I. The Story So Far -- Summary of last Term

A. The Major Question: How do Living Things Work? Or how does 1 cell make two?

B. Answer so far has concentrated on

1. Prokaryotes (mostly) -- 1 intracellular compartment, unicellular

2. Macromolecular level -- emphasis on macromolecules, not on larger structures such as organelles, chromosomes, etc.
3. The "big 5" issues

- structure,
- function
- manufacture (including energy requirements)
- regulation
- (evolutionary) origin.

4. Methods -- the ones used to figure out how living things work, especially the methods used to get at the "big 5."

C. Bottom Line: DNA \rightarrow RNA (& more DNA); RNA \rightarrow protein \rightarrow job, say catalyzing X \rightarrow Y. Protein production & activity are regulated.

We emphasized roles of mRNA, tRNA & rRNA in translation, which are well known. Roles of short regulatory RNAs (micro RNA's, RNAi, etc.) in regulation of translation & transcription were mentioned only briefly, as details are just now being uncovered by the Encode project. For the significance of these regulatory RNA's see http://www.nytimes.com/2012/09/06/science/far-from-junk-dna-dark-matter-proves-crucial-to-health.html

II. Overview of this Term

A. Similarities: Same Major Question; Same "big-5" issues; same stress on methods.

B. Differences -- What's new?

1. Organisms: This term will concentrate on Eukaryotes (mostly) as vs prokaryotes. See Becker table 4-1 for comparison of Eukaryotic and Prokaryotic cells. For pictures, compare Sadava fig. 5.4 & 5.7.

2. Structures: This term will consider many things bigger than macromolecules -- intracellular structures (organelles, etc.) and assemblies of cells (organs, tissues, systems).

<table>
<thead>
<tr>
<th>Last Term</th>
<th>This Term</th>
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<tbody>
<tr>
<td>Organisms</td>
<td>Prokaryotes</td>
</tr>
<tr>
<td>Structures</td>
<td>Macromolecules</td>
</tr>
</tbody>
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3. New Issues: Two big innovations of eukaryotic cells are:

a. Intracellular Compartmentalization -- multiple membrane-bound compartments per cell

b. Multicellularity -- multiple cells per organisms

4. Topics: Most of the topics this term will deal with the consequences of the two big innovations.

<table>
<thead>
<tr>
<th>Last Term</th>
<th>This Term</th>
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<tbody>
<tr>
<td>Biochemistry</td>
<td>Cell Biology</td>
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</table>
C. Major Subjects of Term

1. Cell Bio -- study of consequences/implications of compartmentalization within cells (Lectures 1-9)
   

b. Co-ordination (between different parts of cell) -- in this part, we will emphasize generalized features of all eukaryotic cells but discuss some specialized cell types.
   
(1). How do molecules cross membranes? Lectures 4-6.

(2). How do newly made proteins get to the right place? What is the role of each organelle? Lectures 7-9

c. Regulation of eukaryotic gene expression -- chromosome/euk. gene structure & function -- how is DNA arranged and how is its transcription regulated? Lectures 10 & 11

d. Post Transcriptional Regulation -- How can activity of a transcript and the resulting protein be regulated? Lecture 12.

2. Chemical signaling between cells & some of the consequences -- How chemical signals work & how they are used for co-ordination between different cells in a multicellular organism (as vs co-ordination between the different parts of a single cell). Some examples of how specialized cells communicate and carry out specialized functions will be discussed in lectures 13-14; this will be wrapped up in lecture 17, after development. Note that lecture 17 is on a Friday, to compensate for a lecture session that will be missed on 4/15. How cells communicate electrically will be discussed later.

3. Development -- How do you build a multicellular organism? How do cells get specialized and how they stay that way? These lectures will be by Dr. Alice Heicklen. (Lectures 15 & 16)

For a taste of what's to come, see what Science magazine describes as the #1 'Breakthrough of the Year' for '08 -- IPS cells. For an expanded version of the print magazine special section of 2008, that describes IPS cells, go to http://www.sciencemag.org/btoy2008/ For an expanded version of the print magazine 'breakthrough of the year' special for 2009, go to http://www.sciencemag.org/btoy2009/ (There was no equivalent section for more recent years.)

4. Electrical signaling between cells -- Another way to achieve co-ordination between different cells in a multicellular organism.


b. Organization of nervous system and integration of communication (both types) with Muscle (Lectures 21-22).

5. Physiology -- Study of selected consequences/implications of multicellularity for maintenance of organism as a whole. How do you run a multicellular organism, once you've built it? How organisms make use of communication, specialization, etc. to maintain a relatively constant internal environment and how all the various functions are coordinated. This subject will be divided as follows:

   a. Homeostasis & Hormones: How the various systems (nervous, hormonal, muscular, respiratory, etc.) work to maintain a constant internal environment ('constant internal milieu') of temperature, gas, fluid, salt & water (Primarily lectures 17 & 23) and respond appropriately to changes in external environment.
b. Immunology -- The most specialized system; how a multicellular organism fends off invaders (Lecture 24).

6. Regulation of Cell Cycle & Cancer -- How aberrations in cell-cell communication and/or the cell cycle may cause cancer. (Lect. 25).

III. Eukaryotes -- a closer look.

A. What can you see in the light microscope? Suppose we draw a standard light microscope view of eukaryotic cell in interphase -- what can you see? Can't actually see membranes or details of many organelles. Can see nucleus, nucleolus, chloroplasts and/or mitochondria, Golgi (maybe).

B. Issues of size -- What you can see is limited by the size of the object and the amount of magnification you can get using any particular method. For pictures using the different methods of microscopy, see Becker table 1-1 or Sadava fig. 5.3.

- Limit of Resolution. Useful magnification is limited by the resolution, which depends on the wavelength of the electromagnetic radiation you are using. Limit of resolution = \( \lambda/2 \) = smallest distance you can resolve using electromagnetic radiation of wavelength \( \lambda \). Items closer than \( \lambda/2 \) appear as one object, not two. For more details on microscopy, see Becker appendix.

- Types of Microscopes -- Light microscope, SEM (scanning electron microscope) and TEM (transmission electron microscope). For variations on the light microscope, especially those for detection of fluorescence, see Becker appendix.

<table>
<thead>
<tr>
<th></th>
<th>Limit of Resolution</th>
<th>Useful Magnification</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye</td>
<td>0.1 mm</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Light Microscope</td>
<td>0.2 micron</td>
<td>1000X</td>
<td>Can look at living specimens</td>
</tr>
<tr>
<td>TEM</td>
<td>1-2 nm (in practice)</td>
<td>100,000X or 100X light scope</td>
<td>Detect electrons from source that pass through thin slice of tissue</td>
</tr>
<tr>
<td>SEM</td>
<td>Larger than TEM but gives 3D picture of surface*</td>
<td>Less than TEM</td>
<td>Detect secondary electrons emitted from surface of sample</td>
</tr>
</tbody>
</table>

* For a nice SEM picture see Becker fig. 1-4. For more SEM pictures go to Google images and search for SEM images.

- Sizes of things commonly encountered in biology

\[
\begin{align*}
1/1000 & \quad 1/1000 & \quad 1/1000 & \quad 1/10 \\
m \text{ (meter)} & \quad \text{mm} & \quad \text{micron (\( \mu \))} & \quad \text{nm (nano)} & \quad \text{Angstrom (\( \text{Å} \))}
\end{align*}
\]

mm = millimeter = 10\(^{-3}\) meter
micron (\( \mu \)) = micrometer or 'mu' = 10\(^{-6}\) meter
nm = nanometer = 10\(^{-9}\) meter
Angstrom (\( \text{Å} \)) = 10\(^{-10}\) meter
Sizes of some cells, structures and very small things (for reference):

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical eukaryotic animal cell</td>
<td>50 microns ((\mu)) = 50,000 nm.</td>
</tr>
<tr>
<td>Typical bacterium</td>
<td>1-5(\mu) = 1-5000 nm</td>
</tr>
<tr>
<td>Smallest known bacterium</td>
<td>0.2 (\mu) = 200 nm</td>
</tr>
<tr>
<td>nanobes (for <em>Times</em> article see; for more info see) **</td>
<td>20-150 nm</td>
</tr>
<tr>
<td>ribosome</td>
<td>25-50 nm</td>
</tr>
</tbody>
</table>

** The idea of tiny organisms ('Nanobacteria' or nanobes) caused a lot of excitement, but they are probably NOT living. See Nature archive of *Scientific American*, Jan 2010, article by Young & Martel, for details. If this link doesn't work, go through the Columbia Library eJournals, and search Scientific American for 'nanobacteria.'

For more examples and tables of relative sizes see Becker fig.1-3 & box 1A, or Sadava fig. 5.1.

C. What you can see in the EM? See handout 1A and Becker fig. 4-5 or Sadava fig. 5.7 for pictures of whole euk. cells. Some specific references to pictures of organelles are given below, but all pictures in Ch. 4 of Becker & Ch. 5 of Sadava are well worth it. Methods used to characterize the various parts are described below in IV, but will be discussed as they come up.

1. Membranes. In the EM you can actually see membranes.
   - Membrane = bilayer of lipid plus associated proteins. Entire structure is 6-9 nm across.
   - In the EM, after staining, bilayer appears as a double line. Can also be described as a three layered structure, with two dark layers sandwiching a lighter layer between them. Only the top and bottom layers are visible in the EM = double line.
   - Bilayer (& assoc. proteins) = a single membrane. A 'double membrane' = two complete bilayers. (See nucleus, below.)
   - Detailed structure of membranes (how lipid & proteins are arranged) will be covered next time.

2. Nuclear details -- See figs. 4-4 & 4-10 of Becker or fig. 5.8 of Sadava or go to Google images and search.
   - Nucleolus -- not membrane bounded; staging area for ribosome subunit assembly
   - Nuclear Pores & Nuclear Envelope (Double membrane = 2 bilayers, with holes punched across both layers)
   - Space between two layers of nuclear envelope = Perinuclear Space
   - No whole ribosomes in the nucleus. (Does contain newly made subunits on their way 'out' to the cytoplasm.)

3. Endomembrane System (EMS) -- See Sadava figs. 5.9 & 5.10 (5.10-5.11) or Becker fig. 4-15 to 4-18. For a more detailed picture, see fig. 12-1 of Becker.

   a. Why it's considered to all be part of 'one system'?
   - All parts are made of vesicles and flattened sacs (cisternae). Shapes vary. (See 'b' below for individual components.)
Each vesicle or sac is surrounded by a single membrane (one bilayer)

Each vesicle/sac has a space inside = lumen = space inside a hollow organelle, organ, or tube.

Material can be transferred from one lumen to another -- therefore all lumens are effectively (but not physically) connected -- see 'e' below for how transfer works.

b. Components

- **ER** (smooth & rough) -- continuous, but only rough (RER) has attached ribosomes. Membranes of ER are continuous with outer layer of nuclear envelope.

- **Golgi**

- **Many types of vesicles.** (Some examples: lysosomes, transport vesicles, secretory vesicles, & endosomes.) Name, structure & function of each type will be discussed later -- features of each type depend on role in processing, packaging, transport, etc.

- **Important:** Not all vesicles are part of the EMS!

c. **Major function** = processing, sorting, packaging and transport of proteins to/from outside of cell or proper part of EMS.

d. **Isolation:** See methods section below for how the various components are isolated and/or distinguished from each other.

e. **Exocytosis and endocytosis** -- Unique to eukaryotic cells. See handout 1A for steps

- All internal spaces of endomembrane system (lumens & perinuclear space, but not inside of nucleus) and the outside of the cell are effectively connected -- material can be passed between any of these spaces and the outside of the cell.

- How are these spaces & outside of cell (effectively) connected? Through formation and fusion of vesicles.

- Vesicular traffic (budding off and fusion of vesicles) moves and carries both "cargo" (vesicle contents) and membranes.

- Vesicle traffic can transport cargo two ways (see handout 1A).
  - (a) between one membrane-bound compartment inside the cell to another or
  - (b) between inside and outside of cell (by exo- & endocytosis).

- You need labeling of cargo to determine direction.

4. **Peroxisomes** (See Becker fig. 4-19 & 4-20 or text in Sadava Ch 5)

a. **Peroxisomes & lysosomes have some common features:**

- Are surrounded by a single membrane (unlike nucleus or endosymbionts).

- Contains enzymes in lumen

- Can be about the same size
- Contain reactive materials that can destroy cytoplasmic molecules

b. Are peroxisomes & lysosomes 2 different organelles? How do we know? See methods below.

c. A critical difference: Different enzymes are found inside the 2 organelles

  - Lysosomes contain acid hydrolases
  - Peroxisomes contain oxidases
  - Note: The two organelles arise from different structures as will be explained later. Only lysosomes are part of the EMS.

5. Mitochondria and chloroplasts

  a. Structures -- see texts (Becker fig. 4-11 & 4-14; Sadava fig. 5-11 & 5-12 (5-12 & 5-13.) Note double membranes (2 bilayers), prokaryotic style ribosomes, circular DNA, overall size similar to that of bacteria.

  b. Have own genetic systems (DNA, ribosomes etc. -- everything needed for DNA replication, transcription & translation.) Mitochondrial DNA is often used for identifications (when nuclear DNA is not available) or for tracing inheritance of the female line. Peroxisomes, lysosomes etc. do NOT contain DNA, ribosomes, etc.

  c. Most proteins of mito and chloro are NOT made inside the organelle -- most are encoded in the nucleus, made in the cytoplasm, and transported into the organelle after synthesis.

  d. Major similarities in function between mito & chloro

    - Electron transport in membrane used to pump protons
    - H\(^+\) gradient used to make ATP;
    - Carbon rearrangements carried out in matrix or equivalent

  e. Major Differences in function/location

    - Exergonic process that drives electron transport is different -- light absorption (in chloro) vs oxidation of reduced carbon compounds (in mito).
    - Plants have both chloroplasts and mitochondria. Animals have mitochondria only.

  f. Origins -- probably endosymbionts. Mito. and chloro. were once probably free living bacteria. See Sadava fig. 5.23 (5.25) and/or fig. 27.1 & 27.2 (27.2 & 27.3) or Becker Box 11A.

Q's to keep in mind:

1) What are the major similarities and differences between peroxisomes, lysosomes and mitochondria? How can you tell them apart? It is a good idea to make a table that compares and contrasts the three organelles.
2) How are new organelles made? The answers are different for the 3 types of organelles and will be discussed in detail in future lectures.


  a. Cytoplasmic ribosomes: Two types of ribosomes are making protein in cytoplasm (cytosol) -- "free" ribosomes or ribosomes attached to membranes of ER.

    - Free cytoplasmic ribosomes (attached to mRNA, but not to ER) make proteins for nucleus, cytoplasm, peroxisomes etc. -- all parts of cell except EMS.
• Bound ribosomes on RER make proteins for EMS or outside the cell.

b. **Organellar ribosomes**: Additional ribosomes (prokaryotic in size and function) are found inside mitochondria and chloroplasts. (No ribosomes in other organelles.)

c. **Location**: How the "right" cytoplasmic ribosomes attach to the ER & how the various proteins reach their correct destinations will be discussed at length later.

7. **Cytoskeleton** (see Becker figs. 4-23 & 4-24 or Sadava fig. 5.14 (5.17) -- we will discuss this in more detail next time. **How do you locate proteins that have no enzymatic activity?**

a. **Three components made of protein**

<table>
<thead>
<tr>
<th>Component</th>
<th>abbreviation</th>
<th>shape</th>
<th>Made of:</th>
<th>Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microtubules</td>
<td>MT</td>
<td>hollow</td>
<td>tubulin</td>
<td>25 nm</td>
</tr>
<tr>
<td>Microfilaments</td>
<td>MF</td>
<td>solid</td>
<td>actin</td>
<td>5-9 nm</td>
</tr>
<tr>
<td>Intermediate filaments</td>
<td>IF</td>
<td>cable</td>
<td>Different proteins; all similar (in same family); Found in cells of multicellular organisms only</td>
<td>8-12 nm</td>
</tr>
</tbody>
</table>

b. **How discovered?** By immunofluorescence (see below) using antibodies to tubulin and/or actin.

c. **Functions -- more on structure & function next time**

1. **Support/strength** -- weight bearing, shape determining.

2. **Movement (MF & MT)** -- can change shape of cell (as in muscle or amoeba) and/or help move organelles within cells.

3. **Localization of other factors** -- act as peg board or framework for attachment of organelles, enzymes, etc. (Cytoplasm is not simply a "bag of enzymes.")

IV. Methods -- How do you find out which components are in each cell part? A & B will be discussed in Lecture #1 as we get to them. C will be discussed next time.

**Reminder**: Becker has a guide to all techniques and methods described in the book -- see inside front cover.

A. **"Grind and Find"** (Biochemical separations and assays). See Becker Box 4B and Box12A or Sadava fig. 5.6.

1. **Grind**: Break up cell into parts, and separate (fractionate) parts by ultracentrifugation.

2. **Find**: Find the cell part associated with each particular enzyme. How? Assay (test) each cell fraction for enzymes of interest. (Provide substrate for enzyme; look for disappearance of substrate or formation of product.)

B. **In situ labeling** (Localization of enzymes "in situ" = in place).

1. **Substrate**: Provide solution of substrate. Enzyme substrate is soluble, so it diffuses to site of enzyme.
2. **Product**: Product of enzyme catalyzed reaction is *insoluble*, so it stays in the place where it is made. If cells are washed, unused substrate will be removed, but product will stay put.

3. **Localization**: Insoluble product is produced and precipitates only at location of enzyme -- pin points position of enzyme.

4. **Detection of product**: Can be colored (for detection in light microscope) or electron dense (for detection in EM). For an example, see Becker fig. 12-20.

**C. Immunofluorescence** -- Localization of proteins with no enzymatic activity. Use of labeled antibodies as tags -- direct (one step) and indirect (two step). Details & Handout next time. See Becker table 15-2. For more details, see Appendix, A-8 to A-11. For a picture of a typical result, see Becker table 15-1 or Sadava fig. 5.3. Allows you to visualize location(s) of particular proteins.

*Next time: More on the cytoskeleton, and the structure of cell membranes.*