

Centrosome Biology: A SAS-sy Centriole in the Cell Cycle

Dispatch

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A novel protein in *Caenorhabditis elegans*, SAS-4, is a component of centrioles and is required for centriole duplication. Depletion of SAS-4 results in stunted centrioles and a smaller centrosome, suggesting a link to organelle size control.

The centrosome was first described in the late 1800s by Van Beneden and Boveri (reviewed in [1]) as a small body at the center of fibrous asters in invertebrate eggs. Although always of interest to cell biologists, research on the centrosome languished until the 1990s, when two advances brought the focus back to the centrosome. First, proteins of the centrosome began to be identified, bringing a molecular understanding to centrosome function. Second, a link between the centrosome and cancer was established by the observation that cancer cells often have centrosomes of aberrant size and number. Such a link was first proposed by Boveri 90 years ago, and there is great interest in determining the cause of these centrosome defects, and whether they are behind the genomic instability common in cancer cells.

What is the centrosome? A typical centrosome is approximately $1 \mu\text{m}^3$ in volume and composed of a pair of centrioles surrounded by a matrix of pericentriolar material. The centrioles are among the most highly conserved structures in eukaryotic cells, consisting of a cylinder formed by microtubule structures arranged with perfect nine-fold symmetry. The pericentriolar material is known to contain several large coiled-coil proteins which make up the matrix (reviewed in [2]), as well as the γ -tubulin ring complex (γ -TuRC), which forms microtubule nucleation sites [3].

The centrosome has two remarkable properties that separate it from most other organelles. First, it duplicates precisely once per cell cycle, so that a constant number is maintained. Second, the centrosome is not membrane-bound, and yet differs from the surrounding cytoplasm. These properties raise three interesting questions: How does the centrosome duplicate? How are specific proteins recruited from the cytoplasm to form an assemblage around the centrioles? And how does the centrosome maintain a constant size?

Somatic animal cells have one centrosome in G1 phase of the cell cycle, which duplicates once in S phase to form a bipolar spindle during mitosis. Like DNA, the duplication of centrioles is semi-conservative — the two centrioles in the parental centrosome separate and a new centriole grows adjacent to each to form two complete centrosomes [4]. It is not known what

holds the two centrioles together in their orthogonal orientation, but the action of the kinase Cdk2 and its associated cyclins is required for centriole separation and for the duplication process in general (reviewed in [5]). The classic experiments of Mazia *et al.* [6], along with a more recent study [7], showed that the centriole is the fundamental unit of the centrosome. A single centriole can form a centrosome or spindle pole — the yeast equivalent — but it cannot divide to form two centrosomes unless the centriole structure itself is duplicated.

If the centrioles are the fundamental units, how are the pericentriolar components attached to them? This question is central to microtubule organization, because it is the γ -TuRC and other components of the pericentriolar material that nucleate and anchor microtubules. It should be noted that eukaryotes present a continuum of microtubule organization strategies. In yeast and other fungi, all microtubules grow from highly ordered centrosome-like organelles. In contrast, higher plants lack anything resembling the centrosome, and instead appear to have dispersed cortical nucleating sites. The typical animal cell represents the middle ground between the fungal and plant extremes, with a dynamic centrosome that nucleates and releases microtubules and changes in size during the cell cycle. The centriole is required to organize the pericentriolar material in animal cells; disruption of the centriole by microinjection of antibodies against a modified form of tubulin found in centrioles results in loss of a defined centrosome [8]. There is some evidence that large coiled-coil proteins link the γ -TuRC to the centrioles [9], similar to the situation in yeast [10].

The picture of the centrosome drawn above is one of a discrete structure, the centriole, to which is bound an anastomosing network of other proteins and complexes. All centrosome components examined to date are present in excess in the cytoplasm, as well as at the centrosome. How then does the centrosome maintain a constant size? For example, the tubulin subunits of which centrioles are made are present in great excess and should in theory be able to add to the ends of the existing centriole structure to make it longer, so why is the centriole a consistent length in all cells of a single cell type?

Issues of centrosome duplication and centrosome size are addressed in two recent papers [11,12] on the *sas-4* (spindle assembly defective) gene of the nematode *Caenorhabditis elegans*. The *sas-4* gene was among 133 identified in an RNA interference (RNAi) screen for genes that function in cell-cycle regulation [13]. Depletion of SAS-4 protein from *C. elegans* embryos by RNAi resulted in a normal first division, followed by assembly of monopolar spindles during the second division. Electron microscopy [11] showed that each pole in the first division had only a single centriole, indicating that the centriole pair contributed by the sperm separated, but failed to duplicate. This phenotype is identical to that of depletion of ZYG-1,

the only protein previously shown to be required for centriole duplication in *C. elegans* [14].

SAS-4 is not only required for centriole duplication, it is also a component of the centrioles, as shown by immuno-electron microscopy [11]. Both groups [11,12] examined the incorporation of SAS-4 into centrioles, using a marked-mating experiment in which wild-type males were mated to hermaphrodites expressing SAS-4 linked to the green fluorescent protein (GFP). These experiments showed that the GFP-SAS-4 fusion protein incorporated into new centrioles during duplication, but did not incorporate into the existing sperm centrioles. Photobleaching experiments confirmed that centriolar SAS-4 does not exchange with cytoplasmic SAS-4 [12].

The most intriguing result of these studies came from experiments in which SAS-4 was partially depleted by RNAi [11]. By varying the time between injection of double-stranded (ds)RNA into hermaphrodites and analysis of the newly fertilized embryos, the authors created the equivalent of an allelic series. These embryos often had asymmetric spindles with one normal, robust centrosome and a second centrosome with fewer microtubules and less γ -tubulin, in direct proportion to the reduction in level of SAS-4 at that centrosome. Such partial phenotypes were not observed after partial depletion of ZYG-1.

SAS-4 might be involved in the recruitment and maintenance of γ -tubulin and possibly other PCM proteins to the centrosome, however γ -tubulin was the only pericentriolar protein assayed in the study and it will be important to examine others. Kirkham *et al.* [11] propose that SAS-4 is directly involved in controlling the amount of pericentriolar material bound to the centrioles. We find this argument to be unpersuasive, however, because they also show that centriole structure is compromised when SAS-4 is depleted. Therefore, it seems equally likely that the stunted centrioles observed are less able to recruit pericentriolar components for structural reasons.

Perhaps SAS-4 actually controls centriole length. It is interesting to note that the same issue of length determination is elegantly solved for the tail of lambda bacteriophage by use of a 'ruler' protein, the polypeptide chain length of which determines tail length [15]. Perhaps SAS-4, with its coiled-coil structure, is such a ruler for the centriole. Interestingly, Vidwans *et al.* [16] recently showed that manipulation of cell cycle regulators in *Drosophila* larval cells can result in abnormally long centrioles, suggesting that there are both structural and regulatory components to control the size of this remarkable structure.

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