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Functional Role of Centrosomes in Spindle Assembly and Organization

Hanne Varmark*

Programme of Cell Biology and Biophysics, European Molecular Biology Laboratory, Meyerhofstrasse, D-69117 Heidelberg, Germany

Abstract The centrosome is the main MT organizing center in animal cells, and has traditionally been regarded as essential for organization of the bipolar spindle that facilitates chromosome segregation during mitosis. Centrosomes are associated with the poles of the mitotic spindle, and several cell types require these organelles for spindle formation. However, most plant cells and some female meiotic systems get along without this organelle, and centrosome-independent spindle assembly has now been identified within some centrosome containing cells. How can such observations, which point to mutually incompatible conclusions regarding the requirement of centrosomes in spindle formation, be interpreted? With emphasis on the functional role of centrosomes, this article summarizes the current models of spindle formation, and outlines how observations obtained from spindle assembly assays in vitro may reconcile conflicting opinions about the mechanism of spindle assembly. It is further described how *Drosophila* mutants are used to address the functional interrelationships between individual centrosomal proteins and spindle formation in vivo. J. Cell. Biochem. 91: 904–914, 2004.

Key words: mitosis; meiosis; *Xenopus* egg extracts; *Drosophila* centrosome mutants

The role of centrosomes in cell division is a basic cell biological question that has occupied researchers for more than a century. In 1887 Van Beneden and Boveri formulated the classical view of centrosomes, which places this organelle as the cellular center of division that contains the spatial cues for cell division. Rephrased in modern terms, these authors proposed that centrosomes influence cell division by defining the shape of the bipolar MT spindle that segregates chromosomes and directs the cleavage furrow formation during cytokinesis [Wilson, 1900]. The notion that spindle formation relies on centrosome-associated MTs that bind to chromosomes was re-established by Mazia [1987] in the middle of the 20th century, and later on extended by the formulation of the "search and capture" model of spindle assembly by Kirschner and Mitchison [1986]. According to this model, a spindle forms when highly dynamic astral MTs nucleated by bipolar localized centrosomes get stabilized upon contact with kinetochores on chromosomes. This model predicts that centrosome-containing cells require these organelles for spindle formation. However, this is now being challenged from multiple sides. In this article I summarize and discuss the current concepts related to this intriguing problem associated with the study of cell division: The role of centrosomes in spindle formation.

Centrosome Dependent Astral Spindle Assembly

The centrosome is the principal MTOC in most somatic animal cells [Compton, 2000]. It is composed of stable core structures, the centrioles, which are surrounded by a proteinaceous material containing MT nucleating complexes and proteins that facilitate regulation of centrosome function according to the

Abbreviations used: MT, microtubule; MTOC, microtubule organizing center; PCM, pericentriolar material; γ-TuRC, γ-tubulin containing ring complex.

*Correspondence to: Hanne Varmark, Programme of Cell Biology and Biophysics, European Molecular Biology Laboratory, Meyerhofstrasse 1, D-69117 Heidelberg, Germany. E-mail: varmark@EMBL.de

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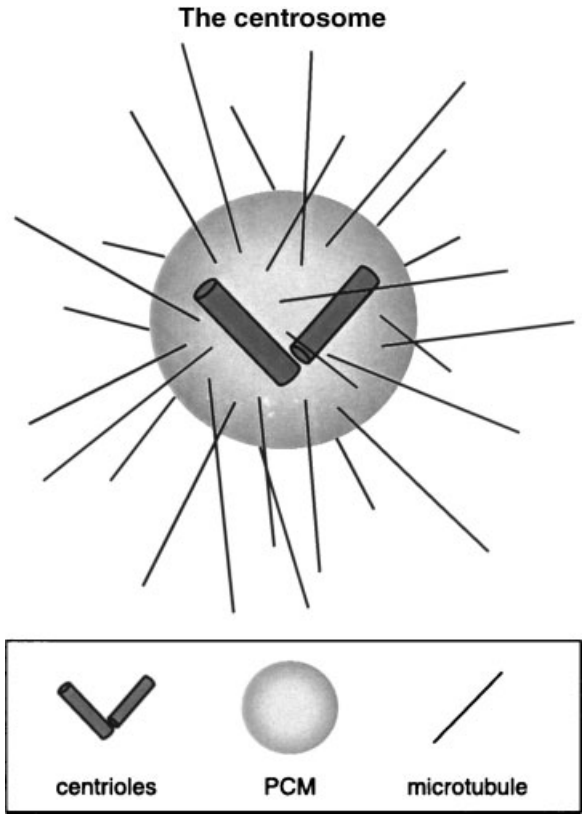

Fig. 1. The centrosome is the MT organizing center in animal somatic cells. It is composed of two centrioles surrounded by a protein matrix called the pericentriolar material (PCM). The PCM is a complex meshwork of proteins, including γ-tubulin containing ring complex (γ-TuRC) that nucleate the polymerization of MTs. For excellent reviews of the molecular composition of centrosomes, its regulation of MT nucleation, and its relation to cell signaling, please refer to Andersen [1999], Palazzo et al. [2000], and Lange [2002], respectively.

cell cycle (Fig. 1) [Andersen, 1999]. Spindles that form in the presence of centrosomes are described as "astral" (since they contain centrosome-nucleated, "astral" MTs at the spindle poles). The initial event in astral spindle assembly is nucleation of MTs from a pair of centrosomes migrating around the nucleus in early prophase. The centrosomes eventually position themselves on opposite sides of the nucleus, and during this process the centrosomes mature and acquire the capacity to nucleate dense arrays of short, unstable MTs known as "asters" (Fig. 2C). Astral MTs continuously grow and shrink from the centrosomes, and following nuclear envelope breakdown, a subset of these MTs attach to kinetochores, which are protein assemblies present on the centromeric DNA of replicated chromosomes. According to the "search and capture" model of spindle assembly, astral MTs get stabilized upon attachment to kinetochores, and a bipolar spindle shape is established by interaction of kinetochores on each replicated chromosome with astral MTs originating at both spindle poles (Fig. 2D,E) [Kirschner and Mitchison, 1986]. Eventually, the chromosomes align at the "metaphase plate," midway between the spindle poles, where their position is fixed by thick bundles of kinetochore MTs. At this stage (metaphase) the spindle is composed of different MT populations, including astral MTs, kinetochore MT fibres, and pole-to-pole MTs formed by anti-parallel overlap of astral MTs at the spindle midzone. The spindle in wild type cells is an inherent polar structure: the minus-ends of the MT polymers are focused at the spindle poles, while the plus-ends point towards the cell equator or are attached at kinetochores. This symmetrical arrangement of two opposing focal points of MT minus-ends is termed spindle bipolarity, and is absolutely essential for spindle function. When bipolarity is compromised, the chromosomes are not distributed equally among the daughter cells. After chromosome segregation in anaphase, the mitotic spindle disassembles and is replaced by the central spindle (Fig. 2H). This is a dense array of crosslinked, antiparallel MTs, which assembles at the equator of the cell and defines the position of the future cytokinesis furrow [Glotzer, 2001 and refs. therein].

Centrosomes and kinetochores provide the spatial cues for the establishment of bipolarity during astral spindle organization, but it is well established that MT motors are essential for spindle morphogenesis [Saunders et al., 1997b; Heald, 2000; Sharp et al., 2000b]. The minus-end directed motor Dynein is involved in the separation of centrosomes during prophase, possibly by pulling on astral MTs from its localization at the cell cortex [Vaisberg et al., 1993; Busson et al., 1998; Robinson et al., 1999; Sharp et al., 2000a]. Activity of plus-end directed, kinesin-like motors such as Eg5 and KLP61F are also required for separation of the spindle poles [Sawin et al., 1992; Heck et al., 1993; Blangy et al., 1995; Wilson et al., 1997]. These "bipolar" kinesins have motor domains at both ends of the molecule, and their plus-end motion along overlapping astral MTs allows the MTs to slide against each other. This activity may be part of the movement required to push the poles apart [Kashina et al., 1996; Sharp et al., 1999].

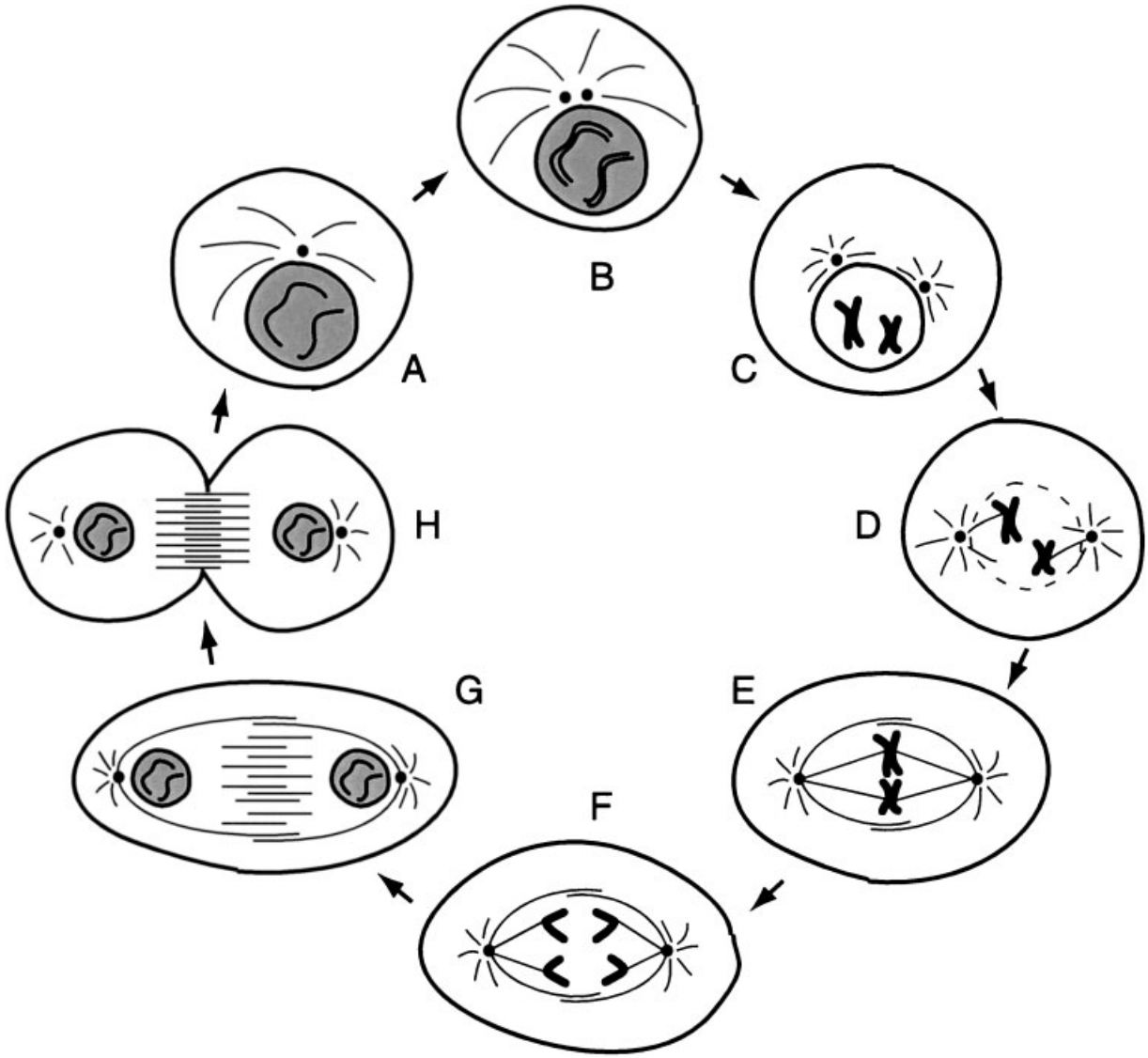

Fig. 2. Spindle organization in centrosome containing cells. Interphase cells contain a single centrosome onto which the long and stable MTs of the interphase cytoplasm focus (**A**). During S-phase, the centrosome duplicates concomitantly with DNA replication (**B**). During prophase, the duplicated centrosomes nucleate dense arrays of astral MTs and migrate around the nuclear envelope until they reach opposing sides of the nucleus (**C**). After nuclear envelope breakdown, elongating astral MTs nucleated from the bipolar localized centrosomes attach to the kinetochores of the condensed chromosomes (**D**). Eventually, each chomosome is attached to MTs originating from both spindle poles, and by metaphase all chromosomes are aligned at the spindle equator (**E**). At this stage, the spindle is composed of astral MTs, kinetochore MTs, and pole-to-pole MT fibres formed by antiparallel overlap of astral MTs from opposite spindle poles. Spindle formation in this figure is presented according to the search and capture model of astral spindle assembly [Kirschner and Mitchison, 1986]. Note however, that an alternative model of astral spindle formation has been proposed (Fig. 3B). After chromosome segregation in anaphase (**F**), the chromosomes decondense to form two new daughter nuclei (Grey nuclei in G, H, A, B represent decondensed chromatin). After anaphase, the mitotic spindle is disassembled, and a belt-like array of anti-parallel MTs called ''the central spindle'' is assembled at the cell equator (**G**). In telophase, the cytokinesis furrow constricts the cell at the site of the central spindle (**H**), and completion of cytokinesis produces two new daughter cells (A).

This separation activity is counter-balanced by the minus-end directed, kinesin-like motor Ncd, which creates a force on overlapping astral MTs that pulls the asters together [Sharp et al., 2000a]. Thus, antagonistic activities of plus-end directed and minus-end directed motors are required to maintain correct spatial organization of spindle poles, although the exact mechanisms remain unclear [Nedelec, 2002]. Motors localizing to chromosomes ("chromokinesins"

such as Xklp1, KLP38B, and XKid) have been reported to be involved in spindle organization and chromosome positioning [Vernos et al., 1995; Molina et al., 1997; Antonio et al., 2000], but how spindle MTs interact with chromosomes during astral spindle assembly is generally poorly understood.

The bipolar spindle structure established by centrosomes, chromosomes, and molecular motors is maintained by proteins that crosslink and stabilize MTs at the spindle poles. The structural protein NuMA is transported to spindle poles by Dynein [Gaglio et al., 1997; Merdes et al., 2000] and is involved in the establishment and maintenance of focused spindle poles, possibly via its MT bundling activity [Yang and Snyder, 1992; Haren and Merdes, 2002; Levesque et al., 2003]. The MT binding protein Msps/XMAP215, which concentrates at the spindle poles by interaction with the centrosomal protein D-TACC, has been proposed to maintain spindle pole bipolarity by stabilizing MTs released from centrosomes [Cullen et al., 1999; Gergely et al., 2000; Lee et al., 2001]. So clearly, the formation of a functional bipolar spindle involves multiple factors. The relative role of centrosomes, chromosomes, MT motors, cross-linking proteins, and proteins that regulate MT stability during spindle assembly is still an open question. This problem has been difficult to address due to the existence of multiple forces that simultaneously act on the MTs during spindle formation. Because of the prominent position of the centrosome in astral spindle morphogenesis, the role of this organelle in spindle assembly and organization has been a classical question in cell biology.

Centrosomes have been reported to be essential for spindle formation in several systems. When centrosomes were removed by micromanipulation during prophase in grasshopper spermatocytes, no spindle assembled even though the chromosomes were shown to have a MT stabilizing activity in these cells [Zhang and Nicklas, 1995a,b]. In sea urchin eggs fertilized under conditions that kept the male and female pronucleus separated, a functional bipolar spindle was formed around the centrosome-containing male pronucleus, while no MTs were organized around the female pronucleus. This suggests that centrosomes are needed for spindle assembly in this mitotic system [Sluder and Rieder, 1985].

Centrosome Independent Anastral Spindle Formation

The requirement for centrosomes in spindle assembly is not universal. In several naturally occurring systems, such as cells of higher plants and some female meiotic systems, spindles form in the absence of any MT organizing center to define the position of the spindle poles [Szollosi et al., 1972; Mahowald and Kambysellis, 1980; Vaughn and Harper, 1998]. Such spindles are anastral, and they assemble via an "inside-out" pathway (Fig. 3A). In *Drosophila* oocytes, the breakdown of the nuclear envelope at the onset of meiotic spindle assembly exposes the condensed chromosomes to a cytoplasm that contains relatively long MTs. The first step in spindle formation is the appearance of MTs of unknown origin around the chromatin, followed by organization of a bipolar MT array around the chromosomes [Theurkauf and Hawley, 1992]. A similar spindle morphogenesis is observed in meiosis I in oocytes of mouse, *C. elegans*, and *Xenopus laevis* [Gard, 1992; Albertson and Thomson, 1993; Brunet et al., 1999].

MT nucleation in the absence of centrosomes is poorly understood, but the appearance of MTs around the chromosomes at the initial stage of spindle formation has led to the proposal that chromatin facilitates MT nucleation in such systems [McKim and Hawley, 1995; Matthies et al., 1996]. Spindle assembly assays using *Xenopus* egg extracts have demonstrated that chromatin can serve as a template for MT nucleation and stabilisation in vitro [Heald et al., 1996]. Moreover, chromosomes in the meiotic cytoplasm of insect spermatocytes have been shown to stabilize MTs [Church et al., 1986; Zhang and Nicklas, 1995a; Fuge, 1999]. Such observations are compatible with a model of anastral spindle assembly, according to which chromosomes are the source of spindle MTs [Karsenti et al., 1984; Steffen et al., 1986]. However, the only currently identified effect that chromosomes have on spindle organization in *Drosophila* female meiosis is via chiasmata (the physical points of crossing over during genetic exchange between bivalent homologs), and the chromokinesin motor Nod. These components facilitate chromosome opposition (proper pairing and alignment), which has been proposed to be required for assembly of the female meiotic spindle [Theurkauf and Hawley,

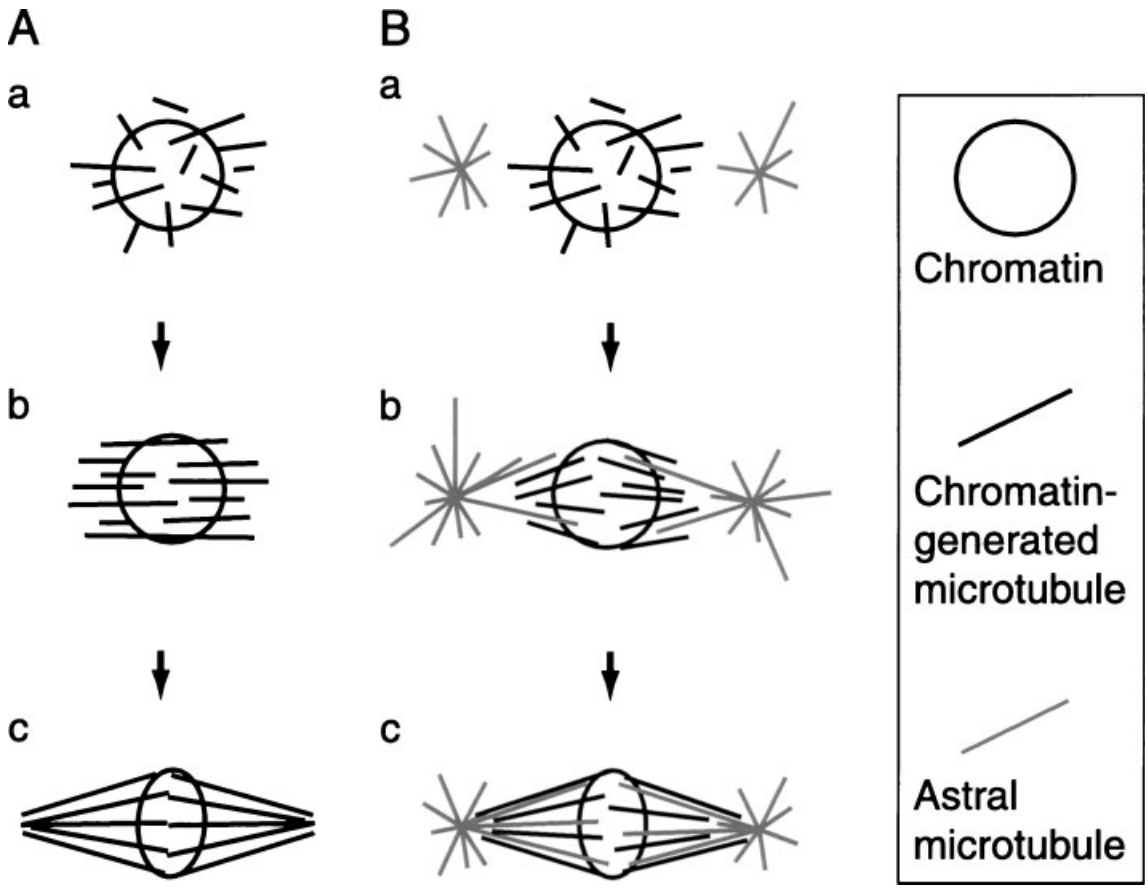

Fig. 3. The current model of anastral spindle assembly (**A**), presented along with a hypothesis of astral spindle formation (**B**). **A**: The current model of anastral spindle assembly is based on spindle assembly assays in *Xenopus* egg extracts and cytological examination of anastral spindle assembly in female meiotic systems. According to this model (A), anastral spindle assembly is initiated by nucleation of MTs in the vicinity of the condensed chromatin (A,a). The MTs then attach to and congress around the chromatin due to activity of molecular motors (A,b). A bipolar spindle is formed by focusing of the terminal ends of the MT array into spindle poles by molecular motors and cross-linking proteins (A,c). **B**: Contrary to the search and capture model of astral spindle assembly (presented in Fig. 2D–E), which proposes that astral MTs are the main source in bipolar spindle establishment, it has been speculated that astral spindles might be a mixture of two MT populations: (1) MTs nucleated in the vicinity of chromosomes (black MTs in B,a) and (2) astral MTs nucleated from the centrosomes (grey MTs in B,a) [Gruss et al., 2002]. According to this model, an astral spindle is formed by the progressive tethering together of chromatin generated MTs and astral MTs into one bipolar structure (B,b–c).

1992; Afshar et al., 1995; McKim and Hawley, 1995]. However, how chromosomes influence MT generation and organization during anastral spindle formation in vivo is currently not clear. Interestingly, several proteins involved in centrosomal MT nucleation and astral spindle pole formation have been identified in acentrosomal systems, even though they were not found to be associated with any discrete MTOC structure. Such proteins include γ-tubulin [Gueth-Hallonet et al., 1993; Tavosanis et al., 1997; Vaughn and Harper, 1998], Pericentrin [Doxsey et al., 1994], and the *Drosophila* proteins Abnormal spindle (Asp) and D-TACC [Cullen and Ohkura, 2001; Riparbelli et al., 2002]. These proteins were either localised to the anastral spindles or shown by genetic analysis to be involved in anastral spindle formation. Such observations suggest that several of the centrosome associated proteins involved in astral spindle organisation might operate in acentrosomal spindle assembly as well, although via mechanisms that currently cannot be fully explained.

Analyses of anastral spindle assembly in *Xenopus* egg extracts and *Drosophila* female meiosis have suggested that MT organisation in the absence of MTOCs rely on motors, several of which also operate during astral spindle assembly. Chromokinesins (like Nod, Xklp1, and Xkid) provide links between the MTs and chromatin, and possibly push the MT minus-

ends away from the chromatin [Afshar et al., 1995; Vernos et al., 1995; Antonio et al., 2000]. Bipolar kinesins like Eg5 crosslink the chromatin-associated MTs into antiparallel bundles and bring the plus-ends of the overlapping MTs together at the spindle equator [Sawin et al., 1992]. The terminal regions of this MT array are then focused into spindle poles by the activity of Dynein and crosslinking proteins [Heald et al., 1996; Walczak et al., 1998]. In *Drosophila* female meiosis, the minus-end directed motor Ncd transports Mini-spindles protein (Msps) to the spindle poles where it is anchored by D-TACC and involved in spindle pole stabilisation [Cullen and Ohkura, 2001].

In summary, two kinds of spindles exist: astral and anastral. The search and capture model describes centrosome dependent, astral spindle assembly, while the model of chromatin driven MT generation (followed by bipolar spindle organisation by motor proteins) traditionally has been assigned to anastral spindle formation in centrosome free systems. However, a key question is whether the centrosome independent spindle assembly pathways found in acentrosomal cells are unique to such systems, or also do operate in centrosome containing systems.

Centrosome Independent Spindle Assembly can be Activated Upon Experimental Removal of Centrosomes

The existence of a centrosome independent spindle formation machinery in centrosome containing cells has been suggested by several observations. When centrosomes were removed by microsurgery from BSC-1 cells before completion of S-phase, or were destroyed by laser-ablation in CVG-2 cells in prophase, functional bipolar spindles were organised in the resulting centrosome free karyoplasts [Khodjakov et al., 2000; Hinchcliffe et al., 2001]. However, 30–50% of the karyoplasts did not complete cytokinesis and all cells arrested in the G1 phase of the following cell cycle [Hinchcliffe et al., 2001; Khodjakov and Rieder, 2001]. In parthenogenetic Sciara embryos, development is initiated in the complete absence of centrosomes. Such embryos assemble functional spindles, but have irregularly spaced nuclei and eventually arrest at early stages of development [de Saint Phalle and Sullivan, 1998]. The establishment of a centriole-free *Drosophila* cell line also suggested that centrosomes can be dispensible for spindle formation in centrosome containing cells [Debec, 1978; Debec et al., 1995]. Such studies demonstrate that centrosome-independent spindle assembly pathways can be activated in some centrosome containing cells, and suggest that centrosomes are needed for cell cycle progression and development, but not for spindle assembly in some systems [Rieder et al., 2001].

Relative Role of Centrosomes and Chromosomes in Spindle Assembly

The presence of centrosome-independent spindle assembly pathways in cells that usually form astral spindles might seem odd. However, such observations can be explained by either of two speculative scenarios, which assign different relative roles to centrosomes in the spindle assembly process. Firstly, it has been suggested that a centrosome independent spindle assembly pathway exists in centrosome containing cells, but is only evident when the centrosomes are removed or non-functional [Heald et al., 1997]. This centrosome independent spindle formation pathway has been proposed to rely on a local MT stabilising environment established in the vicinity of chromosomes by a chromatin induced gradient of Ran-GTP [Carazo-Salas et al., 1999; Gruss et al., 2001; Kalab et al., 2002]. In the presence of centrosomes this pathway might be overridden, since the centrosomes act as kinetically dominant spindle pole organisers [Heald et al., 1997; Hyman and Karsenti, 1998]. This scenario suggests that a centrosome independent spindle assembly pathway exists in centrosome containing cells, but is only active in absence of functional centrosomes.

Alternatively, the centrosome-independent spindle assembly machinery might operate in parallel to the centrosome-dependent spindle formation pathway during astral spindle formation. In other words, capture of astral MTs by kinetochores might happen simultaneously with chromatin-induced nucleation of MTs around the chromosomes. A spindle would then result from the tethering together of these two MT populations into one bipolar structure (Fig. 3B) [Gruss et al., 2002]. If this scenario reflects what happens in vivo, the variation in the relative contributions of these two pathways (centrosomal MT nucleation versus chromatin-driven MT generation) could explain the different requirements of centrosomes observed

among spindle formation in different organisms and cell types. To which extent spindles might be a mixture of chromosome- and centrosome-nucleated MTs is difficult to address by microscopy, since the individual MTs and their origin cannot be resolved within the assembling spindle. It is possible that these two different but simultaneously operating pathways might be influenced by a common factor. It has been proposed that the MT stabilising gradient of Ran-GTP around chromatin could support both MT nucleation in the vicinity chromosomes and facilitate preferential growth of the astral MTs towards the chromosomes [Carazo-Salas et al., 2001]. That is, rather than growing and shrinking randomly in all directions until the astral MTs randomly hit a chromosome, they might be guided to elongate preferentially towards the chromosomes, directed by the field of MT stabilising factors around the chromatin [Carazo-Salas and Karsenti, 2003; Hyman and Karsenti, 1996]. Such a role for Ran-GTP in astral spindle assembly in vivo is supported by studies showing that astral MTs did not organise into a spindle around chromosomes when the Ran-GTP pathway was disrupted by RNAi in *C. elegans* embryos [Askjaer et al., 2002; Bamba et al., 2002].

Drosophila Centrosome Mutants: Addressing the Role of Individual Centrosomal Proteins in Spindle Formation In Vivo

To fully understand the role of centrosomes in spindle formation, the molecular mechanisms by which this organelle influences MT organisation must be characterised in better detail. To this aim, analysis of mutations in centrosomal proteins that affect spindle assembly and organisation in *Drosophila* has been used to dissect out the specific roles of individual centrosomal proteins in spindle formation in vivo.

γ-tubulin is recruited abundantly to the centrosome at the onset of mitosis [Khodjakov and Rieder, 1999] and is part of a complex (the γ-TuRC), which has a well-established role in MT nucleation [Wiese and Zheng, 1999 and refs therein]. Overexpression of γ-tubulin in COS cells produces ectopic MT nucleation in the cytoplasm [Shu and Joshi, 1995], while inhibition or depletion of γ-tubulin in *Xenopus* egg extracts inhibits nucleation of asters from sperm centrosomes [Stearns and Kirschner, 1994]. In yeast, mutations in the γ-tubulin gene has been shown to produce defects in spindle organisation during mitosis [Horio et al., 1991]. Thus, a significant body of cytological, genetic, and biochemical data has demonstrated that γ-tubulin is involved in MT nucleation and spindle organisation.

The *Drosophila* mutant allele γ-Tub23C^{P1} produces severe loss of function of the γ-tubulin gene [Sunkel et al., 1995], and thus provides a system for the study of MT organisation in a genetic background of severe γ-tubulin depletion. Surprisingly, γ-Tub23C^{P1} spermatocytes were able to nucleate a high number of astral MTs even though they lacked detectable levels of γ-tubulin [Sampaio et al., 2001]. However, the initially well separated centrosomes in these cells collapsed together during prometaphase, and the abundant MT population never organised into a bipolar spindle. Thus, in *Drosophila* spermatocytes, wild type levels of γ-tubulin are needed for maintenance of centrosome separation and bipolar spindle formation, but the centrosomes are able to support a high number of astral MTs despite being depleted of γ-tubulin. Similar observations were made in *C. elegans* embryos depleted of γ-tubulin by RNAi [Strome et al., 2001], and in *Drosophila* dd4 mutant spermatocytes, in which Dgrip91 (another γ-TuRC protein) was disrupted [Barbosa et al., 2003]. That is, either γ-tubulin is in vast excess in the wild type, or alternatively, centrosomal MT nucleation might not be entirely dependent on γ-tubulin in these systems.

In cytological studies, γ-tubulin is widely used as a characteristic marker of functional centrosomes. When analysing phenotypes of centrosome mutants, the absence of detectable levels of centrosomal γ-tubulin has frequently been interpreted to indicate a lack of assembly of functional centrosomes. However, γ-tubulin depleted centrosomes in γ-Tub23C^{P1} spermatocytes contained no detectable levels of γ-tubulin, but were structurally well organised and capable of nucleating a high number of astral MTs [Sampaio et al., 2001]. This demonstrates that the lack of detectable levels of γ-tubulin at the centrosomes does not necessarily imply a lack of centrosome function. So far, no viable mutation has been isolated that inhibits centrosome function completely. The mutations in centrosomal proteins generated so far disrupt only some aspects of centrosome structure and function, which should be kept in mind when using mutant phenotypes to draw conclu-

sions about the role of centrosomes in spindle assembly.

Drosophila mutants that eliminate detectable levels of both γ-tubulin and astral MTs have been isolated and are of special interest, since they provide the opportunity to address the role of astral MTs in spindle assembly in vivo. Immunofluorescence studies using fixed material suggest that neuroblasts in the asterless (asl) and centrosomin (cnn) mutants form functional spindles in the absence of γ-tubulin and astral MT arrays [Bonaccorsi et al., 2000; Megraw et al., 2001]. Whether this observation reflects an ability of centrosomes to organise spindles despite severely reduced number of astral MTs, or demonstrates that spindles can assemble in absence of centrosome function, is still an open question since the status of the centrosomes in these mutants has not been fully characterised.

The *Drosophila* protein Asp is a MT binding protein that localizes to centrosomes, spindle poles, and to the central spindle during mitosis [Saunders et al., 1997a; do Carmo Avides and Glover, 1999; Riparbelli et al., 2002]. Asp has been shown to facilitate MT nucleation at mitotic centrosomes, and mutations in Asp produced spindle organization defects [Gonzalez et al., 1990; do Carmo Avides and Glover, 1999; Riparbelli et al., 2002]. The current hypothesis is that Asp is required together with γ-tubulin to organize MT asters, and that it has subsequent functions in the stabilization of spindle poles and the central spindle. The centrosomal function of Asp during mitosis has been proposed to be stimulated by the Polo kinase [do Carmo Avides et al., 2001].

After the mitotic spindle has assembled, the centrosome has been shown to contribute to the maintenance of spindle bipolarity by concentrating MT stabilizing proteins at the spindle poles. Analysis of the *Drosophila* mutants d-tacc and msps has shown that the MT binding protein Msps/XMAP215 interacts with the centrosomal protein D-TACC to stabilize MTs at the spindle poles [Cullen et al., 1999; Lee et al., 2001]. Localization of D-TACC at the centrosome was disrupted in AuroraA mutants [Giet et al., 2002], suggesting that the ability of centrosomes to modulate MT dynamics at the spindle poles is regulated by mitotic kinases.

After anaphase, a central spindle assembles midway between the two asters, which defines the position of the future cytokinesis furrow (Fig. 2G,H) [Giansanti et al., 1998]. asl mutant spermatocytes nucleate severely reduced asters and assemble morphologically normal central spindles and cytokinesis furrows that can be asymmetrically localized with respect to the spindle poles [Bonaccorsi et al., 1998]. Thus in these cells, wild type levels of astral MTs are not needed for initiation of cytokinesis or assembly of a central spindle but may be important for the symmetrical positioning of these structures.

Bipolar Monastral Spindles: Transient Centrosome Disorganization or Differentially Organized Spindle Poles?

Mutations have been identified that uncouple spindle bipolarity from bipolar localization of centrosomes within centrosome containing cells. The mutant alleles ms(1)516, ms(1)RD7 (not cloned), and KLP61F (BimC kinesin-like motor protein) produce bipolar, monastral spindles. Such spindles have one astral, centrosome containing spindle pole, while the second pole is anastral and centrosome-free [Lifschytz and Hareven, 1977; Lifschytz and Meyer, 1977; Wilson et al., 1997]. Formation of bipolar monastral spindles might reflect a dynamic centrosome organization within mutant cells, causing one of the centrosomes to disintegrate temporarily and partially after the spindle bipolarity has been established. This model suggests that the centrosome, rather than being a permanent cellular structure, might be able to disorganize transiently [Wilson et al., 1997]. Alternatively, in mutant cells with a single centrosome (or a cluster of unseparated centrosomes), the chromosomes might first attach to the astral pole in a monastral MT array and subsequently facilitate their own biorientation by inducing the formation of an anastral spindle-half [Fuge, 1999]. This model proposes that the two poles within a bipolar monastral spindle are formed in different ways and therefore are not similar.

Concluding Remarks

Numerous cell biological studies have addressed the requirement of centrosomes in spindle assembly, and observations obtained from different systems suggest mutually incompatible conclusions. This might be attributed to the different experimental strategies applied, or indicate that spindle formation in different systems has varying requirements for centrosomes. While in vitro spindle assembly assays

have allowed for biochemical approaches to identify mechanisms of spindle assembly, analysis of *Drosophila* mutants has begun to dissect the specific roles of the individual centrosomal proteins in spindle organization in vivo. A major limitation in the study of centrosome function is that only a fraction of the centosomal proteins has been identified. Proteomic approaches based on purified centrosomes will hopefully contribute to solving this problem. Since spindle assembly is a dynamic process, a stronger emphasis on real time analysis using improved optics and fast time-lapse acquisition should facilitate better resolution of the assembling spindle and circumvent the potential misjudgments involved in inferring a dynamic process from fixed material. The seemingly opposing conclusions that characterize the field at the moment are an exciting invitation to continue the search for the missing links that will complete the picture. Carefully performed cytological analysis, as initiated by our colleagues more than 100 years ago, in combination with modern genetic and biochemical approaches should eventually provide us with a rather complete view of the functional interrelationships between centrosomes and spindle organization.

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