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The Segmented *Urbilateria*: A Testable Scenario[1]

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SYNOPSIS. **The idea that the last common ancestor of bilaterian animals (*Urbilateria*) was segmented has been raised recently on evidence coming from comparative molecular embryology. Leaving aside the complex debate on the value of genetic evidence, the morphological and developmental evidence in favor of a segmented *Urbilateria* are discussed in the light of the emerging molecular phylogeny of metazoans. Applying a cladistic character optimization procedure to the question of segmentation is vastly complicated by the problem of defining without ambiguity what segmentation is and to what taxa this definition applies. An ancestral segmentation might have undergone many complex derivations in each different phylum, thus rendering the cladistics approaches problematic. Taking the most general definitions of coelom and segmentation however, some remarkably similar patterns are found across the bilaterian tree in the way segments are formed by the posterior addition of mesodermal segments or somites. Postulating that these striking similarities in mesodermal patterns are ancestral, a scenario for the diversification of bilaterians from a metameric ancestor is presented. Several types of evolutionary mechanisms (specialization, tagmosis, progenesis) operating on a segmented ancestral body plan would explain the rapid emergence of body plans during the Cambrian. We finally propose to test this hypothesis by comparing genes involved in mesodermal segmentation.**

INTRODUCTION

The recent advances concerning the phylogeny of metazoans and the identification of their large array of conserved patterning genes have opened a stimulating debate about the morphology of the last common ancestor of the bilaterian animals (*Urbilateria*, de Robertis and Sasai, 1996) and how the bewildering diversity of the bilaterian body plans came into being. A few years ago, some molecular embryology data on amphioxus (Holland *et al.*, 1997) have revived the long forgotten hypothesis (Sedgwick, 1884) that the ancestor of coelomates was a segmented animal (Kimmel, 1996; de Robertis, 1997; Davis and Patel, 1999; Dewel, 2000). On the basis of the emerging molecular phylogeny of metazoans, we have indeed proposed earlier that *Urbilateria* was a complex organism, coelomate and possibly segmented (Balavoine and Adoutte, 1998). Here we seek to develop more thoroughly some morphological, developmental and phylogenetic arguments in favour of a segmented bilaterian ancestor. We will leave aside the currently available genetic arguments since these arguments should be considered in the framework of the heated debate on the value of developmental genetic evidence for demonstrating homology. Our ambition is instead to show that similarities between the morphological and developmental patterns in distant phyla are sufficient to consider seriously the hypothesis of a metameric *Urbilateria* and to suggest some possible axes of comparative embryology research for the future.

THE PHYLOGENETIC LIMITATIONS TO THE RECONSTITUTION OF *URBILATERIA*

Reconstructing the phylogenetic tree of bilaterians is an obvious prerequisite to the question of body plan evolution. Phylogenetic inference using large arrays of metazoan morphological and embryological characters have been proposed (Eernisse *et al.*, 1992; Nielsen *et al.*, 1996; Schram, 1997), recently in combination with molecular data sets (Zrzavy *et al.*, 1998; Giribet *et al.*, 2000; Peterson and Eernisse, 2001). Though these works have applied the apparent rigorousness of the cladistic principles to metazoan phylogeny, it can be said that they have left open many questions concerning the evolution of the bilaterian body plans. The limitations of such approaches in the case of metazoans have been described (Jenner and Schram, 1999). In our opinion, the most damning caveat of this morphology-based phylogeny is the paucity of independent characters available across all bilaterian phyla for testing alternative scenarios of body plan evolution. Most characters also are coded as present or absent and these codings can be misleading since there are good reasons to think that multiple secondary losses of some characters have happened during metazoan evolution.

The rise of the molecular-based estimates of metazoan phylogeny has provided a wealth of new data. The emerging results bearing on the molecular phylogeny of metazoans have been reviewed elsewhere (Adoutte *et al.*, 2000). We want to summarize here the main conclusions of these investigations, carried out mostly with 18S ribosomal RNA (see also Mallat and Winchell, 2002 for 18S and 28S rRNA combined) but also confirmed in some respects with other types of sequences (Erber *et al.*, 1998, 1999; de Rosa *et al.*, 1999; Manuel *et al.*, 2000). Only a few nodes appear robustly resolved but these nodes happen to be quite significant ones (Fig. 1). The bilaterian monophyly is

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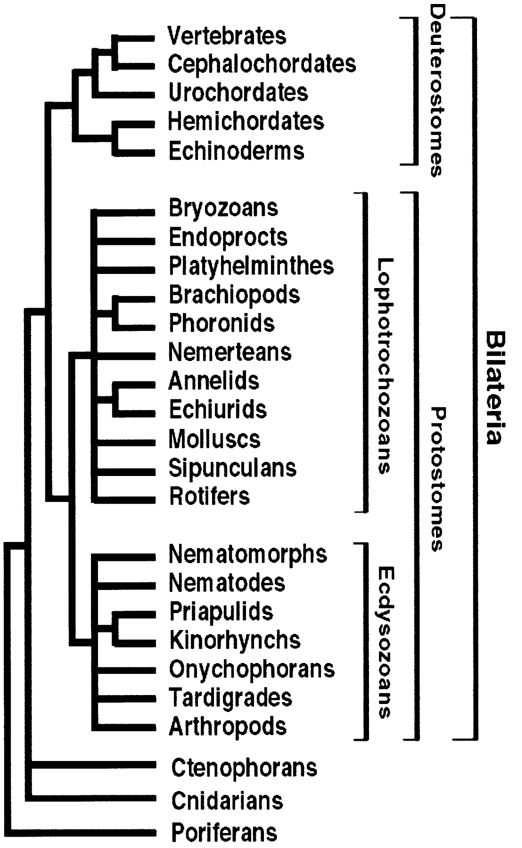

FIG. 1. A phylogenetic tree of metazoans according to recent molecular analyses. This tree should not be seen as a consensus but rather as what we think is our conservative interpretation of current data.

strongly supported. Bilaterians appear to be divided in two large clades: the deuterostomes and the protostomes, and the latter is likewise divided into the ecdysozoans and the lophotrochozoans (or trochozoans). This organization of the tree contradicts some long standing ideas on metazoan phylogeny, notably the paraphyly of protostomes, the articulate clade or the deuterostome affinities of lophophorates. The new topology is extremely important for understanding the comparative reasoning that leads to hypotheses about the morphology of *Urbilateria*. In particular, it implies that:

— the last common ancestor of insects and vertebrates was in fact *Urbilateria*. The remarkable similarities found in the genetics of development between the fruit fly and the vertebrates therefore have their origin in *Urbilateria*.

— the ancestor of annelids and arthropods, whose respective metamerisms have been considered homologous by most zoologists for more than a century, is also the ancestor of all the protostomes. If the ancestor of protostomes displayed a segmentation comparable to annelids and arthropods, this necessarily means that segmentation was lost independently in different protostome lineages.

— the most important consequence is the demise of the idea that some extant metazoan phyla of simple organization represent «living fossils». Platyhelminths, whose relative simplicity of body organization has been considered a remnant from an archaic stock of flatworm-like ancestral bilaterians (Hyman, 1940; Willmer, 1990), are in our view derived trochozoans, whose ancestors have lost the anus, the coelom and the circulatory system (Balavoine, 1998). In partial contradiction to this view, some molecular data, including 18S rRNA (Ruiz-Trillo *et al.*, 1999) and myosin sequences (Ruiz-Trillo *et al.*, 2002) have led some authors to single out the acoelomorphs, a group of very simply organized flatworms, as a potential sister-group to the rest of bilaterians. Although the results in question must be taken seriously, we believe they are misleading. Acoelomorphs share several important morphological synapomorphies with the other platyhelminths and therefore belong with the trochozoans as far as morphology is concerned (Peterson and Eernisse, 2001). Nematodes were also considered primitive because of their pseudocoelomate body organization but they could likewise be viewed as strongly simplified bilaterians.

These latter views were criticized by Jenner (2000) on the account that the reconstruction of ancestral characters we proposed was not based on a rigorous cladistic analysis. In the following section, we want to address these criticisms and demonstrate that cladistics alone does not allow the resolution of the question of ancestral segmentation in our present state of knowledge. The main difficulty of applying a cladistic analysis to the problems of coelom and segmentation ancestry reside in the coding of these characters. Many phyla do not fit easily into the simple categories defined by the most generally accepted definitions of these characters, as we will now explain.

Coelom

A coelom is a body cavity lined by a mesodermic epithelium. According to this simple definition, a large proportion of the bilaterian animals can be described as coelomates (Table 1). The coelom takes vastly different forms and functions between different phyla and also within some phyla. In many annelids, it is considerably developed in the adult animal, thus providing the hydrostatic skeleton, while in molluscs, it is reduced to thin cavities around the heart, gonad and kidneys. In arthropods, the coelom is only a transient feature in embryogenesis and it is entirely replaced in the adult by a large haemocoel. In echinoderms, one of the coeloms forms the complex system of water canals. Nevertheless, whatever a coelom becomes in the adults, it takes at some point during the development the form implied by the definition. The particular significance of this developmental character has been recognized very early on as the presence of the coelom is strongly correlated with the existence of complex organ systems.

Beside coelomate taxa, the so-called ''pseudocoelomate'' groups have always been problematic. Most zoologists agree that ''pseudocoelom'' is an illegitimate catch-all term. The body cavity nature varies considerably in these phyla, from complete absence of body cavity in the entoprocts and gastrotrichs to the priapulid body cavity that is actually interpreted as a true coelom by some authors. In nematodes, nematomorphs and kinorhynchs, the body cavity is supposed to be derived from an embryonic ''blastocoel.'' The loss of a canonical coelomate condition towards these various forms of ''pseudocoelom'' is easy to imagine, especially in minute animals (Budd and Jensen, 2000).

Developmental arguments have often been used to propose the idea that coeloms across bilaterians might not be homologous. According to this view, coelom ontogeneses can be divided in two different ways: enterocoely (pouches forming out of the archenteron wall) in the deuterostomes and schizocoely (hollowing of mesodermal blocks) in the protostomes. Several authors have contested this argument (Remane, 1963) but the most recent and clearest charge against it has been made by Budd and Jensen (2000), with whom we fully agree. In short, the enterocoely/schizocoely dichotomy is an incorrect simplification of the very diverse modes of coelomogenesis. Different modes operate sometimes in closely related animals for giving obviously homologous coeloms and thus this ontogenetic diversity cannot be argued for contradicting the homology of the coelom.

Segmentation

The definition of segmentation is more controversial than the definition of the coelom. Although there is no clear consensus in the literature, we have chosen to use the term metamerism and segmentation as synonymous. An organism is considered metameric (or segmented) when its body is mostly formed of serially repeated anatomical units called metameres (or segments). This definition applies most convincingly to annelids and arthropods. In addition, serially-repeated structures are common among unsegmented bilaterians. Many have sought to make a distinction between full metamerism and mere serial repetition of particular organs. Serial repetitions of organs would be extremely common in evolution and would have little significance when considering phylogeny at a large scale. Full metamerism would be a much more significant phenomenon, since it would require a tremendous amount of morphological, physiological and developmental adaptations to make a viable organism out of repeated units. In our opinion, these definitions are too narrow, especially when applied to cladistic reasoning. The question of the origin of segmentation cannot be addressed rigorously in this manner because the idea that full metamerism and serial repetitions have nothing to do with each other is imposed before any analysis. This restriction is flawed if a metameric organisation of the body can be lost secondarily in a way that will let some organs be repeated. For instance, many arthropods have lost extensive metamerism but still have remnants in the form of repeated organs (such as the limbs in cladocerans and spiders, or the heart ostia in a spider opisthosoma). On the other hand, we cannot rule out that full metamerism evolved as a progressively coordinated repetition of organs and intermediate situation can still exist in extant organisms. As rightly pointed out by Budd (2001), we cannot classify organisms simply as segmented or not. We have to consider the phenomenon of segmentation in all its various manifestations. In Table 1, we extend the definition of segmentation to all forms of periodical repetition of organs along the anterior-posterior axis, without postulating in any way that these are homologous. Table 1 shows that segmentation, far from being restricted to a few metameric phyla, is a very widespread feature of bilaterians.

Two particular problems arise that bring difficulties in defining similarities between phyla:

- organs affected by segmentation are not always the same. Seriation can be acquired or lost selectively in some organs. New organs can also evolve and adopt the general segmented pattern of the body. This is the case for the axial skeleton of vertebrates. Classifying animals as segmented or not cannot be done by simply considering one particular system of organs or one particular germ layer.
- the seriation found in different organs can be disconnected from one another. In amphioxus, there is no apparent relationship between the seriated gill slits and the myotomes. In vertebrates, disconnection is also found between the pharyngeal arches, the somites and the brain segmentation. A classical explanation is that these different segmentation patterns appeared independently but another explanation is possible: ''decoupling'' can occur during evolution and development. For example, anterior somites appear in good correspondance with pharyngeal arches during the embryogenesis of ''basal'' vertebrates such as lampreys (Kuratani *et al.*, 1999) or sharks (Goodrich, 1918; Rylands de Beer, 1922) while they do not correspond in amniotes.

As a consequence of these coding difficulties, both for coelom and segmentation, we do not think that a cladistic optimization analysis of these characters using a molecular tree of metazoans (even if it was entirely resolved, which is far from true in current results) gives presently much insight into how they actually evolve. The outcome of an ancestor reconstruction is entirely dependent on two prior parameters that will inevitably reflect the preconceptions of the investigator: the interpretations of the various forms of seriations described in table 1 and the relative weight accorded to the acquisition and loss of seriation.

One important phenomenon however is confirmed by molecular phylogeny: segmentation has been lost secondarily in some lineages. According to a recent

TABLE 1. *The various forms of body cavities and segmentations found in bilaterians.*

	Body cavity	True coelom	Segmented coelom	External segmentation[1]	Neural segmentation[2]	Segmented organs[3]
Deuterostomes						
Vertebrates	yes	yes	somites		cranial and spinal nerves	myotomes, axial skeleton, pharyngeal arches
Cephalochordates	yes	yes	somites		segmental nerves	myotomes, gill slits, gonads, nephridia
Urochordates	atrium					
Hemichordates	yes	yes	trimeric			gill slits, gonads
Echinoderms	yes	yes	trimeric	(a)	(a)	(a)
Ecdysozoans						
Arthropods	haemocoel	embryonic	yes	segments + appendages	ganglia and nerves	muscles, ostia + vessels, tracheae
Onychophorans	haemocoel	embryonic	yes	appendages	ladder-like cords and nerves	muscles, ostia, tracheae, nephridia
Tardigrades	haemocoel	yes	4 pairs of sacs	appendages	ganglia and nerves	muscles
Nematodes	yes					
Nematomorphes	yes					
Priapulids	yes	disputed		(b)		
Kinorhynchs	yes			‘‘zonites’’	ganglia	muscles
Loriciferans						
Trochozoans						
Annelids	yes	yes	yes	segments + parapodia	ganglia + nerves	muscles, vessels, nephridia, septa
Echiurids	yes	yes			embryonic ganglia + nerves	
Pogonophorans	yes	yes	yes	‘‘Opisthosoma’’	ganglia + nerves	septa
Sipunculans	yes	yes				
Molluscs	haemocoel	yes		(c)	ladder-like	(c)
Brachiopods	yes	yes	2 to 4 pairs of sacs	(d)		
Phoronids	yes	yes	3 pairs of sacs			
Bryozoans	yes	yes				
Endoprocts						
Platyhelminths					(e)	(e)
Nemerteans	yes	yes		(f)		(f)
Rotifers	yes			(g)		
Acanthocephalans	yes					
Unclear affinities						
Gnathostomulids						
Gastrotrichs						
Chaetognaths	yes	yes	trimeric (h)			

[1] *External segmentation*
We mean by external all the structures which are derived from the epidermis, including an exoskeleton or a shell, as well as appendages. The contentious interpretation in this category are the various examples of externally visible segments in echinoderms (stalk of crinoids, arms of ophiuroids, appendage of carpoids), the annulation of the epidermis of priapulidae and various structures in molluscs (eight plate shell of the polyplacophorans, crowns of spicules of larval solenogasters, repeated gills of the polyplacophorans and monoplacophorans). In these cases, there is no certainty on the plesiomorphic nature of the feature because it is found in only a few groups inside the phylum.

[2] *Neural segmentation*
We include in this category not only the animals with repeated ganglia but also the animals with a ‘‘ladder-like’’ central nervous system such as found in many molluscs. A ladder-like CNS is found also in many platyhelminths but there is no certainty on the plesiomorphic nature of this character in this group.

[3] *Internal organs segmentation*
We mostly include in this category the segmented muscles and endoskeletons, but that may also include repeated gonads, nephridia, blood vessels and all mesodermally derived organs. Features that are possibly independent apomorphies include the ‘‘vertebrae-like’’ elements in crinoids and ophiuroids, the segmented muscles of polyplacophorans and monoplacophorans and the repeated gonads in flatworms and nemerteans.

The notes below describe particular cases of seriation.

(a) The stalk of crinoids is segmented based on skeletal elements called ossicles. The arms of ophiuroids have both sub-epidermal and internal segmented skeletal elements called shields and vertebral ossicles. This segmentation extends to the water canals and podia system.

(b) The epidermis of *Priapulus* bears regular rings but there is no associated internal segmentation.

(c) The most overtly segmented molluscs are polyplacophorans and monoplacophorans. Polyplacophorans have a shell divided in eight plates. Pairs of spicules bundles are also often associated in seriated arrangement. Internally, segmental pedal muscles are associated to each shell molecular analysis (McHugh, 1997; reviewed in Halanych *et al.*, 2002), pogonophorans and echiurids would be nested inside the annelid tree, *i.e.*, derived from annelid ancestors. This is hardly surprising for the pogonophorans since the organization of the opisthosoma of these animals, their setae (Bartolomaeus, 1995), as well as their larva (Southward, 1988) show strong polychaete-like characters. Pogonophorans, once considered a phylum of their own, have been downgraded as a family of annelids, the Siboglinidae, in recent systematic literature (Rouse and Fauchald, 1997). More striking is the assumption that echiurids are derived from polychaete ancestors because these animals were described as showing no sign of segmentation, though recent developmental studies have revealed that the nerve chord forms metamerically as in annelids (Hessling and Westheide, 2002). Even Sipunculans, sometimes considered to be close to molluscs, may finally be recognized as derived annelids as suggested by some recent molecular analyses (Boore and Staton, 2002). Siboglinidae and Echiuridae are both remarkable examples of secondary loss of metamerism in animals of relatively large size and these examples make the hypothesis of the repeated loss of segments in several other protostome groups much less extraordinary that it may seem.

As long as phylogeny is concerned, we are thus left with two radically opposed interpretations on the evolution of segmentation: one that postulates that the various forms of seriation appeared convergently in many different bilaterian lineages and the other one that contemplates the possibility that many bilaterian lineages would have lost secondarily the segmentation displayed by a metameric *Urbilateria*. In support for the second scenario, we now want to stress the importance of developmental arguments, especially mesoderm segmentation.

SOMITES: THE MISSING LINK?

Segments are formed in many different ways in bilaterians. Apparently, there is little similarity between the simultaneous subdivision of the blastoderm into parasegments seen in *Drosophila* and the posterior addition of segments from a sub-terminal growth zone seen in many crustaceans. The reasonable assertion that segments in insects and crustaceans are inherited from a metameric arthropod ancestor indicates however that phylogenetic transition between totally different ways of making segments is possible. What is the ancestral way of making segments then? To answer this question, we must focus on the few similarities we can actually find between distant bilaterians and evaluate how likely their parallel evolution is. When considering the phyla that are the most obviously segmented in all three branches of the tree, we find that at some stage, seriated paired coelomic cavities or ‘‘somites’’ are involved. Somites reveal an intimate relationship between the coelom and segmentation and show, in our opinion, that these two characters are not independent. Somites are transient embryonic mesodermal structures. They are usually pockets of epithelium surrounding a coelomic cavity (somitocoel) and formed in paired series along the body axis. The fate of somites varies considerably among bilaterians. In annelids, the whole of the animal body cavity is derived from the large extensions of the somitocoels, while all the mesodermal derivatives in the trunk (including somatic and splanchnic muscles, the septa, the blood vessels, etc.) arise from the mesodermal lining. In arthropods and also onychophorans, the somitocoel, when present, never takes any extension and finally disappears, replaced by the haemocoel as the main body cavity. The epithelium of the somites however still forms most of the mesodermal derivatives. In lancelets (amphioxus), the trunk somites extend and fuse ventrally to give the unsegmented perivisceral coelom. In vertebrates, the mesoderm is divided early on in several components (somites, intermediate and lateral plate mesoderm) and the dorsal somites do not contribute to the adult coelom. Nevertheless, the somitic mesoderm gives rise to the sclerotome (axial skeleton), the myotome (axial muscles) and the dermatome (overlying of the skin). In the segmented organisms in which they occur, somites are always ontogenetically connected to adult segmentation. Each somite pair corresponds to a myotome in vertebrates or to an adult segment in annelids and arthropods. Moreover, somites play comparable roles in the ontogenesis of some seriated organs in distant organims: either these structural units directly originate from the mesodermal epithelium or their seriation is apparently induced by the somites. The organs they produce include segmental muscles, nephridia, gonads and gonoducts (Table 1).

In short, somites as characterized here are present in the most extensively segmented animals of three

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plate (Nielsen, 2001). Monoplacophorans have an undivided shell but a largely segmental organisation with seriated pedal muscles, nephridia, gills and heart atria. Both groups have a ladder-like CNS.

(d) The larvae of craniids and articulates form setal sacs comparable to those found in annelids (Nielsen, 1991).

(e) The CNS of many flatworms is ladder-like, especially in triclads, proseriates and neodermatans. Seriated gonads and gut diverticula are common. The body of cestods is divided in metameric proglottids but these reproductive ‘‘segments’’ are added from an anterior growth zone, in opposition to the general rule of posterior addition.

(f) Seriated gonads and gut diverticula are found in many species of nemerteans. *Annulonemertes* (Berg, 1985) has an external segmentation.

(g) The foot of bdelloid rotifers bears annuli.

(h) The mesoderm of chaetognaths forms as an epithelial outgrowth of the archenteron and becomes later subdivided in three compartments (Shinn and Roberts, 1994).

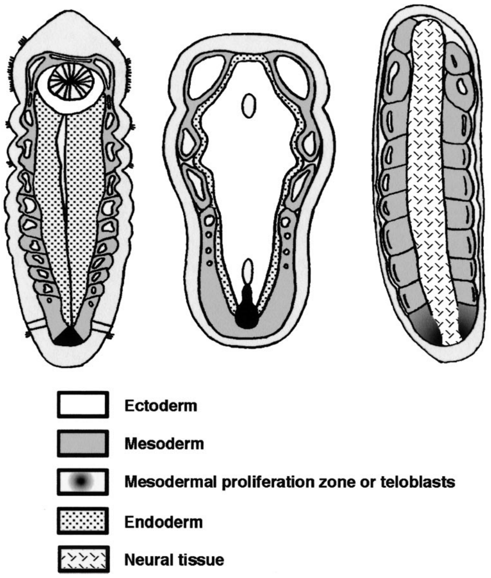

FIG. 2. Comparisons of coelomogenesis and segmentation in three types of bilaterians, belonging to the three great branches of the bilaterian tree. On the left is a frontal section through a metamorphosing larva of the polychaete *Scoloplos* (redrawn from Anderson, 1959); in the middle, a frontal section through an embryo of the onychophoran *Peripatopsis* (redrawn from Manton, 1960) and on the right a frontal section through the embryo of an amphioxus (redrawn from Conklin, 1932). The existence of a genuine posterior growth zone in amphioxus is supported by recent data (Schubert *et al.*, 2001). These pictures have been chosen to emphasize the striking similarities of the processes involved, *i.e.*, sequential splitting of mesodermal somites from a posterior proliferation zone.

most divergent bilaterian lineages and they play similar roles in organogenesis, but is there any potentially homologous similarity in the way they are generated in these distant groups? Indeed, there is: somites in metameric animals usually form sequentially from a posterior growth zone, a process found again in all three branches of the bilaterians (Fig. 2). Of course, there is considerable variation in the structure of these growth zones as well as in the stage when they appear during life history. In amniotes, the growth zone corresponds to the node and later in embryonic development to the tailbud. In short-germ insects, segments are added sequentially in a posterior mass of embryonic cells (Davis and Patel, 2002). In crustaceans such as branchiopods, new segments are added during larval development from a sub-terminal growth zone, in a manner similar to what is seen in annelids. Both embryonic and post-embryonic growth co-exist in the main segmented phyla showing that phylogenetic transitions must have happened between these modes. The growth zone lays out the three germ layers and produces segments in a sequential manner: mesodermal bands elongate posteriorly from the growth zone and become segmented by the sequential splitting of pairs of lateral somites. Shortly later, a somitocoel forms in each somite by schizocoely (enterocoely is encountered mostly in animals that form few coelomic sacs and do not show posterior growth).

In our opinion, the striking similarities illustrated in Figure 2, as well as the similar role played by somites in the formation of some organs are not due to convergence. Somites produced sequentially by a posterior growth zone must have been present in *Urbilateria* and are strong arguments in favour of a metameric *Urbilateria*. The hypothesis of the homology of somites would be greatly strengthened if we could find in ‘‘unsegmented’’ bilaterians remnants of this mechanism for building the body axis, in the same way as embryonic pharyngeal arches have been retained in amniotes despite the disappearance of the adult gill arches. And, in fact, we think it is the case in the so-called ‘‘trimeric’’ or ‘‘archimeric’’ phyla.

Somites in unsegmented bilaterians?

‘‘Trimeric’’ or ‘‘archimeric’’ animals display embryonic features that can be compared to somites but have not generally been interpreted as such. The definition of the trimeric organisation is that the body axis is divided in three parts, each with its own coelomic cavity. Two of these groups (brachiopods and phoronids) belong to the «lophophorates», themselves part of the (lopho)trochozoans, two others are the echinoderms and hemichordates, part of the deuterostomes and the last one is chaetognaths, of still unclear affinities. A trimeric organization has been proposed for the ancestor of bilaterians by a number of authors (Jägersten, 1955; Remane, 1963). More recently, Nielsen (2001) still defends the idea that brachiopods and phoronids are related to the deuterostomes and proposes that a trimeric (or ‘‘archimeric’’) body plan is plesiomorphic in a clade which he calls *Deuterostomia sensu lato.* These scenarios are contradicted however by two sets of arguments: the questionable reality of the tripartite organization in most of these groups and the results of molecular phylogenies.

The coelom of the adult lophophorates (brachiopods, phoronids and bryozoans) has been traditionally said to be divided in three parts: the protocoel (epistome, usually reduced), the mesocoel (lophophore) and the metacoel (trunk). But this arrangement is only seen in phoronids (Emig, 1977 for review). In brachiopods, four pairs of coelomic sacs form in larval *Neocrania* (Nielsen, 1991) but only two pairs in articulates (Conklin, 1902; Percival, 1944). In bryozoans (Ectoprocta), no partition of the coelom is ever observed. Molecular data sets indicate that lophophorate phyla are trochozoans but generally do not support grouping bryozoans with the phoronids and brachiopods. Phoronids might just be a group of derived brachiopods (Cohen *et al.*, 1998; Cohen, 2000). The segmented appearance of the larva of the brachiopod *Neocrania* together with the serial organization of its four pairs of coelomic sacs (Nielsen, 1991) suggests to us that brachiopods may have evolved from a segmented annelid-like ancestor, as proposed by Guttman *et al.* (1978).

In hemichordates, the three adult coelomic cavities are derived from coelomic sacs that form around the

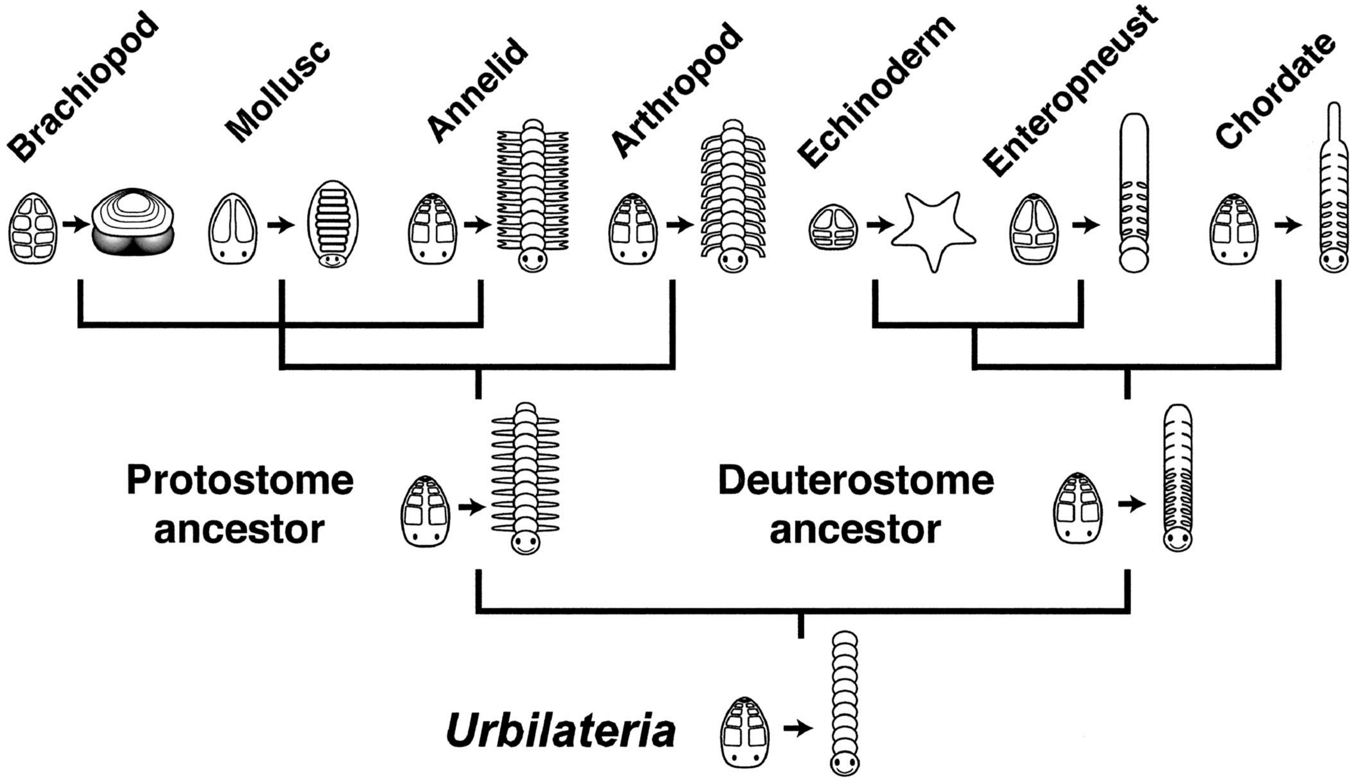

FIG. 3. A scenario on the evolution of a coelomate segmented *Urbilateria*. Only the extant phyla which show some sort of mesodermal segmentation (discussed in the text) are represented. Each organism is represented by a scheme of a key developmental step (embryonic or larval) on the left and a scheme of the adult organization on the right. The developmental sketches show the formation of coelomic cavities and a posterior growth zone (posterior grey band) when present. Together with the adult sketch, it shows some of the possible derivations of the ancestral homonomous ancestor we postulate, including the acquisition of segmental gill slits (chordates and hemichordates), the fusion of segments (enteropneust) and the reduction of segments (molluscs, brachiopods). The terminal posterior piece of the protostome ancestor is an anus-bearing pygidium, also found in extant annelids and arthropods (telson).

gut of the larva (an unpaired protocoel and two pairs of sacs for the mesocoel and metacoel). In echinoderms, a very similar situation occurs with three pairs of sacs forming in the larva (axocoels, hydrocoels and somatocoels). The fate of these sacs is connected to the adult morphology in a complex way (see Peterson *et al.*, 2000*a* for review). The hypothesis that the trimeric organization of the coelomic sacs of larval echinoderms and enteropneusts is a homologous derived trait (*i.e.*, a synapomorphy) is considerably reinforced by molecule-based phylogenies: inside deuterostomes, a clade grouping echinoderms and hemichordates (called ‘‘ambulacrarians’’ in Peterson and Eernisse, 2001) is a likely sister group of the chordates (Wada and Satoh, 1994; Bromham and Degnan, 1999; Cameron *et al.*, 2000). In amphioxus, the coelomic cavity in the anterior-most somites form by enterocoely as it does in echinoderms and hemichordates and an homology is suggested between the anterior-most pair of somites of amphioxus (forming the organ called Hatschek’s pit) and the protocoel (for review: Stach, 2002). This similarity might suggest that the coelomic sacs of ambulacrarians are the remaining anterior sacs of an ancestor with a segmented coelom. Alternatively, the long metasome of the enteropneusts might represent a fusion of the trunk somites of this segmented chordate-like ancestral deuterostome. In support of the latter interpretation, the enteropneust gill slits assume a segmented organization which can be a remnant of a more generalized segmentation.

Finally, in the still poorly known chaetognaths, a head coelom and a pair of trunk coeloms form transiently, the trunk coeloms becoming divided by a septum only later in development (Shinn and Roberts, 1994). Chaetognaths might be related to ecdysozoans (Halanych, 1996; Peterson and Eernisse, 2001).

In short, the so-called «trimeric» organisation of the body, when it truly exists, probably is a derived feature acquired independently in some lophophorates, the ambulacrarians and chaetognaths. However, the pairs of coelomic sacs present in most of these organisms need not be a derived feature. They can be interpreted as being derived from somites and the remnant of a segmented organisation.

THE SEGMENTED *URBILATERIA* AND HOW IT EVOLVED

Taking together all the evidence described above from phylogeny, compared anatomy and embryology, we advocate a coelomate and segmented worm-like ancestor of the bilaterians (Fig. 3). This animal would have to be relatively large since the coelomate condition cannot have evolved in a tiny animal (Budd and

Jensen, 2000). In addition to the coelom, some system of organs which are correlated with it were already probably present such as a blood circulatory system, segmental nephridia (both necessary in an animal of large size) and probably segmental gonads. Additional arguments coming from compared developmental genetics also suggest that *Urbilateria* had simple eyes (Gehring and Ikeo, 1999 but see Arendt and Wittbrodt, 2001 for the particular case of chordate eyes), some kind of appendages (Panganiban *et al.*, 1997) which in a segmented animal may logically have been segmental, a dorsal ''heart'' or heart tube (Bodmer and Venkatesh, 1998) and a centralized nervous system with a regionalized brain and ventral nerve chord (Arendt and Nübler-Jung, 1996, 1999).

Urbilateria must have developed indirectly from a microscopic ciliated larva such as those found in trochozoans or echinoderms. Valuable arguments on the ancestrality of indirect development have been put together by Peterson *et al.* (2000*b*). To us, the strongest fact is that a biphasic life cycle seems to be plesiomorphic in metazoans in general (many sponges and cnidarians have ciliated larvae). This larva must have given rise to a juvenile through a radical metamorphosis. The segmented trunk of this juvenile would have been formed by the process of posterior addition described above. Clearly, the organisms which today resemble this hypothetic *Urbilateria* the most are the annelids. Obviously, annelids are not ''living fossils'' but, in our view, they are the animals whose body-plan (and presumably life-cycle) has derived the least from *Urbilateria*.

How did this ancestral metameric *Urbilateria* give the tremendous diversity of modern body plans? From Table 1 and the above considerations, it is clear that the processes involved must have been diverse and complex. We can tentatively distinguish three major types of processes:

- the first process is the variation of the number of segments. This phenomenon is obvious in most segmented groups. Annelids possess from a few to several hundred segments at adulthood. A similar variation is seen in myriapods or in the number of vertebrae in vertebrates (especially in the tail).
- the second process is the specialization of segments that can lead to the loss of some seriated organs. It is particularly obvious in tagmatized species. Tagmosis is the functional specialization of a group of contiguous segments. This phenomenon is well known in many arthropods but the same process also happens in all other segmented groups. Tagmosis can ultimately lead to the outright fusion of neighbouring segments and the loss of apparent metamerism, as seen for the arthropod head.
- a third major category of processes is the occurrence of progenesis. Progenesis is sexual maturity acquired precociously in an animal that still displays a larval morphology. The effect of progenesis has been best illustrated in some groups of interstitial-living annelids (Westheide, 1987) in which parapodia as well as the coelom are lost and the segmented appearance greatly reduced. Progenesis can be proposed for such animals as rotifers which present some characters of larval trochozoans, including a feeding trocha.

These tentative explanations of the many hypothetical derivations of the ancestral segmentation will show to those who feel uncomfortable with the apparent loss of segmentation in so many modern forms that the real problem is not indeed why it was lost repeatedly but rather why it was retained in some groups. There seems to be such an important trend towards the specialization of body parts that the reason why the original identical units forming these body parts would still be expressed at all does not seem so obvious. This in turn raises an even more fundamental question: why did segmentation evolve in the first place or in other terms, what selective advantages, if any, would have favored its appearance? Some authors have tried to answer this question for particular groups. Clark (1964), basing his reasoning on annelids, has emphasized the crucial advantage that a segmented coelom used as a hydrostatic skeleton may have brought for a burrowing creature. Budd (2001) proposes an evolutionary scenario for the development of a segmented exoskeleton in the ancestors of arthropods (''arthropodization''), based also on adaptative requirements for locomotion. In vertebrates finally, the organization of muscles into myotomes for swimming has long been considered of crucial adaptative importance. All these adaptative explanations certainly have some truth in them as long as the conservation of some aspects of ectodermal or mesodermal segmentations in modern forms is concerned. But they do not provide a response for the initial appearance of segmentation. Dewel (2000) proposes a radical scenario: the evolution of a segmented *Urbilateria* from a colonial cnidarian. Though the idea is not entirely new, Dewel gives this theory new strength by stressing the importance of colony forming as the origin of new ''meta-organisms'' in metazoans and especially in cnidarians. Though her particular point on the correspondance between the body plans of pennatulaceans and bilaterians seems to us a bit too far-fetched, her colonial theory has the merit of giving a possible explanation for the sudden appearance of extensive metamerism.

Testing the Segmented *Urbilateria* Hypothesis

We believe with others (Davis and Patel, 1999) that the idea of a segmented *Urbilateria* is a testable hypothesis through the investigations of comparative molecular embryology. The principle of the test is simple: a genetic machinery for making a segmented trunk must have existed in *Urbilateria* and parts of it may have been conserved in extant organisms. The goal is thus to find similarities in gene expressions and functions between distant segmented organisms belonging to the three great branches of bilaterians. This is illustrated for instance in the similar expressions of *engrailed* and *wingless* in arthropods and the annelid *Platynereis* (Prud'homme *et al.*, unpublished data). This result strongly suggests that *engrailed* and *wingless* were already involved in the ectodermal segmentation of the last protostome ancestor.

The scenario presented here however relies on the hypothesis that the posterior splitting of mesodermal somites is the ancestral mechanism for making segments. If this scenario is true, *Drosophila*, in which the genetic mechanisms of segmentation are by far the best understood, makes its segments in a very derived manner. In the fruit fly, segments are genetically delimitated early and simultaneously, when the embryo still is a blastoderm and the process of segmentation does not involve somites. Vertebrate homologues of most of the fly segmentation genes (*i.e.*, the ''gap,'' ''pair-rule'' and ''segment polarity'' genes) have been identified. It is clear that many of them do not seem to be involved in segmentation processes. In our opinion, these facts do not disprove a common origin of segmentation. They might simply reflect the acquisition of a derived segmentation genetic machinery in insects, in particular in the ''long germband'' insects such as *Drosophila*. What would be a better approach to test our scenario? This scenario leads to the prediction that more similarities are going to be found between the animals which do share posterior growth and mesodermal somites, as illustrated in Figure 2. Among trochozoans, polychaete annelids are an obvious choice for the reasons described above. Choosing appropriate models among ecdysozoans is more problematic. Onychophorans (the example of Figure 2) are probably not the easiest animals to study as long as reproductive biology is concerned. A variety of arthropod species have been used in recent years in comparative developmental biology. Most of them, unlike *Drosophila*, use a posterior addition of segments, such as in spiders (Damen *et al.*, 2000), crustaceans (for example *Triops*, Nulsen and Nagy, 1999) or myriapods (Hughes and Kaufman, 2002). Somite-like structures are present in arthropods (Anderson, 1973) but nothing is known so far on the genetic aspects of their formation. The deuterostomes are of course best represented by chordates. In recent years, a wealth of data has been obtained on the genetic mechanism of mesoderm segmentation (called somitogenesis) in vertebrates (for review, Pourquié, 2000). Somite formation in cephalochordates (amphioxus) as illustrated in Figure 2 is also quite interesting.

Investigating the functions, in annelids and arthropods with posterior growth, of the homologues of the genes involved in vertebrate somitogenesis is certainly a good starting point. Applying a candidate-gene approach of this sort may fail nevertheless to identify an important part of the ancestral segmentation machinery. We hope that the systematic screening for the expressions patterns of large collections of genes, made possible by the growing availability of *in situ* hybridization robots, will in the next few years give a first interesting picture of how all these animals make their segments.

CONCLUSION: SEGMENTED ANCESTORS AND THE CAMBRIAN EXPLOSION

The hypothesis of a coelomate and segmented *Urbilateria* leads us to a new understanding of the Cambrian explosion. We have already briefly mentioned this idea in a previous article (Balavoine and Adoutte, 1998). The basic principle is that it is much simpler to explain the explosive diversification of body plans that apparently occurred over such a short period of time during the Cambrian if we suppose that the common ancestors (*i.e.*, the last common ancestors of trochozoans, ecdysozoans and deuterostomes) of these numerous new lineages were already complex organisms. Many of the features present in Cambrian organisms were already present in these ancestors and did not evolve *de novo*. The last common ancestors of the three superphyla would have been ''large'' animals (*i.e.*, at least centimetre-sized), coelomate and segmented since they would have inherited these features from *Urbilateria*. They could also have borne evolutionary novelties acquired during their post-*Urbilateria* history. In other terms, the ancestor of the trochozoans could have already displayed annelid-like chaetae (they are found today in brachiopods and myzostomids as well as annelids), the ancestor of the ecdysozoans could have borne a sclerotized cuticle (and molted it periodically) and the ancestor of the deuterostomes could have had gill slits and a notochord.

Obviously, the scenario we detail in this article is and will remain controversial in the near future. Some authors (especially Erwin and Davidson, 2002), in direct opposition to us, defend a much more simply organized last common ancestor of bilaterians. We are confident however that this controversy is not a sterile debate and that the ever increasing amount of data obtained in the field of comparative developmental biology in an ever wider panel of metazoans will one day help in getting a better picture of *Urbilateria*.

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