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Beyond the Human Genome

Course Outline

SOUTHERN METHODIST UNIVERSITY (SMU)

Winter 2002

Instructor: Mike Stathis

Course Description for "Beyond the Human Genome"

This course has been created by Mike Stathis with the objectives of providing a one-of-a-kind and highly valuable crash course on biotechnology for biosciences business consultants and patent attorneys seeking to expand their knowledge of the biomedical sciences. Each class will run approximately three hours.

We are experiencing the beginnings of a biomedical revolution that will change the way we live and die forever. It is only through a detailed and comprehensive understanding of normal structure and function of the human body that we may begin to understand both the healthy and diseased states which will enable us to understand and innovate biomedical therapies.

This unique lecture series presents material from chemistry and biochemistry to immunology and pathology in an attempt to provide the layperson a fairly advanced understanding of the basic principles of biology, chemistry, biochemistry, physiology, pathology, immunology, cellular and molecular biology and genetics so that he/she may appreciate the potential power of biotechnology. Much of this course will be taught by use of diagrams obtained from numerous graduate level texts.

If you have always wondered what biotechnology is and what the future holds, join me for this in depth look into the future of medicine.

Class 1: The Foundations of Modern Science: Basic Scientific Principles from Chemistry, Biology, Biochemistry, Genetics, Immunology, and Pathology
Special Topic: human physiology of the organs (as time permits)

Class 2: The Human Genome
Genetic Disease, Diagnosis
Biotech Applications: Gene Therapy
Special Topic: Metabolism (as time permits)

Class 3: Cell Structure and Function
Protein Structure and Function
Biotech Applications: Drug Design

Class 4: Techniques for Biomedical Research
Special Topic: Biochips (as time permits)

Class 5: Immunology, Disease and Aging (part 1)
Biotech Applications: Gene Therapy
Special Topic: nanomedicine (as time permits)

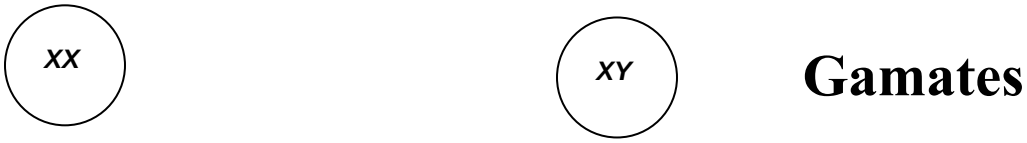
Class 6: Immunology, Disease and Aging (part 2)
Biotech Applications: Vaccine Therapy
Special Topic: telemedicine (as time permits)

Class 7: Final Review and Wrap-up
Agribio—crops and cattle
Bioenergetics
Biopharma
FDA Approval
Biotech in Today's World
Life in the 22nd century

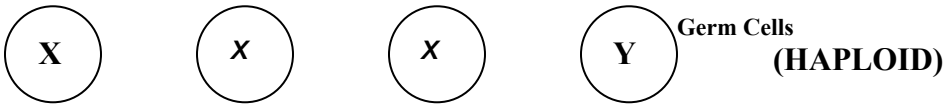
OVERVIEW of "BEYOND THE HUMAN GENOME"

Each cell in our body contains either 46 (for somatic/body cells) or 23 (for germ cells) chromosomes. Somatic cells have a diploid number of chromosomes since they have 2 copies of 22 autosomes + 1 sex chromosome. Germ cells have a haploid number of chromosomes since they have 1 copies of 22 autosomes + 1 sex chromosome.

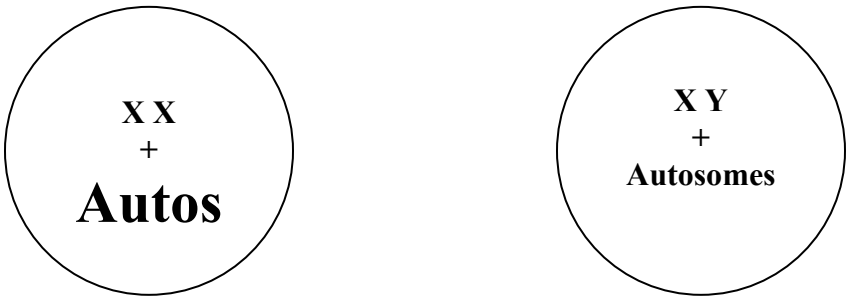
GAMATOGENESIS



MEIOSIS

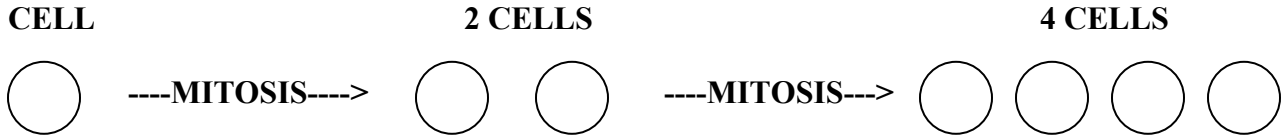


Fertilization



Development

It is important to note while meiosis only occurs during specific periods during our life and only with sex cells, mitoses are occurring in most of our cells most of the time (except for neurons and muscle cells), the rate of which is determined by many factors (growth, sickness, etc). During the first 20 years of our life, mitosis is occurring at tremendous rates so that the number of new cells is >>> than the number of cells dying.



These cells have exact copies of DNA, so that each somatic cell (body cell) contains 2 copies of our 23 chromosomes (22 autosomes + 1 sex chromosome). These cells function to keep our body function in the normal state (homeostasis) by producing various proteins which are responsible for converting food into energy, protecting us from disease, regulating fluid and ion concentrations, etc.

Chromosomes----------> DNA----------> RNA----------> Proteins----------> Processing

Depending on the type of protein processing, proteins are either retained for the cells' own use or exported for use outside the cell.

Proteins for the cells' own use:

1) **Enzymes for metabolism**
2) **Structural proteins for cell membrane integrity**
3) **Functional proteins for cell membrane (Na+/K+ pump) to maintain proper ion conc.**
4) **Cell specific enzymes and other proteins:**
    a) **Antibodies in B cells**
    b) **Collagen in fibrocytes and epithelial cells**
    c) **Digestive enzymes in G.I tract cells**
5) **Regulatory proteins**
    a) **For regulation of transcription and translation**
    b) **For waste disposal (lysosomes)**

Proteins for export and use by other cells and the body:

1) **Blood proteins (compliment, albumin)**
2) **Chemotactants**
3) **Proteins that affect other cells' function**

Each of our organs has several primary functions which are accomplished by organ-specific cells which produce and/respond to organ-specific proteins:

LIVER
--fat, protein, and carbo metabolism
--digestive enzymes
--detoxification
--excretion of toxic byproducts
--blood proteins (complement) and alpha-1-antitrypsin

HEART
--pumps blood to the body and delivers oxygen to cells while excreting CO2

KIDNEYS
--blood filtration
--erythropoeitin
--blood pressure regulation
--maintaining acid-base balance

G.I. TRACT
--digestion→ nutrient absorption
--maintains acid-base balance
--final excretory portal for waste

LUNGS
--respiration→ oxygenates blood
--maintains acid-base balance

SKIN
--barrier to foreign invasion

PANCREAS
--digestive enzymes
--insulin and glucagon production

BLOOD & CIRC. SYSTEM
--organ to organ cellular transport
--delivers O2 to cells via RBCs' hemoglobin
--controls the variable amounts of blood to each organ system which is regulated depending on the physiological state

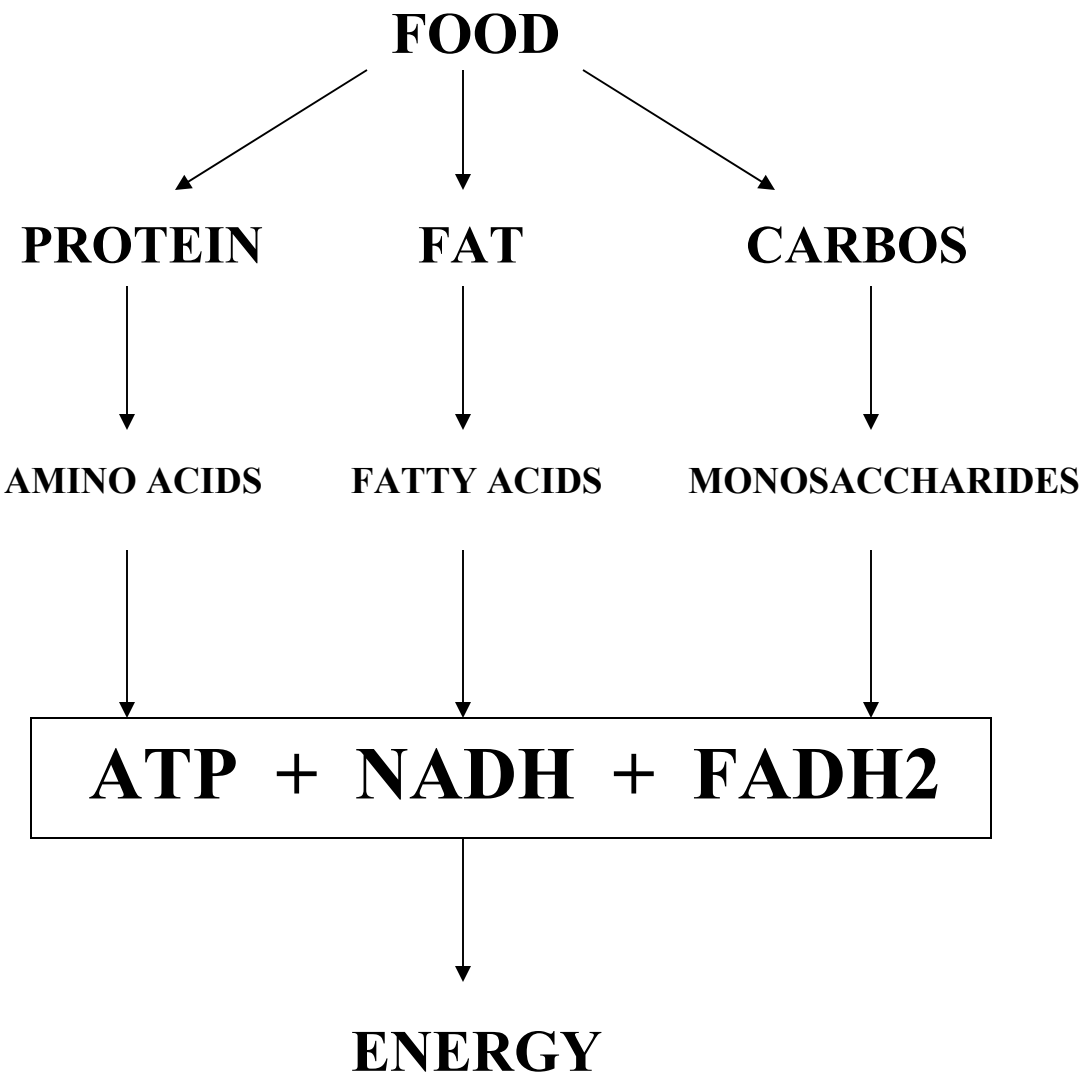
BONE MARROW
--hematopoiesis (RBC & WBC syn)

CENTRAL NERVOUS SYSTEM
--contains a vast interconnected network of sensory and effector neurons which serve to detent and respond to physiological stimuli.
--several different proteins and hormones serve as neurotransmitters which provide the necessary signals for this system to function properly.

More on Metabolism

3 Basic Forms of Nutrients: Protein, Fat and Carbohydrates

--Everything we eat and digest is some combination of these 3 nutrients. Carbohydrates are NOT essential to our diet for survival, but proteins and fat are. Fat and carbohydrates are more efficient forms of energy then proteins. Although fat and carbohydrates are involved in the structure of cells and tissues their main function is energy utilization. For simplicity think of proteins as a car and carbohydrates and fat as the gasoline---that is proteins are usually not used for energy production but for the structural and functional components of the body.



HOMEOSTATIC FUNCTIONS

Heat	Mechanical Energy	Metabolism	
Core Body T	**Cell Motility (Microtubules)** **Est/Maintain Ion Gradients** **Intracellular Transport** **Energy Storage** Tissue Movement (Muscles)	**Anabolic Reactions** (synthesis)	**Catabolic Reactions** (breakdown)

An Example of the Anabolic/Catabolic Cycle

Let's have Mike's breakfast—a Three Musketeer's bar

Contents: Milk chocolate (sugar, chocolate, cocoa butter, lactose, skim milk, milkfat, chocolate processed with alkali, soy, lecithin, natural and artificial flavors), sugar, corn syrup, partially hydrogenated soybean oil.

****Milk chocolate basically contains carbohydrates (sugars); fructose is the main sweetener used in candy (and by the way is not very sweet relative to other sugars). Metabolism of fructose yields ATP and NADH.**

**** Soy and Lecithin are oils, which are made of fatty acids. Lecithin is one of the 2 essential fatty acids we Must have in our diet (i.e. we cannot make these). Fatty acids are catabolized (broken down) to form NADH, which uses its highly reduced state (i.e. the NAD-H vs the NAD+) to indirectly produce ATP. Remember from lecture 1, a molecule is reduced if it gains electrons (in biological Systems, an electron pair usually is transferred via a H atom, i.e. H:).**

So, we have just eaten a candy bar full of sugar and our body is reacting immediately. Enzymes are released in our digestive system which allow the polysaccharides to breakdown into monosaccharides so they can be absorbed. As these sugars are absorbed into the bloodstream, the pancreas is stimulated to release insulin, which helps our cells take up the monosaccharides for use and storage (see previous page).

Now that we have given an example of the catabolic process involved in eating a candy bar let's take a look at an example of some anabolic reactions which occur—Synthesis of DNA by our cells for mitosis. This is an ongoing process that helps increase the number of cells in the body of a developing individual (ages 0-27) and to maintain a constant number of cells in our body (for individuals about 27-35) and to a smaller extent for older individuals. Remember, cells have a finite lifetime and we have millions of cells dying each day.

Let's Review How Our Body Makes DNA for Mitosis

Mitosis requires DNA replication. When we eat our candy bar we have seen that it Is Catabolized to produce monosaccharides which are converted into the energy of ATP and NADH. Some of this ATP is used immediately for normal cell functions while most of it is stored for later use. The NADH is Not a readily available form of energy and must be converted into ATP via a different mechanism. Nevertheless, the energy of ATP is used to fuel the reactions responsible for making DNA. First, the individual bases (A, G, C, T) must be made via purine and pyrimidine synthesis.

Remember, DNA is composed of a Base, a sugar (ribose) and a Phosphate. So once the bases have been made, a ribose is attached, then a phosphate to produce a nucleotide. This ribose-P comes from the catabolism of other carbohydrates in our diet.

Then each nucleotide is added to its complimentary nucleotide on the parent (original) DNA strand to form a daughter (complimentary DNA copy) DNA strand.

Now the amount of DNA has been doubled so that mitosis can occur and results in the formation of 2 cells from one.

Then they are used during DNA replication. And we know very well why how the cells use DNA-----PROTEIN SYNTHESIS.

Now let's look at what happens when a cell has come to the end of its life, due to either some injurious agent (an infectious agent or mechanical injury) or simply is instructed by its own DNA to self-destruct by a process called apoptosis.

During cell death, enzymes are released from within the cell which "chews" up the DNA, as well as other cellular components. And upon lysis (breakage) of the cell membrane, all cell contents are released into the tissue vasculature (blood and/or lymphatic system). Eventually, these molecules are brought to the liver, which serves as the site of purine and pyrimidine metabolism.

Because these DNA bases (as well as all the proteins of the deceased cell) contain nitrogen, We must eliminate these NH_2/NH_3^+ groups from the body or we will die from ammonia toxicity and disruption of acid/base imbalance. (Remember the brief discussion on pH; we must maintain a pH range of about 6.8-7.2 or our body chemistry will be dysfunctional and we will die; also note that NH2- groups add to the basicity of our body, i.e. raises the pH). **The process of excretion of these nitrogen groups from our bloodstream is performed by The liver via purine and pyrimidine catabolism.**

What Can Go Wrong?

1. Genetic
- **a. Inborn Errors of Metabolism---phenyketonuria, Gaucher's disease**
- **b. Autoimmune Disease---Grave's disease, rheumatoid arthritis**
- **c. Immune Dysfunction---thymic hypoplasia (small/no thymus)**
- **d. Increased Predisposition to Certain Diseases—diabetes, cancer, infections**
- **e. Dysfunctional DNA Repair Enzymes---Bloom's syndrome**

2. Environmental Factors—causing mutations and causing cellular dysfunction, immune dysfunction and possibly leading to cancer.

3. Immune Dysfunction—autoimmune disease, disease of chronic infection (AIDS)

How Do We Detect Disease?

1. **Signs and Symptoms---Physical Exam and Patient History**

2. **Cellular---blood workup**

3. **Molecular Markers---RFLP**

4. **Imaging---x-rays, CAT scans, MRIs**

How Can We Treat Disease?

1. **Hormone Replacement Therapy—eg. Synthroid**

2. **Enzyme Replacement Therapy—eg. Lactase, as well as several pharmacological drugs in current use**

3. **Enzyme Inhibitors--- several pharmacological drugs in current use**

4. **Direct Gene Therapy---Human Cloning (replace diseased/defective organs)---gene therapy with stem cells or tissues with high regenerative capacity (bone marrow & liver) so that the normal gene is restored. Then the tissue is grown in-vitro and replaced within the patient.**

How Can We Prevent Disease?

1. **Vaccines**

2. **Chemicals designed to replace/substitute lower/increase activity of a cellular activity**

3. **Chemicals designed to alter genetic mechanisms**

4. **Direct Gene Therapy---Human Cloning to give us "Supergenes"**

5. **Lead a healthy lifestyle—proper balanced diet, exercise, avoidance of adverse environmental elements <=BEST SOLUTION!!!**

Outline for "Beyond the Human Genome"

1. The Development of Biotechnology and Basic Scientific Principles from Chemistry, Biology, Biochemistry, Genetics, Immunology, and Pathology.

A. History of Science---establishing the rules of the scientific process
   1) Scientific Method--the systematic collection of data and formulation and testing of hypotheses based on the data.

   2) Louis Pasteur—disproved spontaneous generation hypothesis

   3) Koch's Postulates—the first proof that bacteria cause disease
      a) The same pathogen (disease-causing agent) must be present in every case of the disease.
      b) The pathogen must be isolated from the disease and grow in pure culture.
      c) The pathogen from the pure culture must cause the disease when it is inoculated into a healthy susceptible lab animal.
      d) The pathogen must be isolated from the inoculated animal and must be shown to be the original organism.

   4) Synthetic Organic Chemists--a routine day at the lab-- The Beginnings of Pharmacology

B. Chemical and Biochemical Breakthroughs (figure 1)

   1) Edward Jenner and Smallpox—the first and most effective vaccination

   2) Watson-Crick Hypothesis
      --what is DNA? Structure, base pairing, bonds, base stacking, double helix

   3) Two Genes One Protein
   4) Lock and Key Hypothesis---the beginning of enzymology
   5) Cell Signaling Pathway--internal cell communication and function
   6) PCR--DNA amplification

C. FDA, Politics and Drug Approval
   1) Role of the FDA

   2) Politics of Drug Approval and Pricing---WHAT ARE WE REALLY PAYING FOR?

   3) Drug Approval Process---1 in 5000 (figure 2)

D. Pharmacology vs. Biotechnology---what's going on?
   1) How are they similar?
   2) How are they different?
   3) How are they complimentary?
      --sales and marketing force vs. R&D
      --clinical trials vs. R&D
      --licensing agreements vs. financial backing throughout the drug approval process

E. Basic Chemistry Concepts

1) Chemical elements (figure 3: periodic table)

2) Chemical Compounds

3) The Atomic Theory and Electronic Configurations (figure 3: periodic table, figure 4)

a) Electrons

b) Protons—positive charged

c) Neutrons—no charge

→ **atoms found in their normal state (ground state) are typically stable**

d) Ions (figure 5)---positive or negative charged atoms

i) Cations—positive charged due to removal of an electron

ii) Anions—negative charged due to addition of an electron

→ **ions are reactive since they either want to accept or donate their electrons**

→ **reactive vs stable**

→ **electronegativity and polarity**

e) Free radicals

→ **free radicals are very reactive**

g) Oxidation-Reduction Reactions (Redox reactions)

i) Oxidation—removal of electron(s) or addition of oxygen

ii) Reduction—addition of electron(s) or removal of oxygen

→ **redox reactions occur together; in biological systems oxygen is usually not directly involved**

4) Chemical Bonds

a) **Intra**molecular Bonds (figure 6)

i) **Covalent Bonds**—formed by the sharing of electron pairs between 2 atoms

→ **covalent bonds are the strongest types of bonds in biological systems**

ii) Special Bonds---**Peptide bond** (figure 6)

iii) Ionic bonds---formed when one atom donates its electrons to the other; not important in biological systems (figure 5)

b) **Inter**molecular (Weak) Bonds

---only significant when several are present within molecules

i) **Hydrogen bonds**—H covalently linked to O or N has a weak attraction to other O and N atoms due to the polarity established within an O—H or O—N covalent bond; these bonds are easily formed and broken (figure 7)

ii) **van der Waals forces** (figure 8)—produced by dispersion

---van der Waals radii = ½ the distance btwn 2 equivalent nonbonded atoms in their most stable arrangement

iii) Electrostatic attractions (figure 7)

5) Acids, Bases and pH

a) Acid is a proton donor (the Acid—H bond is weak)

b) Base is a proton acceptor (the base: ⁻ strongly attracts a proton, H+)

c) pH--the pH is a measure of the dissociation of H+ ions from a substance, hence it's a measure of acidity and basicity.
→ **Biological substances behave differently at different pHs.**

6) Chemical Reactions

Reactants → Products or **Reactants ← Products**

A + B → C or A + B → C + D forward spontaneous reaction

A + B ← C or A + B ← C+ D backward spontaneous reaction

A + B ↞ C or A + B ↞ C+ D equilibrium reaction

---the **speed** of the reaction is catalyzed by **enzymes**. While **enzymes Do Not determine whether a reaction will occur or in which direction**, sometimes they make a reaction so fast that without their presence it might take years to occur.

7) Thermodynamics

a) Enthalpy of Reaction (enthalpy = heat content)
---the change in enthalpy always = enthalpy of products - enthalpy of reactants……i.e energy in the form of heat is either produced or consumed in a chemical reaction (except for equilibrium conditions)
---endothermic vs exothermic reactions

b) Entropy of Reaction (entropy = disorder)

c) Bond Hydrolysis (breaking of bonds using water)

d) Free Energy of a Reaction

F. Basic Biology Concepts (definitions: solvent, solute)

1) Cells (see figure 9)---**all living things are made of cells** (est. by Robert Hooke).

2) Cell-to-Cell Signaling---cells secrete chemicals which influence their own behavior and that of other cells (fig 10)
a) Endocrine Signaling
b) Paracrine Signaling
c) Autocrine Signaling

3) Cell Signaling Pathway---once a cell is contacted by an external chemical (as discussed above) a specific sequence of events is initiated which results in activation of enzymes that alter activity of the cell's DNA. (fig 11)

4) Transport of Molecules into and out of Cells (figure 12)

a) Osmosis (figure 12)
The net movement of solvent molecules across a selectively permeable membrane from an area in which the solvent molecules are highly concentrated to an area of low concentration; serves to maintain equilibrium

b) Passive Transport

The net movement of solvent molecules from an area of high concentration to low concentration; serves to maintain equilibrium; requires no energy (occurs due to Brownian motion)

i) Simple Diffusion--occurs with **small uncharged** molecules (figure 12)

ii) Facilitated diffusion (figure 13)

--the **molecules are too large or have charges** which are not permissive for transport across biological membranes so they are transported by a carrier protein in p.memb

c) Active Transport (see figure 14)

The substance to be transported combines with a carrier protein in the plasma membrane AND is fueled by the energy of ATP. Energy is required because it is used to move the substance against the concentration gradient (i.e., from low concentration to high concentration).

One of the main uses for active transport is the establishment of **Chemical & Electric Gradients** within cell. Gradients are formed when there is a separation between an area of high concentration and low concentration. Gradients are a form of **potential energy** because there is a natural tendency for molecules to move from an area of high concentration to low concentration (potential energy is energy that is stored and ready to be used).

All living cells maintain chemical and/or electric gradients in order to store energy for export and import of substances within the cell.

d) Endocytosis---used to transport macromolecules inside the cell; requires energy (figure 15)

5) Biological Feedback—feedback inhibition A→ B → C → D Final Product

5) Hydrophobic Effect--"fair of water"

a) Any molecules that contain groups capable of forming hydrogen bonds will do so with water (H_2O); they are termed **"hydrophilic"** molecules ("water loving") and water will form caged lattice arrangements with these molecules.

b) Certain molecules do not form caged lattice bonding arrangements with water because they do not possess N-H or O-H groups; these are **hydrophobic** molecules and a disordered (increased entropy) arrangement is observed; eg. Oil in water

7) ATP is the Energy of Life

a) ATP is converted into energy by the hydrolysis of a diphosphate bond (remember, bonds store energy and energy is released when they are broken)

b) The energy of **ATP hydrolysis** is used to fuel a multitude of biological functions

8) Homeostasis----maintenance of the internal environment

G. Basic Concepts in Genetics

1) Mechanisms of Inheritance

a) Autosomal Dominant (figure 16)

---you need only one faulty gene to get the disease so **only one parent** needs to have a faulty gene and that parent will have the disease

---the disease **never skips** generations

b) Autosomal Recessive (figure 16)

---you need 2 faulty genes to get disease so **both parents are carriers** but neither has the disease
---the disease **skips** generation

c) X-linked (figure 17)

2) Mutations—change in genetic material

Mutations are inevitable. Most cells in our body are undergoing continuous rounds of mitosis; therefore DNA is constantly being replicated. In the process of DNA replication there are several possible spontaneous errors, which can and do occur. The key is our cells ability to catch those mistakes and correct them

→ Substances that induce mutations are called **mutagens**

H. Basic Concepts in Immunology

1) Physical Barriers to Entry---skin
2) Chemical Barriers to entry---mucus and cilia, tears (lysozyme), saliva
3) Normal Flora—microorganisms that establish permanent residence in our body but do not produce disease. Some organisms receive benefits from our bodies while we are unaffected by the relationship and vice versa. Other microbes are mutually beneficial. Some are actually necessary---E. coli in large intestine which synthesis vitamin K and B.

---our body contains an average of around 1×10^{13} cells yet there are about 1×10^{14} bacteria within our body!!!!

4) Reaction to Injury—the Inflammatory Response (figure 18)---our body's first reaction to injury---the **Lewis's triple response---redness, heat and pain**
5) Host Response to Tissue Injury (figure 19)

I. Basic Concepts in Pathology (figure 20)

1) Cell Degeneration & Injury ← Nonlethal Injury
   i) energy production and cell membrane is impaired and have metabolic abnormalities

2) Necrosis ←Lethal Injury (irreversible)
   i) energy production stops, cell membrane is lysed and get nuclear pyknosis

3) Apoptosis—programmed cell death (in contrast to necrosis)
   ---important for normal cell function

4) Hypoplasia, Hyperplasia, Dysplasia (figure 21)

5) Atrophy and Hypertrophy

SPECIAL TOPIC: Human Physiology: Organ Systems (as time permits)

2. Genetic Disease, Diagnosis and Gene Therapy

A. Gene Therapy

1) What is Gene Therapy?

a) **Targets of Direct Gene Therapy:** Inheritable Diseases and Vaccines

b) **Targets of Indirect Gene Therapy:** Drug Design to combat Cancer, Infections, AIDS, Cardiovascular Disease, Transplant Rejection, Vaccines

2) Mechanisms and Methods of Direct Gene Therapy

a) Gene Addition—direct delivery of DNA into a cell to compensate for a defective gene

b) Gene Replacement---replacement of the defective gene with a good copy

c) Gene Correction---use of DNA repair enzymes to correct base pairs in defective genes

3) Viral Vectors of DNA Delivery---use of viruses to deliver selected genes to person

Typical Viral Vectors: **Retrovirus, Adenovirus**, Adeno-associated virus, Herpesvirus

4) Genetic Testing for Disease

5) Gene Therapy Clinical Trials—an example of LDL receptor replacement therapy (figure 22)

a) Protocol for Clinical Trials

b) Diseases Currently in Gene Therapy Clinical Trials

X-linked Severe Combined Immunodeficiency
ADA deficiency
Mucopolysaccharidosis
Cystic Fibrosis
Hemophilia B (factor IX deficiency)
Chronic Granulomatous Disease

C. Genetic Disease

1) Relationship Between Chromosomes, Genes, and DNA-----the levels of chromatid structure (figures 23 & 24)

a) Chromosomes contain genes which are found in the cell nucleus and they are responsible for transmitting genetic information from one generation to the next.

b) Each cell contains 22 pairs of chromosomes (autosomes) and either a pair of sex chromosomes----two X chromosomes (for a female) or one X and one Y (for a male), i.e. 46 chromosomes. Thus **each parent contributes 23 chromosomes to each cell (figure 25)**

c) Depending upon the cell type and life cycle, a normal cell will either contain one pair of 23 chromosomes, called **haploid cell** and have **n** number of chromosomes; or two pairs of chromosomes, called **diploid cell**, denoted by **2n**.

i) **Gametes (sperm and eggs) are haploid**. When fertilization occurs, the male and female haploid cells contribute their chromosomes to produce a **diploid cell**.

ii) In general, **gametes (or germ cells) are haploid while somatic cells (cells of the body) are diploid.**

d) Diploid cells are either homozygous (identical DNA on each chromosome) or heterozygous (differences in DNA on each chromosome)

2) Mitosis vs Meiosis (figures 26-28)

a) **Mitosis occurs with almost all Somatic (body) cells** and involves replication of the daughter cells followed by cell division; there are 5 main stages of mitosis:

Interphase---DNA synthesis to form 2 copies of each chromosome, 4n.
Prophase---chromosome condensation and replication of chromosomes forming chromatids (2 copies), 4n
Metaphase---alignment along equatorial plane and **dissolution** of nuclear membrane
Anaphase---chromatids pulled apart **forming chromosomes, 2n**
Telophase---cytokinesis, formation of 2 identical cells each containing 2n chromosomes

b) **Meiosis occurs during gametogenesis and involves cell division without replication**
---the key difference in distinguishing the mechanism of meiosis from mitosis is in mitosis you get cell replication followed by cell division to form 2 exact copies of the same cell; in meiosis you have cell division without replication forming haploid gametes (sperm or eggs) which are then ready for fertilization to create a diploid individual.

3) Patterns of Inheritance—important only for inherited single gene disorders (figure 29)

a) Autosomal Dominant—neurofibromatosis, Huntington's, polyposis coli, congenital Spherocytosis
--**homozygosity is not compatible with life**
--**males & females affected equally**
--at least one parent has disease and disease **never skips** generations
--not associated with consanguineous matings
→ **usually structural proteins via single gene defects are AD**

b) Autosomal Recessive---cystic fibrosis, congenital deafness, mucopolysaccharidoses
--only homozygotes have disease
--**heterozygotes are symptomless carriers**
--males & females equally affected
--**skips generations**
--associated with **consanguineous matings**
→ **usually enzyme defects** are AR…i.e "Inborn Errors of Metabolism"

c) X-linked--- color blindness, hemophilia A, testicular feminization, Fabry's disease, agammaglobulinemia, Fragile X syndrome, muscular dystrophy, factor IX def

→ carried on the X chrom, only males are affected but females are the carriers
--**usually recessive**

4) Somatic Cell Gene Defects—the mutation occurs **after fertilization** of the ovum at any stage in the life of the person, therefore the effects are localized to particular cells and their progeny. (figure 30)

--Sex Cells (Autosomes) vs. Germ Cells (Sex Chromosomes)

i) Neoplasms (cancer)
ii) Harmatomas
iii) Multifactorial Diseases (see below)

5) Inherited Diseases (Germ Cell Defects) figure 31

a) Single Gene Defects
- i) Sickle Cell Anemia, Hemophilia A, Thalessemias, factor IV def, Von Willbrands,
- ii) Fragile X syndrome, Marfans synd, Wilms tumor, Retinoblastoma
- iii) Cystic Fibrosis, Muscular dystrophy, Neurofibromatosis (I), Adult Polycystic Kidney, Osteogenesis Imperfecta,
- iv) Glycogen Storage diseases, Lysosomal storage diseases, Fabrys, G6PD def, Wilson's disease, Galactosemia, Myeloperoxidase def, Familial Hematochromatosis
- v) Phenylketonuria, Albinism, Gaucher's disease, Hurler's disease, Alkaptonuria, Tay-Sachs disease, Familial Hypercholesterolemia

b) Multifactorial Disorders--genetic & environmental components cause disease (figure 30)
- i) Diabetes Mellitus
- ii) Hypertension
- iii) Rhematoid Arthritis
- iv) Cancer
- v) Infections
- vi) Allergies

c) Chromosomal Disorders
- i) Turner's Syndrome
- ii) Down's Syndrome (Autosomal Trisomy) 47XX or XY ←Aneuploidy (figures 32 & 33)
  - --**occurs after fertilization**, hence its autosomal
  - --has an **extra copy** of chromosome 21, hence **trisomy 21**
- iii) Klinefelter's Syndrome (figure 25)

6) Mutations-----What goes wrong? When? And How?

a) Mutations are either a chromosome aberration or a change in the nucleotide sequence (DNA) of a gene.

i) Mutations can have variable degrees of impact

ii) Some mutations are beneficial

iii) Mutations are either Somatic (body cells) or Germline (occurring within germ cells), an important distinction.

b) Types of Mutations (define nucleotide---the building block of DNA)
- i) Nucleotide Substitutions—change in DNA (figure 36)
  - a) Missense mutations—single base change
  - b) Nonsense mutations—single base change forms a stop signal
- ii) Insertions and Deletions---addition or loss of one or more nucleotides
  ---these mutations are also known as Frameshift mutations.

  Eg. Consider the following hypothetical DNA sequence:

a-b-c	d-e-f	g-h-I	j-k-l
codon 1	codon 2	codon 3	codon 4

During protein synthesis nucleotides are read 3 at a time and each group of 3 nucleotides specifies a specific amino acid; several amino acids come together to form a protein.

a) Insertion—addition of a nucleotide, **X**

a-**X**-b	c-d-e	f-g-h	I-j-k	l	
codon 1	codon 2	codon 3	codon 4	*codon 5*	*Thus the amino acids are different because the genetic code is different.*

b) Deletion---removal of a nucleotide, **b**

a-**b**-c	d-e-f	g-h-I	j-k-l	vs	a-c-d	e-f-g	h-I-j	k-l
codon 1	codon 2	codon 3	codon 4		codon 1	codon 2	codon 3	codon 4

iii) Trinucleotide Repeats

7) DNA Damage and Repair
- i) Loss of a base
- ii) DNA strand break
- iii) Guanylmethylation
- iv) Thimine dimers

→ Diseases Associated with Dysfunctional DNA Repair:
1) Bloom syndrome
2) Fanconi's anemia
3) Ataxia telangiectasia
4) Xeroderma pigmentosum

8) Mitochondrial Mutations and Disease

1) Mitochondrial Genome

a) Contains genes that encode for some rRNAs, tRNAs, and 13 polypeptides involved in oxidative phosporylation (which occurs in mitochondria)

b) 5-10 copies of mit DNA, No introns

c) Genetic Code is Broken in Mitochondria

d) Mutation Rates are about 10 times more than in Nuclear DNA

2) Mitochondrial Function—ENERGY

a) Site of the majority of all energy production---Krebs (TCA) cycle, OX. PHOS, and fatty acid oxidation.

b) ATP syn

3) Mitochondrial Genetic Disease—all result in some malfunctions of the respiration chain, therefore get energetic dysfunctions.

-> Maternal Inheritance—only females can pass the disease

-> Heteroplasmy---heterogeneity of mitochondrial populations within the same individual

---results in highly variable symptoms from family to family and within families.

-> Most sensitive tissues are: Nervous system, skeletal muscle, heart muscle, kidney and liver due to reliance on OX PHOS.

a) Leber's hereditary optic neuropathy (LHON)

i) Vison loss, mainly male, begins 20-24 yo

ii) Point Mutation

b) Myoclonic epilepsy and ragged red fiber disease (MERRF)-epilepsy, deafness, dementia, defective skeletal and cardiac muscle.

i) ragged red = appeareance of muscle fibers after tissue stain

ii) Point Mutation

c) Kearns-Sayre syndrome—eye muscle paralysis, dementia and seizures

i) Due to large deletions in mitochondrial DNA

ii) Heteroplasmy occurs (

iii) Thought to be less hereditary, rather random deletions occurring throughout development

C. Genetic Screen, Diagnosis, Counseling and Intervention

1) Prenatal Screening

a) Amniocentesis—removal of 30mL of amniotic fluid (weeks 16-20)

--assay fetal cells from fluid using DNA probes and/or cell multiplication (via culture)

--DISADV---fetal cell multiplication sufficient for chromosome analysis (karyotyping) takes 2-3 weeks, thus little time remaining to abort if needed.

b) Chorionic Villus Sampling (CVS)---taking pieces of chorionic villi tissue during 8-12 weeks of pregnancy.

--ADV---first trimester diagnosis and less need for cell culture since cells are in active growth phase

--DISADV—increased frequency of miscarriage (100% increase; 1% vs 0.5%)

c) AFP and hCG

--AFP screening during weeks 16-18 is routine and screens for neural tube defects

--hCG

2) Neonatal Screening—performed within one month after birth to detect specific genetic disease; done by measuring blood metabolite conc and DNA assays

a) Phenylketonuria (PKU)—A.R.disease missing phenylalanine hydroxylase (cant convert Phe to Tyr); can cause mental retardation if untreated

3) Adult Screening—done to identify 2 defective heterozygotes to prevent an A.R. disease.

a) Tay-Sachs disease

b) Cystic Fibrosis

4) Prenatal Diagnosis---used to detect fetuses which will be born with or later develop a genetic disease; done for abortion decisions; will soon be able to prevent disease by gene therapy.

--analysis of punit squares

--amniocentesis

--chorionic villous sampling

--ultasound

--RFLP

--karyotyping

5) Ethical and Political Considerations

--what to do if a diseased child is detected in-utero?

--what to do if you have a family history of Parkinson's disease?

--at what point are insurance companies and HMOs /PPOs allowed to access this info?

C. Diagnosis, Genetic Counselling and Intervention

1) Methods of Diagnosis

--analysis of punit squares
--amniocentesis
--chorionic villous sampling
--ultasound
--hCG

--RFLP
--karyotyping

2) Diseases Detected
--sickle cell anemia
--Down's syndrome

3) Ethical and Political Considerations
--what to do if a diseased child is detected in-utero?
--what to do if you have a family history of Parkinson's disease?
--at what point are insurance companies and HMOs /PPOs allowed to access this info?

SPECIAL TOPIC: METOBOLISM (as time permits)

3. The Human Genome, Cell Structure & Function, Protein Structure & Function

A. Human Genome

1) Definition of Genome

2) Relative Size of the Human Genome (figure 34)

3) How is Gene Sequencing Done? Five Basic Steps:

a) Subcloning step---breaking the chromosomes into smaller pieces
b) Template Preparation step---each piece is used to make several smaller pieces which differ in length by only one nucleotide
c) Sequencing Reaction step
d) Separation step---by gel electrophoresis (smaller fragments fall to bottom of gel)
e) Base-Calling step---identifying the last nucleotide of each fragment

6) Why Do the Human Genome Project?

All diseases and illnesses are linked to genetics and the way in which an individual responds to disease is influenced to a variable extent by their genetic makeup. Therefore, theoretically, every known disease, illness and condition can be linked to individuals' genes. Once a gene associated with disease is identified and studied, new ways to treat, prevent or correct the disease can be determined. In general this would be accomplished by determining the exact DNA sequence of such genes. Then we will know all of the proteins that are made from those genes and thus we can perhaps replace a bad protein by making a good copy using the DNA sequence.

5) Goal of the Human Genome Project

6) Results of the Human Genome Project

a) Less than 2% of the genome encodes for proteins
b) number of genes in humans
c) Chromosome 1 has the most genes (2968) and Y chromosome has fewest (231)

7) The Next Step—Functional Genomics

a) **Transcriptomics**—studying mRNA to learn more about gene expression
b) **Proteomics**---studying protein expression and function
c) **Structural Genomics**---generating 3-D structures of proteins for drug design
d) **Knockout Studies** (site-directed mutagenesis)---take out a gene and study the effect
e) **Comparative Genomics**---checking for genetic variability and identifying which genes are most/least altered---gives an idea on the significance of the gene

B. The Cell—**each cell is a complex structure composed of a multitude of compounds, each interacting with all the other compounds for the purpose of carrying out specific functions.** (figure 35)

--typical cell size
--number of cells in body

1) Structure (figure 36)

a) Cell Membrane--**Lipid Bilayer (fluid mosaic) Model**

--lipids are made of **amphipathic groups** (positive and negatively charged); the **polar head group** is that portion that contacts the water fluid of the body tissue and it is **hydrophilic**; the **inner portion** of the lipid monolayer is **in contact** with the inner portion of the **other monolayer** and is **hydrophobic**. In general the hydrophilic portion is an alcohol group while the hydrophobic portion is a fatty acid.

--this phospholipid bilayer has **fluid-like consistency** due to the composition of different fatty acids and **each monolayer rotates** in many different directions

--**Membrane Proteins** (see figure)—many different proteins are associated with the phospholipid bilayer of the cell membrane depending on the function of that protein

i) **Integral membrane proteins**—helix spans the entire length of the phospolipid bilayer

Examples: Transport proteins—carrier molecules
Ion channels---Na/K pump

ii) **Peripheral proteins**—don't span entire membrane; van der Waals forces are important

b) Nuclear Membrane—a double membrane
c) Endoplasmic Reticular membrane
d) Lysosomal Membrane
e) Mitochondrial Membrane---a double membrane

2) Function--cell specific for the organs and tissues they serve (figure 35)

Organelles---cells contain several microenvironments separated by membranes, each of these microenvironments carry out different functions and have unique chemical compositions such as different pHs and different ion concentrations.

cytoplasm---
Golgi apparatus---glycoprotein processing
mitochondria—energy generation
endoplasmic reticulum (ER) ---lipid synthesis
rough endoplasmic reticulum (RER)---protein synthesis
nucleus---DNA replication, transfer RNA (tRNA), messenger RNA (rRNA) and nuclear proteins; also contains a "mini nucleus" the nucleolus which is the site of ribosomal RNA (rRNA) synthesis
lysosomes—apoptosis and removal of waste via hydrolytic enzymes
microtubular arrays---necessary for mitosis, meiosis and cell movement

3) Cell Signaling Pathway--intracellular traffic (figure 11)

C. Cell Biology

1) Nucleus--the "brains"--all genetic material is housed in the nucleus (except mitochondria, which has its own DNA)

2) Chromosomes—"warehouses"—the storage units of genes, like books in a box; (figure 23)

DNA within chromosomes is specifically associated with many DNA-binding proteins called **histones** which serve to pack the tremendous length of the chromosomes into tightly packed structures.

3) Genes--**units of DNA storage**—the basic physical and functional units of heredity; they are specific sequences of DNA bases that encode instructions on how to make different proteins

4) DNA--the code—DNA replication → transcription-→>synthesis of RNA (tRNA, rRNA, mRNA, snRNA) → translation → proteins (figure 37)

a) DNA Replication---**Semiconservative** (figures 37-39)

***the 2 strands of a DNA helix are complementary (i.e. base pairing with G-C and A-T) so if we know the base sequence of one strand we can deduce the base sequence of the other.**

→ DNA synthesis must begin with an RNA primer. An RNA primer is formed by RNA polymerase. Then DNA polymerase joins nucleotides to this primer according to the complimentary DNA sequence. As DNA synthesis occurs, Helicase unwinds the double strand.

b) Transcription (RNA Synthesis)---RNA is made from a copy of DNA by the addition of bases via RNA Polymerase; note that instead of T, RNA uses U and no primer is needed (figure 40).

Post-Transcriptional Processing

--RNA processing and ribozymes = **Splicing**

--DNA is composed of groupings of nucleotides called **Exons** and **Introns**

--RNA (once is completely processed) is made only of groupings of nucleosides called Exons (due to RNA splicing)

→ **Exons** determine the type of **proteins** made during translation

→ **Introns** are involved in **gene regulation** and are spiced out of RNA prior to translation

5) Proteins--the product—translation → occurs outside of the nucleus

Proteins are large complex molecules made of smaller subunits called amino acids, linked by peptide bonds. There are 20 different amino acids in humans.

→ **mRNA contains the code for amino acid sequence; every 3 bases on mRNA are called a codon, because they encode for a specific amino acid**

→ **tRNA** contains a region that binds to a specific amino acid. It also contains a different region, which corresponds to the amino acid it binds to called an **anticodon**. The anticodon binds to the complementary **codon** on the mRNA. That is, the complementary base pairing is observed. (figure 41)

→ **Ribosomes are the actual protein synthesis machinery**; they are made of 2 rRNA subunits. The ribosome scans the mRNA reading 3 bases at a time to determine which amino acid tRNA will bind the growing peptide chain; rRNA unit can only hold 2 amino acids at a time so during translation, once the 2 amino acids contained in its unit form a peptide bond, one amino acid is ejected out of the rRNA so that a new space is available for the next approaching amino acid.

--recent studies have shown that translation may also occur within the nucleus

a) Translation (3 Steps):

1) **Initiation**— **rRNA subunits** along with **initiation factors** assemble on mRNA and a specific tRNA (Met—tRNA) binds to the beginning site on the mRNA, which is always the **AUG codon**. (figure 41)

2) **Elongation**---the next **amino acyl tRNA** enters the ribosomal complex and forms a **peptide bond** with methionine residue of the met-tRNA then the met –tRNA is ejected from the rRNA unit making room for the next approaching amino acid tRNA; this process continues until a **termination codon** is reached on the mRNA. (figure 42)

3) **Termination**--- once the ribosome comes across a termination codon (UAG) **translation stops and the ribosome disassembles** into its 2 rRNA subunits. (figure 43)

→ Each step uses energy in the form of GTP. The initiation step also uses ATP.

4) Signal Recognition Particle (SRP) and Docking Protein (figure 44)

b) Protein Processing, Packaging and Targeting to Specific Locations (figure 45 & 46)

6) **Amino Acids (figure 47) all have one thing in common--the same H_3N—CH_2---COO unit. The distinct molecular bonding is determined by the unique side chain group**

7) PCR—DNA Amplification

→ **Taq polymerase** is the key component of this procedure. It is unique in that is resistant to heat, therefore it can withstand denaturing by heat.

Steps in PCR

1) Denature DNA
2) Add Primers (primers are short nucleotide sequences complementary to the DNA of study; they are needed to initiate DNA replication)
3) Add Taq polymerase and nucleotides then incubate (let it set)---DNA replication occurs
4) Heat to 95 (to denature the newly formed DNA into single strands)
5) Recool to 60 and repeat steps 2-5.

D. Molecular Biology

1) Cell Cycle----mitosis vs. meiosis

--DNA synthesis occurs during Interphase
--Cell division occurs during Cytokinesis

a) **Mitosis = Somatic cell replication; get 2 diploid cells from one diploid cell**

---have one pass thru cell cycle: G2 Interphase→Mitosis and cytokinesis → G1Interphase → S Interphase (figure 48)

---Mitosis sequence: Prophase→Metaphase→Anaphase→Telophase & Cytokinesis

DNA replication occurs in S phase
RNA synthesis occurs in G2

b) **Meiosis = Germ-line cell formation; get 4 haploid cells each with a haploid genome from 2 diploid cells (figure 49)**

---2 meiotic divisions but no DNA replication

---get **Recombination during Prophase** 1 b/c homologous chromatids attach to each other forming a bivalent; **accounts for a large degree of genetic divergence**

2) Gene Regulation---turning them on and turning them off

→ Most of the control in gene regulation exists at the level of **transcription**.

a) These control regions are located at certain regions of the DNA chain.

→ Sites on DNA which bind regulatory proteins:

--Promoter
--Operon

→ Proteins which bind to DNA and regulate transcription:
--**Activators**—binds promoter and allows transcription
--**Effectors** ---proteins which bind to activators and repressors and alter their binding affinity to DNA
--**Inducers**---binds repressors and causes them to decrease their binding affinity to operons
--**Corepressors**—binds to repressors and causes them to increase their binding affinity to DNA
--**Enhancers**
--**Repressors**
--**Silencers**

b) Positive Control of Transcription by Activators (figure 50)

c) Negative Control of Transcription by Repressors (figure 51)

d) Methylation and Demethylation
--methylation---usually turns genes on
--demethylation or loss of methylation---usually turns genes off

D. What to Do With The Map---ITS ALL ABOUT THE PROTEINS!!!

1) **The Genetic Code**—each of the 20 amino acids is coded for by a specific sequence of 3 DNA molecules (trinucleotide) figure 52

→ 3 DNA bases => 3 RNA bases=>one amino acid
→ the genetic code is **Degenerate**

a) DNA = repeating units of **sugar + phosphate + base** (figure 53)
--Bases = Adenine, Guanine, Cytosine, or Thymine

b) **DNA double helix** = 2 repeating DNA chains held together by bonds (figures 53 & 54)
--Hydrogen bonds (figure 54)
--N-H bonds (figure 54)
--Base Pair Stacking---van der Waals forces (figure 55)

→ each DNA single chain is linked by **covalent** diphosphate bonds while the joining of the two chains to form a DNA helix is linked by **noncovalent** bonds (H bonds and van der Waals); sugar-phosphate backbone (hydrophilic) located externally while nucleoside bases are internal (hydrophobic). Figure 56

c) **Chargaff's Rules:** A bonds with T (2 bonds), G bonds w/ C (3 bonds)

d) DNA Denaturation (figures 57 & 57A)

e) **Restriction Endonucleases**

f) DNA vs. RNA Structure (cloverleaf, L-shaped; regions of double helix) (figures 53 & 58)

2) Protein Structure and Function

a) What's a protein?

b) Levels of Protein Structure (figure 59)

1) **Primary Structure**—Amino acid sequence

2) **Secondary Structure**

i) **Alpha helix** (figure 60)

--Fibrous proteins

Eg. Collagen----the essential protein of life---makes up skin, tendons, cartilage, bones, blood vessels and important in wound healing and scarring. (figures 61-63)

→ the most abundant protein in humans
→ **left-handed triple helix** (tropocollagen) held together by H-bonding
→ every third amino acid residue is **glycine**
→ **hydroxyproline** is also very common in collagen

Eg. alpha-Keratin (figure 64)
--makes up hair and wool
--3 alpha helices wound together = Protofibril
--9 +2 arrangement of Protofibrils = Microfibril
--several Microfibrils = Macrofibril

ii) **Beta sheet** (figure 65)
--beta-keratins—found in the skin, claws and beaks of birds and reptiles

3) **Tertiary Structure** (figure 66)←the amino acid sequence determines the secondary and tertiary protein structure (shown by denaturation<-> renaturation studies)

**Note: disulfide bonds are not needed for the protein to maintain its globular shape, but its helps stabilize it.

4) **Quaternary Structure**---describes the way that polypeptides associate with each other within the protein

5) **Examples of Globular proteins (most proteins are Globular)**

a) Antibodies
b) Structural proteins in cell walls (membrane proteins)
c) Carrier proteins for selective transport into & out of cells (Na^+/K^+ pump)
d) DNA-binding proteins—for gene regulation
e) Enzymes--basic enzymology

→ **Enzymes only speed up the rate of reaction, they do not determine the direction of a reaction.**

→ enzymes speed up reactions by **lowering the activation energy**, or the energy required to form products from reactants (figure 67)

→ **enzymes are not consumed** in the process

→ reactants (substrate) binds an enzyme at a specific region called the **Active site** (figure 68)

→ the Michalis-Menton model describes enzyme kinetics:

$$E + S \underset{\text{<----------}}{\overset{\text{binding}}{\text{---------->}}} \text{E-S complex} \xrightarrow{\text{catalysis}} \text{E-P complex} \xrightarrow{\text{release}} P + E$$

the release of the product is very rapid so that we can write:

$$E + S \underset{k2}{\overset{k1}{\rightleftharpoons}} E\text{-}S \text{ complex} \xrightarrow{kcat} P + E$$

→ Cooperative Binding—a special case of enzyme binding Allosteric binding sites (cooperative binding in hemoglobin) figure 69

i) Enzyme Inhibitors---enzyme effectiveness can be modified by the type and amount of inhibitors (figure 70).

---Competitive Inhibition—both the substrate and inhibitor fit in the enzyme's active site
→ **reversible** by increasing the concentration of substrate

---Noncompetitive---inhibitor binds at a different site on the enzyme;
→ **irreversible**

---Uncompetitive

ii) **Enzyme Regulation** (figure 71)
1) **Covalent modification** (usually through phosporylation & dephosphorylation)

2) **Synthesis and degradation** (clotting cascade) (figure 72)

3) **Induction and repression of enzyme synthesis** (lac operon)

iii) Enzymes used in clinical diagnosis
--alanine aminotransferace (ALA) / liver
--creatine kinase (CK) / heart
--lactate dehydrogenase (LDH) / heart
--oxaloacetate transaminase (GOT) / heart

6) Proteins in Solution---Protein Folding and Stability
a) Protein Folding (figure 73)
→ **Proteins are in continuous motion in solution**

Globular Protein -----> brief loss of tertiary and secondary structure---> Reassociation into a different secondary and tertiary structure---> Globular Protein

b) Thermodynamics of Protein Folding (figure 74)
i) Forces that favor the folding of proteins in solution:
--Electrostatic bonding with water molecules
--Hydrophobic effect

ii) Forces that disfavor folding of proteins in solution:
--Entropy associated with the protein becoming more ordered

iii) The Hydrophobic Effect and Electrostatic bonding produce enough enthalpy to overcome the negative entropy effect of folding so that the Free Energy of folding is favored.

REMEMBER THE STRUCTURAL HIERARCHY:

CHROMOSOMES → GENES → EXONS & INTRONS → DNA → NUCLEOTIDES → SUGAR-P + BASE

**each cell contains 46 chromosomes (in normal individuals) and within those chromosomes are thousands of genes, each gene being made of exons and introns all linked in a continuous chain of DNA. DNA is a double stranded helix of nucleotides, each nucleotide being composed of a deoxyribose sugar unit attached to one of 4 bases (Adenine, Guanine, Cytosine or Thymidine).

REMEMBER THE SEQUENCE:

←----------Nucleus----------→←-------Cytoplasm------→

DNA --Transcription--> RNA --Splicing--> mRNA, tRNA, rRNA --Translation--> Protein

Biotech Applications: Drug Design

SPECIAL TOPIC: PROTEONOMICS (as time permits)

4. Techniques for Biomedical Research and Diagnosis

A. **Light Microscopy vs. Electron Microscopy (figure 75)**

B. **Centrifugation**
1) Velocity Centrifugation Separates on the Basis of Size and Density (see figure 76)
2) Equilibrium Density-gradient Centrifugation Separates Materials by Density Alone

C. **Electrophoresis**---separation based on charge
1) Gel Electrophoresis (figure 77)
2) Paper Electrophoresis

D. **Gel Filtration (figure 78)—**separation based on size----large molecules pass through, small are retained

E. **Affinity Chromatography**---separation based on binding specificity (figure 78)

F. **Restriction End nucleases** (microscopic scalpels) --enzymes which recognize specific sites on DNA (called restriction sites) and break the phosphodiester bond between certain nucleotides; serves to cut DNA into fragments (figure 79)

G. Genetic Engineering and Recombinant DNA Technology Defined

H. **PCR--DNA amplification (figure 80)**

I. **Northern, Southern, and Western Blotting**------named for the target molecule:

Complementarity and Hybridization
1) DNA-DNA---two single stranded DNA sequences will bind together if they complimentary sequences (G binds C and T binds A, so the sequence ATGTCAG will bind TACAGTC)
2) DNA-RNA---a single stranded DNA sequence will also bind to a complimentary RNA sequence (note that in RNA T is replaced with U, so you get U binding to A)
3) Protein-Protein---an antibody (which is a protein itself) will bind to a specific protein if a specific site on the antibody recognizes contacts a specific 3-D arrangement on the protein.

→ **Preparation of DNA probes----label each nucleotide with H^3-thyminidine**

→ **Preparation of protein probes---inject animal with protein of interest, collect antibodies, label the tyrosine residues with I^{-125}.**

1) Draw blood
2) Isolate cells of interest via centrifuge followed by cell sorter
3) DNA extraction
4) DNA denaturation
5) Blotting-----> DNA of interest has been identified

→ **General Steps in Blotting:**
1) Gel Electrophoresis
2) Transfer to Solid Support
3) Blocking
4) Preparing the Probe
5) Hybridization
6) Washing
7) Detection of Probe-Target Hybrids

→ Types of Blotting (figure 81)

1) **Northern** Blotting—detection of **RNA** using radioactively labeled DNA probe
2) **Southern** Blotting---detection of **DNA** using radioactively labeled DNA probe
3) **Western** Blotting---detection of **proteins** using radioactively labeled antibody probe

J. **DNA Sequencing**

K. **Cloning Overview**

L. **Cloning Vectors**

Cloning vectors are DNA molecules that carry foreign DNA into host cell where it replicates and produces many copies of itself and the foreign DNA.

M. **Monoclonal Antibodies (figure 83)—**an antibody that recognizes only one antigen

N. **ELISA**

O. **Stem Cells, Bone marrow transplant and GVH disease**

P. **Autoradiography**---detection of radioisotopes (figure 84)

Used to locate the synthesis of macromolecules within cells and to map out their subsequent movements within cells; eg incorporation of H^3-thymidine and detection by autoradiography reveals that the site of DNA synthesis is in the cell nucleus.

Q. **Transgenic Animals**---the genetic engineering of an animal's germ cells to produce an animal with a gene defect. You can add or take away a gene in an animal's germ cell then let it develop into an adult.

R. **DNA Fingerprinting**---takes advantage of the fact that the DNA differences in people are concentrated in certain regions called **Minisatellites**, short highly repeated 15-nucleotide sequences

1) Cleave DNA into fragments with restriction endonucleases
2) Separate by size via Gel Electrophoresis
3) Incubate the fragments with radioactive DNA probes specific for minisatellites; if the probe binds the DNA then it is detectable by autoradiography; when two DNA samples are compared (eg. Crime scene evidence and the suspect) and you have two identical sized fragments bound to the probe then you have a match. Accuracy is several billion to1.

S. **Using DNA Probes to Locate a Gene**

DNA probes are short single stranded DNA molecules which are complementary to the sequence on the gene of interest. They are used for disease prediction and diagnosis and for DNA fingerprinting. They are labeled with radioactive isotopes for identification by autoradiography. The probes bind the complementary single stranded DNA and Southern Blotting is done to separate the double stranded DNA into single strands.

Construction of DNA Probes

How do you know what probe to use if you are not sure what gene you are looking for?

Isolating a Gene---what to do if we don't know what gene we are looking for.

T. **Vectors: Delivering Genes to Target Cells**

1) Vectors---an agent for delivering a therapeutic gene to a desired target cell so it can be expressed, and the desired function can be realized.

a) Viral Vectors
---Viruses----what's a virus? (figure 85)

i) RNA Viruses--Retroviruses
---Life Cycle of Retrovirus (figure 85)
ii) DNA Viruses
iii) Adenoviruses
iv) Herpes Simplex Viruses
v) Paroviruses

b) Bacterial Vectors---Plasmids
---Bacteria—What are bacteria? (figure 86)
Plasmids are mobile extrachromosomal circular DNA segments in bacteria that replicate autonomously. They are able to diffuse in and out of bacterial cells (figure 87).

c) Phage Vectors---linear DNA molecules derived from bacteriophage lambda
d) Cosmids---extrachromosomal circular DNA molecule that combines features of phage and plasmids
e) Bacterial Artificial Chromosomes (BAC)
f) Yeast Artificial Chromosomes (YAC)
g) Non-Viral Vectors
i) Naked DNA
ii) Liposomes
iii) Electroporation

2) Use of Vectors

U. Characteristics of Organisms Used for Genetic Studies:

1) Good genetic background (we understand its genetics)
2) Easy to grow
3) Controlled matings possible
4) Can be genetically engineered
5) Funding Available

→ <u>Organisms Used for Genetic Studies</u>:

1) Viruses
2) E.coli
3) Humans---cannot genetically engineered
4) Drosophilia
5) Maize
6) Arabidopsis thaliana

V. **Mass Spectroscopy**

W. **X-Ray Crystallography & Protein Databases**

X. **3D-NMR**

Y. **Enantiomers & Drug Specificity**

SPECIAL TOPIC: BIOCHIPS (as time permits)

5. Immunology, Disease & Aging (Part 1)

A. Basic Immunology

--Definitions: antigens, cytokines, phagocytosis, exocytosis, endocytosis, lymphoid tissue

1) Anatomy & Physiology of the Lymphatic System (figures 88 & 88A)

a) **Only Certain Organs Are Able to Make White Blood Cells**

--Bone Marrow (also makes red blood cells), thymus, spleen

b) **Only Certain Organs House White Blood Cells**

--Lymph Nodes

--GI epithelium

c) **B Cells Mature in the Bone Marrow**

d) **T Cells Mature in the Thymus**

e) Hematopoietic Cell Differentiation (figure 89)

f) Composition of Blood:

i) Blood Plasma vs. Blood Serum

ii) Clotting Cascade and Disease

g) **Immunosurveillance**

2) The Immune Response---What Happens When We Encounter an Infectious Agent? (Figure 90)

a) **Innate (Natural) Immune Response** = the **first line of defense→** compliment, inflammatory response, lysozyme, sebaceous, lacrimal and salivary glands, IgA, mucosal secretions, macrophages, neutrophils, eosinophils, natural killer cells. It is present at birth. (Figures 91 & 92)

b) **Acquired (Specific) Immune Response (figure 93)**

This immune response is **acquired through previous contact** with the antigen and **acts specifically** upon it through binding certain parts of it via receptors and causing a series of killing mechanisms, much of which involves cytokine release for lymphocyte killing stimulation. It is comprised of antibodies and sensitized **B and T cells**.

i) **Humoral Immunity---antibody-mediated immune response**

→ <u>How the Antibody-Mediated Immune Response Works:</u>

--antigen is typically particulate (bacteria)

--bacteria or a portion is phagocytosed by a macrophage and the antigen processed and internally then presented on the macrophage surface for interaction with B cells. The macrophage has receptors that specifically recognize antibodies bound to antigen (the pathogen) unlike in phagocytosis during the innate immune response.

--B cells are activated to respond specifically to the bacterial antigen (i.e. intracellular events take place)

--B cells then produce and secrete antibodies, which recognize the bacterial antigen

--B cells which are fully differentiated and activated to respond to specific antigen are either plasma cells or memory cells; plasma cells are antibody producing variants of mature B cells; memory B cells are not actively secreting antibody but may be activated at some later date to quickly secrete antibody if the same antigen which activated it before appears in the body.

--antibodies bind the antigen cell surface

--complement cascade causes cellular lesions and/ or macrophages specifically recognize antibodies bound to antigen then they bind and phagocytose it; activated by antibodies **(figure 92)**

Components of the Humoral Immunity:

-B cells
-Antibodies
-Compliment
-Macrophages

ii) **Cellular Immunity --uses helper T cells, cytotoxic T cells, natural killer cells and activated macrophages ➔ destruction by cytokine-mediated phagocytosis and cell lysis**

--antigen is typically soluble

--macrophage phagocytoses antigen, selects certain parts of it then and presents those parts on the surface for T cell to bind.

--Th cell binds the antigen piece from the macrophage and this activates the Th cell, which the activates a Tc cell; the Tc cell specifically binds foreign cell which causes secretion of cytokines; these cytokines directly kill the antigen and/or signal other cells to act upon the antigen. (figures 94 & 94A)

Components of the Cellular Immunity:

-B cells (an indirect mediator)
-T cells (3 types):
Th cells (helper T cells)—involved inactivation of B cells for Ab production
Tc cells (cytotoxic T cells)—cause antigen-specific lysis by direct cell to cell contact
Ts cells (suppressor T cells)—inhibition
-Natural Killer cells—non-specific lysis of antigen by direct cell to cell contact
-Activated Macrophages---antigen-specific phagocytosis, B and T cell activation

c) **<u>B Cell Development and Maturation</u>** (figures 95 & 96):
--B cells develop in 2 main stages---Antigen-Independent Differentiation and Antigen-Dependent Differentiation

i) Antigen-Independent Differentiation (figure 97)
---stem cells in the bone marrow differentiate into pre-B/ T cells; pre-T cells enter thymus for further maturation;
---immature B/T cells fully mature in the BM/thymus as they express specific surface receptors

ii) Antigen-Dependent Differentiation (figures 97 & 97A)
---mature B/T cells contact foreign antigen and become activated;
---**Clonal expansion** occurs, lymphocytes recognize the same antigen
--B cells mature into plasma cells characterized by a fully active and developed RER needed as they make antibodies specific for the antigen which activated the B cell

--T cells differentiate into T_h, T_c, or T_s cells and are specific for the antigen which activated the parent T cell

d) Mature B Cells Produce Antibodies

i) Antibody Structure (figure 98)

5 main types of antibodies are specified by the type of heavy chains they contain:

IgG—contains gamma heavy chains
IgA—alpha heavy chains
IgM--mu
IgE--epsilon
IgD—delta

ii) Antibody Function

IgG—compliment activation, opsonization, placental passage
IgA—mucosal (secretory)
IgM—compliment activation
IgE—basophil and mast cell sensitization
IgD—B cell surface receptor involved in binding antigen and activating B cells

3) How Our Body Fights Our Environment Daily

i) Innate Immune Response--protective barriers and normal secretions
ii) Acquired Immune Response--Immune Surveillance→ Lymphatic tissues

4) **Primary vs. Secondary Immune Response** = Naïve vs. Memory cells (figures 99 & 100)

i) **Primary Immune Response**---follows first exposure to antigen and serves to activate B and T cells to specifically recognize and react to the antigen. The antibody response to the antigen takes several days to materialize. The primary antibody secreted is IgM

ii) **Secondary Immune Response**—follows repeat exposure to an antigen and because the B and T cells have been previously activated by the antigen, a faster more intense antibody response occurs. The primary antibody secreted is IgG

5) **Immune Tolerance vs Hypersensitivity**

Immune Tolerance--the ability of our immune system to distinguish between our own cells and tissues and foreign invaders; required to prevent autoimmune diseases (figure 101).
Hypersensitivity—immune cells recognize and destroy our normal cells.

→ **Clonal Deletion / Selection**—all lymphocytes that respond to self tissue are deleted while those that don't are allowed to survive. (figure 102)

6) **Suppressor T Cells**—serve to inhibit immune reactions

7) Genetics of the Immune System (figures 103 & 104)

B. Normal Structure and Function

1) Skin, Vessels, Organs-----disease and infection

C. Wound Healing

1) **Healing by First Intention**---the surgical incision model (figure 105)

2) **Healing by Second Intention** (figures 106 & 107)

a) Inflammatory response (natural immunity→non-specific response)

i) **Acute Inflammation**-generic response occurring w/ virtually any injury/ infection.
--characterized by **pain, redness, heat and swelling** which is caused by dramatic molecular and cellular events resulting in alterations in vascular physiology (figure 108).

--What's the purpose of all this discomfort?

--<u>Sequels of Acute Inflammation (figure 109):</u>

a) **Resolution—minimal cell death and tissue damage**, rapid elimination of the causative agent, removal of fluid and debris from inflammation (figure 109)

b) **Suppuration** (pus formation)--- PMNs and fat globules (figure 110)

c) **Repair and Organization**---due to excessive exudation or necrosis or when removal of exudates and debris is incomplete and have growth of new capillaries into the exudates with migration of macrophages and fibrosis (proliferation of fibroblasts) (figure 111)

d) **Fibrosis—repair of parenchymal cell by connective tissue and results in scarring** (see below—"tissue repair")

e) **Chronic Inflammation**---follows if the causative agent is not removed however usually occurs without a proceeding acute inflammatory reaction; proliferation of fibroblasts (which make collagen) resulting in fibrosis (figure 112)

→ Diseases associated with chronic inflammation:

2) Tissue Repair---regeneration of damaged tissue depends the local physiology, nutrition and the types of cells damaged (figures 113-115)

Labile cells

Stable cells

Permanent cells

4 Main Steps in Tissue Repair:

1.**Angiogenesis**—new blood vessel formation (also important in cancer)

2.**Granulation Tissue formation** (the hallmark of healing) via migration and proliferation or fibroblasts and endothelial cells

3.**Deposition of extracellular matrix**

4.**Tissue remodeling**---maturation and organization of the fibrous tissue

--Tissue injury results in cell death and blood vessel disruption→ blood extravasation into skin→platelet activation and blood coagulation.
--Platelets facilitate promoting hemostasis through self-aggregation and release of extracellular matrix molecules, fibronectin, fibrinogen thrombospondin and von Willebrand factor.
--Blood coagulation→ kallikrein, thrombin, plasmin, fibrinopeptides, fibrin-split products, bradykinin, C3a and C5a (anaphylatoxins) from the complement cascade.
--Within hrs of injury re-epithelialization begins as epithelial cells migrate over wound surface.
--By 1-2 days re-epithelialization has progressed as epidermal cells at the margins of the wound proliferate due to the actions of EGF and TGF-alpha and the ECM is remodeled.
--Angiogenesis occurs as granulation tissue is formed.
--Tissue remodeling is the final stage of wound repair. Type I collagen fibers are remodeled to contain less type III collagen, so the scar tissue has more tensile strength (due to the crosslinking in type I)

* Cells important in wound repair: monocytes, neutrophils, fibroblasts , epidermal cells, macrophages, endothelial cells (figure 116).

* Substances important in wound repair: platelets, kinins, fibronectin, fibrin, thrombin

* Growth factors important in wound repair: TGF-alpha, TGF-beta, PDGF, EGF,

3) Scarring--Elimination of the Scarring Process---the internal fountain of youth?

→ **A scar is a mass of collagen that is the end result of repair by organization and fibrosis (figure 117).**

→ **Complications of wound healing (figure 118):**
Contractures
Excess Granulations
Keloids

D. Infection

1) Susceptibility to Infection (figure 119)
2) Portals of Entry (figure 120)
3) Reservoirs and Vectors
4) Infectious Agents of Humans Classified According to Structure (figure 121)
5) Mechanisms of Cell Damage and Disease Causation in Infectious Diseases (figure 122)
6) Tissue Changes Caused by Host Responses to Infection (figure 123)

7) Mechanisms of Microbial Killing (figure 124)

8) Infectious Organisms
i) Bacterial← current drugs
a) Pathophysiology of Bacterial Infections----secrete toxins or cause direct damage

b) Rx .
--Antibiotics
--Antibiotic Resistance
--Vaccines

ii) Viral← target for biotechnology
a) Uptake of Viruses by Endocytosis and Fusion (figure 16)

b) Classification of Viruses by Replication Mechanism and Relationship of the Genome to mRNA

E. Vaccines
--The smallpox story----Edward Jenner and cowpox--the only complete eradicated virus

1) What Do They do? **Vaccines protect against infection and disease by inducing passive immunity (i.e. an antibody response) and active immunity (i.e. cell mediated immune response) against an organism**

2) How Do They Do it? In general**, an organism or its pathogen** (the disease causing agent eg. A bacterial toxin**) is modified** in one of several ways so that the pathogenic effect of the organism or toxin has been eliminated or diminished. **Cellular immune response and memory are made to the pathogen, yet it causes no illness.**

3) Current Approaches (figure 125)
a) Live Attenuated--measles, mumps, rubella, Sabin, smallpox, Yellow fever
b) Inactivated (killed)—Salk, Rabies, Cholera, (injected) Typhoid
c) Acellular (a portion of the causative agent is used)--HIB
d) Toxoid (inactivated toxin from the organism)--DPT
e) Similar organism--BCG for TB
f) Edible vaccines
g) Recombinant DNA—Hep B
4) Biotechnology and Vaccines
a) Peptides
b) DNA fragments
5) Novel Uses for Vaccines
a) Pregnancy (see figure 126)
b) Cocaine (see figure 127)

Biotech Applications: Gene Therapy

SPECIAL TOPIC: NANOMEDICINE (as time permits)

6. Immunology, Disease, Aging (Part 2)

A. Cancer = **Loss of cell cycle regulation**

1) Cancer Survival Rates (figure 128)

2) Cancer Terminology----classification of tumors (figure 129)

Tumors are Classified by:

a) Tissue Type

i) **Carcinoma**—a neoplasm (new growth) of epithelial cells (skin and tissue that lines organs)—90% of all cancers

ii) **Sarcoma**—solid tumors in connective tissue, muscles and bone—2%

iii) **Leukemias and Lymphomas**---neoplasms is circulatory or lymphatic systems---8%

b) Cell Type

i) Adenomatous cells—ductal or glandular cells
ii) Squamous Cells—flat cells
iii) Myeloid---blood cells
iv) Lymphoid---lymphocytes or macrophages

c) Site of origin

d) Benign or Malignant

i) **Benign Tumors are Slow growing, encapsulated / no filtration, no metastases, non-cancerous**

ii) **Malignant Tumors are rapid growing, infiltrative, metastasize and cause cancer**

e) Clinical Staging

3) **Life Cycle of a Somatic Cell---Cell Cycle Revisited (figure 48)**

Cancer is a cell cycle problem in which the normal controls for the regulation of cell division have been lost and the cancerous tissue develops due to uncontrolled cell growth.

Kinetics of Tumor Cell Growth: a single transformed cell must undergo approx 30 doublings to produce 10^9 cells (weighs 1 gram—smallest size detectable), yet only 10 more doublings are needed to produce a tumor containing 10^{12} cells (1 kg), the maximal size compatible with life.

4) **Protooncogenes and Oncogenes** (figure 130)

Protooncogenes promote normal growth and division of cells by encoding for cellular proteins that relay signals to the cell nucleus, causing genetic changes (DNA replication, transcription factors and protein synthesis) which result in growth stimulation.

a) **Activation of Protooncogenes into Oncogenes**

1) Point Mutation
2) Chromosome Rearrangement
3) Gene Amplification—increase in the number of protooncogenes
4) Viral Insertion

b) **Oncogenes Cause Several Effects on the Cell (figure 131)**

i) Overproduction of Growth Factors
ii) Flooding of the cell with Replication Signals
iii) Uncontrolled Stimulation of Components within the intracellular cascade
iv) Unrestrained cell Growth due to increased number of transcription factors

5) **Tumor Suppressor Genes**

They inhibit cell growth so mutations in tumor suppressor genes cause the cell to ignore the stop signals for cell growth and results in uncontrolled cell growth, i.e. cancer.

6) Cofactors in Carcinogenesis
- a) Heredity
- b) Race
- c) Geography
- d) Age
- e) Hormones
- f) Chronic Irritation

7) Epidemiology Reveals That Environmental Factors Cause Most Cancers
→ Environmental Agents Associated with Cancer:

- a) Viruses—mostly DNA viruses, casually linked to cancer
  - i) **Human papillomaviruses types 16 and 18 / cervical cancer**
  - ii) **Hepatitis B and C viruses / liver cancer**
- b) Tobacco Smoke—associated with 50-60% of cancer deaths in USA
- **c) Food---assoc with 50-60 % of cancer deaths in the USA**
- d) Radiation
  - i) UV-b from sun can damage DNA and is assoc with over 90% skin cancers incl melanomas
  - ii) Radon emitted from earth / lung cancer in ppl who work in mines
  - iii) Radio frequency electromagnetic radiation from cell phones or microwave ovens have not been empirically linked to cancer
  - iv) Nuclear radiation causes ionization of molecules and is therefore carcinogenic
- e) Chemicals
- f) Pollution

8)**Two-Hit Hypothesis**--the development of tumors requires 2 separate mutations.

9) Abnormalities in Cell Growth and Maturation (figure 21)

- a) Metaplasia—Reversible replacement of mature cells of one type to cells of another
- b) Dysplasia---Partially reversible loss of control of cell growth and tissue organization with abnormal differentiation and maturation; shows cytologic abnormalities
- c) Neoplasia---Irreversible complete loss of control of cell growth and tissue organization with abnormal differentiation and maturation, marked increase in cell number; shows cytologic abnormalities
- d) Atrophy---decrease in cell size and/or cell number
- e) Hypertrophy—increase cell size
- f) Hyperplasia---increase in cell number

10) **Multi-step Theory of Carcinogenesis (figure 132)**

a) **Initiation—**carcinogen forms an activated substance (usually pos.) which reacts with DNA (neg) causing mutation; DNA repair mechanisms?

b) **Latent Stage---**struggle of dysplastic cells for cell cycle autonomy

c) **Promotion---**co-carcinogen stimulates activated substance causing increase proliferation; clonal expansion

d) **Malignant Changes---**neoplastic cells evade immune response, increase proliferation

11) **Benign vs Malignant Neoplasms (figures 133 & 134)**

BENIGN **MALIGNANT**

12) Neoplasms with a Known Consistent Chromosome Defect (figure 135)

13) Human Diseases Associated with DNA-Repair Defects (figure 135)

14) Diseases Associated with Increased Risk of Neoplasia (figure 135)

15) Isolation of a Cellular Oncogene from a Human Tumor by Molecular Cloning

16) Immune Response to Neoplasms (figure 136)

17) Clinical Effects of Tumors (figure 137)

a) **Oversecretion of Cytokines → Physical symptomology**

b) **Organ Dysfunction**

18) Mechanisms of Invasion and Metastasis

a) **Malignant Tumors Metastasize** (i.e. form tumor implants discontinuous with the primary tumor)
b) **All Cancers Metastasize** (except Gliomas and Basal Cell Carcinomas)
c) Factors Involved in Tumor Invasion and Spread (Metastasis)
   i) Immune Evasion—receptor downregulation, shedding, mutation
   ii) Changing from Clonal → Differentiated cells
   iii) Size of Emboli
   iv) Penetration of cell basement membrane of host tissue cells—enzymes
   v) Angiogenesis

d) **Carcinomas** usually spread by **lymphatics (figure 138)**

e) Sarcomas spread by **blood (figure 139)—penetration of veins Not arteries**
i) Most Common sites of Spread: Liver & Lungs
ii) Least Common Sites of Spread: Spleen & Moving Tissues (Muscles & Tendons)

19) Cancer Diagnosis and Monitoring---serologic assays
- a) **CEA**—colon, breast and lung
- b) **AFP**---Hepatoma, yolk sac tumors
- c) **Prostatic acid phosphatase (PAP) & prostate-specific antigen (PSA)**—prostate
- d) **CA 125**---Ovarian carcinoma
- e) Specific Hormones---Endocrine Neoplasms and ectopic hormone producing tumor

20) Cancer Gene Therapy (see notes)
- a) Replacement Gene Therapy
- b) Knockout Gene Therapy
- c) Suicide Gene Therapy
- d) Immunomodulatory Gene Therapy
- e) Genetic Profiling

21) Cancer Vaccine Development
- a) Tumors have two main types of antigens which distinguish them from normal cells:
  - i) TSTAs (Tumor-Specific Transplantation Antigens)
  - ii) TATAs (Tumor-Associated Transplantation Antigens)

- b) Current Vaccine Development:
  - i) Whole Cell Vaccines
  - ii) Cell Lysate Vaccines
  - iii) Tumor antigen Vaccines
  - iv) Genetically Modified Tumor Cell Vaccines
  - v) Recombinant Vaccinia Virus
  - vi) DNA Viruses

B. Immunodeficiencies ←causes loss / decreased immune response → prone to infections & Disease

1) Severe Combined Immunodeficiency Syndrome--No T or B cell development (figure 140)

2) DiGeorge Syndrome (thymic hypoplasia)--No T cell development (figure 140)

3) AIDS
- a) Infection of HIV (figures 141-145)
  - --T cells
  - --Macrophages

- b) Diagnosis (figure 146)
- b) Breakdown of the Immune system (figures 147 and 148)
  - --Cancer
  - --Infection
  - --Neurodegeneration

- c) Natural Resistance (figure 149)

- d) Current Therapies (figures 150 & 151)

- e) Vaccine Strategies (figures 152 & 153)

C. Autoimmune Disorders ← causes organ / tissue self destruction or dysfunction (figure 154)

Abnormalities of the immune system can cause the body to attack its own tissues. This can occur in a variety of ways (figure 155):

→ Loss or dysfunction of suppressor T cell function
→ Dysfunctional maturation of lymphocytes and the development of tolerance

1) Hypersensitivity Reactions (figure 156)

a) Type 1(asthma)---IgE mediated, allergic reaction and / or anaphylaxis
b) Type 2 (transfusion rxns)---IgG or IgM mediated; transfusion reactions and hemolytic anemia, myasthenia gravis
c) Type 3 (serum sickness)---IgG or IgM immune complex mediated; serum sickness, rheumatoid arthritis
d) Type 4 (delayed type hypersensitivity)---no antibody involved; T cell mediated; transplant and graft rejections contact dermatitis

2) Rheumatoid Arthritis--- a type 3 mediated response

3) Blood Transfusion Incompatibility (figure 157)---

4) Graves Disease (figure 158)

5) Myasthenia Gravis---type 2 mediated (figure 158)

D. Transplantation Immunology and Graft Rejection

1) Terminology of Transplantation
a) Autograft—grafts from one part of the body to the other
b) Allograft---organ transplant into another person
c) Isografts---grafts between identical twins
d) Xenografts---grafts between different species; pig heart valve to human

→ Autografts and usually isografts are recognized as self tissue and therefore induce no immune response.

2) Methods to Minimize Rejection
a) HLA matching

b) Immunosuppression

c) Perform more blood transfusions

3) Most transplant rejections are hypersensitivity reactions (figure 159)
a) Hyperacute Rejection
b) Acute Rejection
c) Chronic Rejection

E. Cardiovascular Disease ←causes acute loss of blood supply (strokes, myocardial infarction aortic dissection) resulting in sudden death or permanent impaired function, or causing chronically progressive loss of cardiovascular function resulting in diffuse organ dysfunction.

1) Pathophysiology

--cardiovascular system is responsible for delivering nutrients and oxygen to the cells of our body, as well as serving as a vehicle for excretion of toxic by-products of metabolism.

2) Main Causes of Cardiovascular Disease (figure 160)

a) Atherosclerosis---hereditary, cholesterol, hardening of the arteries

b) Hypertension (figures 161 & 162)---hereditary, atherosclerosis, drug-induced

c) Valve Disease---infections, hereditary, lung disease, hypertension

d) Chronic Pulmonary Disease---infections, hereditary, smoking

3) Results of Cardiovascular Disease

i) Organ Compromise

ii) Myocardial Infarction

4) Targets of Genetic Therapy

--Beta Receptors

--Heart Muscle Regeneration

F. Aging ← damaged tissues, decreased organ function, decreased immunity = disease & debilitation

1) Effects of Aging (figure 165)

2) Theories of Aging

a) Free Radical Damage (the main theory)

i) Apoptosis---programmed cell death

b) Cumulative Injury Theory

c) Growth Hormones and Gene expression---DNA Repair

3) Current Aging Decelerators

a) Antioxidants—**superoxide dismutase** is the most common enzyme we have that is responsible for antioxidation reactions

b) Exercise

Biotech Applications: Vaccine Therapy

SPECIAL TOPIC: TELEMEDICINE (as time permits)

7. Final Wrap-Up and Review

- **Agribio—crops and cattle**
- **Bioenergetics**
- **Biopharma**
- **FDA Approval**
- **Biotech in Today's World**
- **Life in the 22nd century**