BE/APH161 – PHYSICAL BIOLOGY OF THE CELL

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Surreal game of telephone – my lecture slides were made by someone else who was teaching out of our book. In some cases, a little more a repeat of book relative to my usual tendency.

http://www.rpgroup.caltech.edu/courses/aph161/2010/index.html

Username: aph161winter2010

Password: bythenumbers

"The most fascinating subject at the time that I was a student was Maxwell's theory" - Einstein.

- ***"His (Weber's) lectures on classical physics were lively, but we waited in vain for a presentation of Maxwell's theory. We knew that it confirmed the identity of transmission of electricity and light and that Hertz's investigations on electric waves had confirmed the theory. Einstein above all was disappointed." – Einstein fellow student L. Kollros quoted in "Einstein: The Formative Years" by Don Howard and Jon Stachel***
- ***What about our time?***

Table 18–1 Classical Physics

Maxwell's equations

I. $\nabla \cdot E = \frac{\rho}{\epsilon_0}$ (Flux of E through a closed surface) = (Charge inside)/ϵ_0

II. $\nabla \times E = -\frac{\partial B}{\partial t}$ (Line integral of E around a loop) = $-\frac{d}{dt}$ (Flux of B through the loop)

III. $\nabla \cdot B = 0$ (Flux of B through a closed surface) = 0

IV. $c^2 \nabla \times B = \frac{j}{\epsilon_0} + \frac{\partial E}{\partial t}$ c^2(Integral of B around a loop) = (Current through the loop)/ϵ_0 $+\frac{\partial}{\partial t}$ (Flux of E through the loop)

[Conservation of charge

$\nabla \cdot j = -\frac{\partial \rho}{\partial t}$ (Flux of current through a closed surface) = $-\frac{\partial}{\partial t}$ (Charge inside)]

Force law

$F = q(E + v \times B)$

Law of motion

$\frac{d}{dt}(p) = F$, where $p = \frac{mv}{\sqrt{1 - v^2/c^2}}$ (Newton's law, with Einstein's modification)

Gravitation

$F = -G\frac{m_1 m_2}{r^2} e_r$

From "Feynman Lectures on Physics"

CLASSIFICATION OF CELLS: THE THREE DOMAINS OF LIFE

- Classification based on similarities and differences in ribosomal RNA seq.:

Cells

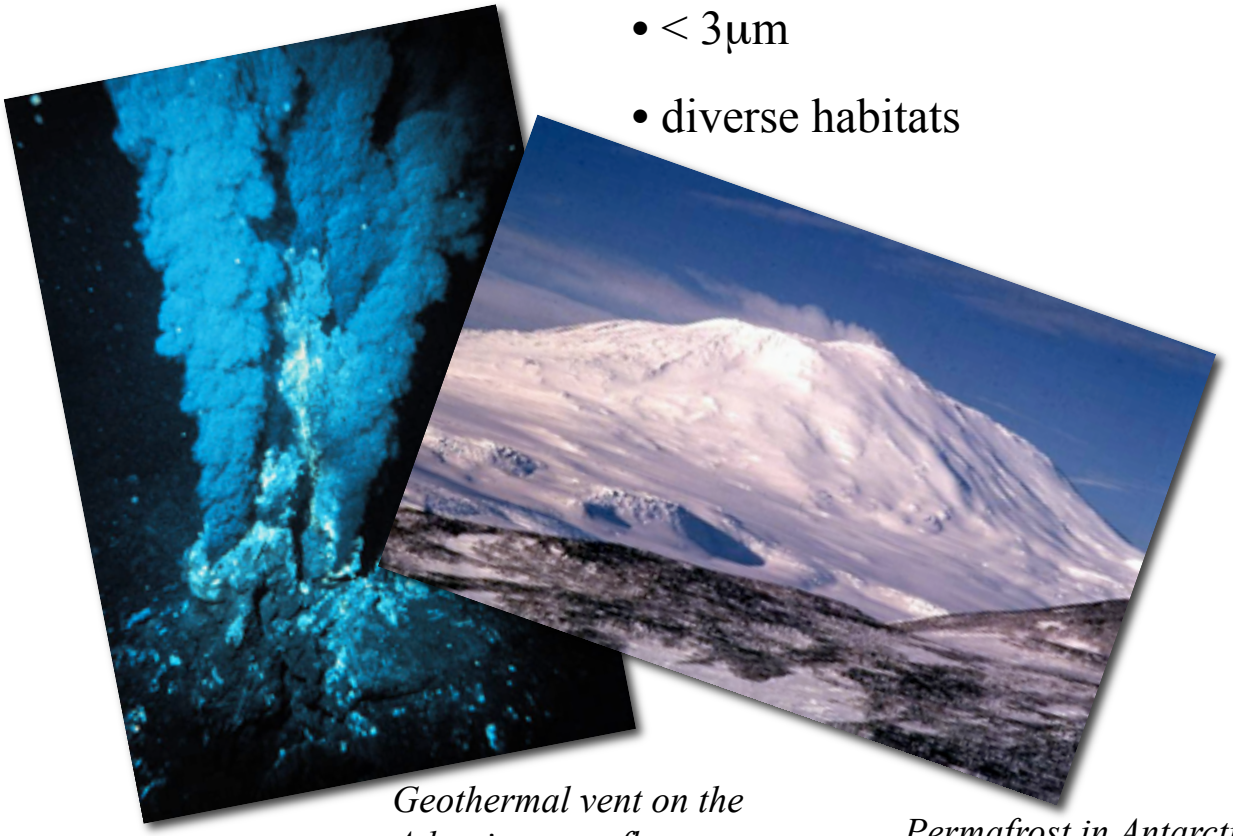
Bacteria

Archaea

- < 3μm
- diverse habitats

Eukarya

- animals, plants, fungi (*e.g., mushrooms, mold*)
- definition: a cell that contains its DNA genome within a membrane-bound nucleus



Geothermal vent on the Atlantic ocean floor

Permafrost in Antarctica

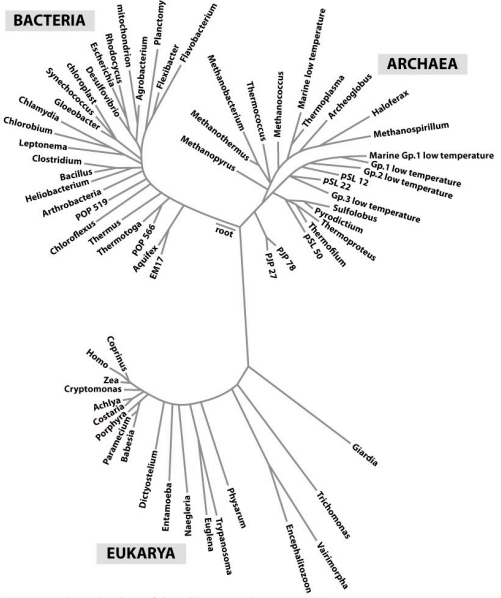
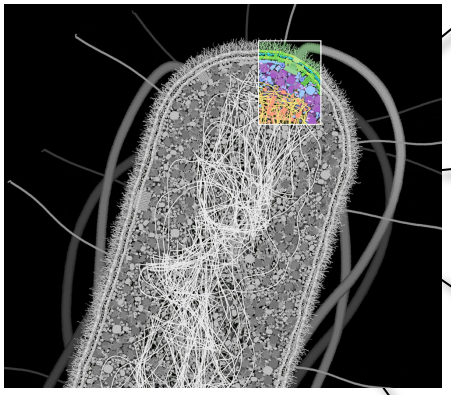


Figure 3.6 Physical Biology of the Cell (© Garland Science 2009)

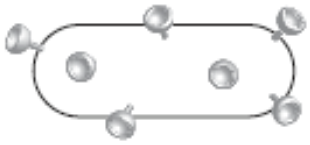
IDEALIZATIONS OF LIVING CELLS

The cell can be modeled as…



E. coli

Receptor array



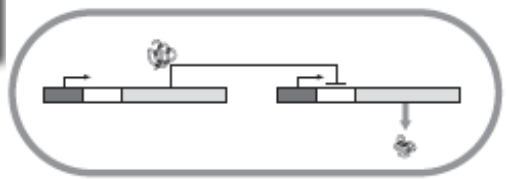
Swimmer

- *how a bacterium swims through water*

Random walk

- *cell's large-scale motion*

Genetic network

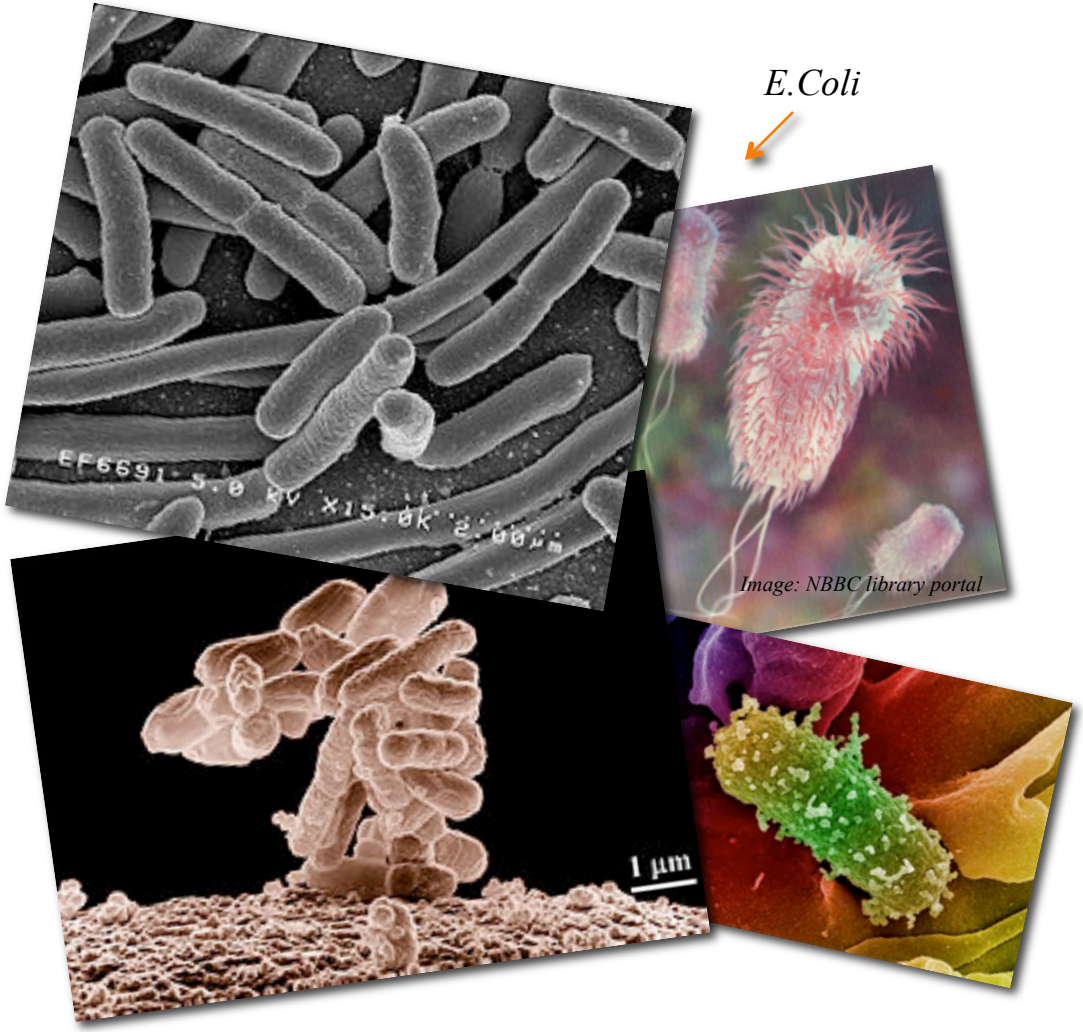


- *how cells alter the expression of their polymer languages in response to changing conditions*

AN ODE TO *E.COLI*

> Although not everyone is mindful of it, all cell biologists have two cells of interest: the one they are studying and Escherichia coli.
>
> F. Neidhardt

• To understand the basic rules governing metabolism and replication (and life in general): focus on a few representatives.

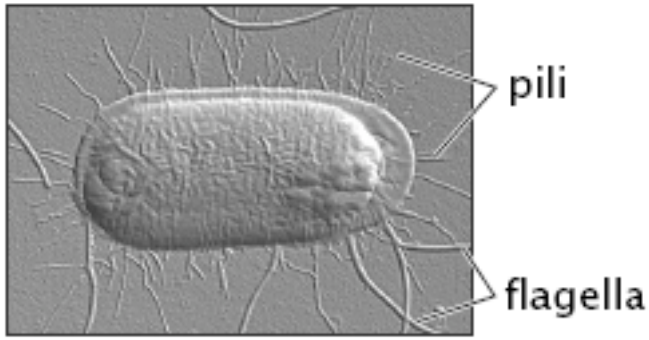


• Bacteria *E.coli*: human intestinal inhabitant

- easy to isolate
- is able to grow well in the presence of O_2 (*unlike most other bacteria*)
- replicates rapidly *in vitro*, easily adjusts to changes in its envir.
- routine to produce mutants (*changes in DNA seq. =>biol. signif. differences,e.g. resistance to antibiotics*)

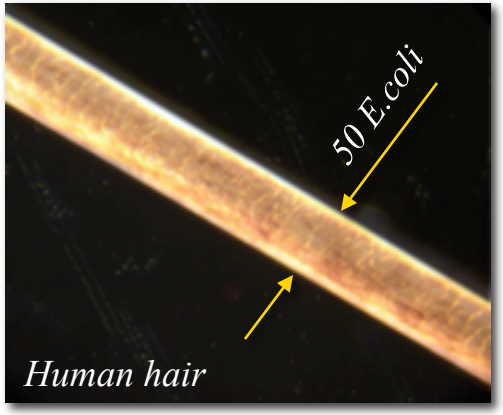
E.COLI AS THE BACTERIAL STANDARD RULER

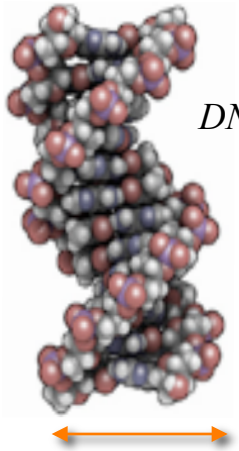
- All cells share with *E.coli* the fundamental biol. directive to convert E_{envir} into struct. order and to perpetuate their species.
- Min requirements for the perpetuation of cellular life, as observed on Earth:
  - DNA-based genome
  - mechanisms for DNA → RNA $\xrightarrow{ribosomes}$ proteins



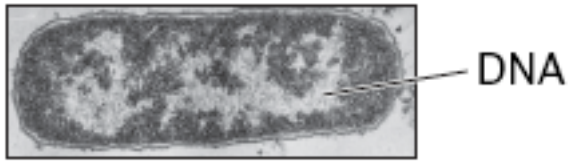
AFM image of an E.coli cell

E.coli as a standard ruler:





1/500 E.coli



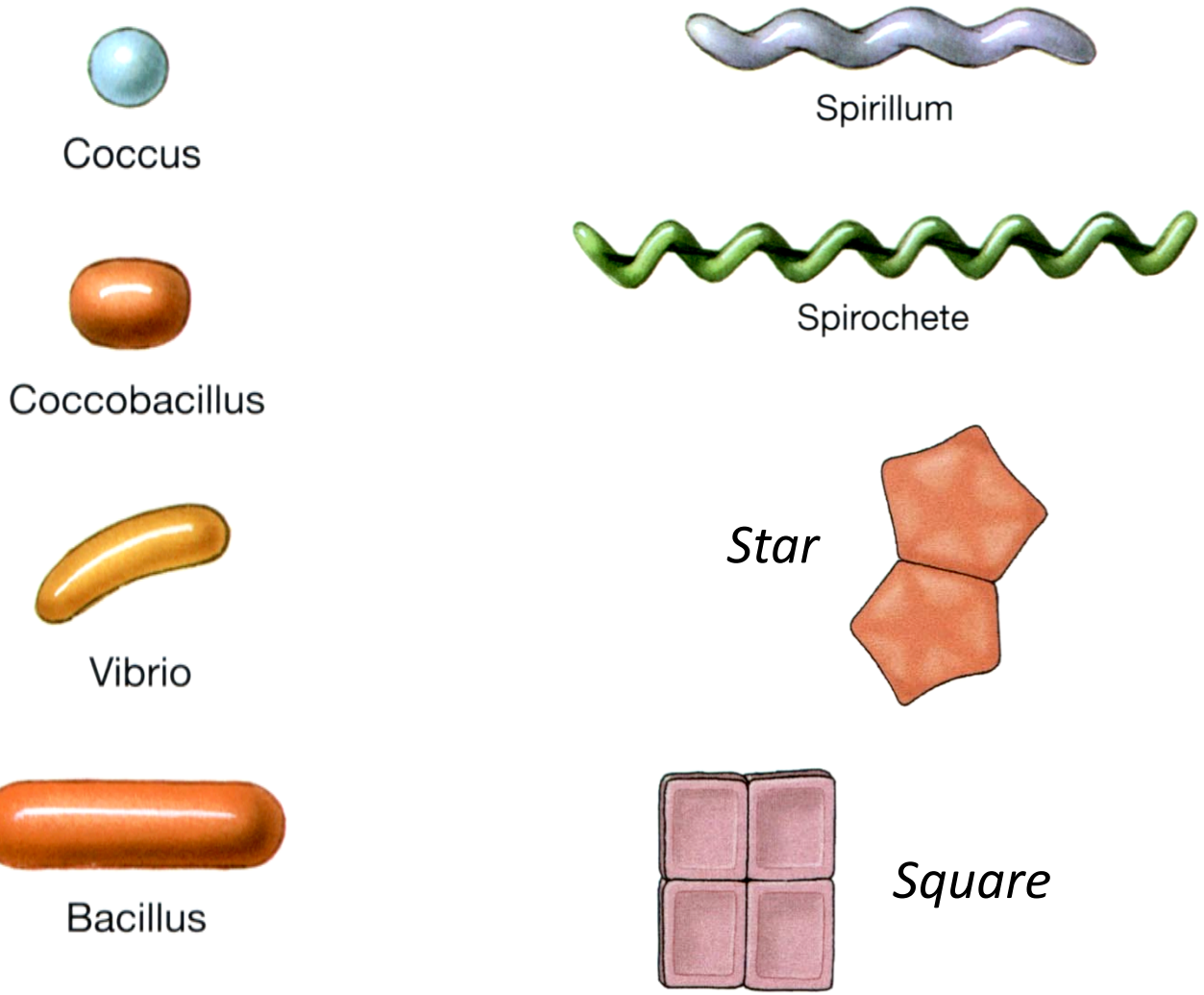
Electron micrograph

- Note: size of *E.coli* depends on the nutrients provided: richer media => larger size.
- Biochem. studies usually use "minimal medium": salts+glucose

the E.coli ruler

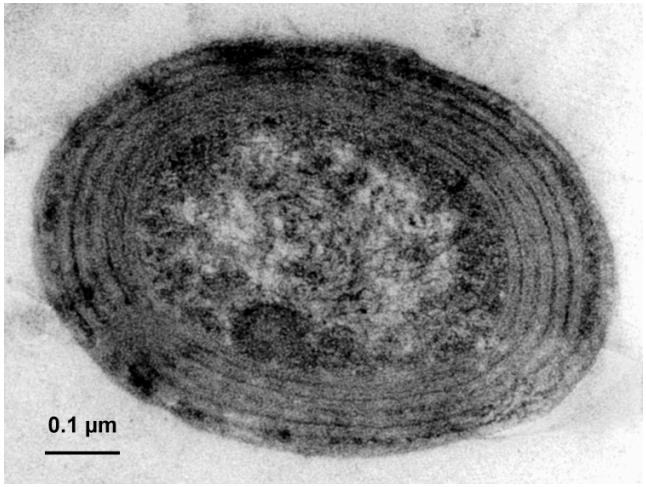
BACTERIAL SHAPES

- Here we simply note that the diversity of cell shapes and sizes is immense.



ARCHITECTURE OF CYANOBACTERIA

- ***Every time I show you a picture of a cell, ask yourself how the architecture works.***
- ***For cyanobacteria, we are going to examine several remarkable specializations related to their ability to perform photosynthesis.***



(Cannon et al.)

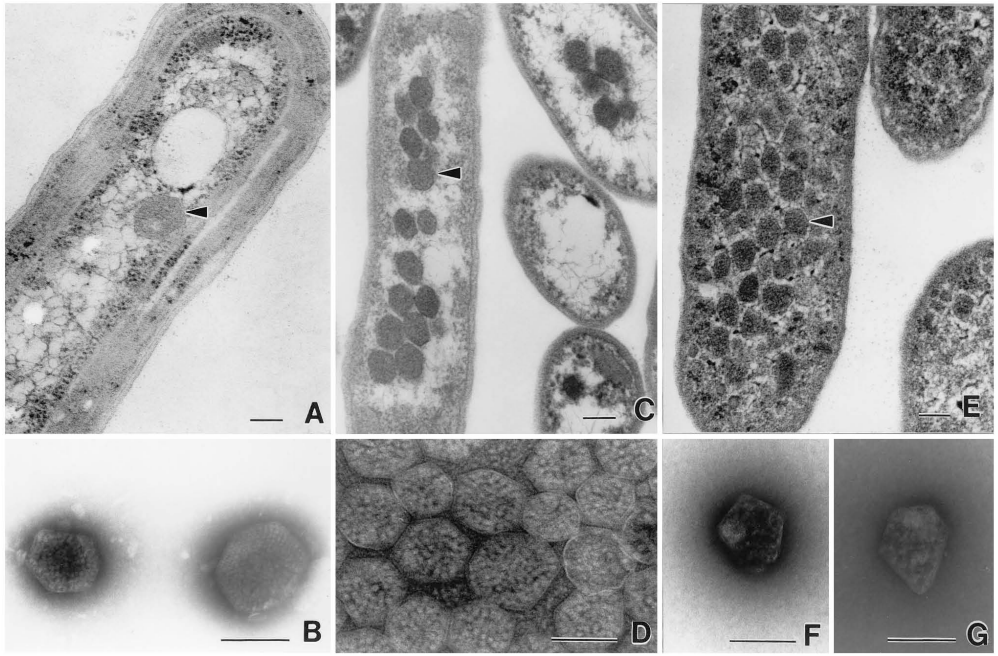


FIG. 1. Transmission electron micrographs of carboxysomes and enterosomes. (A) Thin section of a cell of *Synechococcus* strain PCC7942 (fixed cells kindly supplied by George Espie), showing a typical carboxysome (arrowhead). (B) Negatively stained carboxysomes from lysed cells of *A. nidulans* (now *Synechococcus*). Molecules of RuBisCO are visible inside. Micrograph kindly supplied by Elisabeth Gantt. (C) Thin sections of *H. neapolitanus* grown in air, showing aggregation of carboxysomes (arrowhead) in the nucleoid region of the cell. (D) Negative stain of carboxysomes isolated from *H. neapolitanus*. RuBisCO assemblies are visible inside. (E) Thin section of *S. enterica* serovar Typhimurium LT2 grown on propanediol under aerobic conditions. Many polyhedral bodies (enterosomes [arrowhead]) are visible throughout the cytoplasm. They are less regular than carboxysomes and slightly smaller. (F and G) Negatively stained, isolated enterosomes from *S. enterica* serovar Typhimurium LT2. Note the irregular shape. Contents appear to be of variable sizes. Photographed from preparation kindly supplied by Greg Havemann. Panels A, C, and E are all printed at the same magnification, as are panels B, D, F, and G. Bars, 100 nm.

EUKARYOTIC CELLS: A ROGUE'S GALLERY

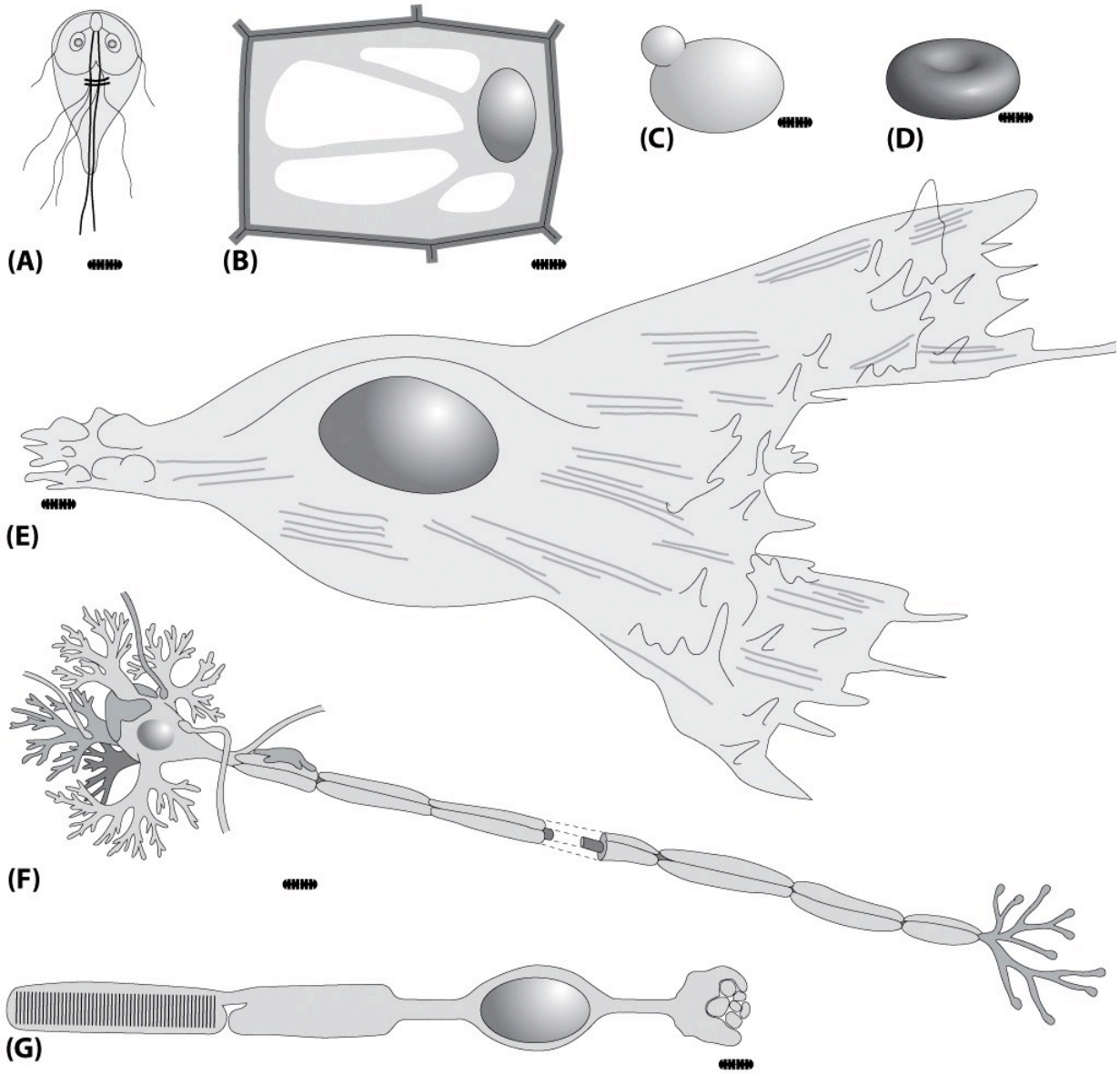
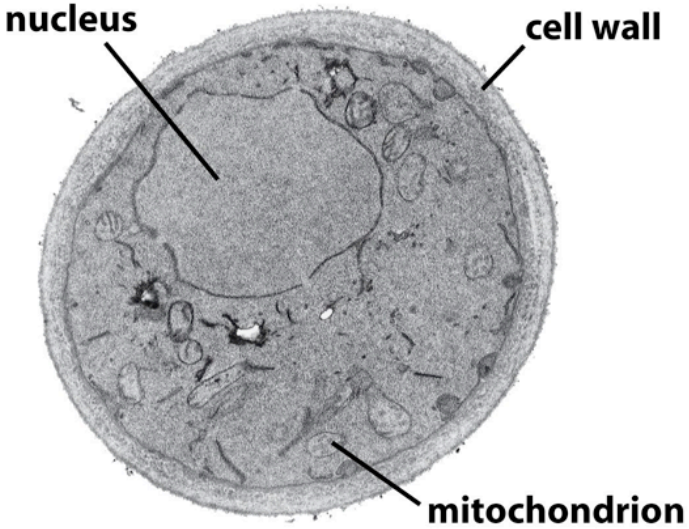


Figure 2.8 Physical Biology of the Cell (© Garland Science 2009)

Yeast as the model eukaryote

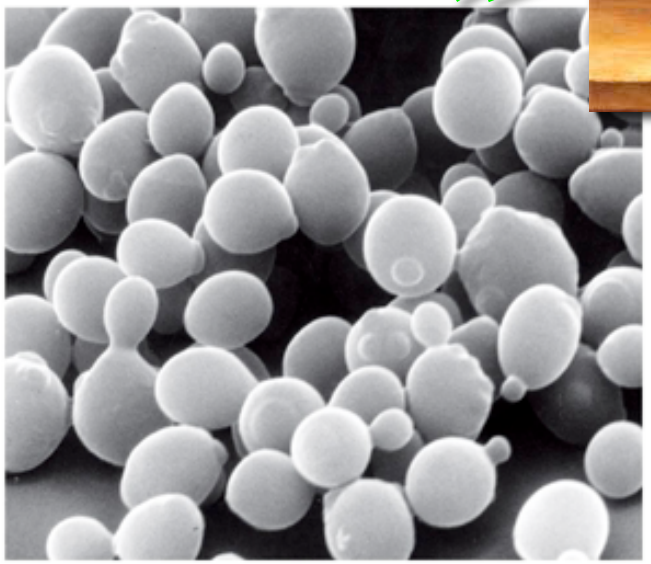
A budding yeast cell: *a model eukaryotic cell*

- *Budding yeast (S. cerevisiae): Fungi - most closely related to animals in terms of evolutionary descent and similarity of protein functions.*
- *Although there are no single-cell animals, there are some single-cell fungi.*
- *~5μm*



Electron microscopy image of a cross-section of a budding yeast cell

Figure 1-42b Molecular Biology of the Cell 5/e (© Garland Science 2008)



A scanning electron micrograph of budding yeast

Figure 1-42a Molecular Biology of the Cell 5/e (© Garland Science 2008)

SIZE AND SHAPE OF FIBROBLASTS

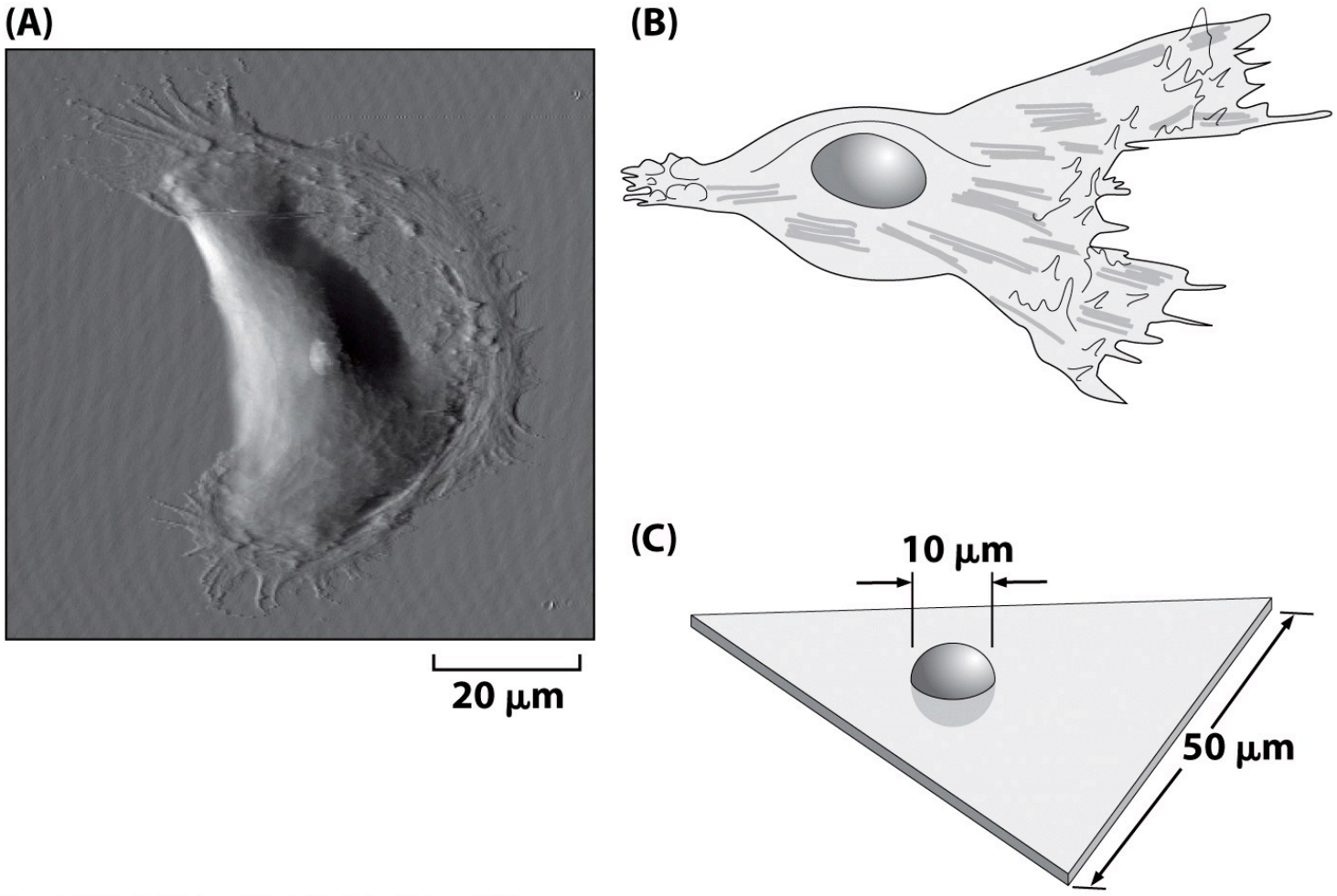
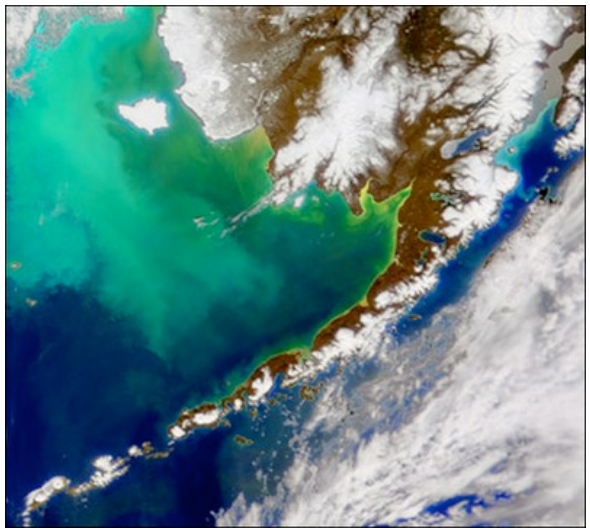


Figure 2.12 Physical Biology of the Cell (© Garland Science 2009)

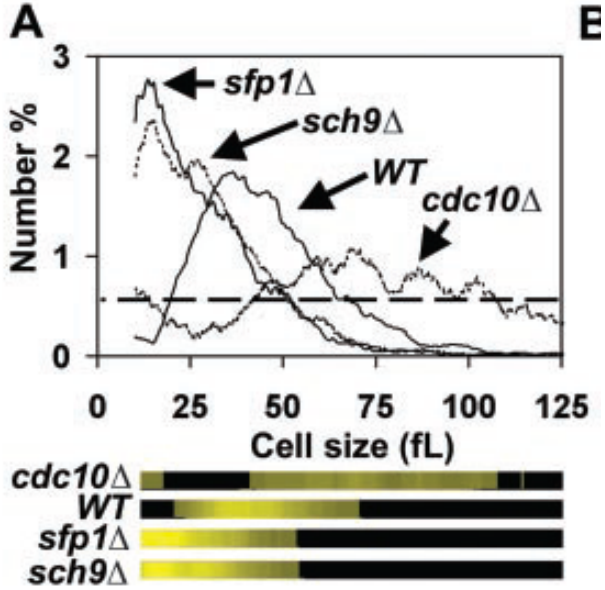
EUKARYOTIC PHYTOPLANKTON

- ***One of my favorite marine organisms is Emiliana huxleyi, a single-celled, eukaryote that performs photosynthesis to make a living.***
- ***These organisms also have a peculiar morphology (mineral shell) that scatters light and gives characteristic appearance to the ocean from space known as a "bloom"***

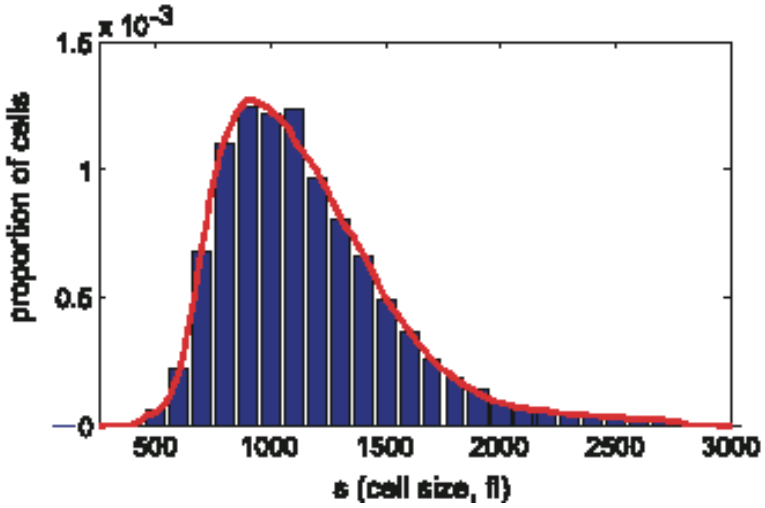


CELL SIZE: BEYOND THE MEAN

- Careful measurements of the size of yeast cells (for different mutants corresponding to different genes being knocked out) reveal a broad distribution of sizes.
- Compare these sizes to the mean sizes of bacterial cells.
- See reading in HW1 for similar characterization of bacteria.



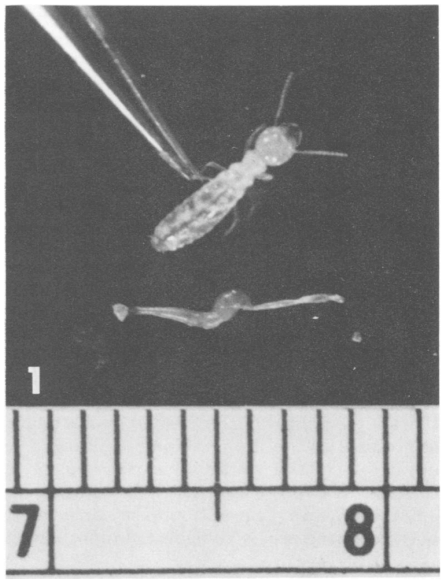
Yeast cell size distribution, Jorgensen et al.



Cell size distribution of mammalian cells, Kirschner et al.

AN ENVIRONMENTAL SAMPLE

- Here we simply note that the diversity of cell shapes and sizes is immense.



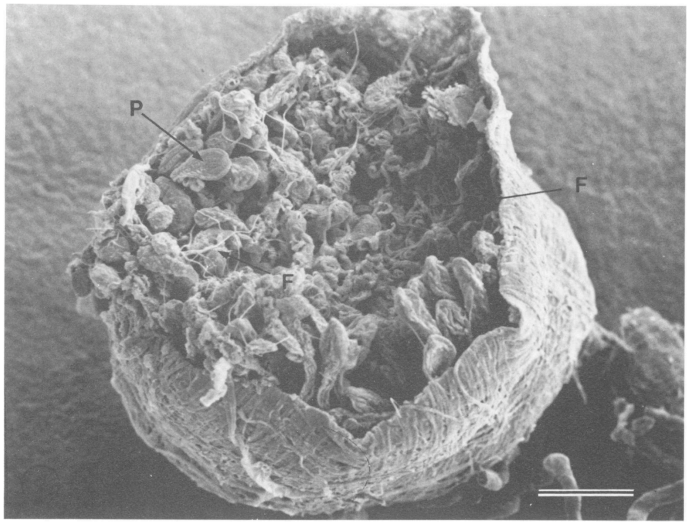


FIG. 2. *Scanning electron micrograph of a cross section through the paunch of an R. flavipes worker. Note the abundance of protozoa, with P. vertens (P) oriented with its anterior (tapered) end toward the epithelium. Bacterial filaments (F) are present among the protozoa. Bar = 100 μm.*

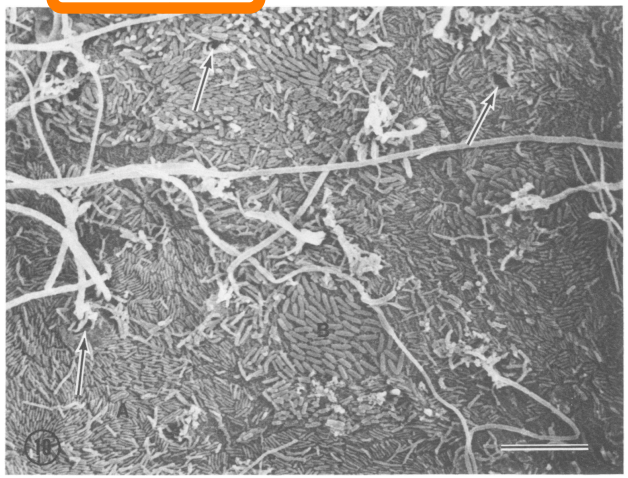


FIG. 10. *Scanning electron micrograph of the paunch surface of R. flavipes after gentle agitation in phosphate-buffered saline. A suggestion of epithelial cups may be seen (arrows). A microcolony of morphotype 1 cells (A) and that of an undesignated morphotype (B) are evident. Bar = 10 μm.*

THE INVENTORY OF CELLS

L2

- Cells: variety of shapes and sizes, yet many common features of their mol. inventories <=> underlying biochemical unity of life.

- Physicists: fundamental unit of matter is the atom
  (*at least for chem. transactions*)

- Life ≡ metabolism + replication

consump. & use of energy from envir. *generating offspring that resemble the orig.*

- Biologists: indivisible unit of life is the cell

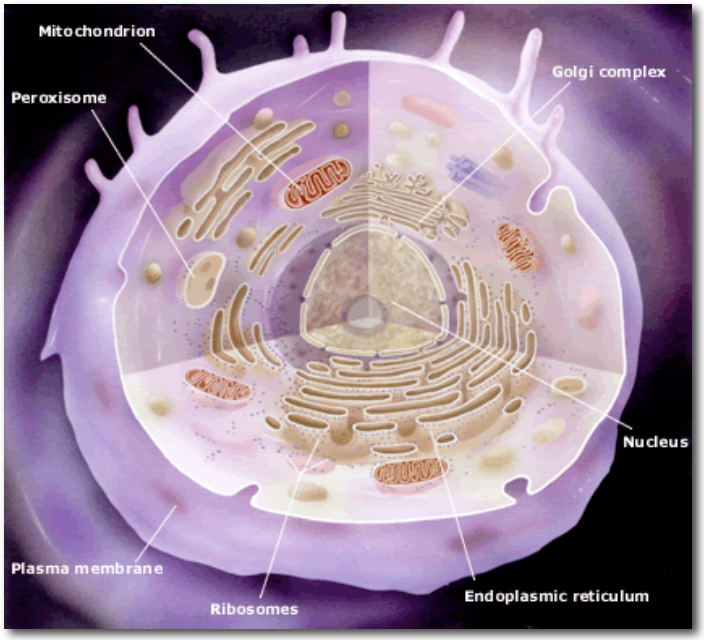
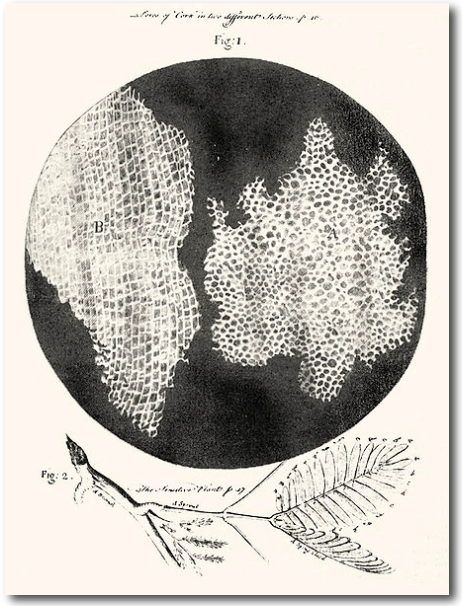
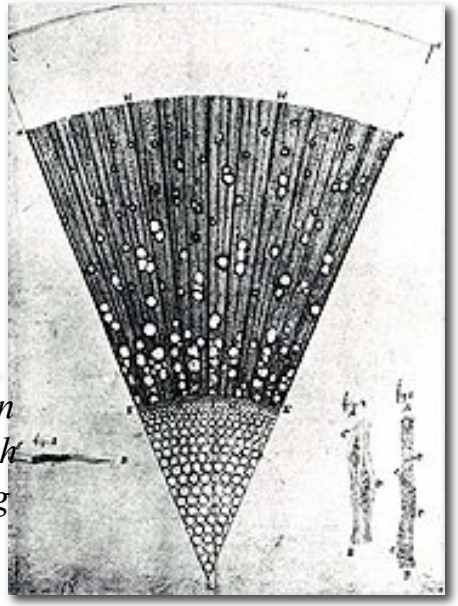


Image: nobelprize.org



Drawing of the structure of cork as it appeared under the microscope to Hooke.

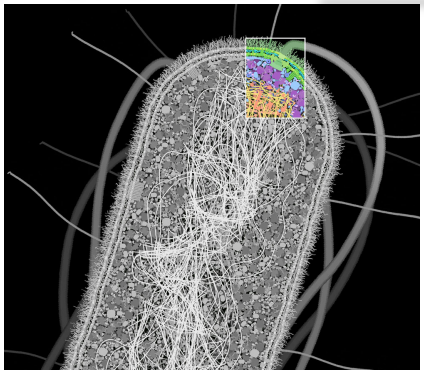


Microscopic section through 1-yr old ash tree wood, drawing by Leeuwenhok

17th cent.: microscopic observations by Hooke and Leeuwenhoek

19th cent.: modern cell theory by Schwann, Schleiden, Virchow; confirmed by Pasteur

TAKING THE MOLECULAR CENSUS OF *E.COLI*: WHY?



- For most cases of interest, it suffices to attribute to *E.coli*

$V_{E.coli} \approx 1\mu m^3 = 1$ fL

$A_{E.coli} \approx 6\mu m^2$

femto = 10^{-15}

- Estimating the number of molecules of diff. kinds that are in an *E.coli* cell…

…Why care about these numbers?

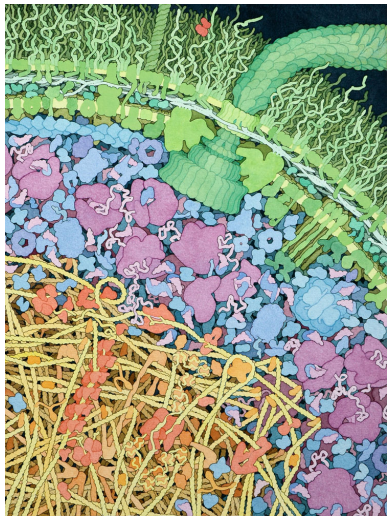
☜ a realistic physical picture of a bio phenomena demands understanding of the individual particles involved + the spatial dimensions over which they can interact (*crowded? dilute? homogeneous?*)

☜ a prerequisite to beginning to answer questions such as: *How fast is a genome replicated? What is the aver. rate of protein synthesis? How do the ribosomes maintain this rate?*

☜ To understand many experiments in biology. Most experimentation is comparative ("normal" behavior vs. "perturbed" behavior, compared by observing some measurable property)

☜ "a lot" vs. "a few" copies of a molecule => describe concentration vs. influence of stochasticity (random chance) on cellular function

SIZING UP *E.COLI*



Estimate $N_{protein}$ in an *E.coli* cell:

- $N_{protein} = m_{total\ protein} / m_{per\ protein}$
- $V_{E.coli} \approx 1$fL, assume $\rho_{E.coli} \approx \rho_{H20} = 1$ g/mL => $m_{E.coli} \approx 1$ pg
- from exp.: cell's dry weight = 30% cell's total weight, protein = 50% of dry weight

  => $m_{total\ protein} \approx 0.15$ pg
- Aver.protein = 300 AA, $m_{AA} \approx 100$ Da => $m_{per\ protein} = 30{,}000$ Da; 1Da $\approx 1.6\times10^{-24}$ g

  => $m_{per\ protein} = 5 \times 10^{-20}$ g
- $N_{protein} = m_{total\ protein} / m_{per\ protein} \approx (15 \times 10^{-14}\ g) / (5 \times 10^{-20}\ g) \approx 3 \times 10^{6}$

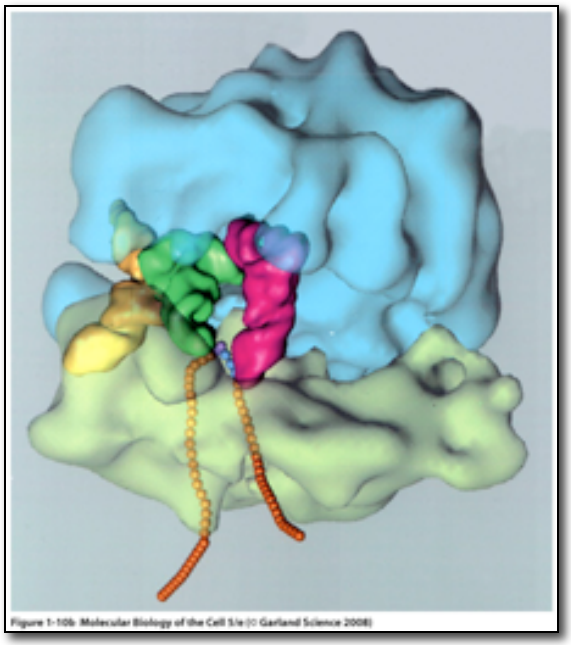
$$N_{protein} \approx 3 \times 10^{6}$$

- 1/3 proteins coded in a typical genome = membrane proteins

  => $N_{cytoplasmic\ protein} \approx 2 \times 10^{6}$, $N_{membrane\ protein} \approx 10^{6}$

SIZING UP *E.COLI*

Estimate $N_{ribosome}$ in an *E.coli* cell:



Ribosome - cellular machine that synthesizes proteins

- $N_{ribosome} = m_{total\ ribosome} / m_{per\ ribosome}$
- facts: ribosomal protein = 20% cell's total protein,

  $m_{per\ ribosome} \approx 2.5$ MDa,

  $m_{per\ ribosome} \approx$ (1/3 protein + 2/3 RNA)

- $N_{ribosome} = m_{total\ ribosomal\ protein} / m_{protein\ per\ ribosome}$

$$\approx (0.2 \times 0.15 \times 10^{-12}\ \text{g}) / (830{,}000\ \text{Da}) \times (1\ \text{Da}) / (1.6 \times 10^{-24}\text{g}) \approx 20{,}000\ \text{ribosomes}$$

20% of cell's protein is ribosomal

15% of cell's mass is protein

cell mass

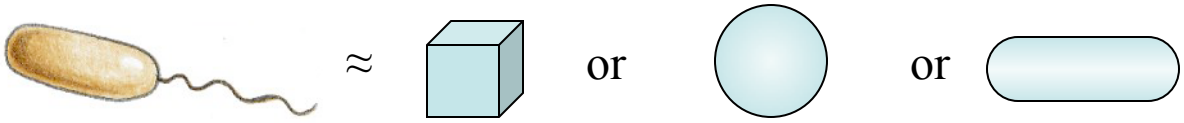
1/3 of the ribosomal mass is protein

$N_{ribosome} \approx 20{,}000$

- $d_{ribosome} \approx 20$ nm => volume taken up by 20,000 ribosomes:

  $V_{total\ ribosome} \approx 10^8\ \text{nm}^3$ <=> 10% of total cell volume

SIZING UP *E.COLI*

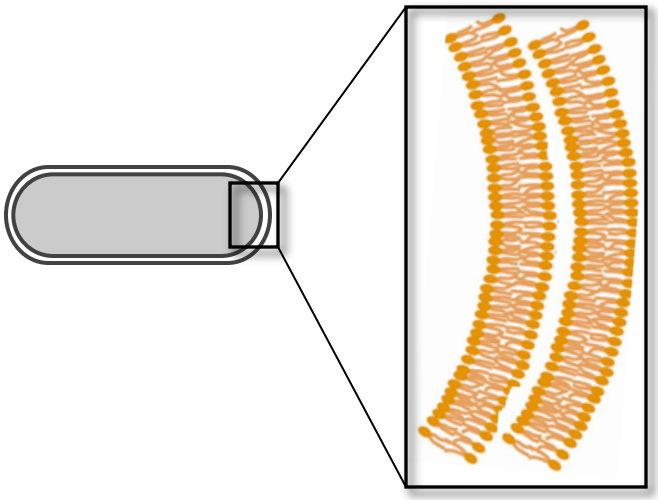


=> surface area $A_{E.coli} \approx 6\ \mu m^2$

Estimate N_{lipid} associated with the inner and outer membranes of an *E.coli* cell:

$$N_{lipid} = 4 \times 0.5 \times A_{E.coli} / A_{lipid}$$

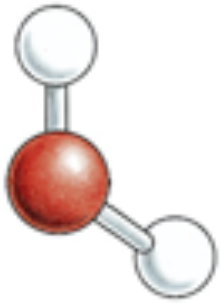
roughly half of the surf. area is covered by membrane proteins rather than lipids



$$\approx 4 \times 0.5 \times (6 \times 10^6\ nm^2) / 0.5\ nm^2 \approx 2 \times 10^7$$

$$N_{lipid} \approx 2 \times 10^7$$

SIZING UP *E.COLI*



Estimate N_{H_2O} in an *E.coli* cell:

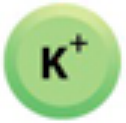
- fact: 70% $m_{E.coli} \approx m_{H_2O}$ => $m_{total\ H_2O} \approx 0.7$ pg

- $N_{H_2O} \approx 0.7 \times 10^{-12}$ g / (18 g/mol) × (6×10^{23} molecules/mol)

  $\approx 2 \times 10^{10}$ water molecules

$$N_{H_2O} \approx 2 \times 10^{10}$$

Estimate $N_{inorganic\ ions}$ in a typical bacterial cell:



- assume a typical concentration of inorganic ions (e.g., K^+) is 100 mM =>

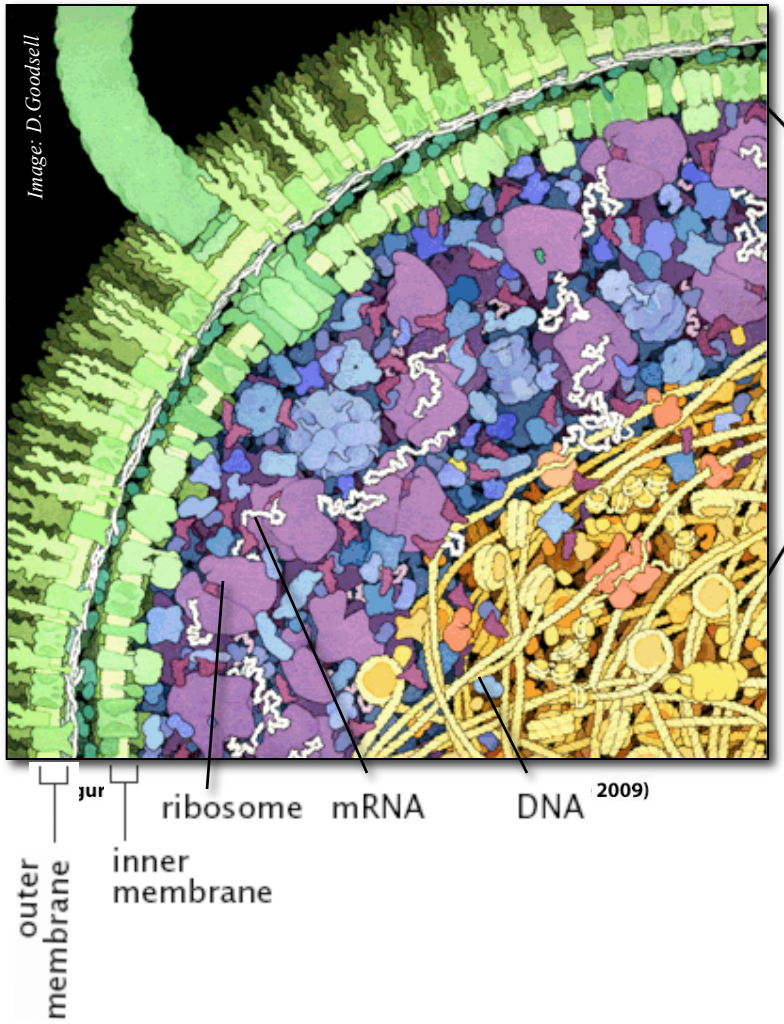
- $N_{inorganic\ ions} \approx (100 \times 10^{-3}\ \text{mol}) / (\underbrace{10^{15}\ \mu m^3}_{1L}) \times (6 \times 10^{23}\ \text{molecules/mol}) \times 1\ \mu m^3$

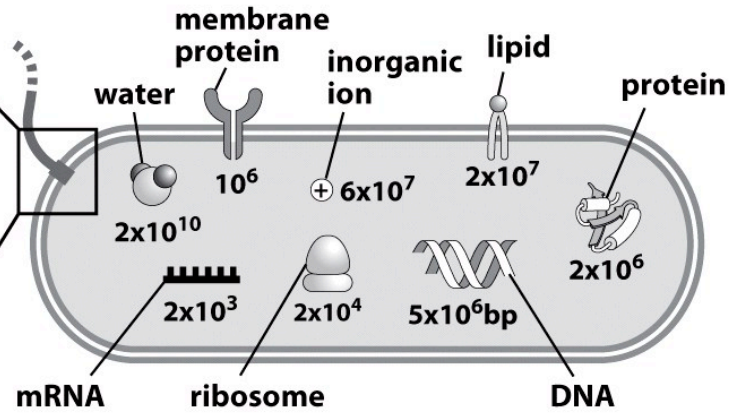
  $\approx 6 \times 10^7$

Cell volume

$$N_{ions} \approx 6 \times 10^7$$

SIZING UP *E.COLI*: AN OUTCOME





Substance	% of total dry weight	Number of molecules
Macromolecule		
Protein	55.0	2.4×10^6
RNA	20.4	
23S RNA	10.6	19,000
16S RNA	5.5	19,000
5S RNA	0.4	19,000
Transfer RNA (4S)	2.9	200,000
Messenger RNA	0.8	1,400
Phospholipid	9.1	22×10^6
Lipopolysaccharide	3.4	1.2×10^6
DNA	3.1	2
Murein	2.5	1
Glycogen	2.5	4,360
Total macromolecules	**96.1**	
Small molecules		
Metabolites, building blocks, etc.	2.9	
Inorganic ions	1.0	
Total small molecules	**3.9**	

Observed molecular census of an E .coli cell. (Data from Neinhardt et al., 1990, and Schaechter et al., 2006)

TAKING THE MOLECULAR CENSUS OF *E.COLI*: HOW?

- An important tool: gel

  - break open cells, keep only protein components
  - separate complex protein mixture into individual molecular species:

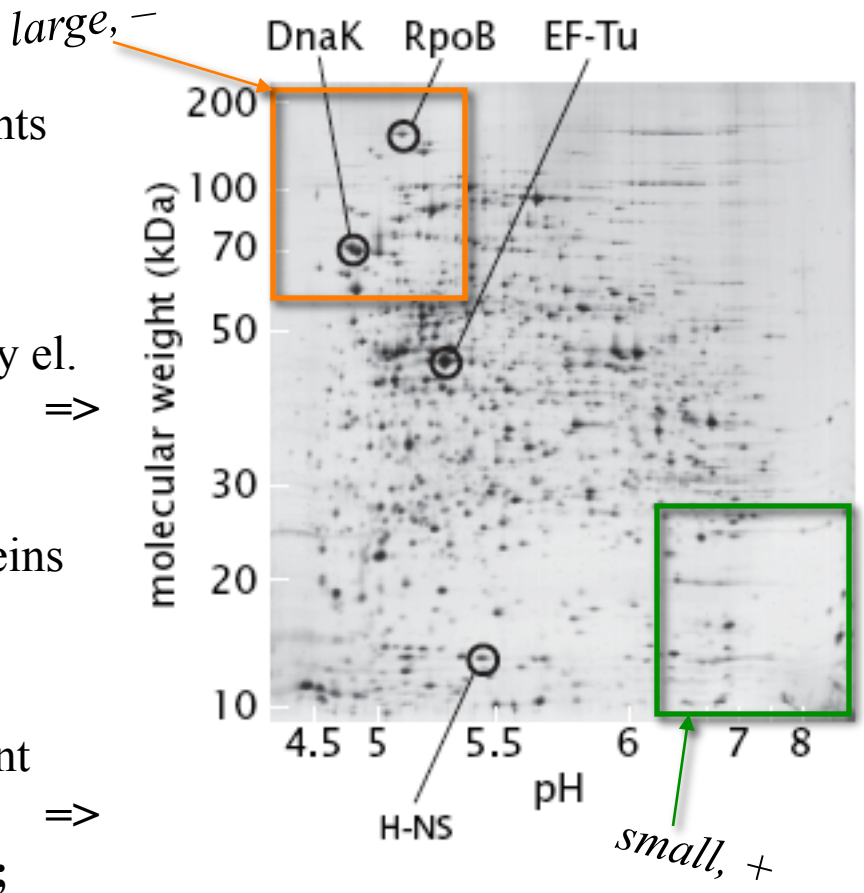
1. Load the mixture at one end of the gel, apply el. field across the gel => **diff. proteins migrate at rates ~ net charge;**

2. Add charged detergent that binds to all proteins => *N* of detergent molecules associated with a protein ~ the protein's size;

3. Apply ⊥ el. field; net charge on the detergent molecules >> original charge of the protein => **diff. proteins migrate at rates ~ protein size;**

  - stain proteins with a non-specific dye to locate
  - cut each spot out, elute protein, determine size and AA-content with mass spectrometry

- Use similar tricks to characterize RNA, lipids, etc.



Protein census of E.coli using 2D polyacrylamide gel electrophoresis. Each spot represents an individual protein with a unique size and charge distribution. (Swiss Inst. of Bioinformatics)

CENSUS IN YEAST

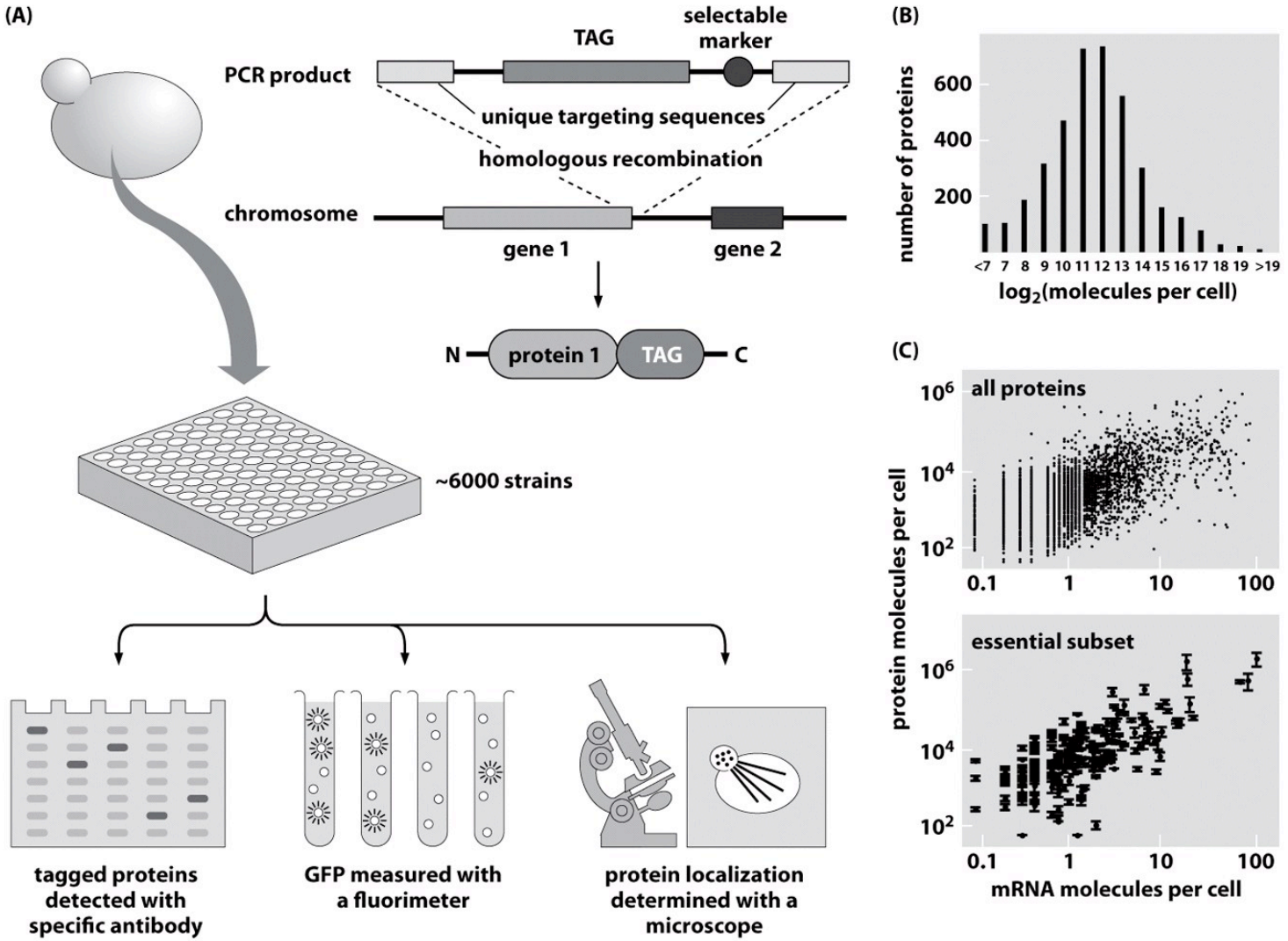
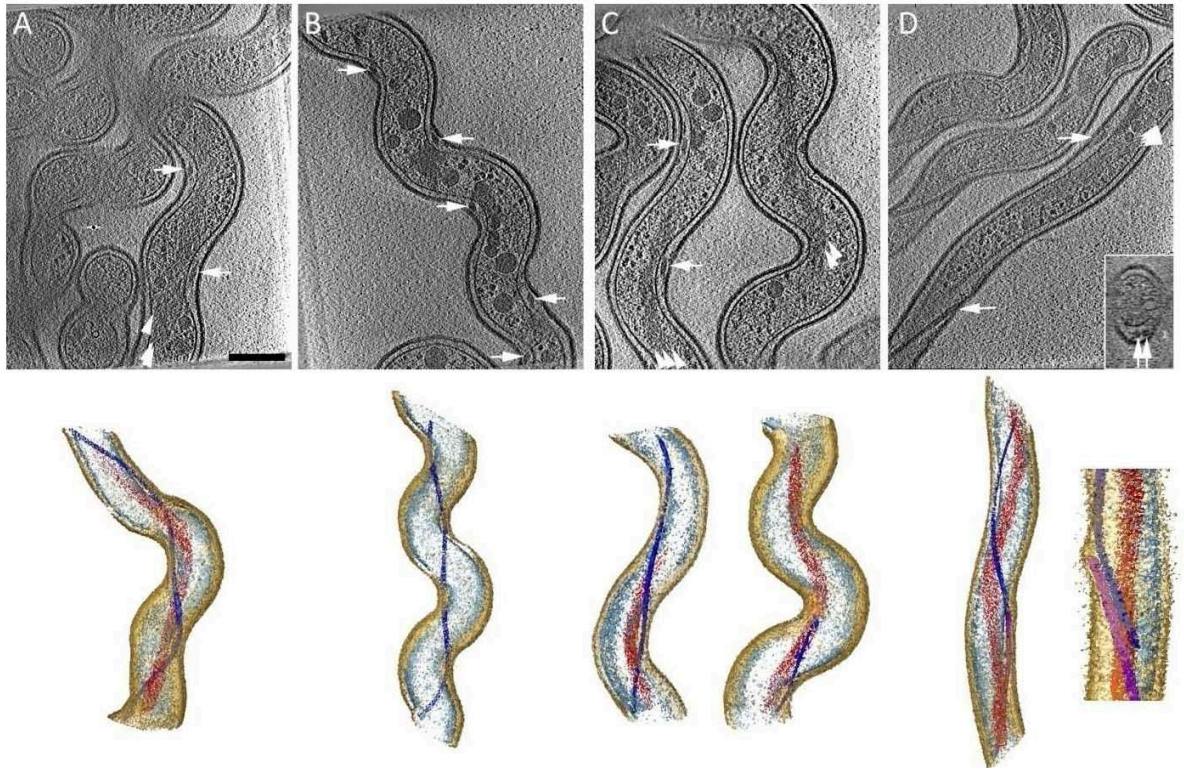


Figure 2.10 Physical Biology of the Cell (© Garland Science 2009)

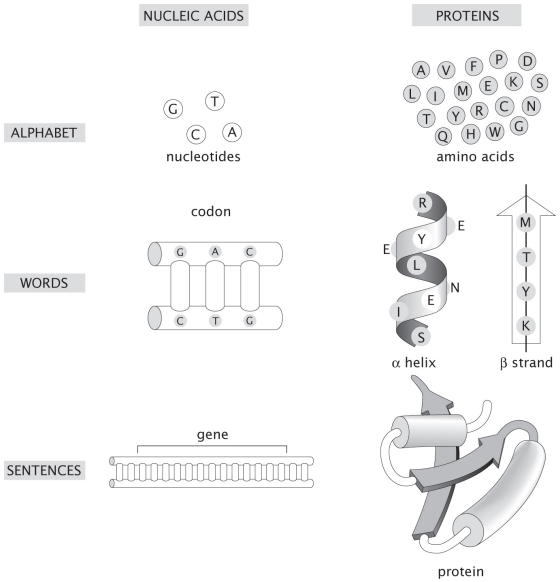
Mass spec and census in spirochete

Supplement: Malmstroem, Beck *et al*. Proteome-wide copies per cell measurements

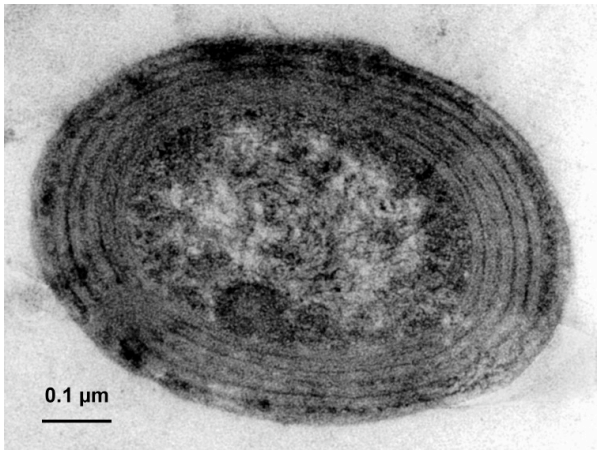


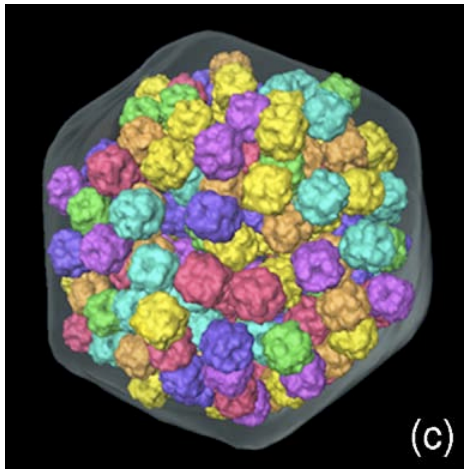
INVENTORIES AND BUDGETS FOR SOME OF THE MOST ABUNDANT ORGANISMS ON EARTH

- ***We think about the microbes that are responsible for 40% of the overall photosynthesis on Earth.***
- ***Ocean water census tells us between 10,000 and 100,000 cyanobacteria per mL. This yields estimate of roughly 10^{26} cyanobacteria doing 10% (ish) of the overall carbon fixation. Conclusion: 10^{4} rubisco per cyanobacterium.***
- ***Using relatively few facts: 1 pg in 1fL with 30% of the mass ``dry''. 30,000 Da "typical" protein tells us 3 x 10^{6} proteins.***



Prochlorococcus





(Iancu et al, JMB, 2007)

ARCHITECTURE OF CYANOBACTERIA (AGAIN)

- ***Every time I show you a picture of a cell, ask yourself how the architecture works.***
- ***For cyanobacteria, we are going to examine several remarkable specializations related to their ability to perform photosynthesis.***

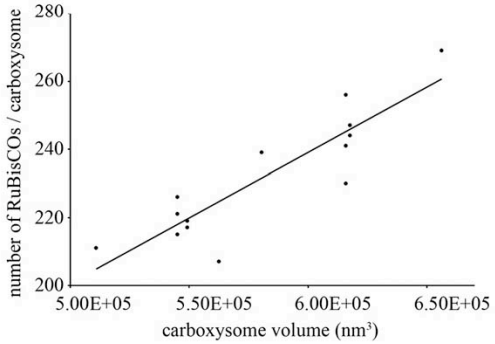


Figure 6. Number of RuBisCOs per carboxysome as a function of carboxysome volume. The number of RuBisCOs was assessed by template matching followed by a customized peak-search algorithm, and the volume calculated as that of the best fitting regular icosahedron.

(Iancu et al, JMB, 2007)

(Cannon et al.)

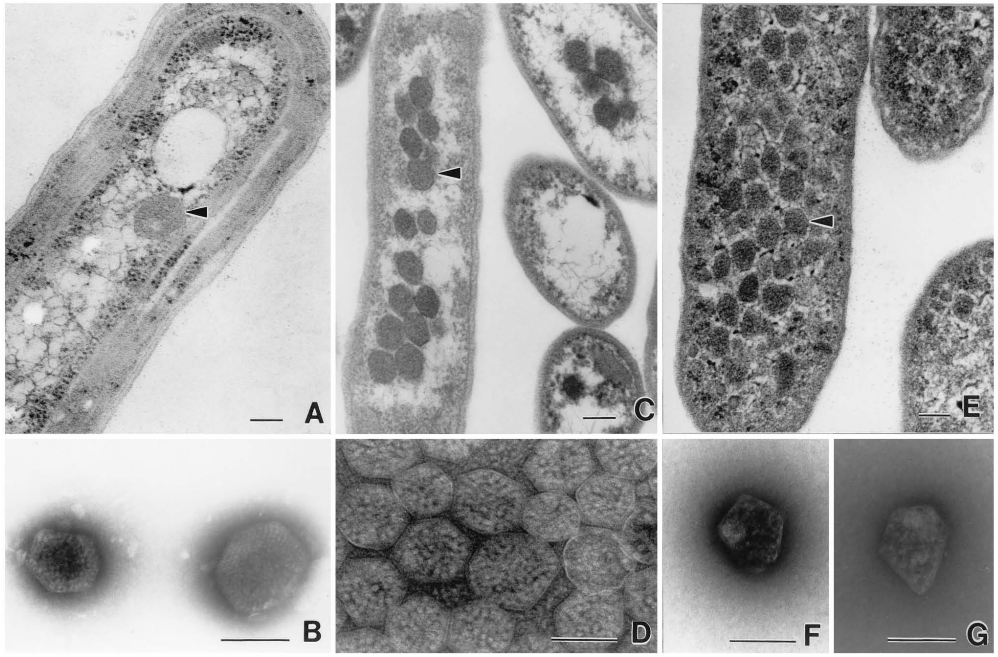
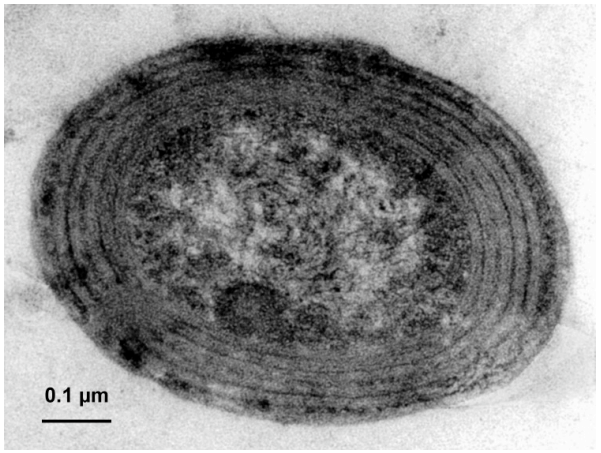


FIG. 1. Transmission electron micrographs of carboxysomes and enterosomes. (A) Thin section of a cell of *Synechococcus* strain PCC7942 (fixed cells kindly supplied by George Espie), showing a typical carboxysome (arrowhead). (B) Negatively stained carboxysomes from lysed cells of *A. nidulans* (now *Synechococcus*). Molecules of RuBisCO are visible inside. Micrograph kindly supplied by Elisabeth Gantt. (C) Thin sections of *H. neapolitanus* grown in air, showing aggregation of carboxysomes (arrowhead) in the nucleoid region of the cell. (D) Negative stain of carboxysomes isolated from *H. neapolitanus*. RuBisCO assemblies are visible inside. (E) Thin section of *S. enterica* serovar Typhimurium LT2 grown on propanediol under aerobic conditions. Many polyhedral bodies (enterosomes [arrowhead]) are visible throughout the cytoplasm. They are less regular than carboxysomes and slightly smaller. (F and G) Negatively stained, isolated enterosomes from *S. enterica* serovar Typhimurium LT2. Note the irregular shape. Contents appear to be of variable sizes. Photographed from preparation kindly supplied by Greg Havemann. Panels A, C, and E are all printed at the same magnification, as are panels B, D, F, and G. Bars, 100 nm.

PHOTOSYNTHETIC MEMBRANES!

- ***One of the intriguing features of these organisms is their membrane disposition. Membrane area is roughly 5 microns^2. This means that the number of lipids in the outer leaflet of the bilayer is roughly 10^7, yielding a total of roughly 10^8. Membrane management: interesting and challenging.***

Prochlorococcus



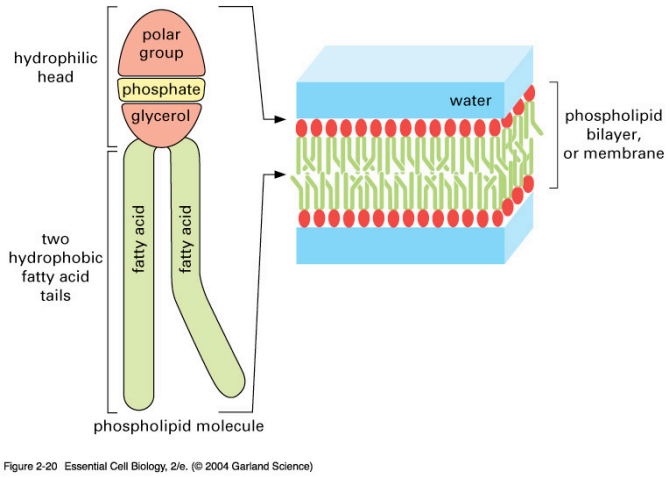
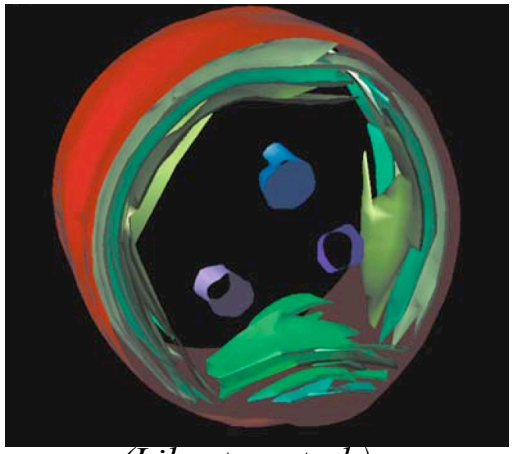


Figure 2-20 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Thylakoid membrane in Synechocystis



(Liberton et al.)