One to only two: a short history of the centrosome and its duplication

Greenfield Sluder

Department of Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, MA 01655, USA

This review discusses some of the history of the fundamental, but not fully solved problem of how the centrosome duplicates from one to only two as the cell prepares for mitosis. We start with some of the early descriptions of the centrosome and the remarkably prescient but then controversial inferences drawn concerning its function in the cell. For more than 100 years, one of the most difficult issues for the concept of the centrosome has been to integrate observations that centrosomes appear to be important for spindle assembly in animal cells yet are not evident in higher plant cells and some animal cells. This stirred debate over the existence of centrosomes and their importance. A parallel debate concerned the role of the centrioles in organizing centrosomes. The relatively recent elucidation of bipolar spindle assembly around chromatin allows a re-examination of the role of centrioles in controlling centrosome duplication in animal cells. The problem of how centrosomes precisely double in preparation for mitosis in animal cells has now moved to the mystery of how only one procentriole is assembled at each mother centriole.

1. Introduction

The centrosome, as the cell’s primary microtubule organizing centre (MTOC), nucleates the interphase microtubule array that is central to many cellular processes [1]. In preparation for cell division, the centrosome duplicates, and in mitosis, the sister centrosomes act in a dominant manner to determine the essential bipolarity of the spindle. Because the purpose of mitosis is to divide a mother cell into two genetically identical daughter cells, the cell must ensure that the centrosome inherited from the previous mitosis doubles once and only once. The presence of more than two centrosomes at mitosis greatly increases the chances that the cell will distribute chromosomes unequally and that some chromosomes may be profoundly damaged [2–4]. Genomic instability can contribute to multi-step carcinogenesis (reviewed in [5–9]).

2. Early days

The first drawings of the centrosome are found in an 1876 study of mitosis in a metazoan parasite of cephalopods by Van Beneden (reviewed in [10]). The centrosomes, labelled polar corpuscles, were drawn as dark dots or small filled circles at each spindle pole. Little attention was given to these structures at the time, but in 1883 and 1887, Van Beneden published studies on fertilization and early cleavage stages of eggs of Ascaris megalacephala, a parasitic nematode worm of horses and pigs. In these studies, he detailed a differentiated sphere of cytoplasm at spindle poles from which astral and spindle fibres emanated. The centre of each contained a more densely staining dot (see fig. 1.2 in [10]). Notably, he drew the doubling and separation of the dark dots during anaphase and telophase. He proposed that what he now called the central corpuscles were permanent self-replicating organelles of the cell.

Shortly thereafter, Theodor Boveri published two similar characterizations of early Ascaris development in 1887 and 1888 (reviewed in [10,11]) describing specializations at spindle poles he called centrosomes (see fig. 1.3 of [10] for...
photographs of dividing *Ascaris* zygotes taken by this author from Boveri’s own slide preparations!). Boveri also proposed the term centriole for the single or double dense staining dots in the centre of these polar specializations. In time, centrosomes were described in interphase, and dividing cells from a wide variety of animal cells, though variations in morphology led to the interchangeable use of the terms centriole and centrosome. Wilson [11] admitted to the difficulty of distinguishing between the centriole and the centrosome and suggested the more non-committal term ‘central body’.

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Further investigation of this intriguing four-way division led to some surprising observations [18]. They treated sea urchin zygotes coming into first mitosis with mercaptoethanol to disassemble the mitotic apparatus and hold the cells in mitosis for roughly the duration of a cell cycle. Upon release from the chemical, each cell immediately assembled a tetrapolar mitotic apparatus (figure 1) and later divided into four blastomeres. During prolonged metaphase, the two spindle poles had split into four functional poles, and after mitosis the four blastomeres each inherited a split pole. In the second cell cycle, each of the four daughter cells assembled a monopolar spindle. Blastomeres inheriting split poles later divided in a bipolar manner suggesting that the inherited split pole had functionally duplicated. If a cleavage furrow failed in a three-way division after release from mercaptoethanol, then two monopolar spindles could come together to form a functional bipolar spindle that supported normal division.

The remarkable aspect of this study was the involved but elegant interpretation of the results. At the outset, Mazia and his co-workers were deliberate in saying that they were using the behaviour of spindle poles to analyse the duplication of ‘mitotic centres’ not morphological structures such as the centrioles, though they were well aware that there might be parallels. In concept, the mitotic centre was not the spindle pole itself, but the seed or organizer around which the pole (centrosome) was elaborated. Thus, the duplication and separation of the spindle poles reflected the duplication of the mitotic centres. Although not said at the time, this abstraction—the mitotic centre—freed them from becoming enmeshed in any uncertainties over the role of centrioles in spindle poles.

The key finding was that a spindle pole could split and do so only once. In addition, a cell inheriting a split pole could make only one spindle pole in the next cell cycle. The reproductive capacity of the centrosome was quantized; it had a ‘valence’ which could be experimentally manipulated. They proposed that functionally each spindle pole contained two linked mitotic centres that could split apart and separate during prolonged mitosis to give a tetrapolar division. Each mitotic centre was capable of elaborating a functional spindle pole. After the four-way division, they proposed that each single inherited mitotic centre duplicates to produce one spindle pole of normal ‘valence’ with two linked mitotic centres—hence a monopolar spindle. In effect, the splitting and duplication of mitotic centres could be temporally uncoupled under their experimental conditions. They proposed that the reproductive cycle of the mitotic centres involved three distinct and separable events: splitting, separation and duplication. Their timing experiments indicated that normally these three events occur at the same time in telophase, a time when DNA synthesis occurs in these rapidly cycling zygotes.

Of course mercaptoethanol is a blunt instrument with many effects. Subsequent investigation revealed that the relevant activity of mercaptoethanol in this context is to prolong prometaphase long enough to allow the spindle poles to split. Exactly the same phenomena, namely centrosome splitting during prometaphase and assembly of monopolar spindles at second mitosis, were observed in sea urchin zygotes when prometaphase was prolonged by colcemid at microtubule-specific doses or by microsurgically cutting the first division spindle into two half spindles thereby maintaining the activation of the mitotic checkpoint [21]. Importantly, continuous observation of zygotes released from mercaptoethanol (or colcemid,
microsurgery) revealed that second division blastomeres with monopolar spindles showed prolonged prometaphase and the monopolar spindles could split to form bipolar spindles that supported bipolar division. At the third mitosis, the daughter cells inheriting split poles again assembled monopolar spindles that in turn could split to form a bipolar spindle during prolonged prometaphase (figure 1). The same was observed for the fourth mitosis. These observations revealed that splitting of poles during prolonged prometaphase was not due to some peculiar action of mercaptoethanol during first mitosis; rather it was something that spontaneously occurs when prometaphase is prolonged—even well after experimental intervention. Spindle pole splitting during prolonged prometaphase is not peculiar to echinoderm zygotes. When prometaphase is prolonged in Chinese hamster ovary (CHO) cells with colcemid, one or both centrosomes can split leading to multipolar divisions in slightly less than half the cells [22,23].

Although Mazia [18,24] indicated that centrioles could be morphological correlates for the mitotic centre, this had not been tested. This was substantiated in a serial section electron microscopy characterization of individual mercaptoethanol-treated sea urchin zygotes that had been previously followed in vivo [25]. After release from mercaptoethanol, each pole of the tetrapolar spindles contained just a single centriole (also see [23]). At second mitosis, the monopolar spindles were found to contain a pair of centrioles. After these monopolar spindles split into bipolar spindles, the poles contained a single centriole apiece. Thus, the functional behaviour of mitotic centres is mirrored by the splitting and later duplication of centriole pairs (diagrammed in figure 1). These results raised the question of whether centrioles were actually the mitotic centres, a possibility supported by observations that microinjection of partially isolated sperm centrioles or Chlamydomonas basal bodies into echinoderm zygotes or frog eggs led to the assembly of supernumerary asters [26–28]. In addition, nature’s own microinjection ‘experiment’—namely polyspermy—was long known to lead to the assembly of extra centrosomes. However, this notion that centrioles were the mitotic centres ran afoul of the need to explain how higher plant and acentrosomal animal cells assembled bipolar spindles without centrioles. At the time, Mazia felt that centrioles, when present, were good ‘advertisements’ for the mitotic centres, not the mitotic centres themselves. Put differently, he thought that even though the mitotic centres may be spatially and mechanically associated with centrioles in most animal cells, they are distinct entities.

4. Centrosomes versus mitotic poles

Starting in the late 1800s and continuing almost up to the present, one of the most nettlesome issues for the concept of centrosomes determining the spindle poles had been to explain why higher plant cells and some animal cells can assemble bipolar spindles without centrioles or evident centrosomes. Neither light nor electron microscopy revealed recognizable specialized structures of the spindle poles of these forms. Mazia [29] said ‘We may as well face the fact that microscopy has not yet confirmed any general portrait of the centrosomes as mitotic poles.’

Mazia [18,24] was careful to separate the concept of the mitotic centre (an activity) from the centriole (a morphological structure in animal cells). Although he did not say at the time why he was so deliberate in doing this, his later writings suggested that he was thinking about integrating his findings with spindle assembly in higher plant and acentriolar animal cells (see [30]). Implicit in his writings and those of others [31,32] was the notion that all cells, even those of higher plants, must have something (presumably an MTOC) at the spindle poles to originate and/or anchor spindle and kinetochore fibres. In animal cells, this is a corpuscular centrosome, whereas in ‘acentrosomal’ cells, this something would be diffuse enough not to be readily apparent. Mazia did not believe that chromosomes could organize spindles, because sea urchin zygotes with only one centrosome assembled a monopolar spindle [29,33]. In addition, zygotes in which pronuclear fusion had been prevented assemble a bipolar spindle at the sperm pronucleus, but no spindle in association with the condensed female chromosomes [34,35]. Other supporting observations (reviewed in [29]) included the classic observation of polar caps in prophase plant cells. These have the appearance of two cones at the nucleus whose tapered ends predict the orientation of the anastral spindle after nuclear envelope breakdown. Mazia thought these polar caps pointed to the MTOCs that would organize the spindle. In addition, in some plant and animal cells the spindle starts as a multipolar figure (polyarchal spindle assembly) that soon resolves into a bipolar configuration. This bundling of multiple spindle poles was interpreted to be due to the presence of diffuse centrosomes not visible by conventional methods.

Mazia pointed out in 1961 that the activity of mitotic centres was the same whether centrioles were present or not. Later, he was more explicit: ‘Mitotic centres are real; they are not abstractions improvised to explain mitosis in the absence of centrioles... As to centrioles, the problem will be to understand how centers make centrioles, rather than the other way around.’ [36]. He went on to say ‘In my opinion the phenotypic centrioles are giving accurate information about the progress of the reproduction of the centrosome that they inhabit’ [29]. These thoughts mirrored those of Pickett-Heaps [32] who said ‘The centrosome is simply one form of the expression of the informational content of MTOCs in the cell’.

5. Centrioles versus centrosomes

In the 1960s and early 1970s, the role of centrioles in organizing centrosome/spindle poles in animal cells was debated. Some felt that, when present, the centrioles themselves were the organizing principle for the centrosome (see [37,38]; reviewed in [16]). Centrioles were at the right location and their duplication/separation tracked the behaviour of sister centrosomes as the cell approached mitosis. In addition, it was long established that introduction of extra centrioles into eggs through polyspermy led to supernumerary centrosomes and multipolar divisions.

However, any simple claim that centrioles were the spindle poles or the centrosomal MTOC in animal cells was open to criticism. Although spindle microtubules converge on the centriolar region, they do not contact the centrioles themselves but rather end in the pericentriolar material. In vitro work with disrupted, isolated centrosomes incubated in tubulin revealed that microtubules grew from bits of pericentriolar material, not naked centrioles [39]. Spindle poles in animal cells are organized by the centrosomal MTOC (term coined by [31]). Nevertheless, some felt that centrioles were
not necessarily irrelevant; Don Fawcett [40] said ‘This does not necessarily mean that the centrioles are inactive during cell division. They may control aggregation of the pericentriolar material or may be involved in its activation at the appropriate time in the cell cycle’.

Other investigators, however, had a very different and provocative point of view. They proposed that centrioles were passive or inert passengers attached to the spindle poles. In their view, the spindle leads the centrioles to its poles and their presence there is a consequence, not a cause, of spindle assembly. This is simply the animal cell’s way of ensuring the equal partitioning of basal bodies to daughter cells—much like the chromosomes [32,41]. In spindle pole formation, the MTOC is the key player, be it focal in animal cells or diffuse—even invisible—in higher plant cells. Significantly, studies by Roland Dietz reported for primary cranefly spermatocytes that the centrioles could be displaced from the late diakinesis nucleus by flattening the cell; nevertheless, such cells assembled functional bipolar spindles (reviewed in [42]). Furthermore, exacting electron microscopy of such cells did not find accumulations of pericentriolar material (PCM) at these spindle poles; the PCM stayed with the centrioles [42]. In addition, for spermatocyte meiosis in the silkworm moth Bombyx mori the centrioles were not associated with astral fibres and were substantially separated from the spindle poles at metaphase [41]. So, even animal cells that normally have centrioles do not need them for bipolar spindle assembly, a conclusion borne out by later work [43–45].

These arguments, however, begged the question of what precisely controlled the number of spindle poles in acentrosomal cells. In grappling with this issue, Mazia [29,33] turned back to MTOCs as the determinant of spindle poles (also see [32]). He proposed in 1987 that ‘The concept of the centrosome as the “germ” or “seed” of molecular dimensions. This gives “germ” or “seed” of molecular dimensions. This gives

6. Centriole duplication: only one daughter

The numerical and spatial specificity of procentriole assembly has historically led to the notion that the mother centriole has only one unique site or ‘template’ that can seed the assembly of the new procentriole (see [55] for a thoughtful discussion of the possible meanings for this ambiguous term). In its simplest form, the term template implies a structure that directly patterns the carwheel structure and the nine triplet microtubules of the procentriole—a ‘rubber stamp’ in the parlance of Fulton. In Paramecium, there is a plaque next to the parent basal body upon which the barrel of triplet microtubules progressively assembles [56] and in Chlamydomonas, there is a looped fibre at the mother basal body containing nine densely staining foci that later elaborate into triplet microtubules [57] (reviewed in [58]). However, it is uncertain whether these structures are the proposed template on the mother basal body or the early assembly intermediates of daughter basal bodies.

Another possibility is that the template is not a patterning structure but rather a tiny seed or singularity at the proximal wall of the mother centrioles that specifies the site where a daughter centriole self-assembles. In the words of Mazia [18] ‘...a body as complex as the centriole contains a reproducing “germ” or “seed” of molecular dimensions. This gives
rise to its like, which in turn direct the growth of a replica of the original body’. This is what Fulton [55] calls a ‘self-replicating entity’ (SRE) that is conceptually distinct from the morphological barrel of nine triplet microtubules. Needless to say, the duplication of this proposed singularity would have to be under tight control to ensure that only one is present at each mother centriole at each cell cycle. Support for this notion has come from images of a focus of gamma tubulin on the mother centriole from which the microtubules of the procentriole grow [59,60] (also see Sonnen [61]). Functional evidence for the existence of such an SRE came from the finding that the amoeboid form of *Naegleria* propagates without centrioles but when stressed, this organism differentiates into a flagellated motile form with the assembly of just two basal bodies (reviewed in [55]). More recently, Collins [62] reported that when assembly of cytoplasmic microtubules and new centriolar triplet microtubules is completely blocked, there is a time-dependent accumulation of precursors that soon elaborate into multiple centrioles after removal of the microtubule inhibitor.

The nature of this proposed self-replicating ‘seed’ was a mystery. Almost 50 years ago, researchers started considering the notion that centrioles, such as mitochondria and chloroplasts, have their own DNA that would serve a local genomic function (see Hall [63] and references therein). Conceivably, the duplication of this DNA would produce a copy around which only one procentriole assembles at each mother centriole. A variant on this theme held that centrioles, such as ribosomes, contain RNA that serves a structural role that could in principle limit the number of daughter centrioles assembled at each cell cycle. These possibilities inspired numerous studies, some of which sought to demonstrate the localization of DNA (or RNA) to centrioles/centrosomes on the assumption that presence indicates function. In the end, these studies have been regarded as inconclusive due to technical limitations and an inability to rule out more mundane explanations, such as the redistribution and differential extraction of nuclear and cytoplasmic nucleic acids. More interesting functional studies reported that the aster inducing activity of injected centrioles or basal bodies was nuclease sensitive. These also were subjected to serious technical concerns that have taken their conclusions out of serious consideration. Another promising lead was the report of RNAase/pronase sensitive fibrogranular inclusions in the lumen of ciliate basal bodies [64]. Later investigation revealed that these luminal structures were alpha-amylose sensitive, indicating that they were glycogen inclusions [65]. Reviews of this subject are found in [17,55,66,67].

The ongoing appeal of the template or singularity hypothesis has come from observations that only one procentriole assembles at each mother despite abundant and complete cytoplasmic pools of centriolar subunits. For example, early sea urchin and frog zygotes contain complete pools of subunits on hand at fertilization to make many centrioles [68,69]. The same holds true for early *Drosophila* embryos that are reported to have subunit pools sufficient to assemble $2 \times 10^{13}$ centriole pairs [70]. Even mammalian somatic cells contain enough centriolar subunits to assemble multiple procentrioles within a single cell cycle when procentriole initiating proteins, such as Plk4 kinase, STIL or SAS-6, are overexpressed [71–74]. There are adequate pools of all other centriolar subunits to match the activity of the individually overexpressed centriole initiating proteins.

However, a number of observations challenge the template or singularity hypothesis. De novo centriole assembly has been demonstrated for a wide variety of cell types, including parthenogenetically activated sea urchin eggs [75], mammalian somatic cells [45,76], *Chlamydomonas* [77], and certain insect eggs when sperm centrioles are not present [70,78] (reviewed in [79]). The presence of a mother centriole and its putative singularity are clearly not needed for the assembly of new centrioles. Another challenge is the experimentally induced assembly of several procentrioles around each mother centriole in cultured cells which argues against the notion that a mother centriole can bear only one singularity or template in any given cell cycle [72,73,80]. A direct challenge comes from the finding that laser ablation of a daughter centriole in S-arrested HeLa and CHO cells results in the formation of a new procentriole at the mother centriole, not always at the exact location of the previous procentriole [81]. Some ablations even took out a bit of the mother centriole which should have removed the putative template. Although S phase is constitutively permissive for procentriole assembly, the presence of a daughter centriole blocks further daughter assembly at that mother centriole. New centriole assembly appears to be controlled at the level of the centrioles and surrounding PCM. Consistent with this notion, increasing the size of the PCM by pericentrin overexpression allows assembly of multiple procentrioles near the mother centriole [81].

If there is no template on the mother centriole, we are left with a messier notion that the PCM together with proteins on the wall of the mother centriole provides a small local environment that promotes the self-assembly of the daughter centriole [81] (also see [70]). This begs the question of how such a permissive microenvironment can be precisely controlled to yield the formation of only one procentriole at each mother with high fidelity in the face of non-limiting pools of centriolar subunits. Perhaps the subunit and regulatory kinase pools in the microenvironment are so limited that there is only enough ‘stuff’ to make one daughter.

Although the concept of a template or singularity at the mother centriole appeared to be moribund, recent work has breathed new life into this idea. Super-resolution immunofluorescence microscopy revealed that Plk4, a kinase necessary for procentriole initiation, is localized to a single small spot on the wall of the mother centriole throughout the cell cycle [61]. This spot colocalizes with Sas-6 as the procentriole starts assembling. This plus the finding that the Plk4 spot is present in G1 before Sas-6 presence indicate that this spot could be the singularity where only one procentriole can assemble at each mother centriole.

If so, we are back to almost full circle to thinking about a pre-existing singularity or initiating complex at each mother centriole that seeds procentriole assembly. The observations that previously argued against a singularity may simply tell us that this one Plk4-containing spot is not determined by an autonomous SRE; there must be different mechanisms in play. Rather than seeing a discrepancy, we can use these observations critical of a singularity as valuable insights into the functional properties of the system that lead to the formation of a singularity. For example, we could consider the possibility that a tightly regulated equilibrium of regulatory kinases, phosphatases and structural proteins in the microenvironment at the centriole wall supports the formation of centriole initiating complexes with kinetics slow
enough that only one forms at a time. Once formed, the newly assembled complex could have an activity that immediately inhibits the formation of other initiating complexes. Overexpression of centriole initiating proteins could perturb this putative equilibrium, so that multiple pro-centrioles are assembled at once. After laser ablation of the daughter centriole, a new Plk4-containing spot could form as it would under normal circumstances. Obviously, there is much still to be learned and our fascination with how centriole duplication is so precisely regulated will continue unabated.

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References


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