Outline

- Universal features of life among bacteria, archeae, eukaryotes
- Gap between data and knowledge
- How is research conducted? Learning to think independently.
- Cell biology integrates several approaches to understand how cells live, respond to stimuli and divide.

Approaches and methods:

Cell biology: microscopy to visualize where and when a process occurs

Biochemistry: purify to demonstrate activity of (a) molecule(s)

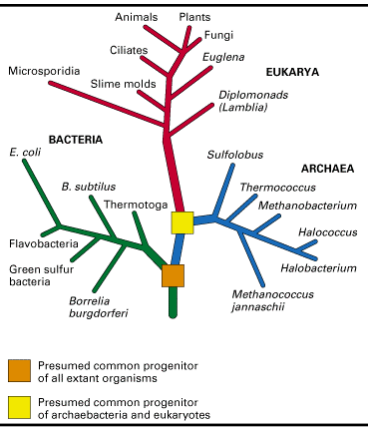
Genetic: study function in organism using mutants

Genetics & biochemistry: study mechanism of molecule activity

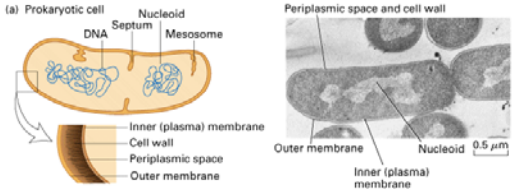
Bioinformatics: Use data of genes, proteins, microarrays, proteomics (protein interactions) to get inferences or clues to function

Universal features of life and of cells

1-5. 3 kingdoms of organisms are related through common sequences of their r-RNAs

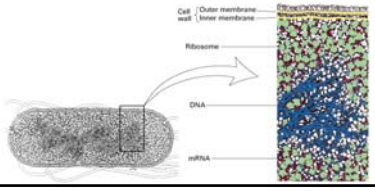


1-21. Alberts



1-2. Prokaryotic cell

1-7



Egg cells from different organisms look pretty similar. Information in egg cell determines nature of organism

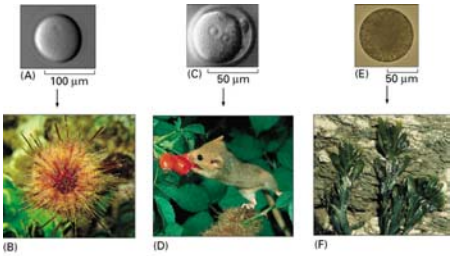
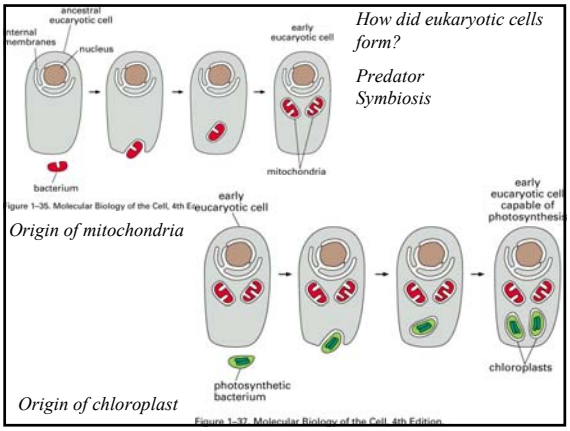


Figure 1–1. Molecular Biology of the Cell, 4th Edition.



How did eukaryotic cells form?

Predator

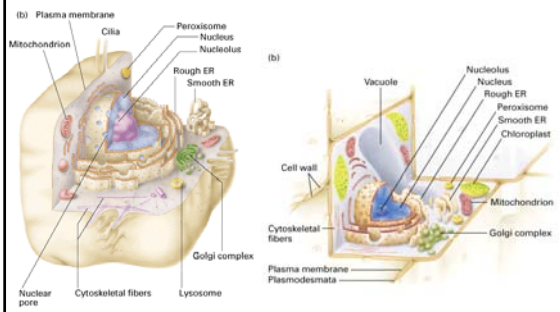
Symbiosis

Origin of mitochondria

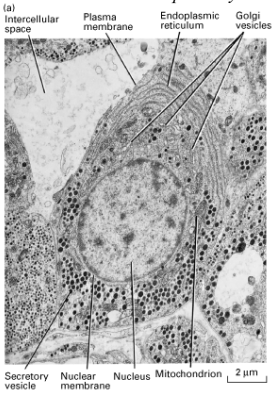
Origin of chloroplast

Figure 1–37. Molecular Biology of the Cell, 4th Edition.

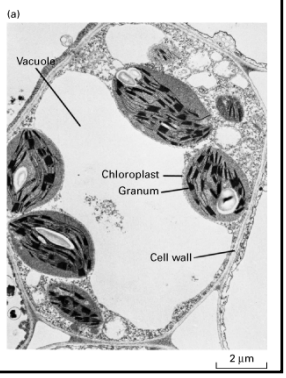
5-42 and 43. Animal and plant cells are very similar in structure



5-42. Animal cell: rat pituitary

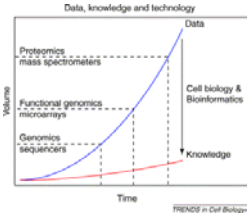


5-43. Plant leaf cell (differentiated)



Brunet et al 2003. TiCB

Gap between data and knowledge. *Proteomics is a powerful tool though other studies are needed to reveal how the dynamic structures interact with one another and remodel in response to stimuli.*



The task of managing, analyzing and visualizing such volumes of data requires the skills of the bioinformaticist, but even more critical is the participation of the cell biologist who must form and evaluate hypotheses based on these data. In this context, two key aspects of bioinformatics in MS-based proteomics can be highlighted. First, how bioinformatics enables the generation of proteomic data by overcoming technical hurdles. Second, how bioinformatics can enable the cell biologist to interpret proteomic data.

Analysis of the cell organelles in various conditions will be needed to understand the dynamic nature of integrated cell functions.

Discovery begins with a question

1. Observation by Microscopy

What is that structure?

What does it do?

How?

2. We have a complete set of genes and predicted proteins for yeast, fly, plant, or human.

What is the function of each gene?

Then one generates a working hypothesis or collect useful information to form a hypothesis

A model or idea of how a process might work.

Test hypothesis.

One chooses an approach & gives a rationale

•*Approach is a way or means to reach a destination.*

e.g. genetic
biochemical

Approach is not = method.

•*Rationale : reason, a logical basis*

Genetics: study of mutants [why?]
Forward genetics
Reverse genetics

Biochemistry: [why?]

Choose a material /organism

Give rationale for choice

And plan the method

e.g. Plan a genetic approach.

a biochemical approach- study of the function of a molecule.

extract & purify

study the chemical activity of the molecule

Cell biological approach

Get results and analyze them. Interpret results

What do results tell you? Interpret independently.

Be a detective, look for clues

Ask if results are convincing.

To interpret results confidently, one needs to know the technique and its limitations

Review of methods used in cell biology

Microscopy, in Alberts ch 9

Biochemical methods

Genetic

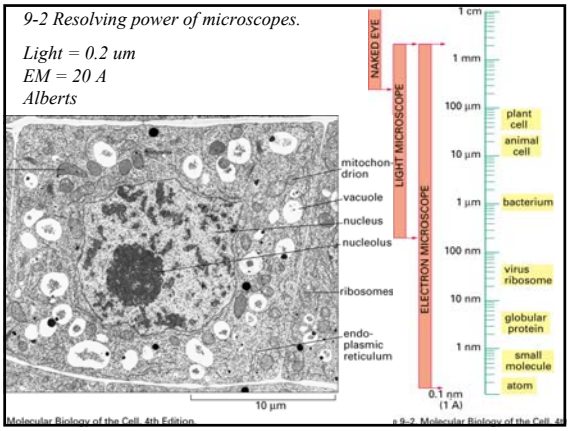
Molecular

Bioinformatics

Read chapters to get an idea. Will see examples in journal discussions.

9-2 Resolving power of microscopes.

Light = 0.2 um
EM = 20 A
Alberts



Molecular Biology of the Cell, 4th Edition

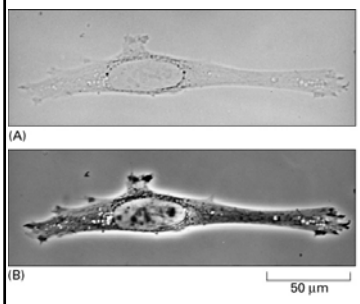
e 9-2. Molecular Biology of the Cell, 4th

Light Microscopy

- **Low resolution**
- **Can view dead and living cells**
- **Bright Field- fixed, sectioned, stained**
- **Fluorescence: fixed or living**
- **Phase contrast- living**
- **DIC or Nomarski- living**
- **Confocal fluorescence- 3D**
- **Image deconvolution- 3D**
- **Visualize location and/or movement of molecules in living cells**

Electron Microscopy

- **High resolution**
- **Detailed structure.**
- **Tissue- fixed, dead**
  - **sectioned**
  - **stained**
- **Specific molecules can be localized with gold-linked antibodies**
- **Rapid freeze cryo-EM**

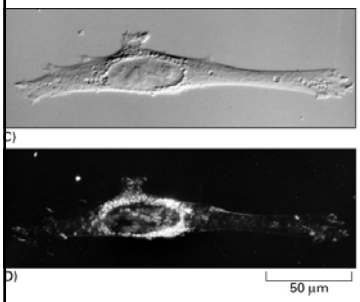


Alberts Fig 9-8

A. Bright field

B. Phase contrast

Figure 9–8 part 1 of 2. Molecular Biology of the Cell, 4th Edition.



C. Nomarski differential interference contrast

D. Dark field

igure 9–8 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Observations raise many Questions:

*What **molecules** **form the structures**?*

*What are the **functions** of the subcellular parts?*

*What **activities** do they have?*

How do cells respond to stimuli?

How does a single cell develop into an organized multicellular organism?

What controls development?

*How do you **approach** these questions?*

1. ***Biochemical**-& **Biophysical***

Find the essential and minimum players (e.g. proteins) responsible for the structure and activity.

2. ***Molecular genetics**- Find the genes responsible for the structure, response. Mutate.*

3. *Cell Biology- Combine them to understand function in a cell and in an organism*

Functional Genomics

How do we discover functions of genes?

Genetics

Biochemistry

Cell Biology

What is the role of a gene in a cell?

What role does it play in the whole organism?

Genes --> Proteins ---> Activities—> in cell

Methods used in Biochemical Approach:

-separate and purify an organelle or protein

-study its specific activity or role

-develop simple cell-free systems to determine the essential components required for activity.

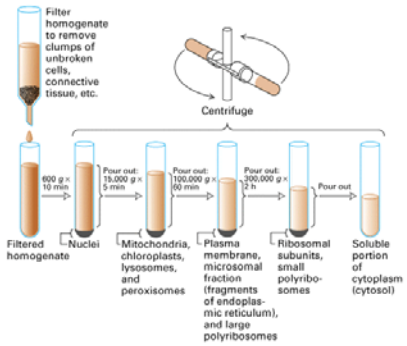
How?

1. **Cell Fractionation**
2. Centrifugation
3. Column Chromatography
4. Electrophoresis
5. Reconstitute activity

5-47. Cell fractionation by differential centrifugation

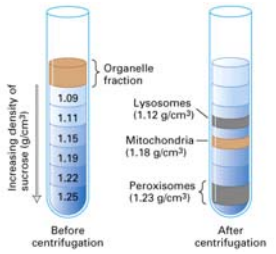
Goal:

separate, purify then measure activity



8-8 Alberts

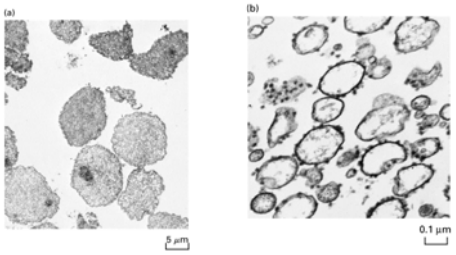
5-24. Separation of organelles by density gradient centrifugation



How do you verify if a fraction contains e.g. mitoch?

8-9 Alberts

5-25. EM of a) nuclei and b) ER purified from rat liver

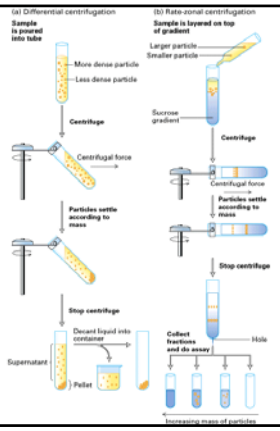


Goal- Verify purity of isolated fractions

a. microscopy
b. Biochemical enzyme activity [marker]
c. Immunostaining with antibody to marker

3-40

Centrifugation to separate particles differing in mass or density



To get highly purified organelles, there are new methods :

Affinity methods

Antibody coated vesicles

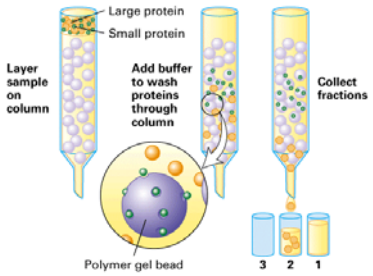
Physical methods

Phagosome containing low-density latex beads

How do you separate a mixture of proteins to find the one you want?

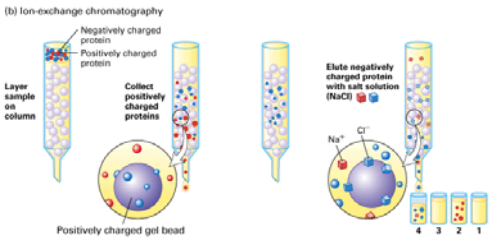
3-43. Column Chromatography to separate proteins in **active** form

(a) Gel filtration chromatography Separate based on size

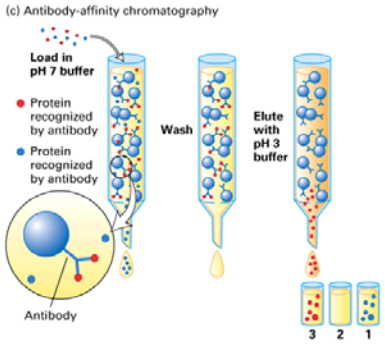


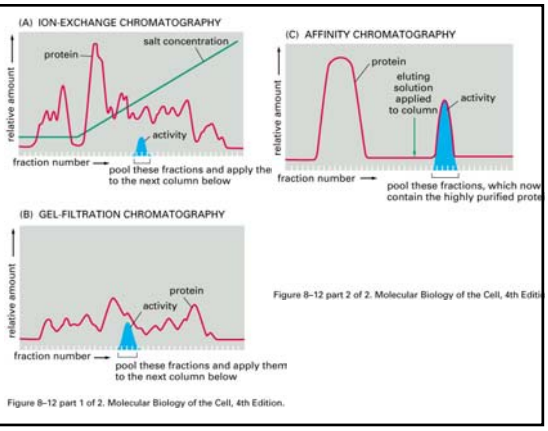
3-43

Separate based on charge

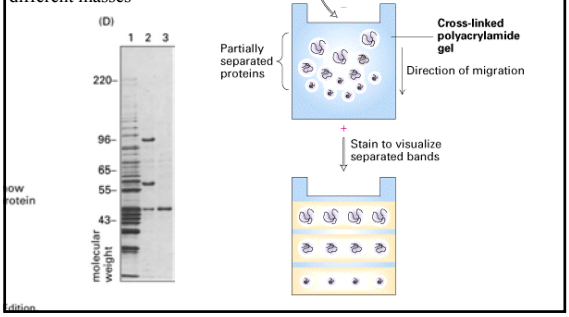


3-43c

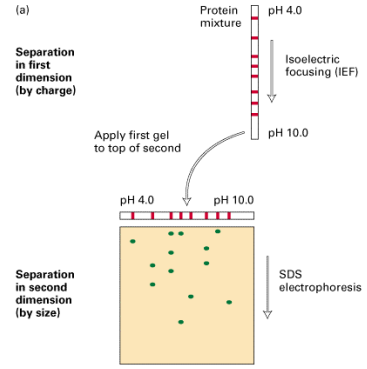




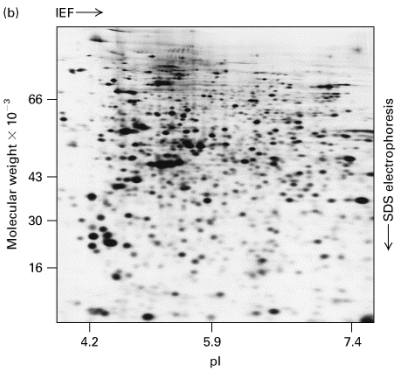
3-41. SDS polyacrylamide gel electrophoresis- to separate proteins of different masses



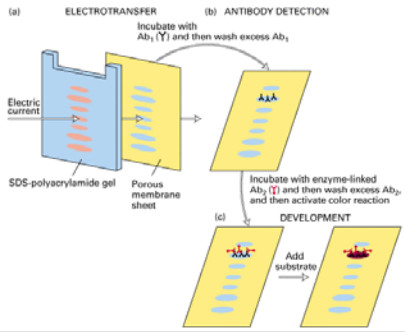
3-42. 2-D gel. A method to separate proteins based on different mass and charge.



3-42b



3-44. Western blot: using a specific antibody to recognize a specific protein. 2nd ab is linked to an enzyme that gives a colored product.



Cell biologists are now using organelle proteomics

to identify and localize all proteins in each organelle

Thus building a cell map.

Ref. Brunet et al M Desjardins 2003. Trends Cell Biol. 13, 629

Method:

1. *Purify organelle or complex*
2. *Run 2-D gel*
3. *Cut out each protein spot*
4. *Digest with trypsin*
5. *Determine mass of peptides, characteristic for each protein.*
6. *Use protein database of sequenced genome to identify protein.*

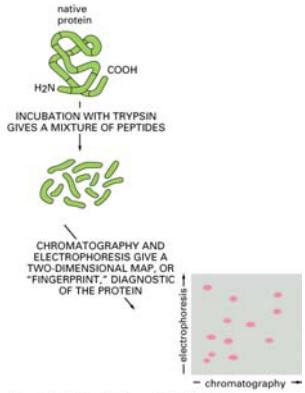


Figure 8–19. Molecular Biology of the Cell, 4th Edition.

Identify proteins by mass spectrometry

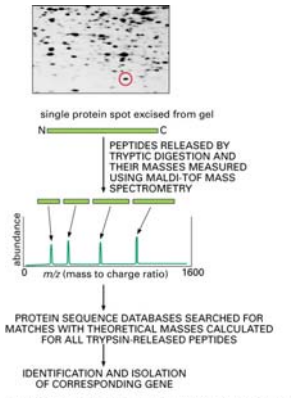
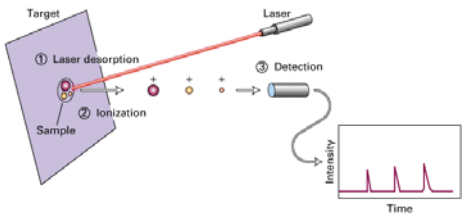


Figure 8–20 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

3-47. Mass measurements by time of flight mass spectrometry.



Determine amino acid sequence of peptides

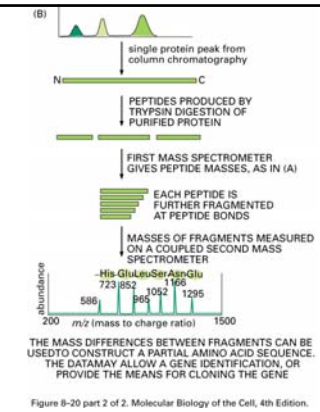
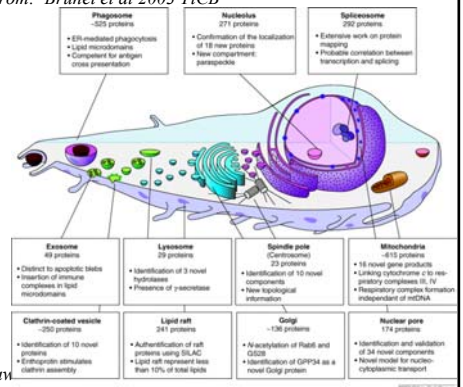


Figure 8–20 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Cell Map. From: Brunet et al 2003 TiCB



What are draw

How can you prove if the proteins are actually on the said organelle?

Brunet et al 2003. TiCB

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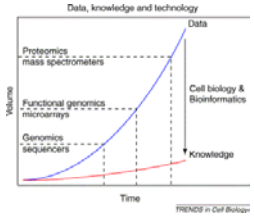

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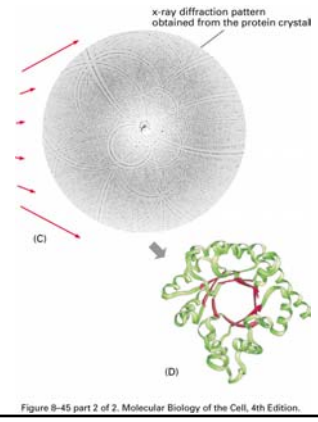

Figure 8-45 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Sequence similarity gives clues about protein function- BLAST

```
 Score =  399 bits (1025), Expect = e-111
 Identities = 198/290 (68%), Positives = 241/290 (82%), Gaps = 1/290

Query: 57  MENFQKVEKIGEGTYGVVYKARNKLTGEVVALKKIRLDTETEGVPSTAIREISLLKELNH 116
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Sbjct: 1   MEQYEKVEKIGEGTYGVVYKALDKATNSTIALKKIRLEQEDEGVPSTAIREISLLKEMNH 60

Query: 117 PNIVKLLDVIHTENKLYLVFEFLHQDLKKFMDASALTGIPLPLIKSYLFQLLQGLAFCHS 176
            NIV+L DV+H+E ++YLVFE+L  DLKKFMD+          LIKSYL+Q+L G+A+CHS
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Figure 8–47. Molecular Biology of the Cell, 4th Edition.