Molecular Characteristics of the Centrosome

Søren S. L. Andersen
Department of Molecular Biology, Princeton University,
Princeton, New Jersey 08540-1014

As an organizer of the microtubule cytoskeleton in animals, the centrosome has an important function. From the early light microscopic observation of the centrosome to examination by electron microscopy, the centrosome field is now in an era of molecular identification and precise functional analyses. Tables compiling centrosomal proteins and reviews on the centrosome are presented here and demonstrate how active the field is. However, despite this intense research activity, many classical questions are still unanswered. These include those regarding the precise function of centrioles, the mechanism of centrosome duplication and assembly, the origin of the centrosome, and the regulation and mechanism of the centrosomal microtubule nucleation activity. Fortunately, these questions are becoming elucidated based on experimental data discussed here. Given the fact that the centrosome is primarily a site of microtubule nucleation, special focus is placed on the process of microtubule nucleation and on the regulation of centrosomal microtubule nucleation capacity during the cell cycle and in some tissues.

KEY WORDS: Centrosome, Microtubule-organizing center, Centriole, Assembly, Cell cycle, Microtubule, Mitotic spindle, Spindle pole body, Microtubule nucleation.

I. Introduction

No element of the cell has aroused a wider interest of late than the remarkable body known as the *centrosome,* which is now generally regarded as the especial organ of cell-division and in this sense the *dynamic centre* of the cell—Wilson (1896, p. 36). More than 100 years later this organelle

still "arouses" as much interest as ever before, as evident by the fact that an international meeting held in 1997 was entirely devoted to the centrosome (Stearns and Winey, 1997).

It is interesting to briefly review the historical development of the centrosome field (Fulton, 1971). Initially, light microscopists observed centrosomes (Fig. 1) until the advent of electron microscopy in the 1950s. Studies during these early phases were mostly descriptive, and the lack of techniques to complete the analysis led to a field characterized by much philosophical and mystical thinking. In particular, the replication process of this organelle was a matter of speculation. Ever since its discovery, it was described as an "autonomous organelle" which implies that it is capable of self-reproduction. The ideas regarding centrosomal self-replication were fueled by the discovery of the DNA double helix, and evidence for the direct involvement of either DNA or RNA as templates during centriole duplication has often been reported (Hall *et al.,* 1989; Heidemann *et al.,* 1977; Huang, 1990; Johnson and Rosenbaum, 1990). However, careful studies on somatic cells have shown that the pericentriolar matrix is practically devoid of nucleic acids (Dirksen, 1991). Today, we can with certainty say that centrioles do not contain DNA or RNA required for their duplication (Dirksen, 1991).

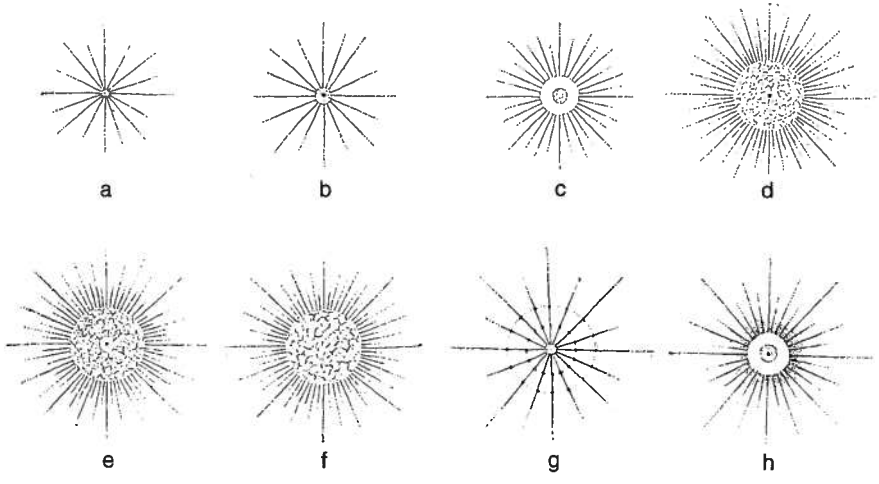


FIG. 1 Diagrams illustrating various descriptions of the centrosome and centrosphere. (a) Simplest type; only a minute centrosome at the focus of the rays (sperm-aster in many forms). (b) Rays proceeding directly from a centrosome of considerable size within which a central granule resides. (c) Rays proceeding from a clear centrosphere, enclosing a centrosome like that in b but with no central granule. (d) An extremely minute centrosome lying in the middle of a large reticulated centrosphere. (e) Like that in d, but with a small spherical body surrounding the centrosome. (f) No centrosome as distinguished from the reticulated centrosphere. (g) The centrosome contains a central granule or centriole (cf. b); outside this is a clear zone (medullary zone of Van Beneden), and outside this is a vaguely defined granular zone, probably corresponding to Van Beneden's cortical zone. (h) The same as g, only according to Boveri (reproduced from Wilson, 1896).

The nomenclature of the centrosome has also been heavily debated through the years (Wilson, 1896, pp. 36 and 232; see also legend to Fig. 1 and Bornens, 1992). The nomenclature adopted here, with which most people would agree, is the following (see also Section II and Figs. 2–6): First, a *centrosome* consists of a pair of centrioles with an associated proteinaceous *pericentriolar matrix* (PCM). Second, a *centriole* is a microscopic structure composed of nine groups of microtubules (MTs) (9+0 configuration). Third, a *basal body* is a centriole bearing a flagellum or cilium (at the transition zone between basal body and flagella two extra MTs originate at the centriole center giving rise to the so-called 9+2 configuration of the flagellum; Fulton, 1971; Gibbons and Grimstone, 1960). Fourth, a MT-organizing center (MTOC) is a structure capable of organizing and nucleating MTs (Pickett-Heaps, 1969; Porter, 1966). In fungi the centrosome is called the "spindle pole body" (SPB) and is the primary MT-nucleating center. All centrosomes are MTOCs, but MTOCs that are not centrosomes are frequently encountered. It is now clear that an MTOC alone can organize the MTs required for cell division (Heald *et al.,* 1997; Lambert, 1993). Thus, a centrosome is not essential for cell division but when present it is dominant over other MTOCs (Heald *et al.,* 1997; González *et al.,* 1998). Therefore, although the centrosome is important it is not "the organ of cell division *par excellence*" (Wilson, 1896, p. 85).

The descriptive phase lasted approximately 100 years until the molecular-biological revolution at the beginning of the 1970s. The past 10 years in particular have been the decade of molecular identification. With increasingly powerful molecular and genetic techniques, more proteins have been described as "centrosomal." The number of centrosomal proteins now form an impressive list (Table I). The latest approach consists of purification of the organelle, separation of associated proteins by gel electrophoresis, and identification by mass spectroscopy via computer-linked gene databases (Wigge *et al.,* 1998). With such techniques new proteins have been identified and added to the already impressive long list of centrosomal proteins (Table I; because this review is not on the yeast SPB only very few SPB components are included in Table I).

The centrosome equivalent in yeast is called the SPB. Because of the ease with which genetics can be performed, yeast has proven very powerful at identifying components and regulatory mechanisms for SPB duplication. It is certain that yeast will continue to yield important information on SPB components and duplication. These discoveries may be applicable to the vertebrate centrosome. At least three aspects of similarities can be com-

pared. First, it is certain that the process of MT nucleation is conserved between the SPB and the centrosome (i.e., the components γ-tubulin, Tub4, Spc97, and Spc98; Table I; see Section III; Stearns and Winey, 1997). Second, the machinery that regulates SPB duplication also shares components with the centrosome duplication machinery just like the components that regulate the cell cycle in yeast and animal cells share components with each other. Third, the biggest differences are probably between the proteins actively duplicating and assembling SPBs and centrioles. Because comparing the SPB and the centrosome is a review in itself, and also because there are recent reviews in this series and elsewhere on the topic (Balczon, 1996; Table II), this review will primarily focus on the vertebrate centrosome.

The most important function of the centrosome is to be a focal dominant point for MT polymerization. In most cells the centrosome is localized at the perinuclear region during interphase. During mitosis the duplicated centrosomes form the poles of the spindle (see Fig. 12). When talking about MTs, it is important to grasp the special polymerization properties of MTs, described by a concept called "dynamic instability." In the dynamic instability model (Erickson and O'Brien, 1992; Kirschner and Mitchison, 1986), MTs polymerize with a certain "growth rate" (v_g) and depolymerize with a certain "shrinkage rate" (v_s). The transition from growth to shrinkage is called a "catastrophe" (McIntosh, 1984) and occurs with a certain frequency (f_{cat}). The opposite event, from shrinkage to growth, is called a "rescue" and occurs with a frequency (f_{res}) (Walker *et al.,* 1988). By modulating these four parameters, and in particular the catastrophe frequency, the cell manages to very efficiently control the amount of MT polymerization across the cell cycle (Verde *et al.,* 1992; Belmont *et al.,* 1990). Recently, the importance of control of MT polymerization via "treadmilling" in addition to dynamics has been demonstrated (Rodionov and Borisy, 1997; Margolis and Wilson, 1998). For example, treadmilling is a way to regulate dynamics of MTs in the cytoplasm after ejection from the centrosome (Rodionov and Borisy, 1997; Keating *et al.,* 1997; see Section V,C). Moreover, spindle MT flux is also dependent on treadmilling (Waters *et al.,* 1996). However, the most powerful mechanism of MT assembly regulation occurs via regulation of the four parameters in the dynamic instability model. This is because regulation of the catastrophe frequency can drastically determine the final average length of a population of MTs (Verde *et al.,* 1992). Treadmilling, MT dynamics, and their regulation will not be discussed further in this context.

Nucleation of MTs is the first step in the formation of MTs and precedes MT assembly. Thus, regulation of nucleation is another important step in MT formation. Although the "nucleation frequency" is not part of the dynamic instability model, this frequency could actually be included as a

fifth parameter. Because of its importance for MT dynamics regulation, much of this review will be devoted to the process of MT nucleation.

It is certain that the centrosome will continue to attract biologists' attention for years to come. Thus, one may ask the following fundamental questions: How does a centrosome assemble? What is the function of the centrioles? How are centrioles duplicated? What is the evolutionary origin of centrosomes and centrioles? How are MTs nucleated by the centrosome? How much is dynamic instability influenced and regulated by the centrosome through regulation of MT minus-end dynamics? These are questions that have puzzled cell biologists since the discovery of the centrosomes by Van Beneden and Boveri 110 years ago (Wilson, 1925). Numerous reviews, some of which are listed here (Table II), have through history summarized results regarding these questions.

In the history of reviews (Table II), this review belongs to the molecular biological era of analyses of centrosome function through the combined power of biochemistry, genetics, and advanced microscopy. This review contains the classical sections encompassing definitions and descriptive morphological studies of the centrosomes. Emphasis is put on centrosomal components, possible molecular mechanisms of centriole and centrosome assembly, and MT nucleation. Readers interested in the *Saccharomyces cerevisiae* SPB should consult other reviews (Table II).

II. On the Origin and Low-Resolution Structure of the Vertebrate Centrosome

Regarding the centrosome structure, it is worth noting that the first studies using light microscopy were rather remarkably accurate (Fig. 1). With electron microscopy it became possible to visualize the centrioles, which are at the heart of the centrosome. Centrioles look like barrels surrounded by proteinatious material, the so-called "pericentriolar matrix" (Fig. 2). In recent years we have gained much insight into the constituents, organization, and regulation of this matrix (see Section III). In Section IV, I discuss how this matrix assembles onto the centrioles and concentrate on centriolar structure.

There are numerous excellent reports on centriole structure, and they are in agreement to a certain level of resolution (Alvey, 1985; Chrétien *et al.,* 1997; Fuller *et al.,* 1995; Kenny *et al.,* 1997; Kuriyama and Borisy, 1981; Paintrand *et al.,* 1992; Rieder and Borisy, 1982; Tournier *et al.,* 1991a; Wilsman and Farnum, 1983). Centrioles are mostly observed in pairs and are never more than 1 μm apart (Fig. 2; Tournier *et al.,* 1991a). The length of the mature centrioles is constant but cell type dependent and in the

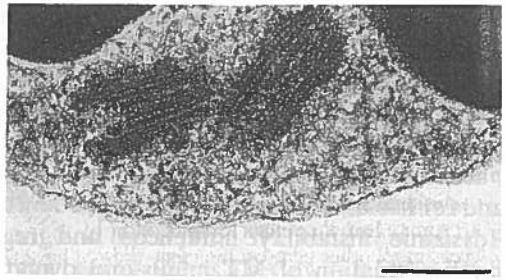


FIG. 2 Electron micrograph showing the structural organization of mouse thymocyte centrioles in centrosomes *in situ*. Scale bar = 0.5 μm. Note the nonlinear arrangement (reproduced from Tournier *et al., The Journal of Cell Biology,* 1991, **113,** 1361–1369 by copyright permission of The Rockefeller University Press).

range of 0.1–0.7 μm (Kenny *et al.,* 1997). The centrioles are linked to each other by filamentous and amorphous material (Figs. 3 and 4). The two centrioles of the pair most frequently adopt a nonlinear conformation with respect to each other. This means that the axis of one centriole is not parallel with the axis of the other centriole (Fig. 2). A centriole is like a barrel, with the sides made of MTs. The diameter of the barrel is 225–335 nm (Chrétien *et al.,* 1997) or 9–13 times the diameter of a single MT. To describe the orientation of the one barrel with respect to the other,

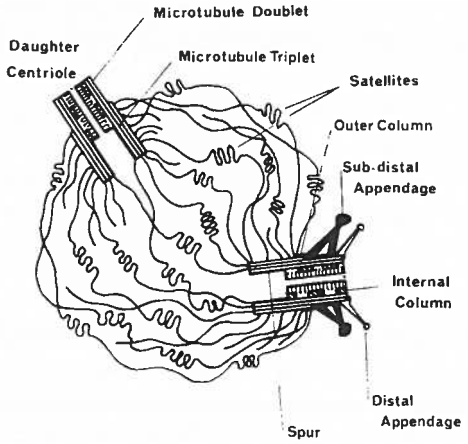


FIG. 3 Schematic representation and nomenclature of centriole organization and architecture. Note the appendages associated with the mother centriole and the filamentous material linking the two centrioles (reproduced from Paintrand *et al.,* 1992, with permission from Academic Press).

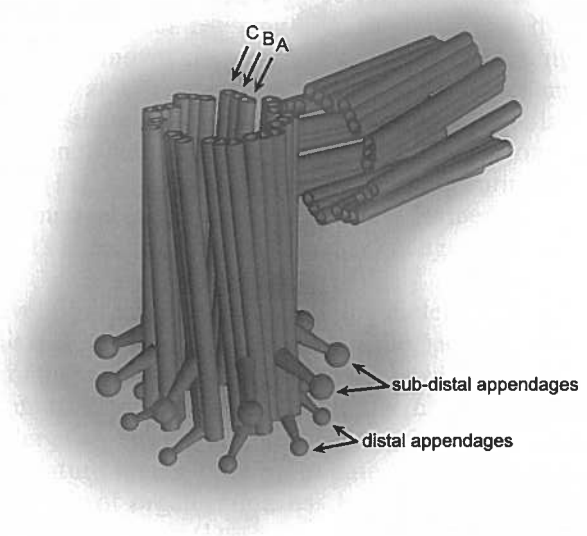


FIG. 4 Schematic simplified three-dimensional representation of the centrioles. Each centriole comprises nine sets of microtubules made of the A–B–C tubules (however, see text and Fig. 5). The mother (mature) centriole carries two sets of appendages at its distal extremity called the distal and subdistal appendages. The daughter (immature) centriole is devoid of appendages. The two centrioles are shown in an orthogonal configuration, with the proximal end of the daughter centriole facing the wall of the mother centriole. The centrioles are surrounded by ill-defined material called the pericentriolar matrix (PCM) (reproduced from Chrétien *et al.,* 1997, with permission from Academic Press).

"proximal" and "distal" have been adopted. Proximal refers to the end of the barrel that is closest to the other centriole (Figs. 2–4). The proximal and distal ends are functionally distinct. Duplication of centrioles commences at the proximal end. The flagellum and cilium originate from the distal end of the centriole barrel. The MTs in the wall of the centriole wall do not form a closed barrel, and the arrangement of the MT in the wall is complicated. At the proximal end the wall consists of nine triplet MTs, as shown in the simplified drawings in Figs. 3 and 4. Toward the distal end this organization changes, and the wall here consists of nine *doublets* of MTs (Fig. 5). To keep track of these MTs a special nomenclature has been adopted. The MT closest to the center of the centriole barrel, an ordinary MT with 13 protofilaments, is called "A." The two others further from the center are called "B" and "C," respectively. There seems to be a continuous change from the triplet A–B–C to the doublet A–B organization from the proximal

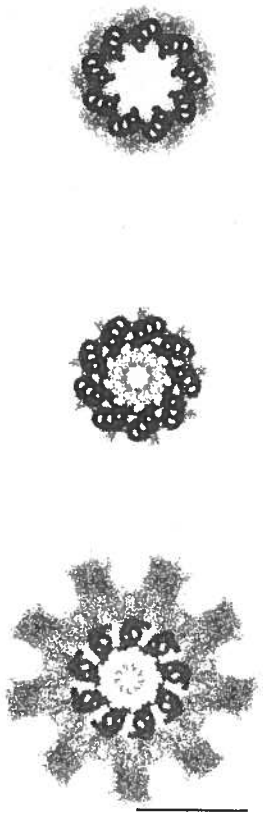


FIG. 5 Electron micrograph showing serial sections of the centriole cylinder from the proximal (top) to the distal (bottom) end. (Top) Note the three A–B–C microtubules as well as their tight connection. (Bottom) Note the doublet structure of the centriole blades and the appendages. Scale bar = 0.2 μm (reproduced from Paintrand *et al.,* 1992, with permission from Academic Press).

to the distal end. This is probably because MTs share walls with each other. In some cases it is observed that the walls of MT C and B are not completely closed and contain 11 and 10 protofilaments, respectively (Tilney *et al.,* 1973). Thus, the centriole cylinder consists of two parts. The proximal part is more like the classical organization of the basal body with nine opened triplets and a distal one which is more reminiscent of the organization of an axoneme with nine doublets. Moreover, the distal end is indeed the end from which a primary cilium and flagellum originate in certain cell types. Thus, the centriole possesses the double organization of basal bodies and cilia.

The triplets/doublets of MTs in the centrioles can be visualized as rafts (Fig. 4). With respect to the tangent to the centriole cylinder, the rafts are tilted like spokes in a wheel. Looking from the distal end, the vector from the A to the B tubule moves clockwise (Fig. 5, top). Interestingly, the angle between the vector and the cylindrical centriole "wall" changes from the proximal to the distal end. At the proximal end this angle is 45°, and it is 20° at the distal end. Thus, there is a twist in the MTs from the proximal to the distal end (Fig. 4). This suggests a strain in the entire centriolar wall. Although not impossible, this seems unlikely. Rather, the change from three to two MTs is the cause of this angular change from the proximal to the distal end.

The next level of organization involves the material associated with the centrioles. This material is often referred to as the "pericentriolar material." The presence of filamentous-like structures has been reported and the name "centrosome matrix" or pericentriolar matrix has for this reason been proposed (Fuller *et al.,* 1995; Paintrand *et al.,* 1992). As discussed in Section IV, this nomenclature seems more up to date than pericentriolar material since a high degree of organization has been observed for some of the proteins present in the matrix (Dictenberg *et al.,* 1998). Moreover, proteinatious appendages are observed with one of the centrioles (the older of the centrioles in the pair). This particular aspect of centrosomes is discussed in detail by Chrétien *et al.* (1997) and Paintrand *et al.* (1992) (see Figs. 3, 4, and 8). Interestingly, it was reported that the distance between the centrioles changed markedly, depending on the presence of Ca^{2+} ions (Paintrand *et al.,* 1992). The calcium-dependent conformational changes may reflect contractile activity in some of the proteins in the centrosome matrix (i.e., centrin) and show that the centriole pair with associated matrix is a dynamic structure.

A particularly puzzling question is the origin of the centrosome. As described in Section IV, this question can be reduced to a question of the origin of the centrioles. Moreover, the question can be addressed both in evolutionary terms and in physiological terms, i.e., having to do with maternal or paternal origin.

With mouse as the only known exception, centrioles are paternally inherited (Schatten, 1994; Simerly *et al.,* 1995). As described in Section IV,B, the pool of maternal proteins complements the paternally delivered sperm centrioles to form a mature centrosome.

If we consider the evolutionary question of centrosome origin, it has been proposed that centrioles were acquired through endosymbiosis much like mitochondria (Sagan, 1967). In Sagan's meticulous theoretical account of the origin of mitosis it is proposed that ingestion of a flagellar-driven motile parasite by an ancestral amoeboid is the origin of eukaryotic flagella. These ancestral ameboids would have gained a selective advantage of an

increased novel form of motility over those that did not have these flagella. The idea seems attractive, and it is true that the basal body at the base of flagella is very much like a centriole. However, as Fulton (1971, p. 211) points out, "The origin of the centriolar pinwheel is obscure. All known centrioles have the definitive morphology [nine-MT triplet morphology]; there are no evolutionary intermediates on which to build even a hypothetical sequence." Later in evolution of mitosis the basal body was retrieved by the nucleus and used during cell division while serving as a base of a primary cilium during interphase (Reinsch and Karsenti, 1994). How and why this happened cannot be answered. Moreover, at some point there must have been a transfer of the genetic material encoding the basal bodies from the originally engulfed "organism" to the nucleus of the host cell since all the material necessary for centriole formation is encoded by the nucleus. Today, the centrioles are an integral part of the centrosome and follow the centrosome's cycle. Remarkably, they can also "break free" of the cycle and serve as basal body for a primary cilium (Reinsch and Karsenti, 1994; Figs. 8 and 13).

III. Centrosomal Proteins

Since 1990 there has been a rapid increase in the number of proteins known to be associated with the centrosome and MTOCs. Several recent reviews have compiled lists of these proteins. In fact, the number of reviews published on the centrosome is so impressive that a table containing some of these reviews was compiled for this review (Table II). Readers knowledgeable about the proteins localized to the centrosome may wish to jump directly to Section IV, which discusses regulation of centriole and centrosome assembly and division.

Assigning a protein as "centrosomal" is not an easy task. Thus, many papers report that proteins are centrosomal because by immunofluorescence they have been localized to the centrosome. However, immunofluorescence alone is not a sufficient criterium for centrosomal localization because the centrosome is a focal point, and any minor amount of protein associated with this point would by immunofluorescence give the impression that the protein is associated with the centrosome. Moreover, since the centrosome is at the center of the cell or serves as a spindle pole focal point during mitosis, many proteins that are not really centrosomal are probably fluxed to the centrosome by motors. Therefore, it seems reasonable to distinguish between three classes of centrosomal proteins: (1) integral centrosomal/centriolar proteins, (2) proteins tightly associated with the centrosome/pericentriolar matrix, and (3) proteins loosely associated with

the centrosome. In order to assign proteins to these groups the proteins must fulfill the following experimental criteria:

Groups 1 and 2: Localization to the centrosome observed with one or several antibodies by immunofluorescence and eventually electron microscopy. In addition, association with purified centrosomes by Western blotting (alternatively, but not as good, is the demonstration of resistance to salt extraction and urea extraction *in vivo* prior to immunofluorescence). In systems amenable to genetic manipulations (such as *S. cerevisiae*), the absence of centrosomal/spindle pole body staining at decreased protein expression is sufficient.
Group 3: Localization to the centrosome by several different antibodies and disappearance of centrosomal staining upon MT depolymerization or centrosomal purification.

For this review a list of proteins published in the 1990s that belongs to these groups was compiled (Table I; organized by decreasing molecular mass). Most of the proteins in the table are integral centrosomal proteins (group 1), and the table is not an exhaustive list of proteins. Especially for the proteins belonging to group 3 only those that may have an important role for the centrosome as MTOC have been included. *Saccharomyces cerevisiae* has proven especially powerful in identifying centrosome/spindle pole body components but only those that are known to be conserved across species are included in the table (for recent reviews see Table II). Thus, Table I is primarily a list of truly centrosomal proteins and not an exhaustive list of MTOC-binding proteins. Moreover, many papers describe some of the proteins listed. The references cited in Table I are therefore the most informative, the most current, or those with the best cross-referencing to other papers. In the following sections, I discuss the properties of the best characterized centrosomal proteins listed in Table I. As far as possible the following description is provided for all proteins: identification, molecular structure data if available, characteristics of centrosomal localization, and proposed function.

A. Integral Centrosomal Proteins

The 55-kDa **γ-tubulin** molecule is a member of the tubulin family that also contains the building blocks of MTs (α- and β-tubulins). Several recent excellent reviews describe γ-tubulin in detail and I shall therefore be brief (Table II). γ-Tubulin was identified as a suppressor gene of a β-tubulin mutation (Oakley and Oakley, 1989). After cloning of the protein it was discovered that it had 30% homology to α- and β-tubulin. Several studies

TABLE I

Centrosomal Proteins

Protein/mass (group)	Source/sequence	Suggested function(s)/comments	Reference
CTR56/350 (2)	Human/no	Ab cross reacts with myosin heavy chain	Bailly *et al.* (1992a)
350 kDa (2)	Dictyostelium/no	Maintenance of the structural integrity of the nucleus-associated body/Ab cross reacts with myosin	Kalt and Schliwa (1996)
MAP1B/325 (1)	Human/yes	Nucleation at the centrosome	Domínguez *et al.* (1994)
Ninein/245 (2)	Mouse/yes	?/Like PCM-1 and centrin, very acidic; extensive coil–coil structure; GTP binding site and EF-hand; PEST sequence	Bouckson Castaing *et al.* (1996)
PCM-1/228 (2)	Human/yes	Centrosome duplication and/or regulation of MT nucleation/not centrosomal during mitosis; very acidic and contains a nucleotide binding site	Balczon *et al.* (1994, 1995)
Pericentrin/220 (1)	Human/yes	MT nucleation/colocalizes with γ-tubulin but has distinct function in pole formation	Dictenberg *et al.* (1998), Doxsey *et al.* (1994)
NuMA/220 (3)	Human *Xenopus*/yes	Pole formation/in a complex with dynein	Merdes *et al.* (1996)
XKLP2/160 (3)	*Xenopus*/yes	Centrosome separation/member of the BimC kinesin family	Boleti *et al.* (1996), Wittmann *et al.* (1998)
LK6/134 (2)	*Drosophila*/yes	Regulation of MTs dynamics/domain ressembling Ca^{2+}; calmodulin-regulated kinases; PEST-rich sequence	Kidd and Raff (1997)
CP190/120 (1) Ident.: Bx63	*Drosophila*/yes	?/In the nucleus during interphase and centrosomal during mitosis; classical N-terminal NLS; central 124-aa MT and centrosomal-binding domain overlap (close to four zinc finger motifs)	Kellogg *et al.* (1989), Oegema *et al.* (1995a, 1997), Whitfield *et al.* (1995)
Basonuclein/120 (1)	Human/yes	Associated with meiotic centrioles, organization/zinc finger motif	Yang *et al.* (1997)
Nuf1p(Spc110p)/110 (1)	*S. cerevisiae*/yes	Essential structural component of the SPB required during spindle formation; spanning the central and inner SPB plaques; central coil–coil and globular ends	Friedman *et al.* (1996)
Spc98p/98 (1) hGCP3 Xgrip109 HsSpc98p	*S. cerevisiae*/yes Human/yes *Xenopus*/yes	γ-TuRC assembly and see Spc97p/mammalian and *Xenopus* homolog	Geissler *et al.* (1996), Murphy *et al.* (1998), Tassin *et al.* (1998), Martin *et al.* (1998)
Spc97p/97 (1) hGCP2 HsSpc97p	*S. cerevisiae*/yes Human/yes	MT nucleation and SPB duplication/ complexed with Spc98p and Tub4p; localized to both inner and outer SPB plaques	Knop *et al.* (1997), Murphy *et al.* (1998), Martin *et al.* (1998)
Cenexin/96 (1)	Human/no	Associated with mature centrioles and perhaps involved in centriole replication	Lange and Gull (1995)
Phosphoinositide reg. subunit/85 (1)	Human/yes	Orienting centrosome during chemotaxis/ direct association with α-, β-, and γ-tubulin stimulated by insulin and PDGF	Kapeller *et al.* (1995)
hsp73/70 (1)	Many/yes	Recovery of centrosome structure and function after heat shock	Brown (1996b)
Tcp1/60 (1)	Mouse, yeast/yes	Chaperone for MT growth of centrosomes/antibodies block centrosomal MT nucleation	Brown (1996a)

(*continues*)

TABLE I (*continued*)

Protein/mass (group)	Source/sequence	Suggested function(s)/comments	Reference
CP60/60 (1)	*Drosophila*/yes	?/Centrosomal during anaphase and telophase; degraded or in the nucleus during interphase; complexed with CP190 and an unknown kinase; MT binding activity	Kellog *et al.* (1995)
Sad1p/58 (1)	*S. cerevisiae*/yes	SPB structure/membrane spanning domain; acidic N terminus	Hagan and Yanagida (1995)
γ-Tubulin/55 (1)	All eucaryotes/yes	MT nucleation and centriole duplication	Fuller *et al.* (1995), Moudjou *et al.* (1996), Pereira and Schiebel (1997)
Tub4p/55 (1)	*S. cerevisiae*/yes	γ-Tubulin homolog	Sobel and Snyder (1995)
Nuf2p/53 (1)	*S. cerevisiae*/yes	SPB separation and duplication/coil–coil structure	Osborne *et al.* (1994)
Tektins/47–55 (1)	Human, molluscan/yes	Link between centrosome and the intermediate filament cytoskeleton, centriole stabilization/ centrosomal localization from prophase to anaphase; coil–coil structure	Steffen *et al.* (1994)
Nek2/48 (1)	Human/yes	Regulation of centrosome separation	Fry *et al.* (1998)
pEg2/46 (1)	*Xenopus*/yes	Unknown/Ser/Thr kinase; member of a growing family of protein kinases	Roghi *et al.* (1998)
Actin-RPV or centractin/ 43 (1)	Human/yes	Unclear: link centrosome to the nucleus; structural; couple dynein motors to vesicles/50% homology with actin; part of dynactin complex	Clark and Meyer (1992), Merdes and Cleveland (1997)
Cdc2/34 (1)	Human/yes	Centrosome architecture/localized to the centrosome throughout the cell cycle	Bailly *et al.* (1989), Pockwinse *et al.* (1997)
PPX/35 (2)	Human/yes	Regulation of the level of centrosomal protein phosphorylation/PP2A homolog	Brewis *et al.* (1993)
CycB1/50 (2)	Human/yes	Regulation of cdc2 kinase and centrosomal protein phosphorylation/centrosomal up to metaphase of each cell cycle	Bailly *et al.* (1992b), Buendia *et al.* (1992)
CycA/50 (2)	Human/yes	Regulation of cdc2 kinase and centrosomal protein phosphorylation/centrosomal from prophase to metaphase	Bailly *et al.* (1992b), Buendia *et al.* (1992)
cAMP RII/49 (1)	Human/yes	?	Keryer *et al.* (1993, 1995)
p23/23 (3)	Chicken/yes	?/Ca^{2+} binding like centrin but no homology	Zhu *et al.* (1995)
Centrin or Cdc31p or caltractin/20 (1)	Human, plant, yeast/yes	SPB duplication and clipping of centrosomal MTs/ member of the calmodulun superfamily; has four Ca^{2+}-binding EF hands; 70% homology between algae and human form	Errabolu *et al.* (1994), Levy *et al.* (1996), Paoletti *et al.* (1996)
PCM-1/PCM-2/PCM-3 (2)	Human/only for PCM-1	Regulation of MT nucleation, centriole assembly/ proteins recognized by autoimmune sera	Balczon *et al.* (1994, 1995), Balczon and West (1991)
Katanin (3)/81 and 61	Sea urchin/yes	Severing of centrosomal MTs/centrosomal association is MT dependent; ATPase activity; heterooligomeric protein	McNally *et al.* (1996), Hartman *et al.* (1998)

TABLE II
Reviews on the Centrosome

Review	Subjects covered	Reference
	Reviews on specific centrosomal components	
"Centrosome–Microtubule Nucleation"	Mechanism of nucleation of microtubules by γ-tubulin	Pereira and Schiebel (1997)
"Cytoskeleton: Anatomy of an Organizing Center"	Components localized to the SPB	Marschall and Stearns (1997)
"Centrosomes and Microtubules: Wedded with a Ring"	The γ-tubulin ring complex (γ-TuRC)	Raff (1996)
"γ-Tubulin and Microtubule Organization in Plants"	γ-Tubulin in plants	Joshi and Palevitz (1996)
"In Search of a Function for Centrins"	Possible conserved function of centrins between species	Schiebel and Bornens (1995)
"Centrin, Centrosomes, and Mitotic Spindle Poles"	Role of centrin in centrosome dynamics	Salisbury (1995)
	Comprehensive reviews on the centrosome	
"Centrosomes and Microtubule Organization during *Drosophila* Development"	Centrosome and centriolar structure and function during development, role of centrosomes in spindle assembly	González *et al.* (1998)
Structure and Function of the Centriole in Animal Cells: Progress and Questions"	See ref.	Lange and Gull (1996)
"The Centrosome in Animal Cells and Its Functional Homologs in Plant and Yeast Cells"	See ref.	Balczon (1996)
"Regulation of Centrosome Function during Mitosis"	See ref.; in particular on *in vitro* reconstitution of centrosome nucleation capacity	Buendia and Karsenti (1995)
"The Centrosome and Cellular Organization"	Centrosomal components, assembly, duplication, and role for cellular organization	Kellogg *et al.* (1994)
"The Centrosome and Its Mode of Inheritance: The Reduction of the Centrosome during Gametogenesis and Its Restoration during Fertilization"	Compilation of the evidence that the centrosome is paternally inherited during fertilization and of organisms devoid of centrosomes	Schatten (1994)
"Microtubules, Centrosomes and Intermediate Filaments in Directed Cell Movement"	Role of the centrosome in cell locomotion	Schliwa and Höner (1993)
"Unravelling the Tangled Web at the Microtubule-Organizing Center"	Review on centrosomal components notably from yeast	Rose *et al.* (1993)
The Centrosome	Book on centrosome definition, components, and structure from different organisms	Kalnins (1992)
"Spindle Poles in Higher Plant Mitosis"	See ref.	Smirnova and Bajer (1992)
"Centriole and Basal Body Formation during Ciliogenesis Revisited"	Compilation of literature on basal body formation and relevance for centriole formation	Dirksen (1991)
"The Chromosome Cycle and the Centrosome Cycle in the Mitotic Cycle"	Historical review on centrosome research	Mazia (1987)
"Microtubule Organizing Centers"	Examination of the centrosome as MTOC	Brinkley (1985)
"Centrosomes and Mitotic Poles"	Historical review on speculations on centrosome function and assembly	Mazia (1984)
"The Centrosome as an Organizer of the Cytoskeleton"	Review on the status of centrosomal research at the molecular level	McIntosh (1983)

showed that γ-tubulin is expressed in all eukaryotes studied so far and localized to MTOCs (Sobel and Snyder, 1995; Zheng *et al.,* 1991). γ-Tubulin is crucial for MT nucleation at the centrosome (Félix *et al.,* 1994; Joshi *et al.,* 1992; Marschall *et al.,* 1996; Shu and Joshi, 1995; Stearns and Kirschner, 1994). However, γ-Tubulin is in a 25S complex with at least seven other proteins, forming the so-called **γ-tubulin ring complex (γ-TuRC)** (Stearns and Kirschner, 1994; Zheng *et al.,* 1995). Most likely, γ-tubulin can only induce MT nucleation as a γ-TuRC. γ-Tubulin is an integral member of the centrosome/centriole and is even observed inside the proximal part of the centriole cylinder (Fuller *et al.,* 1995). Only a fraction of the total amount of γ-tubulin is centrosome associated (Moudjou *et al.,* 1996). How it becomes localized to the centrosome and how it promotes MT nucleation are unresolved issues. One study indicated that γ-tubulin is very tightly associated with the minus end of MTs (Li and Joshi, 1995), but this does not answer the question of how γ-tubulin in a MT-independent way becomes localized to the centrosome (Félix *et al.,* 1994; Stearns and Kirschner, 1994), and whether pure γ-tubulin can nucleate MTs. However, four recent papers provide novel information on this issue and on the components of the γ-TuRC (Murphy *et al.,* 1998; Tassin *et al.,* 1998; Martin *et al.,* 1998; Moritz *et al.,* 1998). Murphy *et al.,* made human cell lines that stably express epitopetagged versions of human γ-tubulin. The tagged γ-tubulin was then immunoprecipiated from cells and associated proteins were analyzed. Of seven proteins (48, 71, 76, 100, 101, 128, and 211 kDa; equivalent to the number of proteins identified in the Xenopus γ-TuRC) the 100- and 101-kDa proteins (hGCP2 and hGCP3; for "human γ-tubulin complex protein") were cloned and found homologous to the yeast Spc97p and Spc98p (Tassin *et al.,* 1998) proteins, respectively (Knop *et al.,* 1997; Marschall and Stearns, 1997). Together with γ-tubulin (also called hGCP1), hGCP2 and hGCP3 form a trimeric complex which is the minimal building unit of the γ-TuRC. This unit is also found free in the cytoplasm, and Murphy *et al.* suggest that the other proteins of the γ-TuRC are involved in gluing together the trimeric complex and attaching the γ-TuRC to the centrosome. (However, see Moritz *et al.,* 1998.) From yeast it has only been possible to isolate the trimeric complex and not the entire γ-TuRC. Tassin *et al.* hypothesize that the large size of the γ-TuRC (compared with the trimeric complex in yeast) may be specific for higher eucaryotes and may have evolved because of a requirement of stabilization of the minus end of cytoplasmic MTs in higher systems (see Section V,C and Fig. 14). Martin *et al.* used *Xenopus* egg extracts to characterize proteins of the γ-TuRC and identified a protein called Xgrip109, homologous to Spc98p from yeast and highly conserved between humans, mice, fish, rice, and flies. Upon treatment of γ-TuRCs with high salt the γ-TuRCs fell apart, and after desalting 50% reassembled (Martin *et al.,* 1998). If Xgrip109 was immunodepleted from salt-disrupted

γ-TuRCs, the γ-TuRCs did not reassemble after desalting, strongly suggesting that Xgrip109 is required for γ-TuRC assembly (consistent with the data of Murphy *et al.,* 1998). Moritz *et al.* (1998) showed *in vitro* that the MT nucleation activity of 2 M KI salt-stripped *Drosophila* centrosomes could be restored by the addition of γ-TuRCs and unknown proteins in *Drosophila* extracts. This study shows that γ-TuRCs are required for MT nucleation and are linked to centrosomes by unknown factors. The factors seem to include a 220-kDa protein that may be pericentrin (Dictenberg *et al.,* 1998; Moritz *et al.,* 1998).

Pericentrin was identified and cloned using an antisera from scleroderma patients (Doxsey *et al.,* 1994; Dictenberg *et al.,* 1998). It is a 220-kDa protein with a large central 150-kDa α-helical domain containing coil– coil domains. The N and C termini are nonhelical and noncoiled and have no homology to other proteins apart from six cdc2 consensus phosphorylation sites. Recruitment of pericentrin to sperm heads strongly promotes MT nucleation off centrosomes in *Xenopus* extracts. However, anti-pericentrin antibodies do not block MT nucleation off mature centrosomes (sperm heads incubated in the extract) *in vitro* (Doxsey *et al.,* 1994). It is unclear whether pericentrin is not required for the association of γ-tubulin onto the centrosome. The data indicate that pericentrin and γ-tubulin are part of two different pathways for components recruitment onto centrosomes and required for MT nucleation. However, recent data suggest that pericentrin forms a complex with γ-tubulin independently of γ-TuRC (Dictenberg *et al.,* 1998).

CP60 and **CP190,** previously called DMAP60 and DMAP190, are two centrosomally located proteins identified in *Drosophila melanogaster.* Both proteins were identified using a combination of MT affinity chromatography and immunocytology in *Drosophila* (Kellogg *et al.,* 1994). CP190 was determined to be identical to the antigen recognized by the Bx63 monoclonal antibody (Whitfield *et al.,* 1988). Immunoaffinity chromatography showed that CP190 is in a complex with approximately 10 proteins, among which are CP60, an uncharacterized kinase, and γ-tubulin (Raff *et al.,* 1993). The functions of CP190 and CP60 are unknown, but the complex with γ-tubulin may indicate that they are required for centrosomal localization of γ-tubulin. The complex between CP60 and γ-tubulin is tight and does not require CP190. In contrast to these results, Moritz *et al.* (1998) showed that CP60 and CP190 do not form a complex with γ-tubulin or the γ-TuRC and are not required for centrosomal MT nucleation.

Oegema *et al.* (1995) studied the molecular requirements for centrosomal localization of CP190. How proteins become localized to the centrosome is a very important question about which only little is know. CP190 is localized to the nucleus in interphase and to the centrosome during mitosis. CP190 has a classical bipartite N-terminal 19-amino acid-long nuclear local-

ization signal (NLS). If the NLS is deleted, CP190 associates with the centrosome throughout the cell cycle. The region required for centrosomal localization is centrally located, 124 amino acids long, and overlaps the region required for MT association but has no homology with other MAP-binding domains. Moreover, this sequence contains one of a total of four zinc finger motifs (Whitfield *et al.,* 1995).

CP60 is especially abundant on centrosomes during anaphase and telophase and is apparently degraded at the onset of interphase up to nuclear cycle 12. Then, CP60 is found localized to the nucleus in interphase and to the centrosome during mitosis. CP60 has no homology with other known proteins but contains six cdk consensus phosphorylation sites and a sequence similar to the "destruction box" in cyclins (Kellogg *et al.,* 1995). It is unknown how CP60 localizes to the centrosome but it may do so indirectly through binding to CP190 (Oegema *et al.,* 1997). The function of CP60 and CP190 remain elusive (Moritz *et al.,* 1998).

PCM-1 was identified using serum from patients with systemic sclerosis and Raynaud's phenomenon (Balczon and West, 1991). With these sera, three proteins of 39, 185, and 220 kDa, called PCM-3, PCM-2, and PCM-1 respectively, were identified. Balczon *et al.* (1994) described the cloning of the 228-kDa PCM-1. PCM-1 has no homology to other known proteins but is very acidic and contains a nucleotide consensus ATP/GTP-binding motif. PCM-1 is associated with the centrosome throughout the cell cycle but there is a decrease in staining intensity at the onset of mitosis. This led to the hypothesis that PCM-1 may be an inhibitor of MT nucleation since release of the protein at metaphase could explain the increased MT nucleation at the onset of metaphase. Considering the acidic p*I* of PCM-1, it is possible that it rejects acidic tubulin subunits from the centrosome and thereby inhibits MT nucleation. However, perhaps it is not involved in MT nucleation but rather in centriole duplication (Balczon *et al.,* 1995).

Centrin is a 20-kDa filamentous protein member of the EF-hand Ca^{2+}-binding superfamily with four helix–loop–helix motifs. It was first discovered in the flagella apparatus of unicellular green algae and is concentrated on centrioles. Like γ-tubulin, only a fraction of the total cellular pool of centrin is centrosome associated (Paoletti *et al.,* 1996). Recent reviews summarize knowledge about centrin from different organisms and its possible functional role (Baron and Salisbury, 1992; Salisbury, 1995; Schiebel and Bornens, 1995). The precise function of centrin is unknown but the yeast homolog cdc31p is essential and involved in SPB duplication. Interestingly, centrin contracts upon exposure to Ca^{2+} and it has been suggested to be important for centriole duplication and excision of the flagella associated with the sperm centriole (Sanders and Salisbury, 1994). Centrin may be responsible for the Ca^{2+}-dependent changes in centriolar structure observed by Paintrand *et al.* (1992) (Fig. 3).

Cenexin is a 96-kDa centriole-associated protein (Lange and Gull, 1995). Cenexin associates with the maturing mother centriole at the G_2-M phase transition (Fig. 8). Cenexin has not been cloned but the data on the association to the new mother centriole suggest that this protein may be directly involved in regulating centriole duplication. Note that the possibility that cenexin represents a posttranslational modification cannot be formally excluded. A recent review describes details on centriole duplication with respect to cenexin (Lange and Gull, 1996).

Tektins were discovered by studies on flagellar and ciliary doublet MTs (Chen *et al.,* 1993; Linck and Stephens, 1987; Steffen and Linck, 1988). Because of the relationship between flagellar MTs and the centriole it was reasonable to assume that the tektins may also be present on centrioles. This has indeed been confirmed biochemically and by immunofluorescence (Steffen *et al.,* 1994; Steffen and Linck, 1988). Tektins form filamentous polymers in the walls of ciliary and flagellar MTs. The central region of the tektins forms a coil–coil rod, consisting of four major α-helical regions that are separated by nonhelical linkers. The tektins have a secondary structure and molecular design similar to that of intermediate filament proteins although the primary sequence homology is low. The function of tektins appears to be stabilization and regulation of the species-specific length of centrioles (Steffen and Linck, 1988; Kenny *et al.,* 1997).

Molecular **chaperones** are proteinaceous structures that are thought to facilitate the folding of proteins *in vivo* (Frydman and Hartl, 1996). Tailless complex polypeptide-1 (TCP-1) and hsp73 have been shown to be integral components of the centrosome and to colocalize with pericentrin (Brown *et al.,* 1996a, b; Nelson and Craig, 1992). However, for both proteins, like centrin, γ-tubulin, and Nek2, only a minor fraction is centrosome associated and the remainder is diffusely localized in the cytoplasm. TCP-1 is a cytosolic chaperonin and exists as a 25S complex with at least eight related subunits (the TRiC complex) and this complex has been shown to chaperone tubulin folding (Tian *et al.,* 1996). hsp73 belongs to the heat shock proteins that are produced in increasing amounts after thermal stress probably to either repair and/or replace proteinaceous components. Apparently, TCP-1 and hsp73 participate in different aspects of the modulation of the structure and function of the centrosome. In the case of TCP-1, antibodies to the protein were capable of blocking the initiation of MT growth off the centrosome. hsp73, on the other hand, facilitates the recovery of centrosomal structure and function after heat shock, indicating that hsp73 may participate in the process of centrosomal assembly following centriole duplication during S phase. The function of chaperones in the centrosome may be to facilitate the movement of proteins into and out of the organelle as well as to catalyze spatial changes in the organization of pericentriolar material during the cell cycle. It seems possible that

chaperones would be required to facilitate the nucleation of MTs at the centrosome. Nucleation sites, especially during mitosis, are very closely packed and could inhibit each other's function without some external help in organizing their position and spatial orientation.

One of the first biochemical characterizations of the centrosomes consisted of the observation that the phosphorylation level of centrosomal proteins increases at the onset of mitosis, during which the nucleation capacity is also increased (Tash *et al.,* 1980). The regulation of this change in phosphorylation involves cell cycle-regulated **kinases** and **phosphatases.** Several studies have shown localization to and functional effects of cyclin A and cyclin B on centrosomes (Bailly *et al.,* 1989, 1992b; Buendia *et al.,* 1992; Maldonado Codina and Glover, 1992), and cdc2 kinase was recently described as constitutively associated with the centrosome throughout the cell cycle (Pockwinse *et al.,* 1997). Recently, two new kinases, LK6 and pEg2, were reported to be centrosomally associated (Kidd and Raff, 1997; Roghi *et al.,* 1998). A mammalian type 2A phosphatase PPX (Brewis *et al.,* 1993) has also been localized to the centrosome. Genetically lowering the dosage of a type 2A phosphatase not associated with the centrosome was shown to uncouple the nuclear and centrosomal cycles (Snaith *et al.,* 1996; Tournebize *et al.,* 1997). In summary, both kinases and phosphatases have been localized to the centrosome. How the balance in their activities determines the final level of centrosomal protein phosphorylation remains unknown. How phosphorylation affects the nucleation and duplication capacity of centrosomes has to be investigated further (see Section V,C).

Nek2 is a kinase of the NIMA family associated with the centrosome. Apart from cyclin A-dependent cdc2 kinase (Buendia *et al.,* 1992), it is the only kinase for which a centrosomal function has been convincingly shown (Fry *et al.,* 1998; Fry and Nigg, 1995). Nek2 localizes to the centrosome throughout the cell cycle. When cells were incubated with taxol for prolonged periods, mitotic arrest and formation of multiple asters ensued. These asters are examples of self-assembled MTOCs (see Section IV,B). In such cells Nek2 was associated only with the two MTOCs containing centrioles. Depolymerization of MTs did not affect the localization to centrosomes. Moreover, Nek2 was found to associate with purified centrosomes. Therefore, Nek2 is a truly integral centrosomal protein. Only 10% of total Nek2 is centrosome associated as reported for centrin, γ-tubulin, and chaperones. Overexpression of Nek2 had two effects. Using γ-tubulin as a marker Fry *et al.* (1998) observed that centrosomes had separated into two foci and that the pericentriolar matrix in many cases was dispersed from the centrosomes. However, transfection with a catalytically inactive Nek2 mutant also caused dispersal of centrosomal material but did not trigger any centrosome separation. The authors conclude that centrosome separation depends on kinase activity but that dispersal is

brought about by a different mechanism. It is suggested that Nek2 activates centrosome separation at the end of G_2 prior to cdc2-activated centrosome migration by motors such as Eg5 and XKLP2 (Blangy *et al.,* 1995; Boleti *et al.,* 1996; Sawin and Mitchison, 1995; Wittmann *et al.,* 1998; see Fig. 10 and Section IV,C).

B. Proteins Concentrated at the Centrosome

Katanin is a heterodimeric (81 and 60 kDa), ATP-dependent, MT-severing protein from sea urchin eggs (McNally *et al.,* 1996). Microtubule-severing proteins promote the disassembly of MTs by generating internal breaks within a MT (Karsenti, 1993; McNally *et al.,* 1996). It is proposed that Katanin is involved in severing MTs at their minus ends and in this way involved in generating spindle MT flux (McNally *et al.,* 1996; Sawin and Mitchison, 1994; Waters *et al.,* 1996). The localization studies showed that MTs are required to localize Katanin to the centrosome. When cells were preextracted prior to immunofluorescence the staining pattern obtained was a large hollow ball of Katanin around the centrosomal area with γ-tubulin at the interior of the ball. These data show that Katanin is not an integral part of the centrosome and can be assigned as a group 3 member of the centrosomal proteins (however, in some cells, nocodazole-resistant centrosomal association has been observed; F. J. McNally, personal communication). Katanin exemplifies the very dynamic interaction between the pericentriolar material and the surrounding cytoplasm and also the experimental difficulties involved with assigning a protein to the centrosome and grasping the large number of activities localized to and present at centrosomes.

Recent investigations cast new light on the issues of centrosomal localization and MT-severing activity by Katanin (Hartman *et al.,* 1998). The 60-kDa subunit is a new member of the AAA family of ATPases and contains the ATP binding site, the ATPase activity, and the severing activity of Katanin. However, the severing and ATPase activity of the 60-kDa subunit were enhanced twofold when complexed to the 81-kDa subunit, and *in vivo* the 60-kDa subunit is exclusively found in a very tight complex with the 81-kDa subunit. Interestingly, rotary shadowing electron microscopy showed that the 60-kDa subunit and the complex between the 60- and 81-kDa subunits forms ring-like structures. Previous studies showed that the heterooligomeric complex can bind to a tubulin subunit in its ADP form. Moreover, the heterooligomeric complex binds to MTs but not to subtilisin-cleaved MTs (in which the C terminus of tubulin is missing; McNally and Vale, 1993. Microtubule binding characterization of the Baculovirus-expressed 60- and 81-kDa subunits was not performed by Hartman *et*

al., 1998.) Surprisingly, subtilisin-treated MTs still stimulated the ATPase activity without MT severing (McNally and Vale, 1993). Previous studies also indicated that Katanin removes single tubulin subunits from the wall of MTs rather than removing oligomeric tubulin complexes (McNally and Vale, 1993). In summary, it is known at present that the C terminus of tubulin, ATP hydrolysis, and binding to MTs are required for Katanin to sever MTs. More work is required to determine how MTs mechanistically are severed, but the ring-like structure of the complex may be a key.

The 81-kDa subunit contains a C-terminal domain that is required for interaction with the 60-kDa subunit. In addition, the N terminus of the 81-kDa subunit contains six "WD40" repeat motifs, and this domain was sufficient to localize a GFP fusion protein to the centrosome in a MT-independent way. This suggests that the WD40-containing domain targets Katanin to the centrosome. Since previous studies showed that MTs are required to target Katanin to the centrosome (McNally *et al.,* 1996), it is possible that the binding characteristics of the entire complex are somewhat different from those of the WD40–GFP fusion protein or that more proteins are involved.

The homology between the 220-kDa human and *Xenopus* **NuMa** is only 37% (Merdes *et al.,* 1996), but both proteins contain a central 150-kDa predicted coil–coil domain and are localized to the centrosome. NuMA is localized to the nucleus during interphase and in a MT-dependent way to the spindle pole/centrosome region during mitosis. Like Katanin, NuMA is not an integral member of the centrosome but a member of the outermost layer of the pericentriolar matrix. Merdes and Cleveland (1997) and Merdes *et al.* (1996) showed that NuMA forms a complex with the MT minus end-directed cytoplasmic dynein–dynactin motor complex and proposed that dynein drives NuMA to the minus end of MTs where it is involved in focalizing MTs by cross-linking. This localization mechanism is attractive and probably applies to more proteins that are localized to the MTOC/centrosome and that are not centrosomally associated (i.e., Katanin, XKLP2, and Eg5).

In addition to the data on CP190, recent data on **XKLP2** (Boleti *et al.,* 1996; Wittmann *et al.,* 1998) also give some insights on the requirements for localization of a protein to the centrosomal area. XKLP2 is a plus-end-directed motor but accumulates at the minus ends of MTs during mitosis. The minus-end-directed motor dynein is required for this localization. This bears resemblance with the localization mechanism of NuMA. Moreover, in the very C terminal of the motor (the tail) a leucine zipper is essential for localization, and a single point mutation in the leucine zipper is sufficient to prevent localization of the tail. The leucine zipper has been shown to interact with a 100-kDa MT-associated protein (TPX2). The dynein complex together with TPX2 somehow target the tail of XKLP2 to MT minus ends (Wittmann *et al.,* 1998). (It remains to be shown how the full length XKLP2 motor targets.)

IV. High-Resolution Analysis of Centrosome Structure and Assembly

In this section I discuss the basic principles that are at work during centrosome assembly. I divide the analysis in three parts. First, I discuss the role of centrioles for centrosome function. Then I examine how a centrosome may assemble "on top" of the centrioles. Lastly, I discuss cell cycle control of centriole and centrosome duplication.

A. Role of Centrioles for Centrosome Function

In the absence of centrioles, a structure that nucleates MTs is a MTOC (Pickett-Heaps, 1969; Porter, 1966). This definition of the centrosome simplifies the discussion on centrosome assembly since the complication arising from the absence of centrioles from many so-called centrosomes is eliminated.

The functional difference between a centrosome and a MTOC can be appreciated from two elegant studies. In the first, the centrosome was removed by microsurgery (Maniotis and Schliwa, 1991) from somatic vertebrate tissue culture cells. Cells without centrosomes continued to grow but failed to divide and the cell cycle was arrested (Sluder, 1992). However, a functional MTOC developed in the cells devoid of centrosomes. Thus, these cells appear to be dependent on centrosomes for cell division, indicating that the centrosome has functions besides being a MTOC. The second study was performed using sea urchin eggs (Picard *et al.,* 1987). Sea urchins oocytes are arrested in interphase. This is different from most other embryos, such as human or *Xenopus,* which arrest at the second meiotic metaphase (Schatten, 1994). Fertilization of *Xenopus* eggs results in a Ca^{2+} wave that causes cdc2 kinase inactivation and reinitiation of the cell cycle (Sagata *et al.,* 1989). In sea urchin a Ca^{2+} wave can, as in *Xenopus,* resume the cell cycle. Contrary to *Xenopus,* however, a centrosome is sufficient to start the cell cycle (in the absence of Ca^{2+}; Picard *et al.,* 1987). This study indicates that the centrosome in sea urchin either contains or assembles components that are involved in regulating the reinitiation of the cell cycle. The observations by Picard *et al.* complement the observations by Maniotis and Schliwa (1991) and suggest that the centrosome can be involved in the regulation of cell cycle progression and cell division. Other experiments

from *Xenopus* show that the centrioles are not required for cell cycle initiation (a Ca^{2+} wave induced by pricking is sufficient) but are required for proper cleavage (Karsenti, 1991).

However, there are numerous examples of cells and eggs that have a cell cycle and that divide without centrosomes. Examples include the early stages of mouse oocyte cleavage, plant cell division, and a mutant *Drosophila* cell line (Debec *et al.,* 1995; Joshi and Palevitz, 1996; Lambert, 1993; Palacios *et al.,* 1993; Schatten, 1994). These exceptions probably reflect alternative ways of solving the "problem" of cell division: Cell division is such an essential process that different pathways must have evolved (Schatten, 1994; González *et al.,* 1998).

What is then the role of the centriole if it is not present in all systems? The short and simple answer is that in systems with centrioles, cell division seems to have become "addicted" to their presence. All vertebrate somatic cells, being rather young from an evolutionary perspective, contain centrioles. It is possible that the centriole developed as an extra regulatory mechanism for vertebrate somatic cell division (Grafen, 1988; Sluder, 1992). However, the function of centrioles and how they organize material for organization around them are unknown. In the words of Lange and Gull (1996, p. 351), "Comparison of the capacity of cells with and without centrioles can be informative, but really the question is: what is the function of centrioles in those cells that possess them?" However, currently this question cannot be answered.

B. Centrosome Assembly

Why is it that we know so little about centrosome assembly, when (perhaps) equally complicated assembly processes, such as ribosome assembly, are well characterized (Nomura and Held, 1974)? This is in part because of the low abundance of centrosomal proteins. One centrosome weighs approximately 10^{-14} g (Bornens *et al.,* 1987), and it is therefore difficult to obtain enough centrosomes to be able to characterize the attached components both morphologically and biochemically. In addition, it is difficult to assess centrosome function both *in vivo* and *in vitro.* For example, *in vitro* experiments show that the centrosome is capable of MT nucleation. However, as discussed in Section IV,A some cells do not divide without a centrosome, although a functional MTOC forms in the absence of centrioles. Centrosomes are clearly more than just MTOCs.

However, MTOCs that lack a centriole pair may provide insight into how centrosomes assemble since they are examples of self-organized MTOCs which in many ways behave like centrosomes. Such acentriolar

centrosomes, MTOCs, are found in plants (Joshi and Palevitz, 1996; Lambert, 1993), the early mouse embryo (Palacios *et al.,* 1993), and an acentriolar *Drosophila* cell line (Debec *et al.,* 1995). In addition, they can "artificially" be induced by taxol or DMSO addition to *Xenopus* egg extracts and in other systems (De Brabander *et al.,* 1986; Sawin and Mitchison, 1994; Verde *et al.,* 1991).

I now discuss the formation of an "artificial" MTOC. The formation of MTOCs in DMSO-treated mitotic *Xenopus* egg extracts first involves *nucleation* of MTs followed by *stabilization* and *growth* by MT-associated proteins (MAPs). The MTs are then *sorted* by motors that *read the polarity* of MTs and MTOCs or asters form (Heald *et al.,* 1996; Heald *et al.,* 1997; Hyman and Karsenti, 1996; Karsenti, 1991; Verde *et al.,* 1991). Thus, these asters assemble by an interplay between motors and MTs. The end result is an aster of MTs with uniform polarity, in which the base of the aster morphologically and compositionally resembles a centrosome (Galio *et al.,* 1996; Heald *et al.,* 1997; Merdes *et al.,* 1996; Verde *et al.,* 1991). It is unknown whether these asters can actively nucleate MTs and thus whether they should be called MTOC. This issue, however, is of minor importance in this context because the motor-dependent MT sorting mechanism also is at work in the presence of a centrosome (Fig. 6) where MTs are attached to the centrosome with their minus end. Therefore, the term "artificial" MTOC is used for these structures. Thus, the motors that convey the sorting during artificial MTOC formation are also working when a centrosome is present. One possibility is therefore that duplication and *de novo* formation of centrioles/centrosomes ensues as a result of local high concentration of proteins obtained by transport on MTs to the center of the centrosome/MTOC (Verde *et al.,* 1991). (However, see the discussion on *de novo* centriole formation kinetics later in this section).

What makes a centrosomes different from a DMSO-induced MTOC is especially that it must have some kind of "protein glue" that tethers the MT nucleating material to the centriolar surface. For example, centrosomes can be purified with the MT nucleation material attached (Bornens *et al.,* 1987), whereas dissociation of DMSO-induced MTOCs is irreversible (i.e., Heald *et al.,* 1997). It is unresolved how a centrosome can maintain the MT nucleation material such as γ-tubulin/pericentrin on the centrosome, and currently there are no candidates for such centrosomal protein glue (Section III.A). Mazia and Schatten suggested hypothetical models for such glue. Thus, the template attached to the centriole that allows stable binding of pericentriolar matrix components is proposed to appear either as a sticky ribbon (for γ-tubulin) or as a crystalline mold into which the bulk of the centrosomal proteins fit (Mazia, 1984, 1987). There seems to be some experimental support for this idea (Dictenberg *et al.,* 1998). Schatten (1994)

proposed that during the penetration of the egg the sperm could acquire some kind of a receptor system that would allow molecules such as γ-tubulin to stably attach to the centrioles and thus provide the starting point for a centrosome. Favorable to the latter idea is that the *Xenopus* egg cortex is full of γ-tubulin (Gard, 1994). However, since complementation directly in the extract is possible, there does not seem to be a need to invoke a cortex-associated receptor for molecules such as γ-tubulin (Félix *et al.,* 1994; Stearns and Kirschner, 1994).

In trying to imagine how a centrosome assembles on top of a centriole, it is useful to think about layers of proteins that attach to the surface of the centrioles in a defined order (Fig. 6). Central to the scheme is that the egg complements the sperm centrioles with proteinatious components.

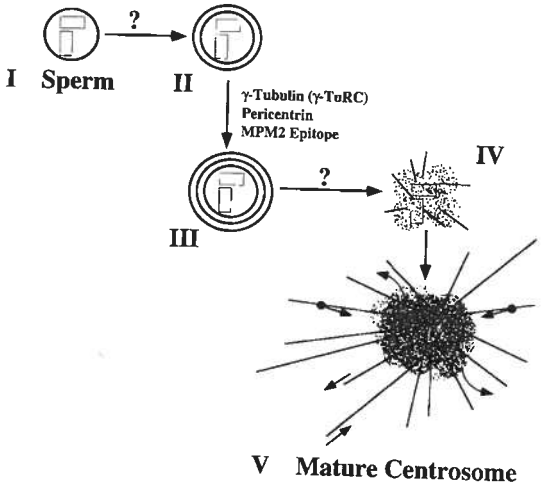


FIG. 6 Schematic representation of the pathyway leading to centrosome assembly in interphase or mitotic *Xenopus* egg extracts (Stearns and Kirschner, 1994; Félix *et al.,* 1994; Buendia *et al.,* 1992). The paternally delivered sperm-associated centrioles have some proteins attached (*I*). Upon entry into the egg some proteins probably immediately associate with the centriole, i.e., during penetration of the egg (transition I–II). During transition II–III, specific proteins attach to the centrosomal "glue" present on the centrioles. These components include the γ-TuRC, pericentrin, and the MPM2 phosphoepitope. At some point MTs start to nucleate on the nascent centrosome. This requires correct orientation and organization of the pericentriolar matrix components (transition III–IV). During transition IV–V, more MTs are nucleated and more pericentriolar material accumulate at the centrosome. Motor proteins drive components to the minus end of MTs localized in the pericentriolar matrix (circle with arrow), proteins have a dynamic attachment to the centrosomal area (curved arrows indicate a certain off-rate), and MTs growing from the centrosome are dynamic (arrows on MTs indicating growth and shortening events). Note: The activity of minus end-directed motors shown here seems to be considerably increased during mitosis and may not exist during interphase.

Imagine an assembly scheme as presented in Fig. 6: (I) Depict paternal proteins associated with the centrioles. At the time of delivery to the egg some proteins immediately attach (transition I–II). After and during transition II–III proteins required for MT nucleation are retrieved from the maternal pool. During transition III–IV the three-dimensional lattice required for MT nucleation is constructed (Dictenberg *et al.,* 1998). During transition IV–V, proteins are transported by MT-associated motors toward the centrosome (as in DMSO-induced MTOCs), terminating the assembly process. Arrows on MTs indicate movement by motors, and otherwise loss of proteins due to their intrinsic on/off rate.

The sequence of association of pericentrin and γ-tubulin has been characterized in detail and can be fitted to the scheme presented in Fig. 6 (Archer and Solomon, 1994; Dictenberg *et al.,* 1998; Doxsey *et al.,* 1994; Félix *et al.,* 1994; Stearns and Kirschner, 1994). Thus, MTs do not nucleate off *Xenopus* sperm nuclei *in vitro* prior to incubation in the *Xenopus* egg extract (Fig. 6, I). After incubation in the extract the sperm nuclei become competent for MT nucleation and this competence requires the recruitment of γ-tubulin (Archer and Solomon, 1994; Félix *et al.,* 1994; Stearns and Kirschner, 1994). The accumulation of γ-tubulin does not require MTs as shown by complementation in the absence of MTs. However, the recruitment of γ-tubulin requires ATP and occurs concomitantly with the recruitment of the phosphoepitope called MPM2 (Davis *et al.,* 1983). Whether the requirement for ATP is due to an energy-dependent step in the recruitment of γ-tubulin to the sperm or related to the simultaneous addition of the MPM2 epitope is unknown (Fig. 6, II and III). Interestingly, γ-tubulin is in a 25S complex (γ-TuRC) indicating that other proteins may be required for the attachment of γ-tubulin to the centrosome (Stearns and Kirschner, 1994; Zheng *et al.,* 1995; Section III.A).

In contrast to γ-tubulin, the proteins pericentrin, centrin, and α-tubulin are all present on the sperm prior to incubation in the extract. However, incubation of sperm heads in extracts containing anti-pericentrin antibodies causes an inhibition of MT nucleation (Doxsey *et al.,* 1994). Additional pericentrin is recruited in the extract that somehow is involved in forming a proper environment for MT nucleation. The recruitment of γ-tubulin to the sperm heads is not abolished by anti-pericentrin antibodies (it is unknown whether γ-tubulin is required to recruit pericentrin to the sperm head in the extract). This indicates that both γ-tubulin and pericentrin are required for MTs to be able to nucleate off centrosomes. Pericentrin may be required for the γ-TuRC to bind to the centrosome since purified γ-TuRC does not bind to salt-stripped (pericentrin-free) centrosomes (Dictenberg *et al.,* 1998; Moritz *et al.,* 1998; Section III.A).

Recent data go one step further in the 3D elucidation of centrosomal structure for MT nucleation (Fig. 6, IV and V). Dictenberg *et al.* (1998) showed that pericentrin and γ-tubulin are part of a complex (separate from the γ-TuRC) that forms large ring-like structures aligned side by side with a circumference of 800–900 nm and a girth of 90 nm when resolved by deconvolusion immunofluorescence microscopy (Fig. 7). Dictenberg *et al.* hypothesize that the rings are made of pericentrin since the primary amino acid sequence of pericentrin predicts a 218-nm linear coil–coil polypeptide. γ-Tubulin could then be attached to this large ring, most likely in the form of a γ-TuRC (since under mild conditions percentrin and the γ-TuRC comigrate on sucrose gradients). It appears from the study by Dictenberg *et al.* that the function of pericentrin may be to spatially bind and modify the orientation of γ-tubulin/γ-TuRC, thus allowing MT nucleation to progress. Dictenberg *et al.* report that the lattice observed on centrosomes is also found at normal MTOCs indicating that this is a basic structure present at all MTOCs. Moreover, Dictenberg *et al.* report on changes in lattice structure during the cell cycle which illustrates that the lattice consisting of pericentrin and the γ-TuRC may regulate the nucleation capacity of the centrosome. However, due to limited resolution, the γ-TuRCs previously observed by electron microscopy could not be visualized in this study (Moritz *et al.*, 1995a, b).

Although with these studies our understanding of centrosome organization and assembly has significantly increased, we still have only a rudimentary idea about this process. A three-dimensional reconstitution of a

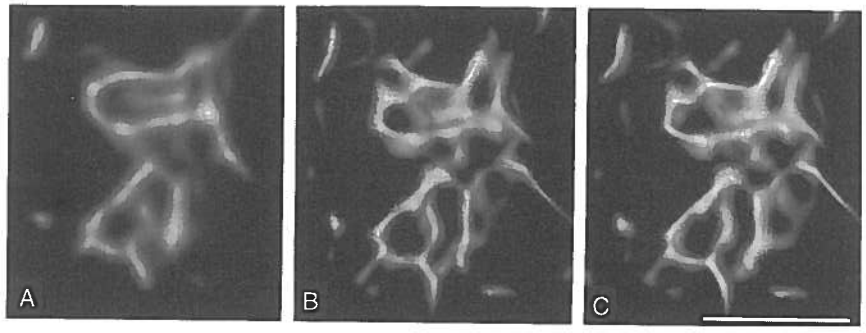


FIG. 7 Pericentrin forms a novel lattice at the centrosome. Centrosomes from a CHO cell were stained with antibodies to pericentrin. (A) A lattice structure observed using commercially available deconvolution software. (B, C) High-resolution stereo images of the same centrosome as in A after utilization of an algorithm. Note the large ring structures of pericentrin. Scale bar = 1 μm (reproduced from Dictenberg *et al., The Journal of Cell Biology,* 1998, **141,** 163–174 by copyright permission of The Rockefeller University Press).

centrosome similar to that of Dictenberg *et al.* (1998) will provide further information on the assembly and nucleation capacity of the centrosome.

It is worth mentioning the variations observed in the assembly processes between different systems. Human sperm cannot be complemented by *Xenopus* egg extracts unless it is primed by disulfide bond cleavage reduction. Moreover, human sperm already has γ-tubulin attached but this γ-tubulin is not apparent until the sperm has been primed. After priming, the human sperm head has some, but not full, capacity for MT nucleation. Thus, the pathway of reconstitution of a human centrosome is not identical to the reconstitution pathway of a *Xenopus* centrosome (Schatten, 1994).

Here I have analyzed centrosome assembly. An equally interesting topic is centrosome disassembly during gamete formation. How is the centrosome specifically destroyed in eggs but maintained in spermatocytes? Although ingenious experiments have been performed to address these questions, there are no molecular data suggesting a specific pathway. Readers interested in these issues are referred to Schatten (1994) and Sluder *et al.* (1989, 1993).

C. Cell Cycle Control of Centriole and Centrosome Assembly

Elucidation of the mechanism of centriolar assembly and duplication is of prime importance for a full understanding of centrosome assembly and function.

Description of an assembly pathway involves morphological and molecular data. However, while data clearly show where and when a centriole assembles, little is known about the molecular machinery or mechanism that makes the assembly possible. I first describe the morphological data and then discuss the possible mechanism of centriole assembly.

There are several excellent morphological studies that report on the cell cycle control of centriole assembly. These studies are summarized in Fig. 8 (Alvey, 1985; Anderson and Brenner, 1971; Chrétien *et al.*, 1997; Kochanski and Borisy, 1990; Lange and Gull, 1995; Tournier *et al.*, 1991a). Centriole duplication starts in G_1, in which protrusions form perpendicular to the proximal ends of the parent centriole pair. For duplication to be possible there is a requirement for a nonperpendicular orientation of the two parent centrioles as illustrated in Fig. 8. Tournier *et al.* (1991a) suggested that the orientation of the centrioles may regulate the progression of the cell cycle and entry into G_0. Anderson and Brenner (1971) and others showed that it is during the previous M phase that the centriole pair loses the perpendicular orientation that is a result of the perpendicular duplication process. The

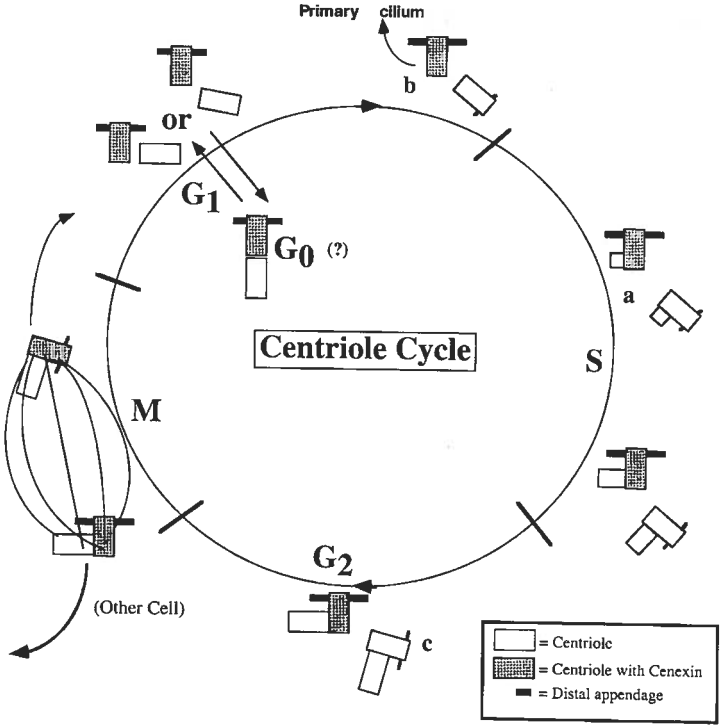


FIG. 8 Schematic representation of the centriole cycle. After cell division a pair of centrioles is delivered to the two daughter cells (G_1). The centrioles have an orthogonal or slightly tilted orientation with respect to each other (filamentous connections are not shown; see Fig. 3). The orthogonal configuration may be lost during M phase (Alvey, 1985). It has also been suggested that the orthogonal configuration is maintained during M phase. This configuration could presumably control and restrict centriole duplication to one duplication event per cell cycle (Tournier *et al.,* 1991b; Hinchcliffe *et al.,* 1998). It has been suggested that one of the morphological differences that follows, and perhaps regulates, entry into quiescent state (G_0) is a close linear conformation of the centriole pair (G_0) (Tournier *et al.,* 1991a, b). Note that one centriole is delivered with distal appendages and has Cenexin associated (mostly at the distal end; Lange and Gull, 1995). It is not clear when and how the distal appendages grow or are being initiated (see Chrétien *et al.,* 1997, for details on appendages and satellites). It is suggested here that the distal appendages are permanent structures that reach their final length sometime during G_1 (b). This centriole (b) corresponds to a "mature centriole" that is able to nucleate a primary cilium or a flagella during interphase (see Fig. 13). The other centriole is "immature" and does not have this capacity. The centriole pair completely loses the orthogonal connection during G_1, and seeding and duplication of the new daughter centrioles starts at the G_1–S transition. If we consider the seeding of the new centrioles as the starting point it can easily be appreciated that the time for formation of a mature centriole (from a to b) takes more than one full cell cycle. It is suggested that the distal appendages start to grow on the immature centriole in late G_1 and that growth continues until M phase. At the end of G_2 the new daughter centrioles have almost reached their final length. However, new data indicate that the daughter cells are significantly shorter than their parents at the end of G_2, suggesting that elongation may continue into G_1 of the subsequent cell cycle (Chrétien *et al.,* 1997). At the G_2–M transition the centriole pairs separate to each end of the bipolar spindle (lines indicate MTs). At the same time the youngest of the parent centrioles acquire Cenexin. Note that MTs in the spindle do not nucleate from the centrioles but from the PCM (not shown).

daughter centrioles grow perpendicularly on the parent centrioles during G_1–S and G_2 phases and the duplication process is semiconservative (meaning that the mother centriole is completely stable and the daughter centriole generated *de novo*; Kochanski and Borisy, 1990). It is unknown when the distal appendages start to grow. Full-size appendages are observed only on the mature mother centriole (Fig. 8, a). Due to experimental difficulties no studies have reported on the speed at which daughter centrioles grow, although it appears to be a continuous process. It is also unknown what determines the fixed length of the centrioles. When the parent centrioles have duplicated, the centrosomes divide at M phase and each centrosome harbors a centriole pair consisting of one parent and one daughter centriole. At the G_2–M transition the mother centriole achieves cenexin. Note that formation of a mature centriole takes almost two more than one cell cycle (Fig. 8, follow the new centriole from a to b).

Studies on the requirements for centriole assembly have shown that centriole duplication can occur in the absence of DNA replication, transcription, and translation (Balczon *et al.,* 1995; Debec *et al.,* 1996; Gard *et al.,* 1990; Kuriyama *et al.,* 1986; Rattner and Phillips, 1973; Sluder *et al.,* 1990). This is quite remarkable and shows that the centrosome cycle to some extent is independent of the nuclear cycle. However, in somatic cells there must be a link between the cell cycle regulating DNA replication and centrosome duplication because, like chromosomes, centrosomes duplicate only once during one cell cycle. There is no explanation for how this is possible. The regulatory machinery must to some extent be autonomous but also share components with the machinery that regulates the cell cycle (Debec *et al.,* 1996; Snaith *et al.,* 1996). Recent results indicate that blocking cyclin E/cdk2 activity in *Xenopus* and sea urchin eggs by injection of the p21 cdk inhibitor protein blocks the continued rounds of centrosome duplication, implicating cyclin E/cdk2 as the kinase driving centrosome duplication (Stearns and Winey, 1997). Moreover, Hinchcliffe *et al.* (1998) used fertilized sea urchin eggs (zygotes) to determine what regulates centrosome duplication. Continuous centrosome duplication was observed when S phase was prolonged by inhibition of DNA polymerase with aphidicolin. Duplication was not affected by Cdk1-B kinase activity (in this system it stably rises to supramitotic levels when DNA duplication is inhibited).

Mitotic arrest (induced by injection of mRNA coding for nondegradable cyclin B) caused failure to duplicate the centrosome although anaphase still occurred. In accord with previous studies, multiple centrosome duplication occurred if protein translation was blocked at fertilization, a condition that also blocks Cdk1-B activation. Thus, regulation of centrosome duplication occurs independently of Cdk1-B activity and the proteolytic events during anaphase. Remarkably, if protein translation inhibitors were applied during prophase of the first cell cycle (rather than at fertilization), the zygotes arrested in the second cell cycle and subsequently duplicated their centrosome only once over an 8-h period (five cell cycles). Analysis of the arrests showed that zygotes treated with translation inhibitors at fertilization were arrested in active S phase (verified by refertilizing zygotes to monitor active DNA replication by BrdU incorporation), whereas no DNA replication occurred in those arrested during the second cell cycle (G_1 equivalent). Thus, genuine S phase arrest is apparently necessary and sufficient to support continuous centrosome duplication. Interestingly, during S phase arrest the time for centrosome reduplication was up to three times longer than one complete cell cycle (this also holds true in somatic cells; see Balczon *et al.,* 1995). A slow S phase-regulated clock may thus ensure that centrosomes duplicate only once during the faster cell cycle. Moreover, during a normal cell cycle the previous S phase may prime the centrioles/centrosome, "licensing" them to duplicate only once during the next cell cycle.

At the molecular level nothing is known about how the complicated 9×3 MT triplet structure assembles perpendicularly on top of the parent centriole. γ-Tubulin was found in the lumen of the centrioles and together with centrin proposed to be involved in centriole duplication (Fig. 9; Fuller *et al.,* 1995; Schiebel and Bornens, 1995). However, considering the 9×3 MT structure of the centriole, the assembly process of centriolar MTs most likely is complex and probably involves more than just γ-tubulin and pericentrin. Previous studies report how treatment of eggs with hypertonic solutions can induce the *de novo* formation of centrioles (Dirksen, 1991). Palazzo *et al.* (1992) described how centrioles could assemble *in vitro* within only 4 min in surf clam extracts. Considering these fast kinetics, it seems possible that the 9×3 MT structure may be initiated by a macromolecular proteinatious template containing γ-tubulin and present in the extract and in the pericentriolar matrix. It is possible that such a "centriolar assembly template" (CAT) to a large extent consists of γ-TuRC linked together at the base of the centriole in pairs of three. Although a CAT could explain seeding of the centriole, it is not easy to imagine how the number and alignment of the triplet MTs relative to one another changes from the proximal to the distal end of the centriole (Figs. 3–5).

The components and structure of this hypothetical CAT are unknown. Considering the low abundance of centrioles in a cell, a genetic approach

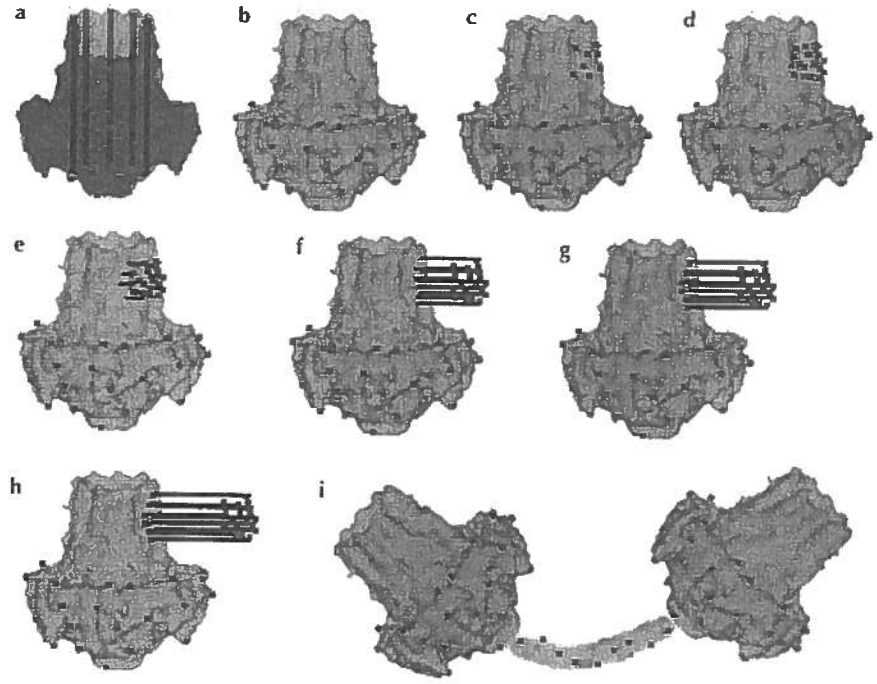


FIG. 9 Centriolar association of γ-tubulin and hypothetical function for centriole duplication. The suggested distribution of γ-tubulin and other components during centriole duplication is shown using a low-resolution centriole reconstruction to indicate positions; γ-tubulin is show in black. The maternal centriole has γ-tubulin (a) in its core as well as (b) on the periphery of the pericentriolar material. The MTs of the centriolar barrel are made up of α- and β-tubulin and are represented as straight black lines. (c, d) Duplication begins with the association of γ-tubulin and other components of the centriolar assembly template (CAT; see text) at a proximal site on the side of the barrel. (e–g) The components of the CAT form a template from which the MTs of the daughter centriole grow. (h, i) Once the daughter's barrel has grown to full length, the centrioles separate but remain linked by a structure that contains γ-tubulin (see also Fig. 3) (partially reproduced from Fuller *et al.,* 1995, with permission from Current Biology Ltd.).

(as opposed to a biochemical) will probably be required to define its components. The most promising organism for this purpose seems to be the biflagellated green algae *Chlamydomonas reinhardtii* (Dirksen, 1991; Dutcher, 1989). Thus, a study on *Chlamydomonas* from Ehler *et al.* (1995) gives hope of identifying molecules involved in centriole assembly and function. Based on a study by Goodenough and St. Clair (1975), Ehler *et al.,* showed that the *bld2–1* mutation in *Chlamydomonas* did not assemble basal bodies, and that the genetic locus did not match with actin, α-, β-, or γ-tubulin, or centrin. However, it was not possible to identify additional

alleles with similar phenotypes and the gene(s) has not been identified (Dutcher and Trabuco, 1998). Interestingly, the cells with the *bld2-1* mutation are viable and form functional spindles, although spindles are mispositioned compared to wild-type strains. Ehler *et al.* (1995, p. 959) conclude:

> It is clear from this study that centrioles/basal bodies are not necessary for the nucleation of cytoplasmic, rootlet/astral, or spindle microtubules during the mitotic cell cycle in *Chlamydomonas.* Rather, it appears that the role of the centriole in some cell types may be to provide an organized cytoskeletal superstructure that is critical for the correct placement of the spindle and the cleavage furrow during cell division.

To understand centriolar function and assembly, one could take a genetic approach using *C. reinhardtii* and *Drosophila* to determine components and functions, and then use biochemical systems, such as *Xenopus* or surf clam, to determine a biochemical pathway of assembly.

The next level one must understand is centrosome separation or division as a process—not the regulation of the process. Thus, how does the cell at prophase manage to separate the pericentriolar matrix surrounding the "double centrosome" into two equally big pools? Two mechanisms have been proposed for how the centrosomes are separated. The first proposes that minus-end-directed motors attaching to astral MTs emanating from the centrosome pull centrosomes apart and also help in the migration of centrosomes during prophase (i.e., Waters *et al.,* 1993). The other model suggests that plus-end-directed motors push centrosomes apart. By sitting on MTs emanating from one centrosome and moving toward the plus end of MTs emanating from the other centrosome, separation would occur (i.e., Boleti *et al.,* 1996, Fig. 10).

In addition to an active motor-driven movement, it seems reasonable to invoke a mechanism that weakens the adhesiveness of the pool of pericentriolar matrix to itself to separate centrosomes at prophase. A recent study on Nek2 supports this idea (Fig. 10). It is proposed that phosphorylation of the pericentriolar matrix by Nek2 leads to a weakening of the interaction/adhesiveness of the pericentriolar matrix. Through the subsequent movement by motors the centrosomes are separated. In the model the plus-end-directed motor Eg5 is proposed to push the centrosomes apart. As indicated in the speculative Fig. 10, recruitment of the motor Eg5 is a step that occurs after Nek2 has loosened the pericentriolar matrix in preparation for centrosome separation. This is based on observations showing that phosphorylation of a single cdc2 site is required to localize Eg5 to the centrosome during mitosis and that this site can be phosphorylated by cdc2 kinase *in vitro* (Blangy *et al.,* 1995; Sawin and Mitchison, 1995). Interestingly, there is evidence that Eg5 associates with p150 (glued), which is a member

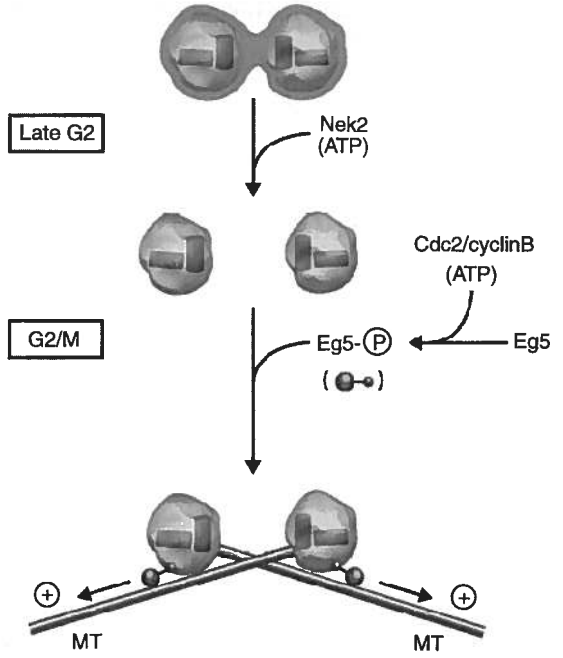


FIG. 10 A two-step model for centrosome separation. In this model, the process of centrosome separation is described in terms of two independent steps which must occur at the onset of mitosis. The first step involves the severing of a connection (e.g., the dissolution of a hypothetical cage) which holds the centrosomes in close proximity throughout interphase. Under physiological conditions, this step is proposed to be triggered in late G_2 by the activation of Nek2 (and/or the inactivation of a counteracting phosphatase). Under the experimental conditions described here, premature separation (i.e., splitting) of centrosomes results from ectopic expression of active Nek2. In both cases, phosphorylation of as yet unidentified centrosomal "glue" proteins may cause their depolymerization or target them for proteolytic degradation. The second step involves several MT-based motor proteins, only one of which is depicted here. This kinesin-related motor, Eg5, is recruited to the centrosome by the dynein complex and in response to phosphorylation by Cdc2/cyclin B, and its activity contributes to the separation of centrosomes along MTs. Note that objects are not drawn to scale, and the precise mode of action of Eg5 remains to be determined (a similar function has been proposed for the motor XKLP2, see text) (reproduced from Fry *et al.,* 1998, with permission from Oxford University Press).

of the dynactin complex that interacts with the minus-end-directed motor dynein (Blangy *et al.,* 1997). These findings indicate that it is possible that localization of a plus-end-directed motor (i.e., Eg5) may be regulated by a minus-end-directed motor (i.e., dynein), and vice versa. This is interesting because it strongly suggests that motors localized by such means do not simply transport cargo around but serve other functions, i.e., push

centrosomes apart (Fig. 10). (A similar function and localization mechanism has been proposed for XKLP2.)

An interesting addition to the regulatory hierarchy of mechanisms that control the frequency of centrosome duplication came with the discovery that the tumor suppressor p53 can influence centrosome duplication (Fukasawa *et al.,* 1996). In a mouse cell line lacking p53, multiple copies of functionally competent centrosomes were generated during a single cell cycle. p53 is a tetrameric transcription factor that can activate or inhibit the expression of groups of genes and the effect on centrosome duplication may therefore be rather indirect. It is unclear how p53 affects centrosome duplication, but it most likely involves a change in the signaling cascades regulating the cell cycle. Since multiple centrosomes lead to missegregation of chromosomes, this may explain how lack of p53 ultimately can lead to development of cancer (Agarwal *et al.,* 1998; Winey, 1996).

V. Microtubule Nucleation

The principal function of the centrosome is to be a site of MT nucleation. Since nucleation is the first step in MT assembly it is a process that is important to understand. The aim of this section is to analyze and compare different mechanisms of MT nucleation. I will distinguish between three types of MT nucleation: self-nucleation, catalyzed nucleation, and centrosome-mediated nucleation (Fig. 11).

A. Bulk Microtubule Assembly: A Thermodynamic Problem

Only the tubulin dimer and GTP are needed to form a MT. A MT is an assembly of approximately 13 protofilaments, consisting of $\alpha\beta$-tubulin heterodimers, closed into a hollow filament 25 nm in diameter. Centrosomally nucleated MTs always contain 13 protofilaments, whereas self-nucleated MTs may have a variable number of protofilaments (12–16). Both α- and β-tubulin can bind GTP but only GTP bound to the β-tubulin subunit can be hydrolyzed. Since the building block is the $\alpha\beta$-tubulin heterodimer, MTs have an intrinsic polarity: a fast growing plus end with β-tubulin at the end and a slow growing minus end with α-tubulin at the end (localized at the centrosome). In the cell we mostly see MTs growing off centrosomes because they ease the nucleation step. However, centrosomes are not strictly required for nucleation and MTs can self-nucleate in the absence of centrosomes. However, self-nucleation of MTs require a much higher tubulin concentration than the concentration required to grow MTs off

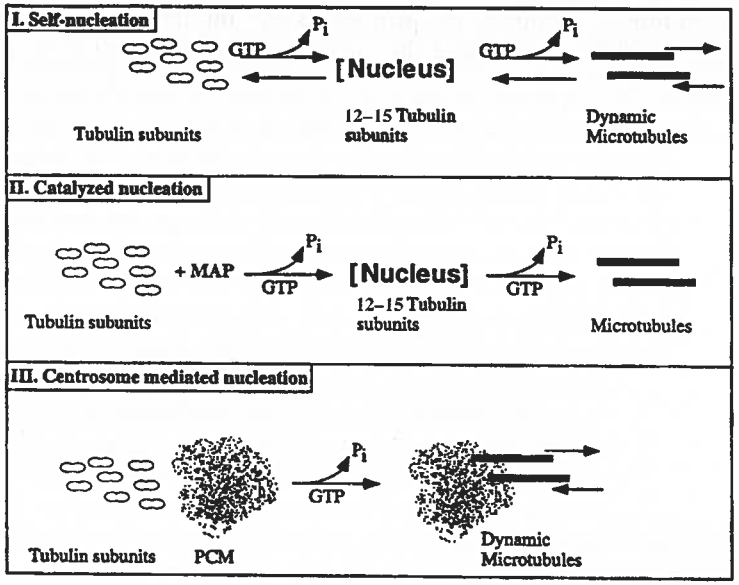


FIG. 11 Microtubule nucleation. (I) Pure tubulin can spontaneously assemble into MTs. This requires tubulin concentrations that are much higher than those *in vivo* and occur via a nucleation core of twelve to fifteen tubulin subunits. Concomitantly with MT assembly, the GTP bound to tubulin hydrolyzes into GDP and P_i. The exact timing of polymerization and phosphate release is unclear (Erickson and O'Brien, 1992). (II) In the presence of MAPs the mechanism of nucleation is the same as that for pure tubulin. However, the MAPs manage (see text) to stabilize the critical nucleation core consisting of five to seven subunits. MAPs that enhance nucleation (i.e., τ) also dampen MT dynamics, and stable MTs are the end result. (III) The pericentriolar matrix contains the components required for nucleation off centrosomes (i.e., γ-TuRC and pericentrin). The γ-TuRC helps in the formation of the first nucleation core, probably in a manner comparable with a template for MT assembly (Pereira and Schiebel, 1997).

centrosomes. The requirement for a higher tubulin concentration reflects the transition energy that has to be surmounted to form the first part of a MT. It is thought that formation of a core of approximately twelve to fifteen tubulin heterodimers is required to form the critical starting nucleus for MT assembly (Carlier *et al.,* 1997; Carlier and Pantaloni, 1978, 1981). The formation of this nucleation core is the rate-limiting step for MTs assembled in solution (Fig. 11, I). Using the second law of thermodynamics, it can be appreciated why formation of the nucleation core is the rate-limiting step (the second law of thermodynamics states that $\Sigma\Delta G = \Sigma\Delta H - T\Sigma\Delta S$. For a process to occur, ΔG must be negative). The entropy (S) of the system that is going to form a MT is high before nucleation because there is a high probability of finding the freely diffusing dimers in a given position

at a given time. In contrast, the probability of simultaneous association of five to seven dimers is low and this corresponds to a decrease in entropy. Thus, the sign of $\Sigma\Delta S$ is negative during nucleation. (Note, however, that if the dimers have water molecules associated that dissociate upon interaction with other tubulin dimers, this will increase the entropy.) The entropy change during MT nucleation and MT growth is therefore most likely unfavorable for the formation of MTs (Inoué and Salmon, 1995). It therefore seems that the driving force must come from a decrease in internal energy of the tubulin dimer during MT polymerization. Where does the energy come from? The β-subunit hydrolyzes GTP to GDP upon MT polymerization. The hydrolysis of GTP results in the release of energy that perhaps could be used for nucleation and polymerization. However, studies from a slowly hydrolyzable GTP analog (GMPCPP) show that MTs can nucleate much better in the absence of GTP hydrolysis (Carlier *et al.,* 1997; Carlier and Pantaloni, 1978; Hyman *et al.,* 1992). Moreover, calculations have shown that the energy of GTP hydrolysis is stored in the MT lattice (Caplow *et al.,* 1994). Thus, GTP hydrolysis cannot be used to fuel the nucleation process. On the contrary, GTP hydrolysis destabilizes the interaction between tubulin subunits and is required for the process of MT dynamics (Chrétien *et al.,* 1995; Erickson and O'Brien, 1992; Hyman *et al.,* 1992). It is possible that tubulin in the GTP state has a conformation favorable for nucleation and that rapid GTP hydrolysis evolved to control the nucleation step. We must conclude that the transition energy for formation of the nucleus cannot be very high, and it remains unclear exactly how the first nucleation core forms during MT self-nucleation. With the structure of tubulin now solved (Nogales *et al.,* 1998) we may soon have answers to these questions. For example, how does the structure of tubulin change upon GTP hydrolysis?

Although the centrosome is the major site of MT nucleation *in vivo,* it has recently been shown that MTs can assemble spontaneously in the cytoplasm *in vivo* (Keating *et al.,* 1997; Vorobjev *et al.,* 1997). However, using very pure tubulin it has been observed that nucleation of MTs in solution does not occur *in vitro* even at tubulin concentrations that are five- to sevenfold higher than those *in vivo.* This suggests that nucleation of cytoplasmic MTs *in vivo* must involve some catalysts. *In vivo* there are two types of catalysts: MAPs and the centrosome (Fig. 11, II–III). In the cytoplasm MAPs most likely are involved in regulating the spontaneous assembly of MTs (Andersen, 1998). For example, the MAP τ is an example of a catalyst that very potently promotes MT polymerization in solution (Brandt and Lee, 1993) and suppresses MT dynamics (in particular by suppressing the catastrophe frequency). How can τ promote MT nucleation? Recent studies have shown that MT binding of τ occurs through two types of sites (Preuss *et al.,* 1997). One site targets the MAP to the

MT or to the MT nucleation unit, consisting of twelve to fifteen tubulin subunits. This site is called a "jaw" since it attaches to the nascent MT. The other site is the repeat domains that are required for inducing MT nucleation and polymerization. It is suggested that MAPs such as τ can cross-link protofilaments and in this way facilitate nucleation. Whether τ or other MAPs influence the hydrolysis rate of GTP is unknown. Most likely τ promotes nucleation simply by stabilizing interaction between tubulin dimers in the nucleation center. As already described and further discussed in Section V,B, centrosome nucleation is distinct from self-nucleation and MAP-catalyzed nucleation. Centrosome-mediated nucleation utilizes the γ-TuRC and always results in MTs with 13 protofilaments.

B. Microtubule Nucleation by Centrosomes

In vitro the centrosome nucleates MTs at very low tubulin concentrations (around 5 μM; Andersen *et al.,* 1994; Andersen and Karsenti, 1997; Bré and Karsenti, 1990; Hill and Kirschner, 1983). As described in Section V,A, it has been known for more than 20 years that MAPs can lower the critical concentration for MT nucleation. However, a search for MAPs that could explain the extraordinary nucleation capacity of centrosomes has been unsuccessful (but see Domínguez *et al.,* 1994). It appears that γ-tubulin in the form of the γ-TuRC is the unit that is responsible for all MT nucleation by the centrosome (Section III.A). Most likely, γ-tubulin requires some additional proteins to be able to induce MT nucleation, some of which may be members of the γ-TuRC and pericentrin. Thus, the γ-TuRC has been purified and shown to induce MT nucleation *in vitro* (Zheng *et al.,* 1995; Moritz *et al.,* 1998). There is no direct knowledge about the physical chemistry of the facilitation of nucleation but a recent review examines two models for how the γ-TuRC serves as a template for MT nucleation at the centrosome (Pereira and Schiebel, 1997).

The next level of complexity for centrosomal nucleation involves regulation of the nucleation capacity. I split the discussion of regulation of the nucleation capacity into two components. First, I shed some light on the biophysical aspects of MT nucleation by the centrosome, and then I discuss cell cycle regulation in Section V.C.

Microtubules are nucleated very closely to one another at the centrosome and a study reports on the possibility of local depletion of tubulin dimers around the centrosome during massive nucleation of MTs off the centrosome (Dogterom *et al.,* 1995). Assuming that the centrosome is a smooth sphere and by changing the number of nucleation sites on the surface, Dogterom *et al.* calculate that diffusion may effectively limit the number of MTs growing off a centrosome. How would this be possible?

The dynamics of MTs is very dependent on the tubulin concentration and by local depletion MTs could become so dynamic that they would not at all polymerize (Fygenson *et al.,* 1994). The result of Dogterom *et al.* (1995) is interesting since it could indicate that it is not the number of nucleation sites that regulates how many MTs can grow off the centrosome but perhaps the diffusion of tubulin to the nucleation sites. However, Méda *et al.* (1997) show that purified centrosomes have a capacity to bind 25,000 tubulin dimers with a K_D of 5 μM and with a half-saturation time of 3 min. Interestingly, this K_D closely matches the critical concentration for MT growth off centrosomes (Andersen *et al.,* 1994). Moreover, if such low-affinity binding sites exist the diffusion limit (Dogterom *et al.,* 1995) for the total number of MTs growing off a centrosome would be annulled since they could fuel the real nucleation sites with tubulin. The composition of the low-affinity binding sites is unknown, but the data by Méda *et al.* (1997) indicate that this binding is not to the γ-TuRC.

The study by Méda *et al.* (1997) is also interesting because it brings a new dimension to centrosome-mediated MT nucleation by suggesting that the nucleation step is a two-step process. The first step consists of local increased concentration at the centrosome of tubulin dimers by an unknown mechanism. The next step consists of nucleation of MTs facilitated by the γ-TuRC. Méda *et al.* make clear that two-step processes are common in biological systems in which a few high-affinity structures (in this case the γ-TuRC) are bound to insoluble structures (in this case the centrosome) since ligand (in this case tubulin) targeting is greatly facilitated under such conditions (e.g., DNA-binding proteins, phages binding to cell membranes, and actin nucleation).

It has been observed that soluble MAPs affect the nucleation capacity of centrosomes *in vitro,* which shows that soluble factors not associated with the centrosome can also influence nucleation (Andersen *et al.,* 1994; Bré and Karsenti, 1990). However, these soluble factors do not directly enhance nucleation of MTs on the centrosome but simply allow more growth off the centrosome through a regulation of MT dynamic instability. This mechanism is thus an indirect way of regulating the nucleating capacity of the centrosome. This type of modulation may be very important *in vivo* in which both soluble and insoluble factors are subject to cell cycle regulation.

C. Regulation of Centrosomal Microtubule Nucleation Activity during the Cell Cycle and Differentiation

Why is it important that the cell regulates the MT nucleation capacity of the centrosome? There are several answers to this question. Here I will

approach this question by contemplating the properties of MTs from a biophysical point of view. A MT is a very stiff polymer. Its resistance toward bending is expressed as "flexural rigidity" and has been measured to be in the range of 2–40 10^{-24} Nm^2, comparable with the stiffness of plast polymers (Feigner *et al.,* 1996; Kurachi *et al.,* 1995; Mickey and Howard, 1995). Thus, such a polymer can be used to generate shape in three-dimensions, like poles holding a tent. In a way, MTs are part of the mechanism responsible for transforming the linear two-dimensional DNA code into the three-dimensional realm of a cell. Because MTs have this function for morphogenesis (Hyman and Karsenti, 1996; Kirschner and Mitchison, 1986), it is important for the cell to regulate the length, the number, and the orientation of MTs. Moreover, MTs are involved in directed vesicular transport, organelle movement, and cell division. Therefore, it is important to regulate MT assembly both spatially and temporally.

An important parameter in this regulation consists of regulation of the nucleation of MTs in general and by centrosomes in particular. The following two examples will illustrate why it is important to regulate the nucleation capacity of the centrosome.

The first example concerns morphogenesis of neurons. Extending axons build an elaborate cytoskeleton mainly composed of MTs (Matus, 1994). There is lively debate about the origin of these MTs (Baas and Brown, 1997; Bray, 1997; Hirokawa, 1997; Hirokawa *et al.,* 1997). One model proposes that tubulin subunits are transported to the tip of the axon where they are polymerized into MTs (Hirokawa, 1997; Hirokawa *et al.,* 1997). The other model suggests that MTs are nucleated by the centrosome in the cell body, released from the centrosome, and then transported out to the tip of the axon (Baas and Brown, 1997; Bray, 1997). In the latter model the centrosome serves as an engine for the generation of MTs. Thus, the ability of the centrosome to nucleate MTs indirectly becomes essential for axon outgrowth. One way to resolve the controversy would be to remove the centrosome by microsurgery (Maniotis and Schliwa, 1991). It remains unresolved which model most faithfully reflects reality.

The second example relates to regulation of MT assembly during the cell cycle. In all cells there is a dramatic rearrangement of the cytoskeletal network from interphase to mitosis. A schematic representation of the transition from interphase to mitosis is shown in Fig. 12. Note the following: (i) In interphase many MTs are not only found associated with the centrosome but also free in the cytoplasm and (ii) in mitosis, all MTs seem to emanate from the centrosome and are almost only observed in the direction of the chromosomes (Fig. 12). How is this rearrangement brought about? What is the origin of the MTs? Are they derived from the centrosomes or spontaneously nucleated in the cytoplasm?

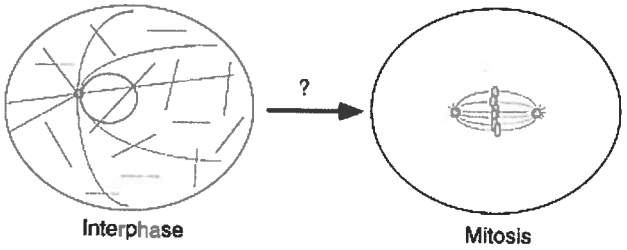


FIG. 12 Schematic representation of the rearrangement of MTs between interphase and mitosis. Interphase MTs (black lines) are long and stable and are found associated with the centrosomes (circle) or assemble freely in the cytoplasm (Vorobjev *et al.,* 1997; Andersen, 1999). In mitosis all free cytoplasmic MTs depolymerize and MTs only polymerize off centrosomes and in the vicinity of the chromosomes (ellipses), forming the elliptical bipolar spindle.

Recent studies have shown that cytoplasmic MTs during interphase are derived both by ejection from the centrosome and from spontaneous assembly in the cytoplasm (Keating *et al.,* 1997; Vorobjev *et al.,* 1997; Andersen, 1998). In mitosis MTs are also ejected from centrosomes, as observed in mitotic *Xenopus* egg extracts, but no spontaneous assembly occurs. Thus, in both interphase and mitosis, MTs are ejected from the centrosome into cytoplasm. Why then are no cytoplasmic MTs observed during mitosis (Fig. 12)? Research during the past decade has shown that MTs become more dynamic during mitosis, primarily due to a dramatic increase in the catastrophe frequency, which subsequently leads to a 10-fold increase of MT turnover from interphase to mitosis (Belmont *et al.,* 1990; Tournebize *et al.,* 1997; Verde *et al.,* 1992). Thus, the increase in MT dynamics during mitosis explains why the cytoplasmic MTs disappear from interphase to mitosis (Fig. 12).

How do the MTs manage to preferentially grow in the area around chromosomes during mitosis if cytoplasmic MTs are so dynamic that they depolymerize? Research during the past 20 years has shown that the mitotic chromosomes change the environment for MT polymerization (Andersen, 1999; Andersen *et al.,* 1997; Heald *et al.,* 1996; Karsenti, 1991; Karsenti *et al.,* 1984; Marek, 1978; Nicklas, 1988; Zhang and Nicklas, 1995). Thus, the mitotic chromosomes somehow stimulate MT polymerization in an area around them, leading to the preferential distribution of MTs around the mitotic chromosomes. These findings may also explain how spindle assembly is possible in some systems devoid of centrosomes (i.e., plants and eggs) because spindle MT assembly may be largely chromosome driven in such systems (Andersen, 1999; Andersen *et al.,* 1997; González *et al.,* 1998).

Regarding specifically the nucleation capacity of centrosomes, it is seen from this analysis that in mitosing vertebrate cells the only source of MTs is the centrosome. All MTs must be generated by the centrosome. Therefore, the centrosomal ability to generate MTs becomes essential for cell division. In order to safely and efficiently build the mitotic spindle, the centrosome as "microtubule source" must work at a maximum rate, i.e., a priori have a higher MT nucleation capacity during mitosis than during interphase. The benefit of an augmented capacity to generate MTs during mitosis is an increased chance of proper chromosome attachment to the MTs.

How does the cell manage to change the nucleation capacity of the centrosome? The first important discovery was that it is the pericentriolar matrix that nucleates the MTs and not the centrioles (Gould and Borisy, 1977). Several studies showed that there is more pericentriolar matrix around the centrioles during mitosis than during interphase, indicating that this could explain the increased MT nucleation activity observed at mitosis (Dictenberg *et al.,* 1998; Rieder and Borisy, 1982; Snyder and McIntosh, 1975). Concurrent with the increase in volume of pericentriolar matrix the MPM2 antibody that recognizes a phosphoepitope shows increased staining of the centrosome (Davis *et al.,* 1983; Vandre *et al.,* 1986). Subsequent studies showed that the increased MT nucleation capacity during mitosis is dependent on the MPM2 epitope and thus phosphorylation events (Centonze and Borisy, 1990). Additional *in vitro* experiments showed that treatment of centrosomes with cyclin A-dependent cdc2 kinase caused a specific increase in MT nucleation. In contrast, cyclin B-dependent cdc2 kinase which had no effect (Buendia *et al.,* 1992; Buendia and Karsenti, 1995). Thus, through a cascade of phosphorylation events, more MTs are nucleated by centrosomes during mitosis than during interphase.

How is this increased nucleation capacity brought about by phosphorylation of centrosomal proteins? There are several possibilities: (i) phosphorylation-dependent removal of inhibitors of MT nucleation; (ii) phosphorylation-dependent binding of activators of MT nucleation; (iii) phosphorylation-dependent reorganization of the pericentriolar matrix, opening up for more nucleation sites; and (iv) regulation of soluble factors involved in MT stabilization. The first possibility seems the least likely since *in vitro* phosphorylation of purified centrosome results in increased nucleation capacity (but see Balczon *et al.,* 1994). Moreover, this first possibility may be thought of as a variation of the third possibility.

The latter three possibilities are probably all at work. Thus, the increase in the amount of pericentriolar material probably to some extent reflects recruitment of material required for MT nucleation. Moreover, it seems likely that reorganization of the pericentriolar matrix can open up otherwise

hidden nucleation sites. With the discovery of the γ-TuRC, and its direct localization to the base of centrosome nucleated MTs, this possibility seems even more attractive (Dictenberg et al., 1998; Moritz *et al.,* 1995a, b; Vogel *et al.,* 1997; Zheng *et al.,* 1995). The proteins associated with γ-tubulin in the γ-TuRC are beginning to become characterized and may be the target of the cyclin A-dependent phosphorylation events leading to increased nucleation capacity (Section III.A). For example, by tilting the γ-TuRC attached to the pericentrin rings (Fig. 7) it may be possible to regulate the number of accessible nucleation sites at the centrosome. Much more work is required to clarify these issues.

As a last example of the regulation of MT nucleation by centrosomes, I discuss the change in MT nucleation pattern during the differentiation of cells into epithelial cell layers. The MT rearrangement shown in Fig. 12 is primarily found in fibroblast cells or cells in suspension. If one examines an epithelial cell layer, the behavior of the centrosome is markedly different (Fig. 13, see color plate). How the cell regulates this change in location and activity of the centrosome during the cell cycle is unknown. It is notably interesting in this example that during interphase the epithelial cells may be said to be devoid of a centrosome but apparently contain numerous apically localized MTOCs.

Where do all these noncentrosomal apical MTs come from? One possibility is that the material mediating MT nucleation (i.e., the γ-TuRC and pericentrin) is still localized to the periphery of the centrioles and that this area serves as a MT factory at the apical side. This would work much like the proposed centrosome-driven MT engine for axon extension. Another possibility is that MTs may nucleate throughout the entire apical side through dispersed γ-TuRCs and pericentrin. Alternatively, MTs may be spontaneously nucleated independently from γ-TuRC and pericentrin through the activity of MAPs and subsequently sorted by motors with their minus end localized to the apical surface (Vorobjev *et al.,* 1997; Andersen, 1998). Which of these possibilities is most likely to work?

Although a study reports localization of dispersed γ-TuRC and pericentrin in some epithelial cells at the apical surface (Meads and Schroer, 1995), others have obtained negative results on this issue (Meads and Schroer, 1995; Mogensen *et al.,* 1997; Reinsch and Karsenti, 1994; Vogl *et al.,* 1995). In the latter three studies, γ-TuRC and pericentrin were only found localized to the material surrounding the apically located centrioles. This could indeed suggest that MTs become generated close to the centrioles through nucleation by γ-TuRC and pericentrin and subsequently transported to their destination at the apical membrane. Figure 14 summarizes how Mogensen *et al.* (1997) imagine that such a MT factory could work. Central to the model is that MTs are nucleated in a γ-tubulin-dependent way (Fig. 14A). The minus end is then stabilized through binding of proteins (Figs.

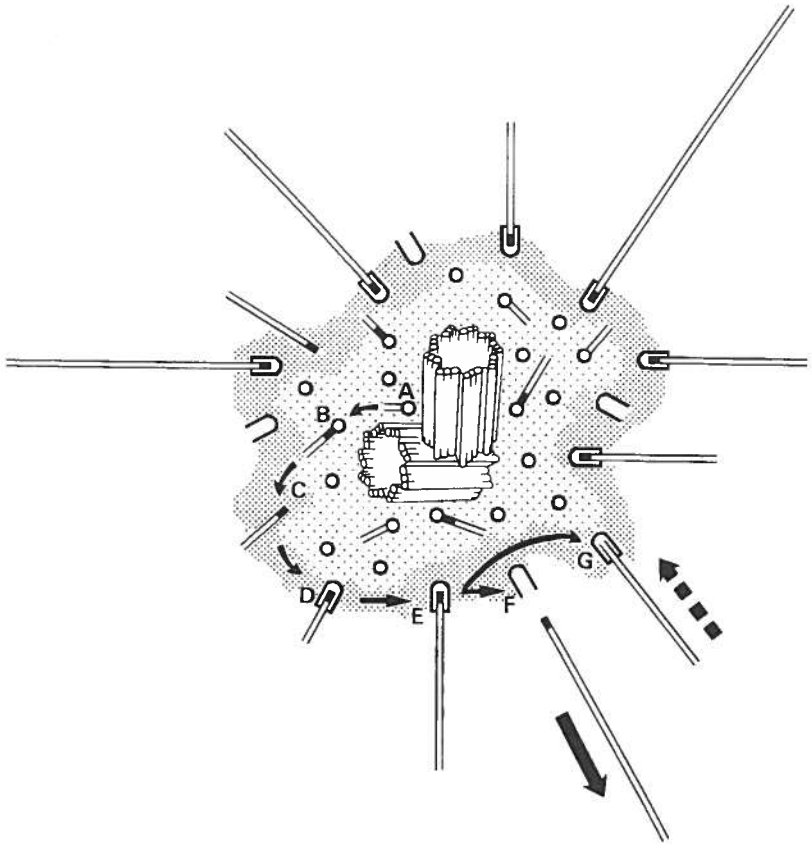
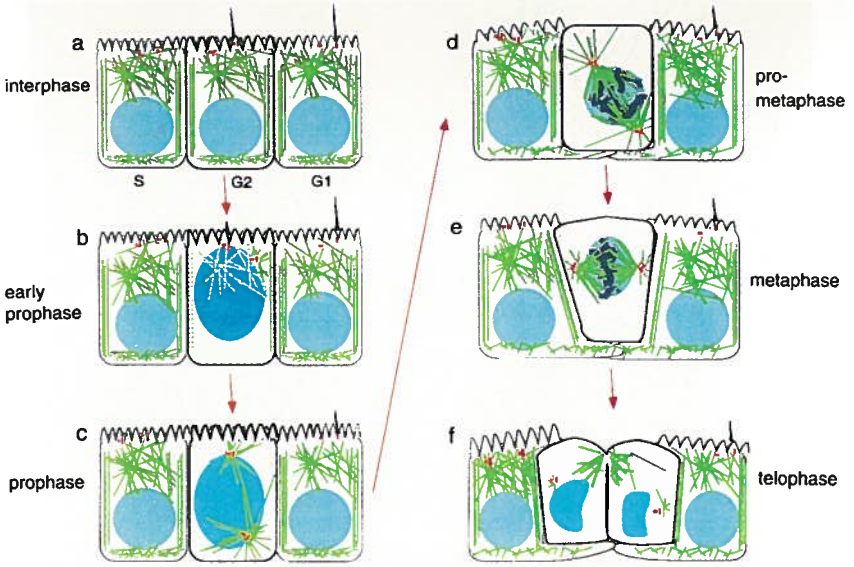


FIG. 14 Schematic diagram of a typical animal cell centrosome outlining the main features of the docking element hypothesis. The pericentriolar matrix is differentiated into a mainly central nucleating domain (lightly stippled) containing nucleating elements (○) and a more peripheral docking domain (densly stippled) with docking elements (U). (A) Nucleation: Microtubule assembly is initiated by a nucleating element. (B) Capping: Addition of a minus-end cap (black stain on MTs). (C) Translocation: Release from a nucleating element is followed by translocation. (D) Docking: A docking element changes its configuration when it effects anchorage of a capped minus end (schematically represented by closure lines at the previously open end of the docking element). (E) Elongation: The proximal minus end remains anchored to the centrosome via a docking element as the MT continues to elongate by addition of tubulin to its distal plus end. (F) Escape: The minus end retains its protective cap as it is released by a docking element. The MT escapes from the centrosome and migrates (arrow) to a new cytoplasmic location. This transport is mediated by motors and has been directly observed *in vivo* (Keating *et al.,* 1997; Vorobjev *et al.,* 1997). (G) Minus-end dynamics: Anchorage by a docking element is maintained after cap loss, which permits minus-end loss of tubulin that can contribute to a flux of tubulin or MT shortening (dashed arrow) (reproduced from Centrosomal deployment of γ-tubulin and pericentrin: Evidence for a microtubule-nucleating domain and a minus-end docking domain in certain mouse epithelial cells, Mogensen, M. M., Mackie, J. B., Doxsey, S. J., Stearns, T., and Tucker, J. B., *Cell Motil. Cytoskel.,*



CH. 2, FIG. 13 Schematic representation of the changes in the organization of MTs (green) and the localization of centrioles (red) and the nuclei (blue) as observed in MDCKII epithelial cells. In interphase the mature centriole is at the base of the primary cilium (a; see also Fig. 8). Microtubules are very concentrated toward the apical surface and many minus ends are found in this location (see also Fig. 14). At the onset of prophase the centrioles start to migrate toward the nucleus to act as poles for the mitotic spindle (b, c). The spindle orients in a way so that cleavage primarily results in expansion of the epithelium, as opposed to the loss of a cell out of the epithelium during division (d, e). The centrosome plays a critical role in the correct positioning of the spindle. After telophase and cytokinesis (f) the cell returns to the interphase situation (a) (reproduced from Reinsch and Karsenti, *The Journal of Cell Biology*, 1994, **126,** 1509–1526 by copyright permission of The Rockefeller University Press).

14B and 14C) which allows the MT to be inserted in so-called docking sites either at the centrosome (Fig. 14D) or elsewhere at the apical surface after transportation (Fig. 14F). In this model the area around the centrioles functions like a MT "factory" and MTs are then transported to other locations (Keating *et al.*, 1997; Rodionov and Borisy, 1997).

VI. Concluding Remarks

The challenges for the future lie in integrating the vast amount of molecular information about the centrosome into a coherent story that mechanistically answers the questions addressed in the Introduction. With regard to the questions surrounding centriole assembly and duplication, we are still in very uncharted territory. We need to understand how centrioles can affect the cell cycle and cell division in some cells. We also need to determine more thoroughly how nucleation is facilitated by the centrosome and how the nucleation capacity of the centrosome is regulated during the life cycle of a cell. It will also be interesting to contrast and compare differences and similarities of MTOCs from different organisms. The following are the major questions in the field: What is the precise function of the centrioles and how do they assemble? The major goals within the centrosome field will be to describe the time and place of the association of centrosomal proteins to the centrioles and to build a dynamic three-dimensional model of the pathway leading to a mature centrosome.

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