

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 XV

List of Contributors XVII

Color Plates XXIII

Part I Microtubule Organization and Dynamics 1

1 Early Studies on Centrioles and Centrosomes 3

Joseph G. Gall

- 1.1 Introduction 3
- 1.2 Pioneering Studies 4
- 1.3 Self-replication versus *De Novo* Formation 7
- 1.4 Centrioles and Basal Bodies 7
- 1.5 Blepharoplasts 9
- 1.6 The Search for DNA 11
- 1.7 On to Self-assembly 12
- References 14

2 The Tubulin Superfamily 17

Tim Stearns

- 2.1 History 17
- 2.2 Family Relations 18
- 2.3 Localization and Function 21
- 2.4 γ -Tubulin 21
- 2.5 δ -Tubulin 22
- 2.6 ϵ -Tubulin 22
- 2.7 Other Members of the Fold 23
- References 24

3 Microtubule Nucleation 27

Michelle Moritz, Luke M. Rice and David A. Agard

- 3.1 Introduction 27

3.1.1	The Nucleation of Microtubules can occur Spontaneously <i>In Vitro</i> , but Requires γ -Tubulin <i>In Vivo</i>	28
3.1.2	Models for the Mechanism of γ -TuRC/Tub4 Complex-mediated Microtubule Nucleation	29
3.2	Kinetic Models of the Mechanism of Microtubule Nucleation	31
3.3	The Involvement of Non- γ -TuRC Proteins in Microtubule Nucleation	36
3.4	Future Directions	37
	Acknowledgments	38
	References	38
4	The Budding Yeast Spindle Pole Body: A Centrosome Analog	43
	<i>Suzanne van Kreeveld Naone and Mark Winey</i>	
4.1	Introduction	43
4.2	Molecular Composition of the Spindle Pole Body	45
4.2.1	The Central Plaque	47
4.2.2	The Inner Plaque	48
4.2.3	The Outer Plaque	49
4.2.4	Nuclear Membrane Factors	49
4.2.5	The Halfbridge	50
4.2.6	Structure Summary	50
4.3	Microtubule Nucleation	51
4.4	Assembly/Duplication of SPBs and Centrosomes	53
4.4.1	Electron Microscopic Description of Duplication	53
4.4.2	Cell Cycle Regulation of Duplication	55
4.4.3	Genetic Analysis of Duplication	55
4.5	Signaling Platform	57
4.6	Developmental Alteration of SPB Function	60
4.7	Parting Thoughts	61
	Acknowledgments	61
	References	62
5	Dissection of Basal Body and Centriole Function in the Unicellular Green Alga <i>Chlamydomonas reinhardtii</i>	71
	<i>Susan K. Dutcher</i>	
5.1	Introduction	71
5.2	Why Study a Green Alga to Learn about Centrioles and Basal Bodies?	72
5.3	Structure of the Basal Body and Centriole in <i>Chlamydomonas</i>	72
5.4	Additional Fibers that Connect Basal Bodies and Centrioles	76
5.4.1	Contractile Fibers	76
5.4.2	Rootlet Microtubules	77
5.4.3	Non-contractile Fibers	77
5.5	Overview of the Cell Cycle of <i>Chlamydomonas</i>	78
5.6	Duplication of Basal Bodies in <i>Chlamydomonas</i>	78
5.7	Role of Tubulin Isoforms in Basal Body Duplication	82
5.8	Timing of Basal Body/Centriole Duplication in <i>Chlamydomonas</i>	83

5.9	Function of Basal Bodies and Centrioles in <i>Chlamydomonas</i>	83
5.10	What Makes a Basal Body Different from a Centriole?	84
5.10.1	Transition Zone and Docking	84
5.10.2	Transition Zone and Autonomy	84
5.10.3	Maturation of Basal Bodies	86
5.11	Conclusion	87
	Acknowledgments	87
	References	88
6	The Centrosome in Evolution	93
	<i>Juliette Azimzadeh and Michel Bornens</i>	
6.1	Introduction	93
6.2	The Centriole/Basal Body Structure is a Derived Characteristic of Eukaryotes	94
6.3	The Basal Body/Axoneme is the Ancestral Structure	94
6.4	Functions Associated with the Flagellar Apparatus	96
6.4.1	Cell Locomotion	97
6.4.2	Sensory Reception	97
6.4.3	Cell Division	98
6.5	The Conservative Mode of Duplication of the Basal Body/Centriole/SPB: An Essential Clue for Cell Morphogenesis	100
6.6	The Centrosome or Central Body	102
6.7	Evolution of Centrosome-associated Gene Products	104
6.7.1	γ -Tubulin	104
6.7.2	Centrin	106
6.7.3	Centrin-binding Proteins	111
6.8	Conclusion: The Centrosome – A Cell Individuation Organ?	113
6.8.1	Survival Value of Coupling Basic Functional Modules on the Same Organ	113
6.8.2	Co-Evolution of the Centrosome and the Cleavage Apparatus	114
6.8.3	The Biological Significance of Having a Cell Center	115
	Acknowledgments	116
	References	116
Part II	The Integration of Centrosome and Chromosome Cycles	123
7	A Proteomic Approach to the Inventory of the Human Centrosome	125
	<i>Christopher J. Wilkinson, Jens S. Andersen, Matthias Mann and Erich A. Nigg</i>	
7.1	Introduction	125
7.2	What is a Centrosome Component?	126
7.3	Composition of the Human Centrosome: A Proteomic Approach	127
7.4	Inspection of Novel Centrosome Proteins by Sequence Analysis	132
7.5	Cell Cycle Changes in Centrosome Composition	135

- 7.6 The Impact of MS on Centrosome Analysis during Cell Cycle and Development 137
- 7.7 Expanding Proteomic Information into Knowledge about Function 138
- 7.8 Conclusion and Prospects 138
 - Acknowledgments 139
 - References 139

- 8 The Role of the Centrosome in Cell Cycle Progression 143**
Andrew M. Fry and Rebecca S. Hames
 - 8.1 Introduction 143
 - 8.2 Cell Cycle Dynamics of Centrosome Structure 144
 - 8.3 Old and New Functions of the Centrosome 145
 - 8.4 The Centrosome in G2/M Control 146
 - 8.5 Initiation of Cyclin B Destruction at the Centrosome 149
 - 8.6 The Contribution of Centrosomes to Cytokinesis 153
 - 8.7 A Role for Centrosomes in G1/S Progression? 157
 - 8.8 In Conclusion 159
 - Acknowledgments 159
 - References 160

- 9 Centrosome Duplication and its Regulation in the Higher Animal Cell 167**
Greenfield Sluder
 - 9.1 Introduction 167
 - 9.2 The Events of Centrosome Reproduction 168
 - 9.2.1 Centriole Disorientation 168
 - 9.2.2 Centriole Duplication 169
 - 9.2.3 Centrosome Disjunction 170
 - 9.2.4 Centrosome Separation 170
 - 9.2.5 Some Proteins Needed for Centrosome Reproduction 172
 - 9.3 Control of Centrosome Duplication 173
 - 9.3.1 Control of Centrosome Number: Intrinsic Mechanisms 173
 - 9.3.2 Block to Re-replication 175
 - 9.3.3 Time of Centrosome Duplication: Extrinsic Controls 176
 - 9.3.4 Cyclin-dependent Kinases in the Control of Centrosome Reproduction 176
 - 9.3.4.1 Zygote Systems 177
 - 9.3.4.2 Mammalian Somatic Cells 177
 - 9.3.5 Targets of Cdk2–Cyclin E Kinase 179
 - 9.3.6 Other Kinases Involved in Centrosome Duplication 180
 - 9.3.7 Ubiquitin-mediated Proteolysis in the Control of Centrosome Duplication 181
 - 9.4 Closing Remarks 183
 - Acknowledgments 183
 - References 183

10	A Synergy of Technologies: Using Green Fluorescent Protein Tagging and Laser Microsurgery to Study Centrosome Function and Duplication in Vertebrates	191
	<i>Alexey Khodjakov and Conly L. Rieder</i>	
10.1	Introduction	191
10.2	Laser Microsurgery	193
10.2.1	A Brief History of Development	193
10.2.2	Utility for Removing the Centrosome	195
10.3	Roles of the Centrosome during Cell Division	199
10.3.1	Role of the Centrosome during Spindle Assembly	199
10.3.2	Role of the Centrosome during Cytokinesis	202
10.4	The Centrosome in the Cell Cycle	203
10.4.1	Role of the Centrosome in Progression through the Cell Cycle	203
10.4.2	<i>De Novo</i> Centrosome Formation	205
10.5	For the Future	208
	Acknowledgments	208
	References	209
11	Centrosome Regulation in Response to Environmental and Genotoxic Stress	211
	<i>Ody C. M. Sibon and William E. Theurkauf</i>	
11.1	Introduction	211
11.2	Heat Shock	211
11.3	Centrosomes and the Unfolded Protein Response	213
11.4	Centrosome Disruption in Response to Genotoxic Stress	215
11.4.1	Centrosome Inactivation in Early Embryos	215
11.4.2	Chk2 is Required for DNA Damage-induced Mitotic Catastrophe	216
11.4.3	DNA Damage and Mitosis in Mammalian Cells	218
11.5	Final Thoughts	219
	References	221
Part III	The Centrosome in Development and Tissue Architecture	225
12	The <i>C. elegans</i> Centrosome during Early Embryonic Development	227
	<i>Laurence Pelletier, Thomas Müller-Reichert, Martin Srayko, Nurhan Özlü, Anne-Lore Schlaitz and Anthony A. Hyman</i>	
	Abbreviations	227
12.1	Introduction	227
12.1.1	<i>C. elegans</i> as a Tool to Study Centrosome Biogenesis	227
12.1.2	The First Cell Division of the <i>C. elegans</i> Embryo	228
12.2	The <i>C. elegans</i> Centrosome	230
12.2.1	The Centrioles	231
12.2.2	The Pericentriolar Material (PCM)	233
12.3	The Centrosome Cycle in <i>C. elegans</i> Embryos	234
12.3.1	Centriole Duplication	236

12.3.2	PCM Recruitment	237
12.3.3	Centrosome Maturation	238
12.4	Centrosome Functions	240
12.4.1	Spindle Assembly and Microtubule Nucleation	240
12.4.2	Determination of Anterior–Posterior Polarity	242
12.4.3	Spindle Positioning	243
12.5	Concluding Remarks	244
	Acknowledgments	245
	References	245
13	Centrosomes in a Developing Organism: Lessons from <i>Drosophila</i>	251
	<i>Jordan W. Raff</i>	
13.1	Introduction	251
13.2	Centrosome and Microtubule Organisation during the <i>Drosophila</i> Life Cycle	251
13.2.1	Oogenesis	251
13.2.2	Spermatogenesis	253
13.2.3	Early Embryogenesis	254
13.2.4	Asymmetric Divisions of Embryonic Neuroblasts	255
13.2.5	Larval Development	256
13.3	<i>Drosophila</i> Centrosomal Proteins	257
13.3.1	Microtubule Nucleation from Centrosomes: γ -Tubulin and the γ -TuRC	257
13.3.2	The Recruitment of the γ -TuRC to Centrosomes: The Potential Roles of Asp, Polo, CNN, Aurora A, and CP309/D-PLP	258
13.3.3	The Interaction between Centrosomes and Microtubules: The Role of D-TACC and Msp	259
13.3.4	Centrosomes and the Organization of the Actin/Myosin Cytoskeleton in Early Fly Embryos: The Role of Scrambled, Nuf, and CP190	260
13.3.5	Centrosomes and Cytokinesis: Studies on <i>asl</i> , <i>cnn</i> , and γ -Tubulin Mutant Spermatocytes	262
13.3.6	Centrosomes and the Cell Cycle	263
13.3.7	Centrosome Dynamics: Inactivation and Flares	264
13.3.8	Microtubule Motors and Plus-end Tracking Proteins at the Centrosome	265
13.3.9	The Interphase Centrosome in Flies: Missing in Action?	265
13.4	The Role of Centrosomes and Centrosomal Proteins <i>In Vivo</i>	266
13.4.1	The Essential Role of Centrosomes In <i>Drosophila</i>	266
13.4.2	The Role of Centrosomal Proteins in Oogenesis	268
13.5	Summary	270
	Acknowledgments	270
	References	270

14 Centrosome Inheritance during Human Fertilization and “Therapeutic” Cloning: Reproductive and Developmental Diseases and Disorders Caused by Centrosome Dysfunction 279

C. S. Navara, C. Simerly and G. Schatten

- 14.1 Introduction 279
- 14.2 Centrosomes during Human Fertilization 281
- 14.3 Centrosome Dysfunction as Causes of Human Infertility 281
- 14.4 Centrosome Functional Assays for Diagnosing Male Infertility 284
- 14.5 Polyspermy in Humans 285
- 14.6 “Dispermy Hypothesis” for the Origins of Genomic Imprinted Disorders 287
- 14.7 Maternal Centrosome Anomalies and Birth Defects 288
- 14.8 Resolving the Special Problem of Parthenogenesis: Roles of Cytoplasmic Motors and NuMA 289
- 14.9 Centrosomes during Cloning, and Centrosomes in Embryonic Stem Cells Derived after Nuclear Transfer 290
- 14.10 Research Challenges for Centrosome Developmental Biologists: Developmental Centrosomopathies 292
- References 293

15 Microtubule Organizing Centers in Polarized Epithelial Cells 299

Mette M. Mogensen

- 15.1 Introduction 299
- 15.2 Centrosomal Microtubule Nucleation 300
- 15.3 Non-centrosomal Microtubule Arrays 301
- 15.4 Microtubule Minus-end Anchorage at Centrosomal and Non-centrosomal Sites 305
- 15.5 Centrosomal Release of Microtubules and Anchoring Complexes 307
- 15.6 Stabilization of Non-centrosomal Microtubules 308
- 15.7 Release and Capture 310
- Acknowledgments 312
- References 312

Part IV Centrosomes in Disease 321

16 Centrosome Anomalies in Cancer: From Early Observations to Animal Models 323

Thea M. Goepfert and William R. Brinkley

- 16.1 Early Observations 323
- 16.2 Origin of Centrosome Anomalies 326
 - 16.2.1 Deregulation of Centrosome Duplication 326
 - 16.2.2 *De Novo* Formation of Centrioles or Uncontrolled Separation of Centriole Pairs 327
 - 16.2.3 Failure to Undergo Cytokinesis 327
 - 16.2.4 Fusion of Cells 327

16.3	Animal Models	328
16.3.1	Centrosome Anomalies and the p53 Pathway	328
16.3.2	Centrosome Anomalies and BRCA 1	329
16.3.3	Centrosome Anomalies and Aurora A	330
	Acknowledgments	333
	References	333
17	Radiation Therapy and Centrosome Anomalies in Pancreatic Cancer	337
	<i>Norihiro Sato, Kazuhiro Mizumoto, and Masao Tanaka</i>	
	Abstract	337
17.1	Introduction	337
17.2	Radiation-induced Cell Death: Apoptosis or Mitotic Cell Death?	338
17.3	Centrosome Anomalies Induced by Radiation	339
17.4	The Mechanism(s) Leading to Centrosome Anomalies after Radiation Treatment	341
17.5	The Consequence of Centrosome Anomalies after Irradiation	343
17.6	Factors Affecting Centrosome Anomalies after Irradiation	345
17.7	Conclusions and Future Directions	346
	References	347
18	Human Papillomavirus Infection and Centrosome Anomalies in Cervical Cancer	353
	<i>Karl Münger and Stefan Duensing</i>	
18.1	Genomic Instability and Malignant Progression	353
18.2	Human Papilloma viruses	354
18.3	Biological Activities of HPV E6/E7 Oncogenes	355
18.4	HPV-mediated Cervical Carcinogenesis as a Model System to Study Genomic Instability and Malignant Progression	357
18.5	Centrosome Abnormalities and Genomic Instability: Cause or Effect?	358
18.6	Induction of Centrosome Abnormalities by HPV Oncoproteins: Boveris Model Revisited	359
18.7	Do HPV E7-induced Centrosome Anomalies Contribute to Carcinogenic Progression?	362
18.8	Mechanistic Considerations	363
18.9	Concluding Remarks	365
	Acknowledgments	366
	References	367

19	Manipulation of Centrosomes and the Microtubule Cytoskeleton during Infection by Intracellular Pathogens	371
	<i>Niki Scaplehorn and Michael Way</i>	
19.1	Introduction	371
19.2	Microtubule-directed Movement of Viruses and Membrane Compartments during Viral Infection	372
19.2.1	Targeting the Nucleus using Motor-proteins and the Microtubule Network: <i>Herpes Simplex Virus</i> , Poliovirus and Retroviruses	373
19.2.2	Hijacking Motor Proteins to Promote Cytoplasmic Assembly and Spread: <i>Vaccinia Virus</i> and African Swine Fever Virus	375
19.2.3	Conclusion	379
19.3	Virus-mediated Damage to the Centrosome and Microtubule Network	380
19.3.1	Viral Disruption of Microtubule Organization	380
19.3.2	Virus-mediated Centrosomal Damage	381
19.3.3	Summary	383
19.4	Viral Disruption of the Centrosome Duplication Cycle and Spindle Checkpoints	383
19.4.1	Early Studies on Centrosome Number: Paramyxoviral Syncytia	384
19.4.2	Multiple Centrosomes: Human Immunodeficiency Virus and the DNA Damage Checkpoint	385
19.4.3	Multiple Centrosomes: DNA Tumor Viruses, Retinoblastoma and Ran GTPase	387
19.4.4	Targeting the Spindle Assembly Checkpoint: Human T-Cell Leukemia Virus-1	388
19.4.5	Summary	389
19.5	Bacterial Manipulation of the Centrosome and Microtubules	390
19.5.1	Bacterial Manipulation of the Microtubule Network	390
19.5.2	Interactions between Bacteria and the Centrosome	393
19.5.3	Summary	394
19.6	Conclusion	394
	Acknowledgments	394
	References	395
20	Basal Bodies and Microtubule Organization in Pathogenic Protozoa	401
	<i>Keith Gull, Laura Briggs and Sue Vaughan</i>	
20.1	Introduction and Appreciation	401
20.2	The “Dispersed” MTOC Complement of Protozoal Cells	403
20.3	The <i>Trypanosoma brucei</i> Microtubule Biology	403
20.3.1	The Spindle and Cell Division	404
20.3.2	Basal Bodies	407
20.4	The Microtubule Biology of the <i>Apicomplexa</i>	408
20.4.1	The Apical Polar Ring: A Unique Cytoplasmic MTOC	408
20.4.2	The Conoid	409
20.4.3	Apicomplexan Basal Bodies	410

20.4.4	The Spindle MTOC	411
20.4.5	Apicomplexan Cell Division and Cell Morphogenesis	411
20.5	Basal Bodies Are More than Just Microtubule Organizers: The Hitchhikers Guide to the Cytoskeleton!	414
20.6	Cytoskeletal Adaptations to Parasitism	416
20.7	Conclusion	419
	Acknowledgments	420
	References	420

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

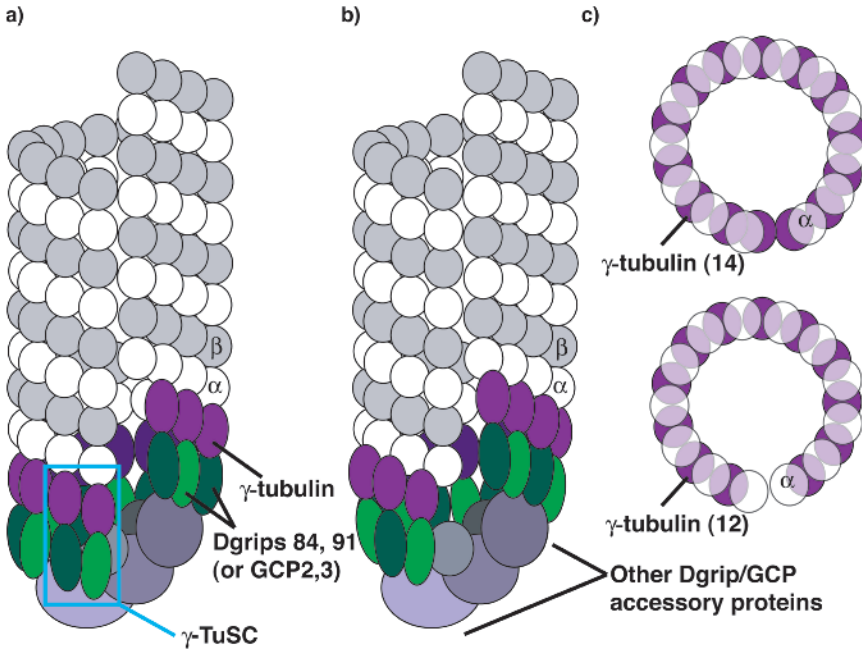


Figure 3.5 Modified template model of γ TuRC-mediated microtubule nucleation. (a) The original template model proposed that γ -tubulins bind to α -tubulins at the minus ends of protofilaments similarly to longitudinal α/β -tubulin binding within a protofilament (reviewed in [15, 17]). (b) The modified 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 γ TuRC containing 12 γ -tubulins is shown associated with the microtubule, but a 14- γ -tubulin γ TuRC 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 γ TuRC containing an even number of γ -tubulins could template a 13-protofilament microtubule, the most common architecture observed *in vivo*.

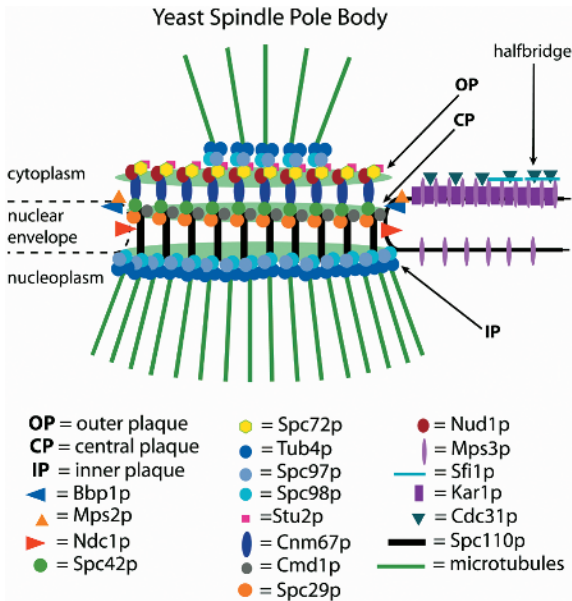


Figure 4.2 Yeast Spindle Pole Body. Shown here is a schematic of the organization of most of the components described in Table 4.1.

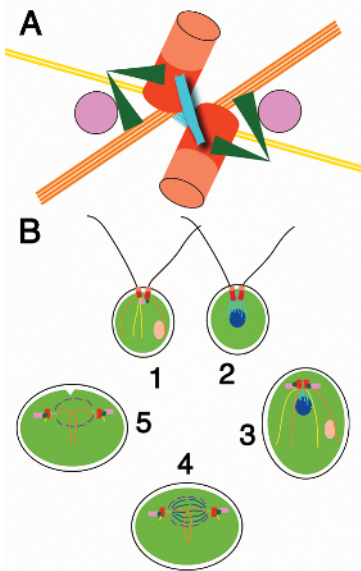


Figure 5.5 The three fiber systems of the basal body complex. (A) The mature basal bodies are shown in red, the transition zones in peach and the probasal bodies are shown in pink. The rootlet microtubules have four microtubules (orange) or two microtubules (yellow) and attach at specific triplet microtubules 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.

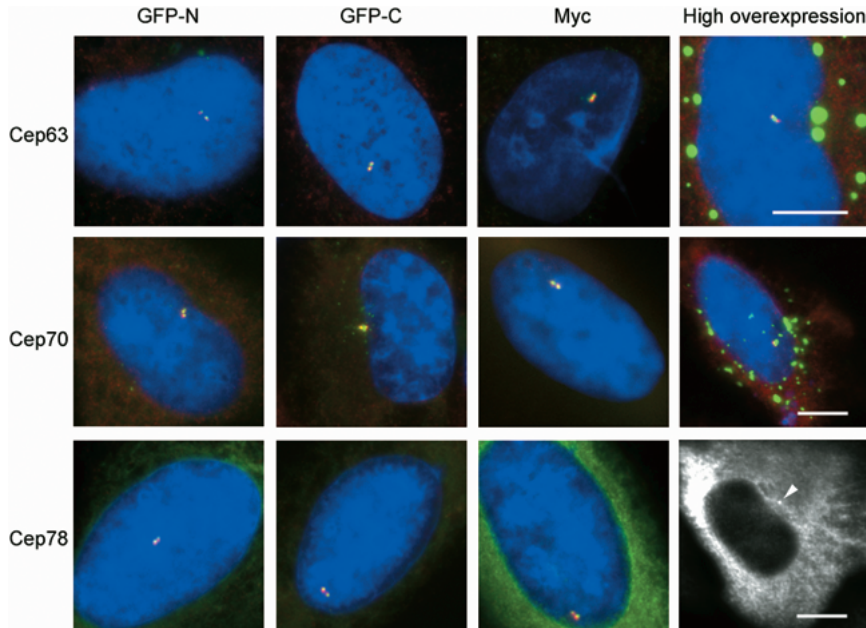


Figure 7.2 A selection of differently tagged, novel centrosome proteins. Rows from top to bottom show Cep63, Cep70 and Cep78. Columns from left to right show N-terminal GFP, C-terminal GFP and N-terminal myc-tagged proteins, respectively. The most right-hand column shows the results of very high overexpression 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 μ m; panels in the three left columns are to the same scale as the top right panel.

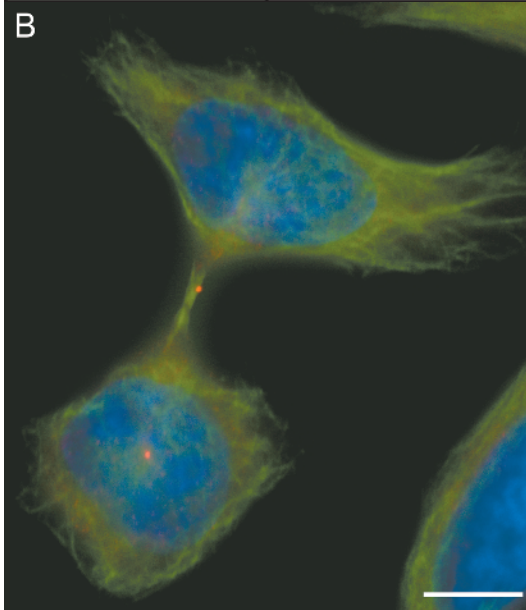
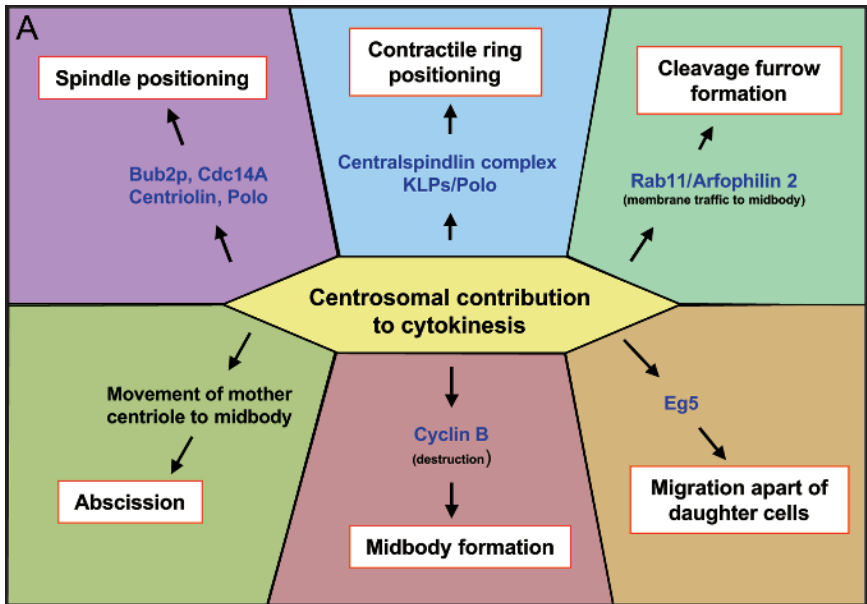


Figure 8.3 Proposed functions for the centrosome in cytokinesis. (A) Centrosomes have been implicated in a number of different processes that ultimately lead to, and in some cases are required for, mitotic exit and cytokinesis. On a temporal basis, these can be divided into mitotic spindle and contractile 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 μ m.

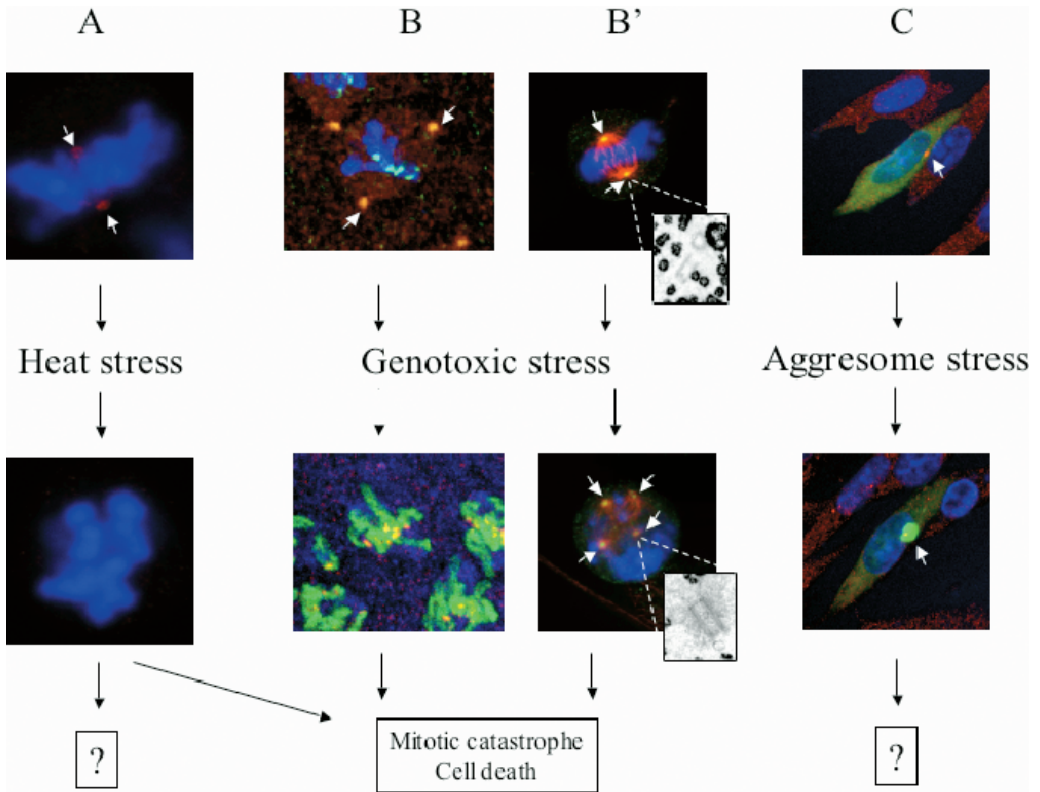


Figure 11.1 Centrosome alterations in response to heat, genotoxic and aggresome stress. In diverse systems, γ -tubulin (red) localizes to centrosomes at the mitotic spindle poles (A, B, B) and close to interphase nuclei (C). In Chinese hamster ovary (CHO) cells, heat stress (A) triggers loss of γ -tubulin 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 tagged 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.

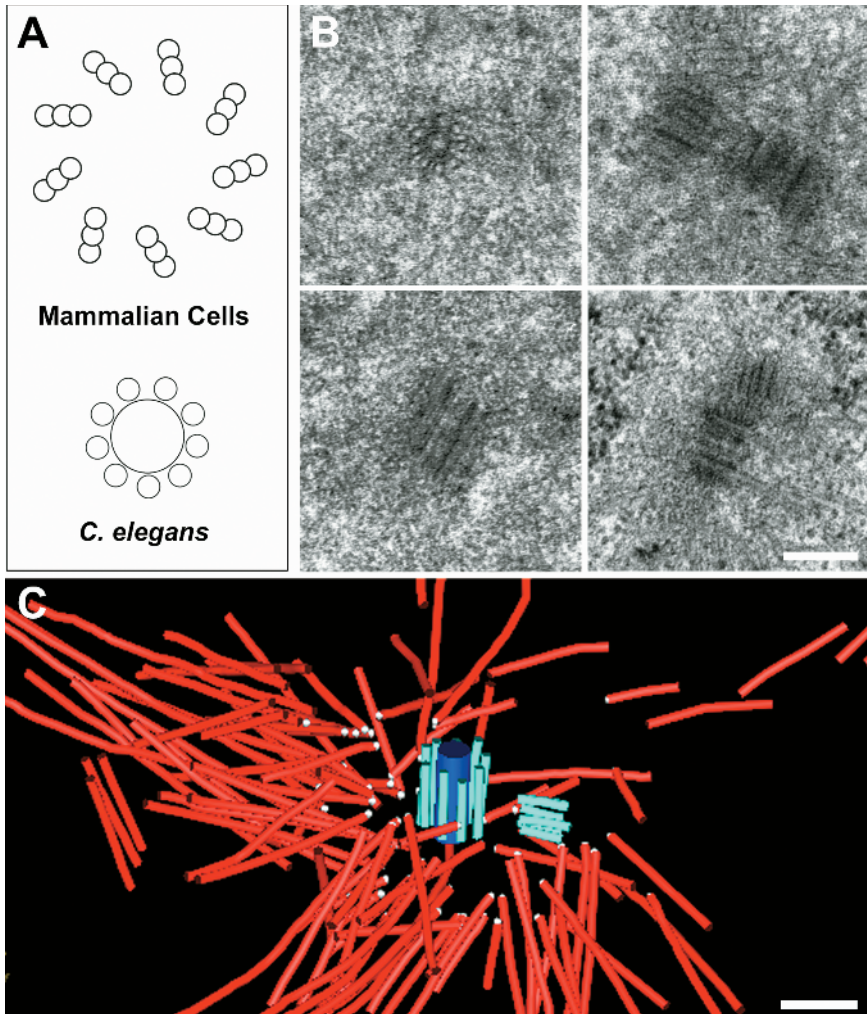


Figure 12.2 The ultrastructure of the *C. elegans* centrosome. (A) Schematic representation of the triplet structure of centrioles found in mammalian cells (top) and the singlet structure observed in *C. elegans* (bottom). (B) Electron micrographs of wild-type centrioles in cross-section and longitudinal 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 μ m. 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-