Heide Schatten *Editor*

The Centrosome

Cell and Molecular Mechanisms of Functions and Dysfunctions in Disease

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ISBN 978-1-62703-034-2 ISBN 978-1-62703-035-9 (eBook)
DOI 10.1007/978-1-62703-035-9
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2012942022

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Preface

The discovery of centrosomes well over 100 years ago has been called as important as the discovery of the nucleus but it was only recently that research on centrosomes has moved this central organelle to the forefront of modern research and has exploded as a result of renewed appreciation, new enthusiasm, and new methods that are now available to study centrosomes on cellular, molecular, and genetic levels. Centrosome functions are critically important for cell cycle regulation while centrosome dysfunctions have been implicated in numerous diseases such as cancer and in disorders such as infertility and other reproductive disorders.

While most frequently described as microtubule organizing centers (MTOCs), the recent recognition that centrosomes are critical for cell signaling coordination and cellular protein degradation, and regulated proteolysis has revolutionized studies into disorders and in the pathogenesis and progression of diseases. To cover the wealth of new data on the significant role of centrosomes in cellular functions and implications in disease, a number of topic-focused review articles have been written and published in various specialized journals, as the demand for understanding the direct and indirect functions of this important organelle has increased in various areas of basic and biomedical research.

This book features a variety of different aspects on classic and modern centrosome research to cover topics of current interest. Each chapter is written by internationally recognized experts in their respective fields who have contributed their unique expertise in specific research areas and include comprehensive and concise reviews of key topics in the field as well as cell and molecular details that are important for the specific subtopics. Cutting edge new information is balanced with background information that is readily understandable for the newcomer and the experienced centrosome researcher alike. In addition, several articles will raise awareness of centrosomes in areas that have not yet considered centrosomes associated with disease including aspects of misguided signal transduction and several others that may find centrosomes as new targets for therapeutic intervention.

The book includes chapters on

- Centriole duplication and inheritance.
- Sperm centrioles and abnormalities underlying sperm pathology and infertility.
- Centrosomal functions and dysfunctions in cat spermatozoa.
- Nuclear-centrosome interactions during fertilization and cell division.
- Human centrosomal dynamics during gametogenesis, fertilization, and embryogenesis.
- Asymmetric centrosome behavior in stem cell divisions.
- Functional associations between the Golgi apparatus and the centrosome.
- The centrosome and its role in regulated proteolysis.
- Regulation of the centrosome cycle by protein degradation.
- Molecular links between centrosome duplication and other cell cycle associated events.
- Regulation of centrosomes by cyclin-dependent kinases.
- Disruption of centrosome duplication control and induction of mitotic instability by the high-risk human papillomavirus oncoproteins E6 and E7.
- Centrosomes, DNA damage, and aneuploidy.
- Centrosome regulation and breast cancer.
- The role of centrosomes in multiple myeloma.
- Centrosomal amplification and related abnormalities.
- Mechanisms and consequences of centrosome clustering in cancer cells.
- The neuronal centrosome as a generator of microtubules for axons and dendrites.
- Centrosomes and cell division in Apicomplexa.
- The centrosome life story in *Xenopus laevis*.
- The role of centrosomes in T cells, and concludes with.
- Thoughts on progress in the centrosome field.

The topics addressed are selected to be of interest to scientists, students, teachers, and to all who are interested in expanding their knowledge related to centrosomes. The volume is intended for a large audience as a reference book on the subject.

It has been a great pleasure and timely to edit this book on centrosomes and I would like to sincerely thank all contributors for their outstanding chapters and for sharing their unique expertise with the centrosome community. I hope that this book will stimulate further advances in centrosome research and contribute new insights and appreciation for the role of centrosomes in the basic and biomedical sciences.

Heide Schatten

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Part I
Centrosomes in Reproduction

Chapter 1
Centriole Duplication and Inheritance in *Drosophila melanogaster*

Tomer Avidor-Reiss, Jayachandran Gopalakrishnan, Stephanie Blachon and Andrey Polyanovsky

Abstract Centrosomes are conserved microtubule-based organelles that are essential for animal development. In this chapter, we highlight key centrosomal proteins and describe the centrosome in the context of several developmental processes in *Drosophila melanogaster*. These processes include fertilization, during which the centrosome mediates the fusion of male and female pronuclei; development of the embryonic syncytium, where centrosomes act as microtubule-organizing centers and participate in nuclear division; and the formation of sensory and motile cilia in the adult, where the centrosome's centrioles template axoneme assembly. The study of these processes in *Drosophila* provides a unique experimental system where classical approaches in genetics and biochemistry can be used to dissect centrosome biology.

1.1 What are the Challenges in Studying the Centrosome and Why Use *Drosophila*?

Like chromosomes and yeast spindle pole bodies (SPB), centrosome numbers in the cell is strictly controlled. Control of centrosome numbers is achieved by a process of duplication in which the preexisting structure is used as a means to

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H. Schatten (ed.), *The Centrosome*, DOI: 10.1007/978-1-62703-035-9_1,

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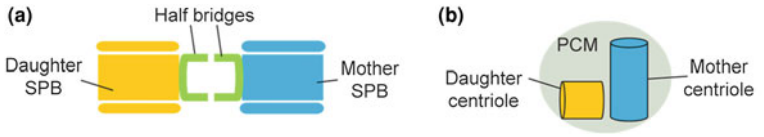

Fig. 1.1 Models for duplication **a** Yeast spindle pole bodies (SPB Duplication); the half bridge of the preexisting (mother) SPB serves as a template and nucleation site for the new (daughter) SPB that is formed in parallel. **b** Centriole Duplication; the new (daughter) centriole is formed perpendicularly to the preexisting (mother) centriole and at a significant distance from the surface of the preexisting centriole

ensure that only a single new structure is formed. Centrosomes consist of two centrioles surrounded by PCM. Centrosome duplication starts after the two preexisting centrioles separate slightly and a new centriole forms near each of the preexisting centrioles. Centrosome duplication is concluded when each centriole pair completely separates, along with some of the PCM of the original centrosome. In each centrosome, the older centriole is also known as the mother centriole and the new centriole is known as the daughter centriole.

The process of centrosome duplication is conceptually similar to how DNA and yeast SPB duplicate (Fig. 1.1). DNA duplication starts by splitting into two strands, which then serve as a template to create a new strand. Yeast SPB duplicate by splitting into two halves. Each half contains a structure known as the half bridge, which then serves to template the formation of another half bridge (Jaspersen and Winey 2004; Jones and Winey 2006). Since, like DNA and SPB duplication, centrosome duplication maintains one preexisting element and creates one new element, it is thought that centrosomes duplicate in a semi–conservative manner.

Although many of the proteins involved in centrosome duplication have been recently identified, the critical question of how the centrioles duplicate remains elusive. Very little is known about the overall mechanism of centriole duplication (Azimzadeh and Marshall 2010; Nigg and Raff 2009). It has become increasingly accepted that centriole duplication does not involve a templating mechanism like in DNA and SPB duplication.

Several lines of observation suggest that de novo formation of the new centrioles takes place at the vicinity of the preexisting centriole.

i) The new centriole is formed perpendicularly to the preexisting centriole.
ii) The new centriole forms at a distance of about 30 nm from the surface of the preexisting centriole (Anderson and Brenner 1971; Phillips 1967),
iii) The new centriole can have a very different structure from the preexisting centriole (Phillips 1967).
iv) Under certain conditions, centrioles can form in the absence of a preexisting centriole (Fulton and Dingle 1971; Rodrigues-Martins et al. 2007b)
v) Several new centrioles can be induced to form simultaneously around the preexisting centriole by overexpressing centriolar components (Kleylein-Sohn et al. 2007).

vi) No proof is available of an intrinsic asymmetry around the preexisting centriole before the onset of centriole duplication

These observations suggest that a yet unidentified mechanism inherent to the centrosome assures that only one new centriole is formed near a preexisting centriole (Sluder and Khodjakov 2010).

Analysis of centriole duplication is challenged by a combination of factors. Centrosomes are essential for development in animals. Centrosomes and centrioles are in low-abundance, found only in one or two copies per cell, thus challenging biochemical approaches. Furthermore, new centriole intermediates are few, small, short-lived, and form too close to the preexisting centriole to be observed as a distinct entity, making it extremely challenging to study centriole intermediates by traditional light microscopy (the internal structure of the centriole is beyond the resolution of standard light microscopy).

Despite these challenges, many proteins involved in centriole duplication have been identified over the past 15 years. The recent identification of many proteins involved in centriole duplication opens new ways to overcome these barriers. One commonly used way is to overexpress the centriolar protein, usually in immortalized cells (in vitro), and observe the consequences using microscopy (Dzhindzhev et al. 2010; Gopalakrishnan et al. 2010; Tang et al. 2009). While sometimes informative, interpreting overexpression data is problematic due to the fact that proteins are studied at non-physiological levels. Also, immortalized cells often have abnormal centrioles, suggesting they already carry mutations that prevent normal centriole duplication. Therefore, to balance these limitations, it is important to develop approaches to study centriole duplication in vivo, when proteins are expressed at physiological levels.

For a number of reasons, *Drosophila* is ideal for developing such a balanced approach for studying centriole duplication and centrosome biogenesis:

First: mutants defective in centrosome biogenesis are available (see Table 1.1). In flies, even null mutations in essential centrosomal proteins are not embryonic lethal and the fly can often develop to maturity. This is due to maternal contribution which allows the embryo to form centrosomes when they are critical for development, namely during early embryonic development. Later during pupal development when the adult fly is forming, maternal contribution becomes depleted but centrosomes are no longer essential for development. This allows extensive characterization of defective centrosome biogenesis in the testes and sensory neurons of pupae (Basto et al. 2006; Blachon et al. 2009; Blachon et al. 2008; Mottier-Pavie and Megraw 2009).

Second: techniques are available to introduce newly-engineered proteins with modified capabilities into a null mutant background, allowing their specific function to be studied with expression at near physiological levels and in the absence of the wild-type protein (Blachon et al. 2008; Gopalakrishnan et al. 2011). This is especially useful in the study of centrosomes, as many centrosomal proteins form multiprotein complexes and may have more than one function. The ability to engineer a mutant that is deficient in one or limited interactions is very insightful

Table 1.1 *Drosophila* genes involved in centrosome biogenesis

Name	CG ID	Ortholog	Localization in *Drosophila*	Phenotype	Additional information	References
Asl	CG2919	Cep152 (MCPH4)	Centriole PCM interphase	Uncoordinated Meiosis defect Nonmotile sperm	Complete block of centriole and centrosome formation in mutant	(Blachon et al. 2008; Bonaccorsi et al. 1998; Varmark et al. 2007)
Ana1	CG6631	KIAA1731	Centrosome	Uncoordinated Meiosis defect Nonmotile sperm	Essential for centrosome formation	(Blachon et al. 2009; Goshima et al. 2007)
Ana2	CG8262	STIL	Centrosome	Unknown	Induces cartwheel-like structures together with Sas-6	(Goshima et al. 2007; Stevens et al. 2010a)
Ana3	CG13162	Rttn	Centrosome	Uncoordinated	Required for centriole structural integrity	(Goshima et al. 2007; Stevens et al. 2009)
Sak	CG7186	Plk4	Centrosome	Uncoordinated Meiosis defect Nonmotile sperm	Essential for centrosome formation	(Bettencourt-Dias et al. 2005)
Sas-4	CG10061	CPAP (MCPH6) TCP10	Centriole and PCM	Uncoordinated Meiosis defect Nonmotile sperm	Essential for centrosome formation Short mc-giant centriole in mutants	(Basto et al. 2006; Blachon et al. 2009; Gopalakrishnan et al. in press)
Sas-6	CG15524	Sas-6	Cartwheel	Uncoordinated Meiosis defect Nonmotile sperm	Centrioles that lack symmetry in mutant; Central tubule protein	(Gopalakrishnan et al. 2010; Rodrigues-Martins et al. 2007a; Stevens et al. 2010b)
D-Plp	CG6735	Pericentrin AKAP450	Centrosome	Uncoordinated Nonmotile sperm	Essential for PCM formation	(Martinez-Campos et al. 2004)
Cnn	CG4832	CDK5RAP2 (MCPH3) Myomegalin	Centrosome, PCM	Meiosis defect Nonmotile sperm	Essential for PCM formation	(Heuer et al. 1995; Li et al. 1998; Megraw et al. 1999; Vaizel-Ohayon and Schejter 1999)

(continued)

Table 1.1 (continued)

Name	CG ID	Ortholog	Localization in *Drosophila*	Phenotype	Additional information	References
Spd-2	CG17286	Spd-2	Centrosome	Uncoordinated Meiosis defect Nonmotile sperm	PCM formation	(Dix and Raff 2007; Giansanti et al. 2008)
Poc1	CG10191	Poc1 Pix1 Pix2	Centrosome	Mail sterile	A short giant centriole in mutant	(Blachon et al. 2009)
Unc	CG1501	OFD1?	Basal body	Uncoordinated Nonmotile sperm	Mutants result in a short giant centriole	(Baker et al. 2004)
Bld10	CG17081	Cep135	Centriole wall	Nonmotile sperm	Mutants result in a Short giant centriole	(Blachon et al. 2009; Mottier-Pavie and Megraw 2009)
CP190	CG6384	Not found	Centrosome	Pupal lethal	Nuclear function	(Butcher et al. 2004)
CP60	CG6384	Not found	Centrosome	Unknown	Forms a complex with CP190	(Kellogg et al. 1995)

in identifying the separate functions mediated by a centrosomal protein (Gopalakrishnan et al. 2011).

Third: it is possible to biochemically isolate centrosomes and centrosomal complexes from *Drosophila* embryos to study them ex vivo (Gopalakrishnan et al. 2010; Gopalakrishnan et al. 2011; Kellogg and Alberts 1992; Moritz et al. 1995). This allows one to study protein interactions under near physiological conditions. This also opens a window to use purified centrosomal proteins, structures, and complexes in cell-free experiments that can investigate the individual steps in centrosome duplication. Ultimately, this can theoretically allow centrosome duplication and function to be reconstituted using purified components.

Finally: *Drosophila* centrosomes are formed using conserved proteins and the overall structure of *Drosophila* centrosomes is very similar to that of other organisms, suggesting that the basic mechanisms of centrosome duplication used in *Drosophila* are similar to those used in other organisms.

1.2 Centrosomes in *Drosophila* Development

Centrosomes in *Drosophila* were studied in some detail in a context of several developmental processes. In this chapter, we will focus on four processes. The first two processes take place during early embryonic development: (1) Fertilization, and (2) Syncytial blastoderm formation. The next two processes occur in differentiated cell types and can be studied during pupal development: (3) Sensory neuron differentiation, and (4) Spermatogenesis. We will summarize key features of the centrosome in each of these developmental processes, highlighting unique properties that have provided insight into the biology of the centrosome.

1.2.1 Fertilization

Fertilization is the process by which the sperm (male gamete) and oocyte (female gamete) are fused to form a zygote, the first cell of a new organism. In general, a key step in fertilization is the migration of sperm and oocyte pronuclei toward each other and their subsequent fusion (Fig. 1.2).

It is generally recognized that in most animals, including *Drosophila,* the oocyte does not contain centrioles (Krioutchkova and Onishchenko 1999; Manandhar et al. 2005; Sun and Schatten 2007). Instead, oocytes have acentriolar centrosomes or microtubule organization centers that participate in female meiosis and in the formation of the female pronucleus (Megraw and Kaufman 2000). While the oocyte does not appear to have centrioles, it does contain a large amount of centriolar and PCM proteins within its cytoplasm, enough to form 2^{13} centrosomes (Rodrigues-Martins et al. 2007b). These proteins, contributed by the mother via the oocyte (maternal contribution), are sufficient to support centrosome

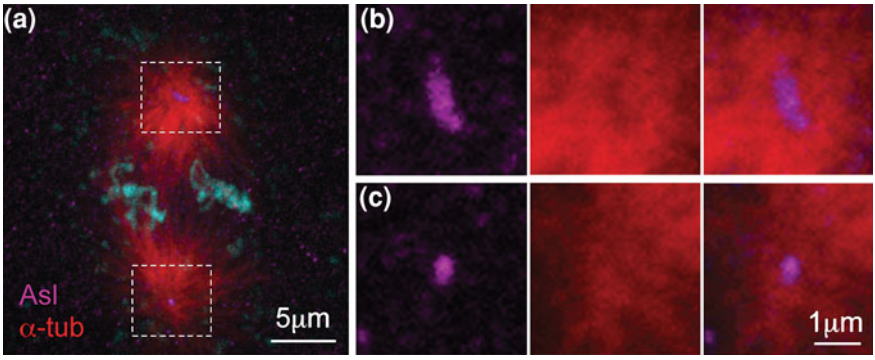

Fig. 1.2 Fertilization during mitosis of the zygote (**a**), the giant centriole (**b**) and a second smaller centriolar structure (**c**) are observed at the two poles. Note that the two pronuclei (*blue*) are not mixed and they divide separately in parallel. A low magnification image with small inset squares outlines the approximate positions of the centriolar structures, which are shown under a higher magnification in (**b** and **c**). Embryos were stained with rat anti-α-tubulin (*red*), and anti–N-ter-Asl (*purple*); 4'-6-diamidino-2-phenylindole (DAPI) stains DNA

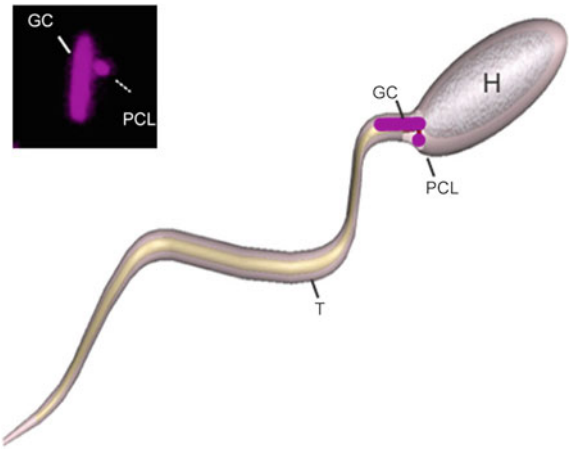

Fig. 1.3 Spermatid with a giant centriole (GC) and a PCL. *Left*, giant centriole and PCL labeled by Ana-1-GFP (*purple*). *Right*, cartoon depicting the relative location of the sperm centriolar structures relative to the sperm head (H) and tail (T). (The panel on the *Left* is modified from Fig. 1.2b in (Blachon et al. 2009)

duplication in early embryonic development, a time when the embryonic genome is not yet fully involved in producing the proteins necessary for development.

On the other hand, the *Drosophila* sperm contains two centriolar structures. The first, termed the "giant centriole" (due to its exceptional length) resembles the distal centriole found in vertebrate sperm and functions to nucleate the sperm flagellum (Friedlander and Wahrman 1966; Fuller 1993; Krioutchkova and Onishchenko 1999; Manandhar et al. 2005; Sun and Schatten 2007). A second centriolar structure associates with the giant centriole and is termed the proximal centriole-like (PCL) structure due to the fact that, like the vertebrate sperm proximal centriole, it does not form a flagellum (Blachon et al. 2009) (Fig. 1.3).

Upon fusion of the sperm and oocyte, the sperm giant centriole recruits PCM proteins from the surrounding cytoplasm and forms a centrosome. The zygote centrosome acts as a microtubule organization center and assembles an aster—a star-like structure consisting of microtubules. These asters are thought to play a role in bringing together the female and male pronuclei and in orchestrating the first cell division (Callaini and Riparbelli 1996). In support for the critical role of centrosomes in zygote biology, it has been reported that interfering with centrosome biogenesis after fertilization inhibits zygote development (Dix and Raff 2007; Stevens et al. 2007; Varmark et al. 2007).

The role of the PCL after fertilization is currently not known; however, one attractive hypothesis is that the PCL later becomes the second centrosome of the zygote. The observation that two centrosomes can be observed after fertilization in mutant oocytes under conditions that block centrosome duplication supports this hypothesis (Stevens et al. 2007).

1.2.2 Syncytial Development

Drosophila early embryonic development takes place in a syncytial blastoderm, a large cell containing many nuclei. In this developmental stage, the embryo undergoes 13 rounds of nuclear duplication and division without forming individual cells surrounded by a plasma membrane; each of these rounds is called a "nuclear cycle". For simplicity, we will refer to each dividing unit that includes a nucleus and its associated centrosomes as a "cell". At the end of syncytial blastoderm development, the nuclei are partitioned into separate cells where they are surrounded by a plasma membrane (cellular blastoderm stage). This partitioning, termed cellularization, is mediated by actin, which forms a cleavage furrow (the structure that mediates the separation of daughter cells).

The syncytial blastoderm is an excellent system where one can do live imaging of centriole duplication in real time (Fig. 1.4). In the embryonic syncytium, the nuclear cycle is very rapid ($\sim$10 min) and many centrioles duplicate synchronously. The nuclear cycle is comprised of two phases: synthesis and mitosis. Early in synthesis phase, each cell has two centrosomes, each containing one centriole surrounded by PCM (Callaini and Marchini 1989; Callaini and Riparbelli 1990, 1996; Riparbelli et al. 1997). During synthesis phase, the centriole within each of the two centrosomes duplicates, generating a new centriole at the vicinity of the older centriole. At the onset of mitosis, the centrosomes move to opposite poles such that each future daughter "cell" will inherit one of the of the mother "cell" centrosomes. During late mitosis, each of the centrosomes that are associated with one of the nuclei splits into two centrosomes. As a result, each of the daughter "cells" inherently contains two centrosomes, each with one of the centrioles from the original centrosome (Fig. 1.5).

The centriole in the *Drosophila* syncytial blastoderm has a very intriguing structure. Unlike classic centrioles, which are made of nine triplets of

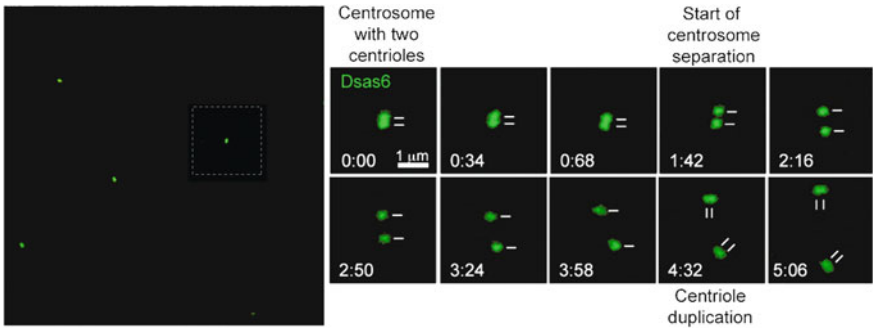

Fig. 1.4 Live imaging of centriole duplication in the early development of an embryo expressing the centriolar marker Sas-6-GFP. The start of centriole separation marks the splitting of the centrosome. The *left* panel shows low magnification of the centrioles, organized in the *right* panels by time. The white square in the panel on the *right* corresponds to the centrioles displayed in the *left* panels. A line distinguishes each centriole. S. B produced this picture

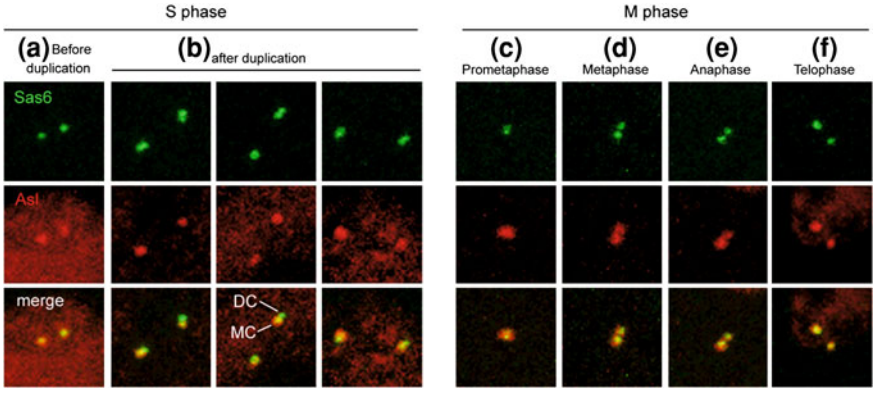

Fig. 1.5 Centriole duplication and centrosome separation in the syncytial blastoderm. The embryo expressed the centriolar marker Sas-6-GFP (*green*) and was stained with an anti-Asl antibody; Asl is a component of the PCM that is found near the centriole (*red*). **a** In early S phase, each of the two centrosomes contain the PCM protein Asl and have a centriole labeled by Sas-6-GFP. **b** During S phase each of the centrioles duplicates to form a daughter centriole (Dc). The daughter centriole is marked with Sas6-GFP but not Asl. **c–f** Only one of the centriole pairs is shown. The mother and daughter centrioles start to separate and the daughter centriole accumulates Asl, finally leading to the formation of two centrosomes. Note that anti-Asl antibody also lightly labeled the nucleus. S. B produced this picture

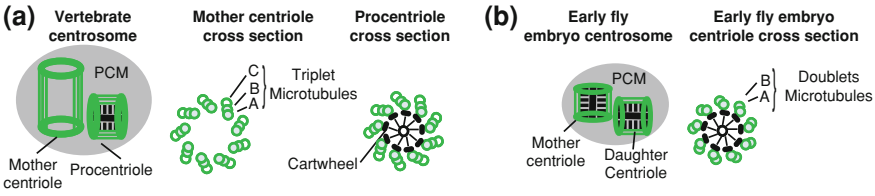

Fig. 1.6 The fly early embryonic centriole resembles a procentriole. (The fig is modified from Fig. 1 in (Avidor-Reiss 2010)

microtubules, centrioles in the syncytial blastoderm are made of nine doublets of microtubules. In addition, syncytial blastoderm centrioles are shorter than classic centrioles: ~200 nm long instead of 400–500 nm long observed in vertebrates. Finally, the centrioles of the syncytial blastoderm have a structure known as the "cartwheel" within their core, which in vertebrate cells is characteristic of a young, developing centriole (procentriole) and is absent from mature centrioles. This raises the hypothesis that syncytial blastoderm centrioles are centriolar structures arrested in the procentriole stage. It is possible that since the syncytial blastoderm nuclei divide rapidly and there is no need for cilia formation (see below), the centrioles do not have the time, nor the need, to develop into their mature states (Fig. 1.6).

1.2.3 The Drosophila Zygote and Syncytial Blastoderm Develop Using Proteins Generated in the Mother

Protein deposition in the oocyte that supports early embryonic development is called maternal contribution. An important implication of the presence of maternal contribution in *Drosophila* development is that, despite the fact that an embryo may be genetically homozygous for a mutation in an essential centrosomal gene, it will still contain the wild-type protein, allowing it to produce normal centrosomes as long as the maternal contribution persists. As a result, studying those mutants cannot reveal the role of centrosomes in early embryogenesis. Indeed, studies using homozygous mutants for an essential centrosomal gene, have demonstrated that the fly embryo can develop normally (Basto et al. 2006; Blachon et al. 2008; Rodrigues-Martins et al. 2007a).

Investigating the role of centrosomes in early embryogenesis requires the study of an embryo that is produced from an oocyte generated in an environment that is also mutated. Since flies with mutations in essential centrosomal proteins are unable to walk, mate, or lay eggs (see below), it is not possible to use embryos produced by homozygous females. However, this obstacle can be overcome using other approaches.

One way is to study centrosomal proteins that are essential for aspects of centrosome function but are not necessary to produce a fertile female. For example, mutations in centrosomin (Cnn) result in flies that are viable but female sterile (Megraw et al. 1999; Vaizel-Ohayon and Schejter 1999). Cnn is a PCM protein that plays an important role in PCM formation and is required for the centrosome's activity as a microtubule organization center (Li and Kaufman 1996). Studies of *cnn* mutants in early embryogenesis reveal an impairment of several aspects of embryo development that depend on the function of the cytoskeleton (Megraw et al. 1999; Vaizel-Ohayon and Schejter 1999). In particular, it appears that Cnn is essential for the organization of actin into cleavage furrows. New information suggests that the centrosome functions as a site where Cnn

interacts with Centrocortin, a protein that is required for cleavage furrow formation and is localized both to centrosomes and to cleavage furrows (Kao and Megraw 2009). It is therefore possible that the centrosome functions as a signaling hub within the cell. At this signaling hub, proteins can interact in order to integrate information and later move to other domains in the cell where they execute their function (Alieva and Uzbekov 2008; Wang et al. 2009).

Another way by which to study centrosomes in early embryogenesis is to study hypomorphic mutations of centrosomal proteins that are essential for centrosome formation and produce a female that can mate. In this case, the studied protein is partially functional and missing an activity that is essential specifically for centrosome function during embryogenesis. An example of one such mutation is asl^{1}, in which the C-terminus of the essential centrosomal protein Asterless (Asl) is truncated (Blachon et al. 2008). While flies homozygous for the severe loss-of-function allele asl^{mecD} are unable to walk and mate, asl^{1} generates viable females that can lay eggs. Analysis of embryonic centrosomes generated from an asl^{1} female finds that they initially form asters, but these asters are not stable and later fall apart ((Varmark et al. 2007) and S. B. unpublished data). In addition, pronuclei fusion does not take place and embryo development is arrested at the zygote stage. Similar results were obtained when a hypomorphic mutation of the *Drosophila spd*-2 gene was studied, while severe loss-of-function *spd-2* mutants are unable to walk and mate (Dix and Raff 2007; Giansanti et al. 2008).

One can also study centrosomal proteins that are essential for centrosome formation and produce a female that can mate using genetic tricks. One way to do so is to make germline clones that lack any particular essential centrosomal protein from maternal contribution (Stevens et al. 2007). This is done using the dominant female sterile (DFS) technique (Chou and Perrimon 1996), a variant of FLP-FRT recombination. In this case, recombination is induced in larvae via a heat shock-inducible flippase (FLP); as adults, the fly produces homozygous mutant oocytes that lack the essential centrosomal protein. Study of oocytes that are mutant for *sas*-4 or *sas*-6 after they are fertilized with a wild-type male finds that they possess two centriolar structures (presumably the giant centriole and PCL) (Stevens et al. 2007). These centriolar structures can nucleate centrosomes and can undergo few nuclear cycles before embryogenesis is arrested presumably because the centrosomes cannot duplicate. This suggests that the PCL can form an independent centrosome and demonstrates that centrosomes are essential for syncytial blastoderm development (after their role in zygote pronuclei fusion).

An alternative method to study centrosomes in the syncytial blastoderm is to inject it with an antibody for a particular centrosomal protein and observe the consequence (Conduit et al. 2010). A potential problem with this approach is that when a centrosomal protein forms a complex, binding of an antibody may not only inactivate its intended protein target, but may also inactivate other proteins found in the complex.

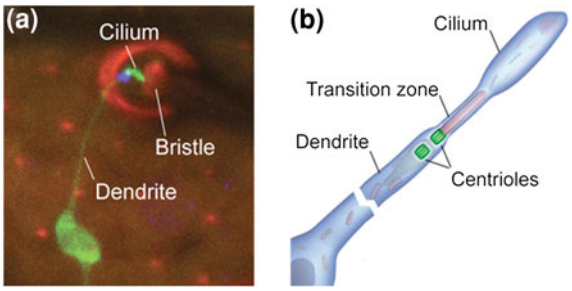

Fig. 1.7 Drosophila mechanosensory neuron morphology. **a** The neuron cell body is filled with tubulin-GFP. The dendrite is lightly labeled by tubulin-GFP. At the dendrite tip, the cilium is strongly labeled by tubulin-GFP. Red labels cuticle structures, including the bristle. Blue labels the Transition zone vicinity. **b** Diagram of sensory cell dendrite and cilium. (The a panel is modified from Fig. 7c in (Avidor-Reiss et al. 2004)

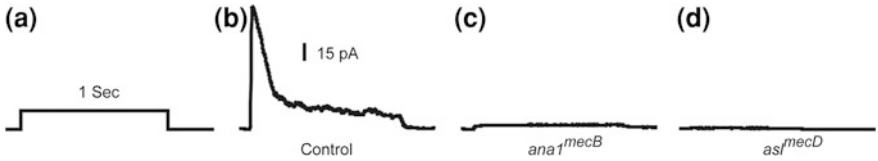

Fig. 1.8 *Drosophila* mutants with centriolar or cilia defects are mechanosensory defective. Displacing a bristle 30 um for one second (**a**) generates a mechanoreceptor current in control flies that adapts over the course of the stimulus (**b**). In contrast, mutations that affect centriole formation (**c** and **d**) and thus cannot form mechanosensory cilia have no mechanoreceptor current. (The a and b panels are modified from Fig. 1.4c in (Avidor-Reiss et al. 2004)

1.3 Centrosomes in Differentiated Cells

1.3.1 Sensory neuron differentiation

In *Drosophila,* the first cells that develop cilia are the type I sensory neurons (Fig. 1.7). These neurons mediate the reception of mechano- and chemo-sensory information and are found in both larvae and the adult fly. One subtype of these sensory neurons is found on the cuticle of the adult fly and mediate touch sensation (Fig. 1.7) (Keil 1997). These neurons are bipolar sensory neurons that extend a dendrite with sensory cilia at their tip in one direction. The sensory cilia are attached to a cutaneous structure termed the bristle. When the bristle moves due to mechanical stimuli, the mechanosensory transduction machinery found in the cilia is activated and a mechanoreceptor current is generated (Fig. 1.8). This current produces an action potential that is delivered to the brain, transmitting information regarding touch sensation or proprioception (Avidor-Reiss et al. 2004; Kernan et al. 1994; Walker et al. 2000).

The sensory neuron is a product of asymmetrical cell division and it inherits a centrosome with two centrioles (Gomes et al. 2009; Keil 1997; Seidl 1991). After the sensory neuron generates a long dendrite, the two centrioles are reorganized and are found in tandem, one of which is attached to a vesicle. This reorganized structure appears to migrate along the dendrite until it reaches the distal end, where the associated vesicle fuses with the plasma membrane to form the sensory cilium (Seidl 1991). The sensory cilium is composed of a transition zone, also called the connecting cilium, and the sensory cilia proper, also called the outer segment (Fig. 1.7b).

1.3.2 Spermatogenesis

Spermatogenesis is the process that takes place in the testes to form mature male gametes and begins when a sperm stem cell divides asymmetrically to form another stem cell and a progenitor spermatogonium. The spermatogonium divides 4 times to form 16 spermatocytes. These spermatocytes grow to ~30 times their original size and ultimately undergo two cycles of meiosis to generate 64 spermatids. The spermatids, which are first round, undergo a dramatic differentiation program, called spermiogenesis. The completion of this differentiation program results in the formation of a sperm cell that is ~2 mm long, a length comparable to that of the fly itself.

Centrosomes in the *Drosophila* testes have several interesting properties:

First, unlike the syncytial blastoderm, the centrosome and centrioles of the adult testes are similar to their vertebrate counterparts (Tates 1971; Tokuyasu 1975). These centrioles have nine triplet microtubules (Fig. 1.9k). This normal centriolar structure correlates with the ability of the spermatogenic centrioles to form cilia and suggests that in ciliated cells, the centriole needs to develop into its mature state. In this regard, sensory neurons that have cilia also contain centrioles with triplet microtubules (Keil 1997).

Second, in spermatogenesis, the centrosomes form two types of cilia. During spermatocyte growth, each of the spermatocyte's four centrioles forms a primary cilium-like structure of unknown function (Fig. 1.9b) (Tates 1971). Later in spermiogenesis, each of these primary cilia is modified to form a motile cilium—the sperm flagellum.

Third, male meiosis is absolutely dependent on centrosomes. Flies that do not have functional centrosomes fail to accurately separate genetic material and mitochondria (Fig. 1.10), one of the reasons why centrosomal mutants are male sterile. It is currently unclear why centrosomal defects cause abnormalities in male meiosis but do not disrupt mitosis. However, it is possible that male-specific meiotic defects are due to the fact that the centrosomes are associated with a ciliary-like structure that requires centrosomal components for their formation.

Fourth, during spermatocyte growth and spermiogenesis, the centriole elongates to ~2.5 um, much larger than centrioles found in any other *Drosophila* tissue or

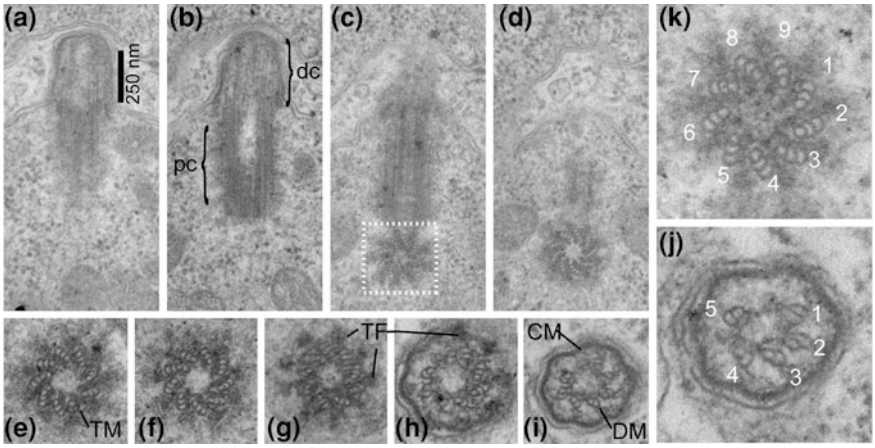

Fig. 1.9 The giant centriole (proximal centriole) and primary cilium (distal centriole) of fly spermatocytes: A-J serial section electron microscopy analysis of a pair of giant centrioles organized in an orthogonal relationship. **k** The cross-section of the daughter centriole from **c** is magnified to demonstrate triplet microtubules. **j** The last cross-section is highlighted to depict the presence of irregular numbers of doublet microtubules in the primary cilium. TF, transitional fibers connecting the centriole to the plasma membrane; TM, triplet microtubules; DM, doublet microtubules; CM, cilium membrane; pc and dc according to Tates: pc, proximal centriole or basal body and dc, distal centriole

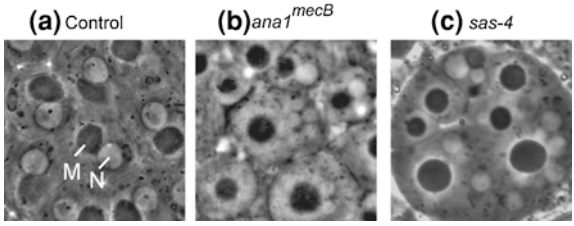

Fig. 1.10 Round spermatids formed imminently after meiosis, contain a white nucleus (N) and dark mitochondria (M) of similar size (**a**). Centriolar mutants in the spermatid state form nuclei and mitochondria of variable size (**b** and **c**)

those of other organisms (Blachon et al. 2009; Mottier-Pavie and Megraw 2009; Tates 1971). At particular stage of spermiogenesis these giant centrioles are surrounded by a long and thick PCM (also referred to as the "centriolar adjunct"). These centrioles provide a very convenient model to study centriole elongation and several mutants that have shortened giant centrosomes have been described. Proteins essential for giant centriole elongation include: Bld10, Poc1, and Sas-4 (Blachon et al. 2009; Mottier-Pavie and Megraw 2009).

Fifth, during spermiogenesis, the spermatid cell forms a centriole precursor-like structure called the PCL (Fig. 1.11a). The PCL has been proposed to be a centriole intermediate that arrests at the stage before the centriolar microtubules are assembled. Therefore, by studying how the PCL forms, one can study the early

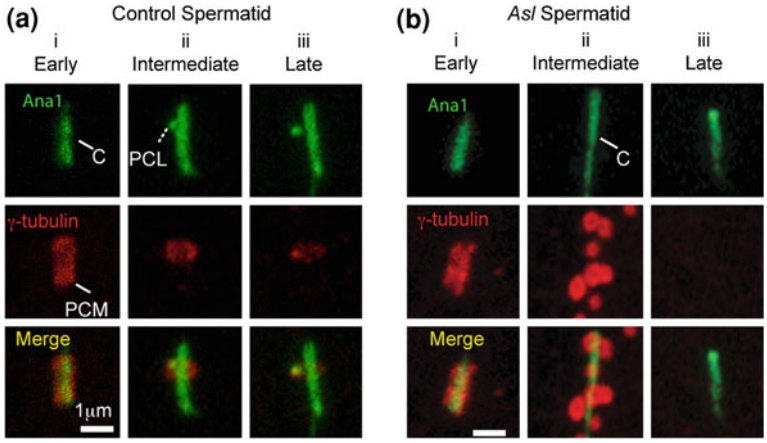

Fig. 1.11 Asl is essential for PCL formation. **a** To determine the relationship between the PCL, the giant centriole (**c**), and the PCM, the localization of Ana1-GFP relative to the centriolar adjunct protein γ-tubulin (Wilson et al. 1997) was analyzed. In early spermatids, γ-tubulin labels the vicinity of the giant centriole along most of its length (i). During the time the Ana1 labelled PCL appears, γ-tubulin assembles a half ring structure around the giant centriole, which touches the PCL (ii). At a later stage, the PCL migrates distally and γ-tubulin labels the PCL. **b** To determine if Asl plays a role in PCL formation we followed maternally contributed giant centrioles in spermatids of the asl^{mecD} mutant. No PCL is observed near the giant centriole, demonstrating an essential role for Asl in PCL formation. However, while in early asl^{mecD} spermatids γ-tubulin localization appears to be normal, many abnormal γ-tubulin rings are found at the vicinity of the maternally contributed giant centrioles in intermediate asl^{mecD} spermatids. (The **a** panel is modified from Fig. 1.2c in (Blachon et al. 2009)

events in centriole biogenesis (Blachon et al. 2009). PCL formation depends on the function of the centrosomal proteins Plk4, Sas-6, and Asl and it contains the following centrosomal proteins: Sas-6, Ana2, Ana1, Sas-4, Bld10, Cnn, and Asl (Blachon et al. 2009; Mottier-Pavie and Megraw 2009; Stevens et al. 2010b) and (Fig. 1.11b).

1.3.3 The Drosophila Adult Sensory Neuron and Testes Develop Using Proteins Generated During Metamorphosis

Unlike early embryogenesis that utilizes maternally contributed proteins, the adult fly develops during metamorphosis by utilizing proteins synthesized from the genome of the fly itself. Therefore, by studying sensory neurons and testes in the pupa or adult, one can study the full impact of a mutation in a centrosomal protein. Interestingly, flies that have mutations in essential centrosomal proteins and have no centrosomes can develop to adulthood but die soon after leaving the pupa

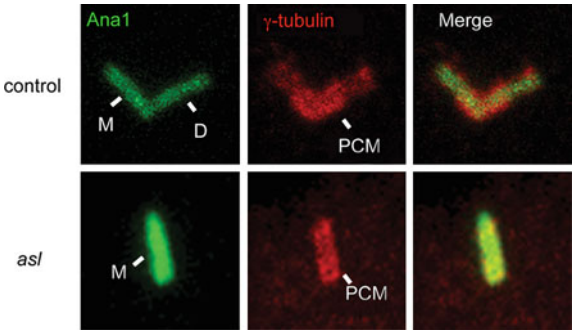

Fig. 1.12 Asl is essential for centriole duplication in vivo. Wild-type cells (control) contain both a mother centriole (M) and its daughter centriole (D). Centrioles are labeled by Ana1-GFP (green); PCM assembled around these centrioles are labeled for γ-tubulin (*red*). In *asl* loss-of-function mutant cells, the maternally contributed centrioles elongate but its duplication is blocked. Modified from (Blachon et al. 2008). (The fig is modified from Fig. 1.5d in (Blachon et al. 2008)

because they cannot stand on their legs, walk, or fly [defects collectively referred to as uncoordination (Kernan et al. 1994)]. This uncoordination results from the fact that flies with centrosomal defects have no mechanosensory cilia and cannot sense the environment or their body parts.

The germline stem cells found in the testes originate from the first group of cells that are generated early in embryonic development (pole cells) (Okada 1998). When germline stem cells divide to form a new stem cell and a spermatogonium, the older centriole (the maternally contributed centriole) stays in the stem cells while the newer centriole is inherited by the spermatogonium (Blachon et al. 2008; Yamashita et al. 2007). A fly that is homozygous mutant for an essential centriole component and cannot form new centrioles will have functional centrioles in the germline stem cells that are made using maternal contribution, but will lack these centrioles in the later progenitors.

Since germline stem cells in the developing *Drosophila* testes have two maternally contributed centrioles, some of the first spermatogonium to form can each inherit one maternally contributed centriole. These centrioles then duplicate and elongate during spermatogenesis and end up in the first spermatids to form. By that time maternal contribution of wild-type proteins becomes depleted. Following maternally contributed centrioles of the spermatogonium and later in spermatogenesis allows one to dissect the role of a particular protein under circumstances where a centriole is present (Fig. 1.12). Using this approach, it was found that maternally contributed centrioles require Sas-4 to elongate but not Asl (Blachon et al. 2009; Blachon et al. 2008). On the other hand, formation of the PCL can form in Sas-4 mutants but not in Asl mutants (Blachon et al. 2009) and Fig. (1.11b).