The centrosome in vertebrates: more than a microtubule-organizing center

Conly L. Rieder, Shamsa Faruki and Alexey Khodjakov

The somatic cells of all higher animals contain a single minute organelle called the centrosome. For years, the functions of the centrosome were thought to revolve around its ability to nucleate and organize the various microtubule arrays seen in interphase and mitosis. But the centrosome is more than just a microtubule-organizing center. Recent work reveals that this organelle is essential for cell-cycle progression and that this requirement is independent of its ability to organize microtubules. Here, we review the various functions attributed to the centrosome and ask which are essential for the survival and reproduction of the cell, the organism, or both.

According to Wilson[1], the term 'centrosome' was coined by Boveri in 1888 to describe a single 'extremely minute body, or more commonly a pair of bodies, staining intensely with haematoxylin… and surrounded by a cytoplasmic radiating aster'. Like the nucleus, this organelle grows and replicates autonomously during the cell cycle, and a single copy is then segregated to each new daughter cell during division through its association with the mitotic spindle. Because the 'poles' that define the essential bipolar nature of the spindle each contain a centrosome, Boveri viewed this organelle as the 'especial organ of cell division' – an opinion that has remained unchallenged until recently.

More than a century of research has revealed that the centrosome, which is not found in higher plants, is a complex organelle that is structurally conserved among all higher animals. Many *bona fide* centrosomal components, such as γ-tubulin, pericentrin and centrin, are also highly conserved. Given its complexity and conservation, the centrosome must perform essential function(s) as, otherwise, it would have been eliminated during evolution by random mutations.

In the cell, the 'radiating aster' described by Boveri defines one clear ubiquitous function for the centrosome: it is the primary microtubule (MT)-organizing center (MTOC) of the cell. When disassembled, MTs rapidly and preferentially re-grow from this organelle during recovery. The conspicuous ability of the centrosome to nucleate

MTs led to a general consensus that its essential functions revolve around this feature. We now know, however, that, during interphase, MT arrays – similar to those organized by a centrosome – can be organized in cultured cells even in the absence of this organelle. Also, as discussed below, many vertebrate cells can complete the entire interphase portion of the cell cycle in the absence of MTs, and centrosomes are not required during mitosis to form functional spindles.

In higher (multicellular) animals, some of the functions of centrosomes are clearly more important for the survival of the organism than for the survival of individual cells. A good example here is the formation of primary cilia in somatic cells and flagella in sperm. These structures are generated directly from the centrosome and are crucial for proper development and reproduction. But some cells in higher animals lack a flagellum or cilium, which means that this centrosomal function is not essential for the survival and reproduction of cells. These observations imply that, even though the MTOC activity of the centrosome might be important for the development, maintenance and reproduction of multicellular organisms, it is not required for cell survival and reproduction. This contention is supported by the fact that, at least in *Drosophila*, stable cell lines, but not flies, that lack a centrosome can be isolated[2].

> '…the centrosome's role as an MTOC is more important for the proper development and maintenance of the organism than for cell viability…'

Here, we summarize the various functions ascribed to centrosomes in higher animals, placing our focus on vertebrates. We ask whether each is vital to the survival and reproduction of the cell, the organism, or both. The views expressed here are our opinions, molded in part from recent work on the topic, and they probably differ from others working in the field. Our goal is not so much to persuade the reader that we are right, as to provoke thought on issues important for the future. Our theses are that, in vertebrates and other higher animals, the centrosome is defined by the centriole, that its role as a MTOC is more important for the proper development and maintenance of the organism than for cell viability and that the centrosome has at least one vital function in the cell that is independent of its role as an MTOC.

Centrioles and the centrosome
We begin our argument with a definition of what a centrosome is in vertebrates. Despite recent

Conly L. Rieder*
Shamsa Faruki
Alexey Khodjakov**
Laboratory of Cell Regulation, Division of Molecular Medicine, Wadsworth Center, New York State Dept of Health, Albany, NY 12201-0509, USA. C.L.R. and A.K. are also at: Marine Biology Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA.
*e-mail: rieder@wadsworth.org
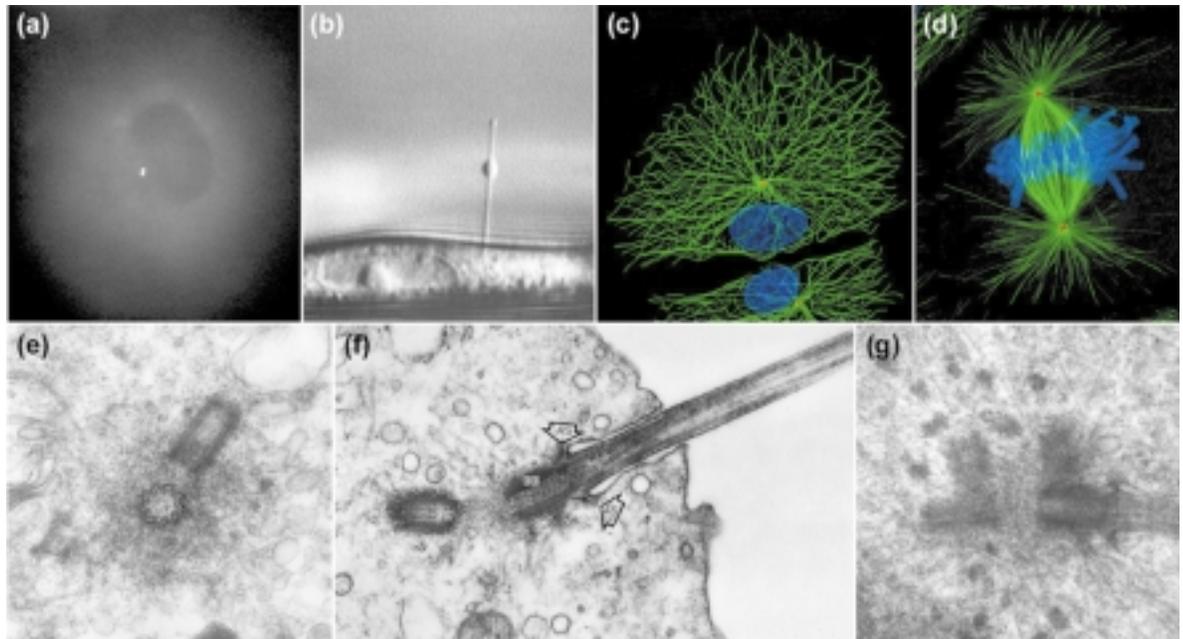**e-mail: khodj@wadsworth.org

Fig. 1. (a) In living cells the centrosome cannot be distinguished from other inclusions unless it is first labeled with a fluorescent probe, such as green-fluorescent protein (GFP)–γ-tubulin, after which it appears as one or two dots generally located near the nucleus. (b) The primary cilium can be easily detected when PtK$_1$ and other types of cell are viewed from the side. Electron microscopy (f) reveals that this cilium is generated from the oldest 'mother' centriole in the cell. (c) During interphase, the cytoplasmic microtubule complex (green) in CV-1 and in other types of cell appears to focus on the centrosome (red dot near blue nucleus). (d) During mitosis, the cytoplasmic microtubule complex is resorbed, and each of the replicated centrosomes (red dots) nucleates a radial 'astral' array of microtubules that separate ultimately to define the spindle poles. The example shown is a newt lung cell in metaphase of mitosis. (e) At the level of the electron microscope, the centrosome in a late-telophase PtK$_1$ cell comprises two associated centrioles surrounded by a diffuse cloud of pericentriolar material. Here, the mature mother centriole is cut in cross-section, whereas the daughter, which has maintained its orthogonal relationship with its parent, is transversely sectioned. (f) In most types of vertebrate cells, the mature centriole templates the formation of a primary cilium sometime in early G1 phase. During this process, the centrioles frequently lose their orthogonal relationship. 'AS' indicates spokes or transition fibers). (g) As the centrosomes replicate near the G1–S boundary, procentrioles are formed in association with the wall of both the mother and daughter centrioles.

advances in light microscopy, in the living cell, this organelle still appears as one or two dots, usually located near the nucleus (Fig. 1a). To appreciate fully its structural complexity, the centrosome must be viewed by electron microscopy. With this technique, it is seen to contain two 'centrioles', each of which comprises a pinwheel of nine triplet MT 'blades' distributed evenly around the perimeter of a compact cylinder (Fig. 1e–g). The centrioles are embedded in a cloud of pericentriolar material that is organized by a relatively insoluble filamentous 'centromatrix'[3]. The functional components responsible for MT nucleation and anchoring are bound to this centromatrix and include, for example, ring-shaped γ-tubulin-containing complexes, pericentrin and ninein[4].

The centrioles themselves also contain several specific proteins, including centrin, cenexin and tektin, and the α–β-tubulin subunits forming the blades are modified, for example, by polyglutamylation[4,5]. Depending on the cell type and the cell-cycle stage, the two centrioles comprising the centrosome can be intimately associated or well separated[6]. Most types of cells in higher animals also possess *in situ* and *in vivo* a single non-motile primary cilium, which is nucleated from the distal end of the oldest 'mother' centriole (Fig. 1b,f). In some cells, such as kidney epithelia, this cilium protrudes many micrometers from the dorsal cell surface[7].

The centrosome replicates during the G1–S-phase transition through a process regulated by the activity of the cyclin-dependent kinase 2 (CDK2)[8]. During this time, a new pro-centriole is formed on the wall of, and at right angles to, each centriole of the diplosome (Fig. 1g).

It has been argued strongly that, in higher animals, the centrosome is defined by the centrioles[8–10]. This is evident because, for example, when centrioles are induced to disassemble by injecting cells with antibodies to polyglutamylated tubulin, the surrounding cloud of pericentriolar material (including γ-tubulin and pericentrin) becomes dispersed[10]. When the antibodies are degraded, however, γ-tubulin and pericentrin once again become concentrated around the centrioles as they reform.

In this view, centrosome replication is regulated by centriole replication that occurs in higher animals only in association with an existing centriole. To our knowledge, there are no data to conflict with this contention but good data to support it: centrosome replication is not compromised when (sea urchin) cells are manipulated to contain a single centriole. But if the

centrosome/centriole is removed from vertebrate cells during any stage of the cell cycle, a new centriole, and thus centrosome, does not regenerate even though a loosely focused MT array can be reformed ultimately[11–13].

There are examples in which centrioles are not formed in association with another centriole. For example, many centrioles form on ill-defined structures called 'deuterosomes' as epithelial cells differentiate to form ciliated tissues[14–16]. We believe, however, that each of these represents an additional centrosome. Like the original centrosome, these new organelles organize cytoplasmic microtubules (from their satellite arms) and also template the formation of a cilium. That they never serve as spindle poles can be attributed to the fact that, in higher animals, this amplification process only occurs in cells that have exited the cell cycle. A *de novo* formation of centrioles also occurs during the early stages of development in rodents[17], and this formation must occur for the organism to develop.

Centrosome functions based on microtubule nucleation

The MTOC activity of the centrosome includes the nucleation and organization of those MTs that form the interphase cytoplasmic MT array (Fig. 1c) and the mitotic spindle (Fig. 1d), as well as cilia (Fig. 1b,f). The first two activities are centred on the nucleation of MTs by sites inside the pericentriolar material, whereas the last activity involves the nucleation of MTs directly from the centriole.

Organization of the interphase cytoplasmic microtubule complex
In interphase, the cytoplasmic MT complex is normally organized by the centrosome. In some cells, such as monocytes and fibroblasts, most MTs emanate from the centrosome (Fig. 1c). In other cells, such as epithelia, few of the microtubules are actually anchored on the centrosome at any one time. In the latter type of cell, the MTs seem to be generated by the centrosome, but then released to wander through the cytoplasm. In either case, interphase cells normally contain a roughly radial array of MTs.

It is now evident that a typical interphase MT array can be formed in vertebrate cells, ranging from fish melanophores[18] to mammalian kidney epithelia[12–13], in the absence of a centrosome. Under this condition, MTs are nucleated randomly within the cytoplasm and then organized progressively into a characteristic array by the action of multivalent MT molecular motors such as cytoplasmic dynein[13,19]. Importantly, these acentrosomal MT arrays persist for many days and support normal intracellular trafficking, including melanosome aggregation and dispersion.

It is not clear whether the 'acentrosomal' MT arrays that form during interphase support all of the MT-mediated functions that are normally characteristic of a particular cell type. For example, such arrays might or might not support the polarized transport of vesicles in epithelia. But, as emphasized above, we believe that such functions are crucial to the survival of the organism but not the cell.

Even highly specialized forms of intracellular motility traditionally associated with the centrosome can occur via an acentrosomal pathway. For example, after the male pronucleus is introduced into the egg at fertilization, it moves to and fuses with the female pronucleus in a process known as syngamy. In most mammals, including humans, the egg lacks a centrosome, and pronuclear migration occurs in association with the sperm centrosome, which is also used in development. Once incorporated into the oocyte, this centrosome nucleates a radial array of MTs, called an aster, which then transports the pronuclei towards one another. This paternal inheritance of the centrosome prompted Boveri to regard this organelle as 'the especial fertilizing element in the spermatozoon, which, when introduced into the egg, endowed the latter with the power of division and development'[1].

Microtubules are clearly involved in pronuclear migration as inhibiting MT formation prohibits this process. However, the requirement for a centrosome is not absolute. In rodents, for example, the centrosome is derived maternally and does not appear until after the fertilized egg has completed the first two zygotic divisions. On fertilization, several MT foci, often associated with the pronuclei, form spontaneously and mediate pronuclear migration[20]. Because mouse zygotes lack centrosomes, these foci are also probably organized by molecular motors that sort randomly nucleated Mts[21]. Thus, although the centrosome clearly facilitates the construction and organization of cytoplasmic MT arrays in vertebrate cells, there seem to be other redundant routes that accomplish the same tasks in its absence.

Formation of the mitotic spindle and cytokinesis
The notion that each pole of the mitotic spindle in animals is defined by, and thus formed from, a centrosome seems so obvious (Fig. 1d) that, until recently, it was seldom questioned. Indeed, throughout his distinguished career, Mazia[22] considered the centrosome to be synonymous with a spindle pole and then sought to explain why poles in the plant and animal kingdoms display so many structural variations – from highly focused centers in animals containing centrioles, to flat broad poles in plants (and animal oocytes) lacking centrioles. His conclusion was that centrosomes are 'flexible linear structures', which, in addition to their roles as MTOCs, 'are organizers of half spindles… and dictate the planes of cytoplasmic division.'[22]

Work based on extracts from *Xenopus* oocytes reveals that functional bipolar spindles can be formed in the absence of centrosomes through a pathway in which antagonistic MT motor molecules (including cytoplasmic dynein and members of the kinesin superfamily) sort MTs nucleated randomly in the vicinity of the chromosomes[23]. This 'acentrosomal' route of spindle formation is also used in some mammalian oocytes (see below) and insect spermatocytes[24], as well as in the acentrosomal *Drosophila* cell line[2]. Surprisingly, when their centrosomes are destroyed in S or G2 phase by laser microsurgery or removed by a microneedle, green monkey kidney (CV-1 and BSC-1) cells also form functional bipolar spindles[13,25].

Thus, a redundant centrosome-independent pathway for spindle assembly is also present in vertebrate cells that, like oocytes, probably employ MT motors and structural proteins instead[26]. It remains to be determined whether this pathway is activated only in the absence of centrosome function, or is always working but normally masked by centrosome activity[27]. The notion that the cell can 'sense' the absence of a centrosome – and then activate such a backup pathway – is not without precedent. Recent work suggests that just such a regulatory mechanism is used by *Chlamydomonas* to suppress the *de novo* formation of centrioles when another centriole is present[28].

'...a redundant centrosome-independent pathway for spindle assembly is also present in vertebrate cells...'

The conclusion that centrosomes are not required for spindle formation in higher animals has an interesting ramification: it validates the early idea, mentioned by Wilson[1] and developed subsequently by others[24,29], that centrioles reside at the spindle poles in animals not because they are necessary for spindle formation but, instead, to ensure that each new cell starts life with a copy of this organelle. The original idea envisaged that the centriole was dispensable for centrosome function, although it did not question the importance of the centrosome in spindle formation. We know now, however, that the hypothesis is validated – not for this reason, but because the centrosome itself is dispensable for spindle formation!

Although centrosomes are not required for segregating chromosomes, they still have a key role in mitosis. It has been known for years that the radial 'astral' arrays of MTs generated by mitotic centrosomes are actively involved in establishing where in the cell the cleavage furrow will form.

We now also know that spindle positioning requires the dynein-mediated interaction of astral MTs with the cell cortex (Fig. 2a)[30,31].

One of the characteristic features of acentrosomal spindles is that they lack astral MTs and thus the ability to reposition themselves as the cell changes its shape (Fig. 2b)[12]. In such cells, cytokinesis often fails when the long axis of the spindle is positioned, at the onset of anaphase, perpendicular to the long axis of the cell (Fig. 3a), because the furrow must function over a distance that might exceed the limit of its action[32]. Thus, although centrosomes in higher animals are not essential for cytokinesis, they do serve the non-trivial function of ensuring the fidelity of this process.

During development, cells of different sizes that are destined for different fates are generated from certain mitoses. This 'asymmetric' cytokinesis is also mediated by centrosomes through their effect on spindle positioning. In the central nervous system of *Drosophila* larvae, neuroblasts divide asymmetrically to produce another neuroblast and a smaller ganglion mother cell (which forms two neurons after its next mitosis). The genesis of this asymmetry correlates with the progressive disappearance of one centrosome and its aster during late anaphase, which induces the spindle to shift closer to one side of the cell[33].

The centrosomal requirement for this process is, however, clouded by recent studies showing that asymmetric divisions occur in *asterless* or *centrosomin* fly mutants that appear to lack astral MTs during mitosis[34,35]. One possibility is that the astral MT arrays in these mutants are attenuated severely but not eliminated completely. Another is that, by chance, the spindle can be positioned favorably in the cell at anaphase so that enough asymmetric divisions occur to ensure (in the case of *centrosomin* mutants) fly viability. But it is also possible that, at least in flies, there is a redundant acentrosomal pathway that can mediate this process[35].

The asters also control spindle positioning in yeast, which possess a cell-cycle checkpoint pathway, centered on the centrosome equivalent (the 'spindle pole body'), that delays the completion of cytokinesis when spindles fail to become properly positioned in the neck bud[36]. Because mitotic mechanisms are highly conserved, a similar centrosome-based 'cytokinesis' checkpoint has been posited to exist in vertebrates[37]. This idea draws support from reports that cells containing acentrosomal spindles frequently fail to complete cytokinesis (see above) and also that the completion of cytokinesis in some cultured cells (including HeLa, L929, CHO and Indian muntjac) is correlated with the motion of one centriole first to, and then away from, the furrow site (i.e. midbody)[38]. Where it has been examined carefully, however, the failure of cytokinesis in cells containing acentrosomal
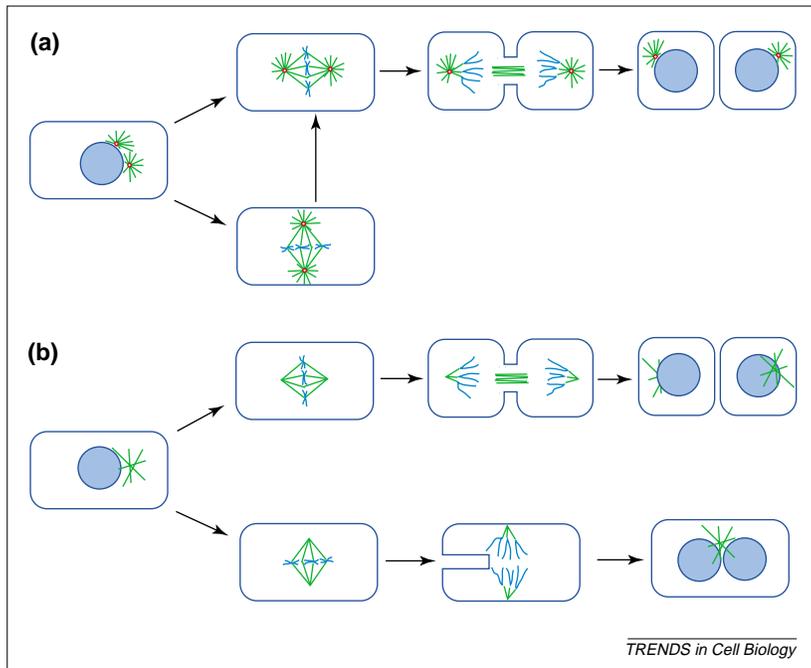
Fig. 2. Schematic diagram depicting a model that explains why cytokinesis fails in some cells lacking centrosomes. (a) In normal cells, the arrays of astral microtubules associated with the centrosomes function to keep the spindle properly positioned in the cell; that is, they constantly reposition the spindle so that its long axis is parallel to the long axis of the cell. In turn, this ensures that cytokinesis is completed to produce two daughter cells. (b) In the absence of centrosomes, asters do not form and spindle re-positioning does not occur. As a result, cells often enter anaphase when the spindle long axis is perpendicular to the cell long axis, and cytokinesis ultimately fails.

spindles has been attributed to errors in spindle positioning (Fig. 2b)[12]. Moreover, cytokinesis rarely fails in cultured cells (including PtK$_1$, CV-1, BHK, LLC-PK) in which the centrioles do not migrate towards and away from the midbody at the end of mitosis.
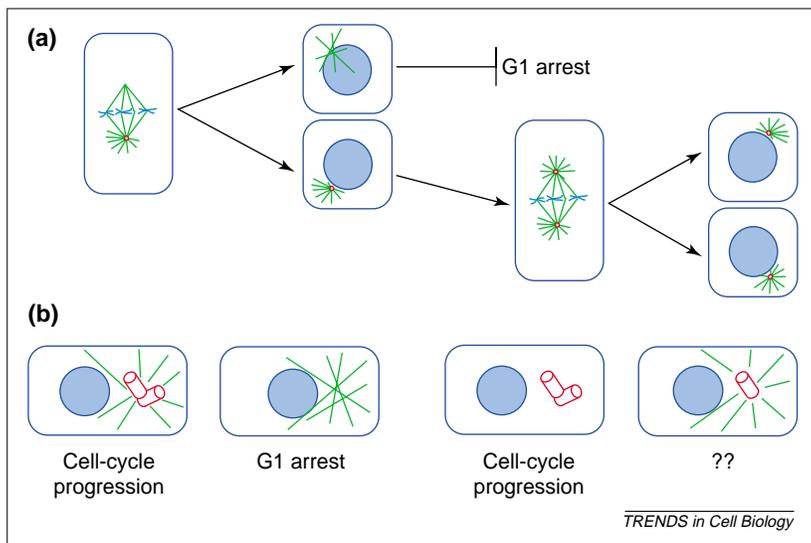

Fig. 3. (a) Diagram depicting the fate of cells born without a centrosome. When a centrosome is destroyed in metaphase of mitosis, the cell completes the division to produce two daughters, one of which lacks a centrosome. The centrosome-containing control completes another cell cycle and enters the next mitosis. By contrast, the cell lacking a centrosome fails to enter the next round of DNA synthesis and arrests in G1 phase. (b) Cells that contain a centrosome progress through the cell cycle, even in the absence of microtubules; however, cells that lack a centrosome arrest during G1 phase. An important issue for the future is whether cell-cycle progression is supported by specific components of a centrosome and, if so, the identification of these components.

Thus, even though the question of why the centrosome is important for cytokinesis remains controversial, there is little doubt that it plays a crucial role in ensuring the fidelity of this process – a role that is clearly important both for the survival and reproduction of the cell and for the organism.

Formation of cilia and flagella
The only MTOC activity that cannot be achieved in higher animals via an acentrosomal pathway seems to be the formation of primary cilia in somatic cells (Fig. 1b,f) and flagella in sperm. Primary cilia are so ubiquitous in vertebrate tissues that it is easier to list the cells that lack them than those that possess them (see http://members.global2000.net/bowser). Because these structures are not found on many cell types *in vivo* or *in vitro*, they are clearly not essential for the survival of the cell, a fact that led early researchers to speculate that they are simply vestigial appendages. Yet, recent studies reveal that, in vertebrates (and probably all other higher animals), primary cilia are required for proper development[39,40] and tissue function[41], and that their derivatives form structures such as the rods and cones of the eye.

The same argument can be made for the flagella of sperm, which are also organized by the centrosome/centriole. At the cellular level, these structures are not required for viability and reproduction, but their formation is essential for the development and reproduction of multicellular organisms. Because the formation of cilia/flagella is essential to higher animals, positioning the replicated centrosomes at the poles of the spindle is an efficient way to ensure that each cell will receive a copy of this important organelle.

Centrosome function(s) unrelated to MTOC activity
When the centrosome is surgically removed from BSC-1 cells during S phase[13], or destroyed in CV-1 or PtK$_1$ cells during G2 phase by laser ablation[12], the cells continue to progress into and complete mitosis, but the resultant progeny arrest in G1 (Fig. 3a). Importantly, this G1 arrest occurs in spite of the fact that the (now) acentrosomal cells appear to contain normal amounts of microtubules that are organized in a way similar to those of controls. Furthermore, when the MTs in CV-1 (Fig. 4) and in other types of vertebrate cell[42,43] are destroyed by drugs, the cells continue to progress through G1, S and G2. BSC-1 cells also undergo many cell cycles when exposed continuously to low concentrations of taxol, a drug that stabilizes Mts[13]. Finally, if the centrosome is not destroyed or removed, but only temporarily dispersed by antibody microinjection, vertebrate cells seem to cycle normally[10].

Together, these findings suggest that progression through G1 in vertebrates requires a factor normally associated with the centrosome that is
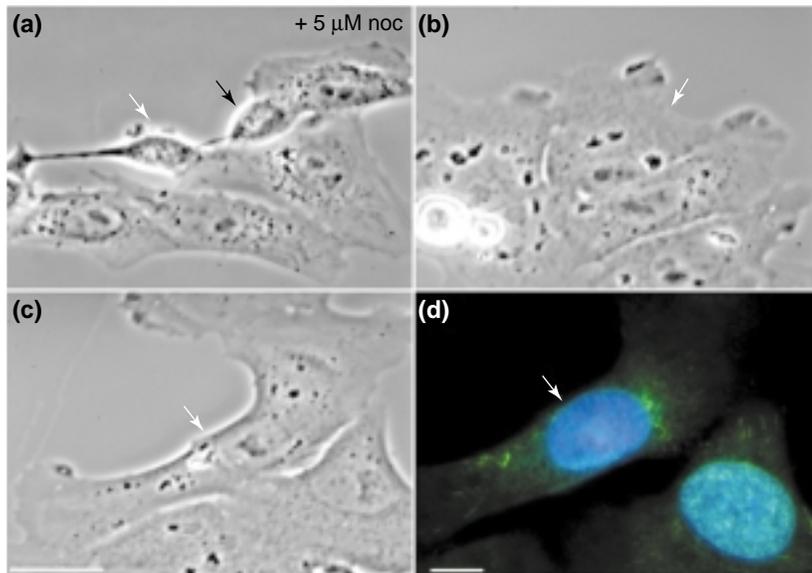
Fig. 4. Vertebrate somatic cells, born lacking microtubules, progress through the cell cycle. In this example, a CV-1 cell was treated with 5 μM nocodazole and 10 mM bromodeoxyuridine (BrdU) as it was completing the division process. After division had finished, one of the progeny [black arrow in (a)] crawled out of the field of view, but the other (white arrow) was followed for several days before fixation (c) and indirect immunofluorescence staining for DNA by BrdU (blue) and microtubules by Nocodazole (green). As evident from the fluorescent micrograph shown in (d), this cell completed G1 phase and entered S (blue nucleus) even though largely depleted of microtubules. Bars: 50 μm (c) and 10 μm (d).

independent of the higher-order structure of the centrosome and its MTOC activity. This might also be true for other multicellular animals such as flies – although the centrosomes in *centrosomin* mutants are reported to lack a MTOC function during mitosis, these organelles are still present during interphase and the cells continue to cycle normally[35].

It remains to be determined what component(s) of the centrosome are required for progression through G1 (Fig. 3b). It is similarly unclear whether removing the centrosome arrests the cell cycle because it is part of a checkpoint pathway, or because it is required, for example, simply to catalyse reactions needed for progression into S phase. The idea that the centrosome triggers a checkpoint is supported by the existence of an acentrosomal (fly) cell line, which provides the 'relief of dependence' required for a checkpoint. In other words, this cell line demonstrates that conditions can be defined for continued cell-cycle progression in the absence of a centrosome.

Alternatively, the idea that the centrosome catalyses crucial reactions required for the G1–S transition is not without merit. Many proteins and macromolecular complexes are concentrated in the centrosome. Some accumulate in the absence of MTs because they have a true affinity for this site (e.g. γ-tubulin[44], proteosomes[45]), whereas others concentrate because the centrosome is the 'terminal hub' of the internal MT-mediated transportation system in the cells. The ability of the centrosome to concentrate various enzymes and their substrates

suggests that it 'is a specialized site for the coordination of complex molecular interactions'[46], which is essential, for example, for the G1–S transition.

In some cells such as megakaryocytes, myocytes and red blood cells of higher animals, the centrosome degenerates. As a rule, however, such cells are terminally differentiated and no longer capable of entering the mitotic cycle. One clear exception is the oocyte, which, although it lacks a centrosome, remains capable of re-entering the cell cycle when a new centrosome is supplied at fertilization.

At first glance, reports that the developmental pathway in eggs can be activated in the absence of true fertilization (i.e. by parthenogenesis) conflict with our contention that centrosomes are required for cell-cycle progression; however, zygotes lack many cell-cycle checkpoint pathways and they contain ample precursors to sustain the first few developmental cleavage divisions[47]. Furthermore, in frogs – where this process has been studied intensively – parthenogenic development is terminated shortly after egg activation unless a centrosome is supplied artificially[9]. In cases where parthenogenic vertebrate eggs do develop in culture to the blastocyst stage, such as in mice and rabbits, a centriole and centrosome forms *de novo* early on within the zygote[17]. Finally, some fish, reptiles and amphibians have even evolved a strategy, termed gynogenesis, in which the sperm appears to contribute the centrosome required for development but not a pronucleus[48]. In nature, such populations arise mostly from hybridization between two closely related species, and most are triploid[49].

Concluding remarks

Traditionally, research on the centrosome in higher animals has focused its ability to nucleate and organize MT arrays during interphase and mitosis. It is now evident, however, that the centrosome is more than just an MTOC. In fact, although the MTOC functions of this organelle are its most visible activity, we argue that they are not essential for the survival and reproduction of individual cells. This is because, with the exception of cilia/flagella formation, functional MT arrays can be organized during interphase and mitosis in the absence of centrosomes by redundant pathways. The centrosome is, however, essential to the cell through its involvement in cell-cycle progression. Furthermore, the MTOC functions of the centrosome that are not essential at the level of the cell are, arguably, crucial for the survival and reproduction of the organism. A major challenge for the future will be to determine specifically what centrosomal component(s) are required for the G1–S transition and how they function in this process.

References

1 Wilson, E.B. (1911) *The Cell in Development and Inheritance*, The MacMillan Company

2 Debec, A. *et al.* (1995) Polar organization of gamma-tubulin in acentriolar mitotic spindles of *Drosophila melanogaster* cells. *J. Cell Sci.* 108, 2645–2653

3 Schnackenberg, B.J. and Palazzo, R.E. (1999) Identification and function of the centrosome centromatrix. *Biol. Cell*, 91, 429–438

4 Andersen, S.S.L. (1999) Molecular characteristics of the centrosome. *Int. Rev. Cytol.* 187, 51–109

5 Bobinnec, Y. *et al.* (1998) Glutamylation of centriole and cytoplasmic tubulin in proliferating non-neuronal cells. *Cell Motil. Cytoskeleton.* 39, 223–232

6 Piel, M. *et al.* (2000) The respective contribution of the mother and daughter centrioles to centrosome activity and behavior in vertebrate cells. *J. Cell Biol.* 149, 317–329

7 Roth, K.E. *et al.* (1988) Flexible-substratum technique for viewing cells from the side: some *in vivo* properties of primary (9+0) cilia in cultured kidney epithelia. *J. Cell Sci.* 89, 457–466

8 Hinchcliffe, E.H. and Sluder, G. (2001) 'It takes two to tango': Understanding how centrosome duplication is regulated throughout the cell cycle. *Genes Dev.* 15, 1167–1181

9 Kuntziger, T. and Bornens, M. (2000) The centrosome and parthenogenesis. In *The Centrosome in Cell Replication and Early Development* (Palazzo, R.E. and Schatten, G., eds), pp. 1–25, Academic Press

10 Bobinnec, Y. *et al.* (1998) Centriole disassembly *in vivo* and its effect on centrosome structure and function in vertebrate cells. *J. Cell Biol.* 143, 1575–1589

11 Maniotis, A. and Schliwa, M. (1991) Microsurgical removal of centrosomes blocks cell reproduction and centriole generation in BSC-1 cells. *Cell* 67, 495–504

12 Khodjakov, A.L. and Rieder, C.L. (2001) Centrosomes enhance the fidelity of cytokinesis in vertebrates and are required for cell cycle progression. *J. Cell Biol.* 153, 237–242

13 Hinchcliffe, E.H. *et al.* (2001) Requirement of a centrosomal activity for cell cycle progression through G1 into S phase. *Science* 291, 1547–1550

14 Sorokin, S.P. (1968) Reconstructions of centriole formation and ciliogenesis in mammalian lungs. *J. Cell Sci.* 3, 207–230

15 Dirksen, E.R. (1991) Centriole and basal body formation during ciliogenesis revisited. *Biol. Cell* 72, 31–38

16 Kubo, A. *et al.* (1999) Centriolar satellites: molecular characterization, ATP-dependent movement towards centrioles and possible involvement in ciliogenesis. *J. Cell Biol.* 147, 969–979

17 Szollosi, D. and Ozil, S.A. (1991) *De novo* formation of centrioles in parthenogenetically activated, diploidized rabbit embryos. *Biol. Cell* 72, 61–66

18 Rodionov, V.I. and Borisy, G.G. (1997) Self-centering activity of cytoplasm. *Nature* 386, 170–173

19 Borisy, G.G. and Rodionov, V. (1999) Lessons from the melanophore. *FASEB J.* 13, S221–S224

20 Schatten, G. *et al.* (1985) Microtubule configurations during fertilization, mitosis, and early development in the mouse and the requirement for egg microtubule-mediated motility during mammalian fertilization. *Proc. Natl. Acad. Sci. U. S. A.* 82, 4152–4156

21 Brunet, S. *et al.* (1998) Bipolar meiotic spindle formation without chromatin. *Curr. Biol.* 8, 1231–1234

22 Mazia, D. (1987) The chromosome cycle and the centrosome cycle in the mitotic cycle. *Int. Rev. Cytol.* 100, 49–92

23 Walczak, C.E. *et al.* (1998) A model for the proposed roles of different microtubule-based motor proteins in establishing spindle bipolarity. *Curr. Biol.* 8, 903–913

24 Dietz, R. (1966) The dispensability of the centrioles in the spermatocyte division of *Pales ferruginea* (Nematocera). In *Chromosomes Today* (Darlington, C.D. and Lewis, L., eds), pp. 161–166, Oliver and Boyd

25 Khodjakov, A. *et al.* (2000) Centrosome-independent mitotic spindle formation in vertebrates. *Curr. Biol.* 10, 59–67

26 Compton, D.A. (1998) Focusing on spindle poles. *J. Cell Sci.* 111, 1477–1481

27 Hyman, A.A. and Karsenti, E. (1996) Morphogenetic properties of microtubules and mitotic spindle assembly. *Cell* 84, 401–410

28 Marshall, W.F. *et al.* (2001) Kinetics and regulation of *de novo* centriole assembly: implications for the mechanism of centriole duplication. *Curr. Biol.* 11, 308–317

29 Pickett-Heaps, J. (1969) The evolution of the mitotic apparatus: an attempt at comparative ultrastructural plant cytology in dividing plant cells. *Cytobios* 3, 257–280

30 Busson, S. *et al.* (1998) Dynein and dynactin are localized to astral microtubules and at cortical sites in mitotic epithelial cells. *Curr. Biol.* 8, 541–544

31 O'Connell, C.B. and Wang, Y. (2000) Mammalian spindle orientation and position respond to changes in cell shape in a dynein-dependent fashion. *Mol. Biol. Cell* 11, 1765–1774

32 Savoian, M.S. *et al.* (2000) The rate of chromosome poleward motion is attenuated in *Drosophila zw10* and *rod* mutants. *Nat. Cell Biol.* 2, 948–952

33 Kaltschmidt, J.A. *et al.* (1999) Rotation and asymmetry of the mitotic spindle direct asymmetric cell division in the developing central nervous system. *Nat. Cell Biol.* 2, 7–12

34 Bonaccorsi, S. *et al.* (2000) Spindle assembly in *Drosophila* neuroblasts and ganglion mother cells. *Nat. Cell Biol.* 2, 54–56

35 Megraw, T.L. *et al.* (2001) Zygotic development without functional mitotic centrosomes. *Curr. Biol.* 11, 116–120

36 Hoyt, M.A. (2000) Exit from mitosis: spindle pole power. *Cell* 102, 267–270

37 Pereira, G. *et al.* (2000) The Bub2p spindle checkpoint links nuclear migration with mitotic exit. *Mol. Cell* 6, 1–10

38 Piel, M. *et al.* (2001) Centrosome-dependent exit of cytokinesis in animal cells. *Science* 291, 1550–1553

39 Nonaka, S. *et al.* (1998) Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking FIG3B motor protein. *Cell* 95, 829–837

40 Pazour, G.J. *et al.* (2000) *Chlamydomonas* IFT88 and its mouse homologue, polycystic kidney disease gene Tg737, are required for assembly of cilia and flagella. *J. Cell Biol.* 151, 709–718

41 Praetorius, H.A. and Spring, K.R.Bending the MDCK primary cilium increases intracellular calcium. *J. Membr. Biol.* (in press)

42 Puck, T.T. and Steffen, P. (1963) Life cycle analysis of mammalian cells. 1. A method for localizing metabolic events within the life cycle, and its application to the action of colcemid and sublethal doses of X-radiation. *Biophys. J.* 3, 379–397

43 Trielli, M.O. *et al.* (1996) Differential Taxol-dependent arrest of transformed and nontransformed cells in the G$_1$ phase of the cell cycle, and specific-related mortality of transformed cells. *J. Cell Biol.* 135, 689–700

44 Khodjakov, A. and Rieder, C.L. (1999) The sudden recruitment of γ-tubulin to the centrosome at the onset of mitosis and its dynamic exchange throughout the cell cycle, do not require microtubules. *J. Cell Biol.* 146, 585–596

45 Wigley, W.C. *et al.* (1999) Dynamic association of proteasomal machinery with the centrosome. *J. Cell Biol.* 145, 481–490

46 Palazzo, R.E. *et al.* (2000) Centrosome maturation. In *The Centrosome in Cell Replication and Early Development* (Palazzo, R.E. and Schatten, G., eds), pp. 449–470, Academic Press

47 Glover, D.M. (1991) Mitosis in the *Drosophila* embryo – in and out of control. *Trends Genet.* 7, 125–132

48 MacGregor, H.C. and Uzzell, T.M. (1964) Gynogenesis in salamanders related to *ambystoma jeffersonianum. Science* 143, 1043–1045

49 Cuellar, O. (1974) On the origin of parthenogenesis in vertebrates: the cytogenetic factors. *Am. Nat.* 108, 625–648

Forthcoming features in *Trends in Cell Biology*:

The November issue will contain 'A Trends Guide to Cancer Biology' – a collection of reviews on the latest advances in cancer research, covering diverse topics ranging from transcription and genomics to apoptosis and angiogenesis.

And the December issue is a special anniversary issue to mark the 10th birthday of *Trends in Cell Biology*. In this bumper issue, several experts in various fields across cell biology review highlights of the exciting developments in their field over the past 10 years.