Chapter 13: Cilia and Flagella
CONTRIBUTORS OF ELECTRON MICROGRAPHS

Dr. John Albright
Dr. David Albertini
Dr. Nancy Alexander
Dr. Winston Anderson
Dr. Jacques Aubert
Dr. Baccio Baccetti
Dr. Michael Barrett
Dr. Dorothy Bainton
Dr. David Begg
Dr. Olaf Behnke
Dr. Michael Berns
Dr. Lester Binder
Dr. K. Blinzinger
Dr. Gunter Blöbel
Dr. Robert Bolender
Dr. Aiden Breathnach
Dr. Susan Brown
Dr. Ruth Bulger
Dr. Breck Byers
Dr. Hektor Chemes
Dr. Kent Christensen
Dr. Eugene Copeland
Dr. Romano Dallai
Dr. Jacob Davidowitz
Dr. Walter Davis
Dr. Igor Dawid
Dr. Martin Dym
Dr. Edward Eddy
Dr. Peter Elias
Dr. A. C. Faberge
Dr. Dariush Fahimi
Dr. Wolf Fahrenbach

Dr. Marilyn Farquhar
Dr. Don Fawcett
Dr. Richard Folliot
Dr. Michael Forbes
Dr. Werner Franke
Dr. Daniel Friend
Dr. Keigi Fujiwara
Dr. Penelope Gaddum-Rosse
Dr. Joseph Gall
Dr. Lawrence Gerace
Dr. Ian Gibbon
Dr. Norton Gilula
Dr. Jean Gouranton
Dr. Kiyoshi Hama
Dr. Joseph Harb
Dr. Etienne de Harven
Dr. Elizabeth Hay
Dr. Paul Heidger
Dr. Arthur Hertig
Dr. Marian Hicks
Dr. Dixon Hingson
Dr. Anita Hoffer
Dr. Bessie Huang
Dr. Barbara Hull
Dr. Richard Hynes
Dr. Atsushi Ichikawa
Dr. Susumu Ito
Dr. Roy Jones
Dr. Arvi Kahri
Dr. Vitaubs Kalnins
Dr. Marvin Kult
Dr. Taku Kanaseki

Dr. Shuichi Karasaki
Dr. Morris Karnovsky
Dr. Richard Kessel
Dr. Toichiro Kuwabara
Dr. Ulrich Laemmli
Dr. Nancy Lane
Dr. Elias Lazarides
Dr. Gordon Leedale
Dr. Arthur Like
Dr. Richard Linck
Dr. John Long
Dr. Linda Malick
Dr. William Massover
Dr. A. Gideon Matolsky
Dr. Scott McNutt
Dr. Oscar Miller
Dr. Mark Mooseker
Dr. Enrico Mognai
Dr. Toichiro Nagano
Dr. Marian Neutra
Dr. Eldon Newcomb
Dr. Ada Olins
Dr. Gary Olson
Dr. Jan Orenstein
Dr. George Palese
Dr. Sanford Palay
Dr. James Paulson
Dr. Lee Peacher
Dr. David Phillips
Dr. Dorothy Pintelka
Dr. Thomas Pollard
Dr. Keith Porter

CONTRIBUTORS OF PHOTOMICROGRAPHS

Dr. Jeffrey Pudney
Dr. Eliio Raviola
Dr. Giuseppina Raviola
Dr. Janardan Reddy
Dr. Thomas Reese
Dr. Jean Revel
Dr. Hans Ris
Dr. Joel Rosenbaum
Dr. Evans Roth
Dr. Thomas Roth
Dr. Kogaku Saito
Dr. Peter Satir

Dr. Manfred Schliwa
Dr. Nicholas Severs
Dr. Emma Shelton
Dr. Nicolai Simionescu
Dr. David Smith
Dr. Andrew Somlyo
Dr. Sergei Sorokin
Dr. Robert Speck
Dr. Andrew Staehelin
Dr. Fumi Suzuki
Dr. Hewson Swift
Dr. George Szabo

Dr. John Tersakis
Dr. Guy de Thé
Dr. Lewis Tilney
Dr. Greta Tyson
Dr. Wayne Vogl
Dr. Fred Warner
Dr. Melvyn Weinstock
Dr. Richard Wood
Dr. Raymond Wuerker
Dr. Eichi Yamada
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 the 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 monotonous 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 abundant harvest of new structural information brought about an unprecedented convergence of the interests of morphologists, physiologists, and biochemists; this convergence has culminated in the unified new 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 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 privileged to participate in one of the most exciting and fruitful periods in the long history of morphology, the young seem to be entering the theater in the middle of an absorbing motion picture without knowing what has gone before. Therefore, in the introduction to each organelle, I have tried to identify, in temporal sequence, a few of 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 dramatis personae are still living. My apologies to any who may feel that they have been overlooked.

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.

The burden of 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. Assembling these micrographs illustrating the remarkable order and functional priority and significance while many of the included 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 privileged to participate in one of the most exciting and fruitful periods in the long history of morphology, the young seem to be entering the theater in the middle of an absorbing motion picture without knowing what has gone before. Therefore, in the introduction to each organelle, I have tried to identify, in temporal sequence, a few of 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 dramatis personae are still living. My apologies to any who may feel that they have been overlooked.

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.

The burden of 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. Assembling these micrographs illustrating the remarkable order and functional proportion 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 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 privileged to participate in one of the most exciting and fruitful periods in the long history of morphology, the young seem to be entering the theater in the middle of an absorbing motion picture without knowing what has gone before. Therefore, in the introduction to each organelle, I have tried to identify, in temporal sequence, a few of 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 dramatis personae are still living. My apologies to any who may feel that they have been overlooked.

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.

The burden of 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. Assembling these micrographs illustrating the remarkable order and functional
CILIA AND FLAGELLA

The minute motile processes that occur on some cell types are designated by different names depending upon their length, the character of their movement, and the number per cell. When there are only one or two and they are long in relation to the size of the cell, they are called flagella. When they are shorter and numerous, they are called cilia. Cilia are 6 to 10 \( \mu m \) long, whereas protozoan flagella range up to 150 \( \mu m \) in length and some sperm tails are several times this length. Flagella beat independently and exhibit an undulating motion that results from the propagation of successive waves of bending from base to tip. Cilia have an oscillating to-and-fro motion in which they are relatively straight and stiff during the forward or effective stroke and more curved and whip-like during the return stroke.

The beating of cilia is highly coordinated and the rate varies on different biological material from 10 to 30 per second. All of the cilia on the same cell may beat in phase, isochronal rhythm, or in an out-of-phase metachronal rhythm. In the latter, which is characteristic of ciliated epithelia, successive cilia along each row in the direction of beat are slightly more advanced in their cycle than the preceding cilium. This results in appearance of waves that sweep over the epithelial surface like the waves that move before the wind across a field of wheat, except that for cilia, the metachronal waves are perfectly regular in their occurrence and they move at a uniform rate. It is not clear whether this is due to sequential activation of the cilia or is imposed upon them by external hydrodynamic and viscous forces of the fluid medium.

Cilia are essential to locomotion in a large class of free living protozoa and to feeding in sessile protozoa. In multicellular invertebrates, they are involved in such diverse physiological processes as alimentation, circulation, and respiration. Flagella propel a large class of protozoa, and flagellate male gametes are involved in reproduction throughout most of the animal kingdom and in some primitive plants.

Few cellular activities have proved more fascinating to cytologists than ciliary and flagellar motion. The occurrence of a continuous flickering movement on the surface of the epithelia of the respiratory tract produced by cilia was first described by Purkinje and Valentin in 1834. Soon thereafter Sharpay (1836) established that the direction of beat of tracheal cilia is always toward the pharynx.

Although with the light microscope cilia appeared devoid of internal structure, Englemann (1868) pointed out that their contractile protoplasm was probably composed of longitudinally oriented fibrillar elements that shortened into globular form during the active phase of the beat. The first actual observation of fibrillar components in motile cell processes is attributed to Jensen (1887) and Ballowitz (1888), who described fraying of the tips of sperm tails into a number of fibrils when the flagella were disrupted by pressure on the coverglass. Dellinger (1909) confirmed the presence of several longitudinal internal fibrils by microdissection of protozoan cilia, and fibrils were reported in stained intact epithelial cilia by Korschekov (1923) and Grave and Schmitt (1925).

Motile microorganisms were among the first biological materials examined when the electron microscope was developed. Disrupted protozoa and spermatozoa were simply dried onto a Formvar substrate. Crude though this method of preparation was, it permitted Harvey and Anderson in 1943 to reaffirm the presence of internal fibrils in cilia and flagella. Studying disrupted sperm flagella with slightly improved technique, Huxley and Hanson (1954) showed that fibrils differed from the other nine in appearance and stability. In a series of comparative studies on plant cilia, Manton and Clarke (1950-1952) also found 11 fibrils and concluded that this number was probably of universal occurrence in plant cilia. They published in 1952 a three-dimensional diagram of the probable arrangement of components based upon their extensive experience with images of disrupted cilia. In this reconstruction, nine fibrils were evenly spaced around a central pair. The nine peripheral fibrils were depicted as though tubular and each divided by a radially oriented septum. Two similar fibrils in the axis of the cilium were enclosed in a membrane and surrounded by a slender helically wound fiber, forming a sheath around the central pair.

Technical progress in fixation, embedding, and microtomy of tissues for electron microscopy in the early 1950s made it possible for Fawcett and Porter (1954) to study epithelia from a wide range of animal species in ultrathin sections. Cross sections of cilia substantiated the general features of Manton's hypothetical reconstruction of their internal organization. From invertebrates to humans, the number of fibrils was 11, with 2 in the center and nine around the periphery. The outer fibrils appeared hollow and had a double-barreled, figure-of-eight cross section. The two central fibrils were single and had a circular cross section. As methods of fixation and staining improved and more components were resolved, Azeflius (1959) and Gibbons and Grimstone (1960) drew attention to the fact that the subunits of the doublets were unequal in size. The smaller designated subfiber A was slightly closer to the axis of the cilium than the larger, called subfiber B. Subunit B had a circular cross section, subunit A a C-shaped cross section closed by a sector of the wall of subunit A. Two small diverging projections, or arms, were restored, from the wall of subfiber A toward the next doublet in the row (Azeflius, 1959), and these were found to project clockwise from the point of view of an observer looking along the cilium from base toward tip. A radial pattern of slender spokes extends from the sheath around the central pair to subfiber A of each doublet.

A return to the study of dissociated cilia and flagella using negative staining with phosphotungstic acid (Pease, 1963; Andre and Thiery, 1963) showed that the contrast medium penetrated into the interior of the axonemal elements, thus establishing that the "fibrils" are in fact microtubules. Their cylindrical wall was seen to be made up of about 13 protofilaments 3.5 nm in diameter. Although the length of the fibrils was reduced by only about 3% by negative staining, the contrast medium provided a useful means of identifying the fibrils and of measuring their spatial arrangement.

Investigation of the mechanism of ciliary motion required a model system that could be subjected to experimental manipulation in vitro. This was provided by Hoffman-Berling (1950) who showed that ciliated cells extracted with glycerin were killed but that addition of ATP resulted in reactivation of their cilia. It was subsequently shown by Child and Satir that glycerination removes the ciliary membrane but leaves the axonemes intact, suggesting that the principal function of the membrane is to maintain around the axoneme the appropriate concentration of ions and ATP to sustain its function. In more recent refinements of this approach, the membrane is removed with detergent, leaving the axonemes accessible to experimental alterations in their environment. On addition of ATP to the bare axonemes of sea urchin spermatozoa, they are induced to swim like living sperm (Gibbons, 1963). Thus, as in other cellular processes, the energy required for ciliary motion appeared to depend upon the hydrolysis of ATP to ADP and phosphoric acid. In further studies on the biochemical dissection of flagella, Gibbons showed that the arms of the doublets could be selectively removed by sequestering magnesium ion with a chelating agent. The protein extracted, called dynein, was shown to have the number of fibrils was about 11, with nine peripheral fibrils defibrillated and the central pair in the form of two adjacent subfiber A's. The arms were restored to the doublets and ATPase activity was again demonstrable in the axonemes.

Skeletal muscle was long believed to shorten as a result of a conformational change in the molecules of its contractile proteins, but it was shown by Huxley and Hanson (1954) that muscle contraction takes place by sliding of interdigitating sets of actin and myosin filaments of constant length. Similarly, it was long assumed that the fibrillar components of the ciliary axoneme were capable of shortening. But if the ciliary fibrils were contractile, those on the concave side during the effective stroke would be expected to shorten. In electron microscopic studies on the tips of cilia in different phases of the beat, Satir (1966) found that the fibrils on this side extended farther into the tips. It was therefore evident that bending occurred without shortening of the fibrils. It was concluded that ciliary and flagellar motion involves a sliding-microtubule mechanism comparable to the sliding-filament mechanism of muscular contraction.
More direct evidence was provided by ingenious experiments of Summers and Gibbons (1971). Segments of flagellar axonemes were briefly exposed to trypsin to release the doublets from their attachment to the central sheath. Upon the addition of ATP to this preparation, tubules rapidly emerged from opposite ends of the axonemal segments as a result of active sliding movements between the doublets. The trypsin treatment evidently digested the radial spokes and interdoublet nexin links, uncoupling the sliding of the doublets from axonemal bending and permitting ATP-energized sliding to propel microtubules out of the ends of the segments of axoneme.

Clinical observations on a congenital form of human infertility have now validated the assumption that dynein is essential for motility of cilia and flagella. A disease syndrome described by Kartagener (1933) is characterized by chronic sinusitis, bronchiectasis, situs inversus viscerum, and male infertility. The ejaculate of such a patient, analyzed by Pedersen and Rebbe (1975), showed normal numbers of spermatozoa but no motility. When the spermatozoa were examined with the electron microscope, they exhibited an absence of arms on the doublets of the axoneme. Biopsy of ciliated cells of the respiratory tract of several such patients has shown a similar defect in the cilia. An absence of ciliary and flagellar motion accounts for the chronic sinusitis, bronchiectasis, and male infertility in these patients, who appear to have an inherited defect in synthesis of dynein (Afzelius et al., 1975; Eliasson et al., 1977).

Current research is concentrated on the structure, composition, and function of the radial spokes and nexin links. It seems likely that these components provide the shear resistance within the axoneme that converts sliding of microtubules into bending. Consistent with this interpretation is the observation that in longitudinal sections of straight regions, the spokes are perpendicular to the doublet microtubules and in bent regions they are oblique as might be expected if they were attached to the doublet at one end and to the central sheath at the other when the doublet was displaced by sliding (Warner and Satir, 1974).
The differences in size and shape of cilia and microvilli are well illustrated by scanning micrographs of the lumenal surface of the epithelium lining the mammalian oviduct. The tufts of cilia associated with individual ciliated cells project several microns above the convex apices of nonciliated cells covered with short microvilli. The number of ciliated cells in this epithelium is under hormonal control by estrogens.

Figure 316. Scanning micrograph of the epithelium of the human oviduct, at mid cycle. (Micrograph from Gaddum-Rosse, Blandau and Tiersch, Am. J. Anat. 138:269-275, 1973.)
The inconsistent orientation of the cilia in scanning micrographs does not accurately reflect their state in the living cell. All of the cilia on a cell would normally be in the same phase of their cycle of beating. Routine methods of fixation are incapable of instantly immobilizing them in the attitude typical of their effective stroke or recovery phase. They therefore tend to come to rest in a more or less straight neutral position.
The ciliated cells of mammals often have microvilli interspersed among the cilia. These transverse sections near the cell surface permit a comparison of the size and internal structure of the two types of cell process. Both are limited by a trilaminar unit membrane and have a cytoplasmic matrix of similar density. The nonmotile microvilli have no resolvable internal structure, whereas the motile cilia have a consistent pattern of nine doublet microtubules uniformly spaced around a central pair of singlet microtubules. This nine plus two complex was visualized by the early light microscopists as a single axial fiber and was therefore called the *axoneme*. The term has been retained even though it does not accurately describe the microtubule complex revealed by the electron microscope.

**Figure 319.** Epithelial cilia and microvilli from rat trachea, fixed in aldehyde and osmium tetroxide and exposed to low molecular weight galloyl-glucoses (tannic acid). (Micrograph from Simionescu and Simionescu, J. Cell Biol. 70:608-621, 1976.)
MATRIX COMPONENTS OF CILIA

The microtubules of the axoneme are adequately preserved by routine fixation for electron microscopy of thin sections. These methods are less satisfactory for their subunits and appendages and have contributed little to our understanding of the organization of the ciliary matrix. Addition of tannic acid to the fixative results in an apparent thickening and a more intense staining of these components. The dynein arms are made more conspicuous, the radial spokes more robust, and the unstained protofilaments in the wall of the microtubules are clearly revealed in negative image.

The upper figure on the facing page is a diagrammatic representation of components of the axoneme and matrix identifiable in high magnification micrographs of tannic acid–treated cilia such as illustrated in the lower figure. The nexin links depicted are seldom clearly visualized. The foreshortening of the radial spokes in the diagram reflects the prevailing uncertainty as to the exact site of linkage of the spoke heads to the central microtubule complex. The central sheath is interpreted by some investigators as a thin filament wound helically around the central pair, but it usually appears in cross-sections as two symmetrical arciform elements joining the walls of the microtubules instead of a circumferential sheath. The distance between the central microtubules is somewhat exaggerated in the diagram and the connecting bridge is therefore longer than it appears in micrographs. Negatively stained preparations of disrupted cilia have proved more informative than sections for visualizing the bridges between the central pair and the appendages of the doublets (see Fig. 320), but the linkage of the spokes to the central microtubule complex is broken in dissociation of the axonemes.
The disposition of the dynein arms along the length of the doublets is studied to best advantage in dissociated cilia examined with negative staining. The upper figure (A) shows parts of three overlapping doublets from a demembranated cilium disintegrated in the presence of ATP. Figure 3205 is a reinforced image of the same micrograph produced by a two-step linear translation. Free dynein arms can be seen projecting from the lowermost doublet at intervals of 24 nm along its length. Somewhat less clearly shown are arms bridging the interspaces between the three doublets. During active sliding, the space between doublets is considerably less than that usually seen in electron micrographs of thin cross sections. The microtubule sliding responsible for ciliary bending is believed to be due to attachment of arms on subunit A of one doublet to subunit B of the adjacent doublet followed by a conformational change resulting in arm deflection.

Figure 321A is a thin longitudinal section of a cilium showing the radial spokes joining subunit A of a doublet to a row of projections along one of the central pair of microtubules. The spokes occur in groups of three with a repeat of 86 nm. Figure 321B is a reinforced image of the same micrograph resulting from linear translation in steps corresponding to 86 nm between successive exposures.

---

**Figure 320.** Negatively stained preparation of dissociated axonemal doublets. (From F. D. Warner and D. R. Mitchell, J. Cell Biol. 76:261-277, 1978.)

**Figure 321.** Longitudinal section of a cilium. (From F. D. Warner and P. Satir, J. Cell Biol. 63:35-63, 1974.)
Our current concept of the complex organization of a doublet microtubule and its appendages is presented in the accompanying diagram based on data from optical diffraction analysis and computer reconstruction of ciliary and flagellar axonemes. The wall of the microtubule is made up of heterodimers of tubulin arranged in tandem to form protofilaments. Two rows of dynein arms project from the A-tubule with an axial repeat of 24 nm. The arms are staggered such that the outer arms are 9 nm nearer the flagellar base than the corresponding inner arm. Three subunits are discernible in each arm. The members of the radial spoke triplets are unevenly spaced 32 nm and 24 nm apart with the longer spacing nearer the flagellar base. The exact sites of attachment of the arms and spokes on the surface lattice of the A tubule have yet to be established.
Cilia and flagella usually occur on motile cells or on the free surfaces of epithelia. However, a solitary cilium is a normal occurrence on the cells lining the intrahepatic bile ducts, the intercalated ducts of the pancreas, the rete testis, and certain segments of the nephron. The functional significance of these processes is not evident, since it seems unlikely that their motility could do more than create local turbulence in the fluid contents of these excretory ducts.

Rudimentary cilia other than those that project from the free surface of epithelia were first observed in electron micrographs of cells in the anterior pituitary (Barnes, 1961) and on fibroblasts and smooth muscle cells of chicks (Sorokin, 1962). They have since been reported in some thirty-six avian and mammalian tissues (Scherft and Daems, 1967) and in several cell lines in tissue culture (Brinkley and Stubblefield, 1967; Rieder et al. 1979; Albrecht-Buehler and Bushnell, 1980).

Especially puzzling is the occurrence of rudimentary cilia on mesenchymal derivatives such as smooth muscle cells and fibroblasts. As illustrated in the accompanying micrograph, these grow out from one of the centrioles in the juxtanuclear Golgi region of the cell. The doublet microtubules of the axoneme terminate a short distance beyond the basal body. The tapering cilium shown here passes out of the plane of section, but its slender terminal portion, devoid of an axoneme, is seen again at the arrow.

Such abortive cilia usually lack the central pair of microtubules and are probably immotile. Because modified cilia are found in photoreceptors and in olfactory epithelia, it has been suggested that these solitary cilia might have a sensory function but there is no evidence to support this speculation. It seems probable that they are merely anomalous, rudimentary structures with no function.
MODIFIED CILIA

The outer segments of the rods and cones of the vertebrate retina and many photoreceptors of invertebrates begin their differentiation as single cilia with a typical basal body and doublet microtubules forming a more or less complete axoneme. In their subsequent development, membranous discs containing the visual pigment accumulate within the confines of the ciliary membrane and become closely packed in parallel array to constitute the outer segment of the photoreceptor cell. Vestiges of the axonemal microtubules (at arrows) persist along one side of the outer segment which is connected to the inner segment by the proximal portion of the shaft of the original cilium.
STEREOCILIA

Histologists using the light microscope recognized a category of epithelial cell processes which had the appearance of cilia but showed no motility. These were called stereocilia to distinguish them from the motile form, or kinocilia. The stereocilia have a rather limited distribution, being found on the epididymal epithelium of mammals, and in certain sense organs, notably the organ of Corti, the crista ampullaris, maculae utriculi, and maculae sacculi of the mammalian inner ear; the lateral line organs of fish; and the sensilla of insects.

With the electron microscope, the stereocilia in these several organs were found to differ in their fine structure. Those of the epididymis were found to be as long or longer than kinocilia but more slender and quite flexible. They have no obvious internal structure other than an axial bundle of actin filaments which often extends some distance downward into the apical cytoplasm. They differ from microvilli only in their length and greater flexibility. Those of sensory organs are relatively thick, stiff bristle-like appendages, with a shaft that is often narrower at the base than in its more distal portion. The interior is filled with closely packed, longitudinally oriented filaments.

Stereocilia in the mammalian epididymis are slender, flexible processes resembling unusually long microvilli, but they are more variable in their orientation and less closely packed than the microvilli of a brush border. They are assumed to be a device for amplifying the surface of this absorptive epithelium.

Figure 325. Stereocilia of the epithelium of rabbit epididymis. (Micrograph courtesy of Roy Jones.)
Cells of sense organs detecting vibration are provided with stiff surface projections, the size of cilia but lacking their internal structure. The accompanying low-power scanning micrograph presents a surface view of the organ of Corti in the third turn of the guinea-pig cochlea. Three rows of outer hair cells are shown, each bearing a V-shaped array of sensory hairs. The inset shows at high magnification the hairs on two cells from the first row in the second turn of the organ of Corti. Their relative size can be judged by comparison with the microvilli on neighboring cells. These specializations do not arise from centrioles and never contain microtubules. Therefore, they cannot be regarded as modified cilia, but appear to be a unique specialization for mechanoelectrical transduction.

Figure 326. Scanning micrographs of guinea pig organ of Corti. (Micrographs courtesy of Kogaku Saito and Kiyoshi Hama.)
In longitudinal section, the sensory hairs are narrower at their base and contain a large number of parallel filaments that converge into a dense bundle in the narrow basal region of the hair and then diverge again as they continue into the cuticular plate in the apical cytoplasm.

Figure 327. Longitudinal section of the apex of a hair cell from the saccular macula of the goldfish, (Micrograph courtesy of Kogaku Saito and Kiyoshi Hama.)

References for Cilia and Flagella and Sperm Flagellum are found at the end of the next part, pp. 638-639.