Microtubules and the Evolution of Mitosis

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Abstract The microtubular cytoskeleton of higher plants diverges considerably from its animal counterpart. This divergence involves a fundamentally different organization with microtubule arrays, which are specific to higher plants, such as cortical microtubules or the phragmoplast. On the other hand, centrioles, which are central organizers of microtubules in cells of animals and lower plants, have been progressively reduced in the course of plant evolution, and are now absent, as in most seed plants. In addition to these structural differences, the molecular composition of associated proteins also deviates to a degree that sequence homologues of important animal microtubule associated proteins (MAPs) seem to be absent in higher plants. The transition towards multicellular plants was intimately linked to rhythmic changes of division axis, and, thus, to the spatial regulation of mitotic microtubule arrays. To understand the peculiarities of plant microtubules, we have to combine cell biology with a more phylogenetic viewpoint. Therefore, this chapter is dedicated to the role of microtubules in the evolution of mitosis. It is shown that the microtubule divergence between plants and animals is already laid down in the prokaryotic ancestors, when the walled eubacteria are compared to the mycoplasms that lack a cell wall. The complex situation in lower eukaryotes can be understood as variations of this theme. The relation between chromatin/nucleus and the so-called nucleus associated organelle (NAO), which organizes microtubules, is central for the realization of mitosis. The extensive variability observed in algae and fungi is then progressively channeled, when, during the evolution of the higher Chlorophyta, most microtubule structures characteristic for higher plants develop. However, the interpretation of these mitotic structures has remained ambiguous, due to possible convergent developments, and due to our limited understanding of the phylogenetic relationships in many taxa of the algae. At the time when plants shifted to a terrestrial lifestyle, the microtubule arrays from higher plants have already been worked out. This was accompanied by a progressive reduction of centriolar functions and the increasing predominance of acentriolar microtubule organization, which could be followed on the structural level and also by a redistribution of microtubule-nucleating proteins, such as γ-tubulin, during recent years. Indicative of the evolutionary processes towards the highly divergent microtubular cytoskeleton of higher plants, interesting evolutionary footprints still exist, and are difficult to interpret merely in terms of cellular function. These include the cytoplasmic occurrence of the tubulin ancestor, FtsZ, in mosses, or the recent discovery of intranuclear tubulin. If one merely attempts to explain them in terms of current function, these phenomena appear to be unusual and difficult to understand. However, they can be readily interpreted as rudiments from a long evolutionary path, driven by the necessity to divide cells that are surrounded by rigid cell walls.

1
The Cytoskeleton as Target of Evolution

For decades, textbooks have cited the cytoskeleton as a classical example for the achievements reached by the eukaryotes. However, this view has been shattered by exciting discoveries in bacterial cells made at the turn of the century. It began with the striking news that FtsZ, a protein essential for the fission of bacterial cells, is the prokaryotic ancestor of tubulin (Löwe and Amos 1998). It was later shown that MreB, a bacterial protein with homology to actin, is required for the maintenance of cell polarity during asymmetric cell division (Gitai et al. 2004), and is, therefore, also functionally equivalent to its eukaryotic counterpart. Eventually, CreS, a coiled-coiled protein, which, similar to intermediate-filament proteins of eukaryotes, can polymerize into filaments, was shown to be responsible for the shaping of bacterial cells, but not their polarity (Ausmees et al. 2003). Thus, precursors of all three major cytoskeletal elements (microtubules, microfilaments, and intermediate filaments) already do exist in prokaryotes, and they seem to fulfill similar functions.

These functions are linked to cell division – to the fission process itself (FtsZ), to its organization in space (MreB), or to the shaping of the daughter cells (CreS). The evolution of the prokaryotic cytoskeleton was most likely driven by the necessity to increase mitotic efficiency. With the transition to multicellularity, additional requirements were posed upon mitosis – in addition to the correct duplication and separation of chromosomes, it became essential to control the separation of the two daughter cells in space and time. This requirement is especially evident in walled cells, where irregularities of cytokinesis cannot be adjusted by movements of the daughter cells. Here, mitosis is actually used to organize the *Bauplan* of the resulting organism.

By rhythmic changes in the axis or symmetry of individual cell divisions, it became possible to produce defined patterns of cell files. This is impressively illustrated by the modular structure, which is characteristic for the transition from higher algae to the primitive land plants (Fig. 1):

In the primary situation (Fig. 1A, 1), an apical stem cell divides asymmetrically, yielding: a proximal cell, which will elongate without dividing further, and a distal progenitor cell, which will repeat the stem-cell program of its mother (unimodal stem cell). The axis of division is aligned with the axis of the cell file leading to the filaments, which is characteristic for many green algae. However, this situation is also found in the protonemata of mosses and fern plants, which seem to recapitulate the development of their ancestors.

In a more advanced situation, the mitotic activity of the proximal daughter cell will not be irreversibly inactivated. It will recover in defined intervals, whereby the division axis is tilted perpendicular to the file axis (Fig. 1A, 2). This will result in branching. If the mitotic activity rhythmically returns in the offspring of such a branching division, a progressively hierarchical system of axes, branches of first and second order, emerges (Fig. 1A, 2–4). This
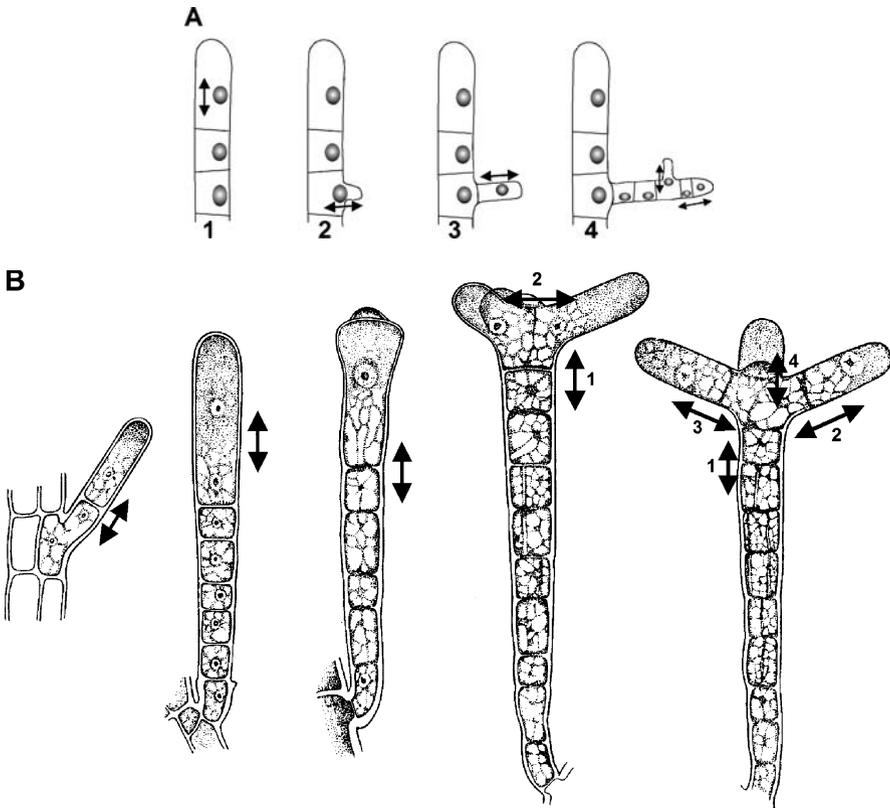
Fig. 1 Impact of spatial regulation of mitosis on the morphogenesis of lower plants. **A** Generation of complex hierarchical morphologies by rhythmic changes in the axis of cell division. *1* filamentous morphology generated by an unimodal stem cell; *2, 3* branching produced by transient stem cell activity in a proximal cell; *4* secondary branching generated by transient stem cell activity in a second-order cell. **B** Hierarchical whorl formation in *Sphacelaria fusca*, according to Zimmermann and Heller (1956)

is observed in many of the higher green, red, and brown algae. Branching in *Sphacelaria fusca* has been a classical object of studies for this aspect (Fig. 1B). By confining this rhythmic appearance of tilted divisions to the primary stem cell, it is possible to generate dichotomous branching, as observed in the brown alga *Dictyota* and in several pteridophytes. When the intervals between the tilting events are reduced and eventually eliminated, one arrives at the bimodal stem cells, characteristic for the prothallia of ferns.

This example illustrates how relatively simple, rhythmic changes in axis and symmetry of cell division produce a panel of diverse body plans. It further demonstrates that cell division, in a certain sense, has to be placed upstream of cell expansion, because it will define the axis of the daughter cells, and, thus, the framework in which expansion can occur.

In addition, cell division in plants can even interfere with cell differentiation, and, thus, with the developmental fate of a given cell. During cell division, there are two aspects that are under tight spatial control in the walled plant cells: the axis of division and the symmetry of division (Fig. 2). In most divisions, the axis does not change and most divisions are symmetrical. However, occasionally, axis and symmetry do change, and this is usually the earliest manifestation of developmental switches, which often occur in response to environmental stimuli. In protonemata of ferns, which is a classical system for the study of unimodal stem cells, the axis of division is parallel to the axis of the protonema, as long as it grows either in darkness or under red light. However, upon irradiation with blue light, this axis is rhythmically tilted by 90° during each of the subsequent divisions, yielding two-dimensional growth and the formation of a prothallium (Mohr 1956; Wada and Furuya 1970). When the cell is returned to red light, the division will assume the initial state, and a new protonema will emerge from the front edge of the prothallium.

Similar changes in division symmetry accompany differentiation events in all land plants. For instance, the formation of guard cells (Bünning 1965), the generation of water-storing cells in peat mosses (Zepf 1952), or the first cell division that separates prospective thallus from prospective rhizoid fate in many spores and zygotes of lower land plants, provide ample evidence for
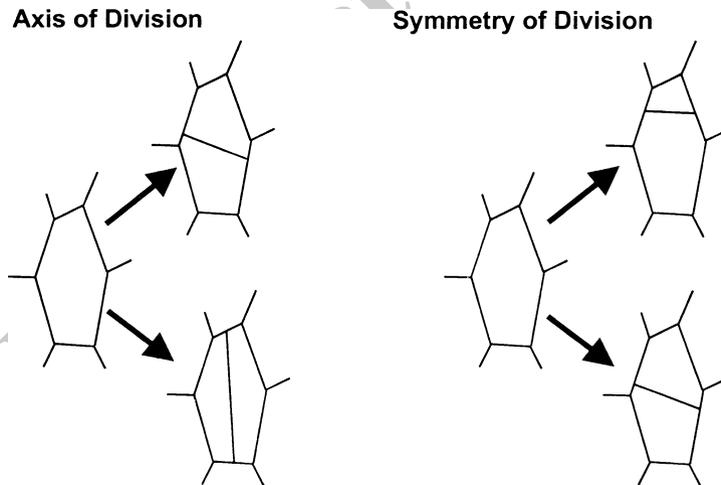
Fig. 2 Impact of division axis and symmetry on the shape of plant cells. In most cases, the division axis is parallel to the main axis of cell expansion (*left*); however, in a few defined cell divisions, it can be tilted by 90°. These tilted divisions in most cases give rise to cell lineages of differing developmental fate. Most cell divisions are symmetric (*right*); however, asymmetries can occur, but are relatively rare and are characteristic for differentiation events, similar to the tilted divisions

such asymmetric divisions. In some cases, it is possible to modify these asymmetric divisions by either pharmacological or genetic manipulation, such that the cells separate symmetrically. This determines whether these asymmetric divisions are formative in character, i.e., whether they assign differential developmental fates to the daughter cells.

As an example, it is possible to shift the first division of the spore in the fern *Onoclea sensibilis* from asymmetry to symmetry by treatment with antimicrotubule compounds (Vogelmann et al. 1981). Instead of the normal separation of a protonema from a rhizoid precursor, this will result in the formation of two protonemata-like organs.

Similar to the situation in lower land plants, the first zygotic division of higher plants is asymmetric as well, and it separates suspensor from embryo fate. By mutation in the *GNOM* gene, it is possible to obtain a symmetric division. The subsequent development is affected by dramatic defects in apicobasal polarity and the differentiation of daughter cells (Mayer et al. 1993). The GNOM protein was later shown to encode an accessory protein that regulates the activity of the small GTPase ARF, which is involved in the budding of vesicles at the endoplasmatic reticulum (Geldner et al. 2003). This finding indicates a link between the spatial regulation of vesicle fluxes, the spatial regulation of cell division, and developmental fate.

Thus, plant mitosis is peculiar, not only with respect to its cellular details, but also because it plays a tremendous role in establishing a developmental pattern. This peculiarity is connected with the presence of a cell wall, excluding cell migration as the morphogenetic mechanism (central to development in animal development). Thus, in order to understand plant mitosis, it is necessary to consider how it evolved parallel and in concert with a plant-specific developmental strategy.

2
The Starting Point – Prokaryotic Mitosis

The evolution of mitosis reaches back beyond the discovery and research on bacteria. Remainders of this time persist today. They suggest that the most primitive cells are those of the mycoplasmas (Wallace and Morowitz 1972), because their genomes are much smaller than those found in other bacteria. They lack many of the genes typically present in bacteria, including those used in wall synthesis, signaling, and even housekeeping activities, such as *de-novo* synthesis of nucleotides and amino-acids (Himmelreich et al. 1996). The typical, large, bacterial chromosomes are thought to derive from mycoplasma-like ancestors by end-to-end annealing, whereas duplications of the genome unaccompanied by such end-to-end annealing gave rise to small and separate chromosomes, which is characteristic for lower eukaryotes. The formation of numerous separate genetic entities was the driving force for the

evolution of more efficient mechanisms of segregation and, thus, for the evolution of mitosis. Moreover, generating a specialized chromosome-containing compartment and creating a specific environment favorable for replication and transcription, would be advantageous in such a multi-chromosome constellation. This might have been the driving force for the development of a nuclear envelope.

The mechanisms of chromosomal segregation in mycoplasm should, therefore, recapitulate some aspects of this very early stage of mitotic evolution. The genome of mycoplasmas is tethered to the plasma membrane by its replication point. In some mycoplasmas, specialized discoid structures adjacent to the membrane have been detected by electron microscopy (Maniloff and Quinlan 1974), and later designated as terminal organelles (for a recent review, see Balish and Krause 2006). Mutant analysis, in combination with in vivo microscopy of fluorescently labeled components of these terminal organelles (Hasselbring et al. 2005, 2006), has shown that they are not only responsible for the adhesion of the parasitic mycoplasms to the host cell, but also for gliding and cell division. The terminal organelles are singular in non-dividing cells, but duplicate prior to division. They constitute a type of polarity marker, because they are situated at opposite poles. Interestingly, the division process is blocked by cytochalasin B, a well-know inhibitor of actin filaments (Ghosh et al. 1978). This indicates that actin filaments are used to push the replicated daughter genomes apart and constrict the two daughter cells. It should be mentioned, however, that FtsZ, a central component of bacterial cell division and ancestor of eukaryotic tubulin, exists in mycoplasmas and is able to functionally complement *E. coli* strains deficient in FtsZ (Osawa and Erickson 2006). Nevertheless, in this very primitive situation, the central role is played by actin, and not by microtubules. This might reflect the hypothetical initial stage in the evolution of mitosis. When the plasma membrane bearing the disc-like structures invaginated this hypothetical ancestor, such that the terminal organelle was internalized, this would generate a typical eukaryotic nucleus with intranuclear genome organizers.

It seems that the mycoplasmic type of mitosis mirrors the primordial situation for those organisms that lack a cell wall. A detailed model for the evolution of mitosis has been elaborated for such cells (Heath 1974) and it is also valid for the evolution of animal mitosis. For walled cells, the controlled segregation of genetic material is only one of the tasks to be solved – in addition, a walled cell has to be divided. A septation of the daughter cells by constriction through the actomyosin system (or its functional homologues in prokaryotes) is not sufficient; walled cells have to establish a new cross wall, and this poses specific challenges to the mitotic machinery. Already walled prokaryotes – such as eubacteria and cyanobacteria – were confronted with this task. They used the FtsZ system to cope with it.

FtsZ is present in almost all recent prokaryotes (including the mycoplasmas). It has been identified as the essential component of the division

machinery (for review, see Errington et al. 2003). Prior to cytokinesis, FtsZ assembles into a ring, accompanying the inner membrane, and it recruits additional proteins to this ring. This ring then progressively constricts and organizes the synthesis of a new cross wall (which is, thus, formed in a centripetal fashion).

FtsZ harbors a GTP-binding activity. It can assemble into polymers, and it shares striking similarities to tubulin in three-dimensional structure (Löwe and Amos 1998). Therefore, it is thought to represent the ancestor of eukaryotic tubulin. The formation of a contractile FtsZ ring underneath the plasma membrane heralds the ensuing bacterial cell division (Sun and Margolin 1998). This FtsZ ring recruits accessory proteins to the division site (for review, see Addinall and Holland 2002), which partially act as stabilizers of FtsZ in the division site, or suppress the assembly of FtsZ at ectopic locations. For instance, the division factors ZipA, FtsA (Pichoff and Lutkenhaus 2002), or the recently discovered protein, ZapA (Gueiros-Filho and Losick 2002), seem to stabilize the FtsZ ring, whereas the MinC and MinD proteins suppress the assembly of FtsZ (Hu et al. 1999), and are, in turn, inhibited by MinE (Fu et al. 2001). MinE forms a ring independently of FtsZ, and, thus, it seems to be the upstream factor that defines the prospective site of cell division (Raskin and De Boer 1997). Thus, the division plane appears to be determined by an interplay between non-linear self-amplifying circuits (FtsZ, ZipA, ZapA) and lateral inhibition (MinC, MinD), which is, in turn, suppressed by the inhibitory activity of MinE heralding the division site (Fig. 3). Consistent with this model, overexpression of MinC and MinD blocks cell division and phenocopies a FtsZ-null mutation, whereas mutation or deletion of MinC or MinD results in ectopic division sites.

In contrast to cell division in eubacteria, relatively little is known about cyanobacterial mitosis (for review, see Hashimoto 2003). The central player
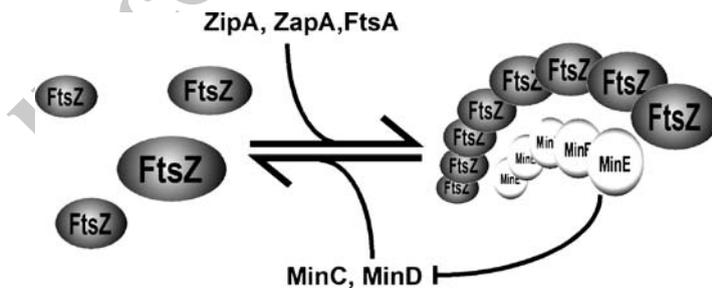
Fig. 3 Control of the bacterial FtsZ division ring by accessory proteins. The assembly of FtsZ is promoted by ZipA, ZapA, and FtsA, but suppressed by MinC and MinD. MinC and MinD, in turn, are suppressed by MinE, which forms a ring at the division site independently of FtsZ. This ensures that FtsZ cannot assemble outside the prospective division site, such that FtsZ monomers are preferentially recruited to the FtsZ ring

seems to be FtsZ (Osteryoung and Vierling 1995). The FtsZ transcription is regulated by DNA-replication. When replication is inhibited by hydroxyurea (a blocker of ribonucleotide reductase) or nalidixic acid (a blocker of DNA gyrase) in the cyanobacterium *Microcystis aeruginosa*, the transcription of FtsZ and cytokinesis are arrested (Yoshida et al. 2005). However, the associated proteins seem to differ. Proteins that are specific for cyanobacteria and plastids, such as ARTEMIS (Fulgosi et al. 2002) or the Arc proteins (Vitha et al. 2003) not found in *E. coli*, exist, whereas MinC seems to be absent from cyanobacteria and plastids. Some of those seem to be functionally equivalent to the eubacterial Min proteins. For instance, the arc6 protein appears to stabilize the FtsZ ring in a manner similar to the eubacterial ZipA protein (Vitha et al. 2003). Since chloroplasts arose in a long-lasting process of endosymbiontic domestication from free-living cyanobacteria (Hashimoto 2003), they are more relevant to our understanding of plastid division than the much more intensively studied division systems in *Escherichia coli* or *Bacillus subtilis*.

Thus, already at the prokaryotic stage, three at least partially different division systems were available. They were adapted to the specific needs of the corresponding cells: (i) The terminal organelles that seem to interact mainly with an actin-related force-generating system in the unwalled mycoplasmas and may have been the ancestral situation for mitosis in primitve eukaroytes; (ii) the FtsZ-Min-ring system that constricts the daughter cells by a converging ring in the walled eubacteria; and (iii) a FtsZ-dependent system utilizing a different set of accessory proteins that evolved in the walled cyanobacteria and was adopted during endosymbiosis for the division of chloroplasts in the plant kingdom. It should be noted that FtsZ, the tubulin ancestor, is present in all three division systems, but differs with respect to its impact on mitosis. The primordial eukaryotic mitosis was probably much more actin-dominated than in modern eukaryotes. This situation changed dramatically when the eukaryotic cells, probably through an endosymbiontic interaction, acquired a new organelle – the flagellum. This acquisition subsequently shaped the evolution of mitosis in many of the lower eukaryotes.

3
Mitosis in Lower Eukaryotes

3.1
Structural Aspects of Mitosis and Cytokinesis

In a recent review, Cavalier-Smith (2006) established an Eukaryote phylogeny partially based on the diversification of the microtubular cytoskeleton. The tree was rooted in the unikonts. Eukaryotic motility was postulated to originate from an ectosymbiosis of a spirochete (Margulis 1993). However, this

hypothesis was recently revised (Li and Wu 2005). Polymers composed of α/β-tubulin dimers observed in epixenosomes, ectosymbionts of *Euplotidium* ciliates (Petroni et al. 2000), place the possible origin of tubulin into the newly discovered bacterial division of Verrucomicrobia (Li and Wu 2005).
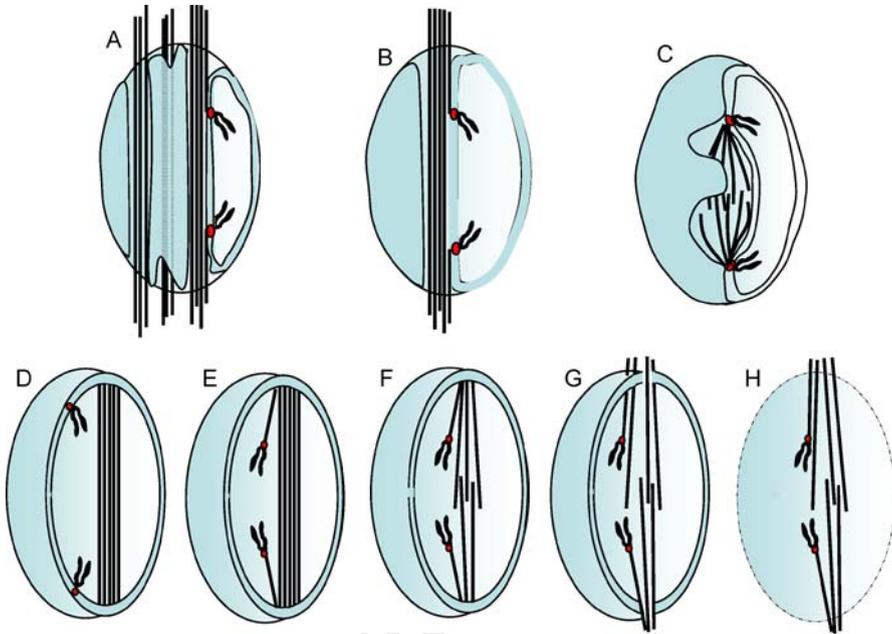
Fig. 4 Organization of the mitotic spindle in lower Eukaryotes. Extranuclear (**A–C**) and intranuclear spindles (**D–H**). **A–B** Cytoplasmic microtubule arrays are formed in one or more grooves of the nucleus. Chromosomes are directly attached to the nuclear envelope and they separate along this axis. Kinetochores and NAOs form the same entity (i.e., *Cryptothecodinium* and *Syndinium*, Dinoflagellata, Alveolata). **C** A cytoplasmic spindle links kinetochores/NAOs to chromosomes and drives karyokinesis (i.e., *Trichomonas*, Parabasilida, Excavata). **D** An intranuclear bundle of continuous contiguous pole-to-pole microtubules forms a central spindle. Its elongation during anaphase splits the chromosomes in two batches (i.e., *Trypanosoma*, Kinetoplastida, Excavata). **E** Kinetochores have dissociated from NAOs and kinetochore microtubules are formed. A central spindle of continuous microtubules elongates during anaphase (i.e., *Polysphondilium*, Myxomycetes, Amobozoa). **F** Elongation of interdigitating polar microtubules allows segregation with or without poleward migration of chromosome. NAOs are part of the nuclear envelope or intranuclear structures (i.e., *Paramecium*, Ciliates, Alveolates). **G** NAOs are cytoplasmic, and the spindle forms through fenestrae within the nuclear envelope which partially opens (i.e., *Euglena*, Euglenida, Discicristates; *Toxoplasma*, Apicomplexa, Alveolates; *Plasmodium*, Phytomyxa, Rhizaria and *Phytophtora*, Oomycota, Stramenopiles). **H** Open mitosis with complete breakdown of the nuclear envelope. When at all present, NAOs take highly variable forms (i.e., *Navicula*, Diatoms, Stramenopiles and *Ochromonas*, Chrysophyta, Stramenopiles; *Pelomyxa*, Rhizopoda, Amoebozoa and *Cyanophora*, Glaucophyta, Rhodophyta)

The putative ancestral, heterotrophic, aerobic, uniciliate eukaryote would first have developed a microtubular cytoskeleton associated with a basal body. This organizer of the flagellar apparatus would have been designed to ensure multiple functions: locomotion, sensory perception, as well as cell division. Given optimal survival value of the primitive eukaryote cells, this integration of three basic processes would have optimally increased the fitness, because increase in size was linked to the acquisition of subcellular compartment (Azimzadeh and Bornens 2004). During evolution, lower eukaryotes largely diversified their spindle apparatus, in order to ensure equational symmetric partitioning of chromosomes (Fig. 4). A detailed review on the structural aspects of mitosis was given by Heath (1980). These morphologic variations bear on polar structures, nuclear envelope, distribution and activity of spindle microtubules, and chromosome behavior.

The evolution of microtubule mitotic structures in relation to actin needs to be considered. For a long time, it has been known that actin microfilaments are present in an acto-myosin ring, which constricts at the cell equator in telophase and is centrally involved in cytokinesis (Guertin et al. 2002). Therefore, the loss of myosin functions affects cytokinesis in budding and fission yeast. In *Dictyostelium*, this may be accompanied by furrowing, which is independent from myosin II, but dependent on adhesion.

Protists, algae, and fungi are polyphyletic. They are dispersed among the five to 11 eukaryotic groups (superkingdoms or kingdoms). We will examine structural mitotic aspects of these groups. The polar structures closely resembling the nine triplet-microtubule arrangement will be named centrioles, whereas for other structures related to the organization of microtubules we will use the term nucleus associated organelle (NAO).

3.1.1
Excavata and Discicristata (Ciliate and Flagellate Protists)

The nuclear membrane remains intact (closed) during mitosis; in the Excavata, the anaphase spindle is cytoplasmic, and it is located in a groove of the nucleus (i.e., *Barbulanympha*). The central spindle extends across one side of the nucleus, with chromosomes connected to microtubules at kinetochores situated within the nuclear envelope; the spindle poles are often associated with "atractophores" (fibrous extensions from specific basal bodies) (i.e., *Gigantomonas herculea*).

Discicristata (i.e., Euglenida, Kinetoplastida) also maintain an intact nuclear envelope, but form an internal spindle, connecting separate nucleoplasmic chromosomes to poles via kinetochore microtubules. The rhizoplast, a striated band, elongates from duplicated flagellar basal bodies (blepharoplasts) to the nuclear envelope at undifferentiated polar regions or at NAOs.

3.1.2
Stramenopila (Heterokonts)

Among other taxa, this phylum includes the Oomycota, Xanthophyta, Chrysophyta, Phaeophyta, and the diatoms. In the majority of studied species, one microtubule per kinetochore is observed, but the chromatin does not condense. Mitotic microtubules form only an intranuclear spindle, without a central non-kinetochore array. This spindle elongates between anaphase and telophase. Chromosomes do not separate; they form a joint mass (i.e., in Cryptophyta and diatoms). In the Xantophyta, chromosomes are well-separated, with exception of one taxon where the chromatin was observed to be uncondensed.

The nuclear envelope disperses and a bundle of closely spaced interdigitating microtubules connect both poles in diatoms. A central spindle is only present in the Bacillariophyta (diatoms) and one taxon of the Xantophyta. In diatoms and chrysophytes, the NAO forms a striated bar or plaque resembling a rhizoplast. In oomycetes, centrioles are aligned in parallel (Fig. 5C), and the nuclear envelope remains intact during mitosis.

3.1.3
Alveolata

Dinoflagellates maintain an intact nuclear envelope. Chromosomes are mostly well-separated, but they sometimes remain aggregated in a joint mass. Non-kinetochore microtubule bundles are located in extranuclear tunnels or channels (i.e., *Cryptothecodinium*). In this species, the chromosomes become temporarily attached to the nuclear membrane surrounding these channels without distinguishable kinetochores. In the Ciliophora (ciliates), polar structures are absent, but spindle microtubules still elongate in anaphase. The micronucleus performs a closed mitosis with an intranuclear spindle and chromosomes with kinetochores. In Apicomplexes, a closed mitosis with an intranuclear spindle is mostly observed. Nuclear pores associated with cytoplasmic NAOs have also been described (i.e., *Plasmodium*). Alternatively, in the absence of any structured NAO, schizogony occurs through nuclear budding (i.e., *Toxoplasma*). In *Eimeria*, a cytoplasmic ring nucleates cortical microtubules, whereas spindle microtubules are nucleated from centrosomes.

3.1.4
Rhizaria and Cercozoa

In a majority of these protists, the nuclear envelope remains intact during mitosis and the spindle is intranuclear.
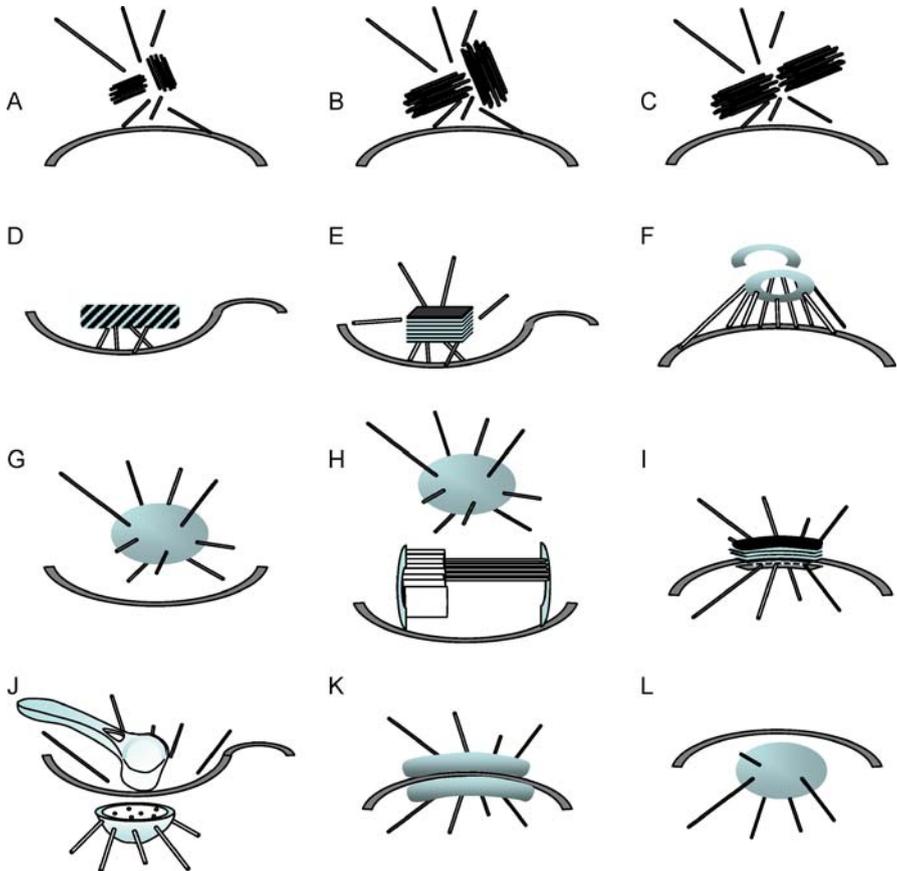
Fig. 5 Organization of centrosome and NAOs in lower Eukaryotes. Extranuclear (**A–H**), mixed (**I–K**), and intranuclear (**L**) microtubule organizing centres are observed. **A** Procentrioles (i.e., *Cladophora* and *Labyrinthula*). **B** Canonical centrosome as found in the chytrids. **C** Aligned, antiparallel centrosomes of the oomycetes. **D** Rhizoplast (i.e., *Ochromonas*) or plaques of Cnidosporidia. **E** Eight-tier core NAO in *Dictyostelium*. **F** Ring–shaped NAO (i.e., *Polysyphonia*, *Polysphondilium*). **G** Attractophore or sphere (i.e., *Filobasidiella*, Basidiomycota, and Zoomastigina). **H** Striated bar appearing beneath a cytoplasmic sphere in the prophase of diatoms (i.e., *Surirella*). It splits into two sets and forms a central spindle as soon as the nuclear envelopes breaks down. **I** Typical spindle pole body of yeast, forming dense plaques within the nuclear envelope. **J** Specialized structured NAO of *Erynia neoaphidis* (Zygomycota, opistokonts). **K** Slightly electron dense material distributed on both sides of the nuclear envelope (i.e., *Mucor*, Zygomycota). **L** Intranuclear NAO organized in a sphere (i.e., *Minchinia*, *Rhizaria*, plasmodial form of *Physarum*, *Tocoplasma tachyzoites* and *Oedogonium*)

In the Phytomyxa (i.e., *Plasmodiophora brassicae*), an unusual type of chromosome segregation, called cruciform division (chromatin divides in a clump and moves to the poles, while the nucleolus elongates), is observed.

In interphase, the centrioles are aligned antiparallel in one axis. The nuclear envelope partially breaks down in prometaphase, and large amounts of membrane cisternae become clearly visible in the interior of the spindle during metaphase (Garber and Aist 1979). In the Radiolaria, adjacent fan-shaped microtubule arrays form simultaneously at the surface of duplicated polar structures (plaque and orthogonal centrioles). Bipolarization occurs in a subsequent step. Acantharia (i.e., *Acantholithium*) develop a central spindle, whereas polycistines (i.e., *Theopilium*) do not.

3.1.5
Amoebozoa

There are three types of mitotic divisions: **promitotic**, with an intranuclear spindle and a persistent nucleolus (i.e., *Naegleria gruberi*, *Entamoeba histolitica*, *Acanthamoeba*); **mesomitotic**, with the absence of centrioles and delayed breakdown of the nuclear envelope and nucleolus (i.e., *Pelomyxa carolinensis*); and **metamitotic**, with absent centrioles and early breakdown of both nuclear envelope and nucleoli. In the Myxomycota (i.e., *Physarum*) and Dictyostela (i.e., *Dictyostelium*), mitosis is closed with an intranuclear spindle that is connected with a ring or a cubical extranuclear NAO, respectively.

3.1.6
Opisthokonta (Including Fungi and Animals)

Orthogonal centrioles are observed in the hypochytrids and chytrids, whereas Ascomycota and Basidiomycota integrated the microtubule-nucleating components closer to the nuclear envelope in various forms of NAOs, such as plaques, spheres, bars, or rings. Fungi and animal cells present mostly well-defined, separate, distinguishable chromosomes. They exhibit a clear central intranuclear pole-to-pole microtubule bundle, which is absent in the hypochytrids and chytrids, but is present in some zygomycetes. The fungi display a great variability with regard to the persistence of the nuclear envelope. Their variability range includes: a nuclear rim that remains intact throughout mitosis; nuclei that are polarly fenestrated (i.e., in the chytrids); as well as a situation where the nuclear envelope becomes entirely dispersed.

Canonical centrosomes are present in all animal classes, with the exception of Euarthropodae (i.e., *Drosophila*), in which they are replaced by cytoplasmic ring-like structures, and nematodes (i.e., *Caenorhabditis elegans*), in which only orthogonal procentriole-like structures are visible. In the zygomycete *Erynia*, the NAO consists of both a saucepan-shaped extranuclear and a saucer-shaped intranuclear plaque.

3.1.7
Lower Plants

Uncondensed chromatin and a central spindle were only described for one taxon of the Rhodophyta. Otherwise, well-defined chromosomes are condensing in G2 phase, and the nuclear envelope breaks down in prophase (i.e., *Cyanophora*, Glaucophyta, Rhodophyta). In the green algae *Cladophora*, the NAO is composed of one centriole and a procentriole, which is only formed by microtubule singlets in place of triplets and persists during mitosis. Otherwise, centrosomes are maintained from green algae to *Gingko* in their motile male gametophytes.

3.2
Molecular Aspects of Mitotic Evolution

Most of the genes involved in mitosis and their control were discovered using **yeast** models. This was possible by the strong conservation, preserved up to mammalian systems, of the major players and regulators of mitosis, due to the fundamental importance of cell division.

3.2.1
Microtubule Motors

Among other functions, the kinesin superfamily participates in chromosome movement and spindle functions during mitosis. The kinesins, which have the catalytic domain located at the N- or C-terminus are responsible for: generating forces for spindle assembly, bipolarity, stabilization, and dynamics; maintaining antagonistic forces within the spindle; and allowing for cross-linking and bundling of microtubules. On the other hand, kinesins, which have the catalytic domain located in the center, link the microtubule end and stimulate its depolymerization.

Kinesins are found in protists, yeasts, fungi, plants, invertebrates, and vertebrates. They are distributed over at least 14 subfamilies. Among the seven kinesins found in *Saccharomyces cerevisiae*, six play a role in mitosis in association with one dynein. In *Caenorhabditis elegans*, 19 kinesins and two dynein havy chains have been characterized. The number of kinesin species raises up to 45 for humans, and even 60 for *Arabidopsis* kinesins. Protozoan kinesins diverge quite considerably. Additional kinesin-like proteins, which could not be attributed to any of the above mentioned 14 classes of canonical kinesins, have been identified in *Cenorhabditis*, *Plasmodium*, *Tetrahymena*, *Giardia*, and *Leishmania*.

Interestingly, kinesins have acquired multiple additonal functions during their evolution from their first ancestors. There are classical microtubule plus-end directed motors, such as kinesin-5 (formerly BimC); microtubule

minus-end directed motors, like kinesin-14 (formerly C-term motors); and I-type kinesins, like kinesin-6 (formerly MKLP1) or kinesin-3 (formerly Unc104/KIF1). All of them are involved in spindle maintenance or cytokinesis. However, there are chromosome-associated kinesins, connecting kinetochores and kinetochore microtubules or associated along chromosome arms, and contributing to chromosome condensation or motility, such as kinesin-7 (formerly CENP-E), kinesin-13 (formerly MCAK/KIF2), and kinesin-4 (formerly Chromokinesin or KIF4), respectively (Miki et al. 2005; Mazumdar and Misteli 2005; Wordeman 2005).

Dyneins are strictly microtubule minus-end motors. They have been first characterized in *Tetrahymena*. Members of this family were found in most phyla, including slime molds, fungi, *Chlamydomonas*, sea urchin, *Drosophila*, or mammals. Among higher plants which have lost cilia and flagella, four dynein heavy chains have been reported only for *Oryza sativa* (King 2002). However, a recent comparative genomic analysis came to the conclusion that dyneins have been lost during the evolution of higher plants (Wickstead and Gull 2007).

3.2.2
Centrosomal Proteins

As mentioned above, the (9 + 2) centriolar structure was acquired early. It was primarily associated with cell motility, sensing, and division (Azimzadeh and Bornens 2005). Although the architecture of microtubule organizing centers has undergone divergent evolution, many of their molecular components have remain conserved.

This is the case for γ-tubulin and the γ-tubulin complex proteins (GCPs), which are the essential components of microtubule nucleation (Erhardt et al. 2002). However, for a long time, the γ-tubulin orthologues of budding yeast and *C. elegans* had not been identified, due to a high divergence in their sequence. This could either be the consequence of rapid evolution rate, or of an adaptation to specific partners associating the γ-tubulin small complex into a larger nucleation complex (like the γ-TuRC found in *Drosophila*, *Xenopus*, humans, and higher plants).

Centrins, a second important group of centrosomal proteins, are closely related to calmodulin and have been proposed to act as regulators. They form two largely conserved subfamilies that are defined by the founding members, Cdc31p of *S. cerevisiae* (Cdc31p) and the centrin of *Chlamydomonas reinhardtii*. The sequence specificities of these subfamilies do not necessarily mirror functional differences, since both centrin types play a role in centrosome duplication, segregation, and cytokinesis. These canonical centrins are complemented by centrin subfamilies in the Alveolata and the angiosperms, which are, apparently, no longer linked with motility (Azimzadeh and Bornens 2005).

3.2.3
Kinetochore Proteins

Kinetochore proteins are multiproteic complexes binding to centromeric DNA and connecting chromatids to the spindle poles via kinetochore microtubules. Phylogenetic and structural analysis of kinetochore proteins (Meraldi et al. 2006) revealed that the kinetochore core proteins have conserved short motives, which have been conserved throughout the eukaryotes, despite a huge divergence within centromeric DNA sequences. The simplest kinetochore has been characterized in *Encephalitozoon cuniculi*, a microsporidial parasite, which reduced its genome under selection pressure. Three of the four multiproteic complexes characterized in higher eukaryotes link DNA-binding components to microtubule binding components (including motors) and regulatory elements (including the kinases, Mps1 and Bub3, both playing a role in the mitotic checkpoint). The proteins constituting these complexes have been identified all over the eukaryotes, from yeast to humans (Meraldi et al. 2006).

3.2.4
Microtubule Associated Proteins

Structural Microtubule Associated Proteins (MAPs) are proteins whose binding to microtubule modulates their dynamic properties (Cassimeris and Spittle 2001). Several classes of MAPs have been implied in microtubule nucleation and spindle organization (for instance, the γ-TuRC components and TPX2); monomer binding (e.g., OP18/stathmin); polymer stabilization (e.g., MAP U); and destabilization or severing (e.g., I-type kinesins and katanin complexes, respectively). Among these, the MAP215/Dis1 family of MAPs are highly conserved microtubule regulators in the mitotic spindle (Gard et al. 2004), and have been described under various names in a large number of organims (Dis1 and Alp14 in *S. pombe*, Stu2 in *S. cerevisiae*, DdCP224 in *D. discoidum*, Zyg9 in *C. elegans*, Msps in *Drosophila*, XMAP215 in *Xenopus*, ch-TOG in humans, MOR1 in *Arabidopsis*, and TMBP200 in tobacco). In the meantime, more than 30 additional related genes have been discovered through database searching. All these homologues are targeted to kinetochores in metaphase and to their respective microtubule organizing center (MTOC) in anaphase.

3.2.5
Mitotic Kinases and Phosphatases

Protein kinases are one of the largest gene families in all eukaryotes, accounting for 2–4% of all genes (Manning et al. 2002). This is to be expected, considering the large number of biological processes that are regulated through

phosphorylation. Mitotic kinases, such as the Cdk/cyclins, Aurora-kinases, polokinases, and the "Never In Mitosis A"-kinases play a role in the condensation of chromatin, the disassembly of nuclear lamina, nuclear envelope, nuclear bodies and nucleoli, as well as in spindle assembly, microtubule-kinetochore attachment, and spindle checkpoint (for review, see Li and Li 2006).

The serine/threonine phosphatases, PP1 and PP2A/CDC55, and the dual-specificity protein tyrosine phosphatases (DUSPs), CDC25 and CDC14, are key regulators of mitosis (for review, see Trinkle-Mulcahy and Lamond 2006). PP1 is involved in the maintenance of chromatin achitecture during metaphase-anaphase (Trinkle-Mulcahy et al. 2006; Vagnarelli et al. 2006). It regulates both chromatid decondensation and nuclear envelope reassembly in telophase (Steen et al. 2003; Landsverk et al. 2005). PP2A participates in the maintenance of chromosome cohesion (Kitajima et al. 2006). It regulates both chromosome segregation (Tang et al. 2006) and mitotic exit (Queralt et al. 2006). Animal and plant PP2A have evolved independently (Terol et al. 2002), but members of this family still share the same functions.

CDC25 is a family of three phosphatases, which are generally involved in all cell-cycle checkpoints (Donzelli et al. 2003). CDC14 has also been conserved from yeast to mammals. Some of their mitotic functions are species specific, but their role in spindle stabilization and cytokinesis seems to be conserved among eukaryotes (Trinkle-Mulcahy and Lamond 2006).

4
Milestones Towards Acentriolar Plant Mitosis

Land plants are generally thought to originate from green algae, where structured NAOs dominate. Centrosomes or organelles deriving from basal bodies are found in the unicellular flagellates of monadoid algae or the spermatozoids of multicellular green algae. The transition from a mobile aquatic life style to a sessile terrestrial life style was accompanied by profound changes of mitosis, which directly influenced morphogenesis. These evolutionary modifications went through a progressive loss of the typical (9 + 2) centrioles, and they were associated with a diversification of body plans, varying across unicellular forms, organisms composed of multinucleated modules, and mononucleated cells, which are organized into functional contexts capable of functional differentiation (first primitive parenchymes, which subsequently developed into differentiated tissues). In all cases, nucleocytoplasmic domains containing microtubule organizing complexes represent the basic cellular unit (Pickett-Heaps et al. 1999).

From green algae up to the Gingkophyta, the canonical centrioles generated de novo in gametes are replaced by a variety of more or less structured NAOs in somatic or meiotic cells. Prior to division of these cells, the micro-

tubule interphase network is usually disassembled and replaced by a polarized mitotic spindle. In terms of evolutionary relationships, emphasizing the structural differences of spindle poles can be more misleading than revealing. Instead, it is more fruitful to consider the proteins or protein complexes involved in spindle assembly and organization.

4.1
Localization of γ-tubulin

Gamma-tubulin has been often used as a marker for microtubule organization centers. It is mostly found to segregate at each mitotic pole. It has been shown to concentrate around the centrosomes at the spindle poles in all cells endowed with centriole-driven mitosis, to mark the periphery of polar plastids, and to label acentrosomal spindle poles. All these different conformations are present in Bryophytes (Shimamura et al. 2004). For example, during the final spermatogenous mitotic division of liverwort *Makinoa crispata*,
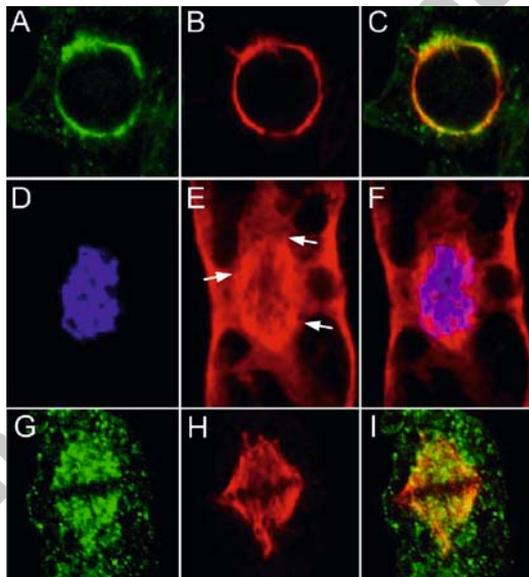
Fig. 6 Dynamic localization of γ-tubulin (*green*), α-tubulin (*red*), and chromosomes (*blue*) in the tobacco cell line, BY-2, during the transition between prophase and metaphase. **A–C** late G2 phase: A majority of perinuclear microtubules is polarized into the future spindle axis and γ-tubulin concentrates into two polar caps. Microtubular plus-ends penetrating into the nucleus are void of γ-tubulin at their tips. **D–F** Breakdown of the nuclear envelope: chromosomes disperse into the cytoplasm and microtubule fibers are not clearly aligned with the division axis (*arrows*). **G–I** During metaphase, the spindle is characterized by broad poles and γ-tubulin is not localized to the microtubular plus-ends at the kinetochores

γ-tubulin surrounds polar Centrosomes. Conversely, during archesporial mitosis of liverwort *Marchantia polymorpha*, γ-tubulin reorganizes from initial foci around discrete microtubule organizing centers in prophase to a broader polar region in metaphase. Monoplastidic meiotic cells of *Dumortiera hirsuta* present periplastidial γ-tubulin in prophase I and II; a focused polar distribution in late prophase I; a broader localization on spindle fibers in metaphase I; and localization to the nuclear rim as well as the minus-ends of phragmoplast microtubules in telophase I and II.

In higher plants, as exemplarily shown for the acentrosomal tobacco BY-2 cells, γ-tubulin is dispersed at the nuclear surface of interphase cells. In late G2, (Fig. 6), the division axis becomes progressively manifested by a corresponding reorientation of perinuclear microtubules. Simultaneously, γ-tubulin is redistributed along perinuclear microtubules, and it concentrates into two polar caps (Fig. 6A). Then, the nuclear envelope breaks down at polar sites and microtubule plus-ends rapidly elongate toward chromatin (Fig. 6B,E). γ-tubulin does not colocalize with these fast growing sites, but it shifts towards the proximal minus-ends of the polar microtubules (Fig. 6A,G).

4.2
Evolution of Cytokinesis

Cytokinesis is the mechanisms leading to cellularization. Usually, it directly follows karyokinesis. Structurally, it is achieved through different mechanisms (Fig. 7): a centripetal cleavage furrow between daughter cells (e.g., *Nephroselmis*, Prasinophyceae, Fig. 7A); a cleavage furrow forming a phycoplast, consisting of a set of microtubules oriented parallel to the plane of the new cell wall (i.e., *Chlamydomonas*, Clamydomonadales, Chlorophycaea, Fig. 7B); a central fusion of vesicles with the phycoplast (i.e., *Oedogonium*, Oedogoniales, *Cylindrocapsa*, Chlorococcales and *Uronema*, Chaetophorales, and Chlorophycaea, Fig. 7C); a cleavage furrow with simultaneous persistence of central spindle microtubules (i.e., *Pyra-mimonas*, Prasinophyceae; *Ulothrix*, Ulvophycaea; *Klebsormidium*, Klebsormidiophyceae, Fig. 7D); or a centrifugally established cell plate organized by a phragmoplast (i.e., *Trentepohlia*, Ulvophyceae; *Spirogyra*, Zygnematophyceae; *Chara*, Charophyceae, *Chlorokybus*, Choleochaetophycaea, and also all Embryophytes, Fig. 7E). In multinucleated cytoplasts, cytokinesis may be delayed after karyokinesis and phragmoplasts form wherever overlapping microtubules are present, i.e., between sister and non-sister nuclei (i.e., sporogenesis of *Conocephalum*, Bryophyta; endosperm of higher plants, Fig. 7F).

It is puzzling that a phragmoplast is found not only in the Streptophyta (e.g., some Zygnematophycaea, Charophycaea, Choleochaetophycaea) and the Embryophyta (e.g., *Bazzania*, Marchantiophytes), but also in Chlorophyta, such as the Trentepohliales (Mattox and Stewart 1984). It either corresponds to a structural convergence evolved in parallel or, in case it is of mono-
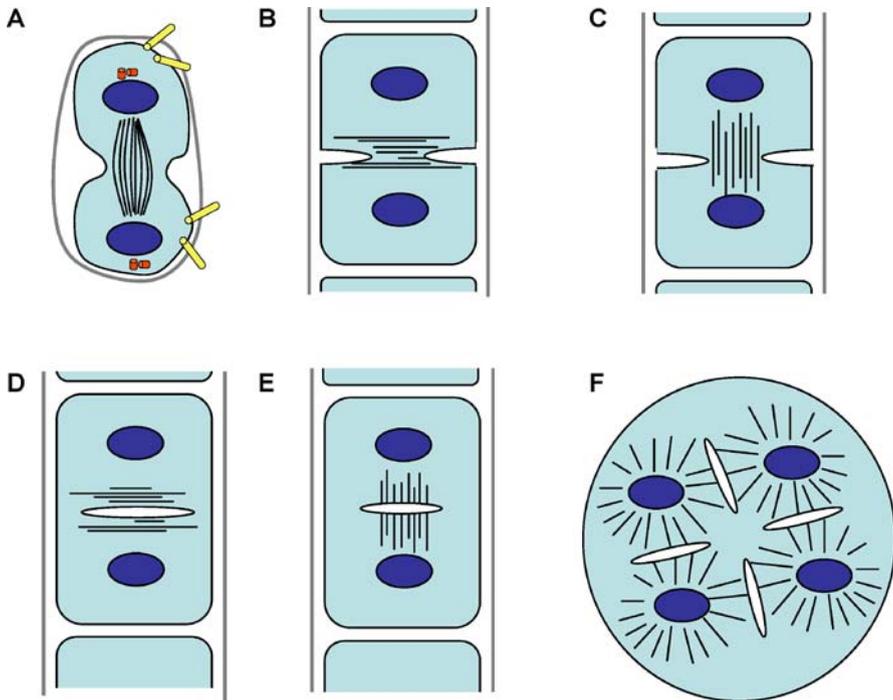
Fig. 7 Variability of cytokinetic mechanisms in algae and embryophytes. **A** Microtubule-independent cleavage furrow in an unicellular organism (e.g. Nephroselmis). **B**, **D** Phycoplast microtubules that are oriented perpendicular to the spindle axis, are involved in cell wall formation either through cell wall ingrowth (**B**) or outgrowth (**D**). **C**, **E** Phragmoplast microtubules that are oriented parallel to the spindle axis associated with a cleavage furrow (**C**) or with centrifugal vesicle fusion leading to the formatkion of a cell plate development (**E**). **F** Cellularization of coenocytes with phragmoplasts developing between sister and non-sister nuclei

phyletic origin, it necessitates a revision of the current views on taxonomy and evolution of the Chlorophyta. To address this question, Lopez-Bautista et al. (2003) analyzed the phylogeny of phragmoplastin, a protein found to play a role in squeezing Golgi-derived vesicles, which fuse at the cell plate and form a tubulovesicular pattern during early cell plate development. This protein could be identified in *Trentepohlia* and *Cephaleuros*, consistent with the model of a putative common ancestor expressing phragmoplastin and using a primitive form of phragmoplast-dependent cytokinesis. However, this data set is still too limited for a conclusive answer. With respect to phragmoplast structure, the Trentepohliales are close to the Charophycea. However, in many other aspects, they behave more like Chlorophyta, such that they seem to represent a chimera comprising traits from both Chlorophyta and Streptophyta.

4.3
Cytokinesis – The Role of Actin and Spindle Polarity

In the absence of microtubules, a ring of actin is involved in the formation of the cleavage furrow (e.g., *Spirogyra*, Goto and Ueda 1988). Moreover, actin filaments are also found in the phycoplasts of the Chlorophyta (e.g., in *Chlamydomonas*, Harper et al. 1992; *Nannochlorys*, Yamamoto et al. 2001). In phragmoplasts, actin filaments are mostly aligned parallel to microtubules, forming a wreath or a belt encircling the extending microtubule ring. Inhibitors of F-actin, such as cytochalasins or latrunculin, impede the centrifugal extension of the microtubule phragmoplast, suggesting that the actin cytoskeleton cooperates with microtubules in the development of the cell plate (Schmit and Lambert 1988).

In walled, sessile cells, the polarity of the mitotic spindles and the subsequent deposition of the new cell walls are of crucial importance for the control of morphogenesis. In higher plants, the phragmoplast is laid down in close proximity to the site where, prior to the mitotic prophase, a cortical preprophase band consisting of microtubules is formed. It is interesting to determine whether the cleavage plane is similarly predictable in lower eukaryotes.

Cortical microtubules encircling the nucleus in higher plants are absent in algae, including *Coleochaete* and *Chara*. In these cells, the polarity of division is mostly determined by polar location of NAOs or plastids.

In primitive Embryophyta, the future plane of cell division is predicted by a microtubule preprophase band (PPB). Actin filaments are associated with this PPB. When the PPB disappears at the onset of the prophase, it leaves behind a cytoplasmic region, which is also depleted of actin. This reorganization of both cytoskeletal components predicts the boundaries of the prospective daughter cytoplasts (Pickett-Heaps et al. 1999). However, the formation of a PPB seems to be confined to somatic cells (Brown and Lemmon 2001), and it seems to be frequently incomplete in the fern *Azolla* (Gunning et al. 1978), and even absent in the filamentous moss protonema (Doonan 1991).

The reproductive lineage of most land plants (Bryophytes, Lycophytes, and certain ferns) utilizes different modes of cellular polarization. In the sporogenesis of monoplastidic cells, a bipolar NAO first appears during early meiotic prophase. Subsequently, a quadripolar microtubule system (QMS) originates from the divided plastids, delineating the position where the four haploid spores will segregate in telophase II (Pickett-Heaps et al. 1999; Shimamura et al. 2004). Recently, Brown and Lemmon (2006) described sporogenesis in liverwort *Aneura pinguis*, in which perpendicular girdling bands of microtubules encircle the prophase nucleus and delimit the prospective cytokinetic partition planes of each individual spore. This organization may correspond to ancestral PPBs. A difference noticed in this case is the as-

Table 1 Phylogeny used in this chapter

Kingdom	Glaucophyta		
	Rhodophyta		
	Chlorobionta		
	Division	Chlorophyta	
	Class	Prasinophycaea	
	Order	Prasinococcales
		Pyramimonadales
		Mamiellales
		Nephroselmidiales
		Pseudoscourfieldiales
		Picocystidales
		Chlorodendrales
	Trebouxiophycaea	
		Trebouxiales
		Microthamniales
		Prasiolales
		Chlorellales
	Chlorophycaea	
		Oedogoniales
		Chaetophorales
		Dunaliellales
		Tetrasporales
		Chlamydomonadales
		Chaetopeltidales
		Sphaeropleales
		Volvocales
		Chlorococcales
		Chlorasarcinales
		Microsporales
		Cylindrocapsales
	Ulvophycaea	
		Ulotricales
		Ulvales
		Trentepohliales
		Cladophorales/Siphonocladales
		Oltmannsiellopsidales
		Bryopsidales
		Caulerpales/Halimedales
		Dasycladales
	Streptophyta	
		Mesostigmatophycaea
		Chlorokybophycaea
		Klebsormidiophycaea
	Phragmoplastophytes	
		Zygnemophycaea
		Zygnematales
		Desmidiales

Table 1 (continued)

Plasmodesmophytes
    Charophycaea
        Charales
    Chaetospheridiophycaea
Parenchymophytes
    Coleochaetophycaea
        Coleochaetales
Embryophyta (land plants)
    Bryopsideae
        Marchantiophytes (Hepatics = liverworts)
        Bryophytes (Mosses)
        Anthocerophytes (Hornworts)
    Tracheophytes (Vascular plants)
        Polysporangiophytes
        Lycophytes
    Euphyllophytes
        Monilophytes
        Sphenophytes
        Filicophytes
    Spermatophytes
        Coniferophytes
            Ginkgophytes
            Pinophytes
        Cycadophytes
        Anthophytes
            Gnetophytes
            Angiosperms

sociated protrusion of four distinct lobes, restricting the cytoplasm earlier than what is found in other Bryophytes and higher plants. The mechanism of lobing remains unknown. However, it seems not to involve F-actin constrictions, as observed in animal cell cleavage (Brown and Lemmon 2006). In the liverwort *Reboulia*, microtubules originating from polar NAOs are organized in a criss-cross pattern, constituting a PPB-like structure coexisting with the prophase spindle (Brown and Lemmon 1990). In flowering plants, a triploid coenocytic endosperm is only later cellularized (e.g. in *Haemanthus*), such that karyokinesis is uncoupled from the largely delayed cytokinesis. These cells do not form PPBs either (Mole-Bajer and Bajer 1963). This is not surprising if one considers that these cells lack cortical microtubules. Instead, the G2 nuclei are efficiently separated by perinuclear microtubules that maintain a cytoplasmic area around the individual nuclei, which enter mitosis in a synchronized manner, such that a wave of division

can be observed to travel through the syncytium (e.g., in *Ginkgo*, Brown et al. 2002).

5
Evolutionary Footprints 1: Division of Plastids and Mitochondria

The qualitative difference between eukaryotic and prokaryotic cells is generally accepted to have derived from multiple endosymbiotic events, whereby a larger "host" cell integrated a prokaryotic prey and failed to digest it. During the subsequent phase of "domestication", numerous genes were transferred from the endosymbiont into the host genome, such that the endosymbiont progressively became dependent on the eukaryotic host. This process, in general, seems to be more advanced for the mitochondria, as compared to the plastids. However, both organelles have preserved their generic identity, because they cannot be assembled by the host de novo. Instead, they arise exclusively ex suis generis. This evokes the question whether or not organelle divisions are coordinated with the division of the host cell. It seems that plastids and mitochondria represent different steps on this evolutionary path. Plastids have not yielded to domestication to the same degree as mitochondria (for review, see Osteryoung and Nunnari 2003).

Mitochondria have lost FtsZ, whereas plastids still utilize the original prokaryotic FtsZ-machinery for division. However, mitochondrial FtsZ can still be detected in primitive Eukaryotes, such as the chromophyte alga *Mallomonas* (Beech et al. 2000). In animals and fungi, the division of mitochondria involves the activity of dynamin-related proteins, i.e., GTPases that can generate force and associate with the cytoplasmic surface of the outer membrane in form of a ring. This indicates that the originally autonomous (FtsZ-based) division function of mitochondria has been completely replaced by a host function. However, dynamin-related proteins have been identified in plastids as well. They participate there in the late phases of division. For instance, the dynamin-related proteins, Arc5 (*Arabidopsis*, Miyagishima et al. 2006) or Dnm2 (Rhodophyta, Miyagishima et al. 2003), interact with the plastid division machinery during later phases of septation. Thus, the dynamin-related proteins represent a general eukaryotic tool for organelle division. In the case of plastids, they interact with FtsZ, whereas, in mitochondria, the function carried by FtsZ has been taken over by other unknown host proteins.

Until relatively recently, it was unknown whether the division of plant mitochondria was driven by dynamin-related proteins, as is the case in animals and fungi. However, a screen for *Arabidopsis* mutants with altered morphology of mitochondria and peroxisomes led to the identification of the dynamin-related protein, DRP3A, which participates in the division of mitochondria and peroxisomes. Mutants of the corresponding gene produce misshaped and partially giant mitochondria and peroxisomes, but contain

perfectly normal chloroplasts (Mano et al. 2004). This suggests an evolutionary scenario, where a GTPase responsible for a host-cell activity (peroxisomal division) has acquired a dual function accepting mitochondrial function as "moonlighting" job.

Similar to mitochondria, chloroplasts have lost most of their genetic autonomy by transfer of genes into the nuclear genome. The cyanobacterium *Synechocystis* encodes more than 3000 proteins, whereas most chloroplast genomes harbor less than 200 coding sequences (Martin and Herrmann 1998), which means that most proteins have to be imported from the cytoplasm into the chloroplast, including FtsZ and other components of plastid division. The first indication for the activity of a prokaryotic type of division machinery came from the identification of a plant FtsZ (Osteryoung and Vierling 1995). When this FtsZ was specifically knocked down in moss *Physcomitrella patens* (Strepp et al. 1998), vermiform and giant chloroplasts were produced, demonstrating that this FtsZ was essential for plastid division. This conclusion was supported by similar findings in *Arabidopsis* (Osteryoung et al. 1998). In contrast to the situation in prokaryotes, where Ftsz is almost exclusively encoded by a single-copy gene, plant FtsZ contain multiple homologues of FtsZ, which cluster into two subfamilies (Rensing et al. 2004). The respective gene products participate in chloroplast division. However, most of the bacterial division proteins could not be identified in plant chloroplasts. Moreover, the cause for the duplication and diversification of the plant FtsZ family has remained unknown.

To gain insight into these questions, it is necessary to enlarge the spectrum of the usual model plants (mostly *Arabidopsis*, rice, and tobacco), by including model plants that are more distant in terms of phylogeny. Mosses, such as *Physcomitrella patens*, are very well suited in this context, because they appeared as early as 450 million years ago (Theißen et al. 2001), and, judging from fossil records, they have remained unchanged, at least in terms of morphology (Miller 1984). Thus, the bryophytes represent the most conservative group of land plants. They are ideal candidates for evolutionary studies.

During a study on the localization FtsZ isoforms in *Physcomitrella patens*, one isoform, FtsZ1-2, was found in the cytoplasm (Kiessling et al. 2004). So far, plant FtsZ isoforms have been exclusively reported in filamentous structures, whereas rings and networks inside the chloroplasts, GFP fusions of FtsZ1-2, could be found in form of rings in both compartments. This peculiar FtsZ isotype is endowed with two alternative start codons, whereby the chloroplast signature present in the longer transcript (which has been demonstrated to be imported into the chloroplast) is absent in the shorter transcript (which forms peculiar filamentous structures in the cytoplasm). The nature of these cytoplasmic filaments has not yet been uncovered. However, they seem to be specific for this FtsZ-isotype, because ectopic expression of a different FtsZ-isotype by removal of the plastidic import signature yielded punctate structures instead of filaments. It is tempting to interpret

FtsZ1-2 as a component with dual function. In addition to its participation in the division of chloroplasts, it might have acquired a second "moonlighting" function that involves an interaction with unknown partners of the host cytoskeleton.

So far, cytoplasmic FtsZ isoforms have not been reported outside the Bryophyta. Therefore, they might represent an exotic footprint of evolution. However, from a functional point of view, proteins of analogous function must exist in the other land plants as well, because the division of chloroplasts has to be tuned with the division of the host cells, in order to ensure that each cell remains with at least a minimal set of plastids. If one takes into account that most components of the prokaryotic division machinery have been replaced by functionally equivalent, but phylogenetically unrelated proteins, carrying the respective functions in organelle division, it is to be expected that the cytoplasmic function carried by FtsZ1-2 in mosses has been taken over by other (still unknown) proteins during the evolution of the cormophytes.

6
Evolutionary Footprints 2: The Plant Nucleus Revisited

As described in detail above, the open mitosis found in animals and higher plants has evolved from mitotic forms where the nuclear envelope persists, at least partially. In organisms that follow this so-called closed mitosis, the entry of tubulin and microtubule-nucleating components into the nucleus at the onset of mitosis, as well as their exclusion at the end, must be under strict cell-cycle control. For example, the spindle pole body of Saccharomyces cerevisiae is assembled in the cytoplasm and subsequently transported in a cell-cycle-dependent manner into the nucleus via the nuclear-localization sequence (NLS) of the Spc98p protein (Pereira et al. 1998). Interestingly, α- and β-tubulins are not found in interphasic nuclei of cells with closed mitosis. Therefore, in these organisms, tubulin has to be transported in and out of the nucleus. Indeed, it has been shown in *Aspergillus nidulans* that tubulin enters the nucleus immediately before the onset of closed mitosis, and it is rapidly excluded at the end (Ovechkina et al. 2003). Although the molecular mechanisms of nuclear tubulin transport remain to be elucidated, these results suggest that the movement of tubulin through the nuclear envelope during the cell-cycle is an active and highly regulated process.

In contrast to closed mitosis, in the open mitosis of animals, higher plants, and Charophyceae, the interaction of (cytoplasmic) tubulin with (intra-nuclear) chromatin occurs spontaneously at the breakdown of the nuclear envelope. Consequently, directed movement of tubulin through the nuclear envelope is not required for formation of the mitotic spindle. After disintegration of the nuclear envelope, the microtubule spindle can exploit the pool

of cytoplasmic tubulin that is no longer withdrawn by compartmentalization. As discussed above, the spindle microtubules are nucleated from centrosomes in animal and algal cells, but from rather diffuse microtubule-organizing centers (MTOCs) in the acentriolar cells of higher plants. In addition, the kinetochores of both animal and plant cells are endowed with a microtubule-nucleating activity (Cande 1990). γ-Tubulin, an indispensable component of both centrosomes and plant MTOCs (Pereira and Schiebel 1997, Stoppin-Mellet et al. 2000), is already interacting with the prospective kinetochore sites during the G2 phase and, thus, prior to the disintegration of nuclear envelope (Binarová et al. 2000). This indicates that, like the SPB of *S. cerevisiae*, the microtubule-nucleating component γ-tubulin must be actively transported through the intact nuclear envelope before mitosis. Again, neither has the molecular mechanism of this nuclear import been identified, nor is it known whether other components of MTOCs persist in the nucleus during the whole cell-cycle or have to be imported as well. A nuclear-localization sequence (NLS) is present in GCP3, one of the three components of the small gamma-tubulin nucleation complex (γ-TuSC), and this protein was detected by immunolabeling in G2 nuclei of BY-2 cells (Schmit, unpublished results). Moreover, sequences conferring targeting to the nuclear envelope have been found in GCP2 and GCP3 of *Arabidopsis thaliana* (Seltzer et al. 2007). This suggests that perinuclear microtubule nucleation initiates spindle formation in early G2, and that, upon breakdown of the nuclear envelope, microtubules are newly assembled around chromosomes from nucleation complexes already present in the vicinity of chromosomes.

Not only nucleation components, but also the bulk tubulins themselves, can be imported into the nucleus. Circumstantial evidence of intranuclear tubulin comes from mammalian tumour lines (Menko and Tan 1980; Walss et al. 1999; Walss-Bass et al. 2003) and *Aesculus hippocastanum* by electron microscopy (Barnett 1991). However, the functional context of nuclear tubulin import remained enigmatic. One possibility might be the sequestration of soluble tubulin heterodimers to circumvent the induction of regulatory circuits that otherwise would downregulate tubulin abundance (see chapter "Plant tubulin genes: regulatory and evolutionary aspects" of this book). If this working hypothesis was correct, the formation of intranuclear tubulin should be initiated by factors that trigger the disassembly of interphase microtubules. To test this idea, tobacco BY-2 cells were subjected to a chilling treatment that efficiently eliminated cortical and radial microtubule arrays (Schwarzerová et al. 2006). In fact, tubulin progressively accumulated in the nuclei with the disassembly of cytoplasmic microtubules. Upon re-warming, this intranuclear tubulin was rapidly, within minutes, excluded from the nuclei, and it immediately polymerized into cytoplasmic microtubules. This cold-induced tubulin accumulation was not only shown by immunofluorescence, but could also be followed in vivo in cells expressing a GFP-fusion of one tobacco tubulin isotype. By confocal microscopy it was verified that

this tubulin is localized inside the nucleus. When nuclei were purified from cold-treated cells and stringently stripped of cytosolic proteins, the tubulin remained in the nuclear fraction, nevertheless. Nuclei from unchilled control cells were void of intranuclear tubulin demonstrating the stringency of this assay. Interestingly, *bona-fide* nuclear-export sequences (NES) could be identified in plant tubulins. Some of them were specific for plant tubulins, which would explain the rapid exclusion of intranuclear tubulin upon return to the permissive temperature.

The exclusion mechanism that sequesters tubulin from the chromatin during interphase in those cells seems to be highly sensitive to temperature. Nuclear import is mediated by nuclear pore complexes residing in the nuclear envelope. Passive diffusion through these pores is almost impossible for proteins exceeding 50 kDa (for review, see Talcott and Moore 1999). Therefore, the import of $\alpha\beta$-tubulin heterodimers (110 kDa) must involve specific interaction with the nuclear transport machinery. The massive increase in the pool of free tubulin dimers in the cytoplasm, as a consequence of cold-induced elimination of assembled microtubules, might simply overrun the highly selective transport through the nuclear envelope, such that tubulin "leaks" into the nucleus. However, when such an excess of tubulin dimers was generated by oryzalin, tubulin did not enter the nucleus (Schwarzerová et al. 2006). Moreover, intranuclear tubulin accumulated against a concentration gradient, suggesting active transport and binding to an unknown intranuclear lattice. The affinity to this lattice seems to be rather strong, since tubulin remained attached even during the isolation of nuclei.

Tubulin is exclusively cytoplasmic during interphase, probably because its presence in the interphasic nucleus would induce serious disorders of cell-cycle regulation. During interphase, when nuclear pores control the transport of molecules, the integrity of the nuclear membrane seems to be the main mechanism that ensures the sequestration of tubulin to the cytoplasm.

However, the interaction of tubulin with chromatin is crucial during mitosis. For closed mitosis, tubulin is imported into the nucleus by an unknown mechanism, whereas for open mitosis, no tubulin transport through the nuclear membrane is required, due to the breakdown of the nuclear envelope. However, when daughter nuclei are formed at the end of mitosis, new nuclear envelopes have to be reestablished and the pool of tubulin molecules from the disintegrated spindle has to be removed from the karyoplasm. At this stage, tubulin is excluded from nuclei, probably utilizing the NES export signals conserved in all tubulins. This exclusion mechanism seems to depend on active metabolism. Therefore, it is impaired during cold treatment, resulting in intranuclear accumulation of tubulin.

This study on intranuclear tubulin suggests that, in spite of substantial differences between open and closed mitosis, some mechanisms must be preserved in all organisms in order to ensure proper progression of the cell-cycle. Some of these mechanisms include the import of cytoskeletal components

regulating polymerization of microtubules at the beginning of mitosis and export of tubulin at the end.

Whereas the biological function of tubulin shuttling in interphase nuclei is still enigmatic, intranuclear actin and even type-I myosin have been reported for mammalian cells to participate in chromatin modeling and the regulation of gene expression (for review, see Pederson and Aebi 2005). This seems to be true for plant cells as well, because silencing of an intranuclear actin-related protein identified in *Arabidopsis* produced marked changes in plant development (Kandasamy et al. 2005). Thus, we have to get used to the idea of an intranuclear cytoskeleton sharing at least some of the components that build cytoplasmic actin filaments and microtubules. As noted in this article, open mitosis represents a highly advanced situation, and it derives from ancestral precursors, where the nuclear envelope persists during mitosis. The evolutionary origin of the eukaryotic nucleus is still under debate (Pennisi 2004). However, recent findings (for review, see López-García and Moreira 2006) seem to rehabilitate the original idea that the nucleus derives from an endosymbiontic event (Margulis 1993), suggesting a scenario where a methanogenic archaeon is ingested by a fermentative myxobacterium. This is, for instance, supported by similarities between nuclear-core complexes and components of coated vesicles (Devos et al. 2004).

If the ancestor of the eukaryotic nucleus was an endosymbiont, it must have been endowed with an autonomous division machinery. The variant forms of mitosis in lower eukaryotes can be understood as variations on the theme of closed mitosis, where the (presumably endosymbiotic) nucleus maintains a high degree of integrity. The original function of the cytoskeleton – mitosis was, therefore, located inside of the nucleus. Intranuclear tubulin and actin might be nothing else than evolutionary footprints, rudiments dating back to the times when eukaryotes began their victorious existence. A few years from now, once molecular cell biology extends from the relatively small group of currently studied model organisms to the more distantly related group of species, we will be able to determine whether this is more than a speculation.

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