In the next few lectures we will....

- Study a specific disease as a vehicle to understand biological molecules and cell function.

Goals

- To learn enough about biological molecules and cell function to figure out what causes a debilitating disease.
- To practice posing scientific questions and developing hypotheses.

Readings in Essential Biology by Campbell, et al

1. Chapter 3: Molecules of Life
2. pp. 78-79: Enzymes
3. Chapter 4: A Tour of the Cell

Gaucher Disease

An inherited condition
1. Affects 1 in 100,000 people
   - 1 in 100 are carriers of the gene
2. Affects 1 in 450 Jews of Eastern European origin (Ashkenazi Jews)
   - 1 in 10 are carriers of the gene!

Gaucher Disease Online Resources

Optional Reading
- http://www.gaucherdisease.org
- http://www.umm.edu/ency/article/000564.htm
- http://www.mssm.edu/gaucher/

Gaucher Disease

Named for French physician Philippe Gaucher (1854-1918)

<table>
<thead>
<tr>
<th>GD Results in...</th>
<th>Caused by...</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Always tired</td>
<td>Anemia (low RBC count)</td>
</tr>
<tr>
<td>- Enlarged abdomen</td>
<td>Huge spleen and liver</td>
</tr>
<tr>
<td>- Bent or curved legs</td>
<td>Weak bones</td>
</tr>
<tr>
<td>- Bruise easily</td>
<td>Low Platelet count</td>
</tr>
<tr>
<td>- Cross-eyed + head back</td>
<td>Neurological problems</td>
</tr>
<tr>
<td>- Premature death</td>
<td></td>
</tr>
</tbody>
</table>

Mandy's Story (www.gaucherkids.org/mandy)

- Diagnosed with GD at age 4
- Tired all the time
  - Didn’t want to play with other kids—would sit and watch others play
- Was very pale
- Really big abdomen due to enlarged spleen.
  - Developed a mass on my neck, just below my left ear
  - Surgeons removed mass and analyzed the cells + many other tests
  - First thought she had cancer
  - Finally identified Gaucher cells.
- Got 1st IV the day when 5 years old
- IV infusions every 2 weeks for last 15 years
- Now just as healthy as other people her age
- Cheerleader in high school
  - Lifted people 3/4 of her own weight (and had them fall on her too!)
  - did rigorous exercise, and gave it my all
- Her disease does not really affect her as long as she gets her IV every two weeks.
- Now a junior at the University of Northern Colorado studying elementary education: healthy and happy, with a lot of friends and a wonderful boyfriend who all understand her disease and what it means.

Mandy age 20
GD is a Genetic Disease

1. A bi-weekly therapy has been developed
   - Costs $100,000 per year.
   - The therapy turned 11 year old boy’s life around.

2. Before therapy
   - anemia threatened his life
   - too weak to walk up stairs.

3. After Therapy
   - RBC count is normal
   - Purple belt in karate
   - Plays basketball and football


X-Rays of a Gaucher Patient....

Before therapy          2 years after therapy

Source: Blood, Cells, Molecules and Disease (1996) 22 (11) June 15: 104-111

Gaucher Disease

Due to the inability of macrophages to digest...

Monomers Build Polymers

Monomers build polymers
- Is a common theme for the four classes of Biomolecules
- What does this mean?

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Monomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>______________________</td>
</tr>
<tr>
<td>Lipids (fats)</td>
<td>______________________</td>
</tr>
<tr>
<td>Proteins</td>
<td>______________________</td>
</tr>
<tr>
<td>Nucleic acids</td>
<td>______________________</td>
</tr>
</tbody>
</table>
Kinds of Carbohydrates

1. **Monosaccharides** (single sugars)
   - e.g. glucose, fructose, galactose
     - **Isomers**: Same formula: \( \text{C}_6\text{H}_{12}\text{O}_6 \), but have different structures

2. **Disaccharides** (double sugars)
   - **Sucrose**: glucose — fructose \( \text{C}_{12}\text{H}_{22}\text{O}_{11} \)
   - **Lactose**: glucose — galactose \( \text{C}_{12}\text{H}_{22}\text{O}_{11} \)
   - **Maltose**: glucose — glucose \( \text{C}_{12}\text{H}_{22}\text{O}_{11} \)

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Examples of Double Sugars

- **Sucrose**
  - Glucose — Fructose
- **Lactose**
  - Galactose — Glucose

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Polysaccharides

- **Poly-sugars** (polysaccharides)
  - Long chains of monosaccharides

Some examples:.... (all are polymers of glucose!)

1. **Starch** — energy storage in plants
2. **Glycogen** — energy storage in animals
3. **Cellulose** — outer wall of plant cells
   - provides support and protection
Glycogen — energy storage in animals

Glucose monomer

Branching occurs here

Lipids: Fats and Waxes

Properties...
- Don't dissolve well in water
- Mostly carbon and hydrogen

Major Functions
- Energy storage
- Transmit signals/messages:
  - Steroid hormones (e.g. human sex hormones)

Fats
- A type of lipid
- Made of ......

Phospholipid – another kind of lipid

Fatty Acids

(a) Palmitic acid

(b) Stearic acid

Oil and water don’t mix! Why?

Nonpolar Oil Molecules have greater attraction for each other than for water molecules.

Polar Water Molecules have greater attraction for each other than for oil molecules.

Oil molecules (triglycerides)

Fatty Acid

Hydrophilic Head

Special region

Phosphate

Hydrophobic Tail

a) Reaction to produce a fat molecule

b) Fat Molecule (a triglyceride)
Role of Phospholipids

Form ______________________________________
- Outermost boundary of animal cells
- Helps to control what can enter & leave a cell

The detailed structure of an animal cell’s plasma membrane

Gaucher Disease

- Associated with a molecule that is half sugar and half lipid.
- It’s called ______________________________
  - Gluco = glucose, the sugar part.
  - Cerebro = cerebrum
    - Part of brain where most higher thought goes on.
    - Those cells have lots of this type of lipid

Proteins

1. Long chain of amino acids
2. Order of amino acids determines 3-D shape
3. Shape determines the protein’s function

The parts of an amino acid

Variable side chain (hydrogen in the case of glycine)

Proteins have many roles inside the cell (1 of 2)

1. ______________________________
   - Speed-up chemical reactions so they can occur at a life sustaining rate
2. ______________________________
   - Carry substances in and out of cells
   - Hemoglobin carries O\textsubscript{2} in R.B.C.’s
3. ______________________________
   - Protect against disease
Proteins have many roles inside the cell (2 of 2)

4. ____________________________________________
   - e.g. muscle fibers, cartilage, tendons

5. **Peptide Hormones**: regulate body processes
   - ________________ → control blood sugar levels
   - ________________ → control size

Role of Proteins in Gaucher’s Disease.....

**Healthy People can Breakdown GC**

1. A specific __________________ breaks down a certain sugar—lipid (GC) in healthy people.

   ![Diagram](image)

2. The enzyme: Glucocerebrosidase.
3. The sugar—lipid is GC = Glucocerebroside

**The Enzyme is defective in GD Patients**

1. Why can’t the defective enzyme break down the sugar—lipid?
   ![Diagram](image)

2. What is the name of the ....
   - sugar lipid? ____________________________
   - enzyme? ____________________________

**The Defective Enzyme in GD Patients**

**Glucocerebrosidase** or **GCase**

- the enzyme that normally breaks down the sugar—lipid molecule (GC) that builds up in people with Gaucher Disease
- Most enzymes have the suffix: -ase.
  - e.g. Sucrase
  - Lactase
  - Maltase
  - Pepidase
  - Lipase

Why do GD patients have the defective enzyme?

1. They inherited a mutant glucocerebrosidase gene
2. A Gene is...
   - segment of DNA in a chromosome
   - Carries information that can be decoded to produce a specific kind of protein
3. DNA is a polymer of ____________________________

**Nucleic Acids: DNA and RNA**

1. **Nucleic acids**
   - are long chains of ____________________________
   - the “genetic molecules”
2. **Nucleotides**
   - the building blocks (monomers) of DNA and RNA
   - As monomers they power almost all processes in all cells
     - e.g. **ATP**
DNA Nucleotides

1. Four Kinds of nucleotides in DNA
   - A = Adenine
   - T = Thymine
   - G = Guanine
   - C = Cytosine

2. Central dogma of Biology
   - The order of nucleotides in a gene determines the order of nucleotides in a protein
   - The order of amino acids in a protein determines the three-dimensional structure of the protein which in turn determines the functions of the protein.

Nucleotide Structure

- Nucleotides are the building blocks (monomers) of DNA and RNA.
- As monomers they transfer energy to power almost all processes in all cells.
  - e.g. ATP

From DNA to Humans

- 80,000 different proteins in the body’s cells
- DNA dictates
- Genotype
- Phenotype

DNA Genetic Code Dictates Amino Acid Identity and Order

- DNA Sequence
- Es
- the Genetic Code
- GCA AGA GAT AAT TGT
- Growing Protein Chain

DNA Sequence Variation in a Gene Can Change the Protein Produced by the Genetic Code

- Gene A from Person
- Protein Products
- GCA AGA GAT AAT TGT
- Gene A from Person
- GCG GAG AAT TGT
- Gene A from Person
- GCA AAA GAT AAT TGT

Human are the product of genetic information encoded by environmental effects.
Gaucher Cells

• Gaucher patients have a peculiar cell type other people don’t have called a Gaucher cell
• Note the large size of the Gaucher cell (arrow) compared to the RBC’s and WBC’s
• Why are they so large?

1. Derived from __________________________
   - a W.B.C. that eats dying cells, invading microbes, and damaged tissue
2. 10 x’s larger than normal macrophage cells
   - Gaucher Cell = 100 μm
   - Macrophage cells = 10 μm

• Why are Gaucher cells 10x’s larger than normal? What is accumulating inside? Let’s learn a bit about cells.....

The 3 Basic Parts of all Eukaryotic Cells

1. Plasma Membrane
   • Controls enters & exits the cell
2. Cytoplasm
   • Entire contents of cell between P.M. and nucleus
   • Where most metabolic activity occurs
3. Nucleus
   • Contains DNA, the genetic material

Eukaryotic Cell Structure

1. Nucleus:
   » Site of DNA, the genetic material
   » Controls cellular activities
2. Smooth Endoplasmic Reticulum:
   » Contains enzymes for making lipids and other substances.
3. Ribosomes:
   » Site of protein synthesis
4. Rough Endoplasmic Reticulum:
   » Membrane bound channel studded with Ribosomes
   » Makes proteins found in other organelles and proteins exported from the cell
5. Golgi Apparatus:
   » Modifies, stores and ships products of the ER
6. Vesicles (or vacuoles):
   » Membrane-enclosed sacs or “balloons” that transport or store substances in cells
**Eukaryotic Cell Structure**

7. **Lysosomes:**
   - Sacs containing enzymes that digest worn out cell parts
   - Digests food ingested by phagocytosis

8. **Cytoskeleton:**
   - Protein fibers that help a cell maintain its shape
   - Some fibers involved with transport of vesicles

9. **Mitochondria:**
   - Harvests energy from organic molecules (e.g., sugars and fats) to produce ATP— the energy "currency" of all cells!

**Molecules involved with GD**

1. What does GCase do?
2. Which organelle contains glucocerebrosidase?
3. Where are digestive enzymes?
4. How GCase get there?
5. Trace the pathway of the enzyme to this organelle.

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**Ribosome on Rough ER Producing a Protein such as GCase**

- A ribosome reads mRNA to produce a protein molecule

**ID of structures...**

1. ______
2. ______
3. ______
4. ______

**Rough E.R. to Golgi Apparatus**

- Proteins modified by Golgi Apparatus are either...
  - Used inside cell
    - e.g. _______________

  - Exported from cell
    - e.g. _______________

**Membrane Bound GC**

- Sugar — lipid molecules (GC) are normally found on the surface of cells.
- GC involved with cell — cell recognition

**Transport from Golgi Apparatus**

- Cell Product(s) inside Vesicle
  - Proteins are modified by Golgi Apparatus
  - Exported from cell
    - e.g. _______________

- Membrane Bound GC
  - Glycolipid (sugar lipid) molecules
  - Outside of cell
  - Cytoplasm (inside of cell)
Synthesis of GC in Cells

1. Where is GC produced within a cell?
   - Hint: where are lipids made in cell?
2. Where are sugars added to newly made biochemicals?
   - Hint: where do chemical modifications occur?
3. Trace the biosynthetic pathway of the sugar—lipid through the cell to its home in the plasma membrane

Digestion of GC

1. Now we’ve got GCase in a lysosome of macrophage cells and GC in the plasma membrane of the cells they eat.
2. How does GC get into Macrophage lysosomes?
3. Let’s take a look at this happens......

Macrophages: “Big Eaters”

- Eat dead, injured, and foreign cells
- Cells consumed have GC in their membrane
- Engulfed cells transported to lysosome for digestion

ID each of the following

1 = __________
2 = __________
3 = __________
4 = __________
5 = __________
Lysosome Fusing with a Vesicle containing a cell

1. Where is digestion occurring?
2. Where is GC?
3. What happens to the GC in...
   a. healthy people?
   b. GD patients?

Gaucher Cells

1. Are Macrophages with Defective GCase
2. Undigested GC builds up in both the
   a. ______________________Membrane, which causes ______________________
   b. ______________________Membrane, which causes ______________________
3. Affected macrophages become ____________ in size and are known as “Gaucher Cells.”

Gaucher Cells before and after therapy

Bone c.s.

GD Therapies

1. What kind of therapy would help patient a with Gaucher Disease?
2. How would it work at the cellular level to overcome disease symptoms?
3. What would be the drawbacks of the therapy?

Enzyme Therapy

1. Why given by intravenous injection and not as a pill?

2. Drawbacks
   a. ______________________
   b. ______________________
3. Obtain the enzyme from genetic engineering in prokaryotes.
   » In the past: from human placentas
Challenges of Enzyme Therapy

1. Must get GCase into the lysosomes of macrophage cells
2. How do we get it there?
   a. A special signal is attached to GCase
   b. The signal is recognized by macrophages
   c. Stimulates macrophages to bring GCase inside via phagocytosis
   d. This signal is a disaccharide called mannose phosphate

Cell Therapy

1. Bone marrow transplant
2. Blood stem cell transplant
3. Both work, but have serious drawbacks

Problems with Cell Therapy

1. ___________________________
2. ___________ risk of fatal complications
3. Cell transplantation therapy does work!
   » The presence of normal stem cells reduces the severity of the disease.
   » How can we make normal cells?

Gene Therapy

1. Isolate ___________________________ from GD patient
2. Insert functional ___________________________ into stem cells using a ___________________________
3. Put these genetically engineered cells back into the patient
4. Patient’s blood stem cells reproduce to form normal ___________________________
5. Gene therapy works because the macrophage cells can now make the normal ___________________________

Potential Problems with Gene Therapy

1. Virus might ___________________________
2. ___________________________ might overreact to the virus
e.g. Jesse Gelsinger (died Fall 1999)
3. GCase gene might insert into the patient's DNA in a place that causes a mutation that results in ___________________________
4. Clinical trials are now being done.