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Segmentation: mono- or polyphyletic?

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ABSTRACT Understanding the evolutionary origins of segmented body plans in the metazoa has been a long-standing fascination for scientists. Competing hypotheses explaining the presence of distinct segmented taxa range from the suggestion that all segmentation in the metazoa is homologous to the proposal that segmentation arose independently many times, even within an individual clade or species. A major new source of information regarding the extent of homology *vs.* homoplasy of segmentation in recent years has been an examination of the extent to which molecular mechanisms underlying the segmentation process are conserved, the rationale being that a shared history will be apparent by the presence of common molecular components of a developmental program that give rise to a segmented body plan. There has been substantial progress recently in understanding the molecular mechanisms underlying the segmentation process in many groups, specifically within the three overtly segmented phyla: Annelida, Arthropoda and Chordata. This review will discuss what we currently know about the segmentation process in each group and how our understanding of the development of segmented structures in distinct taxa have influenced the hypotheses explaining the presence of a segmented body plan in the metazoa.

KEY WORDS: *segmentation, evolution, metazoa*

Introduction

Segmented animals have been highly successful in the animal kingdom and can be found in a broad range of terrestrial and marine habitats. It is unclear, however, whether the segmental nature of all these animals comes from a single evolutionary event. A major focus in trying to resolve this issue in recent years has been on elucidating the developmental mechanisms that give rise to a segmented body plan with the rationale that shared mechanisms reflect a shared evolutionary history. The interpretation of developmental mechanisms must be placed in a phylogenetic context, highlighting the importance of having knowledge of the accurate evolutionary relationships among segmented taxa. Another prerequisite to making intertaxonomic comparisons concerning mechanisms of segment formation among the three largest metazoan clades of overtly segmented animals, the annelids, arthropods and chordates is an understanding of the ancestral condition within each taxon. It is therefore essential to examine the variability within a group in order to make more accurate comparisons among clades. The morphological and molecular details of segmentation within the annelids, arthropods and chordates will each be considered followed by a discussion of comparisons among the three groups.

Segmentation, what is it?

Discussions of the evolution of segmentation are complicated by the fact that there does not appear to be a consensus on what constitutes a 'segmental' body plan. In addition, it is difficult to identify groups where all members of a clade conclusively meet all the criteria for segmentation. Generally, a distinction is made between true segmentation and serial repetition (Willmer, 1990). Serial repetition includes simple repeated structures and it is proposed that there is a tendency towards such repetition in animals. For example, a strobilizing cnidarian (e.g., scyphozoan) is composed of repeated units, each of which will bud off to become a complete individual. Also, rotifers have an annulated outer cuticle and chitons contain serially repeated shell plates (Brusca and Brusca, 1990). 'True' segmentation includes repeated units along the anterior-posterior body axis of an animal and each unit is comprised of a combination of structures from both ectoderm and mesodermal origins such as excretory organs, muscles, gonads, blood vessels, nerves, appendages, coelomic cavities and septa (see Scholtz, 2002). This definition suggests a certain amount of integration of a reiterated developmental program that is not likely to have arisen by fragmentation or simple modification of existing structures. The body plans of annelids, arthropods and chordates are usually distinguished from other animals with serial repetition and are known as the 'eusegmented' animals.

By many views, animals are said to be either segmented or not. In contrast, Budd (2001) has advocated the view that organs should be considered to be segmented, not whole organisms. From this perspective animals can be partially segmented, and Budd argues these are important cases to consider when trying to understand the evolution of segmentation. Such animals may

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represent potential intermediates between an unsegmented organism and a eusegmented animal and consideration of only the eusegmented groups might limit what we can learn about the evolutionary process. This argument assumes that eusegmentation arose from animals that have serial repetition of some organs (pseudometamerism, discussed in (Clark, 1964). As more is learned about the process of making segments and whether there is a common underlying genetic basis for both eusegmented animals and 'partially segmented' animals, it may become easier to define segmentation in a less typological manner, and include intermediate forms.

Proposed origins of segmentation in the Metazoa and relationships to phylogenetic trees

Discussions about the origins of segmentation have a long history and distinct theories have been proposed, many of which have had strong support. Prior to the advent of molecular genetics, most hypotheses explaining the origins of segments were tied to the functional and physiological advantages of a segmented body plan. As the molecular mechanisms of segment formation in *Drosophila* became characterized, there has been a shift of focus to examining the ontological origins of segmentation, specifically at the molecular level. Functional morphological approaches suffer from obvious forces of convergence, while studies of developmental pathways might offer a clearer view of the historical origins of animal forms.

There has been an intricate link between opinions concerning the homology of segmentation and the clustering of taxa in the metazoan tree. Therefore, it is important to consider how our views about the monophyly or paraphyly of segmentation are influenced by and on current phylogenies. Many metazoan phylogenies have utilized segmentation as a heavily weighted character to group taxa and as a single character it has had a disproportionate influence on the branching patterns of the overall metazoan tree. These associations implicitly assume that segmentation is a stable character that does not 'easily change back and forth' and can be applied to the whole animal. By many proposals, the origins of segmentation have been closely linked to origins of the coelom, and this is reflected in trees that contain a segmented trimeric coelomate as a common protostome/deuterostome ancestor (discussed in Willmer, 1990). In a scheme proposed by Gutman (1981), annelids, arthropods and chordates arose from a segmented coelomic ancestral group, the Polymera. The propensity to group segmented animals is best exemplified with the long-term acceptance of the Articulata clade, which includes annelids and arthropods as sister taxa and onychophorans as a possible link between the two.

This grouping was initially proposed by Cuvier (Cuvier, 1817) and is based almost solely on segmentation as a synapomorphic character. In some scenarios (see Barnes, 1987; Brusca and Brusca, 1990; Raff, 1996), the arthropod lineage is proposed to have arisen once or even multiple times from a homonomous annelid-like ancestor (Fig. 1B). Conversely, the deep split at the base of the metazoan tree between protostomes and deuterostomes impacted ideas about the homology of segmentation; somitogenesis in the chordates has been traditionally considered to be independent from the types of segmentation seen in the protostome lineage.

Recent evidence utilizing both morphological and molecular data has offered a very different set of animal associations, dividing the Metazoa into three great clades, the Deuterostomia, Lophotrochozoa and Ecdysosoa (Lake, 1990; Eernisse *et al.,* 1992; Halanych *et al.,* 1995; Aguinaldo *et al.,* 1997; Godwin and Capecchi, 1998; de Rosa *et al.,* 1999; Peterson and Eernisse, 2001). An interesting characteristic of these associations is that this same topology is recovered with and without the use of segmentation as a character. In fact, the annelids, arthropods and chordates are all placed in separate groups, the Lophotrochozoa, Ecdysosoa, and Deuterostomia, respectively, each of which also contains many unsegmented groups (Fig. 1A). Whether or not this metazoan tree is accurate, it is conceptually useful because with many interspersed unsegmented groups more closely aligned with segmented animals, we are required to find supporting evidence for homology of segmentation among groups. It is crucial to keep in mind the assumptions made about the homology of segmentation that are influenced by a metazoan 'tree of choice', which could reflect circular reasoning.

Among the distinct proposals explaining the origins of segmentation is the hypothesis that there is a single origin of segmentation in the Metazoa. In this case, the Urbilateria, the ancestral primitive bilateral animal that gave rise to both the protostomes and the deuterostomes (De Robertis and Sasai, 1996), was a segmented animal. Monophyly of segmentation has been proposed historically (Geoffroy Saint-Hilaire, 1822; Gutmann, 1981) and has recently received support based upon molecular data of developmental characters (Kimmel, 1996; De Robertis, 1997; Carroll *et al.,* 2001). Regardless of whether one considers either the Articulata or the Ecdysosoa hypothesis, segmented groups are interspersed with many more closely related unsegmented ones (Fig. 1). If segmentation is monophyletic, we are faced with the challenge of explaining loss of segmentation in numerous taxa throughout the Metazoa, including the Acoelomorphs, the most basal extant tripoblastic bilaterians (Ruiz-Trillo *et al.,* 1999; Ruiz-Trillo *et al.,* 2002; Telford *et al.,* 2003).

Independent origins of segmentation have also been proposed, in which chordates evolved segmentation independently from annelids and arthropods, which shared a common segmented ancestor. This theory is closed tied to the Articulata hypothesis and has had support through most of the 20th century. Support for an independent origin of segmentation between chordates and annelids/arthropods has been based in part on functional arguments, in which segmentation arose for distinct locomotory purposes in the ancestor of modern day annelids and arthropods and chordates (Clark, 1964). In segmented animals, the body is divided into a series of compartments, each of which can be regulated more or less independently of others. In chordates, a segmented body was considered to give mechanical advantages in swimming, based on transmission of torsional forces. In annelids, a segmented body plan has been cited as advantageous for burrowing, because of hydroskeletal advantages of isolating a subset of segments from the rest of the body. This proposal has received criticism (Giangrande and Gambi, 1998; Willmer, 1990) however, because the arguments were based on details of the anatomy and locomotory behavior of an earthworm, a terrestrial animal that burrows through a hard substrate. This is very different from most annelids that are marine and burrow through a soft substrate. Furthermore, some burrowing marine polychaetes,

such as *Arenicola*, have reduced segmental organization (reduced septa) at their burrowing end. Moreover, many burrowing vermiform animals such as sipunculids and echiurans have reduced or no segmentation, demonstrating that segmentation is not essential for a burrowing life style.

A third hypothesis for the origins of segmentation in the Metazoa is that each of the eusegmented groups has an independent origin. If one considers the Ecdysosoa, Lophotrochozoa and Deuterostomia associations, an independent origin of segmentation in each of the three clades is the most parsimonious interpretation and does not require explanation of a massive loss of segmentation in more closely related sister taxa. For example, within the Lophotrochozoa, the phoronids, nemerteans, bryozoans, brachiopods, platyhelminthes, sipunculids and echiurans are all unsegmented animals more closely related to annelids than are annelids to arthropods. This is not to say that some of these animals could have undergone loss of segmentation, such as sipunculids and echiurans (see below).

Complicating discussions of the evolutionary origins of segmentation is the notion that segmentation could have arisen independently within the same body plan. This has been mainly supported by biomechanical and functional arguments reflecting life history differences (Clark, 1964; Willmer, 1990). Proposals of distinct mechanisms of segmentation in a single animal are based primarily on the influence of the work of Iwanoff (Iwanoff, 1928) who suggested that the larval segments of polychaetes have a distinct ontological origin and structure from adult segments. Furthermore, he observed that in the larval segments of *Hydroides*, the ectoderm becomes segmented prior to the mesoderm, whereas in the formation of the post-metamorphic segments, the mesoderm segments first and the other tissues follow. Iwanoff also made a mechanistic distinction between the formation of naupliar and post-naupliar segments in crustaceans. There are embryological examples of distinct origins of segments within a species. For example in amphioxus, the 8 anterior-most segments form by an outpocketing of the gut (enterocoely), while the remaining posterior segments form from a hollowing of solid blocks of mesoderm (schizocoely) (Holland *et al.,* 1997). Although many researchers do not believe there is grounds for such a difference (Anderson, 1973), Wilmer (Willmer, 1990) supports such a proposal and carries this argument further by stating that *"the fact that two methods of achieving metamerism can occur in the same animal suggests that there may have been repeated inventions of a metameric state by different animals, using whatever method was embryologically feasible at the time"*. Thus, it may be that life history traits have the foremost impact on whether an animal has a segmented body plan or not. Clearly more information is needed about the underlying basis for both larval and adult segment formation of the same species.

In considering the possibility that different parts of the same animal might utilize distinct segmentation mechanisms, there is a notable caveat, however. Some morphologically distinct processes of segmentation might be based on the same fundamental mechanism. Segmentation in the arthropods is generally accepted as being homologous. However, there is intra-taxonomic variation in whether segments are patterned in a syncytium versus a cellular environment, the presence or absence of obvious stem cells, and within insects, the germ type (long and short germband) (Sander, 1976; Davis and Patel, 2002). Molecular characterization of arthropod segmentation in has revealed many similarities that are not obvious from examinations of the morphology. Thus, in considering distinct scenarios for the origins of segmentation, molecular data will in many cases confirm morphological observations and may also reveal developmental relationships that are not otherwise obvious.

A prerequisite for making intertaxonomic comparisons concerning mechanisms of segment formation among annelids, arthropods and chordates to understand the extent of variability within each taxon. This should not only reveal the ancestral state for each group, allowing for appropriate comparisons with other clades, but also reveal the extent of diversity within a group may

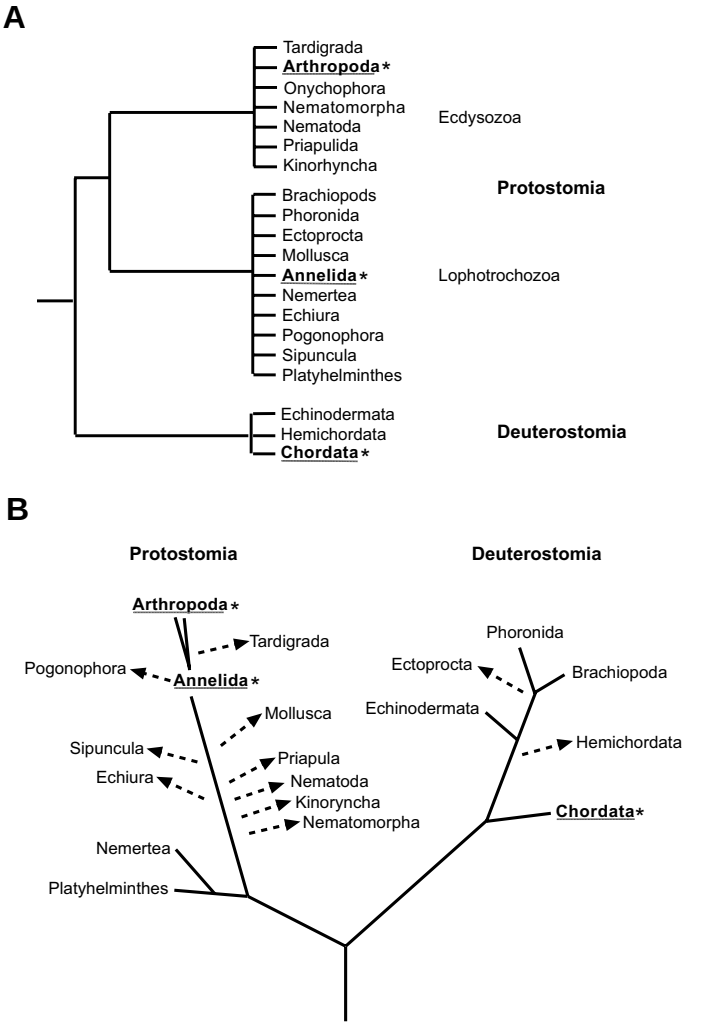

Fig. 1. Two different views of Metazoan relationships and the distinct relative positions of the major segmented phyla. (A) *Recent metazoan phylogenies group bilatereans into three great clades, each of which contains a single eusegmented phylum (adapted from Balavoine and Adoutte, 1998).* **(B)** *Traditional phylogeny showing the close relationship between annelids and arthropods within the protostome branch. Chordates are widely separated from the other segmented taxa on the deuterostome branch (adapted from Kozloff, 1990). Note that in this senerio arthropods arose from a segmented annelid ancestor. The three major segmented clades, (annelids, arthropods and chordates) are underlined and starred in (A) and (B).*

reveal a level of 'evolvability' of the segmentation program. The details of what is known both morphologically and molecularly within the annelids, arthropods and chordates will be considered separately followed by a discussion of comparisons among the three groups.

Segmentation in Arthropods

Arthropods are a speciose group that contains a high level of morphological diversity, within the context of a common segmented body plan. The arthropods contain four major subphyla, the hexapods (e.g. insects), crustaceans (e.g. lobster, shrimp), myriapods (e.g. centipedes, millipedes) and chelicerates (e.g. spiders, mites, scorpions) and the interrelationships among various clades has been a somewhat controversial subject (see Thomas *et al.,* 1997). Recent evidence has placed a close association between insects and crustaceans, grouping them together in a 'pancrustacea'clade with the myriapods and chelicerates as sister taxa (Giribet *et al.,* 2001; Hwang *et al.,* 2001). Thus, characters in common between 'pancrustacea' and the myriapods and/or the chelicerates are likely to be ancestral arthropod characteristics.

Compared with other animal groups, by far the most cellular and molecular information about how a segmented body plan develops comes from the insect model *Drosophila melanogaster*. An initial anterior-posterior gradient is set up by maternal genes, which influence the expression of the zygotic segmentation genes. These genes consist of three classes: the gap (e.g. *hb*), pair-rule (e.g. *runt*, *hairy*, *eve* and Pax group III) and segment polarity genes (e.g. *wg* and *en*), which together act in a hierarchy to sequentially subdivide the ectoderm of the embryo into smaller and smaller units (reviewed in Lawrence, 1992). They all have been defined in a phenotypic manner and the expression patterns of these genes reflect their loss-of-function mutant phenotypes. Gap genes are expressed in broad domains and gap mutants are missing those contiguous groups of segments. Pair-rule mutants fail to form even or odd segments reflecting their expression in every other segment. The pair-rule genes represent the earliest periodic gene expression along the anterior-posterior axis during embryogenesis, and several of them show a later segmental pattern. Segment polarity genes are expressed in a striped pattern in every segment and loss-of-function mutants are missing a region of each segment. The segment polarity genes define the boundary of the parasegment, what appears to be the fundamental metameric unit in flies.

The genetic analysis of *Drosophila* has provided the main defining molecular paradigm in studies of the segmentation process among various metazoans and there have been many studies to test whether homologues of the segmentation genes found in *Drosophila* are used in segment formation of other arthropods. At a morphological level it is not necessarily obvious that all the details of the molecular mechanisms of the segmentation gene cascade would be the same. For example, a lot of the segmental patterning in *Drosophila* occurs in a nuclear syncytium, and many of the patterning signals are transcription factors that diffuse within a common cytoplasm. In most species, including basal arthropods, segmental patterning occurs in the context of a cellular environment in which signals need to cross cell membranes. Furthermore, in *Drosophila* all segments appear almost simultaneously, whereas in most arthropods, including basal insects, segments are formed sequentially, a characteristic more similar to segment formation in vertebrates and annelids and likely to be characteristic of basal arthropods.

There are some interesting variations in the cellular origin of segmental tissues among arthropods. The two extremes are probably represented by the nuclear syncytium in the embryo of *Drosophila* and the teloblastic growth in some malacostracan crustaceans, both of which show a derived pattern of early development. In some malacostracans, the segmental tissues arise from the asymmetric divisions of posteriorly located ectoteloblasts. In many intermediate cases where segments arise from a posterior 'growth zone', the exact cellular basis of the origin of the segmental tissues has yet to be fully characterized. In addition, many arthropods generate segments post-embryonically, such as in some centipedes and crustaceans. Thus, there is much to be learned from studies across the four subphyla arthropod groups.

Comparative gene expression studies

Not surprisingly, virtually all of the segmentation genes isolated in *Drosophila* are also present in distantly related metazoan taxa. Examination of the expression patterns of number of the *Drosophila* segmentation genes across a range of arthropod species suggests that at least some of the molecular aspects of segmentation are conserved within anthropods. The segment polarity genes have the most conserved expression patterns. The homeodomain transcription factor *en* has been the most intensively studied across a diverse group of arthropods, initially owing to the availability of the cross reactive monoclonal antibody 4D9 (Patel *et al.,* 1989b). In *Drosophila,en* is defined functionally as being critical for subsegmental patterning and deficiencies in *en* result in loss of posterior structures of the segment, manifested by an altered denticle pattern in the cuticle. Many studies have shown that in myriapods (Hughes and Kaufman, 2002a; Kettle *et al.,* 2003), insects (Patel, 1994), crustaceans (Patel *et al.,* 1989a; Patel *et al.,* 1989b; Manzanares *et al.,* 1993; Scholtz *et al.,* 1993; Patel, 1994; Scholtz *et al.,* 1994) and chelicerates (Damen, 2002a), *en* is expressed in a striped pattern, in the posterior portion of every segment. The incredible level of conservation of its expression in the developing ectoderm is striking, suggesting the segment polarity function of *en* was canalized at the base of the arthropod lineage.

Wingless (*wg*), another segment polarity gene, encodes a secreted protein which functions in a signaling pathway to pattern the anterior portion of each segment in *Drosophila.* It is expressed in the cells immediately adjacent to and anterior of *en*-expressing cells. It has also been examined in representatives of all four major arthropod groups, and in insects it generally shows a pattern of expression very similar to that observed in *Drosophila* (Nagy and Carroll, 1994; Niwa *et al.,* 2000; Dearden and Akam, 2001), a single ectodermal stripe/segment around the circumference of the embryo. In myriapods, *Lithobius atkinsoni wg* is expressed adjacent to *en* stripes, in a pattern very similar to that of *Drosophila* (Hughes and Kaufman, 2002a). There are some interesting differences in crustacean and chelicerate *wg* expression, however. In chelicerates, a typical *wg* pattern is found but is a comprised of the expression of 2 *Wnt* gene members, *Cs-wingless* and *Cs-Wnt5-1* (Damen, 2002a). In crustaceans, there

is also an indication that the role of *wg* in *Drosophila* is divided. In *Triops*, a *wg* orthologue is found in segmentally iterated pattern; but, it is restricted to the ventral portion of the body and does not appear to play a role in patterning the dorsal portion of segments (Nulsen and Nagy, 1999). In contrast, in the malacostracan *Mysidium columbiae*, *wg* is only present in the dorsal part of the segment (Duman-Scheel *et al.,* 2002). So, although a segmental pattern for members of the *Wnt* gene family is widespread in arthropods, it appears that a segment polarity function may include other orthologues of the *Wnt* gene family instead of just *wg*, as is the case in *Drosophila*.

Unlike the conserved patterns of expression of the segment polarity genes, the pair-rule genes such as *runt*, *hairy*, *eve*, *ftz* and *paired* show pronounced variability in their expression patterns, even within insects. In the grasshopper embryo, a short germ band insect, the genes *ftz* (Dawes *et al.,* 1994) and *eve* (Patel *et al.,* 1992) are not expressed in a pair-rule pattern, and this result initially implied that pair-rule patterning may not play a role in more basal insects where segments are generated in a temporally progressive manner in the context of a cellular environment. More recently, a *paired* homologue from grasshopper, *prby1*, has been shown to have a transient pair-rule pattern, later resolving into a segmental pattern (Davis *et al.,* 2001). The expression of *prby1* in grasshoppers constitutes thus far the only published evidence of pair-rule patterning in basal insects.

Outside of insects, several of the pair-rule genes have a periodic pattern in the developing segments, but do not have obvious pair-rule expression. For example, *eve, runt* and *hairy* have a segmental pattern in a crustacean (see Davis and Patel, 2003). In the chelicerate, *Cupiennius salei*, these same 3 genes have a striped pattern as segments are forming, although it is not clear if they have a pair-rule pattern (Damen *et al.,* 2000). In another chelicerate, the spider mite, *runt* and *pax3/7* show a segmental pattern of expression, although in the prosoma region (head), stripes in even appendage bearing segments appear before the odd stripes (Dearden *et al.,* 2002). The authors cite this temporal difference as evidence for possible pair-rule patterning. In the myriapod *Lithobius atkinsoni* (Hughes and Kaufman, 2002a), *eve* is expressed strongly in the posterior growth zone and in a transient segmental pattern in newly forming segments. Such prominent expression in the posterior region is similar to that seen in grasshoppers (Patel *et al.,* 1992), beetles (Patel *et al.,* 1994; Brown *et al.,* 1997) and spiders (Damen *et al.,* 2000), consistent with the idea that *eve* expression in the posterior of the embryos may be more representative of the basal arthropod condition. *Ftz* does not show a periodic pattern at all in chelicerates and is expressed in a broad posterior band, more like a Hox gene pattern (Telford, 2000; Hughes and Kaufman, 2002b), which is its proposed ancestral arthropod role (see Damen, 2002b). Therefore, it is probable that several of the pair-rule genes have a role in segmentation in basal arthropods, however, it is not clear if they specifically act in a pair-rule manner (versus e.g. a segment polarity function) nor is it clear how basal in the arthropod lineage pair-rule patterning functions.

Gap genes have been less well characterized across arthropods than have been members of the pair-rule and segment polarity genes. In *Drosophila*, the gap gene *hb* is expressed zygotically in a broad anterior domain at the blastoderm stage and also in a limited posterior domain (Tautz, 1988). In several insects such as *Tribolium* (Wolff *et al.,* 1995), *Manduca* (Kraft and Jackle, 1994) *and Bombyx* (Xu *et al.,* 1997), *hb* is expressed in the anterior region of the embryo. In the short germ band insect, grasshopper, *hb* is also expressed in a broad domain of expression in the anterior of the embryo prior to segment formation, characteristics shared with the *Drosophila hb* pattern (Patel *et al.,* 2001). Therefore, in insects at least, there appears to be conservation of gap gene patterning.

Comparative gene expression studies have been quite fruitful in the arthropods, where patterns of gene expression are interpretable within a reasonable phylogenetic context. Current evidence supports the idea that the ancestral arthropod generated segments in a temporal progression, in a cellular environment, with a short germ band mode of development (Davis and Patel, 2002) and had segment polarity patterning probably organized around parasegmental units. The evidence for pair-rule patterning in basal arthropods is less compelling and future studies are necessary to resolve this issue.

Segmentation in Annelids

The annelids, also known as the 'segmented worms', are found in marine, freshwater and terrestrial habitats (Brusca and Brusca, 1990). Traditionally, the Annelida has been divided into the Hirudinida (leeches), Oligochaeta (e. g. earthworms) and Polychaeta. Polychaetes represent by far the most speciose group of annelids and contain the most variability, both in life history, body plan variability, and range of ecological niches. It is generally agreed that polychaetes have retained more ancestral features than oligochaetes or leeches, which share a derived character, the clitellum. Annelid fossils, have been described from the Burgess Shale of the Cambrian (Conway Morris, 1979) and show already diversified body plans. The early fossil record of annelids is variable (see Rouse and Pleijel, 2001) making predictions concerning the details of the possible basal annelid body plan difficult to reconstruct and unfortunately, there is no concurrence concerning the morphology of the basal polychaete body plan (reviewed in McHugh, 2000).

The systematics of the annelids is problematic and is a currently debated issue. A confounding problem is that diagnostic shared derived characters for adult annelids have not been identified, although Dohle (1999) has recently proposed that the highly stereotypic first 7 cleavages of one of the blastomeres (2d) might serve as a synapomorphy for annelids. Based upon both morphological and molecular datasets, polychaetes are likely to be a paraphyletic clade (McHugh, 2000; Rouse and Pleijel, 2001). The annelid radiation may also include the unsegmented echiurans, the deep sea pogonophoran tube worms (reviewed in McHugh, 2000) and perhaps sipunculids (Boore, 2002). These groups each had previously held phylum level status. If these systematic associations are appropriate, they might indicate a level of plasticity of segmentation within the 'Annelid' radiation. It will be important in future studies to examine the deployment of genes orthologous to those genes that function in the segmentation process of polychaetes in these unsegmented taxa.

One of the hallmarks of early development in annelids is its highly stereotypical 'spiral cleavage'program, named as such because of the oblique orientation of the cleavage planes relative to the animal vegetal axis (Shankland and Savage,

1997). This pattern of holoblastic cleavage is also found in other protostomes such as molluscs, nemerteans, polyclad flatworms, echiurans and sipunculids, but is not found in arthropods. Following the first two cell divisions, 4 blastomeres are produced, designated the A, B, C and D quadrants. The spiral cleavage pattern becomes most apparent at the third cell division, when each of the first four blastomeres divides asymmetrically to produce a smaller cell, or micromere, towards the animal pole and macromere at the vegetal pole. The orientation of the cleavage plane that produces the micromeres is at a 45º angle with respect to the animal vegetal axis. In the following division of the macromeres, the cleavage plane is also oriented in an oblique manner but in the opposite orientation. Thus, successive divisions alternate the direction of orientation of the cleavage plane. In many annelid embryos, the D macromere is larger than the other blastomeres and gives rise to the majority of the segmented tissue of the body. Specifically, descriptive studies in polychaetes have shown that the ectoderm arises from one of the D quadrant micromeres (2d) and the mesoderm of the trunk from another (4d) (Anderson, 1966). The highly stereotypical cleavage program of annelids has placed more reliance on the cleavage program to segregate developmental potential than do chordates and arthropods. The ability to identify individual blastomeres in annelids sets it apart from early cleavage stages in many arthropods and chordates, wherein large numbers of cells in chordates and the nuclear syncytium that dominates early development in many arthropods, make these early stages difficult to follow.

The cellular origin of the segmented tissues during development has been well documented in both the leeches and oligochaetes, where highly stereotypical patterns of cleavage have an important role in segmental patterning (Sandig and Dohle, 1988; reviewed in Weisblat and Huang, 2001). Descendants from the 2d micromere give rise to a 'protoectoteloblast' NOPQ, which then undergoes serial divisions to produce the four ectoteloblasts, N, O, P and Q. The mesodermal teloblasts, M, also arise from the D quadrant, as the descendants of 4d, which undergoes a single equal division to give rise to the left and right mesodermal teloblast.

The earliest signs of segmentation in the leech and oligochaete embryos appear with the onset of a series of highly asymmetric divisions of each of the five teloblasts, located at the posterior of the embryo (reviewed in Shankland *et al.,* 1991; Shimizu and Nakamoto, 2001). The teloblasts produce a chain of daughter cells called primary blast cells. The temporal birth order of the primary blast cells relate directly to the final anterior-posterior segmental position in that earliest born cells will occupy a more anterior position than later-born sibling cells. Each primary blast cell also undergoes a series of highly stereotyped divisions eventually giving rise to approximately 70 descendant cells (Weisblat and Shankland, 1985). Exact descendents from each primary blast cell are known and many of the final differentiated cell types can be identified at the single cell level (such as neurons). The primary blast cells from each teloblast lineage have a unique division pattern, and the segregation of cell fate through a number of cell divisions has been described (Weisblat *et al.,* 1988). Among the different teloblast lineages, the M, O and P teloblasts generate one primary blast cell/segment while the N and Q teloblasts generate 2 primary blast cells/segment. The anterior-posterior boundaries of the descendant clones from the segment founder cells M, O and P are positioned such that about half the clone falls anterior to a segmental boundary while the posterior portion of the clone lies posterior to the segmental boundary. In the case of the N and Q lineages, one of the blast cells gives rise to descendants that reside in the anterior half of the segmental repeat and the other resides in the posterior half (Weisblat and Shankland, 1985), although in none of the lineages is there strict correlation between clones and segmental boundaries.

The large morphologically conspicuous teloblasts and bandlets present in leeches and oligochaetes are not found in polychaetes. Unlike the direct development found in leeches and many oligochaetes, most polychaete species pass through a pelagic larval period of variable length and it is during these larval stages when the first segments are generated (Anderson, 1973). The majority of the body segments are formed following metamorphosis during juvenile through adulthood stages. Polychaetes are generally thought to follow a strict temporal anterior-posterior progression in the appearance of segments. In addition to contributions to the segmented ectoderm by 2d, micromeres of the other quadrants of the second quartet and even from the first quartet (1d) form a portion of the segmented ectoderm in some polychaetes. These variants depend upon the size of the embryo and the relative size of the D quadrant with respect to the other quadrants. It has been proposed, and is firmly entrenched in the classic literature, that the segmented ectoderm arises as the result of mitotic activity from a band of ectoteloblasts cells in the posterior region of the mid-body of the larva, just anterior to the telotroch, that gives rise to the ectoderm of the future segmented trunk region (reviewed in Anderson, 1966; 1973). However, there is little convincing evidence of ectoteloblasts in polychaetes.

The mesoderm of the segmented portion of the mid-body of the larva arises from descendants of the 4th quartet micromere of the D quadrant (4d). 4d initially undergoes an equal cleavage to yield M_l and M_r, which then are internalized and undergo teloblastic divisions to give rise to paired ventro-lateral segmental mesodermal blocks. It is reported that in the general case for annelids, the mesoderm segments before the ectoderm. A causal relationship in the mesoderm influencing the segmentation of the ectoderm has been suggested although not directly demonstrated for polychaetes (Anderson, 1973). There are some exceptions to this case, in groups such as the serpulids and the nereids, in which the ectoderm precociously segments relative to the mesoderm in the larval segments.

Although oligochaetes and leeches share a specialized early developmental program in the production of teloblasts and germinal bands to form the segmented tissue, oligochaetes have many characteristics that are more typical of polychaetes. Oligochaetes have an indeterminate number of segments (Hyman, 1940), axial regenerative abilities and many species can also reproduce asexually by fission. These life history characteristics are relevant to studies of segmentation because, unlike leeches that produce all of their segments during embryogenesis, both polychaetes and oligochaetes continue to add segments after embryogenesis. This underlies the fact that leeches pattern their anterior-posterior axis only once during early ontogeny, while oligochaetes and polychaetes are capable of executing the segmentation program throughout their life span.

Expression of 'segmentation' genes in Annelids

The molecular mechanisms of segment formation have been relatively understudied in annelids. Molecular investigations of segmentation in annelids have primarily utilized a candidate gene approach to characterize components of the segmentation pathway as defined in *Drosophila.* Because functional approaches in these animals are still being developed, the focus has been on determining whether any segmentation genes are expressed in spatial and temporal patterns that implicate them in the segmentation process. Within annelids, the leech embryo has served as a developmental model system due to its large size and the ability to maintain them in a laboratory culture. However, leeches are somewhat unique within annelids. They have poor regenerative capacity, an invariant number of segments (32), and undergo direct development, generating all segmental tissue during embryogenesis in an 'acceleration of adult segmentation into embryogenesis' (Shankland and Savage, 1997).

In the leech embryo homologues of representatives from one gap, one pair-rule and two segment polarity genes have been reported to date. The first segmentation gene to be isolated and characterized in the leech embryo was a homologue of the segment polarity gene *en*, *ht-en* from *Helobdella triserialis* (Wedeen and Weisblat, 1991; Lans *et al.,* 1993). Its expression pattern is quite provocative in that *ht-en* is present early during segment formation in all the ectodermal lineages in a transverse banded pattern, initially as a single cell/segment. *Ht-en* is also expressed in the mesoderm, in two cells in each segmental clone. The earliest expression is observed in the O and P ectodermal lineages when the segmental clone contains only five cells, consistent with a potential role in subsegmental patterning in the leech embryo. However, single cell laser ablations of the *en*-expressing cell or its precursor in both the O and P lineage result in normal patterning of the remaining segmental clone (Seaver and Shankland, 2000; Seaver and Shankland, 2001). In addition, ablations of *en*-expressing cells in the N lineage do not result in any defects in separation of the nervous system primordia into discrete ganglia of the CNS (Shain *et al.,* 1998). These experiments provide no evidence for a role of *ht-en* in segmental patterning of the leech embryo.

Another gene originally defined as serving a segment polarity function in *Drosophila*, *hh*, has been characterized in *Helobdella robusta* (Kang *et al.,* 2003), a closely related species to *Helobdella triserialis*. In the *Drosophila* blastoderm, *hh* is co-expressed in the same cells as *en* and the secreted HH protein specifies fates of nearby cells. The initial expression of *Helobdella robusta*, *Hro-hh*, is restricted to the very anterior of the embryo, in an unsegmented region. As the pattern matures, *Hro-hh* transcripts are localized to the developing foregut and midgut. Embryos grown in the presence of cyclopamine, a specific inhibitor of the *hh* signaling pathway, have disruptions in the formation of the crop and proboscis, and mesenchymal cells normally present in the coelom are absent but there is no segmentation defect. The *Hro-hh* transcript is not expressed until well after *ht-en* expression is initiated in a segmental pattern in the germinal band (Lans *et al.,* 1993). *Hro-hh* and *ht-en* are not reported to have overlapping patterns of expression in any spatial or temporal context in the leech embryo. In the unsegmented gastropod mollusc *Patella*, *hh* transcripts (Nederbragt *et al.,* 2002b) also do not co-localize with *en* during development (Nederbragt *et al.,* 2002a). Thus, there is no compelling evidence for a role of *hh* in segmentation or *en* regulation in the Lophotrochozoans.

In the embryo of *Helobdella robusta*, *eve* (*Hro-eve)* transcripts are expressed in teloblasts and primary blast cells for all the ecto- and mesoderm lineages (Song *et al.,* 2002). All primary blast cells express *Hro-eve* instead of alternating blast cells that might be predicted for a pair-rule pattern. At later stages *Hro-eve* is expressed in a subset of neurons in the CNS, a feature found among many diverse metazoans. One of the strengths of the leech embryo as an experimental system is that individual teloblasts can be injected allowing for perturbations specifically directed to localized portions of the embryo. When *Hro-eve* morpholinos are injected into the N teloblast, which produces the majority of CNS neurons, there is a general disruption of gangliogenesis and in addition, primary blast cells undergo premature divisions. Perturbations of the O lineage by *Hro-eve* morpholino injections into the O teloblast result in a cluster of undifferentiated cells. Therefore, it appears that in the leech embryo, *Hro-eve* many have a general role in cell differentiation but not segmentation per se.

Hunchback (hb), a member of the gap group of segmentation genes, has been in characterized by both *in situ* hybridization and immunohistochemistry in the leech embryo (Iwasa *et al.,* 2000; Savage and Shankland, 1996). The transcript, *Lzf2*, is reported to be present during early cleavage stages and broadly and uniformly expressed during stages when segments are forming. The leech *hb* protein (LZF2) is expressed in a subset of micromeres during early cleavage which does not give rise to segmental structures, and later in epithelial derivatives and a subset of neurons in the CNS. It is not detectable in the teloblasts or in the bandlet, consistent with the conclusion that *Lzf2* does not have a role in segmentation in leech.

In oligochaetes, both *en* and *hb* orthologs have also been examined. The *en* transcript (*Pl-en*) has been characterized in *Pristina leidyi* during fission and regeneration (Bely and Wray, 2001). In both processes, expression of *Pl-en* is similar and is found in a small number of cells co-localized with the CNS. There are additional segmentally reiterated cells; however, these do not correspond to positions of previously identified structures. Currently, there is no *en* expression data in developing embryos/larvae for oligochaetes. The expression of the *hb* protein during embryogenesis in the oligochaete, *Tubifex* (Shimizu and Savage, 2003) has been characterized utilizing a cross-reacting antibody generated against the leech ortholog of *hb. Hb* cross reactivity is present in early cleavage blastomeres (in the peri-nuclear region) and in the micromere cap. Interestingly, it is also found in the O, P and Q ectodermal teloblasts in what appears to be a temporally dynamic manner such that at a single time point, immunostaining is apparent in only one teloblast per hemi-segment. This is an intriguing result, but more in depth characterization is necessary to determine if this pattern of expression is related to segmentation. Neither *hb* or *en* expression patterns strongly supports a role in oligochaete segmentation.

It is important to examine expression of the *Drosophila* segmentation genes in polychaetes because it is difficult to know whether the segmentation process in clitellates can be generalized for annelids since at the cellular level, clittelates appear to generate segments in a distinct manner from polychaetes. Most

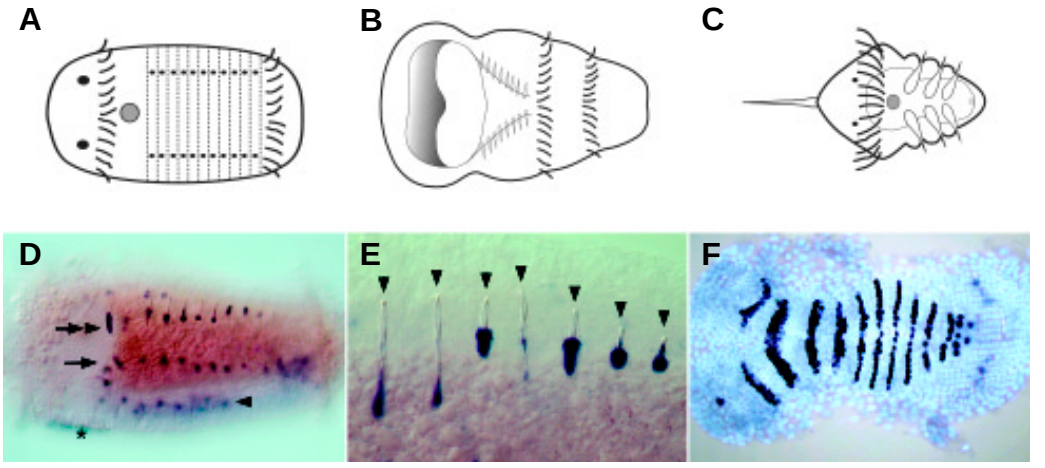

Fig. 2. Variation in larval morphology among polychaete annelids and example of *engrailed* expression in a polychaete and a crustacean. *(A,B,C) Schematic of three different polychaete larval morphologies. The three specimens shown are at comparable developmental stages in which the larval segments have formed. All three are ventral views.* **(A)** Capitella *6 day larva containing 13 segments;* **(B)** Chaetopterus *40 day larva with 15 segments, and* **(C)** Hydroides *3 day larva with three segments. (D,E) In* Capitella, en *is present in segmentally iterated structures such as chaetal sacs and a subset of the neurons in the CNS. Expression is initiated after indications of morphological segmentation and does not appear to be involved in delineating segments from one another.* **(D)** *Lateral view of a Day 6* Capitella *larva. On each side of the animal, there are two rows of chaetae. The notopodial row is located dorso-laterally (double-headed arrow) and the neuropodial row is positioned ventro-laterally (single arrow). Asterisk marks the position of the mouth. Arrowhead points to staining in the ventral nerve cord.* **(E)** *Close up view of the notopodial chaetae in* Capitella. *The* en *transcript is localized to the base of the chaetal sac. Arrowheads point to the distal tip of the forming chaetae. At this stage in each row a single chaetae forms per segment. Note the anterior to posterior developmental progression of chaetal formation from left to right.* **(F)** *Ventral view of an Stage 17 embryo of the amphipod crustacean* Parhyale hawaiensis *showing a striped pattern of engrailed protein expression in the ectoderm, a pattern highly conserved across arthropods. Picture in (F) is provided by William Browne. Anterior is to the left in all panels.*

polychaetes have an indirect life cycle, and it is during larval stages when the first segments are generated (Fig. 2 A-C). Expression patterns of the orthologues of the segment polarity gene *en* have been characterized in several polychaete species with distinct life history traits. In the polychaete *Chaetopterus*, *Ch-en* is expressed in subsets of neurons in the CNS and in the mesoderm of some portions of the larva, but is not expressed in a striped pattern prior to the morphological appearance of segments in the ectoderm or the mesoderm (Seaver *et al.,* 2001). In another polychaete, *Capitella*, *en* is present in a subset of CNS cells, developing chaetal sacs, and bilaterally symmetrical regions of cells delaminating from the ectoderm epithelium (Fig. 2 D,E) (E. Seaver, in preparation). In both *Capitella* and *Chaetopterus*, *en* is found in iterated structures, but expression does not appears as ectodermal stripes or correspond to segmental boundaries. In contrast, Prud'homme and co-workers (Prud'homme *et al.,* 2001) report a striped pattern in the larva and in regenerating adult regions of *Platynereis dumerilii* corresponding with a segmental periodicity that they interpret as being consistent with a possible role in the segmentation process. These conflicting data underlie the importance of performing detailed comparative studies in this complex and speciose group of lophotrochozoans.

Utilizing the cross-reacting antibody generated against the leech *hb* protein, immunoreactivity was examined in the polychaete *Capitella* (Werbrock *et al.,* 2001). As in the leech, the *Capitella hb* cross reactivity is expressed globally in early cleavage blastomeres, in both the micromeres and the macromeres. At later stages, *hb* immunoreativity is seen in the developing gut, in a subset of neurons of the CNS, to the anterior and posterior of the prototroch and telotroch respectively. Its subcellular localization in the blastomeres is distinct from that observed in leech: in *Capitella, hb* is localized to the nucleus. It is likely that the teloblast expression of *hb* in *Tubifex* arose as a separate innovation in the Oligochaete lineage since it is absent in leeches and polychaetes.

Thus, among the three groups of annelids, oligochaetes, leeches and polychaetes, the pattern of *en* expression is not as highly conserved as it is in arthropods. Furthermore, evidence does not support a role for either *hh* or *en* in segment formation in annelids. Cumulatively, the expression of the *Drosophila* segmentation genes, both transcription factors and signaling molecules, annelids do not have patterns that support a homologous molecular pathway of segment formation. Furthermore, the lack of support for conservation of the segmentation pathway between annelids and arthropods cannot be explained by the derived developmental characteristics of the clittelates.

Segmentation in vertebrates

In vertebrates, segmentation is apparent in and limited to the axial skeleton, muscles and nerves. During embryogenesis, the segmental nature of the vertebrate body plan is morphologically obvious by the presence of transient structures called somites. Somites are paraxial mesodermal structures that form as repeated blocks from a large population of cells in the presomitic mesoderm (PSM) and give rise to the segmental tissues in the adult. The formation of somites generally follows a temporal progression from anterior to posterior and has a regular periodicity; the length of each cycle and the number of somites is specific to and varies among species. Many of the molecular details of somite formation vary among the three vertebrate systems that have been most intensely studied (chick, mouse and zebrafish). A detailed description of somitogenesis and discussion of the evolution of somitogenesis is presented by O. Pourquie (Pourquie, this volume). Here we briefly describe salient features of somitogenesis relevant for comparisons with other segmented taxa.

The production of somites involves a molecular oscillator or 'segmentation clock', in which a temporal oscillation is translated into a spatial code with a periodicity of a single somite. The first

molecular evidence for the segmentation clock was described in chick (Palmeirim *et al.,* 1997) with the discovery of repeated waves of expression of the transcription factor *c-hairy1* in the chick presomitic mesoderm (PSM). c-hairy1 is a basic helix-loop-helix transcription factor which functions as a pair-rule gene in *Drosophila*. The *c-hairy1* message has a single wave of expression from the posterior of the PSM to the anterior end for each somite formed. As the wave reaches the anterior of the PSM, its expression becomes stabilized in a band of cells that will become the posterior of the forming somite.

Since these initial observations, other genes in addition to *hairy* have been identified that have expression patterns which cycle through the PSM in a pattern and correlate with the morphological formation of somites (reviewed in Saga and Takeda, 2001). These genes are expressed in a similar manner in frogs, chicks, mice and zebrafish and are collectively known as the cyclic genes. Of the cyclic genes characterized, most is known about members of the Notch/Delta signaling pathway. Notch signaling has a crucial role in vertebrate segmentation, as shown from e. g. mouse knockout experiments, and has been proposed to play a role in synchronizing both the temporal periodicity in the PSM as well as the anterior posterior polarity in the forming somite. The on-and-off expression of the cyclic genes has provided evidence of an intrinsic oscillatory mechanism in the cells of the PSM, and part of the challenge has been to determine which genes are actually part of the clock mechanism itself and which genes have oscillation patterns of expression as part of the output of the clock. A recent report by Dale *et al.,* (2003), implicates *Notch* and *lunatic fringe*, a modifier of Notch activity, in a negative feedback loop that may act as a component of the clock mechanism in the chick embryo. Central to the molecular clock is the expectation that an unstable protein that negatively autoregulates its transcription is at the core of the cycling mechanism, a characteristic found in *lunatic fringe.* The *Wnt* signaling pathway has also recently been implicated in somitogenesis of the mouse embryo and axin2, an inhibitor of *Wnt* signaling, is expressed in a dynamic pattern in the PSM (Aulehla *et al.,* 2003). There is also evidence that the *Wnt* signaling pathway may interact with the Notch signaling pathway.

In addition, a number of other 'noncycling' genes expressed in the PSM have been functionally implicated in establishment of rostral/caudal polarity within the somite and formation of the somite boundary. For example, *fgf8* is expressed at high levels in the posterior of the PSM and gradually decreases towards the anterior end of the PSM. It appears from a number of studies that high levels of *fgf8* expression inhibit the formation of somites, and in the anterior portion of the PSM where Fgf8 levels are low, somites can form. Therefore, FgF8 functions as a positional cue that translates the clock into the placement of segment boundaries. Thus, it is clear that the temporally progressive nature of somitogenesis is reflected by oscillatory molecular mechanisms.

Is there homology among any of the major segmented taxa?

Based upon what we now know about segmentation in each of the annelids, arthropods and chordates, do we currently have enough information to decisively state whether segmentation is monophyletic or polyphyletic in the metazoa? The main assumption in current analyses of the relationships among segmented animals is that shared molecular developmental pathways will reflect shared evolutionary histories. The molecular information we have concerning the segmentation process is based primarily upon a candidate gene approach whereby comparisons in expression patterns are made by utilizing genes defined functionally in a single or small number of organisms as having a role in the segmentation process. There are two 'perspectives' currently available for comparisons: the segmentation genes as defined in *Drosophila* and the genes involved in vertebrate somitogenesis. There has not yet been a 'forward' approach to directly identify genes in the segmentation process in annelids, although genomics approaches such as EST analysis combined with *in situ* hybridization hold promise for the future.

In any discussions considering possible homologies, it is important to identify ancestral character states. A dramatic example of convergence in the segmentation process is the generation of segmental tissue via stereotyped asymmetric cleavages by ectodermal teloblastic cells in clitellates and some malacostracan crustaceans (for discussion see Scholtz, 2002). The presence of such teloblasts is restricted to these two groups, both of which have many derived characters within the annelids and arthropods respectively. These morphologically obvious teloblasts evolved independently in the annelids and arthropods and may suggest that there is a trend to precociously segregate axial patterning information into the cleavage program.

From the *Drosophila* perspective

The expanding body of observations of the segmentation process across a range of arthropods has been invaluable in trying to elucidate how much of the *Drosophila* segmentation program was utilized in the ancestral arthropod, one that has holoblastic cleavage and sequential addition of segments.

In vertebrates, homologues of many the *Drosophila* segmentation genes do not have a role in vertebrate segmentation, however, a few exceptional cases have been made. This has included the expression of a *Drosophila* pair-rule *hairy* homologue in zebrafish (Muller *et al.,* 1996) and the expression of *en* in *Amphioxus* (Holland *et al.,* 1997). In the case of the zebrafish *her1*, the zebrafish homologue of *hairy*, a possible pair-rule patterning in vertebrates was proposed with the observation that *her1* transcripts were expressed in alternate presumptive somites in zebrafish embryos. However, more recent evidence has shown that the stripes of expression correlate with each somite (Holley *et al.,* 2000), although in morpholino studies where *her1* was knocked down enlarged somites result in a pattern that might suggest a pair-rule mechanism. The universality of a pair-rule mechanism outside insects remains uncertain and current evidence from non-insect arthropods is more consistent with a segmental pattern of 'pair-rule' genes, rather than a pair-rule pattern.

The highly conserved *en* gene expression pattern across arthropods has made it a useful character for comparisons with other taxa. In *Amphioxus*, a cephalochordate, *en* is expressed in the eight anterior-most developing somites suggesting a role for *en* in somitogenesis. However, *Wnt1* does not show a segmental pattern in of *Amphioxus* (Holland *et al.,* 2000), suggesting that the *en/wg* signaling network is not conserved. Additionally, in acidians, which are basal to *Amphioxus* in the deuterostome lineage, *en* is expressed in a small number of cells in the anterior of the tadpole but is not in an iterated pattern (Jiang and Smith, 2002).

Taken together, the *Drosophila* segmentation genes do not have an obvious role in somitogenesis in vertebrates. In annelids, it is clear that more data is needed, but from the published expression patterns, the *Drosophila* segmentation genes do not obviously have a role in annelid segmentation.

From the vertebrate perspective

With the deep level split between protostomes and deuterostomes, it has been thought for about a century that segmentation in the chordates is independent from annelid/arthropod segmentation. However, with the recent explosion in our understanding of the molecular mechanisms that control somitogenesis, we are now in a position to ask whether molecular components of the'segmentation clock' could possibly be functioning in protostomes. This is especially appealing because like vertebrates, most arthropods and annelids form segments in a cellularized environment with a anterior-posterior temporal addition of segments.

Initial attempts to implicate the Notch signaling pathway in the segmentation process in arthropods did not produce convincing evidence. First, the Notch signaling pathway has not been shown to play a role in *Drosophila* segmentation, although this is perhaps not a surprising result because most steps of the *Drosophila* segmentation pathway do not need to utilize a cell-cell signaling system. Second, characterization of *Notch* gene expression in grasshoppers has not been compelling. During stages when segments are being formed, *Notch* appears to be expressed ubiquitously throughout the embryo and does not cycle (Dearden and Akam, 2000). Third, the grasshopper *fringe* gene, a homologue of vertebrate *lunatic fringe* and an important component of the vertebrate molecular oscillator mechanism, also does not have an expression pattern that implicates it in segmentation. Although it is expressed in an iterated manner, expression of the message does not appear until after *en* expression appears and after segment boundaries have formed (Dearden and Akam, 2000).

However, a recent study from chelicerates raises the possibility that Notch signaling may be involved in segmentation in arthropods (Stollewerk *et al.,* 2003). Stollewerk and co-workers report the expression of *Notch* and two *Delta* transcripts in the posterior growth zone and also in a banded pattern of forming segments of *Cupinneus salei.* Furthermore, *Delta1* shows a dynamic pattern of expression in the spider growth zone. RNAi disruptions for *Notch* or either of the *Delta* transcripts result in malformed segments and *hairy* stripes become disorganized. The reliance of the proper *hairy* expression on Notch signaling has similarity to vertebrate somitogenesis in which expression of *hairy* orthologues in stripes in the PSM is dependent upon Notch signaling. Although it remains to be seen if *Delta1* in fact has an oscillating pattern, this result is consistent with the idea that the common ancestor of both vertebrates and arthropods was segmented and used the Notch signaling pathway in formation of segments.

Concluding remarks

Over the past five years there has been substantial progress in our understanding of the segmentation process in a variety of metazoans. Within arthropods, examination of segmentation genes has been extended to include all 4 major arthropod groups. This is significant because now our 'arthropod perspective' represents more than studies from insects and a few crustaceans. In vertebrates, there has been an explosion of data describing the molecular mechanisms of somitogenesis, accompanied by a conceptual shift from the candidate gene approach of examining *Drosophila* segmentation genes in vertebrates to characterizing the molecular details of the segmentation clock. However, there is still a paucity of data concerning the molecular mechanisms of segmentation in annelids. Additionally, there may be valuable information gained from sampling additional taxa such as tardigrades, onychophorans, and echiurans. Onychophorans may be especially interesting to examine since they do not have segmented ectoderm, but their mesoderm is segmented, and *en* has been reported to be expressed in repeating mesodermal blocks (Wedeen *et al.,* 1997). The fact that vertebrate somitogenesis is mesodermally driven while *Drosophila* segmentation is ectodermal, represents an issue that also needs to be resolved. Although the classic literature supposes that mesoderm initiates segmentation in annelids (Anderson, 1966), it is likely that both types of segmentation will be found to operate in this clade.

Importantly, we also need to work out what constitutes convincing evidence of homology of segmentation. How do we combine morphological and molecular data? Based on developmental genetic programs, is an argument based on one gene enough? It is hard to imagine that the evolutionary history of such a complex structure as a segment would be revealed by examination of a single gene. Are several components of a genetic network enough? How do we interpret the presence of 'partial' networks? As we weigh the available data, it is also important to make a distinction between transcription factors and signaling pathway components. There are far more transcription factors than signaling pathways and thus it is even more critical when examining signaling pathways to consider the details of the associated cellular context. If *Notch* signaling is associated with a cycling mechanism of segmentation in annelids, arthropods and vertebrates, does this argue for monophyly of segmentation or represent an example of convergence/superficial similarity? As yet there is no persuasive evidence of a universal metazoan molecular segmentation program. However, before we can make a conclusive interpretation of the history of body plan segmentation in the Metazoa, it will be critical to come to a fundamental understanding of development in annelids and other members of the Lophotrochozoa.

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