

Cleavage patterns and the topology of the metazoan tree of life

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ABSTRACT Several major alliances of metazoan phyla have been identified by small subunit rRNA sequence comparisons. It is possible to arrange the phyla to produce a parsimonious distribution of cleavage types, requiring only one change from a radial ancestral condition to spiral cleavage and one other to “idiosyncratic” cleavage; this arrangement is consistent with most of the recent molecular phylogenies. The cleavage shifts are correlated with changes in many of the features that once were used to distinguish Protostomia and Deuterostomia. It is hypothesized that changes in cleavage direction are causally associated with changes in blastomere fates and thus that cleavage type correlates with such features as the identity of mesoderm founder cells, which in turn can constrain the mode of origination of the eucelom. Cleavage changes may also affect the timing of cell fate specification. In a tree that emphasizes cleavage parsimony, radial cleavage, regulative development, and enterocely are ancestral within the Bilateria, and spiral or idiosyncratic cleavages, mosaic development, and schizocely are associated with a change in cleavage direction. Deuterostomy is presumably ancestral and is correlated with radial cleavage for this reason, rather than mechanistically.

The most famous branching on the metazoan phylogenetic tree separates the protostomes from the deuterostomes (1, 2). Despite wildly different phylogenetic scenarios espoused by different workers, the distinction between these branches has been relatively well supported. The characters used to establish or support this classic branching are chiefly developmental: Protostomes have spiral cleavage and usually mosaic development, form the mouth at (or near) the site of the blastopore, form mesoderm from a mesentoblast that is usually 4d, and are schizocelic; deuterostomes have radial cleavage and usually regulative development, form the mouth away from the blastopore, form mesoderm from endodermal cells along the archenteron, and are enterocelic. These basic criteria can be found in almost every invertebrate textbook. Although it has been known from the first that some of the taxa assigned to each of these clades do not display all of these characters in a “pure” form, it has usually been assumed that any anomalies represent secondary modifications to the ancestral conditions. There is, however, no general explanation for the correlation of the characteristic protostome and deuterostome features with cleavage or with each other. Several lines of evidence now suggest that most of these features may be associated with a shift in cleavage planes in early development.

Molecular Trees and Cleavage Patterns

Small subunit rRNA (SSU rRNA) phylogenies generally have supported the protostome–deuterostome (P/D) branchpoint although they have suggested reassignment of some taxa from

one of these clades to the other, e.g., pogonophorans have been reassigned from Deuterostomia to Protostomia (3, 4). SSU rRNA data have suggested that the protostomes may comprise two major branches (3, 5): the Ecdysozoa, including arthropods (6), and the Lophotrochozoa, including annelids (7). Aschelminths appear to be a polyphyletic assemblage of protostomes, some of which are more closely allied to Ecdysozoa with others allied to the Lophotrochozoa (6, 9). Moreover, some molecular data suggest that at least some of the acelomate flatworms are lophotrochozoan protostomes (6, 8, 10).

Among the more interesting features of the relationships suggested by SSU rRNA is the distribution of cleavage patterns among the major, more stable alliances. (Summaries of early development among metazoan phyla are found in refs. 11 and 12; unattributed, general, developmental observations in the following discussion can be documented in those works.) In Fig. 1, the major alliances suggested by SSU rRNA data are preserved as closely as possible, but the phyla are grouped according to the most parsimonious distribution of cleavage patterns. Five major alliances are found: Diploblastica, which are radialian; Deuterostomia, also radialian; Ecdysozoan, with radial cleavage at the deepest branch and idiosyncratic cleavages in the more derived taxa; Lophophorata, which are radialian and are united on strong morphologic grounds as well as on the common cleavage pattern; and the Eutrochozoa (13, 14), which include the classic spiralian but are interpreted here to have radialian members at the deepest branches.

The protostome alliances have the more heterogeneous cleavages. Despite the putative spiralian nature of protostomes, several of the phyla identified as protostomes by 18S rRNA sequences have radial cleavage. Included in the Ecdysozoa are priapulids (radial cleavage) and nematomorphs (“modified radial” cleavage; see ref. 15), both of which branch deeply within that clade (6, 9). Indeed, within the ecdysozoans, characterized by molting habits, there are no classic spiralian. In onychophorans, what seems to be the least derived pattern is a cleavage that produces an envelope of small cells surrounding a yolk; mesoderm proliferates from paired postero–ventral bands (16). Tardigrades exhibit total and equal cleavage but not spiral (17, 18); the origin of their mesoderm is not yet clear. Arthropods themselves display a variety of cleavage patterns (16, 19). Crustaceans exhibit a pattern that has been called modified spiral cleavage, but if the cells are numbered as if homologous with spiralian blastomeres, mesoderm does not arise from 4d. Chelicerates have yolky eggs that cleave superficially to produce a stereoblastula; mesoderm arises from cells along the midline of the ventral blastular surface. The cleavage patterns of myriapods and other terrestrial arthropods (and for that matter other terrestrial ecdysozoans) may be derived with respect to their marine ancestors; at any rate, they are certainly not spiral [their varied developmental patterns are reviewed by Anderson (16)]. Thus, of all of the Ecdysozoa for which data are available, only the crustaceans, which display unusual early development, exhibit something of a cleavage pattern that can be related to spiral cleavage, but this is evidently homoplastic,

Abbreviations: SSU, small subunit rRNA; P/D, protostome–deuterostome.

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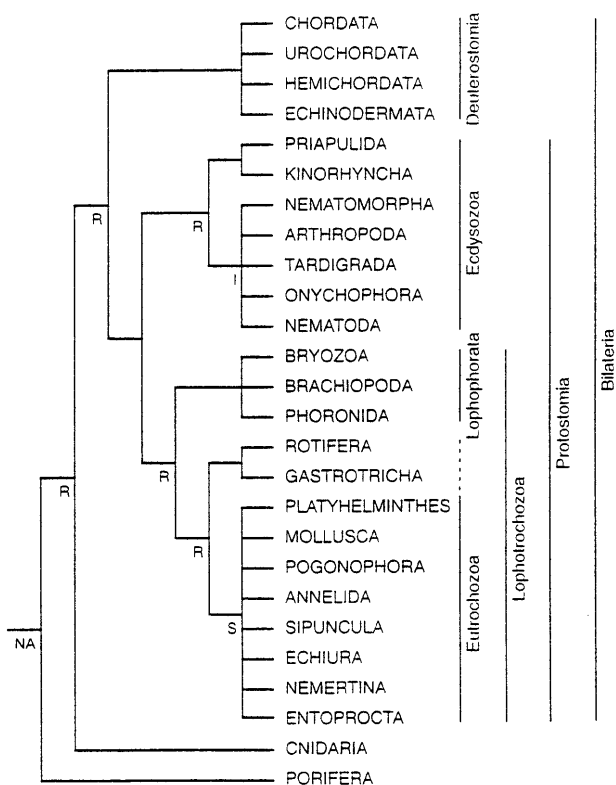


FIG. 1. A phylogenetic hypothesis of some metazoan phyla based on major groupings suggested by SSU rRNA data, modified so as to produce a parsimonious distribution of cleavage types. Although the general topology is stable under a variety of SSU alignments and algorithms, the positioning and branching patterns of the phyla within the major alliances vary somewhat with algorithms, with different associations of taxa, or with different exemplars. (The major clades are recognized from data in refs. 4, 6, 8, 9, 24, and 37–43.) The chief placements that are not directly supported by SSU rRNA data are of Rotifera and Gastrotricha, placed at the base of the Eutrochozoa, and of the Lophophorata, united as a sister group to the Eutrochozoa. It is possible that the Rotifera and Gastrotricha are basal to the entire Lophotrochozoa. (Data for cleavages are from refs. 10 and 11 or as cited in the text.) Cleavage type abbreviations: I, idiosyncratic; NA, not applicable; R, radial; S, spiral.

for so far as is known the deepest ecdysozoan branch has radial cleavage.

The phyla of the lophotrochozoan alliance are divided between radial and spiral cleavages. SSU rRNA branchings within the lophophorates are not stable (20) although these phyla appear to be closely related and to be protostomes. The lophophorates have radial or modified radial cleavage. Bryozoan cleavage is radial, with the form of the blastula varying among the classes. Mesoderm arises from cells that introgress into the blastocel or (in stenolemates) are pinched off the blastula. Brachiopod cleavage is generally radial, producing a hollow blastula; mesoderm is derived from cells along the archenteron after gastrulation (21, 22). Phoronid cleavage is characterized as biradial, and mesoderm also is budded off from cells along the archenteron after gastrulation (21, 23). These developmental similarities, joined with their tripartite body plans and possession of lophophores, have caused lophophorates to be considered as a monophyletic assemblage by most workers, but they have been shuffled back and forth between the deuterostomes and protostomes over the years, until molecular studies finally related them to the Protostomia (3, 7, 24). The Lophophorata are hypothesized here to form a clade within the Lophotrochozoa that is a sister to the Eutrochozoa (Fig. 1). An alternate possibility, that the rotifers and

gastrotrichs branch more deeply than either lophophorates or eutrochozoans, is by no means ruled out.

Within the Eutrochozoa, spiral cleavage and other protostome characters are classically displayed by mollusks, sipunculans, echiurans, and annelids. Nemertines also have spiral cleavage, and evidently most or all have a 4d mesentoblast as well. Cleavage in pogonophorans is reported usually to be spiral (25), but mesoderm derivation is uncertain, early claims of enterocely being unproven. Many platyhelminths are difficult to evaluate by SSU rRNA methods, being long-branched. There is evidence that they are polyphyletic (8); the Catenulida may be a sister group to the rest of the Bilateria, and the Rhabditophora seem allied to the eutrochozoans. Some studies have placed flatworms in a clade that includes the Gastrotricha and Rotifera and that is sister to the Eutrochozoa (9), or in an unresolved relationship within the Eutrochozoa (10). Rotifers and gastrotrichs have radial and “modified radial” cleavage, respectively. To place these two phyla in the position that results in the most parsimonious cleavage arrangement, they should be basal within the Eutrochozoa. It is consistent with molecular, morphologic, and developmental data that the classic spiralian features are derived within the eutrochozoan clade, and that spiralian form a monophyletic subclade within the Eutrochozoa, characterized by spiral cleavage and a 4d mesentoblast (or in the case of flatworms, the mesentoblast is one of two daughters of a 4d division).

With only modest changes from SSU rRNA topologies, a tree can be produced that unites the cleavage groups parsimoniously (Fig. 1). In this tree, there is only one major switch between radial and classic spiral cleavage, from an ancestral radial state already present in diploblastic forms and inherited by the rotifers to the spiral pattern of flatworms, mollusks, annelids, etc. The only other important cleavage switch is from radialian to the idiosyncratic cleavages of the higher Ecdysozoa. Radialians thus lie at the base of each of the major metazoan bilaterian branches—deuterostomes, ecdysozoans, lophophorates, and eutrochozoans. The phyla hypothesized to represent the deepest branches within the ecdysozoans (priapulids) and eutrochozoans (rotifers) are pseudocelomates, and in general acelomates and pseudocelomates occupy the lower branches of those alliances, in accord with available SSU rRNA data.

Correlations Among Deuterostome–Protostome Characteristics

Some of the other major features of protostomy and deuterostomy, such as the position of the mouth relative to the blastopore and the origin of mesoderm and of the celom, are not distributed in exactly the same patterns as cleavage. However, some of the unusual combinations of cleavage with other putative protostome or deuterostome characteristics occur in suggestive positions within the tree. For example, the only cleavage within the Ecdysozoa that can be termed “modified spiral” is found in the crustaceans, but it is spiral only in the sense that it is possible to number the cells according to convention for spiralian (16); the cleavage is not actually spiral, and there are no assurances about cell homologies with classic spiralian. This idiosyncratic cleavage is associated with a nonspiralian formation of mesoderm, which derives from blastomeres that can be numbered 3A, 3B, and 3C instead of 4d (which gives rise to endoderm). Early crustacean development appears to have been significantly modified, ultimately from a radialian condition.

Some of the lophophorates exhibit a decoupling of cleavage, blastopore-site fate, and other features (21). For example, phoronids have radial cleavage, and mesoderm is derived from the archenteron whereas the phoronid celom can be interpreted as a schizocel and the mouth is, literally, protostomous. The mouth is not protostomous in the other lophophorates.

Such occurrences certainly suggest that early development has been modified in lineages leading to these forms. A number of workers have argued strongly that early developmental stages within and among the phyla have been extensively modified (26–28), for, on the level of phyla, developmental stages appear to be nearly or quite as disparate as adult stages although the patterns of disparity are commonly different. The similarity of cleavage patterns when mapped on the SSU rRNA tree, however, suggests that modifications may have remained minimal at the stages of cleavage.

No matter what tree topology is postulated, some evolutionary modifications in cleavage are required among some clades, in addition to the major change at the base of the classic spiralian. These other, less extreme cleavage modifications include the crustaceans mentioned above and several ecdysozoan clades that began as marine clades but have become entirely terrestrial, so that the cleavage changes are probably associated with the loss of their marine larval stage(s) and the presence of abundant yolk in the egg. Marine forms of any clade with very yolky eggs may also display unusual cleavage. Some of the minute lineages, which are commonly eutelic, are still represented in the marine realm and yet have idiosyncratic cleavages, perhaps related to a reduction of cell numbers in the blastula; some minute acel flatworms have so few cleavages before gastrulation that they do not produce a 4d cell, and nematode “rotational” cleavage is quite unique.

Correlates of the Evolution of Cleavage Patterns

There has not been a convincing explanation for the adaptive significance of cleavage pattern in invertebrates although the maintenance of given cleavage types within major invertebrate clades for well over half a billion years certainly implies that there is an important reason that it is conserved. A major aspect of cleavage is that it separates an anisotropic egg into blastomeres whose lineages have distinctively different fates, owing to the sequestering of different gene regulatory factors, chiefly transcriptional regulators, in different blastomeres (28–30). Embryogenesis in the marine invertebrate clades under discussion is of Davidson’s type I (31), in which cell specification occurs chiefly *in situ* and cell lineage is important in organizing the early embryo. In the initial developmental stages, the cleavage planes are chiefly invariant and produce cell differentiation before gastrulation (31). During mitosis, cleavage planes are normal to the spindle axes, and the axes of spindles in radially cleaving organisms are oriented either equatorially or meridionally so that cleavage planes are polar or equatorial, respectively. The axes of spindles in spirally cleaving organisms, however, are inclined with respect to the cell equator so that the cleavage planes are inclined as well and daughter cells are offset with respect to the cell axes (30). The sequestering of cell contents is commonly related to cell axes, so changes in cleavage plane location with respect to cell polarity would result in the sequestration of regulatory factors in different blastomeres, unless there was a reorganization of the distribution of the prepositioned regulatory factors. Barring such repositioning, the cell fates of the blastomeres could be altered. It is therefore hypothesized that general changes in blastomere cell fates such as are observed between classic radialian and spiralian lineages are causally associated with the changes in cleavage patterns.

Among the predictions of this hypothesis is that differences in blastomere fates should have arisen in association with cleavage changes. The origin of mesoderm is a likely example of the correlation of cleavage with early cell fates. Radial cleavage and derivation of mesoderm from the endodermal cells lining the archenteron are presumably ancestral conditions for bilaterians. Lineages wherein cleavage patterns have been significantly altered from that state have had the cell lineage(s) of their presumptive mesoderm altered as well.

Clades that display classic spiralian cleavage have a 4d mesentoblast. Clades with idiosyncratic cleavage, such as the Arthropoda, have unusual mesoderm progenitors. Direct developers with yolky eggs tend toward combinations of both unusual cleavage and unusual mesoderm formation.

Because a mechanism that associates cleavage to mesoderm formation unites two of the main features of the traditional P/D developmental assemblages, it is useful to ask whether other features are correlated as well. One such probable relation is with the origin of celomic space. In classic spiralian with a 4d mesentoblast, the proliferation of mesodermal bands into the blastocoel compartment requires a schizocelic celom; there is no opportunity to capture space from the archenteron. In radialians with mesoderm derivation from endodermal cells along the archenteron wall, enterocely is possible, but schizocely is possible, too, depending on the pattern of mesoderm proliferation. Schizocely is indeed universal among classic spiralian whereas radialians display both types of celom formation, although enterocely appears to be primitive. A shift toward mosaic development may also correlate with cleavage shifts. Radialian cell lines that would have remained totipotent or pluripotent for numbers of cell generations but that are transformed by a novel assemblage of transcription factors owing to cleavage changes may undergo a shift in fate dating from the cleavages that produced them, truncating the period during which their fates were unspecified.

The final general feature that distinguishes protostomy from deuterostomy is the position of the embryonic mouth relative to the blastopore, the condition from which these assemblages of features happen to take their names. Aside from being involved in some general early embryonic reorganization that might arise from changes in early cell fates, there seems to be no particular reason that the location of the mouth should be affected by changes in cleavage. The location of the blastopore is associated with the pattern of gastrulation, and, as Nielsen (32) points out, the blastopore, which forms at the vegetal pole, never represents a functioning mouth. If radialian development is primitive in bilaterians, the growth of the archenteron anteriorly from the posterior vegetal pole automatically produces an anterior mouth, leaving the anus to form in the region of, or actually to be recruited from, the blastopore opening, and most radialians have inherited this pattern. However, especially in nonradialians, there is great variation in the site of formation of mouth and anus (34, 35), presumably related to the growth pattern of the larva or embryo. It seems doubtful that the location of the mouth is functionally related to the earlier developmental features that characterize clades with distinctive cleavage patterns.

There are examples of abnormal shifts in cleavage planes that produce normal adults. In articulate brachiopods, cleavage is radial, usually with the second cleavage plane normal to the first. However, the second cleavage plane is occasionally inclined up to 45° from the axis of the first cleavage (33). The polarity of the resulting cells is not known, and in this case the normal cleavage pattern is immediately restored, and subsequent development is normal (early brachiopod development is probably regulative; see ref. 33). Of course, there have been significant alterations in the early developmental pattern of a great many lineages that have not affected adult morphology to any important extent (28). There must be mechanisms within the cascading hierarchy of developmental regulation to dissociate early ontogenetic changes from later development. The mechanisms responsible for such dissociations are uncertain, but the evidence that they occur is clear. The onset of novel cleavage patterns are not necessarily associated with the rise of new adult body plans, then, but they do seem to be so conservative as to serve as markers for major clades.

The Nature of the Last Common P/D Ancestor

In the latest generation of SSU rRNA trees, all of the bilaterians have been assigned either to the protostomes or the deuterostomes (Fig. 1). It is this arrangement that permits so much harmony between the tree topology and the cleavage patterns, but one result is that it appears that the body plan of the last common P/D ancestor is not represented among living bilaterian phyla. Unfortunately, in attempting to reconstruct this form, it is very difficult to use any of the living phyla as an outgroup; the disparity between the body plans and grades of diploblastic forms and the bilaterians is simply too great to provide much meaningful information. The early metazoan fossil record contains traces that indicate the presence of vermiform bilaterians with roundish bodies at ≈ 565 million years before the present (34, 35). However, the trace makers cannot be placed confidently on the phylogenetic tree, and their morphology, as implied by their activities, cannot be used in place of a formal outgroup. Nevertheless, if it is assumed that features in common among the phyla that are basal to the major clades in Fig. 1 are plesiomorphies, then (taking the diploblastic grade as a starting point and using the fossil record heuristically) it is possible to speculate on the characteristics of the P/D ancestor.

Under these assumptions, the most likely P/D ancestor had isolecithal eggs with holoblastic radial cleavage. The larval mouth formed apically, and thus the larva was deuterostomous. The ancestor was a bilaterian, in that it possessed mesoderm and, in all likelihood, vascular fluid, either in a hemocoel or vessels or both, suggesting that the adult was not flat and was not minute, consistent with the early bilaterian traces. Mesoderm was proliferated from archenteric endoderm, but there was no eucelom that formed a hydrostatic skeleton, although ducts within mesodermal tissues may have been present; if so, some of them may well have arisen by splitting within mesodermal tissues—by schizocely, if such spaces are considered to be celomic. Hydrostatic functions were accommodated by a hemocoel or perhaps by tissues. An organism at this structural grade would be termed a “pseudocelomate.” That pseudocelomates branch deeply in the SSU rRNA tree argues against a celomate ancestry for these forms, as required by the archicelomate group of phylogenetic hypotheses, and suggests that they represent the branches of a major precelomate radiation. This suggestion is consistent with the idea that the P/D ancestor was a pseudocelomate, for it is evidently from forms of that grade that major protostome clades have arisen.

In this scenario, spiral and radial cleavage types may not be adaptive as such but, once established, have been duly inherited, evolving further when associated with marked changes in developmental type, such as from indirect to direct development. It is of course possible that spirality was functionally related to some developmental innovation. Spiral cleavage must have appeared early in eutrochozoan history and persisted within what became a major invertebrate clade, including the highly successful mollusks and annelids and, evidently, flatworms. Celomic cavities evolved independently in deuterostomes and lophotrochozoans, as has been conceded by many authorities (36). It is questionable whether ecdysozoans should be considered as celomates in the same sense that other clades are, but at any rate their intramesodermal spaces were probably evolved independently of the lophotrochozoan celom, which is probably polyphyletic itself. Flatworms are presumably “reduced,” at least from a pseudocelomate condition.

Testing a Tree of Life

Phylogenetic hypotheses at the level of the metazoan phyla are notoriously difficult to disprove, partly because they incorporate so many degrees of freedom. The present hypothesis also

has so many facets that it cannot be completely crushed by a single new fact, but nevertheless it does entail a number of interesting predictions. For example, there are several phyla, chiefly minute forms, whose cleavage is unknown or whose position in the tree is tentative or unstable that will serve as tests of the possible conservatism of cleavage. Two minute phyla that have radial or “modified radial” cleavage are allied to the Lophotrochozoa by SSU rRNA data: the Rotifera and Gastrotricha. If their cleavage is correctly represented and is any guide to their relationships, they should branch from the ancestral eutrochozoans. Such a position is by no means ruled out by available molecular data (6, 9). The Kinorhyncha are placed among the Ecdysozoa both by their molting habit and by SSU rRNA data, which suggests that they are sisters to the radially cleaving priapulids (6). Kinorhynch cleavage is unknown, but if the parsimony of cleavage is to be preserved, and the kinorhynchans remain in that clade, it is predicted to be nonspiral. Perhaps experiments that examine the positioning of regulatory factors relative to cleavage planes will throw light on the evolution of cleavage and its consequences.

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