Annals of Botany **94**: 9–32, 2004
doi:10.1093/aob/mch109, available online at www.aob.oupjournals.org

INVITED REVIEW

Eukaryotic Cells and their *Cell Bodies*: Cell Theory Revised

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Received: 9 January 2004 Returned for revision: 20 February 2004 Accepted: 2 March 2004 Published electronically: 20 May 2004

• *Background* Cell Theory, also known as cell doctrine, states that all eukaryotic organisms are composed of cells, and that cells are the smallest independent units of life. This Cell Theory has been influential in shaping the biological sciences ever since, in 1838/1839, the botanist Matthias Schleiden and the zoologist Theodore Schwann stated the principle that cells represent the elements from which all plant and animal tissues are constructed. Some 20 years later, in a famous aphorism *Omnis cellula e cellula*, Rudolf Virchow annunciated that all cells arise only from pre-existing cells. General acceptance of Cell Theory was finally possible only when the cellular nature of brain tissues was confirmed at the end of the 20th century. Cell Theory then rapidly turned into a more dogmatic cell doctrine, and in this form survives up to the present day. In its current version, however, the generalized Cell Theory developed for both animals and plants is unable to accommodate the supracellular nature of higher plants, which is founded upon a super-symplasm of interconnected cells into which is woven apoplasm, symplasm and super-apoplasm. Furthermore, there are numerous examples of multinucleate coenocytes and syncytia found throughout the eukaryote superkingdom posing serious problems for the current version of Cell Theory.

• *Scope* To cope with these problems, we here review data which conform to the original proposal of Daniel Mazia that the eukaryotic cell is composed of an elemental *Cell Body* whose structure is smaller than the cell and which is endowed with all the basic attributes of a living entity. A complement to the *Cell Body* is the *Cell Periphery Apparatus*, which consists of the plasma membrane associated with other periphery structures. Importantly, boundary stuctures of the *Cell Periphery Apparatus*, although capable of some self-assembly, are largely produced and maintained by *Cell Body* activities and can be produced from it *de novo*. These boundary structures serve not only as mechanical support for the *Cell Bodies* but they also protect them from the hostile external environment and from inappropriate interactions with adjacent *Cell Bodies* within the organism.

• *Conclusions* From the evolutionary perspective, *Cell Bodies* of eukaryotes are proposed to represent vestiges of hypothetical, tubulin-based 'guest' proto-cells. After penetrating the equally hypothetical actin-based 'host' proto-cells, tubulin-based 'guests' became specialized for transcribing, storing and partitioning DNA molecules via the organization of microtubules. The *Cell Periphery Apparatus*, on the other hand, represents vestiges of the actin-based 'host' proto-cells which have become specialized for *Cell Body* protection, shape control, motility and for actin-mediated signalling across the plasma membrane.

Key words: Actin, Cell Body, Cell Periphery Apparatus, Cell Theory, coenocytes, cytoskeleton, nucleus, plasma membrane, plasmodesmata, polarity, syncytia, tubulin.

MULTICELLULARITY VERSUS SUPRACELLULARITY

Supracellular plants do not fit with the classical Cell Theory

'. . . something truly fundamental is missing in our image of the cell . . .' Daniel Mazia (1987)

The cell doctrine is firmly embedded in all biological disciplines and acts as a general paradigm of organismal and tissue construction and function (Wolpert, 1995; Mazzarello, 1999; Nurse, 2000). Mainstream biologists take this concept for granted and use it to underpin sophisticated reductionistic approaches by which to understand the molecular basis of cellular development (Pollard, 2003). However, those who are aware of the most recent advances in plant cell biology (see also Rustom *et al*., 2004) are convinced that Cell Theory, as it now stands, is absolutely incompatible with a cell-based organization of higher plants (Fig. 1) and requires an update (Box 1). Indeed, formulation of organismal theory of plant development, in which it is stated that it is not the cell but the whole multicellular organism that is the primary unit of plant life (Kaplan, 1992; Sitte, 1992; Barlow, 1994; Korn, 1999; Niklas, 2000; Wojtaszek, 2001; Tsukaya, 2002), has precipitated a crisis for Cell Theory as applied to plants. Organismal theory is an idea whose formulation and reformulation occurs with each successive generation of biologists (e.g. Sinnott, 1960; and before him all the way back to de Bary, 1864; see also Barlow, 1982). Furthermore, after a hundred years of discussion, the endosymbiotic concept of cell organization and evolution is now finally widely accepted (Margulis, 1993; McFadden, 1999; Martin

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† PWB dedicates his contribution to this paper to his friend and mentor, Professor Paul E. Polani FRS, on the occasion of his 90th birthday, 1 January 2004.

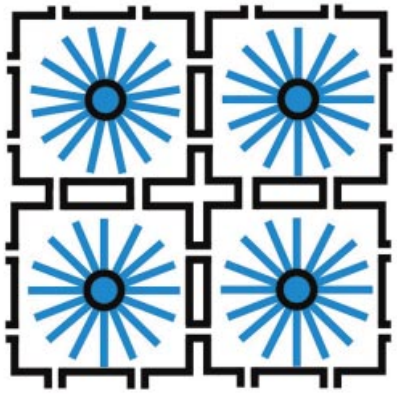

FIG. 1. The supracellular nature of higher plants is incompatible with the current version of Cell Theory. Plant cells are not physically separated. Cytoplasms of 'cells' are interconnected via plasmodesmata and endoplasmic reticulum into supracellular assemblies bounded by a plasma membrane. Enclosed within discrete cytoplasmic domains are unitary complexes of nucleus and perinuclear microtubules. Each complex we term a *Cell Body* in accordance with Daniel Mazia's conception of this structure. Cortical microtubules are not shown in this highly simplified scheme.

et al., 2001; Gray *et al*., 2001; Cavalier-Smith, 2002*a*). The implication of this concept is that present-day eukaryotic cells represent assemblages of 'cells within a cell'. Other even more obvious examples of 'cells within a cell' are the sperm cells of higher plants (Mogensen, 1992; Palevitz and Tiezzi, 1992; Southworth, 1992), endosperm of higher plants (Olsen, 2001; Brown *et al*., 2004) and spores within yeast mother cells (Knop and Strasser, 2000; Nickas *et al*., 2003; Shimoda, 2004). Interestingly in this respect, and relevant to our further argumentation, is that sperm cells of higher plants do not contain any F-actin but do have prominent microtubules (Palevitz and Tiezzi, 1992), suggesting that the actin cytoskeleton is neither essential for eukaryotic cellular life nor for cell divisions (Palevitz and Tiezzi, 1992; for a similar conclusion on somatic plant cells see Baluška *et al*., 2001*c*; Vantard and Blanchoin, 2002). Concerning the last-mentioned point, genetic and pharmacological evidence convincingly document that it is the microtubular cytoskeleton which is essential for cell division and the formation of multicellular organisms (for plant cells see Mayer *et al*., 1999; Mayer and Jürgens, 2002).

All these problems with Cell Theory were forecast by Thomas Henry Huxley in 1853, who was convinced that cells were not anatomically independent but that they were interconnected into supracellular assemblages (Richmond, 2001). Therefore, for Huxley, cells could not be the elementary units of life. In fact, current advances in plant cell biology reveal that this view is correct for all higher plants (Fig. 1). Strictly speaking, higher plants are supracellular organisms because almost all the cells of a given plant organism are interconnected via cell-to-cell channels known as plasmodesmata (Lucas *et al*., 1993; Zambryski and Crawford, 2000) that form primarily across the division wall at cytokinesis, and secondarily across selected, already established walls (Ehlers and Kollmann, 2001). Their mode of development attests to the necessity of direct cell–cell communication during plant development. These complex, communicative and contractile channels (Blackman *et al*., 1999; Zambryski and Crawford, 2000; Baluška *et al*., 2001*b*) are not only lined with the plasma membrane but are also traversed by endoplasmic reticulum. This latter feature, together with the well-known continuity between endoplasmic reticulum elements and nuclear envelopes, means that all nuclei of a given plant are potentially in direct contact and are part of a structurally integrated supracellular network of nuclei interconnected via endoplasmic reticulum elements (Lucas *et al*., 1993). It is not possible to interpret this phenomenon correctly using cell doctrine as it stands now because this is based on the belief that cells are physically separated and structurally independent. In fact, recent advances in animal cell biology also reveal that cells are also not isolated from each other in some situations (Rustom *et al*., 2004). We are, however, still far away from understanding how individual nuclei of a supracellular network of plant nuclei might communicate with each other via the intervening cytoplasmic channels.

A consequence of the fact that the cytoplasms of plant cells are interconnected via plasmodesmata is that the individuality of the cell is given up in favour of an integrated and corporate cytoplasm that benefits the whole organism. This supracellular, or organismal, approach towards multicellularity seems to have allowed sessile plants to adapt to life on land and to evolve even within hostile environments. The continuity of cellular units allows potentially unrestricted exchange of information throughout the plant body, the informational signals being used to rapidly coordinate genome transcription that can either neutralize or take advantage of environmental challenges (Baluška *et al*., 2004). Thus, whereas animals and humans are perhaps truly multicellular organisms, higher plants are composed of communicative cytoplasms.

The current crisis of the Cell Theory in plants (Kaplan and Hagemann, 1991; Kaplan, 1992; Korn, 1999; Wojtaszek, 2001) is quite paradoxical if we consider that Robert Hooke in 1665 and Nehemiah Grew in 1682 discovered cells from observations on higher plant tissues (Wolpert, 1995; Harris, 1999; Nurse, 2000). It took more than 250 years until the Cell Theory was definitely accepted for animals and humans, neurons being the last type of cell to be definitely defined as such (Mazzarello, 1999). Plants also served as useful objects for the discovery of the nucleus, the plasma membrane, cell cycle and cytokinesis (Harris, 1999; see also Boxes 2–4). Thus, plants seem always to have been at the forefront of Cell Theory, even now when it needs updating in order to accommodate the supracellular nature of higher plants. Numerous examples of multinucleate cells (Fig. 2) in almost all eukaryotic organisms, direct cytoplasmic continuity in some animal cells (Rustom *et al*., 2004), as well as the ability to form the plasma membrane *de novo* (Shimoda, 2004)—all these suggest that the Cell Theory is in crisis elsewhere too, and that it is not solely a plant-specific problem.

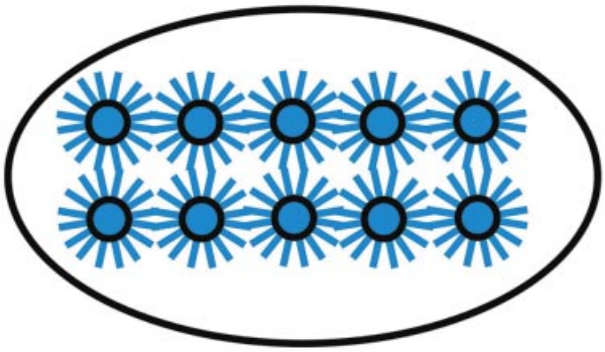

FIG. 2. *Cell Bodies* are obvious in multinucleate coenocytes and syncytia, structures which have been reported in almost all major taxonomic groups of eukaryotes. Importantly, perinuclear radiating arrays of *Cell Body* microtubules are critical for the regular spacing of nuclei and *Cell Bodies* in the multinucleate cytoplasmic community.

Unique organization of microtubules and Golgi apparatus in multinuclear syncytia–coenocytes of animals and lower plants resembles situations in supracellular plants

There are several well-known examples where not only plant cells but also several animal cell types do not conform to the traditional view of cells as the smallest unit of life. Mention can be made of the many examples of multinucleate coenocytes and syncytia throughout the eukaryotic kingdom (Fig. 2). Coenocytes are formed as a result of the uncoupling of mitosis from cytokinesis. Whereas mitosis is a conservative and persistent living process, cytokinesis appears to be less conservative, more sporadic, and can even be absent; this results in situations where numerous nuclei come to be present within the confines of a 'mother' cell. Besides the already mentioned yeast spores (Shimoda, 2004), good examples of coenocytic plants are the multinucleate algae (Woodcock, 1971; Goff and Coleman, 1987; McNaughton and Goff, 1990) and also the male and female gametophyte tissues of higher plants (Brown and Lemmon, 1992, 2001; McCormick, 1993; Reiser and Fischer, 1993; Russell, 1993; Brown *et al*., 1994*a*, *b*, 1996; Huang and Sheridan, 1994, 1996; Smirnova and Bajer, 1998; Otegui and Staehelin, 2000, 2003; Ranganath, 2003). In animals, well-studied examples of the coenocytic state are found in oogenesis and in the early embryogeny of *Drosophila* (St Johnson and Nüsslein-Volhard, 1992; Foe *et al*., 2000; Mazumdar and Mazumdar, 2002). The simplest coenocyte would be a cell with two or four nuclei, as occurs in plants in the anther tapetum and in the liver of many rodents (D'Amato, 1977). There are also several examples of coenocytes elicited by mutations that prevent cytokinesis (Sipiczki *et al*., 1993; Adam *et al*., 2000).

A syncytium, another multinucleate form, derives from uninucleate cells that have fused together. Examples of homotypic cell fusion and hence of homokaryotic multinucleate syncytium formation in animal systems are myotubes, which are essential for muscle differentiation, multinucleate osteoclasts, which are active in bone resorption and homeostasis, and the syncytiotrophoblast, which is characteristic of the mammalian placenta (Cross *et al*., 1994; Solari *et al*., 1995; Shemer and Podbilewicz, 2000, 2003; Taylor, 2002). There are also examples of fusions between different animal cell types: neurons and bone marrow-derived stem cells can both form stable heterokaryons (Kozorovitskiy and Gould, 2003; Weimann *et al*., 2003). Moreover, huge multinucleate syncytia can be induced by viruses such as HIV and measles (Sylwester *et al*., 1993; Cathomen *et al*., 1998). Intriguingly, animal syncytia behave like single cells, mimicking their polar integrity and showing pseudopod extensions and actin-based motility (Lewis and Albrecht-Buehler, 1987; Sylwester *et al*., 1993). In plants, syncytia are formed by means of the enlargement of plasmodesmata, dissolution of the original cell walls and consequent merging of neighbouring cytoplasmic domains (Fink, 1999). In some cases, syncytium formation is the normal mode of plant cellular development, like articulated laticifers (Mahlberg and Sabharwal, 1966); in other cases, it is a response to a challenge from organisms that burrow into plant tissue and convert it into the nutritive syncytial nurse cells of insect and nematode galls (Jones and Northcote, 1972).

A major hallmark of plant cells is that they organize their microtubules from sites upon a nuclear surface (Lambert, 1993; Mizuno, 1993; Baluška *et al*., 1996, 1997*a*; Schmit, 2003). Often they also organize microtubules at the cell cortex from the secondary microtubule organizing centres (MTOCs) which have been derived from primary MTOCs that lie on the nuclear surface (Baluška *et al*., 1997*a*). In the case of those animal cells which embark upon coenocytic or syncytial developmental pathways, the typical centrosome-based organization of their microtubules is abandoned and the whole nuclear surface starts to organize microtubules, as is known from plant cells (Tassin *et al*., 1985*a*; Sylwester *et al*., 1993; Lu *et al*., 2001; Mulari *et al*., 2003). In this way, the animal coenocyte or syncytium is similar to the individual plant 'cell', suggesting that this type of animal 'cell', too, may be a supracellular continuum of many nuclei and cytoplasms.

The above suggestion can be followed using another line of evidence involving the Golgi apparatus. For animal cells, it is well known that localization of the Golgi complex is dependent on microtubules while, at the same time, the Golgi complex acts as a microtubule-organizing organelle (Tassin *et al*., 1985*b*; Kronenbusch and Singer, 1987; Ho *et al*., 1989; Cole *et al*., 1996; Bloom and Goldstein, 1998; Burkhardt, 1998; Chabin-Brion *et al*., 2001). But in the case of the animal cell syncytium, the Golgi apparatus undergoes a dramatic reorganization and acquires features that correspond to what is found in supracellular higher plants where numerous small Golgi stacks are closely associated with endoplasmic reticulum export sites (Boevink *et al*., 1998; Brandizzi *et al*., 2002). For instance, during myogenesis in animals, similarly to cells devoid of microtubules (Cole *et al*., 1996), perinuclear Golgi apparatus re-arranges into numerous small Golgi stacks that are closely associated with the endoplasmic reticulum exit sites (Ralston, 1993; Lu *et al*., 2001; Ralston *et al*., 2001). Golgi mini-stacks and microtubules organized around nuclei were also reported for maturing mouse oocytes (Moreno *et al*., 2002). Thus, the plant microtubular and Golgi apparatus organizations are

directly related to their supracellular nature in both plants and animals.

Coenocytic and syncytial nuclei organize cytoplasmic domains via radiating microtubules and they obey the cytonuclear rule

One characteristic feature of the majority of syncytia and coenocytes is that their nuclei are regularly spaced within the cytoplasm (Goff and Coleman, 1987; McNaughton and Goff, 1990; Bresgen *et al*., 1994; Bruusgaard *et al*., 2003) and this is apparently due to the assembly of perinuclear radiating microtubules (Woodcock, 1971; Brown and Lemmon, 1992, 2001; Brown *et al*., 1994*a*, *b*, 2004; Huang and Sheridan, 1994, 1996; Otegui and Staehelin, 2000, 2003). Each individual nucleus of both syncytia and coenocytes controls a cytoplasmic domain (Fig. 2), the size of which depends on the DNA content and volume of that nucleus. These nucleo-cytoplasmic domains, despite lacking any obvious physical borders, behave like independent structural entities (Goff and Coleman, 1987; McNaughton and Goff, 1990; Brown and Lemmon, 1992, 2001; Brown *et al*., 1994*a*, *b*, 1996; Reinsch and Gönczy, 1998; Pickett-Heaps *et al*., 1999). Distinct nucleo-cytoplasmic domains are organized also in animal syncytial myotubes (Hall and Ralston, 1989; Bruusgaard *et al*., 2003), where the individual nuclei even maintain their own transcription and translation domains (Rotundo and Gomez, 1990; Ralston and Hall, 1992). Individual nuclei of multinucleate muscle fibres exert control also over distinct cell surface domains (Rossi and Rotundo, 1992). Thus, characteristic cytogenetic patterns could theoretically be set up within a coenocytic structure without the need for any defining cell membranes or wall boundaries, the cytoplasmic domains being patrolled by the microtubules radiating from the nuclear surface.

In plants, there are numerous studies showing that radiating perinuclear microtubules are essential for the regular spacing of nuclei (Goff and Coleman, 1987; McNaughton and Goff, 1990; Brown and Lemmon, 1992, 2001; Brown *et al*., 1994*a*, *b*, 1996, 2004; Baluška *et al*., 1996, 1997*a*, *b*, 1998; Pickett-Heaps *et al*., 1999). An important feature is that the whole nuclear surface is active in the initiation and maintenance of minus-ends of microtubules, while dynamic plus-ends exert pushing/pulling forces when contacting the cell boundary, or when approaching plus-ends of microtubules radiating from other adjacent nuclei. This phenomenon allows each nucleus to actively conquer and maintain its own unique cytoplasmic space which does not encroach upon the spaces controlled by neighbouring nuclei (Strasburger, 1893; Hertwig, 1903; Trombetta, 1939; Pickett-Heaps *et al*., 1999; Gregory, 2001*a*, *b*).

The nuclear spacing is often in the form of regular hexagonal arrays, this feature being indicative of the isomorphic space-claiming force of individual nuclei-MT complexes. Interestingly, correct patterning and polarity are expressed throughout animal syncytia and plant coenocytes (St Johnston and Nüsslein-Volhard, 1992; Boisnard-Lorig *et al*., 2001; Sørensen *et al*., 2002; Brown *et al*., 2004). This is perhaps an expression of precisely regulated 'cell-like' domains of varying strength, each maintained by precisely regulated activities of perinuclear radiating microtubules (Goff and Coleman, 1987; McNaughton and Goff, 1990; Brown and Lemmon, 1992, 2001; Bresgen *et al*., 1994; Brown *et al*., 1994*a*, *b*, 1996; Baluška *et al*., 1996; Pickett-Heaps *et al*., 1999; Bruusgaard *et al*., 2003).

THE CELL BODY CONCEPT

Cell Body *represents the smallest autonomous and self-reproducing unit of eukaryotic life*

'The Cell Body pervades the whole interphase cell and condenses into a mitotic apparatus during mitosis' Daniel Mazia (1993)

The supracellular nature of higher plants, as well as of coenocytes and syncytia found in almost all eukaryotes, implies that it is not the cell but some subcellular structure which represents the elementary unit of eukaryotic life. In fact, such ideas have often been expressed in the past. The cytoskeleton was unknown in these early times, and so these ideas were doomed to be forgotten (Harris, 1999). But already the very early studies on plant microtubules revealed that these structures controlled the spatial distribution of chromosomes during mitosis (Ledbetter and Porter, 1963) and of whole nuclei during interphase (Kiermayer, 1968; Woodcock, 1971). These features were also confirmed for animal cells (Slautterback, 1963; Aronson, 1971). However, the close connections between DNA and tubulin molecules throughout the cell cycle as well as in postmitotic eukaryotic cells became obvious only later (see Box 4), providing a completely new perspective upon what came to be known as the cytoskeleton.

Daniel Mazia was the first to realise that a close connection between DNA and tubulin molecules would have an immediate impact upon Cell Theory. He was also the first to suggest that the nucleus with its associated microtubules formed a composite structure which he called *Cell Body* (Mazia, 1993; Epel and Schatten, 1998). Although this concept was left almost unnoticed, we revealed that it is obviously also valid for plant cells (Baluška *et al*., 1997*a*, 1998). Importantly, *Cell Body* represents the smallest unit of life which is capable of self-organization, self-reproduction and of responsiveness to diverse external stimuli (Mazia, 1993; Baluška *et al*., 1997*a*, 1998, 2000*b*, 2001*a*; Epel and Schatten, 1998).

This new perspective improves our understanding of several, at first sight unrelated, phenomena like the C-value enigma and the related nucleotypic effect of DNA molecules, irrespective of their encoded informational content (Bennett, 1972; Gregory, 2001*a*, *b*). *Cell Body* concept also provides insight into cancer which results from impaired genome–centrosome stability (Lingle *et al*., 1998; Anderson *et al*., 2001; Brinkley, 2001; Maser and DePinho, 2002; Nigg, 2002). The association between DNA and tubulin allows an unprecedent expansion of genome size (Gregory, 2001*a*, *b*) because it enables a high fidelity of segregation, motility and propagation of large DNA-based structures like mitotic chromosomes and even whole nuclei (Mazia, 1984, 1987; Inoue and Salmon, 1995; Reinsch and

Gönczy, 1998; Adames and Cooper, 2000; Compton, 2000; Tran *et al*., 2001; McIntosh *et al*., 2002; Kusch *et al*., 2003). This unique molecular coupling between DNA and tubulin allows DNA-based structures, including individual chromosomes and whole nuclei, to express motility and exploratory behaviour.

Nucleus as the most ancient endosymbiont of eukaryotic cell

The *Cell Body* concept permits an understanding of cellular organization of eukaryotes from an evolutionary perspective. As happens in science, after a long time in oblivion, the endosymbiotic theory of Constantin Mereshkowsky has finally, after almost 100 years of discussion, become widely accepted for both of these organelles (Mereshkowsky, 1905, 1910; Margulis, 1993; Rizzotti, 2000; Martin *et al*., 2001; Cavalier-Smith, 2002*a*). Current advances in molecular and cellular biology have provided conclusive evidence that eukaryotic cells are composite structures that incorporate ancient and originally free-living cells (Gray *et al*., 2001; Martin *et al*., 2001; Timmis *et al*., 2004). This feature is especially obvious in plant cells containing both mitochondria and plastids (McFadden, 1999). Even peroxisomes seem to have endosymbiotic origins (de Duve, 1996; Katz, 1999).

In contrast, the evolutionary origin of nuclei remains obscure and serves as a matter of hot debate (Margulis, 1993; Lake and Rivera, 1994; Margulis *et al*., 2000; Martin *et al*., 2001; Cavalier-Smith, 2002*a*; Dolan *et al*., 2002). In his original theory, Mereshkowsky proposed that nuclei were also of endosymbiotic origin (Mereshkowsky, 1905, 1910; Martin *et al*., 2001). Now, in the last 10 years, the first strong data have been published in line with this idea that the nucleus could be the vestige of an originally free-living proto-cell (Gupta *et al*., 1994; Gupta and Golding, 1996; Horiike *et al*., 2001; Dolan *et al*., 2002; Hartman and Fedorov, 2002). Several authors consider as almost accepted that the nucleus is of endosymbiotic origin, the only disputed point being the identity of the 'guest' and 'host' proto-cells (Margulis *et al*., 2000; Horiike *et al*., 2001; Dolan *et al*., 2002; Hartman and Fedorov, 2002). Such an origin of the nucleus would also explain the unexpected finding of RNA-to-protein translation within the nucleus (Hentze, 2001). Intriguingly, this nuclear translation seems to be dependent upon ongoing DNA-to-RNA transcription, a situation resembling that which occurs in prokaryotes (Iborra *et al*., 2001; Pederson, 2001).

If the nucleus is the most ancient example of a 'cell within cell', then the *Cell Body* concept is in the right position to explain why there is a subcellular unit of eukaryotic life, composed of nucleus and perinuclear microtubules, capable of autonomous existence reproducing itself once per cell cycle. The *Cell Body* concept can also cope with the well-known fact that the nucleus–microtubule complex often divides independently of the cell in which it resides, thus resulting in the coenocytic condition found in all eukaryotes. Looking at this problem from the opposite end, the supracellular nature of higher plants, as well as the existence of coenocytes and syncytia throughout the eukaryotic superkingdom, can be understood much better if nuclei are considered as vestiges of originally free-living pro-eukaryotic cells. A legacy of these ancient symbiotic interactions is that eukaryotic cells continue to show tight links between nuclei, centrosomes and microtubules in the form of *Cell Bodies*. This legacy may also be reflected in the epixenosomes, unique bacterial ectosymbionts located at the cell periphery of hypotrich ciliates (Petroni *et al*., 2000). These organelles consist of tubulin-based tubules and DNA/basic proteins complexes resembling eukaryotic chromatin (Jenkins *et al*., 2002) and possessing some of the characteristics of the predecessors of eukaryotic *Cell Bodies*.

It is well-known that coenocytic and syncytial organisms, such as, for example, slime-molds and *Acetabularia*, propagate from uninucleate spores. This feature might also be relevant for the surprising observation that naked nucleo-cytoplasmic aggregates released from cut siphonous algae can regenerate *de novo* the lost plasma membrane (O'Neil and La Claire II, 1984; Pak *et al*., 1991; Kim *et al*., 2001; Kim and Klotchkova, 2001; Ram and Babbar, 2002). This ability can be used for propagation, in this case via the formation of nucleated but envelope-less protoplasts which, after their release, form a plasma membrane *de novo* (Kim and Klotchkova, 2001). In yeast cells, too, the plasma membrane is formed *de novo* during spore formation (Shimoda, 2004). Similarly, the nuclei of syncytial osteoclasts can form uninucleate cells by means of a budding process during which individual nuclei (in reality, *Cell Bodies*) are enclosed within a regenerating plasma membrane (Solari *et al*., 1995). It is important to mention in this respect that cytokinetic plant cells also form a plasma membrane *de novo.* This involves the active participation of daughter *Cell Bodies* following their division at mitosis. Use is made of the *Cell Body*-based radiating microtubules (Baluška *et al*., 1996) to position new plasma membrane (Pickett-Heaps *et al*., 1999; Brown and Lemmon, 2001) arising from homotypic fusions of endosomes containing internalized cell wall pectins (F. Baluška, unpubl. data). This process resembles a large-scale repair of a damaged cell periphery, which is also based on homotypic fusions of endosomes and lysosomes (McNeil and Terasaki, 2001; Reddy *et al*., 2001; McNeil *et al*., 2003). In a similar fashion, the final stage of animal cytokinesis is based on *de novo* fomation of the plasma membrane (Bowerman and Severson, 1999) via the interdigitating microtubules known as the midbody. Closure of the midbody requires the presence of a mother centriole to close the intercellular bridge (Doxsey, 2001; Khodjakov and Rieder, 2001; Piehl *et al*., 2001). Interestingly, centrosomes and their microtubules drive cytokinesis in brown algae (Nagasato and Motomura, 2002).

Several features of centrosomes suggest that these structures might be considered as highly reduced vestiges of a putative endosymbiont which, having reduced its content and structure, retains only the centrosomes and microtubules (Margulis, 1993). This idea receives support from recent data on nucleomorphs (Cavalier-Smith and Beaton, 1999; Keeling *et al*., 1999; Gilson, 2001) where the extreme reduction of endosymbiotic cells has led to the evolution of certain almost vanishingly small organisms. Other data document that, in some situations, centrosomes

can behave independently of nuclei and chromosomes (Balczon *et al*., 1995; Fukasawa *et al*., 1996; Piehl *et al*., 2001; Rieder *et al*., 2001; Burakov *et al*., 2003; Malone *et al*., 2003). In fact, centrosomes emerge as a real command centres for cellular control (Doxsey, 2001), an idea forecast by Theodore Boveri in 1888 (Boveri, 1888; Mazia, 1987).

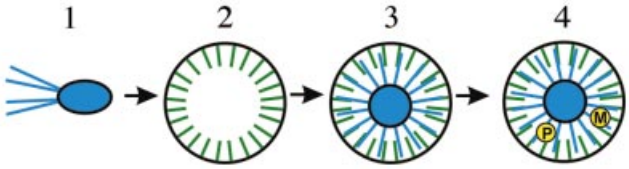

FIG. 3. Hypothetical formation of a eukaryotic cell from two proto-cells differing in their life-style. A more active and motile tubulin-based proto-cell with a rigid surface (blue, 1) is hypothesized to penetrate a rather static, large and actin-based proto-cell with a soft surface (green, 2). The tubulin-based proto-cell became transformed into the nucleus within the ancient predecessor of the eukaryotic cell (3). Later, during phylogenesis, phagocytosis of other prokaryotic cell types allowed the acquisition of plastids (P) and mitochondria (M) to form the contemporary eukaryotic cells (4).

Cell Body*: cell within a cell*

If the case is strong for the endosymbiotic origin of the eukaryotic nucleus, then the question is this: how could primitive proto-cells have accomplished such a fusion? Unfortunately, these fusion events took place in such ancient times that they are nearly beyond scientific imagination based on any human experience. Consequently, proposed scenarios, models and answers can only be speculations and visions (Forterre and Philippe 1999; Woese, 2002; Brooke and Holland, 2003). Nevertheless, analysis of extant cells can give some clues.

Phagocytosis is often considered as the only possibility of acquiring endosymbionts (Cavalier-Smith, 2002*a*). However, it is not necessary to rely on this quite complex process for the earliest merging of two ancient pro-eukaryotic cells. In any case, phagocytosis is not helpful in solving this mystery as these primitive proto-cells would have lacked the complex and signalling-competent actin-based cytoskeleton which is necessary for the phagocytosis-like uptake of a 'guest' cell by a 'host' cell. Importantly, phylogenetic analysis of small GTPases suggests that phagocytosis developed relatively late in eukaryotic evolution, after the nucleus and secretory pathway were already well-established (Jékely, 2003).

There are, however, other possible scenarios, among which the most preferable is that two fundamentally different types of proto-cells merged by a more direct mechanism, whereby a small tubulin-based proto-cell with a rigid surface penetrated a large actin-based proto-cell with a soft surface (Fig. 3). In fact, there is a nice example of this process when predatory bacteria of the genus *Daptobacter* invade the cells of its bacterial prey in the genus *Chromatium* (Guerrero, 1991). This quasi-sexual encounter of sperm-like and egg-like proto-cells is suggested, therefore, to be the basis of contemporary eukaryotic life. On the other hand, it is important to keep in mind that these ancient proto-cells have no more to do with currently living prokaryotic cells than they do with extant eukaryotic cells; the only common point is that all contemporary cells, whether prokaryotic or eukaryotic, are descendants of these hypothetical proto-cells. For the sake of argument, we propose that these two types of proto-cells were contemporaries and developed in parallel.

In order to attain an active life-style based on physical forces prior to the hypothetical fusion event suggested above, one proto-cell line had already invented actin polymerization while the other proto-cell was structurally based upon polymerized ancient tubulin. This would be in accord with the notion that forces based on polymerization are very ancient whereas motor molecules are a much later acquisition of eukaryotic life (Mitchison, 1995). Merging of these two types of proto-cells apparently occurred due to their predator/prey interactions, as these are inherently associated with endosymbiosis (Guerrero, 1991; Kooijman *et al*., 2003), as part of the search for food. During further phylogenesis, some of the large actin-based proto-cells might eventually have succeeded in sealing off their penetrated surfaces, thus trapping within themselves the raptor tubulin-based proto-cells. Some of the trapped 'guest' proto-cells may have escaped from the 'digestive' activities of the 'host' proto-cells, allowing them to persist within the 'host' cells. In fact, predator/prey relationships are obvious elsewhere in eukaryotic life and result in secondary and tertiary endosymbiotic events, accomplished in the present-day eukaryotic kingdom by phagocytosis (Cavalier-Smith, 2002*b*; Bhattacharya *et al*., 2003). For instance, predator/prey endosymbiosis events represent the major force shaping algal evolution (Bhattacharya *et al*., 2003). Of course, this fusion between the two types of proto-cell may also have occurred entirely accidentally within their shared environment.

After the tubulin-based 'guest' cells became symbionts within the 'host' cells, they might have progressively accumulated 'host' DNA via horizontal transfer of DNA (Doolittle, 1998; Jain *et al*., 1999; Timmis *et al*., 2004). This process allowed acquisition of a single ancient nucleus which then became specialized for storage and segregation of DNA while the rest of the cellular functions were taken over by the actin-based 'host' proto-cells. In strong support of this endosymbiotic concept of nuclear origin, it has been found that there are two basic types of genes within eukaryotic nuclei, suggesting that the nuclear genome is, in fact, a chimeric mixture of genes having two distinct origins (Ribeiro and Golding, 1998; Rivera *et al*., 1998).

Summarizing the above: we hypothesize that the eukaryotic lineage started with a predator/prey-based and penetration-mediated fusion between a small, motile tubulin-based swimmer having a rigid surface, and a large, less motile and actin-based amoeba-like prey with a soft surface (Fig. 3). This hypothetical scenario of a receptive 'host' and a raptor 'guest' would have great implications for understanding the cytoskeleton of both ancient and current eukaryotic cells. The actin- and tubulin-based cytoskeletons are proposed to have evolved independently in the two proto-cell lines. The bringing together

of actin and tubulin within the same cell resulted in a new quality due to the fact that these at first unique pro-eukaryotic cells were equipped with a more complex cytoskeleton. This feature endowed these ancient pro-eukaryotes with tremendous advantages, resulting in an explosive evolution of early eukaryotic life. It might also have allowed these new cells to survive the most critical phases of evolution in which extremely harsh conditions could cause bottlenecks for the predecessor proto-cell populations yet allow the pro-eukaryotes to flourish. This scenario also gives a possibility of understanding the cytoskeleton of eukaryotic cells from a completely new prespective.

Tubulin-based flagellate sperm cells, lacking F-actin, penetrate into large actin-based egg cells to generate plant Cell Bodies

A hypothetical penetration or fusion event between two ancient proto-cells can explain not only the origin of the eukaryotic nucleus but can also serve as a useful paradigm for understanding sexual reproduction of present-day multicellular organisms where, invariably, two haploid cells fuse together to form a diploid zygote (Fig. 4). The proto-cell fusion event is also reminiscent of the ancient Chinese Yin/Yang concept. The tubulin-based sperm cell is small and motile (Yang), whereas the large, actin-based egg cell (Yin) is non-motile and lacks a centrosome. These structural features, as well as the mode of sexual cell fusion, might resemble the ancient fusion event which may have given rise to the pro-eukaryotic cell.

Higher plants seem not to fit completely into this scheme as they do not have obvious motile sperm cells equipped with flagellae (Fig. 4A). However, plant sperms lost their flagellae only secondarily (Poort *et al*., 1996) as a result of their adaptation to life on land. In this situation, actin-driven tip growth of pollen tubes (Äström *et al*., 1995; Raudaskoski *et al*., 2001; Laitiainen *et al*., 2002) provides the actual vehicle for the tubulin-based sperm cells' transport (Fig. 5) towards the egg within the female gametophyte (Silflow and Lefebvre, 2001). Tip growth in plants is represented not only by pollen tubes but also by root hairs, where it is driven by actin polymerization and is tubulin-independent (Bibikova *et al*., 1999; Gibbon *et al*., 1999; Baluška *et al*., 2000*a*; Raudaskoski *et al*., 2001; Vidali *et al*., 2001; Foissner *et al*., 2002; Laitiainen *et al*., 2002; Šamaj *et al*., 2002).

Sperm cells of higher plants have not only lost their flagellae, but they are also devoid of F-actin (Pierson *et al*., 1986; Heslop-Harrison *et al*., 1988; Palevitz and Tiezzi, 1992). In fact, higher plant sperm is the only known example of a plant cell that lacks F-actin. On the other hand, sperm cells are equipped with a prominent tubulin-based cytoskeleton in the form of bundled microtubules (Pierson *et al*., 1986; Palevitz and Liu, 1992; Palevitz and Tiezzi, 1992) whose assembly is directed by γ-tubulin (Palevitz *et al*., 1994). From the cytoskeletal point of view, the sperm cell resembles a mitotic spindle (mitotic *Cell Body*) which represents the most basic form of *Cell Body* (Mazia, 1993; Baluška *et al*., 1998).

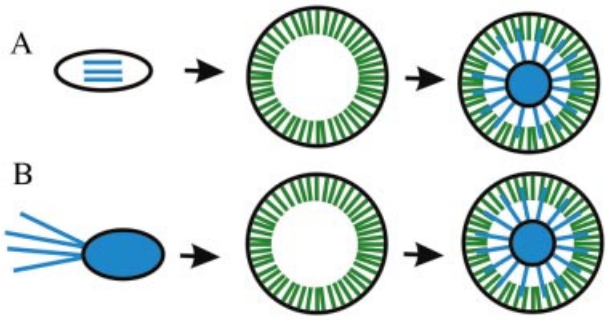

FIG. 4. Sexual reproduction of current eukaryotic organisms is based on similar sequences of events with the tubulin-based sperm cells penetrating the actin-based oocytes. Sperms of most higher plants (A) are non-flagellated, and thus lack active tubulin-based motility, as is the case in most other eukaryotic organisms (B). However, this is a secondary trait associated with the adaptation of plants to life on land. This has enforced a 'dry' mode of pollination in contrast to the motile 'wet' mode of gamete penetration still found in lower plants and some primitive gymnosperms (cycads, Gingko).

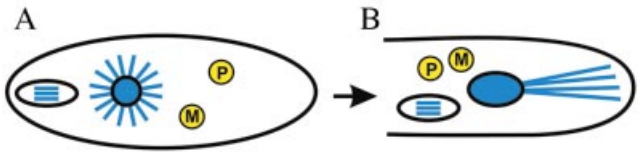

FIG. 5. Pollen (A) and pollen tubes (B) of higher plants constitute a good example of 'cells within a cell', a mode of organization which is not compatible with the current version of Cell Theory. The sperm cell is immobile and lacks F-actin, but it contains abundant microtubules (blue). In contrast, the vegetative nucleus forms an active *Cell Body* with radiating perinuclear microtubules and assembles a dense F-actin cap (not shown) which drives tip-growth of the pollen tube.

Nevertheless, lower plants do still possess flagellate sperm cells (Li *et al*., 1989; Vaughn *et al*., 1993; Renzaglia and Garbary, 2001; Silflow and Lefebvre, 2001; Sakaushi *et al*., 2003), and these cells closely resemble the motile sperm cells of other eukaryotic organisms, not only with respect to their tubulin-based flagellae but also on account of the importance of centrin for their MTOCs (Vaughn *et al*., 1993; Hart and Wolniak, 1998). For instance, the most ancient gymnosperm species, cycads and *Ginkgo biloba*, release from their pollen tubes multiflagellated sperm cells which actively swim towards the egg cells using tubulin-based flagellae (Li *et al*., 1989; Renzaglia and Garbary, 2001; Silflow and Lefebvre, 2001).

CELL BODY VERSUS CELL PERIPHERY APPARATUS

Tubulin-based mitosis versus *actin-based cytokinesis from the* Cell Body *perspective: divisions of 'guest' and 'host' cells?*

It is undisputable that mitosis and cytokinesis, although tightly coupled in most cells, can often be uncoupled, suggesting that these two processes are actually independ-

ent, even though they usually cooperate to bring about cell division. The very nature of these processes implies that they are based on different principles. It is obvious that mitosis represents the division of the tubulin-based 'guest' cell (now in the form of *Cell Body*), whereas cytokinesis corresponds to the division of the actin-based 'host' cell.

It is well known that nuclear division (*Cell Body* division or mitosis) is an extremely conservative process driven solely by the microtubular cytoskeleton (Pickett-Heaps, 1969; Hyman and Karsenti, 1996). In contrast, cytokinesis, which divides the cytoplasm as well as the cell boundary complex, is less conservative (Ueda and Nagasaki, 2004), and is driven mainly by the actin cytoskeleton, although it also requires the cooperation of microtubules (Hyman and Karsenti, 1996; Glotzer, 1997; Hales *et al*., 1999; Karsenti and Vernos, 2001; Guertin *et al*., 2002). Moreover, mitosis not only precedes cytokinesis temporally but also instructs cytokinesis spatially (Glotzer, 2004). This more conserved nature of mitosis and less conserved nature of cytokinesis, combined with the many examples of mitosis not followed by cytokinesis, suggests that mitosis is much more important for eukaryotic life. Importantly, the plasma membrane can form *de novo* during cytokinesis, and this process is then instructed and regulated by *Cell Bodies* (for sporulation in yeast see Knop and Strasser, 2000; Nickas *et al*., 2003; Shimoda, 2004).

The coenocyte-like nature of higher plants deviates from this scheme slightly because here cytokinesis is based more on microtubules than on actin filaments (Staehelin and Hepler, 1996; Assaad, 2001; Baluška *et al*., 2001*c*; Bednarek and Falbel, 2003). Owing to the evolutionary loss of the compact centrosomes and the acquisition of abundant cortical micortubules (Mazia, 1987; Baluška *et al*., 1997*a*), plant cytokinesis has undergone dramatic changes during the evolution of supracellular higher plants. For example, cytokinesis in lower plants is either partially or fully actin-dependent (McIntosh *et al*., 1995; Sawitzky and Grolig, 1995; Höftberger and Lütz-Meindl, 1999; Karyophyllis *et al*., 2000), whereas in higher plants it is directed preferentially by the microtubular *Cell Body*. Under stress situations, however, plant cells sometimes revert to a cleavage-like cytokinesis resembling animal cytokinesis (Herth and Meyer, 1978; Sonobe, 1990; Cleary, 2001). It is as though the basic and ancient cytokinetic process is still embedded in contemporary plant cells and can reassert itself as a default upon severe challenge when all other division systems are prone to failure.

On the other hand, animal cells experimentally made devoid of centrosomes also fail to complete a true cytokinesis, leaving the daughter cells coupled by cytoplasmic bridges (Doxsey, 2001; Khodjakov and Rieder, 2001; Piehl *et al*., 2001) resembling plasmodesmata. Interestingly in this respect, in higher plants, centriole and centrosome-based centrin localize to both plasmodesmata (Blackman *et al*., 1999) and cytokinetic cell plates (Del Vecchio *et al*., 1997; Harper *et al*., 2000). Moreover, plant cells lack myosin II (Reichelt and Kendrick-Jones, 2000). The significance of this is that, in animal as well as yeast mutant cells devoid of class II myosins, there are aberrations in the final phases of their cytokinesis, with a failure to separate the daughter cells (Bi *et al*., 1998; Tolliday *et al*., 2003). This, in turn, suggests that the coenocyte-like higher plants perhaps evolved their apparent multicellularity by processes that resulted from the loss (or the non-acquistion by evolution) of myosin II and compact centrosomes. Moreover, remains of MTOCs might have become trapped within cell-to-cell channels which failed to constrict due to the absence of myosin II. Intriguingly, centrin and plant-specific myosin VIII are found at contractile cell-to-cell plasmodesmatal channels in plants (Blackman *et al*., 1999; Baluška *et al*., 2001*b*). This finding is potentially very relevant because centrioles are known to be essential for the final stage of animal cytokinesis (Khodjakov and Rieder, 2001; Piehl *et al*., 2001).

Actin-based Cell Periphery Complex versus *tubulin-based* Cell Body*: Yin and Yang principles imply sexual nature of the cytoskeleton*

Vasiliev (1987) was the first to propose that eukaryotic cells are based on a symbiosis-like coexistence of two co-operating, yet competing domains: an actin-based cell periphery termed *actinoplast*, and a tubulin-based *tubuloplast* (see also Figs 3, 4), an idea that clearly foreshadows Mazia's *Cell Body* concept. These two cellular domains segregate completely during mitosis when the tubulin-based mitotic spindle, or naked *Cell Body*, is divested of actin and the cells revert to the primitive nature that is characteristic of the early eukaryotic cells (Fig. 6). As discussed above, this feature is also a characteristic of sperm cells of higher plants. In contrast, plant cells entering into interphase deploy their microtubules at the cell periphery (Baluška *et al*., 1997*a*) while actin and diverse actin-binding proteins accumulate within their nuclei and participate in the organization of nuclear structure and chromatin activities (like DNA transcription) as well as in the maturation and transport of RNA molecules (Olave *et al*., 2002; Pederson and Aebi, 2002; Kandasamy *et al*., 2003; Kraus *et al*., 2003; Shumaker *et al*., 2003).

Obviously, both actin and tubulin are important for the organization of eukaryotic cells and therefore it is not surprising that both these proteins are among the most conserved of eukaryotic proteins. Strikingly, tight parallels exist between this symbiotic-like organization of the actin-based *Cell Periphery Apparatus* and the tubulin-based *Cell Body*, both assemblies being the vestiges of an ancient hypothetical actin-based 'host' cell and a tubulin-based 'guest' cell (Fig. 3). As mentioned above, this sequence of events is recapitulated during the sexual reproduction of eukaryotic organisms when, invariably, fusion between a tubulin-based sperm cell and an actin-based oocyte gives rise to a new multicellular organism (Fig. 4). After fusion of the tubulin-based sperm cell with the actin-based oocyte, followed by the fusion of their haploid nuclei (*Cell Bodies*), the centrosome-less oocyte acquires the sperm centrosome which then takes control of the spatial arrangement of microtubules in the fertilized zygote.

This sexual background to the current cytoskeleton, and the joining of the two ancient and Yin-Yang-like cytoskeletal systems into one cell, may explain the extreme

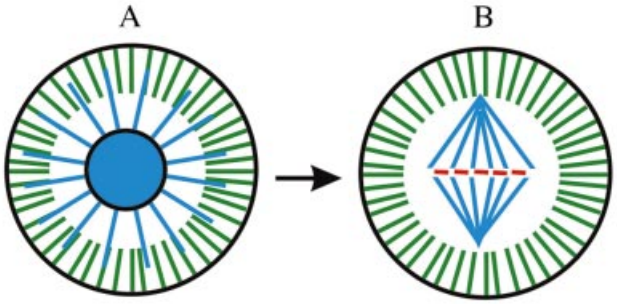

FIG. 6. During mitosis, all microtubules (blue) retract from the actin-rich (green) cell periphery and participate in the assembly of the mitotic spindle, which represents the most basic form (or transformation) of the *Cell Body*. In this state, the *Cell Body* is specialized for the segregation of large amounts of chromosomal DNA (red). Mitosis is one of the most conserved process found within the eukaryotic superkingdom.

rapidity of the prokaryotic–eukaryotic switch and the consequent lack of fossil records of 'transition' organisms (Dacks and Doolittle, 2001). The actin cytoskeleton remained associated preferentialy with the flexible cell boundary which thereby drives an actin-based motility (Pantaloni *et al*., 2001), whereas the microtubular cytoskeleton evolved, together with DNA and associated proteins, into the *Cell Body*. Both basic types of cytoskeleton exert mechanical forces via polymerization and depolymerization of their respective polymers, resembling the force generation of present-day prokaryotic life, which is also based on actin-like and tubulin-like proteins (van den Ent *et al*., 2001*a*, *b*; Ben-Yehuda and Losick, 2002; Carballido-López and Errington, 2003; Daniel and Errington, 2003). On the other hand, more advanced force-generating systems, such as molecular motors which use actin- and tubulin-based polymers as tracks, are true eukaryotic inventions accomplished only as a consequence of the increased complexity of eukaryotic cells (Mitchison, 1995; Vale, 2003). Interestingly, not only present-day cellular parasites but also endosomes and phagosomes (Merrifield *et al*., 1999; Taunton *et al*., 2000; Zhang *et al*., 2002; Fehrenbacher *et al*., 2003; Southwick *et al*., 2003) use actin polymerization as a driving force for their motilities (Machesky, 1999; Maly and Borisy, 2001; Pantaloni *et al*., 2001; Pollard and Borisy, 2003).

Actin- and tubulin-based cytoskeletal systems can support cellular and subcellular movements independently of each other. Cellular fragments containing portions of cell periphery and an actin polymerization machinery, but lacking nuclei and microtubules, are still capable of autonomous directional motility (Albrecht-Buehler, 1980; Euteneuer and Schliwa, 1984; Malawista and Chevance de Boisfleury, 1984; Verkhovsky *et al*., 1998; Maly and Borisy, 2001). On the other hand, tubulin-based *Cell Bodies* are also inherently motile. The characteristic motility of *Cell Bodies* within eukaryotic cells (Baluška *et al*., 2001*a*) strongly implicates the independent nature of this part of the eukaryotic cell. As mentioned above, perinuclear microtubules, capable of both pushing and pulling forces, act as effective instruments to allow *Cell Bodies* to claim a certain amount of the cytoplasmic space. If one of them is less effective in this activity, then unequal daughter cells of a division are the result; the weaker *Cell Body* has a smaller influence and gains a correspondingly smaller cytoplasmic space (Pickett-Heaps *et al*., 1999; Brown and Lemmon, 2001).

A nice example of this situation is the first mitotic division of a pollen nucleus to produce a large vegetative cell, which supports pollen tube growth, and a small generative cell designed to form sperm cells devoid of F-actin. Such rudimentary *Cell Bodies* of the sperm cells are inactive and are fully dependent upon the metabolic activities of the vegetative nucleus and pollen tube. Another example of such a 'tug-of-war' between *Cell Bodies* having different strengths is the first division of the fertilized zygote, which is often asymmetric and thereby defines the anterior–posterior body axis of most multicellular organisms (Wallenfang and Seydoux, 2000; Lyczak *et al*., 2002; Wodarz, 2002). Smaller cells typically give rise to the posterior/shoot poles of multicellular organisms, and then ultimately they become specialized for the development of sexual organs and organs of movement. The larger cells produce, again via asymmetric division, anterior/root poles specialized for the uptake of nutritive substances and for neuronal-like activities (for plants see Jürgens, 2000, 2003; Baluška *et al*., 2004).

Centering of tubulin-based Cell Body *and its modulation via actin-based* Cell Periphery Apparatus

Recently, we reviewed data reporting that the actin-based cell periphery participates in the positioning of the *Cell Body* by means of interactions between the dynamic plus-ends of microtubules, which emanate from the *Cell Body*, and the actin-rich *Cell Periphery Apparatus* (Baluška *et al*., 2000*b*, 2001*a*). In the most typical situation, the *Cell Body* settles at the geometrical centre of the cell as a result of a centripetal pushing force directed from the cell periphery. Dynamic microtubules lacking association with centrosomes and nuclei, but equipped with microtubular motors, are also capable of this centering phenomenon if the minus-ends of microtubules focus upon cellular inclusions, such as melanophores, while their plus-ends radiate towards the cell periphery (Rodionov and Borisy, 1997). Centrosomes released from their inherent nuclear association use the same mechanism for positioning and centring (Rieder *et al*., 2001; Euteneuer and Schliwa, 1992; Burakov *et al*., 2003).

Cell Bodies make use of interactions with the cell periphery-enriched actin cytoskeleton (Pruyne and Bretscher, 2000) to maintain their positions (Burakov *et al*., 2003). Dynamic microtubules explore the surrounding perinuclear cytoplasmic space (Holy *et al*., 1997; Faivre-Moskalenko and Dogterom, 2002). The property of microtubule instability, which is affected by reaching the cell boundary, is crucial for this explorative behaviour (Komarova *et al*., 2002). It allows mitotic spindles and interphase nuclei to perform rotations in the cytoplasm, these movements also being navigated by the actin cytoskeleton which accumulates under the plasma membrane (Reinsch and Gönczy, 1998; Adames and Cooper,

2000; Tran *et al*., 2001; Burakov *et al*., 2003; Kusch *et al*., 2003). The identity of critical molecules that link the plus-ends of microtubules with the actin cytoskeleton at the cell cortex has recently been illuminated in yeast and animal cells (Goode *et al*., 2000; Pruyne and Bretscher, 2000; Glynn *et al*., 2001; Ishizaki *et al*., 2001; Gundersen, 2002; Kodama *et al*., 2003). Interestingly, plant cells express a homologue of Kar9p (Gardiner and Marc, 2003) which is responsible for linking *Cell Body* microtubules to the actin-rich cell cortex (Segal *et al*., 2002).

Accumulations of actin at distinct cell periphery domains attract and stabilize nearby microtubules, and these ultimately polarize the *Cell Body* (Baluška *et al*., 2000*b*, 2001*a*). The centring and polarizing properties of *Cell Bodies* are essential not only for division of unicellular yeast cells (Pruyne and Bretscher, 2000) but also for cell-to-cell communication, as evidenced by actin-based synaptic contacts both in animal and plant cells (Dustin and Colman, 2002; Baluška *et al*., 2003*a*, *b*, *c*; Barlow *et al*., 2004). In plants, polar transport of auxin is inherently linked to the overall polarity of the *Cell Bodies* (Baluška *et al*., 2003*a*, *b*, *c*; Barlow *et al*., 2004). This in turn leads to a preferred orientation of mitotic division. *Cell Bodies* of animal cells are also polarized via immunological synapses (Sancho *et al*., 2002). In fact, in what seems to be part of a cellular 'arms race', active *Cell Bodies* organizing lysosome-based secretion of lytic substances can be considered to behave as some sort of 'killer machines' (Bossi *et al*., 2002; Clark *et al*., 2003).

From the Yin/Yang perspective, mitosis might be viewed as a phase in which the two types of cytoskeleton are separated from each other, and revert back to the ancient configuration of the cytoarchitecture (Fig. 6). Mitotic segregation of DNA-based mitotic chromosomes is organized and driven solely via microtubules, which retract from all cellular areas and are then free to build up the spindle apparatus. Conversely, the actin cytoskeleton retracts from the cell's interior and associates preferentially with the *Cell Periphery Apparatus* (Fig. 5). When mitotis and cytokinesis are both concluded, tubulin and actin-based cytoskeletons interpenetrate again and form the integrated cytoskeletal network of eukaryotic cells (Goode *et al*., 2000; Kodama *et al*., 2003).

Cell Body-*based exocytosis* versus Cell Periphery-*based endocytosis*

From a phylogenetical perspective, the *Cell Body* concept gives us some clues to speculate on how it came about that eukaryotic cells developed two quite contrasting pathways for vesicular membrane trafficking. The secretory pathway is organized by the *Cell Body*: it starts at the nuclear envelope (Vorísek, 2000; Matynia *et al*., 2002), continues via endoplasmic reticulum and Golgi apparatus, and culminates with secretory vesicles fusing with the plasma membrane (Fig. 7). Secretion is tightly coupled with nuclear organization (Nanduri and Tartakoff, 2001) and is under the spatial control of the *Cell Body* microtubules (Bloom and Goldstein, 1998; Müsch, 2004).

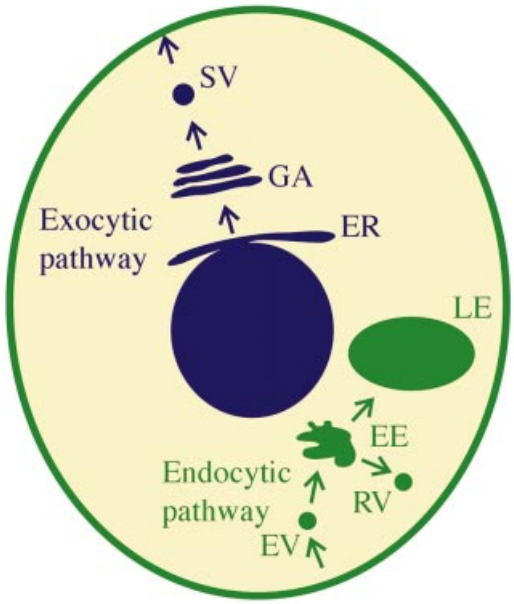

FIG. 7. The *Cell Body* organizes the exocytic secretory pathway (blue), which is composed of endoplasmic reticulum (ER), Golgi apparatus (GA) and secretory vesicles (SV). In contrast, the *Cell Periphery Apparatus* organizes the endocytic secretory pathway (green), which is composed of endocytic vesicles (EV), recycling vesicles (RV), early endosomes (EE) and late endosomes (LE).

Importantly, the outwardly directed exocytic pathway is phylogenetically older than the inwardly directed endocytotic pathway (Jékely, 2003), which is organized by the *Cell Periphery Apparatus* (Fig. 7). The endocytic pathway starts at the plasma membrane (Conner and Schmid, 2003) with actin-dependent internalization steps (Engquist-Goldstein and Drubin, 2003), and proceeds deeper into the cytoplasm via different types of endosomes (Fig. 7) propelled by comet-like actin tails (Merrifield *et al*., 1999; Taunton *et al*., 2000; Zhang *et al*., 2002; Fehrenbacher *et al*., 2003; Southwick *et al*., 2003). This pathway, which is evolutionarily speaking a more recent one, is a vestige of the activities of the ancient actin-based 'host' proto-cell which represents a transformation of its actin-based plasma membrane. These internalization pathways, including primitive versions of phagocytic and endocytic pathways, allowed the symbiotic acquisition of further organelles of eukaryotic cells; these acquired organelles were the forerunners of the present-day mitochondria and plastids (McFadden, 1999; Gray *et al*., 2001). Nowadays these endocytotic pathways are hijacked by viruses and bacteria, allowing them to intrude into eukaryotic cells (Brock *et al*., 2003; Stamm *et al*., 2003; Wang *et al*., 2003) and then, after entering the cell, to exploit the actin cytoskeleton for their intracellular, as well as cell-to-cell, migration (Goldberg, 2001; Fehrenbacher *et al*., 2003; Stamm *et al*., 2003).

Using the actin cytoskeleton, the most primitive eukaryotic cells exploited this second, endocytotic pathway of vesicular trafficking not only for cellular nutrition (Conner and Schmid, 2003) but also for complex cell-to-cell

signalling pathways which have now become a prevalent feature of multicellular organisms (Gundelfinger *et al*., 2003; Stevens, 2003). The best examples here are adhesion domains specialized for vesicular cell-to-cell communication in neuronal, immunological and plant synapses (Dustin and Colman, 2002; Barlow *et al*., 2004). Moreover, besides the endosymbiotic acquisition of the power-houses of eukaryotic cells—the mitochondria and chloroplasts (McFadden, 1999; Gray *et al*., 2001) – there were secondary endosymbiotic events in which one primitive eukaryote enclosed a second eukaryote (Cavalier-Smith and Beaton, 1999; Douglas *et al*., 2001; Gilson, 2001; Cavalier-Smith, 2002*b*). This reveals that there is an inherent tendency for endosymbiosis which has operated throughout the evolution of biological systems.

Small GTP-binding proteins from the Cell Body *perspective: the unique status of Ran family*

Besides the nucleus, cytoskeleton and vesicle trafficking machinery, all eukaryotic cells are characterized by the Ras superfamily of small GTPases that are key regulators of both cytoskeletal dynamics and vesicular traffickings. The phylogenetic analysis of small GTPases reveals that the most ancient eukaryotic cells were equipped with a secretory machinery but, as mentioned above, lacked the molecules which would support endocytosis and phagocytosis (Jékely, 2003). The nuclear envelope is part of the exocytic pathway (Vorísek, 2000; Matynia *et al*., 2002; Shimoda, 2004) that is organized along *Cell Body* microtubules radiating from the nuclear envelope towards the cell periphery. It is probable that the symbiotic origin of the nucleus (Gupta *et al*., 1994; Gupta and Golding, 1996; Horiike *et al*., 2001; Hartman and Fedorov, 2002) is inherently linked with the acquisition of this pathway.

Most small GTPases localize to the membranes of eukaryotic cells where they act as biological switches, activating or terminating biological processes. Particular subcellular localizations of these membranous targets are specified by post-translational modifications with farnesyl, palmitoyl, myristolyl and geranylgeranyl lipid groups (Takai *et al*., 2001; Vernoud *et al*., 2003). Members of the Ras family are predominantly localized to the plasma membrane where they activate stimulus–responsive serine/threonine kinases (Takai *et al*., 2001; Vernoud *et al*., 2003), while members of the Rho family organize a cytoskeleton in association with phagocytic and endocytic membranes (Ridley, 2001; Etienne-Manneville and Hall, 2002). Members of the Rab family organize endocytic pathways (Zerial and McBride, 2001) while Arf members localize preferentially to endoplasmic reticulum and Golgi apparatus (Pasqualato *et al*., 2002; Spang, 2002). Interestingly, cell fusion is regulated by the plasma membrane-associated GTPase ARF6 (Chen *et al*., 2003; Taylor, 2003).

Of all the known families of small GTPases, only the Ran family members lack lipid attachment modules. They are thus not localized to membranes but are, instead, abundant within the nucleus. Ran GTPases shuttle to the cytoplasm and organize diverse nuclear features and processes, such as nuclear architecture (Clarke and Zhang, 2001), the assembly of nuclear pores (Ryan *et al*., 2003), the sorting out of the nuclear envelope as a specialized domain of endoplasmic reticulum (Hetzer *et al*., 2002; Mattaj, 2004), nucleocytoplasmic transport (Görlich and Kutay, 1999), targetting of nuclear proteins (Narayanan *et al*., 2003), as well as centrosome activity (Di Fiore *et al*., 2003; Keryer *et al*., 2003), kinetochore function (Arnaoutov and Dasso, 2003), nuclear chromatin- and chromosome-driven polymerization of microtubules (Carazo-Salas *et al*., 1999; Ohba *et al*., 1999; Wilde *et al*., 2001; Kalab *et al*., 2002) and spindle checkpoints (Li *et al*., 2003). Because Ran GTPases also regulate DNA synthesis (Moore, 2001; Yamaguchi and Newport, 2003) and cell-cycle progression (Moore, 2001), this class of small GTPases emerges as a central organizing component of the *Cell Body*, linking together DNA- and tubulin-based structures.

INHERENT DNA–TUBULIN INTERACTIONS

'Happy marriage' or 'master–slave' relationships?

The evolutionary transition from prokaryotes to eukaryotes, which is still one of the greatest puzzles for contemporary biology, was marked by the unprecedented molecular coupling of tubulin with DNA. In prokaryotes, DNA is associated with membranes which thereby allow its replication and partitioning, whereas eukaryotes use exclusively microtubules to partition huge amounts of DNA with high fidelity. Recently, DNA segregation in bacteria was shown to rely on polymerization of actin-like ParM protein (Møller-Jensen *et al*., 2003) This suggests that DNA has an inherent tendency to enslave cytoskeletal molecules, irrespective of its association with either prokaryotic or eukaryotic cellular organization.

The association of DNA with nuclear proteins, especially histones (Malik and Henikoff, 2003), as well as the association of chromatin with tubulin-based microtubules, are the most characteristic features of eukaryotic cells (Baluška *et al*., 1997*a*). Double-stranded (but not the single-stranded) DNA binds to the microtubule-associated protein tau, which somehow protects the DNA double helix (Hua and He, 2003; Hua *et al*., 2003). This latter feature together with those processes that drive mitosis indicate that DNA is perhaps the dominant partner in this molecular relationship. This would imply some kind of molecular slavery relationship between tubulin and DNA, the latter playing the role of master.

Importantly, this 'master–slave' relationship allows the *Cell Bodies* to exhibit exploratory properties in space and time (Kirschner and Gerhart, 1998; West-Eberhard, 1998; Baluška *et al*., 2001*a*). These properties are essential for driving cellular polarities (Pruyne and Bretscher, 2000; Baluška *et al*., 2001*a*) as well as for pattern formation, morphogenesis and development of complex multicellular organisms (Baluška *et al*., 2003*b*). Due to the abandonment of the inherent association between DNA and membranes, which is the hallmark of prokaryotes, eukaryotic DNA became free to engage in extensive proliferation. Using specialized nuclear proteins that direct tubulin polymerization (Oegema *et al*., 1997; Wittmann *et al*., 2000; Du *et al*.,

2001; Keryer *et al*., 2003; Rabitsch *et al*., 2003; Raemaekers *et al*., 2003; Schatz *et al*., 2003; for a review see Baluška *et al*., 1997*a*), DNA enslaved the microtubular cytoskeleton (see also Box 2) and exploited it as the vehicle to move large DNA assemblages, such as mitotic chromosomes or even whole nuclei. *Cell Bodies* clearly manifest this master–slave relationship.

The inherent relationship between eukaryotic DNA and microtubules is so strong that even the extremely reduced nucleomorphs, which have undergone up to 1000-fold reduction of their genomic DNA mass (Cavalier-Smith and Beaton, 1999; Gilson, 2001), still retain genes for α-, β- and γ-tubulins, although they lack most other proteins typical of eukaryotic cells (Keeling *et al*., 1999). As mentioned, a very strong argument for an inherent association between DNA molecules and tubulins can be found in the unique nature of epixenosomes, which are ectosymbionts located on the surface of marine ciliates (Petroni *et al*., 2000). They might be considered to be highly reduced symbiotic *Cell Bodies* consisting only of DNA and tubulins (Jenkins *et al*., 2002).

MAZIA'S VISION OF FLEXIBLE LINEAR CENTROSOMES IN PLANT CELLS: GAMMA-TUBULIN, EB1 AND SPC98P HOLD THE KEY

One outstanding mystery of higher plant cells, which has perplexed scientists for many years, is the apparent absence of corpuscular centrosomes. This failure to identify any definite centrosome in the cells of higher plants stimulated Mazia to propose, in an entirely speculative manner, the concept of a 'flexible' linear centrosome which should be able to modify its three-dimensional arrangement (Mazia, 1987). He was proposing a 'flexible string' composed of discrete units which are, in a way similar to DNA, capable of folding and hence attaining secondary and tertiary orders of structural organization.

At the time of Mazia's suggestion, γ-tubulin was not known (Oakley and Oakley, 1989), and no MTOC component had been identified in plant cells, despite the fact that the concept of a MTOC had already been proposed for them (Pickett-Heaps, 1969). Now, however, numerous data are accumulating that indicate that plant γ-tubulins, together with other proteins, correspond to the putative discrete units which represent the flexible linear centrosome of higher plant cells (Liu *et al*., 1993; Joshi and Palevitz, 1996; Canaday *et al*., 2000; Panteris *et al*., 2000; Dibbayawan *et al*., 2001; Dryková *et al*., 2003; Horio and Oakley, 2003; Kumagai *et al*., 2003; Schmit, 2003; Shimamura *et al*., 2004). In addition to γ-tubulin, very recent advances have identified the tubulin plus-end binding protein EB1 (Rehberg *et al*., 2002; Rogers *et al*., 2002) which, in plant cells, also marks the mobile minus-ends (Chan *et al*., 2003). These findings strengthen Mazia's view of a flexible and dispersed centrosome in plant cells (Chan *et al*., 2003; Lloyd and Chan, 2004). This interpretation is supported by the situation known from *Dictyostelium discoideum* where EB1 is an integral part of the centrosome, is independent of microtubules (Rehberg *et al*., 2002), and emerges from centrosomes on tips of growing microtubules (Piehl *et al*., 2004). SPC98p and SPC97p are other well-known components of MTOCs, and they are part of a ubiquitous microtubule nucleator complex with a molecular mass of about 280 kDa (Moritz and Agard, 2001). SPC98p has also been identified in plant cells (Erhardt *et al*., 2002) where it localizes to three distinct sites: intranuclear dots, the nuclear surface, and at sites near the plasma membrane (Seltzer *et al*., 2003). All this conforms very well with the *Cell Body* concept as elaborated in this review.

OUTLOOK

Genome evolution is associated with a huge variation in nuclear DNA amounts. Eukaryotic organisms at either the same or different levels of complexity differ considerably in their DNA amounts. The genome of *Amoeba*, for example, is about 200 times larger than the human genome, representing one of the most astonishing examples of the C-value enigma (Gregory, 2001*a*). In the plant genus *Luzula*, whose species are difficult to distinguish morphologically from one another, diploid DNA values vary 15-fold and chromosome numbers 10-fold (species with low chromosome numbers having higher DNA amounts) (Barlow and Nevin, 1976). Not many molecular biologists are aware of the fact that coding DNA comprises only <10 % of the whole genome, the rest of the DNA being of unknown function (Cavalier-Smith and Beaton, 1999). Importantly, the non-coding DNA has an effect on cell size via its so-called nucleotypic influence (Bennett, 1972; Gregory, 2001*a*, *b*), although there is no plausible explanation for this phenomenon (Gregory, 2001*a*).

Fortunately, the *Cell Body* concept is poised to explain these enigmas. DNA-binding proteins stored within nuclei often regulate tubulin polymerization (Baluška *et al*., 1997*a*), while the dynamism of cytoplasmic microtubules regulates the access to the nucleus of proteins sequestered within the cytoplasm (for transcription factors see Oegema *et al*., 1997; Wittmann *et al*., 2000; Du *et al*., 2001; Keryer *et al*., 2003; Rabitsch *et al*., 2003; Raemaekers *et al*., 2003; Schatz *et al*., 2003; for a review see Baluška *et al*., 1997*a*). This, in turn, regulates the binding of these proteins to the DNA, irrespective of its coding capacity, and influences the assembly of the nuclear matrix (Baluška and Barlow, 1993; Baluška *et al*., 1995*a*, *b*, 1997*b*).

Dynamic microtubules also stabilize the nucleocytoplasmic ratio (Trombetta, 1939), this being a measure of the efficiency with which the microtubules of the *Cell Body* patrol the cytoplasmic domain surrounding the nucleus. DNA can independently increase or diminish in amount, and a given nucleocytoplasmic ratio can be maintained so long as the microtubules radiating from the nucleus dominate the surrounding cytoplasmic domain in proportion to nuclear volume. As mentioned above, nucleomorphs are not relict nuclei, as generally assumed (Cavalier-Smith and Beaton, 1999; Gilson, 2001), but are relict *Cell Bodies*. This is evidenced by their expression of tubulin genes, although this does not lead to the formation of microtubules (Keeling *et al*., 1999). Similarly, epixenosomes represent another example of highly reduced *Cell Bodies* composed only of tubulins and DNA (Jenkins *et al*., 2002). Importantly, the miniaturized genomes of nucleomorphs do not scale with

the cell size of their hosts (Gilson, 2001). Because nucleomorphs lack non-coding DNA, which in most eukaryotic genomes is much more abundant than coding DNA (Gregory, 2001*a*, *b*), one could propose that the non-coding DNA is relevant for the *Cell Body* on account of its interaction with tubulin molecules via diverse tubulin/DNA-associated proteins, of which NuMa is the most instructive example (Levesque *et al*., 2003; Tulu *et al*., 2003). Recently, a putative plant homologue of NuMa was reported for *Arabidopsis* (Gardiner and Marc, 2003).

In the framework of the *Cell Body* concept, the non-coding DNA could control nuclear structure via its ability to control both internal nuclear architecture and the availability of nuclear proteins that have tubulin-polymerizing activity (Baluška *et al*., 1997*a*). This would be in a full agreement with the proposition that non-coding DNA acts as nucleoskeletal DNA (Cavalier-Smith and Beaton, 1999). On the other hand, the dynamic properties of cytoplasmic microtubules can make a direct impact on nuclear architecture. They can either exert pushing forces on the nuclear surface or sequester, within the cytoplasm, proteins critical for structuring the nuclear chromatin and for regulating genome expression. In support of these notions, we have reported elsewhere upon the close relationships between nuclear size, chromatin structure and dynamicity of cytoplasmic microtubules (Baluška and Barlow, 1993; Baluška *et al*., 1995*a*, *b*, 1997*b*).

Thus, the *Cell Body* concept not only predicts that DNA regulates tubulin assembly within the cytoplasm but also that the assembled microtubules control the availablity of nuclear proteins, sequestered within the cytoplasm, for the decondensation of chromatin which controls DNA replication and transcription (Oegema *et al*., 1997; Du *et al*., 2001; Wittmann *et al*., 2000; Keryer *et al*., 2003; Rabitsch *et al*., 2003; Raemaekers *et al*., 2003; Schatz *et al*., 2003; for a review see Baluška *et al*., 1997*a*). From the point of view of the *Cell Body*, there is no difference between coding DNA and non-coding DNA; both are predicted to interact, directly or indirectly, with the sequestered nuclear proteins. However, uncovering those proteins which interact directly with the non-coding skeletal DNA (Cavalier-Smith and Beaton, 1999) will require concentrated activity from molecular biologists. Unfortunately, this will take some time because current scientific efforts are focusing solely on the genetic coding properties of DNA.

The importance of non-coding DNA for interactions with the tubulin-based cytoskeketon is well known from centromeres (repetitive non-coding DNA sequences), which organize, via associations of numerous DNA binding proteins, kinetochores that are specialized for the attachment of mitotic chromomes to microtubules of the mitotic spindle (De Wulf *et al*., 2003). Recent advances in studies on centromeres and kinetochores reveal a lack of DNA sequence specificity for the establishment and maintenance of centromere DNA identity, as well as kinetochore assembly (Sullivan *et al*., 2001; Amor and Choo, 2002).

Clearly, the centromere–kinetochore complex is assembled and maintained via self-propagating epigenetic mechanisms based on chromatin structures, but independent of DNA sequences (Amor and Choo, 2002). These findings are exciting in that they reveal the need for the *Cell Body* concept, especially because of the unique power of this concept to explain the role of non-coding DNA as a central player responsible for the linkage between the DNA-based nuclear chromatin with the tubulin-based cytoskeleton. This feature allows the *Cell Body* to couple genomic information (encoded within DNA sequences and handed over to RNA molecules) with epigenetic information (embodied within the inherent physical properties of DNA structures, which can store and propagate this information via complex DNA–protein and protein–protein templating processes) (Gregory and Herbert, 1999; Zuckerkandl, 2002). The crucial question to answer is what molecules accomplish the inherent DNA/tubulin-based cytoskeleton interactions. The first clues are emerging in this respect. First, linker histone H1 was reported to exert a dual function, acting as some kind of microtubule-associated protein which stabilizes preformed microtubules (Multigner *et al*., 1992; Saoudi *et al*., 1995; Kaczanowski and Jerzmanowski, 2001). Second, microtubule-associated protein tau binds to double-stranded, but not single-stranded, DNA and does so reversibly in the presence of histones (Hua *et al*., 2003). It also apparently protects the double helix structure from damaging free radicals (Hua and He, 2003).

Cell Bodies can sense electric and magnetic fields, and use them as cues for the orientation of mitotic divisions (Denegre *et al*., 1998; Zhao *et al*., 1999; Song *et al*., 2002; Valles, 2002). Relevant in this respect are the microtubules which, in this sensory context, have even been proposed to act like the 'nerves' of cells (Albrecht-Buehler, 1998) due to their ability to perceive and transmit light (Albrecht-Buehler, 1992, 1994, 1998). The orientated self-organization of microtubules is, in some circumstances, also dependent upon gravitational fields (Tabony and Job, 1992; Papaseit *et al*., 1999, 2000; Tabony *et al*., 2001). Moreover, dynamic microtubules can sense another critical physical parameter of environment: temperature. This may be via Ca^{2+} liberated from cell walls (Plieth *et al*., 1999) and from the endocytotic components of the *Cell Periphery Apparatus*. Sensing the physical environment is inherently combined with the ability of microtubules to organize into radial arrays of the *Cell Body* that scan the plasma membrane boundaries of the cytoplasm, exerting either a pushing or a pulling force on any object or boundary to which they are attached. Thus, the combination of all these properties allows the microtubular mitotic spindle (specialized form of the *Cell Body* optimized for its multiplication) not only to act as an ideal tool for separating large amounts of DNA molecules with high fidelity but also to endow the otherwise passive DNA-storing nuclei with sensory and exploratory properties (Kirschner and Mitchison, 1986; Kirschner and Gerhardt, 1998). Clearly, microtubules are central for these abilities of *Cell Bodies* to, first, sense physical properties of their environment and, second, to use this information directly in morphogenesis (Kirschner and Mitchison, 1986; Hyman and Karsenti, 1996; Papaseit *et al*., 1999). These two phenomena are especially critical for sessile higher plants (Baluška *et al*., 1997*a*, 1998; Wasteneys, 2002).

CONCLUSIONS

In conclusion, the *Cell Body* concept proposed here is useful for understanding eukaryotic cells in their whole complexity. This concept not only explains how eukaryotic cells came to be formed from their proto-cellular predecessors, and in particular how the eukaryotic nucleus was formed and why it is so intimately linked with centrosomes and microtubules, but it also explains why mitosis and cytokinesis can be accomplished independently of each other. Moreover, this concept reveals the fundamentally sexual nature of the eukaryotic cytoskeleton, and explains differences between exocytosis and endocytosis from an evolutionary point of view. Last, but not least, the *Cell Body* concept allows, for the first time, an explanation of the C-value enigma from the perspective of nucleotypic DNA–tubulin interactions.

A FINAL NOTE

This review is dedicated to the memory of Daniel Mazia (1912–1996) who was well aware of the unique nature of DNA–tubulin interactions. The apparent absence of a corpuscular centrosome in cells of higher plants was a puzzle to be solved, and Mazia approached this enigmatic problem by proposing the existence of a thread-like flexible centrosome which would be in a position to attain higher-order structures. Daniel Mazia discovered and isolated the mitotic apparatus of sea urchins (Mazia and Dan, 1952) even before microtubules were known. Following this, and with the legacy of Theodore Boveri in mind (Mazia, 1987), he devoted almost his whole life to understanding how centrosomes, microtubules and nuclei (or mitotic chromosomes) interact to build the structural and functional unit which he termed the *Cell Body* (Mazia, 1994). Unfortunately, his death prevented him from formulating this concept to the full. Here, we make an attempt to do this, employing a holistic approach that embraces both the evolutionary as well as the structural and functional aspects of eukaryotic life. We find that these approaches can be satisfactorily integrated into the miracle of the *Cell Body*.

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BOX 1

Updated cellular doctrine

The *Cell Body* is a primary element of organismal structure. The plasma membrane, being a component of the complex *Cell Periphery Apparatus*, encloses the *Cell Body* to form a complete *Cell.* The *Cell Periphery Apparatus* is a secondary, largely self-assembled structure that is guided and maintained in its position by the *Cell Body*, providing it with both a protective layer and a mechanical support. Evolutionarily, the actin-based portion of the outer boundary represents the vestige of an actin-based 'host' proto-cell that was penetrated by a tubulin-based 'guest' proto-cell. The latter proto-cell, in tight coordination with the 'host' DNA, was subsequently transformed into a composite DNA/tubulin-based *Cell Body* on account of the great affinity of DNA for tubulin molecules. Importantly, the active *Cell Body* of contemporary eukaryotic cells can participate in elaborating the plasma membrane *de novo*. This can happen either occasionally during cell wounding or regularly during cytokinesis and meiosis. On the other hand, the *Cell Body* cannot be formed *de novo* and can be formed only from a pre-existing *Cell Body*. Therefore, the *Cell Body* represents the smallest autonomous and self-reproducing unit of eukaryotic life.

BOX 2

Cell Body *manifesto: four basic principles*

I. 'Templating' Principle
Two basic types of templates store and propagate information (with *Positive Feedback Loops* operating between them).

1. Molecular templates based on complementarity of molecules (e.g. DNA and RNA);
2. Structural templates based on topological order of vectorial structures (arrangement of microtubule organizing centres and the microtubules which arise from them, non-coding DNA interacting with specific proteins).

II. 'Molecular Slavery' Principle
DNA is proposed to act as a 'master' molecule with cytoskeletal molecules acting as its 'slaves'. The assembly and spatial distribution of cytoskeletal polymers is directly (with tubulin as a primary 'slave') and indirectly (with actin as a secondary 'slave') controlled by DNA molecules and their associated proteins which together assemble into chromatin.

1. DNA enslaves tubulin, the primary slave, in order:
- to gain motility (microtubules move large DNA-based structures such as nuclei and mitotic chromosomes);
- to bring about the evolution of its structure and base sequence, and to gather environmental information (microtubules provide DNA with a sensory apparatus optimized to gather and process information via the properties of dynamically unstable microtubules).

2. DNA enslaves actin, the secondary 'slave', via nuclear–cytoplasmic shuttling of G-actin, profilin and actin-depolymerizing factor, all of which are intrinsically linked to the plasma membrane and derived membrane-enclosed compartments (endosomes). The actin-associated elements together form part of the *Cell Periphery Apparatus*.
3. For survival, the *Cell Body* participates in the organization of the *Cell Periphery Apparatus* (cell boundary composed of plasma membrane and extracellular matrix/cell wall). These boundary structures enable the *Cell Body*:
- to be protected from the hostile outside world;
- to accumulate information about the outside world;
- to interact with the outside world directly via endocytosis, allowing the *Cell Body* to 'taste' its surroundings.

III. Cytoskeleton as a Force Generator
The cytoskeleton is specialized for the conversion of chemical energy into mechanical energy. It generates two types of force:

1. A primitive force based on the polymerization of tubulin and actin: i.e. microtubules push or pull DNA-based structures and membranes, actin filaments push membranes.
2. A more advanced type of force based on molecular motors (myosins, kinesins, dyneins) which drag membranous cargoes along tracks based on polymerized microtubules and actin filaments.

IV. Principle of Membrane Boundary and Compartmentalization
The *Cell Periphery Apparatus* that encloses *The Cell* is a boundary that represents a vestige of the 'host' cell. It is specialized for the protection of the *Cell Body*. A major portion of cellular DNA is stored within nuclei separated from the rest of the cell by a nuclear envelope which, similar

to other endosymbiotic organelles, is composed of two membranes (that are probably descended from the ancient boundary membranes of 'host' and 'guest' cells).

BOX 3

The Cell Body *and its history*

The history of the *Cell Body* starts in 1892 with Julius Sachs who, when faced with the curious internal structure of several species of siphonous coenocytic algae, concluded that the nucleus organizes a distinct cytoplasmic domain, even in the absence of any obvious cytoplasmic boundary. Sachs coined the term 'energid' to describe the nucleus with an associated portion of cytoplasm. He then postulated that the algal siphons, which he was examining at that time, were polyenergids as opposed to the more usual monoenergidic cells of higher plants with only one nucleus (Sachs, 1892; see also Sitte, 1992). In fact, similar views had been proposed some 30 years before those of Sachs, by Max Schultze studying multinucleate muscle cells of animals (Schultze, 1861; cited in Harris, 1999), and by Anton de Bary working on multinucleate plasmodia of slime moulds (de Bary, 1864; cited in Harris, 1999). Influenced by these and other multinucleate situations, Eduard Strasburger proposed, in 1893, the concept of the karyoplasmic ratio (now known as the nucleocytoplasmic ratio), stating that there is a positive interrelationship between nuclear and cellular sizes.

More than 100 years after Julius Sachs and Eduard Strasburger, Daniel Mazia tackled many of these same problems from the perspective of centrosomes, microtubules and their roles in partitioning the mitotic chromosomes within which the whole genome resides (Mazia, 1993; Epel and Schatten, 1998). Mazia proposed that the eukaryotic cell is a confederation of two independent units: a tubulin-based *Cell Body* composed of nucleus and a complement of perinuclear microtubules, and an actin-based *Cell Periphery Apparatus* organized at the plasma membrane (Mazia, 1993). Although this proposal was formulated for the unitary cells that compose animal organisms, we have shown that the *Cell Body* concept is also valid for the supracellular 'confederation' of interconnected cells that comprise plant organisms (Baluška *et al*., 1998, 2000*b*, 2001*a*). Obviously, this concept is of general applicability throughout the eukaryotic superkingdom. We conclude that the DNA-based nucleus and its associated tubulin-based microtubules form the *Cell Body*, and that this item represents the smallest autonomous and self-reproducing unit of the eukaryotic life.

BOX 4 *Milestones on the path towards the* Cell Body *concept. A story of two dominant molecules in eukaryotic life, DNA and tubulin, interacting together and using a large battery of supporting proteins to build up the* Cell Body*—the smallest self-replicating and autonomous unit of eukaryotic life. Discoveries and concepts made using plants are highlighted in bold.*

1665:	**R. Hooke discovers cells in plants** (Harris, 1999).
1830:	J. Purkinje and G. Valentin discover cells in animals (Harris, 1999).
1831:	**R. Brown discovers the nucleus in plant cells** (Harris, 1999).
1838/1839:	**M. Schleiden and Th. Schwann annunciate a general Cell Theory** (Harris, 1999).
1871:	F. Miescher discovers nuclein, which was later identified as DNA (Harris, 1999).
1875–1882:	**W. Flemming and E. Strasburger discover chromosomes, mitosis and cytokinesis.**
1879:	**E. Tangl describes 'open communications' for direct cell-to-cell transport in plant cells, which E. Strasburger (1901) named plasmodesmata.** Recent discovery reveals also that animal cells can be linked together via direct cytoplasmic channels, which even transport organelles (Rustom *et al.*, 2004).
1888:	Th. Boveri discovers centrosomes as well as the individuality and continuity of chromosomes.
1892:	**J. Sachs, studying coenocytic algae, postulates the energid as a nuclear unit equipped with a portion of cytoplasm (the earliest version of the *Cell Body* concept applied to coenocytes).**
1893:	**E. Strasburger postulates the karyoplasmic ratio (now known as the nucleo-cytoplasmic ratio) in which a nucleus claims a certain amount of surrounding cytoplasmic space by means of an unspecified influence. This 'sphere of influence' is under the control of the nucleus (the earliest version of the *Cell Body* concept applied generally to multicellular organisms).**
1901:	Th. Boveri discovers the cyclical nature of centrosomes and their link to chromosomes as units of heredity (chromosomal theory of heredity).
1905/1910:	**C. Mereshkowsky proposes that the nucleus was the first endosymbiont of eukaryotic cells.**
1950:	**H. Swift defines DNA amounts of haploid (1C-value) and diploid (2C-value) nuclei.**
1952:	D. Mazia and K. Dan isolate intact mitotic spindle apparatus.
1953:	**A. Howard and S. Pelc report that DNA synthesis is restricted to the discrete period of interphase, later known as S-phase, and thus lay the basis for the cell cycle concept.**
1953:	J. Watson and F. Crick discover the double-helix structure of DNA molecules, which allows DNA to self-replicate and to serve as a template which instructs the formation of RNA molecules.
1963:	**M. C. Ledbetter and K. Porter, as well as D. B. Slautterback, discover microtubules in plants using electron microscopy and propose that they form mitotic spindles.**
1968:	R. Weisenberg, G. Borisy, and E. Taylor discover tubulin, a protein from which microtubules are formed.
1968:	**O. Kiermayer reports that perinuclear microtubules position nuclei in differentiating cells of the desmid, *Micrasterias denticulata*.**
1969:	**J. Pickett-Heaps proposes the concept of Microtubule Organizing Centre (MTOC), according to which microtubules and their orientation are seeded by a structural template.**
1971:	**C. L. F. Woodcock shows that coenocytic nuclei of *Acetabularia* caps are positioned by perinuclear microtubules.**
1972:	R. Weisenberg succeeds in polymerizing microtubules from tubulin *in vitro*.
1972:	**M. Bennett postulates the *Nucleotype Concept*, according to which DNA, irrespective of its informational content and role in heredity, determines the size of the cell.**
1984:	T. Mitchison and M. Kirschner discover the dynamic instability of microtubules.
1987:	D. Mazia proposes that chromosome- and centrosome-based cycles of mitotic cells are closely associated and mutually interdependent.
1987:	J. M. Vasiliev proposes that the eukaryotic cell consists of two co-operating and competing systems: an actin-based cell periphery called *actinoplast*, and a tubulin-based *tubuloplast*. The cell is viewed as some kind of symbiotic association between these two types of cytoplasmic organization.
1992:	**R. M. Brown and B. E. Lemmon study the development of female gametophytes of higher plants and postulate the *Cytoplasmic Domain* as a cytoplasmic space controlled by a given nucleus via radiating arrays of microtubules from which new plasma membranes may be formed at the line of interaction between neighbouring arrays of microtubules which are also radiating from the surfaces of adjacent nuclei. This domain corresponds to the 'sphere of influence' postulated by Strasburger in 1893 (see above).**
1993:	**A.-M. Lambert and K. Mizuno postulate and show that the whole nuclear surface acts as a MTOC in plant cells. K. Mizuno identifies proteinaceous nuclear factors essential for the MTOC nature of nuclear surface. Also, radiating arrays of microtubules are formed around small purified nuclear particles.**
1994:	D. Mazia postulates the *Cell Body* concept for animal cells.
1997:	**F. Baluška, D. Volkmann and P. W. Barlow review the many nuclear, DNA- and chromatin-associated nuclear proteins which stimulate polymerization of microtubules when released into the cytoplasm. Their controlled release into the cytoplasm during interphase, and their bulk release during mitosis, is proposed to regulate the distribution of microtubules. *Cell Body* is postulated to underlie the nucleo-cytoplasmic ratio.**
2003:	**J. Chan, G. Calder, J. H. Doonan and C. W. Lloyd show that EB1 marks the elusive MTOCs of plant cells as mobile sites, supporting the Daniel Mazia's idea of a flexible plant centrosome.**
2004:	**F. Baluška, D. Volkmann and P. W. Barlow postulate that the *Cell Body* represents the smallest autonomous unit of eukaryotic life, and that it can be structurally uncoupled from the *Cell Periphery Apparatus* (cell boundary) and functionally uncoupled from cytokinesis.**