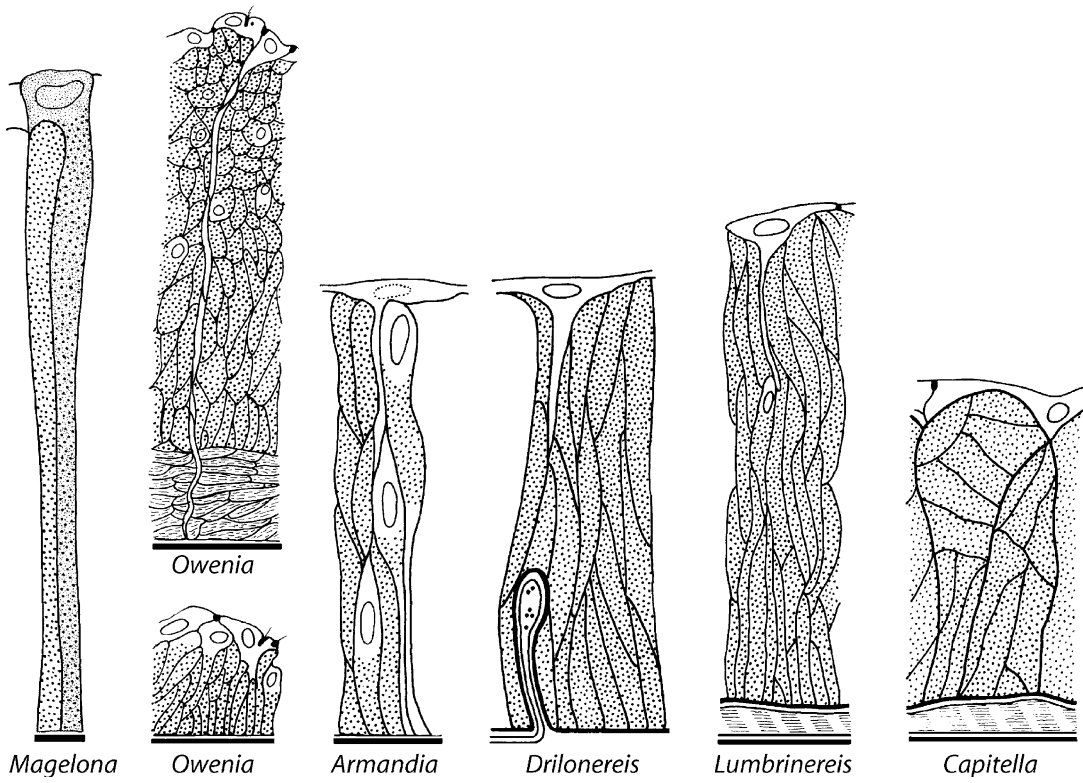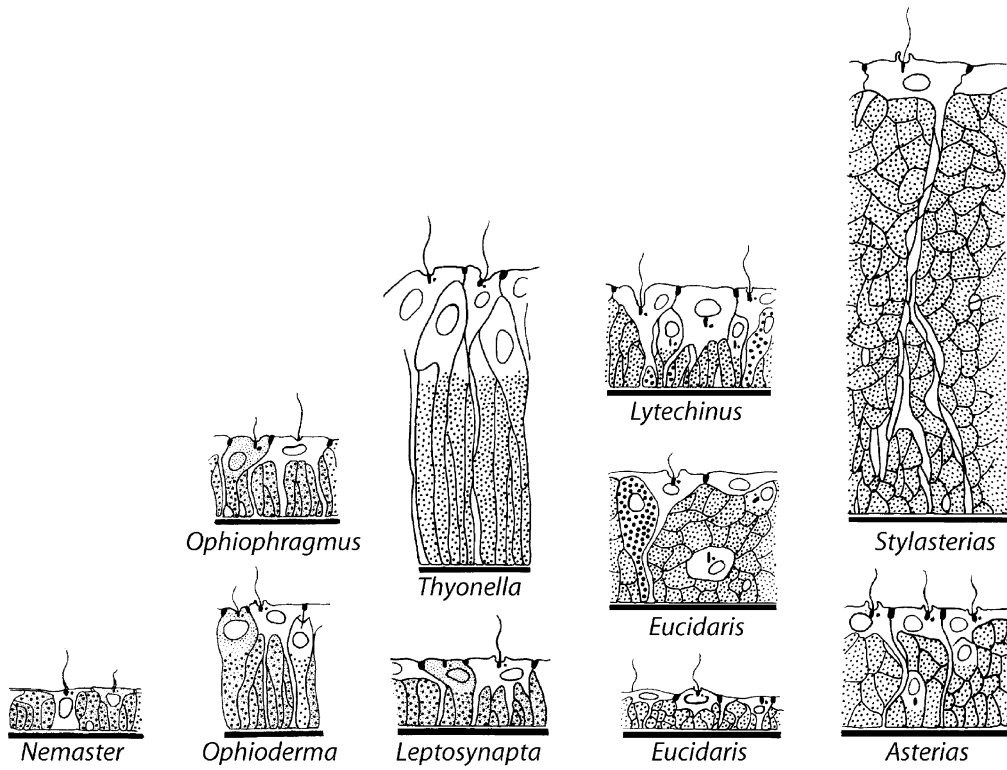
Ophiophragmus

Thyonella

Lytechinus

Stylasterias

Nemaster

Ophioderma

Leptosynapta

Eucidaris

Eucidaris

Asterias

Magelona

Owenia

Owenia

Armandia

Drilonereis

Lumbrinereis

Capitella

within the Bilateria from a coelomate stock (Fig. 2C).

In other proposals a microscopic organism such as a pseudocoelomate larvae of the Annelida are seen to represent the stem species of the Spiralia. The idea of a pseudocoelomate larva as ancestral body plan for the Bilateria has been recently revived by Davidson and co-workers (e.g., Davidson et al., 1995; Peterson et al., 1997, 2000; Peterson & Davidson, 2000). Adult structures such as the coelom would be a feature originating in this pseudocoelomate micrometazoan from a set of special cells (set aside cells), perhaps a kind of stem cells.

Finally, the Trochaea-theory suggests the origin of the spiralian phyla from a pseudocoelomate larva which became adapted to benthic life and developed either into an acoelomate adult ancestor for the Spiralia or a coelomate adult ancestor in the deuterostomes (Nielsen, 2001).

Histological organization of the coelomic lining in Annelida

The coelomic lining in annelids is either a myoepithelium, or a peritoneum, or a combination of these two epithelial configurations (e.g., Rieger, 1986; Fransen, 1988; Gardiner, 1992; Bartolomaeus, 1994; terminology in Rieger & Lombardi, 1987). Epithelio-muscle cells, fibre-type muscle cells, peritoneocytes and podocytes constitute the main epithelial cell types in this lining (Fig. 3). All muscle cells in coelomates may have originated in epithelia (Rieger & Ladurner, 2003).

Apical junctional complexes which are similar to zonulae adhaerentes have been described between all different epithelial cell types (e.g., Fransen, 1988; Gardiner, 1992; Bartolomaeus, 1994). Proximal to these junctions septate junctions may also occur (Fransen, 1988), but published images of these structures are lacking in Annelida (but see, e.g., Fig. 9 in Rieger & Lombardi, 1987).

A special, electron dense layer of the extracellular matrix (ECM) at the base of the coelomic lining and often an additional fibrous layer of various thickness have been found in many cases (Fransen, 1980, 1988; Gardiner, 1992; Bartolomaeus, 1994). This electron dense limiting layer of the ECM (Fransen, 1982) represents the basal lamina of the basal matrix (Rieger, 1985, 1986).

The known conformations of coelomic linings in annelids and those found in echinoderms can be aligned in a transformation sequence with a single layered myoepithelium on one end and a peritoneum with subperitoneal musculature on the other end (Rieger, 1986; Rieger & Lombardi, 1987; Stauber, 1993; Bartolomaeus, 1994). Such a transformation sequence including annelid species is shown here (Fig. 3). In annelids simple myoepithelia may occur, as well as pseudostratified and stratified myoepithelia containing all of the somatic and the splanchnic musculature (Bartolomaeus, 1994). When a non-muscular squamous peritoneum is differentiated, the somatic musculature, and in some cases also the splanchnic musculature, is found below the epithelial layer.

In echinoderms, the mechanism of the musculature sinking into the connective tissue below the epithelial level is well clarified (Stauber, 1993). It is most probable that the simple myoepithelium occurred first, followed by stepwise transformations to a non-muscular peritoneum plus subperitoneal musculature. The mechanism of myoepithelial muscle cells sinking below the level of the coelomic epithelium in annelids is less well understood (Rieger, 1986). The interpretation of a reading direction from a myoepithelium to the subperitoneal musculature is therefore less definite (but see Rieger, 1986, pp. 38–39, points A–E for general arguments in favour of that reading direction among Bilateria). A simple myoepithelial lining is currently regarded to represent the primary condition in Annelida (see Bartolomaeus, 1994 for discussion). Such transformation sequences from simple myoepithelia to separate somatic musculature and peritoneum are to be observed during development in several annelids: Coelomic cavities often arise from solid blocks of cells which become separated probably by fluid accumulation leading
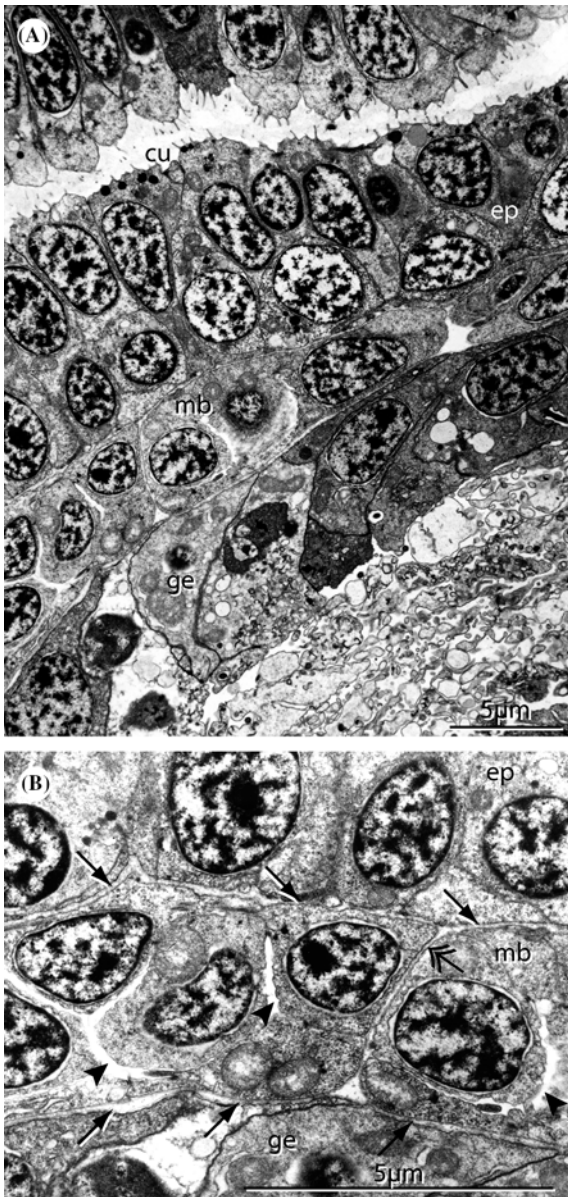
Figure 4. Ultrastructure of early mesodermal bands (mb) in a young mitraria larva of *Owenia fusiformis* (Oweniidae) in sagittal section showing their epithelial nature. (A) Low magnification to show location of mesodermal band (mb) between epidermis (ep) and gut epithelium (ge), cu cuticle. (B) Enlargement of left part of mesodermal band shown in A. Lumen of coelom represented by narrow spaces between the cells (arrowheads) which are joined by zonulae adhaerentes (double arrow). Arrows point to ECM between the different tissue layers. Micrographs: R. M. Rieger.

to an epithelial coelomic lining. Later these cells develop into peritoneal cells and muscle cells (Anderson, 1973; Gardiner, 1992; Bartolomaeus,

1994). Moreover, this pattern follows the increasing functional differentiation of cell types during evolution. Following this hypothesis, a peritoneum with underlying musculature should have evolved several times independently within Annelida.

Homology of the epithelial organization of coelomic linings in annelids

Generally the homology of the coelom in this taxon is not put into question (but see Minelli, 1995 for discussion). However, the characterization of the coelomate organization of annelids requires also strong arguments for a unique evolution of their specific epithelial differentiations. The probability of a unique evolution of myoepithelial coelomic linings in annelids, or coelomates in general, depends on the structural details defining them as 'true' epithelia, namely the apical junctional complex and the structure of the basal lamina (Rieger, 1994). Regrettably, no lanthanum preparations or freeze fracture images of apical junctional complexes in the coelomic lining of annelids have been published. These structures have been studied in other annelid epithelia (e.g., Green, 1981; Green & Bergquist, 1982). Junctions between cells in coelomic linings could now also be investigated with molecular markers (e.g., for cadherins, beta-catenin: Takeichi, 1991; Tepass et al., 2000; Tyler, 2003; B. Hobmayer, personal communication). This would be especially useful for investigating their formation during coelomogenesis. Similarly, differentiations such as basal laminas which have so far been identified with conventional TEM, could be investigated with immunocytochemical and molecular methods (see Pedersen, 1991; Kleinig & Maier, 1999; Schiebler & Schmidt, 2002; Tyler, 2003). The molecular substructure of these basal laminas could then be compared with the complex molecular networks known from basal laminas in ectodermal and entodermal epithelia of vertebrates (Fawcett, 1994; Kleinig & Maier, 1999) and other bilaterians (Tyler, 2003). Elucidating structural details of apical junctional complexes and basal laminas of the coelomic lining would yield a better understanding of coelom organization and of coelom formation. It would then be possible with increased confidence to postulate a
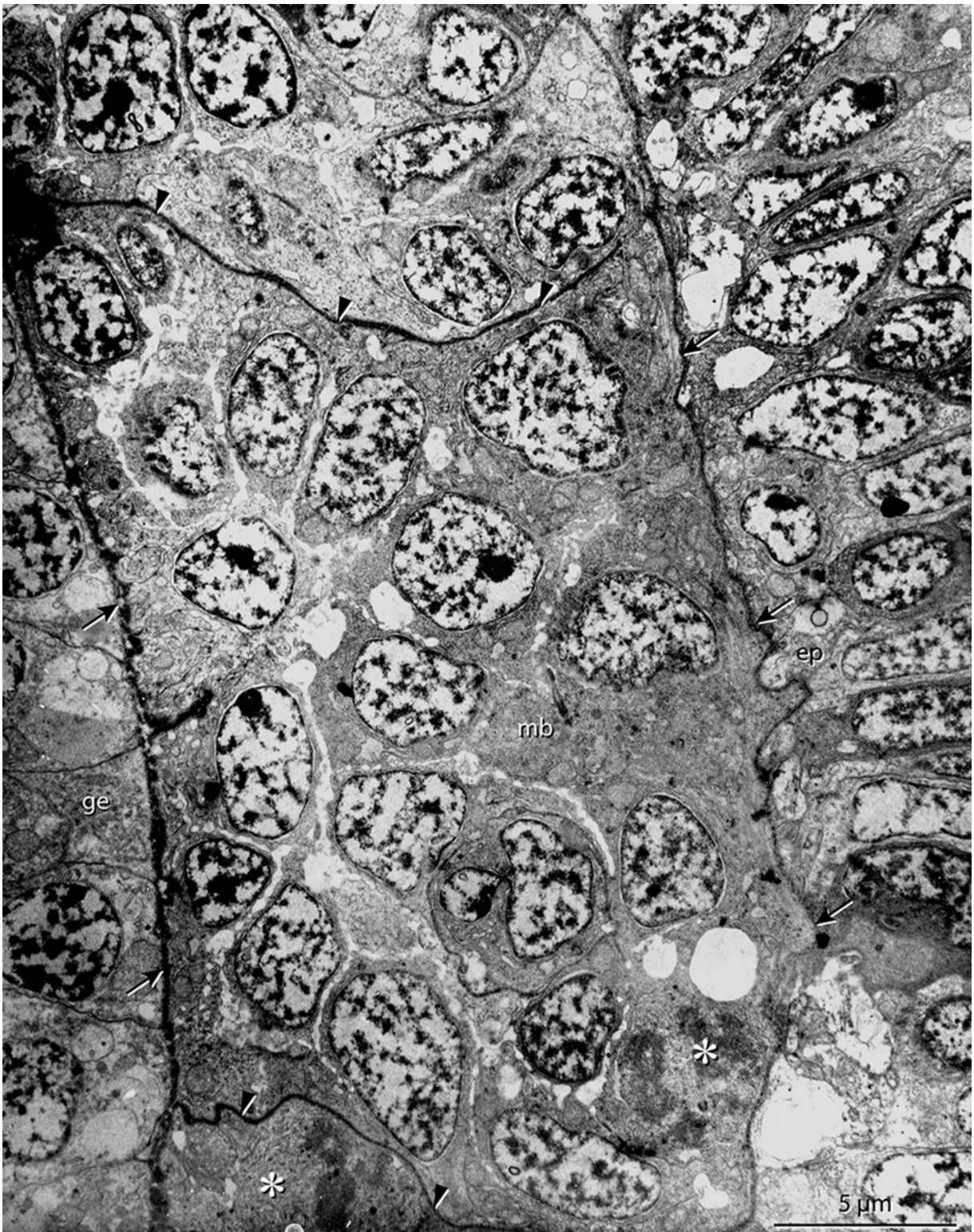
Figure 5. Ultrastructure of early mesodermal band (mb) in a early larva of an unidentified species of Oweniidae. Mesoderm shows a mesenchymal organization; arrows point to ECM between epidermis (ep), mesoderm band and gut epithelium (ge), arrowheads to ECM between adjacent epithelia in presumptive septa. Note dividing mesodermal cells (asterisks). Figure oriented perpendicular with respect to Figure 4B. Micrograph: R. M. Rieger.

monophyly of the coelomic lining in annelids, in other Spiralians and possibly even deuterostome phyla, as information about the substructures always enhances the probability of homology (e.g., Rieger & Tyler, 1979; Tyler, 1988; Haszprunar, 1996).

Origin of the histological design of the coelomic lining (coelomogenesis)

The coelom in Annelida is formed by mesodermal cells of the blastoporal region and is derived from the mesoblast 4d (Anderson, 1973; Nielsen, 2001). These cells form bilateral mesodermal bands, in which the epithelial nature of a lining of the secondary body cavity (coelom) is established at quite different times during embryogenesis (Potswald, 1981; Heimler, 1981a, b, 1983, 1988; Turbeville, 1986; Rieger, 1986; Rieger & Ladurner, 2003). In some species a mesenchymal organization is evident for a longer time in development (Fig. 5), whereas cells are arranged in epithelial configuration apparently almost from the onset of differentiation and cell proliferation in other (Fig. 4A, B), even closely related species. In the latter case coelomic cavities with collapsed lumen (Rieger, 1986) are surrounded by the epithelium (Fig. 4B). Also intermediate tissue organization, that is neither true epithelial tissue nor mesenchymal, can be found in mesodermal bands of certain annelids (Rieger, 1986). This variations of mesenchymal and epithelial conformations during coelomogenesis, and the different time points at which mesodermal bands form true coelomic epithelia allow to derive acoelomates from coelomates by progenesis without postulating the reduction of the coelom (Fig. 2A–C; Rieger, 1986; Smith et al., 1986). Other mesodermal tissues in such progenetic polychaetes can exhibit the same histological organization as do the mesodermal bands prior to the formation of the coelomic lining. The same argument has been recently proposed for deriving acoelomates from juvenile enteropneusts (Tyler, 2001). Comparative data about the differentiation of the coelom within the mesodermal band in annelids will certainly be needed for a better understanding of the evolution of the annelid body plan.

Origin of the segmented condition in annelids

Two groups of hypotheses for the origin of the Bilateria are still discussed, depending on whether bilaterians originally where coelomates or acoelomates/pseudocoelomates (Balavoine, 1998; Rieger & Ladurner, 2001):

(1) Based on the assumption that all bilaterians are coelomates having developed coelomic linings from gastrodermal pockets of coelenterate ancestors, Remane (1950, 1954, 1963a, b) proposed a model in which a vermiform coelomate bilaterian stem species developed serial subdivisions in the posteriormost region of three pairs of coelomic cavities (see model and critique in Hartmann, 1963). Using the arguments of Clark (1964), this evolution of segmentation (known as tritomery) can be seen as an adaptation for borrowing in mobile substrates. Annelid segments would have originated either from septa dividing existing coelomic cavities, or within the differentiating mesodermal bands. Examples that 'solid' mesodermal bands actually may reveal coelomic epithelial organization have been shown above.

Without addressing the question of whether bilaterians were originally coelomates, Westheide (1997) has pointed to another mechanism as being a possible key factor in the evolution from an unsegmented, coelomate ancestor to the segmented annelids. According to this model transverse septation of the coelom developed as a necessary prerequisite for transverse blood vessels to cross the body in recurring intervals and thereby ensure uniform, repeated blood supply for all body regions in larger vermiform coelomate animals. Because the blood vascular system and the coelomic organization are so intimately related (see Ruppert & Carle, 1983) the suggestion that blood circulation was a main functional factor for the development of segmentation in annelids seems most reasonable.

(2) Alternative theories propose that original bilaterians were acoelomates or pseudocoelomates, and segmentation evolved gradually (pseudometamerism hypothesis sensu Clark, 1964), together as the coelomic organization arose. Although such hypotheses have been thought to be refuted (see Remane, 1963a; Clark, 1964) they are presently discussed especially for the origin of segmentation within the arthropods (e.g., Budd,

2001). One example for pseudometamerism as origin of the segmented coelom was the gonocoel theory, which was particularly elaborated by Goodrich (1946). Pseudometamerism suggests that multiple substructures became organized in complex, sequentially arranged segments. Iterative structures such as cuticular setae, nephridia or gonads increasingly co-established iterative organs and thus segmentation, resembling the annelid body plan, arose gradually within an acoelomate or pseudocoelomate vermiform bilaterian stem species. This idea is of special significance for the Ecdysozoa-concept (e.g., Schmidt-Rhaesa et al., 1998; Budd, 2003; Garey, 2003; Schmidt-Rhaesa, 2003) which considers annelid and arthropod segmentation to have evolved in parallel. Scholtz (2003) has summarized the evidence concerning the issue of Ecdysozoan- vs. Articulata-concepts, and has argued in favour of the Articulate-hypothesis and of a homology of segmentation in annelids and arthropods.

Discussing the primary tissue organization of the mesoderm in Bilateria, Rieger and Ladurner (2003) have recently suggested that, if a model for the gradual origin of segmentation in annelids is envisioned, one driving force might be found in the strict repetitive pattern observed in the embryonic development of the circular musculature of small acoelomates such as *Convoluta pulchra* (Ladurner & Rieger, 2000). While data are lacking on myogenesis in most other spiralians (but see e.g., Reiter et al., 1996, for Macrostomorpha, Wanninger & Haszprunar, 2002, for Mollusca), the identical distances between circular muscle cells seen during early embryogenesis of *C. pulchra's* circular muscles may be due to the same or similar molecular mechanisms acting during early processes in segmentation in other protostomes and in deuterostomes (e.g., Davis & Patel, 1999; Shankland & Saever, 2000; Jouve et al., 2002).

Summary

With this paper we have tried to demonstrate the need for more detailed comparative ultrastructural and molecular analysis of the formation and of the adult organization of mesodermal tissues, in particular the histological organization and the origin of the coelomic lining in macroscopic and microscopic annelids. Although information on this subject has been accumulated during the 80s and 90s of the last century, especially detailed ultrastructural studies on apical junctional complexes and basal laminas are extremely rare and molecular information is still missing by and large. Comparisons of the formation and organization of the muscle system in spiralians usually considered as primary acoelomates (Platyhelminthes and Gnathostomulida) with that of secondary acoelomates (as so often seen among interstitial Annelida) would produce new insight into the question what makes primary acoelomates distinct from secondary ones and which of the present hypotheses might better explain the origin of the segmented coelom of Annelida. Without investigations of the features defining the 'true' epithelial organization of coelomic linings, a critical evaluation of the phylogenetic significance of the extremes of bilaterian body cavity organization (acoelomate/pseudocoelomate, coelomate) will not be possible.

Acknowledgements

References

Adoutte, A., G. Balavoine, N. Lartillot & R. de Rosa, 1999. Animal evolution the end of the intermediate taxa? Trends in Genetics 15: 105–108.

Adoutte, A., G. Balavoine, N. Lartillot, O. Lespinet, B. Prud'homme & R. de Rosa. 2000. The new animal phylogeny: reliability and implications. Proceedings of the National Academy of Sciences, USA 97: 4453–4456.

Anderson, D. T., 1973. Embryology and Phylogeny in Annelids and Arthropods. Pergamon, Oxford, 495 pp.

Ax, P., 1996. Multicellular Aminals. A New Approach to the Phylogenetic Order in Nature. Springer, Berlin, Heidelberg, New York, 225 pp.

Balavoine, G., 1998. Are Platyhelminthes coelomates without a coelom? An argument based on the evolution of *Hox* genes. American Zoologist 38: 843–858.

Bartolomaeus, T., 1994. On the ultrastructure of the coelomic lining in Annelida, Echiura and Sipuncula. Microfauna Marina 9: 171–220.

Budd, G. E., 2001. Why are arthropods segmented? Evolution and Development 3: 332–342.

Budd, G. E., 2003. Arthropods as ecdysozoans: the fossil evidence. In Legakis, A., S. Sfenthourakis, R. Polymeni & M. Thessalou-Legaki (eds), The new Panorama of Animal Evolution. Proceedings XVIII International Congress of Zoology, Pensoft, Sofia: 479–487.

Clark, R. B., 1964. Dynamics in Metazoan Evolution. The Origin of the Coelom and Segments. Clarendon, Oxford, 313 pp.

Davidson, E. H., K. J. Peterson & R. A. Cameron, 1995. Origin of adult bilaterian body plans: evolution of developmental regulatory mechanisms. Science 270: 1319–1325.

Davis, G. K. & N. H. Patel, 1999. The origin and evolution of segmentation. Trends in Genetics 15: M68–M72.

Fawcett, D. W., 1994. A Textbook of Histology. Chapman & Hall, New York, 964 pp.

Fransen, M. E., 1980. Ultrastructure of the coelomic organisation. I. Archiannelids and other small polychaetes. Zoomorphologie 95: 235–249

Fransen, M. E., 1982. The role of ECM in the development of invertebrates: a phylogeneticists view. In Hawkes S. & J. L. Wang (eds), Extracellular Matrix 478. Academic, New York: 177–181.

Fransen, M., 1988. Coelomic and vascular system. In Westheide W. & C. O. Hermans (eds), The Ultrastructure of Polychaeta. Microfauna Marina 4: 199–213.

Gardiner, S. L., 1992. Polychaeta. General organization, integument, musculature, coelom, and vascular system. In Harrison, F. W. & S. L. Gardiner (eds), Microscopic Anatomy of Invertebrates Vol. 7 Annelida. Wiley-Liss, New York: 19–52.

Garey, J. R., 2003. Ecdysozoa: the evidence for a close relationship between arthropods and nematodes. In Legakis, A., S. Sfenthourakis, R. Polymeni & M. Thessalou-Legaki (eds), The new Panorama of Animal Evolution. Proceedings XVIII International Congress of Zoology, Pensoft, Sofia: 503–509.

Goodrich, E. S., 1946. The study of nephridia and genital ducts since 1895. Quarterly Reviews of microscopical Science, New Series 86: 113–392.

Green, C. R., 1981. A clarification of the two types of invertebrate pleated septate junction. Tissue & Cell 13: 173–188.

Green, C. R. & P. R. Bergquist, 1982. Phylogenetic relationships within the invertebrata in relation to the structure of septate junctions and the development of 'occluding' junctional types. Journal of Cell Sciences 53: 279–305.

Hartmann, W., 1963. A critique of the enterocoele theory. In Dougherty, C. E. (ed.), The Lower Metazoa Vol. 5. University California, Berkeley: 55–77.

Haszprunar, G., 1996. The Mollusca: coelomate turbellarians or mesenchymate annelids? In Taylor, J. (ed.), Origin and Evolutionary Radiation of the Mollusca. Oxford University, Oxford: 1–28.

Heimler, W., 1981a. Untersuchungen zur Larvalentwicklung von Lanice conchilega (Pallas) 1766 (Polycheata, Terebellomorpha). Teil I: Entwicklungsablauf. Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere 106: 12–45.

Heimler, W., 1981b. Untersuchungen zur Larvalentwicklung von Lanice conchilega (Pallas) 1766 (Polycheata, Terebellomorpha). Teil II: Bau und Ultrastruktur der Trochophora-Larve. Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere 106: 236–277.

Heimler, W., 1983. Untersuchungen zur Larvenentwicklung von Lanice conchilega (Pallas) 1766 (Polychaeta, Terebellomorpha) Teil III: Bau und Struktur der Aulophora-Larve. Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere 110: 411–478.

Heimler, W., 1988. Larvae. In Westheide, W. & C. O. Hermans (eds), The Ultrastructure of Polychaeta. Microfauna Marina 4: 353–371.

Hyman, L. H., 1951. The Invertebrates: Platyhelminthes and Rhynchocoela. The Acoelomate Bilateria. McGraw Hill. New York, 550 pp.

Jouve, C., T. Iimura & O. Pourquier, 2002. Onset of the segmentation clock in the chick embryo: evidence for oscillations in the somite precursors in the primitive streak. Development 129: 1107–1117.

Kleinig, H. & U. Maier, 1999. Zellbiologie. Gustav Fischer, Stuttgart, Jena, 534 pp.

Ladurner, P. & R. M. Rieger, 2000. Embryonic muscle development of Convoluta pulchra (Turbellaria-Acoelomorpha, Platyhelminthes). Developmental Biology 222: 359–375.

Minelli, A., 1995. Body cavities and body segmentation: problems of homology and phylogenetic reconstruction. In Lanzavecchia, G. R. Valvassori & M. D. Candia Carnevalli (eds), Body Cavities: Function and Phylogeny. Selected Symposia and Monographs, U.Z.I., 8, Mucchi, Modena: 69–73.

Nielsen, C., 2001. Animal Evolution. Interrelationships of the Living Phyla. Oxford University, New York, 563 pp.

Pedersen, K. J., 1991. Structure and composition of basement membranes and other basal matrix systems in selected invertebrates. Acta Zoologica (Stockholm) 72: 181–201.

Peterson, K. J. & E. H. Davidson, 2000. Regulatory evolution and the origin of the bilaterians. Proceedings of the National Academy of Sciences of the USA 97: 4430–4433.

Peterson, K. J., R. A. Cameron & E. H. Davidson, 1997. Set-aside cells in maximal indirect development: evolutionary and developmental significance. Bioessays 19: 623–631.

Peterson, K. J., R. A. Cameron & E. H. Davidson, 2000. Bilaterian origins: significance of new experimental observations. Developmental Biology 219: 1–17.

Potswald, H., 1981. Abdominal segment formation in Spirorbis moerchi (Polychaeta). Zoomorphology 97: 225–245.

Reiter, D., B. Boyer, P. Ladurner, G. Mair, W. Salvenmoser & R. Rieger, 1996. Differentiation of the body wall musculature in Macrostomum hystricinum marinum and Hoploplana inquilina (Plathelminthes), as models for muscle development in lower Spiralia. Rouẋs Archives of Developmental Biology 205: 410–423.

Remane, A., 1950. Die Entstehung der Metamerie der Wirbellosen. Zoologischer Anzeiger, Supplementband 14: 16–23.

Remane, A., 1954. Die Geschichte der Tiere. In Herberer, G. (ed.), Die Evolution der Organismen. Vol. 2, 2nd edn. Fischer, Stuttgart: 340–422.

Remane, A. 1963a. The enterocelic origin of the coelom. In. Dougherty, C. E. et al. (eds), The Lower Metazoa. University of California, Berkeley, CA: 78–90.

Remane, A. 1963b. The evolution of the Metazoa from colonial flagellates vs. plasmodial ciliates. In Dougherty, C. E. et al. (eds), The Lower Metazoa. Comparative Biology and Phylogeny. University of California, Berkeley, CA: 23–32.

Rieger, R. M., 1985. The phylogenetic status of the acoelomate organization within the bilateria. a histological perspective. In Conway-Morris, S., J. D. George, R. Gibson & H. M. Platt (eds), The Origins and Relationships of Lower Invertebrates. Clarendon, Oxford: 101–122.

Rieger, R. M., 1986. Über den Ursprung der Bilateria: die Bedeutung der Ultrastrukturforschung für ein neues Verstehen der Metazoenevolution. Verhandlungen der Deutschen Zoologischen Gesellschaft 79: 31–50. (English translation at http://www.umsci.maine.edu./biology/labs/original/)

Rieger, R. M., 1994. Evolution of the 'lower' Metazoa. In Bengtson, S. (ed.), Early Life on Earth. Nobel Symposium no. 84. Columbia University, New York: 475–488 (references on 517–598).

Rieger, R. M., 1991a. Neue Organisationstypen aus der Sandlückenfauna: die Lobatocerebriden und Jenneria pulchra. Verhandlungen der Deutschen Zoologischen Gesellschaft 84: 247–259.

Rieger, R. M., 1991b. Jennaria pulchra, nov. gen. nov. spec., eine den psammobionten Anneliden nahestehende Gattung aus dem Küstengrundwasser von North Carolina. Berichte des Naturwissenschaftlich Medizinischen Vereins in Innsbruck 78: 203–215.

Rieger, R. M. & P. Ladurner, 2001. Searching for the stem species of the Bilateria. Belgian Journal of Zoology, 131 (Supplement 1): 27–34.

Rieger, R. M. & P. Ladurner, 2003. The significance of muscle cells for the origin of mesoderm in Bilateria. Integrative & Comparative Biology 43: 47–54.

Rieger, R. M. & J. Lombardi, 1987. Comparative ultrastructure of coelomic linings in echinoderm tube feet and the evolution of peritoneal linings in the Bilateria. Zoomorphology 107: 191–208.

Rieger, R. M. & S. Tyler, 1979. The homology theorem in ultrastructure research. American Zoologist 19: 654–666.

Ruppert, E. E. & K. J. Carle, 1983. Morphology of metazoan circulatory systems. Zoomorphology 103: 193–208.

Salvini-Plawen, L. v. & T. Bartolomaeus, 1995. Mollusca: mesenchymata with a coelom. In Lanzavecchia, G. R. Valvassori & M. D. Candia Carnevalli (eds), Selected Symposia and Monographs U.Z.I., 8, E. Muncchi, Modena: 75–92.

Schiebler, T. H. & W. Schmidt, 2002. Anatomie. Springer, Berlin Heidelberg, 892 pp.

Schmidt-Rhaesa, A., 2003. Integrative approaches to phylogenetic relationships of arthropods: introduction to the symposium. In Legakis, A., S. Sfenthourakis, R. Polymeni & M. Thessalou-Legaki (eds), The new Panorama of Animal Evolution. Proceedings XVIII International Congress of Zoology, Pensoft, Sofia: 461–466.

Schmidt-Rhaesa, A., T. Bartolomaeus, C. Lemburg, U. Ehlers & J. R. Garey, 1998. The position of the Arthopoda in the phylogenetic system. Journal of Morphology 238: 413–418.

Scholtz, G., 2003. Is the taxon Articulata obsolete? Arguments in favour of a cose relationship between annelids and arthropods. In Legakis, A., S. Sfenthourakis, R. Polymeni & M. Thessalou-Legaki (eds), The new Panorama of Animal Evolution. Proceedings XVIII International Congress of Zoology, Pensoft, Sofia: 489–501.

Shankland, M. & E. C. Seaver, 2000. Evolution of the bilaterian body plan: what have we learned from annelids? Proceedings of the National Academy of Sciences of the USA 97: 4434–4437.

Siewing, R., 1985. Lehrbuch der Zoologie Vol. 2, Systematik. Fischer, Stuttgart, New York, 1107 pp.

Smith, J. P. S., J. Lombardi & R. M. Rieger, 1986. Ultrastructure of the body cavity lining in a secondary acoelomate, Microphthalmus cf. listensis Westheide (Polychaeta, Hesionidae). Journal of Morphology 188: 257–271.

Stauber, M., 1993. The latern of Aristotle: organization of its coelom and origin of its muscles (Echinodermata, Echinoida). Zoomorphology 113: 137–151.

Takeichi, M., 1991. Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251: 1451–1455.

Tepass, U., K. Truong, D. Goudt, M. Ikura & M. Pfeifer, 2000. Cadherins in embryonic and neural morphogenesis. Nature Reviews of Molecular Cell Biology 1: 91–100.

Turbeville J. M., 1986. An ultrastructural analyis of coelomogenesis in the hoplonemertine Prosorhochmus americanus and the polychaete Magelona sp. Journal of Morphology 187: 51–60.

Tyler, S., 1988. The role of function in determination of homology and convergence – examples from invertebrate adhesive organs. In Ax, P., U. Ehlers & B. Sopott-Ehlers (eds), Free-living and Symbiotic Plathelminthes. Progress in Zoology 36: 331–374.

Tyler, S., 2001. Origin and relationship of lower flatworms. In Littlewood, D. T. J. & R. A. Bray (eds), Interrelationsships of the Platyhelminthes. Taylor and Francis, New York: 3–12.

Tyler, S., 2003. Epithelum – the primary building block for metazoan complexity. Integrative and Comparative Biology 43: 55–63.

Wanninger, A. & G. Haszprunar, 2002. Chiton Myogenesis: Perspectives for the development and evolution of larval and adult muscle systems in molluscs. Journal of Morphology 251: 103–113.

Westheide, W., 1987. Progenesis as a principle in meiofauna evolution. Journal of Natural History 21: 843–854.

Westheide, W., 1997. The direction of evolution within the Polychaeta. Journal of Natural History 31: 1–15.

Westheide, W. & R. Rieger, 1996. Spezielle Zoologie. Teil 1: Einzeller und Wirbellose Tiere. Gustav Fischer, Stuttgart, 909 pp.

Willmer, P., 1991. Invertebrate Relationships. Patterns in Animal Evolution. Cambridge University, Melbourne: 400 pp.

Willmer, P., 1995. Modern approaches to the phylogeny of body cavities. In Lanzavecchia, G., R. Valvassori & M. D. Candia Carnevalli (eds), Body Cavities: Function and Phylogeny. Selected Symposia and Monographs, U.Z.I., 8, Mucchi, Modena: 23–39.