Protozoan Organelles of Locomotion

Secondary article

Michael A Sleigh, *University of Southampton, Southampton, UK*

Structures responsible for locomotion of single-celled protozoa are referred to as organelles because they comprise only part of a cell that is performing all of the functions of a complete organism. Organelles that produce motion may move the whole organism from place to place, or may change the shape of the cell or the position of components within it.

Article Contents

Pseudopodia, Cilia, Flagella and Other Means

Locomotion of protozoa is generally produced by changes in shape of the cell or of projections from it which either move the cell by exerting forces on sites of attachment to a substratum, i.e. creeping, or by propelling fluids past the cell, i.e. swimming. These changes in shape may involve the whole of the cell or only limited structures within it or projections from it. Most of the types of structure responsible for these forms of locomotion of protozoa are also found in metazoan animals, where they serve comparable functions, although they show greater diversity and versatility among protozoa. A few protozoa show gliding movements in which no changes in body shape can be seen as the cell progresses smoothly over a surface.

Amoeboid locomotion is characterized by flowing movements of internal cytoplasm, usually aided by attachments of the membrane to the substratum, which result in the protrusion of temporary extensions of the cell named pseudopodia. Pseudopodia have different forms in different protozoa, and participate in locomotion in a variety of different ways, as well as assisting in food collection.

Cilia and flagella are permanent projections from the cell surface containing a fibrous core. Their thickness is about 0.25 μm and their length generally between 5 and 30 μm. They actively and rapidly change in shape by bending and in so doing they propel the surrounding water, enabling the cell to swim. The beating patterns of cilia and flagella differ, and these and the mechanism by which bending is produced will be described later. Many protozoa also extract food particles from the water current created by ciliary or flagellar beating.

Many protozoa show changes in body shape as an avoidance response to a disturbing stimulus; in general these do not result in locomotion, only retraction of part of the body from the site of stimulation. Avoidance responses, by their nature, are generally rapid, and are brought about by sudden changes of shape of fibre systems such as myonemes.

Internal Cyclosis

The cytoplasmic organelles within cells of all types are moved to the sites where their functions are required to be performed. Generally specific organelles are moved individually at suitable times in their development or in the life of the cell. Some protozoa show a more rapid mass movement of a major part of the internal cytoplasm of the cell. Typically this takes the form of a circulation or cyclosis of the cytoplasm and any organelles that lie free within it. Cyclosis of cytoplasm around the cell is clearly seen in many ciliates, and spectacular examples of cyclosis are seen in some algal cells, e.g. *Nitella*.

Mechanisms of Movement

Movement by pseudopodia

The shape of a pseudopodium and any additional characteristic features it may possess give rise to the names given to pseudopodia of different types. Thus, the large pseudopod which develops as an extensive lobe during the movement of *Amoeba proteus*, and which contains a substantial part of the cytoplasm, is called a lobopodium. When the pseudopodium emerges as a broad flat tongue it is a lamellipodium, and when it takes the form of a long, narrow projection it is a filopodium. Filopodia may branch outwards, but the foraminiferan pseudopodial system takes the form of a highly dynamic, and constantly moving, branching network of very slender projections containing conspicuous granules – granuloreticular pseudopodia. Long, slender projections like these require some internal support unless they move along a surface; this support is provided by internal fibres. There are only a few fibres in foraminiferan reticulopods, but they form a very thick bundle in the stiff radiating axopodia of the Heliozoa and Radiolaria. The extensive branching network of cytoplasmic tubes that forms the body in slime moulds, which show affinities with amoebae, is called a plasmodium (not to be confused with the genus *Plasmodium*, to which the malarial parasites belong).

If the formation and extension of a lobopodium is to result in progression over a surface, the membrane surrounding the pseudopod must attach to the substratum so that cytoplasm may be pushed forwards and the posterior part of the cell may be drawn forwards towards the new attachment. There have been a number of theories of the mechanism of this form of amoeboid movement, but the present consensus is that the early (1926) explanation of Mast is probably near the truth (Grebecki, 1986). The flow of cytoplasm in the formation of a lobopod is assumed to be produced by the contraction of elements within the stiffer cortical layer of cytoplasm (ectoplasm) which lies just beneath the membrane exerting pressure upon the more fluid endoplasm. A squeezing action by the ectoplasm at the posterior end, a holding tension of the ectoplasm of the lateral walls and a thinning and weakening of the ectoplasm at the tips of advancing pseudopods together direct the flow forwards (**Figure 1**). Stimulation of an advancing tip, for example by a bright light, will stiffen the ectoplasm there and cause the emergence of a pseudopodium at another place and in another direction. Alternatively, contact with a food particle will encourage the formation of lateral pseudopods which extend around the food particle to surround it and form a food cup and subsequently a food vacuole. In the posterior contraction region the ectoplasm becomes fluid as it is converted to endoplasm, while at the advancing tip of the pseudopod the flowing endoplasm transforms to ectoplasm to extend the wall of the pseudopod forwards. This 'pressure-flow' mechanism contrasts with a 'fountain zone' theory that was popular several decades ago, in which contractile activity near the anterior end of the advancing pseudopod was thought to pull a stiff central core of cytoplasm forwards until it spread out like a fountain to form the extending ectoplasm just behind the pseudopod tip (Allen, 1973).

The same type of pressure-flow mechanism is held to be responsible for the shuttling flow of syncytial endoplasm within the tubes of the slime mould plasmodium. Here the endoplasm flows away from a region of the network where contraction of the ectoplasm is constricting the tubes and towards a region where the tubes are relaxing and dilating. After a time another part of the network will contract and endoplasm will flow in new directions, and so on with frequent changes in the pattern of flow. In addition to such shuttle flow in older parts of the network, the pressure will cause the extension of the network at its periphery by the formation of new tubes. Contraction and constriction appears to cause thinning of the walls of the tubes as ectoplasm is converted to endoplasm, while in the dilating regions the walls of the tubes are becoming thicker as endoplasm is converted to ectoplasm.

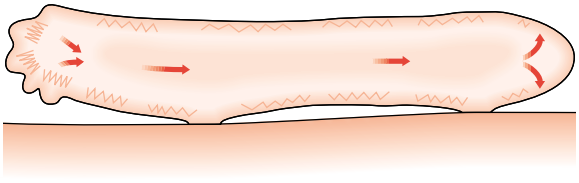

Figure 1 Diagram to illustrate the principles believed to drive the movement of *Amoeba*. Actin filaments exist in the cortical ectoplasm, and are capable of exerting contractile pressure on the more fluid endoplasm. Contraction of ectoplasm at the tail end (left) causes solation of the cytoplasm and depolymerization of the actin filaments. The pressure created propels endoplasm towards the right through a tube whose walls exert enough tension that endoplasm only escapes at the tip of the extending pseudopod (right end). Here endoplasm spreads and gelates to extend the ectoplasmic tube, as actin filaments repolymerize. (Modified from Sleigh, 1989.)

The fibres supporting reticulopods and axopods are microtubules. These microtubules are arranged in loose groups in reticulopods but in precise crosslinked arrays which are characteristic of particular taxa in the case of axopodia. Although the length of reticulopods and axopods is maintained by these internal fibres, both of these types of pseudopod are constantly changing in length because the microtubule bundle can be shortened and extended by removal or addition of subunits at the end of the protein fibre. Generally these movements are slow, of the order of a few micrometres per minute, but in some cases the retraction of axopodia takes place by almost instantaneous collapse of the microtubule fibres within a few milliseconds and is followed by a slow reextension. Change in length of the reticulopods of foraminiferans enables them to extend their feeding net to new areas, and, in the case of benthic forms, to explore new surfaces. Heliozoans and radiolarians use axopod retraction in feeding, for prey organisms that swim into axopods are caught by sticky secretions on the axopod membrane and drawn towards the main cell surface by shortening of the axopod prior to enclosure of the prey in a food vacuole.

Movement of cilia and flagella and related organelles

All cilia and flagella are supported by an axoneme with the same characteristic and highly uniform '9 + 2' arrangement of longitudinal microtubular fibres surrounded by cytoplasm within a slender extension of the cell membrane, and anchored within the cell by root fibres (Warner, 1974). The fact that cilia and flagella have identical structure has led to attempts to use terms like 'undulipodia' to cover both forms of organelle. However, cilia are functionally different from flagella – which is why the two forms of organelle were recognized as being different by the early microscopists; almost universally, therefore, authors have continued to use the two separate terms with their specific meaning. It is true that the bending motion which is responsible for fluid propulsion by organelles of both types takes the form of undulations which pass along the organelle from one end to the other, and the molecular

basis of bending by the production of active sliding between microtubules is the same in both, but differences in the timing of bending in cilia and flagella result in differences in the direction of propulsion of the surrounding fluids.

There is typically more than one wavelength of the bending pattern within the length of a flagellum (**Figure 2a**). As the bends are propagated along the organelle they push on the surrounding water symmetrically at the two sides, with a net propulsion of water parallel to the length of the flagellum, perpendicular to the cell surface, and (normally) in the direction in which the waves move (Sleigh, 1991). The beat of a cilium is asymmetrical, with generally less than one wavelength of the bending pattern within its length (**Figure 2b**). In the bending of the whole organelle to one side it sweeps through a large arc, but the bending back to the other side quickly follows, and a region of bending passes up the cilium to the tip. The first swing propels fluid perpendicular to the axis of the cilium and the propagated bend carries little fluid back, so that the net motion of water around a beating cilium is parallel to the cell surface (Sleigh, 1984). This difference in the way organelles of the two types propel water means that a single flagellum can achieve effective locomotion on its own, but the beating of a single cilium will only rotate the cell which bears it; hence flagella commonly occur singly or in small numbers on cells (though some cells bear thousands of them, typically inclined at an angle to the cell surface), while cilia typically occur in large numbers on cells, or occasionally work, symmetrically opposed, in pairs ('breast-stroke' fashion). It also means that flagella tend to be longer than cilia, but examples of long cilia and of short flagella are common. Wherever cilia or flagella occur in groups, they beat in coordination with one another, as a result of viscous forces between adjacent moving organelles, the pattern of coordination depending on the timing and extent of these hydrodynamic forces (Sleigh, 1984).

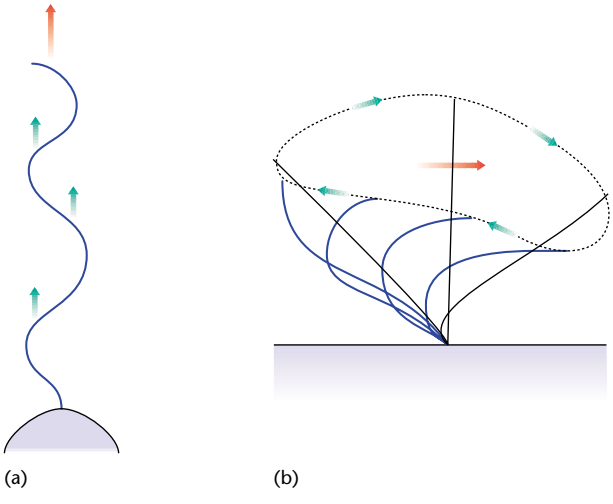

Figure 2 Comparison of the propulsion of water (red arrows) by a flagellum (a) with that by a cilium (b) as a result of motion by these organelles indicated by blue arrows (see text). (Modified from Sleigh, 1974.)

Flagella frequently bear hairs or scales on the outside of the membrane, cilia almost never have these. There is some benefit, in terms of water propulsion, for a flagellum to be somewhat thicker than the basic organelle; this extra thickness is provided by slender hairs wrapped around the axis, or small scales attached to the membrane. Thicker stiff hairs around 2 μm long, called mastigonemes, which are borne in two rows along opposite sides of the flagellum, serve a different hydrodynamic function. As undulations pass along the flagellar axis, these mastigonemes act as minute oars sweeping water in the opposite direction to the passage of the undulations. These hairs therefore reverse the direction of propulsion of water around a beating flagellum, enabling such a hairy flagellum to pull its cell body through the water, or draw a food-bearing water current towards the cell surface, when undulations pass from base to tip along the flagellum (Sleigh, 1991). This mode of flagellar functioning characterizes the flagellate cells of the Chromista, including unicellular forms and the gametes and zoospores of brown algae and oomycete fungi. The lack of additional structures on cilia reflects the fact that they gain great functional diversity by the way they are grouped together, either to make compound structures in the form of plates (membranelles) or conical tufts (cirri) or to interact by the coordination of their beating in the formation of a diversity of patterns of metachronal waves.

The haptonema of the prymnesiophyte (= haptophyte) flagellates looks like a third slender flagellum emerging between two normal flagella; it has a simpler internal structure of about seven microtubules and shows coiling movements but does not beat like a flagellum. These structures are adhesive and can function in anchoring the cell to a substratum or capturing food particles and transferring them to the posterior side of the cell where food vacuoles are formed.

Shape changes of microtubular organelles within the body of various protozoa are responsible for other forms of motion. The axostyle of *Saccinobaculus*, a large flagellate living within the hindgut of termites, is a massive bundle of microtubules. Undulations pass along the length of this axostyle and, by distorting the body of the flagellate, they cause it to writhe around, presumably aiding exchange of materials with the surrounding gut contents. Relatively slow length changes of the ciliate *Stentor*, and some other large ciliates, but not the very rapid shortening (see below), are thought to be caused by active changes in length of bundles of microtubules forming ciliary rootlet fibres in the cortex of the ciliates.

The motion of organelles like mitochondria or food vacuoles within the cytoplasm probably occurs in all

protozoa. In some cases these organelles move close to, and parallel with the length of, groups of microtubules in the cytoplasm, e.g. the movement of granular organelles alongside the microtubular axis of heliozoan or radiolarian axopodia or foraminiferan reticulopodia, or the motion of food vacuoles along a microtubule bundle or sheet leading into the cytoplasm from the cell mouth in many ciliates. The cyclosis of cytoplasm in some cells may be driven by such active motion along microtubules, but in other cases it is likely that cytoplasmic flow is powered by a mechanism more like that which forms pseudopodia in *Amoeba* or drives the reversible flow of cytoplasm in slime mould plasmodia.

There is probably a link between the types of motion just described and some reports of gliding locomotion among protozoa. Various flagellates glide smoothly over substrata with only their flagella in contact with the surface, or can carry attached granules along the outside of the flagellum. In these cases there is no undulation of the flagella which could be responsible for the motion. A similar gliding of the cell over a substratum and transport of particles along the cell membrane occurs on the axopodia of heliozoans. Gliding of cells along surfaces without change in shape is a characteristic of the sporozoites of apicomplexans (sporozoans), where microtubules lie just under the surface membrane, although it is also well demonstrated by large growing cells of species of *Gregarina* in the gut of locusts where microtubules are not evident in the surface ridges of the cell (King, 1981).

Myonemes and similar structures

Various protozoa exhibit extremely rapid contractions resulting from the shortening of internal fibre systems composed of filaments which differ from those causing the motion of cilia and flagella or amoeboid shape change. In ciliates the contractile coiling of the stalk of peritrichs like *Vorticella*, as a result of shortening of a spiral 'spasmoneme' thread within the stalk excited speculation because of its speed (Amos, 1975). The sudden contraction of the body of *Stentor* or *Spirostomum* by shortening of myoneme fibres which take the form of a basket-work of filament bundles in the cortex of these large ciliates is nearly as quick. These large myonemes may be merely a condensation and enlargement of a system of cortical filaments present in many ciliates. Sudden folding of the flagellum of the dinoflagellate *Ceratium* is the result of shortening of a striated fibre alongside the 9 + 2 axoneme within the flagellum, and root fibres associated with the flagellar basal bodies of such flagellates as *Tetraselmis* show a similar contractility. Twitching movements of the surface of the acantharian *Acanthometra* are associated with the shortening of myoneme fibres connected to the skeletal rods.

Molecular Bases

Motion in protozoa is associated with filamentous proteins of three known types, the first two of which have similar roles throughout eukaryotes. Microtubules are cylindrical fibrils around 25 nm in diameter and of indefinite length, whose walls are composed of 13 longitudinal rows of tubulin molecules, and which extend unidirectionally from some form of microtubule organizing centre, such as a ciliary basal body. Since these fibrils are tubular, they have some resistance to longitudinal compression and lateral distortion as well as to longitudinal extension (like a straw); they can be used to push as well as pull, and form strong skeletal elements, especially in crosslinked bundles. By contrast, microfilaments, which are about 7 nm thick and composed of two strands of actin filaments twisted around one another, can resist extension but have limited resistance to longitudinal compression or lateral distortion (like a string); these can be used to pull but not to push, unless they form part of an extensively crosslinked structure. No special structure is required to nucleate the formation of a microfilament. Microtubules interact with dynein adenosine triphosphatase (ATPase) proteins to cause movement, while microfilaments interact with myosin ATPases. Myonemes are constructed of protein filaments which differ from microfilaments and microtubules in that they do not need an associated ATPase protein for contraction. Although proteins of the class present in myonemes are found in higher animals, they do not appear to produce comparable contractions.

Microtubules and motility

Slow motion is produced by the polymerization and depolymerization of the tubulin molecules, at the ends of microtubules. At least part of the movement of chromosomes during mitosis is believed to be based on these processes. In protozoa slow movement of heliozoans by change in length of axopods depends on these changes in length of microtubules (principally at their distal 'plus' ends), but the sudden collapse of the axoneme in some species is thought to require simultaneous depolymerization throughout the length of the microtubule, probably mediated by change in calcium ion concentration.

The more important roles of microtubules in protozoan movement result from their interaction with dynein ATPase. Dyneins are large molecules composed of several subunits and with two anchorage points, one sensitive to the presence of ATP and the other ATP-insensitive. A dynein is typically permanently attached to one microtubule (A) by its ATP-insensitive (basal) end and can form a transient link to another microtubule (B) in an ATP-dependent manner at the other (distal) end (**Figure 3a**). The distal end of a dynein molecule is released from its attachment to a B microtubule when an ATP molecule

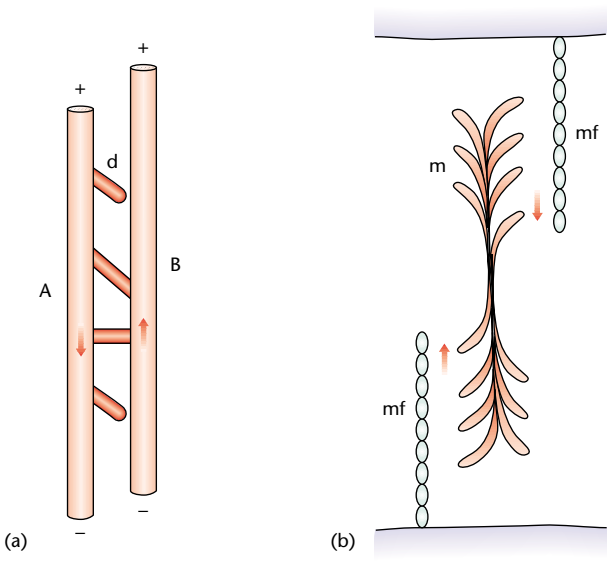

Figure 3 Comparison of (a) the action of dynein molecules (d) in generating sliding motion between two microtubules, A and B, with (b) the action of a myosin filament (m) in drawing together the attachment points of two actin microfilaments (mf) (see further description in the text). (Modified from Sleigh, 1989.)

binds to the dynein. The binding of ATP changes the shape of the dynein so that it tilts towards the minus end of the A microtubule. The ATP is rapidly split to adenosine diphosphate (ADP) and phosphate, which remain bound to the dynein, which is now in an activated state. The activated dynein is ready to bind to a tubulin of the B microtubule, and in doing so it releases the ADP and phosphate. At the same time it also changes shape once again so that its tip moves towards a position perpendicular to the A microtubule, and, since it is now bound to the B microtubule, it pushes the B microtubule towards its plus end. Repeated cycles of attachment and detachment, linked with a supply and splitting of ATP, result in unidirectional relative sliding between the A and B microtubules, such that the B microtubule slides in a plus direction relative to the A microtubule. Such relative sliding has now been clearly proved to be the basis of the bending movements of cilia and flagella, and is probably the cause of the undulations of flagellate axostyles and sliding of cytoplasmic structures along microtubules, including some forms of cyclosis and gliding.

In cilia and flagella the peripheral doublets of the axoneme consist of one A microtubule and one B microtubule. The dyneins permanently attached to the A microtubule of one doublet form transient bonds with the B microtubule of the adjacent doublet, and in doing so they cause sliding of the adjacent doublet tipwards in relation to the first doublet (Satir, 1974; Warner and Mitchell, 1978). The dyneins on doublets at one side of the axoneme are active in order to achieve bending of the axoneme in one direction, then later dyneins on doublets at the other side actively generate sliding to bend the axoneme back again. Dyneins which are not active during each half of the bending cycle must allow the doublets at the inactive side to slide passively. It is assumed that additional structures in the axoneme, such as the peripheral links and the radial spokes which can form links with the central complex including the two separate microtubules, serve functions in relation to the translation of sliding into bending, the plane of bending and the control of which dyneins are active at particular parts of the bending cycle. Controls of this type determine the beat shape – flagellar or ciliary, or more subtle variants of these principal patterns.

The contractile action of cytoplasmic actin microfilaments requires myosin in the same way as in muscle contraction, but without the same precise pattern. In amoebae it is thought that much of the actin is depolymerized, and only polymerizes to form filaments in the cortex as required for purposes of contraction. Two actin filaments of opposite polarity can be drawn together by a bridging filament of myosin in which the heads of the myosin molecules have opposite polarity at the two ends of the myosin filament (**Figure 3b**). The myosin heads change in shape cyclically with ATP binding and splitting in a precisely comparable manner to dyneins, the active motion of the myosin head pulling an attached actin microfilament towards the centre of the myosin filament. Actin microfilaments are anchored to membranes or cytoplasmic organelles at their plus ends. If the heads on the two ends of a myosin filament 'walk' along two actin filaments of opposite polarity towards their plus ends, then these two attachment sites will be drawn together and contribute to contraction of the cytoplasm. The simultaneous contraction of many actin and myosin filaments in different parts of the cortex of an *Amoeba* can propel cytoplasm to form pseudopodia and contribute to locomotion.

Different patterns of locally organized actin filaments can create a variety of contractile shape changes. Thus, they can locally cause membrane invagination to form a food vacuole, and indeed to constrict its neck to pinch it off from the cell membrane. They can also constrict the narrowing connection between two daughter cells during cell division. In addition, actin filaments attached at the cell cortex can be used to propel cell organelles to which other actin filaments are attached, and in this way may generate cyclosis of cytoplasm.

Myonemes are formed of filaments about 10 μm thick, consisting of a single type of protein named centrin (or spasmin, according to its origin) (Amos, 1975). The molecules of this protein can exist in two states, according to the calcium concentration (Salisbury, 1983). It is thought that when centrin binds calcium ions the molecule changes shape, so that filaments of the protein suddenly shorten, only to reextend again when the calcium ions are withdrawn once more. The contraction of these filaments

can be made to occur repeatedly without any need for ATP if the calcium concentration is raised and lowered. In life, the cell uses ATP to pump calcium ions across a membrane out of the cytoplasm and into storage vesicles. Stimulation of the cell causes release of calcium ions from the stores and almost instantaneous shortening of the centrin filaments.

Hypotheses and Facts Regarding Structures and their Functions

The proteins involved in the three types of molecular mechanism described here have now been extensively studied, and their properties are well understood. Microfilaments and microtubules are believed to function in basically similar ways in all types of eukaryote cells. However, a great variety of movements is exhibited by different protozoa, and much remains to be discovered about the way in which these proteins interact and are controlled to produce the observed diversity of motion.

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