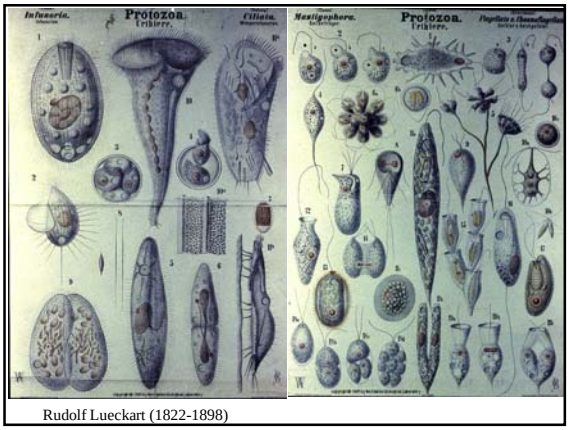
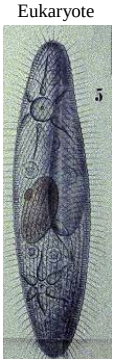


Rudolf Lueckart (1822-1898)

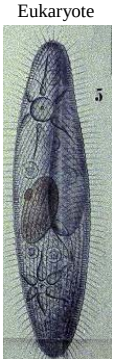
Eukaryote Prokaryote (bacteria or archaea)



(to scale)

Paramecium

Eukaryote Prokaryote (bacteria or archaea)

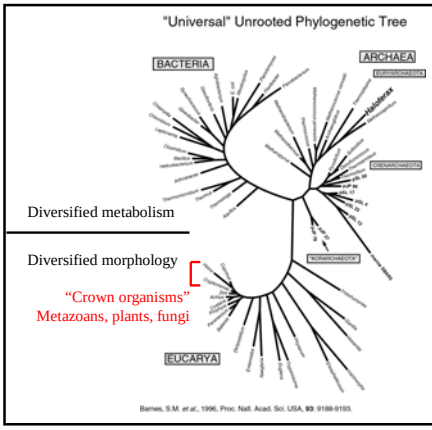


(to scale)

Paramecium

How are eukaryotes different from prokaryotes?

- Membrane-enclosed nucleus
  - …uncoupled transcription and translation
- Extensive internal membrane systems and membrane-bound organelles
- Expanded genome with multiple, large chromosomes
- Much larger cell size (1-5 μm ⇏ 20-200 μm)
- High degree of subcellular compartmentalization
- Endosymbionts (mitochondria and chloroplasts)
- Better, bigger, fancier multicellular organisms



Q:Why? A:(~1990) The cytoskeleton

Membrane-enclosed nucleus…uncoupled transcription and translation

Extensive internal membrane systems and membrane-bound organelles

- INTRACELLULAR MEMBRANE TRANSPORT WITH MOTOR PROTEINS ON MICROTUBULES CAN DRAW PLASMA MEMBRANE INSIDE, MODIFY SHAPE, LOCATION
- NUCLEAR LAMINS STABILIZE NUCLEAR MEMBRANE

Expanded genome with multiple, large chromosomes

- MITOTIC SPINDLE (MICROTUBULES, MOTORS) CAN SEGREGATE ACCURATELY, EFFICIENTLY

Much larger cell size

- DIRECTED INTRACELLULAR TRANSPORT FREES THE CELL FROM THE DIFFUSION LIMIT

High degree of subcellular compartmentalization and specialization

- MICROTUBULE ORGANIZING CENTER SETS UP A UNIVERSAL COORDINATE SYSTEM FOR CELL POLARITY

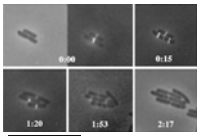
Endosymbionts (mitochondria and chloroplasts)

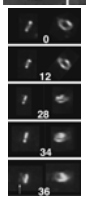
- ACTIN CYTOSKELETON ENABLES PHAGOCYTOSIS, ALLOWING SELFISH PREDATION AND CAPTURE OF ENERGY-PRODUCING SERVANTS

Better, bigger, fancier multicellular organisms

- ACTIN AND INTERMEDIATE FILAMENTS COOPERATE IN GENERATING STRONG, FLEXIBLE CELL-CELL JUNCTIONS IN METAZOANS
- CYTOSKELETON COORDINATES CELL WALL AND ECM DEPOSITION IN METAZOANS, FUNGI AND PLANTS

The plot thickens… Bacteria have tubulin (FtsZ)





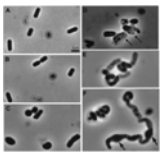
GFP-FtsZ

FtsZ (required for cell division):

- -Is a GTPase with limited sequence similarity to tubulin (Mukherjee et al., 1993)
- -Assembles into filaments in a GTP-dependent manner (Mukherjee and Lutkenhaus, 1994)
- -Crystal structure is superimposable with either α or β-tubulin (Lowe et al., 1998; compare Nogales et al., 1998)

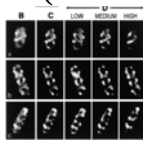
Sun and Margolin, 1998

And bacteria have actin (several kinds)

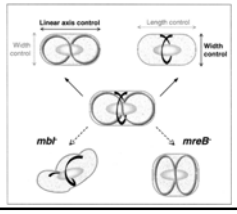


A, B wild-type *B. subtilis*
C, D *mreB* mutant
E, F *mbl* mutant

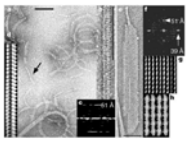
Jones et al. 2001



Mbl protein helices in cells

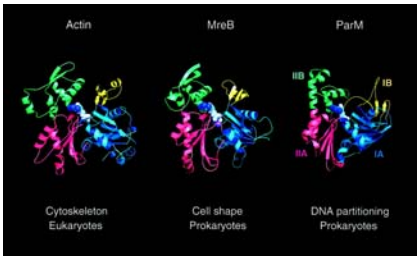


MreB filaments in vitro



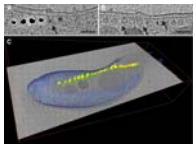
van den Ent et al, 2001

Strong structural conservation among actin superfamily

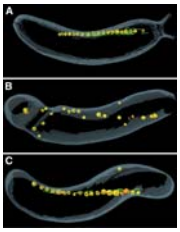


van den Ent et al., 2002

Actin homolog used to organize magnetosomes



Wild-type



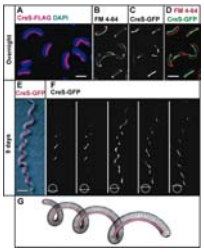
ΔmamK

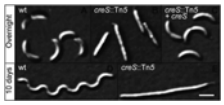
mamK-gfp

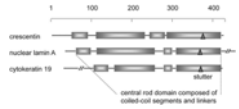
Komeili et al., 2006

And some may have intermediate filaments….

Caulobacter crescentus







Ausmees et al., 2003

Q2: If bacteria have a cytoskeleton, why don't they do something more interesting with it?

Hypothesis: The central feature of the cytoskeleton necessary to cellular life, large-scale cell organization, and cell division is the dynamic assembly and disassembly of helical protein filaments

-Eukaryotes enhance these features with specialized cytoskeleton-associated factors: NUCLEATORS and MOLECULAR MOTOR PROTEINS

-Corollary: Prokaryotes lack nucleators and molecular motor proteins (Q3: Why?)

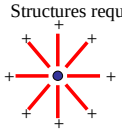
Cell biological basis for the hypothesis

A. Self-assembling structures not requiring motors or nucleators:

Single filament
(may include multiple protofilaments)

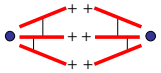
Mixed-polarity bundle

B. Structures requiring localized nucleation and/or motor activity:



Aster

Parallel bundle



Spindle

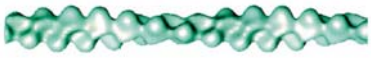
Assertion:
NO TYPE B STRUCTURES HAVE BEEN FOUND IN BACTERIA

Structural encoding: how to make a helix

On any (asymmetrical) protein surface, one of the possible pairs of interaction sites will yield the most favorable energy on binding. Therefore, any pure protein at high concentration will have some tendency to aggregate helically. Secondary favorable interactions will stabilize helices with multiple protofilaments.

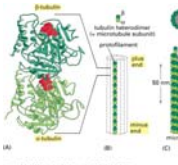
Crane, 1950

Pauling, 1953

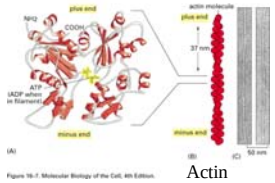


Actin filament reconstruction - Amy McGough

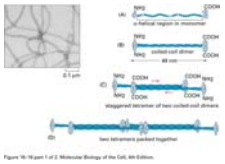
Cytoskeletal filament structures



Microtubule

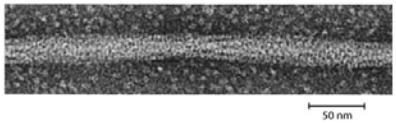


Actin



Intermediate filament

The accidental polymer: Hemoglobin S forms helical filaments (14 protofilaments)



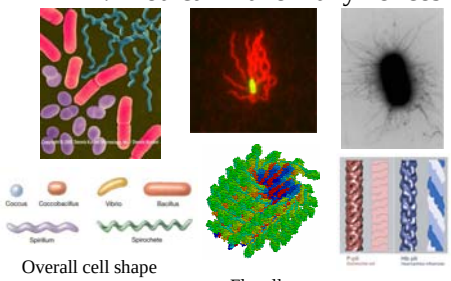
G. Dykes, R.H. Crepeau, and S.J. Edelstein. *Nature* 272(1978):509.

Protein structure considerations

- It is easy to make a helical polymer
- It is even easy to make a POLARIZED helical polymer
- Dynamic behavior requires energy input
  - Harnessing nucleotide hydrolysis gives treadmilling and dynamic instability
- Hypothesis: Large-scale cellular organization in eukaryotes depends on breaking helical symmetries
  - (Type A structures vs. Type B structures)

Design principles for bacterial cells:

1. You can only make helices
2. You can make many helices



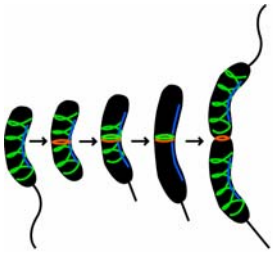
Overall cell shape

Flagella

Pili

The Cytoskeleton of *Caulobacter crescentus*

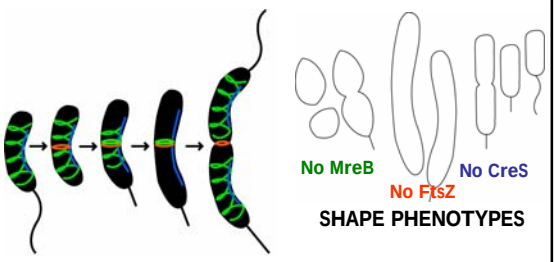
MreB=Actin **FtsZ=Tubulin** **CreS=Intermediate Filament**



LOCALIZATIONS

The Cytoskeleton of *Caulobacter crescentus*

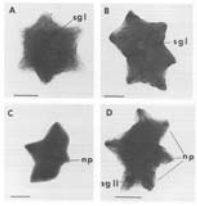
MreB=Actin FtsZ=Tubulin CreS=Intermediate Filament



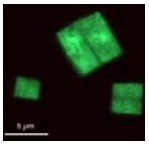
LOCALIZATIONS

SHAPE PHENOTYPES

Favorite exceptions



Stella humosa
Bacterium
Flat, star-shaped



Haloquadratum walsbyii
Archaeon
Flat, square

Epulopiscium fishelsonii
Bacterium
HUGE (>600 µm)

The universal cytoskeleton

What common design principles are shared by all cells on Earth?

How are eukaryotic cells so morphologically complex, while prokaryotic cells are (mostly) morphologically simple?

What was the cytoskeletal organization of the last common ancestor of all cells on Earth, and what were the key events in the evolution of morphologically distinct clades of cells?