Parallel evolution of segmentation by co-option of ancestral gene regulatory networks

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Different sources of data on the evolution of segmentation lead to very different conclusions. Molecular similarities in the developmental pathways generating a segmented body plan tend to suggest a segmented common ancestor for all bilaterally symmetrical animals. Data from paleontology and comparative morphology suggest that this is unlikely. A possible solution to this conundrum is that throughout evolution there was a parallel co-option of gene regulatory networks that had conserved ancestral roles in determining body axes and in elongating the anterior-posterior axis. Inherent properties in some of these networks made them easily recruitable for generating repeated patterns and for determining segmental boundaries. Phyla where this process happened are among the most successful in the animal kingdom, as the modular nature of the segmental body organization allowed them to diverge and radiate into a bewildering array of variations on a common theme.

Keywords: body plans; evolution; fossil record; gene regulatory networks; segmentation

Introduction

A segmental organization of body parts is a very efficient scheme for making an animal. In a segmentally organized animal, several types of units at the cell, tissue or organ level are repeated along the anterior-posterior (A/P) axis, and units of different types are grouped together in individual segments. These units can include muscles, skeletal units (internal or external), clusters of nerve cells (ganglia), excretory organs, sensory organs, and locomotory organs (legs, wings, or paddles).(1) A segmented body offers a modular ground plan, which can be modified by the forces of evolution to generate a bewildering array of variations on a common theme. Indeed, three of the most speciose and ecologically diverse animal phyla present such a body plan. The arthropods (insects, crustaceans, spiders, centipedes, and their kin), the annelids (segmented marine worms, earthworms, leeches), and the chordates (lancelets, sea squirts and vertebrates, including mammals and within them our own species) are all built of repeated units including some or most of the organ systems detailed above.

While it is the inherent modularity of the segmented body plan that has made these three phyla so successful, this is probably not the original driving force for its evolution. The selective forces for evolving a segmented body were probably originally related to movement.(2) An animal with a hard exoskeleton would need joints to allow lateral movements of the body, and these joints could form the external basis of the segments. In contrast, a relatively soft-bodied animal would need segmentally organized muscles to allow it to push through the substrate in a burrowing lifestyle. An alternative selective force could be the need to organize nervous tissue (or germ tissue) into clusters along the axis of a long-bodied animal. These clusters would form the basis of reiterated ganglia (or gonads), and thus a precursor to full segmentation.

There is some confusion in the literature regarding what is actually meant by segmentation, as it can refer both to the *process* of generating segments and to the segmented body plan (the *outcome* of the process). It is now generally accepted that a segmented body plan is not a single all-or-nothing trait, but a complex suite of traits, with many examples of intermediate or partial segmentation.(1,2) Throughout this paper, I use the term "segmentation" as a convenient shorthand for the phenomenon in which the body plan includes multiple body systems that are repeated along the A/P axis, and organs of different types are parceled together in morphological units – segments – that are generated individually during embryogenesis. In essence, I am referring to the maximal manifestation of the segmented body plan, as it is found in the three aforementioned phyla. There are many examples of lesser segmentation (sometimes referred to as "metamerism"(3)), where only a few body systems are repeated, there is only external annulation, or repeated units are not parceled in segments. These have been reviewed elsewhere,(4) and are beyond the scope of the current discussion, except as specific examples. When discussing the process I explicitly use the term "segmentation process" to refer to the totality of the embryological events generating a segmented body plan, including cell movements and shape changes, gene expression and signaling.

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Embryological research, carried out over the past decade on representatives of the three segmented phyla, has revealed remarkable similarities in the molecular mechanisms involved in the generation of the segmented body plan. These similarities have led to the seemingly obvious conclusion that the common ancestor of arthropods, annelids, and chordates exhibited segmental repetition of several body parts, and that the entire process of generating this segmented body plan was inherited from the common ancestor. Since these three phyla belong to three separate major subdivisions of the animal kingdom,(5) a common origin for the segmentation process in these phyla would mean that the last common ancestor of all extant bilaterian animals, the so-called "Urbilateria," was a segmented animal.(6,7) Although this is not always stated explicitly, the implication is that Urbilateria was a complex animal exhibiting full segmentation in multiple systems.(7,8) However, on close inspection, there are a number of problems with this scenario.(9) A corollary to the segmented Urbilateria hypothesis would be that segmentation has been lost or degenerated in all but three animal phyla. This would mean multiple losses of a complex organizational trait that has significant adaptive value. Furthermore, as our knowledge of the early fossil record of bilaterians improves, it is becoming increasingly difficult to accommodate a segmented ancestor in the early evolution of extant unsegmented animal phyla.

I start by presenting and discussing the data that have led many researchers to suggest a common origin for segmentation, and follow this with a discussion of the data that make this scenario unlikely. There are problems with many of the arguments in both directions, and I also discuss these. I then present an evolutionary developmental scenario that is consistent with the highly conserved molecular pathways we see in segmentation, but does not assume a segmented common ancestor.

The case for a common origin of segmentation

Until the beginning of this decade, our understanding of segmentation was mostly based on two systems, vertebrate somitogenesis(10) and arthropod segmentation, as exemplified by the fruitfly *Drosophila melanogaster*.(11) The first is known from work on a number of traditional model species, *i.e.*, the chick, zebrafish, mouse, and clawed toad (*Xenopus*). Somites are the repeated mesodermal units that give rise to segmental structures in vertebrates. They are formed sequentially from posterior undifferentiated tissue known as the pre-somitic mesoderm (PSM), through cyclical expression patterns of genes from the HES family(12) under the control of Notch signaling.(13) More recent work has identified additional players in the process, including wnt signaling,(14) FGFs,(15,16) and others, to give a complex and highly integrated clock(10,17,18) that ticks off somites during development.

In contrast, segmentation in *D. melanogaster* is seen as a hierarchical process that proceeds in a number of linked stages, encompassing the entire length of the embryo, rather than a posterior segmentation zone.(19) With the exceptions of *hairy* (an HES family member), and *wingless* (a *wnt* family member), there are no known overlaps with vertebrate somitogenesis either in the nature of the process or in the molecular players involved. However, when the segmentation process of additional arthropod species was studied, it rapidly became clear that the *Drosophila* pattern is highly derived and not representative of arthropods in general.(19) In most arthropods, segments are generated sequentially from a segmentation zone – similar to the PSM of vertebrates. The surprising discovery of Notch-Delta signaling in spider segmentation(20) brought arthropod segmentation and vertebrate somitogenesis a step closer. It has since emerged that Notch signaling is involved in the segmentation of centipedes,(21) and of cockroaches,(22) strongly suggesting that its involvement is ancestral to arthropods, and has been secondarily lost in *Drosophila*. Furthermore, the existence of a clock-like mechanism that is involved in cyclical generation of segments through a signal emanating from a posterior focus has been suggested for centipede segmentation,(23) and possibly for spider segmentation as well(24) – again very similar to the process in vertebrates.

Segmentation in annelids is less well known. There are large differences in the segmentation process in different species studied to date, making general statements difficult. In addition, there are no reliable molecular markers for segment formation, further complicating the possibility of functional analyses. Most of the information comes from the leech *Helobdella robusta*,(25,26) and this species has recently been joined by the polychaete worms *Platynereis dumerilii*,(27) *Hydroides elegans*, and *Capitella*.(28,29) While in all these cases segmentation proceeds by terminal addition, as it does in all vertebrates and in most arthropods, it is unclear to what degree the molecular players are conserved. The recent demonstration that Notch signaling might play a role in segmentation of the leech(30) suggests that this signaling pathway may also be a participant in the segmentation process of the third major segmented phyla. However, attempts to demonstrate an involvement of Notch signaling in *Capitella* have proved inconclusive.(31)

In addition to the apparent conservation of the Notch signaling pathway in segmentation of all three phyla, there are other genes that have been found to be active in segmentation in more than one case. One such example is the homeobox-containing transcription factor *engrailed*. Widely conserved in arthropod segmentation, *engrailed* is often used as a general marker to identify segment boundaries in studies of arthropod development. Expression of the *engrailed* gene transcript or of the protein product has been examined in a number of chordate and annelid embryos,(26–28,32–35) revealing that it has multiple dynamic and complex expression patterns, often involving expression in the developing nervous system, but no obvious segmental pattern. There are several exceptions to this. One is in the non-vertebrate chordate amphioxus, where *engrailed* is expressed in the posterior of the first eight somites in early development,(32) similar to its expression in the posterior of developing segments in arthropods. Because amphioxus is thought to represent a primitive developmental mode, it has been suggested that this apparently conserved role is ancestral in chordates, and has been lost in the lineage leading to vertebrates. A second example of segmentation-linked engrailed expression is in the annelid *P. dumerilii*(27) where not only is *engrailed* expressed in the posterior of segments, it is expressed in conjunction with the secreted protein *wingless* (*Wnt1*), which in arthropods acts together with *engrailed* to define segment boundaries. Again, this is interpreted as evidence for the conservation of segment boundary formation mechanisms throughout bilaterians.

Although a lot of the data suggesting a common origin for segmentation come from recent molecular developmental studies, there are classical morphological data supporting the traditional "Articulata" hypothesis, *i.e.*, the idea that annelids and arthropods are closely related and united in a single segmented super phyletic assemblage. Scholtz(1) has discussed the morphological arguments supporting a common origin for segmentation (at least in arthropods and annelids) based on numerous criteria, most notably the complexity of segmental structures and substructures. He concluded (in 2002) that it may be too early to dismiss the Articulata hypothesis, although the phylogenetic data assembled in the past few years(36,37) make such an assemblage even less likely today than it was at that time. Similarly, Nielsen(38) has attempted to reconcile the morphology-based Articulata concept with recent molecular phylogeny, and has placed the entire radiation of the Ecdysozoa within the Articulata, with the loss of segmentation as a single event. Although this proposal neatly solves many of the problems associated with the evolution of segmentation, it is, in fact, not concordant with any of the current phylogenetic datasets, and must therefore be dismissed.

The case against a common origin of segmentation

One of the main objections to a common origin of segmentation comes from the principle of parsimony. It is seemingly more parsimonious to assume three independent attainments of a character than 30-odd losses of the same character. This is of course an over-simplification. Unsegmented phyla are grouped into larger clades, so segmentation could have been lost only a few times at the base of large phyletic radiations. It has been argued that character losses are common and their importance is often underestimated relative to the importance of character gains,(39,40) further weakening the parsimony-based argument. However, even given these caveats, the widespread loss of segmentation (assuming an urbilaterian with complex, multi-system segmentation), with its manifold selective advantages seems highly unlikely, and currently lacks a convincing explanation. There are, indeed, many well-known cases of segmentation being lost, but the specifics of each case cannot be generalized to explain all unsegmented phyla.

Whole body segmentation has been lost, complete with all segmented organ systems, several times within one phylum, namely, the annelids.(41,42) Two unsegmented groups, the sipunculids and the echiurans, previously regarded as phylum level taxa, have now been shown to be nested within polychaete annelids.(43) Both taxa retain transient vestiges of a segmented body plan during their development.(44,45) In both cases, the apparent selective force leading to the loss of segmentation is a transition from an active life style to a sessile one. Simplification of body structure, including possible loss of segmentation, can also occur as a result of miniaturization, or as a result of a parasitic lifestyle.(40) While these evolutionary processes can explain some cases of loss of segmentation, they cannot be applied to all, and are not concordant with what we know of the evolutionary history of Bilateria (see discussions of the fossil record below).

An additional line of arguments against a common origin of segmentation points at the differences in the identity and arrangement of the segmented tissues between the three groups. These arguments are not without caveats, mostly since we do not always know what the ancestral arrangement was. Most notably, segmentation in chordates is primarily mesodermal, whereas in arthropods and annelids it is primarily ectodermal, with mesoderm being segmented secondarily. There is, however, a suggestion that the primitive state for arthropods is mesodermal segmentation.(2,46) Annelids have segmental coelomic cavities and clear septa separating adjacent segments; segmental borders in arthropods are mainly cuticular; and chordates do not have any clear boundaries between segments. Many annelids generate segments through specialized stem-cell like cells known as teloblasts,(9) and this is may be the ancestral pattern for this group.(1) While there are some arthropods that generate segments through teloblasts,(47) this is a derived state, and the ancestral state is more likely to be generation of new segments through cell rearrangements in a growth zone.(1,48)

The third line of evidence comes from an integrated phylogenetic analysis of living and fossil taxa. The wealth of material being uncovered in Cambrian Lagerstätten, and in Ediacaran assemblages has improved our knowledge of the early stages in the evolution of Bilateria. If there were indeed a segmented common ancestor to all bilaterians, we would expect to see some evidence of such an animal in early Cambrian or late Proterozoic fossils. At the very least, we would expect to see evidence of segmentation in the earliest known stem groups of deuterostomes, lophotrochozoans, and ecdysozoans, the three main branches of bilaterian animals..

Deuterostomia

The living cephalochordate *Branchiostoma* (amphioxus) represents a body plan that may be similar to that of early deuterostomes. In amphioxus there are said to be two distinct levels of segmentation(4): segmental gill slits, complete with branchial arches, and hemal system, often referred to as branchiomery; and somitic segmentation, encompassing muscle blocks, and nervous system, referred to as myomery. The early fossil record of deuterostomes includes putative chordates and hemichordates with signs of segmentation,(49,50) in the form of segmental gill slits in the head region, often with somites in the trunk, and tail. Echinoderms are found in the earliest Cambrian,(51) and possibly even in the Ediacaran,(52) although this is disputed. All extant echinoderms and most of the basal echinoderms are not segmented in any way. However, one group of fossil echinoderms, the stylophorans, are notable in having structures that have been interpreted as gill slits(53) (an interpretation not universally accepted(54)), thus representing a potential intermediate between echinoderms and other deuterostomes with gill slits.(55) Current phylogenetic analyses place hemichordates as a sister group to echinoderms.(56) This suggests that the ancestors of echinoderms and hemichordates had, at the very least, segmentally reiterated gill slits, and that the echinoderm body plan is derived through dramatic transformations.(55) A group of early Cambrian deuterostome fossils, the vetulicolians, is interpreted as being basal to the deuterostome radiation.(57) Since the vetulicolians are reconstructed as having gill slits and whole body segmentation (somites), the conclusion is that segmentation is primitive in crown group deuterostomes. However, the placement of vetulicolians at the base of the deuterostomes has been questioned,(58) and there has even been some doubt as to whether they are deuterostomes at all!(59,60) The fourth deuterostome phylum contains one species: the very simple, unsegmented, worm-like *Xenoturbella*.(56) If this simplicity is primitive, it would argue against a segmented deuterostome ancestor. However, the position of *Xenoturbella* in current phylogenies(56) suggests its simple body plan is secondary, and thus does not contribute to the discussion.

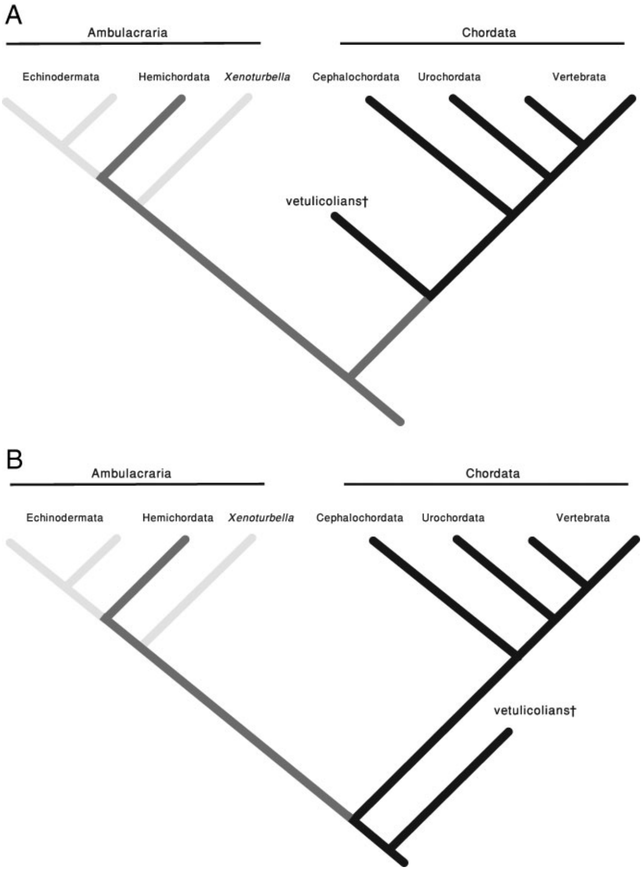

Figure 1. Schematic phylogeny of Deuterostomia showing the origin of segmentation under two scenarios: **A**: Vetulicolians are basal chordates (the preferred scenario here). **B**: Vetulicolians are basal deuterostomes. Light gray, no segmental structures; dark gray, segmental gill slits; black, segmental gill slits and trunk somites. According to the preferred reconstruction, segmental gill slits existed in the common ancestor of all extant deuterostomes and were lost during the evolution of echinoderms and in *Xenoturbella*. Segmental mesodermal somites in the trunk appeared during the evolution of chordates. In the alternative and less likely scenario the deuterostome ancestor had gill slits and mesodermal somites. Somites were lost at the base of the ambulacraria, and gill slits were lost in echinoderms and in *Xenoturbella*. Phylogeny based on Refs. (36,56)

The inconclusive and controversial early fossil record of deuterostomes(61) offers two possible reconstructions of the evolution of segmentation in the group (Fig. 1). The more conservative reconstruction would suggest anterior segmentation, in the form of pharyngeal gill slits (branchiomery) as primitive to all deuterostomes. Under this reconstruction, somites (myomery) evolved in the stem lineage of extant chordates. The more radical reconstruction (based on the controversial placement of vetulicolians as basal deuterostomes) has gill slits and somites as primitive, both being lost in echinoderms and in *Xenoturbella*, and somites being lost in hemichordates.

Ecdysozoa

The case for Ecdysozoa seems more straightforward, as their fossil record is better. The earliest known arthropods are fully segmented, at least as far as we can tell from external anatomy. The paraphyletic lobopods, believed to include the sister group to the arthropods, show varying degrees of segmentation. Most have segmental appendages, but not in all cases are these accompanied by obvious ectodermal segmentation. Some have external annulations that do not necessarily correspond to segments. Similarly, the living onychophorans, or velvet worms, have segmental limbs, without corresponding external segmentation. Segmental motoneurons and muscles correspond to the limbs, and there is some debate as to whether the rest of the nervous system is primitively segmented or not.(62,63) Moving away to further outgroups within the Ecdysozoa, we do not see evidence of complete segmentation. External annulations, sometimes continuing deeper into the body, are seen in several fossil taxa within the Scalidophora(64) (the group comprising several phyla of marine worms, such as priapulids, kinorhynchs, and loriciferans), whereas extant members have varying levels of annulation, and repeated structures.(4) Other ecdysozoan taxa show no evidence of segmentation or annulation either in fossils or in living forms. Given the patchy spread of repeated structures in the ecdysozoan fossil record, it is difficult to determine where these arose and identify primitive versus derived states. The ancestral state in ecdysozoans may have included some sort of metamery, or repeated structures may have evolved independently a number of times. However, it is nearly certain that the full segmentation seen in arthropods evolved stepwise and was not found in the common ancestor of Ecdysozoa(2,63,65) (Fig. 2).

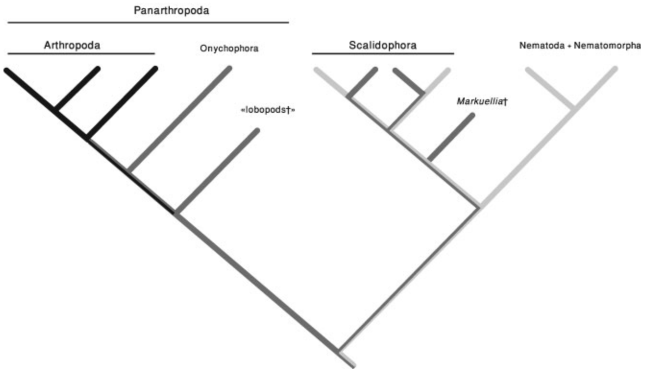

Figure 2. Schematic phylogeny of Ecdysozoa tracing the evolution of segmental structures. Light gray, no segmental structures; dark gray, external annulation and/or segmentally organized central nervous system; black, segmental structure of multiple systems. According to the portrayed reconstruction the common ancestor of all ecdysozoans may have had limited repeated structures. If so, then this state forms the ancestral state for Panarthropoda, and full segmentation evolved gradually along the branch leading to arthropods. Partial segmentation within the Scalidophora may have been retained from the ancestral ecdysozoan condition (implying a loss at the base of Nematoda + Nematomorpha), or may have evolved independently several times in different lineages. Phylogeny based on Refs.(36,64)

Lophotrochozoa

The third super phylum Lophotrochozoa, which includes the segmented annelids, has a much poorer fossil record than Deuterostomia or Ecdysozoa, and the interpretation and affinity of early lophotrochozoans is hotly debated. However, the earliest known probable bilaterian, the Ediacaran *Kimberella*, is thought to be a member of the Lophotrochozoa, and is clearly unsegmented.(66) An additional series of relevant Cambrian fossils is included in the sclerite-bearing taxon Halwaxiida.(67) These fossils are all unsegmented and have variously been interpreted as stem lophotrochozoans, stem molluscs, or stem annelids,(68) whereas some researchers dispute their assembly into a single clade.(69) Interpreting these unsegmented fossils either as stem-group annelids or as stem-group lophotrochozoans virtually rules out the possibility of a segmented lophotrochozoans ancestor (Fig. 3). Various other interpretations allow for such a possibility, but make it unlikely. While the debate about the interpretation of the phylogenetic relationships of these fossils is bound to continue (and has only been reviewed briefly here), it is generally accepted by most of the authors cited

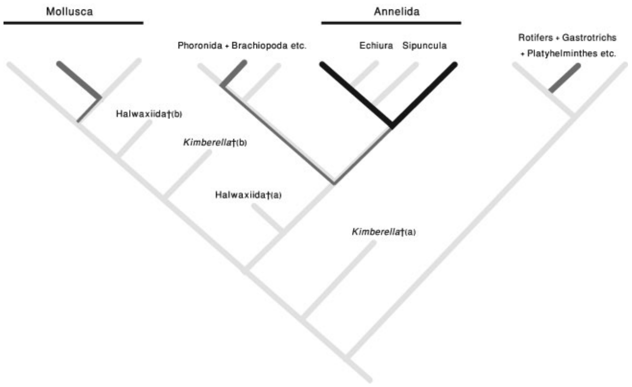

Figure 3. Schematic phylogeny of Lophotrochozoa tracing the evolution of segmental structures. Light gray, no segmental structures; dark gray, segmental organization of a small number of systems; black, segmental structure of multiple systems. The common ancestor of lophotrochozoans is reconstructed here as unsegmented. Segmentation of multiple body systems arose only in the lineage leading to annelids and was secondarily lost there. The annelid ancestor probably had partial segmentation, and vestiges of this state are found in other closely related phyla. Segmental organization of a few systems arose in the molluscan group including polyplacophorans and monoplacophorans, but may have existed deeper in the ancestry of molluscs and were secondarily lost. Two possible positions each are given for the fossil halwaxiids and for the Precambrian *Kimberella*. This does not affect the reconstruction of the lophotrochozoan ancestor. Phylogeny based on Refs. (36,67)

above that already in the Cambrian, segmentation was a trait distinguishing annelids from other lophotrochozoans.

Within extant lophotrochozoans there are several examples of repeated structures,(4) but many of these are probably taxon-specific novelties (*e.g.*, in tapeworms). Specifically, repeated structures are found in several molluscan taxa, and it has been postulated that the ancestral state for molluscs is of a segmented body plan. According to this view, extant "primitive" molluscs maintain evidence of this state, *e.g.*, in the form of repeated polyplacophoran shell plates and serially repeated gills, nephridia and muscles in monoplacophorans.(4) It has recently been suggested that these two groups are related, and nested deep within molluscan phylogeny, such that the relatively complex segmentation they show is apomorphic for one small clade rather than plesiomorphic for molluscs.(70) It is nonetheless possible that the ancestral molluscan body plan did include some sort of metamerism.

In summary, this analysis of the status of segmentation in the ancestors of the three animal super-phyletic assemblages suggests that the ancestor of Ecdysozoa had an unsegmented body but may have had some form of metamery, most interpretations of early lophotrochozoans fossils suggest an unsegmented ancestor, and the most likely reconstruction of common ancestor of deuterostomes has segmentally repeated gill slits in the head and no body segmentation. Thus, the possibility of full segmentation in the common ancestor of all bilaterians is not supported. We can add the Acoela,(71) the presumed sister group to all bilaterians, to this analysis. Acoels have a very simple body plan with no repeated structures, lending support to the alternative view of an unsegmented urbilaterian.(72) Couso(3) has suggested that each of the three groups had some form of metamery in its common ancestor. While this cannot be ruled out, the putative ancestral metamery in each of the groups seems very different, and is unlikely to have arisen from a common source.

Reconciling contradicting datasets

We are left with a conundrum. The bulk of the evidence makes it highly unlikely that the common ancestor of all bilaterians was segmented. On the other hand, we cannot ignore the many similarities in the developmental process generating segments in the three major segmented phyla. I suggest that a solution to this conundrum can be found by assuming parallel recruitment of pre-existing gene regulatory networks in the three phyla. However, before discussing what these networks are, we must first discuss the generation of a segmented body plan and the processes involved therein.

In most segmented animals, segments are added sequentially from the posterior pole of the embryo. Generating a repeated pattern in sequentially segmenting animals requires three distinct processes(48): Initially, the anterior and posterior poles must be defined in the early egg and embryo. This is usually inherent in a basic asymmetry of the egg, but requires the activity of specific mRNA molecules and proteins to effect the axial differentiation of the embryo further on in development. Next, the axis has to be elongated, with the elongation occurring from the end that was defined as the posterior pole in the previous step. Finally, a segmented pattern has to be overlain on the extending posterior tissue. The first two of these processes occur in unsegmented animals as well as in segmented animals, and probably took place in the common ancestor of all bilaterians.(73) Indeed, inasmuch as they have been studied, many of the molecular players and interactions in these two processes are conserved throughout the Bilateria. It is these conserved networks that I suggest were recruited independently to generate the segmented pattern.

Common networks – independent recruitment

Conserved pathways in axis determination and elongation

One of the genes that are widely conserved in axis determination, even outside of the Bilateria, is *nanos*.(74–77) It acts as a posterior determinant, and is usually localized to the posterior pole. An additional conserved axial determinant is *caudal*, found as a marker for the posterior pole in several different phyla.(8,78–81) The conservation of these two genes strongly suggests an ancient common ancestry for the process of axis determination in all bilaterians. Unfortunately, there are almost no data on the role of these genes outside of phyla that exhibit a segmented body plan.

The *caudal* gene has a conserved role in the process of axis elongation, in addition to its role in axis determination.(8,78,82,83) Knocking down the gene in several model species leads to complete truncation of the body axis. A similar phenotype has been reported in knocking down the homeobox gene *even-skipped*,(8,84–86) suggesting a conserved role for it as well. Yet another central player in axis elongation in several phyla is the wnt signaling pathway.(87) It has been shown to be crucial for axial elongation in arthropods(83,88,89) and vertebrates,(82,90) probably in concert with *caudal*(82,83); *wnt1* is expressed in the posterior of elongating annelid embryos,(28) suggesting a possible involvement in this phylum as well. Many processes in embryonic development include close interactions between the wnt signaling pathway and the Notch signaling pathway, leading to the suggestion that they are functionally linked pathways.(91) Thus, it is possible that *Notch* also holds a central and conserved role in axial elongation. Once again, data on axial elongation from unsegmented phyla are completely lacking.

Axis genes and segmentation

Many of the genes detailed above, which have roles in axis determination and axial elongation, are also involved in generating a segmental pattern in at least some phyla, as detailed in the first section of this paper. Their participation in axis patterning means that they are deployed at the right place and at the right time to be readily recruited into the segmentation process. The same gene networks could have been recruited several times throughout evolution because of inherent characteristics of the network that make them pre-adapted for a role in segment generation.

Notch signaling involves cell-cell communication that activates a signal transduction cascade culminating in the expression of transcription factors that activate or repress a series of downstream genes. Among the genes repressed by Notch signaling are some of the Notch ligands themselves. This type of negative auto-regulation can form the basis of a cellular oscillator. The combination of a cycling oscillator with cell-cell communication offers the possibility of generating a traveling wave,(3) as is indeed seen in the somitogenesis process of vertebrates,(10,17,18) and probably also in the segmentation process of centipedes.(21,92) A superficially similar type of traveling wave is mediated by a negative auto-regulatory loop, including Notch signaling, in early endomesoderm differentiation during sea urchin development.(93)

The wnt signaling pathway has a role in boundary formation in many developmental processes.(94) Thus, it could readily have been recruited to form segment boundaries during the segmentation process. In arthropods, *wingless* interacts with *engrailed* to define segment boundaries before parasegmental grooves can be seen.(95) In annelids, the role of *engrailed* and *Wnt* in boundary formation seems to be later, after overt segmentation is visible.(27) It has been suggested that the ancestral role of *engrailed* is in generating skeletal boundaries,(96) though *wnt* is not discussed in this context. Intriguingly, in an onychophoran, *engrailed* is expressed before segmental boundaries are visible, but *wingless* is only expressed in the boundaries of fully formed segments,(62) and it is not clear whether they are involved in defining segmental boundaries at all. This could be a derived feature for onychophorans, or it could represent the ancestral state in the arthropod + onychophoran group.

Gene regulatory network recruitment

In practical terms, the recruitment of a gene regulatory network can be as little as the addition of a few transcription factor recognition sites to the control elements of one of the key genes in the network. Complete networks are known to have been recruited in the evolution of echinoderms, where the skeletogenic pathway of the adult is redeployed in the larvae to form skeletal spicules.(97) If a network is already active, or in other words, genes involved in the network are already being transcribed in the correct area of the embryo, they can be redeployed for additional functions. This type of recruitment of an entire network would lead to the apparent conservation of numerous genes, following a singular evolutionary event.

According to the current suggestion, the Notch pathway and the wnt pathway had ancient roles in axis elongation and were active in the posterior of the embryo of many bilaterian phyla (Fig. 4A). Somewhere in the evolutionary path leading to arthropods, the Notch pathway started generating a cycling pattern of gene expression, which was used for generating repeated morphological units (Fig. 4B). The *engrailed* gene probably had an original role in patterning the nervous system, but since the nervous system may have been one of the first to be segmentally arranged, it acquired a segmentally reiterated expression pattern, and was then secondarily recruited to act in boundary formation together with wnt pathway genes (Fig. 4C). Similarly, *even-skipped* was involved in axial elongation and was recruited to the segment generation cascade.

In the evolutionary lineage leading to vertebrates a similar recruitment of *Notch* occurred, but using wnt pathway genes to define an axial gradient, which was interpreted by the cycling machinery and used to carve out mesodermal somites (Fig. 4D). The recruitment of the Notch pathway in annelids may have occurred only in the lineage leading to clitellates (oligochaetes and leeches), thus it is absent in segmentation of polychaetes. However, the wnt pathway genes were perhaps recruited to define segment boundaries in other annelids as well.

An additional isolated example can serve to illustrate the point further. In the centipede *Strigamia maritima* the axis elongation gene *caudal* has acquired a novel role, and its expression pattern suggests that it is involved in generating a repeated two-segment periodicity,(23) and in splitting this periodicity to single segmental repeats.(21) This role for *caudal* is clearly derived, but its recruitment to this role would have been simple, as it is already expressed in the segmenting tissue at the correct time during development.

This suggestion bears some resemblance to previously suggested models, but expands on them. Arthur *et al*(98) have discussed co-option of previously existing genes into the segmentation process, but not in the context of axial determination and elongation, and in less detail than presented here. The idea of ‘‘deep homology’’(99,100) is used to explain the appearance of homologous genes in similar structures, while the structures themselves were not present in a common ancestor, and are therefore not truly homologous. The resulting ‘‘deeply homologous’’ structures are in

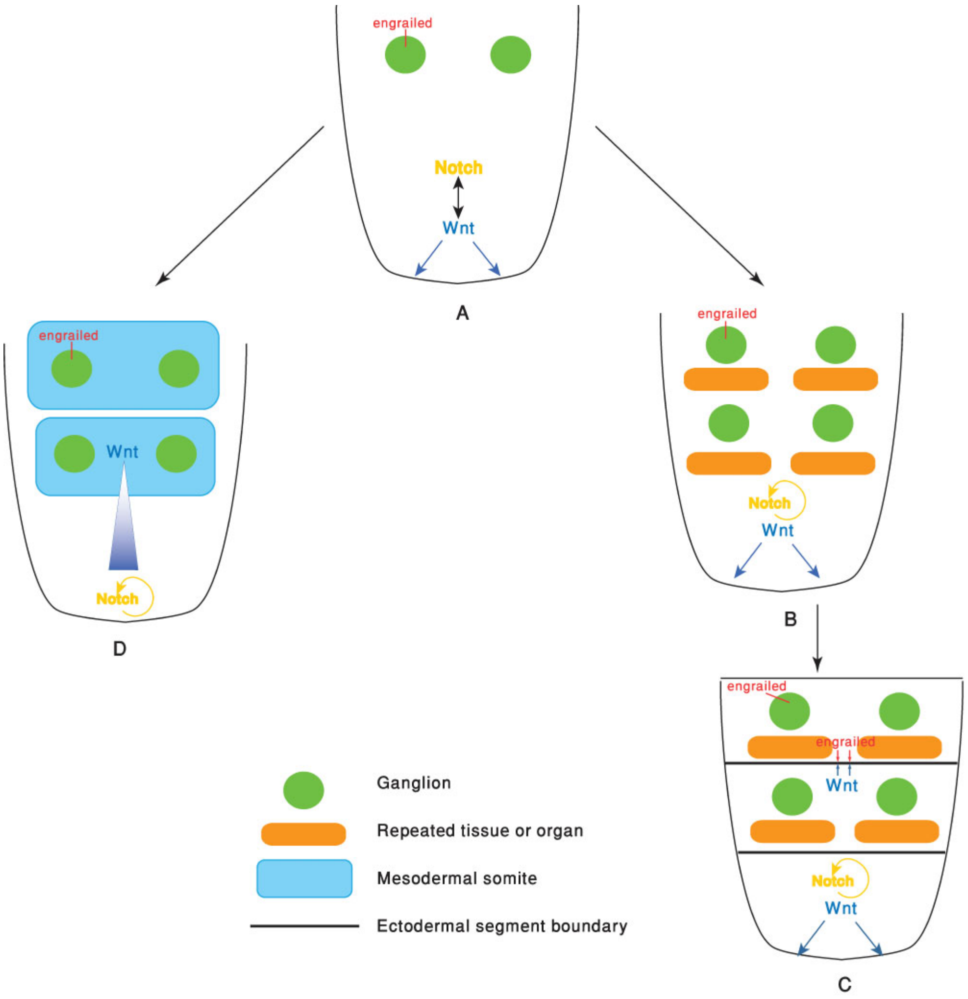

Figure 4. Simplified possible scenario for the recruitment of gene regulatory networks during the evolution of segmentation. Only a small number of genes are indicated, representing complete gene regulatory networks. **A**: Hypothetical bilaterian ancestor where neuronal differentiation is controlled by engrailed and axial elongation is controlled by the Notch and wnt pathways. **B**: In the lineage leading to arthropods the Notch pathway has evolved a cycling pattern, which is recruited for generating repeated morphological units of different kinds. wnt maintains a role in axial elongation. **C**: A later stage in the evolution of arthropod segmentation, in which engrailed and the wnt pathway have been recruited for an additional function, setting up ectodermal segment boundaries, while continuing to retain their ancestral functions. **D**: In the lineage leading to vertebrates Notch has evolved a cycling pattern in parallel with arthropods. The wnt pathway has evolved an expression gradient, which interacts with the cyclical Notch pattern to generate mesodermal somites. No explicit hypothesis is given for annelids, since very little is known about the common features of the segmentation process in this group. However, the starting point for the evolution of annelid segmentation is assumed to be (**A**) as in the other segmented phyla.

fact, as discussed above, not comparable structures but a similar organizational feature that evolved gradually using independently recruited networks. Finally, a very similar analysis has been published almost contemporaneously with the current paper.(3) Couso suggests that the ancestral bilaterian state is of an unsegmented body plan, containing certain metameric features that were the basis of parallel evolution of true segmentation. My analysis of the spread of repeated structures within Bilateria leads me to different conclusions to those of Couso regarding the bilaterian ancestral state, and I therefore suggest axis elongation rather than metamery as the primitive process that gave rise – in parallel – to the segmentation process in arthropods, annelids, and chordates.

Conclusions

There is a wealth of data coming from comparative developmental biology and paleontology regarding the question of homology of segmentation in different animal phyla. These data lead to confusing and sometimes contradicting conclusions. In this paper I have argued that the apparent homology of molecular processes involved in segmentation does not necessarily imply that the segmental body plan itself is homologous. Rather, we should think of it as something akin to deep homology,(99) where the common ancestor of all segmented animal phyla – and by implication, of all bilaterian animals – was unsegmented, but had most or all of the relevant gene regulatory networks in place with a role in axial elongation. The recruitment and assembly of the different networks into the process of generating segments occurred a number of times independently. Many possible driving forces for segmentation can be postulated. The driving force could have been movement facilitation in an animal with an exoskeleton,(2) or regionalization of the nervous system. Segmentation could have started as external annulations only, or within a single body system, and the reiterated process generating this simple pattern could have then been recruited to drive metamerism in other organ systems as well. Wherever this occurred, the advantages of the modular segmental body plan allowed dramatic radiations and significant evolutionary success. The obvious similarities in the segmentation processes of the highly successful arthropods, chordates, and annelids are testimony to the ancient origins of the molecular pathways. The no-less obvious differences are evidence of the independent recruitment of the pathways.

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