Biology 3102 – Microbial Eukaryotes
Supplementary Course Material #2, chapter 2
Fall 2020

LOCOMOTION

Swimming using flagella or cilia

The action of flagella/cilia

Eukaryotic flagella (and cilia) are a remarkable motor system that has been retained throughout the evolutionary history of eukaryotic cells. Although some variation exists, the basic structure of the flagellar axoneme is very similar across most living eukaryotes, even those most distantly related to one another.

The flagellar axoneme is fundamentally a flexible tube of nine microtubule doublets that interact along their length via the motor protein dynein: Dynein arms anchored to one doublet interact with one of the adjacent doublets. With the expenditure of ATP, the dyneins attempt to slide one microtubule doublet 'along' the other, such that if the doublets were not all anchored together, the flagellum would pull itself apart. However, the doublets are rigidly connected together at their base by the basal body structure, and are constrained to remain 'in formation' by connections between adjacent doublets (the Nexin-Dynein Regulatory Complexes; N-DRC; Beeby et al. 2020). Because of these connections the sliding motion is limited, and is translated into a bending motion.

How do flagella manage to control this bending motion to produce a regular beat? Obviously only some of the dyneins can be active at any one instant. In principle, if the activity of dyneins cycled regularly around the axoneme, the flagellum would have a helical beat (Beeby et al. 2020). This simple beat pattern is indeed seen in some cells. However, many flagella and cilia in nature have beat patterns that show a flattened beat envelope (e.g. cilia, many flagella – see below), or are essentially beating entirely in one plane (uniplanar). This requires that dynein activity alternates between opposite sides of the flagellum. There is good evidence (e.g. from studies of mutants, and inhibition studies in *Chlamydomonas*) that such an alternation of dynein activity is controlled in part by interactions with proteins attached to the central pair microtubules, mediated by the radial spokes that point inwards from each microtubular doublet (See Part 2, Chapter 1).

It is important to remember also that the dynein arms are arrayed along the length

of each microtubule doublet in the axoneme. Different sequences of dynein activity along the length of the microtubule doublets can result in different wave propagations along the flagellum (e.g. base-to-tip waves, vs tip-to-base waves – see below). The distribution of dyneins also allows cells to produce beat patterns that rely on the tip of the flagellum behaving differently to its basal portion, for example.

Swimming and the small cell

Compared to the objects that we commonly see travelling through water (fish, ships, human swimmers), most microbial eukaryotes are extremely small. This has profound consequences for how they swim.

There are two primary considerations when moving through fluid that need to be taken into account – *inertia* (associated with moving mass), and *viscosity* (resistance to flow, or internal friction of the fluid). When humans swim in water, for example, inertial forces dominate, and viscous forces are relatively insignificant. The opposite is true for very small organisms, such as microbial eukaryotes, where viscosity dominates.

For a body moving through a fluid, the relative importance of inertial and viscous forces is expressed by the Reynolds number (Re).

$$Re = \rho v l / \mu$$

Where:

ρ = density of the fluid,
v = velocity,
l = length scale (related to diameter/length of the body), and
μ = the viscosity of the fluid.

Thus, for a given fluid, large objects travelling at high speeds have high Reynolds numbers. Their movement is governed by inertial forces, with viscous forces being relatively trivial. For very small objects travelling at low speeds, Reynolds numbers will be low, meaning that inertia will be insignificant and viscous forces will be all-important.

One way to think about life as a small swimming microbial eukaryote is to imagine your human self swimming in a fluid that has a similar density to water, but a much higher viscosity, such as syrup. This massively increases the denominator in the equation above, thus giving you a similar Reynolds number to that of a small microbial eukaryote in water (strictly speaking, you would also have to move your arms extremely slowly, and slow down your perception of time correspondingly, but this is mind-bending to think about).

Consequences for cells with flagella and cilia

1) For microbes, there is no 'coasting' – they come to an almost-instant halt once they stop doing work. Compare this to a motorboat in water, which continues to move forward after the motor is turned off. Or consider a human swimmer using breast-stroke, who will continue to coast forwards between each stroke. Again, to have an intuition about life at low Reynolds numbers, imagine the motorboat or the breast-stroke swimmer in syrup rather than water (or imagine how fast you would stop if you dove into a lake full of syrup).

2) Tiny objects are effectively surrounded by a clinging 'layer' of water (note - not a sharply-defined layer) that is thick relative to the size of the object. This means that there will be a larger envelope of fluid all around a flagellum or cilium that is 'transported' along with the flagellum/cilium when it moves relative to the cell (though see note below). The envelope of transported fluid around a flagellum or cilium is much larger than the flagellum/cilium itself. This helps explain why cilia and flagella can work as 'oars' without actually being paddle-shaped.

By the same token, however, the cell body itself is also surrounded by a clinging envelope of fluid. Sometimes this is referred to as the 'no-slip zone' or 'boundary layer'. Fluid close to the cell surface will tend to remain 'with' the cell, rather than with the cilium or flagellum. As a consequence, the effectiveness of cilia/flagella increases with distance from the cell body, and the envelope of fluid transported by a cilium (for example) can be visualized as a diffuse volume shaped like a single-scoop ice cream cone.

Note: In the discussion above we refer to fluid being 'transported' (i.e. moved) by a beating flagellum or cilium, and we will continue to use this convenient description in the sections below. This is indeed what happens if the cell is fixed and unable to move - the action of the flagellum will transport fluid past the cell. However, when the cell *is* free to move the cell will be 'pushed' though the fluid in the opposite direction with relatively little effect on the surrounding fluid. This is again a consequence of low Reynolds numbers. Compare this to a motorboat, in which the propeller thrusts large amounts of fluid backwards as it propels the boat forward - you would feel this thrust if you were a little way behind the boat. By contrast, if you were an equivalent relative distance behind the beating flagellum of a swimming cell you would not experience an equivalent force. Cells really 'drag' or 'screw' their way through water.

How do ciliary and flagellar beat?

In many cases cilia and flagella can be thought of as cylinders (albeit flexible ones). Although many flagella bear hairs and other accretions of various kinds, many flagella and cilia are naked, and thus relatively smooth. We will consider smooth flagella/cilia first:

At low Reynolds numbers, the viscous drag on a long, thin, smooth cylinder is about two times greater perpendicular to the long axis of the cylinder than parallel to it. This difference in drag relates to the difference in the amount of fluid transported by the moving flagellum or cilium: In other words much more fluid will be transported along with a cylinder being moved perpendicular to its long axis than would be transported by the same cylinder being moved parallel to its long axis.

Ciliary beat

The ciliary beat is relatively intuitive in operation– the cilia goes through an 'effective' oaring stroke, followed by a recovery stroke in the opposite direction. Forward motion depends on the effective stroke transporting more fluid than the recovery stroke.

During the effective stroke the cilium is held stiffly and is moved through the fluid tangentially to its long axis. This maximises the amount of fluid moved with it. During recovery, the cilium curls back on itself, such that most of its motion is parallel to the long axis of the flagellum, and thus transports less fluid than the effective stroke (see previous section). The amount of fluid transported by the recovery stroke is further reduced by the

fact that the cilium is very close to the surface of the cell during the return stroke, and thus is within the no-slip zone.

The classic ciliary beat is seen in ciliates, which have hundreds or thousands of individual cilia, and also in some flagellates, including many Chloroplastida. The model chloroplastid alga *Chlamydomonas*, for example, has two 'isokont' flagella that (most of the time) beat in synchrony, with a similar ciliary beat. This is rather like a human swimmer doing breast-stroke, except that the cell stops moving forward at the end of each effective stroke because of viscous drag, rather than continuing to coast forwards between effective strokes like a breast-stroking swimmer. *Chlamydomonas* actually backs up a little during the recovery stroke. Because the flagella beat dozens of times a second, the forward motion appears smooth to the human eye, but it is actually jerky, moving in dozens of 'two steps forward, one step back' jerks every second.

Ciliates really do swim smoothly, but this is because they have large numbers of cilia, and these cilia do not move in unison. At any instant there are always some cilia in the effective stroke, propelling the cell forwards.

Flagella

The 'typical' flagellar beat is that performed by animal sperm and swimming choanoflagellates (to pick simple examples). In 'typical' flagellar beating there are regular waves passing along the length of the flagellum. The waveform varies from uniplanar (the waves travel in one plane) to a helical beat.

Consider a single planar wave propagating along an anchored flagellum – we'll call the end where the wave started the 'base' and the other end the 'tip'. At any one instant, the segment of the flagellum on the front of that wave is being moved obliquely relative to its axis and will transport fluid with it towards the tip. However, at the same time, the flagellum is being 'pulled back' through the fluid – thus if you look at the segment of flagellum at the apex of the wave it is being pulled back towards the base parallel to its axis. The flagellum achieves a net flow of fluid in the direction that the waves are propagating because the viscous drag on the flagellum is lowest parallel to its axis. [This is much easier to demonstrate in class than describe in words!].

We will consider cells with a single flagellum to begin with. Some, such as choanoflagellates (or animal sperm) have the flagellum directed posteriorly, and base-to-tip waves pass along it. Others, such as trypanosomatids (Discoba: Kinetoplastida) and pelobionts (Amoebozoa; Archamoebae), hold their flagellum directed anteriorly, and pass tip-to-base waves along it (i.e. the waves start at the tip of the flagellum and travel towards the cell). In both cases the cell swims in the opposite direction to the direction that the waves are propagated - being 'pushed' by the flagellum in the first case, being 'pulled' by the flagellum in the second case.

Stramenopile flagella

So far we have considered smooth flagella. However, many flagella in nature carry various sorts of hairs or scales. Most of these (it can be presumed) have effects on the viscous drag on the flagellum in all directions, but do not drastically alter its hydrodynamic behaviour. The hairs of stramenopiles are a striking exception, however.

In typical stramenopiles, one flagellum is directed anteriorly and has two rows of long hairs. These hairs are stiff and stick out nearly perpendicularly to the long axis of the flagellum. These hairs drastically increase the viscous drag parallel to the long axis of the flagellum such that it is actually higher than the drag perpendicular to the long axis, at least in the plane in which the hairs lie (one estimate is ~1.8 times higher). This is the opposite of what is seen with a smooth flagellum (see above). As a consequence, a uniplanar beat of the hair-bearing flagellum will cause a net movement of fluid in the *opposite* direction the propagating waves. If the cell is free, this will cause the cell to swim in the *same* direction as the waves. Thus, a typical stramenopile is 'pulled forward' by base-to-tip waves travelling along the anterior flagellum.

Anisokont (heterodynamic) flagella

Most flagellates actually have two (or more) flagella, not one, and in most cases the flagella are anisokont (i.e. different in length and/or augmentations and/or behaviour), and thus 'heterodynamic' (literally 'differently moving'). Typically one flagellum is directed posteriorly and the other laterally or anteriorly. In most cases, we do not have a clear idea of the relative contributions of the two flagella to forward motion. In some groups, it is likely that most of the power comes from one flagellum and the other imparts control rather than power, for example, acting to govern the degree of rotation of the cell (see below).

One group with strongly anisokont flagella that have been studied to some extent is the dinoflagellates. Most dinoflagellates have one posteriorly directed longitudinal flagellum and one laterally directed 'transverse' flagellum. This transverse flagellum usually runs around the cell, more-or-less at right angles to the main axis of the cell, within a groove called the cingulum. Both flagella produce base-to-tip waves. In the case of the transverse flagellum this wave is a tight helix and causes cell rotation. However, despite being directed laterally, the transverse flagellum also contributes heavily to forward motion. It is generally thought to provide half or more of the power to the cell, with the longitudinal flagellum providing the remainder. Hydrodynamic models of one species indicate that the transverse flagellum actually provides almost *all* of the power for forward motion.

Viscous coupling

Another situation to be considered is that of cells with many cilia or flagella located close together, for example, the cilia within a complex ciliary organ of a ciliate (i.e. a membranelle or cirrus). Cilia that are very close to one another lie within each other's envelopes of transported fluid. Consider two such cilia: if, hypothetically, only one were to beat, the other would tend to move passively in synchrony, 'dragged around' with the transported fluid envelope of the first. If both beat, this phenomenon leads to a mutually reinforcing tendency to beat in synchrony *without* any outside co-ordination. This is called 'viscous coupling', and it explains why complex ciliary structures are cohesive and behave in a concerted fashion without any structural connection between the cilia, and without complex external control. In fact, the same phenomenon of viscous coupling can be seen in completely ad-hoc concentrations of spirochaete prokaryotes, for example.

How fast do cells swim?

Flagella and cilia typically beat at up to 50 cycles per second (50 Hz), though up to 100 Hz

is seen in various cases (and an amazing 250 Hz has been recorded during 'backwards' swimming of one unusual ciliate; Ueyama et al. 2005). This activity is able to drive some 'normal' flagellates at speeds up to 200 μm/sec, although most flagellates are considerably slower. Dinoflagellates are often rather faster –moving at up to 500 μm/sec. Many ciliates can travel at speeds around 1 mm/sec, or even faster.

Interestingly, speed is essentially a function of the organization of the flagella and cilia, and is largely *independent* of cell body size – for example, ciliates that are 20 μm long can move at comparable speeds to ciliates that are 1 mm long. This is related to the very finite lengths possible for flagella and, especially, for individual cilia. Individual cilia cannot be longer than a certain length or they would buckle during the power stroke. Thus, a large ciliate (with a somatic ciliature consisting of individual cilia rather than cirri) can get more power only by having more small cilia on its surface, not by making each cilium longer. The number of cilia that a ciliate can have is related to the surface area of the ciliate, which increases with the square of linear dimensions, however viscous drag on the cell also increases with the square of length, balancing the additional power.

'Walking'

Some cells with flagella or cilia use them like legs, rather than oars, and will 'walk' on surfaces. This is most common in certain groups of ciliates that have a number of specialised cirri (compound cilia) on one surface of the cell. The cirri beat in a fashion that resembles a modified typical ciliary beat, with the tip of the cirrus in contact with the substrate in the effective stroke, and lifted clear during the return stroke. The return stroke is usually quicker than the effective stroke, unlike in a typical ciliary beat.

Amoeboid Locomotion

Many types of eukaryotes produce pseudopodia of various kinds, which are underlain by different arrangements of cytoskeletal proteins. We briefly discussed these in Part 1, and in Part 2, chapter 1. These pseudopodia are frequently used for surface-associated locomotion of the cell, often in addition to roles in feeding. Here we will discuss locomotion in several types of actin-based pseudopodia: broad-but-flat 'lamellipodia', fine 'filopodia', broad-rounded 'lobopodia' and, eruptive pseudopodia/'blebs'.

Of these types of pseudopodia, we know most about **lamellipodia**. They are comparatively easy to examine by microscopy because of their flatness, and they are produced by various kinds of motile animal cells. The trophic cells ('myxamoebae') of the cellular slime mold *Dictyostelium* are relatively small amoebae that are more-or-less lamellipodial, and are also studied as a model of amoeboid locomotion.

In amoebae that produce a lamellipodium the cell surface is supported by an actin network, however at least one edge of the cell is extended out into a thin, flat area that is devoid of normal cytoplasmic organelles – this is the lamellipodium. The leading edge of the lamellipodium is supported by a dense, branching actin network based on ARP-complex-nucleated microfilaments (see Part 2.1). This network is polar, with the plus ends of microfilaments directed toward the margin of the lamellipodium. It is polymerization of this network that extends the lamelipodium – the actin microfilaments extend by incorporating new g-actin (actin monomers) at their plus ends, and this pushes the margin

of the lamelipodium forwards. In other words, the lamellipodium advances without the use of a separate molecular motor, although there is energy used, not least because the addition of an actin monomer to a microfilament ultimately represents the commitment of an ATP molecule. As the lamellipodium moves forwards it forms small areas of attachment to the substrate that connect to the actin network. In *Dictyostelium* at least some of the attachment proteins are probably homologous to cell-adhesion proteins in animal cells (that connect animal cells to the extracellular matrix).

So far we have described only *extension* of the lamellipodium – in order for the cell to achieve movement, rather than just stretching out, the rest of the cell has to *follow up* the advancing lamellipodium. There are two processes at work here. Firstly, as it is extending forwards by polymerization, the actin network is simultaneously depolymerising at the minus end. In effect, this is a sort of 'treadmilling', although it is occurring across a whole microfilament network, rather than in a single microfilament. Since the lamellipodium attaches to the substrate near its forming end, depolymerisation at the junction between the lamellipodial actin network and the rest of the cell, or at the posterior end of the cell, tends to pull the rest of the cell forward. Secondly, it is not just the lamellipodium that is actin-rich; amoeboid cells have a peripheral/cortical network of actin microfilaments underneath (and connected to) the whole expanse of the cell membrane. This peripheral actin network is contracted by the action of myosins, especially at the posterior end of the cell. This activity is a bit like contraction of muscle fibres by actin-myosin interactions, but carried out over a 2D network rather than in a parallel bundle. This has the effect of squeezing cytoplasm forward, and also 'pulling up' the trailing edge of the cell. Actin treadmill-type activity and the actin-myosin contraction system both seem to contribute to locomotion in individual *Dictyostelium* amoebae; for example, mutants of *Dictyostelium* with non-functional myosin can still locomote under certain conditions, but the cells move only slowly (and see 'eruptive/blebbing pseudopodia' below for a further motility role played by the actin-myosin system in *Dictyostelium*).

Filopodia are similar to lamellipodia, except that they are fine structures supported by parallel microfilaments rather than the branching array organization. They also extend through actin polymerization. Filopodia are often not used for locomotion by themselves – some filopodia-producing organisms use them only for feeding.

Lobopodia are broad, blunt-ended pseudopodia that often contain typical cytoplasm, at least in their centre. They are characteristic of many medium-sized and large Amoebozoa. Each cell can form one to many lobopodia, depending on the species. As in lamellipodial amoebae, the outer portion of the cell cytoplasm is gel-like and houses an actin network. In large lobopodial amoebae this material is sometimes referred to as the 'ectoplasm', while the main cell cytoplasm, containing the various large cell organelles, is called the 'endoplasm'. The advancing ends of the lobopodia are usually distinguished by having a distinct 'hyaline cap' of cytoplasm, without organelles (similar in this respect to lamellipodia, as discussed above). Finally, in many amoebae, the opposite end of the cell includes a bulb with a highly ruffled profile called the 'uroid'.

Amoeboid motion using lobopodia involves the mass movement of endoplasm forward into the hyaline cap region, where it pushes the pseudopodium forward, and gels to form new ectoplasm. This transition involves addition to the microfilament network in the ectoplasm. Meanwhile, ectoplasm is recycled back into new endoplasm (i.e. the actin

network is depolymerised) at the posterior end of the cell. According to the most popular model, the important activity powering this cycle is similar to the 'pulling up' mechanism in lamellipodial cells: Actin-myosin interactions within the ectoplasm cause the microfilament network to contract, especially in the posterior end of the cell. This puts the endoplasm under hydrostatic pressure, and it is 'squeezed' forwards, thus pushing the advancing pseudopodium forwards. The pseudopodium moves forwards, rather than rest of the cell moving backwards, because rest of the cell is attached to the substrate at several points (and/or is pressed hard against the edges of a narrow gap within a substrate), while the new pseudopodium attaches only *after* it has extended.

Eruptive/blebbing pseudopodia: Some amoebae can produce broad pseudopodia through a process that is very different to that described above for 'lobopodia'. A rounded hyaline extension will appear rapidly, e.g. it will extend several micrometres in less than a second (peak velocities of 20 μm per second have been measured in *Entamoeba*). In some species, the extension can also spread laterally along the cell for several micrometres. After this the pseudopodium does not extend further, but gradually returns to the character of the rest of cell; this process typically taking 10-20 seconds. In the meantime the cell may produce more of these structures to continue extending, although, obviously, progression in a particular direction tends to be very stop-start. This general phenomenon is usually called 'eruptive' in the amoeba literature, and 'blebbing' in the cell biology literature (that focuses on animal and cancer cells); they are probably two manifestations of the same process, and we will cautiously assume this is the case. This eruptive/blebbing process has been documented as a supplementary form of amoeboid activity in the more-or-less filopodial/lamellipodial amoebae of *Dictyostelium* (see above), but other amoebae use it as a major process in their motility. This is most famous/widespread in the Heterolobosea (the name invokes their difference from typical lobopodia-producing amoebae), but is best studied in the mostly parasitic Entamoebae (Amoebozoa; Archamoebae).

The eruptive blebbing process begins with a delamination event, where a region of the cell membrane separates from its underlying actin network. The bleb is then forced outwards rapidly by the hydrostatic pressure imparted by myosin-mediated contraction of the actin network (discussed above). This leaves the originally underlying actin network behind, but it presumably still prevents large organelles from flowing into the expanding bleb, leading to its initially hyaline nature. Once the bleb has stabilized it begins to 'heal', with a new actin network established under the membrane at the margin of the bleb. This accounts for the 'rapid extension but long refractory period' patten that is typical of these pseudopodia. The original patch of actin network (now marooned within the cytoplasm) is gradually depolymerized.

Gliding

Gliding is a very common motility mechanism in the microbial eukaryote world. It is used as the primary form of locomotion by many heterotrophic flagellates as they scour surfaces for prey. Many good swimmers also have a supplementary ability to glide, including *Chlamydomonas* (although *Chlamydomonas* is a very slow glider).

Gliding mechanisms are much less well understood than the action of beating eukaryotic flagella, or (some forms of) amoeboid locomotion. They are also quite

heterogenous. Gliding doubtless evolved many times in eukaryote evolution and different groups use very different molecular mechanisms to achieve gliding motility.

Components of a (hypothetical) gliding system:
One important consideration with gliding is that the mechanism must somehow couple an external substrate to an intracellular molecular motor: Molecular motors like dynein, kinesin and myosin run on ATP, and would not work if attached to the outside the cell (there isn't much free ATP in pond water…).

One can think of a gliding motility system as consisting of the following components:

* The cytoskeleton (e.g. actin filaments or microtubules)
* A motor (that can travel along the cytoskeleton)
* A transmembrane element
* An element that adheres to the substrate

Note: The transmembrane element and adhesion functions could, in principle, be fulfilled by a single protein complex.

In the lecture slides there are two hypothetical models for gliding systems depicted. In one, the motor protein is connected directly to the transmembrane element and adhesion element, and will interact with an internal cytoskeleton element. This is loosely analogous to classic intracellular transport (e.g. of vesicles) along microtubules. In the other model, the motor is connected to the internal cytoskeleton and interacts with a second cytoskeletal element that is connected to the transmembrane element. This second model is loosely analogous to the sliding filament model of flagellar beating. In both cases, the action of the motor will cause the transmembrane elements to move relative to the cytoskeleton (remember that the cell membrane has fluidity). However, since the transmembrane elements are connected to the adhesion elements that are anchored to the substrate, this motion will actually cause the cell to move relative to the substrate.

We will look at three different forms of gliding in eukaryotes, which seem to have different cell biological and molecular bases. They are (i) flagellar gliding, (ii) gliding in apicomplexan parasites, and (iii) gliding by pennate diatoms.

Flagellar gliding
Flagellar gliding is probably the most significant type of gliding, ecologically speaking, but is not well studied. Some flagellates can glide with surprising speed. For example, the large phagotrophic euglenid *Peranema* has been reported to glide at up to 50 μm/sec. We will hopefully explore the properties of flagellar gliding in euglenids in a lab exercise.

The best-studied flagellar gliding mechanism is actually the system in *Chlamydomonas*. *Chlamydomonas* has two flagella and usually swims with a ciliary beat (see above), but can also attach to surfaces and glide on its flagella, albeit quite slowly (~1.5 μm / sec). The flagella attach such that they usually point in different directions and the cell moves in the direction of one or other flagellum. Only the flagellum pointed in the direction of movement appears to be 'driving', while the other trails passively.

Adhesion to the surface is caused by a particular glycoprotein that is embedded in

the flagellar membrane. These glycoproteins also attach to a molecular motor that walks along the microtubules of the axoneme, from the tip to the base of the flagellum. Since this is towards the minus end of the axonemal microtubules, the motor in question is likely to be a dynein. Such motors are present within the *Chlamydomonas* flagellum as part of the intraflagellar transport (IFT) system, which uses particular kinesins and dyneins to translocate protein complexes towards the tip of the flagellum and towards the base of the flagellum respectively (see Part 2, Chapter 1). These processes are referred to as 'anterograde IFT' and 'retrograde IFT'. Intraflagellar transport occurs inside the flagellar membrane, but TEM images of particles being transported by IFT indicate that they are associated with the flagellar membrane as well as being associated with the axonemal microtubules. This adds plausibility to the idea that flagellar gliding is similar to IFT, but with the molecular motor connected (not necessarily directly) to the adhesive flagellar membrane glycoprotein, rather than a transported protein complex. In fact, most *Chlamydomonas* mutants in which retrograde IFT is non-functional are also unable to perform (experimental proxies of) gliding behavior. Furthermore, some inhibitors of dyneins (including the retrograde IFT motor) also reduce the effectiveness of flagellar gliding. Live-cell imaging experiments demonstrate that instances where a cell begins to glide coincide with the flagellar glycoprotein co-localising with a retrograde IFT train, and being 'picked up', fixing the IFT train to the substrate, and causing subsequent motion to pull the cell 'forward' in space, rather than the IFT train moving 'backwards' (Shih et al. 2013).

Gliding in Apicomplexa

Apicomplexa (e.g. the malaria parasite *Plasmodium*, and *Toxoplasma*, which causes toxoplasmosis) are a major group of parasitic organisms, most of which are intracellular, meaning that they must invade host cells in order to complete their lifecycles. All Apicomplexa lack flagella during most of the life cycle, and are not able to swim (except for the flagellated 'male' gametes produced by some species). Nonetheless, many apicomplexan cells are capable of gliding along substrates, such as the surfaces of host cells, and use this same general gliding ability to help invade host cells by 'pushing' their way in. Gliding is unidirectional – cells glide forwards, although they often rotate slightly while doing so, or glide in circular paths when on a glass slide.

The mechanism for gliding in Apicomplexa is complex but is ultimately based on the motor myosin, which interacts with actin microfilaments. Attachment to the substrate (usually the surface of host cells) is achieved by a protein complex that includes adhesins that attach to the substrate (TRAP in the malaria parasite *Plasmodium*; the equivalent protein in *Toxoplasma* is called MIC2), and transmembrane proteins. These constituents of the complexes are secreted at the anterior end of the cell from the specialised secretory organelles called *micronemes*, which in turn are part of the apical complex.

How does myosin interact with the adhesion/transmembrane complexes? One could imagine a simple system where myosin was somehow bound to the transmembrane complexes and then 'walked along' actin microfilaments within the static cytoskeleton of the cell. However, this is not what happens in apicomplexan parasites, in fact: Instead, the myosin motors used in gliding are linked to the alveoli – stable membrane sacs that are anchored in turn to the underlying cytoskeletal matrix (i.e. to alveolins and ultimately the longitudinal microtubules). A series of gliding-associated proteins (GAPs) are associated

with the myosins, and some of these are known to also be embedded in the alveoli. The adhesion/transmembrane complexes, meanwhile, become connected to short, transient actin microfilaments. These short microfilaments interact with the anchored myosins, such that the adhesion/transmembrane complexes attached to the actin microfilaments are transported towards the *rear* of the cell. But since the complexes are also attached to the substrate, this transport causes the cell itself to move *forwards* over the substrate.

Obviously, this mechanism will result in accumulation of adhesion complexes and actin microfilaments at the posterior end of the cell. The microfilaments depolymerise and the actin monomers can then be recycled. The adhesion complexes are not recycled – they are cleaved and remain adhering to the substrate. This means that the gliding cell leaves a trail of discarded attachment complex proteins on the substrate. This trail can be visualised using fluorescently labeled antibodies raised against the adhesion complex proteins.

Pennate diatom gliding

Pennate diatoms are non-flagellated unicellular algae that typically live on substrates. Most can adhere strongly to the substrates, and many can glide on the substrates as well. Gliding is bi-directional – forwards or backwards – parallel to the long axis of the cell.

In understanding diatom adhesion and gliding it is important to remember that diatoms are essentially enclosed within an extracellular box of silica - the frustule. Thus, unlike *Chlamydomonas* flagella or apicomplexan cells, the cell membrane is not in close contact with the substrate. However, in pennate diatoms that are capable of gliding, there is an elongate longitudinal slit – the raphe – on one or both valves of the frustule, running parallel to the long axis of the cell. The raphes mediate attachment of the cell to the substrate. An organic mucilage (likely to be glycoprotein) is secreted from the cell along the raphe and forms strands that pass through the raphe to attach the cell to the substrate. Presumably there is a transmembrane element that connects the mucilage strands to the cell membrane, although this hasn't been identified.

During motion, the mucilage strands are transported one way or the other along the raphe. If the strands are connected to a solid substrate this results in motion of the cell. As with apicomplexan gliding the adhesive components (the mucilage strands) that accumulate at the posterior end of the raphe are discarded – they snap off and leave a trail behind the moving cell.

How is the motion powered? Previously, some researchers suggested that the motion was a form of 'jet propulsion', with mucilage being directionally secreted from the cell at the raphe, and then expanding by conformational change brought on by hydration, thereby pushing the cell in the direction opposite its expansion after it contacts the substrate (note - directional mucus secretion *is* used as a motility mechanism in some cyanobacteria, and perhaps some other microbial eukaryotes). However, other researchers had noted that there are 'cables' of actin microfilaments associated with each raphe, and had suggested that gliding was powered by an actin-myosin system. In fact, gliding in diatoms is inhibited by drugs that cause disassociation of actin microfilaments, *and* by drugs that prevent myosin from cycling. The preferred model now is that myosin is somehow connected through the cell membrane to the mucilage strands, and interacts with the actin cables alongside the raphes. This would make diatom gliding similar to apicomplexan gliding in terms of the molecular motility system used (actin-myosin), but more similar to models of flagellar gliding in *Chlamydomonas* in terms of the basic architecture of the system (i.e.

with the motor attached to the adhesion system, rather than to the static internal cytoskeleton).

Other motility systems

Other swimming mechanisms.

There are swimming mechanisms other than flagella or cilia that are used by some microbial eukaryotes. Adult Radiolaria lack flagella and are normally incapable of movement other than by buoyancy regulation (see below). They have long, stiff pseudopodia called 'axopodia' that are supported by bundles of linked microtubules, but these are not normally used in locomotion. Nonetheless, one radiolarian – *Sticholonche* – is a swimmer. *Sticholonche* is a large cell that has numerous axopodia ~150 micrometres in length. Unusually, these axopodia are highly motile and articulate against the surface of the nucleus. Unlike flagella these axopodia are stiff and move like oars. They have a fast effective stroke and slower recovery stroke. The motor is not dynein (and the axopodial microtubules do not slide relative to each other), nor is it an actin-myosin system. Instead, the motor appears to be a calcium-binding contractile protein fibre, comprised of centrin (or a similarly-functioning protein). The contractile fibre is attached near the base of each axopodium, like a muscle attached to a bone in a vertebrate, with the other end of the fibre connecting to the surface of the nucleus.

While barely counting as swimming, members of one group of bizarre dinoflagellates – warnowiids – have a 'piston' that rapidly elongates 'backwards', violently throwing the rest of the cell forwards in the process.

'Squirming' of various kinds; Euglenoid motion.

Some microbial eukaryotes are able to undergo drastic active body deformations that, when in contact with a substrate, can actually result in purposeful movement. **Oxymonads** are a group of anaerobic metamonad excavates that live in animal guts. They have a central cytoskeleton component called the 'axostyle' that is composed of a bundle of microtubules. In some oxymonads, such as ***Saccinobaculus,*** the axostyle can flex from side to side (the scientific name of this organism means 'snake in a bag'). This flexing clearly involves the sliding of adjacent microtubules within the axostyle, and is probably powered by motor proteins that interact between the microtubules, perhaps dyneins. Thus, the flexing of this axostyle is mechanistically similar to bending of the flagellar axoneme.

Many **euglenids** are capable of profound active deformation called '**euglenoid motion**' (or 'metaboly'). Euglenoid motion involves the sliding of adjacent long, thin strips within the pellicle (these strips lie immediately under the cell membrane). When the strips are arranged with a very steep helical pitch (e.g. close to parallel to the long axis of the cell) the cell will be long and skinny. When the strips are arranged in a shallow-pitched helix the cell (or that part of it) will be short and fat. Some species (including the model species *Euglena gracilis*) are capable of holding most of the cell in a long-skinny configuration while passing a short-fat bulge from one end of the cell to the other, in a peristalsis-like motion. Euglenids that normally locomote by flagellar swimming will tend to undergo

euglenoid motion specifically when they are confined or crowded, suggesting that euglenoid motion can be involved in surface motility. Indeed, the extreme 'peristalsis-like' form of euglenoid motion is demonstrably effective as a surface motility system when the 'moving bulge' can press close against the confining surfaces, with a series of bulges passing from the anterior of the cell to the posterior, moving the cell 'forwards', or *vice versa* (Noselli et al. 2019). Speeds of ~2.5 micrometres per second are recorded for *Euglena gracilis* confined within glass capillaries (Noselli et al. 2019), which is comparable to the best speeds sustained by many amoebae (while being much slower than the flagellar gliding motility seen in many other euglenids – see above).

The molecular basis for euglenoid motion is not understood. The strips are underlain by microtubules, including microtubules that attach to one pellicular strip but lie very close to an adjacent strip. This suggests the possibility of a microtubule-walking motor connected directly or indirectly to one pellicular strip and interacting with one or more of these microtubules associated with the adjacent strip. Consistent with this, studies of a *Euglena* species found that ionic dissociation of a particular microtubule (and its directly associated proteins) that lies 'between' the armature of one strip and the edge of the adjacent strip, would abolish euglenid motion (Murata & Suzaki, 1998). Obvious candidates for a motor would include dyneins and/or kinesins. Interestingly, typical general inhibitors of dynein (at least) do not seem to inhibit euglenoid motion, while extracts thought to derive from material connected to the dissociated microtubule are rich in kinesin-sized proteins. Other possibilities exist, however, and more work is needed to understand this motility system.

Ciliate contraction

While not resulting in active motion, one other system deserves brief mention. Some ciliates are capable of profound and rapid contractility. Stentorid ciliates (e.g. *Stentor*) are elongate cells that can contract to a more-or-less rounded shape in around 10 milliseconds. The process of re-elongation following contraction is much slower. The contraction process is powered by the contraction of longitudinal fibres called 'myonemes' that lie nearby the ciliary basal bodies. Contraction of the myonemes is calcium-dependent (rather than ATP-dependent), and is powered by centrin-family protein in the myonemes. Re-elongation likely involves 'post-ciliary ribbons', which are long microtubular roots that originate associated with each pair of basal bodies in the somatic ciliature, and are aligned in a parallel, overlapping fashion along the kineties. These 'post-ciliary ribbons' are telescoped together by the contraction process so that at any one point there is a large stack of overlapping ribbons; Microtubule sliding (perhaps dynein-powered?) then pushes the ribbons along one other to return the cell to the elongated state.

Buoyancy regulation

Many microbial eukaryotes found in the water column lack the capacity to swim, but nonetheless can control their buoyancy, and thus move along a vertical axis. This ability is important for many photosynthetic cells in particular, as the depth of greatest nutrient availability is often lower than the optimum for photosynthesis, and cells may benefit if they are able to migrate between these depths. Cells may also sink rapidly to the sediment at the onset of dormancy, or migrate back to the photic zone after dormancy.

In the absence of buoyancy regulation or swimming, 'normal' eukaryotic cells are almost invariably more dense than freshwater or seawater, and will thus tend to sink. The velocity of sinking of a tiny particle is governed by the relative densities of the particle and the fluid, and by the viscous drag on the particle. It is described by Stokes' Law:

Stokes' Law: Velocity $(v) = 2gr^2(\rho 1-\rho 2)/9\mu$

Where:

g = acceleration due to gravity
r = equivalent radius of a particle (i.e. represents 'size')
$\rho 1$ = density of the particle
$\rho 2$ = density of the fluid
μ = the viscosity of the fluid

In other words, all other things being equal, sinking velocity is proportional to the *square* of the equivalent radius of a particle. Thus, base sinking rates are (much) higher for big cells than for small ones. In fact, typical sinking rates for very large cells (of the order of 100+ μm) are higher than the maximum observed speeds for swimming flagellates using conventional flagella (see above). This may help explain why the large cells found in the plankton are either dinoflagellates or ciliates (both of which swim faster than normal flagellates - see page 6, above), or are cells that lack flagella/cilia altogether, and use mechanisms other than swimming for staying suspended.

Negative buoyancy is increased in some cells by the presence of a heavy mineral test or skeleton (notable examples include centric diatoms, Radiolaria, planktonic Foraminifera). Silica and calcium carbonate - the two most common compounds used to construct mineral scales and similar structures - both have densities >2 (g/cm^3), whereas the density of seawater is around 1.025. Under some circumstances cells may increase the rate of sinking by constructing substantial mineral structures. Conversely, it has been proposed that the extremely long spines found on some planktonic cells, notably some centric diatoms, might increase viscous drag enough to significantly retard sinking (relative to a round cell of equivalent density and volume).

Positive buoyancy can be achieved by accumulating less dense materials within the cell, such as lipids, or extracellular mucilage. However, seawater is denser than freshwater due to the dissolved salts. Cells, especially those with pressure-resistant cell walls or equivalent (such as diatoms), can 'lighten' themselves relative to seawater by maintaining a somewhat hypo-osmotic state. It is also possible for cells to exploit the fact that some isotonic solutions will be less dense than seawater - for example, an ammonium chloride solution has a slightly lower density than the equivalent sodium chloride solution. Some planktonic marine organisms have a large vacuole that occupies much of their volume. Examples include large centric diatoms and the giant dinoflagellate *Noctiluca*. Radiolaria, meanwhile often have a highly vacuolated ('frothy') cytoplasm outside their central capsule. These cells preferentially accumulate 'light' solutes (i.e. solutes that will yield solutions that are less dense than seawater when at an equivalent concentration) within these vacuoles, and exclude 'heavy' solutes like sulfate, magnesium ions and calcium ions.

The maintenance of an absolute reduction in osmolyte concentrations, or of highly

skewed relative concentrations of some osmolytes, requires the expenditure of energy to support the pumping of ions out of the cell. The importance of such *active* buoyancy regulation in planktonic cells is underscored by observations that dead, metabolically poisoned, or otherwise stressed cells tend to sink much faster than healthy cells.

Some prokaryotes (especially cyanobacteria) achieve buoyancy using gas vesicles, while many macroalgae use gas-filled floats or bladders. Nonetheless, gas accumulation does not appear to be a documented buoyancy mechanism in unicellular eukaryotes.

Going in the right direction

Going straight

In many cases cells will want to swim consistently in a particular direction for a period of time, rather than looping round and round in circles. A cell that swims without any rotation (roll) will swim in circles if it doesn't swim exactly straight (i.e. if it swims with any pitch or yaw). Many swimming cells avoid this by 'deliberately' rotating while they swim, and thus progress in a helical path around a straight axis. Rotation is a natural consequence of swimming powered by a single flagellum with a helical beat. In many biflagellated cells with heterodynamic flagella, it is likely that one flagellum functions primarily to impart rotation (or control the rate of rotation) rather than generating forward motion. Ciliates can achieve rotation by having the cilia beating with their power strokes directed slightly off-axis relative to the direction of swimming (*Paramecium* does this, for example).

Changing direction

Cilia and flagella are typically capable of more than one form of beat activity. For example, while the chlorophyte alga *Chlamydomonas* usually swims with both flagella performing a 'ciliary beat', a shock (e.g. very bright light) will cause both flagella to switch to a typical 'flagellar beat', which results in the cell swimming backwards. There are also less drastic changes possible in which the flagella will maintain a ciliary beat, but one of the flagella will behave less effectively than the other, which will cause the cell to turn. The ciliate *Paramecium* also shows a 'shock' response when it collides with an object (for example). In this response, the cell swims backwards by maintaining a ciliary beat, but reversing its direction. Usually the cell will swim backwards for a brief period before slowing to a halt, turning in a new direction, and progressing forwards again. Such reversals will also happen spontaneously with some frequency.

The switch for turning on these alternative beat patterns is calcium. The concentration of calcium ions in the flagellum/cilium (and the rest of the cell) is normally very low. When calcium is present it interacts with some ciliary proteins, affecting the control of ciliary beating. To use the *Paramecium* collision behaviour as an example, the mechanical stimulus of collision is sensed by mechanoreceptors and induces a membrane depolarisation. This causes voltage-gated calcium channels to open and allow calcium into the cilium, triggering the ciliary reversal. As the calcium is pumped back out of the cilium the normal beat is restored. The 'turning' behaviour of the cell corresponds to the time at which some cilia, but not all, have switched back to the conventional beat pattern, due to small variations in the rate of calcium removal around different cilia.

Small variations in intraflagellar calcium can more subtly affect flagellar beating.

For example, *Chlamydomonas* controls phototaxis (orienting to light) by manipulating intraflagellar calcium, which has different effects on the mechanical effectiveness of its two flagella, causing turning.

Response to the environment (kineses and taxes)

Microbial eukaryotes can respond to many of the same stimuli as animals, including light, chemicals, contact, gravity, temperature, and so on. The response is often to move towards or away from the stimulus. 'Taxis' refers to the situation where an organism orients itself towards a stimulus (or away from it). 'Kinesis' refers to the situation where an organism responds to a stimulus not by 'steering', but by altering its motility behaviour in a non-directional manner.

Taxes require either that the stimulus be directional (e.g. electromagnetic radiation; mechanical forces), or that the organism can detect a gradient across its body (i.e. in space and not time). Microbial eukaryotes are too small to directly detect spatial gradients in temperature and chemical concentrations (including dissolved gases), and kinesis mechanisms are employed to respond to these sorts of stimuli (see below).

True phototaxis (taxis in response to light) is relatively common in microbial eukaryotes. It is best studied in *Chlamydomonas*. *Chlamydomonas* cells have a light-sensing organ called the *stigma*. This structure includes a photoreceptor that is receptive to light from one side of the cell only, as it is shielded on other sides by light-absorbing pigments in the stigma. Therefore, as the cell rotates during normal swimming, the photoreceptor continuously 'scans' the lateral environment. In response to moderate light the photoreceptor transmits a signal that results in a small increase in flagellar calcium and a reduction in the effectiveness of the flagellum nearest the photoreceptor (by contrast, very bright light causes an increase in flagellar calcium sufficient to elicit the shock response in *both* flagella). In consequence, the cell turns ('yaws') in the direction of the photoreceptor, and hence is pointed more towards the light.

Photoreceptors with associated shading pigment regions are common in motile algae, but have rather different operations in different groups – for example, photosynthetic euglenids and many photosynthetic stramenopiles have photoreceptors, but they are associated with different flagella. The photoreceptor in green euglenids is associated with the anterior flagellum (F2; see Part 2, Chapter 1), which is usually the dominant, or only, provider of swimming power in these cells. The photoreceptor in most photosynthetic stramenopiles, meanwhile, is associated with the posterior flagellum (F1), which is usually short and does not contribute to forward motion.

Ciliates are usually sensitive to gravity. Free-swimming ciliates like *Paramecium* presumably orient relative to gravity to counteract sinking, and therefore tend to swim in the opposite direction to the gravity force vector (see above). Some ciliates (but not *Paramecium*) have complex organelles containing (dense) barium sulfate crystals that resemble the gravity-sensing 'statocysts' of some animals, and are likely gravireceptors.

Kineses represent a way for cells to detect and respond to stimulus gradients by sampling over time as they move through them. This gets around the problem of being too small to detect stimulus gradients instantaneously by observing different signal strengths in different parts of the cell (i.e. over distance). There are two kinds of kinesis mechanisms used by microbial eukaryotes – cells can vary the frequency of direction changes (klinokinesis) or vary their swimming speed (orthokinesis).

Consider a cell that is swimming in straight lines punctuated by random changes in direction that occur at some frequency – this cell would perform a 'random walk' through the environment. However, if the cell increases the time between direction changes as it experiences increasing strength of some stimulus (e.g. the concentration of a chemical), and vice versa, the random walk will be biased and the cell will tend, on average, to move towards the source of the stimulus, and then remain in its vicinity. The reverse relationship between the timing of direction changes and stimulus strength will result in the organism tending to move away from the source of the stimulus. In the first case the stimulus would be an attractant, in the second case, a repellant. Either way, these behaviours by the cell are forms of *klinokinesis* (note that this account would apply equally to prokaryotic or eukaryotic cells – many bacteria use this form of klinokinesis based on 'run and tumble' locomotion to swim toward chemical attractants).

Increasing or decreasing the speed of swimming (i.e. *orthokinesis*) will have a similar effect to increasing or decreasing the length of time between direction changes. Orthokinesis is supposedly distinctive for eukaryotic cells. Some cells (e.g. *Paramecium*) respond to chemical stimuli by combined klinokinesis and orthokinesis: When heading towards an attractant they will swim in straight lines for longer, *and* will swim faster.

In many cases a stimulus will be attractive at low concentrations, but repel at high concentrations. This means that cells will tend to congregate in regions of optimal stimulus strength, rather than the stimulus source.

Note: Shock responses such as those described above for *Paramecium* and *Chlamydomonas* represent discrete responses to a stimulus threshold, rather than a graded response to changes in stimulus intensity, and thus represent their own class of response, rather than constituting a 'kinesis'.

Relevant readings (encouraged but not required):
Hausmann et al., 2003: 206-213; 216-222; 284-295.

Important sources:
Beeby M. et al. (2020) FEMS Microbiology Reviews, 44:253–304
Boyd C.M. & Gradmann, D. (2002) Marine Biology 141:605-618
Cachon et al. (1977) Journal of Cell Biology 72:314-338
Fujita S. et al. (2014) Biophysical Journal 107:336-345
Fukui Y. (2002) Cell Biology International 26:933-944
Ginger, M.L. et al., (2008) Nature Reviews Microbiol. 6:838-850
Hausmann K. et al., (2003) Protistology (3rd Ed.)
Higgins et al. (2003) J. Phycol. 39:1181-1193
Heintzelman M.B. (2015) Seminars in Cell & Developmental Biology 46:135–142
Lammermann T. & Sixt, M. (2009) Curr. Opin. Cell. Biol. 21:636-644
Linck R.W. (2015) Cilia and Flagella. In: eLS. John Wiley & Sons, Ltd: Chichester.
Maugis B. et al. (2010) Journal of Cell Science 123:3884-3892
Mitchell D.K. (2000) Journal of Phycology 36:261-273
Murata K. and Suzaki, T. (1998) Protoplasma 203:125-129.
Nicastro D. et al. (2006) Science 313:944-948
Noselli G. et al. (2019) Nature Physics 15:496-502
Odo et al. (2014) Journal of Cell Biology 204:807-819
Poulsen et al. (1999) Cell Motility and the Cytoskeleton 44:23-33
Raven, J.A. & Waite A.M. (2004) New Phytologist 162:45-61
Saito, A. et al., (2003) Cell Motility and the Cytoskeleton 55:244-253
Shih S.M. (2013) eLife 2:e00744
Sibley L.D. (2004) Science 304:248-253
Sleigh M.A. (1991) Protoplasma 164:45-53
Ueyama S. et al. (2005) Cell Motility and the Cytoskeleton 60:214–221
Yoshida K. & Soldati T. (2006) Journal of Cell Science 119: 3833-3844