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Centrosome Duplication: A Centriolar Pas de Deux

Minireview

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The microtubule organizing center, or centrosome, is an unusual organelle. Unlike most organelles, it is not bounded by a membrane, yet it is distinct from the surrounding cytoplasm. It is at the center of important processes in animal and fungal cells, yet many plant cells completely lack it. And perhaps most perplexingly, the centrosome duplicates precisely once per cell cycle, yet the molecular mechanism of duplication remains obscure. This is in stark contrast to chromosomes—the Watson and Crick DNA structure of 1953 was among the greatest "Eureka!" moments in science because it revealed in an instant how our genetic material is duplicated each generation. Chromosomes and centrosomes are the only known cell components that are precisely duplicated each cell cycle, but the structure of the centrosome does not clearly reveal a mechanism like that of DNA. It is only in the last few years that substantial progress has been made in understanding centrosome duplication, progress that is continued in a paper published in this issue of *Cell* (O'Connell et al., 2001). In this brief review, I will focus on these recent results. I apologize to the many colleagues whose work could not be discussed or referenced here due to length constraints. In particular, although this review will deal only with the animal cell centrosome, I note that many of the paradigms for centrosome duplication come originally from work on the yeast equivalent, the spindle pole body (reviewed in Adams and Kilmartin, 2000).

The centrosome is most often defined by morphology, and since this morphology changes with the cell cycle, we will consider as the general case the centrosome of an animal cell in G1, prior to the initiation of centrosome duplication. As defined by electron microscopy, the centrosome occupies a volume of approximately one cubic micron, and consists of a pair of short cylindrical centrioles surrounded by dense fibrillar pericentriolar material (shown in cartoon form in Figure 1). Centrioles are among the most beautifully symmetrical structures in nature, with a 9-fold axis of symmetry derived from the arrangement of nine microtubule triplets into a cylinder. In most centrosomes, the centrioles are oriented perpendicular to each other, although the significance of this unusual orientation is still unknown.

The pericentriolar material of the centrosome contains elements that nucleate and organize microtubules. The pericentriolar material has traditionally been considered to be amorphous and homogeneous, but this is almost certainly not the case given the large number of proteins that localize there, and the dynamic changes that occur there during the cell cycle. In centrosomes that have been stripped of most of their proteins, the pericentriolar material can be seen to consist of a fibrous core, to which other proteins and complexes are attached. Microtubule nucleation is accomplished by the γ-tubulin complex, a large ring-shaped multiprotein complex that localizes to the pericentriolar material and provides a template for the addition of tubulin subunits to make a microtubule. The pericentriolar material must have specific interactions with the centrioles and with functional components, such as the γ-tubulin complex, but these interactions have not been characterized, nor have the proteins making up the structural core of the pericentriolar material been identified. If the pericentriolar material is doing most of the heavy lifting with respect to microtubule nucleation and organization, of what use are the centrioles? One role for the centrioles is to organize the pericentriolar material into a discrete, stable structure. Centrioles can be caused to disassemble by injection into cells of an antibody against a modified form of α-tubulin that is found predominantly in centrioles (Bobinnec et al., 1998). In cells without centrioles, the pericentriolar material disperses, and the centrosome structure is lost.

Before we can discuss centrosome duplication, we must understand the origin of the first centrosome in development. In most animals, the centrosome and centrioles of the oocyte degenerate prior to the meiotic divisions. In contrast, sperm typically contain two centrioles and a subset of pericentriolar material proteins. After fertilization, the sperm centrioles recruit centrosomal components from the egg cytoplasm and form the first centrosome. It is the paternal centrioles, and not the DNA, that are the essential component of the sperm for the cell divisions of early development. The researchers working on cloning various mammals by injection of nuclei from somatic cells into eggs would do well to consider the source of centrioles in their experiments. It seems possible that the high failure rate of many of these efforts is due to the lack of a functional centrosome in the injected eggs.

How does centrosome duplication occur? Figure 1 shows the known steps for a typical somatic cell. Cells enter G1 with a single centrosome, with two centrioles. One of the two centrioles is older, generated at least two cell cycles prior, whereas the other is newer, generated in the previous cycle. The long axis of the older centriole (labeled "1") is intersected by the long axis of the newer centriole (2). The older centriole has appendages at its distal end, farthest from the newer centriole. In many cell types, the older centriole also serves as the basal body for a nonmotile primary cilium. At the G1/S transition, the centriole pair splits, and new centrioles (3 and 4) begin to grow from the sides of the two original centrioles (1 and 2). The parallels with DNA replication are striking: centrosome duplication and DNA replication are initiated at the same time in the cell cycle, and, like DNA replication, centrosome duplication can be considered to be semiconservative, with each centrosome receiving one old centriole and one new centriole.

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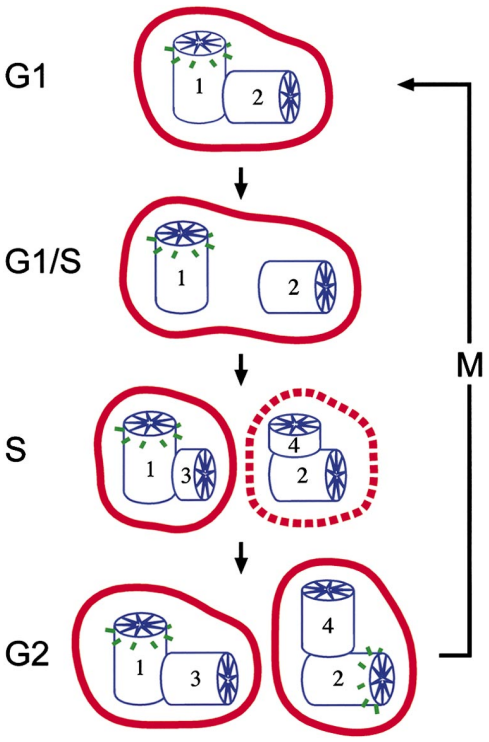

Figure 1. The Centrosome Duplication Cycle

Centrioles are indicated as blue cylinders, and are numbered. Distal appendages are shown in green on centrioles 1 and 2 (G2/M only). The pericentriolar material is indicated by the red line encircling the centrioles. The dotted red line on the S phase centrosome bearing centrioles 2 and 4 indicates the lack of ϵ-tubulin on this centrosome, as referred to in the text.

The earliest point at which there are two centrosomes, defined as two discrete bodies of pericentriolar material, occurs during S phase, although the exact timing of this transition from one centrosome to two is not clear. At this point the centrosomes differ in two ways: (1) one centrosome contains the oldest centriole, marked by the presence of distal appendages, and the cenexin protein (Lange and Gull, 1995), and (2) the pericentriolar material of the centrosome with the oldest centriole contains the recently discovered ϵ-tubulin (Chang and Stearns, 2000), whereas the other does not (indicated by the dashed line). During G2, the new centrioles (3 and 4) elongate to their full length, the pericentriolar material of the centrosome bearing centrioles 2 and 4 acquires ϵ-tubulin, and at the G2/M border, centriole 2 acquires both distal appendages and cenexin. At the start of M phase, the amount of pericentriolar material increases, along with the number of nucleated microtubules. The two centrosomes separate from each other, driven by microtubule motor-mediated sliding of microtubules, and ultimately form the two poles of the mitotic spindle. Completion of mitosis results in two daughter cells, each with a single centrosome containing two centrioles, one centriole newly formed in the previous generation and the other at least two generations old.

What are the control points in centrosome duplication, and what molecules are involved in their regulation? The earliest known regulated step is the splitting of the two parental centrioles. In another parallel with the initiation of DNA replication, splitting of the two centrioles requires the activity of the cell cycle kinase Cdk2. Experiments with *Xenopus* eggs and embryos showed that inhibition of Cdk2 either in vivo or in extract systems blocks centrosome duplication (Hinchcliffe et al., 1999; Lacey et al., 1999). Although it is possible that Cdk2 is required for several steps in the duplication process, the earliest step that fails in the absence of Cdk2 activity is splitting of the centrioles (Lacey et al., 1999). Similar experiments in cultured mammalian cells showed that Cdk2 is also required for centrosome duplication in somatic cells (Matsumoto et al., 1999; Meraldi et al., 1999). Cyclin A and cyclin E are both potential partners of Cdk2, and either will serve to drive centriole separation in *Xenopus* embryos (Lacey et al., 1999). Cyclin A appears to be more active than cyclin E in catalyzing centrosome duplication in mammalian somatic cells (Meraldi et al., 1999). However, these experiments did not differentiate between the stages of duplication; thus, it is possible that either Cdk2/cyclin E or Cdk2/cyclin A is required for centriole splitting, but that only Cdk2/cyclin A can efficiently complete some later step.

What does Cdk2 phosphorylate to drive centrosome duplication? Okuda et al. (2000) showed that the protein nucleophosmin is localized to the centrosome prior to centrosome duplication, is a substrate of Cdk2/cyclin E, and is released from the centrosome upon phosphorylation. Most importantly, a mutant of nucleophosmin that removes the Cdk2/cyclin E phosphorylation site at Thr199 (Tokuyama et al., 2001), blocks centrosome duplication when expressed in vivo. Cells expressing this dominant mutant form of nucleophosmin had a single centrosome with two centrioles, consistent with the requirement for Cdk2 to drive centriole splitting.

A simple model for nucleophosmin function is that it is involved in maintaining cohesion of the two centrioles within a centrosome, and that it must be removed to allow splitting of the centrioles. This raises the question of what holds the centrioles together in their characteristic orthogonal orientation. This is not known, but several groups have reported observing fibers linking the centrioles in purified centrosomes. The existence of such a proteinaceous link suggests an analogy with chromosome separation at mitosis. Replicated sister chromatids are held together by the cohesin protein complex, which is removed by proteolysis to allow chromosome separation in anaphase (Uhlmann et al., 2000). Similarly, the centriole pair might be held together by a link that is proteolyzed at the G1/S transition. This model is supported by the finding that the SCF complex, a ubiquitin-conjugating machine that is active at the G1/S transition, is localized to the centrosome, and that inhibition of the proteasome, the protease that degrades ubiquitinylated proteins, blocks splitting of the centrioles (Freed et al., 1999). In addition, mutations in slimb, a *Drosophila* SCF complex component, result in aberrant centrosome duplication (Wojcik et al., 2000). From these results, it seems likely that centriole cohesion is regulated by cell-cycle-specific proteolysis, and a current challenge is to identify the molecules making up the link.

Once the centrioles have separated, growth of new centrioles is initiated. Each new centriole grows perpendicularly from the side of one of the parental centrioles.

It is one of the remarkable oddities of centrosome duplication that the complex centriole structure is assembled on the side of a parental centriole, where there is no obvious template, rather than from the end, where it could extend from the existing structure. What little was known about the regulation of new centriole formation has now been expanded greatly by work from John White's lab reported in this issue of *Cell* (O'Connell et al., 2001). O'Connell and colleagues show that the *C. elegans* ZYG-1 protein is a protein kinase that is required for new centriole formation. The *zyg-1* gene has been identified in several genetic screens, most recently as a mutant with defects in early embryonic cell divisions (O'Connell et al., 1998). *zyg-1* mutants were shown to form monopolar mitotic spindles, with only a single centrosome, but not to affect progression of the cell cycle in general. This phenotype suggested that *zyg-1* mutants might have a defect in some aspect of centrosome duplication.

The authors have now examined the single centrosomes in mutant embryos by electron microscopy and found that they contain only one centriole rather than the usual pair (note that worm embryo centrioles have the 9-fold symmetry common to all centrioles, but have only singlet microtubules rather than the usual triplets). A reasonable interpretation is that ZYG-1 protein is required for formation of new centrioles. Centrosomes with single centrioles would result from splitting of the centrioles in the previous cell cycle, making two centrosomes, each with a single centriole. After mitosis, each of the daughter cells would have one centrosome with only one centriole. Without the ability to make a new centriole, these centrosomes would be a dead end for duplication, and could only assemble a monopolar spindle.

Cloning of *zyg-1* revealed that it encodes a protein that is related to protein kinases, and has kinase activity in vitro. However, ZYG-1 is sufficiently different from known kinases that it was not possible to determine which kinase subfamily it belongs to. There are no obvious ZYG-1 orthologs in other organisms, although I wouldn't count this as evidence against the possibility that ZYG-1 is a universal regulator of centriole formation, since worm centrosome proteins tend to be quite divergent. A kinase could exert its effect on a process such as centriole formation at many possible levels, from direct phosphorylation of centrosome components to action in an upstream pathway. Localization of a protein can often provide spatial and temporal clues to function, and being at the right place at the right time is a strong indicator of local action. O'Connell et al. (2001) report that ZYG-1 in *C. elegans* embryos is localized to the centrosome for a brief period in the cell cycle, mostly from anaphase of mitosis to the beginning of the G1 of the next cell cycle. This might seem like the wrong time to be present if ZYG-1 is to act directly, since in a typical somatic animal cell centrosome duplication cycle, the new centrioles do not begin growing until S phase. However, in many rapidly dividing embryos, the process is advanced so that by the end of mitosis, each pole of the spindle already has two centrosomes (for *Drosophila* example, see Vidwans et al., 1999). ZYG-1 thus appears to be at the centrosome when centriole duplication is taking place.

Can cells generate centrioles de novo? Most somatic cells are not able to generate centrioles de novo, whereas some embryonic cells are able to do so. There are some interesting exceptions to this rule. Ciliated epithelial cells in the airway and in the oviduct typically have more than 100 cilia on their apical surface, each with a centriole, called a basal body in that context, at the base. These basal bodies arise de novo from concentrated dense material, and not from repeated duplication of the existing centrioles. If this capacity for de novo centriole generation were widespread, it would create serious problems, since each new centriole is potentially one new centrosome. Recent experiments in *Chlamydomonas* suggest that a mechanism exists to temper de novo assembly. Marshall et al. used *Chlamydomonas* mutants defective in centriole segregation to show that cells born without a centriole are often able to regenerate centrioles within a single cell cycle (Marshall et al., 2001). They propose that this de novo pathway is normally repressed by the presence of a centriole, favoring the normal centriole-associated pathway.

The last step of the centrosome cycle is separation of the duplicated centrosomes and segregation to daughter cells. In most cell types, the duplicated centrosomes remain associated through G2, then separate at prophase. During mitosis, the two centrosomes define the ends of the bipolar spindle, and are segregated along with the chromosomes at the completion of division. There is still some debate about whether the centrosomes typically found associated in G2 cells are held together by a specific mechanism, or whether they tend to be together because they are at the center of a large dynamic microtubule array. It has long been known that simple depolymerization of microtubules with anti-microtubule drugs, such as nocodazole, often leads to separation of centrosomes from each other, arguing against a specific attachment. However, Erich Nigg and colleagues have identified a protein kinase, Nek2, that when overexpressed results in premature centrosome separation. They report that a putative substrate of this kinase, C-Nap1, is localized to the centrosome in a manner consistent with a centrosome-linking activity, and that interfering with C-Nap1 function also causes premature centrosome separation (Mayor et al., 2000). These results suggest that there is a linkage between duplicated centrosomes, and that it is regulated.

Future Directions

I see two large challenges on the horizon for centrosome research. First, to truly understand centrosome duplication, we must define the components of the centrosome. The current state of our knowledge reminds me of the situation in developmental biology a decade ago—a "theory of everything" would be proposed, based on knowledge of a small fraction of the total number of the important players, only to require weekly revision as new players were discovered. In the beautiful work of John Kilmartin and colleagues, the yeast equivalent of the centrosome was shown to have on the order of 50 proteins (Wigge et al., 1998). It seems likely that the animal cell centrosome will be more complex by several-fold. Because there is only one small centrosome per cell this will be a challenging project, but the combination of extremely sensitive mass spectrometry techniques with complete genome sequences makes a large-scale as-

sault on the centrosome feasible. Once we have most of the components in hand, the task of determining how they fit together and how they are regulated will be greatly simplified.

The second challenge will be to understand the relationship between the centrosome and genome stability. There have been many reports in recent years of a correlation between aberrant centrosome number or structure and gross aneuploidy of the sort seen in many cancer cells (reviewed in Lingle and Salisbury, 2000). It is still not clear whether centrosome abnormalities are a cause of genomic instability, or a consequence of an altered cell cycle due to other changes in the cancer cell. Clarification will require identification of specific regulators of centrosome duplication, and genetic manipulation in model systems. Just as it has been possible to demonstrate that mutations in DNA mismatch repair genes can be a primary cause of cancer, it should be possible to test whether manipulation of centrosome number and structure in vivo leads to aneuploidy and cancer. ZYG-1 and the other regulators discussed here are almost certainly just the tip of the centrosome iceberg.

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