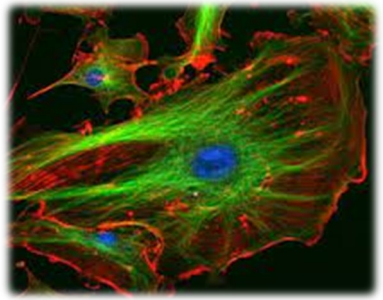
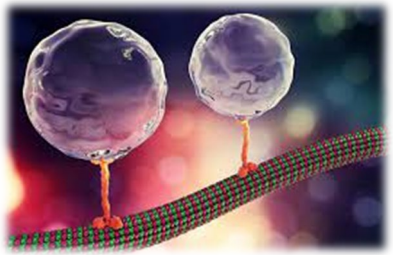
Cell-Structure Support and Motility Systems

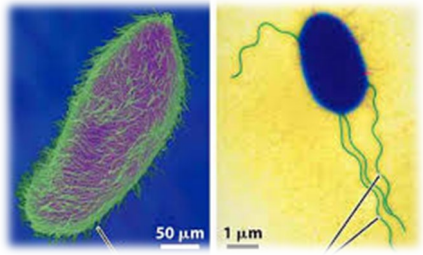
- Cytoskeletal proteins.



- Cell walls and cell shape.

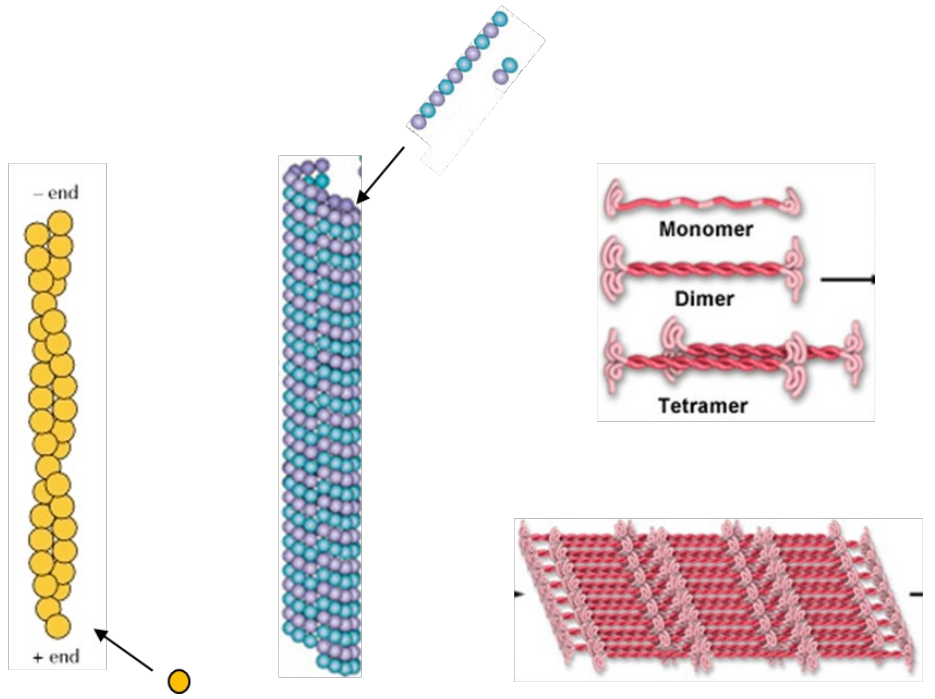


- Molecular motors.
- Swimming motility.



Evolution of Cytoskeletal Proteins

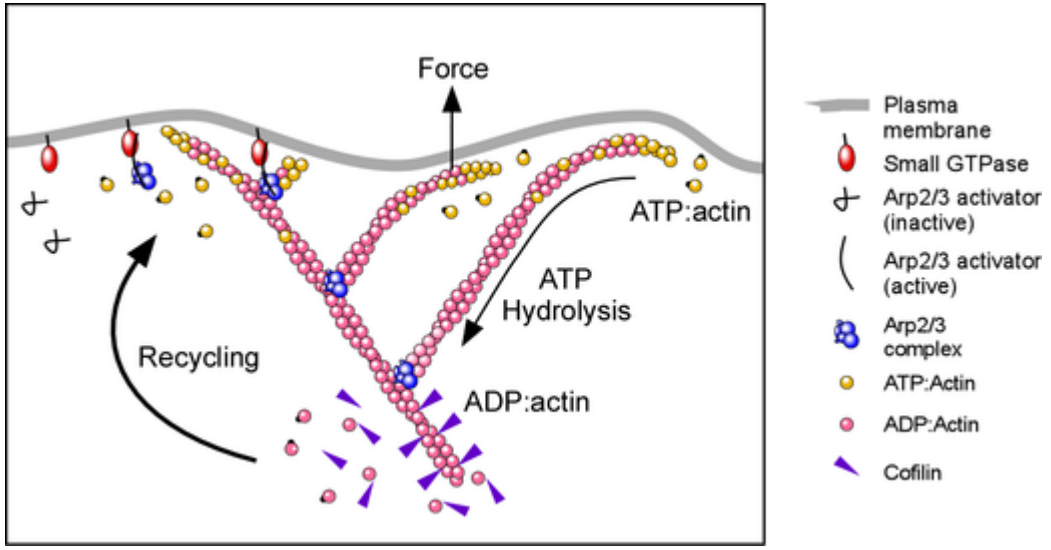
- Filaments / fibrils evolved at least two times prior to the emergence of eukaryotes: actins and tubulins.
- Diversification to novel functions within eukaryotes, and shifts in functional roles relative to prokaryotes.
- Energetic costs of investment.

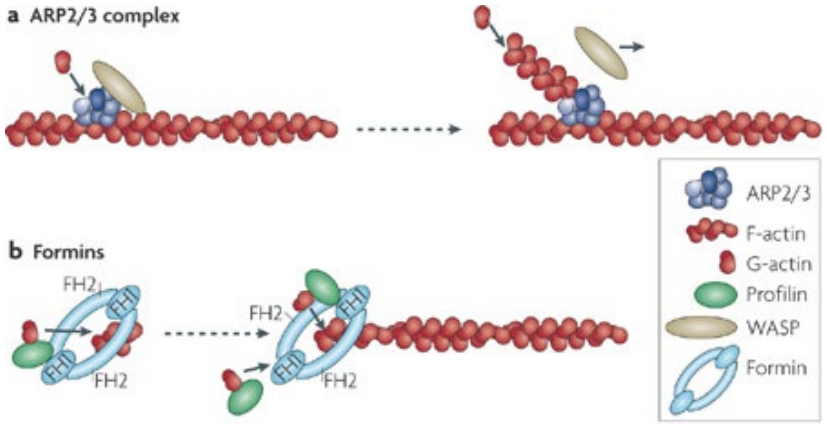


Actin Tubulin Intermediate Filaments

Actin Filaments

- Assembles into homopolymeric double-filaments via ATP hydrolysis.
- Treadmilling – addition of monomeric subunits at the plus (barbed) end, combined with removal at the minus end results in “apparent” movement of a filament; there is a critical concentration of monomers beyond which the two rates are equal, and there is no net growth.
- Functions include organelle motility, cell division, cell signaling, and maintenance of cell shape.
- Amino-acid sequences are highly conserved across all of eukaryotes (only 10% AA-sequence divergence between yeast and mammal).
- Many accessory proteins determine how and when microfilaments assemble, e.g., heptameric ARPs.





Pieta et al., 2008, Nature Reviews Mol. Cell Biol.

Evolutionary Diversification of Actin Functions in the Ciliated Protozoan, *Paramecium tetraurelia*

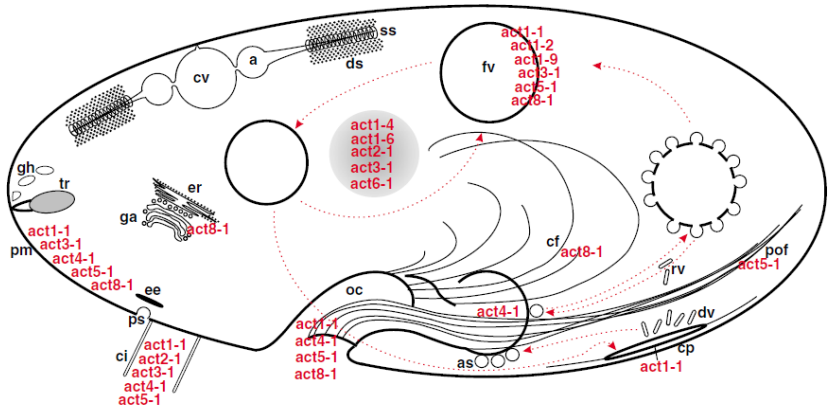
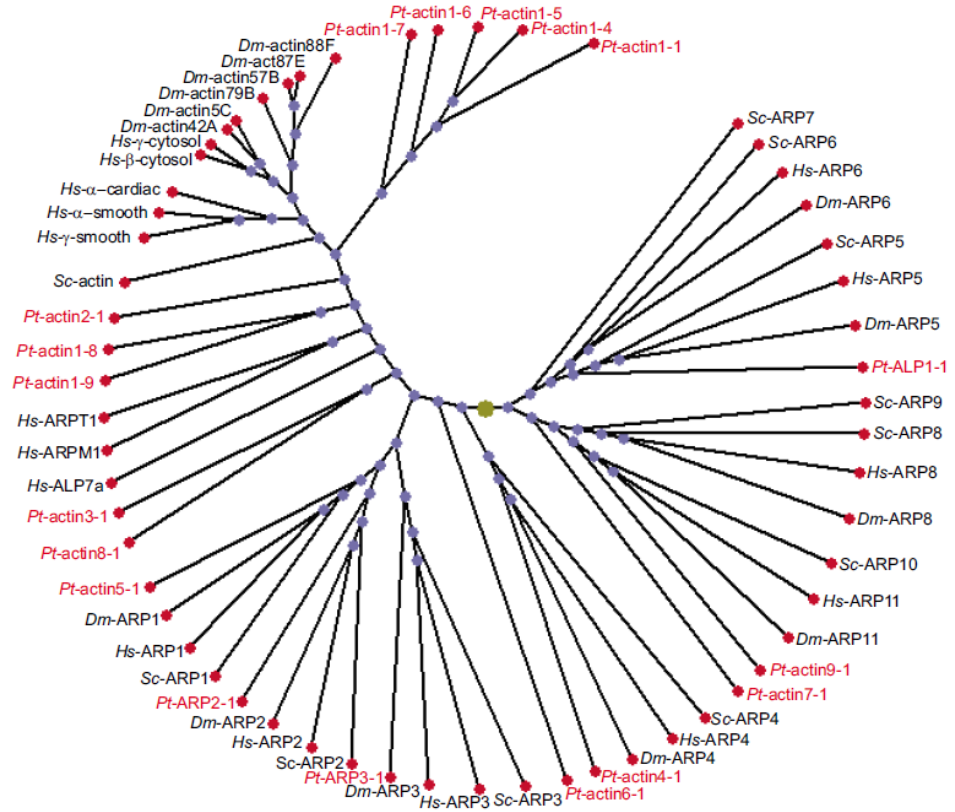


Fig. 11. Schematic distribution of actin isoforms in the *Paramecium* cell, as outlined in Table 1. The trafficking scheme is based on published reviews (Fok and Allen, 1990; Allen and Fok, 2000; Plattner and Kissmehl, 2003). Actin distribution is based mainly on the present data obtained with GFP localization in vivo and with antibody labeling, but also takes into account data from previous work (Tiggemann and Plattner, 1981; Kersken et al., 1986a; Kersken et al., 1986b; Kissmehl et al., 2004). The scheme contains elements of the osmoregulatory system (a, ampula; cv, contractile vacuole; ds, decorated spongiome; ss, smooth spongiome), though consistently unlabeled, of the phagosomal apparatus (as, acidosomes; cf, cytopharyngal fibers; cp, cytoproct; ci, cilia) dv, discoidal vesicles and other recycling vesicles, rv; ee, early endosome; er, endoplasmatic reticulum; fv, food vacuole; ga, golgi apparatus; gh, ghosts (from released trichocysts); oc, oral cavity; pm, plasma membrane; pof, post oral fibers; ps, parasomal sacs; tr, trichocysts; gray background, cytosolic.

Table 2. Localization of the different actin isoforms

	GFP localization								
	actin1-2	actin1-4	actin1-6	actin1-9	actin2-1	actin3-1	actin5-1	actin6-1	actin8-1
Cortex	–	–	–	–	–	+	+	–	+
Oral cavity	–	–	–	–	–	–	+	–	+
Oral filaments	–	–	–	–	–	–	+	–	+
Phagosomes	+	–	–	+	–	+	+	–	+
Cilia	–	–	–	–	+	+	–	–	–
Cytosolic compartment	–	+	+	–	+	+	–	+	–
Cleavage furrow	–	–	–	–	–	–	–	–	–



Sehring et al., 2007, J. Cell Science

Tubulin Microtubules

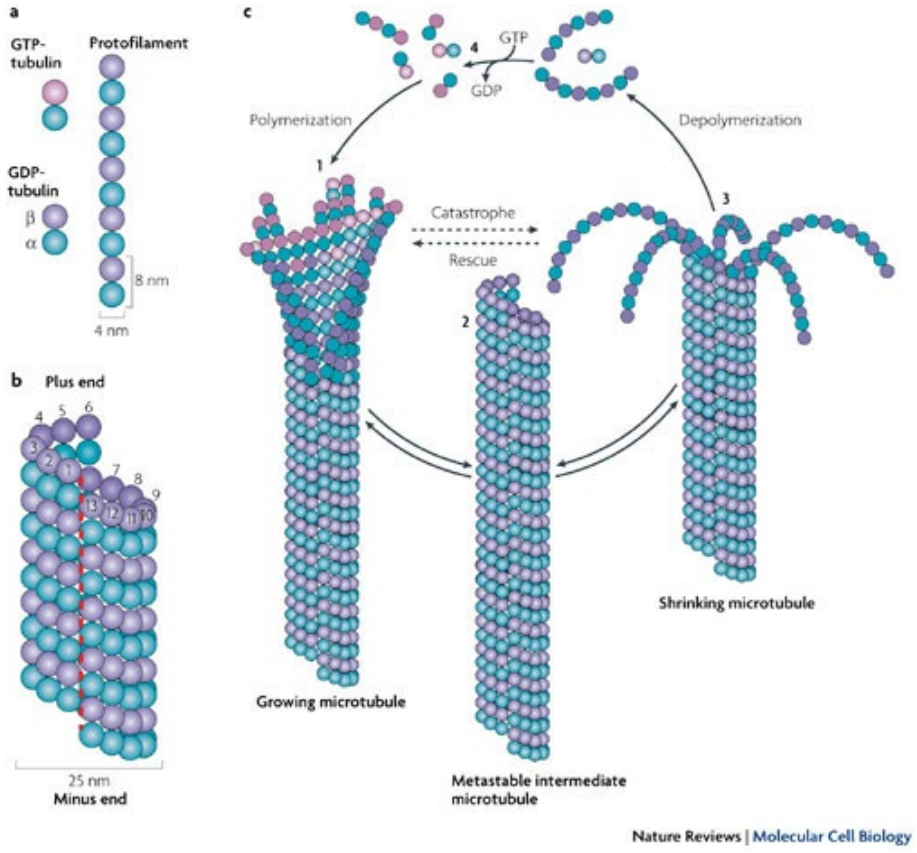
- The “elementary particles” are heterodimers of α- and β-tubulin, which assemble into higher-order structures called protofilaments, which in turn assemble into tubes of 13 laterally connected filaments.

- Assembly involves GTP hydrolysis.

- Assembly dynamics and bending makes the filaments cytomotive, and specific motors use them as highways for organelle transport.

- Roles in maintenance of shape, cell division (mitotic spindle), and motility (cilia and flagella).

- Post-translational modifications: the Tubulin Code in the C-terminal tails marks subpopulations of microtubules for specific downstream functions.



Intermediate Filaments

- Differ from microtubules and microfilaments in structure and phylogenetic distribution.

- Not dynamic, and are primarily used for mechanical strength.
    - Nuclear lamins, which support the nuclear envelope, and keratins are intermediate filaments.

- Largely eukaryotic, an exception being crescentin in *Caulobacte*r, which causes the helical cell shape.

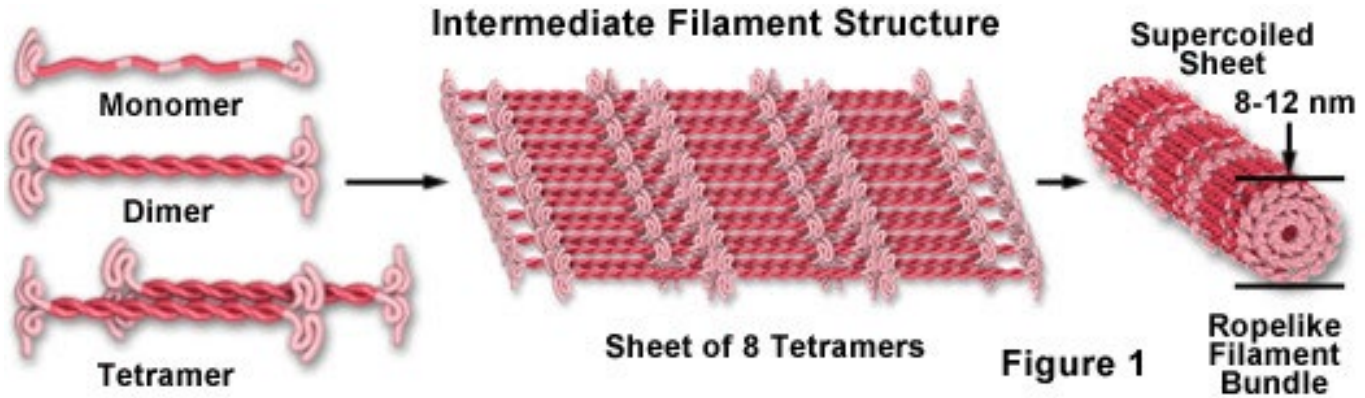
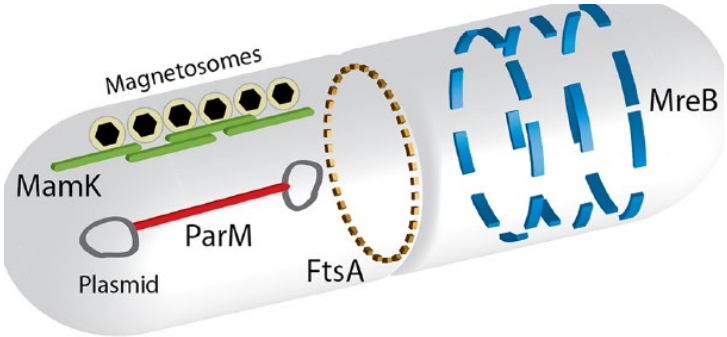
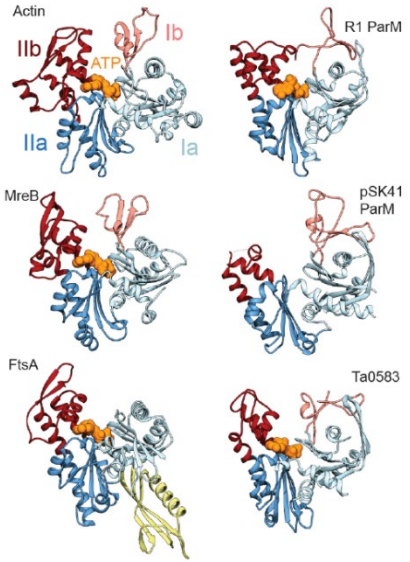


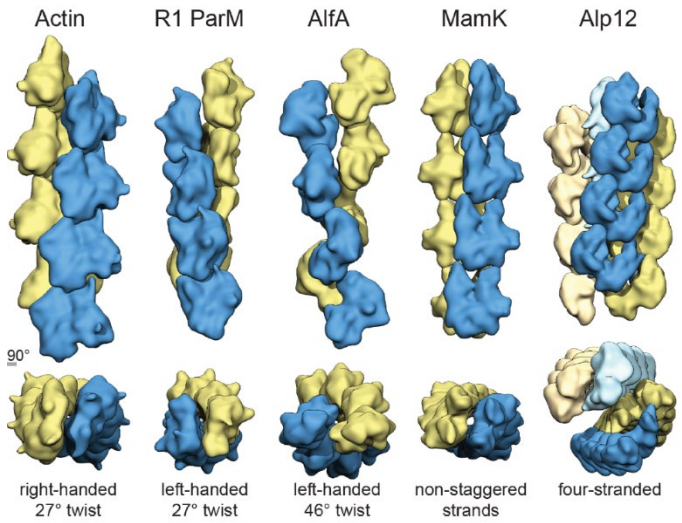
Figure 1

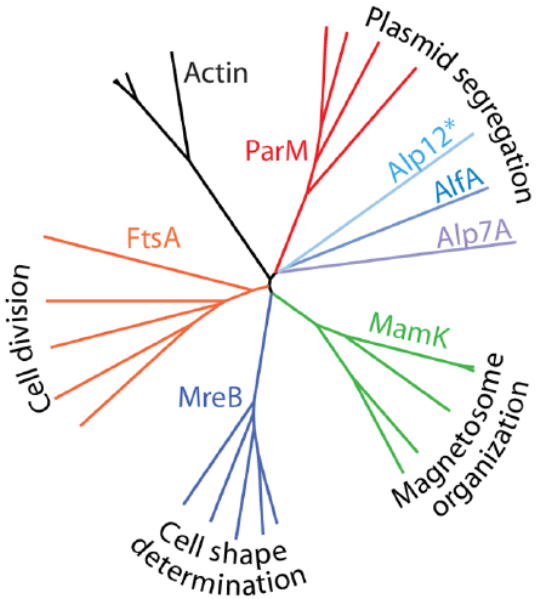
Diverse Functions of Actins in Prokaryotes

- Cell-shape sculpting: MreB.
- Plasmid replication partitioning: ParM.
- Magnetosomes: MamK organizes magnetite crystals used in orientation.







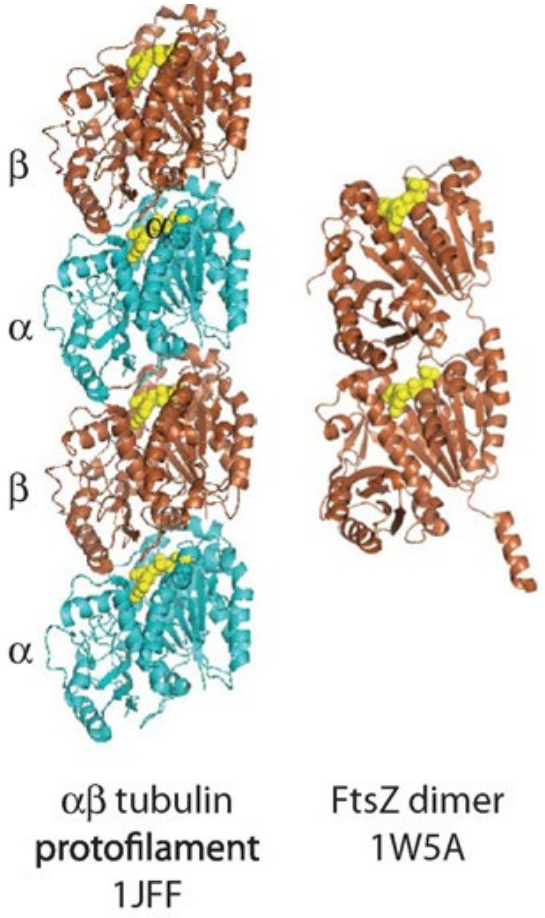


Architectural variants: bacterial actins all share the core actin structural fold.

Ozyamak et al., 2013, Biochem.

Tubulin-Like Filaments in Bacteria

- Cytokinesis in bacteria often involves constriction of an internal ring consisting of the FtsZ protein.
- Although the structure of FtsZ subunits is very similar to that of tubulin, they are only 10% similar at the AA-sequence level.
- Despite this sequence divergence, FtsZ and tubulin are identical at key sites involved in ATP binding, suggesting common ancestry.
- Unlike tubulin, which consists of heterodimeric subunits that form tubes, FtsZ is homopolymeric and forms filaments, not tubes.



Wickstead and Gull (2011); Lowe and Amos (2009)

Some Members of the Verrucomicrobiales Genus *Prosthecobacter* make Microtubules

- Two tubulin-like genes, BtubA and BtubB, make proteins that form heterodimers, which in turn polymerize into five-filament microtubules.
- BtubA/B are very closely related to eukaryotic tubulin – acquired by horizontal transfer or the source of eukaryotic subunits?

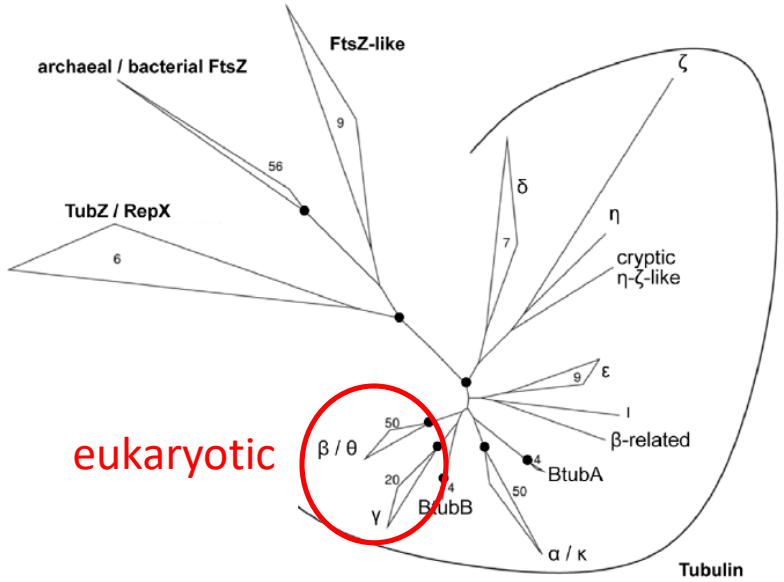


Figure 6. BtubA and BtubB represent two novel tubulin subfamilies in the eukaryotic clade of tubulins. In global phylogenetic analyses of the FtsZ/Tubulin superfamily, BtubA and BtubB stably clustered within the clade of eukaryotic tubulin subfamilies (i.e., the Tubulin family). A second stable group of sequences comprised bacterial and archaeal tubulin homologues (FtsZ, FtsZ-like, TubZ, RepX). The relationships between tubulin subfamilies were instable (except β-θ and α-κ). Here and in further phylogenetic analyses (Figure S11, Tables S1 and S2, and Materials and Methods) no stable associations between BtubA or BtubB and any tubulin subfamily were detected, in agreement with a previous less comprehensive study [11]. Shown is one representative maximum likelihood tree calculated using a 10% minimum similarity filter. A black circle indicates that the respective node/group was stable in different trees. Bar represents 1% estimated evolutionary distance. Numbers indicate how many sequences were included in a closed group.
doi:10.1371/journal.pbio.1001213.g006

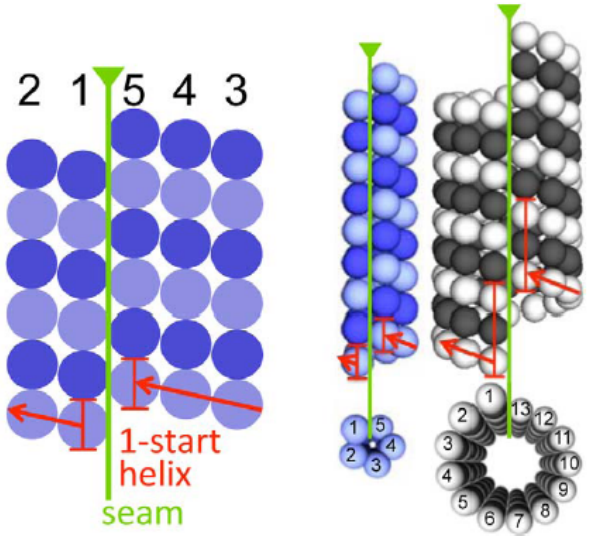


Figure 4. Structural model of "bacterial microtubules." (A) 2-D schematic of the proposed architecture of bacterial microtubules built from BtubA (dark-blue) and BtubB (light-blue). Protofilaments are numbered 1–5. (B) 3-D comparison of the architectures of a bacterial microtubule (left; BtubA in dark-blue; BtubB in light-blue) and a 13-protofilament eukaryotic microtubule (right; β-tubulin in black; α-tubulin in white). Seams and start-helices are indicated as in (A).
doi:10.1371/journal.pbio.1001213.g004

Pilhofer et al. (2011, PLoS Biol.)

Role Reversal

- Bacteria employ a ring of tubulin-related protein in cytokinesis, whereas eukaryotes employ a ring of actin.
- Bacteria use actin-like proteins in chromosome separation, but eukaryotes deploy spindle microtubules for such purposes.
- At least three divergent mechanisms of cell division exist within the archaea:

  one bacterial (FTsZ)-like,

  one eukaryotic (actin)-like,

  one based on an ESCRT-related system (endosomal sorting complex required for transport; used in vesicle partitioning in eukaryotes).

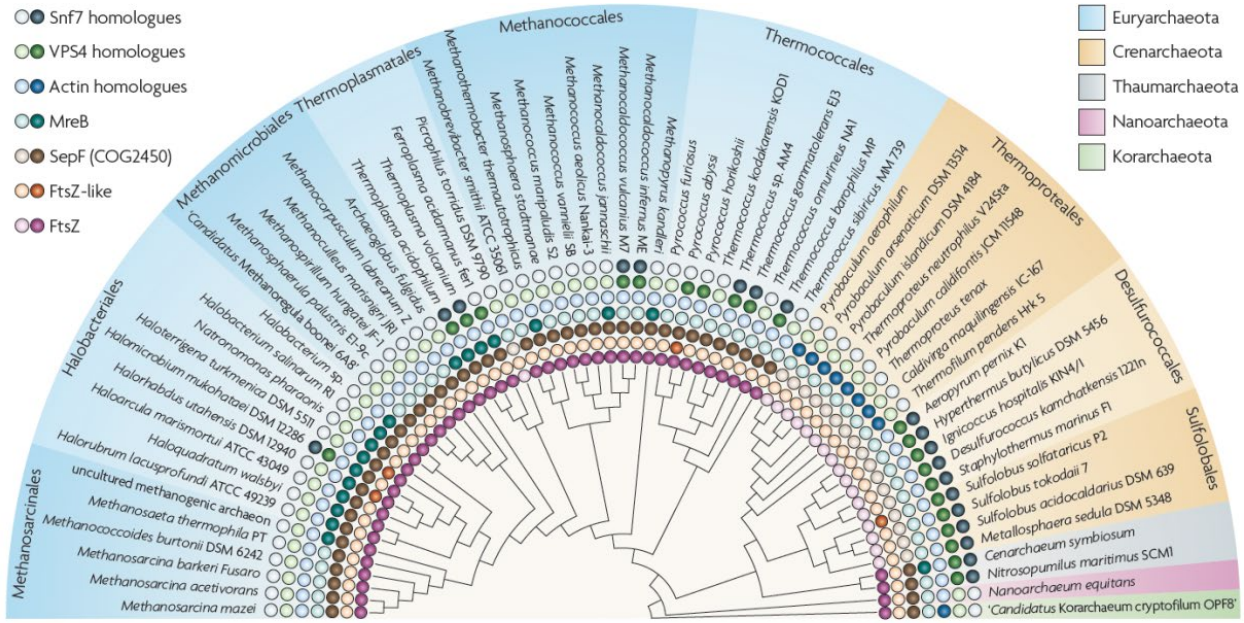


Figure 2 | **The distribution of the key components of membrane manipulation systems among the Archaea.** The phyletic patterns for indicated proteins are represented by filled circles to show the presence of the proteins and empty circles to show their absence. This is arranged according to the archaeal phylogenetic tree, the topology of which is a consensus based on several recent analyses[63–65]. Species are coloured by phyla and then subdivided into orders. VPS4, vacuolar protein sorting 4.

(Makarova et al. 2010, Nature Rev. Microbiol.)

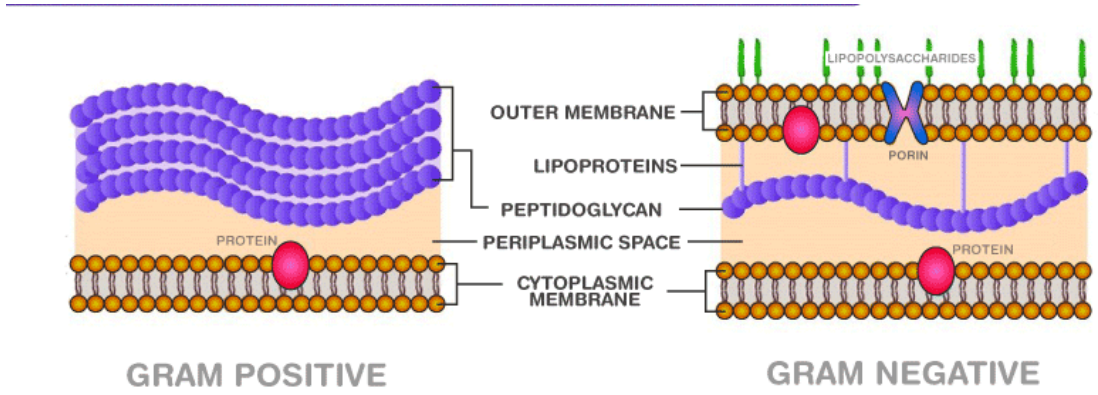
Energetic Investment in Actin Filaments and Tubulin Microtubules

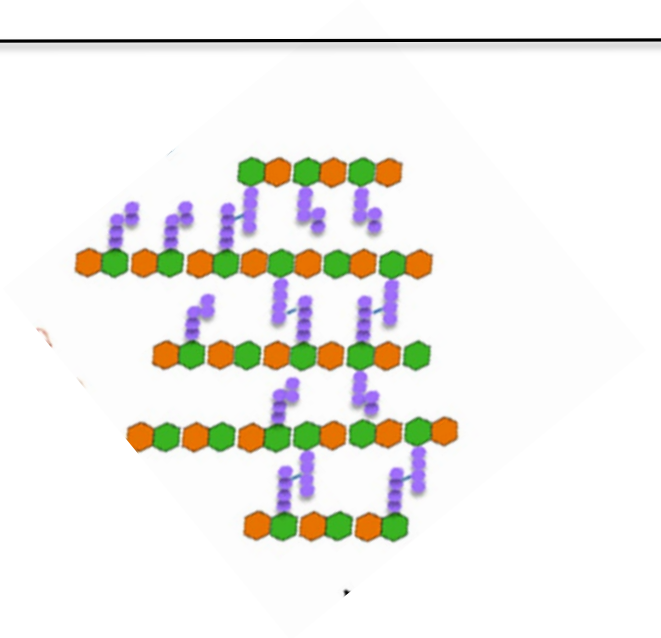
Fraction of Whole-Cell Construction Costs:

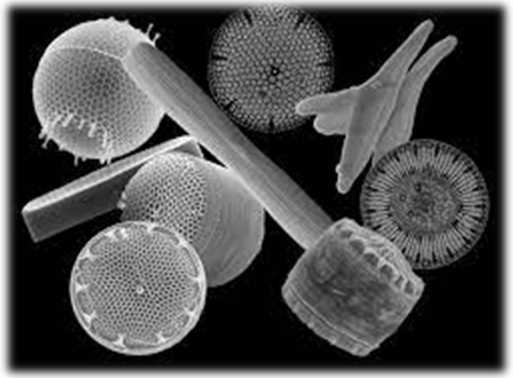
	Actin	Tubulin
Saccharomyces cerevisiae	0.0007	0.0022
Schizosaccharomyces pombe	0.0031	0.0007
Mouse fibroblast cells	0.038	0.019
Human HeLa cells	0.001	0.005

	FtsZ	MreB
Escherichia coli	0.0016	0.0004

Cell Walls and Cell Shape

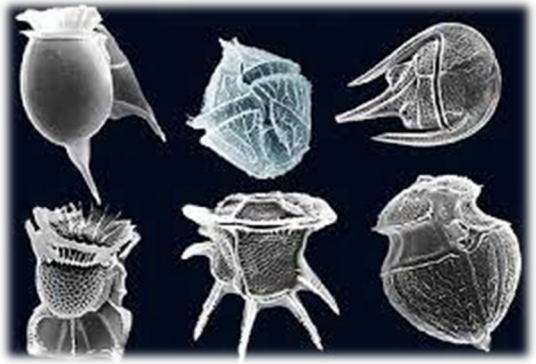






Diatoms
(silicon dioxide)

Haptophytes
(calcium carbonate)



Dinoflagellates
(cellulose)

Benefits and Costs of Cell Walls and Shapes

- Hard walls resist turgor pressure, eliminating the need for a contractile vacuole.
- High surface area : volume ratio can enhance nutrient uptake.
- Flatter, more elongate shapes reduce sinking velocities.
- Protection against predators / herbivores.

- Rod-shaped bacteria tend towards (SA : V) = $2\pi V^{-1/3}$, when grown at different nutrient levels or compared between phylogenetic groups (Ojkic et al. 2019).
  - This implies a nearly constant aspect ratio (length : width) ≈ 4.

- Single amino-acid substitutions in the sculpting protein MreB can generate major changes in cell shape.

Realtor's Rule of Thumb

The yeast *Saccharomyces cerevisiae*: cost of entire cell ≈ 5 x 10^{10} glucose molecules.

- Cell wall is 4% protein, 30% mannose, 60% glucan, 1% chitin.

  Mannose and glucans impose a requirement of 1.2 x 10^{10} glucose molecules.

  With a total of 1.7 x 10^{6} proteins, an average cost of synthesizing and concatenating amino acids of 34 ATP hydrolyses each, and an average protein length of 400 amino acids, the cost of proteins (in glucose equivalents) is 7.2 x 10^{8}.

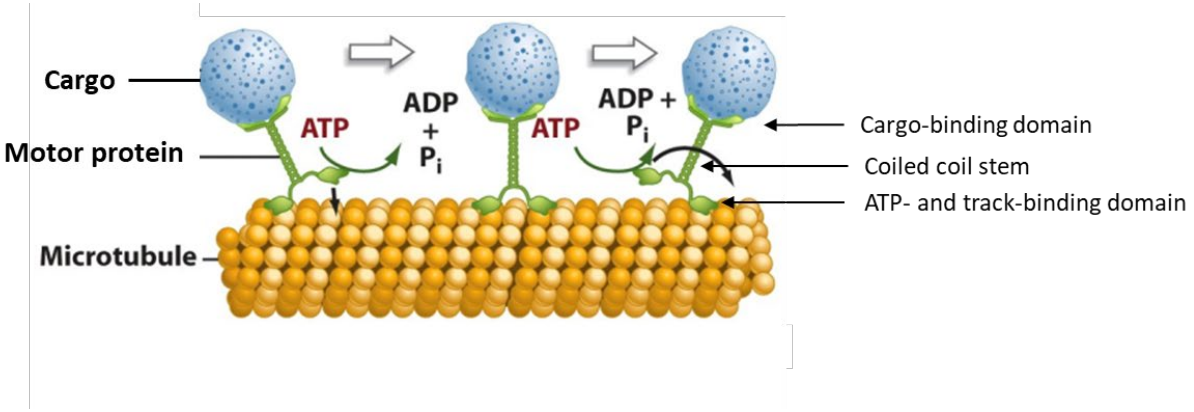
- Total fractional cost associated with the wall ≈ 26%.

- Cell membrane constitutes an additional 5% of the cell budget.

Entire cell envelope cost ≈ 31% of the total cell budget.

- Gram-negative *E. coli* and Gram-positive *Bacillus subtilis*: cost of the wall is less than half that of the membrane(s), but the total envelope cost is still ≈ 30% of the total cell budget.

Molecular Motors: a unique eukaryotic invention.



- All work by the same mechanism – the transduction of chemical energy into mechanical force, via the hydrolysis of ATP – each hydrolyzed results in one step forward.
- Each has an ATP-binding site, a track-binding site, and a tail domain involved in cargo attachment.
- Mechanical transport of cargos is carried out by three types of cytoskeletal motors – dynein, kinesin, and myosin – all of which have diverged into multiple families with diverse functions.
- Myosin travels exclusively on actin filaments; kinesins and dyneins on microtubules (tubulin filaments).
    - Commonly exploited by bacterial pathogens as transport mechanisms.
    - Involves coevolution between motors and tubulin/actin surface residues.

Recognition capacity and rate of progression of molecular motors (kinesins) is defined by sequences of C-terminal tubulin tails.

- Another example of molecular-language evolution.

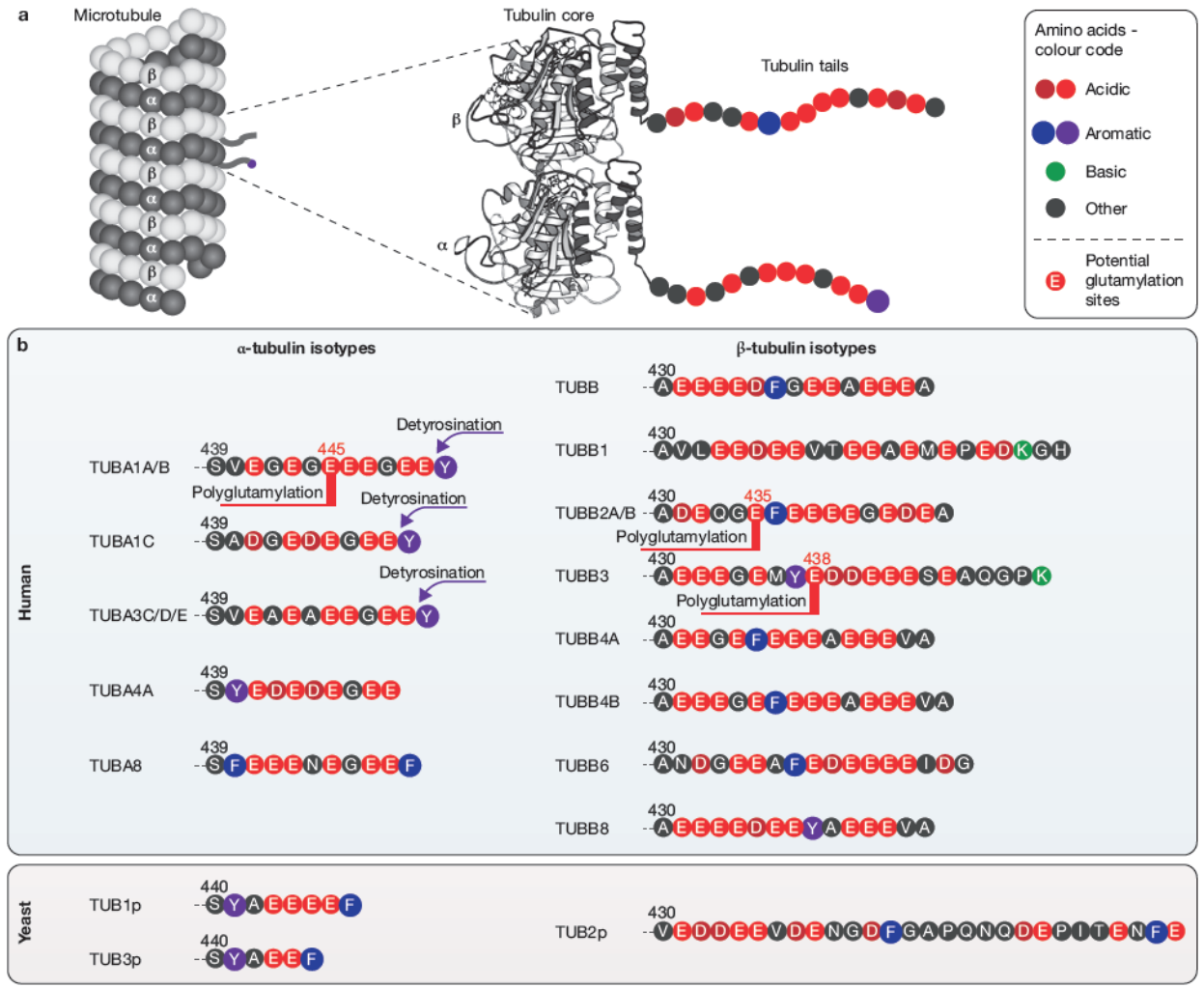
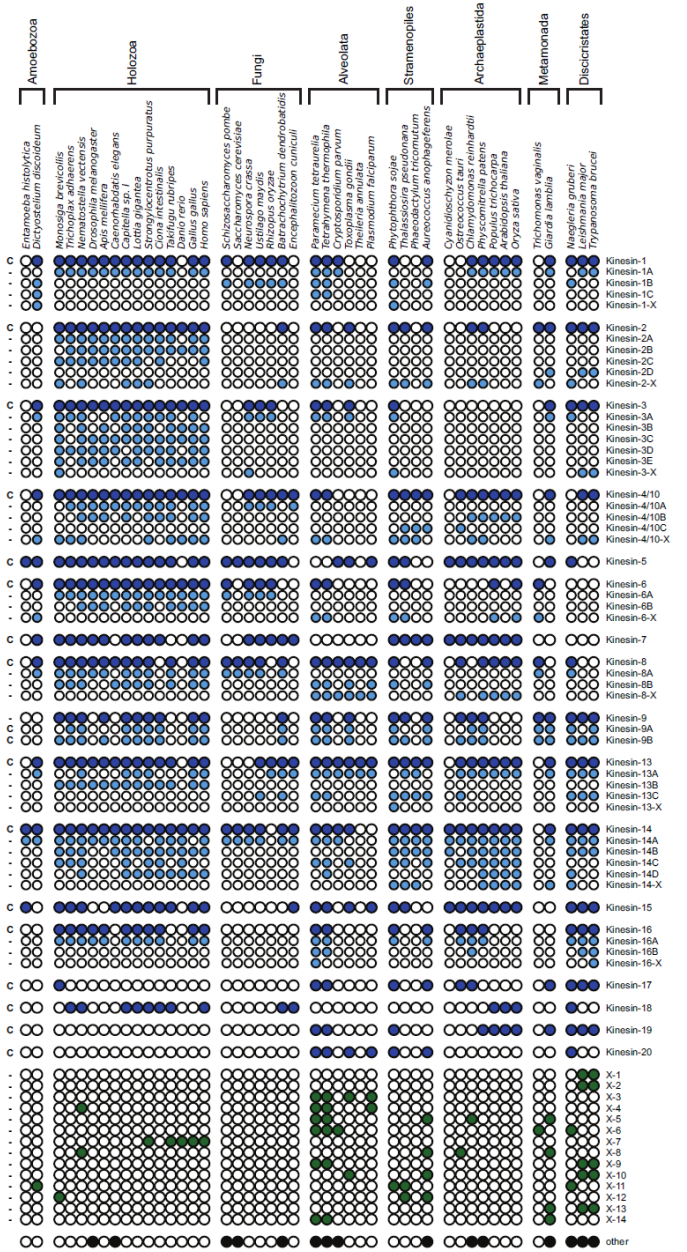


Figure 1 Diversity of C-terminal tubulin tails. (**a**) Schematic representation of the localization of C-terminal tubulin tails (CTTs) on the microtubule lattice. Left: schematic representation of a microtubule, with α- and β-tubulins shown as dark and light grey spheres, respectively. Right: structure of the α- and β-tubulin dimer, with added CTTs (which interact with microtubule-associated proteins and motors). Adapted with permission from ref. 5 © 2000 Elsevier. (**b**) Comparison of CTTs from all human and yeast (*S. cerevisiae*) tubulin isotypes. Previously identified polyglutamylation sites[13–15] are labelled. Generally, all glutamate residues within these tails could serve as potential polyglutamylation sites.

Wehenkel and Janke (2014, Nature Cell Biol.)

Kinesins: monomeric, dimeric, and tetrameric forms; both homo- and heteromeric.

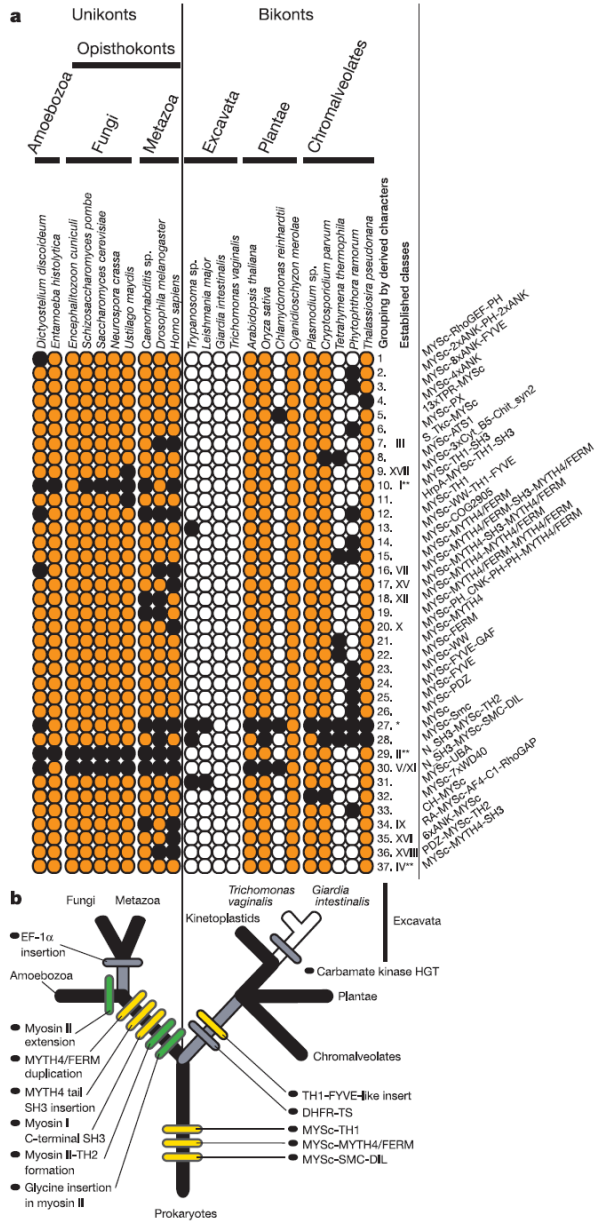
- Ubiquitous to all eukaryotes.
- Massive diversity across today's eukaryotes; an estimated 11 families in LECA.
- Multiple functions, including mitosis, meiosis, and cargo transport.
- Most move towards the "plus" end of microtubules, from cell centers to edges.



Wickstead et al. (2010, BMC Evol. Biol.)

Myosins: monomeric and dimeric forms exist.

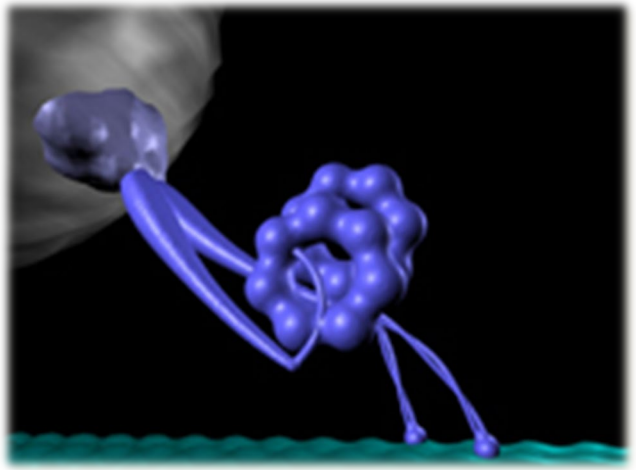
- Have diversified into a large number of classes, with numerous functions (e.g., vesicle transport, motility, muscle contraction).
- Myosin and kinesin may have evolved from a common ancestor – substantially different in size and sequence similarity, but have highly similar 3-D structures.
- Apparently absent from red algae, *Giardia*, and *Trichomonas*.
- Three ancestral genes likely in LECA.
- The modern use of these three families are consistent with LECA having a cilium and a mechanism for moving by pseudopodia.



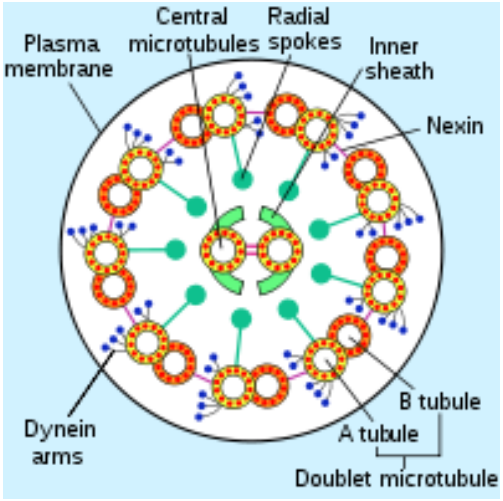
Foth et al. (2006, PNAS)
Richards and Cavalier-Smith (2005, Nature)

Dynein: the world's largest protein.

- Have a substantially different structure than kinesins/myosins, including an intramolecular hexameric ring, resulting from an ancient internal duplication.
- A huge molecule, >4500 amino-acid residues – so huge that perhaps every protein contains a transcription or translation error.
- Six AAA (ATPases Associated with cellular Activities) are encoded in a single gene (most other AAA proteins are homohexamers).



- All but one family member is associated with the flagellum: responsible for ciliar movement, causing microtubules to slide with respect to each other.
- Usually walk toward the minor ends of microtubules, towards the cell center.
- At least nine deeply diverging lineages, most of which go back to LECA.
- Were lost in the lineage leading to land plants and red algae (Wickstead and Gull 2007).



The Axoneme

The Bacterial Flagellum: the Icon of “Intelligent Design” or “Irreduceable Complexity”

- Flagella appear to have evolved independently at least three times across the Tree of Life.

- The same basic structure can be found in one or more species in all major bacterial groups, implying its presence in the ancestral bacterium.

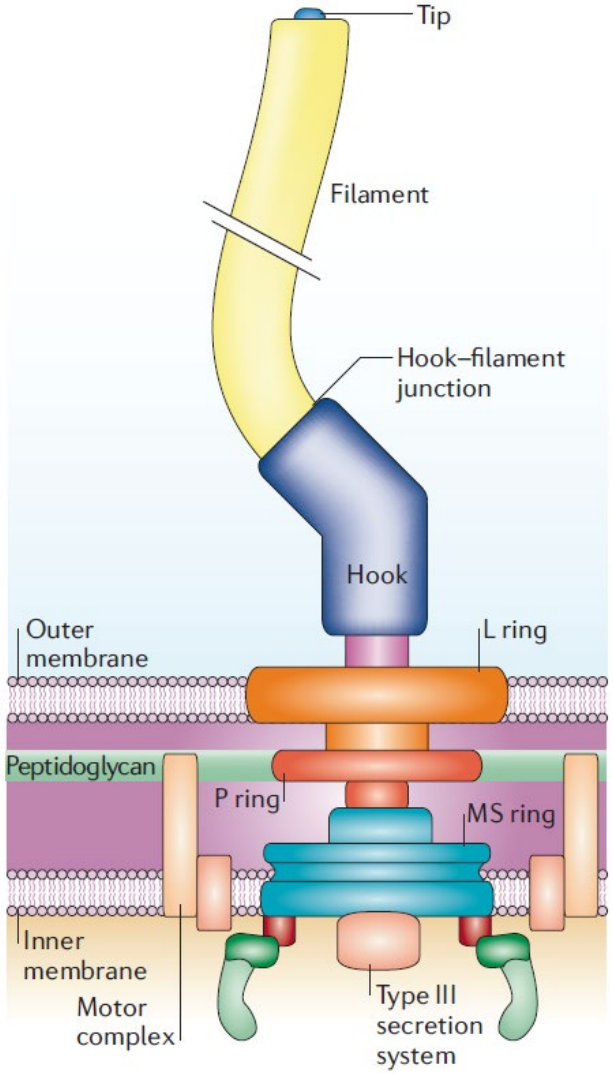
  - Many losses have occurred.

    - Cost of motility can be high: in a stirred culture of *S. typhimurium*, flagellated cells disappeared in ~10 days (Macnab 1992).

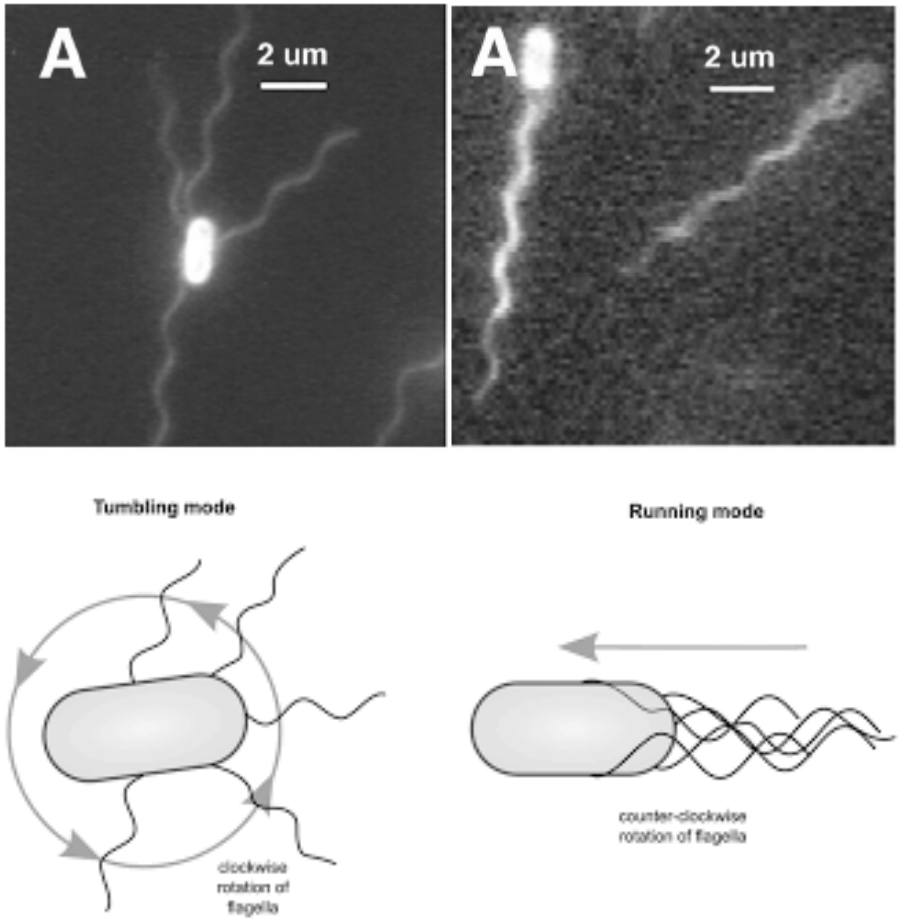
- Among bacteria, there are numerous variants:

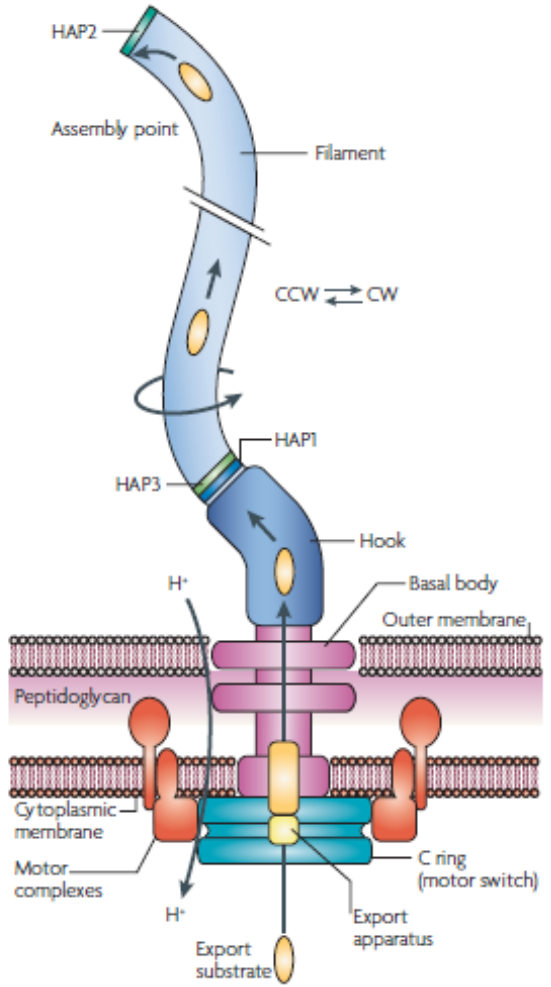
  - in some species, rotation is unidirectional, in others bidirectional;
  - some use a proton pump, others a sodium pump;
  - some species have just a single distal flagellum, others have many.

- Flagellar protein export leads to growth from the inside out, with exported proteins moving through a central lumen to the tip.



Flagellar Motility: run and tumble in *Escherichia coli.*

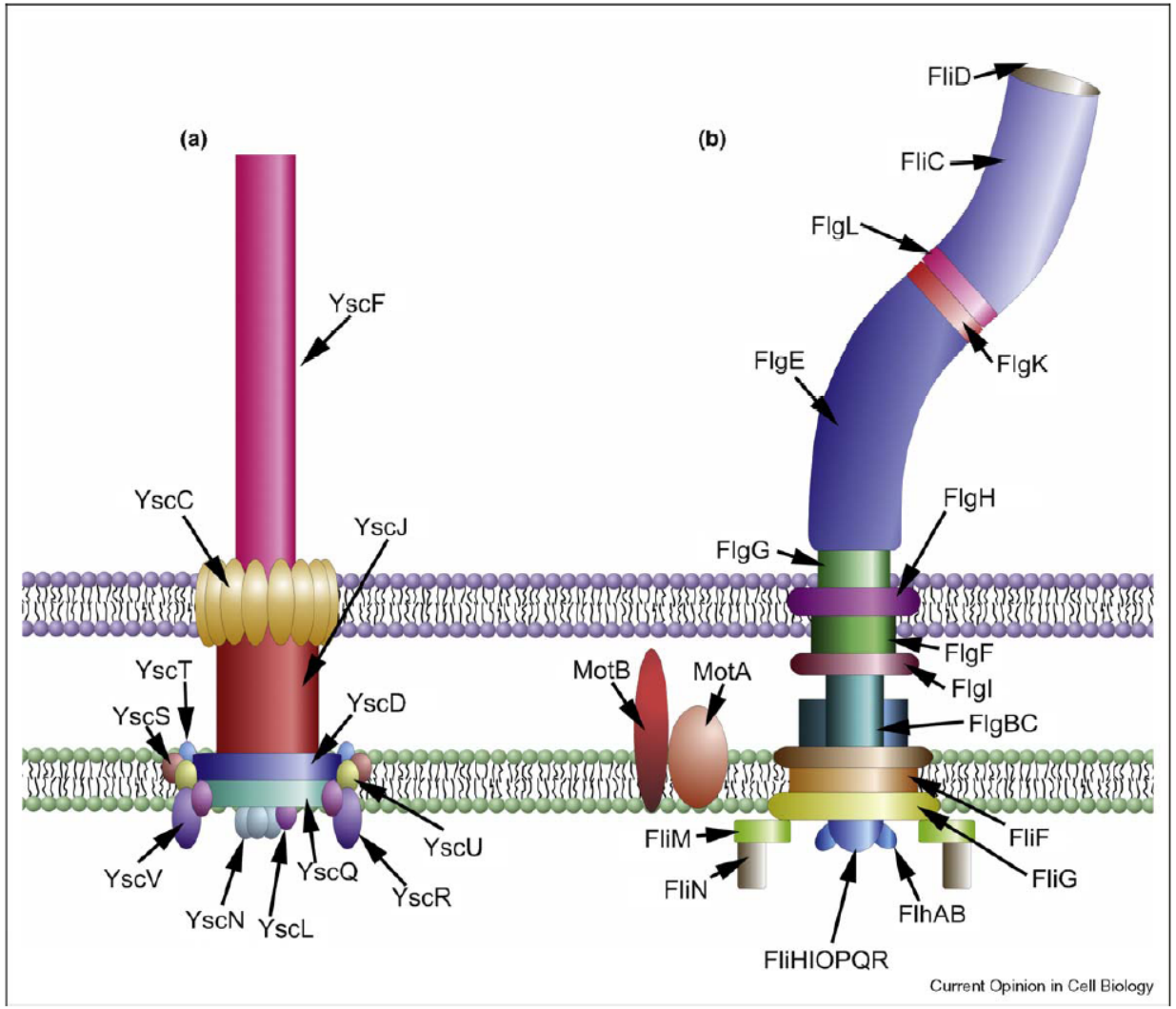




Turner, et al. (2000) Real-Time Imaging of Fluorescent Flagellar Filaments
Jarrell and McBride (2008) The surprisingly diverse ways that prokaryotes move

Type III Secretion System: antecedent form or descent from a common ancestor?

- T3SSs are present largely in bacterial species that interact with animal or plant hosts; either as pathogens or endosymbionts.
- Used to inject “effectors” into the host cell.



A cartoon showing the structural similarities between **(a)** the T3SS and **(b)** the flagellum. Reproduced with permission from Desvaux *et al.* [12]. The nomenclature used is from *Yersinia*. In the T3SS **(a)**, YscJLNPRSTUV have been shown to have homology with the corresponding proteins in the flagellar system. In the flagellar system **(b)**, FliFHIKNPQR and FlhAB have been shown to have homology with the corresponding proteins in the T3SS [12].

Egelman, 2010, Curr. Opin. Cell Biol.

The Bacterial Flagellum Consists of Components Thought to be Derived Via Gene Duplication

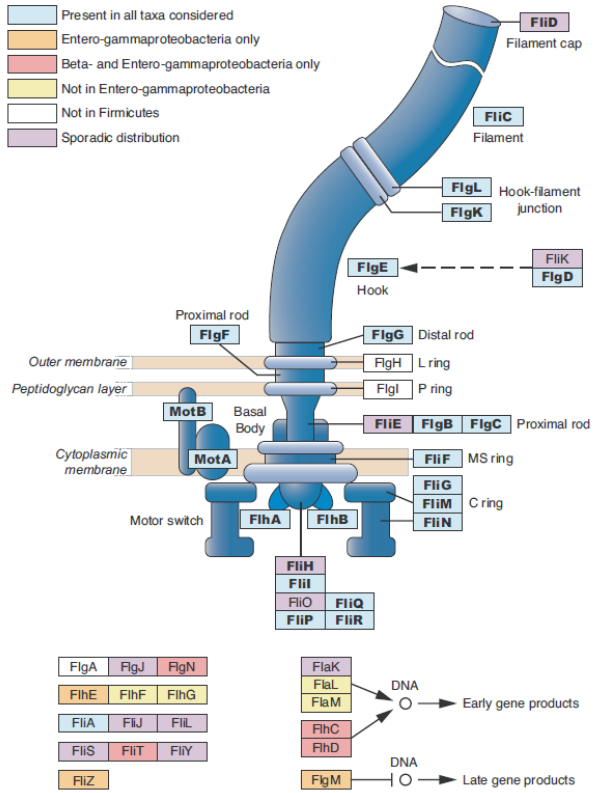


Fig. 1. Distribution of flagellar proteins (excluding chemotaxis proteins) among flagellated bacterial species. Those proteins encoded by the core gen designated in bold. This figure is redrawn with permission from that appearing in the KEGG pathway database (www.genome.jp/kegg/pathwa eco02040.html).

- Based on the relationships of the underlying proteins, an "inside-out" model is suggested, with the earliest proteins arising on the cytoplasmic side, and later proteins being located more distally.

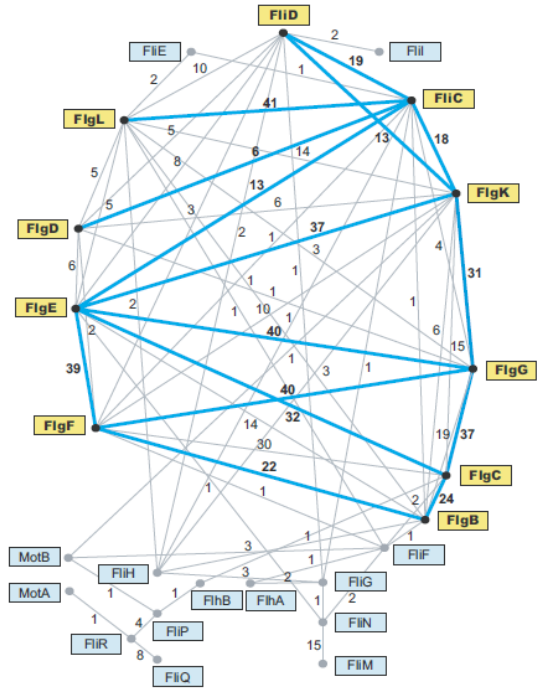


Fig. 3. Network of relationships among flagellar core proteins. Above each link is the number of genomes for which homology between a particular protein pair was detected by pairwise comparison at a cutoff value of 10^{-4} or lower. Blue lines linking yellow-boxed proteins portray the homology network revealed when core proteins of *E. coli* were subjected to pairwise comparisons.

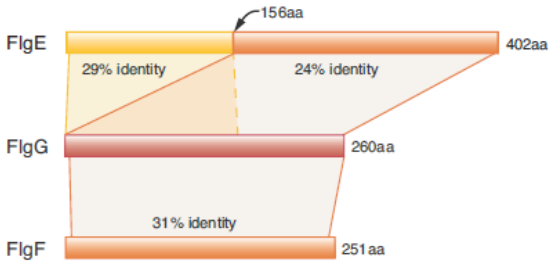
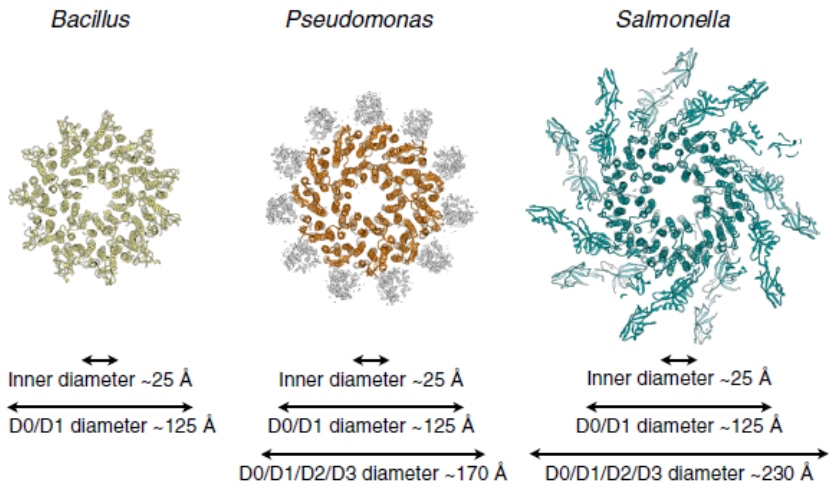


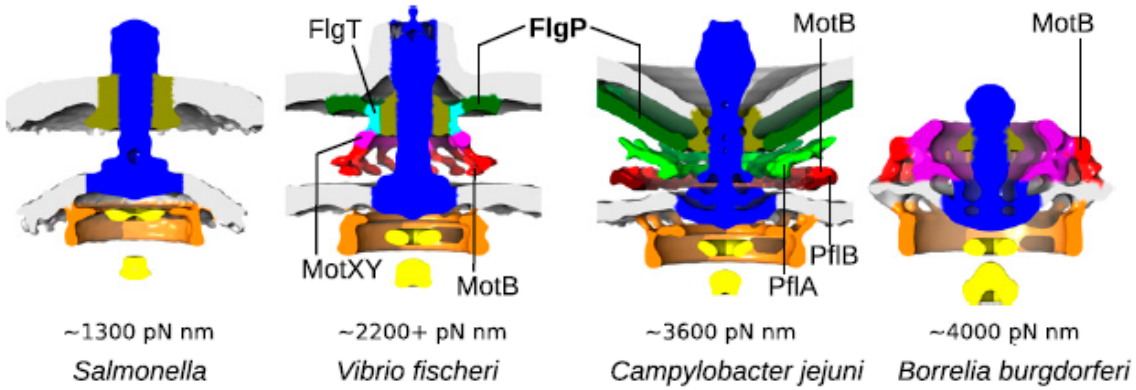
Fig. 4. Protein sequence similarity among the proximal rod protein FlgF, the distal rod protein FlgG, and the hook protein FlgE in *E. coli*. Whereas FlgF and FlgG are homologous over their entire lengths, FlgE contains an intragenic duplication at its N terminus.

Liu and Ochman, 2007, PNAS

Variation in flagellar filament thickness and number of “protofilaments”

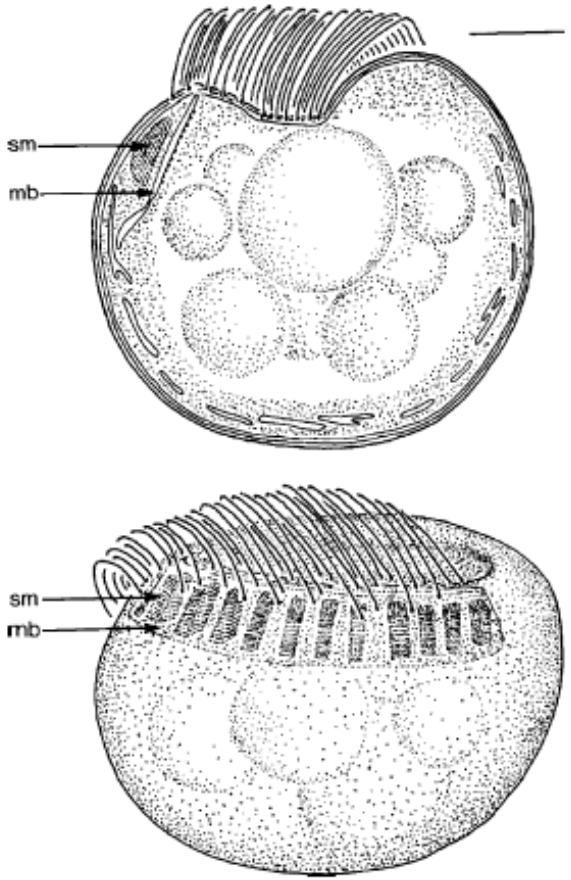


Variation in flagellar base structure



Beeby, et al. (2016); Wang, et al. (2017); Thomson, et al. (2017)

Some Bacteria Have Large Numbers of Flagella



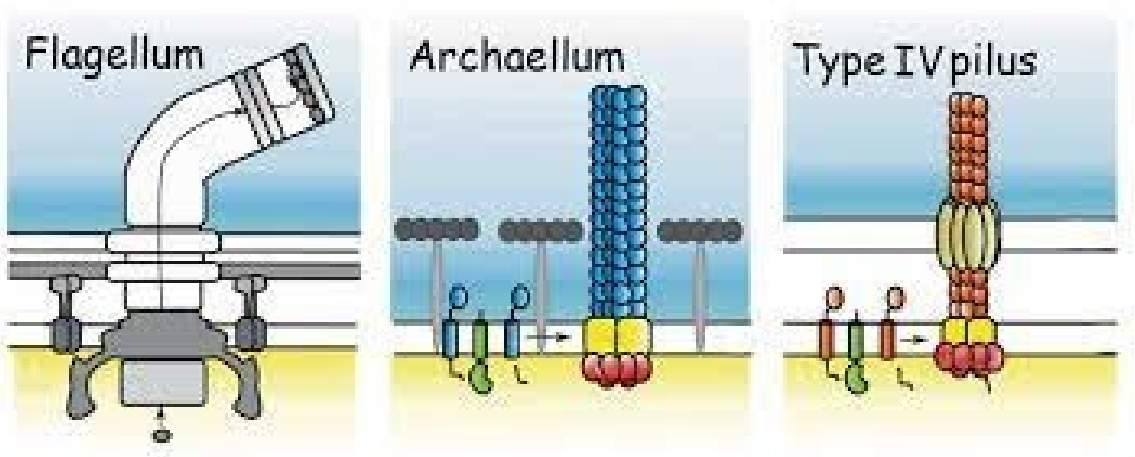
~ 400 flagella

Scale bar: 1 µm

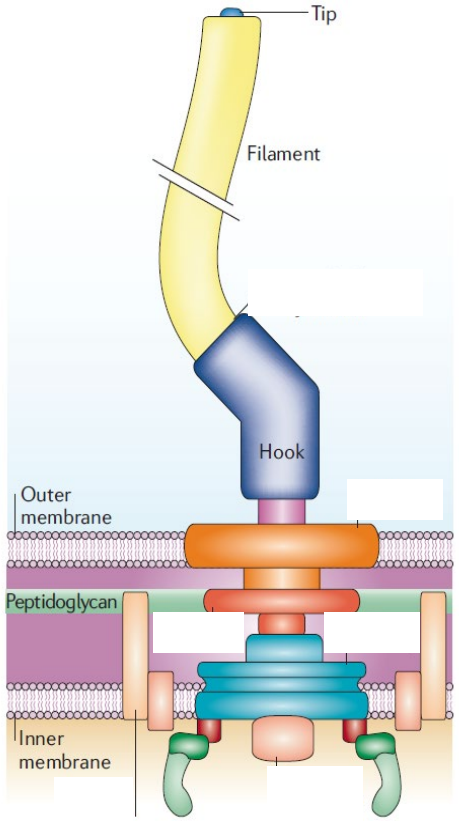
Fenchel and Thar (2004) ‘‘Candidatus Ovobacter propellens’’: a large conspicuous prokaryote with an unusual motility behaviour

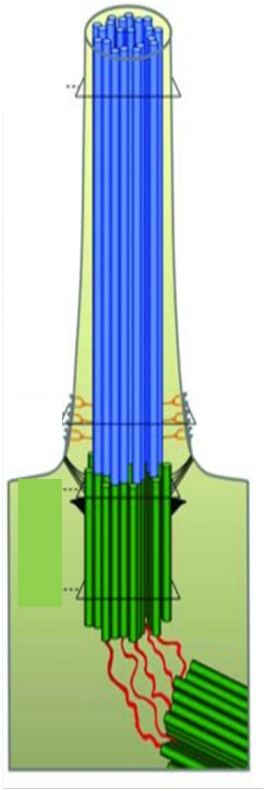
The Archaeal Flagellum

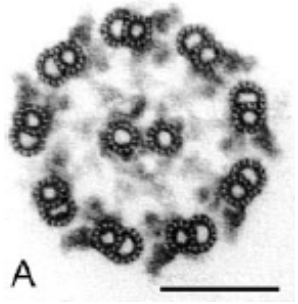
- In contrast to bacterial flagella, there is no lumen within the flagellum, and assembly is by addition of subunits at the proximal end.
- Rather than being driven by a proton-motive force, ATP hydrolysis drives rotation of the archaellum.
- The components of the whip, archaellins, appear to be unrelated to bacterial flagellins.
- There is no hook structure.
- Structurally similar to bacterial Type IV pili, which are used in twitching motility, adhering to surfaces and retracting.



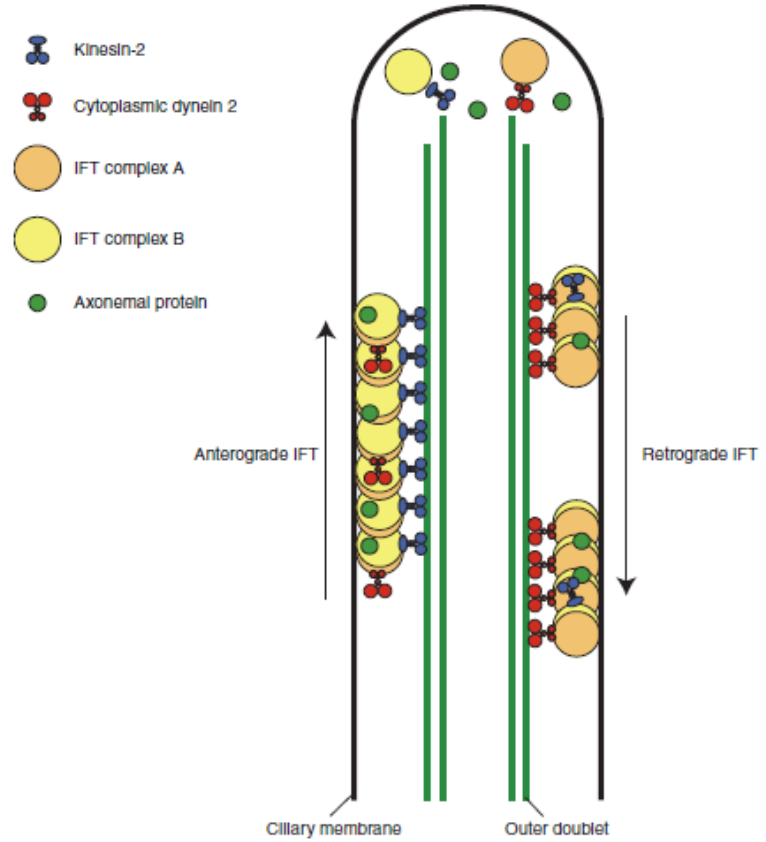
The Eukaryotic Flagellum: the axoneme







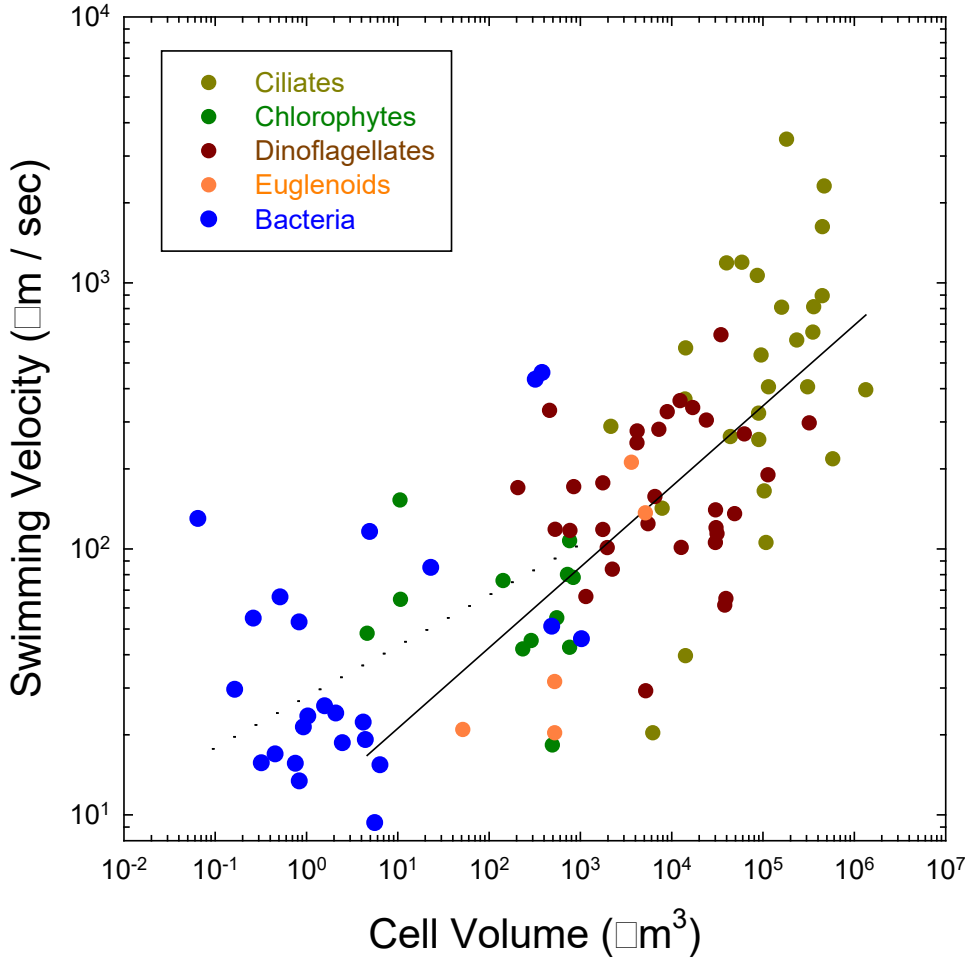
Axoneme (9 + 2)



- The axoneme – structure around which all eukaryotic cilia and flagella are formed; nine microtubule doublets around two central singlet microtubules.
- At least 100 associated proteins, none of which have obvious prokaryotic orthologs.

Ishikawa and Marshall (2017)

Positive Scaling of Average Swimming Speed and Cell Size



Scales as ≈ $10V^{0.3}$

≈ average 6 cell diameters / sec

17 to 35 lengths / sec for the largest to smallest bacteria

Fastest fish ≈ 15 lengths / sec

Michael Phelps ≈ 1 length / sec

Cost of Swimming at Low Reynold's Numbers (ratio of inertial to viscous forces)

- Is this trivially small, as suggested by Purcell and others?

- Efficiency of conversion of chemical energy into mechanical swimming with flagella is uniformly low, ~1%, owing to Brownian motion, rotational diffusion, flagellar flexibility, helical motion, etc.

- The cost of swimming is less than the cost of building flagella.

Using Stokes' Law to Estimate the Energy Required for Moving a Sphere of Radius r at Speed v

Power: $P = 6\pi \cdot \eta \cdot r \cdot v^2 /$ (efficiency of conversion)

- η is the fluid viscosity.
- Hydrolysis of 1 mol ATP ≈ 50 kilojoules.

The green alga *Chlamydomonas reinhardtii* (~150 um^3):

- Cost of swimming ≈ 0.8% of total cell budget
- Construction cost of two flagella ≈ 2.5% of total cell budget

The bacterium *Escherichia coli* (~1 um^3):

Total cost of a cell ≈ 3×10^{10} ATP hydrolyses

- Cost of swimming ≈ 54×10^6 ATP / hour

  Five hours / cell division → 2.5×10^8 ATPs

  ≈ 0.8% of total cell budget

- Construction cost of individual flagella ≈ 10^8 ATPs

  Five flagella / cell → 5×10^8 ATPs

  ≈ 1.6% of total cell budget