PERSPECTIVES

OPINION

Microtubule-organizing centres: a re-evaluation

Jens Lüders and Tim Stearns

Abstract | The number, length, distribution and polarity of microtubules are largely controlled by microtubule-organizing centres, which nucleate and anchor microtubule minus ends in a process that requires γ-tubulin. Here we discuss recent evidence indicating that γ-tubulin-dependent formation of new microtubules is not restricted to conventional microtubule-organizing centres. These findings suggest that the spatio-temporal control of microtubule nucleation is more complex than previously thought, leading us to a re-evaluation of the concept of the microtubule-organizing center.

Microtubules are tubular polymers that are composed of two proteins, α- and β-tubulin. The orientation of these proteins in the polymers gives microtubules an intrinsic polarity. Microtubules are involved in many cellular processes, including the transport of proteins and organelles, the establishment of cell polarity and the formation of the meiotic and mitotic spindles. In most cell types, microtubules form highly organized, polarized arrays; in proliferating cells, these arrays undergo cell-cycle-dependent reorganization to form the bipolar spindle in mitosis. These properties of the microtubule cytoskeleton led to the notion of an organizer that promotes the formation and the ordered arrangement of microtubules. Various terms were used to describe this hypothetical organizer until Pickett-Heaps coined the now widely accepted term microtubule-organizing centre (MTOC)[1].

The concept of an MTOC was based on microscopy studies and referred to all cellular structures that seemed to assemble and organize microtubule arrays. Initially, the nature of this material was "totally obscure" and the term 'centre' remained "deliberately vague"[1]. Two structures that could easily be visualized by electron microscopy were identified as important MTOCs, and these became a research focus in subsequent years. These structures are the centrosome in animal cells and the spindle pole body in yeast.

The tools of modern genetics and cell biology allowed the analysis of the molecular nature of these organelles and the identification of a still-growing list of proteins that are involved in their function. However, the resulting centrosome-centric view has drawn attention away from other sites in cells that also nucleate and organize microtubules.

Here, we describe the recent rediscovery of sites that nucleate and organize microtubules and discuss why the analysis of these sites might be the key to an understanding of microtubule nucleation and organization. We will focus on how, where and when microtubules are made. For a discussion of the roles of microtubule-associated proteins and motor proteins in the generation of organized microtubule arrays, we direct the reader to excellent recent reviews[2,3].

The centrosome — a central organizer

Since its description by Theodor Boveri more than 100 years ago, the centrosome has attracted special attention because of its prominent location at the centre of the microtubule cytoskeleton and because it contains one of the most recognizable structures found in eukaryotic cells, the centriole. Each of the two centrioles in a typical centrosome, oriented perpendicularly to each other, is a barrel-shaped cylinder that is composed of nine microtubule triplets arranged radially and with a precise symmetry. Centrioles were initially thought to be the microtubule-organizing structure responsible for assembling the mitotic spindle. Although this view was challenged when the concept of MTOCs was introduced[1], centrioles continue to intrigue cell biologists and are often still described as an essential component of the mitotic spindle poles.

However, many eukaryotes, including fungi and higher plants, completely lack centrioles yet still organize a bipolar mitotic spindle, clearly showing that centrioles are not fundamentally required for this function. Moreover, when centrioles are removed from animal cells by microsurgery or are destroyed by laser ablation, the resulting karyoplasts can still assemble a mitotic spindle[4,5]. Therefore, it seems likely that the "vital function of centrioles lies in their ability to become … basal bodies which produce flagella or cilia."[1] It took almost four decades

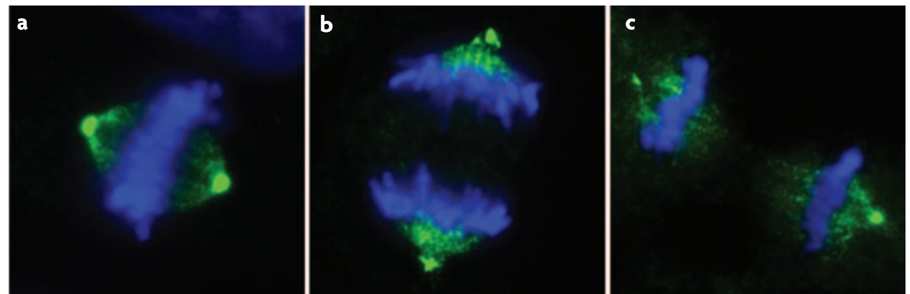


Figure 1 | **Localization of γ-tubulin in the mitotic spindle.** Human U2OS cells were fixed at different stages in mitosis. γ-tubulin (green) localizes to the two spindle poles and to the region of kinetochore microtubules during metaphase and anaphase (panels **a** and **b**, respectively). During late anaphase–early telophase (panel **c**), γ-tubulin localizes both to the region of kinetochore microtubules and to the region between the separated chromosomes. DNA is shown in blue.

© 2007 Nature Publishing Group

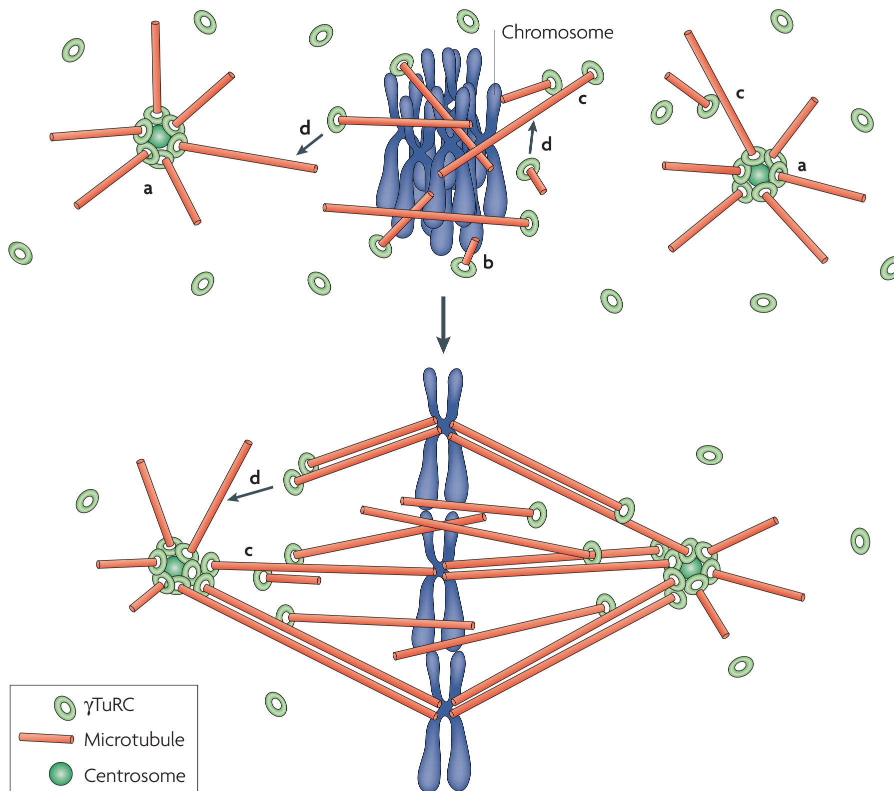


Figure 2 | **Amplification model for the function of the γ-tubulin ring complex in spindle assembly.** Microtubules are nucleated by the γ-tubulin ring complex (γTuRC) at the two centrosomes (**a**) and near the mitotic chromatin (**b**). A third nucleation pathway depends on γTuRC bound to the sides of existing spindle microtubules (**c**). This pathway amplifies the process of spindle assembly by nucleating additional microtubules, where needed, in the forming spindle. Correct orientation is achieved by directional nucleation or by motor-mediated orientation soon after nucleation. Minus-end-bound γTuRC facilitates the capture of free microtubules by directing their ends to the sides of other microtubules (**d**).

to obtain direct experimental support for this interpretation: mutant flies that lack centrioles develop into morphologically normal animals, but they suffer from defects in cilia-dependent functions[6]. If centrioles are dispensable for the function of MTOCs, what are the components that promote the formation and organization of microtubules? The answer lies in the proteinaceous material that surrounds the centriole pair, the pericentriolar material (PCM).

The microtubule-nucleating material. Many protein components of the PCM have been identified, but only a subset of these are involved in the function of the centrosome as an MTOC. One of these proteins, γ-tubulin, is the linch-pin of microtubule nucleation and is therefore a prime player in microtubule organization. γ-tubulin was first identified in *Aspergillus* and was shown to be required for the formation of microtubules at the spindle pole body[7,8]. γ-tubulin nucleates the polymerization of microtubules from α- and β-tubulin subunits, but is not itself incorporated into that polymer. γ-tubulin forms a complex with members of a conserved protein family. The human proteins are named gamma complex proteins (GCPs, numbered by increasing molecular weight, with γ-tubulin as GCP1). In budding yeast, the γ-tubulin complex contains only two GCP family members, the orthologues of human GCP2 and GCP3, whereas in most organisms γ-tubulin complexes contain additional GCP subunits. This large γ-tubulin complex is referred to as the γ-tubulin ring complex (γTuRC) because of its characteristic ring shape when viewed by electron microscopy. In cases in which it is not clear whether the full γTuRC is involved, we will use the term γ-tubulin complex. A new component of the γTuRC, Dgp71WD, was recently identified in *Drosophila melanogaster*[9]. Dgp71WD contains N-terminal WD40 repeats and is not related to the GCP family. Its orthologue in humans, GCP-WD (also known as NEDD1), was shown to be a targeting factor for the γTuRC[10,11].

Although the mechanism of nucleation is still unknown, γ-tubulin, in its several forms, is considered to be the main microtubule nucleator. γ-tubulin has pericentriolar localization, but it is also found in the centriole cylinder, consistent with it having a possible function in the nucleation of the centriole microtubules[12].

Microtubule stabilization and anchoring. In most cells, microtubules are organized such that the minus end is proximal to the centrosome and the more dynamic plus end extends away from the centrosome. To achieve this organization, the centrosome must both stabilize microtubule minus ends and anchor them at the centrosome. The γTuRC stabilizes microtubules by capping the minus end[13], thereby preventing depolymerization. Although the anchoring function could, in principle, also be fulfilled by centrosome-bound γTuRC, it apparently requires additional proteins, including ninein, which is found on specialized appendages of the centriole[14,15]. The mechanism of microtubule anchoring and the mechanism by which microtubules are released from the centrosome to form non-centrosomal microtubule arrays[16] are unknown.

Decentralized microtubule formation

The centrosome and the spindle pole body are multifunctional organelles; they control microtubule organization, but they also function as control centres that integrate signalling pathways to regulate cellular processes. This diversity of function is reflected in their associated parts lists, which include many proteins without obvious roles in microtubule organization such as known cellular regulators or large structural molecules of unknown function[17]. The diversity of function also complicates the analysis of centrosomal MTOC function, as defects potentially affect many processes. Therefore, it is useful to consider non-centrosomal MTOCs, which might lack these other functions, for insights into microtubule nucleation, anchoring and stabilization. Such sites were first described in higher plants, which do not have a centrosome-like microtubule organizer, but they have now been identified in many organisms and cell types.

Making microtubules without a central organizer. Despite the absence of a centrosome-like organelle, higher plants do have discrete, highly organized microtubule arrays. These arrays include the radial array,

© 2007 Nature Publishing Group

which is associated with the nuclear envelope, the cortical interphase microtubules, the cortical preprophase band, the spindle and the phragmoplast, which might be analogous to the midbody in animals. At least some of these structures involve MTOCs (for example, both the nuclear envelope and the cell cortex nucleate microtubules)[18,19]. γ-tubulin localizes to all of these non-centrosomal MTOCs; in the absence of functional γ-tubulin, these MTOCs either do not form or are unable to nucleate and organize microtubules, resulting in lethality early in development[20,21].

γ-tubulin in plants is not restricted to regions of microtubule minus ends, but instead seems to be present along the entire length of microtubules. Murata *et al.* recently showed that γ-tubulin in tobacco cells is recruited to the sides of cortical microtubules where it nucleates new microtubules that branch from existing ones[22]. Together with previous observations, this observation indicates that the microtubule-nucleating complexes of plants can associate with different cellular structures, including existing microtubules, to promote microtubule nucleation and organization. Such nucleating complexes might be similar in their composition to the microtubule-organizing material found in the PCM of the animal centrosome and, therefore, might constitute a flexible centrosome in plant cells, as proposed by Mazia[23].

Such plasticity of the organizing material is also observed in some animal cells. For example, during skeletal muscle differentiation, myoblasts fuse to form multinucleated myotubes. During this process, proteins that are involved in microtubule organization are redistributed from the centrosome to the nuclear envelope, which then functions as an MTOC in differentiated myotubes[24]. Pericentrin, ninein and γ-tubulin are among the redistributed proteins, and blocking γ-tubulin function by antibody injection prevents microtubule formation at the nuclear envelope[25,26].

Distributed organizing material is also present in the apical domain of multiciliated epithelial cells, in which many cilia are formed from basal bodies at the apical membrane. Each basal body is structurally similar to the centriole in centrosomes, and, like the centriole, each has appendages that are associated with γ-tubulin-containing material[27]. Although it is not known whether this material has microtubule-nucleating activity, it is associated with the ends of cytoplasmic microtubules[28].

Table 1 | **Known and potential microtubule-organizing centres and their properties**

Structure (organism)	Composition	Properties
Centrosome (animals, some lower plants); spindle pole body (yeast)	Proteinaceous matrix	γ-tubulin-dependent nucleation of microtubules; anchoring of microtubule minus ends
Basal bodies (animals)	Proteinaceous matrix	Ends of cytoplasmic microtubules bound to electron-dense material at basal foot; presence of γ-tubulin, but nucleation has not been shown
Cortical membrane (plants)	Membrane	Nucleation of microtubules depends on γ-tubulin bound to the sides of existing microtubules; anchoring of microtubules by lateral interaction
Cortical basal membrane of polarized epithelial cells (animals)	Membrane	Dynamic microtubules; presence of γ-tubulin, but nucleation has not been shown; anchoring of microtubules by lateral interaction
Centrosome and apical membrane in polarized epithelial cells (animals)	Proteinaceous matrix, membrane	γ-tubulin-dependent nucleation of microtubules at centrosome; anchoring of microtubule minus ends at apical membrane
Nuclear envelope in myotubes (animals); nuclear envelope (plants)	Membrane	γ-tubulin-dependent nucleation of microtubules; anchoring of microtubule minus ends
Golgi complex (animals)	Membrane	Nucleation and anchoring of microtubules; presence of γ-tubulin
Interphase MTOCs at nuclear envelope and along microtubules (fission yeast)	Membrane, proteins	γ-tubulin-dependent nucleation of microtubules from nuclear envelope or from existing microtubules; anchoring of microtubule minus ends
Midbody (animals); phragmoplast (plants); equatorial MTOCs (fission yeast)	Proteinaceous matrix	γ-tubulin-dependent nucleation of microtubules at cytokinesis; anchoring of microtubule minus ends
Mitotic spindle (animals)	Proteinaceous matrix	Non-centrosomal, γ-tubulin-dependent nucleation of microtubules; anchoring of microtubules by end and lateral interaction
Mitotic chromosomes (animals)	DNA, proteins	γ-tubulin-dependent nucleation of microtubules; capture and anchoring of microtubule plus ends by kinetochores

MTOC, microtubule-organizing centre.

Secondary MTOCs. Some cells that have centrosomes or their equivalents also have secondary MTOCs, which co-exist with the primary centrosome MTOC. Such secondary MTOCs are present in fission yeast[29]; γ-tubulin complexes localize to the spindle pole body as well as to interphase and equatorial MTOCs. Interphase (i)MTOCs nucleate and anchor microtubules at the nuclear surface. In mitosis, these structures disappear and microtubules are nucleated by the spindle pole body. The equatorial (e)MTOC forms at the beginning of cytokinesis in association with the contractile ring at the site of cell division. γ-tubulin-complex components also localize to, and move along, interphase microtubules[30]. There, they nucleate new microtubules, resulting in the formation of bipolar microtubule bundles[31]. However, anucleate fission yeast cells, which lack both spindle pole bodies and nuclear-associated iMTOCs, can assemble a normal interphase microtubule array[32,33]. This process depends on the γ-tubulin-complex associated protein Mto1, indicating that mobile cytoplasmic γ-tubulin complexes are involved in the nucleation of these microtubules.

The results from experiments with plants and fission yeast present an obvious question: is γ-tubulin-mediated microtubule nucleation in animal cells restricted to the centrosome, or does it also occur at secondary MTOCs? Several examples indicate that animal cells have secondary MTOCs and that they are important in microtubule organization. Polarized epithelial cells have an extensive non-centrosomal microtubule network, and few microtubules associate with the centrosome. The apico-basal array in these cells consists of parallel microtubules with their minus ends anchored at the apical membrane. In addition, these cells have a cortical microtubule network

© 2007 Nature Publishing Group

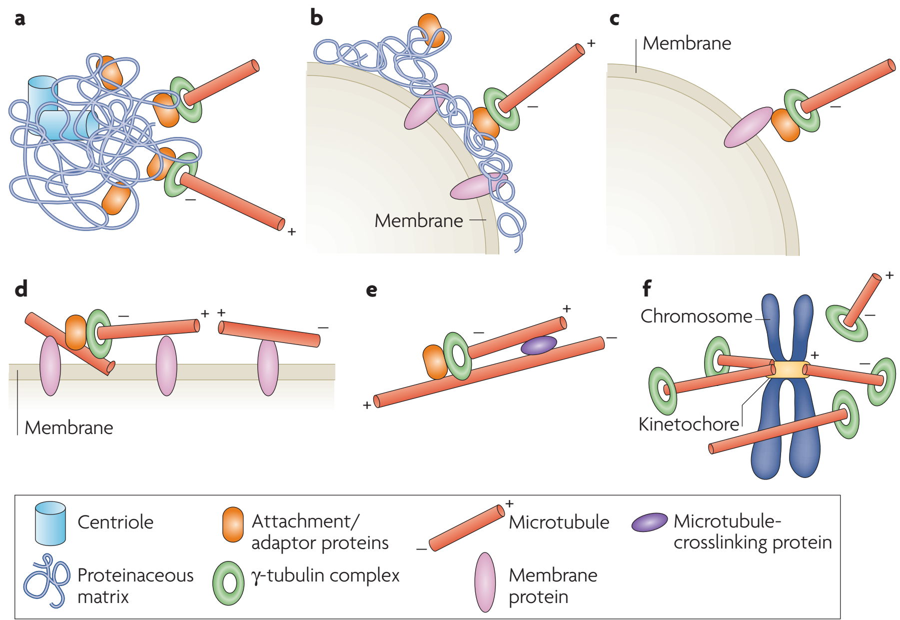


Figure 3 | **Models for the organization of γ-tubulin complexes at microtubule-organizing centres.** Microtubules are nucleated by γ-tubulin complexes (green) that are either targeted to the microtubule-organizing centre (MTOC) by attachment or adaptor factors (orange; **a**–**e**) or are free in the cytoplasm (**f**). Microtubules (red) interact with the MTOC with their minus ends (**a**–**c**,**e**), plus ends (**f**) or sides (**d**–**f**). **a** | γ-tubulin complexes are embedded in a proteinaceous matrix (grey) that interacts with attachment factors and, in the case of centrosomes, is associated with centrioles (light blue). **b** | A similar matrix might localize to the cytoplasmic surface of certain membranes by interacting with membrane proteins (for example, at the nuclear envelopes in myotubes and in plant cells). **c** | The interaction of γ-tubulin complexes with MTOCs could occur by direct interaction in the absence of other centrosomal material. Such an interaction might be sufficient to establish interphase MTOCs at the nuclear envelope in fission yeast. **d** | γ-tubulin complexes nucleate microtubules while bound to the sides of pre-existing microtubules that are laterally tethered to membranes. The new microtubules become part of the membrane-associated network. This organization has been described for the cell cortex in plants. **e** | Pre-existing microtubules can function as MTOCs. γ-tubulin complexes bind laterally and nucleate new microtubules, which become bundled by microtubule-crosslinking proteins (purple). Transport of γ-tubulin complexes along parallel microtubules contributes to microtubule organization. During interphase in fission yeast cells, this results in the formation of antiparallel microtubule bundles with overlapping minus ends. **f** | γ-tubulin-dependent nucleation of microtubules occurs near mitotic chromatin. After nucleation, microtubules are organized into bundles and their plus ends are captured by kinetochores (yellow). In addition, lateral interaction of microtubules with chromosome arms occurs.

that is associated with the basal membrane. These non-centrosomal microtubules could be derived by nucleation and release from the centrosome, by assembly in the cytoplasm independent of the centrosome or by nucleation from a secondary MTOC[16]. Evidence for the involvement of a secondary MTOC comes from work showing that adenomatous polyposis coli protein (APC) bound to the basal cortex functions as a template for microtubule-network formation[34]. Moreover, γ-tubulin localizes to the cortex on basal membrane patches, along cortical microtubules and at microtubule branch points[35]. However, γ-tubulin-dependent nucleation of microtubules at the basal cortex of polarized epithelial cells has not yet been demonstrated. Another example of a secondary MTOC is the Golgi complex, which nucleates and anchors microtubules at its cytoplasmic face[36]. Rios *et al.*[37] found that the Golgi protein GMAP210 targets the γTuRC to Golgi membranes and promotes Golgi positioning by linking Golgi membranes to microtubules. However, this result has been questioned[38] and the role of Golgi-nucleated microtubules remains unclear.

One of the characteristics of secondary MTOCs is that they are non-centrosomal sites of γ-tubulin accumulation. Where else does such accumulation occur? In mitotic animal cells, γ-tubulin is found at the two centrosomes at the spindle poles, but it also localizes to spindle microtubules outside the centrosome area. In metaphase, γ-tubulin localizes to the spindle between the chromosomes and poles; in late anaphase–early telophase, it also localizes between the separated chromosome masses[39] (FIG. 1). A common interpretation of this localization is that it reflects binding of the γTuRC to the many microtubule minus ends in the spindle[40,41]. However, the data from plants and yeast discussed above indicate that γ-tubulin can also bind laterally to microtubules. Might these spindle-associated γTuRCs be involved in spindle formation? To answer this question, we next consider how microtubules are nucleated during mitosis.

Making microtubules during mitosis

At least two independent microtubule-nucleation pathways operate in mitotic animal cells. The centrosomal nucleation pathway depends on centrosome-bound γTuRCs and promotes the separation of the two duplicated centrosomes, their migration to opposite sides of the nucleus and the formation of two microtubule asters at the beginning of mitosis. After nuclear envelope breakdown, a second nucleation pathway, which is controlled by the small GTPase Ran, becomes active[42]. A chromatin-bound nucleotide-exchange factor for Ran generates a high local concentration of RanGTP, which activates spindle-assembly factors and promotes the formation and organization of microtubules in the vicinity of the chromosomes. This pathway also requires γ-tubulin *in vitro*[43] and *in vivo*[44], as well as the targeting factor GCP-WD[10]; however, it is not clear whether the γTuRC is directly regulated by RanGTP.

Does the γTuRC that is localized to the spindle also have a role in making spindle microtubules? It was recently shown that the localization of γ-tubulin to the spindle is required for proper spindle formation and depends on mitotic phosphorylation of the γ-tubulin-targeting factor GCP-WD[10]. This indicates that a mitosis-specific activity links the γTuRC to spindle microtubules. This spindle-bound γTuRC might be involved in an amplification mechanism for spindle assembly (FIG. 2). In this model, the forming spindle itself becomes a self-reinforcing MTOC. γTuRCs bound laterally to microtubules would contribute to the formation of an ordered, bipolar array by nucleating additional microtubules along existing microtubules, ensuring that new microtubules are made where needed. These microtubules might be oriented correctly either by oriented nucleation from the γTuRC, or by the action of motor proteins soon after nucleation. This model is supported by the aforementioned data from fission yeast

© 2007 Nature Publishing Group

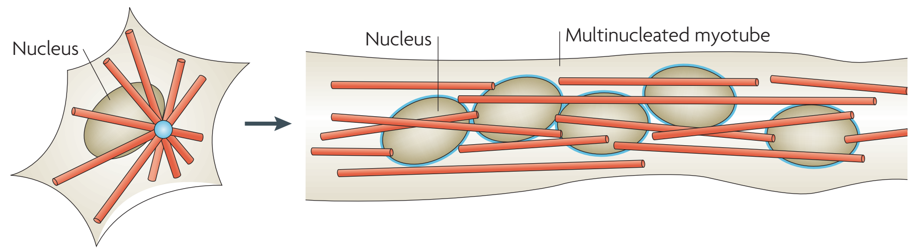
in which such a mechanism promotes the formation of microtubule bundles during interphase. In addition, γTuRCs on the minus ends of chromatin-derived microtubules might facilitate the incorporation of those microtubules into the forming spindle by lateral interaction with existing microtubules. Such capture of free minus ends of kinetochore-bound microtubules by astral microtubules of the centrosome during spindle formation was recently observed[45,46]. Moreover, most of the minus ends of interpolar microtubules in spindles of Ptk1 cells terminate in the bundles of kinetochore microtubules[40], consistent with the possibility that they interact laterally.

Does microtubule nucleation occur in the mitotic spindle? Mahoney *et al.*[44] visualized microtubule growth in mitotic spindles of *D. melanogaster* cells using end binding-1 (EB1)–green fluorescent protein (GFP), which labels growing microtubule plus ends. They found many microtubules were growing from the spindle poles towards the metaphase plate even when centrosomes were detached from the spindle by RNA interference (RNAi) depletion of the centrosome protein centrosomin. Although the growth of microtubule plus ends does not directly indicate nucleation of new microtubules, the data are consistent with the mitotic spindle functioning, independently of the established centrosomal and chromatin-mediated nucleation pathways, as a microtubule-nucleating structure.

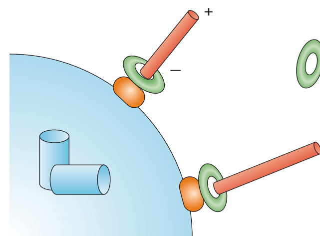
Redefining MTOCs

Since the discovery of γ-tubulin as a microtubule-nucleating component of the centrosome and the yeast spindle pole body, these two organelles have been studied intensively in the hope of better understanding microtubule organization. The focusing of research efforts on these organelles has largely been at the expense of other organizing structures. As a result, the term MTOC is often used synonymously with centrosome. However, many if not all of the non-centrosomal microtubule-nucleation sites described above seem to have at least some properties of MTOCs as originally defined by Pickett-Heaps[1] (TABLE 1). We propose a redefinition of the term MTOC that takes into account the original, broader definition and includes a modern understanding of microtubule nucleation and organization. We propose that MTOCs are plastic, often transient, structures that can catalyse γ-tubulin-dependent microtubule nucleation and that can anchor microtubules by interacting with their minus ends, plus ends or sides (FIG. 3).

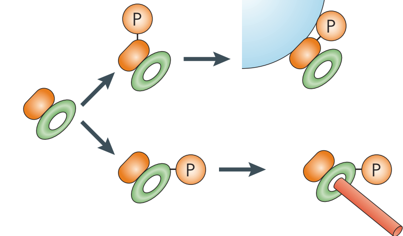
a Morphology and distribution of MTOCs



b Targeting/activation factors



c Covalent modification



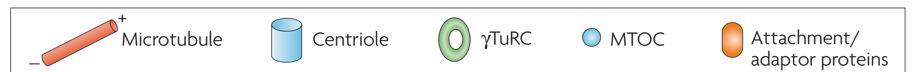


Figure 4 | **Regulation of microtubule nucleation. a** | Morphology, number and distribution of microtubule-organizing centres (MTOCs; blue) control the organization of the associated microtubule array (red). This is illustrated by comparing a single MTOC, the centrosome, at the centre of a radial microtubule array in an interphase cell (left) with multiple MTOCs, the nuclear envelopes, embedded in a dense array of parallel microtubules in differentiated, multinucleated myotubes (right). **b** | γ-tubulin ring complex (γTuRC)-interacting proteins (that function as attachment or adaptor proteins; orange) control the localization and/or activity of the γTuRC (green). In the example shown, this regulation results in spatially restricted nucleation of microtubules at the centrosome. **c** | Post-translational modification of a γTuRC-interacting protein, for example by phosphorylation (P), controls the localization of the γTuRC to an MTOC (top). Phosphorylation of the γTuRC itself regulates γTuRC activity (bottom). The two pathways, depicted separately for clarity, are not mutually exclusive.

Controlling where and when

MTOCs are not only structurally diverse, they are also highly dynamic. Control of microtubule organization during the cell cycle and during differentiation depends in part on the distribution and activity of the microtubule-nucleating material (see FIG. 4 for three regulatory themes for this control). First, the morphology, number and distribution of MTOCs affect the organization of the associated microtubule array. In myotubes, for example, the nuclear envelopes of multiple nuclei function as MTOCs and organize a longitudinal array of parallel microtubules with no apparent focus (FIG. 4a).

A second regulatory mechanism involves proteins that mediate the interaction of γ-tubulin complexes with the MTOC and/or regulate its activity (FIG. 4b). In budding yeast, γ-tubulin complexes are attached to the nucleoplasmic and cytoplasmic faces of the spindle pole body by the attachment factors Spc110 and Spc72, respectively[47,48]. In fission yeast, the proteins Mto1 and Mto2 cooperate to recruit γ-tubulin complexes to cytoplasmic MTOCs, including the sides of microtubules[31,49–53]. By contrast, the Mto1-related protein Pcp1 might be involved in γ-tubulin-dependent nucleation at the spindle pole body during spindle formation[49].

In animals, the proteins abnormal spindle-like (ASP)[54], CG-NAP (also known as AKAP450)[55], ninein-like protein (NLP)[56], centrosomin[57] and kendrin (also known as pericentrin)[58] have all been implicated in the attachment of the γTuRC to the centrosome. A conserved region of the *D. melanogaster* protein centrosomin is also present in the fission yeast proteins Mto1 and Pcp1 (REF. 49). In mammals, two centrosomin-related proteins, CDK5RAP2 and myomegalin (also known as PDE4DIP), contain this region, but their function in γ-tubulin targeting has not been investigated.

The γTuRC component GCP-WD has unique properties, which indicate that it might be the attachment factor most proximal to the γTuRC[10,11]. GCP-WD has the properties of a true γTuRC subunit, yet it can localize to the centrosome even in the absence of intact γTuRC. Most importantly, the depletion of GCP-WD by RNAi results in mislocalization of γ-tubulin without affecting other centrosome proteins and impairs centrosomal

© 2007 Nature Publishing Group

microtubule nucleation during interphase and during mitosis. Centriole-duplication defects observed in GCP-WD-depleted cells[11] might also be a result of impaired targeting of the γTuRC, assuming that it is indeed required for the nucleation of centriole microtubules. The targeting function of GCP-WD is not likely to be limited to the centrosome, as GCP-WD is also involved in the binding of the γTuRC to spindle microtubules and it is conserved in plants, which lack centrosomes[10]. γTuRC subunits and associated proteins might also regulate γTuRC function. Although such regulatory activities have not yet been identified, it is interesting that orthologues of the GCP4, GCP5 and GCP6 γTuRC subunits are not essential for microtubule nucleation itself, either at spindle pole bodies in fission yeast[59] or at centrosomes in *D. melanogaster*[60]. However, they might be involved in the anchoring of microtubules at non-centrosomal sites[61].

The third regulatory mechanism involves post-translational modifications that potentially affect the function of γ-tubulin complexes (FIG. 4c). In budding yeast, phosphorylation of γ-tubulin is important for proper microtubule organization[62]. Phosphorylation of γ-tubulin in *D. melanogaster* has also been reported[63], but the functional significance of this modification is unknown. Phosphorylation of GCP-WD controls targeting of the γTuRC to spindle microtubules[10]; this is the first description of a functionally important mitosis-specific phosphorylation of a γTuRC component[10,11]. Another modification that could confer regulation is ubiquitylation. The breast-specific tumour suppressor BRCA1 and its binding partner BARD1 constitute an E3 ubiquitin ligase that is involved in the DNA-damage response. Recently, this protein complex has been implicated in the regulation of centrosomes, based on BRCA1–BARD1-dependent monoubiquitylation of centrosome proteins, including γ-tubulin, *in vitro*[64]. However, it is not known whether γ-tubulin is ubiquitylated *in vivo*.

Concluding remarks

Many of the centrosomal proteins that are involved in microtubule organization have been identified, but the molecular functions of most of these proteins have yet to be determined. To simplify this task, it will be necessary to define the minimal set of components that are required to constitute an MTOC as defined above. Comparisons of the molecular organization of different MTOCs, including non-centrosomal structures, will help in distinguishing the essential from the non-essential components in the existing long list of candidate proteins. Because spatial and temporal dynamics are the essence of MTOC function, future research must focus on understanding the molecular control of these properties and will include the identification of regulatory molecules and their substrates. Understanding microtubule organization is truly a systems biology problem, involving structural and regulatory components undergoing dynamic changes, with the MTOC at the centre of it all.

Jens Lüders is at the Department of Biological Sciences, Stanford University, and Tim Stearns is at the Department of Biological Sciences, Stanford University, and the Department of Genetics, Stanford University Medical School, Stanford, California 94305, USA.
Correspondence to T.S.
e-mail: stearns@stanford.edu

doi:10.1038/nrm2100

1. Pickett-Heaps, J. D. The evolution of the mitotic apparatus, an attempt at comparative ultrastructural cytology in dividing cell plants. *Cytobios* **1**, 257–280 (1969).
2. Wittmann, T., Hyman, A. & Desai, A. The spindle: a dynamic assembly of microtubules and motors. *Nature Cell Biol.* **3**, E28–E34 (2001).
3. Galjart, N. CLIPs and CLASPs and cellular dynamics. *Nature Rev. Mol. Cell Biol.* **6**, 487–498 (2005).
4. Khodjakov, A., Cole, R. W., Oakley, B. R. & Rieder, C. L. Centrosome-independent mitotic spindle formation in vertebrates. *Curr. Biol.* **10**, 59–67 (2000).
5. Hinchcliffe, E. H., Miller, F. J., Cham, M., Khodjakov, A. & Sluder, G. Requirement of a centrosomal activity for cell cycle progression through G1 into S phase. *Science* **291**, 1547–1550 (2001).
6. Basto, R. *et al.* Flies without centrioles. *Cell* **125**, 1375–1386 (2006).
7. Oakley, C. E. & Oakley, B. R. Identification of γ-tubulin, a new member of the tubulin superfamily encoded by *mipA* gene of *Aspergillus nidulans*. *Nature* **338**, 662–664 (1989).
8. Oakley, B. R., Oakley, C. E., Yoon, Y. & Jung, M. K. γ-tubulin is a component of the spindle pole body that is essential for microtubule function in *Aspergillus nidulans*. *Cell* **61**, 1289–1301 (1990).
9. Gunawardane, R. N., Martin, O. C. & Zheng, Y. Characterization of a new γTuRC subunit with WD repeats. *Mol. Biol. Cell* **14**, 1017–1026 (2003).
10. Luders, J., Patel, U. K. & Stearns, T. GCP-WD is a γ-tubulin targeting factor required for centrosomal and chromatin-mediated microtubule nucleation. *Nature Cell Biol.* **8**, 137–147 (2006).
11. Haren, L. *et al.* NEDD1-dependent recruitment of the γ-tubulin ring complex to the centrosome is necessary for centriole duplication and spindle assembly. *J. Cell Biol.* **172**, 505–515 (2006).
12. Fuller, S. D. *et al.* The core of the mammalian centriole contains γ-tubulin. *Curr. Biol.* **5**, 1384–1393 (1995).
13. Wiese, C. & Zheng, Y. A new function for the γ-tubulin ring complex as a microtubule minus-end cap. *Nature Cell Biol.* **2**, 358–364 (2000).
14. Mogensen, M. M., Malik, A., Piel, M., Bouckson-Castaing, V. & Bornens, M. Microtubule minus-end anchorage at centrosomal and non-centrosomal sites: the role of ninein. *J. Cell Sci.* **113**, 3013–3023 (2000).
15. Delgehyr, N., Sillibourne, J. & Bornens, M. Microtubule nucleation and anchoring at the centrosome are independent processes linked by ninein function. *J. Cell Sci.* **118**, 1565–1575 (2005).
16. Mogensen, M. M. Microtubule release and capture in epithelial cells. *Biol. Cell* **91**, 331–341 (1999).
17. Andersen, J. S. *et al.* Proteomic characterization of the human centrosome by protein correlation profiling. *Nature* **426**, 570–574 (2003).
18. Schmit, A. C. Acentrosomal microtubule nucleation in higher plants. *Int. Rev. Cytol.* **220**, 257–289 (2002).
19. Ehrhardt, D. W. & Shaw, S. L. Microtubule dynamics and organization in the plant cortical array. *Annu. Rev. Plant Biol.* **57**, 859–875 (2006).
20. Binarova, P. *et al.* γ-tubulin is essential for acentrosomal microtubule nucleation and coordination of late mitotic events in *Arabidopsis*. *Plant Cell* **18**, 1199–1212 (2006).
21. Pastuglia, M. *et al.* γ-tubulin is essential for microtubule organization and development in *Arabidopsis*. *Plant Cell* **18**, 1412–1425 (2006).
22. Murata, T. *et al.* Microtubule-dependent microtubule nucleation based on recruitment of γ-tubulin in higher plants. *Nature Cell Biol.* **7**, 961–968 (2005).
23. Mazia, D. Centrosomes and mitotic poles. *Exp. Cell Res.* **153**, 1–15 (1984).
24. Tassin, A. M., Maro, B. & Bornens, M. Fate of microtubule-organizing centers during myogenesis *in vitro*. *J. Cell Biol.* **100**, 35–46 (1985).
25. Musa, H., Orton, C., Morrison, E. E. & Peckham, M. Microtubule assembly in cultured myoblasts and myotubes following nocodazole induced microtubule depolymerisation. *J. Muscle Res. Cell Motil.* **24**, 301–308 (2003).
26. Bugnard, E., Zaal, K. J. & Ralston, E. Reorganization of microtubule nucleation during muscle differentiation. *Cell Motil. Cytoskeleton* **60**, 1–13 (2005).
27. Hagiwara, H., Kano, A., Aoki, T., Ohwada, N. & Takata, K. Localization of γ-tubulin to the basal foot associated with the basal body extending a cilium. *Histochem. J.* **32**, 669–671 (2000).
28. Hard, R. & Rieder, C. L. Muciliary transport in newt lungs: the ultrastructure of the ciliary apparatus in isolated epithelial sheets and in functional Triton-extracted models. *Tissue Cell* **15**, 227–243 (1983).
29. Sawin, K. E. & Tran, P. T. Cytoplasmic microtubule organization in fission yeast. *Yeast* **23**, 1001–1014 (2006).
30. Zimmerman, S., Tran, P. T., Daga, R. R., Niwa, O. & Chang, F. Rsp1p, a J domain protein required for disassembly and assembly of microtubule organizing centers during the fission yeast cell cycle. *Dev. Cell* **6**, 497–509 (2004).
31. Janson, M. E., Setty, T. G., Paoletti, A. & Tran, P. T. Efficient formation of bipolar microtubule bundles requires microtubule-bound γ-tubulin complexes. *J. Cell Biol.* **169**, 297–308 (2005).
32. Daga, R. R., Lee, K. G., Bratman, S., Salas-Pino, S. & Chang, F. Self-organization of microtubule bundles in anucleate fission yeast cells. *Nature Cell Biol.* **8**, 1108–1113 (2006).
33. Carazo-Salas, R. E. & Nurse, P. Self-organization of interphase microtubule arrays in fission yeast. *Nature Cell Biol.* **8**, 1102–1107 (2006).
34. Reilein, A. & Nelson, W. J. APC is a component of an organizing template for cortical microtubule networks. *Nature Cell Biol.* **7**, 463–473 (2005).
35. Reilein, A., Yamada, S. & Nelson, W. J. Self-organization of an acentrosomal microtubule network at the basal cortex of polarized epithelial cells. *J. Cell Biol.* **171**, 845–855 (2005).
36. Chabin-Brion, K. *et al.* The Golgi complex is a microtubule-organizing organelle. *Mol. Biol. Cell* **12**, 2047–2060 (2001).
37. Rios, R. M., Sanchis, A., Tassin, A. M., Fedriani, C. & Bornens, M. GMAP-210 recruits γ-tubulin complexes to *cis*-Golgi membranes and is required for Golgi ribbon formation. *Cell* **118**, 323–335 (2004).
38. Barr, F. A. & Egerer, J. Golgi positioning: are we looking at the right MAP? *J. Cell Biol.* **168**, 993–998 (2005).
39. Lajoie-Mazenc, I. *et al.* Recruitment of antigenic γ-tubulin during mitosis in animal cells: presence of γ-tubulin in the mitotic spindle. *J. Cell Sci.* **107**, 2825–2837 (1994).
40. Mastronarde, D. N., McDonald, K. L., Ding, R. & McIntosh, J. R. Interpolar spindle microtubules in PTK cells. *J. Cell Biol.* **123**, 1475–1489 (1993).
41. Buster, D., McNally, K. & McNally, F. J. Katanin inhibition prevents the redistribution of γ-tubulin at mitosis. *J. Cell Sci.* **115**, 1083–1092 (2002).
42. Gruss, O. J. & Vernos, I. The mechanism of spindle assembly: functions of Ran and its target TPX2. *J. Cell Biol.* **166**, 949–955 (2004).
43. Wilde, A. & Zheng, Y. Stimulation of microtubule aster formation and spindle assembly by the small GTPase Ran. *Science* **284**, 1359–1362 (1999).
44. Mahoney, N. M., Goshima, G., Douglass, A. D. & Vale, R. D. Making microtubules and mitotic spindles in cells without functional centrosomes. *Curr. Biol.* **16**, 564–569 (2006).
45. Khodjakov, A., Copenagle, L., Gordon, M. B., Compton, D. A. & Kapoor, T. M. Minus-end capture of preformed kinetochore fibers contributes to spindle morphogenesis. *J. Cell Biol.* **160**, 671–683 (2003).

© 2007 Nature Publishing Group

46. Maiato, H., Rieder, C. L. & Khodjakov, A. Kinetochore-driven formation of kinetochore fibers contributes to spindle assembly during animal mitosis. *J. Cell Biol.* **167**, 831–840 (2004).
47. Knop, M. & Schiebel, E. Spc98p and Spc97p of the yeast γ-tubulin complex mediate binding to the spindle pole body via their interaction with Spc110p. *EMBO J.* **16**, 6985–6995 (1997).
48. Knop, M. & Schiebel, E. Receptors determine the cellular localization of a γ-tubulin complex and thereby the site of microtubule formation. *EMBO J.* **17**, 3952–3967 (1998).
49. Sawin, K. E., Lourenco, P. C. & Snaith, H. A. Microtubule nucleation at non-spindle pole body microtubule-organizing centers requires fission yeast centrosomin-related protein mod20p. *Curr. Biol.* **14**, 763–775 (2004).
50. Venkatram, S. *et al.* Identification and characterization of two novel proteins affecting fission yeast γ-tubulin complex function. *Mol. Biol. Cell* **15**, 2287–2301 (2004).
51. Samejima, I., Lourenco, P. C., Snaith, H. A. & Sawin, K. E. Fission yeast mto2p regulates microtubule nucleation by the centrosomin-related protein mto1p. *Mol. Biol. Cell* **16**, 3040–3051 (2005).
52. Venkatram, S., Jennings, J. L., Link, A. & Gould, K. L. Mto2p, a novel fission yeast protein required for cytoplasmic microtubule organization and anchoring of the cytokinetic actin ring. *Mol. Biol. Cell* **16**, 3052–3063 (2005).
53. Zimmerman, S. & Chang, F. Effects of γ-tubulin complex proteins on microtubule nucleation and catastrophe in fission yeast. *Mol. Biol. Cell* **16**, 2719–2733 (2005).
54. do Carmo Avides, M. & Glover, D. M. Abnormal spindle protein, Asp, and the integrity of mitotic centrosomal microtubule organizing centers. *Science* **283**, 1733–1735 (1999).
55. Takahashi, M., Yamagiwa, A., Nishimura, T., Mukai, H. & Ono, Y. Centrosomal proteins CG-NAP and kendrin provide microtubule nucleation sites by anchoring γ-tubulin ring complex. *Mol. Biol. Cell* **13**, 3235–3245 (2002).
56. Casenghi, M. *et al.* Polo-like kinase 1 regulates Nlp, a centrosome protein involved in microtubule nucleation. *Dev. Cell* **5**, 113–125 (2003).
57. Terada, Y., Uetake, Y. & Kuriyama, R. Interaction of Aurora-A and centrosomin at the microtubule-nucleating site in *Drosophila* and mammalian cells. *J. Cell Biol.* **162**, 757–763 (2003).
58. Zimmerman, W. C., Sillibourne, J., Rosa, J. & Doxsey, S. J. Mitosis-specific anchoring of γ tubulin complexes by pericentrin controls spindle organization and mitotic entry. *Mol. Biol. Cell* **15**, 3642–3657 (2004).
59. Anders, A., Lourenco, P. C. & Sawin, K. E. Non-core components of the fission yeast γ-tubulin complex. *Mol. Biol. Cell* **17**, 5075–5093 (2006).
60. Verollet, C. *et al. Drosophila melanogaster* γ-TuRC is dispensable for targeting γ-tubulin to the centrosome and microtubule nucleation. *J. Cell Biol.* **172**, 517–528 (2006).
61. Vogt, N., Koch, I., Schwarz, H., Schnorrer, F. & Nusslein-Volhard, C. The γTuRC components Grip75 and Grip128 have an essential microtubule-anchoring function in the *Drosophila* germline. *Development* **133**, 3963–3972 (2006).
62. Vogel, J. *et al.* Phosphorylation of γ-tubulin regulates microtubule organization in budding yeast. *Dev. Cell* **1**, 621–631 (2001).
63. Stumpff, J., Kellogg, D. R., Krohne, K. A. & Su, T. T. *Drosophila* Wee1 interacts with members of the γTURC and is required for proper mitotic-spindle morphogenesis and positioning. *Curr. Biol.* **15**, 1525–1534 (2005).
64. Starita, L. M. *et al.* BRCA1-dependent ubiquitination of γ-tubulin regulates centrosome number. *Mol. Cell. Biol.* **24**, 8457–8466 (2004).

Acknowledgements
We thank M.-F. Bryan Tsou for critical reading of the manuscript and E. Vladar for helpful comments.

Competing interests statement
The authors declare no competing financial interests.

DATABASES
The following terms in this article are linked online to:
UniProtKB: http://ca.expasy.org/sprot
Centrosomin | CG-NAP | GCP2 | GCP3 | GCP6 | kendrin | Mto1 | Mto2 | NEDD1 | ninein | Pcp1

FURTHER INFORMATION
Tim Stearns's laboratory: http://stearnslab.stanford.edu
Access to this links box is available online.

INNOVATION

Towards the plant metabolome and beyond

Robert L. Last, A. Daniel Jones and Yair Shachar-Hill

Abstract | Methods for network-wide analysis are increasingly showing that the textbook view of the regulation of plant metabolism is often incomplete and misleading. Recent innovations in small-molecule analysis have created the ability to rapidly identify and quantify numerous compounds, and these data are creating new opportunities for understanding plant metabolism and for plant metabolic engineering.

Plant biologists hope to harness the availability of genome-wide data on DNA, RNA and proteins to develop an integrated understanding of the behaviour of complex metabolic networks. This is an increasingly important goal for our global economy as we rely on plant-derived materials to replace fossil fuels as energy sources and industrial feedstocks, and for their traditional and crucial roles in food, fibre and pharmaceuticals. Reaching such an understanding will require cataloguing all the metabolites that are synthesized, identifying the enzymes that are responsible for the synthesis of each product, and characterizing the dynamic behaviour of the metabolic pathways and networks. Developing a predictive level of understanding of metabolism is an especially daunting task in plants because they produce orders of magnitude more metabolites than animals.

Although the analysis of metabolites in plants has a long history, the development of high-throughput methods for measuring large numbers of compounds has arisen in less than a decade. This is a result of rapid improvements in mass spectrometry (MS)-based methods and in computer hardware and software that is capable of interpreting large datasets. Studies in which bioinformatics and data-analysis methods are applied to such data are beginning to contribute to functional genomics studies and to generate new hypotheses about how plant metabolism is controlled, but such systems approaches are still in their infancy.

The use of high-throughput analytical strategies to identify and measure the concentration of as many metabolites as possible is termed metabolomics. For systems-wide analyses, accurate identification and quantification of small molecules are the essential first steps. Despite much recent progress, metabolomics of plant systems is not yet '-omic' in scale, so it is useful to consider the prospects for progress in this direction.

This article primarily focuses on recent innovations in MS-based analytical chemistry that have created the opportunity to measure the abundances of hundreds of metabolites and also provides information on the powerful and complementary techniques of nuclear magnetic resonance (NMR) (BOX 1). The challenges of using the large amounts of data that are generated by metabolomics are discussed. We then show, by using examples from the recent literature, how metabolomics can be used for discovering new metabolic genes and enzymes.

Measuring molecules: analytical methods

There are three general approaches to analyse small molecules, and these differ in the number of compounds they analyse, in the level of structural information they obtain, and in their sensitivity[1–4]. The most common approach, metabolite profiling, is the analysis of small numbers of known metabolites in specific compound classes (for example, lipid, amino acid or sterol). At the other extreme, metabolic fingerprinting detects many compounds, but their structures are rarely identified. Metabolomics methods typically allow the measurement of hundreds of molecules, with a small number being definitively

© 2007 Nature Publishing Group