Centrosome composition and microtubule anchoring mechanisms
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Centrosomes of animal cells and spindle pole bodies of fungi are the major microtubule nucleating centers. Recent studies indicate that their capacity to organize microtubule arrays rests on elaborate control of the anchoring and release of the nucleated microtubules. Although common molecular mechanisms are likely to be involved in both cases, the centrosome from animal cells shows considerable complexity and flexibility, which contrasts with the simple laminar organization of spindle pole bodies in fungi. The role of the centriole pair in controlling both the structural stability and the activity of the centrosome in animal cells is now becoming clearer. The potential use of the generational asymmetry of centrosomes or spindle pole bodies for controlling cell polarity is also a growing theme.

Introduction

When dealing with centrosome components and microtubule-anchoring proteins from animal cells, one must distinguish between growing or quiescent cells. In growing cells, the behavior of the centrosome is markedly different in early G1 cells than once S phase has been initiated. The duplication of the centrosome, which blurs somewhat the early G1 cells than once S phase has been initiated. The crossover point in G1 cells is becoming clearer. The potential use of the generational asymmetry of centrosomes or spindle pole bodies for controlling cell polarity is also a growing theme.

Abbreviations

DC daughter centriole
MC mother centriole
MAP microtubule-associated protein
NuMA nuclear mitotic apparatus protein
PP1 protein phosphatase 1
SPB spindle pole body

The isolated centrosome and the in situ centrosome

Isolated centrosomes conserve all the basic structural and functional properties of in situ centrosomes: they contain a centriole pair linked by a matrix (Figure 1) and are able not only to nucleate microtubule tubulin assembly below the critical concentration, but also to replace the sperm centrosome in triggering cellularization and embryo development in frogs [6]. The in situ centrosome boundary depends also on microtubules nucleated in the vicinity of the centriole pair: additional centrosomal components from the cytoplasmic pool can move along these microtubules in a dynein-dependent manner [7,8*] and concentrate around the centriole pair (Figure 2). Depending on whether these microtubules are anchored at, or released from, the centrosome, the extent and distribution of centrosomal components is likely to vary considerably depending on the somatic cell type. Unattached cells like lymphoblasts have most of their microtubule-anchoring components concentrated on the centriole pair: in these cells, all microtubules are anchored at the centrosome, even when cells are treated with taxol (Figure 3). In contrast, in epithelial cells in which a major fraction of anchoring proteins are released from the centrosome together with microtubules [9,10], taxol treatment induces the assembly of centrosome-free microtubules that eventually form into bundles according to their polarity.

Centriole-based assembly of the centrosome: exploiting microtubule polarity to build the microtubule-nucleating center

The structural stability of the centrosomes from vertebrate cells and their reproduction depend upon centrioles [11,12**]. The nine proximal triplets of each centriole provide a cluster of microtubule minus ends that, owing to their considerable stability with respect to that of cytoplasmic microtubules [13], could successfully compete for minus-end-binding proteins, including γ-tubulin-containing complexes, and concentrate them. Alternative and more selective mechanisms are also possible: recent results suggest that splice variants of the centrosomal protein AKAP450 bind constitutively to the centriole walls in the presence of microtubule-disrupting drugs whereas other variants are recruited to the centrosome in a microtubule-dependent manner (W Kemmner and G Keryer, personal communication).
The stability of centriole microtubules also results in heavy polyglutamylation of centriole tubulin, which apparently confers long-term stability to centrioles [11,14]. Polyglutamylation might control the association of specific centrosomal microtubule-associated proteins (MAPs) to the centriole wall, in a manner similar to that which has been shown for the binding of brain MAPs to tubulin (see [11] and references therein). Centrioles could therefore trigger the assembly of the centrosomal matrix through two subsets of microtubule-binding proteins, one able to bind to their proximal ends and another able to bind to their walls in a tubulin polyglutamylation-dependent manner (Figure 1b). If these two subsets of proteins were able to interact with each other, this would form a matrix and create additional nucleating sites for microtubule assembly and could provide a mechanism for regulating their number (Figure 4). Whether this would suffice to form a physical link between the two centrioles able to resist centrosome isolation and further KI or urea treatment [15,16] is not known.

In agreement with the a critical role of the centriole microtubule in the assembly of the pericentriolar matrix, most of the substructures (Figure 1a) and the characterized centrosomal proteins (Figure 5) are associated with centrioles according to their proximo-distal polarity. The notion that the centriole/basal body cylinder acts as a critical organizing structure is familiar to specialists of unicellular flagellates and ciliates [17,18].
Centriole stability

How are the dynamics of centriole microtubules reduced to a level that gives them a longer half-life than the cell cycle duration? Proteins such as tektins are likely to play a role, as they do in the stability of the axoneme doublets [19]. Microtubule triplets (addition of a C tubule to the doublets) are observed only in centrioles and basal bodies, at their proximal side. Such a structure is more stable than the distal doublet structure, as observed when centrosomes are subjected to heat (45°C) or to hydrostatic pressure [20]. The presence of the C tubule depends upon the presence of a distinct tubulin protein, the δ-tubulin, which was first characterized as the product of the gene UNI3 from *Chlamydomonas reinhardtii* and which appears necessary for the stability of basal bodies [21]. This gene is conserved from unicellular algae to mammalian organisms [22•,23•], a result which could parallel the evolutionary conservation of the triplet organisation of centriole microtubules. Gene-specific silencing of δ-tubulin in *Paramecium* strongly supports this view [24•] and, furthermore, shows a role of the C tubule in the stability of the centriole and in its interaction with surrounding structures rather than in its...
duplication. Conversely, inactivation of the \( \gamma \)-tubulin gene [25], or the mutation \( sm19-1 \) in a new member of the tubulin superfamily, the so-called \( \eta \)-tubulin [26••], leads to the absence of basal body duplication. Surprisingly, another member of the tubulin superfamily behaves as a marker of centrosome maturation: \( \varepsilon \)-tubulin associates preferentially with the older centriole until late G2 [22 •]. In addition to functional studies, a more precise localization of \( \varepsilon \)-tubulin will be necessary to determine the significance of this result, which suggests a role for \( \varepsilon \)-tubulin in differentiated functions of the centrosome, such as microtubule anchoring, rather than in centriole stability. In any case, centriole/basal body structure obviously requires several specific and very rare tubulin proteins for its assembly and stability.

Centriole stability could depend upon the activity of the protein kinase Nek2, which localizes to the proximal end of centrioles (even in the absence of cytoplasmic microtubules) [27] where it interacts with the large coiled-coil protein C-Nap1 [28] and a protein phosphatase, PP1 [29•]. The role of Nek2 in centrosome assembly or stability was demonstrated both in the early embryo [30**,31•] and in somatic cells [27]. The localization of the ternary complex Nek2/C-Nap1/PP1 at the proximal end of both centrioles (Figure 5) could control the docking of the intercentriolar link, and therefore the distance between both centrioles [32]. Another possibility could be that C-Nap1 acts as a minus end-capping protein, and needs proper control of its phosphorylation state by Nek2 and PP1 to protect proximal ends from disassembly.

**Generational asymmetry of the centriole pair: impact on microtubule nucleation, anchoring and release activities of the centrosome**

The assembly of a new centrosome involves the duplication of the centriole pair. During this process, which is not the
The focus of this review is on the pro-centriole, a structure that forms from a bud or generative disc close to the proximal end of each centriole. This process takes place over more than one cell cycle (Figure 1a). Initially, licensing of the pro-centriole involves duplicating its components, including γ-tubulin, leading to the acquisition of two sets of nine appendages that help identify the older centriole in a pair. This maturation of the centriole is significant for the microtubule-organizing activity of the centrosome as it results in a specific role for each centriole [2**]. More cryptic differential features are likely to arise from the age difference between the two centrioles. If the level of polyglutamylation increases with the lifetime of centriole microtubules, one would expect the centrioles to be significantly different in that respect, as the turnover of centriole microtubules is very slow [13]. In support of this view, cells with only one centriole were observed during cell cycles.

Figure 4

Hypothetical model of how centrioles could organize the centrosome matrix. Minus-end-binding proteins, including γ-tubulin-containing complexes, are concentrated at the proximal end of centrioles (-), whereas tubulin polyglutamylation of the centriole walls controls the binding of other proteins acting as microtubule-stabilizing proteins. The two sets of centriole-interacting proteins are in turn capable of binding and accumulating matrix proteins in a specific manner. The matrix is in turn capable of binding and concentrating γ-tubulin containing complexes, as well as regulatory activities. A release and capture mechanism could control the overall activity of the centrosome. Once nucleated, microtubules could be further capped at their minus ends and either released away from the centrosome or anchored at specific sites.
the course of centriole disassembly (or reassembly) after loading cells with antipolyglutamylated tubulin antibodies [11].

**Anchoring/capping versus nucleation/capping protein**

There is increasing evidence that the dynamics of both ends of microtubules are regulated by specific proteins in vivo [4]. At the minus end, it is not easy to distinguish between nucleation, capping or anchoring activities. The γ-tubulin ring complex (γ-TuRC) could act as a capping complex [33,34]. As is the case for actin, productive nucleation is likely to need a stabilising component. This could be the role of the Stu2 protein in yeast and of its homologs in other species [35]. Recent work on the Drosophila homolog Msps has provided new insights [36*,37**]: Msps, by interacting with the Drosophila transforming acidic D-TAAC protein (which is essential for mitotic spindle function in the early embryo [38]), forms a γ-tubulin-free complex, associating microtubule-promoting (Msps) and anchoring (D-TAAC) activities that are able to regulate microtubule behaviour during mitosis. Interestingly, the polar localization of Msps mediated by D-TAAC (and the kinesin Ncd) looks like a large particle: this apparently gives the acentrosomal meiotic spindle its typical tapered poles, and is in marked contrast to the barrel shaped acentrosomal spindle in other species. An apparent paradox is that Msps homologs such as XMAP215 were first shown to influence microtubule plus-end dynamics, an activity which is opposed by members of the Kin I subfamily of kinesins (see [39*,40] and references therein, see also Update). Interestingly, some evidence that the Msps/D-TAAC complex could control microtubule dynamics at both minus and plus ends and that it could be conserved among divergent species has been reported [37**]. A family of

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**Figure 5**

Some examples of centrosomal proteins that are associated with centrioles according to their proximo-distal polarity. (a) Non-matrix proteins; γ-tubulin and centrin are two classical markers of the centrosome where they have specific localizations, but the major part (80–90%) is not centrosome-associated. (b) Matrix proteins: they are concentrated at the centrosome, with specific localizations and the size of the cytoplasmic pool is small. The localizations of γ-tubulin [66], centrin [57], ninein [1*,57**] and C-Nap1/Nek2 [27,28] have been reported whereas that of AKAP450 or pericentrin have not yet been reported at the ultrastructural level.

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human centrosomal TAAC proteins exists [41]; its precise localization in the centrosome structure has yet to be revealed. The involvement of DdCP224 in centrosome duplication and cytokinesis in Dictyostelium discoideum [42] also suggests other functions for this protein family.

Centrosomal anchoring complexes, distinct from the γ-tubulin nucleating complexes, have been proposed following studies on cochlear epithelial cells, in which the vast majority of the microtubule minus ends are associated with apical noncentrosomal sites [1*,43]. The centrosomal protein ninein is a strong candidate for participating in the anchoring complex. It localizes primarily to the post-mitotic-mother centriole (MC), and concentrates at the appendages surrounding the MC and at the minus ends of noncentrosomal microtubule bundles associated with apical noncentrosomal sites. Evidence that ninein is released from the centrosome together with the microtubules suggests that it may have a dual role as a minus end capping and anchoring protein, and could participate in modular complexes that are able to dock at different sites. Other candidate anchoring proteins have been reported to localize at the subdistal appendages, such as ODF2 [44*]. ODF2, which might be related to cenexin (P March, K Gull, personal communication), has yet to be precisely localized at the subdistal or the distal appendages.

The molecular mechanisms by which capping and anchoring proteins exert their effect on microtubule ends, as well as the regulation of these activities, will have to be worked out in detail. In any case, dynein and dynactin are actively involved. Their significance in the regulation and turnover of the microtubule network as a whole in differentiated cells is clearly important [2••,9,45••]. For obvious reasons, the regulation of the microtubule network in brain tissue has long been of particular interest. A wealth of reports (for a review, see [46]) have established the existence of a highly conserved pathway (the ‘nuclear distribution’ or nud pathway), in organisms from fungi to fly and human brain that is able to regulate dynein motor function and is primarily involved in nuclear migration (for a review, see [46]). LIS1, the product of a gene mutated in the human neuronal migration lissencephaly, is a member of this pathway which binds to and regulates dynein motor function. Two mammalian homologs of another fungus Nud gene, mNudE and NUDEL, interact with LIS1 and colocalize with it and with dynein heavy chain at the centrosome in dividing embryo neuroblasts [47**,48**].

The interphase centrosome and the mitotic centrosome: a switch in anchoring activity?

From the onset of mitosis up to the onset of anaphase, the centrosome, like any other cellular compartment, undertakes a dramatic reorganization. The nucleating activity of the centrosome increases significantly and an accumulation of γ-tubulin at the centrosome takes place in a microtubule-independent manner [49]. The stability of centrioles themselves might have to be upregulated during mitosis: a significant increase in the polyglutamylation of centriole tubulin takes place during mitosis [14]. C-Nap1 apparently dissociates from the centrosomes during mitosis [28], whereas the kinase Nek2A is largely degraded (RSHames, AM Fry, personal communication).

The anchoring activity is likely to be dramatically modified during a short temporal window: the subdistal appendages are no longer visible and are replaced by a halo once centrosomes have migrated to opposite locations. Thus, while centrosomes become more efficient microtubule nucleators, nuclear mitotic apparatus protein (NuMA), together with other proteins [50], is ensuring minus-end binding and stabilization on the centrosome side facing the chromosomes, resulting in the cross-linking of spindle microtubules. Such a switch in anchoring activities might be coordinated with the nuclear envelope breakdown by the liberation of Ran, which triggers the dissociation of minus-end-interacting proteins such as NuMA and TPX2 from the importins with which they are complexed in the interphase nucleus [51**, 52**,53**].

On the cytoplasmic side facing the cortex, the aster of microtubules is anchored to the centrosome as soon as anaphase onset is triggered, and plays an important role in orienting the spindle within the cell. At this stage, the centrosome, in which the centriole pair has a compact orthogonal configuration owing to its replicative origin, is reminiscent of the yeast SPB activity: nuclear and cytoplasmic microtubules are apparently controlled independently [54]; stathmin/Op18, the phosphorylation of which is critical for its tubulin-sequestering and catastrophe-promoting activities and depends upon microtubule assembly [55**], accumulates in part at the spindle poles but only on the side facing the cortex [56]. How this differential regulation is brought about is not known in any detail.

As soon as the centriole pair disassociates, following the onset of furrowing, anchoring distal appendages reform around the parental centriole and this is critical for the behavior of the centrosome until the completion of cytokinesis [57**]. In addition, transient microtubule nucleating and anchoring sites distinct from the centrosome can exist during specific phases of the cell division process. Part of γ-tubulin is known to accumulate in the midbody during cyto-kinesis. It is also the case for ninein (M Abal, M Bornens, unpublished data).

Conclusions

Besides microtubule nucleation, microtubule anchoring mechanisms are likely to be a major way to control the microtubule array and centrosome movements during cell locomotion and division. Strikingly different microtubule arrays are observed, for example, in cytoplasts containing either the anchoring MC or the non-anchoring DC [2**]. The accumulation of regulatory complexes at the centrosome, as well as centrosome inheritance, also depends upon microtubule anchoring. Several reports have shown
the role of the SPB or centrosome in regulating late mitotic events [58]. The yeast system has revealed how the SPB can accumulate regulatory complexes and, owing to its function in microtubule nucleation and anchoring, and to the dynamics of the centrosome–cortex interactions, can link temporal decisions with the spatial redistribution of cell compartments. The dual function of core components of the SPB in microtubule organization and mitotic control have been demonstrated (for example, see [59**]).

In unicellular biflagellates, the generational asymmetry of the flagellar apparatus and its mode of inheritance are essential clues for maintaining cell polarities during cell division [60]. A similar role might exist for the centrosome in animal cells as well as in yeast [57**]. The regulation of microtubule anchoring at the centrosome is necessary for such a role.

The release of microtubules from the centrosome seems to be an active process, mediated by the severing protein katanin [62]. Microtubule organization in differentiated cells could be controlled by redistributing anchoring proteins from the centrosome to other sites [1*]. Released microtubules could be transported by dynein motors, for example, as it was shown that perturbing dynactin activity greatly disturbed the organisation of the microtubule array. It has also been proposed that the dynein–dynactin complex could have a role in anchoring the microtubules at the centrosome [7,63] and in the centrosome interaction with the cell cortex [64]. The molecular mechanisms by which anchoring and stabilising activities are produced and regulated are not yet known in any detail, but the increasing list of candidate proteins will lead to rapid progress in that direction.

**Update**

Recent work has shown that XPAP215 could span up to eight tubulin dimers and forms tubulin/XMAP215 rings at 4°C [68].

**Acknowledgements**

I thank G Keryer for critical reading of the manuscript and useful suggestions.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


Localization of ninein in the mouse cochlear supporting epithelial cells suggests that it is not involved in microtubule nucleation but is released from the centrosome, translocated with the microtubules, and is responsible for the anchorage of microtubule minus ends to the apical sites. In the L929 cells, ninein concentrates at the subdistal appendages surrounding the MC.


This paper describes the in vivo behavior of centrioles during the vertebrate somatic cell cycle, using time-lapse fluorescence microscopy and serial section electron microscopy. The data reveal that the mother and daughter centrioles differ in their behavior and in their respective contributions to forming the interphase microtubule array.


8. Young A, Dictenberg JB, Purohit A, Tuft R, Dosey SJ: Cytoplasmic δ-tubulin and pericentrin bind to the nascent microtubule aster at the centrosome in a dynein- and dynactin-dependent manner (see also [7]).


This paper describes the characterization of human δ-tubulin and ε-tubulin. It shows that ε-tubulin associates preferentially with the older centriole whereas δ-tubulin is most prominent between the centrioles.

This paper describes the characterization of mouse δ-tubulin and shows that it is highly expressed in testsis. The protein is shown at specific loci in the spermatids and sperm cells, whereas it is both nuclear and cytoplasmic in somatic cells and enriched at the mitotic poles.


The depletion of C-tubulin leads to the loss of C-tubule in all basal bodies without any effect on ciliogenesis or on basal body duplication, but perturbs the cortical cytoskeleton.


The small 19-1 mutation of Paramecium specifically inhibits basal body duplication and cell growth and causes deolocalization of γ-tubulin. The SM19 gene is shown to encode another new member of the tubulin superfamily.

27. Fry AM, Meraldi P, Nigg EA: A centrosomal function for the human Nek2 protein kinase, a member of the NIMA family of cell cycle regulators. EMBO J 1998, 17:470-480.


This paper demonstrates an interaction between Nek2 and PP1 and provides evidence for the mutual regulation of both enzymes and the formation of a ternary complex with C-Nap1.


The authors show that Nek2B, a maternal and stable form of Nek2, plays an important role in the assembly or maintenance of the centrosome in the early Xenopus embryo.


The depletion of Nek2B from Xenopus extracts delays both the assembly of microtubule astral and the accumulation of γ-tubulin on the sperm centrosome.


This paper shows that the acenstorosomal poles in the female meiotic spindle of Drosophila contain Msps and D-TAC proteins, which interact with each other and are both required for maintaining the bipolarity of the acenstorosomal spindles.


This interaction between Msps and D-TAC at the centrosome strongly influences microtubule dynamics in Drosophila embryos, and suggests that this interaction is evolutionarily conserved and could act on both minus and plus ends.


This paper shows that XMAP215 is a major stabilizing factor in Xenopus egg extracts.


This paper shows that outer dense fiber 2 protein, known as a sperm outer dense fiber specific component, is a widespread centrosome component preferentially associated with the mother centriole.


The expression of cadherins in centrosome-free cytoplasts changes the behavior of microtubules from treadmill dynamics to instability and this effect depends on the formation of cell-cell contacts.


The authors identify mNuD as a centrosomal protein interacting with LIS1 and several other centrosomal proteins. Its overexpression dissociates γ-tubulin from the centrosome and disrupts microtubule organization.


The authors demonstrate that LIS1 directly interacts with the cytoplasmic dynein heavy chain and with NuDEL, a murine homolog of the Aspergillus nidulans nuclear migration mutant nuDE.


This paper shows that importin β inhibits spindle formation and sequesters an aster-promoting activity that consists of several independent factors. One of them is the protein NuMA.


This paper shows that importin β is an inhibitor of aster formation in Xenopus egg extracts and that Ran regulates the interaction between importin β and NuMA.


This paper shows that TPX2, a microtubule-associated protein that targets the motor protein Xklp2, is inactivated by binding to importin α and is displaced from importin α by RanGTP.


Now in press

The work referred to in the text as (RS Hames, AM Fry, personal communication) is now in press: