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[REVIEW]

A Developmental Basis for the Cambrian Radiation

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As extant bilaterian phyla emerged during the Lower Cambrian, these clades acquired morphological features that separated them from their stem groups. At the same time, morphological variants on the body plan within a phylum emerged that we recognize as classes and subphyla. In many cases, the emergence of body plan variants within a phylum is associated with major changes in patterns of early regional specification. Subsequently these different patterns of regional specification were stabilized, probably because later developmental events depended on them. As a consequence, the frequency of new body plan variants involving early development declined in these lineages at later periods during their history. This hypothesis is explicated here by examining the process of early regional specification in the different subphyla of brachiopods and in pairs of species from the same subphylum belonging to different clades that originated during the Jurassic, Ordovician, and Lower Cambrian.

Key words: Cambrian, cladogenesis, conserved pattern, brachiopod, development, diversification, evolution, regional specification

INTRODUCTION

Representatives of extant bilaterian phyla first appear in the fossil record during the Lower Cambrian. At this time, fossils from a number of different phyla with mineralized structures (arthropods, brachiopods, echinoderms, molluscs) first appeared during an interval of about 5–10 million years. Because phyla are defined as a community of descent, stem groups of extant bilaterian phyla must have existed prior to the Cambrian; molecular clock data suggest that the origin of these stem groups may predate the onset of the Cambrian by about 100 million years (with a large standard error) (Douzery *et al.,* 2004; Peterson *et al*., 2004). Most fossils from the late Neoproterozoic cannot be related to extant bilaterian phyla because they lack one or more key features of their body plans, suggesting that the Lower Cambrian was a period of rapid body plan evolution. One other feature of cladogenesis during the Lower and Middle Cambrian involved diversification within phyla; these modifications of a body plan are manifest in the origin of a number of higher taxonomic categories such as subphyla and classes (*e.g*., Runnegar, 1996 for Mollusca; Williams *et al*., 1996 for Brachiopoda). Frequently these modifications involved changes in the placement of organs, the origin of novelties, or the disappearance of body-plan components.

A phylum is not only a community of descent, but it also represents a body plan (Valentine, 2004). The foundation for this body plan is laid down during embryogenesis via a process of regional specification. These early regional specification events occur prior to or during gastrulation. There are two complementary views that define the elements that operate during regional specification. One view examines this process at a cellular level. It begins by defining a fate map for the early embryo that establishes what a given region of the embryo will form under normal circumstances. Then different regions of the embryo are isolated at different times to find out when they acquire the ability to differentiate only the structures normally formed by that region. Some regions may already be specified when they are isolated at the earliest possible period during development; they are autonomously specified. Autonomous specification is frequently based on the presence of unique localized mRNAs or proteins that give the cells that inherit them a special set of properties. It is more common for a given region to be conditionally specified. In this case, signaling between cells, which already differ because of the presence or absence of given mRNA or protein, may lead to regional differences (Slack, 1990). By doing various kinds of recombination and isolation experiments involving cells from different regions of the embryo, these inductive interactions and the spatial domains where they operate can be defined.

The other view examines regional specification at a genetic-network level. Davidson and Erwin (2006) have argued that there are basal gene regulatory network circuits, referred to as GRN "kernels", required for the process of regional specification. These regulatory genes encode transcription factors that maintain the regulatory circuit and activate or repress downstream genes that are responsible for regional specification. The kernels are only used during regional specification, and interference with the function of

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This paper is dedicated to the memory of Hidemi Sato, Director of Sugashima MBL and Professor of Biology at Nagoya University.

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any gene in a kernel will destroy the function of the circuit. These networks are regarded as difficult to alter, because a change in the network would lead to a disruption of the process of regional specification upon which the rest of embryogenesis is based. At present it is not clear to what extent these two views of regional specification are congruent.

There are a number of ways to think about changes or the lack thereof in early regional specification with reference to the origin of and diversification within phyla: 1) It could be that either prior to or during the period when a crown group emerged during the Lower Cambrian, a pattern of early regional specification was set up and that this initial pattern was largely conserved during the subsequent history of the phylum, including the periods when new subphyla and classes originated, even when these emerged during the Cambrian. Presumably events associated with the origin of higher taxa within a phylum involved the modification of later developmental processes. This scenario is based on the notion that once the foundation for early development is laid down, subsequent developmental events are constrained because these events depend on them (Arthur, 1988; Wimsatt and Schrank, 2004). 2) During the period shortly after a phylum had emerged when the radiation was occurring to generate differences at higher taxonomic levels within that phylum, modifications of the early regional specification program brought about these higher taxonomic changes. These changes in the process of regional specification were no different from those that generated the different animal phyla in the first place, and in most cases they occurred shortly after the appearance of the first definitive members of the phylum in the fossil record. Only after these large initial changes in regional specification generating different variants of the body plan did subsequent changes in the process of early regional specification stop. 3) The pattern of early regional specification may have initially been quite uniform in the different higher-level clades that make up a phylum, but subsequently, as a consequence of the separate histories of these subclades, the process of early regional specification in each clade would have slowly been modified to different extents. One way to think about this process assumes that development is controlled by a scale-free network of interacting nodes where the most important nodes have a large number of functional links and a situation exists where links can slowly change between nodes. Because links are constantly changing, the pattern of regional specification can change over time during early development while supporting a secondary set of nodes that are responsible for the phenotype (Barabási and Albert, 1999). These are possible large-scale trends in animal evolution that deserve more investigation (McShea, 1998).

Information from phylogenetic and developmental studies can be brought together to find out whether or not there is a unique pattern of early regional specification or multiple modes of regional specification operating in a phylum. This requires studies on the process of regional specification in representatives of all of the extant classes that make up a phylum. Even after 100 years of comparative experimental developmental biology, the process of regional specification has not been adequately documented in a single monophyletic group. In those cases where comparative studies have been done, frequently different investigators have studied different classes within a phylum using different procedures and asking slightly different questions, making it difficult to compare patterns of regional specification. Because members of different classes frequently have different anatomies and life histories, it can be difficult to homologize different regions of early embryos, making statements about regional specification problematic. Terms like phylum, class, and order are relative and only have meaning in a particular phylogenetic context. When two classes are compared within a phylum, it is important to establish when a major morphological difference reflects an early developmental change and when it reflects a later developmental change.

In order to interpret patterns of regional specification within a phylum, it is necessary to have a robust reconstruction of the history of the phylum indicating when new subphyla, classes, and orders originated and the groups from which they originated. At a practical level, this means that one has to work on a phylum with a good fossil record using cladistic studies on fossils coupled with stratigraphic data in order to define the sequence of morphological changes within a phylogeny (Smith, 1994). For those parts of a phylum where there are extant species, it should be possible to check parts of the cladistic phylogeny based on fossils with a molecular phylogeny based on their descendants.

If one is going to argue that the generation of new classes within a phylum during the Cambrian is a consequence of changes in the pattern of regional specification during embryogenesis, one has to be able to asses how patterns of regional specification are organized in Cambrian embryos. In a literal sense this is impossible, because our inferences about patterns of regional specification depend on experimental studies on living embryos. Many phyla have classes originating during the Cambrian that include extant species. By comparing patterns of regional specification in embryos of extant animals from two different Cambrian classes and regional specification in embryos of two members of the same classes that belong to different lineages that diverged during the Paleozoic, one can use embryos of extant animals as surrogates for embryos of Cambrian animals. An example of this kind of analysis is presented here for brachiopods.

THE FOSSIL RECORD AND PHYLOGENETICS OF BRACHIOPODS

Because of their shells, brachiopods have an excellent fossil record that extends from the Lower Cambrian (ca. 530 mya) to the present. Recently a higher-order classification of this phylum has been prepared based on cladistic principles (Williams *et al*., 1996) (Fig. 1A). This classification divides the phylum into three subphyla: Linguliformea, Craniiformea, and Rhynchonelliformea, composed of eight classes. There are living representatives of three classes, each of which originated during the Lower Cambrian; each class with living representatives is in a different subphylum. Linguliformea is composed of two classes. One class is extinct; the other class Lingulata is composed of three orders; one of these orders, Lingulida, has extant members that belong to two families: Lingulidae and Discinidae. The fossil record indicates that the stocks that each family was derived from were present during the Lower Cambrian (Chen *et al*., 2006). Within the Craniiformea, there are living representatives of

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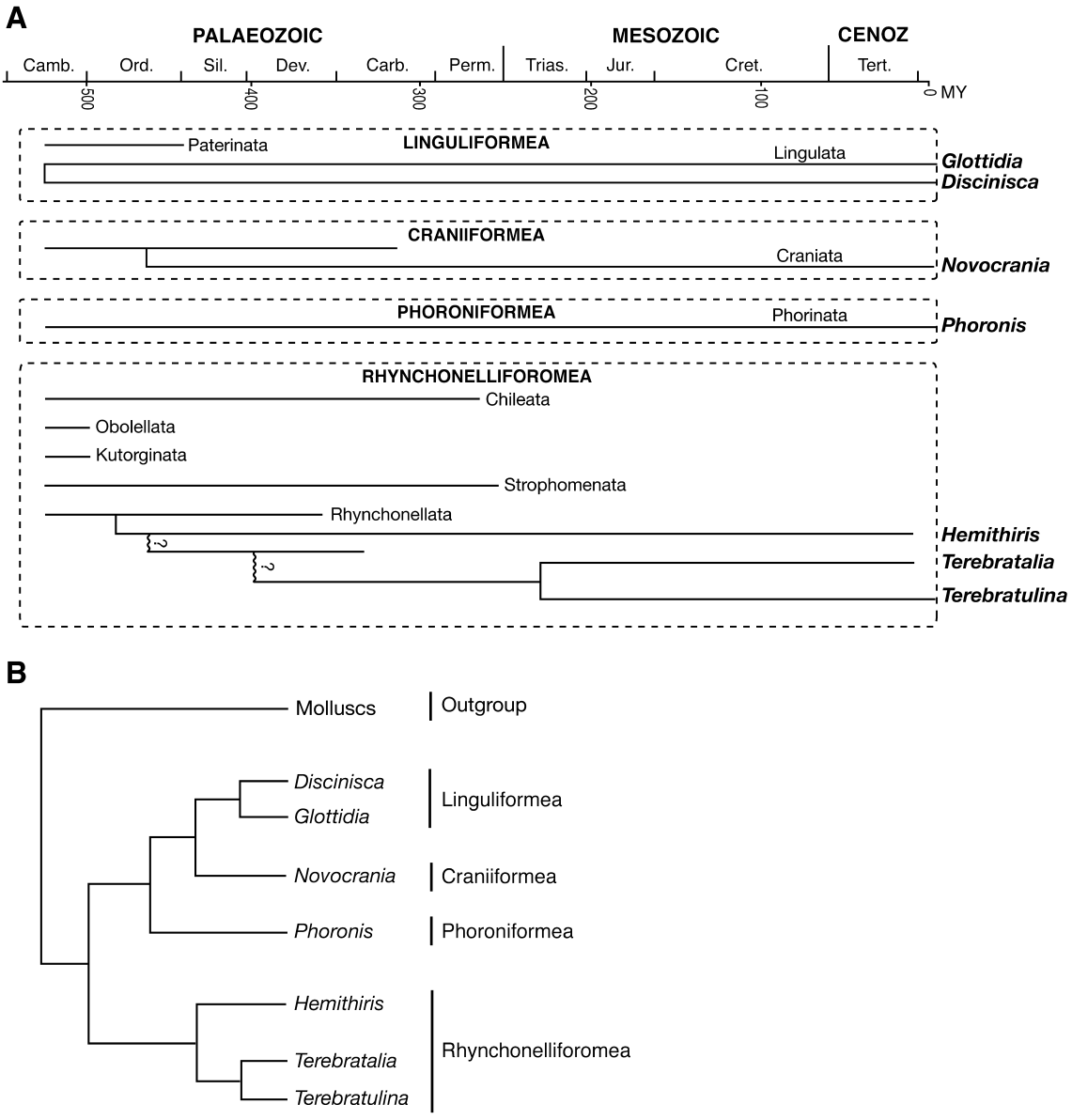


Fig. 1. **A)** Subphylum- and class-level classification of the Brachiopoda (including the Phoroniformea) superimposed on a geological time line. The right side of the chart gives the names of the extant genera used to determine the pattern of regional specification. Their distributions and affinities in the four brachiopod subphyla are indicated. Each subphylum is enclosed in a dashed box. Each class is represented by a solid line indicating time of first appearance and time of extinction. For extant genera, the time when the order they belong to first appeared and any earlier orders that gave rise to the order they reside in are indicated (adapted from Williams *et al*., 1996). **B)** Molecular phylogeny for extant brachiopod subphyla based on the small subunit of rDNA and partial large subunits of rDNA (adapted from Cohen and Weydmann, 2005).

one family in the order Craniida. The fossil record indicates that the stock that gave rise to this family originated from the order Craniopsida during the Lower Cambrian (Skovsted and Holmer, 2005). Living members of the Rhynchonelliformea are all members of the class Rhynchonellata; the four other classes in this subphylum are extinct. The species that we will deal with belong to two orders: Rhynchonellida and Terebratulida (there is a third order with extant representatives: Thecideida). The Rhynchonellida first appear in the fossil record during the Lower Ordovocian. The Terebratulida first appear in the fossil record at the Silurian-Devonian boundary. They appear to have been derived indirectly from the Rhynchonellida via one or more intermediate extinct orders, starting possibly as early as the Middle Ordovician (Carlson and Leighton, 2001). Subsequently the Terebratulida split into two sub-orders, Terebratulidina and Terebratelidina, in the Middle Triassic. Most brachiopods that are extant today are descendants of the Rhynchonellida and Terebratulida that survived and radiated after the mass extinction at the end of the Permian period.

At the same time that the new classification scheme for brachiopods was developed, a molecular phylogeny based on the small subunit of rDNA was generated (Cohen and Gawthrop, 1997). This molecular phylogeny is largely congruent with the cladistic classification; however, it also showed the that phylum Phoronida, which had been used as

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the outgroup for the cladistic classification, nested with the Linguliformea and Craniiformea within the phylum Brachiopoda (see Halanych, 2004 for a discussion of earlier work on the phylogenetic affinities of the Phoronida). This gave a phylum with three closely related subphyla, Linguliformea, Craniiformea, and Phoroniformea, that made up one major clade and the Rhynchonelliformea that made up the other major clade. Subsequent work by Cohen and Weydmann (2005) using both the small subunit and partial large subunits of rDNA sequences showed that the Phoroniformea were the basal members of its clade and that the Linguliformea and Craniiformea were the derived members of that clade (Fig. 1B). Extant phoronids are a small phylum composed of two genera. Because they lack a skeleton, they have a poor fossilization potential; however, well-preserved soft-bodied fossils that have been interpreted as phoronids have been found in the Lower Cambrian Chengjiang fauna (Chen and Zhou, 1977).

Because there are living representatives of four subgroups of the Brachiopoda, each of which presumably attained the status of a crown group during the Lower Cambrian, it is possible to examine patterns of regional specification in each group in the context of the molecular phylogeny for the phylum, to see how much they differ. In the cases of the classes Lingulata and Rhynchonellata, changes in regional specification within a class can be compared by examining members of different families or orders that diverged at different times.

REGIONAL SPECIFICATION IN BRACHIOPODS

Comparative anatomy of larvae

Brachiopods have bilaterally symmetrical adults and larvae (Fig. 2A). The larvae of extant Linguliformea and Phoroniformea are planktotrophic, while the larvae of Craniiformea and Rhynchonelliformea are lecithotrophic; however, during the Cambrian, the larvae of Craniiformea and Rhynchonelliformea were also planktotrophs (Freeman and Lundelius, 1999, 2005). All of these larvae undergo settlement and metamorphosis to become sessile adults. In the Linguliformea, Craniiformea, and Rhynchonelliformea, the larva does not undergo an extensive reorganization during metamorphosis. The Phoroniformea undergo a radical reorganization; during metamorphosis many regions in these larvae cytolize, and there is a change in the axial organization of the metamorphosed juvenile with reference to the larva (Zimmer, 1997). In all four subphyla, the larvae have a similar plan that appears to consist of three homologous regions; in the Linguliformea, Craniiformea, and Rhynchonelliformea these are referred to as the apical, mantle, and pedicle lobes. The anterior apical lobe functions as a sensory structure and locomotory organ, and in planktotrophic larvae as a feeding structure. The mantle lobe contains the alimentary canal, and the mouth forms ventrally at the junction between the apical and mantle lobes; it also contains the mantle that shell will be deposited on after metamorphosis. The pedicle lobe forms the attachment site of the larva during and after metamorphosis. In the Linguliformea, this lobe forms during the larval period as a ventral outgrowth along the mantle lobe anterior to the posterior anus. The Craniiformea lack a pedicle lobe, and in the Rhynchonelliformea it is posterior to the mantle lobe.

Homologizing larval regions in the Phoroniformea versus the other three subphyla of brachiopods is more difficult than homologizing body regions among the brachiopods. The most anterior region of the phoronid larva is the pre-oral hood; it has a sensory function, but lacks the locomotory and feeding structures of a brachiopod apical lobe. In phoronids, the mouth forms in a ventral position between the pre-oral hood and trunk, as it does in brachiopods. The trunk contains the alimentary canal; it also has a set of tentacles that function in locomotion and feeding. Just prior to metamorphosis, the trunk of the larva forms a ventral extension, the metasomal sac, anterior to the anus. This sac is in the same position as the pedicle lobe of the Linguliformea. It also plays a role in attaching the larva to the substrate during metamorphosis; unlike the pedicle lobe of the Linguliformea and Rhynchonelliformea, it contains part of the alimentary canal.

Early embryogenesis and fate maps

The egg diameters of the brachiopod species whose patterns of regional specification will be described vary in diameter from 65–70 μm for *Discinisca* to 190–200 μm for *Hemithiris*. Planktotrophs have smaller eggs than most lecithotrophs. Fertilization of eggs in each of these subphyla leads to polar body formation, which defines the animal pole of the egg. Oogenesis in all four subphyla occurs on a germinal epithelium. In *Glottidia* (Linguliformea), the future animal pole is located at the apical pole of the oocyte furthest from the germinal epithelium (Freeman, 1995). A similar arrangement may exist in the other subphyla. The first two cleavages occur along the animal-vegetal axis in all of these embryos. After the eight-cell stage, the fourth cleavage can either be bilateral or radial (Fig. 3A). Bilateral cleavage occurs in the Linguliformea (Yatsu, 1901). It is either bilateral or radial in the Phoroniformea; some eggs in a batch will exhibit a radial and some will exhibit a bilateral blastomere configuration (Zimmer, 1997). In the Craniiformea (Nielsen, 1991) and Rhynchonelliformea (Zimmer, 1997), cleavage is radial. In some cases these cleavage differences are related to the pattern of regional specification.

Following cleavage, a hollow blastula forms and gastrulation occurs. Gastrulation can occur by simple invagination (Fig. 3B1) as it does in the Linguliformea (Freeman, 1995, 1999) and Craniiformea (Nielsen, 1991), or it can also involve involution of surface cells at one side of the blastopore (Fig. 3B2) as it does in the Phoroniformea (Ikeda, 1901) and Rhynchonelliformea (Conklin, 1902). These differences in the process of gastrulation are also related to the pattern of regional specification. After gastrulation is over, different regions of the larva begin to form.

Fate maps have been made for representatives of each subphylum (Freeman, 1991, 1993, 1995, 1999, 2000, 2003). The animal and vegetal poles of the uncleaved egg and different blastomeres of early cleavage-stage embryos have been labeled, and these labeled embryos have been followed. When the animal poles of representatives of these subphyla are labeled, two patterns are seen. In the Linguliformea, the dorsal surface ectoderm of the apical and mantle lobes is labeled. In the Phoroniformea, Craniiformea, and Rhynchonelliformea, the pre-oral hood/apical lobe regions are labeled (Fig. 2B1). The vegetal pole becomes the site of

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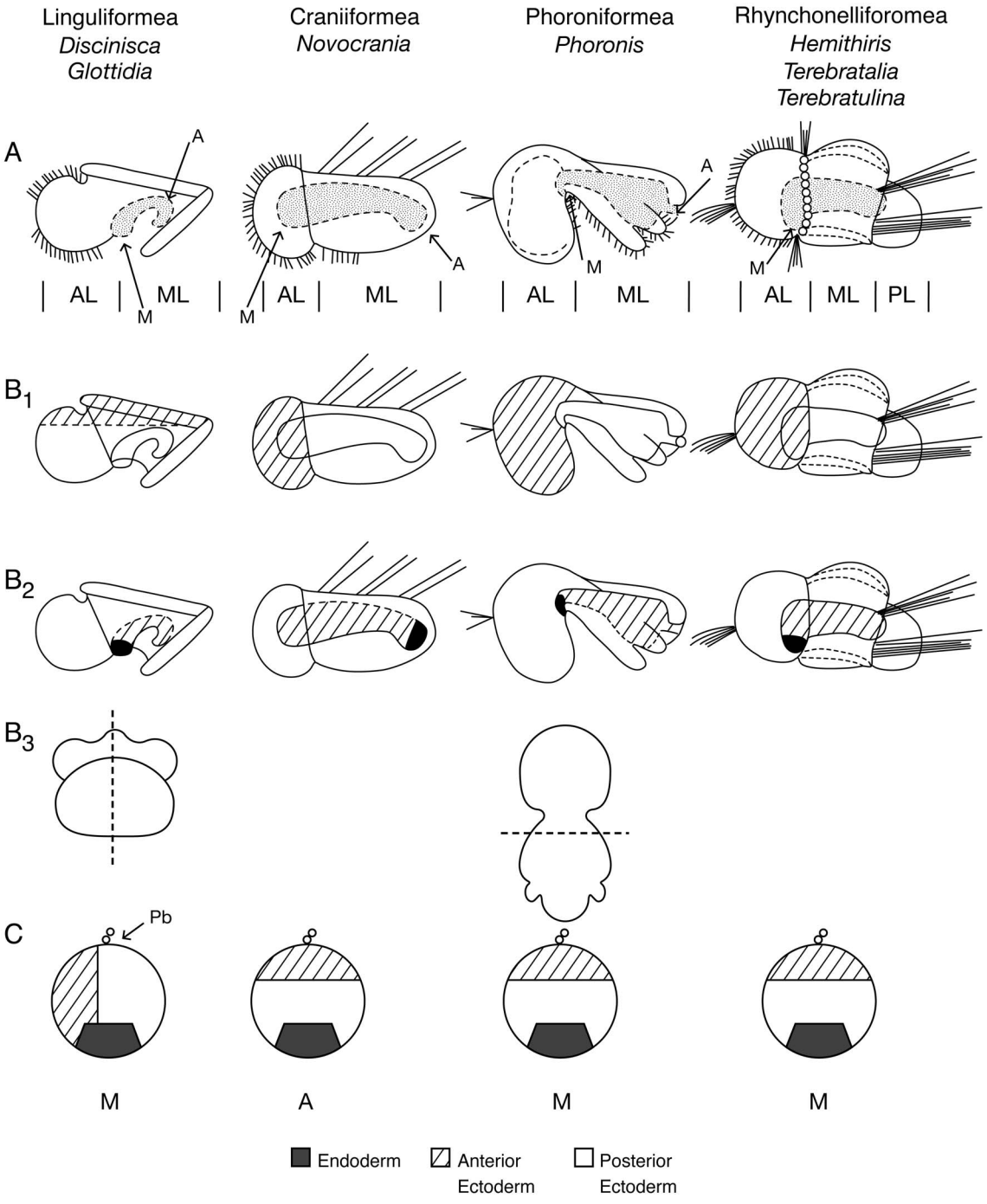


Fig. 2. **A)** Comparisons of larvae of the four brachiopod subphyla. The top of the figure indicates the species analyzed in each subphylum. A lateral view of each larva is shown; dorsal is up and anterior is to the left (AL, apical lobe; ML, mantle lobe; PL, pedicle lobe; M, mouth; A, anus; pb, polar bodies). In the larvae of lecithotrophs, a mouth and anus will not form until after metamorphosis; the Rhynchonelliformea lack an anus. Young larval forms of *Glottidia* and *Phoronis* shown here have yet to form a pedicle lobe/ metasomal sac. **B)** Results of fate mapping on early embryos. **B1)** Animal pole marked (dashed lines). **B2)** Vegetal pole marked; the site of gastrulation is the black circle; the dashed lines indicate endoderm; mesoderm is not shown. **B3)** The plane of the first cleavage is marked by a dashed line. These larvae are viewed from the dorsal side; the anterior end is up. **C)** Projection of the fate maps on fertilized uncleaved eggs. The codes for the different cell fates are given below. M and A indicate the structures formed at the site of gastrulation.

gastrulation in each subphylum. In the Craniiformea, this site becomes the future anal region of the postmetamorphic juvenile; however, in the other subphyla this region becomes the future mouth (Fig. 2B2). During early gastrulation, the label on the surface of the vegetal pole disappears uniformly as invagination occurs so that there is a ring of label around the blastopore where future endoderm and mesoderm are invaginating. In the Phoroniformea and Rhynchonelliformea, the label then disappears locally at a sector at one side of the blastopore as the blastopore elongates with one end at

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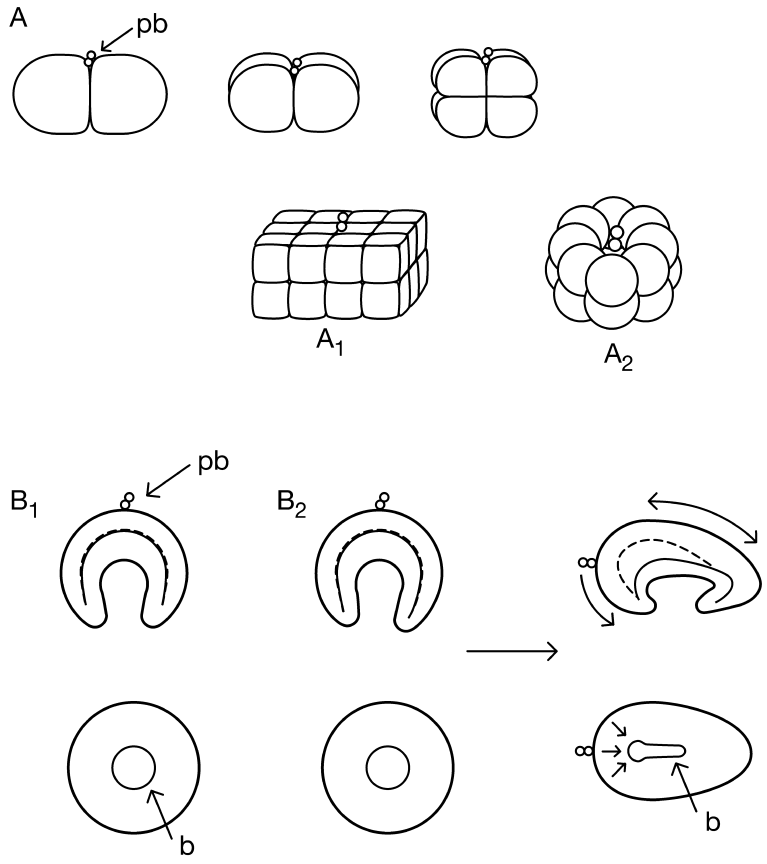


Fig. 3. **A)** Bilateral and radial cleavage; the top of the figure shows the first three cleavages. **A1)** Bilateral 16-cell stage. **A2)** Radial 16-cell stage (pb, polar body). **B)** Gastrulation by invagination, or by invagination and involution. **B1)** Simple invagination. The top panel shows a gastrula that has been cut away. The bottom panel shows a vegetal view of the external surface of an early gastrula, including the blastopore (b). **B2)** Invagination and ingression. The first panel shows an early gastrula undergoing invagination. This is followed by a later stage (after arrow), when the involution of ectoderm in one sector moves the polar body from its apical position to a lateral position as the ectoderm in one sector of the embryo undergoes involution. At the same time, the blastopore becomes a slit. Arrows indicate involution and epiboly.

the involuting side. This site marks the plane of bilateral symmetry. As a consequence of involution the animal pole, ectoderm moves along the larva's future anterior posterior axis to a region adjacent to the future mid-ventral side of the larva where the blastopore will form the future mouth. In *Phoronis*, marking experiments show that cells just outside the blastopore but in close proximity to it also ingress beneath the surface ectoderm and become nerve and mesodermal cells (Freeman and Martindale, 2002). Comparable marking experiments have not been done on other members of the subphyla. In the Linguliformea, the plane of the first cleavage corresponds to the plane of bilateral symmetry. The second cleavage separates the future anterior and posterior domains of the larva. In the Phoroniformea, in 70% of the cases, the plane of the first cleavage separates the anterior from the posterior region of the larva; in the other cases, the plane of cleavage can have any other orientation. In those cases where the first cleavage separates anterior from posterior, the second cleavage occurs along the plane of bilateral symmetry. In the Craniiformea and Rhynchonelliformea, there is no relationship between the first cleavage and the plane of bilateral symmetry.

Fig. 2C projects these fate maps on uncleaved eggs. There are three distinct fate maps that reflect different heterotopies. The Linguliformea and Craniiformea each have different fate maps, while the Rhynchonelliformea and Phoroniformea have the same fate maps. In the case of the Rhynchonelliformea and Linguliformea, where multiple genera were examined, the fate maps for each subphylum were identical.

Regional specification

Regional specification occurs in a species when a given region acquires the ability to differentiate according to its fate map. The timing of specification is established by isolating the same region of the embryo from species in each subphylum at comparable stages of development and rearing them to determine how they will develop. The process of specification can be completed during a single stage, or it can occur over several stages of development. The time course of this process may provide clues that partially define how this process has occurred.

Animal-half isolates (Fig. 4A). In the Linguliformea, the animal half forms the dorsal ectoderm of the apical and mantle lobes. In *Discinisca* and *Glottidia*, the apical lobe is specified before the mantle lobe is; the time when both structures form is considered the time of regional specification. In *Discinisca*, specification has begun by the blastula stage, and it has occurred in over 50% of the cases by the

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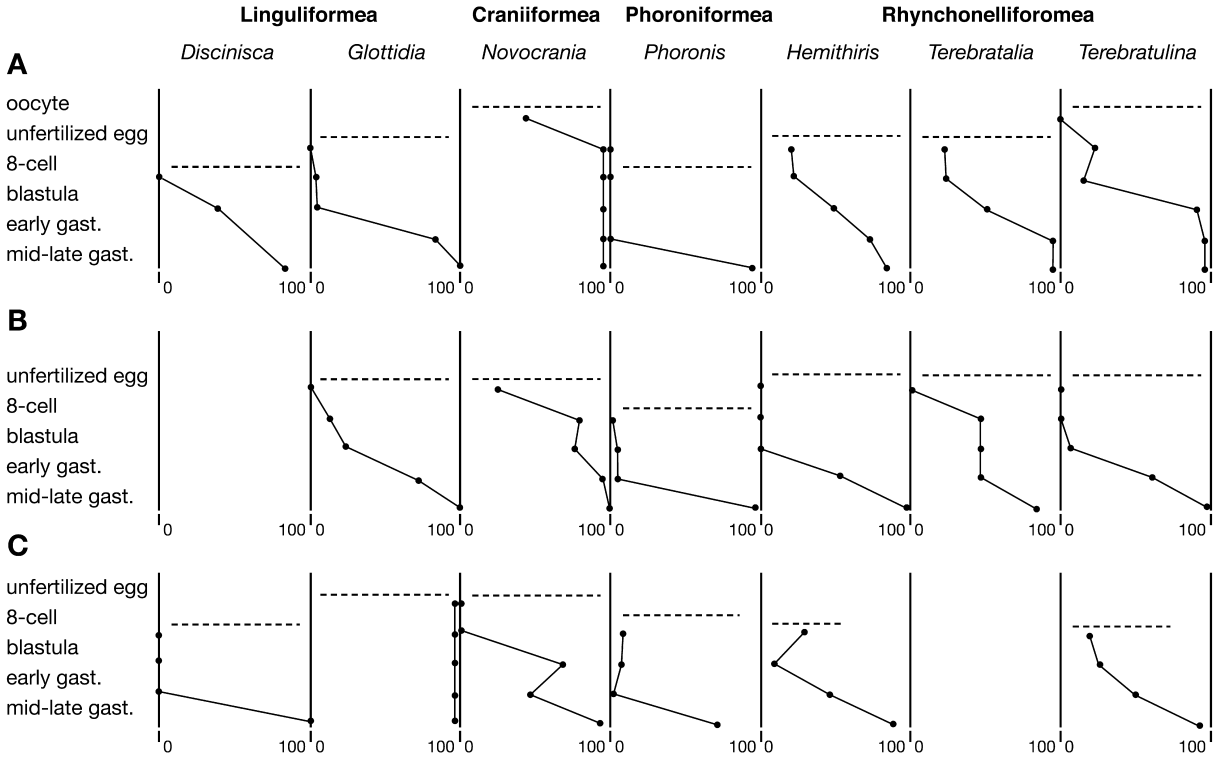


Fig. 4. Graphs showing the timing of regional specification for **A)** the animal region; **B)** the vegetal region; and **C)** the establishment of the plane of bilateral symmetry, for representatives of the four brachiopod subphyla. The dashed line indicates the first developmental stage analyzed for each region of each species. 0–100% at the bottom of the column indicates the percentage of cases showing regional specification. The criteria used for describing regional specification for each species are given in the text.

mid-gastrula stage (Freeman, 1999). In *Glottidia*, specification has begun by the eight-cell stage and has occurred in over 50% of the cases by the early gastrula stage (Freeman, 1995). In *Novocrania,* animal-half specification involves formation of the apical lobe. Animal halves of oocytes that are matured and fertilized form either a normal larva or an apical lobe. When animal halves are generated from unfertilized eggs that are then fertilized, these cases form only apical lobes, and no cases gastrulate and form larvae. The early specification of this region suggests that it depends on localized cytoplasmic factors and that there is a redistribution of these factors during oocyte maturation (Freeman, 2000). In *Phoronis,* the animal region of the embryo forms the pre-oral hood, which includes a nerve plexus that contains catecholamines. This structure is not specified until mid-gastrulation (Freeman, 1991). In the Rhynchonelliformea, the animal half of the embryo forms the apical lobe with a small apical tuft of non-motile sensory and locomotory ciliary cells. When animal halves are isolated at an early stage of development, the entire isolate frequently forms apical tuft cilia, and locomotory cilia formation is suppressed; however, when animal halves are isolated at later developmental stages, the frequency of normal-sized apical tufts increases and locomotry cilia form. The presence of a normal apical tuft has been used as a criterion for apical-lobe specification. In all three species, there is a low level of apical-lobe specification in animal halves from unfertilized eggs that have been fertilized. In *Terebratulina* where animal halves have been isolated from oocytes that have then been matured and fertilized, all of the cases have a large apical tuft. In both *Terebratalia* and *Terebratulina,* the apical lobe has been specified in over 50% of the cases by the blastula stage (and by the early gastrula stage in *Hemithiris*) (Freeman, 1991, 2003). The pattern of apical-lobe specification suggests that it depends at least in part on an inductive signal from the vegetal region of the embryo that inhibits the size of the apical tuft.

Vegetal-half isolates (Fig. 4B). In all four subphyla, the vegetal region of the egg is the site of gastrulation and endoderm and mesoderm formation; it also forms part of the ectoderm. In the Linguliformea, the ectoderm forms the ventral regions of the apical and mantle lobes. In *Discinisca,* the lack of suitable dorsal markers makes it difficult to asses the time of vegetal-half specification. The vegetal half gastrulates and forms both an apical and a mantle lobe by the eight-cell stage. The only structure typical of the apical lobe is the dorsal apical tuft, which is missing. In *Glottidia*, when vegetal halves of unfertilized eggs are fertilized they go on to gastrulate and form both an apical and a mantle lobe. The mantle lobe has both a dorsal and a ventral mantle. When vegetal halves are isolated from unfertilized eggs, both mantles are present; however, as vegetal halves are isolated at later stages of development, the frequency of cases with a dorsal mantle decreases to less than 50% by the early gastrula stage. In *Novocrania*, the vegetal half of the embryo forms the mantle lobe. When vegetal halves are isolated from unfertilized eggs that are then fertilized, the majority of cases have normally proportioned apical and mantle lobes,

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and a minority of cases have either only a mantle lobe or a small apical lobe and normal-sized mantle lobe. By the eight-cell stage, the majority of cases have only a mantle lobe or a small apical lobe and a mantle lobe. In *Phoronis*, the vegetal region of the embryo forms the larval trunk. When vegetal halves are isolated at the eight-cell stage, the majority of cases form a normal larva. Only a few of the vegetal halves from the blastula stage gastrulate. When vegetal halves are isolated from mid-late gastrulae, these cases only form the trunk region of the larva. In the Rhynchonelliformea, all of the vegetal halves form ectoderm of the mantle and peduncle lobes of the larva. In all of the species tested, in a majority of cases, vegetal halves isolated at early stages of development formed larvae with apical, mantle, and pedicle lobes. The only feature of the apical lobe that was missing was the apical tuft. As vegetal halves were isolated at later developmental stages, the apical lobes became smaller and disappeared, giving larvae with only mantle and pedicle lobes in a majority of cases.

One common feature of vegetal halves in each of these subphyla was that when they were isolated at early developmental stages, they regulated to form structures normally formed by the animal region of the egg, while vegetal regions isolated at later stages formed structures typical of their fate map in the majority of cases. This pattern suggests that signals from the animal halves necessary for vegetal specification stopped functioning when vegetal halves were isolated. The timing of vegetal-half specification varied in the different subphyla. Specification occurred early in *Novocrania* and quite late in *Phoronis* for both halves.

Bilateral symmetry (Fig. 4C). In the three subphyla that have been tested, an animal-vegetal axis exists prior to fertilization. The Phoroniformea is the only subphylum where this issue has not been examined. The vegetal pole is specified as the site of endoderm-mesoderm formation, while the animal pole is specified as the site of apical tuft (sensory neuron) formation. Since brachiopods are bilaterians, a plane of bilateral symmetry has to be specified which acts in conjunction with the animal-vegetal axis to establish the anterior-posterior ends and the dorsal-ventral sides of the larva. These regions begin to become morphologically apparent during and following gastrulation.

In the Linguliformea, the plane of the first cleavage is correlated with the plane of bilateral symmetry. The plane of the second cleavage separates anterior from posterior. The animal domain forms the dorsal and the vegetal domain forms the ventral region of the embryo. If four-cell *Glottidia* embryos are separated into two halves along the plane of the first cleavage, both halves will form normal larvae. When four-cell embryos are separated into two halves along the plane of the second cleavage, one half forms the apical lobe and the other half forms the mantle lobe. If unfertilized *Glottidia* are cut into lateral halves along the animal-vegetal axis and each half is fertilized, about 50% of the halves from a given egg each form a normal larva, while in the other 50% of the cases one half forms primarily the apical lobe while the other half forms primarily the mantle lobe. This means that the anterior-posterior difference is set up prior to fertilization, most probably as a consequence of the establishment of different cytoplasmic domains that also entrain the lateral positioning of the plane of the first cleavage. When this same kind of experiment was done in *Discinisca,* presumptive anterior and posterior halves developed as normal larvae when they were isolated at early stages and did not develop as anterior and posterior halves until the mid- to late gastrula stage. The timing of bilateral symmetry specification is very different in the two linguliforms; the mechanism of regional specification must also be quite different. There is evidence that it depends on inductive interactions in *Discinisca* (Freeman, 1999). In *Novocrania*, the anterior-posterior axis of the larva corresponds to the animal-vegetal axis of the embryo. The establishment of the plane of bilateral symmetry specifies the dorsal and ventral sides of the embryo. The dorsal side differs from the ventral side of the larva because it has six groups of setae arranged in two rows of three setae, one on the left and one on the right side of the mantle. When cleavage-stage embryos are cut into lateral halves along their animal-vegetal axis, and both halves are reared, pairs with three left setae and three right setae form. Pairs where one half has no setae and the other member has setae, indicating that the plane of bilateral symmetry has been set up, do not appear in the majority of cases until the blastula stage (Freeman, 2000). In *Phoronis*, the plane of the first cleavage separates the future anterior from the future posterior region of the larva, and the plane of the second cleavage corresponds to the plane of bilateral symmetry. The animal-pole region of the egg will become the anterior end of the larva after it moves to a lateral position during gastrulation. At all stages of development, when embryos were cut into halves along the plane of the second cleavage, the majority of the cases formed a normal larva. When larvae were cut into halves along the plane of the first cleavage from first cleavage through the early gastrula stage, the majority of the cases formed normal larvae; however, by the mid-gastrula stage, half of the cases formed only a pre-oral hood while the other half of the cases formed a trunk, indicating that the anterior-posterior axis is not set up until late development (Freeman, 1991). In the Rhynchonelliformea, there is no relationship between the early planes of cleavage and the plane of bilateral symmetry; however, the members of this subphylum are similar to the Phoroniformea, because the animal pole region will form the apical lobe of the larva after it moves to a lateral position during gastrulation. The best markers are four groups of setae, two on the left and two on the right side of the mantle lobe. When embryos are cut laterally along the animal-vegetal axis into two lateral halves at different stages of development, both halves frequently form all three larval lobes, or the apical and mantle lobes. When a pair of halves from the same embryo were isolated early during development, each half had three to four sets of setae; when pairs of halves were isolated between early and mid-gastula, the number of setal sets went down, so that each member of a pair had a maximum of two setae. This data set has only been collected for *Hemithiris* and *Terebratulina* (Freeman, 2003). A similar data set has been developed for *Terebratalia* based on the fact that there are paired rows of eye spots on the left and right sides of the apical lobe (Freeman, 1993).

PUTTING REGIONAL SPECIFICATION IN A PHYLOGENETIC CONTEXT

In the Rhynchonelliformea, the fate maps, the mode of

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gastrulation (which is associated with the establishment of the plane of bilateral symmetry), and the timing of regional specification for the three species examined are essentially identical. Since the Rhychonellida (*Hemithiris*) and the Terebratulida (*Terebratulina, Terebratalia*) began to diverge between the Middle Ordovician and the Silurian, there has effectively been no change in the process of regional specification in these two orders in spite of the long period that has elapsed and the radiation within the two orders following the mass extinction at the end of the Permian. This is also seen after the split of the Terebratulida into the sub-orders Terebratulidina and Terebratalidina, indicating that patterns of regional specification can be stable over long periods of geological time. The Phoroniformea, the basal member of the Phoroniformea-Craniiformea-Linguliformea clade, shares the same fate map and mode of gastrulation with the Rhynchonelliformea; however, regional specification in the animal and vegetal domains occurs much later in *Phoronis.* It appears that the animal domain of the embryo needs endomesoderm in order to differentiate properly, while the vegetal region of the embryo needs animal ectoderm in order to gastrulate; this is not the case in the Rhynchonelliformea. Late regional specification is associated with basal members of classes or subphyla (Goldstein and Freeman, 1997). The similarities involving the fate maps and mode of gastrulation in the Phoroniformea and Rhynchonelliformea probably reflect the fact that these are sister groups.

In the clade that includes the Linguliformea, Craniiformea, and Phoroniformea, the fate maps of each subphylum differ. There are heterotopies involving the site of origin of the apical lobe (pre-oral hood) and the relationship between the blastopore and the mouth or anus. There are also major differences in the establishment of the axis of symmetry, mode of gastrulation, and the timing of early regional specification. The genera *Discinisca* and *Glottidia* in the Linguliformea have similar patterns of regional specification, indicating that that these processes have been largely conserved since the Lower Cambrian. The only difference between these linguliforms involves the establishment of the plane of bilateral symmetry. In *Glottidia*, this plane is set up by localized maternal factors, while in *Discinisca* it depends on interactions with adjacent blastomeres that are put in place at the 16-cell stage and act during gastrulation (Freeman, 1995, 1999). It is not clear when this difference in the process of regional specification occurred in these two lineages. Since the stocks these genera evolved from were present during the Lower Cambrian, this difference could have been established then or during later geological periods.

REGIONAL SPECIFICATION PATTERNS IN OTHER PHYLA

While studies on early regional specification have not been done on all major clades in other phyla, there are data sets that support the ideas and observations reported here, indicating that there may have been diverse modes of early regional specification in classes and subphyla that originated during the early Cambrian. Cellular-level analysis of regional specification in urochordates and cephalochordates that are descendants of stocks that originated during the Lower Cambrian (Chen *et al.,* 2003 for ascidians; Mallatt and Chen, 2003 for cephalochordates) indicates that many features of the pattern of regional specification in these two subphyla are quite different (Satoh, 1994 and Nishida, 2005 for ascidians; Whittaker, 1997 for lancets; Swalla, 2004 for both groups). Recently a gene regulatory network for early development in ascidians was published for *Ciona* (Iami *et al.,* 2006). This gene regulatory network is very different from the network for the vertebrate *Xenopus* (Loose and Patient, 2004). The problem with this vertebrate-urochordate comparison is that amphibians originated well after the Cambrian. This comparison would have been much more apt if it involved a basal vertebrate such as a cyclostome that belongs to a clade that originated during the Lower Cambrian.

There are also studies that appear to contradict the conclusions presented here. Recently Hinman *et al.* (2003) reported that that echinoid and asteroid echinoderms have similar gene regulatory networks that mediate early regional specification. One has to bear in mind that, while the Echinodermata first appeared in the fossil record during the Lower Cambrian, the classes Echinoidea and Asteroidea first appear during the Ordovician (Sumrall, 1997). Molecular and morphological evidence indicates that these two classes are more closely related to each other than they are to the Crinoidea (Littlewood *et al.,* 1997), which is a more appropriate group for a study of the comparative biology of gene regulatory networks, because it originated earlier and via a different set of early Cambrian ancestors than the Echinodea-Asteroidea pair (Sprinkle and Collins, 1998).

Slack *et al.* (1993) dismissed early developmental stages as highly variable and have argued that biologists should concentrate on the phylotypic stage of development, when the body plan begins to emerge at an anatomical level. These investigators failed to appreciate that in most animal phyla, the period prior to and during gastrulation is the stage when the body plan is specified.

POSTSCRIPT

When work on the relationship between development and evolution began during the 19th century in response to the “The Origin of Species”, the initial questions that were the focus of attention dealt with macroevolutionary issues, such as the origin of body plans (Beeson, 1978). Subsequently much of this work was dismissed because the ideas put forward were vague and untestable. Much of the current work in the field of evolution and development deals with microevolutionary issues; however, it is clear that the macroevolutionary questions posed during the 19th century are still important and do not have adequate answers. Many of the contemporary attempts to address these questions have involved developmental comparisons between ecdysozoans and deuterostomes (*Drosophila* versus *Mus*). Much of this work has not been very illuminating because the phylogenetic context in which it was done is too broad to allow investigators to make meaningful conclusions. The conclusions presented here need to be tested by doing similar comparative analytical developmental studies on other phyla. In order to successfully do this kind of comparative work, one has to make judicious choices about which groups of animals to study. This involves paying careful attention to the work of paleontologists who are reconstructing the origins of animal phyla and molecular evolutionists who are reconstructing within-phylum phylogenies.

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