Fundamentals of Life

Chapter II.3

Early diversification of organisms in the fossil record

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Among the recent main contributions of palaeontology to the present understanding of the course of evolution are discoveries of fossil organisms transitional between basically different major groups of the living world. Thus, an almost complete transition from round worms to arthropods was documented with Cambrian fossils. This anticipated subsequently assembled molecular phylogenetic evidence that the ability of shedding out the chitinous cuticle is a very ancient trait defining the great superphylum Ecdysozoa. Another idea based on molecular evidence, that pelagic larvae are secondary adaptations in marine animals, the Lophotrochozoa, has found support in the Early Cambrian embryos with direct development within the egg covers. A surprising implication of the recent findings of chordates in the Early Cambrian is that these may represent the basalmost deuterostomes which possibly share some archaic aspects of their body with the nemerteans, Ediacarian dipleurozoans, and ctenophores. The latter were in the Cambrian more anatomically complex and diverse than today, and may have derived from the sedentary Ediacarian petalonameans. The main weakness of the available fossil evidence is its inability to document the common roots of animals and plants within the protists. Various Precambrian unicellular flagellates represented by cysts and cell scales, as well as multicellular algae, await an evolutionary interpretation. The cyanobacteria range in the fossil record prior to the formation of oxygen-rich atmosphere and molecular evidence suggests that they may be ancestral to all extant prokaryotes. This would mean that most of the bacterial evolution was a morphological simplification which followed their adaptation to heterotrophic and parasitic modes of life; also thermophily of the archaeobacteria being secondary.

1. Introduction

Life is an ability to evolve. In such sense it is not just a regular physical or chemical phenomenon but a historical process. The process which is extremely extended in time – which proceeds in the geological time scale. There is thus hardly any reasonable way to understand the present complexity and diversity of life without referring to its history. We must know the real course of evolution to know what is life.

The way of reasoning by retrodiction, that is back in time, is the most powerful method of inference on the course of evolution. This applies both to anatomical and physiological evidence.[1] Retrodiction (postdiction) in palaeontology enables falsification of contradictory theories on ancestor–descendant relationship.[2] This method of studying evolution is not only methodologically preferable. It is practical, because in palaeontology (similarly as in archaeology) we base our knowledge mostly on observations of the present world. With an increasing depth of our search in the past, the evidence becomes more and more fragmentary. For these reasons, in this review, the phylogenetic tree will be traced from its top towards roots and the course of the evolution is described in the direction opposite to that generally used: back in time. I will start the story from tracing the most primitive of our known animal ancestors in the fossil record. Then a review of the oldest evolutionary events in other great divisions of the living world will follow, either palaeontologically documented or only hypothesised on the molecular basis. The general attitude will be constructively critical, that is, I will try to arrange the available evidence in a possibly consistent scheme of the evolutionary tree instead of exposing its unavoidably chaotic and equivocal nature.

2. Early diversification of animal phyla

The progressing re-evaluation of the Cambrian fossil record of multicellular animals, the Metazoa, has recently resulted in a rather fundamental rearrangement of the metazoan phylogenetic tree. It was initiated by the revision of fossil worms from the Middle Cambrian Burgess shale by Conway Morris.[3] Contrary to earlier expectations, it appeared that the annelids, allegedly ancestral to all segmented invertebrates, were virtually missing in the Cambrian but, instead, priapulid nemathelminthes and (as it later appeared) lobopodians, both of controversial zoological affinities, were very diverse. The subsequent discovery of the Early Cambrian Chengjiang fauna made this picture even sharper. Perhaps the most unexpected aspect of the oldest lobopodian, the Baltic Early Cambrian *Xenusion*, was that its fossils represent strongly dorsally sclerotized exuvia three-dimensionally preserved in the sandstone.[4] An anatomical simplicity of *Xenusion*, contrasting with its large size, and a similarity to the Cambrian round worms led to the idea that the xenusiid lobopodians originated from a nemathelminthan ancestor by changing its mode of life from an infaunal to epifaunal. The hydraulic mode of locomotion, with retrograde peristaltic wave moving along the body, was modified into a simple gait with serially arranged appendages adapted to the walking function.[4] The xenusians were thus ancestral not only to the extant onychophorans and tardigrades but also to arthropods. The proximity of the xenusians to the arthropods was subsequently confirmed by finding an almost complete series of transitional forms[5] which enables connection of the Xenusia with the anomalocaridids and then with typical arthropods *(Figure 1)*. This means that the ability to secrete periodically shed out cuticle is much more ancient than the origin of the arthropods. It is shared by several phyla forming a great clade. Independently of the palaeontological evidence, the monophyly of the clade, named Ecdysozoa, was documented with molecular data.[6]

The ancestor of the Ecdysozoa was a priapulid-like worm with an annulated body covered with cuticle. It lacked pelagic larva and the development was direct, within the egg covers. Embryos of this kind are known from the Early Cambrian.[7] The Recent priapuloids have preserved this anatomy with little modification.[8]

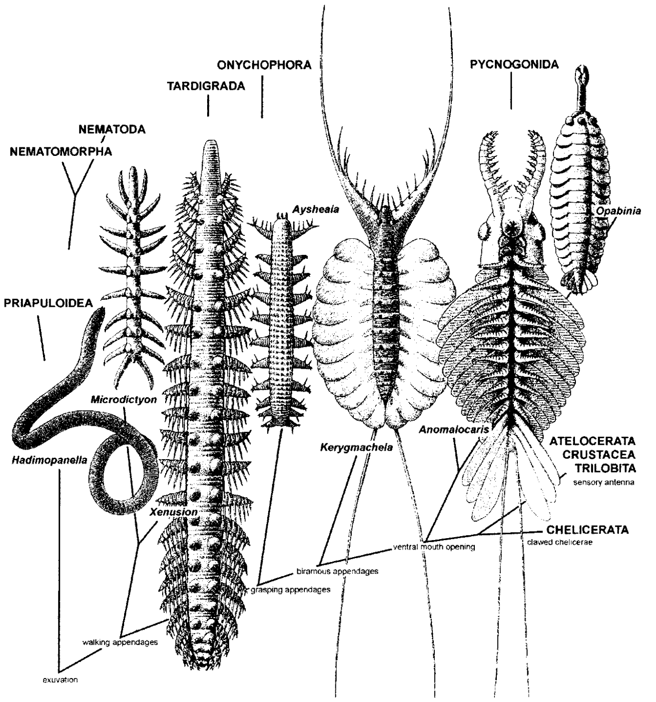

Figure 1. Relationships of the Cambrian ecdysozoans. Note an almost smooth transition between the xenusiid ancestors of the Recent lobopodians (*Xenusion, Aysheaia*) and the anomalocaridid ancestors of the Arthropoda (*Kerygmachela, Anomalocaris*). A possibility which emerges from this diagram (a modification of that in Dzik & Krumbiegel 1989) is that the compound eye originated before separation of the clade of arthropods

This moves us to those great subdivisions of the animal kingdom which now appear to be recognisable not only on the developmental, but also molecular basis. The classic molecular method of establishing relationships by analysing similarities in the nucleotide or amino acid sequences have been powerfully supplemented by a more precise analysis of homologous genes. In fact, much of the recent advance in evolutionary biology[9] is owed to identification of a widespread homology of the homeotic genes, that is genes containing a highly conserved sequence of about 180 nucleotide pairs (homeobox). These nucleotide triplets code 60 amino acids (homeodomain) in a corresponding protein. The homeotic genes are regulatory genes and the homeodomain helices work by binding the protein to the DNA strands.[10] They are responsible for developmental processes and this is why their mutations result in strange modifications of organs. This impressed already in 1894 the founder of genetics, William Bateson, who named such abnormalities the homeotic transformations.

Thus, rather unexpectedly, the palaeontological evidence, molecular phylogenies, and data of evolutionary developmental biology have met in a new unorthodox view on the early phylogeny of organisms. Subdivision of the higher Metazoa into the three main clades: the Deuterostomia, Lophotrochozoa, and Ecdysozoa seems now well established.[11] Of them, perhaps the most troublesome from the palaeontological point of view is the pattern of relationships within the lophotrochozoan clade. For zoologists, it is especially difficult to accept separation of the annelids from the ecdysozoan clade.[12] The polychaete cuticle shows a complex pattern of layered collagen cords which is virtually identical with that known in the Recent nematomorph and in the Early Palaeozoic palaeoscolecid nemathelminthan.[13] A convergent origin of this similarity is possible but not very likely. The body plan of the earliest, Middle Cambrian polychaetes seems to be derivable from that of the hypothetical nemathelminthan ancestor of the xenusian lobopodians.[4] At least on anatomical and palaeontological grounds the annelids seem to occupy a transitional position between the ecdysozoan and lophotrochozoan clades.

The Lophotrochozoa includes the protostomian invertebrates which have a trochophora type pelagic larva, probably invented only once in the evolution.[14] One may envision a common ancestor of the lophophorates and mollusc with a dorsal sclerotized cuticular structure, as was the case with *Xenusion*, but with clearly delimited margins, secreted during whole life and not shed out. Perhaps the mollusc ancestor had chosen the direction of its evolution opposite to that of the lobopodians. The molluscs mastered a peristaltic mode of locomotion, which resulted in a profound differentiation of the dorsal and ventral sides of the body: the venter lost completely its cuticular cover, whereas the dorsum developed a rigid protective shell, secreted marginally by an incipient mantle. Such an ancestral mollusc has not been yet identified in the fossil record, although there are various tracks of Ediacarian age left by peristaltically crawling animals[15] and even possible radular marks.[16]

The shell secreting mantle is shared by both molluscs and lophophorates. Another very specific structure which is common for at least the annelids and the lophophorates are setae. The oldest known lophophorates had a cuticular skeleton of either circular lobate (Eldonioidea) or bivalved (Brachiopoda) shape. In the Bryozoa, non-colonial ancestors of which were definitely represented in the Cambrian, the marginally produced skeleton was tubular.[17] The origin of the lophophorates would thus be connected with their adaptation to the sessile mode of life with their mouth dorsally oriented and equipped with a gradually more and more complex tentaculate filtrating apparatus. Perhaps the Eldonioidea are closest to the ancestral status.[18,19] It has to be noted however, that the very concept of Lophotrochozoa is questionable on embryological grounds and the lophophorates, with their enterocoelic[20] coelom, are believed to be related rather to the Deuterostomia.[21]

We humans, together with other chordates belong to the deuterostomian clade of the Metazoa. Our oldest direct ancestor identified with a reasonable degree of confidence is the Early Cambrian *Yunnanozoon*.[22] It is represented by numerous excellently preserved fossils, labelled with various taxonomic names, from two localities of the Chengjang fauna in southern China. The recently discovered nearby Haikou locality offers even more precise replication of details, enabling restoration i.a. the blood circulation system.[23] Some obliquely compressed in the sediment specimens most likely belonging to *Yunnanozoon* have been described as vertebrates.[24] *Yunnanozoon* is a few centimetres long laterally compressed animal with a dorsally located metameric probably muscular unit. It was identified as a chordate based on the presence of a fusiform notochord in the centre (documented by both a black staining of its contour and an increased resistance to compression in variously preserved specimens) and a pharyngeal cavity below, with seven branchial arches.[22b]

The most phylogenetically important and controversial aspect of the body plan of *Yunnanozoon* is the occurrence of the probable muscular unit above the notochord. Its relatively dark stain indicates a high organic matter content and is suggestive of a thick basement membrane and muscular walls being present there. This would fit rather well the original chambered organisation of the axial musculature in the chordates inferred from the embryology of Recent *Amphioxus*. In fact, Bjerring[25], in his attempt to restore the body plan of an ancestral craniate, presented an organism with dorsally originating metameric muscular chambers. Somewhat surprisingly, the hypothetical ancestor of Bjerring[25] with its dorsoventrally compressed oval body bearing the dorsal metameric chambered unit and intestine with lateral caeca (subsequently transformed into the branchial openings), exactly fits the body plan of the Late Vendian (Ediacarian) dipleurozoan *Dickinsonia*.[26]

Based on a different way of inference from the anatomy of possibly the oldest chordates equipped with the mineral skeleton in their mouth apparatuses *Dickinsonia* was independently proposed to be a relative of *Yunnanozoon*.[22,27] Another unexpected conclusion from that search for the ancestry of the mineral skeleton was that the most primitive palaeontologically documented chordate phosphatic skeleton closely resembles stylets arming the protrusible proboscis of the nemerteans. Most interestingly, the nemertean proboscis internal cavity is the only dorsally located coelomic chamber known in non-chordate invertebrates. It is thus tempting to suggest a parallel homology between chordates and nemerteans in respect to both their dorsal coelomic cavities and oral denticles. *Dickinsonia*, with its intestine bearing serial caeca (as the Nemertini) and metameric dorsal coelomic cavity chambers (as the Chordata) may then serve as a connecting link between these phyla.[27] In *Yunnanozoon*, the dorsal metameric unit tends to separate from the less resistant rest of the body while decaying,[28] similarly as the dorsal 'quilt'of *Dickinsonia*.[27] The evolution from a *Dickinsonia*-like common ancestor would then proceed divergently: in one direction towards an increasing anatomical complexity of the chordates, whereas in the other, nemertean–platyhelminthan lineage, a secondary simplification would dominate *(Figure 2)*. Probably already within the clade of chordates a fundamental change in the mode of locomotion took place. *Yunnanozoon*, like more advanced chordates, was apparently propelled by lateral waving of the body.

This is just a hypothesis which awaits testing by more palaeontological evidence, but it offers explanations for several anatomical and developmental traits, which are otherwise difficult to derive from purely zoological evidence. For instance, the dorsal location of the locomotory apparatus of chordates, from its origin based on the dorsal hydraulic skeleton explains the dorsal location of the main neural system strands, in opposition to the majority of invertebrates, in which the locomotory apparatus is ventrally located. There is thus no need to assume a dorsoventral axis reversal in the evolution of deuterostomes.[29] Also the origin of the serial branchial slits can be explained as being derived from the original intestinal caeca. The chordates have preserved some very ancient anatomical traits which make them more primitive than most of the invertebrates, for instance the innervation of muscular cells with their protrusions (similarly as olfactory sensory cells). The evolutionary separation of the chordates from the nemertean–platyhelminthan stock was definitely an ancient event, it is thus unlikely to find a good support in the zoological evidence.

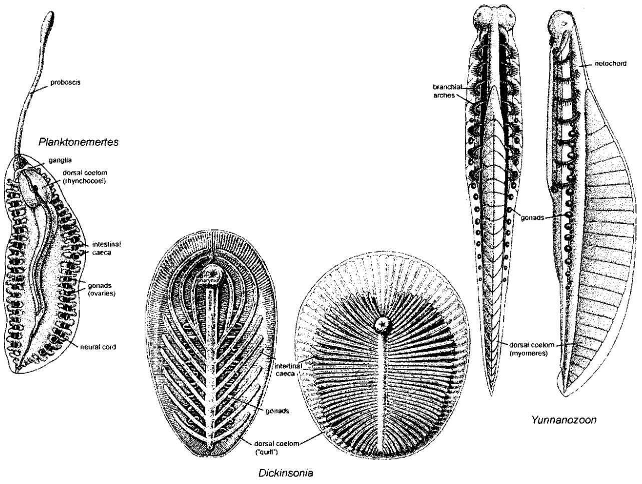

Figure 2. Comparison of the Vendian dipleurozoan body plan (exemplified by *Dickinsonia*; based on Dzik 2000 and Dzik & Ivantsov, in preparation) with those of the Recent nemertean *Plauktonemertes* and the Early Cambrian chordate *Yunnanozoon*. Note that the chordate branchial arches separate branchial slits which derived from the intestinal caeca (e.g., Bjerring 1994). The proboscis of the nemerteans is a hydraulically evertible extension of the unpaired dorsal coelomic cavity (retracted by a muscle constriction) innervated with a dorsal nerve cord. Despite possible anatomical homologies there is a rather fundamental difference in locomotory mechanisms between *Yunnanozoan* and *Dickinsonia* that requires transitional stages which have not been yet identified

Turbeville and Ruppert[30] have already suggested that the nemerteans originated from anatomically complex coelomates. Their idea may extend also to the Turbellaria and other flatworms, traditionally believed to be intermediate between the coelenterates and the nemerteans. This is supported also by studies on the sperm morphology in the invertebrates. The Nemertodermatidae[31] are anatomically closest to the Nemertini. The origin of the Turbellaria from a common ancestor with the Recent Nemertini is likely, a deep secondary simplification being probably involved in this process. This explains also why flatworms are missing in the fossil record. The molecular evidence suggests a secondary loss of coelom at the origin of flatworms and places them in proximity to the nemerteans[11] and annelids.[32]

Within the Ediacarian faunas there are several forms which may be related to the common roots of chordates and the nemertean–platyhelminthan stock.[33] The most surprising result of this palaeontologically-based reasoning is that chordates are probably the most primitive of all the deuterostomians. That echinoderms are derived in respect to them is easy to accept, but this seems to refer also to hemichordates,[22] traditionally believed to be a link with the invertebrate world. Perhaps even more unexpected is a possible connection between the chordate ancestors similar to *Dickinsonia* and the benthic ancestors of the ctenophores.

Recent findings show that the ctenophores were surprisingly diverse in the Cambrian *(Figure 3)*. These were organisms of a more robust appearance than their Recent relatives, with numerous comb organs usually arranged around the body axis (as today) but pinnately in the most complex of them, in *Fasciculus*.[34] Similarly as in the Recent ctenophores, the Cambrian comb organs were fusiform in shape with their flagellate cells connected into regularly transverse rows. In the Middle Cambrian Burgess shale, the frond-like organism *Thaumaptilon*[35] had similar in shape pinnately arranged fusiform units but these units do not show any transverse striation, being covered with irregularly distributed dark spots. These may represent a kind of macrocilia and the units may still be homologous with the ctenophoran comb organs.[33] Despite preservational differences, there are some features of the Burgess shale fossil which substantiate its comparison also with the Ediacarian frond-like petalonameans *Rangea* or *Charnia*, organisms earlier believed to be relatives of the extant pennatularian octocorals. They all have a rachis, basal bulb, pinnately distributed organs, and at least *Rangea* bore radial vanes similar to those of the Early Cambrian ctenophore *Maotianoascus*. This may mean that the pelagic Cambrian ctenophores are direct successors of the Vendian benthic petalonameans.[33]

Interestingly, the still fragmentarily known body plan of the Vendian Petalonamae shows an intriguing similarity to that of the coeval *Dickinsonia*, with the petalonamean basal bulb resembling the pharyngeal cavity of the dickinsoniids, the axial cavity similar to the intestine, and pinnate units which emerge from it resembling the intestinal caeca. Even the dickinsoniid dorsal quilt is of the same construction as the chambered structures known in *Swartpuntia*, *Ernietta*, *Pteridinium* and other radially organised Ediacarian organisms.[36] The Petalonamae may thus appear to be sedentary, radial relatives of the bilateral, probably free-living dickinsoniids.

The picture of the earliest evolution of the Metazoa, which emerge from the above reviewed evidence *(Figure 4)* is very distant from the traditional view of progressively increasing complexity. In fact, our thinking about the first stages in the evolution of multicellular animals is still too much influenced by the old,

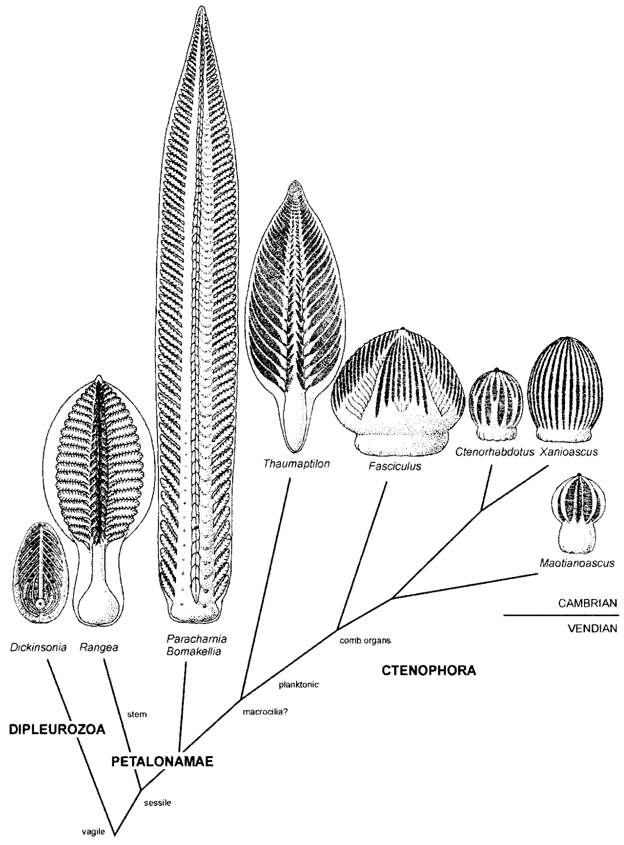

Figure 3. Diagram showing possible relationships between the Vendian petalonameans and the Cambrian ctenophores

dated 1874, idea of Ernst Haeckel that the earliest animal ontogeny recapitulates the earliest stages in the animal phylogeny.[37] The first stages complex enough to be identified with the body plan of Recent organisms would then be a single-layered spherical blastea and double-layered gastrea equipped with the mouth opening.

Cnidarians are traditionally the best candidates to be direct successors of gastrea. However, the most primitive of the Recent cnidarians are those which

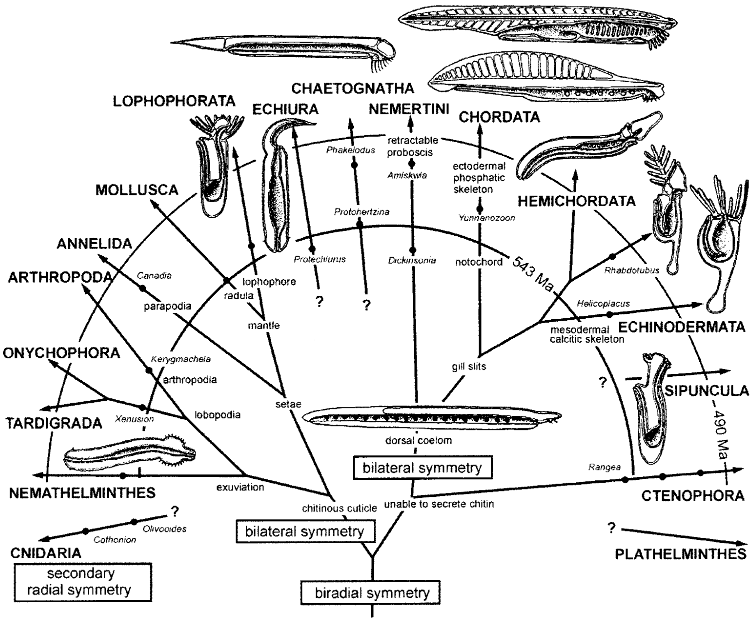

Figure 4. Possible relationships among the metazoan phyla and their palaeontological record (Dzik 33b, modified). Diagrams illustrate body plans of some of the phyla. Boundaries of the Cambrian (543 and 490 Ma) indicated

are anatomically the most complex, the corals.[38] The oldest undoubted corals from the Middle Cambrian[39] are the most sophisticated anatomically, with the presence of mysterious septate opercula suggestive of their nematocysts being non-functional or missing.[40] It is possible that the nematocyst is a secondary acquisition of the cnidarians, by endosymbiosis with dinoflagellates.[41] The observed diversity of the homeotic genes in sea anemones may be a result of their successors of bilaterally organised animals.[42] Also in this case the ancestral members of the phylum appear to be more complex anatomically than their extant members.

Among possible relatives of the cnidarians in the Early Cambrian, the hexaconulariid *Olivooides* seems to be the most plausible. Its development is known, owing to phosphatized specimens, starting from the stage of blastula.[43] The development was direct, with the surface of hemispherical larval stage of the tube covered with conical structures resembling the flagellar cones of Recent cnidarians. Other hexaconulariids show distinctly biradial symmetry, making them similar in this respect to the ctenophores and larval priapulid worms. The annulated postlarval cuticular tube was able to fold radially its aperture. This closely resembles the 'origami' aperture closure in later putative sedentary scyphozoans, the conulariids.[40,44] Some Ediacarian sac-like body fossils (*Kimberella*) and calcareous tubes (*Cloudina*) may represent ancestral cnidarians. Too little is known on the internal anatomy of all these organisms to be confident of their cnidarian affinities. The origins of the cnidarians remains a mystery and the discrepancy between expectations and the true fossil record is especially shocking in this case.

The collagenous lamina basalis which enable proliferating cells to make epithelium, and thus making gastrula-like anatomies, is the fundamental evolutionary achievement (synapomorphy) of the Metazoa. Going even closer to the roots of the metazoan evolutionary tree, it is highly doubtful if any of the living organisms are direct derivatives of blastea. The 'phylum' Mesozoa, once playing such a role included certainly secondary simplified organisms, probably being derived from parasitic flatworms.[45]

Sponges are probably the only multicellular animals which originally do not have any ability to produce collagen-lined epithelia ('Parazoa'). Their most characteristic structures are rather individual specialised cells, choanocytes, with flagella surrounded basally by a palisade of microvilli glued together with a mucopolysaccharide secretion. The microvilli act as a filtratory basket, and choanocytes may or may not be homologous to similar cells occurring in the metazoans.[46] Porocytes, forming linings of the canal openings in the sponge body, resemble endothelial cells of higher animals in their ability to flatten and built cylindrical structures stiffened with their cytoskeleton. The extracellular matrix with collagen (spongin) is the diagnostic evolutionary achievement of sponges inherited after them by the Metazoa. The fossil record of the evolution of sponges *(Figure 5)* is rather good owing to their siliceous or calcareous skeleton, although reports on Precambrian sponges[47] are not completely convincing. In the Early Cambrian the evolutionary roots of the cellular demosponges, with monoaxial siliceous spicules (diactins; e.g., *Leptomitus*), and hexactinellids, with diaxial spicules (stauractins; e.g., *Protospongia*), seem to join together. The most primitive members of these classes had at that time a rather simple skeleton with only a thin layer of spicules near the surface of the body, their axes being oriented along the body or surrounding it. The oldest hexactinellids, which today differ from other sponges in being syncytial, had spicules of the same kind as the larvae of their Recent successors, initially of a normal multicellular organisation.[48]

All the animals originated from a single multicellular ancestor. This is supported by both anatomical and molecular evidence.[49] The first evolutionary event which can be detected on the basis of the distribution of characters among Recent animals was the formation of special sperm cells with condensed chromatin and mitochondria. Sponges share their collagenous extracellular matrix with other animals but also the abilities to transfer signals between cells using neuronal-like receptors. Still, it remains unclear how the body plan of sponges originated and whether the present unicellular or colonial organisation of the Choanoflagellata, their possible relatives among protists, is original or perhaps they are rather secondarily simplified sponges.

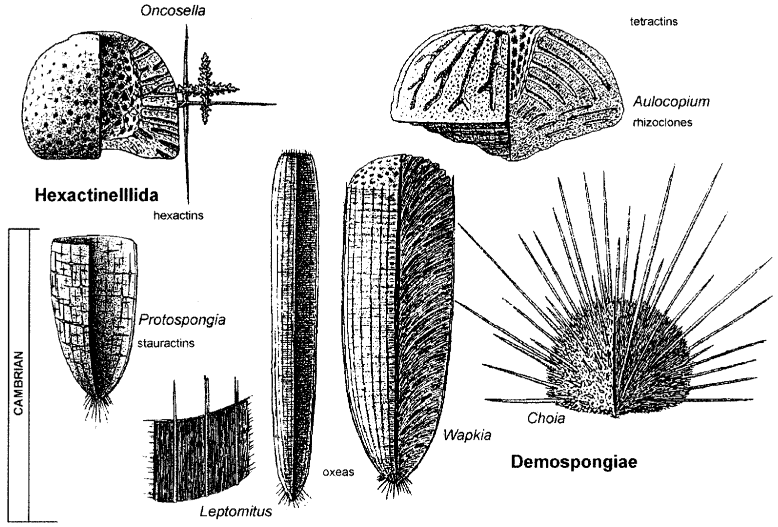

Figure 5. Semi-diagrammatic presentation of skeletal organisation of the oldest and anatomically simplest siliceous sponges (one fourth of the body cut off on the right to show extend of the skeleton and distribution of spicules). Note that in the Cambrian hexactinellid *Protospongia* and the co-occurring demosponge *Leptomitus* spicules are restricted to the surface of the body. In *Wapkia*, this ectosomal skeleton is supplemented by radially arranged spiculation within the whole body, and in *Choia* the ectosomal skeleton is missing. In the subsequent evolution of both the hexactinellids and demosponges a rigid skeleton developed, as exemplified by the Silurian *Oncosella* and the Ordovician "lithistid" *Aulocopium*. Tetractin probably represents a late Palaeozoic evolutionary invention

Choanoflagellates and metazoans share posteriorly oriented flagella with flagellate fungi. Molecular evidence supports monophyly of the group Opisthocontae composed of all these organisms.[50]

3. Ancestry of the land plants

In the development of higher plants, regulatory genes with a conservative short sequence (MADS-box, coding 57 amino acids) are acting. Homologues of these genes are known also in animals. Apparently, such regulatory genes originated in the common ancestor of animals and land plants, in which they performed a different function. This offers a potentially deep insight into the phylogeny of plants, but it is not easy to match the analyses of the homeotic gene evolution with the palaeontological evidence.[51] The early phylogeny of the vascular land plants is relatively well documented with fossils beginning from the earliest Devonian.[52] Already at that time, the dominant generation in their life cycle was the diploid sporophyte stage, the main function of which is dispersal of spores. The sporophyte must elevate sporangia high above the ground to allow a wind dispersal of the spores. This results in a selection pressure on the evolution of a stiff skeletal tissue and an efficient system of water distribution. The xylem (wood) forms such a water transport system, based on dead cells (tracheids), internally strengthened with lignin-impregnated annuli. The other, haploid generation of the gametophyte bears sexual reproduction organs. As long as the flagellate gametes of primitive land plants have to be transferred in water, the gametophyte cannot be too tall, at least in continental environments of normal humidity. Several such gametophytes of the Early Devonian land plants have been identified.[53]

Spores of the vascular land plants show a unique character, which enables their easy identification. In the meiotic cell division, the chloroplast of the original diploid cell splits into four and forms spindle microtubule nucleation centres, working analogously to the centromeres in the animal cells. The cell membrane is then constricted and attains a geometry of four incomplete spheres. Their surface is immediately impregnated with sporopollenin but the walls separating four meiotic cells receive such cover only after completing the division. In result, the contact wall of the tetrade spores are of different ornamentation and form a trilete mark ('Mercedes logo') with three radiating ribs.[54] Spores with trilete marks are known beginning from the Late Ordovician[55] but their diversification started not earlier than in the Middle Silurian.[56] The oldest sporangiophores containing trilete spores (*Cooksonia*) and having epidermis with stomata, but lacking tracheids, are of Latest Silurian age.[57]

Some Early Devonian and Latest Silurian sporangia with bifurcating shoots, thus of morphologies similar to those of the vascular plants, contain tetrads of spores that do not show trilete marks.[58] These were small plants, relics of the Ordovician and Early Silurian vegetation.[56] Such spores (cryptospores) frequently occur in permanent pairs (dyads; unknown in extant lower land plants) or tetrads enveloped in an additional membrane. Their walls are similar in structure to those of spores of Recent liverworts and probably they were produced by an Early Palaeozoic ancestor of the bryophytes.[59] Some Ordovician cellular scraps may represent remains of plant sporophytic tissues homologous with sporangial epidermis of bryophytes.[60] This extends back the fossil record of liverworts to at least the Early Ordovician, from which the oldest cryptospores are known[61], and suggests that the unbranched sporophyte of liverworts and mosses are secondarily simplified.

The external surface of a land plant has to be covered with cutin and waxes, the polymers protecting it against desiccation, which makes respiration difficult. This, in turn, requires a special automatic mechanism of regulation of the water balance. The stomata perform this function. The presence of stomata is shared by vascular plants and bryophytes and they probably developed at the origins of liverworts, some time in the Ordovician. Vegetative cell walls, to some degree resistant to microbial decomposition or desiccation, occur also in the Charophyceae[62], the closest relatives of liverworts among the green algae.[63] Their Ordovician ancestors were probably already terrestrial.[64] Even in the simplest charophycean *Coleochaete* the zygote is nourished by a multicellular reproductive organ, similarly as in the bryophytes. Protective cell walls are developed in this organs, again similarly to bryophytes. The Early Devonian *Parka* is frequently invoked as a fossil relative of *Colechaete* but the spores it contains do not show any tetrad associations.[65]

The charophycean algae share with the bryophytes and primitive vascular plants their unique organisation of the microtubular axial skeleton (spline) in biflagellate sperm cells,[66] an open mitotic spindle, with the nuclear membrane disintegrating in a similar manner as in multicellular animals, and the way they secrete transverse walls separating cells in filaments. Such a wall forms within cisterns (phragmoplast) in the middle between the newly formed cells, where vesicles containing polysaccharides migrate from the Golgi apparatus along the microtubuli of the mitotic spindle. The charophyceans miss the diploid stage (except for a dormant zygote), which differs them from the land plants. The most anatomically advanced of the charophyceans, the Charales, already in the Late Silurian[67] were anatomically distant from the land plants.[68]

The freshwater epiphytic *Chaetosphaeridium* similar to *Coleochaete* connects microscopically the charophyceans with the scaly green prasinophycean flagellate *Mesostigma* in molecular phylogenies.[69] The Prasinophyceae are mostly marine flagellates and coccoid unicellular algae of the nanno- and picoplankton. Their resistant cell walls representing a semidormant vegetative stage (phycoma, lacking flagella and with walls impregnated with sporopollenin) are known from the beginning of the Cambrian (*Cymatiosphaera*). Various unicellular and thalloid algae were represented in the Latest Precambrian.[70] Those from the 750–700 Ma strata of the Svanbergfjellet formation of Spitsbergen are known in microscopical details.[71] Some of them resemble morphologically cladophoran green algae but without any data on reproductive structures it is difficult to prove their affinities. The alleged animal embryos[72] from the Latest Vendian Doushantuo formation in China were also of chlorococcacean relationships.[73] Somewhere among those early protists are thus roots of the land algae which gave rise to both the aquatic charophyceans and higher land plants.

4. Origins and early diversification of eukaryotes

The controversy on the phylogenetic position of the dinoflagellates well exemplifies how difficult it may be to match the molecular phylogenetic evidence with the fossil record. Dinoflagellates in several respects are primitive eukaryotes (organisms having chromosomes separated with double nuclear membrane from the rest of the cell interior). Their nuclear chromosomes remain condensed during the whole cell cycle and in their chloroplasts separate circular chromosomes occur for each of the coded enzymes.[74] The mitochondrial genome shows that dinoflagellate cytoplasm is closely similar only to that of the parasitic apicomplexans[75] but both plastid and mitochondrial genomes have ultra-rapid substitution rates.[76] The internal cell organisation of the dinoflagellates is generally believed to be of ancient origin. This is not supported by the palaeontological evidence, at least in respect to the specific tabulation of their cells.

The fossil record shows that they are geologically young. Although some putative relatives of the dinoflagellates have been reported from the Permian[77] they are not older than the Late Triassic[78] and their main groups developed gradually in the Early Jurassic. This unexpected evidence is also supported by the geochemical data. Products of the degradation of sterols (dinosterols), which nearly exclusively originate from the dinoflagellates, are ubiquitous in the Late Triassic through Cretaceous marine rocks being absent in the Late Paleozoic.[79] However, for some unknown reason the triaromatic dinosteroids are represented also in rocks of Precambrian and Devonian ages. Possibly there were other organisms which produced sterols degradable to similar compounds. To make the picture more foggy, some Silurian cysts (*Arpylorus*) are claimed to resemble those of the dinoflagellates. It has to be admitted that palaeontological studies on the evolution of unicellular algae are on a rather initial stage.

Unfortunately for palaeontologists, most of the Recent flagellates have their cells enveloped in a polysaccharide (mostly cellulose) wall of a very low fossilisation potential.[80] Only those cells which are covered with more resistant polymers (sporopollenin) are likely to survive intact enough to be extracted from the rock as fossils. Such cell walls are formed to protect resting cells (cysts). The encystment is a common phenomenon in the protists, being controlled by the universal mechanism of nuclear signalling, with indoleamine as the inducing factor.[81] During the cyst formation the cytoplasm reduces its volume and produces a new, more resistant and thicker wall built mostly of carbohydrate polymers (sporopollenin). The sporopollenin-rich fossilised eukaryotic cell walls or cysts of obscure taxonomic affinities are conventionally classified as acritarchs. Members of both the great division of protists with chlorophyll *b* (chlorobionts: prasinophycean phycoma stage) and with chlorophyll *c* (chromobionts: dinoflagellate cysts) are thus represented among acritarchs. Fossil resting stages of early eukaryotes, with characteristic tubular processes connecting the cyst with the original cell walls are known from strata at least 950 Ma old. It remains unclear, however, whether their common ancestor can be identified among the acritarchs. The Precambrian unicellular flagellates are documented also with cell scales.[82]

Unlike the metazoans, the algae do not seem to undergo any profound evolutionary transformations near the Precambrian–Cambrian boundary. The Ediacarian acritarchs were more or less similar to those of the Cambrian, with sizes generally not exceeding 100 μm in diameter, thus typical also for Recent unicells. They had an epoch of their greatest morphologic diversity, which preceded the great Laplandian glaciation 600 Ma ago.[83] Very large (more than 0.8 mm) and ornate forms are known from strata deposited that time. Possibly these large photosynthesising organisms were wiped out with the origin of predatory unicellular eukaryotes. The first great diversification of the acritarchs is palaeontologically documented to occur about 1.0 Ga ago.[84] Close to this time first weakly ornamented acritarchs appeared. The oldest unquestionable fossilised eukaryotic cell walls were morphologically simple spheres identified as eukaryotic exclusively on the basis of their large size (in excess of 60 μm). These have been reported from strata which are 1.7 Ga old.[83] Sexual processes (i.e., periodic fusion of nuclei, recombination of chromosomes, and their subsequent segregation) seem to have originated early in the evolution of eukaryotes.[85] Their fossilizable effects are meiotic tetrads.

Among the eukaryotes bearing the typical flagellum with a microtubular internal skeleton, the cryptomonads (cryptophytes) possess the most primitive chloroplasts.[86] No doubt that, similarly to the heteroconts, haptophytes, and dinoflagellates, they inherited their chloroplasts from the red algae.[87] The phycobilisomes (grains on the thylakoid photosynthesising membrane composed of proteins and the phycobiline photosynthetic pigment) are exclusive for the cyanobacteria, rhodophytes and cryptophytes. It is thus tempting to assume that the invention of such a sophisticated and highly ordered cytoskeletal structure as the flagellum was introduced only once: in the course of evolution between these branches of the eukaryotic evolutionary tree. This is complicated, however, by claims that the cryptophyte chloroplasts were acquired from a red alga by a secondary symbiosis.[88]

The chloroplasts of the cryptomonads, brown algae, diatoms, other chromophytes and chlorarachniophytes are enveloped in four membranes. This is generally assumed to be of no special functional importance but rather a result of a historical constraint. The more than two membranes are thus explained as an effect of secondary endosymbiosis with a reduced chloroplast-bearing eukaryote which lost its nucleus.[88,89] Consistently with this idea, the presence of a DNA aggregation (nucleomorph) in the periplastidial space between the inner and outer plastid membrane pairs of the cryptomonads and chlorachniophytes is interpreted as a remnant of the first host nucleus, which underwent degeneration after the secondary symbiosis. The nucleomorph of the cryptomonads is composed of three minute linear chromosomes virtually without introns and with no genes for metabolism but with codes for two essential chloroplast proteins, FtsZ and rubredoxin. This explains why the nucleomorphs persists.[90] The chlorarachniophytes are believed to have evolved from a single secondary endosymbiosis between a protozoan and an ulvophycean green alga.[91]

This is in some inconsistency with the reports that the two external membranes in chloroplasts enveloped with four membranes merge with both the endoplasmic reticulum and nuclear membrane.[92] In the dinoflagellates and euglenoids, in which three plastid membranes occur, the external one connects with the nuclear membrane. The higher-plant, green and red algal chloroplasts are surrounded by a double membrane envelope. In such chloroplasts, the external membrane is in continuity with the endoplasmic reticulum (covered with ribosomes) and imported proteins have to pass through the reticulum before entering the chloroplast.[93] This indicates a far-reaching integration of the chloroplast membranes with the rest of the cell. Undoubtedly, much more information on function of the chloroplast membranes is needed to understand their phylogenetic meaning.

The physiologically most primitive eukaryotes are the rhodophytes (red algae). They, similarly to the fungi, do not have any centrosome and their equivalent is the spindle pole body anchored to the nuclear membrane. Consistently with this simplicity of the cell division apparatus, the red algae are the oldest palaeontologically documented eukaryotes *(Figure 6)*. The first filamentous bangiophycean alga from the Hunting formation of the Sommerset Island in Canada was at least 900 Ma old.[94] More complex thalli, with reproductive structures within fountain-like arrays of the parenchymatous cells, excellently preserved owing to phosphatization, occur in the Doushantuo formation in the Guizhou province of China, probably being Latest Vendian in age.[72]

The most primitive stage in the chloroplast evolution is probably represented by the plastids (cyanelle) of the enigmatic glaucocystophytes. There is a peptidoglycan wall in-between their external membranes apparently homologous to the wall of the Gram-negative bacteria. The substrate and metabolite transport goes through this wall.[95,93] Peptidoglygans are missing in chloroplasts of other eukaryonts.

No doubt that all the structural and physiologic aspects of the chloroplasts are homologous to those of the blue-green algae (cyanobacteria). Plastids inherited from the cyanobacteria their typically prokaryotic mechanism of cell division with the tubulin related FtsZ proteins involved in the membrane constric-

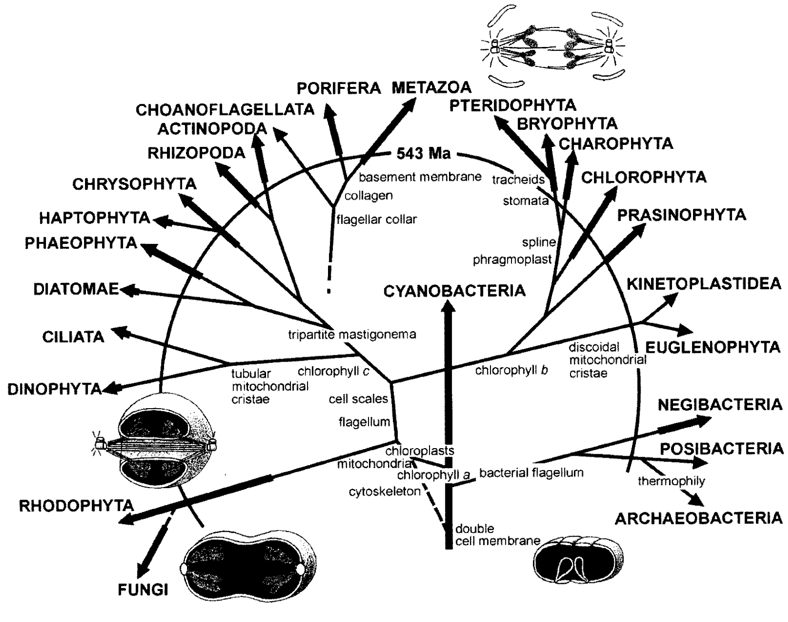

Figure 6. Possible pattern of relationships between the main groups of prokaryotes and eukaryotes based on the controversial idea that all the recent prokaryotes derived from an ancestor equipped with both photosystem I and II and with Gram-negative cell organisation. Diagrams show chromosome segregation in a Gram-negative bacterium, in nucleus of a red alga or fungus (with mitotic spindle organising centre), a dinoflagellate (with centrioles controlling development of external spindle), and in a multicellular animal or land plant (open mitotic spindle). Extend of the fossil record (thick lines) and the Precambrian–Cambrian boundary (543 Ma) indicated. Note that molecular evidence suggests a close affinity between the fungi, metazoans, and land plants (Baldauf et al. 2000)

tion.[96] The eukaryotes are believed to have originated by an endosymbiotic acquisition of photosynthesising cyanobacterial cells by a hypothetical heterotrophic ancestor with nucleus. The symbiotic theory of the origin of chloroplast, introduced as early as 1905 by Konstantin S. Mereschkovsky, is now generally accepted. Chloroplasts of all the Recent photosynthetic organisms share a common ancestry[97], there is thus no need to assume their repeated symbiotic acquisition. The main algal groups appear also directly related to each other, based on the mitochondrial DNA and elongation factor sequences.[98]

More and more homologies with the blue-green algae are shown not only by chloroplasts, but also by mitochondria[99] and the eukaryotic cytoplasm. For instance, cyanobacteria possess ion channels in their cell membrane which are homologous with the eukaryotic glutamate-gated channel, of crucial role in the transmission of signals along the cell membranes.[100] The concept of the autogenic origins of organelle by compartmentation cannot thus be completely forgotten.

Even if the other eukaryotic cell organelle with a partial genetic autonomy, that is the mitochondria, originated by symbiosis, there is no extant descendant of the stage of the evolution preceding this hypothetical event. Those Recent eukaryotes which lack mitochondria lost them secondarily in an effect of adaptation to life in anoxic environments. In some of them, mitochondria evolved into hydrogenosomes, organellae adapted to the anaerobic metabolism.[101] Mitochondria have specific pore proteins (mitochondrial carrier family, MCF) which evolved already before the separation of the main groups of Recent eukaryotes. This shows their close ties with the rest of the eukaryotic cell. Even if they originated by symbiosis, this was a very ancient and unique event. Notably, oxidative metabolism, the crucial function of mitochondria, is also performed by cyanobacteria and their cytochrome oxidase is similar to that of the mitochondria.

The gap in anatomical diversity between the eukaryotes and prokaryotes is not as wide as it may seem. Some Recent prokaryotes bear structures which are at least analogous to the membrane and cytoskeleton-based compartments of the cell interior. A nucleus-resembling nucleoid bounded by two membranes occurs in some bacteria.[102] The bacterial cell division protein FtsZ is homologous to the eukaryotic tubulin, one of the main components of the cytoskeleton. Actually, the process of chromosome segregation in prokaryotes is not much less ordered and precise than that in eukaryotes[103] and several bacteria have more than one chromosome.[104]

Paradoxically enough, the cyanobacteria are thus good candidates for being ancestors of all the basic compartments of the eukaryotic cell: the cytoplasm, chloroplasts, and mitochondria. The transition from the original cyanobacterium to the ancestral eukaryote could have occurred either in separate cell lineages unified by symbiosis or, instead, by a differential evolution of partially genetically autonomous compartments within the same cell. Effects would be the same. There is hardly any possibility to disprove any of these alternative scenarios, which would require a direct palaeontological evidence. Such evidence may be very difficult to obtain, if possible at all.

5. Polarity in the evolution of prokaryotes

Because the quality of the fossil record of the earliest stages of evolution is unavoidably poor, it is extremely important for the palaeontological research to have a precise working hypothesis on the course of evolution. We want to know exactly what is expected to be found in the rock. Similarly as it was the case with higher organisms, also in the prokaryote evolution the traditional assumption that ancient means simple is probably wrong. A secondary simplification is there as common as an anatomical complication.

In fact, the molecular phylogenetic evidence tends to show a pattern of relationships more and more departing from the old scheme of a gradually increasing anatomical complexity being expressed in the diversity of Recent prokaryotes. For instance, it appears that among bacteria the simplest coccus shape has arisen and accumulated independently multiple times in separate lineages. It is rather a degenerative form of the rod[105] than a truly primitive morphology. Apparently, the double membrane is an unnecessary barrier for the food transport in heterotrophs and it tends to disappear together with the cell growth and division mechanisms which make more primitive bacteria rod-like.[106] Moreover, the analysis of the distribution of conserved insertions and deletions in various prokaryote proteins by Gupta et al.[107] gives a surprisingly linear pattern of relationship of the main groups, but at the same time a seemingly chaotic distribution of the photosynthesis reaction centres (RC-1 of the photosystem I and and RC-2 of photosystem II). The most reasonable way to escape from this chaos is to accept the presence of both reaction centres in the common ancestor of Recent prokaryotes. This is consistent with the apparently derived nature of the whole photosystem I in respect to the oxygen-producing photosystem II,[108] on the contrary to earlier assumptions. This means that the common ancestor of Recent prokaryotes had full photosynthetic abilities and it is likely that it was equipped with the photosystem II. Also inferred nitrogen fixation gene duplications predate the appearance of the last universal common ancestor of all Recent organisms.[109] Thus, it was on the cyanobacterial level of physiological organisation.

This fits well the extremely ancient fossil record of the cyanobacteria *(Figure 7)*. Their well preserved fossil colonies are not rare in early diagenetic chert nodules within Precambrian carbonate sediments deposited in littoral environments. Perhaps the most impressive of these microfloras are those of the Bitter Springs formation in the Amadeus basin of Australia, which is 850 Ma old,[110] and the about 2.0-Ga-old Gunflint formation of Ontario.[111,110]

Paradoxically, the most anatomically complex of Recent prokaryotes may thus appear the most primitive and phylogenetically ancient. Much of the prokaryotic evolution identifiable on the basis of Recent materials would thus express a secondary anatomical simplification. However, the structural simplification was undoubtedly connected with more and more sophisticated physiologies enabling bacteria to exploit extreme environments, not penetrable to eukaryotes. These were also most probably not accessible to early prokaryotes. The common assumption that a small size and anatomical simplicity reflects an ancient origin may thus be completely misleading. An additional suggestion emerges from this, namely that the earliest living beings could hardly survive the harsh extraterrestrial conditions.

Equally misleading may be the tempting assumption that the temperature preferences of the Recent prokaryotic organisms are inherited after their ancestors living in geological epochs when such conditions were dominating over the whole Earth. As it is generally accepted that in the earliest Archaean the Earth surface was much warmer than today, the hyperthermophiles have attracted much attention as possible direct successors of the earliest organisms. Among them those which show a separate location on the 16S/18S rRNA phylogenetic tree, the Archaeobacteria (Archaea), look especially well designed for such a role. Moreover, they abound near hydrothermal oceanic thermal vents, which allegedly preserved to our days the Archaean conditions.

The Archaeobacteria were defined by Carl R. Woese[112] exclusively on the basis of their position on the rRNA evolutionary tree and there are hardly any morphologic or physiologic characters which could be used to identify them. If there are such characters, they definitely represent adaptations to extreme thermal conditions of their life. Such are the ether bounds in their membrane lipid molecules, methylated ribose, and complexly folded DNA. The enzyme responsible for the DNA folding, the reverse gyrase, originated from a fusion of heli-

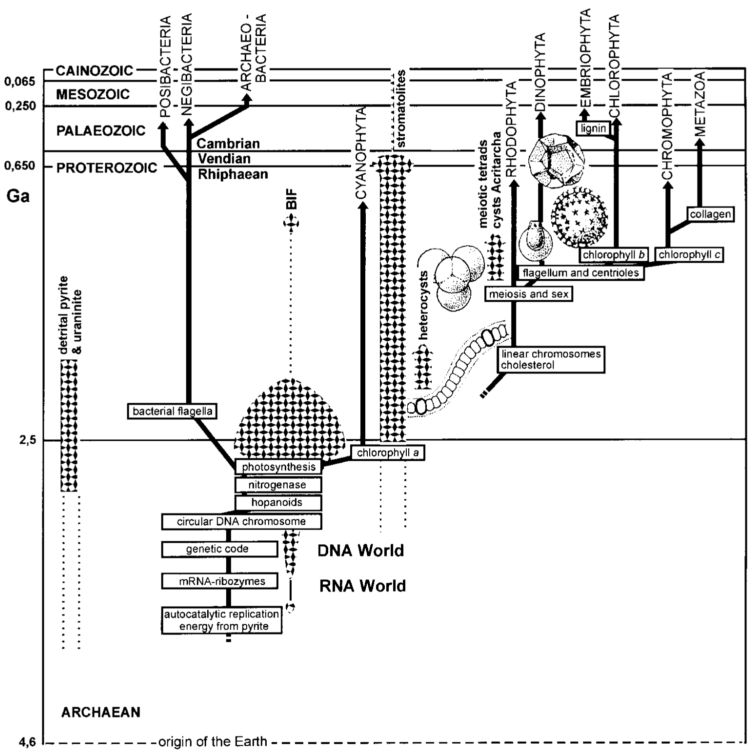

Figure 7. Origins and the evolution of life in the fossil record. The time extent of the pyritic sand formation delimits the anoxic atmosphere, protection of nitrogenase within the cyanobacterian heterocysts documents incursion of oxygen to the environment. The sequence of evolutionary events in the Archaean is purely conjectural

case and topoisomerase units, homologous with those of other bacteria.[113] Otherwise archaeobacteria are equipped with all the main components of the high-organised bacterial cells, including the basic respiratory enzymes[114] and the tubulin-like cell division protein FtsZ apparatus.[115] Most probably, the position of the Archaeobacteria close to the phylogenetic tree roots is due to a long-branch attraction artefact in result of an increased rate of the nucleotide substitution in their extreme life environment.[116] They may appear related to, and even more derived than, the Gram-positive bacteria.[117]

Moreover, the Archaeobacteria bear flagella,[118] perhaps the most complex anatomical structure in the bacteria, being a part of the double cell membrane Gram-negative anatomy.[119] The bacterial flagellum is built of special fibrillar protein[120] and has nothing to do with the eukaryotic flagellum, the skeleton of which is composed of the globular protein (tubulin). Motility of cyanobacteria is very limited. To glide slowly over surfaces they use slime secreted by a specific junctional pore complex.[121] Thus the flagellum seems to be a novel structure in the evolution of non-cyanobacterian bacteria and may possibly demarcate their evolutionary origins. Its independent formation in the evolution of different prokaryotes seems unlikely.

The adaptation to heterotrophic and parasitic modes of life in the evolution of bacteria was probably the main factor leading to the secondary reduction of the bacterial cell wall and finally to the cell membrane being replaced with a sterol-based membrane in the parasitic mycoplasmae. As the rather inflexible prokaryotic cell membrane cannot be used to phago- or pinocytosis, the main way of bacterial feeding is by specific pore systems within the cell membrane. Through this pore apparatuses not only simple compounds and proteins can be taken from the host, but also nucleic acids.[122] This may explain the susceptibility of heterotrophic and parasitic bacteria to a lateral gene transfer but makes such a mechanism unlikely to work extensively in primitive autotrophic prokaryotes. Other ways of gene acquisition and transfer are probably evolutionary late, although, ironically, the cyanobacteria *Synechocystis* seems to be the most infected with foreign genes among better known prokaryotes.[123]

It is not easy to identify and document palaeontologically the evolution of the oldest prokaryotes. In the geologically oldest sedimentary strata, unavoidably metamorphosed and tectonically deformed, organic structures cannot be expected to represent anything more than dark stains of a carbonised organic matter of the extracellular walls and sheaths. To prove that a dark structure visible within the cherty rock matrix is truly a fossil, its morphology has to be specific enough to exclude any possibility that a seemingly organic structure is mimicked by an inorganic growth of mineral structures. This means that the evidence is virtually never convincing. Possible fossils of coccoidal bacterial cells are too simple to be distinguished from inorganic aggregates. Filamentous structures are less common to be produced in an inorganic way.[124] All this has to be kept in mind while use is made of the data on Archaen fossils to test hypotheses on the evolution.

Geological sequences in which the earliest record of life can be potentially preserved in a relatively unchanged state are well represented only in two places, in South Africa and Australia; originally they were parts of the same geotectonic unit split by subsequent continental drift. Dark filaments, which are interpreted as remnants of prokaryotic colonies, are known from both areas. The oldest fossil structures which may represent carbonised sheaths of cells arranged linearly in filaments are those from the Apex chert of the Archaen Pilbara block in north-western Australia, which are almost 3.5 Ga old.[125] The structures, to which several Linnean names have been applied, show a multimodal cell width frequency which roughly fits the size diversity within the Recent cyanobacteria. Their preservation (if they truly represent cyanobacterian filaments) is poor and perhaps it would not be reasonable to trust this evidence too much. In the same area pyritic filaments have been reported from 3.2-Ga-old chert intercalations within a deep-sea volcanogenic heavy metal sulphide deposit. These were interpreted as thermophilic chemotropic prokaryotes.[126] If true, this would mean that almost immediately after the origin of life, organisms developed for the prokaryotes, a highly advanced, colonial filamentous organisation and thick cell walls (now virtually exclusive for the cyanobacteria) and were able to adapt to extreme environments containing heavy metal ions which were normally toxic.[1] The alternative is that this interpretation is a kind of reductio ad absurdum and that these are rather inorganic aggregates of pyrite.[127]

If taken seriously, the evidence that filamentous cyanobacteria occurred already 3.5 Ga ago and filamentous chemotrophs 3.2 Ga ago has truly too far-reaching and evolutionarily troublesome implications.[128] It gives less time for the origins of life, its transformations through the RNA world, invention of the genetic code, translation factory, enzymes, formation of the double-membrane Gram-negative cell wall, and the invention of photosynthetic systems, than it passed to now since the origin of the terrestrial vertebrates. After these fundamental physiologic attributes of life developed, the evolution would have to slow for the next two billion years to preserve virtually unchanged cyanobacterian anatomy and physiology to recent times. This is truly difficult to accept.

The morphology of the oldest microfossils is hardly specific enough to provide any decisive evidence in this respect. Also the generally cyanobacterial in origin calcareous structures (stromatolites) may be mimicked by abiotic sedimentary structures.[129] It may thus easily appear that the structural fossil record starts with the invention of the cyanobacteria and with the income of the oxygen-containing atmosphere 2.2–2.0 Ga ago which they actually produced with their manganese-containing protein of the photosystem II.[130] This may explain why the main oxidative metabolism enzymes appear to be so widespread among Recent prokaryotes.[131] Cyanobacteria are likely to be the most ancient of them.

6. The RNA world

Various kinds of nucleic acids are functionally specialised to different function even in the most simple of the cells. The information storage and its reading are separated from each other. The more stable deoxiribose-based DNA takes the storage function, whereas catalysing functions are performed by special kinds of RNA (tRNA and rRNA), together with associated polypeptides. In fact, today the polypeptides (enzymes) are virtually the only biological catalysts. The production of specific enzymes with repeatable structure requires special mechanisms of translation of the information stored in DNA into the information used by enzymes. Translation requires thus a readable genetic code, the code which is based on nucleotide triplets.

The genetic code itself has its evolutionary history. In the classic attempt to uncipher it, J. Tze-fei Wong[132] superimposed codes of particular amino acids on the pattern of their precursor-product relationships. The degree they fit is really astounding.[133] Notably, the congruence is better in respect to prokaryotic than to eukaryotic metabolic paths in cases they differ. Most of the steps from one to another amino acid correspond to single nucleotide alterations of the code. The only systematic departure of the code triplet relationships from the amino acid metabolic pathways refer to simple and widespread amino acids, which are located in the centre of Wong's diagram. This discrepancy can be removed if one assumes that the original code was more degenerated (more numerous triplets meant the same) than today, and that some of the codes initially synonymous were subsequently used to newly emerging functions.

The main evolutionary conclusion from the Wong co-evolution theory of the genetic code is that there were initially much less numerous amino acids in the process of translation. This may, in turn, imply that the amino acids gradually replaced nucleotides in catalysing molecules.[134] Otherwise the early enzymes would appear to be too simple structurally to perform the needed functions. The co-evolution theory provides thus a strong support to the RNA world hypothesis.[135] This hypothesis predicts that there was a stage in the evolution of life when both the information storage and catalytic functions were performed by ribonucleic acids. Polypeptides, if present, were simple and synthesised without using the mechanism of translation. Possible original non-catalytic function of polypeptides is suggested by the way in which cyanophycin (the cyanobacterial reserve material), a polymer of two evolutionarily ancient amino acids, is synthesised outside the genetic code translation mechanism.[136] Also the most ancient fermentation reactions could have been based on simple amino acids.[137]

One may guess that the evolutionary development of the mechanism of translation, which enabled replacement of the rather inefficient ribozymes with much more sophisticated enzymes preceded the invent of the DNA. Its synthesis and replication requires such enzymes. The genetic code, together with the whole translation factory, is thus older than DNA. Enzymes most probably originated gradually by insertion of polypeptide segments in nucleotide chains of the ribozymes. Finally, the polynucleotide segments disappeared in most of enzymes, with some biochemical 'living fossils' preserved[138], like coenzyme-A (composed of one nucleotide and two amino acids). Almost certainly the DNA developed in the evolution from a less resistant single strand RNA to perform specifically the information storage function with reduced risk of degradation. The RNA world could not have occurred in hot waters because ribose in too sensitive of thermal degradation. This may impose some limits on the possible oldest geological age of the ribonucleotide-based early living beings.

7. The origins of the cell

As the earliest possible fossils reproduce only the external cell morphology, they are of limited value in studies on the early evolution of life. To be identifiable, the morphology of fossilised organisms has to reflect a high degree of internal compartmentalisation and complexity in the morphology of secreted skeletal structures. These are obviously features of evolutionarily advanced beings. Instead of morphology, one has thus to refer to identification of specific products of the metabolisms of early organisms.[139]

Perhaps the most spectacular case of using this way of inference is the recent report on identification of cholestane in 2.7-billion-year-old shales from the Pilbara craton in Australia, the compound interpreted as being a product of degradation of eukaryotic membrane lipids.[140] This is in contradiction with the generally accepted dependency of lipid biosynthesis factory (squalene epoxydase) on free oxygen.[141]

To base any evolutionary inference on palaeobiochemical evidence requires that the products of metabolic activity of organisms are still ubiquitous in the rock, after some billion years of its diagenesis and metamorphism. The original biological productivity of those organisms had to be high enough. This hardly refers to the earliest stages in the evolution of life on the Earth, when it was definitely spatially restricted to special environments and of a low metabolic efficiency (thus of low productivity).

As the morphologic evidence of the earliest life is hardly decisive and the biomarker studies may not be specific enough, the data on carbon isotope depletion in the oldest sedimentary strata are generally accepted to provide the unquestionable evidence for a very early origin of life. An enrichment in isotopically light carbon has been reported for organic compounds in an 3.8-Ga-old banded iron formation in Greenland.[142] Whatever is their exact dating,[143] these are actually the oldest identifiable (although metamorphosed) sediments on Earth. This means that, in fact, sedimentary carbon of any age is always enriched in the light isotope ^{12}C. This leaves virtually no time for the earliest life to develop after the end of ocean-vaporising bolide impacts.[128] However, although the carbon isotope depletion is a powerful method of inference on ancient ecosystems, it can hardly be used alone as the method to distinguish biotically produced compounds from those of abiotic origin. There are some industrial technologies of organic synthesis proceeding in conditions close to those in the earliest Archaen, such as the Fischer–Tropsch catalytic process of gasoline synthesis from CO and H_2 at relatively low temperatures (200–250 °C) and pressure. It is well known[144] that CO_2 synthesised in this process is enriched in the heavy isotope ^{13}C ($\delta^{13}C$ 35 ‰), whereas the more complex organic compounds of the synthetic gasoline are enriched in ^{12}C ($\delta^{12}C$ 33 ‰) in respect to the original CO. Similarly as in biotically synthesised compounds, isotope fractionation is different in respect to different carbon atom locations in the molecule. For instance, the abiotic synthesis of organic compounds containing metoxyl group results in light isotope enrichment of up to 90 ‰. Also the biogenic methane formation results in the same isotopic fractionation as typically observed for the microbial methane.[145] This means that isotope fractionation has to be determined at specific points within the molecule to distinguish abiogenic reduced carbon compounds from those of truly organic origin.[146] This is hardly achievable with the highly degraded Archaen kerogene.

Thus, at present there is hardly any reliable method of reasoning available which could allow us to use any direct geological evidence to test speculations on the RNA world and so more on the earliest pre-cell evolution of life.

8. Conclusions

The calibration of molecular phylogenetic trees with reliable palaeontological evidence suggests that the first divergence of the metazoan lineages took place not earlier than 790 Ma ago.[147] The eukaryotes developed not earlier than the aerobic conditions emerged on Earth about 2.0 Ga ago. There is a possibility that the 'last universal common ancestor'of all Recent organisms was an Early Proterozoic cyanobacterium. Its successors evolved either towards a higher and higher anatomical complexity, and this gave rise to the eukaryotes, or towards adaptations to extreme environments. Prokaryotes living in such environments are hardly primitive. The oldest stages of the evolution, starting from the 'prebiotic soup' or pyrite-based 'pizza'[1,148] and ending at the cyanobacteria, with virtually all basic metabolic mechanisms already formed, are thus hardly possible to be restored by arranging a 'ladder of beings' built of the Recent prokaryotic organisms. Such organisms are no longer in existence and the apparent simplicity of some extant bacteria is rather a result of their secondary simplification and specialisation of certain aspects of their physiology. The only way to construct hypotheses which could be tested by the Archaean palaeontological and geological evidence is thus to restore the most ancient physiologies by retrodiction from the available biochemical evidence.

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