# Biology 3102 – Microbial Eukaryotes
# Supplementary Course Material #2, chapter 1
# Fall 2020

## THE CYTOSKELETON OF PROTISTS



**Cytoskeleton - Structural proteins**
There are many structural proteins in eukaryotic cells. The most important are microtubules, made from tubulin, and actin microfilaments. You should already know quite a bit about microtubules and actin microfilaments from Cell Biology classes/units. Here we will briefly summarise some relevant information about these cytoskeletal proteins, and others that they interact with, before moving on to new information.

**Microtubules**
Microtubules are hollow tubes ~23 nm across. They are made from dimers of two related proteins - alpha tubulin and beta tubulin. The walls of the tube are 13 dimer-widths around (i.e. constructed of 13 parallel protofilaments, each one dimer wide). Microtubules are polar, with a 'minus' end and 'plus' end. The dimers associate in a head-to-tail fashion (beta tubulin toward the plus end). This polarity affects the interactions with other proteins (for example, different molecular motors travel along microtubules in the minus direction and the plus direction – see below). This polarity is particularly important for understanding the growth and orientation of microtubules in cells.

Microtubules usually originate from regions of the cell called microtubule-organising centres (MTOCs). Each originating microtubule forms in association with a small complex of proteins that includes another type of tubulin (gamma tubulin) and grows by the addition of tubulin dimers. The growing end of the microtubule is the 'plus' end. Prior to addition to a microtubule, both subunits of the dimer are complexed with the nucleotide GTP (which, like ATP, is an energy carrier). After incorporation of the dimer into a microtubule, the GTP bound to beta-tubulin tends to be hydrolysed to GDP. Because of this, the formation of microtubules from tubulin dimers and eventual recycling of tubulin dimers will ultimately cost the cell energy.

Many microtubules in the cell are relatively unstable – they are subject to alternating rapid depolymerisation and regrowth at the plus end, and these are the types of microtubules that are usually emphasised in cell biology courses. However, the stability of microtubules can be greatly enhanced by association with certain microtubule-

associated proteins (MAPs) (note – there are also MAPs that promote microtubule INstability). Post-translational modification of tubulins (chemical alteration of the protein after the formation of the polypeptide) also greatly increases the stability of certain microtubules. One example is the acetylation of alpha tubulin, which has been documented in some microtubule assemblies in studied model protists. In the end, some microtubules are very stable, and structures made from them persist throughout the interphase portion of the cell cycle, or even across multiple cell generations. These include basal bodies (and centrioles), and certain other cytoskeleton components of many microbial eukaryotes, for example, flagellar microtubular roots (see below).

**Actin**

Actin microfilaments are thinner than microtubules (around 7 nm). A microfilament is made of two protofilaments, each consisting of a linear chain of actin monomers (polymerised actin in microfilaments is called 'F-actin', while the non-polymerised monomers are called 'G-actin'). Like microtubules, actin microfilaments are polar. Actin monomers are added to a microfilament much more readily at the plus end than the minus end, and thus microfilaments tend to grow at the plus end. As with microtubules, assembly of microfilaments ultimately represents a commitment of energy – there is an ATP molecule tightly bound to the actin monomer that is hydrolysed to ADP after polymerization.

Actin filaments may be stabilised by 'capping proteins' at either end (the minus-end capping proteins can be those that promote the nucleation of the actin filament in the first place – e.g. i) formin, or ii) the 'ARP' complexes discussed below). In the absence of capping proteins, depolymerisation may happen from either end of the microfilament. The net rate of depolymerisation tends to be higher at the minus end, because the rate of polymerisation is lower (for a given concentration of actin monomers), and because the rate of depolymerisation is higher for actin bound to ADP than for actin bound to ATP.

Under certain conditions a phenomenon called 'treadmilling' (or 'actin cycling') can result, in which an actin microfilament is undergoing net growth at the plus end, while simultaneously undergoing net depolymerisation from the minus end. This has the effect of moving the actin filament as a whole, even though the individual actin monomers in the microfilament can be stationary in space. Actin treadmilling can be used by cells to do useful work, and we will look at a couple of examples in this section.

**Higher-order structures**

Microtubules tend to exist individually, or to form parallel arrays, often with MAPs (see above) linking adjacent microtubules together and maintaining a specific spacing between them (this is known primarily from animal cells, but presumably applies also to the microtubular arrays in structures like axopodia – see below).

A wider range of organizations is displayed by actin microfilaments. Actin microfilaments may also form parallel bundles in which the filaments are closely packed or are more widely spaced. Antiparallel arrangements occur as well. Closely packed parallel bundles are seen, for example, in the microvilli of choanoflagellate collars, as

well as more temporary filopodia (see below). Actin bundles are stabilized by actin cross-linking proteins, specifically bundle-forming types, which are roughly analogous to cross-linking MAPs in microtubule arrays. The cross-linking proteins fimbrin and α-actinin are ubiquitous in eukaryotes. Interestingly, another actin cross-linking protein associated with parallel actin bundles in animal cells, fascin, is also found in choanoflagellates and other close relatives of animals, but not in more distantly related protists. Fascin is now known to be localized to the microvilli of the choanoflagellate collar (Sebe-Pedros et al., 2013).

Actin microfilaments may also form branching networks, or webs in which fibres are crosslinked orthogonally. Branching arrays of actin that have a polarity (i.e. the plus ends of the actin microfilaments are pointing mostly in one direction) are formed by nucleation of new actin microfilaments by 'ARP complexes' that attach at a broad-but-acute angle to the side of an existing microfilament.

**Motors**

Motor proteins associate with both microtubules and actin filaments. They form the basis of most transport of organelles within the cell, changes in cell shape and movement of the cell through the environment (though see actin cycling, above, for an example of motility that is based on filament polymerization rather than a motor protein).

The motors that associate with microtubules are dyneins and kinesins. **Dyneins** are complexes of several different polypeptides that form 2-3 'legs' that interact with a microtubule on one side, while the other side of the complex attaches to various possible 'cargos'. The cargo can range from complexes of proteins to vesicles, or even other cytoskeleton elements (as in flagella – see below). The legs of dyneins 'step' along microtubules, hydrolysing ATP in the process, and thus move their cargo along the microtubule. Dyneins 'walk' towards the minus end of microtubules.

Typical **Kinesins** are of different structure to dyneins, but of similar function, except that they move towards the plus end of microtubules, expending ATP in the process. Therefore, a system including both dyneins and kinesins can move cargo in both directions along the microtubular skeleton of a cell.

The motor that interacts with microfilaments is **myosin**. Typical motility myosins have a head region that interacts with actin, and this head region is connected to a tail region that may be long. One function of the tail is to link multiple myosin units together to form various kinds of motility complexes (e.g. a pair of myosins connected flexibly together). Almost all myosin subtypes progress towards the plus end of actin microfilaments. In single myosins this is achieved by alternating changes in the angle between head and the tail associated with the hydrolysis of ATP and the release of ADP.

**Other cytoskeletal elements (including centrin)**

There are a large number of other cytoskeletal proteins in eukaryotic cells. Examples include various 'intermediate filaments', spectrin and others. We will discuss only one

other type of 'universal' protein in any detail – Calcium-binding contractile proteins, of which the best studied is **centrin**. Centrin-family proteins are probably present in all eukaryotic cells (they are integral components of eukaryotic flagella and other structures). Some cells, however, contain thick fibrous structures (tens or hundreds of nanometres broad) in which centrin is a major component. These centrin-rich fibres contract in the presence of calcium ions. The presence of calcium causes centrins to undergo small conformational changes, in turn apparently causing the fibres they are in to supercoil, and thus shorten. In contrast to the motor protein activities described above, this contraction of centrin-rich fibres itself does *not* require ATP (nor GTP). Presumably energy is expended returning the fibres to their uncontracted state. This expenditure would include the cost of pumping calcium back out of the cell cytoplasm (into the lumen of calcium-storing endomembrane compartments, or out of the cell altogether).

While a heavy reliance on the microtubule-based and/or actin-based cytoskeleton is typical across most eukaryotes, there is also substantial variability amongst microbial eukaryotes in the array of other proteins that are major cytoskeletal components. Many groups of eukaryotes display various other cytoskeletal elements that can be seen by electron microscopy, and are clearly not microtubular, nor composed of actin. In many cases these elements are large or extensive, and are clearly important parts of the cell cytoskeleton. These include bundle-like fibres, many types of fibres with regular lateral striations ('striated fibres') and protein lattices. Many of these are associated with the flagellar basal bodies and/or 'flagellar microtubular roots', and discussed again in a later section ('**Non microtubular elements of the flagellar apparatus')**. For the most part the protein compositions of these structures are unknown, or only poorly understood. Where they have been studied in detail, it is very often the case that non-microtubular elements are assembled from proteins with no clear affinity to cytoskeletal proteins in animals. For example, the distinctive paraxonemal rods of Euglenozoa are large lattice-like protein structures that are composed predominantly of two structural proteins called paraxonemal rod proteins 1 and 2 (PAR1 and PAR2). These proteins are related to one another (the genes encoding them arose by a gene duplication event in a common ancestor of Euglenozoa), but their evolutionary relationships to other proteins with defined functions are unknown.

One general area that has received some attention is the 'membrane skeleton' (i.e. internal support for the cell surface) in groups of microbial eukaryotes that have clearly defined shapes. In studied examples the membrane skeleton in such organisms is very different at the protein level from that of animal cells, and 'novel' proteins are major components. For example, the characteristic cortical alveoli of alveolates (e.g. ciliates, dinoflagellates and apicomplexans) is connected to an underlying protein-rich layer. In ciliates this layer is especially conspicuous; it is called the **epiplasm** and is arranged as a series of plates with a stereotyped relationship to the somatic cilia. This layer appears to have a major structural role: In ciliates, detergent-extracted 'ghosts' consisting of the cell membrane, alveoli and attached epiplasm can typically still maintain cell shape. Proteins that are associated with this protein layer include the so-called '**alveolins**'. Alveolins appear to have a structural role, perhaps connecting alveoli to other cytoskeletal elements. Major components of the ciliate epiplasm include 'epiplasmins' and '**articulins**', proteins that share similarities to each other, and to alveolins, but are not related to known

cytoskeletal proteins in animals (for example). There is immunological evidence that epiplasmins and articulins are also present in dinoflagellates, and genes predicted to encode such proteins are present in the genomes of diverse alveolates (Goodenough et al. 2018). More surprisingly, perhaps, they are also present in euglenids. Euglenids belong to the group Euglenozoa (in Discoba; one of the two main groups of excavates), and are not at all closely related to alveolates (as it happpens, articulins were characterized first in *Euglena* and later in ciliates). Articulins form a major part of the characteristic pellicle of euglenids, which comprises a series of parallel strips immediately under the cell membrane underlain in turn by microtubules (i.e. similar in position to the ciliate epiplasm, if the intervening alveoli are discounted). Articulins in the euglenid *Euglena* have been shown connect to integral cell membrane proteins, and can also be localized to the pellicle strips using immuno-electron microscopy. Multiple articulin-like proteins are also predicted from the genomes of cryptophytes, raising the possibility that the cryptophyte periplast system (which resembles the euglenid pellicle in being a protein layer tightly connected to the cell membrane) may have a similar protein basis (Goodenough et al. 2018). Cryptophytes are not closely related to either alveolates or euglenids, making this possibility highly intriguing in terms of evolutionary history.

**The eukaryotic flagellum**

One of the most complex and widespread cytoskeletal structures in eukaryotes is the eukaryotic flagellum (or cilium). Most microbial eukaryotes have flagella or cilia, and use them for motility. Many of those that don't have functional flagella nonetheless retain the basal structure of the organelle – the 'basal body' (also known as the 'kinetosome', or 'centriole' when not supporting a flagellum, especially in animal cells). Basal bodies are most often arranged in small clusters called 'kinetids' that contain a defined number of basal bodies. Most commonly, there is only one cluster per cell, and there are two basal bodies per cluster, although other numbers are observed (4, 1 and 8 being the most common alternatives, probably in that order). There are exceptions: Diplomonads, for example, have two clusters per cell (usually each with four basal bodies), while the 'somatic' ciliature of ciliates typically contains hundreds of clusters, of one or two basal bodies each.

The eukaryotic flagellum is composed of several hundred different proteins (and surrounded by a membrane that is continuous with the cell membrane) but is a microtubular structure at its core. The microtubules are arranged with their minus ends to the basal region of the structure - the basal body. The basal body is essentially a cylinder consisting of nine microtubular 'triplet' structures. Each triplet includes one 'complete' microtubule (the 'A' microtubule) connected in parallel to two 'incomplete' microtubules ('B' and 'C'). The very bottom of the basal body generally has a more complex structure known as the 'cartwheel', which includes spokes connecting each triplet (via a 'pinhead' structure) to a central tubule-like structure called the 'hub' (note – this 'central tubule' is *not* a microtubule – see below). The basal body is located within the cell proper – it is often thought of as the structure that helps to anchor the rest of the flagellum into the cell.

The main structure of the flagellum – the flagellar 'axoneme' – is almost always an array of nine microtubular doublets surrounding a 'central pair' of two individual microtubules. The nine doublets are continuations of the triplets of the basal bodies (one of the incomplete microtubules from each triplet, C, does not continue into the axoneme proper). There are two types of dyneins – inner and outer - that are attached to each doublet and are directed towards one of the adjacent doublets. The action of dyneins against the microtubular doublets provides the motive force for the flagella (as will be discussed in more detail later in the course). Interestingly, outer dynein arms appear to be absent in some flagellum-bearing members of the taxon Amoebozoa (these cells can still swim, albeit not especially rapidly). Adjacent doublets are also connected by 'nexin' bridges. There is also a row of 'radial spokes' connected to the inner side of each doublet and directed inwards towards the central pair microtubules. These radial spokes are not to be confused with the cartwheel spokes at the base of the basal body (see above). The radial spokes include a couple of dozen different proteins, and are not homogeneous – each is actually a repeating array of 2-3 different spoke assemblies running along the length of each axonemal doublet. The radial spokes, and the central pair with its surrounding projections, play a role in regulating flagellar motion (later section). The junction between the basal body and the axoneme itself is called the 'transition zone', and usually coincides with the boundary between the cell body and the flagellum. The transition zone is quite variable among different groups of eukaryotes, but usually includes structures that strongly anchor the doublets to the cell membrane, and to each other, as well as material associated with the origin (minus end) of the 'central pair'.

**Absolute orientation**

Both the basal body and axoneme have a consistent 'handedness' (chirality) that is conserved throughout eukaryote diversity – the triplets within the basal body are always inclined in the same direction, while the dyneins attached to the axonemal doublets always point in the same direction. If you look at a flagellum from the 'tip down' (i.e. from the plus end of the microtubules towards the minus end), the dyneins point towards the doublet on the anticlockwise side of the doublet they are anchored to. This feature is sometimes useful for trying to reconstruct the morphology of cells from ultra-thin-section TEM data.

**Producing new basal bodies and flagella**

The production of a new flagellum begins with the formation of a new basal body. The negative-end structure of the basal body or centriole is the 'cartwheel' (see above), and this element plays an important role in early basal body assembly. The core protein of the hub-and-spoke structure of the cartwheel is 'SAS-6', which has a globular N-terminal domain, followed by a long coiled-coil segment. Dimers of SAS-6 align in parallel, and each dimer connects to two others by their N terminal domains, forming flat rings of nine dimers. These rings stack on top of one another to form the cartwheel, with the N-terminal domains forming the hub, and coiled-coil domains forming the basis of the spokes. Other proteins are associated with outer portions of the spokes. Only later do the microtubular structures form, with the complete 'A' tubules forming first, each one associated with the end of one spoke. Thus, the cartwheel seems to serve to correctly position and space the microtubular elements of the basal body and axoneme.

Interestingly, the cartwheel is actually absent – i.e. discarded - from fully mature basal bodies in a few protist taxa, suggesting that it might not have an essential function after basal body assembly is completed.

In most eukaryotes, each new basal body or centriole forms alongside an existing basal body/centriole. For example, in flagellates with two flagella, one new basal body will form alongside each existing basal body at some time prior to mitosis and cell division. This process is called 'templated' synthesis, as a contrast to the (rarer) de novo synthesis described below. Templated synthesis has the effect of regulating flagellum number across generations: the same number of new basal bodies is produced as the number of existing basal bodies, such that each daughter cell from a round of cell division will have the same number as the parent (see below). It also acts to locate the new basal bodies reliably in space. It is important to note, however, that the **structure** of the new basal bodies is not literally templated from the existing basal bodies, merely their number. In other words, the 'templating' metaphor is being used here in a more limited sense than in transcription or DNA replication, for example. The alternative term 'mentored' has been proposed.

In some microbial eukaryotes the flagella (and indeed the entire flagellar apparatus – see below) can originate entirely de novo. For example, this is seen during the production of flagellated male gametes by taxa that lack basal bodies and centrioles in the main life cycle phase, such as centric diatoms, and apicomplexan parasites. De novo production of a flagellar apparatus *without* reproduction is seen in Heterolobosea (a group of excavates). Most Heterolobosea are amoebae that seem to entirely lack basal bodies and microtubular roots while in the amoeba state. Yet under appropriate conditions many heterolobosean amoebae can transform into flagellates, which can have up to four basal bodies and one or more flagellar microtubular roots (see below), and well as a large non-microtubular fibre. The best-studied heterolobosean, *Naegleria,* has two flagella in the flagellate stage. Both flagella are produced almost simultaneously, in other words, there is no flagellar transformation (see below). Nonetheless, it was recently shown that one of the two basal bodies is produced a few minutes after the other, directly alongside the earlier-formed unit. Therefore, it appears that only the first basal body is formed truly de novo, and the second has a 'templated' origin (Fritz-Laylin et al. 2016). How the cell knows where to form the first basal body is not clear; it has been reported that each *Naegleria* amoeba has a single small structure (not at all basal body-like) that serves as the focus of basal body production if the amoeba transforms to the flagellate stage, but this is controversial.

The growth of a new flagellum from the cell poses a technical challenge. The axonemal microtubules that form the basis of the flagellum must be elongated at their plus ends, i.e. by addition at the tip. However, this means that tip gets ever further away from the cell cytoplasm, where the proteins that make up the structure of the flagellum are actually synthesised. One solution to the problem is '**intraflagellar transport**' or 'IFT'. In IFT, particles consisting of complexes of proteins required for flagellar growth are transported along the flagellum between the axonemal microtubules and the flagellar membrane. The IFT particles are connected to molecular motors that use the axonemal microtubules as their tracks. Kinesins are used to move IFT particles towards the (positive) tip of the flagellum; this is so-called 'anterograde' IFT. There is also a 'retrograde' component to

IFT where particles can move down the flagellum using dyneins (note – retrograde IFT uses a *different* population of dyneins to the flagellar dyneins that are connected to the axonemal microtubules.). The IFT system also operates in completed flagella, not just during their assembly. Evidence from studies of *Chlamydomonas* indicates that anterograde IFT runs along the 'B' microtubule of the axoneme, while retrograde IFT uses the 'A' microtubule (Stepanek & Pigino, 2016); this allows both systems to operate simultaneously on the same axonemal doublet without head-on collisions.

Interestingly, some apicomplexan parasites that produce flagellated microgametes (including *Plasmodium*, the malaria parasite) have lost the ability to perform IFT; for example, they lack genes for the IFT-specific forms of kinesin and dynein, and other proteins. In these species the flagellar axoneme is assembled completely within the cytoplasm during microgamete development, and then 'pops out' from the side of the cell, taking with it a portion of cell membrane to serve as the flagellar membrane.

**Other microtubular structures – flagellar microtubular roots**

The region around the basal bodies usually acts a microtubular organising centre (MTOC) for other microtubular systems (i.e. in addition to the axonemal microtubules themselves). This is often the major (but not necessarily the only) MTOC in the cell. In animals the region around the centrioles – the centrosome - acts as an MTOC for radiating individual microtubules in interphase cells, and also for the spindle microtubules that act to separate sister chromatids during mitosis. Centrioles/basal bodies play similar roles in most other eukaryotic cells as well. However, in most types of microbial eukaryotes, especially 'flagellates', the basal body region also acts as an MTOC for one or a few groups of stable microtubules. These groups usually each consist of a single ribbon-like row of parallel microtubules that are butted up against each other (but are nonetheless all complete, unlike the 'B' and 'C' microtubules of basal bodies). These groups are called 'flagellar microtubular roots', or just 'microtubular roots'. Often they are associated with various non-microtubular elements that adhere to or otherwise interact with the root microtubules (see below). There are a fixed number of microtubular roots in a species, and each is usually distinctive, having a specific position and orientation relative to the basal bodies and containing a fixed number of microtubules, as well as being associated with distinctive non-microtubular fibres in many cases (see below). In general, each appears to have a different structural role in the cell. The flagella, plus these flagellar microtubular roots and associated non-microtubular structures are collectively called the '**flagellar apparatus**'.

Flagellar microtubular roots are often very important in defining the basic morphology of microbial eukaryote cells. Here are just a few examples:

i) The most conspicuous feature of 'typical excavates' is a broad feeding groove on one side of the cell. Each of the two edges of this groove is supported by one of the three main microtubular roots in these cells.

ii) Small phagotrophic stramenopiles (e.g. bicosoecids, chrysophyceans) have a large microtubular 'hoop' that surrounds the site where food particles are ingested – this hoop is mostly a continuation of one of the main microtubular roots in these cells.

iii) The microtubular fibres that connect the kinetids of ciliates along kineties, as well as between adjacent kineties, are also, effectively, flagellar microtubular roots.

iv) In many groups of microbial eukaryotes, microtubules that support most of the cell membrane originate from an MTOC near the edge of one particular microtubular root (see below).

**Non microtubular elements of the flagellar apparatus**

The set of non-microtubular elements of the flagellar apparatus differ widely between different groups of eukaryotes and have diverse appearances when viewed by high-resolution transmission electron microscopy. Some common patterns include: (i) one to several fibres, often with approximately symmetrical patterning about their midpoint, that connect different basal bodies by joining to specific microtubule triplets; (ii) fibres that run alongside a flagellar microtubular root, and appear in cross-section as a series of parallel vanes or a lattice, with each vane (or lattice base) connecting to one microtubule; (iii) 'striated fibres' that have a regular pattern of banding when viewed in longitudinal section, either running alongside a flagellar microtubular root, or taking a separate path through the cell. Examples of all three of these categories can be seen within a single cell.

Basal body connectors (i.e. 'i' in the list above) have been examined in a few of microbial eukaryotes and are partly composed of centrin (see above; Harper et al. 1995; Dutcher and O'Toole, 2016). There is huge variability within categories 'ii' and 'iii', indicating a wide variety of compositions at the protein level. For example, the pattern of striation is different in different striated fibres, and their periodicity (length of each unit in the repeating pattern) varies considerably. Our overall understanding of these elements is poor. The best studied such structures are the 'striated microtubule-associated fibres' of (chlorophyte) green algae. These originate near the basal bodies, then run alongside each of the flagellar microtubular roots, and exhibit a periodicity of ~30 nm. The protein making up these fibres has been characterised in several green algae, and is called 'striated fibre assemblin' (SF-assemblin; Lechtreck & Melkonian, 1998). This is an elongate protein that assembles into long protofilaments, likely due to the individuals units joining together in an overlapping head-to-tail fashion. The whole 'striated microtubule-associated fibre' is then envisaged as a regularly organized parallel offset array of these protofilaments. SF-assemblin seems to have no detectable relationship with any cytoskeletal protein in animals. It is related, however, to 'β-giardin', an abundant cytoskeleton protein in the diplomonad *Giardia*. β-giardin also attaches to microtubules, as part of long vane-like structures along the microtubules that support the 'adhesive disk' (Elmendorf et al., 2003). Interestingly, proteins related to SF-assemblin are also encoded by the genomes of diverse alveolates and stramenopiles, suggesting that SF-assemblin-like proteins might be widespread among microbial eukaryotes (Harper et al. 2009).

**Replicating the flagellar apparatus – flagellar transformation**

Cells that have basal bodies or centrioles must create new basal bodies / centrioles as part of the process of cell division. In most flagellates the basal-body-centred MTOC nucleates the microtubules of the mitotic spindle, and one MTOC is needed for each spindle pole. Therefore, the replication of the flagellar apparatus (including the production of new basal bodies) to form the second MTOC is generally an early event in the process of mitosis and cytokinesis (as is the replication of centrosomes in animal cells, which are effectively homologous to the flagellar apparatus MTOC). In fact, the creation of new basal bodies is usually one of the first conspicuous preparations for cell division that the cell will make. As discussed above, new basal bodies usually form alongside existing basal bodies, and thus the process of flagellar apparatus replication starts from the existing flagellar apparatus.

We will first consider division of a cell that has two flagella and two basal bodies during interphase, with each basal body associated with two microtubular roots. In this example the two flagella and their connected basal bodies are functionally different from each other and are distinguishable (They are called '1' and '2' by convention), and the flagellar microtubular roots associated with each basal body are distinguishable and perform different roles. When the two new basal bodies are created, one originates more-or-less alongside each of the existing basal bodies. This results in the cell having four basal bodies in a single cluster. The cluster breaks into two pairs of two basal bodies in preparation for mitosis. Each pair consists of one old basal body and one of the newly-formed basal bodies. Thus, one pair includes basal body 1 and the other pair includes basal body 2. This disrupts the flagellar apparatus of the cell, and in fact, the microtubular roots may retract back to short structures at this stage (Note - each new basal body has often grown a short flagellum by this point, while the flagella associated with the old basal bodies often shorten up during cell division, and regrow later).

As cell division progresses, the flagellar apparatuses of the daughter cells are reorganised. In the pair that includes flagellum 1, this flagellum and basal body keeps performing the function of flagellum 1, and its microtubular roots return to the same functional roles. The new basal body and flagellum takes on the role of flagellum 2, including growing the microtubular roots normally associated with basal body 2. However, in the other pair, the flagellum that used to be flagellum 2 **transforms** into a flagellum 1, and its microtubular roots transform into (or are replaced by) the microtubular roots associated with basal body 1. The new flagellum becomes the flagellum 2 for this daughter cell.

If we follow the process from the point of view of a basal body – a new basal body is created in one cell, and when that cell divides, becomes the basal body '2' of one of the daughter cells. When this cell divides in turn, this basal body becomes the basal body '1' of one of the daughter cells. In all future cell divisions it remains as basal body '1' in whatever cell it is passed to. Thus, we can say that basal bodies in biflagellated cells take two cell generations to fully 'mature' (i.e. to transform to their final state).

The process is similar in a cell with four flagella in the interphase state, except that basal bodies take even more cell generations to mature. In this case the basal bodies are called '1', '2', '3' and '4'. In general there are still only four or fewer large microtubular roots in cells with four basal bodies, and they are all most closely associated with basal bodies 1 or 2 (and not basal bodies 3 or 4, with a few exceptions). Prior to

division, newly created basal bodies form alongside existing basal bodies, so that there are, briefly, eight basal bodies per cell. Then the cell divides such that basal bodies 1 and 4, plus two new basal bodies, end up in one daughter cell, and basal bodies 2 and 3, plus two new basal bodies, end up in the other daughter cell. The old basal body 2 transforms into a basal body 1 (as in the biflagellated cell), while the old basal bodies '3' and '4' both transform into basal body '2's. The new basal bodies become either '3's or '4's. To trace the fate of one basal body; it is created new in one cell, becomes a '3' or '4' in the next generation, then a '2' in the generation after that, and finally a '1' in the generation after that, and in all subsequent generations. Thus, in species with four flagella, basal bodies take three cell generations to fully mature.

Although flagellates from across most of the diversity of eukaryotes have flagellar apparatuses with two (or more) basal bodies and multiple flagellar roots, the orientation of these flagella and the appearance and roles of the microtubular roots vary greatly in different groups. Traditionally it has been very difficult to establish which basal body or microtubular root in one taxonomic group is homologous to a particular basal body or microtubular root in another taxonomic group (imagine being in a situation where it was difficult to tell whether wings of birds were homologous to the arms of humans, or the legs of humans). Fortunately, the maturity behavior of the flagellar apparatus, when known, can be used to establish homologies - For example, the 'mature' (eldest) basal body in different groups should be directly homologous. Gradually, researchers are trying to apply a 'universal terminology' to as many flagellated eukaryote groups as possible. In this universal terminology the mature basal body is called 'basal body 1', the next most mature is 'basal body 2'. In cells with four flagella, the remaining (least mature) basal bodies are '3' and '4' (This is the terminology we used in the examples above). Interestingly, in almost all anisokont flagellates where flagella transformation has been studied it is the most posteriorly directed flagellum that is the eldest, suggesting that this arrangement might have been inherited from an ancient common ancestor of most or all living eukaryotes.

**Support for the cell membrane.**

Many microbial eukaryotes (especially many flagellates) have a cell membrane that is supported and given shape primarily by assemblages of microtubules that lie immediately under the cell membrane (or nearly so). This contrasts with typical animal cells, in which microtubules play a smaller role in supporting the membrane. These supporting microtubules are typically separated, rather than forming ribbons of adjacent microtubules, as in microtubular roots, though they may be interlinked. They usually do not directly originate from an MTOC centred on the basal bodies, instead they typically form from different sites that function as MTOCs. One common pattern is that these microtubules will form near the edge of one of the microtubular roots ('R3') associated with basal body 2. This is seen in many stramenopiles and many excavates, for example, despite these two groups being distantly related. This may also represent an extremely ancient aspect of the organization of the cytoskeleton within eukaryotes.

In other groups of eukaryotes the microtubules originate from an MTOC at the anterior end of the cell that is not connected to the flagellar apparatus. In Apicomplexa, for example (which lack flagella except, sometimes, as male gametes) longitudinal

microtubules that support the cell membrane originate around the apical complex from a structure called the 'polar ring'.

**Pseudopodia**

Pseudopodia are conspicuous extensions of the cell, usually of a more temporary and/or variable nature than components like the flagellar apparatus. They are used for locomotion and feeding, and are typical for amoebae. A considerable number of flagellates also produce small/fine pseudopodia specifically for feeding. Pseudopodia also appear in other contexts, for example, diatoms recovering from cell shrinkage due to immersion in a hypertonic fluid (plasmolysis) may use fine pseudopodia to reform the appropriate connections to the frustule.

Pseudopodia come in several different subtypes, most of which have clearly evolved more than once in the history of eukaryote cells. Most pseudopodia are fundamentally actin-based structures, although some types are supported by microtubules. Large, broad pseudopodia containing substantial volumes of cytoplasm are called **lobopodia**. Their structure is complex, but is essentially based on actin networks within the region under the cell membrane. Fine, pointed forms are called **filopodia** and are supported by arrays of parallel actin microfilaments. The ability to produce filopodia is particularly widespread across eukaryotes. Some filopodia-like pseudopodia are able to branch. **Lamellipodia** are broad, but very flat, and are supported by branching actin networks (see above). The structure of actin-supported pseudopodia will be examined in more detail later in the 'locomotion' section of the course.

The most widespread forms of microtubule-supported pseudopodia are the **axopodia**. These are superficially similar to long, stiff filopodia, but tend to be larger, and more constant in length and do not normally branch. Axopodia are supported by regular arrays of parallel microtubules, with the precise substructure of the array varying between groups. This variation reflects the multiple independent origins of axopodia over the evolutionary tree of eukaryotes. **Reticulopodia**, which are characteristic of foraminifera, are another form of pseudopodia that are supported by microtubules, but are distinguished by extensive branching and anastomosing (i.e. network-forming) behaviour. Reticulopodia can be extremely dynamic, with the ability to rapidly extend and change conformation. There is also a particularly rapid intracellular-transport-like system of bidirectional movement of particles along the reticulopodial network. This dynamism is facilitated in part by sliding of the microtubules *within* the parallel bundles that support the pseudopodia. It is also helped greatly by the ability of the microtubules to transform to unusual alternate arrangement of tubulin dimers – a coiled, flexible 'helical filament' form - instead of undergoing classic microtubule disassembly (discussed in Habura et al. 2005). The transformation between microtubules and 'helical filaments' is rapid and reversible, allowing the rapid shortening and elongation of microtubules. For example, organised bundles - 'paracrystals' - of the helical filament form can be transported along the reticulopodial network; this then allows the extremely rapid formation of new microtubule bundles at remote sites within the network. An ability to shorten extremely rapidly is also a feature of some axopodia (see above).

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