Eukaryotes and Multicells: Origin

Secondary article

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Article Contents

Among the most important evolutionary events of all time are the origins of eukaryotic cells and of multicellularity. Both the evolution of eukaryotes and the origins of multicellularity (convergently evolved in numerous taxa) can be studied using insights from both palaeontology and molecular biology.

Eukaryotes: Basic Definitions

The defining feature of eukaryotic cells is the presence of a membrane-bound nucleus containing the genome. In all eukaryotes, the genome is composed of linear chromosomes, instead of the circular genome typical of prokaryotes. The DNA is complexed with specialized proteins, the histones. Furthermore, eukaryotic cell division is fundamentally different from prokaryotic fission: most eukaryote nuclei divide by mitosis. Mitosis depends on the presence of microtubules, made up of specific tubulin proteins, which make up the motility system that pulls chromosomes apart. These microtubules are often, though not always, organized by small organelles known as centrioles. Microtubules also make up part of the cytoskeleton, a network of intracellular filaments that is much more complex than anything seen in prokaryotes. Besides microtubules, the cytoskeleton includes microfilaments and intermediate filaments, also made of specialized proteins.

The vast majority of known eukaryotes contain other membrane-bound organelles, notably mitochondria, the sites of oxidative respiration. The few eukaryotes that lack mitochondria may represent very early branches of the eukaryote tree, although some have probably secondarily lost their mitochondria. Photosynthetic eukaryotes carry out photosynthesis in additional organelles, the plastids. Also characteristic of eukaryotes are dictyosomes (also known as the Golgi apparatus), the endoplasmic reticulum (a complex network of infolded membranes), and vacuoles for endocytosis and exocytosis. These collectively make up the endomembrane system for synthesis, uptake, and transport of large molecules. Finally, many eukaryotes bear undulipodia – elongated motility organelles, with an internal structure of nine pairs of microtubules surrounding two inner tubules (‘9 + 2’ structure). These are rooted by basal bodies, which have the same structure as centrioles; both centrioles and basal bodies are known as microtubule organizing centres (MTOCs) (**Figures 1, 2a**).

Cell biological evidence

The closest living relatives to eukaryotes are probably the prokaryotes in the domain Archaea. The Archaea, once referred to as Archaebacteria, include many members that inhabit extreme environments. Archaea are metabolically most like true Bacteria, with which they were grouped for a long time, but share many similarities with the Eukaryota. These include histone proteins; similar nucleic acid replication proteins, including polymerases and TATA-binding proteins; and similar processing of tRNA introns (Edgell and Doolittle, 1997; Olsen and Woese, 1997; Pace, 1997). At least some archaeans – notably *Thermoplasma*, a wall-less hyperthermophile – show further similarities with eukaryotes, such as flexible cell membranes containing sterols; actin-like cytoplasmic filaments; homologues of typical eukaryote proteins such as calmodulin and superoxide dismutase; uniquely shaped ribosomes; and a glycoprotein coat resembling a eukaryote glycocalyx (Dyer and Obar, 1994; Kandler, 1994).

It is not settled yet whether eukaryotes and archaeans are monophyletic sister taxa, or whether archaeans are paraphyletic and eukaryotes are the sister taxon to a subgroup of thermophilic archaeans, the Eocytes or Crenarchaeota. The bulk of molecular phylogenetic evidence seems to favour the former hypothesis (Pace, 1997; Woese, 1998; but see Forterre, 1997). However, archaeans also share some features with true Bacteria, such as operonic genome organization, restriction endonucleases and various metabolic features (Ouzounis and Kyrpides, 1996; Olsen and Woese, 1997). Archaeans also have a number of ‘orphan’ features not found anywhere else, such as ether-linked membrane phospholipids. Archaeans are not ‘Eukaryota in miniature’. Nor are archaeans necessarily ‘archaic’, having evolved, adapted and diversified for over 3.5 billion years (Forterre, 1997).

What is certain is that a number of ‘typical eukaryote’ features appear to go back to the archaean–eukaryote

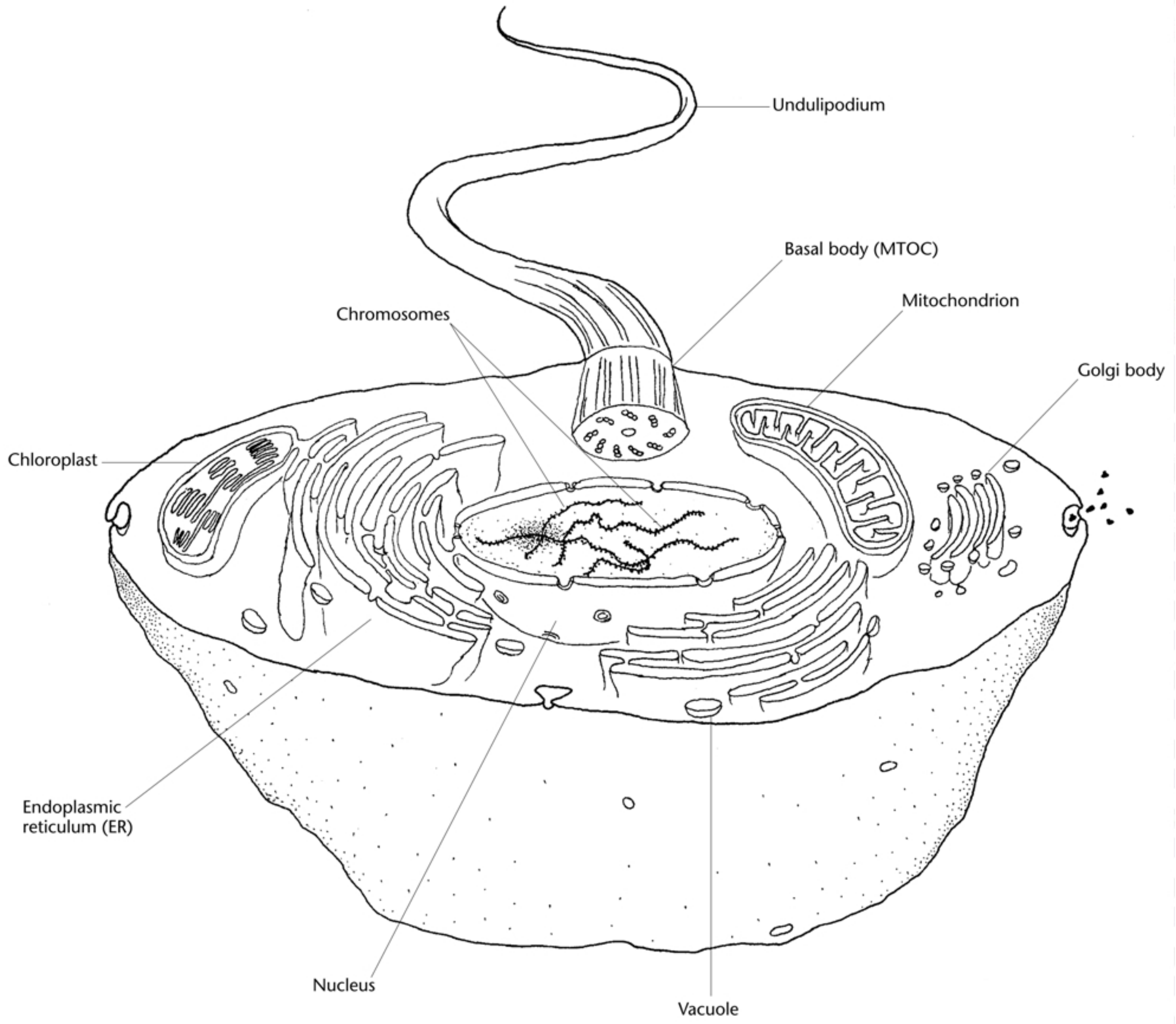


Figure 1 Semidiagrammatic drawing of a generalized eukaryote cell. The connections between the endoplasmic reticulum, the organelles and the nuclear membrane would not necessarily exist permanently in a living cell; they have been included to show the topological arrangements of the organelles and membrane system. The cytoskeleton has been omitted for clarity.

common ancestor, if not further (Olsen and Woese, 1997). Discrepancies among gene-based phylogenies may be explained by horizontal gene transfer, still widespread among prokaryotes and possibly the dominant evolutionary dynamic in the earliest living systems (Pace, 1997; Woese, 1998). It is noteworthy that the earliest branches of both the Archaea and the Bacteria are thermophilic (Pace, 1997). Archaean features that originally were adaptive for life at high temperatures, such as membrane sterols, may have been co-opted for other functions in early eukaryotes. Many prokaryotes have complex folded membranes within their cells. It is plausible that such a system was present in eukaryote ancestors – perhaps increasing surface area for metabolic functions – and was modified to produce a nuclear membrane and endoplasmic reticulum. Sterols increase membrane fluidity, and were probably a necessary precondition for these evolutionary steps (Knoll, 1983).

The circular genomes of prokaryotes are apparently constrained in size: prokaryotic genomes typically fall between 10^6 and 10^7 base pairs, whereas eukaryotes range from 10^7 to 10^{11} base pairs. Linear chromosomes may have permitted this increase in genome size. Yet their actual origins are problematic: how did DNA evolve a linear form from a circular ancestral state? According to Woese (1998), it may not have. The earliest 'progenotes' may have had genomes consisting of small, linear DNA segments distributed randomly at division, since the rudimentary replication and repair mechanisms available could not have handled larger chromosomes. Selection at the subcellular level for more efficient replication would have made it possible to handle larger DNA segments, allowing the evolution of larger chromosomes, whether linear or circular. The evolution of the centromere, telomeres and mitosis might have been driven by strong selection for efficient distribution of copies of all genes to offspring (Margulis, 1981; Woese, 1998). There are nonmitotic mechanisms for distributing genes, such as the fission of the unique achromosomal macronucleus in ciliates (Lynn and Small, 1990), which may be analogous to the fission of the earliest progenotes. These mechanisms, however, are inefficient compared with mitosis (Margulis, 1981).

Endosymbiosis and eukaryote evolution

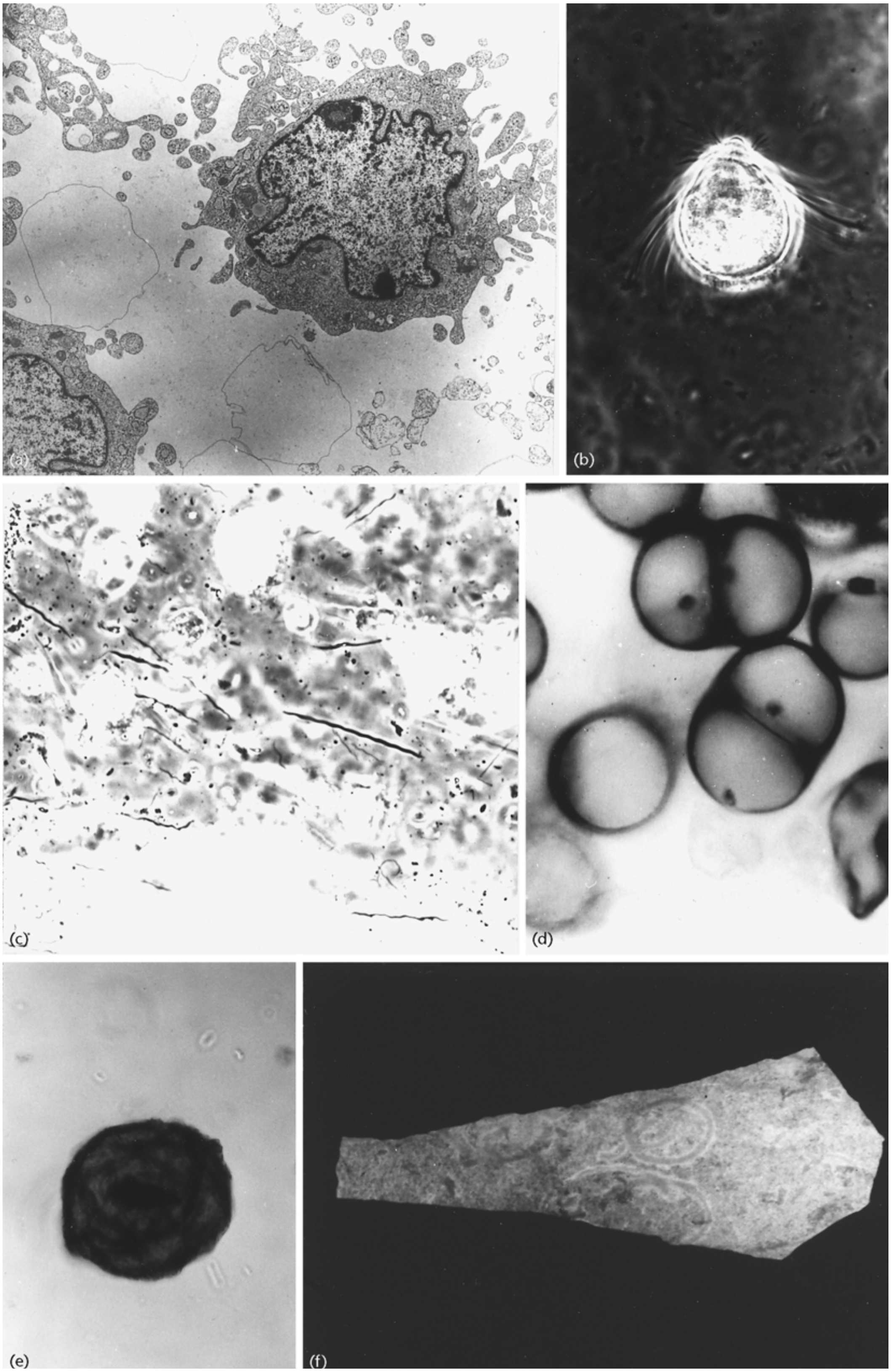
By far the best-supported theory for the origin of complex eukaryote organelles is serial endosymbiosis, which states that organelles are descended from symbiotic prokaryotes that colonized the cytoplasm of early eukaryotes. This theory goes back to the late nineteenth century, but was rejected by most biologists until its revival in the 1970s, primarily by Lynn Margulis (e.g. Margulis, 1981). The evidence for serial endosymbiosis is abundant. Both mitochondria and plastids are bounded by a double membrane, whose outer layer is continuous with the cell membrane; topologically, both are outside of the cell (**Figure 1**). Both retain ribosomes of prokaryotic size. Both are inhibited by antibiotics that specifically target prokaryotic cell processes, such as chloramphenicol and spectinomycin. Both replicate by fission, and cannot be synthesized *de novo* by the cell. Both also retain their own naked, circular genomes, although a number of their genes have been transferred to the eukaryote nuclear genome. Their gene sequences are phylogenetically closest to bacterial homologues. Mitochondria are related to the α-proteobacteria, a group that also includes rhizobacteria, rickettsias and other bacteria that penetrate into eukaryote cells. Plastids in different taxa were once thought to have originated from different prokaryotes, but now all are thought to have descended from cyanobacteria (Pace, 1997; Dyer and Obar, 1994).

Support also comes from the many living eukaryotes that have symbiotic prokaryotes inside their cytoplasm instead of – or in addition to – the typical organelles. The giant freshwater amoeba *Pelomyxa* is a well-known protistan example; it lacks mitochondria but contains at least three types of prokaryotes that carry out oxidative respiration (Whatley and Chapman-Andresen, 1990). Parabasalian protists, such as *Trichomonas* and *Trichonympha* (**Figure 2b**), have lost their mitochondria but contain abundant bacterial endosymbionts, whose function is not clear (Dyer, 1990). A number of protists contain endosymbiotic cyanobacteria, ranging from symbionts that can be cultured independently to wall-less forms incapable of independent growth (Dyer and Obar, 1994). Obligate bacterial–eukaryote endosymbioses have even been created in the laboratory (Jeon, 1991). Interestingly, some eukaryotes contain plastids derived from other eukaryotes. Dinoflagellate and euglenid plastids are surrounded by a triple membrane and apparently came from 'captured' eukaryote plastids. Cryptomonad flagellates contain plastids with a DNA-containing 'nucleomorph', surrounded by four membranes. These apparently were derived from a complete, endosymbiotic eukaryote (Dyer and Obar, 1994).

Other eukaryotic organelles may also be symbiotic in origin, although the evidence is less clear. A claim that DNA exists in the basal bodies of the undulipodia of the single-celled green alga *Chlamydomonas* remains unconfirmed and contentious at best. Nonetheless, microtubule-like structures in some spirochaete bacteria (**Figure 2c**); similarities between eukaryotic tubulins and certain spirochaete proteins; peculiar linkage of undulipodia-related genes in *Chlamydomonas*; RNA associated with basal bodies; and extant protists that move by means of attached symbiotic bacteria, all may support a symbiotic origin for both undulipodia and centrioles (Dyer and Obar, 1994). This is especially important because microtubules are also essential for mitosis and for the cytoskeleton, and no close homologue of tubulins has been found in archaeans (Edgell and Doolittle, 1997). Hydrogenosomes, found in several unrelated anaerobic taxa, reduce pyruvate and malate to acetate, producing hydrogen and carbon dioxide. They lack genomes, but structurally and chemically resemble mitochondria, and are probably modified mitochondria and/or descendants of related bacterial symbionts (Embley *et al.*, 1997).

Palaeontological evidence

Eukaryotes originated well back in the Precambrian, but tracing their earliest evolution is difficult because cell contents rarely fossilize recognizably. Many archaeans lack cell walls and, presumably, so did the earliest eukaryotes; such organisms would rarely if ever form recognizable fossils. Eukaryotes are typically larger than prokaryotes – in part because they have a cytoskeleton – but there is enough overlap between the two to make it hard to identify fossil eukaryotes on size alone (Schopf, 1992). Precambrian fossils exist with nucleus-like inclusions, but these may be shrunken remains of bacterial protoplasts (Knoll, 1983; **Figure 2d**). Fossilized cell tetrads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have been interpreted as products of eukaryotic meiosis, but tetrahedral arrangements are also found in cyanobacteria, and cannot be considered definitive evidence for eukaryote affinity (Knoll, 1983). Another problem has been misidentification of artefacts or contaminants. For instance, inclusions that superficially resemble yeasts have been documented from rocks 3.8 billion years old (nearly the oldest on Earth), but almost all palaeontologists consider these to be abiogenic (Schopf and Walter, 1983). This is not to say, however, that the Precambrian fossil record is useless in tracing eukaryote evolution. Although it probably cannot show the origin of eukaryotes, it can provide data on their early diversification and on the environments in which they diversified.

Until recently, the oldest probable eukaryotes were single-celled microfossils known as acritarchs (**Figure 2e**). Acritarchs resemble the spores and cysts produced by many living algae, and are usually taken to be algal protists of unknown types. The oldest known acritarchs are between 1.8 billion and 1.9 billion years old, from China (Knoll, 1992). In 1992, Han and Runnegar described macroscopic, coiled ribbon-like organisms called *Grypania spiralis* from 2.1 billion-year-old rocks of Michigan, USA (Han and Runnegar, 1992; **Figure 2f**). These are now widely accepted as the oldest known eukaryotes. Though still scanty, the fossil record is consistent with eukaryotes originating before 2 billion years ago, with several taxa diversifying by 1.8 billion years ago.

Grypania coincides with a global increase in atmospheric oxygen, inferred from several lines of geochemical evidence to have happened about 2 billion years ago (Holland, 1994). Furthermore, global climate and crustal dynamics were relatively stable, and nutrient levels relatively low, between 2.0 and 1.0 billion years ago (Brasier and Lindsey, 1998). It is tempting to see these conditions as driving forces behind eukaryote evolution. Perhaps rising oxygen levels created selective pressure for protecting the genome against oxidation, resulting in the evolution of the nucleus (Dyer and Obar, 1994). Low nutrient levels could have favoured the evolution of the host–plastid symbiosis, by selecting for improved recycling of limiting nutrients (Brasier and Lindsey, 1998).

Chemical fossils of steranes – derivatives of the sterols characteristic of eukaryotic cell membranes – provide yet another line of evidence for eukaryote history. Until recently, the oldest known steranes were 1.69 billion years old, from northern Australia (Brasier and Lindsay, 1998), but steranes have been discovered in 2.7 billion-year-old rocks in Australia (Brocks *et al*., 1999). If these steranes were derived from eukaryotes, they pose a problem for the scenario outlined above, in which eukaryote evolution was coupled to geochemical changes. However, since some archaeans are thought to have membrane sterols, it is conceivable that these molecular fossils came from an archaean or 'proto-eukaryote' lineage. Certainly more work is needed to evaluate this finding fully.

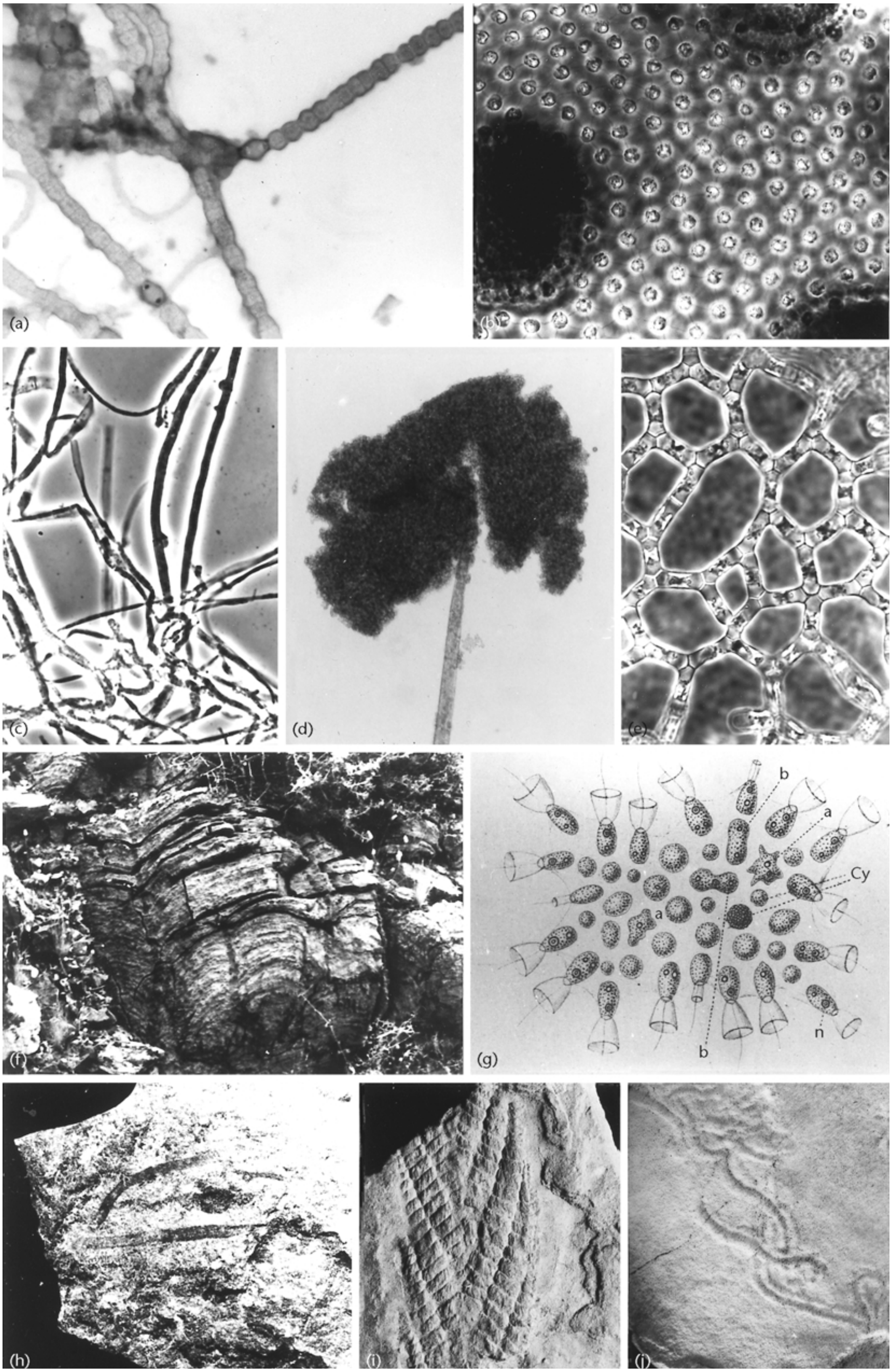
In any case, both acritarchs and multicellular eukaryotes underwent a major phase of diversification beginning about 1 billion years ago (Knoll, 1992) – a date consistent with molecular clock dates for the radiation of the eukaryotic 'crown group'. It is possible, although speculative, that this diversification was tied to the ending of the period of global stability. Perhaps habitat fragmentation plus a suite of new selective pressures drove the radiation of the eukaryote crown group.

Multicellularity: Basic Definitions

If a 'multicellular' organism is simply one that consists of several cells, then multicellularity is as old as the oldest known fossils. However, most biologists would distinguish between colonial organisms and truly multicellular ones, although it is difficult to make a precise distinction. Truly multicellular organisms show some degree of cell interdependence, and have more than one cell type, each specialized for different functions. Not all cells retain totipotency. Cells are physically held together by some kind of extracellular matrix (ECM), and have various means of communication. They can and do influence each other's developmental fates. Endosymbiosis is usually excluded from this definition: although a ciliate, for instance, might contain mitochondria, bacteria and symbiotic algae, and thus consist of many cells, it would not usually be considered 'multicellular'.

Even using this restricted definition, multicellularity has arisen many times in both prokaryotes and eukaryotes. Even bacterial 'colonies' are multicellular: individual cells communicate and differentiate, subgroups of cells coordinate specialized behaviours such as swarming, and colonies undergo a kind of morphogenesis (Shapiro, 1995). Cell differentiation is also seen in cyanobacteria, actinomycetes and myxobacteria, as well as in animals, plants, fungi, and

Figure 2 (a) Transmission electron micrograph of eukaryotic cells – mouse fibroblasts. Note prominent nuclei with a few nuclear pores visible; mitochondria and endoplasmic reticulum are also present. Photo reproduced courtesy of S. Runge. (b) *Trichonympha*, member of the Parabasalia, a very early branch of the Eukarya. *Trichonympha* lacks mitochondria, but has many types of bacterial symbionts, both intracellular and extracellular. It is itself a symbiont, living in the hindgut of termites. (c) Unidentified spirochaete bacteria. These are also gut symbionts of termites. (d) *Glenobotrydion* from the Bitter Springs Formation of Australia, approximately 850 million years old. Note inclusions that may or may not be nuclei or organelles. (e) An acritarch, *Leiosphaeridia*, from the late Precambrian of the St Petersburg region, Russia. Reproduced from the University of California Museum of Paleontology (UCMP) collection. (f) *Grypania spiralis* from the Negaunee Dolomite of Michigan, USA, 2.1 billion years old. If this is a eukaryote, it would be the oldest currently known. Reproduced from the University of Central Arkansas (UCA) collection.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at least eight major multicellular protist taxa (Bonner, 1998; **Figure 3a**). Only a few well-studied cases can be covered here.

Why has multicellularity arisen so many times? Bonner (1998) lists a number of ways in which multicellularity is adaptive, including protection from a hostile environment by production of a regulated internal environment, increased swimming speed, and size increase as a defence against predators. Multicellularity also made possible the evolution of a number of new features, including improved dispersal of propagules and division of labour among cells. Margulis (1981) has pointed out that most eukaryotes use the same MTOCs as centrioles and as basal bodies. This sets up a basic constraint: an undulipodiate eukaryote cannot divide, and a dividing eukaryote cannot move. Some eukaryotes have developed amitotic division, others have lost their undulipodia completely, others have gained multiple MTOCs, and still others transform back and forth between reproductive and motile states. A fifth solution is multicellularity: an organism with both undulipodiate and nonundulipodiate cells can grow and move simultaneously, and may also produce motile gametes or larvae.

Cell biological evidence

All multicellular organisms are not created equal. Multicellularity can arise in at least four ways (**Table 1**; **Figures 3b–e**). Symbioses between unicellular or colonial organisms, leading to a chimaeric origin of multicellularity, are usually not thought of as 'individual' organisms. However, some such associations, such as fossil stromatolites, show consistent enough morphologies that they routinely receive Linnean form binomials and can be used in biostratigraphic correlation (**Figure 3f**). Lichens are another example of 'chimaeric' multicellular organisms, although here at least one of the partners in the symbiosis – the fungus – is already multicellular.

Most theories of the origin of animal multicellularity have derived animals from multicellular colonial protists. Living choanoflagellates appear to be excellent models for the origin of animal multicellularity. These small protists, nearly identical to the choanocytes of sponges, include colonial forms such as *Proterospongia*, which show differentiation between motile and reproductive cells, both of which are embedded in a jellylike extracellular matrix (**Figure 3g**). Crucial for animal multicellularity was the evolution of collagen, a key component of the metazoan ECM (Morris, 1993). Also crucial was the evolution and duplication of homeobox genes, which are 'master switches' that initiate cascades of gene expression; they establish the major features of the body plan (Valentine *et al.*, 1996).

Hadzi (1963) proposed a competing theory, the syncytial theory of animal multicellularity. According to Hadzi, the earliest multicellular animals were small flatworms, derived from a ciliated protist that developed multiple diploid nuclei and later cellularized. According to this scenario, cnidarians are secondarily derived from flatworm-like ancestors, and sponges had a completely separate origin from all other animals. It is true that a number of small flatworms are partially syncytial. However, ciliates are not plausible metazoan ancestors: they have distinctive cortical structures and unusual achromosomal macronuclei that have no counterparts in animals (Lynn and Small, 1990). Ciliates also do not appear close to animals on any molecular phylogenies (e.g. Schlegel, 1994). Furthermore, both sponges and other metazoans contain collagen, integrin receptors, lectins, homeobox genes, and choanocyte-like cells (Müller, 1998) Sponges also form a monophyletic group with other metazoans in recent molecular phylogenies (e.g. Collins, 1998; Müller, 1998). There is no reason to think that the sponges had a separate origin from the rest of the animals.

The situation is different in land plants, fungi, and many algae. Many fungi and algae, including those clades thought to be basal, are completely coenocytic (Butterfield *et al.*, 1994). Even in organisms that do show cell partitioning, intercellular connections allow for much more sharing of cytoplasm than is usual in animals. This is especially true of fungal hyphae, which are either coenocytic, or septate but with septal pores of varying degrees of complexity. Land plant cells appear more discrete, but the plasmodesmata – organelles crossing the cell walls – allow cytoplasm to be shared over wide areas of the plant. The opening or closing of plasmodesmata is important in plant cell differentiation (Zambryski, 1995).

Figure 3 (a) *Anabaena flos-aquae*, a filamentous cyanobacterium. Note the presence of heterocysts, cells specialized for nitrogen fixation. (b) *Volvox* (Chlorophyta, Volvocales). A close-up of this large, spherical, coenocytic alga. Note the network of fine cytoplasmic connections between cells. (c) *Coprinus* hyphae (Fungi, Basidiomycetes). Note the 'clamp connections' between cells, typical of basidiomycete fungi. (d) Fruiting body of *Dictyostelium.* The cellular slime moulds produce stalks and spore masses by aggregation of free-living amoebae. (e) *Hydrodictyon* (Chlorophyta, Chlorococcales). This net-like structure is produced by failure of cells to separate after cytokinesis. (f) Stromatolites, the fossilized remains of cyanobacterial communities. These are approximately 600 million years old, from the lower Johnnie Formation, Nopah Range, California, USA. (g) Drawing of *Proterospongia haeckelii*, an extant colonial choanoflagellate with cellular differentiation. Key: a, amoeboid cell; b, dividing amoeboid cell; cy, reproductive cyst; n, nucleus. Reproduced from Bütschli O (1883–87) *Klassen und Ordnungen des Thier-Reichs. Erster Band: Protozoa.* Leipzig: CF Winter. (h) 'Carbon film' of a possible eukaryotic algal blade. Lower Cambrian of Siberia. Reproduced from the UCMP collection. (i) *Charnia*, one of the 'Ediacaran' organisms, plausibly though not unanimously interpreted as a metazoan. Late Precambrian, White Sea coast, Russia. Reproduced from the UCMP collection. (j) *Aulichnites*, a trace fossil produced by an unknown metazoan. Late Precambrian, White Sea coast, Russia. Reproduced from the Paleontological Institute of the Russian Academy of Sciences collection.

Table 1 Modes of metacellularity

Mode of multicellularity	Examples
Lack of cytokinesis produces coenocytic organism, with or without later cellularization	Fungi; many algae; plasmodial slime moulds; hexactinellid sponges; some animal tissues (e.g. digestive tracts of bdelloid rotifers and acoel flatworms, tapeworm epidermis, insect embryos)
Failure of cells to separate after complete cytokinesis	Some algae; most animals; cyanobacteria
Agglomeration of normally independent conspecific cells	Cellular slime moulds; myxobacteria; one ciliate
'Chimaeric' symbiosis between unrelated unicellular lineages	Stromatolites; lichens

Palaeontological evidence

The oldest plausible fossil eukaryote, *Grypania spiralis*, is thought to have been coenocytic (**Figure 2f**). It is also the oldest of a type of fossil known as 'carbon films', two-dimensional compressions found worldwide in Proterozoic rocks. 'Carbon film' fossils are often difficult to interpret. Some are probably bacterial sheaths or films, and thus not truly multicellular at all; others have unusual microstructure and cannot be referred to any known group (Butterfield *et al.*, 1994; Hofmann, 1994). Still others, however, had blades and stipes, and resemble seaweeds, although most cannot be definitely connected with any living algal taxon (**Figure 3h**). The oldest multicellular eukaryote definitely referable to a modern taxon is a bangiophyte red alga, between 1200 and 750 million years old (Butterfield *et al.*, 1990). Multicellular chlorophytes, xanthophytes and chrysophytes, as well as more problematic multicellular algae, were present by 700–750 Ma, and in some cases even earlier (e.g. Butterfield *et al.*, 1994).

Controversial molecular clock data suggest an origin of animal phyla about 1.0–1.2 billion years ago, coinciding with the crown-group eukaryote radiation; however, other interpretations of the molecular clock place the origin of animals at about 750 Ma (see Conway Morris, 1997, for a review of the controversy). Reasonable fossil evidence for animals is lacking until 600 million years ago, the time of appearance of an assemblage of mostly unmineralized fossils known as the 'Ediacara biota'. Tantalizing reports of pre-Ediacaran animals (e.g. Seilacher *et al.*, 1998) are not accepted by all palaeontologists. If present, pre-Ediacaran animals may have been microscopic and unlikely to leave fossils. The Ediacara biota diversified between about 565 and 540 million years ago. Their evolutionary relationships remain contentious, but at least some probably belonged to modern animal phyla. Furthermore, trace fossils from the same time period confirm the presence of several types of mobile bilaterian animals (Narbonne, 1998). Very recent finds of remarkably preserved sponges and animal embryos from rocks 570 ± 20 million years old (Li *et al.*, 1998; Xiao *et al.*, 1998) have further expanded the known animal fossil record, and point to as-yet unknown ancestors even further back in time.

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