CHAPTER

17

Cilia and Flagella

Euplotes with its fringe of waving cilia is attempting to consume *Paramecium* in the upper left corner.

The cilia (L.,*cili*=eye lash) and flagella (L., Little whip) are microscopic, contractile and filamentous processes of the cytoplasm which create food currents, act as sensory organs and perform many mechanical functions of the cell. Morphologically and physiologically, the cilia and flagella are identical structures but even then both can be distinguished from each other by their number, size and functions. Their distinguishing features are as follows :

1. The flagella are less (1 or 2) in number than the cilia which may be numerous (3000 to 14000 or more) in number.
2. The flagella occur at one end of the cell, while the cilia may occur throughout the surface of the cell.
3. The flagella are longer (up to 150 µm) processes, while the cilia are short (5 to 10 µm) appendages of the cytoplasm.
4. The flagella usually beat independently, while the cilia tend to beat in a coordinated rhythm.
5. The flagella exhibit undulatory motion, while the cilia move in a sweeping or pendular stroke.

STEROCILIA AND KINOCILIA

The cell sometimes gives out immobile cytoplasmic extensions known as **sterocilia**. The sterocilia differ from the true cilia which are known as **kinocilia**. The sterocilia occur in most epithelial cells of the epididymis and macula and crista of the internal ear. Sterocilia of the hair cells of the inner ear are

responsible for the transduction of sound. These and other sterocilia do not contain microtubules. They contain, however, about 3000 microfilaments which are disposed longitudinally but have a definite polarity and a helical symmetry, with cross-bridges around the filaments (**De Rosier**, 1980).

DISTRIBUTION OF THE CILIA AND FLAGELLA

The flagella occur in the protozoans of the class Flagellata, choanocyte cells of the sponges, spermatozoa of the Metazoa and among plants in the algae and gamete cells. The cilia occur in the protozoans of the class Ciliata and members of other classes and ciliated epithelium of the Metazoa. The cilia may occur on external body surface and may help in the locomotion of such animals as the larvae of certain Platyhelminthes, Nemertines, Echinodermata, Mollusca and Annelida. The cilia may line the internal cavities or passages of the metazoan bodies as air passage of the respiratory system and reproductive tracts. The nematode worms and arthropods have no cilia.

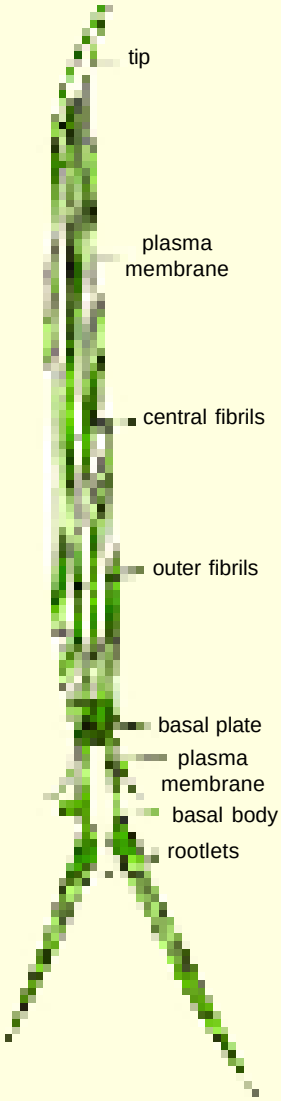


Fig. 17.1. A ciliary apparatus in L.S. showing fundamental structure of cilium or shaft, basal bodies and ciliary rootlets.

Except for sperm, the cilia in mammalian systems are not organelles of locomotion. But their effect is the same, that is, to move the environment with respect to the cell surface.

STRUCTURE OF THE CILIA AND FLAGELLA

The ciliary apparatus is composed of following basic components (1) the **shaft** or **cilium**, which is the slender cylindroid process that projects from the free surface of the cell; (2) the **basal body** or granule, a centriole like cellular organelle from which the cilium originates ; and (3) in some cells fine fibrils—called **ciliary rootlets**. Basal body remains separated from cilium by a **ciliary** or **basal plate** which has two functions : termination of the C Tubule of each triplet of basal body ; and beginning of two central microtubules. The cilia and flagella are extremely delicate, permanently formed, thread-like extension of cytoplasm and their thickness is often at the limit of the resolving power of light microscope.

ISOLATION AND CHEMICAL COMPOSITION OF CILIA AND FLAGELLA

The first detailed chemical analysis of the protein components of the cilia of *Tetrahymena pyriformis* was conducted by **I. R. Gibbons** (1963). Ciliary movements can be analyzed easily by scraping the pharyngeal epithelium of a frog or toad with a spatula and placing the scrapings in a drop of physiological salt solution between a slide and a coverglass. By certain recent techniques, a flagellum can be severed from a cell by a laser beam and ciliary membrane can be peeled off by detergent treatments.

Axoneme of cilia has a variety of proteins such as α and β **tubulins** in the micortubules, **dynein** (the microtubule ATPase), **nexin** and others (see Table 17-1).

ULTRASTRUCTURE OF THE CILIA AND FLAGELLA

An eukaryotic cilium or flagellum is composed of three major parts : a central axoneme or shaft, the surrounding plasma memberane and the interposed cytoplasmic matrix (Fig. 17.1).

1. Ciliary Membrane

Though the ciliary membrane (9.5 nm thick) is physically continuous with plasma membrane of the cell, but it contains far less amount of proteins than the latter (*i.e.*, it is atypically protein poor; **Satir**, 1977). Further, some of the proteins present in the ciliary membrane are specific to it and have a role as the barrier against the loss of ATP and certain essential ion that are required at appropriate concentrations to provide the energy for the ciliary movement.

Ciliary necklace. An unusual feature of the membranes of all somatic cilia is the presence of multiple strands (2 to 6 and up to 11) of particles, called **ciliary necklace**, at the base of the organelle. These particles can be seen in the electron micrographs of freeze-fractured cilium (see **Fumi Suzuki**). The ciliary necklace is found at a region in the cilium where microtubules and basal bodies make contact with the membrane. According to **Thorpe** (1984), ciliary necklace may have following two functions : 1. It may position the underlying basal body from which the cilium is originated. 2. It may help in the differentiation of ciliary membrane; *i.e.*, the rings of particles may retain proteins that would otherwise diffuse out and be incorporated into ciliary membrane.

2. Matrix

The bounded space of the cilium contains a watery substance known as **matrix**. In the ciliary matrix are embedded eleven microtubules of axoneme and other interconnecting proteins.

3. Axoneme

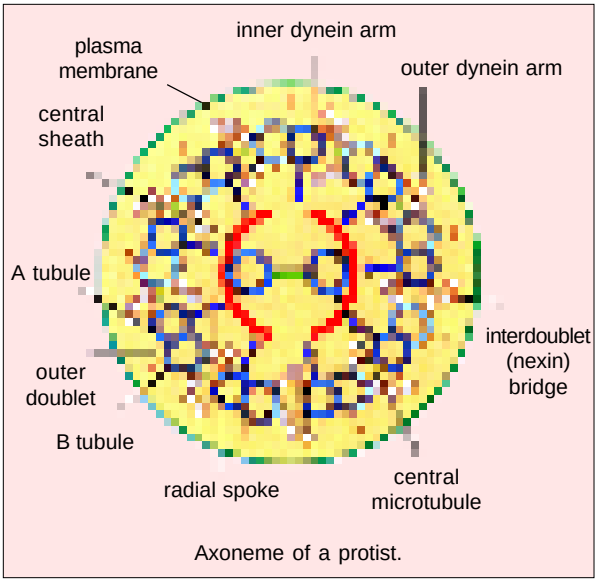
The axial basic microtubular structure of cilia and flagella is called **axoneme**. It is the essential motile element of these organelles. The axoneme is about 0.2 to 10 µm in diameter and may range from a few microns to 1 to 2 mm in length. The cilia may be thicker at the base and may become thinner gradually along the length.

Table 17.1. **Major protein structures of the axoneme of the cilia and flagella (Source : Alberts *et al.*, 1989).**

Axoneme component (periodicity along axoneme)	Function
1. Tubulin (8 nm)	Principal component of microtubules.
2. Dynein (24 nm)	Project from microtubule doublets and interact with adjacent doublets to produce bending.
3. Nexin link (86 nm)	Hold adjacent microtubule doublets together.
4. Radial spokes (29 nm)	Extend from each of the nine outer doublets inward to the central pair.
5. Sheath projections (14 nm)	Project as a series of side arms from the central pair of microtubules ; together with the radial spokes these regulate the form of the ciliary beat.

The axonemal elements of nearly all cilia and flagella (as well as the tails of sperm cells) contain the same 9 + 2 arrangement of microtubules. In the centre of the axoneme are two.**singlet microtubules** or fibrils that run length of the cilium. Each of the central microtubules (25 nm in diameter) is composed of 13 protofilaments. The central fibrils, each has a wall of 6 nm thick and are located 35 nm away from each other. Both central fibrils are connected by a bridge and are enclosed in a common central **sheath**. A plane perpendicular to line joining the two central tubules divides the axoneme into a right and a left symmetrical half. It is generally accepted that the plane of the ciliary beat is perpendicular to this plane of symmetry.

Nine **doublet microtubules** (each 36 nm in diameter) surround the central sheath; they remain separated from each other by a distance of 20 nm and from the ciliary membrane by a distance of 25 nm. Each peripheral doublet consists of two microtubules or subfibres (18 to 25 nm in diameter), one is smaller (A) and complete, having 13 protofilaments of tubulin and lying closer to the axis; the other

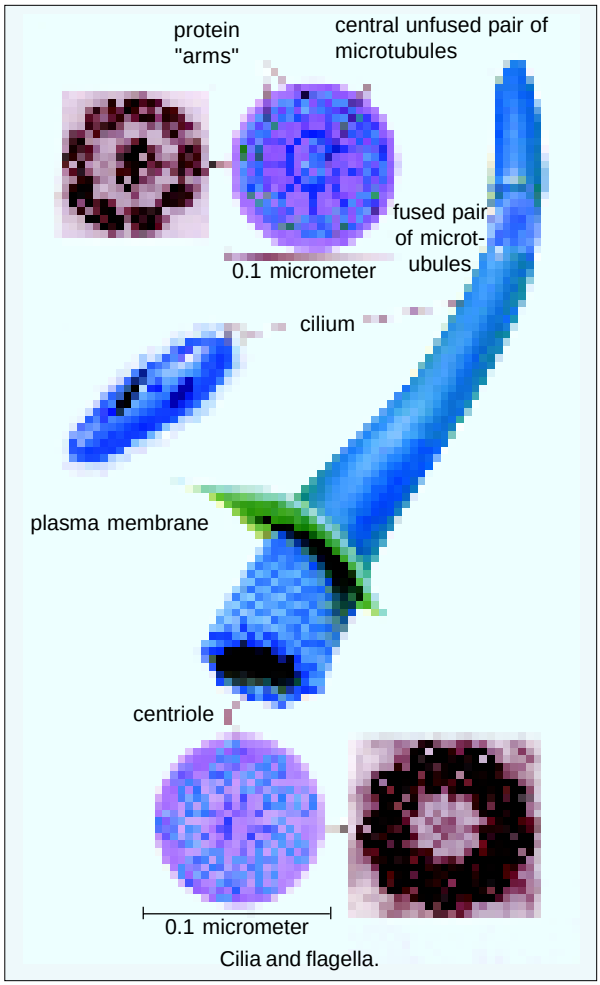


Axoneme of a protist.

subfibre (B) is larger and incomplete, having only 11 protofilaments. The B subfibre lacks the wall adjacent to A subfibre and is skewed at about 10° away from the axis. Other associated structures of the doublets are the following :

1. Dynein arms. Extending from each A subfibre are two **dynein arms**—an **outer arm** and an **inner arm**, that are oriented in the same direction in all microtubules (*i.e.*, peripheral doublets). This orientation is clockwise when the axoneme is viewed from base to tip. The arms contain **dynein**, which is large protein complex (nearly 2 million daltons) composed of 9 to 10 polypeptide chains, the largest of which are about 450,000 daltons. Dynein is a Mg^{2+} and Ca^{2+}-activated ATPase enzyme which after solubilization can recombine at the same position on the A microtubule. Dynein contains two or three elliptical or globular **heads** (depending on the source) linked to a common **root**, **foot** or **base** by the thin flexible **strands** or **stalks** (Fig. 17.3). Thus, the base of the dynein molecule attaches only to A subfibre, leaving the heads free to make contact with the adjacent B tubules of neighbouring doublet. It indicates that B tubule has different structure than the A tubule, so that, the base of dynein cannot attach to it. The resulting asymmetry is required to prevent a fruitless tug-of-war between the neighbouring microtubules, which presumably explains why each of nine outer microtubules is an A-B doublet (see **Alberts** *et al.*, 1989).

2. Nexin links. Adjacent doublets are joined or linked by **peripheral**, **interdoublet** or **nexin links** ; the nexin links have a periodicity of 86 nm. Nexin links extend from A tubule of one doublet to B tubule of adjacent doublet. Nexin protein has a molecular weight of about 150,000 to 160,000 daltons. Nexin links are highly elastic : their normal length is 30 nm, but they can be stretched to 250 nm without breaking (see **Darnell**, 1986). They are thought to function like the rubber bands



Cilia and flagella.

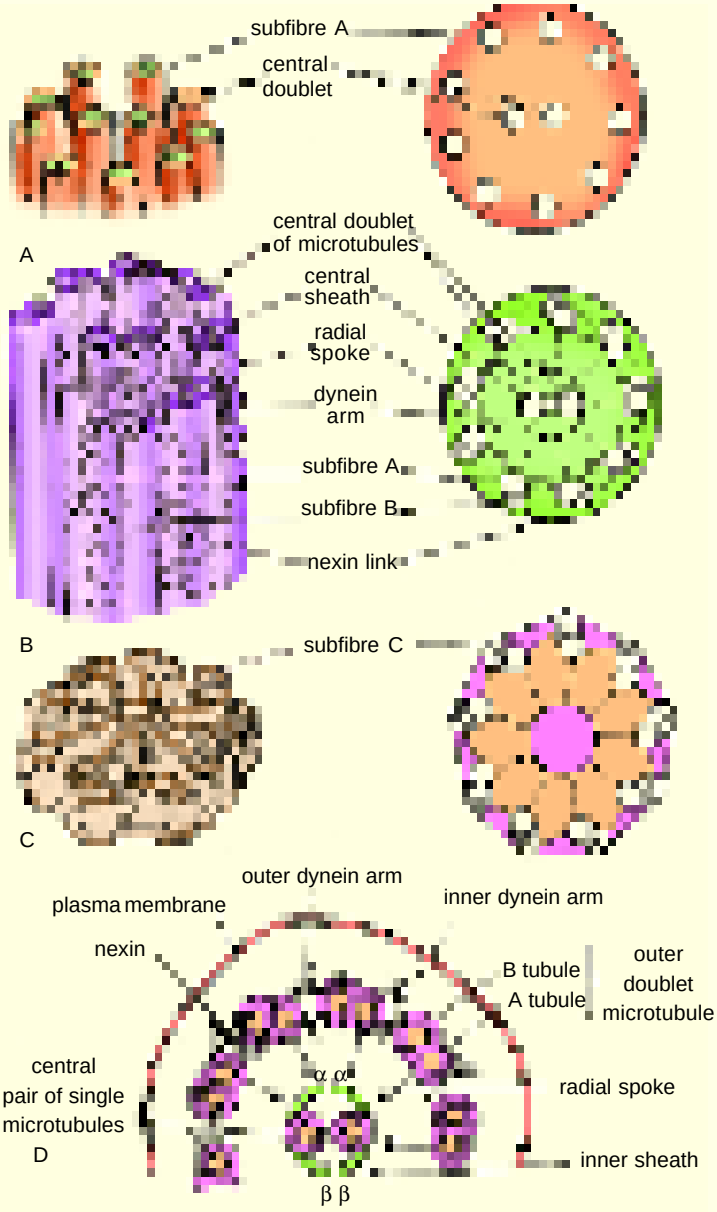


Fig. 17.2. Schematic diagram of a flagellar axoneme, showing relationships between its components at the flagellar tip (A), in the middle of the flagellum (B), and at the basal body (C). More detailed picture of flagellum in the cross section (after Berns, 1983 ; Alberts *et al*., 1989).

to resist the sliding between adjacent double microtubules (*i.e.*, they maintain integrity of the axoneme during the sliding motion).

3. Radial spokes. There are 36 nm long radial bridges or links between the A subfibre and the sheath containing the central microtubules. These spokes terminate in a dense **knob** or **head**, which may have a fork-like structure. Earlier observation of **Warner** and **Satir** (1974) that the spokes are attached perpendicularly to the ciliary axis where it is straight and that they are relatively detached in bent or tilted regions of the axis has led to the hypothesis that *they may be active in the conversion of active sliding between outer doublets into local axial bending*.

Lastly, the structures of a cilium at the base and tip are slightly different from features described above (Fig. 17.2).

PHYSIOLOGY OF CILIARY MOVEMENT

The cilia and flagella serve many purposes and their movements propel the organism. The cilia are contractile structures and in them two types of rhythms known as **metachronic** and **isochronic** or **synchronous rhythms** produce the wave of contractions in the cilia. In the **metachronic** type of rhythm the cilia of a row beat one after the other, while in the **synchronous** or isochronic rhythm, all the cilia of a row beat simultaneously. In contrast to the cilia, the flagella exhibit undulant motion and beat independently.

The beating of cilia or flagella is caused by the intraciliary excitation which is followed by the interciliary conduction. **Hayashi** (1961) has reported that the two inner filaments of a cilium transmit excitation and the nine outer filaments are the seat of ATP splitting. The movement of cilia may be under nervous or cytoplasmic control. In a few invertebrate embryos the cilia are probably under nervous control since their movement may be stopped upon stimulation of the embryo. In ciliates they are thought to be coordinated by a neuromotor centre near the mouth since destruction of the fibres connecting the centre to the cilia results in uncoordinated movements (**Taylor**, 1920). However, **Okajima** (1966) reported coordinated movements in *Euplotes* even after complete dissection of the neuro-motor fibres. Recent studies have shown that cytoplasm is necessary for the ciliary movements. The ATP provides necessary amount of energy for the motion of the cilia and flagella. Four types of ciliary movements have been recognized which are as follows :

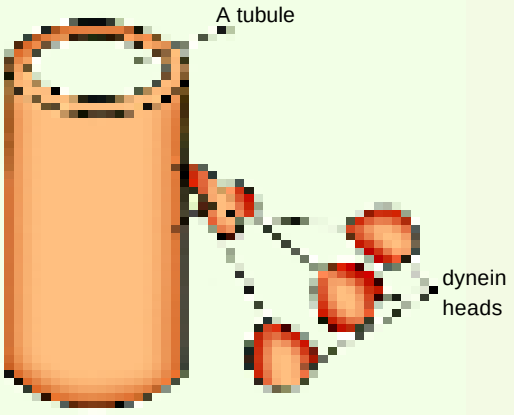


Fig. 17.3. The base of the dynein molecule binds tightly to an A tubule in an ATP-independent manner, while the large globular heads have an ATP-dependent binding site for a second microtubule (B tubule) (after Alberts *et al.*, 1989).

1. The pendulus ciliary movement. The pendulus type of ciliary movement is carried out in a single plane. It occurs in the ciliated protozoans which have rigid cilia. In such cases the movement of the cilia is carried out by a flexion at its base.

2. The unciform ciliary movement. The unciform (hook-like) ciliary movement occurs commonly in the metazoan cells. In such type of movement, when the cilia contract it becomes double and acquires a hook-like shape.

3. The infundibuliform ciliary movement. The infundibuliform ciliary movement occurs due to the rotary movement of the cilium and flagellum. In this case, the cilium or flagellum is passed through three mutually perpendicular planes in the space and makes conical or funnel-shaped shape.

4. The undulant movement. The undulant movement is the characteristic of the flagellum. In undulant movement the waves of the contraction proceed from the site of implantation and pass to the border.

Each beat of cilium or flagellum involves the same pattern of microtubule movement. Each cilium moves with a whip-like motion and its beat may be divided into two phases : 1. The fast **effective stroke** (or forward active stroke or power stroke) in which the cilium is fully extended and beating against the surrounding liquid (*i.e.*, it is like the action of an oar in a rowboat ; Fig. 17.4B). 2. The slow **recovery stroke**, in which the cilium returns

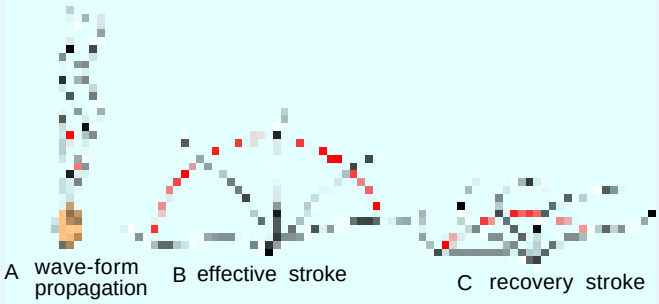
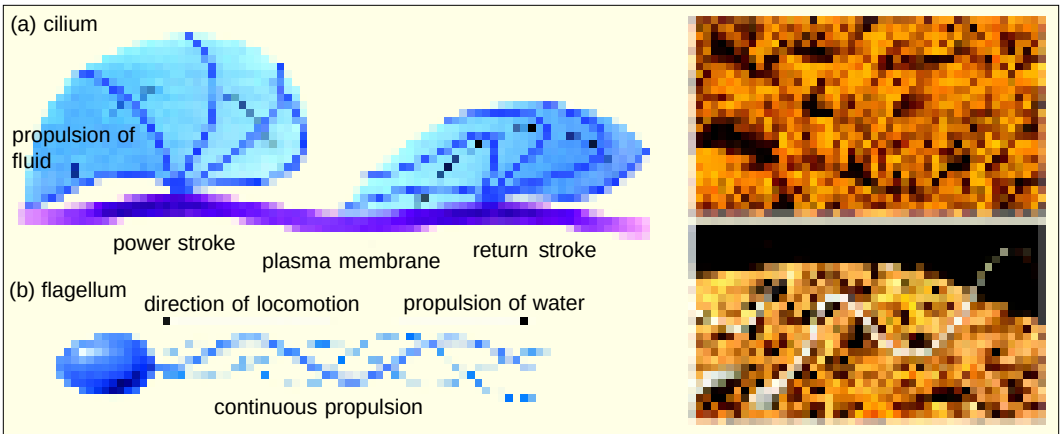


Fig. 17.4. Movement of cilia and flagella. A—Typical wave-form propagation of a flagellum, such as that found in sperm ; B—Effective stroke common with cilia of all types ; C—Recovery of cilium prior to the next effective stroke (after Dyson, 1978).

to its original position with an unrolling movement that minimizes viscous drag (Fig. 17.4B). The cycles of adjacent cilia are almost but not quite in synchrony, creating a wave-like pattern.

The flagellum instead of making whip-like movements, propagates quasi-sinusoidal waves (Fig. 17.4A), *i.e.*, successive waves move toward the tip of the flagellum, propelling the cell (*e.g.*, sperm) in the opposite direction.

The mechanism of force and movement (bending) by the flagellum has recently been studied extensively. It is well established now that the ciliary movement is generated by the microtubules and the associated structures of the flagellum. It was shown that the cell free flagella can be caused to move by adding an energy source such as ATP. Even broken pieces of cilia or isolated axoneme itself continue to beat, suggesting the role of microtubules in the movement. The contractile **axostyle** of some



How cilia and flagella move.

microorganisms such as *Metamonadida* (a dinoflagellate that lives in the gut of termite; Fig. 17.5) is another example of microtubule mediated motile process (see **Berns**, 1983). In fact, bending force is produced by the sliding of microtubules. This has been shown by exposing isolated axonemes to proteolytic enzymes, which disrupts both the nexin links and the radial spokes but leaving the dynein arms and the microtubules themselves intact. If such a partially digested structure is exposed to as little as 10 µM ATP, the axoneme elongates until it is up to nine times its original length, the component microtubules in the axoneme telescoping out of the loosened structure. It seems that the adjacent peripheral doublets can actively slide against each other once they have been freed of their lateral cross-links (such as those made of nexin). Apparently, in intact structure this sliding movement is converted to bending. Further, since the adjacent outer doublets actively slide against each other, a force must be generated between them. This force is

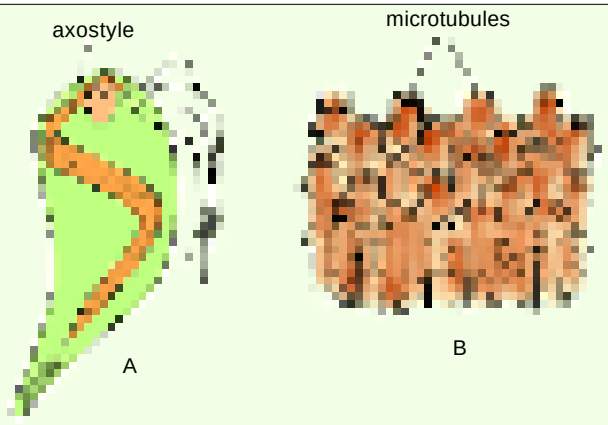


Fig. 17.5. Axostyle of *Metamonadida* flagellate. A—The relationship of the axostyle with the cell body; B—Schematic representation of the arrangement of microtubules into parallel sheets with protein cross bridges between them as seen in a T. S. of axostyle (after Berns, 1983).

apparently generated by dynein arms which 'walk' along the doublets, as has been suggested by **Peter Satir's** (1968) sliding filament hypothesis.

Sliding Filament Hypothesis

Recent experimental work on ciliary motion has shown notable similarities with the sliding mechanism involved in the interaction of actin and myosin in muscle. The dynein arms attached to subfibre A have been compared with the cross bridges of myosin and it has been postulated that they form intermittent attachments, by which one doublet (N_1) is able to push the adjacent one (N_1 + 1) toward the tip of the axoneme (Fig. 17.6). Under normal conditions, the attachment of subfibre A of N to subfibre B of N + 1 by dynein arms is not observed in an intact cilium. Only when the ciliary membrane is extracted with a detergent, the axoneme enters in a state of **rigor** in which the attachment is produced (Fig. 17.6 A). Addition of ATP to axonemes in the state of rigor restores motility and causes release of the dynein arm (Fig. 17.6 B). In this mechano-chemical cycle, the next step would be **reextension** of the dynein arm (Fig. 17.6C) and its **rebinding** at an angle, with a new, more proximal site on subfibre B (Fig. 17.6 D). This step involves the hydrolysis of ATP to ADP + Pi. In the last step, the arm returns to the rigor position and displacement of the doublets results (Fig. 17.6 E).

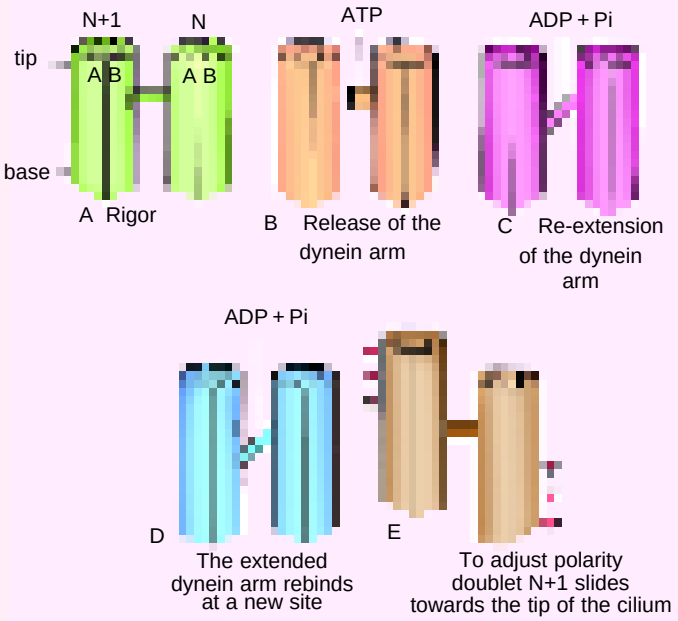


Fig. 17.6. Schematic representation of the mechanochemical cycle involved in sliding of filament in ciliary movement (after De Robertis and De Robertis, Jr., 1987).

Force is generated when dynein arms move. The movement of sliding is converted to bending by virture of radial spokes that bridge each other doublet to the inner pair of microtubules (**Warner and Satir**, 1974 ; **Huang** *et al.*, 1981). The wave that is generated by sliding is propagated down the organelle from base to tip, with the cell generally moving in a direction opposite from that of wave propagation.

An eukaryotic flagellum.

Immotile Cilia Syndrome (Kartagenre's Syndrome)

Ciliary motion can be affected by many deficiencies in the protein composition of the organelle. For example, in immotile cilia syndrome, a condition characterized by severe respiratory difficulty (chronic bronchitis and sinusitis) and male sterility, the underlying genetic defect is the

absence of inner and outer dynein arms on the peripheral doublets of both cilia and flagella. The symptoms of this syndrome result from the immobility of cilia in the respiratory tract and of the flagella in the sperm.

In *Chlamydomonas* several mutational defects have been studied in the axoneme of flagellum which may lead to paralysis of the flagellar function (**Luck**, 1984).

Other Functions of the Cilia and Flagella

1. The ciliary or flagellar movement provides the locomotion to the cell or organism.
2. The cilia create food currents in lower aquatic animals.
3. In the respiratory tract, the ciliary movements help in the elimination of the solid particles from it.
4. The eggs of amphibians and mammals are driven out from the oviduct by the aid of vibratile cilia of the latter.

Thus, the cilia and flagella serve many physiological processes of the cell, such as locomotion, alimentation, circulation, respiration, excretion and perception of sense.

ORIGIN OF CILIA

The newly formed basal bodies become aligned in rows beneath the apical plasma membrane and each basal body may then produce satellites from the side, a root from its base and a cilium from its apex.

The formation of the cilia from the basal bodies is started by the formation of a vesicle-like structure of the cytoplasm towards the distal end of the basal bodies. The walls of the vesicle are invaginated due to rapid growth of ciliary shaft. The walls of the vesicle are temporary and are replaced by the new and permanent ciliary sheaths.

DERIVATIVES OF CILIA

The cilia are modified into a variety of structures such as the rods and cones of the retina, crown cell of saccus vasculosus of third ventricle of fishes, primitive sensory cells of the pineal eye and cnidocil of the nematocysts of the coelenterates.

REVISION QUESTIONS

1. Differentiate between cilia and flagella. Describe the structure of the axoneme.
2. Explain sliding microtubule hypothesis of ciliary movement.
3. Flagella and cilia, though identical in structure, commonly exhibit a quite different pattern of movement. Describe the two patterns and conditions under which one or the other would be more appropriate.
4. Write short notes on the following :

   (a) axoneme ; (b) sterocilia ; (c) ciliary necklace ; (d) axostyle ; (e) sliding filament hypothesis ; (f) Kartagenre's syndrome