Centrosomes in Development and Disease

Edited by Erich A. Nigg

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Cover Illustration
The cover illustration is based on an immunofluorescence picture showing a mitotic mammalian cell (BSC-1) with a monoastral spindle (courtesy of Dr. Thomas Mayer, Max-Planck-Institute of Biochemistry, Martinsried, Germany). Centrosomes are shown in yellow, spindle microtubules in green and chromosomes in blue. In this cell, centrosome separation was blocked by treatment with monastrol, a small molecule inhibitor of the centrosome-associated kinesin-related motor Eg5.

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Contents

Preface

Much like the smile on Mona Lisa's face: beautiful and mysterious…

Ever since the centrosome was discovered more than a hundred years ago, many aspects of its structure, function and reproduction have been shrouded by mystery. However, new information is now rapidly leading to a better understanding of this fascinating organelle, particularly with regard to its role in reproduction, development and disease. The centrosome is a tiny organelle intimately involved with the organization of the microtubule cytoskeleton. Hence, it governs most microtubule-related functions, including intracellular transport, cell motility and polarity, as well as the segregation of chromosomes during cell division. Importantly, the centrioles – cylindrical structures embedded within the animal centrosome – are evolutionarily related to basal bodies. These in turn give rise to cilia and flagella which perform key functions not only in specialized epithelia and motile gametes, but also in many unicellular organisms, including parasites. Thus, wherever centrioles/basal bodies have been conserved in evolution, they are indispensable for cell cycle progression, cell motility or sensory perception. Likewise, the spindle pole body (SPB) of yeast, a microtubule organizing center (MTOC) functionally analogous to the centrosome, is essential for cell viability.

Many of the fundamental problems in centrosome biology, notably its mode of reproduction and its relevance to human development and cancer, were already introduced by Theodor Boveri (1862-1915), the eminent scientist who pioneered the study of centrosomes at the end of the 19th century. However, the centrosome had proven refractory to molecular analysis for decades, largely due to its low abundance and small size. Thanks to modern techniques and the application of complementary research strategies to several distinct organisms, answers to long-standing questions about the centrosome (and related microtubule-organizing centers) are now beginning to emerge. In particular, forward and reverse genetics, mass spectrometry-based proteomics approaches, and the combination of live-cell imaging and laser microsurgery have yielded important new information on the composition of the centrosome, its duplication and its role in the cell division cycle. These results also set the stage for new enquiries into the role of the centrosome in the etiology of cancer and other human diseases, its impact on stem cell biology,

human reproduction and infertility, and last but not least, its relevance to the propagation of intracellular parasites. From this perspective, I hope that this book will serve as a rich source of information for a wide audience, experienced centrosome-researchers and newcomers alike.

My sincere thanks go to all authors for contributing excellent, comprehensive and authoritative chapters, to Ms Alison Dalfovo for expert secretarial assistance and to Dr. Andreas Sendtko and his colleagues at Wiley-VCH for a very pleasant collaboration throughout the preparation of this book.

Erich A. Nigg
Martinsried, June 2004

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Color Plates

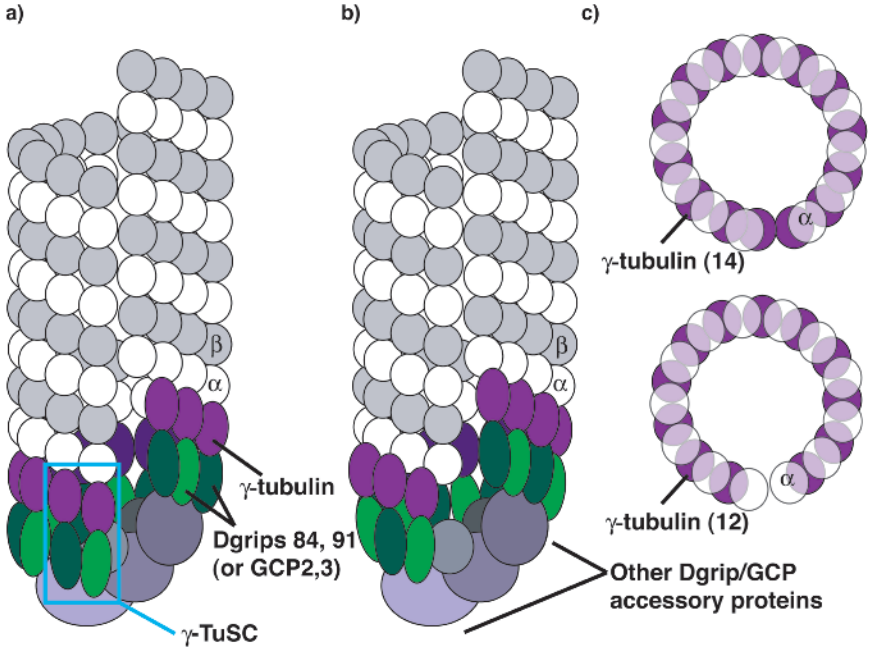

Figure 3.5 Modified template model of γTuRC-mediated microtubule nucleation. (a) The original template model proposed that γ-tubulins bind to α-tubulins at the minus ends of protofilaments similarly to longitudinal α/β-tubulin binding within a protofilament (reviewed in [15, 17]). (b) The modified template model takes into account physical properties of γ-tubulin and the mechanism of γ-tubulin-mediated microtubule nucleation by proposing that γ-tubulin binds between protofilaments [26]. A γTuRC containing 12 γ-tubulins is shown associated with the microtubule, but a 14-γ-tubulin γTuRC could also be accommodated. (c) Cross-sectional views illustrating the proposed binding sites for γ-tubulins between the α-tubulins at the minus end of each protofilament. This mode of binding provides an explanation for how a γTuRC containing an even number of γ-tubulins could template a 13-protofilament microtubule, the most common architecture observed *in vivo*.

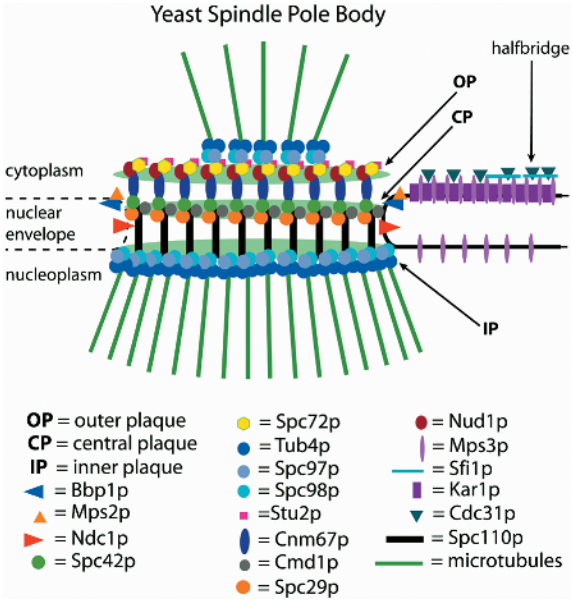

Figure 4.2 Yeast Spindle Pole Body. Shown here is a schematic of the organization of most of the components described in Table 4.1.

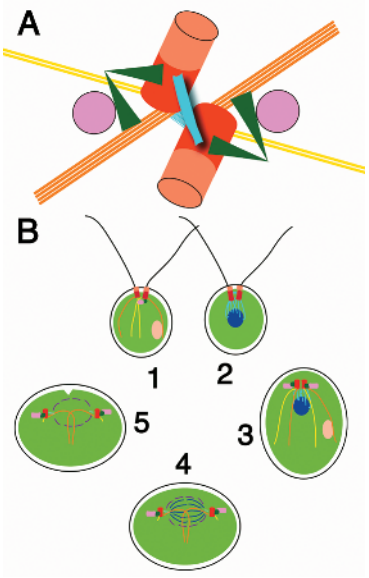

Figure 5.5 The three fiber systems of the basal body complex. (A) The mature basal bodies are shown in red, the transition zones in peach and the probasal bodies are shown in pink. The rootlet microtubules have four microtubules (orange) or two microtubules (yellow) and attach at specific triplet microtubules of the basal body. The distal (solid) and proximal (striped) striated fibers are shown in light blue. They connect the two mature basal bodies at the two ends. The lateral fibers are shown in green. They connect the mature basal body to its daughter probasal body across the rootlet microtubules. (B) Changes in the fiber systems during the cell cycle. 1, During interphase the basal bodies and transition zones are continuous with the flagella. The rootlet microtubules are adjacent to the plasma membrane. One of the four-membered rootlet microtubules lie adjacent to the eyespot (rose). 2, Another view of interphase cells illustrates that the basal bodies are connected to the nucleus and to each other by centrin fibers. 3, At preprophase, the flagella are lost. The probasal bodies elongate. The distal and proximal striated fibers are lost. 4, The two-membered rootlet microtubules shorten. The centrioles (without transition zones) are found at the poles of the spindle. The four-membered rootlet microtubules arc over the spindle. The eyespot is disassembled. 5, Cytokinesis is initiated at one end of the cell. This will be followed by extension of the two-membered rootlet microtubules, the striated fibers, and assembly of new rootlet microtubules and of a new eyespot in association with the new four-membered rootlet microtubules.

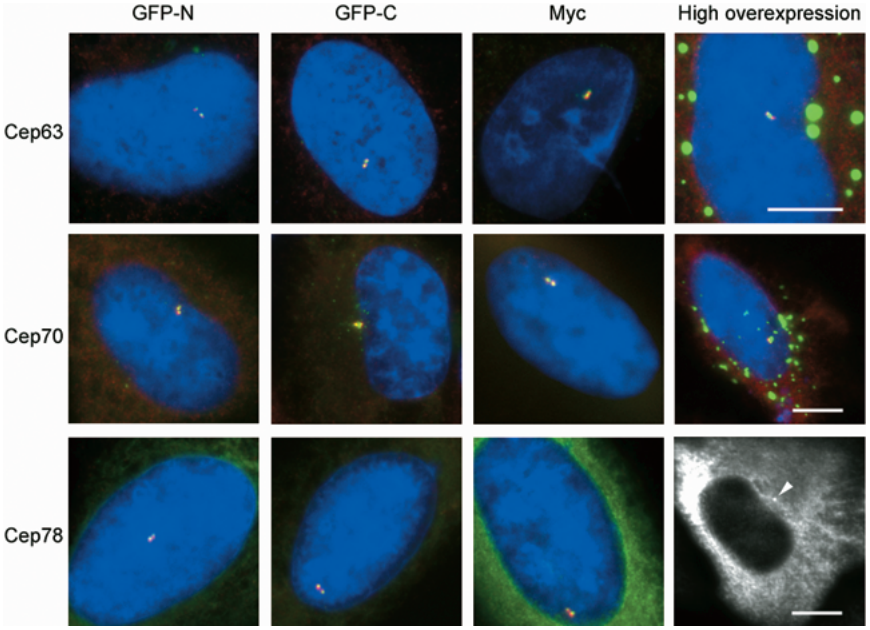

Figure 7.2 A selection of differently tagged, novel centrosome proteins. Rows from top to bottom show Cep63, Cep70 and Cep78. Columns from left to right show N-terminal GFP, C-terminal GFP and N-terminal myc-tagged proteins, respectively. The most right-hand column shows the results of very high overexpression of these proteins (tagged at the N-terminus with GFP), generating large aggregates or a high cytoplasmic background. Green, ectopically expressed centrosomal proteins; red, γ-tubulin; blue, DNA (DAPI). The arrowhead points to the position of the centrosome. Scale bars, 10 μm; panels in the three left columns are to the same scale as the top right panel.

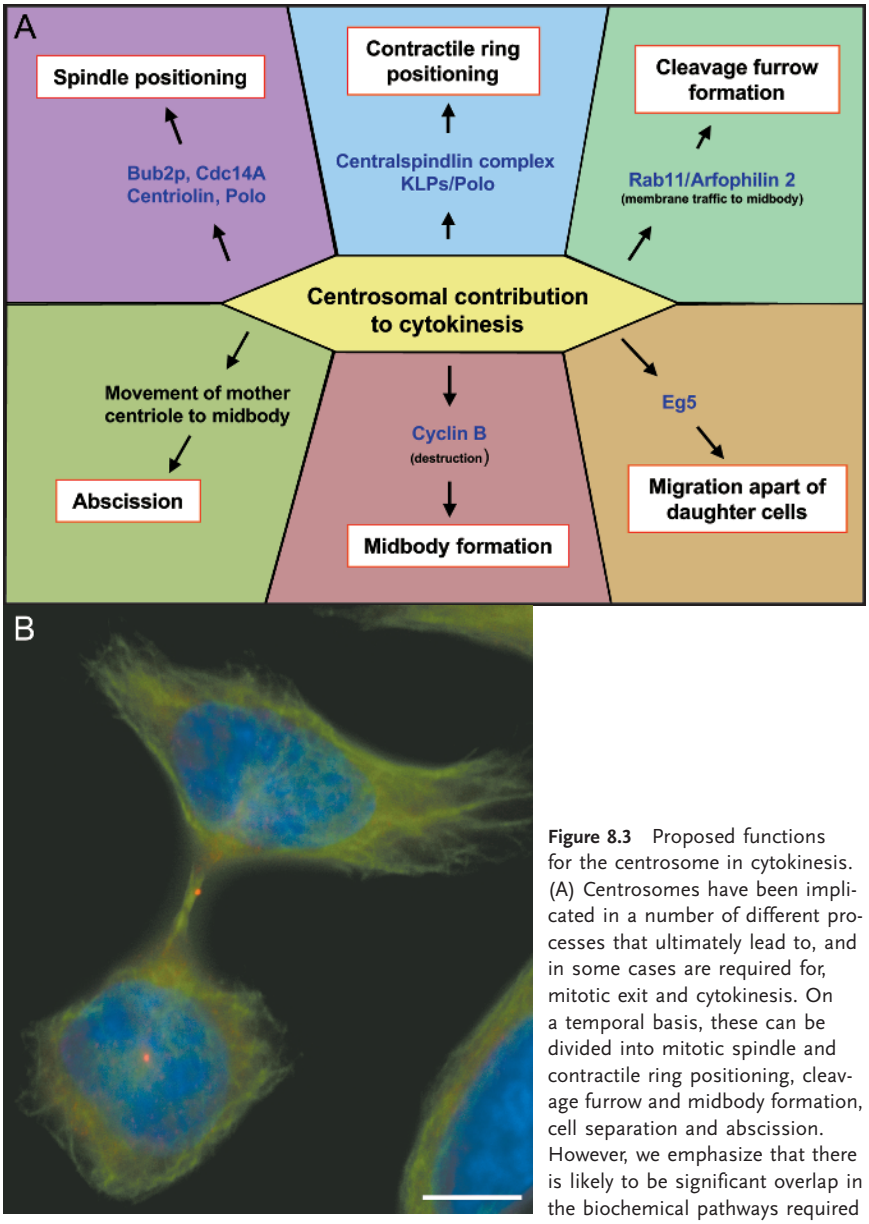

Figure 8.3 Proposed functions for the centrosome in cytokinesis. (A) Centrosomes have been implicated in a number of different processes that ultimately lead to, and in some cases are required for, mitotic exit and cytokinesis. On a temporal basis, these can be divided into mitotic spindle and contractile ring positioning, cleavage furrow and midbody formation, cell separation and abscission. However, we emphasize that there is likely to be significant overlap in the biochemical pathways required for each of these endpoints. Examples of proteins that localize to mitotic centrosomes and are implicated in these pathways are indicated in dark blue. (B) One of the most intriguing questions relating to the role of the centrosome in cytokinesis is why the mother centriole migrates towards the midbody prior to cell abscission. HeLa cells are shown following methanol fixation and staining with antibodies against α-tubulin (green) and γ-tubulin (red). DNA is stained with Hoechst 33258 (blue). Scale bar, 10 μm.

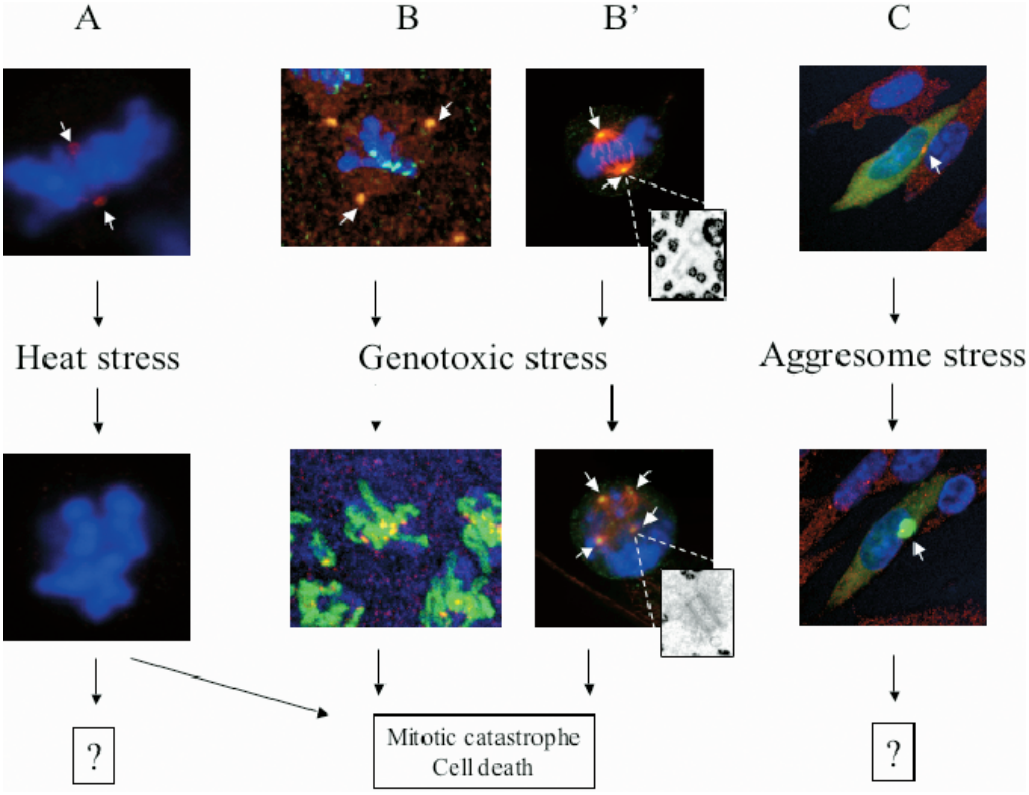

Figure 11.1 Centrosome alterations in response to heat, genotoxic and aggresome stress. In diverse systems, γ-tubulin (red) localizes to centrosomes at the mitotic spindle poles (A, B, B) and close to interphase nuclei (C). In Chinese hamster ovary (CHO) cells, heat stress (A) triggers loss of γ-tubulin localization to the poles (courtesy of H. Hut) while genotoxic stress (B) leads to mitotic centrosome fragmentation. Electron microscopic examination demonstrates that the centrosome fragments contain single centrioles (insets). In response to heat shock and genotoxic stress, centrosome disruption is associated with failures of mitotic division and mitotic catastrophe. In *Drosophila* embryos, genotoxic stress also leads to dissociation of γ-tubulin from the spindle poles (B) and mitotic catastrophe. Over-expression of a mutant form of GFP taggered the Huntingtin protein (green) in hamster cells (C), leads to aggresome formation around interphase centrosomes (courtesy of F. Salomons and M. Rujano). The significance of aggresome formation is not known, but this structure may contribute to neurodegeneration in a number of pathological conditions. In all panels, γ-tubulin is in red and DNA is in blue. In B, the kinetochore marker MeiS332 is in green. In C the Huntingtin-GFP protein is in green.

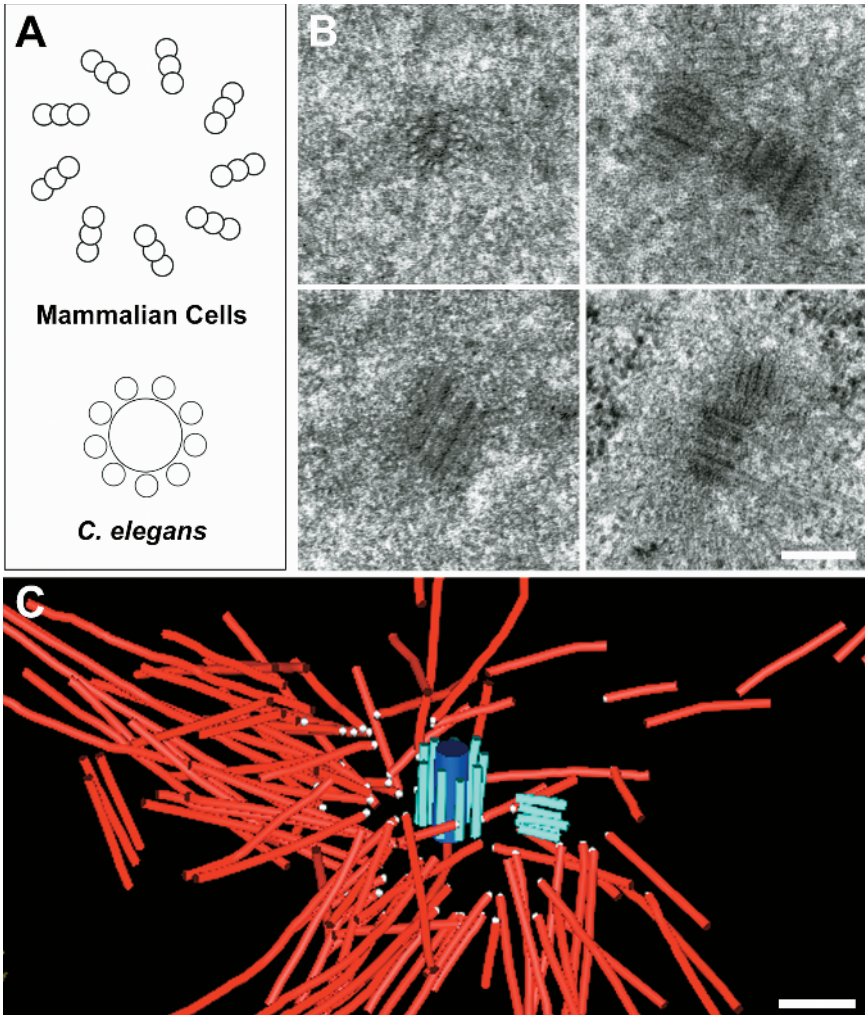

Figure 12.2 The ultrastructure of the *C. elegans* centrosome. (A) Schematic representation of the triplet structure of centrioles found in mammalian cells (top) and the singlet structure observed in *C. elegans* (bottom). (B) Electron micrographs of wild-type centrioles in cross-section and longitudinal orientation (left) and wild-type centriole pairs in orthogonal orientation (right). (C) 3-D model of a centriole pair during prometaphase derived from a tomographic reconstruction. Microtubules (red) are organized mainly around one centriole (blue), referred to as the mother centriole. Note that the minus ends of the microtubules do not come in contact with this centriole. Scale bars = 250 nm.

Figure 12.3 PCM recruitment and spindle assembly in *C. elegans*. Early embryos at different stages of the cell cycle were fixed and labeled for DNA (blue), microtubules (green) and γ-tubulin (red). Z-stacks through entire embryos were acquired, the images deconvolved and shown as two-dimensional projections. Scale bar = 10 μm. The anterior is to the left in all the images. (a) An acentrosomal meiotic spindle can be observed soon after fertilization (arrow). At this stage the centrosome contributed by the sperm has yet to separate. (b) At the beginning of pronuclear migration, the sperm-derived centrosome has separated and recruited some γ-tubulin therefore increasing the amount of microtubules it is able to nucleate. (c) At the time when the pronu- ▶