enhanced by mutations in the plasma membrane H^+-ATPase. *J. Biol. Chem.* 272, 26145–26152
37 Gaxiola, R.A. *et al.* (1999) The *Arabidopsis thaliana* proton transporters, *AtNhx1* and *Avp1*, can function in cation detoxification in yeast. *Proc. Natl. Acad. Sci. U. S. A.* 96, 1480–1485
38 Garrett-Engele, P. *et al.* (1995) Calcineurin, the Ca^{2+}/calmodulin-dependent protein phosphatase, is essential in yeast mutants with cell integrity defects and in mutants that lack a functional vacuolar H^+-ATPase. *Mol. Cell. Biol.* 15, 4103–4114
39 Schumacher, K. *et al.* (1999) The *Arabidopsis det3* mutant reveals a central role for the vacuolar H^+-ATPase in plant growth and development. *Genes Dev.* 13, 3259–3270
40 Allen, G.J. *et al.* (2000) Alteration of stimulus-specific guard cell calcium oscillations and stomatal closing in *Arabidopsis det3* mutant. *Science* 289, 2338–2342
41 Gogarten, P. *et al.* (1992) The use of antisense mRNA to inhibit the tonoplast H^+ ATPase in carrot. *Plant Cell* 4, 851–864
42 Wang, R. *et al.* (2000) Genomic analysis of a nutrient response in *Arabidopsis* reveals diverse expression patterns and novel metabolic and potential regulatory genes induced by nitrate. *Plant Cell* 12, 1491–1509

Motile plant cell body: a 'bug' within a 'cage'

František Baluška, Dieter Volkmann and Peter W. Barlow

Analysis of the cytoskeleton in morphogenetically active plant cells allows us to propose a unified concept for the structural organization of eukaryotic cells. Their cytoarchitecture is determined by two principal structural complexes: nucleus–microtubule-based cell bodies ('bugs') and plasma-membrane–F-actin-based cell periphery complexes ('cages'). There are dynamic interactions between each of these entities in response to extracellular and intracellular signals. In the case of the cell body, these signals determine its polarization, rotation and migration. Interactions between cell body and cell periphery complexes determine cell growth polarity and morphogenesis throughout the eukaryotic kingdom.

František Baluška* **Dieter Volkmann**
Institute of Botany, Rheinische Friedrich-Wilhelms University of Bonn, Dept Plant Cell Biology, Kirschallee 1, D-53115 Bonn, Germany.
*e-mail: baluska@uni-bonn.de

Peter W. Barlow
IACR – Long Ashton Research Station, Dept Agricultural Sciences, University of Bristol, Long Ashton, Bristol, UK BS41 9AF.

In spite of the enormous amount of data accumulated during the past few decades indicating a high level of cytoskeletal organization in eukaryotic cells, there is still a tendency within mainstream cell biology to describe these cells as though they were no more than random macromolecular assemblages, where diffusion is the principal means of creating conditions that enable a cell to change its state. However, it is becoming increasingly clear that besides genomic information, there is another source of information that resides in almost every cellular structure. This epigenomic structural information is required to drive development through various levels of complexity[1–4].

Our current knowledge of the genome (the sum of all DNA molecules organized in the form of complementary nucleotide base pairs) and the genetic basis (RNAs) of eukaryotic cell construction (both components here comprise genomics) is the result of revolutionary technological progress during the latter part of the past century. By contrast, the actual level of understanding of the structural organization of eukaryotic cells seems 'frozen' somewhere at the beginning of that century. This discrepancy is evident, even taking into account the discovery of self-organizing cytoskeletal elements: microtubules (MTs) in the 1960s, and actin filaments (AFs) in the 1970s. These cellular elements were only dimly perceived before these dates, but we know now that they represent major structural components of all eukaryotic cells. In contrast with the well characterized macromolecular structural elements of the cytoplasm, analogous structures that mechanically support and bring order to the interior of the nucleus (nuclear matrix) are highly elusive. After half a century of extensive research, it is possible to say only that the nuclear matrix is built up from complex heteropolymers, including ribonucleoproteins[4].

Structurally, the cytoskeleton is known to be composed of relatively simple polarized, proteinaceous homopolymers built of tubulin (the MTs) and actin (the AFs). In this review, we consider the cytoskeleton from phylogenetic and functional viewpoints. Besides considering its well known roles in both vectorial intracellular motility and the support of the dynamic cytoarchitecture of eukaryotic cells, we shall present the view that the properties of all known cytoskeletal elements are integrated in the form of a coherent superstructure, which pervades the whole cytoplasm. Membranous elements, as well as the extracellular matrix (or cellular exoskeleton), are also important components in defining the limits of this integrated superstructure. At present, the absence of a satisfactory conceptual basis for cell structure means that there is no way of making sense of much of the current avalanche of bewildering biochemical and genetic advances. Our aim is to put forward general principles of the structural organization of eukaryotic cells that should allow present and future genetic and biochemical findings to be incorporated within a coherent structural concept of the eukaryotic cell.

A new structural view of the eukaryotic cell: cell body and cell periphery complex

A primary endosymbiotic event between a Gram-negative eubacteria (host) and a prokaryotic eocyte (guest) is thought to have given rise to the eukaryotic cell, whose interior was then subdivided into a host-derived cytoplasm (plus cell periphery) and a guest-derived nucleus[5,6]. The additional acquisitions of

 1360-1385/01/$ – see front matter PII: S1360-1385(00)01862-8

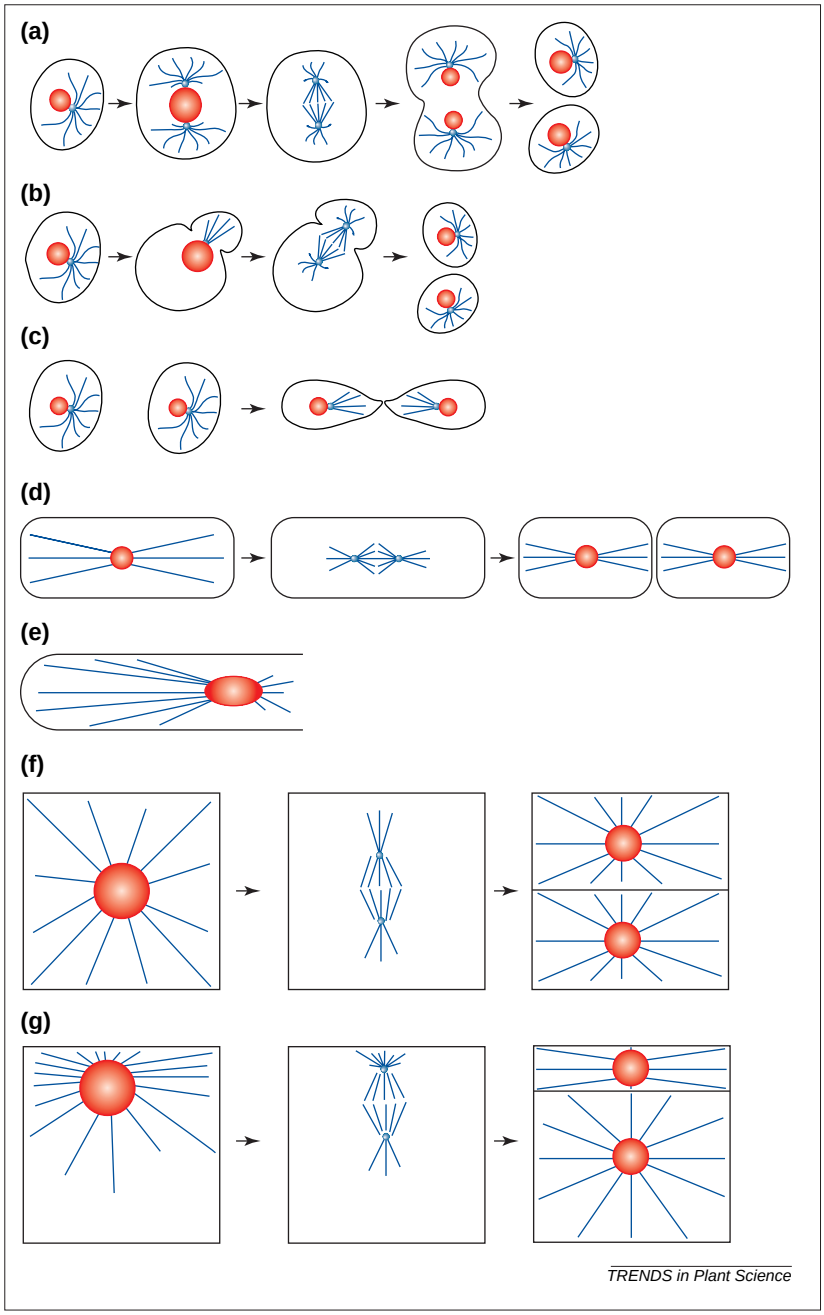

Fig. 1. Cell bodies (red–blue 'bugs' with red nuclei and blue microtubules) enclosed by cell periphery complexes (black envelope-like 'cages') of diverse eukaryotic cell types. (a) Dividing animal cells (amoeba cells would also be similar). (b) Dividing cells of budding yeast. (c) Mating cells of budding yeast. (d) Dividing cells of fission yeast. (e) Tip-growing plant cells. (f) Symmetrically dividing plant cells (having their pre-mitotic nuclei settled at the geometrical cell centre, also Fig. 3). (g) Asymmetrically dividing plant cells (having their pre-mitotic nuclei settled at the cytoskeletal cell centre, see also Fig. 3). The actin cytoskeleton (not shown here), accumulates at cell periphery domains, which attract (polarize) cell bodies in (b) (buds of budding yeasts), (c) (mating projections of budding yeasts), (d) (in fission yeasts, two opposite actin domains hold their cell bodies within the geometrical cell centre), (e) (tip-growing plant cells such as root hairs and pollen tubes) and (g) (specialized higher plant cells, such as dividing pollen and developing stomata).

symbiotic plastids and mitochondria are well accepted[5] and their partial ingestion by the host might account for the double membranes of these organelles. The cytoskeleton co-evolved within emerging eukaryotic cells, their actin-based cytoskeleton becoming closely associated with a plasma membrane[7] and their tubulin-based cytoskeleton intimately associating with the nucleus[8]. In cells of all multicellular eukaryotes, MTs radiate from sites close to the nuclear surface (Fig. 1). In animal cells, these associations include the perinuclear MT-organizing centres (MTOCs), also known as centrosomes[9,10], whereas in other eukaryotic cells MTOCs are associated with the nuclear envelope[8,11,12]. Soon after cytokinesis within a higher plant cell, many of the perinuclear MTs migrate to the cellular cortex[13,14], where they adopt the form of cortical MTs, or tubulin-based cell periphery apparatus[15,16]. Cortical MTs represent the secondary arrangement of the MTs of plant cells[16] and are responsible for the spatial ordering of nascent cellulosic microfibrils. Although typical plant cells contain most of their MTs at cellular peripheries, this seems to be only a secondary feature (reviewed in Ref. 16). Morphogenetically active plant cells assemble abundant endoplasmic MTs around nuclei, which allows nuclear centering (e.g. pre-mitosis, mitosis, cytokinesis and tip growth).

The evolution of the eukaryotic nucleus has been tightly coupled to that of the tubulin-based cytoskeleton[8]. The current status of this co-evolution is a close structural association between the nucleus and MTs during interphase, which becomes even more prominent during mitosis when all cellular MTs are organized in the form of the mitotic spindle (Fig. 1). This reproductive form of the cell body drives the segregation of genomic information (organized in the form of mitotic chromosomes) and, in addition, efficiently partitions the population of tubulin molecules. In fact, mitosis is one of the most conserved cell body-based activities and is performed by all eukaryotic cells in an identical manner[17]. Centrosomes and chromosomes both perform independent structural cycles during the cell cycle[18,19], and these cycles are interdependent with respect to nuclear functional impacts[8]. This points to an example of the close interaction between genome and structure.

Inspired by work published by Daniel Mazia[19,20], we recently proposed that walled plant cells are composed of two primary structural entities: cell bodies ('bugs'), represented by the nucleus and radiating perinuclear MTs (Ref. 12), and cell periphery complexes ('cages') composed of actin-based plasma membrane domains[16]. The terms 'bugs' and 'cages' also reflect the symbiotic origin of the eukaryotic cells mentioned earlier. One of the most crucial nuclear properties, central to the very concept of the cell body, is that numerous molecules are stored within the interphase nucleus which, when released into the cytoplasm, control the organization of MTs (Ref. 8). For example, GTP-bound Ran, a Ras-like nuclear small GTPase, was recently identified as a nucleus-based molecule crucial for MT polymerization in the form of radiating mitotic arrays[21]. It is from this molecule that the MT-based cytoskeleton acquires its primary form during mitosis.

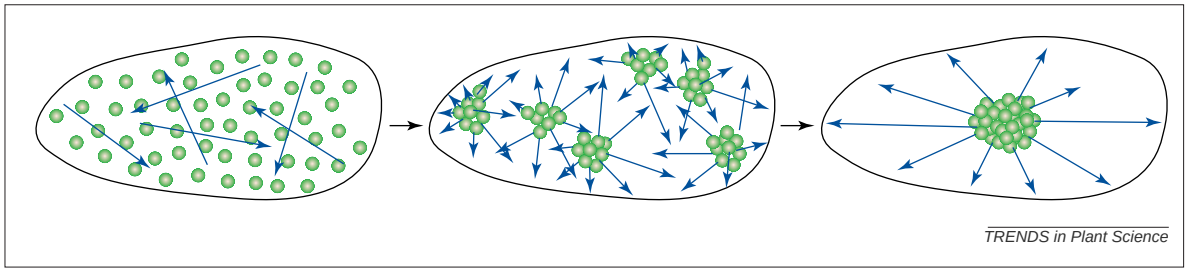

Fig. 2. Self-centering microtubules in cytoplasmic fragments (devoid of nuclei and centrosomes) of fish melanophores. After their isolation, pigment granules and microtubules were distributed homogeneously throughout these cytoplasmic fragments. With time, several microtubular asters formed that progressively transformed into one large aster positioned exactly at the geometrical centre of the cytoplasmic fragments. *Adapted from Ref. 25.*

Motility is one fundamental feature of cell bodies in all eukaryotic cells that can be expressed during both interphase and mitosis. Numerous examples of cell-body-related motilities have been shown in yeast and animal cells[9,22,23]. Nevertheless, this phenomenon is particularly intriguing in the case of walled plant cells as they too have an MT-based cell body, yet are generally regarded as immobile. There are plenty of examples where cells of both gametophyte and sporophyte plant generations are motile. Two examples from each respective generation are pollen tubes and root hairs. Other motile plant cells are present in secondary vascular tissues (fibres), primary root cortex (trichosclereids and the cells of some aerenchyma). According to the cell body concept, one can view plant cellular life from another perspective: the stiff cellulosic 'cage' of the cell exterior imprisons the active cell body ('bug') and thus a sessile mode of life prevails, which in turn permits plants to serve as ready food for mobile animal cells.

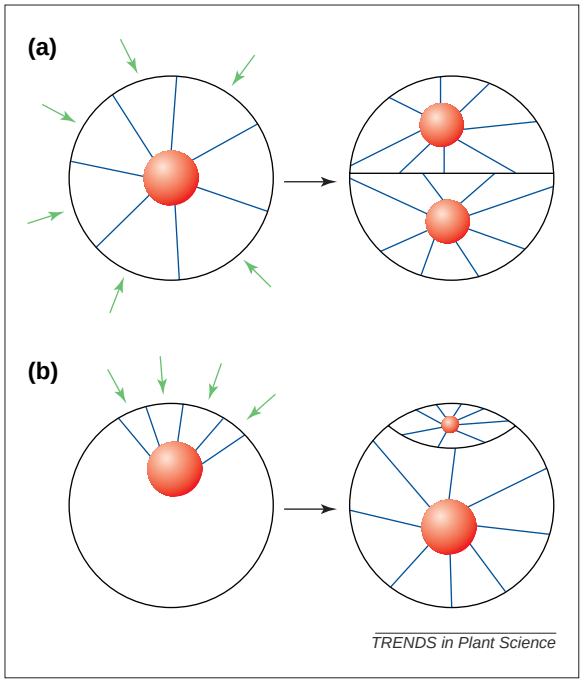

Fig. 3. Premitotic cell body positioning determines cellular fate. (a) Cell body at the geometrical cell centre leads to symmetrical division producing two daughter cells with similar fates (most cell divisions in apical meristems of plant organs). (b) Cell body at the cytoskeletal cell centre leads to two different daughter cells with contrasting cellular fates. Such asymmetrical divisions are typical for morphogenetically specialized plant cells, such as dividing pollen grains[46,48], zygotes[47] and developing stomata[45]. Peripheral F-actin (green arrows) accumulates at domains that attract cell bodies.

Nucleus-associated self-centering microtubules are at the 'heart' of cell body positioning

Besides their strong inherent association with nuclei[8], polymerizing MTs display a unique self-centering activity *in vitro*[24,25] (Fig. 2). To give a simple example of this activity, radiating MTs (asters), assembled *de novo* within artificial microchambers from purified centrosomes, will perform self-centering within their microchambers[26]. Obviously, the process of MT polymerization provides intracellular forces[27] powerful enough to push the whole aster into the geometrical centre of a microchamber. This behaviour is sufficient to indicate that some type of cytoarchitectural self-organization of eukaryotic cells could be based on inherent behaviour of tubulin proteins.

Geometrical versus cytoskeletal cell centre

In the case of cell body positioning, MT-derived forces impinge upon whole MTOC-nucleus complexes and move nuclei to an intracellular site of equilibrium[22]. We call this equilibrium site the 'cytoskeletal centre': it can, if the forces are isotropic, coincide with the geometrical centre of the cell (Fig. 3a). Here, homogeneous distribution of the cell periphery F-actin plays a crucial role. The self-centering process depends not only upon dynamic instabilities of the MTs at their growing (plus) ends and their physical relationship with the cell periphery complex, but also requires that the resultant pushing action is transmitted to the non-growing (minus) ends of the MTs at the points where they embed into the perinuclear MTOC. Numerous observations have been published (reviewed in Ref. 27) that indicate MT polymerization can generate forces enabling MTs to push against moveable cellular objects.

There are many examples where cell bodies actively shift away from the cell's geometrical centre (Fig. 3b). In all these situations, the cell bodies move towards an actin-based cell periphery domain. Intriguingly, the processes underpinning the movement of active cell bodies appear to include the

selective stabilization of their MTs (Ref. 28) at these F-actin enriched peripheral domains. It is within these domains that there is a force-generating response to the presence of MTs, which leads to the repositioning of the cell body. MT-based self-centering towards the geometrical cell centre occurs when cortical F-actin is homogeneously distributed around the plasma membrane. Conversely, accumulation of F-actin at distinct peripheral domains attracts active cell bodies and then maintains them at the cytoskeletal cell centre. New data from studies on budding yeast suggest that the interplay between MT-based cell bodies and the actin-enriched cell periphery domains also involves pulling forces[29]. Specifically, Kar9p associates in an actin-dependent fashion[30] with the plasma membrane and captures cell body MTs that are shortened using motor protein Kip3p (Ref. 31). Pulling forces exerted by the Kip3p move the nucleus towards the Kar9p anchorage site into the bud of mitotic budding yeast cells[29,31,32]. Indeed, it is possible to explain all the motile behaviours of plant cell bodies in terms of their displacements, accomplished via a combination of both pulling and pushing forces[33], to new cytoskeletal centres because of actin enrichment of local sites on the cell periphery[16]. In plants, these nuclear (or cell body) movements have been described as rotations and migrations of nuclei[12,16,34,35].

The motile behaviour of cell bodies might seem to be at variance with the self-centering principle, especially in the case of plant cells that grow at morphologically distinct 'tips', such as root hairs and pollen tubes. In such cases, the cell bodies leave their original intracellular locations and follow the emerging and growing tips at an approximately constant distance[12,16]. These cell bodies move from the cell's geometrical centre (Fig. 3a) towards an actin-enriched cell periphery domains (Fig. 3b). Thus, AFs and MTs act as complementary structural assemblies that maintain a balance between their respective physical actions. In this dynamic system, the actin-based cell peripheries play a leading role in providing motility, whereas the tubulin-based cell bodies use their self-centering activities (regaining the cytoskeletal centre) as a guide for positioning the nucleus appropriately within the cytoplasm.

Centering of mobile plant cell bodies

Plant cells differ from most other eukaryotic cells by the absence of perinuclear centrosomes, these being functionally replaced in plants by MTOCs located on the nuclear envelope[8,11]. It is this characteristic of MT organization that contributes to the efficient self-centering of the plant cell body (Figs 1f,g). In contrast with non-elongating and non-growing walled plant cells, which have inactive cell bodies displaced from the cell centres by turgid vacuoles[12], meristematic and tip-growing plant cells display active cell bodies. These are positioned either at the cell's geometrical centre (Fig. 1f) or, in the case of asymmetrically dividing (Fig. 1g) and tip-growing (Fig. 1e) cells, at the cytoskeletal centre in proximity to actin-enriched cell periphery domains[12,16]. In either case, abundant MTs are initiated upon, and radiate from, the nuclear surface. Thus, the plant cell bodies are positioned at either the geometrical or cytoskeletal centre according to whether the distribution of actin-enriched domains at the cell periphery is symmetrical or asymmetrical.

Pre-mitosis

As they prepare for mitosis, most plant cells position their pre-mitotic nuclei in the geometrical centre of the cell (which corresponds to the cell's cytoskeletal centre, if actin is symmetrically distributed around the cell periphery, Fig. 3a). Mitosis is accomplished by means of a centrally positioned mitotic spindle. Besides MT-based forces, actomyosin can also be implicated in the positioning of pre-mitotic plant nuclei. Clive Lloyd and Jan Traas[36] described AFs connecting spindle poles with the cell cortex domains in carrot suspension cells, which are enriched with both AFs and myosin VIII in maize root apices[37]. The centering of the spindle is particularly prominent in large vacuolate cells because their nuclei are actively moved away from the cell periphery[38]. To achieve this, two MT arrays, the phragmosome and the preprophase band, are assembled. They structurally support the repositioned pre-mitotic nuclei in locations prepared for their subsequent division[34–36,38–41]. Analogous pre-mitotic cell-body-centering occurs in lower organisms[33]. In budding yeast, pre-mitotic nuclei leave the geometrical cell centre and move towards a new cytoskeletal cell centre by means of search-and-capture interactions between cell body MTs and actin-based cell periphery domains associated with cortical sites[42,43] (Fig. 1b). By contrast, fission yeast have two opposite actin-enriched domains and have symmetrical divisions, with their pre-mitotic nuclei remaining at the cell's geometrical centre[44] (Fig. 1d). A similar situation occurs in mitotic root cells of maize where F-actin redistributes from the mitotic spindle region and accumulates at opposing cell periphery domains on the cross-walls facing the spindle poles[37] (Fig. 4).

Although the pre-prophase band of MTs is generally considered to be localized at the cell periphery, there are numerous other MTs that radiate from the nuclear surface and associate with the pre-prophase band[14]. Moreover, both assembly and disassembly of pre-prophase bands require the presence of adjacent nuclei[40,41], suggesting that the pre-mitotic nuclei directly regulate these processes, perhaps by releasing molecules that affect MT polymerization. The connection between the pre-mitotic nucleus and the cortical part of the pre-prophase band via MTs suggests that the nucleus–pre-prophase band complex is the plant-specific form of a morphogenetically active cell body[12].

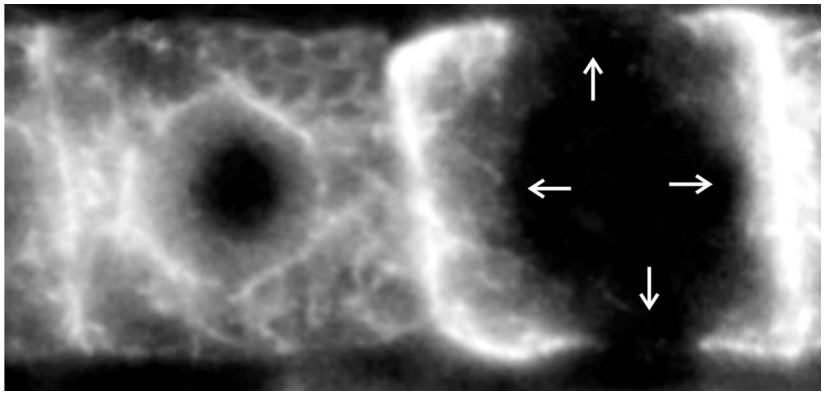

Fig. 4. Redistribution of F-actin during mitosis in maize root cells. The left-hand cell is in interphase and shows F-actin networks homogeneously extending from the nuclear surface towards the cell periphery. The right-hand cell is in mitosis and shows dramatic depletion of F-actin in the spindle region and accumulation of F-actin at cell periphery domains facing the spindle poles (horizontal arrows, indicating root axis too). Note that the cell periphery domains marked by the pre-prophase band of microtubules during the preceding G_2 phase are also depleted of F-actin (vertical arrows).

In most instances, pre-mitotic plant nuclei reside at the geometrical cell centre and, in due course, an equal division occurs. But if pre-mitotic nuclear movement takes place enabling the nucleus to move from the geometrical cell centre to reach the cytoskeletal cell centre (Figs 1g and 3b) as a result of asymmetrical F-actin deployment[45,46] at the cell periphery complex, then an unequal division would occur (Figs 1g and 3b). Important morphogenetic consequences flow from whether or not mitotic division of a given cell is asymmetrical, because this feature regulates the developmental fate of the daughter cells[45–48]. This is possibly because of distinct cytoplasmic domains in which daughter nuclei enter under the influence of the cell periphery domains. Genetically determined alterations to the timing of pre-mitotic nuclear movement, of which some of the most dramatic examples are seen in mutants affecting pollen mitosis I (Ref. 48), might reflect alterations in the timings of the developmental changes that normally affect actin distribution at the cell periphery. The morphogenetic consequences seen in these mutants might stem from alterations to cytoplasmic polarity, which go hand-in-hand with alterations to the actin distribution at the peripheral domains.

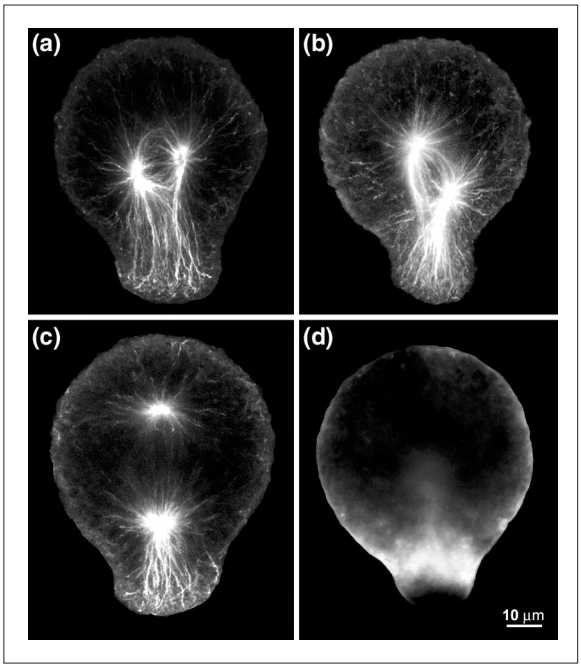

Fig. 5. Rotation of mitotic cell bodies during the first asymmetrical division of *Pelvetia compressa* zygotes. (a–c) Consecutive stages of mitotic spindle rotation visualized with labelling of microtubules with α-tubulin antibody. (d) Actin-enriched cell periphery domain, labelled with actin antibody, interacts with and attracts the leading spindle pole, which becomes larger during interactions with the actin-based domain. Photographs (a–c) were kindly provided by Sherryl Bisgrove, and photograph (d) by Whitney Hable (both at University of Utah, USA).

Mitosis

The mitotic spindle is one of the most conservative forms of cell body organization[12]. It is designed for the exact division of a parent cell body into two daughter cell bodies. In spite of the fact that diverse eukaryotic cell types assemble almost identical mitotic spindles, the respective cells at interphase can show marked differences in the distribution of their cortical and radiating MTs. Another characteristic and important feature of mitotic spindles is that, similar to other cell body forms, they are inherently motile. Mitotic spindles respond to cues issuing from the cell periphery, whereupon they display one of several motility responses. These include simple rotations around the nuclear axis and more complex migrations of whole mitotic spindles within the confines of the parent cytoplasm (resembling 'a bug within the cage'). An excellent example of mitotic plant cell body behaviour is found within the zygote of the brown alga, *Pelvetia compressa*, where the mitotic spindle rotates around its axis during mitosis in response to environmental signals[49] (Fig. 5). Other examples of cell body migrations and rotations during mitosis and cytokineses in eukaryotes have also been reviewed[34,50].

Walled plant cells assemble only a few astral MTs at the poles of their mitotic spindles, a feature that might allow the spindles to shift during metaphase. Of course, it could be that the spindles are too bulky to maintain the orientations assigned to them during pre-mitosis and that they are easily displaced by cytoplasmic motions within the dividing cell. If so, there could be mechanisms for correcting mitotic spindle position by means of interactions between the cell bodies and the actin-based cell-periphery-complex domains (Fig. 4) throughout the ensuing phases of chromosome separation and cell division.

Cytokinesis

Higher plant cytokinesis is a process based on the function of phragmoplast MTs, and in this respect differs from the modes of cytokinesis found in other eukaryotic cell types. Cell bodies remain clearly visible and active during cytokinesis[12]. A unique feature of

plant cytokinesis is that specialized cell-to-cell channels (plasmodesmata) remain open, allowing direct interactions among adjacent cell bodies via ER elements and MTs (Ref. 16). In contrast with wall-less plant cells[51], the current literature on cytokinesis of walled plant cells seems to suggest that phragmoplast MTs are not in physical contact with the surfaces of the newly separated post-mitotic sister nuclei. However, our analysis of mitosis in the cells of maize root apices strongly suggests that these MTs belong to the category of cell body-based, radiating MTs (Ref. 14).

Phragmoplast MTs are organized in the form of two interdigitating sets of MTs that radiate from the opposing surfaces of the two daughter telophase/early interphase nuclei. At this stage, the phragmoplast can be isolated from plant cells as a coherent structural unit[52]. The plane of interdigitation of cell body MTs precisely defines the border between the newborn adjacent daughter cell bodies. This phenomenon is also pertinent for cell body MTs radiating from both sister and non-sister nuclei within a common cytoplasm, as observed in coenocytic plant cells[51,53]. Whether or not a tissue or organ is multicellular or coenocytic makes little fundamental difference to the concept of the cell body. If cell body division is not accompanied by deposition of cell wall precursors within the phragmoplast, then coenocytic development occurs, but when phragmoplasts are associated with cell wall precursor deposition, this becomes the foundation for the multicellular state. A further characteristic of plant cytokinesis *sensu stricto* is that a new cell periphery domain[16] is formed *de novo* among the interdigitating cell body MTs of the phragmoplast.

The cell body administers, by means of its perinuclear MTs, a cytoplasmic domain[51] termed 'cytoplast'[54], which represents a basic unit of the cytoplasm. Coenocytes contain hundreds of such domains forming hexagonal arrays[53]. In fact, the concepts of cell body and cytoplast can be traced back to Strasburger's idea of a 'sphere of nuclear influence' (Wirkungsphäre) and also to the much-studied nucleus-to-cytoplasm ratio[55].

Tip growth

Plant cell bodies are inherently motile and confer this property upon the cytoplasts for which they are responsible. This is evident in two examples that involve tip growth, root hairs and pollen tubes. In these situations, the cell bodies not only polarize and/or rotate but they also migrate to a position behind the advancing F-actin-enriched tip of the cell[16] (Fig. 1e). Significantly, experimental disassembly of F-actin shows that this cytoskeletal element is absolutely essential for the advancement of the tip of root hairs[56] and pollen tubes. However, even though tip growth continues for a short time when all MTs are disassembled[56], MTs are needed for sustained tip growth. Root hair MTs are deployed in the form of longitudinal arrays that anchor the nucleus to the advancing tip[57], and they could serve as guides for the supply of ribosomes and mRNAs[56] to the tip. This feature seems to be relevant for continuous renovation of the tip-growth machinery at the extreme tip. In the case of the pollen tube, the migration of cell bodies (both vegetative and generative) is essential for sexual reproduction, ensuring that sperm cells are delivered to the egg cell buried deeply in female tissue.

With respect to the inherent mobility of cell bodies, nuclear migrations behind the tips of growing plant cells[16,57] closely resemble processes in migrating amoebae and the motile fibroblasts of mammalian tissues, where the centrosome–nucleus complex of the cell body follows the leading edge of the peripheral domain by means of MT-mediated connections between these two cytoskeletal units[9]. Moreover, budding yeast exposed to pheromone gradients are induced to form actin-based mating projections[58] that in turn bring about a polarization of the nuclei by means of the radiating cell-body-based MTs (Fig. 1c). These mating projections are similar to the bud sites already mentioned in that the MTs of the cell body participate in search–capture and pushing–pulling interactions with the actin-rich sites of the cell periphery[58]. In fact, the yeast mating projections could be considered to be analogous to pollen tubes in higher plants.

Conclusions

Eukaryotic cells emerged some billion years ago as a result of a key endosymbiotic event that led to the compartmentation of the emerging eukaryotic cell into nucleus (guest) and cytoplasm (host). The current evolutionary outcome of the structural specialization within this endosymbiotic cell is a motile cell body, represented by the nucleus plus its radiating MTs, enclosed within a cell periphery complex formed by the plasma membrane and its underlying actin cytoskeleton. An intrinsic property of the cell body MTs is self-centering. This process results in the active positioning of cell bodies at the geometrical cell centre. For cell body self-centering, F-actin associated with the cell periphery serves as a reference point. Cells whose peripheral F-actin is assembled asymmetrically polarize their cell bodies as these become attracted towards the F-actin-enriched cell periphery domains. During this process, cell bodies search for, and settle at, the cytoskeletal cell centre, a point of force equilibrium between the actin- and tubulin-based cytoskeletons. Recent data suggest that cell bodies not only respond to F-actin enriched cell periphery domains, but that they can also actively instruct the F-actin assembly at these domains[59].

In spite of the limitation set upon the mobility of plant cells because of the resistance of their walls to deformation and the diffuse nature of their growth, the cell bodies of dividing and tip-growing plant cells are inherently motile and perform rotations and

migrations. This is in line with the characteristics of cell bodies of all other eukaryotic cells and allows us to put forward a new structural concept valid for any eukaryotic cell: they are composed of motile cell bodies ('bugs') enclosed within flexible cell periphery complexes ('cages').

Perspectives

Our short review summarizes various aspects of a large body of evidence and reveals that the basic internal structural organization of walled plant cells corresponds well with that of other types of eukaryotic cells. This similarity enables us to propose a new unified concept for the organization of eukaryotic cells (Fig. 1). After identification and characterization of cell bodies and actin-enriched domains of cell periphery complexes, it will become crucial to identify the key components (e.g. Refs 29,32) involved in the dynamic interactions between these two structural units of cellular organization and to test predictions of this unified concept of the structural organization of the eukaryotic cell. Genetic and mutational approaches should play crucial roles in achieving these goals. Centering of cell bodies can monitor cell sizes and shapes because whenever nucleus-associated MTs hit the cell periphery, their dynamic ends switch from growth to shrinkage[60]. The cell body concept might even turn out to be useful for interpreting diverse physiological problems. For instance, the recent discovery that a circadian clock is inherently localized in the individual cells of a metazoan[61] might be explained by the unique properties of the MTs of the cell body, which can obviously perceive light pulses and transmit these directly into the nucleus[62], thereby influencing patterns of gene activity.

References

1 Albrecht-Buehler, G. (1990) In defense of non-molecular cell biology. *Int. Rev. Cytol.* 120, 191–241
2 Penman, S. (1995) Rethinking cell structure. *Proc. Natl. Acad. Sci. U. S. A.* 92, 5251–5257
3 Barlow, P.W. (1998) Gravity and developmental plasticity. *Adv. Space Res.* 21, 1097–1102
4 Pederson, T. (2000) Half a century of 'the nuclear matrix'. *Mol. Biol. Cell* 11, 799–805
5 Gupta, R.S. and Golding, G.B. (1996) The origin of the eukaryotic cell. *Trends Biochem. Sci.* 21, 166–171
6 Lake, J.A. and Rivera, M.C. (1994) Was the nucleus the first endosymbiont? *Proc. Natl. Acad. Sci. U. S. A.* 91, 2880–2881
7 Mitchison, T.J. (1995) Evolution of a dynamic cytoskeleton. *Philos. Trans. R. Soc. London Ser. B* 349, 299–304
8 Baluška, F. *et al.* (1997) Nuclear components with microtubule organizing properties in multicellular eukaryotes: functional and evolutionary considerations. *Int. Rev. Cytol.* 175, 91–135
9 Schliwa, M. *et al.* (2000) Centrosomes, microtubules and cell migration. *Biochem. Soc. Symp.* 65, 223–231
10 Schliwa, M. (1992) Cell polarity and centrosomes. In *The Centrosome* (Kalnins, V.I., ed.), pp. 331–351, Academic Press
11 Lambert, A-M. (1995) Microtubule-organizing centers in higher plants: evolving concepts. *Bot. Acta* 108, 535–537
12 Baluška, F. *et al.* (1998) The plant cell body: a cytoskeletal tool for cellular development and morphogenesis. *Protoplasma* 202, 1–10
13 Nagata, Y. *et al.* (1994) The origin and organization of cortical microtubules during the transition between M and G1 phases of the cell cycle as observed in highly synchronized cells of tobacco BY-2. *Planta* 193, 567–572
14 Baluška, F. *et al.* (1996) Symmetric reorganizations of radiating microtubules around pre- and post-mitotic nuclei of dividing cells organized within intact root meristems. *J. Plant Physiol.* 149, 119–128
15 Barlow, P.W. and Parker, J.S. (1996) Cytoskeleton and root morphogenesis. *Plant Soil* 187, 23–36
16 Baluška, F. *et al.* (2000) Actin-based domains of the 'cell periphery complex' and their associations with polarized 'cell bodies' in higher plants. *Plant Biol.* 2, 253–267
17 Schrader, F. (1953) *Mitosis. The Movement of Chromosomes in Cell Division*, Columbia University Press
18 Barlow, P.W. (1978) The interrelationship of the cycles of chromosome condensation and reduplication in cell growth processes. *Nucleus* 21, 1–11
19 Mazia, D. (1987) The chromosome cycle and the centrosome cycle in the mitotic cycle. *Int. Rev. Cytol.* 100, 49–92
20 Mazia, D. (1993) The cell cycle at the cellular level. *Eur. J. Cell Biol.* 61, 14
21 Ohba, T. *et al.* (1999) Self-organization of microtubule asters induced in *Xenopus* egg extracts by GTP-bound Ran. *Science* 284, 1356–1358
22 Reinsch, S. and Gönczy, P. (1998) Mechanisms of nuclear positioning. *J. Cell Sci.* 111, 2283–2295
23 Goode, B.L. *et al.* (2000) Functional cooperation between the microtubule and actin cytoskeletons. *Curr. Opin. Cell Biol.* 12, 63–71
24 Rodionov, V.I. and Borisy, G.G. (1997) Self-centering activity of cytoplasm. *Nature* 386, 170–173
25 Borisy, G.G. and Rodionov, V.I. (1999) Lessons from the melanophore. *FASEB J.* 13 (Suppl.), S221–S224
26 Holy, T.E. *et al.* (1997) Assembly and positioning of microtubule asters in microfabricated chambers. *Proc. Natl. Acad. Sci. U. S. A.* 94, 6228–6231
27 Inoue, S. and Salmon, E.D. (1995) Force generation by microtubule assembly/disassembly in mitosis and related movements. *Mol. Biol. Cell* 6, 1619–1640
28 Gundersen, G.G. and Bulinski, J.C. (1988) Selective stabilization of microtubules oriented toward the direction of cell migration. *Proc. Natl. Acad. Sci. U. S. A.* 85, 5946–5950
29 Beach, D.L. *et al.* (2000) The role of the proteins Kar9 and Myo2 in orienting the mitotic spindle of budding yeast. *Curr. Biol.* 10, 1497–1506
30 Miller, R. *et al.* (1999) The cortical localization of the microtubule orientation protein, Kar9p, is dependent upon actin and proteins required for polarization. *J. Cell Biol.* 144, 963–975
31 Yeh, E. *et al.* (2000) Dynamic positioning of mitotic spindles in yeast: role of microtubule motors and cortical determinants. *Mol. Biol. Cell* 11, 1–13
32 Bloom, K. (2000) It's a kar9ochore to capture microtubules. *Nat. Cell Biol.* 2, E96–E98
33 Grolig, F. (1998) Nuclear centering in *Spirogyra*: force integration by microfilaments along microtubules. *Planta* 204, 54–63
34 Gunning, B.E.S. (1982) The cytokinetic apparatus: its development and spatial regulation. In *The Cytoskeleton in Plant Growth and Development* (Lloyd, C.W., ed.), pp. 229–292, Academic Press
35 Gunning, B.E.S. and Wick, S.M. (1985) Preprophase bands, phragmoplasts, and spatial control of cytokinesis. *J. Cell Sci.* (Suppl.) 2, 157–179
36 Lloyd, C.W. and Traas, J.A. (1988) The role of F-actin in determining the division plane of carrot suspension cells. Drugs studies. *Development* 102, 211–221
37 Baluška, F. *et al.* (2000) Actin and myosin VIII in developing root cells. In *Actin: A Dynamic Framework for Multiple Plant Cell Functions* (Staiger, C.J. *et al.*, eds), pp. 457–476, Kluwer Academic Publishers
38 Flanders, D.J. *et al.* (1990) Nucleus-associated microtubules help determine the division plane of plant epidermal cells: avoidance of four-way junctions and the role of cell geometry. *J. Cell Biol.* 110, 1111–1122
39 Katsuta, J. *et al.* (1990) The role of the cytoskeleton in positioning of the nucleus in premitotic tobacco BY-2 cells. *J. Cell Sci.* 95, 413–422
40 Mineyuki, Y. (1999) The preprophase band of microtubules: its function as a cytokinetic apparatus in higher plants. *Int. Rev. Cytol.* 187, 1–49
41 Murata, T. (2000) Preprophase band in fern protonemata: mechanism of development and comparison with flowering plants. *J. Plant Res.* 113, 111–118
42 Adames, N.R. and Cooper, J.A. (2000) Microtubule interactions with the cell cortex causing nuclear movements in *Saccharomyces cerevisiae*. *J. Cell Biol.* 149, 863–874
43 Lee, L. *et al.* (2000) Positioning of the mitotic spindle by a cortical-microtubule capture mechanism. *Science* 287, 2260–2262
44 Chang, F. and Nurse, P. (1996) How fission yeast fission in the middle. *Cell* 84, 191–194
45 Cleary, A.L. (2000) Actin in formation of stomatal

complexes. In *Actin: A Dynamic Framework for Multiple Plant Cell Functions* (Staiger, C.J. *et al.*, eds), pp. 411–426, Kluwer Academic Publishers
46 Zonia, L. (2000) The actin cytoskeleton during differentiation of microspores to mature pollen. In *Actin: A Dynamic Framework for Multiple Plant Cell Functions* (Staiger, C.J. *et al.*, eds), pp. 361–371, Kluwer Academic Publishers
47 Jürgens, G. *et al.* (1997) Establishment of cell polarity during early plant development. *Curr. Opin. Cell Biol.* 9, 849–852
48 Twell, D. *et al.* (1998) Asymmetric division and cell-fate determination in developing pollen. *Trends Plant Sci.* 3, 305–310
49 Bisgrove, S.R. and Kropf, D.L. (1998) Alignment of centrosomal and growth axes is a late event during polarization of *Pelvetia compressa* zygotes. *Dev. Biol.* 194, 246–256
50 Rhyu, M.S. and Knoblich, J.A. (1995) Spindle orientation and asymmetric cell fate. *Cell* 82, 523–526
51 Brown, R.C. and Lemmon, B.E. (1992) Cytoplasmic domain: a model for spatial control of cytokinesis in reproductive cells of plants. *EMSA Bull.* 22, 48–53
52 Kakimoto, T. and Shibaoka, H. (1988) Cytoskeletal ultrastructure of phragmoplast-nuclei complexes isolated from cultured tobacco cells. *Protoplasma* (Suppl.) 2, 95–103
53 Goff, L.J. and Coleman, A.W. (1987) The solution to the cytological paradox of isomorphy. *J. Cell Biol.* 104, 739–748
54 Pickett-Heaps, J.D. *et al.* (1999) The cytoplast concept in dividing plant cells: cytoplasmic domains and the evolution of spatially organized cell division. *Am. J. Bot.* 86, 153–172
55 Trombetta, V.V. (1939) The cytonuclear ratio in developing plant cells. *Am. J. Bot.* 26, 519–529
56 Baluška, F. *et al.* (2000) Root hair formation: F-actin-dependent tip growth is initiated by local assembly of profilin-supported F-actin meshworks accumulated within expansin-enriched bulges. *Dev. Biol.* 227, 618–632
57 Lloyd, C.W. *et al.* (1987) Endoplasmic microtubules connect the advancing nucleus to the tip of legume root hairs, but F-actin is involved in basipetal migration. *Cell Motil. Cytoskel.* 8, 27–36
58 Maddox, P. *et al.* (1999) Microtubule dynamics from mating through the first zygotic division in the budding yeast *Saccharomyces cerevisiae*. *J. Cell Biol.* 144, 977–987
59 Waterman-Storer, C.M. *et al.* (1999) Microtubule growth activates Rac1 to promote lamellipodial protrusion in fibroblasts. *Nat. Cell Biol.* 1, 45–50
60 Drummond, D.R. and Cross, R.A. (2000) Dynamics of interphase microtubules in *Schizosaccharomyces pombe*. *Curr. Biol.* 10, 766–775
61 Whitmore, D. *et al.* (2000) Light acts directly on organs and cells in culture to set the vertebrate circadian clock. *Nature* 404, 87–91
62 Albrecht-Buehler, G. (1998) Altered drug resistance of microtubules in cells exposed to infrared light pulses: are microtubules the 'nerves' of cells? *Cell Motil. Cytoskel.* 40, 183–192

Significance of epidermal fusion and intercalary growth for angiosperm evolution

John A. Raven and Jonathan D.B. Weyers

The ancestral angiosperm flower probably had many separate elements in each floral whorl (sepals, petals, stamens and carpels). Derived character states include 'fusion' of elements within a whorl (cohesion) and fusion between whorls (adhesion), as well as epigyny and the emergence of the other floral elements from the apex of the fused carpels. This article considers the roles of epidermal fusion and intercalary growth in the phylogeny and ontogeny of fused floral elements, and the importance of fusion for angiosperm evolution.

John A. Raven*
Jonathan D.B. Weyers
Division of Environmental and Applied Biology, School of Life Sciences, University of Dundee, Dundee, UK DD1 4HN.
*e-mail: j.a.raven@dundee.ac.uk

In a recent article, Neelima Sinha[1] discusses the importance in plant biology of the responses of epidermal cells to contact. Here we extend the arguments of Sinha[1], and put them in an evolutionary context, following Robert Kuzoff and Charles Gasser[2], Todd Barkman *et al.*[3] and William Crepet[4].

Sinha[1] points out that epidermal adhesion and fusion are essentially confined to higher plants. As an example of the importance of epidermal contact in sexual reproduction, Sinha[1] uses the fusion of floral organs within a whorl, which occurs relatively late in floral development. Such ontogenetic fusions give rise to some examples of corolla (petal) or calyx (sepal) tubes. These ontogenetic fusions also give rise to some examples of syncarpy[1], with fusion of the ovaries, and usually also fused styles and stigmas. Syncarpy can arise from appression of carpels late in development (postgenital fusion), with occlusion of intervening spaces by secretions or ('true' syncarpy) by epidermal fusion[1]. 'True' syncarpy can also involve the absence of epidermal identity in the fused regions at all stages of development (congenital fusion), and might have evolved from occlusion or fusion.

Without denying the ontogenetic significance of epidermal fusion in many cases of 'true' syncarpy, it is not certain that it was a necessary stage in the evolution of true syncarpy with congenital fusion, or other cases of congenital fusion[5]. Allometric changes in development can be responsible. Here, greater growth proximal to the point of organ separation and decreased growth distal to that point might account for the evolution of congenital fusion, with no intermediate stage of postgenital epidermal fusion[5–9]. Studies of certain mutants (*LEUNIG* and *AINTEGUMENTA*) of *Arabidopsis* have implications for congenital organ fusion; among the characteristics of these two mutants is partial separation of the carpels of the normally syncarpous, bicarpellary, gynoecium[10,11].

Implicit in Sinha's[1] analysis, and the discussion above, is that the apocarpous (unfused) state is ancestral in flowering plants. Apocarpy and syncarpy are, of course, only applicable to flowers with more than one carpel. Before molecular genetic analyses of phylogeny became available, the possession of more than one carpel per flower, and having these carpels separate rather than fused, were considered to be ancestral characters[5–9]. The ancestral nature of these characters is generally supported by molecular phylogenetic analyses[4,8,9]. Thus, of the three families that form the sister group to all other flowering

http://plants.trends.com 1360-1385/01/$ – see front matter PII: S1360-1385(00)01809-4