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THE CELL

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The history of morphological science is in large measure a chronicle of the discovery of new preparative techniques and the development of more powerful optical instruments. In the middle of the 19th century, improvements in the correction of lenses for the light microscope and the introduction of aniline dyes for selective staining of tissue components ushered in a period of rapid discovery that laid the foundations of modern histology and histopathology. The decade around the turn of this century was a golden period in the history of microscopic anatomy, with the leading laboratories using a great variety of fixatives and combinations of dyes to produce histological preparations of exceptional quality. The literature of that period abounds in classical descriptions of tissue structure illustrated by exquisite lithographs. In the decades that followed, the tempo of discovery with the light microscope slackened; interest in innovation in microtechnique declined, and specimen preparation narrowed to a nonmonotonous routine of paraffin sections stained with hematoxylin and eosin.

In the middle of the 20th century, the introduction of the electron microscope suddenly provided access to a vast area of biological structure that had previously been beyond the reach of the compound microscope. Entirely new methods of specimen preparation were required to exploit the resolving power of this new instrument. Once again improvement of fixation, staining, and microtomy commanded the attention of the leading laboratories. Study of the substructure of cells was eagerly pursued with the same excitement and anticipation that attend the geographical exploration of a new continent. Every organ examined yielded a rich reward of new structural information. Unfamiliar cell organelles and inclusions and new macromolecular components of protoplasm were rapidly described and their function almost as quickly established. This plentiful harvest of new structural information brought about an unprecedented convergence of interests of morphologists, physiologists, biochemists, and cytochemists; this convergence has culminated in the unified field of science called cell biology.

The first edition of this book (1966) appeared in a period of generous support of science, when scores of laboratories were acquiring electron microscopes and hundreds of investigators were eagerly turning to this instrument to extend their research to the subcellular level. At that time, an extensive text in this rapidly advancing field would have been premature, but there did seem to be a need for an atlas of the ultrastructure of cells to establish acceptable technical standards of electron microscopy and to define and illustrate the cell organelles in a manner that would help novices in the field to interpret their own micrographs. There is reason to believe that the first edition of The Cell: An Atlas of Fine Structure fulfilled this limited objective.

In the 14 years since its publication, dramatic progress has been made in both the morphological and functional aspects of cell biology. The scanning electron microscope and the freeze-fracturing technique have been added to the armamentarium of the microscopist, and it seems timely to update the book to incorporate examples of the application of these newer methods, and to correct earlier interpretations that have not withstood the test of time. The text has been completely rewritten and considerably expanded. Drawings and diagrams have been added as text figures. A few of the original transmission electron micrographs to which I have a sentimental attachment have been retained, but the great majority of the micrographs in this edition are new. These changes have inevitably added considerably to the length of the book and therefore to its price, but I hope these will be offset to some extent by its greater informational content.

Twenty years ago, the electron microscope was a solo instrument played by a few virtuosos. Now it is but one among many valuable research tools, and it is most profitably used in combination with biochemical, biophysical, and immunocytochemical techniques. Its use has become routine and one begins to detect a decline in the number and quality of published micrographs as other analytical methods increasingly capture the interest of investigators. Although purely descriptive electron microscopic studies now yield diminishing returns, a detailed knowledge of the structural organization of cells continues to be an indispensable foundation for research on cell biology. In undertaking this second edition I have been motivated by a desire to assemble and make easily accessible to students and young investigators some of the best of the many informative and aesthetically pleasing transmission and scanning electron micrographs that form the basis of our present understanding of cell structure.

The historical approach employed in the text may not be welcomed by all. In the competitive arena of biological research today investigators tend to be interested only in the current state of knowledge and care little about the steps by which we have arrived at our present position. But to those of us who for the past 25 years have been privy to the history of the young and the major contributors to our present understanding of its structure and function. In venturing to do this I am cognizant of the hazards inherent in making judgments of priority and significance while many of the dramatic personae are still living. My apologies to any who may feel that their work has not received appropriate recognition.

It is my hope that for students and young investigators entering the field, this book will provide a useful introduction to the architecture of cells and for teachers of cell biology a guide to the literature and a convenient source of illustrative material. The sectional bibliographies include references to many reviews and research papers that are not cited in the text. It is believed that these will prove useful to those readers who wish to go into the subject more deeply.

Most of the micrographs in this book are in black and white; magnifications for each of the micrographs will no doubt draw some criticism. Their inclusion was impractical since the original negatives often remained in the hands of the contributing microscopists and micrographs submitted were cropped or copies enlarged to achieve pleasing composition and to focus the reader's attention upon the particular organelle under discussion. Absence was considered preferable to inaccuracy in stated magnification. The majority of readers, I believe, will be interested in form rather than measurement and will not miss this datum.

It is a source of pride that nearly half of the contributors were students, fellows or colleagues in the Department of Anatomy at Harvard Medical School at some time in the past 20 years. I am grateful for their stimulation and for their generosity in sharing prints and negatives. It is a pleasure to express my appreciation for the forbearance of my wife who has had to communicate with me through the door of the darkroom for much of the year while I printed the several hundred micrographs; and for the patience of Helen Déacon who has typed and retyped the manuscript; for the skill of Peter Ley, who has made many copy negatives to gain contrast with minimal loss of detail; and for the artistry of Sylvia Collard Keene whose drawings embellish the text. Special thanks go to Ello and Giuseppina Raviola who read the manuscript and offered many constructive suggestions; and to Albert Meier and the editorial and production staff of the W. B. Saunders Company, the publishers.

And finally I express my gratitude to the Simon Guggenheim Foundation whose commendable policy of encouraging the creativity of the young was relaxed to support my efforts during the later stages of preparation of this work.

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Boston, Massachusetts
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In suitably stained histological sections examined with the light microscope a pair of short rods, the centrioles, are seen in a specially differentiated region of the juxtanuclear cytoplasm called the centrosome, or cell center. The position of the centrosome was long used as an indication of the polarity and symmetry of the cell, and a line passing through the center of the nucleus and through the centrosome was defined as the cell axis. In secretory epithelial cells the pair of centrioles (diplosome) is often located in the supranuclear cytoplasm partially surrounded by the Golgi apparatus. The position of these organelles was believed to determine the polarity of the cell and the direction of its secretion.

The centrioles were observed to double in number immediately before cell division, one pair migrating to the opposite pole of the nucleus. After the breakdown of the nuclear envelope the mitotic spindle developed with a diplosome at each pole. The centrioles were thus considered to play an important role in cell division, serving as centers for organization of the spindle apparatus. It was logical to attribute the doubling of the centrioles immediately before cell division to a process of division comparable to binary fission of bacteria. They were therefore regarded as self-duplicating organelles exhibiting continuity from one cell generation to the next. A diploid cell generally had a single pair of centrioles, but in polyploid cells there was a pair for each chromosome set so that megakaryocytes of bone marrow or multinucleate giant cells of bone may have 30 or more. An exception to this correspondence between ploidy and number of centrioles was recognized in the case of ciliated epithelial cells. A remarkable proliferation of centrioles was observed as an initial event in the differentiation of cilia. The resulting centrioles took up a position immediately beneath the plasmalemma and served as the basal bodies of the developing cilia.

Electron microscopy demonstrated that centrioles were hollow cylindrical structures with nine evenly spaced triplet microtubules in their walls (de Harven and Bernhard, 1956; Bessis et al., 1968). Their duplication in the mitotic cycle and their continuity in the daughter cells were confirmed but the traditional views on the mechanism of centriole duplication proved to be incorrect.

Diagram of centriole replication prior to cell division. Each member of the original pair (A) induces formation of a new centriole perpendicular to a specific region of its wall (B and C). The two diplosomes so formed take up positions at opposite poles of the mitotic spindle (D). In B and C the centrioles are depicted in longitudinal section.
Centrioles do not undergo transverse division nor do they split longitudinally. Instead, the new centriole develops in end-to-side relationship to a specific region of a preexisting centriole but separated from it by a narrow electron lucent space. The annulus from which it develops, called the procentriole, is an annular condensation of dense material approximately the same diameter as a mature centriole but initially devoid of microtubules. The procentriole elongates by accretion of material to its free end, and the pinwheel arrangement of triplet microtubules gradually appears within the previously homogeneous dense ring. The forming centriole maintains its orientation perpendicular to the parent centriole. After reduplication the original members of the diplosome separate and each, with its newly formed centriole, moves to one pole of the division figure. Thus in the cell cycle, centrioles are duplicated but not, as previously believed, by division. A template mechanism cannot be invoked, since the new centriole arises perpendicular to its precursor and not in intimate contact with it. The preexisting centriole seems to act merely as a site of induction of nucleation for self-assembly of the new centriole from precursors synthesized elsewhere in the cell.

Studies of ciliogenesis have shown that centrioles can arise apart from preexisting centrioles (Steinmann, 1968; Steinmann and Cirelli, 1965; Steinmann, 1968; Stockinger and Cirelli, 1968). The great majority of the basal bodies develop around dense spherical bodies variously called deuterosomes, or procentriole organizers. These in turn arise by condensation of smaller aggregations of filamentous material (filosomes). Several annular procentrioles may arise radially around the same procentriole organizer. They elongate rapidly and acquire the microtubular internal structure typical of centrioles. When they have attained their definitive length they dissociate from the organizer and move to the cell surface, where each initiates polymerization of the nine doublet microtubules forming the axoneme of a cilium.

In its role as basal body of a cilium or flagellum, the centriole can be considered to serve a template function, since the polymerization of tubulin takes place directly on the distal end of the triplet microtubules in its wall and the ninefold symmetry of the centrioles is expressed in the nine doublets of the axoneme. For reasons that remain obscure only two members of each centriolar triplet nucleate the assembly of tubulin under these conditions, since doublet and not triplet microtubules are formed as peripheral elements of the axoneme.

Assembly of single microtubules evidently does not require a preexisting site of nucleation, for the axial pair of single microtubules in the axoneme arises without a corresponding structure in the axis of the centriole. The nucleating function of centrioles for microtubule assembly has been demonstrated in vitro using centrioles isolated from human cells and tubulin extracted from bovine brain (McGill and Brinkley, 1975).

The important role traditionally assigned to the centrioles in formation of the mitotic spindle seems not to have been valid. Mitosis occurs with formation of a typical spindle in both lower and higher plants in the absence of centrioles (Heaps, 1969, 1971). In electron micrographs of animal cells, the spindle microtubules do convege upon the centriolar region but they rarely contact the centrioles themselves. They terminate in the pericentriolar cytoplasm in dense spherical granules called centriolar satellites. Selective damage to the pericentriolar material by laser microbeam irradiation in prophase results in failure of the chromosomes to separate at anaphase. This occurs with little or no morphological evidence of structural damage to the centrioles themselves (Berns et al., 1977). Investigative attention has therefore shifted to the surrounding specialized zone of cytoplasm which constitutes the major component of the centrosome. It has been possible to isolate centrosomes from certain strains of cultured cells that have been treated with colchicine to block spindle formation. When these are incubated in vitro with tubulin extracted from brain, large numbers of microtubules polymerize around each centrosome. These emanate from the pericentriolar material and not from the centrioles (Gould and Borisy, 1977). It is concluded therefore that centriolar satellites and possibly other components of the pericentriolar cytoplasm can initiate assembly of microtubules both in vivo and in vitro. This does not necessarily mean that the centrioles are inactive during cell division. They may control aggregation of the pericentriolar material or may be involved in its activation at the appropriate time in the cell cycle. The interrelations of the several components of the centrosome have yet to be worked out.

The ubiquitous occurrence of centrioles, their replication, and their continuity from generation to generation of cells prompted the speculation that they might possess their own DNA, as do mitochondria and chloroplasts. In several early cytochemical and biochemical analyses of basal bodies isolated in bulk from protozoa, the presence of DNA was reported (Seaman, 1960; Randall and Disbrey, 1965). This could not be confirmed in later investigations with purer fractions (Flavell and Jones, 1970). Further cytochemical studies using acidine orange staining suggested, however, that RNA was associated with basal bodies of cilia (Hartman et al., 1974). RNA in centrioles and in their satellites was also reported on the basis of the sensitivity of certain of their components to digestion with ribonuclease (Stubblefield and Brinkley, 1967; Brinkley and Stubblefield, 1970; Dippel, 1976).

Evidence of a functional role for RNA in centrioles has been presented more recently. When basal bodies isolated from protozoa are injected into Xenopus eggs, they induce the formation of conspicuous radial arrays of microtubules (asters). The aster-inducing activity of basal bodies was eliminated by prior treatment with ribonuclease, but this treatment did not interfere with their capacity to serve as templates for polymerization of microtubules at their ends (Heidemann et al., 1977). It was concluded that centrioles contain RNA and that this component is necessary for initiation of aster formation. Other interpretations of the observations are possible and
arguments based upon analysis of partially purified preparations have the inherent weakness of possible contamination with components of the neighboring cytoplasm. Although the evidence is highly suggestive of the presence of RNA, a resolution of this problem will require further study.

Centrioles serving as basal bodies for cilia or flagella usually retain their simple cylindrical form (A), but their shape may be modified by the development of lateral appendages and one or both ends may be closed (B, C, D). In many invertebrates and some vertebrates, cross-striated ciliary rootlets extend from the lower end of the basal body for variable distances into the apical cytoplasm (C, D). In rare instances in protozoa (viz., Euplotes), the central pair of microtubules of the axoneme may form a loop extending into the central cavity of the basal body (E). In cells with a single flagellum, a second centriole is often at right angles to the one serving as basal body (F). (From Fawcett, in The Cell, Vol 2. J. Brachet and A. Mirsky, eds., Academic Press 1961.)

Centrioles are usually positioned so that their long axes form a right angle. Their perpendicular orientation is maintained even though they may be half a micrometer or more apart and have no visible structural elements connecting them. The nature of the long-range forces or organization of the centrosomal cytoplasm that are responsible for this relationship are unknown. Departures from the usual orthogonal arrangement are occasionally encountered in normal cells and are reported to be common in malignant tumors.

Centrioles vary somewhat in length from one cell type to another but are usually about 0.5 \(\mu\text{m}\) long and 0.2 \(\mu\text{m}\) in diameter. In the accompanying micrograph a pair of centrioles near the lumenal surface of an epithelial cell shows the usual perpendicular orientation. The discrepancy in length of the two members of this pair is unusual. The two ends of a centriole can often be distinguished in that one is slightly narrower and appears to be closed and the other appears open.

Figure 301. Intestinal epithelium of a chicken embryo. (Micrograph courtesy of Sergei Sorokin.)
The centrioles replicate early in cell division and take up positions at either pole of the division figure. Concurrently with the condensation of the chromosomes and breakdown of the nuclear envelope, microtubules polymerize, extending from the chromosomes to the poles and from pole to pole, to form the mitotic spindle.

In the accompanying micrograph made before introduction of aldehyde fixatives, the microtubules have not been well preserved. The plane of section is a fortunate one, however, in that three of the centrioles are included. The second centriole at the lower pole is in a plane perpendicular to this section and is not seen. Because the two diplosomes usually differ in their orientation, all four centrioles are rarely, if ever, included in the same thin section.

Figure 302. Centrioles of dividing spermatocyte from cock testis. (Micrograph courtesy of Toshio Nagano.)
In transverse section the cylindrical wall of the centriole is made up of nine longitudinally oriented triplet microtubules. A dense material of unknown nature occupies the interstices between the triplets. Each triplet is at a constant angle of about 40 degrees to its respective tangent. A pattern is thus formed that resembles the vanes of a turbine or the charges of a pyrotechnic "pinwheel." The innermost microtubule of each triplet has a circular cross section and is designated subunit a and the other two b and c. The latter two each share a segment of the wall of the adjacent microtubule and therefore have a C-shaped profile instead of a circular cross section. Subunit a has two short diverging projections resembling the arms on the doublets of the ciliary axoneme. One of these is directed inward along a radius and appears to have a free end pointing toward the center of the centriole. The other projection connects with subunit c of the next triplet. The successive triplets are thus linked together a to c around the circumference of the centriole by a series of linear densities. It is not known whether these latter correspond to the dynein arms or nexin links of flagellar axonemes.
Images of radially symmetrical periodic structures can be intensified and made more coherent by a technique involving rotation between each of several successive exposures in the enlarger. The cross section of a centriole at the upper left (A) has been intensified by summation of fractional exposures through nine steps of rotation to produce the image at the upper right (B). This has succeeded in accentuating the hub-and-spokes pattern of densities in the interior of the centriole. This pattern is clearly visible in some cross sections of centrioles and absent in others. It is not clear whether there are real differences among centrioles in this respect or whether this variation in their appearance in electron micrographs reflects differences in preservation or different planes of section along the length of the centriole.

The cross section of a centriole stained with tannic acid in the lower figure shows the protofilaments of the triplets in negative image. Like other microtubules, the wall of subunit a has 13 tubulin protofilaments, while b and c each have 10. The core structure and radiating spokes are illustrated with unusual clarity. Although the dimensions of the hub are similar to those of a microtubule, no protofilaments are detectable in its wall.

Figure 305. Centriole from H3 polyoma tumor. A, Original image. B, Image reinforced by rotation. (Micrographs courtesy of Etienne de Harven.)

Figure 306. Centriole fixed in glutaraldehyde and tannic acid. (Micrograph courtesy of Vitauts Kalnins.)
In interphase cells, microtubules commonly radiate from the centrosome, and in dividing cells the microtubules of the mitotic spindle converge toward a diplosome at either pole of the division figure. The accompanying micrographs of dividing cells show numerous microtubules radiating from the immediate vicinity of a centriole. Such images have led to the assumption that the centrioles serve as nucleation sites for microtubule assembly. However, detailed studies of this region suggest that the microtubules do not actually contact the centrioles but end in a specialized pericentriolar zone of cytoplasmic matrix and are often associated with small dense bodies called centriolar satellites (indicated by arrows).

Laser microbeam irradiation of acridine orange–treated living cells results in a disorganization of the pericentriolar material but causes no detectable alteration of the centrioles. This treatment disturbs formation of interpolar microtubules and interferes with anaphase movement of chromosomes. It is concluded that although centrioles may not be directly involved as sites of nucleation of microtubules, they may nevertheless contribute indirectly to spindle mechanics as agents of synthesis or organization of the pericentriolar material.

The plane of a thin section only rarely happens to coincide with the long axis of both members of a pair of centrioles. It is more common for one to be cut longitudinally and the other transversely, as in the accompanying micrographs. In both of these examples, the centrioles are oriented at right angles even though they are some distance apart.

The pair of centrioles in the upper micrograph on the facing page is closely associated with the Golgi apparatus and occupies a concavity in the nucleus. This is a common relationship in the hemopoietic cell line and in other cell types as well.

The lower figure illustrates the special character of the pericentriolar cytoplasm which often contains numerous satellites. The labeled microtubules end in one of the satellites.

Figure 309. A pair of centrioles and the associated Golgi complex in a myelocyte from guinea pig bone marrow.

Figure 310. Centrosomal region of an ascites tumor cell. (Micrograph from Guy de Thé, J. Cell Biol. 23:265-275, 1964.)
In ciliogenesis, newly formed single centrioles, serving as basal bodies, are arranged in rows and oriented perpendicular to the cell surface. The end that is adjacent to the membrane functions as a site of nucleation for microtubule protein which polymerizes on subunits a and b of the triplets to form the nine doublet microtubules of the axoneme. The centrioles arrive at the cell apex at different times and the polymerization of tubulin evidently begins as soon as the end of the organelle is juxtaposed to the membrane. Thus in the accompanying micrograph four developing cilia are seen in successive stages of growth.

Flagella usually arise from one member of a pair of centrioles positioned at right angles. The axoneme grows out from the one perpendicular to the membrane. The lower figure on the facing page shows early stages in formation of a sperm flagellum. The differing lengths of the distal centrioles illustrated is attributable to the fact that the micrographs are not all from the same species. In one example (B) the second centriole is out of the plane of section. In sperm tail development the centrioles are involved in organizing other components in addition to the axoneme. In the most advanced stage shown (D), both centrioles are sectioned longitudinally. The thin line above the proximal centriole (*) is the anlage of the capitulum which will later articulate with the implantation fossa of the sperm nucleus. The periodic densities in the bracket (**) represent an early stage in assembly of the cross-striated columns of the connecting piece. The adjunct has begun to develop at the other end of the proximal centriole (at arrow). The centrioles thus appear to be involved in the organization of four distinct structures — axoneme, capitulum, centriolar adjunct, and connecting piece.
During formation of a cilium or flagellum, only subunits \( a \) and \( b \) of the centriolar triplets serve as nucleation sites for formation of the doublets of the axoneme. A unique behavior of the proximal centriole during spermatogenesis demonstrates, however, that subunit \( c \) can serve as a site for nucleation of tubulin during elongation of the triplets to form the transient organelle known as the centriolar adjunct.

Early in mammalian spermiogenesis, the pair of centrioles takes up a position in the peripheral cytoplasm with the end of one member of the pair adjacent to the cell membrane. This relationship initiates the development of the flagellum at the end of the distal centriole (A). Soon thereafter, fine filamentous material gathers around the distal end of the proximal centriole (B). The centriole then increases in length by accretion and polymerization of this material at its end. This process continues for some time after the centrioles and base of the flagellum have moved inward and become lodged in the implantation fossa in the caudal pole of the nucleus (C). As will be illustrated in subsequent micrographs, this newly formed appendage is not identical to the centriole from which it arises.

The centriolar adjunct may attain a length two or three times that of the centriole. It then disappears, leaving no residue in the mature spermatozoon (D). It has been found in all mammalian species thus far examined but its function remains unknown.

Figure 313. Diagram of formation of the centriolar adjunct. (From Fawcett, in Genetics of the Spermatozoon, Bogtrykkeriet Forum, Copenhagen, 1971.)
At the base of the flagellum of a mammalian spermatid, the juxtanuclear proximal centriole is continuous (at the arrow) with a structure of similar configuration, the centriolar adjunct. At the end of the latter, there is an accumulation of loose textured material that appears to be condensing to contribute to elongation of this organelle. The cavity in the centriolar adjunct is narrower than that of the centriole proper.

At the lower left a transverse section through the centriole (at the level of the white line in the upper figure) shows the familiar pinwheel arrangement of closed triplet microtubules. At the lower right a comparable section through the adjunct (at the level of the black line in the upper figure) reveals some distinctive structural differences. Subunit $a$ of the triplets is a typical closed microtubule, but $b$ and $c$ are usually open, presenting a free edge that has failed to fuse with the wall of the adjacent microtubule. In addition, the centriolar adjunct has a lining layer of complex ultrastructure which is lacking in the centriole. This accounts for the differences in diameter of their central cavities.

The formation of atypical triplet microtubules in the centriolar adjunct of spermatids attests to the capacity of subunit $c$ of the centriolar triplets to serve as a site for nucleation of microtubule protein. Why it is inactive during generation of an axoneme so that only doublets are formed remains unexplained.

Figure 314. Implantation fossa, centriolar adjunct, and base of the flagellum in a chinchilla spermatid. (From D. W. Fawcett and D. M. Phillips, Anat. Rec. 165:153-184, 1969.)
The dense material that forms the capitulum and the cross-striated columns of the connecting piece in spermatozoa is formed in intimate association with the proximal and distal centrioles, respectively. These versatile organelles are believed to play a dominant role in the organization of these components of the spermatozoon. The cross-striated columns of the connecting piece are probably homologous to the cross-striated rootlets that often extend downward from the basal bodies into the cytoplasm of ciliated epithelial cells.

Traditionally the basal bodies were regarded as kinetic centers responsible for initiating the beat of cilia and flagella. This interpretation is no longer tenable. It has been shown that sperm tail movements continue after laser microbeam destruction of the centriolar region (Lindemann and Rikmenspoel, 1972). Moreover, electron microscopic studies of spermatogenesis have shown that the distal centriole which initiates development of the flagellum later disintegrates and is no longer present in the mature spermatozoon. The juxtanuclear centriole usually persists, occupying a niche just beneath the capitulum that attaches the tail to the sperm head. In some species, however, this centriole also disintegrates during sperm maturation (Woolley and Fawcett, 1973). Thus centrioles are essential for development of cilia and flagella but are not required to initiate and maintain their beat. Observe in the accompanying micrograph that no distal centriole can be identified at the base of the axoneme.
REFERENCES


centrioles


