The origin and evolution of segmentation

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Arthropods, annelids and chordates all possess segments. It remains unclear, however, whether the segments of these animals evolved independently or instead were derived from a common ancestor. Considering this question involves examining not only the similarities and differences in the process of segmentation between these phyla, but also how this process varies within phyla, where the homology of segments is generally accepted. This article reviews what is known about the segmentation process and considers various proposals to explain its evolution.

To most of us who are segmented, the advantages of a segmented body plan are perhaps not immediately obvious. However, segments are of fundamental evolutionary and developmental importance to several metazoan phyla, including the anterior–posterior axis of a structural unit that comprises a suite of characters involving the entire body1. Animals and plants, which evolved multicellularity independently, possess simple serial rep-
not force us to concede that segments evolved independently (Fig. 1a); any hypothesis of segment homology is less parsimonious because it would require several instances of segmentation loss among the other 20 or so protostome phyla (Fig. 1b,c).

How do we discriminate between these different hypotheses? One way is to rely on the assumption that homologous structures are more likely to employ the same developmental tools than structures that have evolved independently. Thus, the first hypothesis, that segmentation was acquired independently in all three phyla (Fig. 1a), would predict that similarities in the developmental process of segmentation in these different groups should be minimal or merely superficial. The second hypothesis, that segments are homologous within the protostomes, but that chordates evolved segments independently (Fig. 1b), would predict significant differences between chordates and arthropods in the way that they make segments, but significant similarities in the way arthropods and annelids make segments. The third hypothesis, that segmentation is homologous throughout the Bilateria (Fig. 1c), has the heaviest burden of proof because it would predict universal similarities in the process of segmentation that are sufficient to outweigh obvious differences. Additionally, the second and third hypotheses would also predict that, despite the loss of overt segmentation in many phyla, we ought to be able to detect molecular or developmental vestiges of an ancestral segmented state during the embryogenesis of at least some unsegmented phyla.

How do we begin to look for significant similarities and differences in the mechanisms of segmentation in various species? At the molecular and genetic level, we understand the most about segmentation in many phyla, we must first consider the extent to which this process varies across different groups of organisms. In particular, genes of the segment-polarity and pair-rule classes have received the most attention—partly because their expression patterns correlate most closely with the later morphological manifestations of segmentation.

**FIGURE 1.** Three hypotheses for the evolution of segmentation among the Bilateria. Blue indicates deuterostome phyla, green indicates protostome phyla. Both the acquisition of segmentation (solid black blocks) and the loss of segmentation (solid white blocks) are also indicated. (a) Hypothesis 1 proposes three independent acquisitions of segmentation. (b) Hypothesis 2 asserts the homology of segmentation among protostomes and thus requires later loss of the segmented state. (c) Hypothesis 3 asserts the homology of segmentation across the Bilateria and thus requires even more loss. Exactly how much loss occurs in (b) and (c) and, thus, exactly how much less parsimonious these scenarios are will require further phylogenetic resolution.
within a single clade, whose members all possess segments that are undoubtedly homologous. It would be premature to compare segmentation in Drosophila, fleas and chickens without having some idea of how representative each of these organisms is of the general (and presumably ancestral) mechanisms of segmentation within the arthropods, annelids and chordates, respectively.

Segmentation in insects

All insect embryos begin as a syncytium in which the majority of nuclei eventually migrate to the periphery to form a blastoderm, whereas the remaining segments of the thorax and abdomen form progressively from a posterior growth zone. Initial differential fates and segmentation of short- and intermediate-germ embryos take place in a cellular environment. This, of course, raises questions as to the mechanisms employed by short- and intermediate-germ insects, which show the progressive addition of segments in a cellular environment.

Embryos representative of all three modes of embryogenesis, the segment-polarity genes are expressed just before and during the overtly segmented germ-band stage in a segmentally reiterated pattern. These two that have been most widely studied are engrailed and wingless. Each is expressed as a single stripe within each individual segment, and these patterns have thus far been found in all insects examined. In contrast to the segment-polarity genes, orthologues to the pair-rule class of genes have tended to exhibit more-divergent patterns. The three that have been studied most widely are even-skipped (Fig. 3), hairy and fushi-tarazu. In Drosophila, all three of these genes are expressed in stripes before the onset of gastrulation, with a two-segment periodicity. In addition, even-skipped also shows a segmental pattern of expression immediately following gastrulation. The expression of all three of these pair-rule genes is conserved in the beetle Tribolium castaneum (Order: Coleoptera)9–13. In the case of even-skipped, its pair-rule function appears to be conserved as well, because chromosome-assisted laser inactivation (CALI) of the protein results in a pair-rule phenotype14. However, a deletion mutant of the Tribolium Hex complex that includes the hexa-saturnus ortholog does not exhibit any pair-rule defects15, indicating that at least this gene appears to be functioning differently compared with its Drosophila orthologue. Additionally, a recent genetic screen in Tribolium has revealed at least two mutants that display pair-rule phenotypes15.

In the more phylogenetically primitive grasshopper Schistocerca (Order: Orthoptera), pair-rule expression has been particularly elusive. Neither the even-skipped nor fushi-tarazu orthologues are expressed in stripes in the early embryo, but rather both are expressed in broad, Hox-like, posterior domains16,17 (Fig. 3). In light of the phylogenetic position of Schistocerca, it is tempting to view these posterior expression domains as ancestral, existing prior to the evolutionary recruitment of these genes to play a role in segmentation17. This is perhaps most reasonable for even-skipped in even-skipped orthologues are linked to the Hex clusters in vertebrates18,19, and even-skipped orthologues of vertebrates and nematodes are expressed in posterior Hox-like domains20,21,22. However, the observation that both even-skipped (Order: Dermaptera) and (Order: Orthoptera) possess stripes of a one-segment periodicity (P. Moore, R. Dawes and N. Patel, unpublished), suggests that a pattern of segmental stripes is the ancestral even-skipped expression pattern for insects and that the grasshopper perhaps represents a reversion to an even more ancestral pattern shared by nematodes and vertebrates.

Segmentation in non-insect arthropods

In attempting to understand the ancestral expression patterns and function of segmentation genes in arthropods, it will be especially important to investigate segmentation in other arthropod groups, such as crustaceans (brine shrimp, crayfish, etc.), chelicerates (spiders, scorpions, etc.) and myriapods (millipedes and centipedes) (Fig. 2). In many species of crustaceans, most of the thorax and abdomen develops in a cellular environment from a posterior growth zone following gastrulation and thus, at least superficially, resembles the development of short-germ insects. In the case of the crustacean examined so far, the expression of even-skipped is similar to that seen in insects.23 We also know that engrailed is expressed in segmental stripes in spiders23. It is now important to characterize the expression patterns of pair-rule genes in various non-insect arthropods in order to establish the ancestral function of these genes within this group. For now, though, the available data on arthropods reveal that, within this group, the process of segmentation is quite similar at the pair-rule level and yet fairly conserved at the segment-polarity level.

Are segments homologous across phyla?

In spite of its prevalence, variation at the pair-rule level is not enough to shake the widely held conviction that the segments of all arthropods are derived from a common segmented ancestor. Taking this variation into account, though, is important in weighing evidence for either the homology or independent evolution of segments between phyla. What then, are the similarities and differences in the processes of annelid and chordate segmentation when compared with those of arthropods?

Segmentation in annelids (evaluating hypothesis 2)

In the case of annelid worms, the most detailed embryological and molecular data we have come from the leech. In these animals, development proceeds by the anterior-to-posterior progression of
stem cells, which lay down the founder cells for each segment by a series of longitudinal asymmetric divisions, a pattern that is at least superficially similar to that of some crustaceans. The molecular data we have for annelids concerns an orthologue of the segment-polarity gene engrailed from the leech Helobdella triptolema. The expression pattern of this gene appears to be consistent with a role in segmentation because cells that have or will express engrailed 1 occupy a narrow stripe in the posterior of each segment, just anterior to the segmental furrow. More detailed analysis of the N lineage (which gives rise to a large proportion of the morphological segments) in the leech Theromyzon rude revealed that engrailed expression appears only after the ganglionic primordia have separated, raising doubt on its functional role in the segment causing process. Thus, a firm conclusion on the possible homology of arthropod and annelid segments will require future study, both of leeches and ideally of the more basal annelid groups, such as oligochaetes and polycheates, to provide a clearer picture of the mechanisms used by these organisms to make segments.

Segmentation in chordates (evaluating hypothesis 3) What about our third hypothesis – the global homology of segments (Fig. 1c)? Are the similarities between segmentation in arthropods and chordates enough to outweigh the differences? At first glance, the most obvious aspect of vertebrate segmentation – somitogenesis – appears similar to the progressive anterior to posterior formation of segments that we observe both in short-germ insects and in crustaceans. As for genes from the fly segmentation hierarchy, one putative orthologue of zebrafish even-skipped is expressed in segmentally reiterated stripes, including the zebrafish engrailed in Drosophila has been localized to the posterior of the first eight somites, and the expression is reported to precede morphological segmentation. However, the simple observation of stripes might be misleading. One analysis of the expression patterns of randomly selected cDNAs from Drosophila revealed that 87% are expressed in segmentally reiterated stripes, including ‘housekeeping’ genes such as those encoding ribosomal proteins. Indeed, segmentally reiterated patterns of gene expression are exactly what one would expect during the development of a segmented organism, but it is likely that only a subset of these genes actually function in segmentation. Thus, it will be important to test experimentally whether the segmentally repeating patterns of engrailed in chordates are involved in the actual establishment of segments and not just the specification of cell type.

Even more excitement has been generated by a result from zebrafish in which transcripts of her1, a putative orthologue of the pair-rule gene hairy, were localized to domains that correspond to future alternating zones before overt morphological segmentation, thus mimicking a pair-rule pattern. These results have led to the suggestion that the common ancestor of protostomes and deuterostomes was segmental, and that segments across the Bilateria are homologous (Fig. 1e). However, one problem with the zebrafish result is that it is not yet clear that her1 is indeed an orthologue of Drosophila hairy, and it is crucial that we deal with true orthologues if we intend to interpret them to homologize a structure or process. Additionally, while a description of the her1 mutant is not yet available, a zebrafish double mutant that indistinguishably diminishes expression of her1 fails to exhibit any segmentation defects.

In chick, too, the situation differs substantially from Drosophila. Here, another putative orthologue of Drosophila hairy, c-hairy1, is expressed in a cyclical segmental pattern and might be involved in the periodic genesis of somites. The c-hairy1 expression pattern, however, does not appear to possess any sort of two-segment periodicity. Thus, it will be especially important to isolate a chick or mouse orthologue of zebrafish her1 to determine whether this pair-rule pattern is conserved in other vertebrates.

It is also clear that much of the impetus to homologize comes from a conviction that the pair-rule patterning mechanism is not a ‘logical’ way to make segments and that, if we find such a mechanism acting in vertebrates, it is unlikely to have evolved independently. However, this argument relies on the assumption that we can judge accurately what is and what is not an obvious evolutionary solution at the level of developmental mechanism. Finally, it is important to note that several vertebrate genes have been implicated in somitogenesis whose Drosophila orthologues (Notch, Delta and fringe) play no identifiable role in segmentation.

For many, these differences in the process of segmentation, whether molecular or embryological, are easily dismissed as ‘negative’ data in the face of astonishing similarities. But excessive focus on isolated similarities, rather than a more balanced consideration of all the evidence, runs the risk of mistaking independent evolution for common ancestry. On the other hand, it is entirely conceivable that more than 600 million years of evolution has left us with only isolated similarities of a once-shared, ancestral segmentation mechanism.

Concluding remarks

It is clear that we need far more data, including more genes analyzed in more species spanning a range of phyla, as well as tools to study gene function in non-model systems, to answer the question we have posed. Although some of the similarities in patterning found between distantly related organisms are indeed surprising, it is perhaps too soon to conclude that segmentation is homologous between the various phyla. Indeed, the opposite conclusion of convergence at the level of developmental mechanism is perhaps more intriguing.

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Millennium issue
were they right?

In 1993, Trends in Genetics invited five eminent geneticists to speculate on what genetics in the year 2000 would hold. As the quotes below show, they wrote with both humour and insight.

Some brave souls will be embarking on the analysis of polygenic systems. It will still be easier to make a worse mouse (or cow) than a better one.

Anne McLaren

The torrent of new books on ethical issues related to human genetic engineering will have settled down to a steady trickle of one per month, the filled of new journals on molecular medicine will have caused a world shortage of paper, and electronic publishing will be the name of the game. A DNA-sequencing laboratory will have to turn off its lights while the High Court hears a claim for sexual harassment by a robot.

David J. Weatherhall

Understanding morphogenesis will therefore require searching for evolutionary invariance. The emerging picture is going to be more akin to a cubist painting than to a realistic portrait. But in that, biology merely falls in line with the abstract hard sciences.

Antonio Garcia-Bellido

I almost forgot to say that genetics will disappear as a separate science because, in the 21st century, everything in biology will become gene-based, and every biologist will be a geneticist.

Sydney Brenner

References