



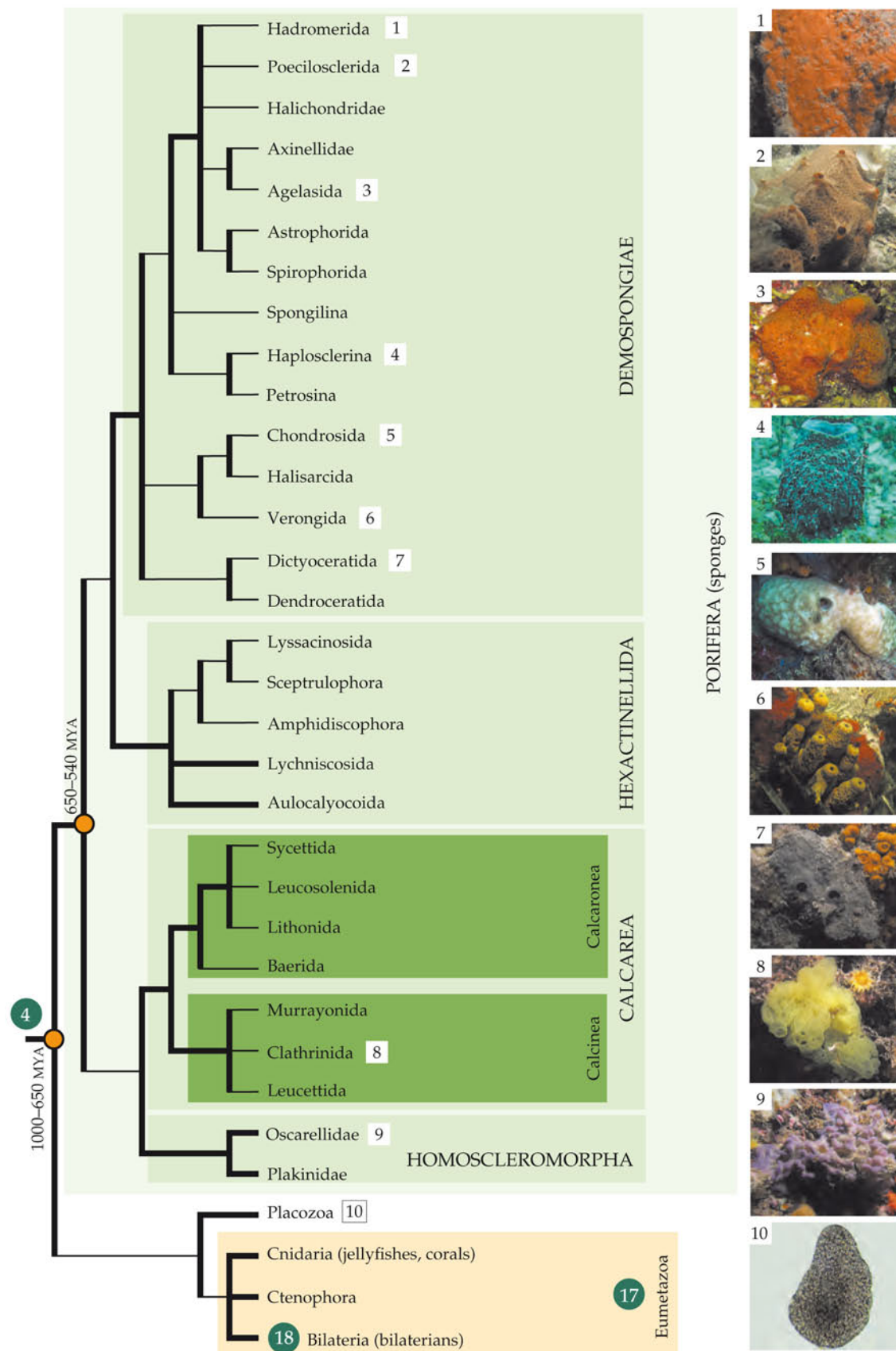
The Tree of Life

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Tree of Metazoans showing the phylogenetic relationships among the most significant groups. Colored boxes indicate high taxonomic ranks. Branches with thick lines indicate robust clades, and branches with thin lines less-supported clades (see alternative hypotheses in this chapter). Each number in a green circle indicates the chapter in which the particular clade is also treated. Orange circles mark some nodes and their age. Photographs illustrate principal clades; boxed numbers associate photographs with clades.

Metazoans

The Rise of Early Animals

Manuel Maldonado

SUMMARY This chapter describes the most important aspects of early evolution of animals (over 1 million species recognized). The origin of animals (metazoans) and the phylogenetic relationships among the early diverging clades (sponges, placozoans, cnidarians, and ctenophores) pose highly controversial evolutionary questions. The most accepted, but not the only, hypothesis proposes that animals originated between 1.2 billion and 700 million years ago, probably from a unicellular ancestor common to modern choanoflagellates. Recent studies have demonstrated that several genetic systems traditionally assumed to be the innovations of animals already existed in a preliminary form in the genome of unicellular organisms or in the most recent common ancestor to choanoflagellates and metazoans, or in both. The main obstacle to providing a definitive description of the evolutionary path taken by animals at the time of their origin is the fact that different regions of the genome seem to have experienced noticeably different evolutionary histories, thus producing conflicting phylogenetic signals. The most pertinent question revolves around whether the first diverging phylum is Porifera, as suggested by the classic analyses of morphological information as well as some phylogenetic analyses; or Placozoa, as suggested by the phylogenetic signal of mitochondrial DNA; or even Ctenophora, as proposed from some recent phylogenomic analyses. There is also debate over the relationships between ctenophores and cnidarians and over which of these groups shared a common ancestor, if any, with bilaterian animals. Several data sources agree in suggesting that the most likely phylogeny would be that sponges diverged first, followed by the placozoans, the cnidarians, and lastly the ctenophores and the bilaterians. Nevertheless, definitive resolution of the evolutionary path followed by animals at the time of their origin probably will need refined computational approaches, both for the reconstruction of characters and for comparative analyses involving larger collections of genes or even complete genomes.

THE EVOLUTIONARY TRANSITION FROM A UNICELLULAR state to the multicellular state that characterizes modern animals or metazoans (**Boxes 16.1** and **16.2**) is a burning scientific question that has been answered only partially. A series of genetic and cytological innovations facilitated the increase in the number of cells that formed the bodies of animals. More importantly, these bodies, by improving the regulation of gene expression, acquired the ability to perform multiple

complex vital functions simultaneously, because different cells, despite possessing identical genetic information, began to develop differentiated characteristics (cellular specialization) and group themselves into supracellular functional units or tissues. The structuring of tissues in animals can already be perceived during the early stages of embryonic development through a reorganization and differentiation process of the embryonic cells known as gastrulation. This first cellular

What is a metazoan?

The conceptual origin of the name for the taxon Metazoa (from the Greek *meta* [between, after, beyond] and *zoon* [animal]) proposed by Haeckel in 1874 to denominate the members of the animal kingdom traces back to *Systema Naturae* by Linnaeus (1735), which established an Animalia kingdom (heterotrophic organisms known at the time)

in opposition to the Plantae kingdom (autotrophic organisms). Today, the phylum Metazoa (animals) includes a set of multicellular organisms built by eukaryotic cells lacking both cell walls and chloroplasts, and capable of organizing themselves into functional groups (tissues) to carry out specific functions.

BOX 16.1 Unique morphological characters of the metazoans

- Multicellular organization comprising cells without walls or chloroplasts, developing through a process of zygote cellular division known as cleavage and a subsequent process of embryonic reorganization and cell predifferentiation known as gastrulation
- Sexual reproduction through spermatozoa and ova
- Presence of collagen in the intercellular matrix
- Distinctive set of insertions and deletions in genes coding for proteins in the mitochondrial genome

reorganization produces an embryo that can be diploblastic, formed by an external cellular layer (the ectoderm) and an internal layer (the endoderm); or even triploblastic, formed by an ectoderm, an endoderm, and an intermediate cellular layer known as the mesoderm. Most animals are characterized by triploblastic embryos already anticipating a rough draft of bilateral symmetry. For this reason, they are generically designated as Bilateria (see Box “*Radiata*” and *Bilateria*). Only the phyla Porifera, Placozoa, Cnidaria, and Ctenophora (see corresponding boxes in this chapter), collectively known as the *lower invertebrates* (a name completely lacking taxonomic or phylogenetic validity) exhibit nontriploblastic embryos. In Cnidaria and Ctenophora the ectoderm and the endoderm are homologous to those in triploblastic animals, given that the embryonic ectoderm forms the external wall of the adult body and its associated organs, while the embryonic endoderm forms the wall of the digestive tube and its associated organs. Tissues of ectodermal and endodermal origin in cnidarians and ctenophores are separated by an intermediate mesenchyme, the mesoglea. This dense matrix is rich in collagen and other macromolecules and contains several types of amoeboid cells in low density. Because of the poor cellular compaction of the mesoglea and the fact that its cells rarely join to form multicellular structures of a higher complexity, this *pseudotissue* is not considered to be a true mesoderm, which is why cnidarians and ctenophores have traditionally been considered diploblastic. In the case of Porifera and Placozoa, it has not been possible to establish a convincing homologous relationship between the embryonic epithelia and those of other animals, and so the terms *gastrulation*, *ectoderm*, and *endoderm* were virtually eliminated from the zoological terminology relating to these groups during the twentieth century. Consequently, Porifera and Placozoa (but Porifera in particular) were regarded to represent a pre-diploblastic, and therefore lower, organizational level compared to that of other animals (Eumetazoa; **Figure 16.1**).

BOX 16.2 Metazoans by the numbers

- Number of species: the number of extant animal species has been estimated at approximately 1,162,000, of which 900,000 are insects
- Phylum with the highest representation: arthropods lead the ranking with more than a million species, followed at a distance by molluscs, with 100,000 species
- Phyla with the lowest representation: Cyclophora, Placozoa, and Xenoturbellida have two officially described species
- Smallest metazoan: several members of the group of harpacticoid acarids, the tardigrades (waterbears), and the nematodes have species in which adult specimens do not exceed 150 μm in length
- Largest metazoan: the blue whale (*Balaenoptera musculus*), measuring up to 33.2 m long and weighing up to 190 tons
- Longest-living metazoans: a *Corallistes* demosponge was estimated, by means of isotope analysis of its skeleton, to be between 135 and 160 years old. Harriet, a female Galápagos giant tortoise (*Chelonoidis nigra*) was collected live by Darwin and died in 2006 at the age of 176; other members of this species have reached 188 years of age. Unofficial dating studies on the valves of the *Arctica islandica* clam suggest that this species can live more than 400 years. Estimates of the age of the giant barrel sponge (*Xestospongia muta*) based on the relationship between size and growth rate suggest lifespans that might exceed 2000 years.
- Shortest-living metazoans: Male *Acarophenax tribolii* mites develop inside the body of the mother, fertilize developing females of the same litter, and die even before leaving the mother's body, all in a period of a few hours. Some species of gastrotrichs live only three days.
- Oldest metazoan: Despite extensive controversy over the interpretation of several fossils, the oldest one is estimated to be between 650 million and 1.5 billion years old.
- Oldest bilaterian metazoan: *Vernanimalcula guizhouena*, a microscopic fossil species that has been interpreted (not without controversy) as possessing a triploblastic structure and an estimated age ranging between 580 and 600 million years

Figure 16.1 The traditionally accepted phylogeny for illustrating the relationships between basal metazoa, inferred from morphological characters. The external group, Choanoflagellata, refers only to members of one of the five classes that form the paraphyletic phylum “Choanozoa.”



Basic terms

Blastomere: Undifferentiated embryonic cell that forms the body of the early embryonic stages of animals.

“Coelenterate”: Term traditionally used in zoology to jointly designate cnidarians and ctenophores, under the assumption that both groups have a body formed by only two cell layers and structured around a large internal cavity (coelenteron). The external layer forms the protective wall on the surface of the body, and the internal layer lines the coelenteron that functions as a digestive cavity and communicates to the exterior through a single orifice that functions both as mouth and anus.

EST: Abbreviation of expressed sequence tag, which refers to a short fragment of DNA unambiguously derived from a cDNA library and therefore from a sequence that has been transcribed in a certain tissue or during a certain process of embryonic development, and that can be mapped to its position in the genome, thus serving to identify the locus of that gene.

Housekeeping genes: Nuclear genes that are expressed at a relatively constant level in the cells of metazoans because their products are essential for maintaining the cell’s life cycle.

Lecithotrophic larva: Metazoan larva that is unable to process particulate food (but not dissolved organic

matter), generally lacking a mouth or digestive tube or both, and feeds on reserves accumulated in its interior as vitellum during embryonic development.

Macromere: Blastomere or undifferentiated embryonic cell, larger than the rest because it generally has a slower rate of division.

Micromere: Blastomere or undifferentiated embryonic cell smaller than the rest because it generally has a faster rate of division.

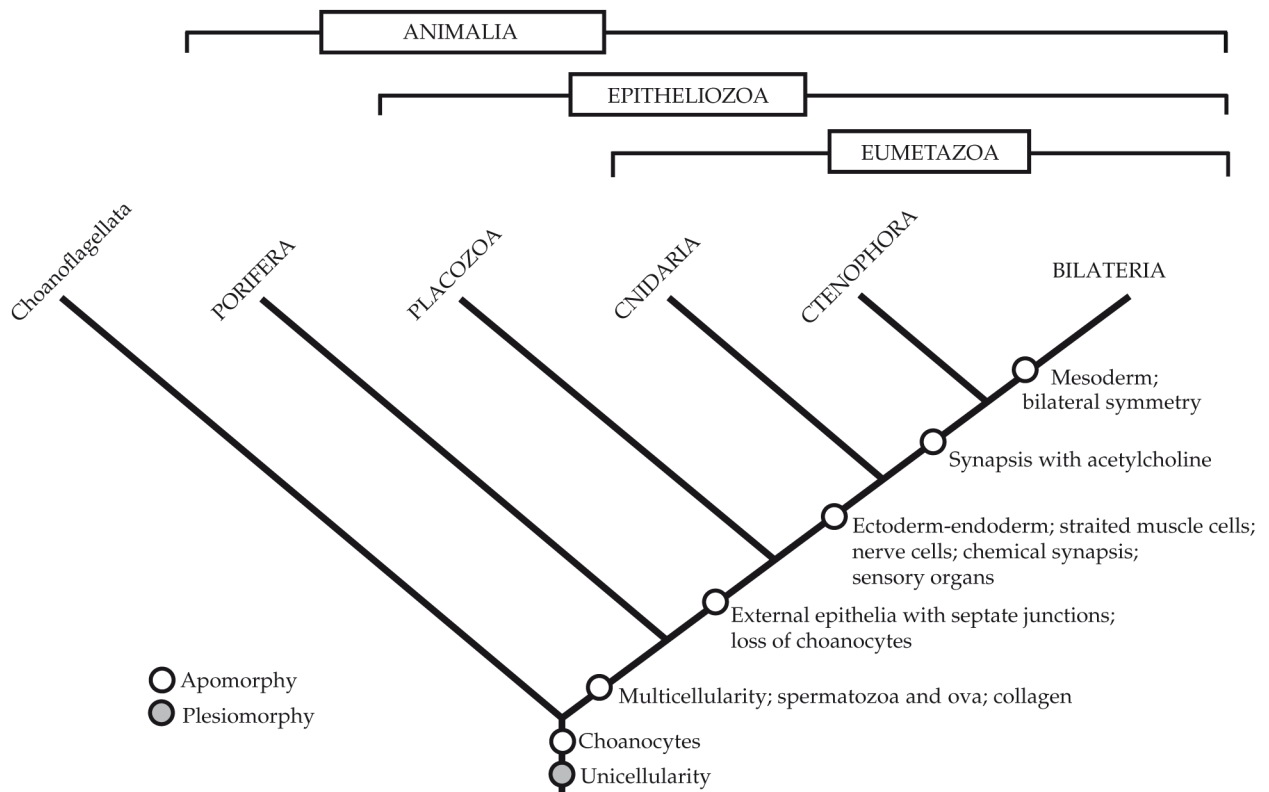
Microphage: Organism that feeds on very small particulate matter (approximately 1 μm).

MicroRNA: Short length single-stranded RNA (between 21 and 25 nucleotides) that has the ability to regulate expression of other genes through various processes.

Opisthokont: Monophyletic group of eukaryote organisms including animals (metazoa), fungi, and choanozoans (choanoflagellates and several other groups of unicellular “protists” related to animals and fungi).

Phylogenomic: Reconstruction of a phylogenetic tree based on the combined analysis of information obtained from many genes or complete genomes.

Protein domain: Part of the sequence and the structure of a protein that can develop, function, and exist independently from the rest of the protein chain.

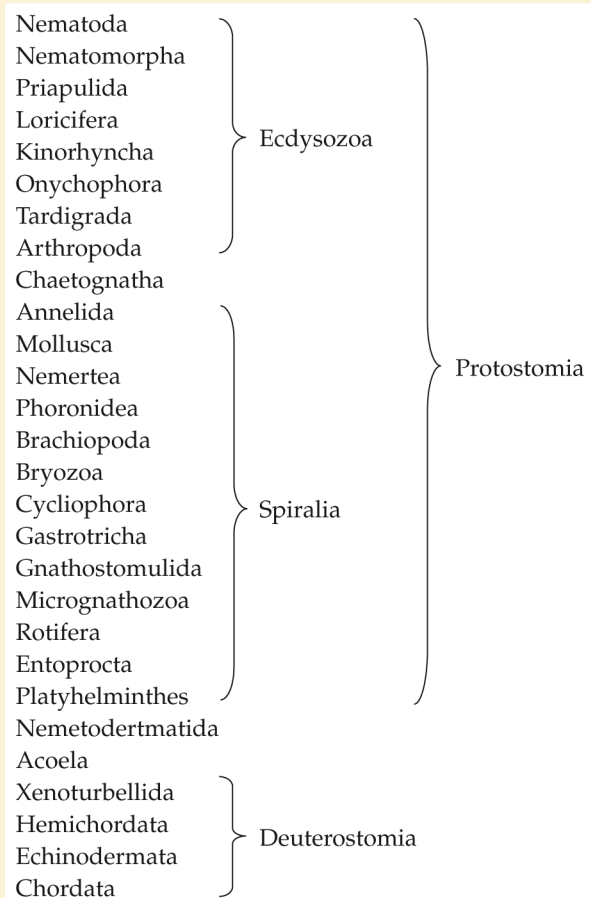


“Radiata” and Bilateria

The terms “Radiata” and Bilateria are used to designate the taxa that include diploblastic and triploblastic animals, respectively, and refer to that fact that the anatomical archetypes of cnidarians and ctenophores exhibit radial symmetry with respect to an oral–aboral axis, while triploblastic organisms exhibit bilateral symmetry, with an antero–posterior axis and a dorso–ventral axis. The Radiata form a paraphyletic group with respect to Bilateria (see the Tree of Metazoans). Bilateria include at least three major clades that are well founded on the basis of morphological and molecular data (see Figure). These clades correspond to:

1. Deuterostomia (Chapter 37): animals in which the embryonic blastopore forms the adult anus, while the mouth appears as a neof ormation.
2. Ecdysozoa (Chapter 24): animals that molt their integument (arthropods, nematodes, tardigrades, and priapulids, for example) and in some cases have a ring-shaped brain around the pharynx (Cycloneurallia).
3. Spiralia (Chapter 20): animals that feed through a structure called a lophophore (for example: brachiopods, bryozoans, and phoronids) or have a spiral segmentation and trochophore larva (for instance, annelids, molluscs, echiurans, sipunculids, nemerteans, platyhelminthes, etc.), as well as several other groups lacking both lophophore and trochophore, but which have been placed in this group on the basis of strong molecular support.

The members of clades 2 and 3 are considered to be Protostomia (Chapter 19) or animals in which the embryonic blastopore forms the mouth in adults.



Bilateria groupings. This book describes the following clades: Deuterostomia and Protostomia (Spiralia or Lophotrochozoa + Ecdysozoa).

Characteristics of Metazoan Genomes

The genomic information of animal cells consists of the DNA contained in the nucleus and in the mitochondria. Nuclear DNA is associated with proteins and is structured in the form of chromosomes, which generally appear in pairs. Mitochondrial DNA is also associated with certain proteins, although these differ from the nuclear proteins and typically (but not in all animals) form a circular molecule.

In metazoans there is no clear correlation between the complexity of organisms and the quantity of nuclear DNA per cell. The human species has a genome of 3.3×10^9 bp, which is smaller than that of amphibians of the genus *Salamandra* (10^{11} bp) and larger than that of the cnidarian *Nematostella vectensis* (4.5×10^8 bp). There is also no apparent relationship between the quantity of DNA and the number

of genes. The human genome contains around 25,000 genes, a significantly lower number than that of certain actinopterygian fishes (the *Takifu gurubripes* pufferfish has 38,000 genes) and only slightly more than that of the nematode *Caenorhabditis elegans* (19,099 genes). The number of chromosomes also varies dramatically in metazoans, with no well-defined pattern. For example, the diploid chromosome number for mammals ranges between 6 and 92 chromosomes, depending on the species. It is illustrative that a similar range of variation is also found in the ant genus *Myrmecia*, whose diploid number ranges between 4 and 76, depending on the species. Certain actinopterygians, such as sturgeons, have up to 276 chromosomes, many of which are microchromosomes. The sponge *Suberites domuncula* possesses 32 chromosomes, which is a larger count than that of certain marsupial mammals and placozoans (6 pairs) and similar to that of several sauropsids.

Porifera

Porifera (sponges) is a phylum that includes approximately 8521 living species, most of them marine. The phylum is subdivided into four classes (Homoscleromorpha, Hexactinellida, Calcarea, and Demospongiae), the phylogenetic relationships of which are a matter of intense debate. Demospongiae represent around 83.8% of living sponges, Calcarea 8%, Hexactinellida 7.2%, and Homoscleromorpha 1%. Sponges of the class Hexactinellida have a syncytial organization, while the rest have a conventional cellular structure. The anatomical archetype of a sponge is that of a sessile filter-feeding animal, with a vase-like or oblate body crossed by a system of canals that communicate to the outside at both ends, and through which a current of environmental water flows. This flow transports the bacterioplankton and dissolved compounds that nourish the sponge while providing oxygen and eliminating products of excretion and digestion. It also functions as a vehicle for the entry and exit of gametes and embryos. The histological archetype of a sponge consists of two epithelial layers of cells (pinacocytes), an external layer that forms the wall of the body, and an internal layer that forms the wall of the aquifer canals. The canals include chamber-shaped expansions, in which the walls are coated with pseudocylindrical cells possessing a flagellum surrounded by a collar of microvilli at the distal pole. These cells, called choanocytes, phagocytose bacteria from the water passing through the chambers; they are the most distinctive feature of the phylum Porifera. Between the epithelium of the canals and the external epithelium, there is a mesenchyme-like zone (called a mesoglea or mesohyl in Demospongiae, Calcarea, and Homosclero-

morph, and a mesolamella in Hexactinellida) that is rich in collagen and is populated by different groups of amoeboid cells that wander at low densities. These cells perform functions such as the production and maintenance of the intercellular matrix, defense and immune response, transporting food and gametes, the production and assembly of skeletal material, replacing aged cells, elimination of metabolic wastes, and so on. This archetypical organization is slightly different in the class Hexactinellida, which has syncytial epithelia, and also in some genera of carnivorous demosponges that have lost both their aquiferous system, and the choanocytes. In the latter group, the archetypical sponge feeding based on pumping ambient seawater through the aquiferous canals and engulfing the suspended picoplankton by means of the choanocytes has been replaced by a novel feeding system based on hook-like spicules arranged at the body surface to entrap microinvertebrates, which are subsequently digested extracellularly with the cooperation of symbiont bacteria. Sponges reproduce asexually by fragmentation, budding, or producing gemmules, and sexually by externally- or internally-fertilized gametes. The spermatozoa are generally primitive, lacking an acrosome. Nevertheless, the group has recently been described to include highly modified spermatozoa equipped with an acrosomal complex with perforatorium (see Figure 16.5D,E). The zygote, whether developed internally or externally, undergoes complete segmentation (even or uneven, depending on the group) and develops into a **lecithotrophic larva** by means of an embryonic development that varies depending on the group (see Figure 16.6).

With respect to the mitochondrial genome, there are noticeable differences between early divergent metazoans and Bilateria. In Bilateria, the mtDNA organizational pattern is relatively similar in all groups: a comparatively small genome (approximately 16 Kb) that is highly compacted and virtually lacking in introns as well as in regulatory and spacer sequences between the genes, which typically number 37. In addition and with few exceptions, the mtDNA of Bilateria is characterized by its own translation-initiation codons, highly modified structures for transfer RNA and ribosomal RNA, a relatively high rate of evolution of sequences compared to a low rate of gene reorganization, and the presence of a single, large, noncoding control region. It should also be pointed out that in Bilateria, the mitochondria translate their proteins by means of their own genetic code, which incorporates several modifications with respect to the standard code, depending on the group. These code changes have allowed Bilateria to reduce to 22 the number of

genes needed to produce the tRNA required for decoding the possible 62 codons in the mRNA. By way of comparison, the Archaea use 46 genes, while Bacteria employ 33 and the chloroplasts of higher plants, 31.

Available information concerning the mitochondrial genome of sponges, placozoans, cnidarians, and ctenophores indicates that their mitochondrial genome exhibits major deviations with respect to the standard pattern found in Bilateria. The early divergent metazoans are characterized by a lesser degree of compaction, with several intergenic regions causing the total quantity of mtDNA to be comparatively larger, with an average of 18.2 Kbp in Cnidaria, 19 in Hexactinellida, 20.6 in Demospongiae, and 37 in Placozoa. Furthermore, these groups exhibit enormous intragroup gene variability and may contain genes that do not exist in Bilateria. For example, several cnidarians contain the gene *MutSK*, probably transferred from the nucleus, which codes for a protein that repairs *mismatch* mutations. In addition, many demosponges and hexac-

Placozoa

Placozoa is a phylum created in 1971 to group only two animal species: *Trichoplax adhaerens* (Schulze 1883, *Zoologischer Anzeiger* 6: 92–97) and *Tetroplox reptans* (Monticelli 1893, *Rendiconti Lincei* (5)II: 39–40). Nevertheless, a study of 31 specimens of *T. adhaerens* has revealed significant interindividual variation in the ribosomal DNA, suggesting the presence of at least six additional cryptic species. Placozoans are organisms measuring between 0.5 and 3 mm in diameter. They are compressed along the plane of the substrate, have an amoeboid appearance, and lack recognizable axes of symmetry. They creep in no preferential locomotive direction and feed on protozoans and benthic diatoms. Structurally, they are the simplest metazoans, as they are formed by only four or five types of cells organized in three layers: (1) one upper (dorsal) epithelium formed exclusively by T-shaped monocoliated cells with intercellular junctions sealed by septate desmosomes; (2) a lower (ventral) epithelium formed by a combination of noncoliated glandular cells full of vacuoles which secrete digestive enzymes over the substrate and cylindrical monocoliate cells that have the ability to incorporate products of external digestion; and (3) a net of syncytial fibrous cells of a tetraploid nature, which occupies the interepithelial area and which can produce pseudopods that reach the exterior by finding their way between the epithelial cells. This syncytium contains actin fibers and has a strong ability to contract, which is probably the cause of the sudden changes in placozoan body shape and the amoeboid nature of its movements. The syncytium expansions are compartmentalized by means of electron-dense discoidal structures that could be

temporary, and that in some cases are similar to the perforated intracellular perforated plates (plugs) described in the syncytial structure of hexactinellid sponges. Some authors suggest that the intermediate cells are protomuscular and protoneuronal because they have the capacity to contract and conduct stimuli; others affirm that no convincing evidence of muscle cells or nerve impulse transmission has been found in placozoans. Nevertheless, antibody labeling has demonstrated that certain yet-unidentified cells (which could constitute a fifth cell type) situated near the margin of the body contain RFamide, a neuropeptide that is characteristic of nerve cells and synapses in eumetazoa. This intermediate body region, which appears to be equivalent to the mesoglea in sponges and cnidarians, lacks any visible extracellular matrix. Usually these organisms reproduce asexually through binary fission, budding, or both. Nevertheless, the high degree of polymorphism detected in the DNA of *T. adhaerens* suggests that populations are maintained over the long term by means of sexual reproduction processes. It has been observed in the laboratory that if the specimen density increases and the condition of the matrix begins to degrade, the individuals produce oocytes from cells of the ventral epithelium, which are released when the tissue degrades. No spermatozoa have been observed, but it has been proposed that certain small, round, flagellated cells in the intermediate layer could be male gametes. It has been observed that presumably fertilized ova form a membrane similar to the fertilization membrane in other metazoans, and that they begin the first stages of segmentation, but later degenerate.

tinellid sponges include the gene *atp9*, which codes subunit 9 of ATP synthetase. There are also substantial differences in the tRNA count; for example, of the 22 genes that code tRNA in Bilateria, only two at most are present in Cnidaria and between five and 15 in Demospongiae, depending on the species. At the same time, the mtDNA of certain early divergent metazoans contains additional tRNA genes not present in Bilateria. Furthermore, it also contains type I introns and its translation (except in hexactinellid sponges) employs a minimally modified code that is identical to that of certain fungi and to that of the choanoflagellates sequenced to date. It is also interesting to note that calcareous sponges or cnidarians of the Medusozoa clade (Cubozoa, Scyphozoa, Hydrozoa) have linear mitochondrial genomes unlike those of most of Bilateria, which are circular molecules. Among the most unusual mitochondrial genomes is that of the calcareous sponges. This genome is distributed among six linear chromosomes and uses a modified code for the translation of mitochondrial proteins in which the UAG co-

don, which originally specified termination, has been reassigned to tyrosine, and the CGN codon for arginine has been reassigned to glycine. In addition, the RNA genes that code for the large and small ribosomal subunits are fragmented and distributed among several chromosomes, as has been similarly described in Placozoa. It is clear, therefore, that the transition from the diploblastic to the triploblastic state involved a general reorganization, compaction, and stabilization of the mitochondrial genome, as well as the specialization of its genetic code.

Phylogenetic Results Contrasted with Previous Classifications

The phylogenetic relationships between the early divergent metazoan groups have not yet been clearly established. The pattern of relationships varies depending on whether the phylogeny is inferred from information derived from cytological and ultrastructural studies, from rDNA genes, from mitochondrial

Cnidaria

Cnidaria is a phylum with about 11,000 living species, most of them marine. Cnidarians are divided into two classes: the first is characterized by forms that have a life cycle in which a polyp stage is followed by a jellyfish or medusa stage (Medusozoa: Scyphozoa + Cubozoa + Hydrozoa); the second includes forms with only a polyp state (Anthozoa). The morphological organization of the archetypical adult cnidarian is vasiform, with a protective external epithelium and a digestive epithelium lining the internal cavity. The orifice connecting the internal cavity with the exterior, and which derives from the embryonic blastopore, functions in adults as both anus and mouth and establishes the oral–aboral axis on which the basic radial symmetry of the group is defined. Cnidarians are characterized by having cnidocytes in their epithelia, a cell type harboring an organelle called a nematocyst, which is the most distinctive feature of cnidarians. The nematocyst is a capsule loaded with a toxic fluid, which is connected to a modified cilium (cnidocilium). The nematocyst wall is invaginated at a point, in the form of a hollow filament that is coiled inside the capsule. When the cnidocilium makes contact with a foreign organism (typically prey or an aggressor), the filament is violently disinvaginated such that it pierces the other organism and injects the poison. The epithelia of cnidarians are consistently characterized by sealing junctions (septate and belt desmosomes) between their cells, as well as a conspicuous basement membrane. Between the external epithelium and the internal epithelium is a very thin

mesoglea that includes epitheliomuscular cells, as well as smooth and striated muscle cells in the medusa stage of some groups. Cnidarians have sensory cells that form organs such as ocelli and statocysts, and they also have nerve cells organized in plexuses in both in the larva, the polyp stage, and the medusa stage. The nervous system is intraepithelial and is formed by networks of neurons that can be condensed at certain points, forming rings and ganglia. The neurons can also be merged to form neural syncytia. The interneuronal and neuromuscular junctions are equipped with gap junctions formed by innexins to establish electrical synapses in addition to the chemical ones mediated by neurotransmitters (FMRFamide, serotonin, and noradrenaline) and can be either bidirectional or unidirectional. Acetylcholine has been detected in the group, but not in connection with synapses. Cnidarians reproduce asexually by budding of the polyp or the jellyfish. Sometimes the buds are not released and remain joined to the parent, forming colonial structures. Fertilization is typically external, but in some species there is internal development. Segmentation produces a hollow or solid blastula, depending on the group. The embryo develops by means of a variable gastrulation process (emboly, unipolar and multipolar ingression, delamination, epiboly), depending on the group and the blastula. Finally, a ciliated larva is formed that can be planktotrophic or lecithotrophic, but always includes anatomical outlines of the ectoderm, the endoderm, and the gastric cavity of the adult.

genomes, from families of genes for nuclear proteins (e.g., Hsp70, Hsp90, α -tubulin and β -tubulin, elongation factors, signaling proteins, adhesion proteins, genes from several superfamilies of development transcription factors, housekeeping genes, EST collections, etc.) or from comparison of the few available complete genomes.

The hypothesis that has been most widely represented in cytological–morphological phylogenies over the past two decades proposes that sponges constitute the first diverging group of metazoans (see Figure 16.1) and that placozoans are the sister group of eumetazoa (cnidarians, ctenophores + triploblastic organisms; see Chapter 17). The basal position of Porifera was founded on the belief that sponges lack the following: (1) an embryonic ectoderm and endoderm comparable to those of other animals, (2) true sealing epithelia, and (3) the capacity to generate behavioral responses consistent with the absence of muscle (effector), sensory, and nerve cells. Placozoans also lack muscle and nerve cells, as well as a basement membrane in their epithelia, but unlike Porifera, they have sealing junctions between their external epithelial cells.

The basal position of sponges in the Tree of Metazoans was corroborated by the early molecular phylogenies based on ribosomal RNA (mostly rRNA 18S). However, such phylogenies produced controversial results with respect to the position of other groups, identifying Ctenophora as a sister group of Cnidaria + Bilateria (Figure 16.2A,B). In addition, placozoans have frequently been identified as a sister group to the cnidarians or even a subset of that group (close to Medusozoa), thus suggesting that this phylum could have arisen from the simplification of the medusa stage of an ancestral scyphozoan. Some of those early rDNA analyses, depending on the number of taxa considered and the sequences selected, also suggested that placozoans could be a sister clade to all Bilateria or to Cnidaria + Bilateria. Now it is questionable whether analyses of rDNA alone can resolve the derivation of the deepest metazoan nodes. It has been discovered, among other problems, that the phylogenetic signals of the rRNA chains in the large subunit (LSU) and the small subunit (SSU) conflict in such a way that the inferred phylogenies vary depending on whether the tree is established on the basis of the SSU sequences

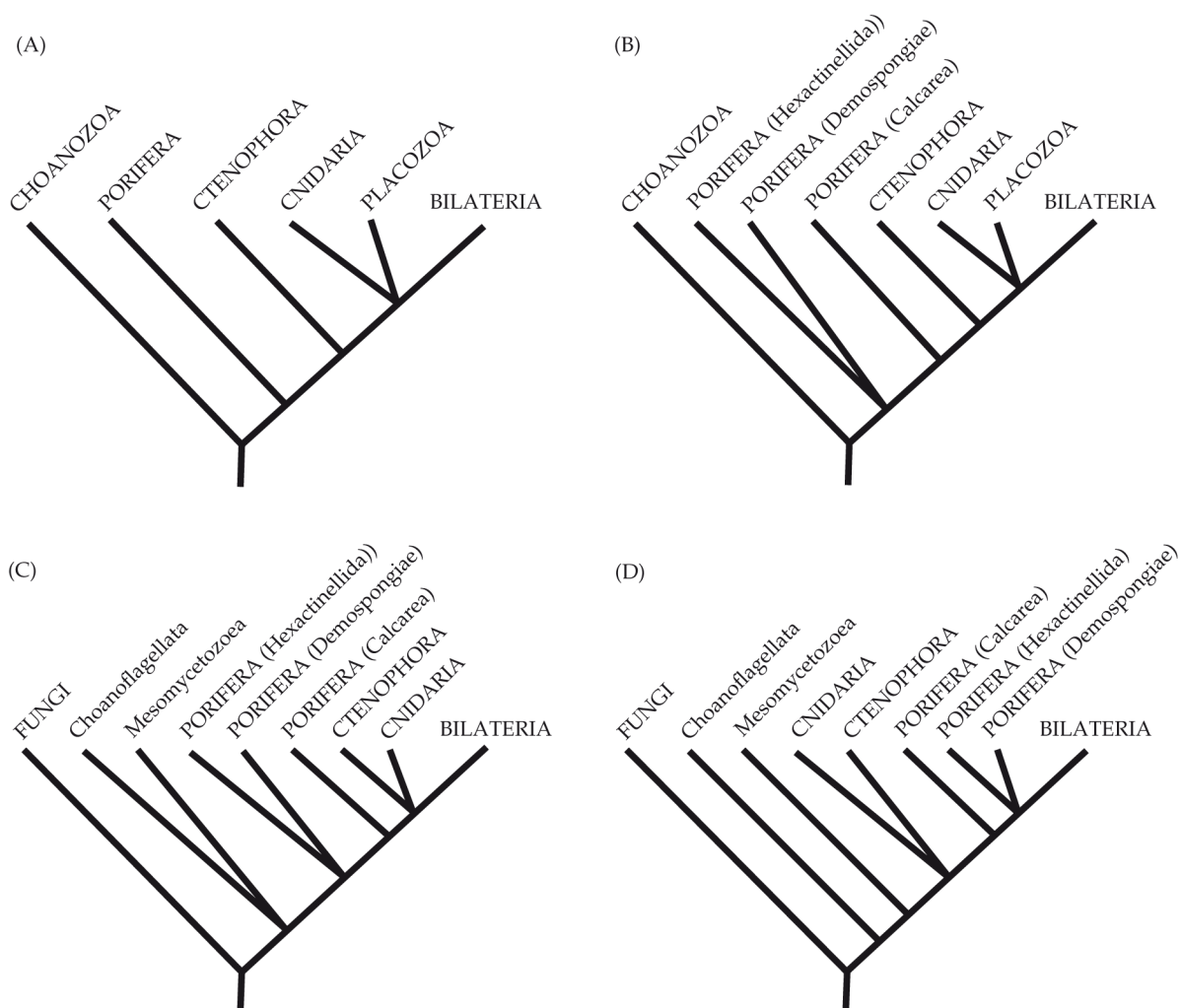


Figure 16.2 (A) The metazoan phylogeny most commonly proposed by early ribosomal DNA studies with sufficient representation of taxa and sequences. (B) The phylogeny most commonly proposed by the first DNA studies that identified a paraphyletic relationship for the 3 classes of the phylum Porifera recognized at that time. In both trees, the outgroup “Choanozoa” represents Choanoflagellata, Mesomycetozoea,

Ministeriida, Corallochytrium, and Cristicoidea. Differences in the pattern of phylogenetic relationships when inferred from genes in the small (C) and large (D) ribosomal units. The phylum Placozoa was not taken into consideration in these analyses, but Fungi and two classes of “Choanozoa” were used as outgroups.

(**Figure 16.2C**), the LSU sequences (**Figure 16.2D**), or a combination of both.

The analysis of complete mitochondrial genomes, which took place after the majority of studies on ribosomal genes, suggests phylogenetic relationships that are different from those proposed by rDNA analyses and also from those inferred from morphological data. The majority of phylogenetic trees based on amino acid sequences from mitochondrial genomes agree in their positioning of placozoans (and not sponges) at the base of the Tree of Metazoans and the hexactinellid sponges as a sister clade to Bilateria (**Figure 16.3A,B**).

The validity of using the phylogenetic signal of mtDNA alone for resolving the relationships between the early metazoans is currently under discussion, due to the immense intragroup variability that is be-

ing uncovered among early divergent metazoans. The great number of singular characteristics found in the mitochondrial genome of calcareous sponges has already been noted. The hexactinellid sponges also exhibit characteristics in their mtDNA that are very similar to those of Bilateria and surprisingly different not only from the rest of the known sponges but also from other early divergent metazoans. The hexactinellid sponges and Bilateria share almost all of their protein-coding genes; the presence of a single, large noncoding control region; a similar modification of the genetic code for the synthesis of mitochondrial proteins; and highly derived genetic structures for tRNA and rRNA, including a characteristic secondary structure for one of the tRNAs of serine. At first glance, the similarities between the mtDNA of hexac-

Ctenophora

Ctenophora is an exclusively marine phylum that comprises about 150 living species. They are carnivorous organisms, basically planktonic, which move by beating eight rows of multiciliated cells (ciliated combs) distributed over the surface of the body. Their general organization is similar to that of the cnidarians, with an external epithelium of ectodermal origin that includes glandular and characteristic adhesive cells (colloblasts) for capturing prey, and a gastric endodermal epithelium in the form of a blind sac that communicates to the exterior through a single orifice, which functions as both mouth and anus. It is assumed that the archetypal body plan is an oblate form, with two tentacles and biradial symmetry in the oral–aboral plane and that of tentacles, which are equipped with colloblasts. Nevertheless, there are tentacleless species, also flat, benthic species, and even a sessile species, which architecturally separate from the archetype. Between the two epithelia, which are made up of cells with specialized junctions (septate and gap) and basement membranes, there is a mesoglea, with muscle cells organized in a subepithelial parietal musculature as well as fascicles of syncytial muscle cells embedded in the mesoglea. Most muscle cells are smooth, but striated muscle has also been reported in some species. A polygonal network of nerve cells innervates the epithelia, the ciliated combs, the muscle cells of the mesoglea, and the tentacles (when existing), and connects to an apical ganglion situated immediately below a statocyst-type sensory organ. The nerve cells are

of a primitive (isopolar) type in which any part of the membrane can establish a synapse with another neuron or an effector. Nevertheless, and although RFamide has been detected, synapsis seems to function basically with acetylcholine, as is typical with triploblastic organisms; unlike in cnidarians, bilaterians and, at least a sponge, serotonin has not been detected. Interneuronal and neuromuscular gap junctions have also been observed. Ctenophores are typically hermaphrodites, having clearly differentiated gonads formed from the endoderm, which open to the exterior through gonopores. In some cases, the reproductive organs become more complex, and it is possible to differentiate accessory cavities, such as incubation chambers and seminal receptacles. The spermatozoon, although its head is round, has a true acrosome with a perforatorium. Fertilization is external, with polyspermy. Segmentation is total but uneven, forming an embryo with **macromeres** and **micromeres**, which have a predetermined location in the adult. Gastrulation is typically produced by cell invagination, and eventually the micromeres give rise to the ectoderm, the associated glandular cells, the apical sensory organ, the nerve cells, the muscle cells, and part of the mesoglea, while the macromeres give rise to the gastric cavity and associated structures. The embryogenesis typically produces by direct development a free-swimming stage (cydippid) that shows the basic architecture of the adult body plan; that is, there is no known larval stage.

tinellid sponges and of Bilateria might suggest that sponges constitute a polyphyletic phylum (see Figure 16.3B). To date none of the mitochondrial genes of tRNA that are present in demosponges and placozoans can be found in hexactinellid sponges, which in addition possess only those tRNA genes found in Bilateria. Additionally, demosponges, placozoans, and cnidarians have conventional tRNA instead of derivatives. These features together could be used to suggest Porifera as a possible polyphyletic phylum, with Hexactinellida being a possible sister clade to Bilateria (see Figure 16.3B). Nevertheless, the scenario provided by mtDNA turns out to be even more complex, because the mitochondrial genome of all demosponges and hexactinellida sequenced to date contains a common characteristic, the *atp9* gene, which does not appear in any other metazoan group and emerges as a major synapomorphy of Porifera, thus weakening the argument for the group as being polyphyletic. In the demosponge *Amphimedon queenslandica* and in the calcareous sponge *Clathrina clathrus*, this gene appears to have been recently transferred to the nucleus—a transfer that could contain an important phylogenetic sig-

nal if it can eventually be demonstrated that the gene has been inherited by Cnidaria and the other animals.

In summary, the available information on the mitochondrial genome of early divergent metazoans suggests the presence of accelerated genetic variability in these groups with respect to Bilateria. For example, it is worth mentioning that in two sponges of the same family (Plakinidae), one of the species (*Oscarella carmella*) contains the largest number of genes (44) known in an animal mitochondrial genome to date, while the other (*Plakortis angulospiculatum*) contains fewer than half that number (20 genes). Such variability between sister genera also suggests caution when using any of the features of the mitochondrial genomes to infer the phylogeny of the deepest metazoan nodes. The size of the mtDNA and its genetic contents are also seen to be less informative than initially expected, because certain evidence suggests that a parallel loss of genes (homoplasy) has occurred in markedly distant groups. In view of these general considerations (and in the absence of further data on calcareous sponges and ctenophores), a conservative phylogenetic analysis of the mitochondrial genome can only translate into a poly-

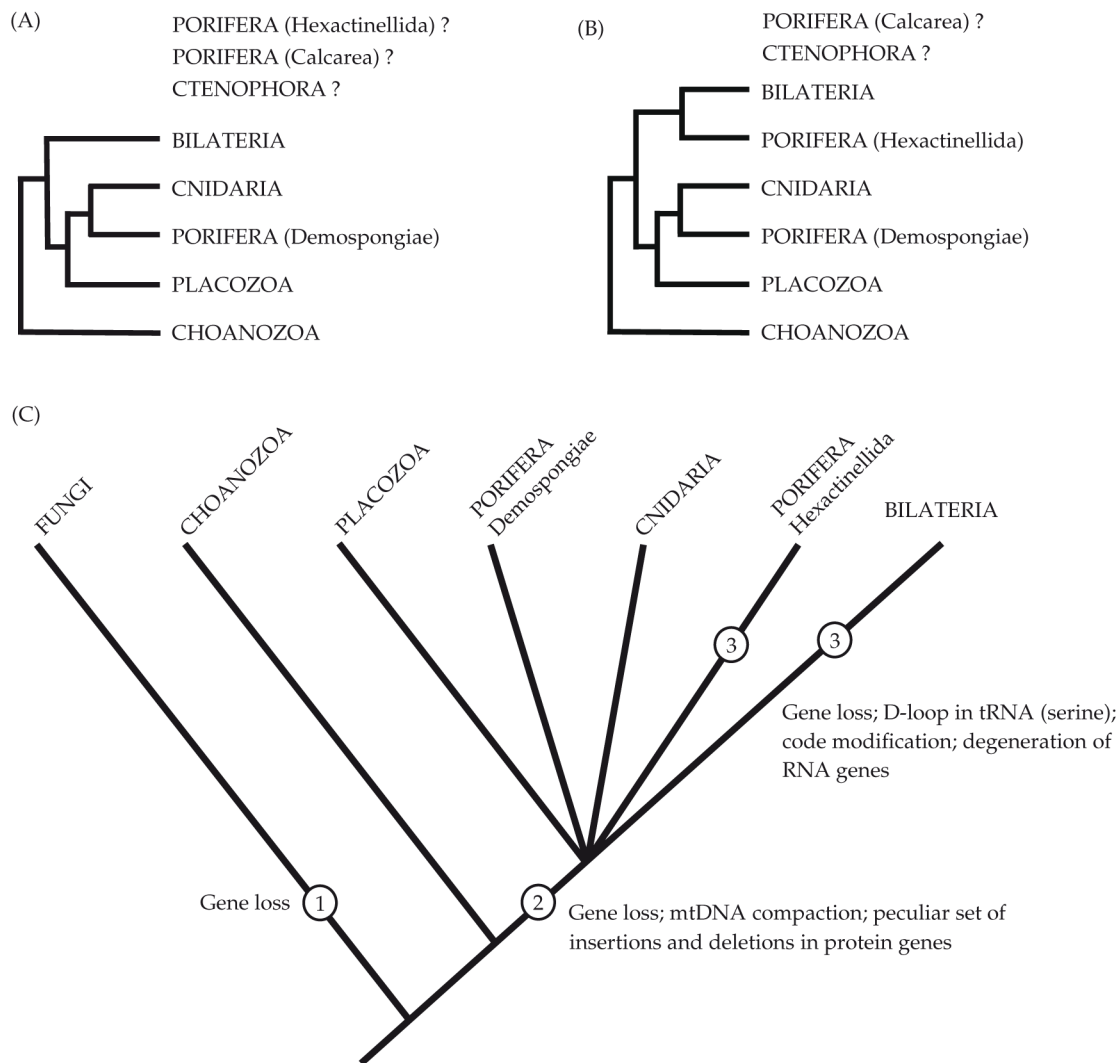


Figure 16.3 (A) Maximum likelihood tree, obtained in 2006 from amino acid sequencing of complete mitochondrial genomes for several early divergent metazoans, among which only one of the three classes of Porifera provided analyzable data. Also note the absence of analyzable data for Ctenophora at that time. (B) Maximum likelihood tree diagram, obtained in 2007 from amino acid sequencing of complete mitochondrial genomes for several basal metazoa, in which two Hexactinellida sponges were included for the first time. Note that Hexactinellida is shown as a clade completely isolated from

the rest of Porifera but being sister to the clade of bilaterians. Analyzable data for sponges of the class Calcarea and for Ctenophora were still lacking at that time. (C) A conservative phylogenetic tree derived from an analysis of the information available on the evolution of mtDNA in Metazoa in 2007. Note that in the absence of data for calcareous sponges and ctenophores at that time, the phylogenetic signal contained in the mitochondrial DNA did not make it possible to resolve the relationships among early divergent metazoans.

tomic tree that is incapable of resolving the relationships among the early divergent metazoans (**Figure 16.3C**).

The problems arising from the use of rDNA and mtDNA have led to the view that the reconstruction of phylogenetic relationships between clades with divergences as deep as those found among the early divergent metazoans (more than 600 million years, approximately) could be resolved more efficiently by comparing nuclear genes that are highly preserved and that can be clearly identified as orthologous. With this aim in mind, some authors have explored tran-

scription factor–coding gene families along with several proteins that are fundamental to the operation of multicellularity in animals, such as the proteins in the main intercellular signaling pathways, as well as those related to adhesion, stress, intercellular matrix production, cell cycle (e.g., housekeeping genes), the neurotransmission process, and so on. In most cases, components of these gene families have been found in numerous animal phyla (both basal and derivative), and even among choanoflagellates and other **opisthokonts** relatively related to metazoans. Such findings indicate that the components of several gene families

that were assumed to be evolutionary innovations of metazoans indeed predate the divergence of the first diverging metazoan, and lack any explanatory power to resolve the phylogeny of the early animals (see *Evolution of Characters*, pp. 194–198). In some cases, as when using a set of housekeeping genes, sponges were placed at the base of the tree, but making a markedly paraphyletic group rather than a clade (Figure 16.4A).

A phylogenetic signal partially consistent with traditional morphological views emerged from a **phylogenomic** study by the Hervé Philippe group (2009), which

analyzed partial sequences (EST) of 128 nuclear protein genes. In the inferred phylogeny, sponges are shown to be the earliest group diverging from the branch of metazoans, followed by the placozoans (Figure 16.4B). Cnidarians and ctenophores may have emerged later, but as a subunit that would correspond to the classical concept of “coelenterata.” From this perspective, cnidarians and ctenophores would be phylogenetically related at the same level with respect to Bilateria.

Since 2008, several phylogenomic approaches have also suggested either Ctenophora or Placozoa to be the

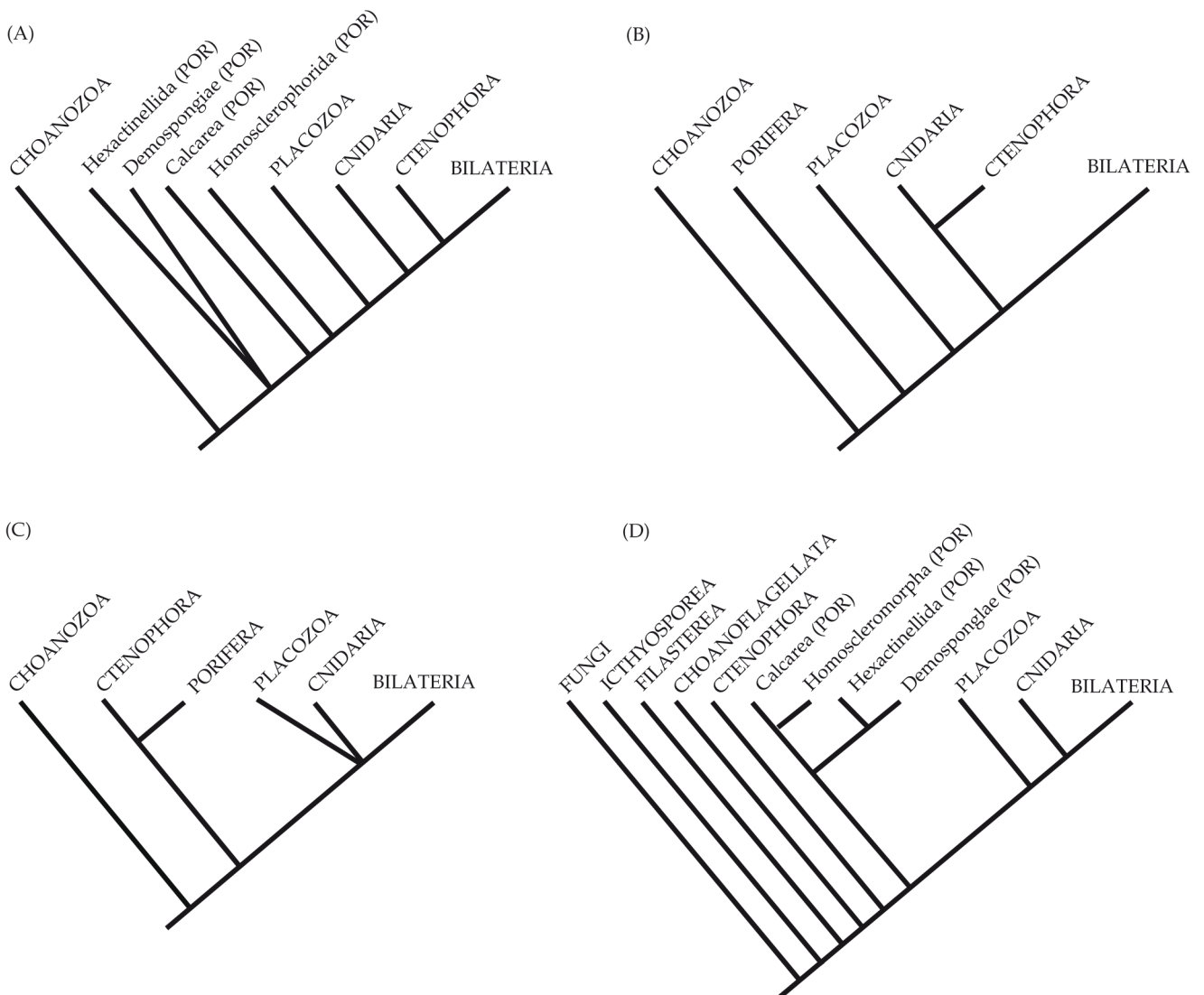


Figure 16.4 (A) Summary of the phylogenetic relationships of a phylum Porifera (POR) with a paraphyletic structure and the rest of the lower metazoans and bilaterians, as proposed based on a maximum likelihood Bayesian analysis of constitutive (housekeeping) nuclear genes. (B) Summary of the phylogenetic relationships for the early divergent metazoans inferred from a phylogenomic study based on data from 128 nuclear genes and with a significant representation of species in each of the invertebrate phyla included in the analysis. (C) Summary of the Bayesian analyses of 13 complete animal ge-

nomes (19.6% missing information), including the ctenophore *Mnemiopsis leidyi*, supporting a Ctenophora + Porifera clade as sister group to all other Metazoa (sensu Ryan et al. 2013). (D) Summary of the phylogenetic relationships inferred by Ryan et al. (2013) from the maximum-likelihood analyses of the EST set that included partial genomic data from 58 animals (242 genes; 104,840 amino acid characters; 64.9% missing data). Ctenophora are displayed as the first diverging animal group, followed by a monophyletic Porifera (POR); Cnidaria were consistently well supported as the sister clade to Bilateria.

earliest diverging animal clade, instead of sponges. Those conflicting metazoan phylogenies, which also differed from each other in their taxon and gene sampling and in models of amino acid substitution, were subsequently revised and found to be affected by contaminations, alignment errors, and reliance on suboptimal evolutionary models, which could account for their shocking analysis outcome. Nevertheless, a more recent comparative analysis by Ryan and co-workers (2013) of amino acid positions and gene content in the first complete genome of a ctenophore (i.e., *Mnemiopsis leidy*) insisted on the hypothesis that ctenophores are the first animal clade diverging from the metazoan root. Neither Bayesian nor maximum-likelihood analyses of the ctenophore genome data supported the morphologically-postulated concepts of eumetazoa (see Figure 16.1 and Chapter 17) or “coelenterate” (i.e., a cnidarians + ctenophores clade; see Figure 16.4B). Rather, the Bayesian analyses supported an early diverging clade of Porifera + Ctenophores (Figure 16.4C), while the maximum-likelihood analyses preferentially supported a placement of the Ctenophora as sister group of all other Metazoa, followed by Porifera (see Figure 16.4C). The four classes of the Porifera were consistently found to make a monophyletic unit. Interestingly, despite the choanocyte-lacking Ctenophora being placed at the base of animal tree, this analysis also positioned choanoflagellates as the “protist” group closest to metazoans (see Figure 16.4C). Such a tree topology forces one to consider a completely new scenario of gains and losses for choanocyte-like cells, sensory cells, nerve cells, muscle cells, neurotransmitting compounds, and so on in the deep nodes of the animal tree (see sections *Evolution of Characters and Evolutionary Tendencies*, p. 200).

In summary, the most recent molecular studies on ctenophores complicate further the evolutionary scenario of early animals. A definitive resolution of the phylogenetic relationships may require not only considering extensive collections of slowly evolving genes and a broad representation of the taxa involved, but also new and more realistic computational models, as concluded from a recent study by Nosenko et al. (2013). These models should incorporate the ability to vary the rates of evolution over time as well as the substitution types in order to be able to control the artifacts arising from the long branch attraction (LBA) effect and the high levels of substitutional saturation in proteins, particularly in noncoding proteins. To efficiently retrace the intricate evolutionary paths that occurred during the early diversification of animals, it may also be necessary to incorporate new markers with sequence-independent phylogenetic signals. Such markers would include the architecture of **protein domains**, the order of genes, gene fusions, duplications, deletions, genetic code variations, intron positions, and, ideally, involving comparisons of entire genomes. Altogether,

it would result in an increased ability to differentiate subcollections of data having contradictory phylogenetic signals, which would in turn increase the reliability of the phylogenetic inference process.

Evolution of Characters

Epithelialization, neuralization, and other derived features

Sponges and placozoans, unlike cnidarians and ctenophores, do not seem to have complex sealing epithelia, nerve cells, or muscle cells, although they do have certain genetic components related to such cellular systems. Due to the lack of these cellular elements, these groups have traditionally been considered to be structurally simpler and evolutionarily older than the rest. Nevertheless, recent ultrastructural studies are highlighting the presence in sponges and placozoans of a mosaic of characteristics in which simple features (which would represent the ancestral condition of metazoan characters) coexist with other relatively complex features (derived conditions) that had traditionally been assumed to be absent in the phyla. In adult sponges, for example, it has been possible to identify epithelia with basement membranes (Figure 16.5A) and that incorporate type VI collagen, laminins, and septate junctions (Figure 16.5B) with claudin, as well as epithelia that are able to secrete skeletal elements (Figure 16.5C). In addition, in sponge larvae, there are examples of epithelia that consist of multiciliated cells and others that incorporate photoreceptive cells to build a photosensorial organ-like structure.

Many derived cytological and histological characters are also found in connection with the reproductive process of sponges—for example, the presence in certain groups of highly modified spermatozoa equipped with an acrosomal complex with perforatorium, condensed chromatin in spiral form, and long striated ciliary roots (Figure 16.5D–F). In addition, a varied pattern of cell migrations and reorganizations during the embryogenesis of several groups of sponges (Figures 16.6 and 16.7) has been described, and is believed to be a process clearly homologous to gastrulation in other animals (Maldonado 2004). Furthermore, there are certain cellular elements and compounds in sponges and placozoans that are related to those of the neuromuscular systems of higher animals. For example, several cell types of sponges show a relative contractile capacity and contain myosin or cytoskeletons of actin filaments, or both. Acetylcholine and cholinesterase have been reported (and not without controversy) in some of these cells. Serotonin has also been found in larvae and juveniles of at least a sponge species. Nevertheless, there is no evidence to date that these substances, which

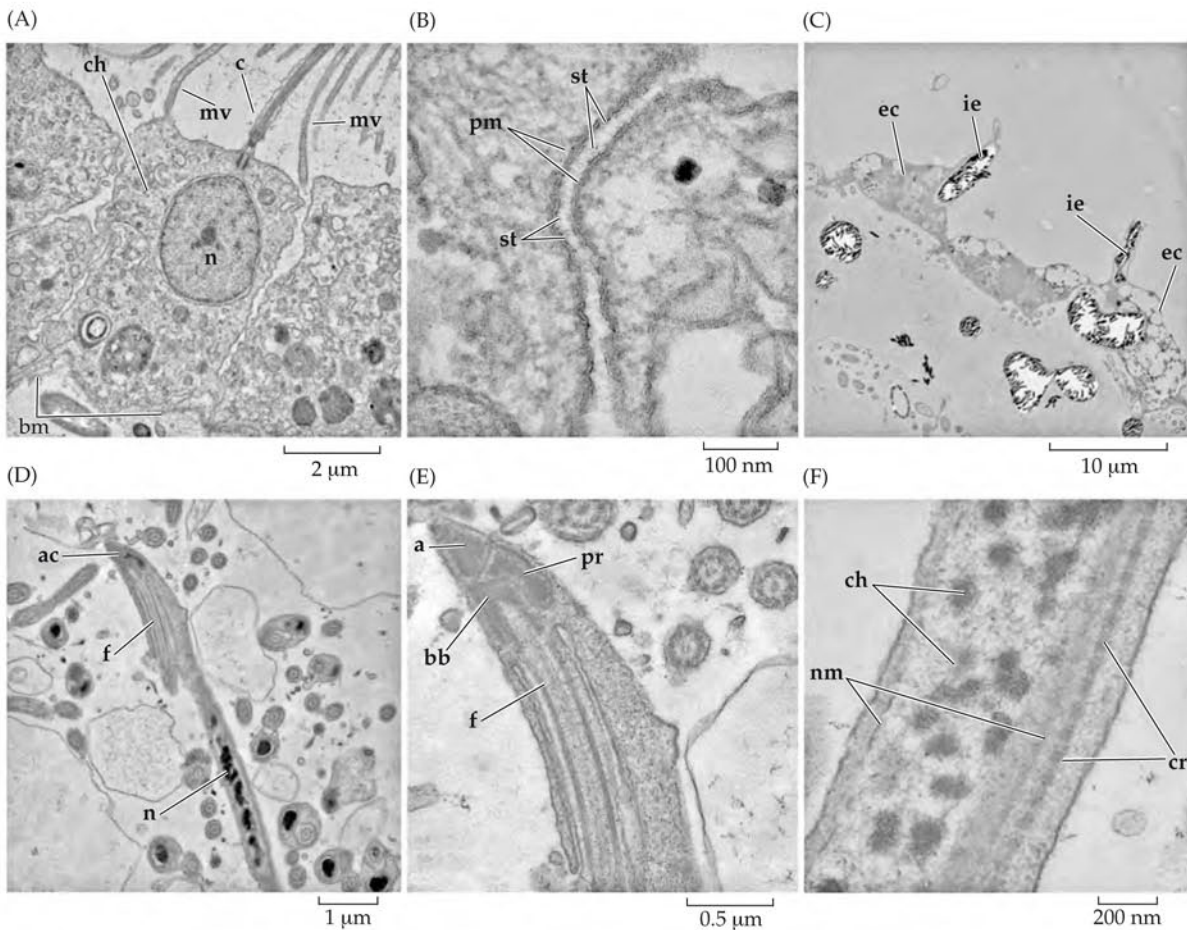


Figure 16.5 (A) Epithelium of choanocytes (ch) from *Corticium candelabrum*, a homosclerophorid demosponge in which a subepithelial basement membrane (bm) is observed. Note that the view of choanocytes, in longitudinal section, shows their nuclei (n), the cilium (c), and the collar of microvilli (mv). (B) Detail of the *C. candelabrum* larva epithelium, where a darkening of the plasmalemma membrane (pm) is seen in the contact area between two adjacent epithelial cells. Note that the intercellular space in the contact area between membranes contains material in the form of septa (st). External septate epithelia had traditionally been considered to be an evolutionary acquisition subsequent to the emergence of Porifera (see Figure 16.1). (C) View of the external epithelial cells (ec) of an adult *C. candelabrum*, in which intraepithelial

spicules (ie) can be observed. (D–F) Details of a spermatozoon (D,E) and a spermatid (F) of the demosponge *Crambe crambe*. Note that a flagellum (f) with its basal body (bb) is inserted in the anterior part of the spermatozoon next to the acrosomal complex (ac). The acrosomal complex is comprised of an acrosome (a) and a perforatorium (pr). The nucleus (n) is elongated, and long striated ciliary roots (cr) run next to the nuclear membrane (nm). The chromatin is coiled into a spiral inside the mature spermatozoon (D) while it is granular (ch) in the previous spermatid state. The acrosome with perforatorium and the helical compaction of the chromatin had traditionally been considered to be evolutionary acquisitions subsequent to the appearance of sponges, placozoans, and cnidarians (F).

function as neurotransmitters in other metazoans, are involved in nerve-type intercellular communication processes in sponges. Nevertheless, significant changes in body contraction patterns of some sponges have been triggered by extracorporeal application of diverse neurotransmitters (adrenaline, nitric oxide, serotonin) and messengers of signal transduction pathways characterizing higher animals. It has been demonstrated that the syncytial epithelia of hexactinellid sponges have the capacity to conduct electrical impulses in the absence of synapses. This preneuronal conduction is far slower than that which typically oc-

curs between the nerve cells of other animals, probably because the transmission seems to be conducted by employing calcium channels, as happens in plants, rather than sodium channels, as typically occurs in neurons. It is remarkable that certain demosponge cells react by modifying the concentrations of intercellular calcium in the presence of the excitatory amino acid glutamate and in the presence of the metabotropic receptor agonists of glutamate. In these cells, a receptor protein has been cloned that is quite similar to the metabotropic glutamate receptor and to the B receptor of gamma-aminobutyric acid (GABA), two

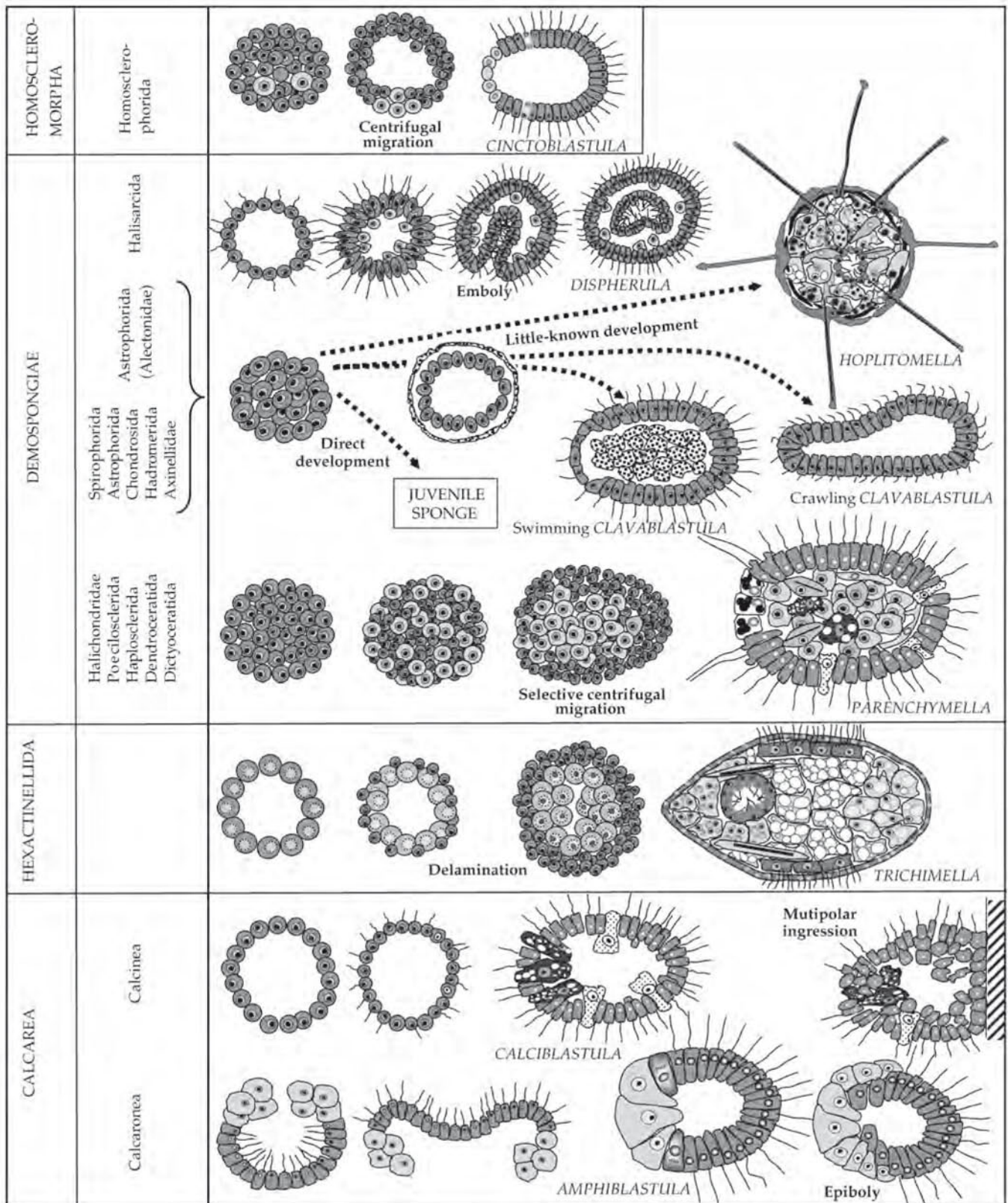


Figure 16.6 Diagram showing the different types of cell reorganizations detected during embryonic development in the different taxonomic orders of the four classes of sponges (Homoscleromorpha, Demospongiae, Calcarea, and Hexactinellida).

The embryonic reorganizations described are proposed as equivalents to the principal types of gastrulation (nomenclature in bold) known in other animals. (After Maldonado, 2004.)

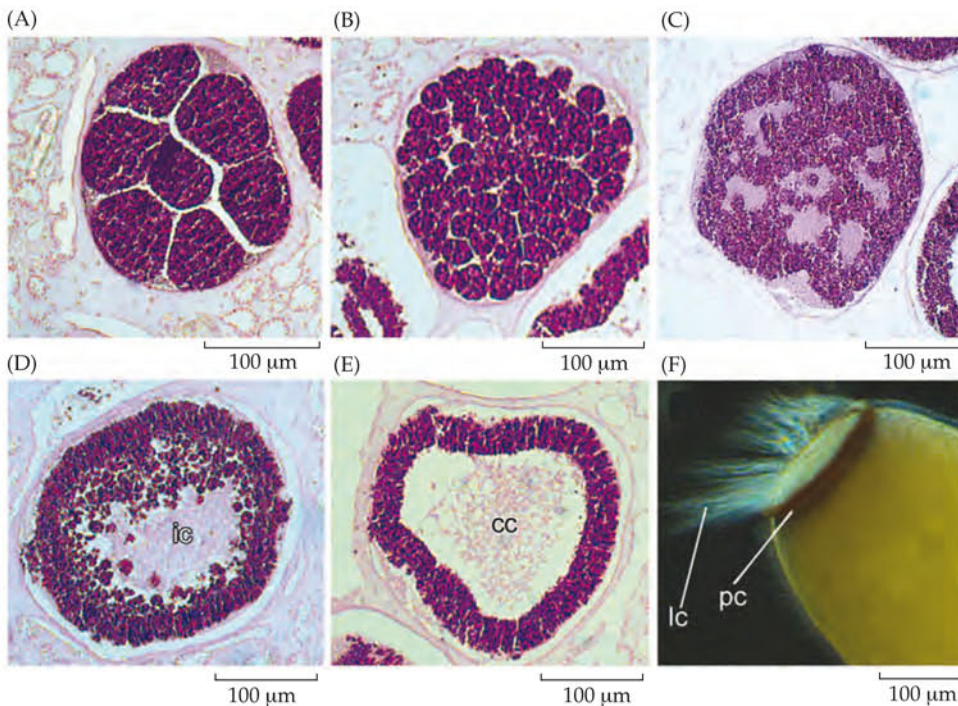


Figure 16.7 (A–E) Histological sections showing the gastrulation of a homosclerophorid sponge embryo in which a solid morula (stereoblastula) turns into a hollow gastrula through a process of migration of the inner **blastomeres** to the periphery of the embryo in order to form an inner cavity (ic) equivalent to a gastrocoele. (F) Posterior part of a larva of demosponge *Ircinia oros* showing the set of pigmented photoreceptor cells (pc) arranged in a ring-shaped photoreceptor organ that controls movement of the long cilia (lc) at the posterior larval pole in response to illumination conditions, and which serves as a rudder for the larva.

neurotransmitters involved in neuronal excitation and inhibition in higher animals. In the demosponge genome, it has also been possible to locate postsynaptic genes similar to those that code elements associated with synaptic cell membranes in neuralized animals. Some of these genes seem to be expressed in pyriform, distally monociliated cells with a proximal abundance of vesicles that appear in the epithelium of the larva and could be the evolutionary precursors of neurons or, more probably, neurosensory cells.

A case similar to that of sponges occurs in Placozoa, which also lack muscle and nerve cells, but in which immunolabeling has revealed that certain yet-unidentified cells located on the margin of the body contain RFamide, a neuropeptide that is characteristic of nerve cells in other animals. Furthermore, the sequencing of the complete *T. adhaerens* genome has revealed that there are several types of genes in placozoans that are orthologous to those expressed in eumetazoa during the formation of the nervous system (*Trox2*, *Pax-B*) and the neuroendocrine system (POU family). Despite the limited cellular diversity characteristic of the placo-

zoans, their genome also contains a comprehensive group of transcription factors that were believed to be unique to eumetazoa, many of which are involved in cellular differentiation, cellular specialization during embryonic development, gastrulation, and the early determination of a cellular germline.

Compared with sponges and placozoans, the cnidarians and the ctenophores exhibit relatively complex epithelia and obvious neuronal systems. In Cnidaria, the nervous system is subepithelial and is formed by networks of neurons that can be condensed at certain points, forming rings and ganglia. The neurons can also fuse to each other to form neural syncytia. The interneuronal and neuro-muscular junctions are equipped with gap junctions that establish electrical synapses as well as chemical synapses by means of neurotransmitters. Nevertheless, the gap channels are not formed by connexins as they are in higher metazoans, but rather by innexins, as is typical of invertebrates. Interneuronal chemical synapses are mediated by RFamide, serotonin, and noradrenaline, and they can be both bidirectional and unidirectional, the latter being the typical model for higher metazoans. Acetylcholine has also been detected in the group but not in relation to synapses. The structural complexity is even greater in the case of Ctenophora, which has traditionally caused this group to be considered as a sister group to Bilateria (see Figure 16.1), from a morphological standpoint. Among ctenophores, a mesoglea can be observed between the external epithelium (ectoderm) and the gastric epithelium (endoderm), both characterized by special intercellular junctions (septate and gap)

and a basement membrane; the mesoglea has muscle cells organized in a parietal musculature underlying the epithelia. There are also fascicles of syncytial striated muscle cells embedded in the mesoglea. A polygonal network of neurons innervates the epithelia, the ciliated combs on the surface of the body, and the muscle cells of the mesoglea, and connects to an apical ganglion that is located immediately below a statocyst-type sensory organ. The nerve cells are of a primitive (isopolar) type in which any part of the membrane can establish synapsis with another neuron or an effector. Although RFamide has been detected, the synapses seem to function basically with acetylcholine, as typically occurs with triploblastic organisms. Gap-type interneuronal and neuromuscular junctions have also been observed.

With regard to sensorialization, no sensory cells have been detected either in placozoans or in adult sponges to date, but the larva of certain demosponges has photoreceptor cells that are interconnected by cytoplasmic bridges and are organized in an annular supracellular structure that is equivalent to a larval photoreceptor organ (see Figure 16.7F). It should be noted that, although the only sensory cells known in the phylum Porifera are these larval photoreceptors, both larvae and adults react with movement in response to environmental stimuli other than light, such as contact with prey (in carnivorous sponges), contact with their own kind, lack of food, excess sedimentation, the presence of currents, the physicochemical characteristics of the substrates they are in contact with, and so on. It is also remarkable that phototactic responses have been described in placozoans in the absence of known photosensitive cells. Cnidarians and ctenophores are more sensorially complex because they have sensory cells that are organized in photoreceptor organs and statocysts, generally innervated by subepithelial nerve plexuses or by branching of the ganglia.

However, the traditional view (summarized above) of a gradual, progressive complication of nervous systems across the evolutionary gradient of sponges-placozoans-cnidarians-ctenophores has recently been challenged by suggestions that ctenophores are the earliest diverging animal group, preceding sponges. Therefore, if the well-neuralized ctenophores were the first diverging animal clade, it has to be assumed that most of the genetic machinery for a nervous system was already present in the metazoan ancestor and that a functional neuralization was secondarily lost in Porifera and Placozoa. Many, but not all, of the genes that are crucial for the neuralization of cnidarians and bilaterians already occur in the genome of the sponge *Amphimedon queenslandica*, despite sponges lacking a recognizable nervous system. Consequently, it was commonly argued that a functional nervous system

developed only when animals acquired those neural elements that sponges still lack. However, the genome of the well neuralized ctenophore *M. leidyi* both contains and lacks virtually the same neural components that are also present and absent, respectively, from the genome of the sponge *A. queenslandica*. They share the presence of the ionotropic glutamate neurotransmitter receptors, transcription factors involved in Bilaterian neural development, neural differentiation RNA binding genes, and some axon guidance genes (neurexin, semaphorin, plexin, and ephrin receptor). Additionally, they share the absence of other axon guidance genes (netrin, slit, and unc-5) and a set of synaptic scaffolding genes (regulators of synaptic signaling pathways) that characterize Cnidaria and Bilateria. This pattern of shared occurrences and absences is striking and makes it difficult to explain why sponges, unlike ctenophores, do not have a recognizable nervous system. A secondary reduction of the ancestral nervous system and muscle cells in Porifera and Placozoa emerge as a plausible explanation. Indeed, prior to the publication of the first complete ctenophore genome, it had already been suggested that, in the case of Placozoa, the known genome components of the nervous system could be the remnants of a secondary simplification process from a true eumetazoan state, or they could be coding mechanisms that are operative only in certain life cycle states that are yet unknown. Likewise, it had also been proposed that at least a part of the cytological and architectural simplicity of sponges may have been derived from secondary simplification processes (Maldonado 2004). An alternative explanation would be that both nervous systems and muscle cell systems arose independently in ctenophores and in the clade leading to cnidarians and bilaterians. This view would be further supported by the fact that virtually none of the genes involved in bilaterian mesoderm development have been found in ctenophores to date.

In contrast, when sponges—rather than ctenophores—are regarded as the first diverging animal clade, the existence of gene families in Porifera and Placozoa that are associated with the formation of the nervous system, striated muscle, complex epithelia and other derived features characterizing higher animals (i.e., eumetazoans) is hard to be explained. Often, it has been interpreted as evidence that some of the basic utensils of epithelialization, neuralization, and the multicellular functioning of eumetazoans already existed at earlier evolutionary stages, where they were used for either similar or different purposes. Under this scenario, it is generally proposed that the preexisting cellular and genetic machinery in the unicellular relatives of animals and/or Porifera and Placozoa was repurposed for new functions by means of changes in the processes of genetic regulation.

Genetics of Embryonic Development

Embryogenesis, a previously nonexistent process, emerged as an indisputable innovation of the metazoans. Studies conducted on Bilateria have demonstrated that the genes for numerous signaling pathways and transcription factors involved in the regulation of gene expression in embryonic development are well preserved. Their orthology is easily identifiable, which could turn out to be useful for inferring the relationship pattern across the early divergent metazoans. Among sponges, placozoans, and cnidarians, recent descriptions have been provided of orthologous genes (either complete or partial) that were originally believed to be limited to Bilateria (e.g., *helix-loop-helix*, *MEF2*, *FOX*, *SOX*, *T-box*, *ETS*, *REL/NF- κ B*, *bZip*, *Smad*, and several other homeobox genes including *ANTP*, *Prd-like*, *Pax*, *POU*, *LIM-HD*, *Six*, and *TALE*). Furthermore, although sponges apparently lack a predetermined germline, they exhibit several germline markers characteristic of metazoans, such as *vasa*, *nanos*, *piwi*, and so forth. These findings suggest that many of the mechanisms activated during the formation of gametes and in the embryonic development of bilaterians to produce the required cellular diversity and to establish the principal axes of body architecture already existed in the genome of the last common ancestor of the metazoans.

Certain studies that have explored the phylogenetic signal contained in four subclasses of Antennapedia-class (ANTP) homeobox genes, such as Hox genes, Parahox, EHGBox, and NK, which contribute decisively to establishing the main organization of the body of triploblastic animals (Bilateria), indicate that these subclasses of genes seem to have emerged evolutionarily in metazoans through *cis*-duplication processes. In line with the pattern observed in other superfamilies of genes, the contents of homeobox genes in the only sponge whose complete genome is known (*A. queenslandica*) are noticeably fewer than those described for Cnidaria and Bilateria. The sponge has NK genes but no Hox, Parahox, or EHGBox genes. The NK group, which had been detected previously in other sponges, forms a package of five genes with an organization similar to that observed in triploblastic organisms. This suggests that the gene package in question must have been present in the ancestor of metazoans before the divergence of Porifera, while Hox genes (existent in cnidarians and placozoans but absent in sponges and ctenophores) could have emerged later in the evolution of lower metazoans. According to this interpretation, the small number of Hox and NK genes existing in Placozoa would represent an initial stage in the increasing complexity of the homeobox system that took place after the divergence of sponges. This inter-

pretation would imply that the axial polarity (antero-posterior or oral-aboral) of the first animals could be determined by factors other than that of colinearity in the expression of Hox genes that operates in bilateria.

Nevertheless, some analyses based both on complex mitochondrial genomes and phylogenomic approximations suggest (not without controversy) that the placozoans could be at the base of the metazoans and that they could have emerged before the divergence between Porifera and all other animals (see Figure 16.3A,B). If this were true, the absence of Hox genes in sponges and ctenophores would have to be explained as a secondary loss, since at least one Hox gene should be present in the ancestral metazoan in order to explain the presence of Hox genes in extant placozoans. It is worth noting that available data for Cnidaria and Eumetazoa suggest that the ancestral metazoan immediately before the divergence between these two phyla should have between 56 and 58 homeobox genes. However, the count found in the only sponge sequenced to date, with only 31 genes, comes to less than half of the predicted figure. For this reason, some authors have proposed that this limited number of genes could have been the result of a loss of several genes of the NK family and at least one of the Hox family. In this regard, a few clear cases of gene loss have already been recognized. For example, the brachyury gene, universally employed in the embryonic gastrulation of animals, seems to have been secondarily lost in the demosponge *Amphimedon queenslandica*, while it is present in the genome of other demosponges and also in Calcarea and Hexactinellida, and even in unicellular groups that evolutionarily predate the metazoans.

It has also been recently demonstrated that the orthologues of several other transcription factors that operate in Bilateria (*Pax*, *PO*, *LIM-HD Sox*, nuclear receptor, Forkhead, *T-box*, *Mef2*, and *ETS*) are expressed during the embryogenesis of the demosponge *A. queenslandica*, in addition to the ANTP genes. A detailed phylogenetic analysis of the helix-loop-helix gene superfamily, which is also present in fungi and plants (although lacking embryonic control functions), has revealed that most of the genes present in Bilateria have orthologues in Cnidaria, while only a few families are present in the sponge *A. queenslandica*; Ctenophores and placozoans have not been studied yet. Again, the number of genes in this superfamily that appear in sponges is significantly lower than expected, according to available data on Cnidaria. Cnidarians, or at least some of the more widely studied anthozoans, such as *Nematostella vectensis*, are not simple organisms from the standpoint of embryonic development regulation. The gene contents of the transcription factors of *N. vectensis* (Cnidaria) are very similar to those of Bilateria and are about two or three times greater than those

known for sponges (*A. queenslandica*) and placozoans (*T. adherens*). The anthozoan *N. vectensis* has between 130 and 139 homeobox genes, including seven Hox genes, 14 Sox, 15 Fox, 12 WNT, 6 TGF- β , and numerous antagonists of these genes. In several gene families, *Nematostella* has more genes than those possessed by other, clearly more sophisticated, invertebrates, such as the insect *Drosophila melanogaster* or the nematode *Caenorhabditis elegans*.

Hence, in the absence of sufficient information on placozoans, ctenophores, and several classes of Porifera, it is very difficult to retrace the likely evolutionary path taken in the early divergent metazoans by the gene families involved in the control of embryonic development. In addition, there are reasonable arguments against discarding the hypothesis that certain groups (Porifera, Placozoa) could have undergone an extensive process of gene simplification (i.e., secondary losses) in their origins, while others (Cnidaria) could have undergone an expansion.

Evolutionary Tendencies

The leap toward the multicellularity of animals

Several data sources suggest that the ancestral metazoan and the extant choanoflagellates (one of the five classes of unicellular organisms in the phylum Choanozoa) derived from a common ancestor. This phylogenetic relationship between choanoflagellates and metazoans, which was initially formulated based on the extraordinary similarity of the cytology of choanoflagellates and choanocytes of sponges (see Figure 16.5A), is currently the most widely accepted view, although several less-widely accepted alternatives (not described in this chapter) have been proposed.

The comparison of the known mitochondrial genomes of sponges, placozoans, and cnidarians with those of the solitary choanoflagellate *Monosiga brevicollis* and the colonial choanoflagellate *Salpingoeca rosetta*, along with the comparison of the filasterean *Capsaspora owczarzaki* (the extant group most similar to the choanoflagellate–metazoan superclade), indicates that the acquisition of multicellularity in animals involved a process of compaction of the mitochondrial genome (see Figure 16.3C), with a dramatic reduction of introns and noncoded intergenic regions, as well as a loss of about 10 genes with respect to the average number present in nonbilaterians. The mtDNA of *M. brevicollis* measures 76.7 Kbp (about 3.8 times the average found in Porifera) and contains 1.5 times as many genes as the average metazoan. It codes slightly modified rRNA and tRNA molecules similar to those of bacteria, and employs minimally modified genetic code. Information

available as of the date of this writing indicates that the mtDNA of choanoflagellates lacks a set of insertions or deletions (indels) in different protein-coding genes that, on the other hand, appear to be well preserved in the principal metazoan groups. The absence, in choanoflagellates, of these indels that are characteristic of metazoans (see Figure 16.3C), along with the absence of the *atp9* gene (so far observed only in Porifera), rules out the possibility of interpreting choanoflagellates as sponges reduced to a unicellular state through a process of secondary simplification.

The sequencing of the complete nuclear genome of two choanoflagellates and one filasterean amoeba has revealed that the leap towards the multicellularity of animals involved several genetic modifications with respect to the previous unicellular state. Three aspects are particularly noteworthy:

1. It is estimated that metazoans evolved about 1235 orthologous clusters and lost about 718 since their divergence from choanoflagellates.
2. Metazoans evolved new and more complex systems for the regulation of gene expression (for example, new transcription factor families, **micro-RNA**, etc.) which, while controlling in time and space the expression of genes that directly code phenotypic features, allow for cells with identical genotypes to develop different features and tissues; and
3. Metazoans evolved new adhesion and intercellular communication mechanisms (for example, adhesion proteins, new intercellular signaling pathways, etc.), allowing for the enhancement of physical attachment and delimitation of an internal intercellular medium, as well as coordination between tissue cells and the functional integration of different tissues.

Nevertheless, part of the gene material that made such innovations possible in metazoans seems to have been already available in the genome of the last common ancestor to metazoans and modern choanoflagellates and, surprisingly, even in the common ancestor to choanoflagellates and filasterean amoebas. The first sequenced choanoflagellate (*M. brevicollis*) had a genome of 49.6 Mbp, that is, on the order of 10^3 bp smaller than that of early divergent metazoans. It is a genome that is relatively rich in genes (9200) and introns; although it belongs to a unicellular organism, the genome codes a surprising array of transcription factors (RUNX, p53, brachyury) of metazoans, as well as protein domains related to adhesion between cells (cadherins) but not to the adhesion of cells to the intercellular medium (integrins). However, integrin has been reported in the colonial choanoflagellate *Salpingoeca rosetta*. The known choanoflagellate genomes barely code elements of

the main intercellular signaling pathways known in animals (with the exception of tyrosine kinase), which suggests that the transport systems and the channels expanded later. No evidence has been found in the choanoflagellate genome of either micro-RNA or of the relevant components of the different transcription factors that make up the regulatory scaffolding for embryonic development in animals. It should be pointed out that placozoans also lack micro-RNA (which controls the posttranscriptional repression of mRNA), while these same regulatory molecules, which are considered to be a relevant evolutionary advance, appear in Porifera and Cnidaria. The absence of micro-RNA in Placozoa could be the result of a secondary loss similar to that proposed for other components of their genome.

In the filasterean *Capsaspora*, genes have been identified that are orthologous to β -integrin, as well as to certain transcription factors and to the G protein signaling family, which have been secondarily lost, either in full or in part, in the choanoflagellates. Therefore, the transition towards the multicellularity of animals involved the appearance of numerous new protein domains (about 235 estimated from the *Capsaspora* genome), but also the expansion of many of the pre-existent domains, along with the total or partial loss of others, as shown in certain clades of choanoflagellates.

In sum, the available information suggests that the initiation of genetic (nuclear and mitochondrial) systems that facilitated the establishment of the multicellularity of animals probably involved a diversity of processes in the preexisting genetic material that affected diverse regions of the genome in different ways, including genetic expansions (duplications, etc.) in some cases and compactions in others. It seems that there were also processes that created new genes. However, we still have no detailed explanation of the mechanisms by which new genes originated nor of those that facilitated their integration into the growing network of signaling and transcriptional regulation systems that characterized the emergence of the multicellularity of animals.

The leap from an archetypical ancestral metazoan to the first metazoan diversification

In order to understand the transition between the ancestral metazoan and the early groups of modern metazoans, it is necessary to establish definitively which is the first diverging metazoan group: sponges, placozoans, or ctenophores. This subject is still under debate. For instance, the phylogenetic signal of the mitochondrial genome and other characters (for example, the absence of micro-RNA, the absence of an extracellular matrix in the mesoglea, limited cellular diversity, etc.) may suggest that the placozoans diverged from

the main ancestral metazoan clade before sponges and other animals (see Figure 16.3). To make the evolutionary scenario of the early diversification of animals even more complicated, several phylogenomic approaches have also suggested that the first diverging animals were neither sponges nor placozoans, but ctenophores (Figure 16.4D).

Nevertheless, any evolutionary scheme proposing either placozoans or ctenophores as the first diverging animal clade would make it difficult to explain how the common ancestor to the choanoflagellates and metazoans, which supposedly had choanocyte-like cells, could have given rise to placozoans or ctenophores, which lack choanocyte-like cells. In order to explain the distribution of this character (presence/absence of choanocyte-like cells) in the phylogenetic tree, it would be necessary to propose parallel but independent losses of this character in either the placozoans or ctenophores and in other groups of invertebrates that diverged after the sponges. Alternatively, it would be necessary to assume the loss of choanocyte cells in the ancestral metazoan (which could not be any longer derived from an choanoflagellate-like “protist” ancestor) and their subsequent reappearance at the base of the evolutionary branch of sponges. Such schemes are not very parsimonious. In addition, if placozoans or ctenophores were basal, the ancestral metazoan could be proposed under the placula theory—that is, as a reptant predator, **microphagus**, lacking in choanocytes but equipped with digestive cells and nerve cells. However, nearly all modern molecular phylogenetic analyses are consistently positioning choanoflagellates as the sister “protist” clade to metazoans.

It has already been pointed out that if ctenophores are the first diverging animal clade, then secondary losses of genes coding for functional nervous and muscle systems have to be assumed in sponges and placozoans. Alternatively, if sponges were at the base of the Tree of Metazoans, the distribution of the choanocyte-like cells feature would be explained by a simple continuity of the feature in Porifera and a subsequent loss in the metazoan branch. Likewise, the distribution of features accounting for nervous, sensory, and muscle systems would be easily interpreted as a progressive complexity of preexisting elements from sponges to ctenophores and bilaterians.

Regardless, a reliable reconstruction of the phylogeny within Porifera, which has not been achieved to date (see Tree of Metazoans, Figures 16.2B, 16.3B, 16.4B, and Figure 16.8), is an essential requirement for refining current inferences concerning the characteristics of the ancestral metazoan and of how the losses and gains of the choanocytes-like cell in the metazoan branch could have occurred. Today, there is an intense debate over whether sponges are a mono-

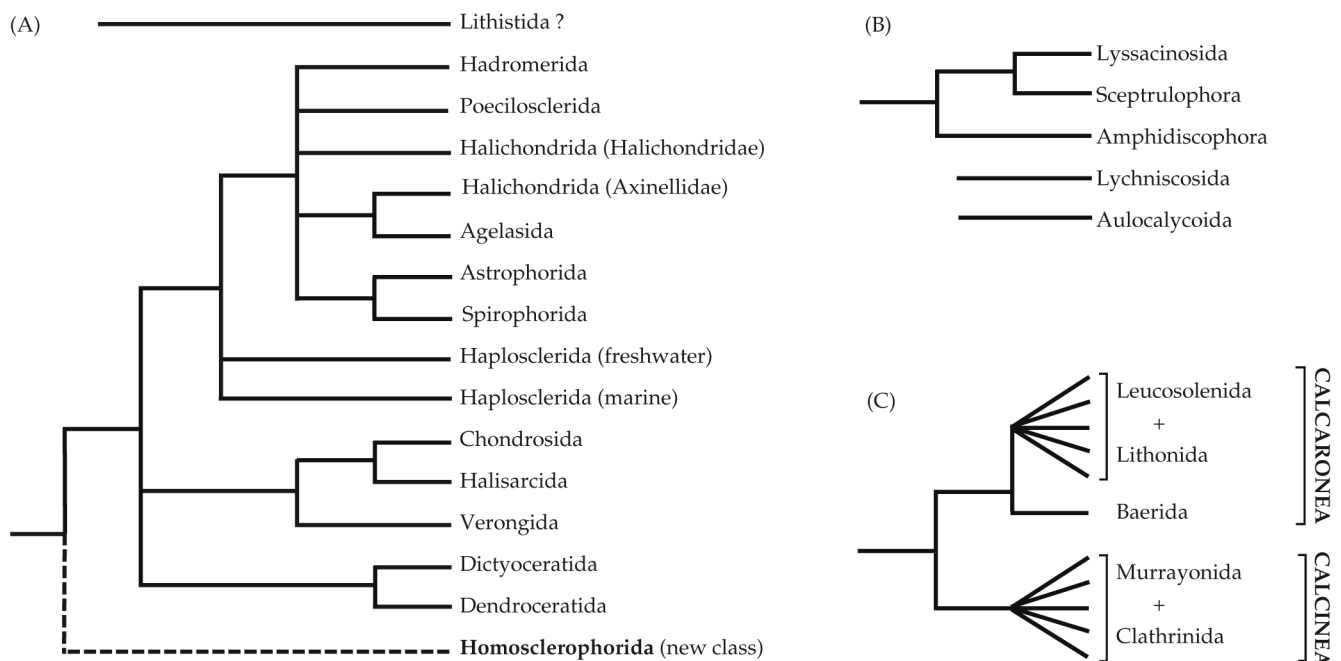


Figure 16.8 Hypotheses on the phylogenetic relationships in Porifera sponges. (A) Summary topology of the most probable relationships between the orders of sponges in the class Demospongiae. Note that the controversial and presumably polyphyletic group “Lithistida” has rarely been included in molecular phylogenies. The position of the formerly order Homosclerophorida shown in the tree is the one that has traditionally been inferred on the basis of mitochondrial DNA, but when other data sources are used (ribosomal DNA or certain families of nuclear genes), this group is located outside the class Demospongiae and is more closely related to the class Calcarea (see Figure 16.4B), which is why the homosclerophorida have recently been established as a fourth class (Ho-

moscleromorpha) within phylum Porifera. (B) Summary of the phylogenetic relationships among the main groups of sponges in the class Hexactinellida, inferred by ribosomal DNA analysis. Note that the difficulty of collecting sponges of this class, which typically appear in bathyal and abyssal habitats, has led to the exclusion of several orders from the phylogenetic analyses carried out to date. (C) Molecular phylogenies of the sponge class Calcarea are still poorly developed. The main problem lies in the fact that while there is significant molecular support for the subclasses Calcinea and Calcaronea, most of the traditional taxonomic orders are recognized as polyphyletic groups in ribosomal DNA studies.

phyletic, paraphyletic, or polyphyletic group. Given the extensive differences between Calcarea, Hexactinellida, Homoscleromorpha, and Demospongiae in terms of organization and mitochondrial genome content, virtually all of the analyses based on mtDNA show sponges as a polyphyletic group in which hexactinellida would be the sister clade of Bilateria (see Figure 16.3B). Even with limited mitochondrial data for sponges of the Calcarea class, the proposal of polyphyly of its mtDNA is not very convincing, because sponges have several strong morphological and molecular synapomorphies (including the mitochondrial gene *atp9*), which virtually rule out the possibility of polyphyly. Even more problematical are proposals based on different rDNA markers (28S, 18S) and certain families of constitutive (**housekeeping genes**) that, apart from conflicting with each other regarding many of the relationships inferred across the orders of demosponges, suggest that sponges could be a paraphyletic group (see Figures 16.2B and 16.4B) with the Calcarea class and a new class (Homoscleromorpha) recently split from Demospongiae (see Figure 16.4B,

16.8A), being more closely related to other early divergent metazoans than to the rest of the sponges.

A recent study (Nosenko et al. 2013) suggests that a matrix analysis of slowly evolving genes, such as those involved in the protein translation processes, generally provides a monophyletic structure in the Porifera tree, while matrices that include data from numerous genes that are not involved in protein translation and that usually exhibit high degrees of substitutional saturation, provide paraphyletic trees. Additionally, the most recent phylogenetic study incorporating data from the first complete genome for ctenophores also depicts Porifera as a monophyletic unit, although not positioned at the base of the animal tree (see Figure 16.4D).

The leap from diploblastic state to triploblastic state

From the standpoint of mtDNA, the origin of Bilateria reflects a remarkable reorganization of the mitochondrial genome of its predecessors (the early divergent metazoans), involving multiple changes in the genetic

code, the loss of tRNA genes, certain genetic innovations, and an accelerated rate of sequence evolution.

Nonetheless, the results of certain studies of genetic expression have undermined the traditional concept of the diploblastic organism, posing serious doubts as to whether cnidarians and ctenophores have cells that are homologous with the characteristic mesoderm of Bilateria (triploblastic organisms). The medusa stage of certain cnidarians has smooth and striated muscle cells formed from a small cell mass (entocodon) that is differentiated from the ectoderm during embryogenesis. During the differentiation of these muscle cells in the medusa stage of the hydrozoan *Podocoryne*, it has been possible to detect the expression of genes that are orthologous to the families *brachyury*, *Mef2*, and *snail/slug*, as well as a homologue of *MyoD*. The expression of these genes, typically associated with the formation of mesodermal derivatives in triploblastic organisms, suggests homology between cnidarians and triploblastic organisms in the construction of the muscular system, which is a typically mesodermal tissue. Likewise, ctenophores have not only epitheliomuscular cells but also true muscular cells that seem to form from micromeres that are differentiated from the ectoderm during gastrulation, in a process similar to that seen in many triploblastic organisms. These recent findings have demonstrated that the basic elements of triploblasty would have become operational prior to the appearance of triploblastic animals. In this connection, recent descriptions have been published of unicellular opisthokonts containing *MyHC* genes that code for type II myosin heavy chain, the motor protein that is characteristic of vertebrates. In addition, sponges have been found to have additional genes that are orthologous to those of the striated muscular system of Bilateria, despite the fact that they lack muscle cells. Cnidarians and ctenophores also possess *MyHC* orthologues, but they lack crucial components of striated muscle found in Bilateria, such as genes coding for titin and tropomyosin. This fact must be considered along with the finding that jellyfish possess Z-disc protein orthologues but, unlike Bilateria, they are expressed ubiquitously and unrelated to striated muscle. For this reason, it has been suggested that the striated muscle of diploblastic organisms (cnidarians and ctenophores) could have evolved independently from that of bilateria, with new and different protein systems being added in each case to the common pre-existing contractile apparatus.

Prior to the above perspective that supports a clear distinction between diploblastic organisms and bilaterians, some authors had proposed that the medusa stage of many cnidarians and ctenophores could actually constitute a triploblastic organism. However, in ctenophore tissues of a presumably mesodermal nature, it has not been possible to detect the expression of NKL genes, which are typically associated

with the development of the mesoderm and the neuroectoderm in triploblastic organisms. This lack of expression could indicate both the absence of a real mesoderm and the existence of a mesoderm-like tissue that would not be strictly homologous with that of other animals. The completion of the first ctenophore genome has shown that most of the genes coding for structural components of the mesodermal cells are present, while many of the genes involved in bilaterian mesodermal specification appear to be absent, further fostering the idea that mesodermal cells may have independently evolved in ctenophores, cnidarians, and bilaterians (Ryan et al. 2013). The expression of some gene families, such as the decapentaplegic system (*dpp*) involved in the specification of the dorsoventral axis of the body in higher invertebrates and vertebrates, had suggested previously that the boundary between diploblastic and triploblastic organization is relatively vague. During the embryogenesis of the cnidarian *Acropora*, a gene of this family is expressed in the ectodermal layer next to the end where the blastopore opens (the orifice that is formed by invagination of the epithelium of the blastula that gives rise to the digestive endoderm of the embryo and functions as an embryonic mouth; it subsequently becomes the mouth of the adult polyp). Some previous studies of genetic expression in Cnidaria and Ctenophora also suggested a possible homology between the oral–aboral axis of “Radiata” and the dorsoventral axis of Bilateria.

Molecular clocks versus the fossil record

Several independent estimates based on calibrated rates of divergence of molecular sequences suggest that the origin of metazoans would have been no later than 1.2 billion years ago, while the appearance of Bilateria is dated at 1.2 billion to 600 million years ago, with major points of disagreement between authors and the methods employed. According to many authors, the remains of the oldest metazoans are represented by sponges, either in the form of molecular fossils corresponding to sterols with 30 carbon atoms, showing that a member of the demosponges could have existed 630 million years ago; or in the form of demosponge spicules that date back 580 to 599 million years; or as body remains that can be unequivocally attributed to a hexactinellid sponge described as *Paleophragmodictya reticulata* and having an estimated age of 543 to 650 million years. Therefore, the evolutionary events that took place in the approximately 600 million years elapsed between the presumed origin of metazoans and the earliest remains of sponges would remain unsupported by fossil records. Nevertheless, some authors have proposed, not without controversy, that not only are there metazoan trace fossils from that period, but that they correspond to organisms that are architecturally

more complex than sponges. For example, fossil remains have been described that consist of a stolon up to 30 cm long, upon which is found a regular arrangement of cones that could have hosted zooids. These fossils, which are abundant in Western Australia, China, and Montana in siliciclastic rock 1.5 billion years old, were originally interpreted to be the first benthic colonial metazoans (*Horodyskia moniliformis*), although they have been interpreted by other authors as possible colonial “protists” related to foraminifera. Furthermore, in the strata of Russian fossil fields dating back 1 billion years, 80 fossils were collected that measured 6 cm in length and 2.5 cm in diameter and had segmentation marks. Those remains were interpreted to be, in principle, an ancestor of the annelids (*Parmia*), but some authors have suggested that they could correspond to “algae.” In general, the majority of neoprotozoic fossils (more than 540 million years of age) are very simple prints or molds, which in every case are hard to interpret without resorting to speculation that frequently give rise to an intense scientific debate.

Not even the phosphorite formation in Doushan-tou (South China), dated at 580 to 599 million years, where neoprotozoic fossils have been preserved with great detail, has aided in inferring the evolutionary pattern of the early metazoans. In strata of virtually identical age, the oldest known remains of demosponge spicules were found, but so were fossilized embryos and eggs that could belong to cnidarians and triploblastic organisms, as well as the remains of *Vernani-malcula guizhouena*, probably the first bilateral organism known. These latter remains consist of 10 fossils measuring between 100 and 200 μm in size that appear to belong to an organism with a triploblastic organization, equipped with coelom, digestive tube, anus, and pairs of cavities that could accommodate sensory organs around a mouth. The discovery of these minute triploblastic organisms suggests that the initial radiation of basal invertebrates could have occurred before they even reached a macroscopic size.

Nevertheless, some authors have suggested the possibility that the remains interpreted as embryos of cnidarians and triploblastic animals were actually *Thiomargarita*, a colonial sulfur bacteria. It has also been suggested that the traces of organs indicating bilateral symmetry in *V. guizhouena* could actually be artificial marks formed during the process of fossilization. If we could rule out the polemical—but intriguing—remains of cnidarians and triploblastic organisms from the Doushantou fossils field, the subsequent sequence of metazoan remains would be:

1. Different sponge remains dated around 600 million years ago;
2. A pedunculated globular organism dated between 550 and 543 million years ago and considered to be a cnidarian (*Namacalathus hermanastes*);

3. Remains of vendobionts, leaf-shaped organisms equipped with ciliated branches, which seem to represent the ancestral shape of the ciliated combs of ctenophores, dating to the Early Cambrian; and
4. Bilaterian animal traces dating back more than 555 million years.

Under this interpretation, the fossil records would support the notion that the first metazoans with fossil remains are those of sponges; cnidarians, ctenophores, and bilaterians would have appeared later.

Biogeography and Biodiversity

Living metazoans (not fossils) are distributed among 35 to 37 phyla, depending on the author. Furthermore, there are different proposals for subdividing phyla that could be polyphyletic (Bryozoa, Platyhelminthes, etc.) or for merging other phyla that are similar. For example, the acanthocephalans can be considered an independent phylum or they could be included within Rotifera (this book); the pterobranchs and enteropneusts can be considered separate phyla or included in Hemichordata (this book). Likewise, there are various phyla with only a few, poorly studied representatives, such as the gnathostomulids, cyclophorans, xenoturbella, and micrognathozoans, among others, whose classification as independent phyla may change based on the results of future studies.

Today, the number of species of classified living metazoans (not including fossils) is around 1,162,000, of which more than a million (approximately 86%) are arthropods. Chordates (vertebrates, ascidians, amphioxus, etc.) are represented by only some 45,000 species (3.8%). In fact, this limited representation of chordates is actually overestimated because, for many non-vertebrate phyla, there is a notable difference between the number of species classified to date and those that actually exist. Nematodes are a case in which there is a notable difference between the number of species extant and the number that have been classified: about 15,000 species have been described, but it is estimated that there are 100,000 yet to be described.

The metazoans probably had a marine origin and, similarly, the four groups of the most basal metazoans (Porifera, Placozoa, Cnidaria, and Ctenophora) also seem to have arisen in the ocean. Only Porifera and Cnidaria have freshwater representatives, but in both cases these are represented by only a few genera and a fossil record suggesting that colonization of continental waters began with ancestral marine forms. After their radiation, metazoans colonized virtually all habitats on the planet, from abyssal marine depths to the tallest mountain peaks and from equatorial to polar latitudes. Some groups or species have undergone surprising adaptations that have made it possible for them to live in extreme habitats, such as on atmospher-

ic dust granules and in caves and chasms, without substantial interaction with the external environment; in thermal freshwater springs and marine hydrothermal vents; in methane and sulfur chimneys in the ocean's crust; and as parasites of other metazoans or plants. Certain species of several groups, such as the tardigrades, actinopterygian fishes, and amphibians, have even developed cryptobiotic capabilities, temporarily suspending metabolic processes when environmental conditions become extreme.

Differentiation and Speciation

Differentiation and speciation patterns are very different in the four groups of early divergent metazoans, as evidenced by the known diversity of each of the groups: Porifera with about 8521 living species, Placozoa with only two, Cnidaria with 11,000, and Ctenophora with around 150. It is still unknown whether these differences in diversity between the groups are due to differences in the impact of extinction processes or to different rates of intragroup speciation. Extinct clades are known in every group except the placozoans. In the case of sponges, the most recent massive extinction process seems to have resulted from the expansion of diatoms during the Cretaceous. Diatoms are avid consumers of marine silica, and their expansion over ocean surface waters led to the extinction of numerous sponges, which also require this nutrient for building their silica skeletons. The competition for silica between these two groups from the Cretaceous to the present day was not only a cause of initial extinction but also seems to have served as a major environmental pressure that may have conditioned the skeletal evolution of sponges, favoring the appearance of new groups in which the silica skeleton has been replaced progressively by organic materials derived from collagen. It could have also been that the extinction of reef-forming siliceous sponges during the Cretaceous might have favored the subsequent evolutionary expansion of modern corals, which found an empty niche.

Studies of the genetics of populations have frequently shown that species traditionally assumed to have had a cosmopolitan distribution are actually subsets that have emerged through allopatric speciation—that is, they are genetically identifiable but morphologically cryptic. This situation affects all four groups of early divergent metazoans, but especially the placozoans, in which the presumably cosmopolitan species *Trichoplax adhaerens*, one of the two species that form the phylum, appears to be a complex of at least six cryptic species, as suggested by the remarkable interindividual variation of four molecular markers of rDNA.

Principal Questions Remaining

- What genomic diversity exists within modern choanoflagellates, and which subgroup would be more closely related to the ancestral metazoan?
- Are sponges a monophyletic, paraphyletic, or polyphyletic group?
- Do sponges have embryonic layers that are truly homologous with the ectoderm and endoderm of other animals?
- Why are placozoans a group of such limited diversity?
- What is the nature of segmentation, gastrulation, and embryonic development in placozoans?
- Could a part of the anatomical simplicity of sponges and placozoans derive from secondary simplification processes?
- Will it be possible to resolve the phylogenetic relationships between the groups of the most basal metazoans without considering the analysis of complete nuclear genomes of several members of each phylum?

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