REVIEW ARTICLE

Fossils, molecules and embryos: new perspectives on the Cambrian explosion

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SUMMARY

The Cambrian explosion is named for the geologically sudden appearance of numerous metazoan body plans (many of living phyla) between about 530 and 520 million years ago, only 1.7% of the duration of the fossil record of animals. Earlier indications of metazoans are found in the Neoproterozic; minute trails suggesting bilaterian activity date from about 600 million years ago. Larger and more elaborate fossil burrows appear near 543 million years ago, the beginning of the Cambrian Period. Evidence of metazoan activity in both trace and body fossils then increased during the 13 million years leading to the explosion. All living phyla may have originated by the end of the explosion. Molecular divergences among lineages leading to phyla record speciation events that have been earlier than the origins of the new body plans, which can arise many tens of millions of years after an initial branching. Various attempts to date those branchings by using molecular clocks have disagreed widely. While the timing of the evolution of the developmental systems of living metazoan body plans is still uncertain, the distribution of *Hox* and other developmental control genes among metazoans indicates that an extensive patterning system was in place prior to the Cambrian. However, it is likely that much genomic repatterning occurred during the Early Cambrian, involving both key control genes and regulators within their downstream cascades, as novel body plans evolved.

Key words: Cambrian explosion, Fossil record, Metazoan phyla, Molecular phylogeny, Developmental evolution, Molecular clock

INTRODUCTION

The explosive appearance near the beginning of the Paleozoic Era of diverse fossils representing disparate animal clades has long been a puzzle to paleontologists and evolutionary biologists. A vigorous paleontological and geological research program to investigate this 'Cambrian explosion' has yielded a host of new fossils and much greater resolution for the timing of events during this extraordinary interval. At the same time, advances in molecular systematics are resolving the topology of the clades involved in this event. Further, the origin of the disparate body plans has become appreciated as an important developmental problem, just as remarkable gains are being made in understanding the molecular underpinnings of pattern formation across the metazoan phyla. The stage is thus set for a deeper understanding of this dramatic evolutionary episode. However, interpretations of these new data have been controversial. Some workers claim that the data indicate that the explosion was of little biological importance, all the significant events in metazoan evolution having occurred much earlier. Others view the data as confirmation of a major evolutionary episode in the establishment of the Phanerozoic biosphere. As we show here, the new data certainly do contribute to understanding the complex temporal structure of early metazoan evolution, but they do not muffle the explosion, which continues to stand out as a major feature in early metazoan history.

THE FOSSIL RECORD

The earliest metazoan fossils

Over the past five years, a firm temporal framework has been established in which the early fossil appearances can be ordered. Relatively abundant, mineralized fossil remains that include living phyla appear in the record at about 530 million years ago (Ma), and for the next 9 or 10 million years, phyla make their appearances in geologically rapid succession (Fig. 1; Bowring et al., 1993). By the end of that time, all but one of the phyla with easily fossilizable skeletons had appeared (the Bryozoa appear in the Early Ordovician, but this group is rich in unmineralized representatives today, and the oldest
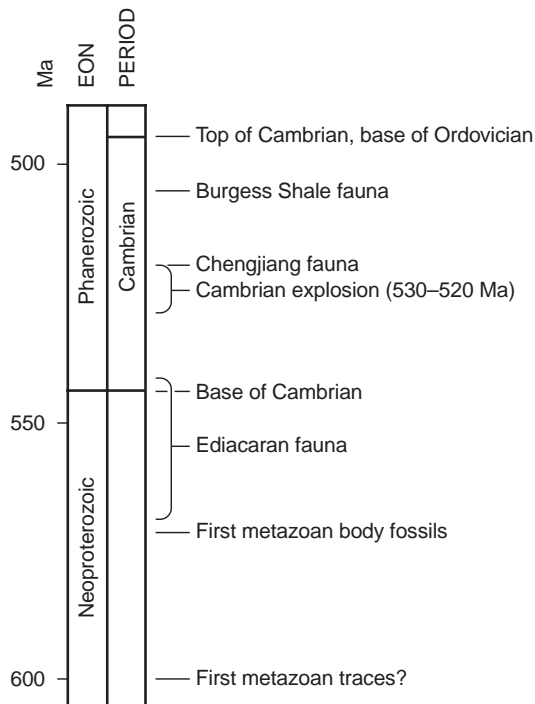
Fig. 1. Time scale and stratigraphic terms for late Neoproterozoic and Cambrian events mentioned in text.

fossils are sufficiently derived to suggest a deeper evolutionary history; P. D. Taylor, personal communication June 1998). It is this relatively abrupt appearance of living phyla that has been dubbed the 'Cambrian explosion.' This event, which lasted less than 2% of the time from the base of the Cambrian to the present day, certainly represents a geologically explosive appearance of animal body plans. Whether or not the explosion coincides with the evolutionary origin of those body plans is still hotly debated.

Records of animals do precede the explosion and they provide evidence of earlier phases of metazoan evolution during late Neoproterozoic time. Among the earliest indications of metazoans are small circular discs from the MacKenzie Mountains, northwestern Canada, that date from between 610 and 590 Ma (Hoffman et al., 1990) or perhaps a bit younger. These discs probably represent pre-bilaterians. Creeping bilaterians, and some cnidarians (sea anemones; Collins et al., 1998), leave shallow trails that can be preserved as 'trace fossils.' Creeping traces are described from rocks that date to about 600 Ma (Brasier and McIlroy, 1998) that may contain faecal strings. If confirmed, these fossils would set a minimum age for the evolution of complete guts, found only in bilaterians. There have been a number of reports of still earlier animal fossils but none is yet confirmed; most have proven either not to represent animals or to have been misdated. The age of trace fossils from India inferred to date to over 1.1 billion years ago (Seilacher et al., 1998) requires confirmation by modern dating techniques.

The remarkable discovery of exquisitely preserved Neoproterozoic eggs and embryos (Xiao et al., 1998) has introduced an entirely new line of evidence into the early history of Neoproterozoic animals. These embryos are the oldest unequivocally metazoan body fossils known; they have been recovered from phosphorite deposits on the Yangtze Platform in southern China that date to 570±20 Ma. The embryos are particularly compelling, exhibiting well-defined cleavage stages (Fig. 2) and blastomere clusters that comprise stereoblastulae (Xiao et al., 1998). The presence of oogenesis and cleavage implies the establishment of adult metazoans with sequestered germ cell lines and differentiated somatic cell lineages. Unfortunately, the identity of the adults cannot be inferred from the embryos, which are interpreted as possible direct developers, and therefore could have been sponges, diploblastic forms, bilaterians or even early members of the enigmatic Ediacaran fauna (Fig. 4G-I; Glaessner 1958), a widespread assemblage of body fossils that appeared in Neoproterozoic rocks about 565 Ma and ranged into the earliest Cambrian (Jensen et al., 1998) and perhaps later (Conway Morris, 1993; Crimes et al., 1995).

The affinities of the Ediacaran body fossils are disputed. Some of these fossils are remarkably large (up to 1 m) but they are preserved only as impressions that commonly lack phylogenetically informative anatomical detail. Many of these forms are either frond-like or discoidal, recalling living cnidarians, yet some share an unusual, quilted structure and have other unique constructional features. This assemblage may well include an extinct 'vendobiont' phylum (Seilacher, 1992; Narbonne et al., 1997; Narbonne, 1998) together with both radiate (Buss and Seilacher, 1994) and bilaterian (Fedonkin and Wagner, 1997) metazoans. However, the relations of any of these fossils to Cambrian bilaterians remains uncertain and awaits further collecting and critical analysis.

Undoubted fossil trails and burrows accompany the early Ediacaran assemblages (Fig. 4F). Although associated body fossils are lacking, these traces are widely believed to record bilaterians (e.g. Crimes, 1974, 1992; Brasier and McIlroy, 1998). The animals that produced these traces are likely to be early members of lineages involved in the Cambrian radiation, but the traces are consistent with the behavior of a variety of vermiform organisms (see Collins et al., 1998), so that body plans cannot be reconstructed in detail. Carefully studied Neoproterozoic trace assemblages differ significantly from those of the Cambrian (Crimes, 1992; Droser et al., 1998). Compared with Early Cambrian traces, for example, Neoproterozoic traces are minute (chiefly near 1 mm though ranging to 5 mm), rare and of low diversity; there are few penetrating burrows and the sediment was not churned ('bioturbated') to any appreciable degree – the traces are chiefly or entirely surface trails (Droser et al., 1998). Cambrian
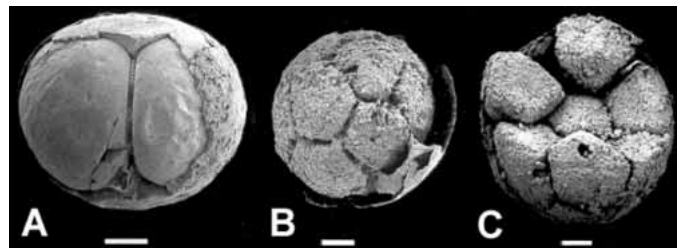

Fig. 2. Phosphatized fossil embryos from the Neoproterozoic, 570±20 million years old. (A) 2-cell stage. (B,C) Later cleavage stages, with internal geometry of cells visible in C. From Xiao et al. (1998).

traces, by contrast, are commonly measured in cms, some are penetrating burrows and they are accompanied by the earliest ichnofabrics – sedimentary textures that result from the activity of organisms (Droser et al., 1998). The earliest mineralized skeletons, which are minute tubes, cones and goblets of uncertain affinity, finally appear in the late Neoproterozoic (Grotzinger et al., 1995).

The Cambrian explosion

An important change in the definition of the Cambrian has often been overlooked. The Cambrian Period, the earliest Period of the Paleozoic Era and of the Phanerozoic Eon (Fig. 1), is now defined to begin with the first occurrence of a particular trace fossil – a type of burrow named *Treptichnus pedum* (formerly *Phycodes*; see Jensen, 1997) – in a section of rocks now exposed in southeastern Newfoundland (Landing and Westrop, 1998) at a horizon dated to about 543 Ma (Bowring et al., 1993). Probably the burrower was a worm of some kind; we are not sure just what it looked like, but whatever animal left that earliest trace in Newfoundland began life in the Proterozoic and died in the Phanerozoic, achieving a special place in history. The newly defined base of the Cambrian considerably lengthens the duration of the Cambrian, adding 13 million years below the diverse skeletonized fossils of the explosion. During this earliest Cambrian time, trace fossils form the chief evidence of metazoan life, harbingers of the coming explosion (Fig. 1). Beginning with *T. pedum*, the traces document an increase in maximum body size and a broadening range of activities (Zhu, 1997; Droser et al., 1998). Mineralized skeletons – mostly small tubes, cones and isolated sclerites – begin to occur in some numbers during the last 3 to 6 million years of this interval, gradually increasing in diversity until the classic explosion began (Brasier et al., 1996; Kaufman et al., 1996).

This emerging portrait of the Cambrian explosion differs significantly from the one that we had just a few years ago. Prior to 1993, the Ediacaran fossils were believed to date to 650 Ma, with a gap of tens of millions of years leading up to a Cambrian explosion, the onset of which was dated at anywhere from 540 to 600 Ma. Now the Ediacaran interval is known to extend to within about 13 million years of the start of the classic explosion, and the intervening rocks bear traces and other evidence that metazoan life was picking up evolutionary momentum.

The diverse assemblages of marine invertebrate skeletons that signal the explosion include the first appearances of body fossils of living bilaterian phyla, represented by numerous, chiefly extinct, classes and orders (see Lipps and Signor, 1992; Bengtson, 1994). Furthermore, an exceptional fossil locality in Yunnan Province, China, has yielded a rich assemblage of soft-bodied organisms, the Chengjiang fauna (Fig. 4A-E), which establishes the presence of many unskeletonized major animal groups, living and

extinct (Hou et al., 1991; Chen and Zhou, 1997). Both the fossil record and molecular phylogenies (Fig. 5) are consistent with the origination of all living animal phyla by the close of this 10 million year explosion interval. Following this time, new appearances of major groups are rare, even though rich faunas abound and some exceptional later faunas, such as from the famous Burgess Shale locality, have been carefully studied. On the face of it, the Cambrian explosion was an impressive evolutionary event from which emerged many of the distinctive body plans that characterize modern groups.

A number of analyses have quantified evolutionary changes in metazoan morphologies independently of detailed taxonomic or phylogenetic data. These studies have found the early Paleozoic to be typified by morphological bursts, in contrast to the more gradual expansions seen later in the histories of major groups (although there was perhaps a secondary pulse following the massive end-Paleozoic extinction; see Foote, 1997 for review). Certainly the Cambrian witnessed a burst; Thomas and Stewart (1995) found that the Middle Cambrian Burgess Shale fauna alone utilized 83% of the possible elements in a multidimensional 'skeleton space' that attempts to capture the important features of skeletal design found in living and extinct animals. Considering that the pre-explosion skeletons exhibited a limited range of form, this result is consistent with the taxonomic evidence of an important Cambrian diversification.

The significance of the explosion has nevertheless been questioned on a number of grounds. For example, Wills et al. (1994) argue that taxonomic data overestimate the morphological magnitude of the Cambrian explosion. By quantifying aspects of

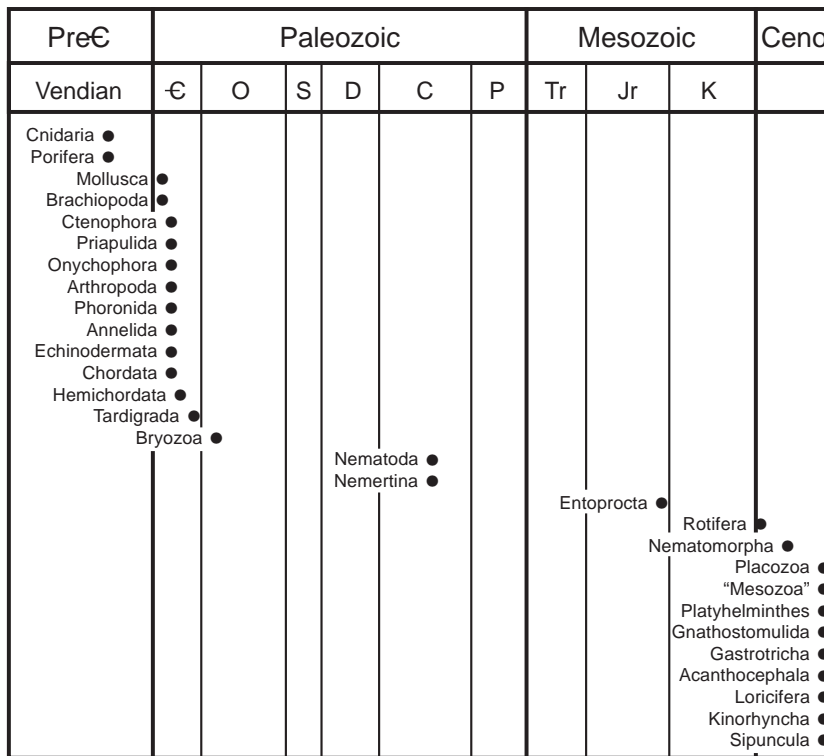| Pre€	Paleozoic						Mesozoic			Ceno
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Fig. 3. The earliest appearance of body fossils of living phyla in geologic time. All well-skeletonized phyla except Bryozoa are known from the Early Cambrian. First records of soft-bodied forms are scattered through time from localities where unusually favorable preservation has occurred. Modified from Valentine (1995).
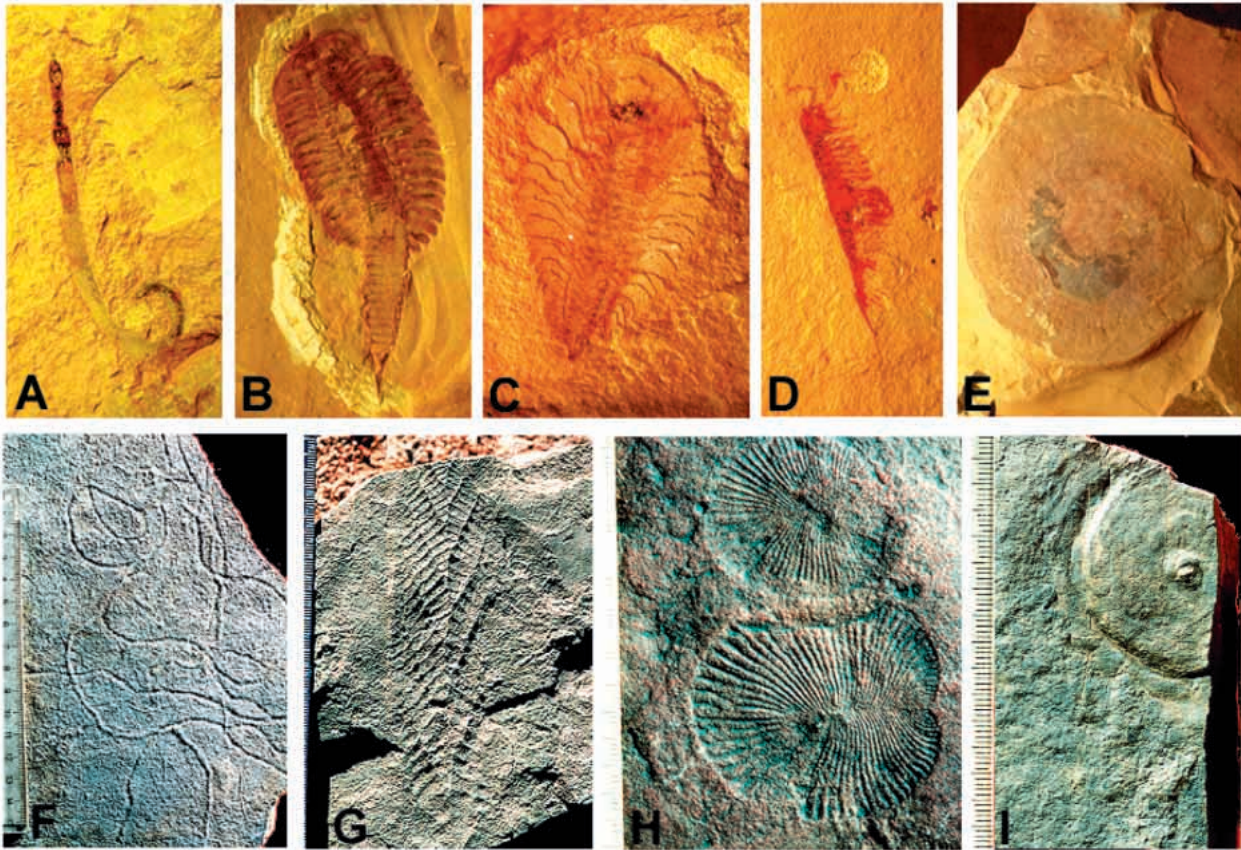
Fig. 4. Representative metazoan fossils from the post-explosion Lower Cambrian (upper row, A-E) and the pre-explosion Neoproterozoic (lower row, F-I). (A). *Maotianshania*, a priapulid. (B) *Fuxianhuia*, probably a rather basal arthropod; (C) *Xandarella*, probably allied to the arachnomorph arthropods. (D) *Jiangfengia*, one of the 'great appendage' arthropods. (E) *Eldonia*, a pelagic form of uncertain affinities; note the curved digestive tract. Forms A-E, from the Chengjiang Formation, Yunnan, China, are between 1.7 and 6 cm in greatest dimension (see Chen and Zhou, 1997; figures courtesy of the US National Museum of Natural History). (F) *Helminthoidichnites*, fossil trails; Blueflower Formation, Mackenzie Mountains, northwestern Canada (from Narbonne and Aitken, 1990). (G). *Charnia masoni*, a quilted organism; Ust Pinega Formation, White Sea, Russia (from Fedonkin, 1981). (H) *Dickinsonia*, possibly a quilted organism; Ediacara member, Rawnsley Quartzite, Australia (from Narbonne, 1998). (I). *Ediacaria*, a benthic cnidarian-like form, basal view to show stem, Sheepbed Formation, Mackenzie Mountains, northwestern Canada (from Narbonne and Aitken, 1990). Body fossils of these forms can be large; *Dickinsonia* may reach 1 m. Photos F-I courtesy of Guy M. Narbonne.

arthropod morphology, they established that the living arthropod fauna is just as disparate morphologically as that of the Cambrian. However, the Recent arthropod fauna has benefitted from a half-billion years or so of subsequent diversification, while the Cambrian fossils are close in time to the initial arthropod radiations (no undisputed arthropods have been found that are older than 530 Ma, the onset of the explosion). Therefore, these data simply reveal that arthropod disparity was achieved early and has not been exceeded even today.

A subdued explosion has also been inferred from a phylogenetic analysis of the morphology of Cambrian and Recent arthropods (Wills et al., 1994; Conway Morris, 1998). In the resulting tree, many bizarre Cambrian forms nest within the arthropods, and thus are held to lie within the morphological limits of the group. These results provide welcome phylogenetic structure to early arthropod evolution, but the tree is constructed on the basis of shared characters, while disparity results in large part from taxon-specific traits that arise as lineages diverge. Thus, tree topology captures neither the rate nor the extent of the divergences.

MOLECULES AND CLOCKS

A new view of metazoan phylogeny

In recent years, molecular evidence has produced a new view of metazoan phylogeny, prompting new analyses of morphological, ultrastructural and developmental characters. The usual metazoan phylogeny placed sponges at the base, followed by cnidarians, then flatworms, and then a number of acoelomates and pseudocoelomates (including nematodes), sometimes grouped as 'aschelminths' or 'paracoelomates'. This sequence of taxa was thought to represent increasingly complex grades, often depicted as the trunk of an evolutionary tree. The trunk then underwent a major divergence that established the deuterostome and protostome branches of the 'higher metazoans.' Lophophorates (brachiopods, phoronids and bryozoans) were sometimes placed in one, sometimes in the other, and sometimes in between those branches. The new data, primarily from 18S rRNA but more recently from additional molecules, indicate a very different configuration (Fig. 5). Although resolution of the branching pattern within

the major groups has been a challenge (though this difficulty is not in itself convincing evidence for a rapid evolutionary burst; see Abouheif et al., 1998), the new basic framework appears robust. Some changes should be expected in the placement of groups within the framework, especially groups wherein relatively few taxa have been sampled.

Four major metazoan alliances can now be identified: the pre-bilaterians (a paraphyletic grouping that includes sponges, ctenophores, cnidarians, and placozoans) and three bilaterian clades consisting of the deuterostomes, ecdysozoans and lophotrochozoans (Aguinaldo et al., 1997; Giribet and Ribera, 1998; Valentine, 1998; Balavoine, 1998). The deuterostome branch (echinoderms, hemichordates and chordates) has been pared by the removal of a number of phyla now assigned elsewhere. The protostome branch has been subdivided into two major branches, each with more phyla than the deuterostomes. The ecdysozoans consist of molting animals in a clade that includes the arthropods, tardigrades, onychophorans and also some 'aschelminths' – priapulids, nematodes and their allies (Aguinaldo et al., 1997). The lophotrochozoans include the classic spiralians such as molluscs, annelids and their allies (the Eutrochozoa: Ghiselin, 1988; Eernisse et al., 1992), some aschelminth phyla, the lophophorates (Halanych et al., 1995; Cohen et al., 1998) and rhabdocoel flatworms (Halanych et al., 1995; Balavoine, 1997; Carranza et al., 1997). The aschelminths have thus been split into at least two groups, one within the Ecdysozoa and the other within the Lophotrochozoa (see Winnepenninckx et al., 1995; Wallace et al., 1996). A number of important problems remain regarding the relationships of metazoan phyla, especially of the former aschelminth phyla and
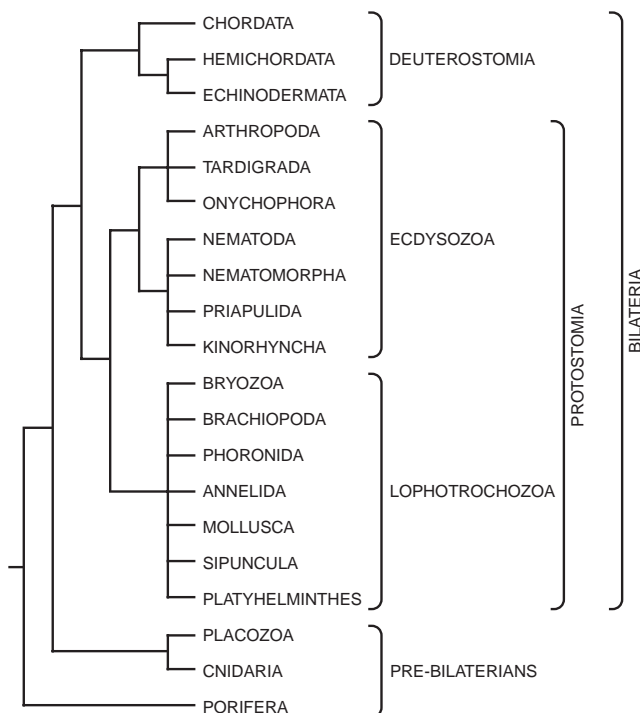
of some flatworms (see Carranza et al., 1997, and Campos et al., 1998). A few taxa with markedly diverged 18S rRNA sequences, such as acoel flatworms, continue to defy confident assignment to any of the alliances. Finally, a large morphologic gap remains between the pre-bilaterians and the living bilaterians, a gap that is also apparent in 18S rRNA (Wainright et al., 1993) and *Hox* gene data (Schierwater and Kuhn, 1998). A fifth, paraphyletic alliance, chiefly of primitive bilaterians, probably fell within this gap; those taxa are presumably extinct, although some living forms now assigned to flatworms may possibly belong there.

Of particular interest to developmental biologists in this new view of metazoan phylogeny are the shifts of once-basal bilaterian taxa to positions higher on the tree, with the nematodes now in the Ecdysozoa and most, perhaps all, flatworms in the Lophotrochozoa. The topologies of the relationships within the alliances remain less certain. The difficulties probably stem from the necessary use of slowly evolving molecules, such as 18S rRNA, to investigate events of over half a billion years ago. For lineages that branched in rapid succession, there may not have been enough time between branchings for informative molecular differences to accumulate, or to be preserved over more than half a billion years of subsequent evolution. Unusual substitution rates or patterns can also obscure the phylogenetic signal in the molecules. Additional molecules are being brought under study, however, and promise to provide increasing resolution of metazoan relationships.

The present molecular phylogeny suggests some key points in metazoan history that are marked by interesting changes in regulatory genes, despite the phylogenetic uncertainties and the limitations of PCR-based studies (Fig. 6). The ancestral ecdysozoan had at least six *Hox* genes, and thus the small and aberrant four-gene *Hox* cluster of nematodes has evidently been reduced and modified from a larger cluster. Other evolutionary changes in *Hox* gene assemblages have been
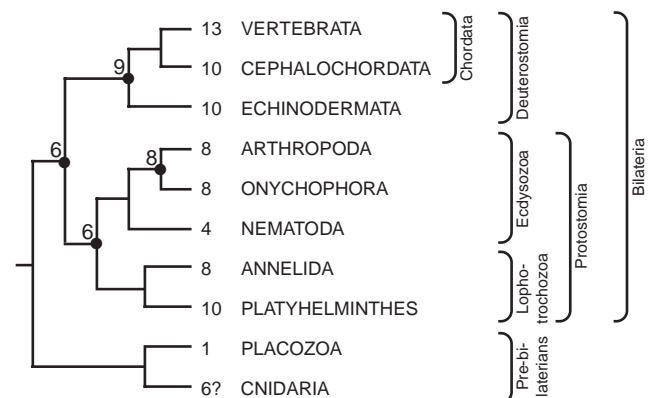
Fig. 5. A metazoan phylogeny based chiefly on SSU rRNA data and on early developmental features. The pre-bilaterians may not be sisters. (From Halanych et al., 1995, Aguinaldo et al., 1997, and various sources cited in Valentine, 1998).

Fig. 6. Numbers of *Hox* genes found in some metazoan phyla, and their implications for the minimum numbers of *Hox* genes at some ancestral bilaterian nodes in the phylogenetic tree based on their orthologous relations; both gains and losses of *Hox* genes are implied. Working up the tree, additional genes must be paralogs, while fewer genes indicate loss of orthologs. Exceptions are the pre-bilaterians, which may not be sisters, and whose *Hox* genes are not known to be orthologs of any given bilaterian *Hox* genes. From data in Kenyon and Wang (1991); Garcia-Fernandez and Holland (1994); Krumlauf (1994); Balavoine and Telford (1995); Grenier et al. (1997); Irvine et al. (1997); Mito and Endo (1997); Schierwater and Kuhn (1998); and references therein.

identified on the ecdysozoan tree; for example, a paralogous split of an ancestral *Hox* gene produced the sister genes *Ubx* and *abdA* in the onychophoran/arthropod clade (Grenier et al., 1997). Important evolutionary changes have clearly occurred in *Hox* genes, both losses and gains, among most other phyla; indeed the usual (but not universal) case is that each phylum has a distinctive *Hox* gene suite. It is possible that many of those changes were intimately associated with the evolution of novel body plans and, therefore, were implicated in the Cambrian explosion. The presence of the large *Hox* array in the protostome-deuterostome ancestor, well before the explosion, indicates that the simple assembly of a suite of *Hox* genes was not sufficient to trigger a metazoan radiation. When the radiation came, the repatternings of body plans seem to have been associated with modifications in the *Hox* assemblage, and with many regulatory changes downstream of those genes as well (see Gellon and McGinnis, 1998).

The involvement in the Cambrian explosion of many of the other developmental control genes that are conserved across broad phylogenetic distances seems certain and it will be fascinating as the details of this history are worked out. However, functional roles have not always been conserved; so for example, *Pax-6* function in eyes of both deuterostomes and ecdysozoans (Quiring et al., 1994; Halder et al., 1995) does not require that their last common ancestor had eyes (see Erwin, 1998). Working out of the body plans of extinct, ancestral taxa will require input from fossils and phylogenetics.

When did major lineages diverge?

The lineages leading to extant body plans could have diverged well before the acquisition of the distinctive characters that now differentiate them. Thus, the dating of divergence times must be estimated indirectly. Most such estimates use molecular clocks, which assume that mutations accumulate linearly over time. The average rate of sequence change within a molecule is calibrated against well-dated branching events from the Phanerozoic fossil record. This rate of change is then extrapolated to estimate the time of divergence of phyla. Unfortunately, molecular evolution has not been clock-like: rates of change in DNA sequences vary widely among taxa (for a general evaluation see Hillis et al., 1996). For example, rDNA rates appear to vary by a factor of three for different clades of sea urchins (Smith et al., 1992), a factor of five for different clades of seastars (Lafay et al., 1995) and by up to two orders of magnitude for different clades of foraminifera (Pawlowski et al., 1997). Different genes in the same clades can evolve at radically different rates (Ayala, 1997), different parts of genes evolve at different rates and, most importantly, rates within clades have changed over time (Goodman et al., 1987; Lake, 1990; Ayala, 1997). A variety of corrections has been proposed in attempts to deal with these and other rate inconsistencies, and molecular clock estimates have been applied to many major events in metazoan history (for example Runnegar, 1982; Wray et al., 1996; Nikoh et al., 1997; Ayala et al., 1998; Bromham et al., 1998).

Molecular clock dates yield divergence times of metazoan clades significantly earlier than the Cambrian explosion. The oldest dates are implied by Bromham et al. (1998); their data contain outlying points but tend to suggest a protostome-deuterostome split before 1,500 Ma. Wray et al. (1996) applied a molecular clock to the evolutionary rates of six protein-coding genes and the 18S rRNA gene. Their techniques yielded ages of about 1,200 Ma for the protostome-deuterostome split and 1,000 Ma for the echinoderm-chordate divergence. These estimates have been criticised on several grounds (e. g. Nikoh et al., 1997; Ayala et al., 1998). Nikoh et al. (1997) used two molecules believed to be reasonably clock-like and found considerably younger ages for the branching of sponges from other metazoans (940 Ma), and for the cephalochordate-vertebrate split (near 700 Ma, with the protostome-deuterostome split occurring shortly before). Ayala et al. (1998) examined 18 protein-coding loci among a number of lineages, discarding data that did not display clock-like behavior. The age of the protostome-deuterostome divergence was estimated at about 670 Ma and of the echinoderm-chordate divergence at about 600 Ma. Clearly, the accuracy of molecular clocks is still problematical, at least for phylum divergences, for the estimates vary by some 800 million years depending on the techniques or molecules used.

Two general issues are fundamental to the interpretation of molecular clock dates. First, even accurate clocks cannot pinpoint the origin of body plans or other events in the evolution of development; they can provide only maximum estimates. At the time of a molecular divergence, the newly separated lineages are represented by sister species with identical body plans; the species differences will not be related to the larger evolutionary transformations of interest here (Valentine et al., 1996, Erwin et al., 1997; Valentine and Hamilton, 1997). The molecular clock has begun ticking, in its irregular fashion, but millions or tens of millions of years may pass before the accumulation of the derived features that characterize the descendant body plans. Conversely, the fossil record tracks those novel morphological features, or even their functions, and provides minimum estimates on their origins, but is silent on the actual nodes indicated by the molecular divergences. Therefore, substitution rates within molecules will appear high when tied to the appearances of body plans in the fossil record, but low when tied to the nodes of lineage divergences. Thus, even if the older molecular clock dates for the divergence of animal phyla were verified, it would in no way diminish the remarkable morphological evolution recorded during the Early Cambrian. Such early dates would, however, indicate a deep decoupling of the branching of major clades from the acquisition of body plans.

A second issue involves dating Neoproterozoic nodes by a molecular clock calibrated in the Phanerozoic. The extensive morphological evolution associated with the Cambrian explosion may have been accompanied by enhanced rates of substitution, which would cause their divergences to appear to be earlier than they were, when calibrated by a Phanerozoic clock. Such bursts of increased substitution have already been documented within 18S rRNA genes for various elements of metazoan body plans (Cavalier-Smith et al., 1996) and for the basal radiation of Dipteran clades (Friedrich and Tautz, 1997), though they may be less likely for protein-coding genes. When interpretations of rates involve extrapolations beyond calibration points, adequate rate testing becomes crucial. Optimally, tests should be based on the clades under study and should involve outgroup references to close phylogenetic neighbors. However, it is not clear that molecular clock dates can ever be applied reliably to such geologically remote events as Neoproterozoic branchings within the Metazoa.

ANCESTRAL BODY PLANS OF THE CAMBRIAN FAUNA

Some authors have suggested that all of the body plans that appear during the Cambrian explosion first evolved as minute, interstitial or planktonic forms well before their appearance in the fossil record (e.g. Fortey et al., 1996; Vermeij, 1996; Cooper and Fortey, 1998). The body plans of explosion taxa are, however, generally unsuited for interstitial life unless significantly modified. Among macrofaunal lineages that have been miniaturized, many body plan features have become reduced or obsolete, because they are encumberances or are simply not needed (Swedmark, 1964; Higgins and Thiel, 1988). As for planktonic progenitors, such features as the arthropod limb, the annelid parapodium and the molluscan foot are not adaptations for minute organisms in the pelagic realm, but clearly evolved for life in benthic environments. Such adult features persist in modified guises in many clades that have subsequently invaded the pelagic realm. The body plans of all of the major explosion phyla except the chordates are adapted for life within the macrobenthic realm, where they first appear during the Cambrian.

One proposal that small-bodied phyla preceded the larger benthic bilaterians has been suggested on the basis of a developmental scenario. In a number of bilaterian phyla, many elements of adult bodies develop from cells set aside from involvement in the larval body, such as imaginal disks in arthropods. Set-aside cells are arguably homologous among some phyla, leading to the hypothesis that the common ancestors of these phyla had body plans similar to an ancestor of their larvae, and the more complex present phyla arose through the evolution of specifications for adult body plans within set-aside cells (Davidson et al., 1995; Peterson et al., 1997). The body plans of the minute ancestors would resemble those of minute acoelomates and pseudocoelomates. The hypothesized body-size increases are consistent with the Cambrian fossil size record, but the scenario is not consistent with present phylogenies, which indicate that minute pseudocoelomate and acoelomate phyla arose after the divergence of protostomes and deuterostomes and probably after that of ecdysozoans and lophotrochozoans. If living phyla in those different alliances have descended from minute forms, the rise of their body plans would be independent and the homology of set-aside cells evolved in a number of independent lineages is brought into question.

SUMMARY AND PROSPECTS

In sum, the fossil record: (1) indicates that metazoans certainly originated significantly earlier than 570 Ma and probably earlier than 600 Ma, but is otherwise silent on this point, (2) suggests that minute bilaterians were present by at least 565 Ma and probably earlier, (3) indicates that larger bilaterians were present by 543 Ma, and (4) suggests that a number of the body plans that today characterize major taxa first appear during or 'shortly' before the interval from about 530 to 520 Ma, when the range of activities of benthic organisms increased markedly. Beyond this information, interpretations of the events in early metazoan history are based on the topology of the phylogenetic tree, the pathways of morphological change implied by the fossils and by the constraints imposed by our understanding of evolutionary processes.

While the time of origin of the Metazoa is not known, an age of 700 Ma or less would not conflict with the evidence now at hand, though it may have been significantly earlier. Is 170 million years long enough for the evolution of the Cambrian fauna from the earliest animals? Clearly, much of body-plan evolution was accomplished by changes in patterns of gene expression. Many genes that mediate the development of disparate phyla are conserved after over half a billion years of independent evolution in lineages that have evolved independent architectures. Gene regulatory elements were probably the most important actors in this process. The rapidity of this sort of evolution has not been formally evaluated, but the use and re-use of established signaling pathways (Gonzalez-Crespo and Levine, 1994) and other regulatory cascades (Warren et al., 1994) seem likely to provide evolutionary shortcuts in the production of novel morphologies. We have every reason to believe that the pace of evolution as suggested by plausible interpretations of the fossil record could easily be achieved.

Understanding the events surrounding the Cambrian explosion remains a major challenge. The stream of extraordinary fossil discoveries has illuminated new suites of characters for phylogenetic analysis and has generated new insights into the breadth of the morphologic innovations associated with the explosion, which are being evaluated by newly developed quantitative techniques. As increasing numbers of precise dates become available for the fossiliferous horizons, the rates of morphologic change can be determined with greater rigor (Bowring and Erwin 1998). The advent of durable skeletons is particularly notable, both in enhancing the visibility of the explosion and because the origins of so many classes of marine invertebrates, including most within Mollusca, Echinodermata, Brachiopoda and Arthropoda, involve the exploration of design opportunities opened by the possession of hard skeletons (see Bambach, 1985; Valentine and Erwin, 1987). However, non-skeletal evidence also attests to the reality of the Cambrian explosion; the onset and the breadth of this event is recorded by numerous soft-bodied forms, by trace fossils, and even by the microplankton (Vidal, 1997).

Several factors are prerequisites for the explosion, necessary but not sufficient. Owing to the new metazoan phylogeny it is possible to reconstruct minimum *Hox* gene arrays, and they suggest that much of the basic gene-regulatory machinery that is required to set up metazoan body plans was in place significantly before the explosion (Valentine et al., 1996). Oxygen concentration must have risen to levels at which active macrobenthic animals can be supported; however, appropriate levels may have been reached over 100 million years before the explosion (Knoll, 1996). Climate fluctuations have also been implicated (Hoffman et al., 1998), although direct causal links are difficult to specify and even harder to test. The effects of competition, predation, and other ecological interactions launched by the evolution of new sorts of organisms, are similarly difficult to evaluate and remain important unresolved questions.

Among the chief challenges of the Cambrian explosion are to understand the evolution of the developmental systems required to produce the novel and disparate body plans, to find the reason(s) that so many different lineages produced novelties in such concert, and to understand why the explosion

was such a brief episode rather than continuing as an ongoing flood of major novelties. Surely one of the pressing questions in evolutionary developmental biology is under what circumstances highly conserved control sequences are also functionally conserved (Shubin et al., 1997). Comparative anatomy of extant organisms is not sufficient on its own to infer the morphology of extinct body plans. The new phylogenetic trees, and fresh perspectives on paleontological evidence, are providing a much-needed basis for establishing patterns of conservation and innovation in developmental regulation across body plans, and for more rigorously constraining the possible ancestral forms. Understanding the differences and similarities in how developmental systems, inherited from a common ancestor, were redeployed in the three great branches of bilaterian evolution, each representing an independent experiment in body plan diversification, will be a major step in understanding the interplay of development and evolution.

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