Part I
Microtubule Organization and Dynamics
1 Early Studies on Centrioles and Centrosomes

Joseph G. Gall

1.1 Introduction

From its discovery in 1876 through the early decades of the 20th century, the centriole occupied the attention of many investigators, who showed that it played a key role in organizing fibrillar structures in the cell, including the spindle and asters during mitosis, cytoplasmic filaments in interphase cells, and cilia and flagella in everything from the tracheal epithelium of mammals to the flagellated sperms of lower plants. In a broad sense, the function of the centriole as an organizer or controller of fibrillar structures was established at the outset, long before any hint of its molecular composition or even its fine structure was known. Centriole replication also attracted attention from the very beginning. Because of its regular doubling during the cell cycle in animals, the centriole was first thought to be an autonomous, self-replicating structure with properties similar to those of chromosomes. As evidence against this view began to accumulate, interest in centrioles waned, reaching a low point in the middle of the 20th century. A resurgence of interest accompanied the introduction of electron microscopy into cell biology in the 1950s, which at last revealed the fine structure of the centriole and established even more clearly its relationship to cilia, flagella, and other microtubule-based structures. But little progress was made on the problem of centriole replication, and still nothing was known about its molecular composition. The modern era, encompassing roughly the last 20 years, began with the introduction of specific immunofluorescent staining, and has picked up ever-increasing speed with improvements in microscopy, especially confocal microscopy and the use of GFP-tagged proteins in living cells. Once again, attention is focused on the replication problem, this time backed by the full force of modern molecular and microscopical techniques. In this introductory chapter I will review the history of centriole research during the past 125 years, concentrating heavily on the problem of centriole replication, which has dominated so much of the thinking about this fascinating cell organelle.
1.2 Pioneering Studies

In 1876 Édouard Van Beneden, then a 30-year-old Professor of Zoology at Liège, provided the first tentative glimpse of the centrosome [1]. His paper was not about centrosomes or even about mitosis, which had not yet been accurately described. Instead, it concerned the anatomy and development of an obscure group of parasites, the Dicyemidae, which live in the kidneys of squids and octopuses. Van Beneden noticed that something unusual happened to the nucleus during the first few cleavages of the egg. He made drawings of what he saw, the details of which are still difficult to interpret. Nevertheless, at the poles of what we can now recognize as the mitotic spindle he drew a small dot or circle, and labeled it the polar corpuscle (Figure 1.1). Seven years later, while examining fertilization in eggs of the nematode *Ascaris megaloccephala* (now called *Parascaris equorum*), he published much more accurate and detailed observations on centrosomes [2]. But again his major interest lay elsewhere, for in this momentous 375-page mono-

![Figure 1.1](image) Van Beneden's drawings of mitosis during the first cleavage division of the mesozoan *Dicymella*. At the poles of the spindle Van Beneden drew a small dot or circle, which he called the polar corpuscle. Van Beneden’s original paper was published in the *Bulletin of the Royal Belgian Academy* [1]. The figures reproduced here were taken from a reprinting of Van Beneden’s plate that appeared in 1877 in the *Quarterly Journal of Microscopical Science*, vol 17 (new series), plate 10. To save space I have rearranged the images so that Figures 10 and 11 are next to Figures 12–15.

---

1 It was Boveri, not Van Beneden, who introduced the terms centrosome [4] and centriole [5]. As originally conceived, *centriole* referred to the tiny granule at the very center of the astral configuration, whereas *centrosome* included the larger area of differentiated cytoplasm around it – the pericentriolar material of recent authors. From the beginning, however, the terms were used interchangeably, and only from the context can one be sure what any particular author was referring to.
graph Van Beneden concentrated on the behavior of the chromosomes during meiosis and fertilization, laying the foundation for the chromosome theory of inheritance. In 1887 he published a second and much shorter paper on *Ascaris* in which he dealt with fertilization and the first two cleavage divisions [3]. Here at last he paid careful attention to the centrosomes, showing their now-familiar association with the poles of the mitotic spindle and, even more remarkably, their duplication at telophase and separation during early interphase (Figure 1.2). He speculated that centrosomes, which by then he called central corpuscles, were permanent self-replicating cell organelles that acted as the insertion site for the astral rays.

While his second *Ascaris* paper was being readied for publication, Van Beneden learned that Theodor Boveri at the Zoological Institute in Munich had independently submitted a manuscript on the same subject. Boveri was soon to become one of the dominant cell biologists of his time, but in 1887 he was a young postdoctoral fellow just beginning the studies on *Ascaris* that brought him worldwide acclaim. In the short paper he published in 1887 [6] and in a longer monograph that appeared in 1888 [4], Boveri came to two major conclusions about centrosomes. First, in agreement with Van Beneden, he suggested that the centrosome is a permanent cell organelle endowed with the property of self-replication. Second, he postulated that a major function of the sperm during fertilization was to supply a functional centrosome to the egg – the egg having lost its centrosome during the divisions leading to formation of the polar bodies. Boveri's theory of fertilization provided a backdrop for later studies by other investigators, but was gradually abandoned as the complexities of fertilization became apparent over the next 10–20 years (see the discussion in [7]). On the other hand, the idea that the centrosome is a permanent self-replicating organelle became widely accepted. Indeed, this hypothesis was so attractive on theoretical grounds and so cogently argued by its

![Figure 1.2](image_url)

**Figure 1.2** Van Beneden’s drawings of the first cleavage division in the egg of the nematode worm *Ascaris megaloecephala* (*now Parascaris equorum*). Van Beneden clearly depicted doubling of the centriole at anaphase and separation of the two resulting centrioles during early interphase. Presumably, doubling actually occurs prior to metaphase, but Van Beneden could not resolve individual centrioles until anaphase. These figures were taken from Van Beneden and Neyt [3], rearranged to bring Figure 4 next to Figures 2 and 3.
supporters that it persisted until quite recently, in the face of much contrary evidence.

Boveri's drawings of centrioles, chromosomes, and other features of the *Ascaris* egg are among the most elegant products of late 19th and early 20th century cytology. Fortunately, a number of Boveri's original microscope slides are still in existence, permitting us to examine the very material with which he worked (Figure 1.3).

**Figure 1.3** Photographs taken by the author from one of Boveri's own slides of *Ascaris* eggs. This slide, which was probably made before 1910, was almost certainly stained with Heidenhain's iron hematoxylin method. (A) Metaphase of the first cleavage division in *Ascaris megaloccephala* (now *Parascaris equorum*). Compare with Van Beneden’s Figure 2 (Figure 1.2). (B) End of the first cleavage division. Two centrioles lie next to one of the two nuclei formed by the first mitosis. Compare with Van Beneden’s Figure 4 (Figure 1.2).
1.3

Self-replication versus De Novo Formation

In 1925 E. B. Wilson began his discussion of centrioles and centrosomes [7] with the comment, “...we must admit that there is a certain presumption in favor of the conclusion of Van Beneden, Boveri and their followers that the division-center (centriole) may be regarded as a permanent and autonomous cell-organ that arises only by the division of a preexisting body of the same kind”. He went on to marshal the evidence for and against this model, but in the end he was swayed by those cases in which centrioles appear to arise de novo, and therefore cannot be permanent and autonomous. Wilson based his argument heavily on the formation of the so-called cytasters that appear in the cytoplasm when the eggs of various marine invertebrates are induced to develop parthenogenetically. These cytasters often contain distinct centrioles, which, of course, cannot have been derived from a sperm centriole. However, it could be imagined that the egg contains an undetected centriole that undergoes rapid division during induction of the cytasters. Wilson argued against this possibility, but it was ultimately the inability to rigorously exclude “undetected” or “invisible” centrioles that plagued all discussions about their possible de novo origin.

From an early date it was recognized that mitosis in somatic cells of mosses, ferns, and seed plants differs from the typical condition in animal cells. Specifically, centrioles and asters are missing from the poles of the spindle, which itself has a more rounded configuration than that in animals. It thus became clear that, even if centrioles were self-replicating and permanent organelles in some animals, they were not even present in most plants. However, the situation in plants was complicated by the existence of giant centrosome-like structures (blepharoplasts) at the poles of the spindle during the last divisions of the male gametophyte in mosses, ferns, cycads, and even Ginkgo. I will return to these interesting cases shortly, but first I will discuss the relationship between centrioles and the basal bodies of cilia and flagella.

1.4

Centrioles and Basal Bodies

Sperm formation was a favorite topic of investigation during the early years of the 20th century. All of the cell organelles – nucleus, mitochondria, Golgi apparatus, and centrioles – undergo striking changes during the transformation of the round spermatid into the elongated sperm with a single flagellum. It was relatively easy to follow the fate of the centriole from the end of the second meiotic division right through to the mature sperm, and to see that the centriole forms the basal body of the flagellum. Meves [8] even described a remarkable case in the moth Pygaera in which flagella grow out from the centrioles while they are still situated at the poles of the first meiotic spindle (Figure 1.4). This case showed conclusively that one and the same structure could function simultaneously as the basal body of
a flagellum and as the pole of a spindle. At about the same time Henneguy [9] and Lenhossék [10] proposed that the basal bodies of cilia might also be identical to centrioles. They reached this conclusion from a study of ciliated epithelial cells, which apparently lacked the two centrioles that normally reside next to the nucleus during interphase. They suggested that these two centrioles migrate to the cell surface, where they multiply to form a cluster of centrioles. These multiple centrioles then line up at the surface of the cell and function as the basal bodies for the cilia.

Because centrioles and basal bodies were structures near the limit of resolution of the light microscope, lingering doubts remained about their structural identity. These doubts were completely overcome by electron microscopic observations made in the 1950s, which showed that basal bodies and centrioles are identical to each other in fine structure, and both are related to the axoneme of cilia and flagella [11–16]. The axoneme consists of nine doublet microtubules surrounding two central microtubules, whereas basal bodies and centrioles have nine triplets in their walls but lack the central microtubules.

Figure 1.4 Metaphase of the first meiotic division in a primary spermatocyte of the moth *Pygaera*. Note that the centrioles serve simultaneously as the poles of the meiotic spindle and as basal bodies for the precociously formed flagella. Drawing created by Friedrich Meves [8].
1.5 Blepharoplasts

Some of the most striking cases of de novo centriole formation take place during spermiogenesis in lower plants, including mosses, ferns, fern allies, cycads and Ginkgo. All of these plants have flagellated sperms, sometimes with as few as one or two flagella but in many cases with 100 or more (Figure 1.5). The champion in this respect is the cycad sperm – a huge sphere some 250–300 μm in diameter – with up to 25000 flagella arranged along a spiral band. Each flagellum has a basal body, and the question is: where do these basal bodies come from? Light microscopic studies undertaken in the late 19th and early 20th century showed that they arise from the breakdown of a spherical structure, the blepharoplast, which resides in the cytoplasm of the spermatid [17–19] (Figure 1.5). The size of the blepharoplast varies from species to species more or less in proportion to the number of basal bodies that it will produce. Thus the blepharoplast of the cycads is a gigantic structure up to 25 μm in diameter. During the last gametophyte mitosis (which gives rise to the spermatids), two blepharoplasts occupy the poles of the mitotic spindle. These two blepharoplasts are produced by still smaller structures that can be found in the cytoplasm during the preceding interphase. However, most cells of the gametophyte completely lack anything that looks like a blepharoplast or centriole, and so the origin of the blepharoplast itself remains a mystery.

Figure 1.5 Stages in the formation of the flagellated sperm of the horsetail Equisetum. A small blepharoplast arises de novo in the cytoplasm during the interphase preceding the last gametophyte mitosis. This blepharoplast divides into two blepharoplasts that occupy the poles of last mitotic spindle. In the spermatid the blepharoplast breaks up into a row of centrioles which line up at the cell surface and create the multiple flagella of the sperm. The drawings are by Lester W. Sharp [18]. Sharp’s original figures were rearranged to give the sequence shown here.
Reproduced from L. Sharp, Introduction to Cytology, 1934, Figure 122, page 205. ©McGraw-Hill.
Figure 1.6  Blepharoplasts in the water fern *Marsilea*. (A) Electron micrograph of one of the two blepharoplasts in the cytoplasm of a haploid gametophyte cell. It consists of somewhat irregular tubules that display nine-fold symmetry in cross-section. One blepharoplast will occupy each of the poles of the last mitotic division (see Figure 1.5). It will then transform into a cluster of procentrioles, which elongate and function as the basal bodies for the multiple flagella of the motile sperm. (B) A diagram showing stages in the formation of basal bodies from the blepharoplast. The blepharoplast begins as a spherical mass of somewhat irregular tubules (1), each of which has nine-fold symmetry in cross-section. The blepharoplast eventually resolves into a cluster of procentrioles arranged on the surface of the sphere (2). The procentrioles lose their regular arrangement (3) and elongate to form full-length basal bodies (4). Reproduced from I. Mizukami and J. Gall [20], The Journal of Cell Biology 1966, 29, Figures 7 and 14, pages 102 and 103. ©The Rockefeller University Press.
EM studies show that the earliest blepharoplasts consist of curious tubular structures, whose nine-fold symmetry in cross-section is clearly related to centrioles (Figure 1.6A). By the time the blepharoplast comes to occupy the pole of the last mitotic division, it has become hollow, and now consists of many short centrioles (procentrioles) covering the surface of a somewhat amorphous sphere [20, 21]. Once released, these procentrioles elongate into full-length centrioles which migrate to the surface of the cell, and function as basal bodies for the flagella (Figure 1.6B).

As in other cases of de novo origin of centrioles, the very earliest stages in formation of the blepharoplast remain obscure. Nevertheless, lower plants provide a clear case of de novo centriole formation, at least in the limited sense that the blepharoplast arises in the absence of preexisting centrioles.

1.6 The Search for DNA

Undaunted by the evidence for the de novo origin of centrioles, or perhaps unaware of it, proponents of self-replication hoped to find DNA in centrioles and basal bodies. In 1924 the German chemist Robert Feulgen introduced the first staining procedure that was specific for DNA in cytological preparations [22]. Feulgen's method involved mild acid hydrolysis of a tissue followed by staining with a decolorized solution of the dye basic fuchsin. It gave a deep magenta color that in most tissues was strictly limited to interphase nuclei and to the chromosomes during mitosis. Just 1 year after Feulgen published his cytochemical test for DNA, Bresslau and Scremin [23] applied the method to several species of the flagellated protozoan Trypanosoma, including T. brucei, the causative agent of sleeping sickness. They saw not only the expected staining of the nucleus but also a clearly positive reaction in a small body at the base of the flagellum, which was known in the protozoological literature as the kinetoplast. For many years it was assumed that the kinetoplast of Trypanosoma was identical to the basal body of other ciliated and flagellated cells. The kinetoplast appeared to provide strong support for the idea that basal bodies contain DNA and hence might be self-replicating structures like chromosomes. The truth turned out to be quite different. When trypanosomes were finally examined with the electron microscope in the 1950s, the kinetoplast was found to be a highly modified mitochondrion closely associated with the basal body of the flagellum, but distinct from it. Kinetoplast DNA is thus mitochondrial DNA – albeit a most unusual type of mitochondrial DNA [24].

Nevertheless, the search for basal body DNA continued. For many years the basal bodies of ciliated protozoa, such as Tetrahymena and Paramecium, provided fertile ground for speculation. The cilia in these protozoa are not randomly arrayed on the surface of the organism, but instead occur in precisely defined rows and patches, whose patterns provide key taxonomic characters on which individual species are recognized. When these organisms undergo cell division, the ciliary patterns are faithfully replicated in the offspring, starting with replication of the
rows of basal bodies. In an influential book published in 1950 Lwoff argued that the basis of the faithful replication of the ciliary rows is self-replication of the basal bodies themselves [25]. This theme was pushed to its logical extreme in a study published in 1965 by Randall and Disbrey [26], who claimed that the basal bodies of *Tetrahymena* contain DNA. They provided two lines of evidence: staining with the fluorescent dye acridine orange and incorporation of [³H]-labeled thymidine. Acridine orange binds to both RNA and DNA in cytological preparations, but its fluorescence is different in the two cases – more orange in combination with RNA and more greenish with DNA. Randall and Disbrey published an image of a *Tetrahymena* pellicle in which the rows of ciliary basal bodies appeared greenish. They stated that the fluorescence was removed by prior treatment with DNase. They also showed an autoradiograph of a pellicle isolated from an animal that had been incubated in [³H]-thymidine. The silver grains in the emulsion appeared in rows roughly corresponding to the ciliary rows of the pellicle, suggesting that the basal bodies had incorporated the DNA precursor during replication. An alternative interpretation of the thymidine experiment was provided by the observations of Miller [27], who noted that rows of mitochondria lie under the ciliary rows and that these mitochondria incorporate a detectable amount of [³H]-thymidine in experiments like those performed by Randall and Disbrey. The color shift with acridine orange staining thus remained the major evidence for DNA in the basal bodies of *Tetrahymena*.

The last major claim for DNA in basal bodies – in the flagellated alga *Chlamydomonas* – was made in 1989 by David Luck and his associates [28]. Their case rested on a combination of genetic data, *in situ* hybridization, and fluorescent staining, this time with the much more sensitive and highly specific DNA dye 4',6-diamidino-2-phenylindole (DAPI). However, the putative basal body DNA itself was not isolated and characterized. Luck estimated that the amount of DNA in the basal bodies was 6–9 Mb – more than in the *E. coli* genome – an amount that could have been seen earlier, even by the Feulgen reaction. A detailed critique of the DNA data was subsequently published by Johnson and Rosenbaum [29, 30], who could find no evidence for DNA in the *Chlamydomonas* basal body.

Evidence for unique physical or chemical properties of the putative basal body DNA was notably lacking in all of these accounts. The first claims that mitochondria and chloroplasts contain DNA were also based on staining, but these were soon backed up by isolation of the DNA and demonstration of its unique physical properties and sequence specificity (reviewed in [31]). Any credible claim for basal body DNA should have met the same stringent criteria.

1.7

**On to Self-assembly**

Viewed in historical context, the search for DNA in basal bodies and centrioles was entirely logical. After all, there seemed to be four self-replicating organelles in the cell – chromosomes, mitochondria, chloroplasts, and centrioles – and three of
them contained DNA. Moreover, the semi-conservative replication of the DNA molecule provided a stunning explanation for the duplication of chromosomes, and there was every hope that it might do the same for centrioles and basal bodies. In many ways the duplication of centrioles during the mitotic cell cycle (at least in animal cells) mirrors the duplication of chromosomes much more closely than it does the somewhat random multiplication of mitochondria and chloroplasts: chromosomes and centrioles both go from one to two to four copies in a synchronized fashion under tight cell-cycle control, as first seen by Van Beneden and Boveri. Nevertheless, there is now general consensus that centriole duplication differs fundamentally from chromosome duplication, and that the search for centriole DNA, although undoubtedly a necessary step in our understanding, was largely a diversion from the real issues.

What is the fundamental difference in mode of duplication? When a chromosome duplicates, the DNA replicates semi-conservatively, resulting in the formation of two essentially identical daughter chromosomes. However, when a centriole duplicates, the two products are not identical. Instead, the original centriole, the mother, remains intact and a new centriole, the daughter, is formed next to it. At first the daughter, termed a procentriole, is shorter than the mother, but eventually grows to full length. Curiously, the procentriole is oriented perpendicular to the mother and lies at the “old” end of the mother. Like so much else in centriole biology, these fundamental facts have now been demonstrated elegantly with molecular markers, but they were clearly presaged by observations dating back many decades. One of the earliest such observations is shown in Figure 1.7, taken from a study of spermatogenesis in the hagfish Myxine, published in 1905 by A. and K. Schreiner [32]. For some reason the centrioles in the hagfish spermatocytes are unusually long; hence, one can see not only two mature centrioles but also two shorter daughters, all four retaining orthogonal orientations with respect to each other. The increased resolution of the electron microscope showed that the daughter procentriole shares the same nine-fold symmetry as the mature centriole and

![Figure 1.7](image-url)
established the topological relationships between the mother and daughter [15, 20, 33].

So what can we learn from this brief history? First and foremost, centrioles and basal bodies are identical structures that can arise in the absence of preexisting centrioles or basal bodies. If we want to know how they replicate, we need to know what they are made of and how their components self-assemble. A good start has been made on this problem in recent years, and subsequent chapters in this book will bring us up to date on these issues. Although self-assembly must be at the heart of centriole replication, it is equally clear that old centrioles have a profound influence on the site at which new centrioles arise. The old centriole may simply serve as a place where precursors are concentrated, or it could be more actively involved as a catalyst or template for some steps that might otherwise be extremely slow or rate-limiting. In addition to assembly issues per se, there is clear evidence that in many animal cells centriole replication is tightly coordinated with the mitotic cycle – the very feature that so impressed and to a certain extent misled Van Beneden and Boveri. When centriole replication and the cell cycle are not properly coordinated, there are dire consequences for the cell, and this time Boveri correctly predicted that cancerous growth might be one of them [34].

After several ups and downs in popularity over more than a century of study, centrioles and centrosomes have once again moved to center stage in cell biology research. The remaining chapters in this book will summarize where we now stand and where we hope to be in the near future, in understanding these fascinating structures.

References
