

Centriole Duplication: Centrin in on Answers?

Dispatch

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Many aspects of centriole biology remain mysterious. A new study has shed light on the role of the centriolar protein centrin-2: reducing levels of centrin-2 in HeLa cells has been found to block centriole duplication, eventually leading to cell death.

The centrosome is the microtubule organizing center of most higher eukaryotic cells, and is generally described as having two orthogonal centrioles surrounded by pericentriolar material. This description highlights the important role of centrioles as organizers of the pericentriolar material [1] that is primarily responsible for coordinating the nucleation of microtubule assembly. Indeed, initiation of centriole replication is one of the earliest events in centrosome duplication. Centrioles are multifunctional: in addition to organizing the centrosome, they have recently been shown to play an important role in cytokinesis and cell cycle progression [2,3]. Questions about centriole biology abound, but answers remain elusive. A recent study aimed at understanding the function of centrin-2 indicates that inhibition of synthesis of this centriolar protein in human cells blocks centriole duplication [4].

Why has it been so difficult to identify and understand the myriad functions of centrioles? One reason has been the lack of a suitable model system amenable to rapid genetic manipulation and analysis. The microtubule organizing center of yeast cells, the spindle pole body, lacks centrioles and forms a structure quite unlike that of the centrosome (reviewed in [5]). The morphological differences between centrosomes and spindle pole bodies are reflected in compositional differences. While the protein composition of the spindle pole body has been characterized in detail [6], the vast majority of the proteins that make up centrioles and centrosomes remain unknown. There are a few families of proteins known to localize to spindle pole bodies and to centrosomes, but most of them are involved in microtubule nucleation at the periphery of these structures and not as architectural components [7,8]. Genetic and biochemical approaches will eventually fill in the details of centriole structure and function, but for now details are few.

Centrins are one of the relatively few proteins known to localize to centrioles [9]. They are small, calcium-binding proteins belonging to the calmodulin superfamily. Centrins have attracted interest, not least because they are a rare example of a centriolar protein with a known homolog in the yeast spindle pole body, in this case Cdc31p. Cdc31p localizes to a substructure

of the spindle pole body called the 'half-bridge' [10], and temperature-sensitive yeast mutants in Cdc31p show defects in spindle pole body duplication [11]. Are centrins required for centriole duplication? Recent evidence, including important new work published recently in *Current Biology* by Salisbury *et al.* [4], suggests they are.

Salisbury *et al.* [4] used RNA interference (RNAi) to reduce centrin-2 expression over tenfold in HeLa cells [4] — the first time that the consequences of eliminating centrin function have been investigated in animal cells. They observed a marked decrease in centriole content: after 72 hours, more than 70% of cells lacked centrioles entirely. Cells with a single centriole at each spindle pole were able to complete at least one round of mitosis and cytokinesis. The authors concluded that the loss of centrioles was caused by a block at an early stage of centriole duplication (Figure 1). The progressive decrease in centriole number eventually led to defects in mitosis and cytokineses, and ultimately to cell death.

Earlier studies of centriole or basal body assembly in other systems had emphasized a requirement for centrin function in the creation of new centrioles. Characterization of a mutation in the centrin gene of the unicellular green alga *Chlamydomonas reinhardtii* showed a defect in centriole duplication [12]. Examination of basal body assembly during spermiogenesis in the water fern *Marsilea vestita* revealed that interfering with centrin translation blocked formation of a structure related to centrioles, the basal body, at a very early stage [13].

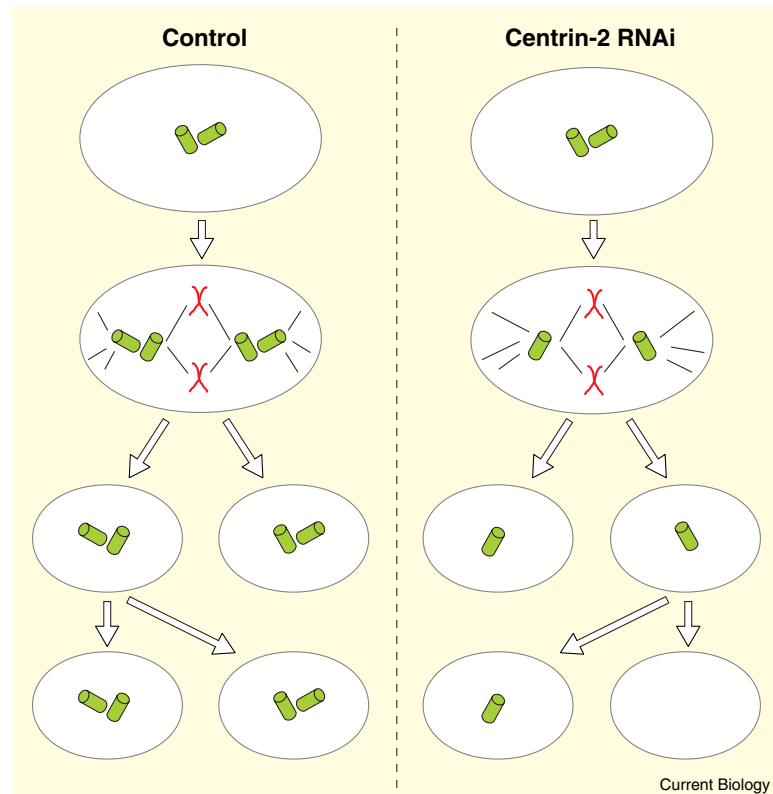
How might centrin proteins function in centriole duplication? While the answer is not known, some notable properties of centrins offer intriguing possibilities. In some organisms, centrins assemble into fibrous structures emanating from centrioles [14]. The yeast centrin Cdc31p localizes to the half-bridge, a substructure of the spindle pole body that spans the gap between the original and nascent spindle pole body. By analogy, it is possible that the role of centrins in centriole duplication is to participate in formation of a structure that facilitates subsequent steps in the assembly of a daughter centriole. Alternatively, as centrins are members of the calmodulin protein superfamily their role might be to act as regulators, modulating the activities of other proteins in response to as yet unknown signals. Either way, for a coherent picture of centriole duplication to emerge, the role of centrins will need to be placed into the larger context of the complex regulatory mechanisms that control centrosome duplication [15].

Given the compelling results of Salisbury *et al.* [4], extending the RNAi approach to other known centriolar proteins will be invaluable in determining their role(s) in the complex biology of the centriole. In the absence of other protein families conserved between yeast spindle pole bodies and centrioles, it seems

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Figure 1. A model to account for the effect of reducing centrin-2 production on centriole duplication [4].

Centrioles are depicted in green, mitotic chromosomes in red, and microtubules in black, and the cell wall in grey. Left and right columns show the centriole/centrosome duplication cycle in control and RNAi treated cells, respectively. Top: initial interphase cells with one centrosome containing two centrioles. Middle: mitotic cell with two centrosomes organizing a bipolar spindle. The control cells have completed a round of centriole duplication, so each mitotic centrosome has two centrioles; the centrin-2 RNAi treated cells fail to duplicate centrioles and the two mitotic centrosomes have only one centriole each. Bottom: interphase daughter cells. The control cells each have two centrioles; the RNAi treated cells each have a single centriole that does not duplicate. Subsequent rounds of cell division will generate uni-centriolar or a-centriolar daughters. Continued centriole dilution ultimately leads to cell death.



clear that identifying and characterizing centrin-interacting proteins in the centriole will be a key next step in an approach targeted at advancing the current rudimentary understanding of centriole duplication.

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