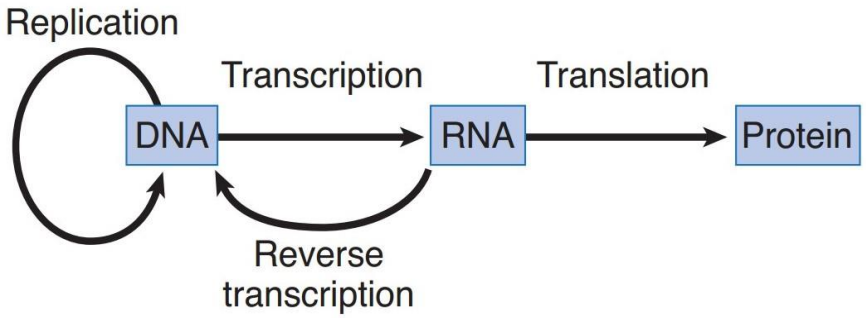
CHAPTER 1

Molecular Biochemistry

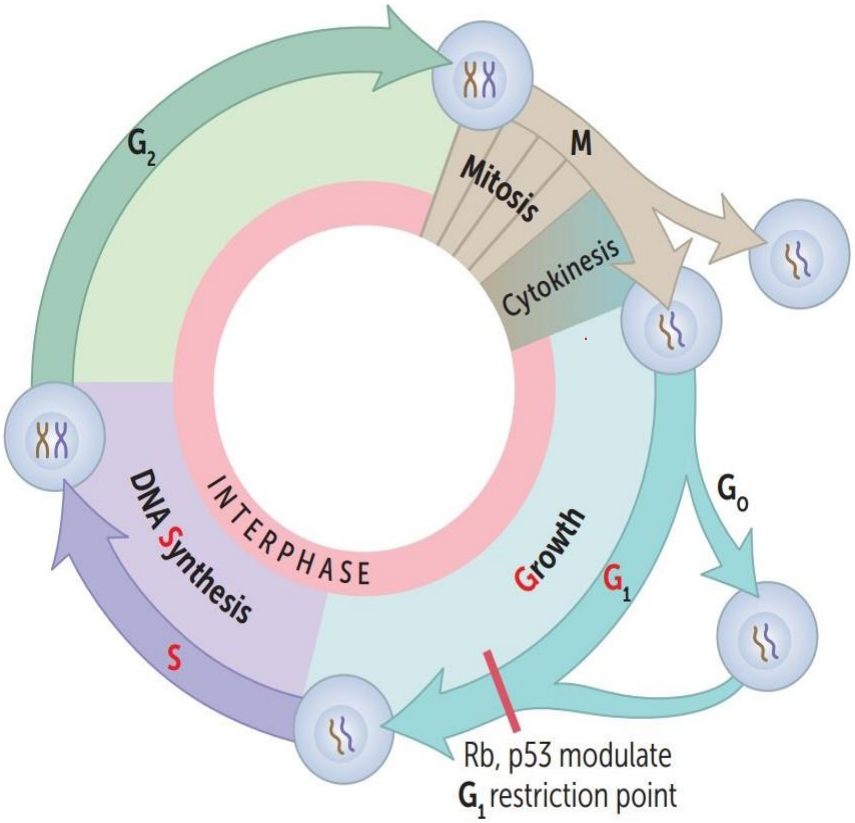
- An organism must be able to store and preserve its genetic information, pass that information along to future generations, and express that information as it carries out all the processes of life.



- When cells divide, each daughter cell must receive an accurate copy of the genetic information. DNA replication is the process in which each chromosome is duplicated before cell division.

- Transcription, the first stage in gene expression, involves transfer of information found in a double-stranded DNA molecule to the base sequence of a single-stranded RNA molecule. If the RNA molecule is a messenger RNA, then the process known as translation converts the information in the RNA base sequence to the amino acid sequence of a protein.

Cell cycle phases



- The concept of the cell cycle can be used to describe the timing of some of these events in a eukaryotic cell.

- The M phase (mitosis) is the time in which the cell divides to form two daughter cells.

- Interphase is the term used to describe the time between two cell divisions or mitoses. Gene expression occurs throughout all stages of interphase. Interphase is subdivided as follows:

A. G_1 phase (gap 1): is a period of cellular growth preceding DNA synthesis. Cells that have stopped cycling, such as muscle and nerve cells, are said to be in a special state called G_0.

B. S phase (DNA synthesis): is the period of time during which DNA replication occurs. At the end of S phase, each chromosome has doubled its DNA content and is composed of two identical sister chromatids linked at the centromere.

C. G_2 phase (gap 2): is a period of cellular growth after DNA synthesis but preceding mitosis. Replicated DNA is checked for any errors before cell division.

Cell types

1. Permanent cells:

- Remain in G_0, regenerate from stem cells.

- EX: Neurons, skeletal and cardiac muscle, RBCs.

2. Stable (quiescent) cells:

- Enter G_1 from G_0 when stimulated.

- EX: Hepatocytes, lymphocytes.

3. Labile cells:

- Never go to G_0, divide rapidly with a short G_1.

- Most affected by chemotherapy.

- EX: Bone marrow, gut epithelium, skin, hair follicles, germ cells.

Regulation of cell cycle

- Control of the cell cycle is accomplished at checkpoints between the various phases by strategic proteins such as cyclins and cyclin-dependent kinases.

- These checkpoints ensure that cells will not enter the next phase of the cycle until the molecular events in the previous cell cycle phase are concluded.

A. Cyclin-dependent kinases: Constitutive and inactive.

B. Cyclins: Regulatory proteins that control cell cycle events; phase specific; activate CDKs.

C. Cyclin-CDK complexes: Phosphorylate other proteins to coordinate cell cycle progression; must be activated and inactivated at appropriate times for cell cycle to progress.

D. Tumor suppressors: p53 induces p21, which inhibits CDKs → hypophosphorylation (activation) of Rb → inhibition of G_1-S progression.

- Mutations in tumor suppressor genes can result in unrestrained cell division (Li-Fraumeni syndrome).

Nucleotide structure and nomenclature

- Nucleic acids (DNA and RNA) are assembled from nucleotides, which consist of three components: a nitrogenous base, a five-carbon sugar (pentose), and phosphate.

A. Five-Carbon Sugars:

- Nucleic acids (as well as nucleosides and nucleotides) are classified according to the pentose they contain:
  - If the pentose is ribose, the nucleic acid is RNA (ribonucleic acid).
  - If the pentose is deoxyribose, the nucleic acid is DNA (deoxyribonucleic acid).

B. Bases:

- There are two types of nitrogen-containing bases commonly found in nucleotides: purines and pyrimidines.

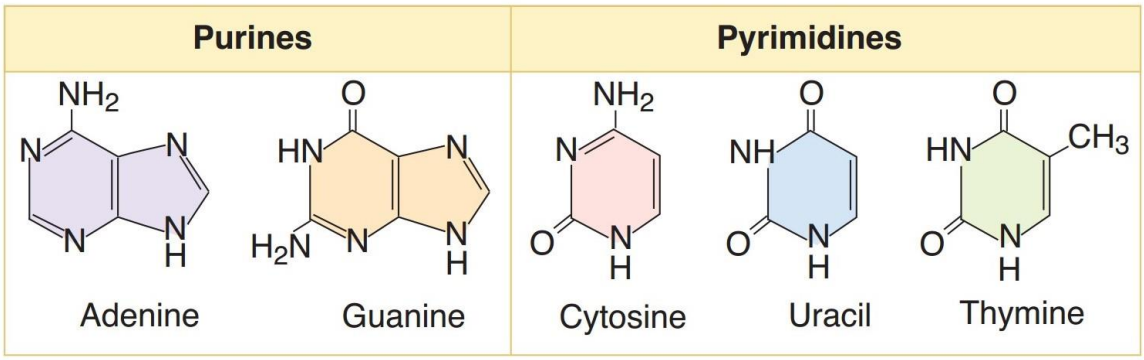
- Purines:
  - Contain two rings in their structure.
  - The two purines commonly found in nucleic acids are adenine (A) and guanine (G).
  - PURe As Gold.
  - Both are found in DNA and RNA.
  - Amino acids necessary for purine synthesis are Glycine, Aspartate, and Glutamine (GAG).

- Pyrimidines:
  - Have only one ring.
  - Cytosine (C) is present in both DNA and RNA.
  - Thymine (T) is usually found only in DNA (Thymine has a methyl), whereas uracil (U) is found only in RNA.
  - CUT the PY (pie).

- Deamination of cytosine makes uracil.

- Deamination of adenine makes hypoxanthine. Deamination of guanine makes xanthine.

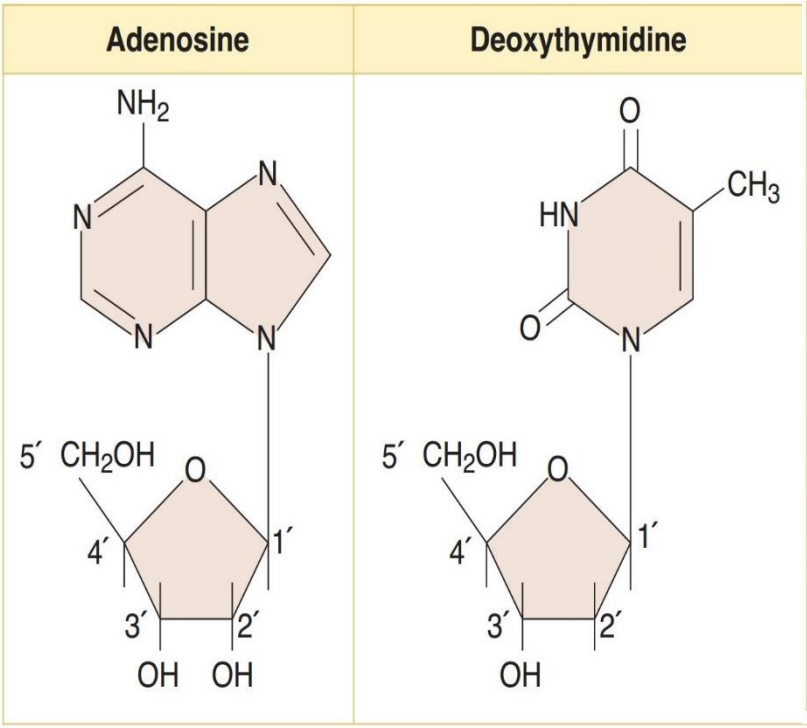
- Methylation of uracil makes thymine.

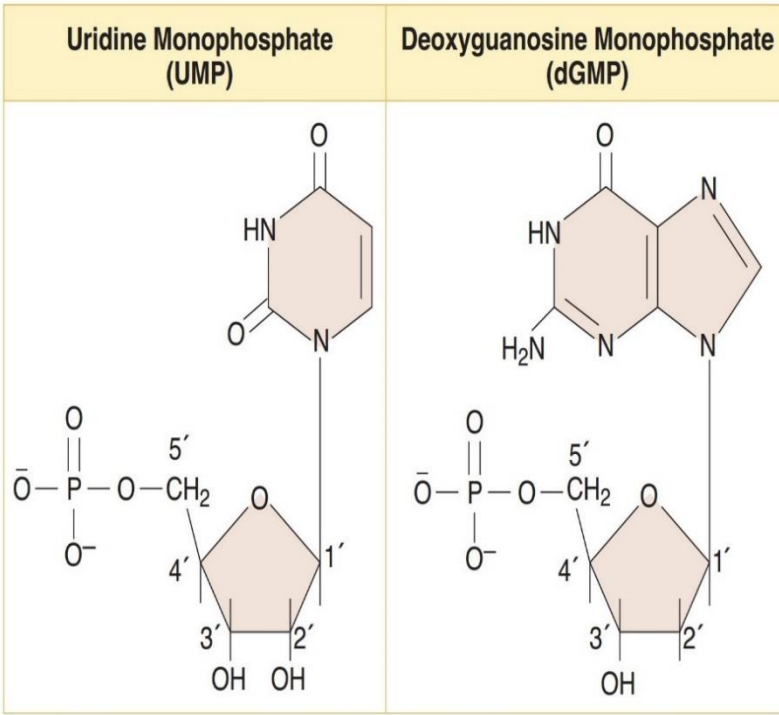


C. Nucleosides and Nucleotides:

- NucleoSide = base + (deoxy)ribose (Sugar).

- NucleoTide = base + (deoxy)ribose + phosphaTe; linked by 3′-5′ phosphodiester bond.

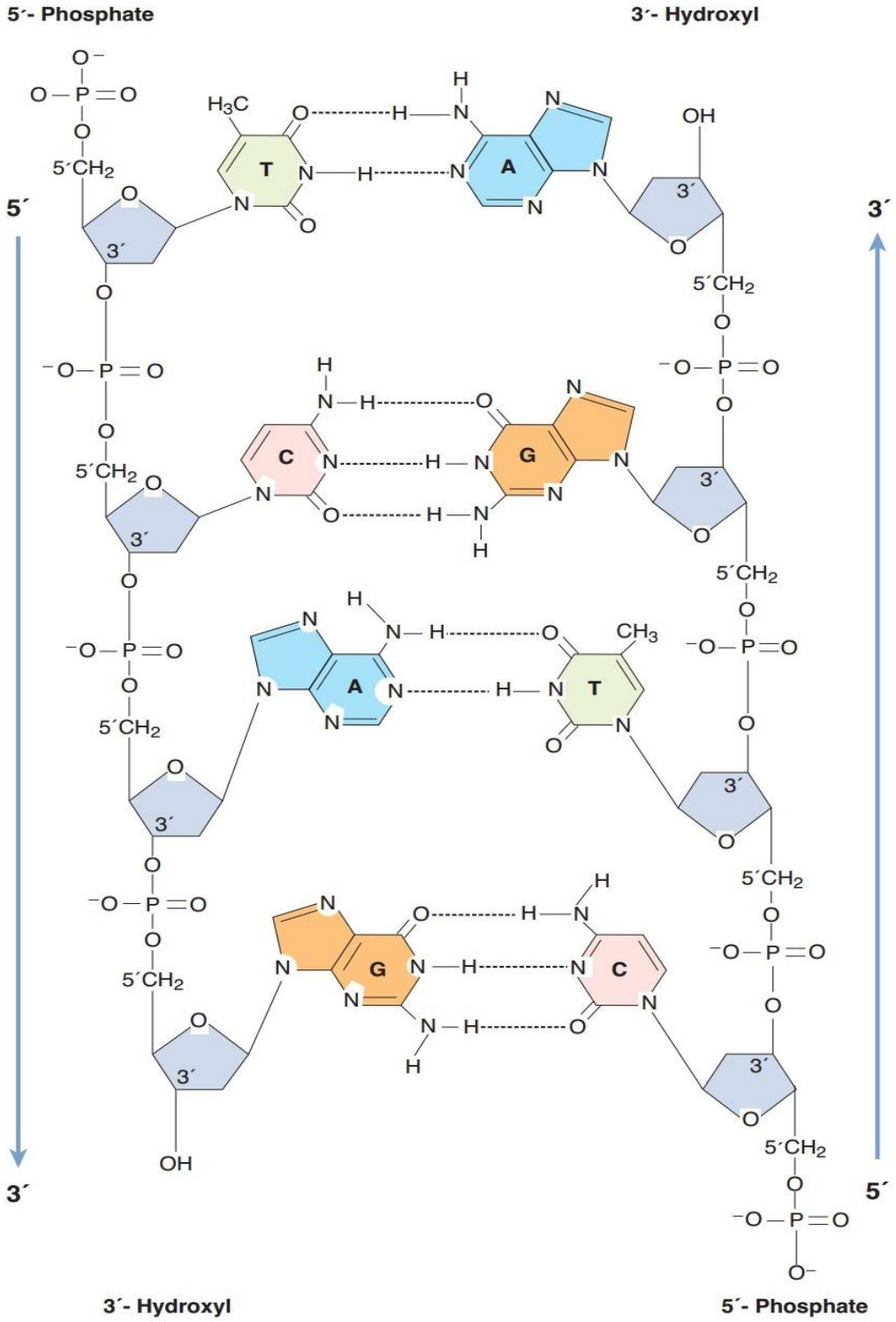




- Nucleic acids are polymers of nucleotides joined by 3′, 5′-phosphodiester bonds; that is, a phosphate group links the 3′ carbon of a sugar to the 5′ carbon of the next sugar in the chain. Each strand has a distinct 5′ end and 3′ end, and thus has polarity.

- A phosphate group is often found at the 5′ end, and a hydroxyl group is often found at the 3′ end.

- The base sequence of a nucleic acid strand is written by convention, in the 5'→3' direction (left to right). According to this convention, the sequence of the strand on the left in the figure below must be written: 5'-TCAG-3' or TCAG.



DNA structure

- Some of the features of double-stranded DNA include:

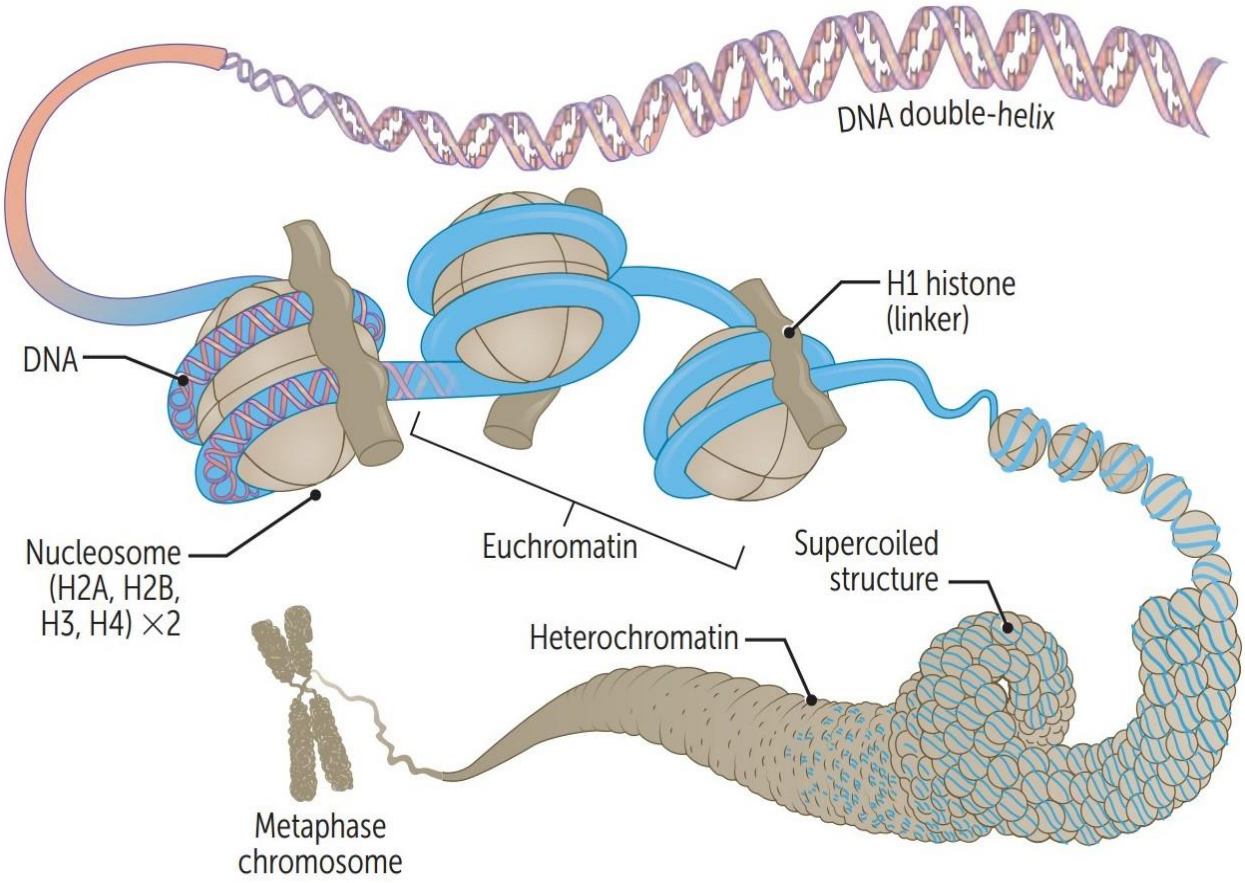
- The two strands are antiparallel (opposite in direction).

- The two strands are complementary. A always pairs with T (two hydrogen bonds), and G always pairs with C (three hydrogen bonds). Thus, the base sequence on one strand defines the base sequence on the other strand.

- 5′ end of incoming nucleotide bears the triphosphate (energy source for the bond). Triphosphate bond is target of 3′ hydroxyl attack.

- G-C bond (3 H bonds) stronger than A-T bond (2 H bonds). ↑ G-C content → ↑ melting temperature of DNA (C-G bonds are like Crazy Glue).

Chromatin structure



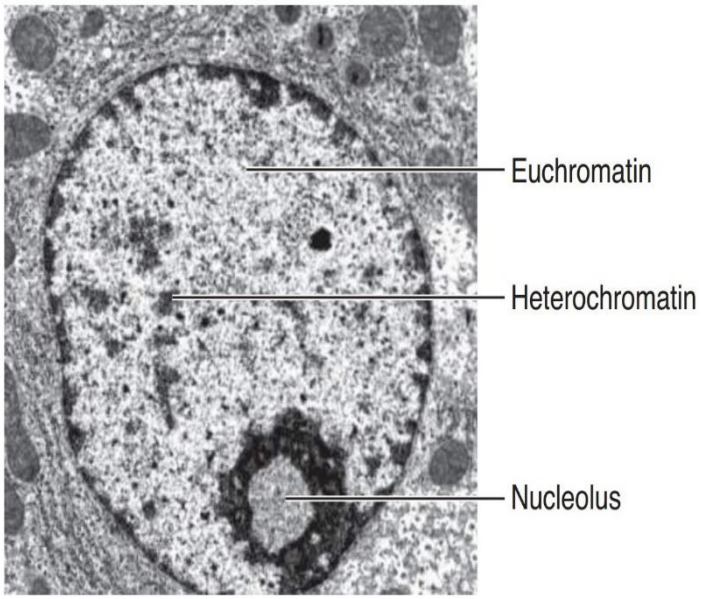
- DNA exists in the condensed, chromatin form in order to fit into the nucleus.
- Nucleosomes are structural subunits present inside the nucleus composed of nuclear proteins called histones.
- There are five major subtypes of histones: H1, H2A, H2B, H3, and H4.
- The nucleosome core is composed of two molecules each of H2A, H2B, H3, and H4, making eight total histone proteins in each nucleosome core. Histones are rich in the amino acids lysine and arginine.
- Phosphate groups give DNA a ⊖ charge. Lysine and arginine give histones a ⊕ charge.
- During the initial steps of DNA packaging into chromatin, negatively charged DNA loops twice around positively charged histone octamer to form nucleosome, but in contrast to the other histone proteins, H1 histones are not part of the nucleosome.
- Histone H1 is located outside of the nucleosome core and helps to package nucleosomes into more compact structures by binding and linking DNA between adjacent nucleosomes.
- The association of DNA with histones gives the appearance of a "beaded chain" as this structure undergoes further rounds of coiling and association with other structural proteins, such as nuclear scaffold proteins, before ultimately forming chromosomes.
- In mitosis, DNA condenses to form chromosomes. DNA and histone synthesis occur during S phase.

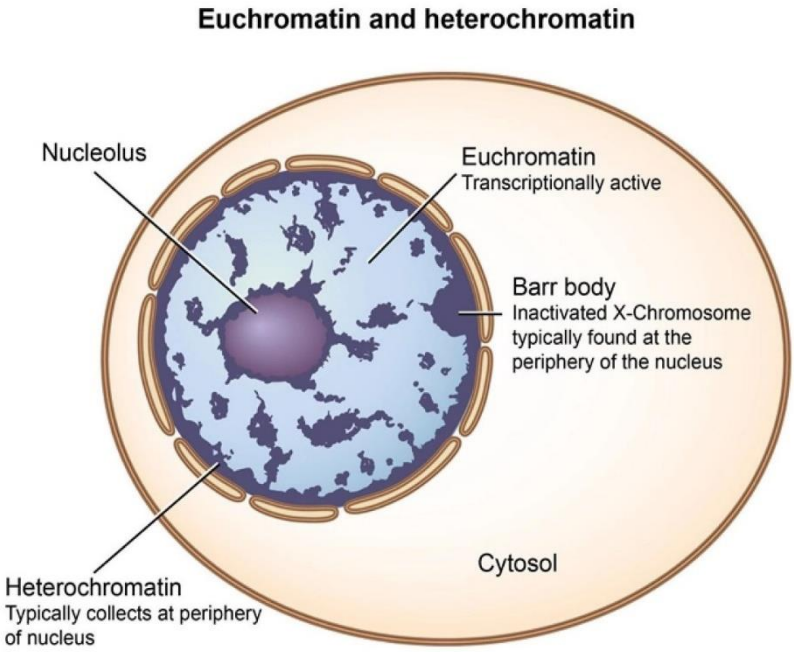
- <u>Heterochromatin:</u>

- Condensed, appears darker on EM. HeteroChromatin = Highly Condensed.
- Transcriptionally inactive, sterically inaccessible. ↑ methylation, ↓ acetylation.
- Barr bodies (inactive X chromosomes) are heterochromatin.

- <u>Euchromatin:</u>

- Less condensed, appears lighter on EM.
- Transcriptionally active, sterically accessible. Euchromatin is Expressed.
- Eu = true, “truly transcribed”.





- Regulation of transcription occurs in part due to the presence of histones, small proteins that complex with DNA to help compact the strands. Histones can undergo a variety of modifications (methylation, acetylation, phosphorylation) that affect the accessibility of the genome for transcription.

- Histone methylation:

- Usually reversibly represses DNA transcription but can activate it in some cases depending on methylation location.

- Histone Methylation Mostly Makes DNA Mute.

- Histone acetylation:

- Relaxes DNA coiling, allowing for transcription.

- Histone Acetylation makes DNA Active.

- Histone deacetylation:

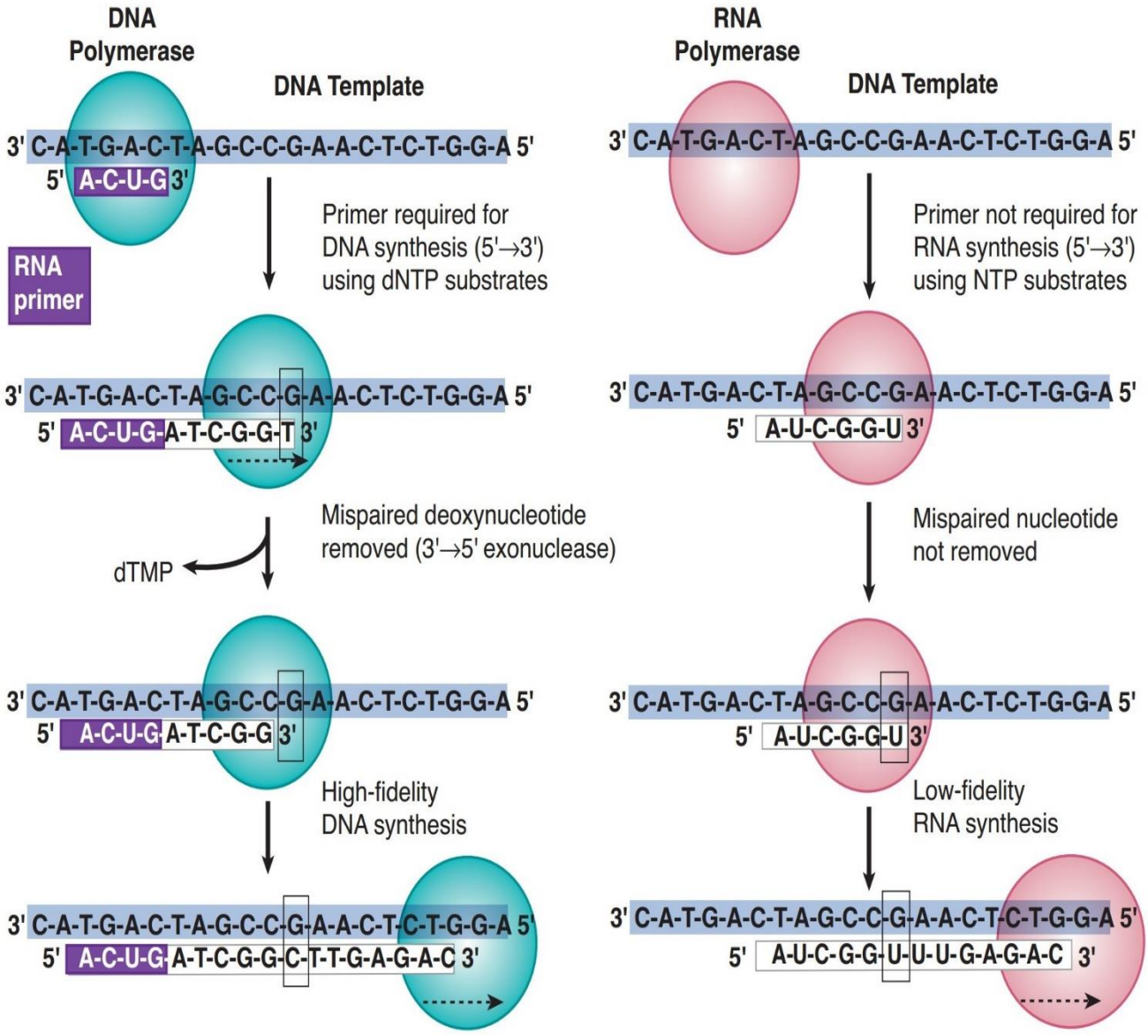
- Removal of acetyl groups → tightened DNA coiling → ↓ transcription.

- In Huntington disease, abnormal huntingtin causes increased histone deacetylation, silencing the genes necessary for neuronal survival. As a result, one of the treatment options under investigation includes histone deacetylase inhibitors that help upregulate survival genes.

DNA Replication

- DNA replication occurs during the S-phase (synthesis phase) of the cell cycle.
- Genetic information is transmitted from parent to progeny by replication of parental DNA, a process in which two daughter DNA molecules are produced that are each identical to the parental DNA molecule.
- During DNA replication, the two complementary strands of parental DNA are pulled apart. Each of these parental strands is then used as a template for the synthesis of a new complementary strand (semiconservative replication). During cell division, each daughter cell receives one of the two identical DNA molecules.

Comparison of DNA and RNA synthesis



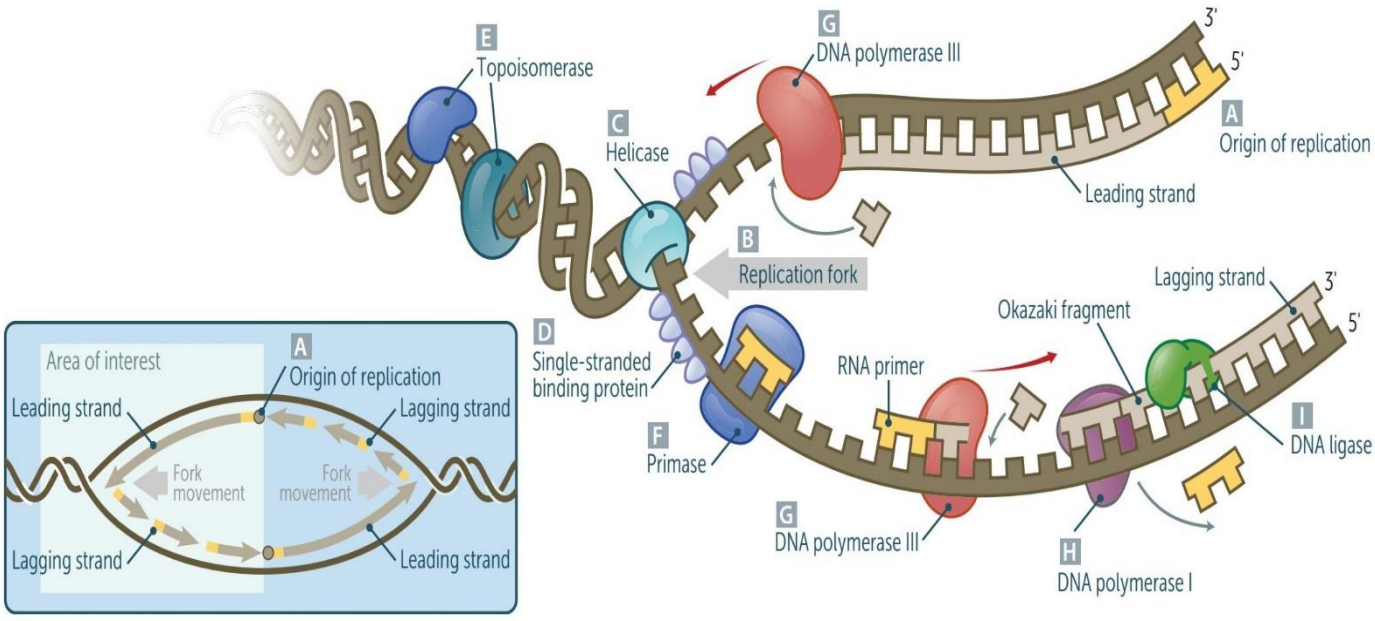
- Similarities include:

- The template strand is scanned in the 3′→5′ direction.
- The newly synthesized strand is made in the 5′→3′ direction.
- The newly synthesized strand is complementary and antiparallel to the template strand.
- Each new nucleotide is added when the 3′ hydroxyl group of the growing strand reacts with a nucleotide triphosphate, which is base-paired with the template strand. Pyrophosphate (PPi, the last two phosphates) is released during this reaction.

- Differences include:

- The substrates for DNA synthesis are the dNTPs, whereas the substrates for RNA synthesis are the NTPs.
- DNA contains thymine, whereas RNA contains uracil.
- DNA polymerases require a primer, whereas RNA polymerases do not.
- That is, DNA polymerases cannot initiate strand synthesis, whereas RNA polymerases can.
- DNA polymerases can correct mistakes ("proofreading"), whereas RNA polymerases cannot. DNA polymerases have 3′ → 5′ exonuclease activity for proofreading.

- DNA replication can only occur on single stranded DNA; therefore, unwinding and dissociation of the parent DNA strands is necessary before replication can proceed.



A. Origin of replication:
- Particular consensus sequence of base pairs in genome where DNA replication begins.
- May be single (prokaryotes) or multiple (eukaryotes).
- AT-rich sequences (such as TATA box regions) are found in promoters and origins of replication.

B. Replication fork: Y-shaped region along DNA template where leading and lagging strands are synthesized.

C. Helicase: Unwinds DNA template at replication fork.

D. Single-stranded binding proteins: bind to the ssDNA and stabilize it, preventing premature reannealing of the ssDNA to dsDNA.

E. DNA topoisomerases:
- Create a single- or double-stranded break in the helix to relieve supercoiling tension of the dsDNA strand caused by the unwinding action of helicase.
- Irinotecan/topotecan inhibit eukaryotic topoisomerase I.
- Etoposide/teniposide inhibit eukaryotic topoisomerase II.
- Fluoroquinolones inhibit prokaryotic topoisomerase II (DNA gyrase) and topoisomerase IV.

F. Primase: A DNA-dependent RNA polymerase which makes an RNA primer on which DNA polymerase III can initiate replication.

G. DNA polymerase III:
- Prokaryotes only.
- DNA polymerases cannot begin synthesis of daughter strands without a free 3'-hydroxyl group, which is provided by an RNA primer.
- Elongates leading strand by adding deoxynucleotides to the 3′ end.
- Elongates lagging strand until it reaches primer of preceding fragment. 3′ → 5′ exonuclease activity "proofreads" each added nucleotide.
- DNA polymerase III has 5′ → 3′ synthesis and proofreads with 3′ → 5′ exonuclease.
- In contrast to the continuous synthesis of the leading strand, lagging strand synthesis occurs discontinuously and is composed of short stretches of RNA primer plus newly synthesized DNA

segments (Okazaki fragments). As a result, lagging strand synthesis requires the repetitive action of DNA primase and DNA ligase.

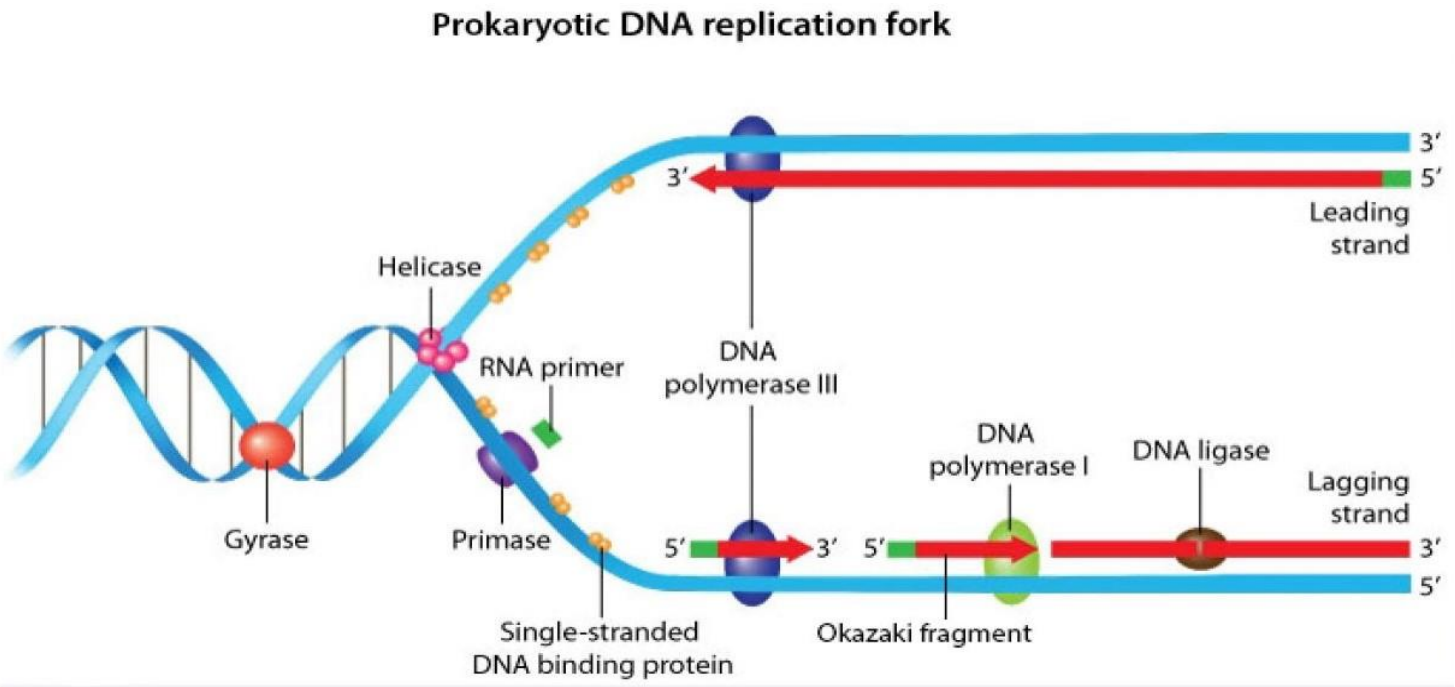
- Drugs blocking DNA replication often have a modified 3′ OH, thereby preventing addition of the next nucleotide "chain termination".

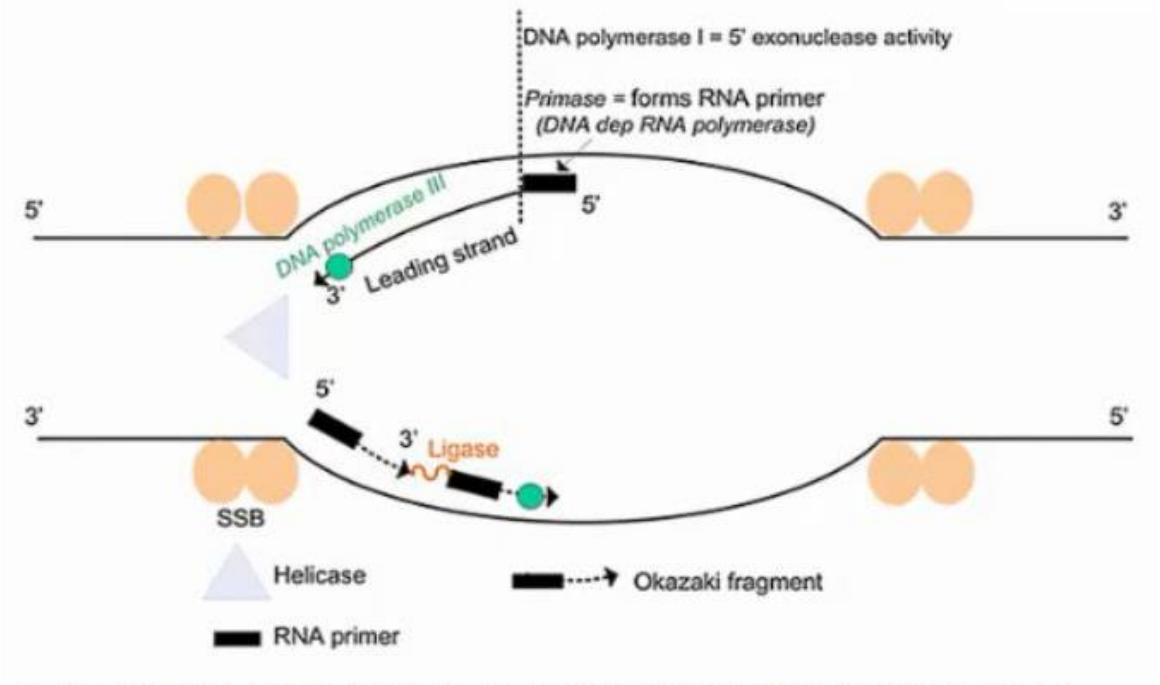
H. DNA polymerase I:

- Prokaryotic only.
- Degrades RNA primer; replaces it with DNA.
- Same functions as DNA polymerase III, also excises RNA primer with 5′ → 3′ exonuclease.

I. DNA ligase:

- Catalyzes the formation of a phosphodiester bond within a strand of double-stranded DNA.
- Joins Okazaki fragments.

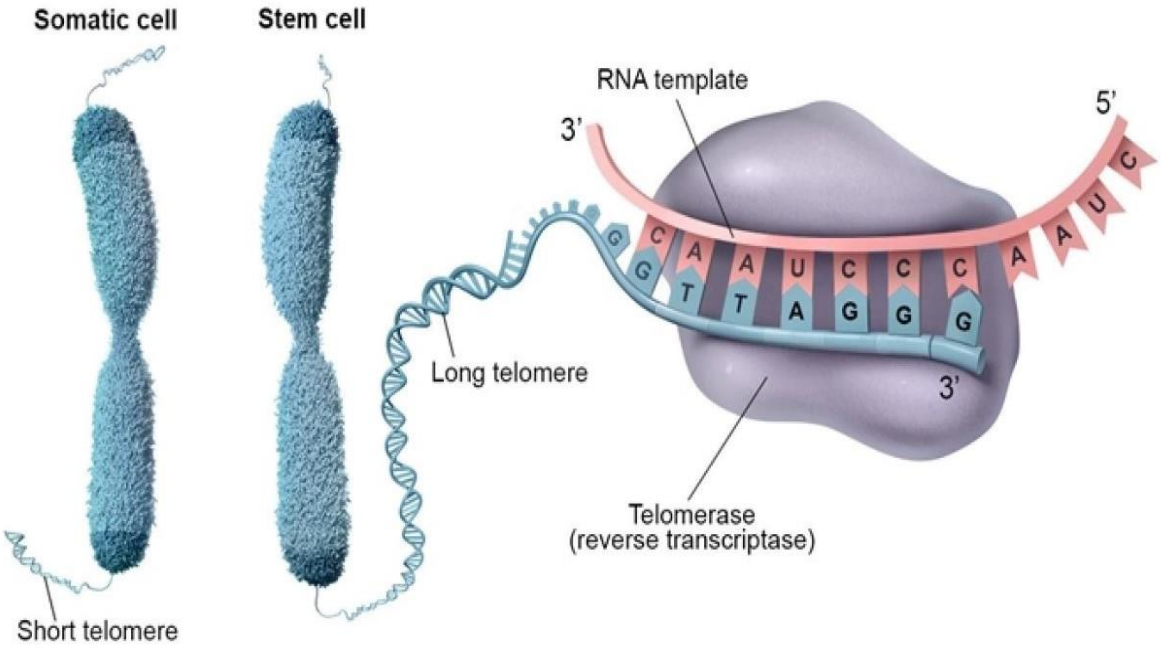




Proteins & their function in prokaryotic DNA replication	
Helicase	Unwinding of double helix
Topoisomerase II (DNA gyrase)	Removal of supercoils
Single-stranded DNA-binding protein	Stabilization of unwound template strands
Primase (RNA polymerase)	Synthesis of RNA primer
DNA polymerase III	5' to 3' DNA synthesis & 3' to 5' exonuclease ("proofreading") activity
DNA polymerase I	Same as DNA polymerase III Also removes RNA primer (5' to 3' exonuclease activity) & replaces it with DNA
DNA ligase	Joining of Okazaki fragments (lagging strand)

❖ Telomerase:

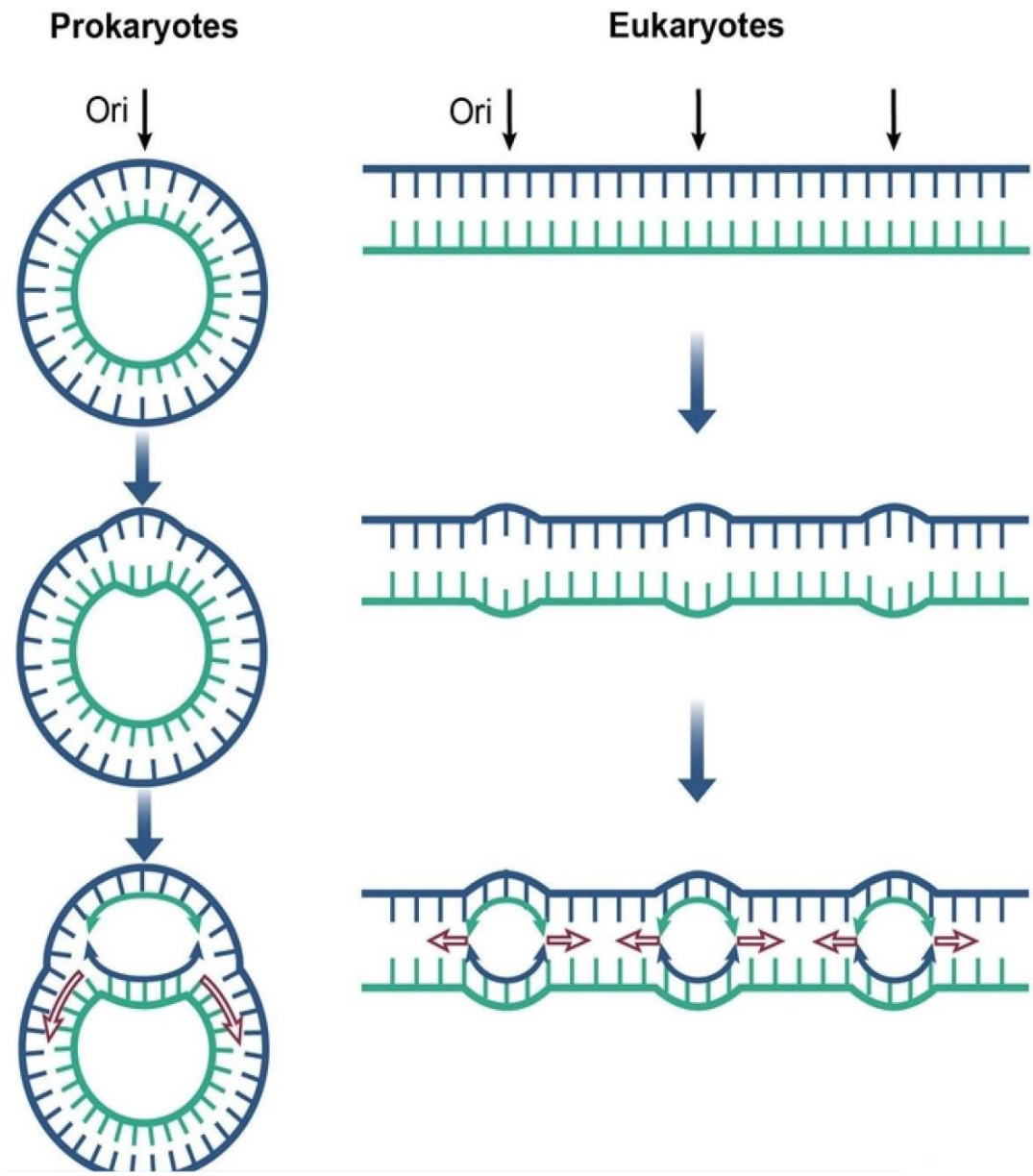
- Eukaryotes only.
- An RNA-dependent DNA polymerase that adds DNA to 3' ends of chromosomes to avoid loss of genetic material with every duplication.
- Stem cells have very long telomeres and active telomerase but with every cell division, the length of telomeres progressively shortens. Terminally differentiated adult somatic cells have very short telomeres.
- Critical shortening in telomere length is thought to be one signal for programmed cell death. On the other hand, cancer cells up-regulate their telomerase activity, preventing cellular death by maintaining the length of their telomeres. Cancer cells are considered immortal because these cells continue to divide without aging or shortening of their telomeres, thus, telomerase is a potential target for the treatment of cancers. Syndromes of premature aging such as Bloom syndrome are associated with shortened telomeres.



❖ N.B:

1. All three prokaryotic DNA polymerases have proof reading activity and remove mismatched nucleotides via 3' to 5' exonuclease activity.
   - Only DNA polymerase I has 5’ to 3’ exonuclease activity which is used to excise and replace RNA primers and damaged DNA sequences.

2. Multiple origins of replication make eukaryotic DNA synthesis quick and effective despite the large size of the genome compared to that of prokaryotic organisms.



Mutations in DNA

- DNA and RNA are composed of sequences of four bases arranged into codons composed of three sequential bases.

- Each codon calls for a particular amino acid except for one codon that signals the initiation of protein synthesis (AUG) and three that stop protein synthesis (UAA, UAG, and UGA).

- Changes in the DNA sequences are called mutations. Some changes in the genetic code can result in the formation of altered proteins, such as enzymes, channels, and structural proteins, which may lead to serious clinical manifestations.

Base substitutions (point mutations)

- Where one base is substituted with another base, are the most common type of mutation.

- These point mutations can be of three types:

1. Silent mutations:

- These mutations result from codon base substitutions which code for the same amino acid.

- For example, a single base substitution in UCA to UCU will not result in any change in protein structure because both code for the same amino acid, serine.

- Silent mutations do not cause amino acid changes within proteins.

- Often base change in 3rd position of codon (tRNA wobble).

2. Missense mutations:

- These mutations are characterized by base substitutions that result in the placement of an incorrect amino acid in a protein sequence.

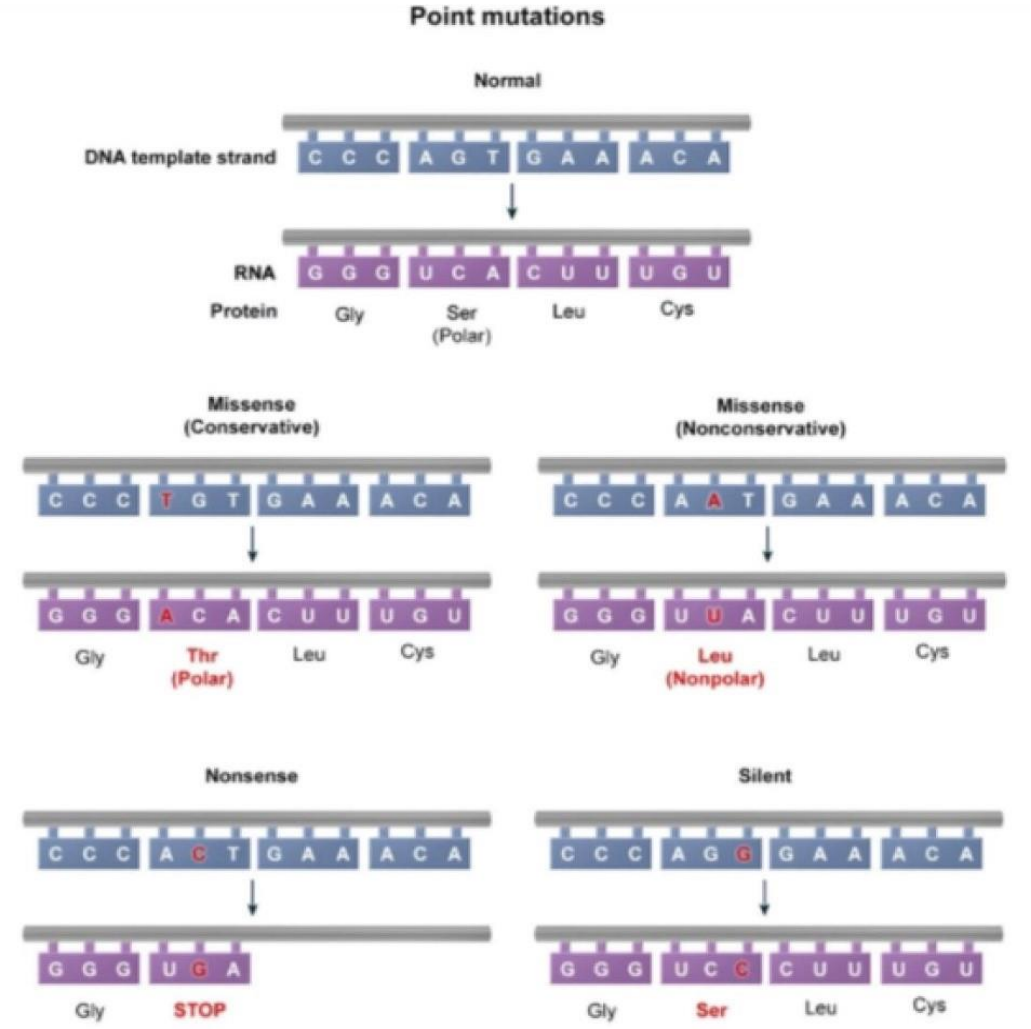
- For instance, a change in the code from UUU to UCU changes the translated amino acid from phenylalanine (UUU) to serine (UCU).

3. Nonsense mutations:

- These mutations introduce a stop codon within gene sequences, resulting in the formation of shorter, truncated proteins.

- An example of a nonsense mutation is a mutational change in the codon UCA (serine) to UAA (stop codon).

- For point (silent, missense, and nonsense) mutations:
  - Transition: purine to purine (A to G) or pyrimidine to pyrimidine (C to T).
  - Transversion: purine to pyrimidine (A to T) or pyrimidine to purine (C to G).



4. Splice site mutations:

- Mutation at a splice site → retained intron in the mRNA → larger protein with impaired or altered function.

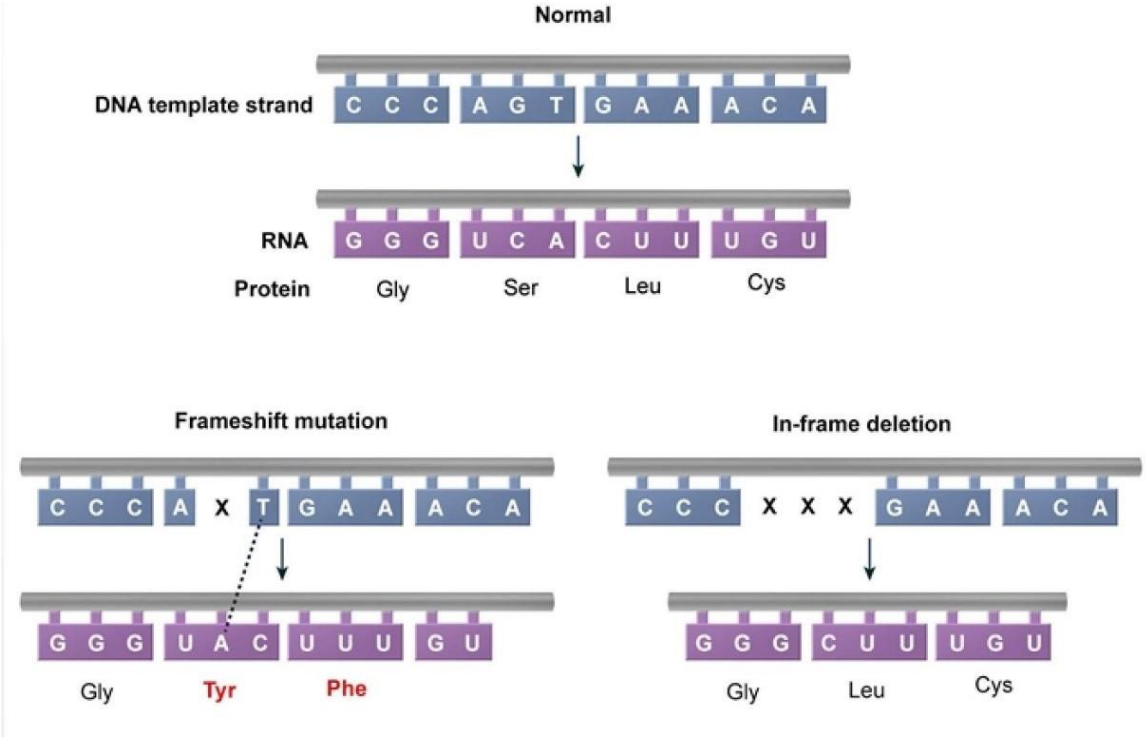
- Rare cause of cancers, dementia, epilepsy, some types of β-thalassemia.

Frameshift mutations

- Result from deletion or insertion of bases that are not a multiple of Three.

- As their name implies, frameshift mutations alter the reading frame of the genetic code, resulting in the formation of non-functional proteins.

- Severity of damage: silent << missense < nonsense < frameshift.



DNA Repair

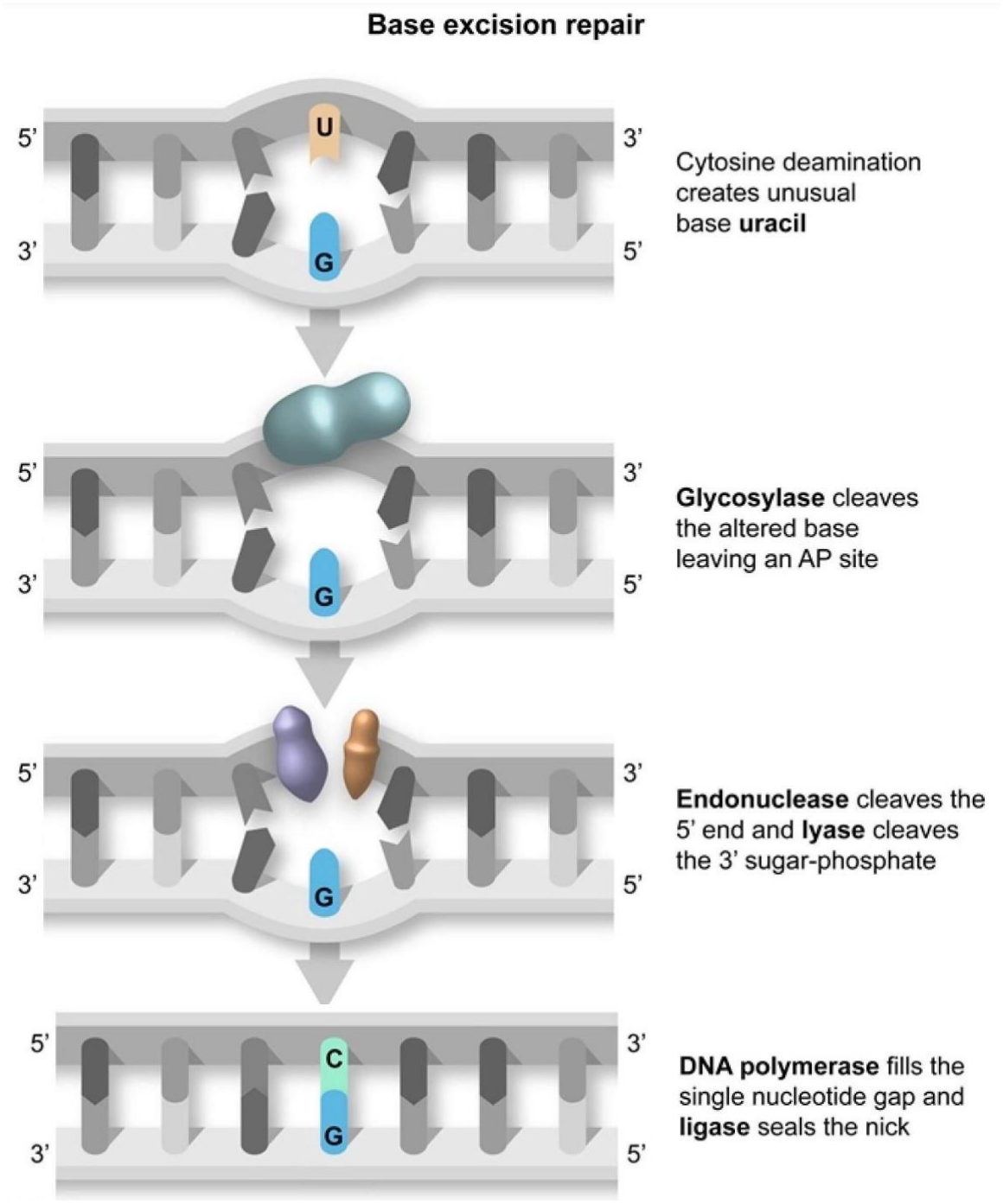
Single strand

1. Nucleotide excision repair:

- Specific endonucleases release the oligonucleotides containing damaged bases; DNA polymerase and ligase fill and reseal the gap, respectively.
- Repairs bulky helix-distorting lesions.
- Occurs in G_1 phase of cell cycle.
- Defective in xeroderma pigmentosum, which prevents repair of pyrimidine dimers that are formed as a result of ultraviolet light exposure.

2. Base excision repair:

- Base-specific Glycosylase removes altered base and creates AP site (apurinic/apyrimidinic).
- One or more nucleotides are removed by AP-Endonuclease, which cleaves the 5' end. Lyase cleaves the 3' end. DNA Polymerase-β fills the gap and DNA Ligase seals it.
- Occurs throughout cell cycle.
- Important in repair of spontaneous/toxic deamination.



3. Mismatch repair:

- Newly synthesized strand is recognized, mismatched nucleotides are removed, and the gap is filled and resealed.

- Occurs predominantly in S phase of cell cycle.

- Defective in Lynch syndrome (hereditary nonpolyposis colorectal cancer [HNPCC]).

Double strand

1. Nonhomologous end joining:

- Brings together 2 ends of DNA fragments to repair double-stranded breaks.
- Sister chromosomes not used as a template.
- Some DNA may be lost.
- Defective in ataxia telangiectasia.

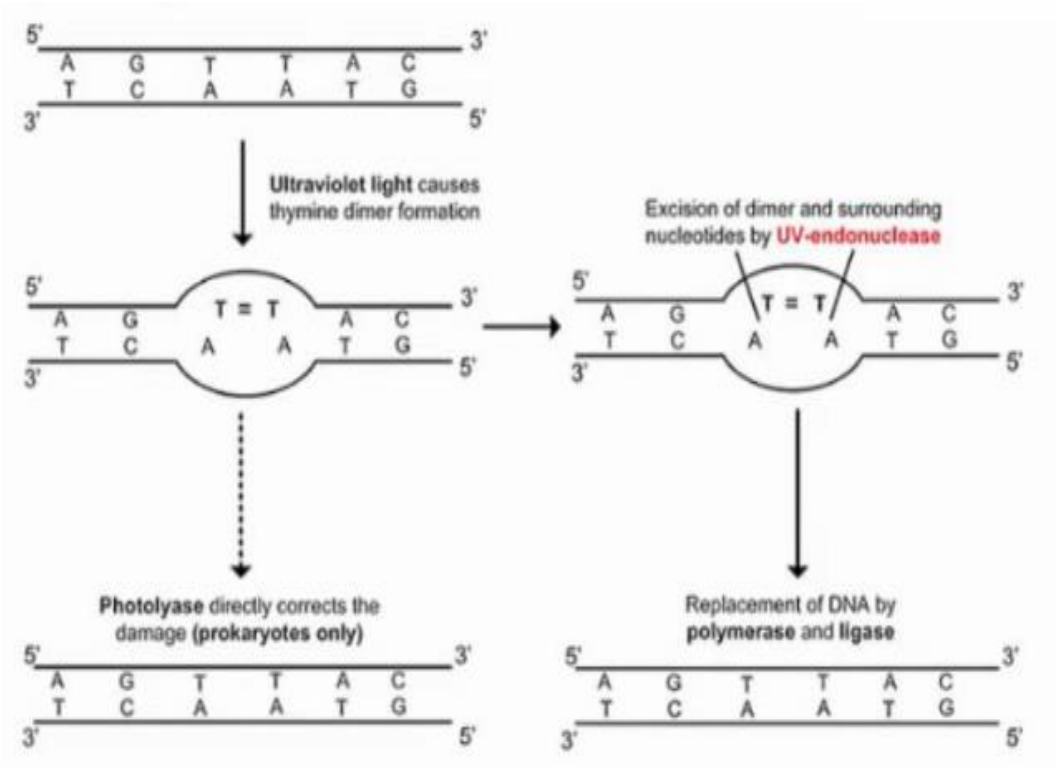
2. Homologous recombination:

- Requires two homologous DNA duplexes.
- Sister chromosomes used as a template.
- A strand from the damaged dsDNA is repaired using a complementary strand from the intact homologous dsDNA as a template.
- Restores duplexes accurately without loss of nucleotides.
- Defective in breast/ovarian cancers with BRCA1 mutation and Fanconi anemia.

❖ N.B:

1. DNA can be damaged by ultraviolet rays, which leads to the formation of thymine dimers from two adjacent thymine residues.

- The most common defect that causes xeroderma pigmentosum is the absence of UV-specific endonuclease.
- This UV-specific endonuclease recognizes distortions in the structure of DNA caused by thymine dimers, and subsequently excises stretches of single stranded DNA which contain these defects.
- The gap created following this excision is then filled in by DNA polymerase, which uses the opposite DNA strand as a template. The new strand of DNA is then joined on both ends to the existing DNA by the enzyme ligase.
- Remember that xeroderma pigmentosum (XP) like most enzymatic disorders, is an autosomal recessive disease. Patients suffering from xeroderma pigmentosum (XP) exhibit photosensitivity, poikiloderma, and hyperpigmentation in sun-exposed areas and also possess a markedly increased risk of developing skin cancers.



2. Lynch syndrome (hereditary nonpolyposis colon cancer) is an autosomal dominant disease caused by defective DNA mismatch repair.

- DNA replication occurs with a high degree of fidelity because mismatched nucleotides are repaired through the proofreading activity of DNA polymerases delta and epsilon. However, this proofreading functionality is not infallible.
- It is the function of the DNA mismatch repair system to fix these errors shortly after the daughter strands are synthesized. The mismatch repair system involves several genes, including MSH2 and MLH1, which code for components of the human MutS and MutL homologs. Mutations in these 2 genes account for around 90% of cases of Lynch syndrome.

RNA Transcription

- The first stage in the expression of genetic information is transcription of the information in the base sequence of a double-stranded DNA molecule to form the base sequence of a single-stranded molecule of RNA.
- For any particular gene, only one strand of the DNA molecule, called the template strand, is copied by RNA polymerase as it synthesizes RNA in the 5′ to 3′ direction.
- Because RNA polymerase moves in the 3′ to 5′ direction along the template strand of DNA, the RNA product is antiparallel and complementary to the template.
- RNA polymerase recognizes start signals (promoters) and stop signals (terminators) for each of the thousands of transcription units in the genome of an organism.

RNA polymerases

A. Eukaryotes:

- RNA polymerase I makes rRNA (most numerous RNA, rampant).
- RNA polymerase II makes mRNA (largest RNA, massive).
- RNA polymerase III makes 5S rRNA, tRNA (smallest RNA, tiny).
- No proofreading function, but can initiate chains. RNA polymerase II opens DNA at promoter site.
- I, II, and III are numbered in the same order that their products are used in protein synthesis: rRNA, mRNA, then tRNA.
- α-amanitin, found in Amanita phalloides (death cap mushrooms), inhibits RNA polymerase II (halting mRNA synthesis). Causes severe hepatotoxicity if ingested.
- Actinomycin D inhibits RNA polymerase in both prokaryotes and eukaryotes.

B. Prokaryotes:

- 1 RNA polymerase (multisubunit complex) makes all 3 kinds of RNA.
- Rifampin inhibits DNA-dependent RNA polymerase in prokaryotes.

Comparison of RNA Polymerases

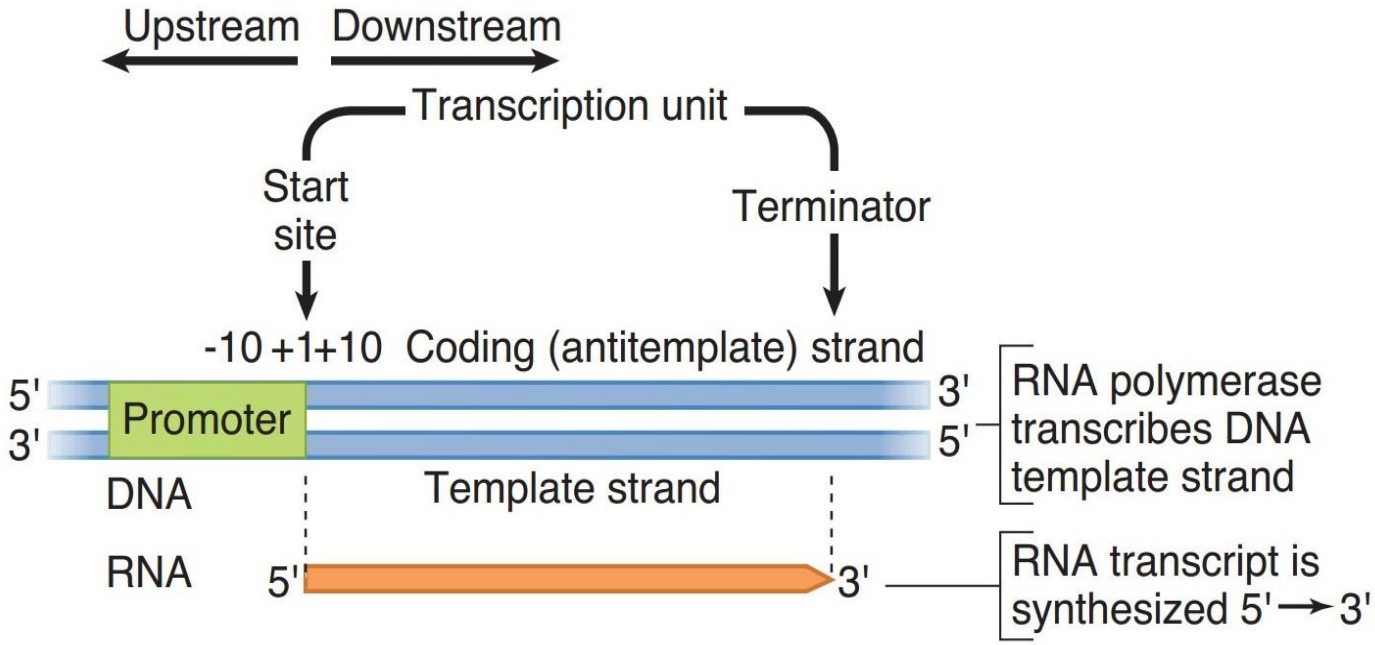
Prokaryotic	Eukaryotic
Single RNA polymerase (α_2 ββ')	RNAP 1: rRNA (nucleolus) Except 5S rRNA RNAP 2: hnRNA/mRNA and some snRNA RNAP 3: tRNA, 5S rRNA
Requires sigma (σ) to initiate at a promoter	No sigma, but transcription factors (TFIID) bind before RNA polymerase
Sometimes requires rho (ρ) to terminate	No rho required
Inhibited by rifampin Actinomycin D	RNAP 2 inhibited by α-amanitin (mushrooms) Actinomycin D

Important concepts and terminology

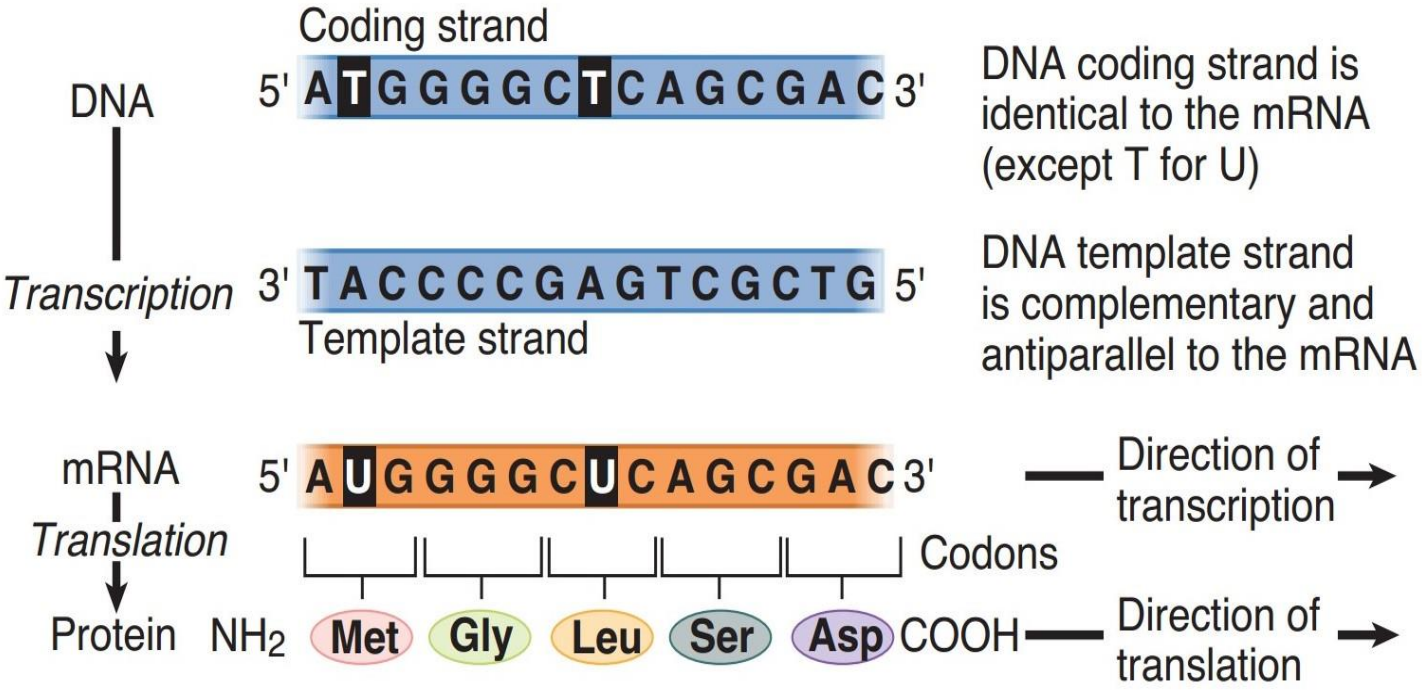
- RNA is synthesized by a DNA-dependent RNA polymerase (uses DNA as a template for the synthesis of RNA).
- RNA polymerase locates genes in DNA by searching for promoter regions.
- The promoter is the binding site for RNA polymerase. Binding establishes where transcription begins, which strand of DNA is used as the template, and in which direction transcription proceeds. No primer is required.
- Though promoters are not directly translated into protein, promoter mutations can cause abnormal gene expression by altering the ability of RNA polymerase II and transcription factors to bind.
- RNA polymerase moves along the template strand in the 3′ to 5′ direction as it synthesizes the RNA product in the 5′ to 3′ direction using NTPs (ATP, GTP, CTP, UTP) as substrates. RNA polymerase does not proofread its work. The RNA product is complementary and antiparallel to the template strand.
- The coding (antitemplate) strand is not used during transcription. It is identical in sequence to the RNA molecule, except that RNA contains uracil instead of the thymine found in DNA.
- By convention, the base sequence of a gene is given from the coding strand (5′→3′).
- In the vicinity of a gene, a numbering system is used to identify the location of important bases. The first base transcribed as RNA is defined as the +1 base of that gene region:

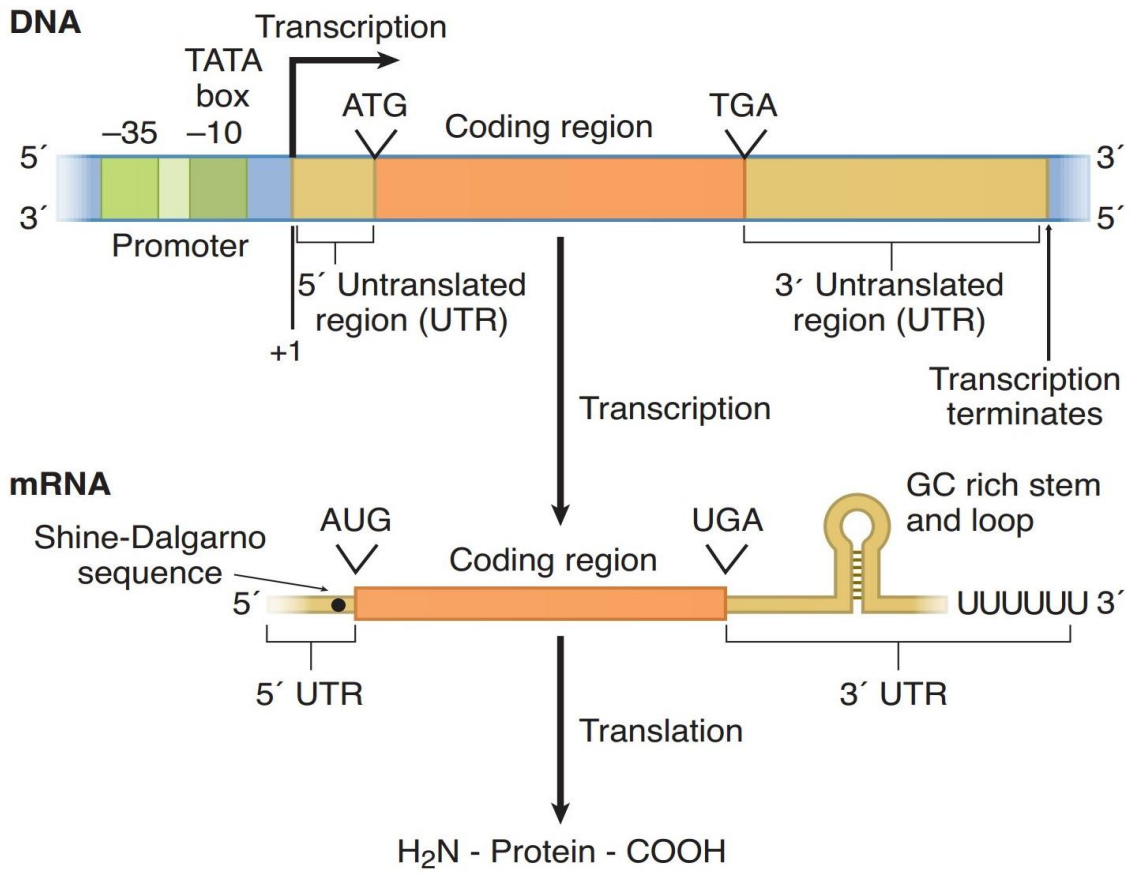
- To the left (5′, or upstream) of this starting point for transcription, bases are –1, –2, –3, etc.
- To the right (3′, or downstream) of this point, bases are +2, +3, etc.

- Transcription ends when RNA polymerase reaches a termination signal.

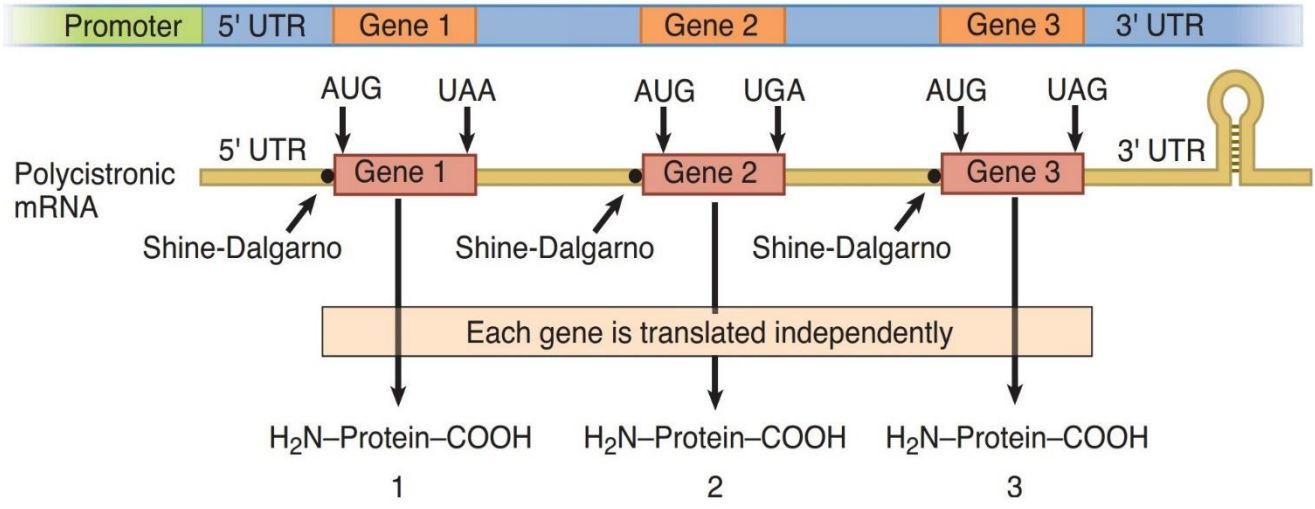


- Messenger RNA is synthesized in the 5′ to 3′ direction. It is complementary and antiparallel to the template strand of DNA. The ribosome translates the mRNA in the 5′ to 3′ direction, as it synthesizes the protein from the amino to the carboxyl terminus.





Expression of a Prokaryotic Protein Coding Gene



Polycistronic Gene Region Codes for Several Different Proteins

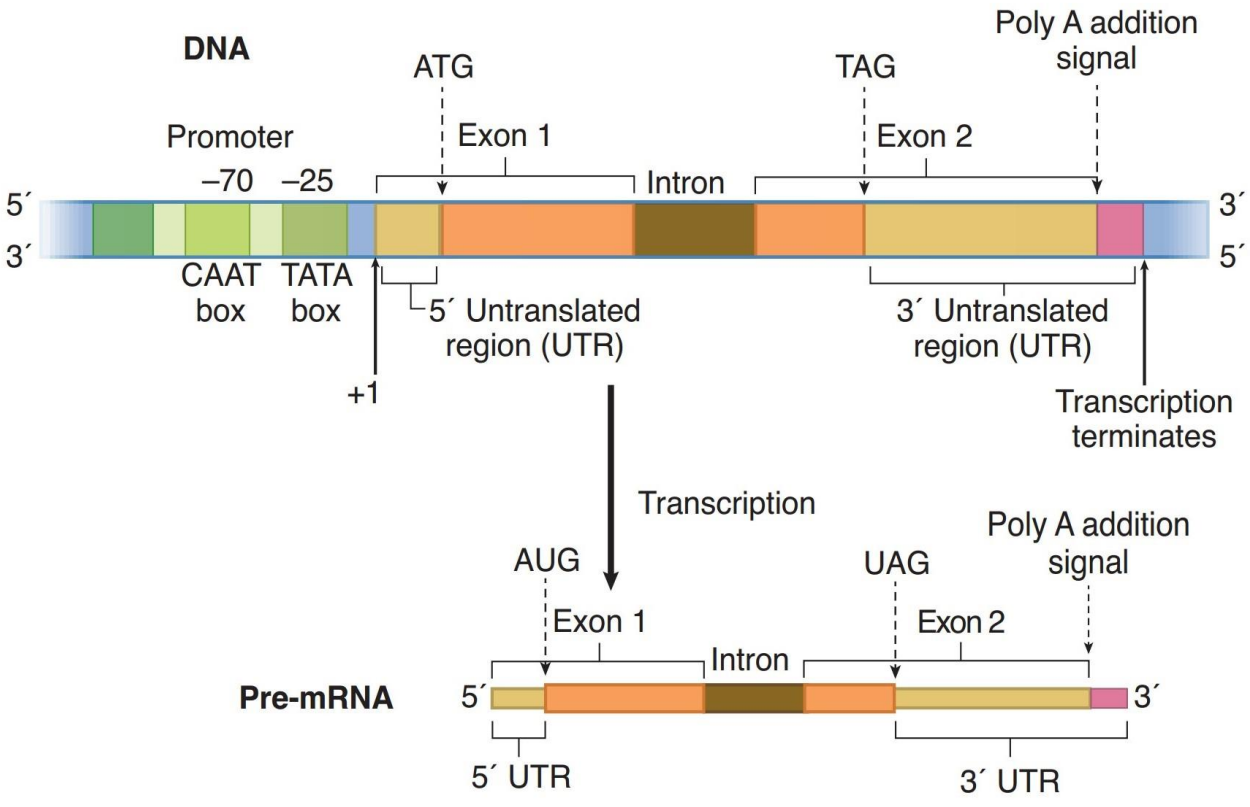
Production of Eukaryotic mRNA

- In eukaryotes, most genes are composed of coding segments (exons) interrupted by noncoding segments (introns). Both exons and introns are transcribed in the nucleus. Introns are removed during processing of the RNA molecule in the nucleus. In eukaryotes, all mRNA is monocistronic. The mature mRNA is translated in the cytoplasm.

- With the help of proteins called transcription factors, RNA polymerase II recognizes and binds to the promoter region. The basal promoter region of eukaryotic genes usually has two consensus sequences called the TATA box and the CAAT box.

- RNA polymerase II separates the strands of the DNA over a short region to initiate transcription and read the DNA sequence. The template strand is read in the 3′ to 5′ direction as the RNA product (the primary transcript) is synthesized in the 5′ to 3′ direction. Both exons and introns are transcribed.

- RNA polymerase II ends transcription when it reaches a termination signal.



A Eukaryotic Transcription Unit

Processing of Eukaryotic Pre-Messenger RNA

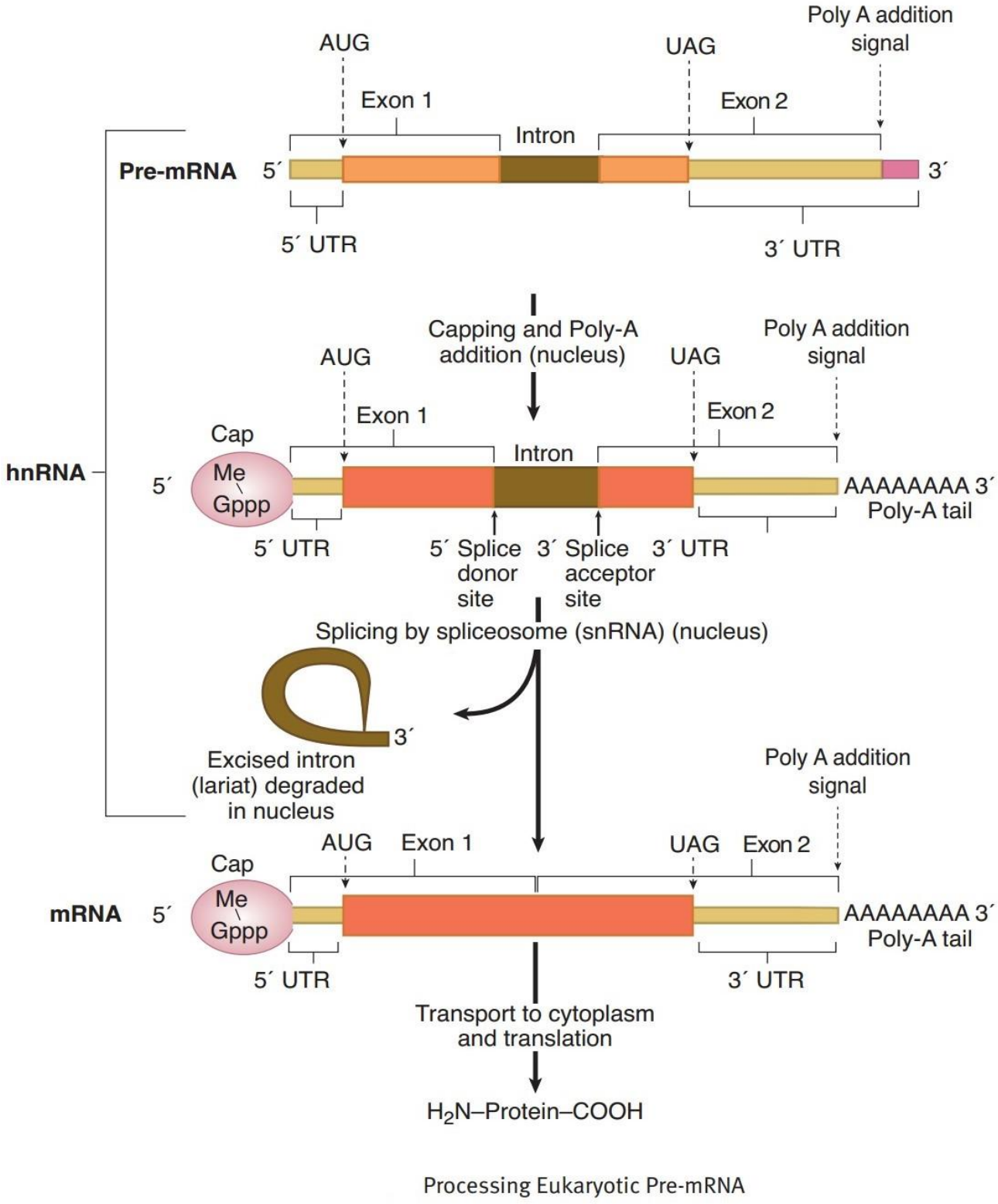
- The primary transcript must undergo extensive posttranscriptional processing inside the nucleus to form the mature mRNA molecule. These processing steps include the following:

1. A 7-methylguanosine cap is added to the 5′ end: while the RNA molecule is still being synthesized. The cap structure serves as a ribosome-binding site and also helps to protect the mRNA chain from degradation.

2. A poly-A tail is attached to the 3′ end: In this process, poly-A polymerase adds the poly-A tail (about 200 As) to the new 3′ end. The poly-A tail protects the message against rapid degradation and aids in its transport to the cytoplasm.

3. Introns are removed from hnRNA by splicing: accomplished by spliceosomes (also known as an snRNP, or snurp), which are complexes of snRNA and protein. The hnRNA molecule is cut at splice sites at the 5′ (donor) and 3′ (acceptor) ends of the intron. The intron is excised in the form of a lariat structure and degraded. Neighboring exons are joined together to assemble the coding region of the mature mRNA.

- Capped, tailed, and spliced transcript is called mRNA.
- The mature mRNA molecule is transported to the cytoplasm, where it is translated to form a protein.
- Antibodies to spliceosomal snRNPs (anti-Smith antibodies) are highly specific for SLE. Anti-U1 RNP antibodies are highly associated with mixed connective tissue disease (MCTD).
- Mutations in splice sites can lead to abnormal proteins. For example, mutations that interfere with proper splicing of β-globin mRNA are responsible for some cases of β-thalassemia.



Processing Eukaryotic Pre-mRNA

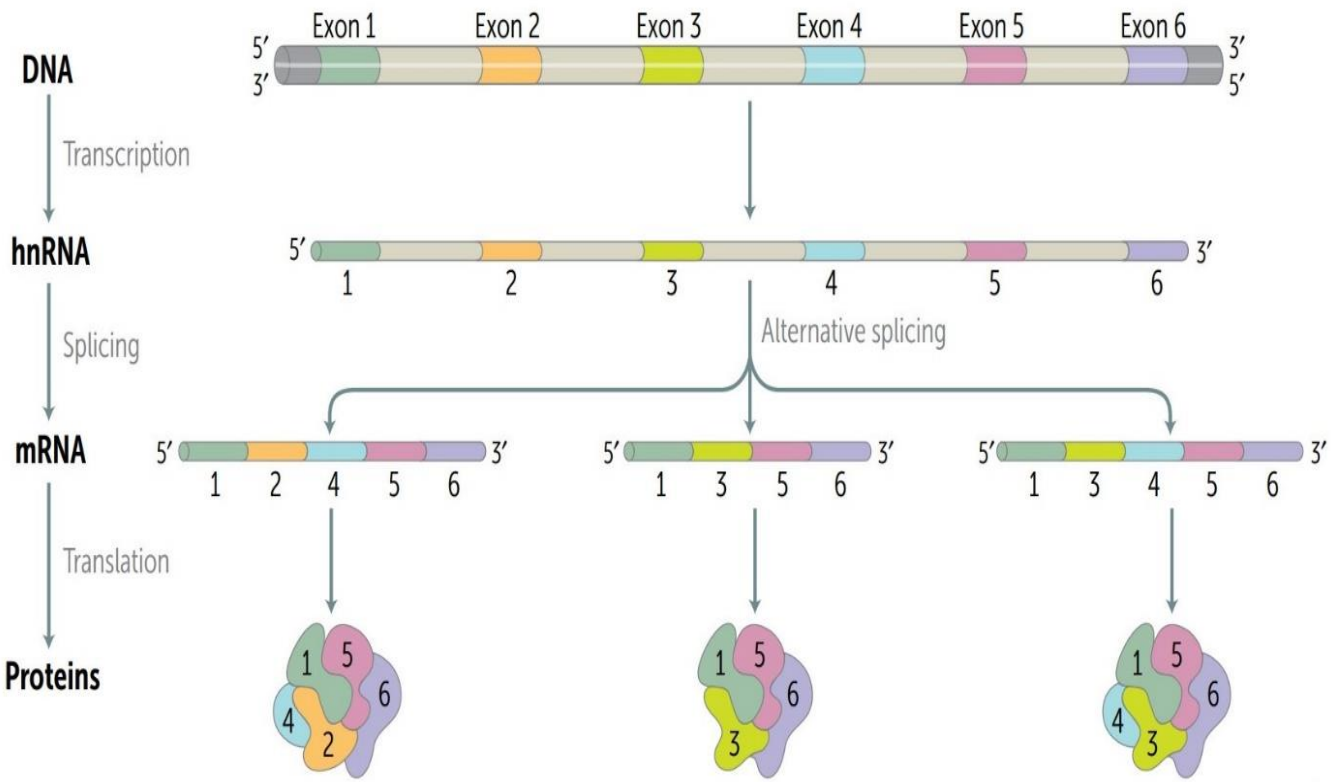
Alternative splicing of Eukaryotic primary pre-mRNA transcript

- For some genes, the primary transcript is spliced differently to produce two or more variants of a protein from the same gene. This process is known as alternative splicing. Variants of the muscle proteins tropomyosin and troponin T are produced in this way.

- The primary transcripts from a large percentage of genes undergo alternative splicing. This may occur within the same cell, or the primary transcript of a gene may be alternatively spliced in different tissues, giving rise to tissue-specific protein products. By alternative splicing, an organism can make many more different proteins than it has genes to encode.

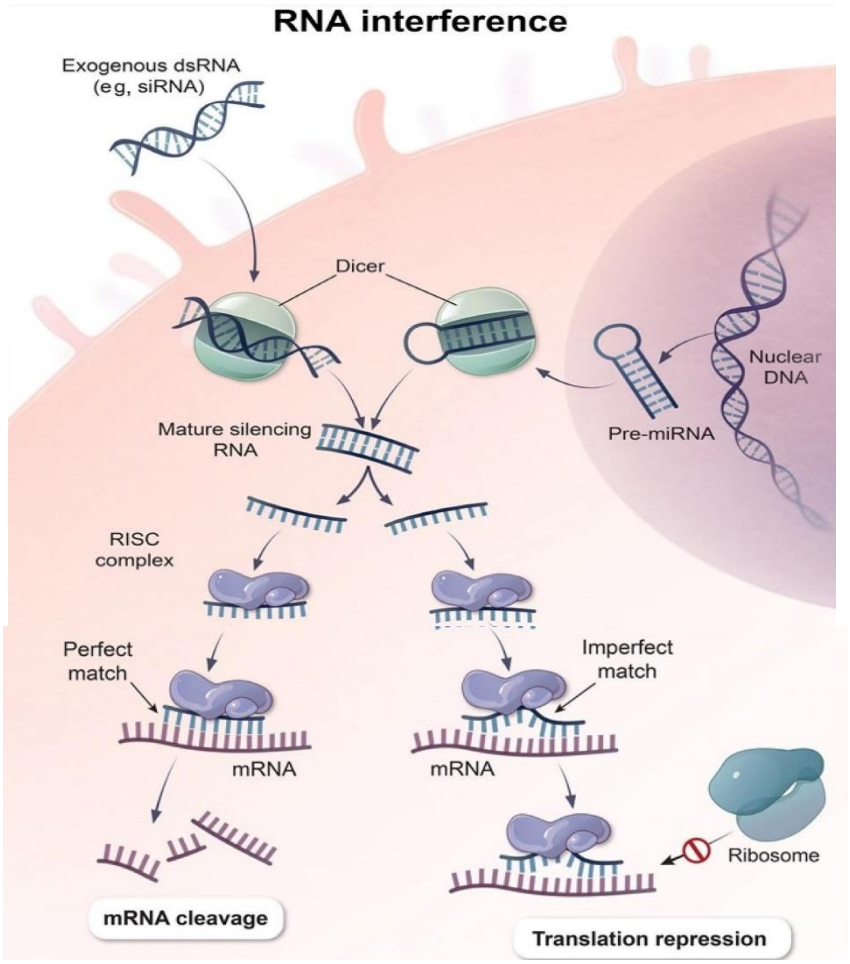
- It has also been implicated in various human diseases. Cancers in particular can use alternative splicing to evade innate defense mechanisms. The Fas receptor-Fas ligand interaction drives programmed cell death via the cytotoxic T-cell mediated extrinsic pathway.

- Cancer cells may develop the ability to splice out a particular exon that codes for the transmembrane domain of the Fas receptor (FasR), converting it to a soluble form that is not expressed on the cell surface, which allows the cells to evade apoptosis.



❖ N.B:

- Once mRNA is finalized, it leaves the nucleus bound to specific packaging proteins.
- Upon entering the cytoplasm, these mRNA complexes often associate with ribosomes to undergo translation. However, certain mRNA sequences instead associate with proteins that are found in P bodies.
- P bodies are distinct foci found within eukaryotic cells that are involved in mRNA regulation and turnover. They play a fundamental role in translation repression and mRNA decay, and contain numerous proteins including RNA exonucleases, mRNA decapping enzymes, and constituents involved in mRNA quality control and microRNA-induced mRNA silencing.
- P bodies also seem to function as a form of mRNA storage, as certain mRNAs are incorporated into P bodies only to be later released and utilized for protein translation.
- RNA interference is an important mechanism by which short (20-30 base pair) non-coding RNA sequences induce posttranscriptional gene silencing.
- Types of silencing RNA include small interfering RNA (siRNA) and microRNA (miRNA).
- MicroRNAs (miRNA) are small, conserved, noncoding RNA molecules that post-transcriptionally regulate gene expression by targeting the 3' untranslated region of specific mRNAs for degradation or translational repression.
- Altered expression of even a few miRNA genes can lead to cellular dysregulation and has been implicated in the development of many diseases. Including hematologic and solid malignancies. In addition, synthetic siRNA sequences can be introduced into cells to silence specific pathogenic genes (c-Myc oncogene) and are being explored as possible therapeutic agents.



Regulation of gene expression

A. Promoter Site:

- Where RNA polymerase II and multiple other transcription factors bind to DNA upstream from gene locus (AT-rich upstream sequence with TATA and CAAT boxes).

- Promoter mutation commonly results in dramatic ↓ in level of gene transcription.

B. Enhancer: DNA locus where regulatory proteins ("activators") bind → increasing expression of a gene on the same chromosome.

C. Silencer:

- DNA locus where regulatory proteins ("repressors") bind → decreasing expression of a gene on the same chromosome.

- Enhancers and silencers may be located close to, far from, or even within (in an intron) the gene whose expression it regulates (variable locations).

Lac operon

- The lac operon consists of a regulatory gene (lac I), a promoter region (lac p), an operator region (lac o), and three structural genes (lac Z, lac Y, and lac A).

- The lac Z gene codes for β-galactosidase, which is responsible for the hydrolysis of lactose to glucose and galactose.

- The lac Y gene codes for permease, which allows lactose to enter the bacterium.

- The lac p (promotor region) is the binding site for RNA polymerase during the initiation of transcription.

- The Lac I repressor protein is the product of the lac I gene and is constitutively expressed. Repressor proteins, when bound to the operator region, prevent binding of RNA polymerase to the promoter region, thus decreasing transcription of the lac Z, lac Y, and lac A genes.

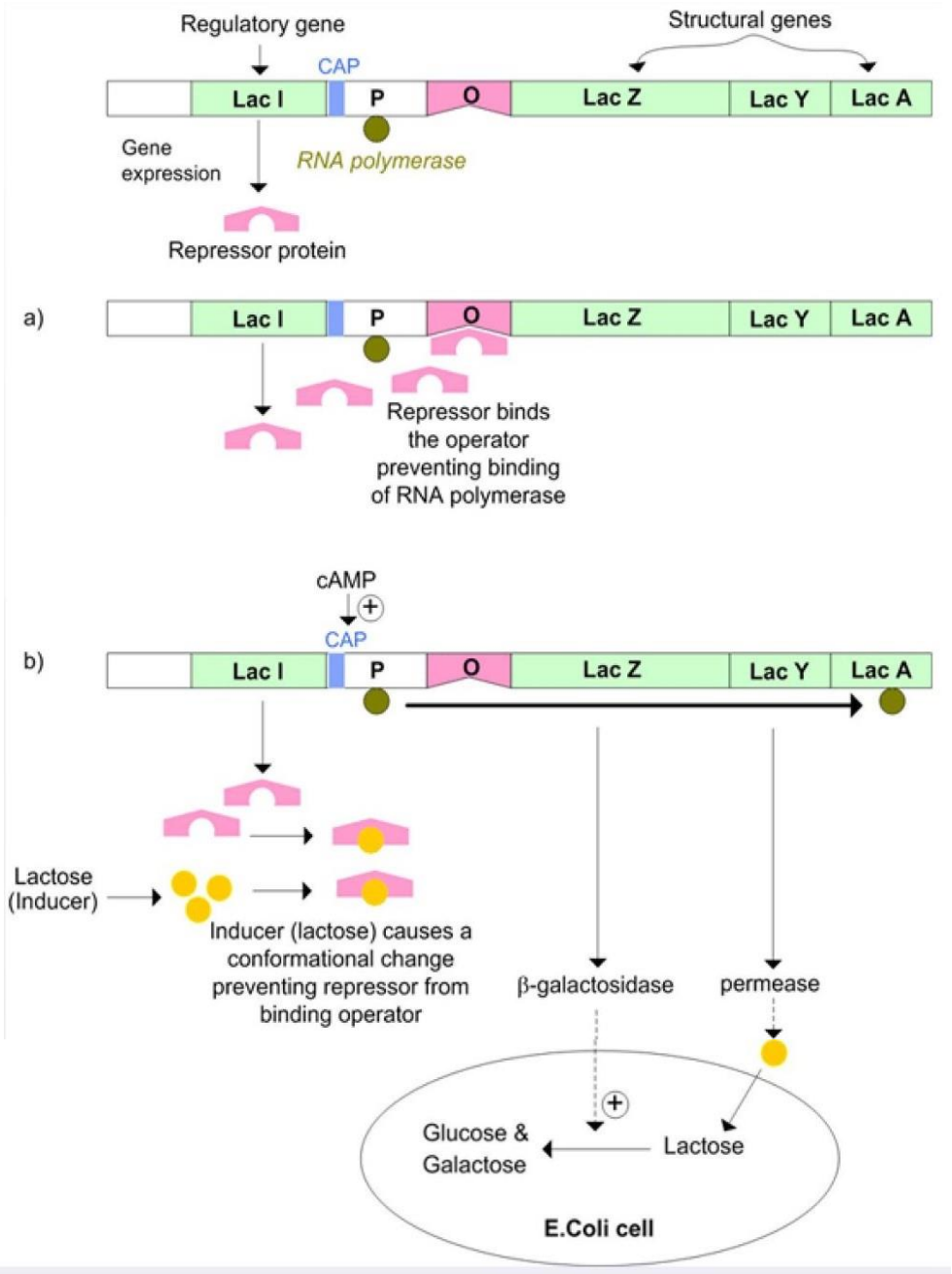
- Culture of E coli in lactose-containing media causes a conformational change in the repressor protein, preventing its attachment to the operator region and increasing transcription of the lac operon structural genes.

- Culturing E coli in media containing glucose results in reduced expression of the lac operon, even when the media contains lactose as well. This occurs because the lac operon is positively regulated by the binding of catabolite activator protein (CAP) to a site slightly upstream from the promoter region. This

only occurs when cAMP concentrations are high. Since glucose decreases the activity of adenylyl cyclase (reducing intracellular cAMP), the lac operon is repressed in high-glucose conditions.

- In summary, the lac operon is regulated by 2 distinct mechanisms:

- Negatively by binding of the repressor protein to the operator locus.
- Positively by cAMP-CAP binding upstream from the promoter region.
- High lactose → unbinds repressor protein from repressor/operator site → ↑ transcription.
- Low glucose → ↑ adenylate cyclase activity → ↑ generation of cAMP from ATP → activation of catabolite activator protein (CAP) → ↑ transcription.



tRNA

- Structure:

- 75-90 nucleotides, 2º structure, cloverleaf form, anticodon end is opposite 3' aminoacyl end. All tRNAs, both eukaryotic and prokaryotic, have CCA at 3' end along with a high percentage of chemically modified bases.

- The amino acid is covalently bound to the 3' end of the tRNA. CCA Can Carry Amino acids.

- T-arm: contains the TΨC (ribothymidine, pseudouridine, cytidine) sequence necessary for tRNA-ribosome binding. T-arm Tethers tRNA molecule to ribosome.

- D-arm: contains dihydrouridine residues necessary for tRNA recognition by the correct aminoacyl-tRNA synthetase. D-arm Detects the tRNA by aminoacyl-tRNA synthetase.

- The anticodon: is located on the opposite end of the tRNA molecule. It recognizes and binds the mRNA codon and assures placement of the proper amino acid in the growing polypeptide chain.

- Acceptor stem: the 5'-CCA-3' is the amino acid acceptor site.

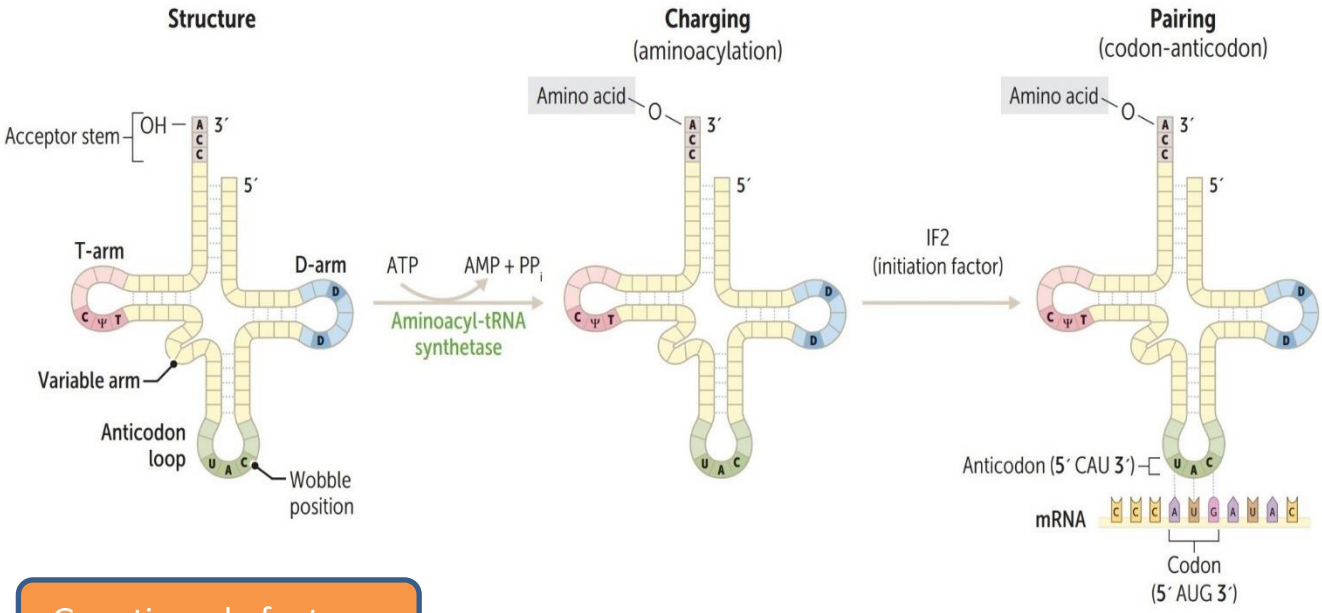
- Charging:

- Amino acid activation and attachment to the 3' end of tRNA is catalyzed by aminoacyl-tRNA synthetases (AA-tRNA synthetases, uses ATP).

- Each amino acid/tRNA pair has a specific AA-tRNA synthetase that links them together. These enzymes are responsible for ensuring that each amino acid binds to the tRNA with the proper anticodon.

- Aminoacyl-tRNA synthetase activation and binding sites are highly specific for their correct amino acids and tRNA molecules. Additionally, some AA-tRNA synthetases can "proofread" their specific tRNA molecules and hydrolyze the amino acid bond when their tRNAs are incorrectly charged. The error rate for AA-tRNA synthetases is thus very low at less than 1 error per 10 charges.

- The sequence of amino acids in a growing polypeptide chain is dictated by the interaction of the mRNA codon with the tRNA anticodon. tRNA that is mischarged with the incorrect amino acid (and not corrected by AA-tRNA synthetase proofreading) will incorporate the wrong amino acid into the growing polypeptide chain, as there is no amino acid proofreading during protein translation.



Genetic code features

- Unambiguous: Each codon specifies only 1 amino acid.
- Degenerate/redundant: Most amino acids are coded by multiple codons.
- Commaless/nonoverlapping: Read from a fixed starting point as a continuous sequence of bases.
- Universal: Genetic code is conserved throughout evolution.

❖ N.B:

1. There are 64 codons in the genetic code, the majority of which code for amino acids. Because there are only 20 amino acids, most amino acids have more than one codon. For example, GUU, GUC. GUA and GUG all code for valine. In addition, there are codons that call for the initiation and termination of protein synthesis.

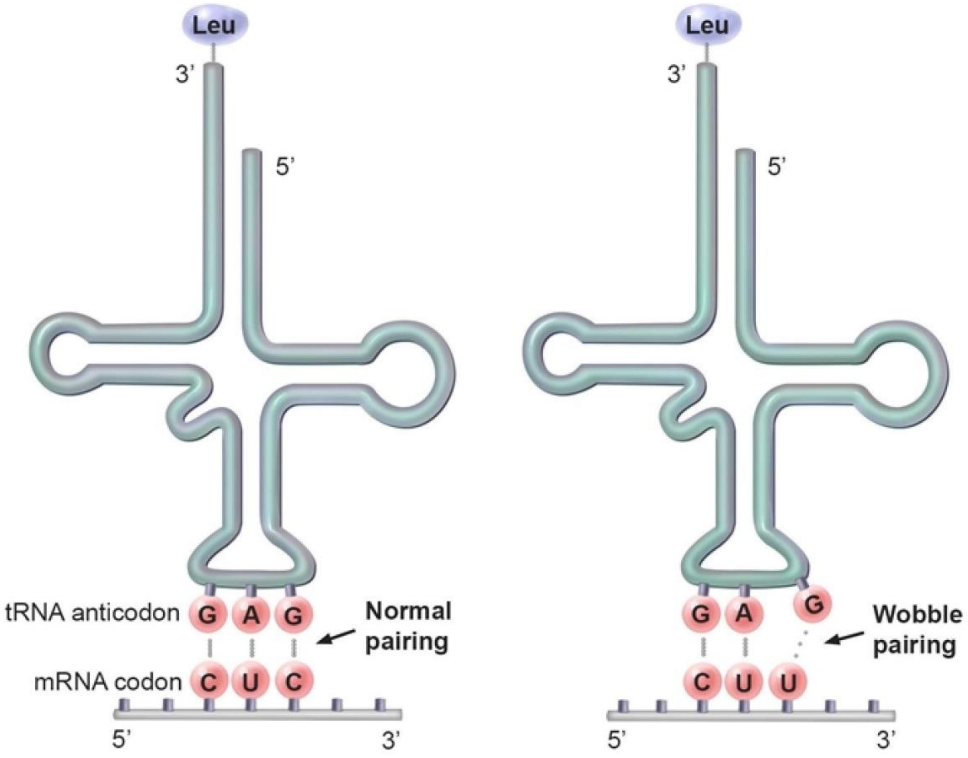
- AUG, which codes for methionine, is the universal start codon. UAA, UAG and UGA are stop codons.
- The stop codons do not code for amino acids. Instead, when the ribosome encounters a stop codon, releasing factors bind to the ribosome and stimulate release of the formed polypeptide chain and dissolution of the ribosome-mRNA complex.

2. There are 61 codons that code for amino acids, but only 20 amino acids used in protein synthesis. The genetic code is thus considered "degenerate" because more than one codon can code for a particular amino acid. For instance, the codons GGU, GGC, GGA and GGG all correspond to the amino acid glycine

- Individual tRNA molecules are specific for certain amino acids and recognize the mRNA codons corresponding to that amino acid.
- Because of the degeneracy of the code, certain tRNA molecules can recognize multiple different codons coding for the same amino acid, a phenomenon explained by the "wobble" hypothesis.

First Position (5' End)	Second Position U	Second Position C	Second Position A	Second Position G	Third Position (3' End)
U	UUU, UUC: Phe; UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr; UAA, UAG: Stop	UGU, UGC: Cys; UGA: Stop; UGG: Trp	U C A G
C	CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His; CAA, CAG: Gln	CGU, CGC, CGA, CGG: Arg	U C A G
A	AUU, AUC, AUA: Ile; AUG: Met	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn; AAA, AAG: Lys	AGU, AGC: Ser; AGA, AGG: Arg	U C A G
G	GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp; GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly	U C A G

Wobble hypothesis



Protein Translation

- Translation occurs in the cytoplasm of both prokaryotic (Pr) and eukaryotic (Eu) cells.
- The process of protein synthesis occurs in 3 stages: initiation, elongation, and termination.
- Special protein factors for initiation (IF), elongation (EF), and termination (release factors), as well as GTP, are required for each of these stages.

A. Initiation:

- Initiated by GTP hydrolysis.
- Eukaryotic ribosomal subunits are 60S and 40S. They join during protein synthesis to form the whole 80S ribosome.
- The large and small prokaryotic ribosomal subunits are 50S and 30S, respectively. The complete prokaryotic ribosome is a 70S particle.

Eukaryotes: 40S + 60S → 80S (Even).

PrOkaryotes: 30S + 50S → 70S (Odd).

- The small ribosomal subunit binds to the mRNA:
  - In prokaryotes, the small subunit binds to the Shine-Dalgarno sequence in the 5' untranslated region of the mRNA.
  - In eukaryotes, the small subunit binds to the 5' cap structure.
- The charged initiator tRNA becomes bound to the AUG start codon on the message through base pairing with its anticodon. The initiator tRNA in prokaryotes carries f-met, whereas the initiator tRNA in eukaryotes carries Met.
- The large subunit binds to the small subunit, forming the completed initiation complex.
- There are 3 important binding sites on the ribosome called the P site, the A site and the E site:
  - The peptidyl site (P site) is the site on the ribosome where (f)met-tRNA initially binds. After formation of the first peptide bond, the P site is a binding site for the growing peptide chain.
  - The aminoacyl site (A site) binds each new incoming tRNA molecule carrying an activated amino acid.
  - E site: holds Empty tRNA as it Exits.

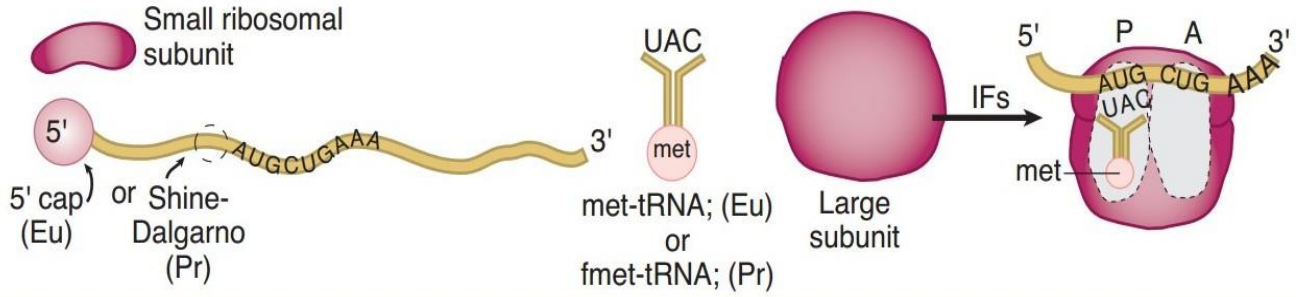
B. Elongation:

- Aminoacyl-tRNA binds to A site (except for initiator methionine).
- rRNA ("ribozyme") catalyzes peptide bond formation, transfers growing polypeptide to amino acid in A site.
- Ribosome advances 3 nucleotides toward 3' end of mRNA, moving peptidyl tRNA to P site (translocation).
- Each cycle uses 4 high-energy bonds (2 from the ATP used in amino acid activation to charge the tRNA, and 2 from GTP).
- ATP: tRNA Activation (charging).
- GTP: tRNA Gripping and Going places (translocation).

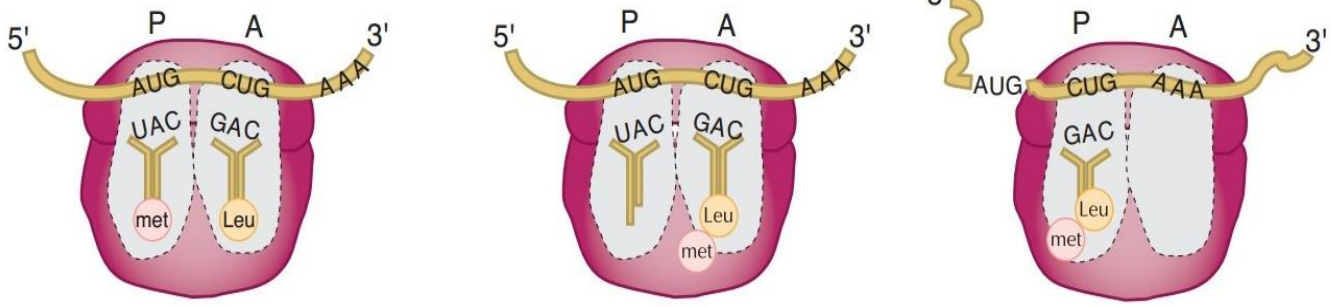
C. Termination:

- Eukaryotic release factors (eRFs) recognize the stop codon and halt translation → completed polypeptide is released from ribosome.
- Requires GTP.

INITIATION



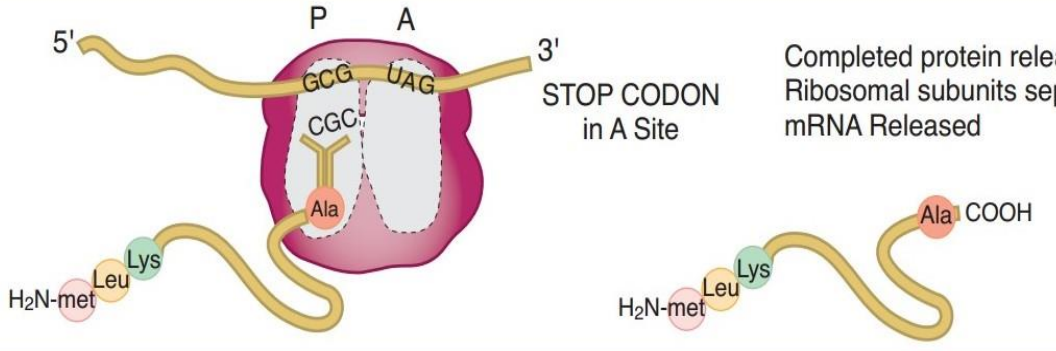
ELONGATION



1. Aminoacyl-tRNA binds to A site
   GTP
   EF-TU and EFTS (Pr)
   eEF-1(Eu)
   Shiga toxin inhibits (cuts 28S rRNA)
2. Peptide bond forms. Peptidyl transferase in large subunit
3. Translocation of ribosome 3 nucleotides along the mRNA
   GTP
   EF-G(Pr)
   eEF-2(Eu)
   Pseudomonas and *diphtheria* toxin inhibit (ADP-ribosylation)
   eEF-2

Elongation cycle repeats for each amino acid added

TERMINATION



Posttranslational modifications

A. Trimming: Removal of N- or C-terminal propeptides from zymogen to generate mature protein (trypsinogen to trypsin).

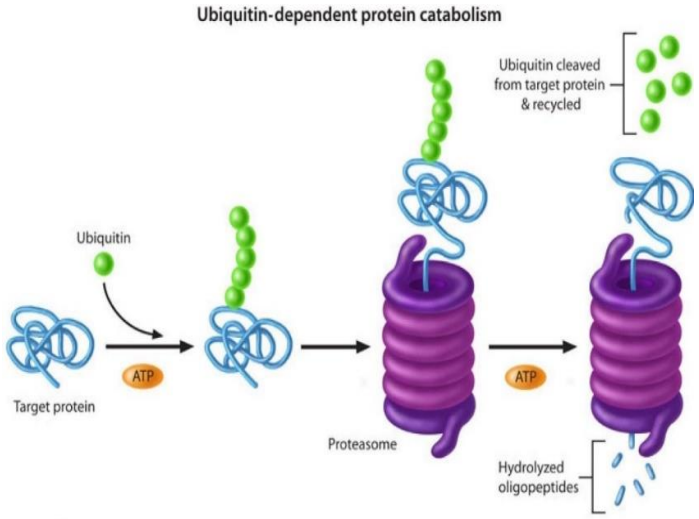
B. Covalent alterations: Phosphorylation, glycosylation, hydroxylation, methylation, acetylation, and ubiquitination.

Chaperone protein

- Intracellular protein involved in facilitating and/or maintaining protein folding.
- For example, in yeast, heat shock proteins (HSP60) are expressed at high temperatures to prevent protein denaturing/misfolding.

Proteasomes and Ubiquitin

- Whenever protein synthesis occurs in a cell, a few copies of a particular protein may not fold correctly. These defective copies are covalently marked for destruction by the addition of multiple copies of ubiquitin.
- Polyubiquinated proteins are directed to proteasomes for destruction. Proteasomes are large, cytoplasmic complexes that have multiple protease activities capable of digesting damaged proteins to peptides.
- Proteasomes also play a role in producing antigenic peptides for presentation by class I MHC molecules.
- Defects in the ubiquitin-proteasome system have been implicated in some cases of Parkinson and Alzheimer's disease.



CHAPTER 2

Cellular Biochemistry

Rough endoplasmic reticulum

- Site of synthesis of secretory (exported) proteins and of N-linked oligosaccharide addition to many proteins.
- Nissl bodies (RER in neurons): synthesize peptide neurotransmitters for secretion.
- Free ribosomes: unattached to any membrane; site of synthesis of cytosolic and organellar proteins.
- Mucus-secreting goblet cells of the small intestine and antibody-secreting plasma cells are rich in RER.

Smooth endoplasmic reticulum

- Site of steroid synthesis and detoxification of drugs and poisons.
- Lacks surface ribosomes.
- Liver hepatocytes and steroid hormone-producing cells of the adrenal cortex and gonads are rich in SER.

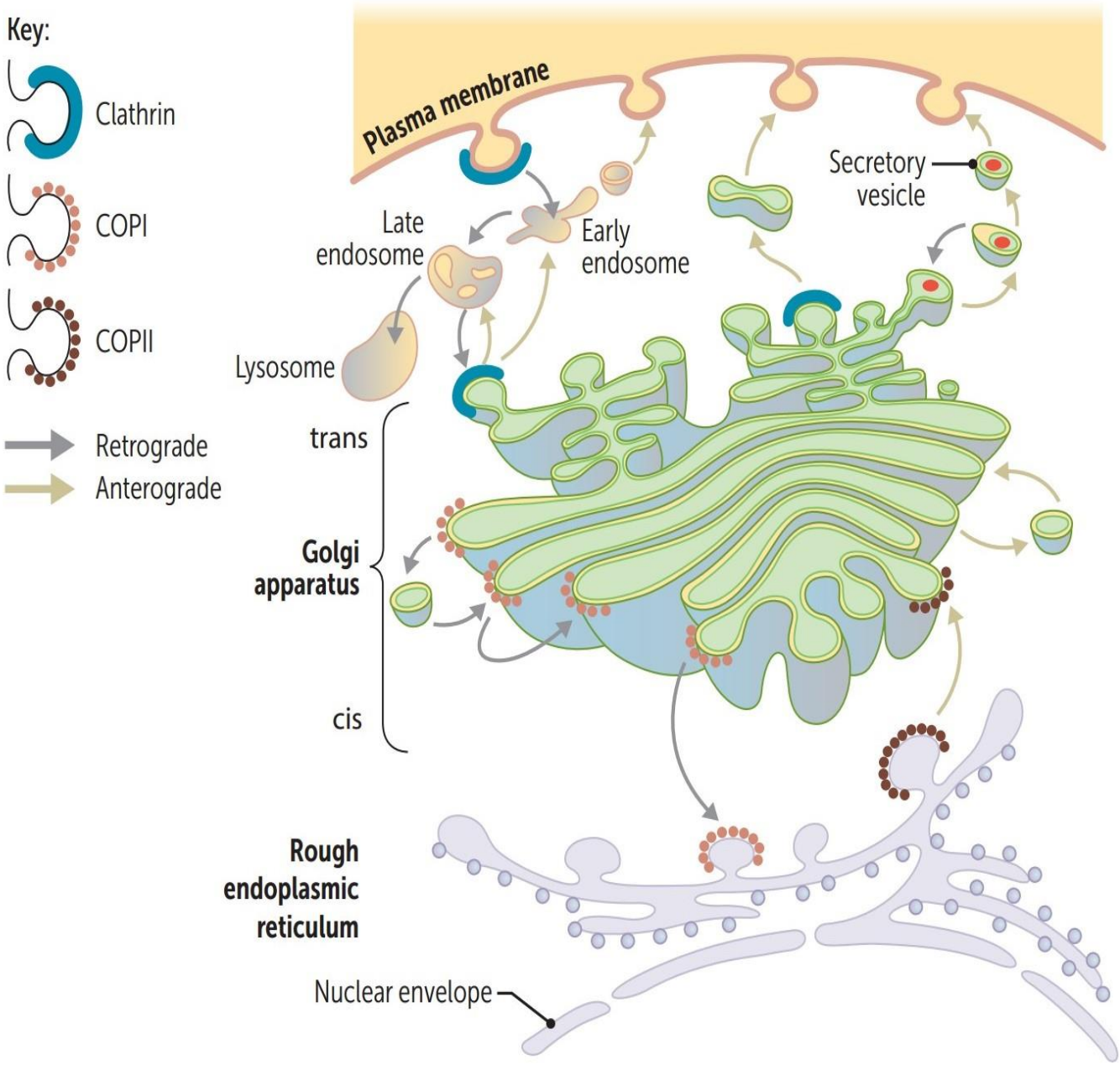
Cell trafficking

- Translation begins on free cytoplasmic ribosomes, but after translation of the signal sequence, the ribosome is positioned on the ER (now RER) with the help of a signal recognition particle.
- Signal recognition particle (SRP): Abundant, cytosolic ribonucleoprotein that traffics proteins from the ribosome to the RER. Absent or dysfunctional SRP → proteins accumulate in the cytosol.
- Golgi is the distribution center for proteins and lipids from the ER to the vesicles and plasma membrane.
- In transit through the ER and Golgi, most proteins acquire oligosaccharide side chains, becoming glycoproteins.
- N-glycosylation refers to the addition of sugar chains to the nitrogen of asparagine residues (N-linked).
- O-glycosylation refers to the addition of sugar chains to the hydroxyl group of either serine or threonine residues of the protein, and it occurs exclusively in the Golgi.
- Lysosomal enzymes are glycosylated and modified in a characteristic way. Most importantly, when they arrive in the Golgi apparatus, specific mannose residues located in their N-linked oligosaccharide chains

are phosphorylated by N-acetylglucosamine-1 phosphotransferase, forming a critical mannose-6-phosphate in the oligosaccharide chain.

- This phosphorylation is the critical event that removes them from the secretion pathway and directs them to lysosomes.

- Genetic defects affecting this phosphorylation produce I-cell disease in which lysosomal enzymes are released into the extracellular space, and inclusion bodies accumulate in the cell, compromising its function.



- Vesicular trafficking proteins:

- COPI: Golgi → Golgi (retrograde); cis-Golgi → ER.

- COPII: ER → cis-Golgi (anterograde).

- "Two (COPII) steps forward (anterograde); one (COPI) step back (retrograde)."

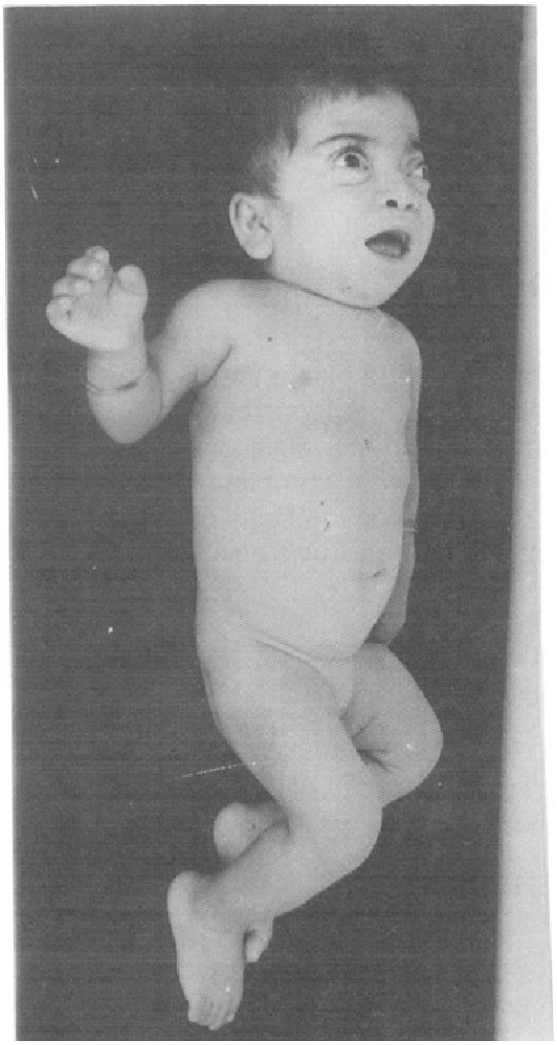
- Clathrin: trans-Golgi → lysosomes; plasma membrane → endosomes (receptor-mediated endocytosis.

❖ I-cell disease (inclusion cell disease/mucolipidosis type II):

- Inherited lysosomal storage disorder; defect in N-acetylglucosaminyl-1-phosphotransferase → failure of the Golgi to phosphorylate mannose residues (mannose-6-phosphate) on glycoproteins → proteins are secreted extracellularly rather than delivered to lysosomes.

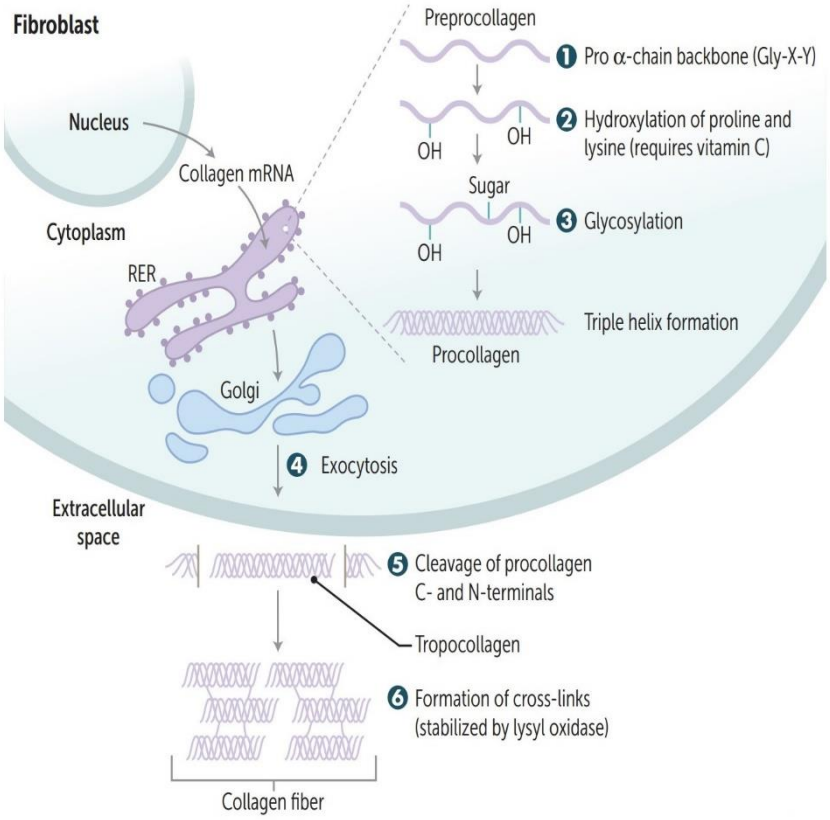
- Results in coarse facial features, clouded corneas, restricted joint movement, claw hand deformities, kyphoscoliosis, and high plasma levels of lysosomal enzymes.

- Often fatal in childhood.



Collagen synthesis and structure

Collagen synthesis and structure



1. **Synthesis**—translation of collagen α chains (preprocollagen)—usually Gly-X-Y (X and Y are proline or lysine). Glycine content best reflects collagen synthesis (collagen is ⅓ glycine).
2. **Hydroxylation**—hydroxylation of specific proline and lysine residues. Requires vitamin C; deficiency → scurvy.
3. **Glycosylation**—glycosylation of pro-α-chain hydroxylysine residues and formation of procollagen via hydrogen and disulfide bonds (triple helix of 3 collagen α chains). Problems forming triple helix → osteogenesis imperfecta.
4. **Exocytosis**—exocytosis of procollagen into extracellular space.
5. **Proteolytic processing**—cleavage of disulfide-rich terminal regions of procollagen → insoluble tropocollagen. Problems with cleavage → Ehlers-Danlos syndrome.
6. **Cross-linking**—reinforcement of many staggered tropocollagen molecules by covalent lysine-hydroxylysine cross-linkage (by copper-containing lysyl oxidase) to make collagen fibrils. Problems with cross-linking → Ehlers-Danlos syndrome, Menkes disease.

- Most abundant protein in the human body. Extensively modified by posttranslational modification. Organizes and strengthens extracellular matrix.

- Types of collage:

A. Type I:

- Most common (90%). Bone (made by osteoblasts), Skin, Tendon, dentin, fascia, cornea, late wound repair.

- Type I: bone.

- ↓ production in osteogenesis imperfecta type I.

B. Type II:

- Cartilage (including hyaline), vitreous body, nucleus pulposus.

- Type II: cartwolage.

C. Type III:

- Reticulin, skin, blood vessels, uterus, fetal tissue, granulation tissue.

- Type III: deficient in the uncommon, vascular type of Ehlers-Danlos syndrome (ThreE D).

D. Type IV:

- Basement membrane, basal lamina, lens.

- Type IV: under the floor (basement membrane).

- Defective in Alport syndrome; targeted by autoantibodies in Goodpasture syndrome.

Osteogenesis Imperfecta

- Genetic bone disorder (brittle bone disease) caused by a variety of gene defects (most commonly COL1A1 and COL1A2).

- Most common form is autosomal dominant with ↓ production of normal type I collagen.

- Manifestations can include:

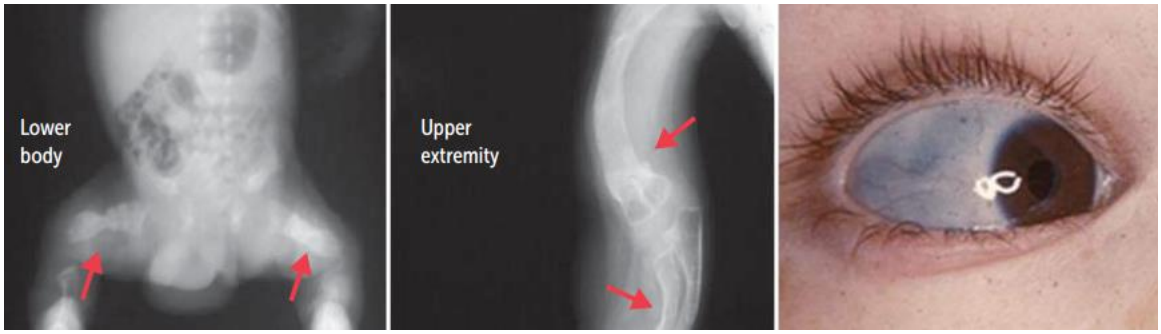
- Multiple fractures with minimal trauma; may occur during the birth process. May be confused with child abuse. Treat with bisphosphonates to ↓ fracture risk.

- Blue sclerae due to the translucent connective tissue over choroidal veins.

- Some forms have tooth abnormalities, including opalescent teeth that wear easily due to lack of dentin (dentinogenesis imperfecta)

- Hearing loss (abnormal ossicles).

- Patients can't BITE:
  - Bones = multiple fractures.
  - I (eye) = blue sclerae.
  - Teeth = dental imperfections.
  - Ear = hearing loss.



Ehlers-Danlos syndrome

- Ehlers-Danlos syndrome is a group of rare hereditary disorders characterized by defective collagen synthesis.
- It can be caused by procollagen peptidase deficiency, which results in impaired cleavage of terminal propeptides in the extracellular space.
- Faulty collagen synthesis causing hyperextensible skin, tendency to bleed (easy bruising), and hypermobile joints.
- Multiple types. Inheritance and severity vary.
- Can be autosomal dominant or recessive.
- Maybe associated with joint dislocation, berry and aortic aneurysms, organ rupture.
- Types:

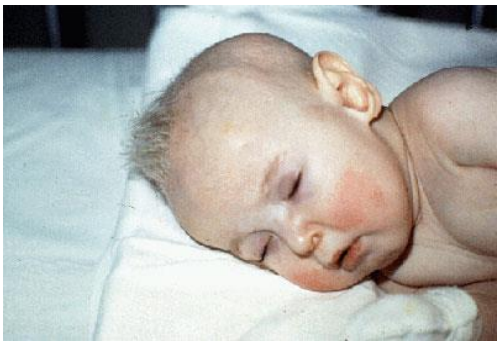
A. Hypermobility type (joint instability): most common type.

B. Classical type (joint and skin symptoms): caused by a mutation in type V collagen (COL5A1, COL5A2).

C. Vascular type (vascular and organ rupture): deficient type III collagen.

Menkes disease

- X-linked recessive connective tissue disease caused by impaired copper absorption and transport due to defective Menkes protein (ATP7A).
- Leads to ↓ activity of lysyl oxidase (copper is a necessary cofactor).
- Results in brittle, "kinky" hair, growth retardation, and hypotonia.

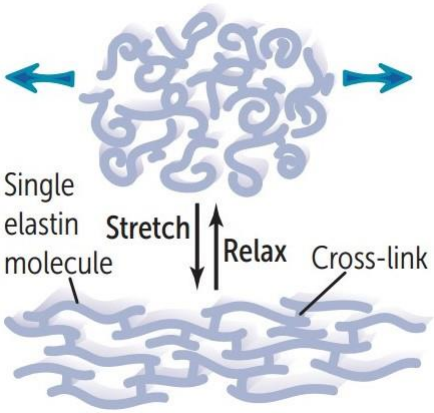


Elastin

- Elastin is a fibrous protein in the connective tissue that gets its name because of its elastic properties.
- Elastin fibers can be stretched to several times their length but recoil back when stretching forces are withdrawn.
- Stretchy protein within skin, lungs, large arteries, elastic ligaments, vocal cords, ligamenta flava (connect vertebrae → relaxed and stretched conformations).
- <u>Elastin differs from collagen in a number of ways:</u>

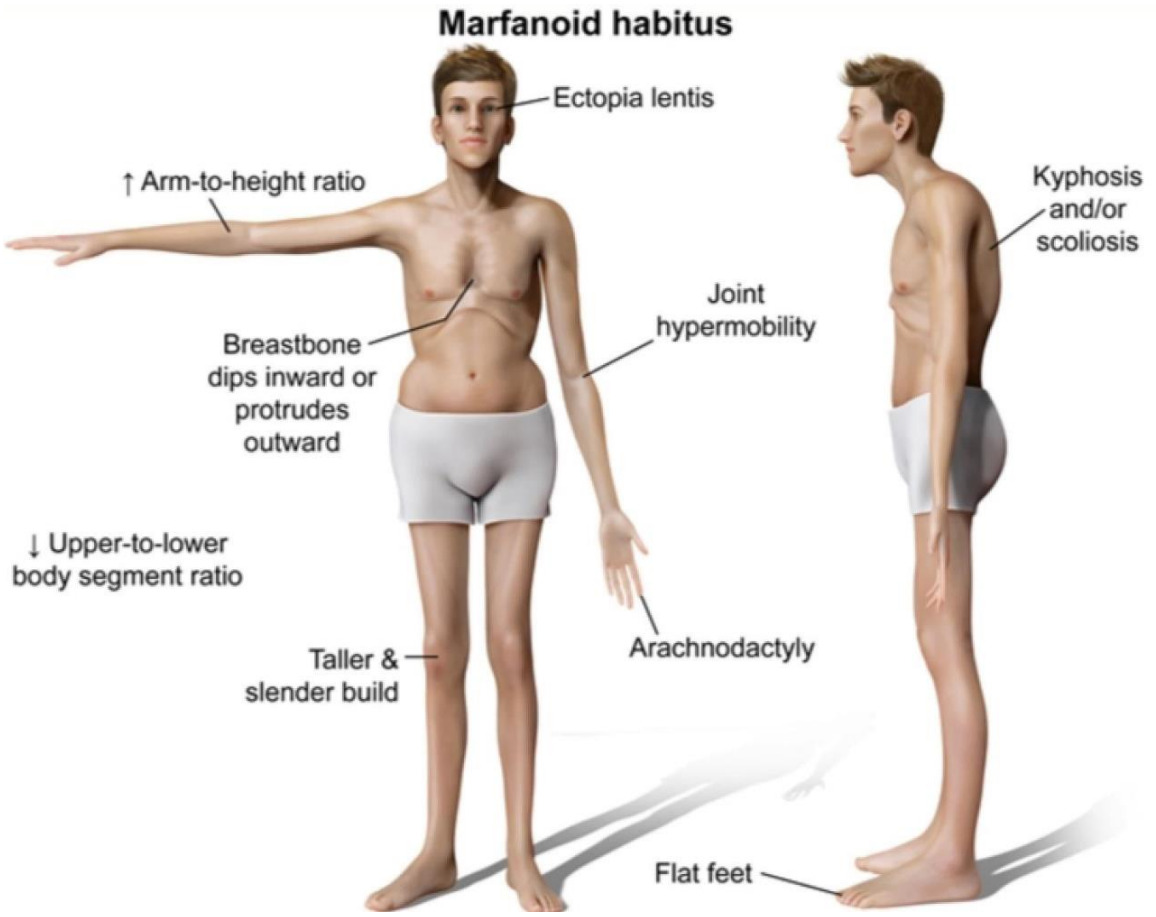
- Very few proline and lysine residues are hydroxylated in elastin.
- Whereas triple helix formation is the basis of the collagen molecule, elastin does not form triple helices.
- Triple helix formation in collagen is initiated by hydroxylation, glycosylation and interchain disulfide bridges at the C-terminus of procollagen molecule. These modifications do not occur in the formation of elastin molecules.

- Elastin's plasticity and ability to recoil upon release of tension is attributable to a unique form of desmosine crosslinking between four different lysine residues on four different elastin chains. This crosslinking is accomplished by the action of extracellular lysyl hydroxylase.
- Cross-linking takes place extracellularly and gives elastin its elastic properties.
- Wrinkles of aging are due to ↓ collagen and elastin production.
- A number of endogenous enzymes called proteinases hydrolyze and destroy such proteins. For elastin, the most important proteinase is neutrophil-secreted elastase. α1-antitrypsin inhibits the action of these endogenous proteolytic enzymes, thereby preventing damage to essential structures within organs. A congenital deficiency of α1-antitrypsin results in excessive degradation of elastin in the lungs and liver, causing panacinar emphysema and cirrhosis, respectively.



Marfan syndrome

- Autosomal dominant connective tissue disorder affecting skeleton, heart, and eyes.
- FBN1 gene mutation on chromosome 15 (fifteen) results in defective fibrillin, a glycoprotein that forms a sheath around elastin.
- Findings: Tall with long extremities; pectus carinatum (more specific) or pectus excavatum; hypermobile joints; long, tapering fingers and toes (arachnodactyly); cystic medial necrosis of aorta; aortic incompetence and dissecting aortic aneurysms; floppy mitral valve. Subluxation of lenses, typically upward and temporally (vs downward and medially in homocystinuria).
- The cardiovascular lesions are the most potentially life-threatening. The two most common cardiac abnormalities are mitral valve prolapse and cystic medial degeneration of the aorta.
- Cystic medial aortic degeneration (Myxomatous changes in the media of large arteries) may lead to aortic dilatation and dissection. Aortic dissection is the cause of death in 30% to 45% of patients with Marfan syndrome, followed by cardiac failure (which may be secondary to mitral and/or aortic regurgitation).
- The average age at death in Marfan syndrome is between 30 and 40 years.



Cytoskeletal elements A network of protein fibers within the cytoplasm that supports cell structure, cell and organelle movement, and cell division.

TYPE OF FILAMENT	PREDOMINANT FUNCTION	EXAMPLES
Microfilaments	Muscle contraction, cytokinesis	Actin, microvilli.
Intermediate filaments	Maintain cell structure	Vimentin, desmin, cytokeratin, lamins, glial fibrillary acid proteins (GFAP), neurofilaments.
Microtubules	Movement, cell division	Cilia, flagella, mitotic spindle, axonal trafficking, centrioles.

Immunohistochemical stains for intermediate filaments

STAIN	CELL TYPE	IDENTIFIES
Vimentin	Mesenchymal tissue (eg, fibroblasts, endothelial cells, macrophages)	Mesenchymal tumors (eg, sarcoma), but also many other tumors (eg, endometrial carcinoma, renal cell carcinoma, meningioma)
DesMin	Muscle	Muscle tumors (eg, rhabdomyosarcoma)
Cytokeratin	Epithelial cells	Epithelial tumors (eg, squamous cell carcinoma)
GFAP	NeuroGlia (eg, astrocytes, Schwann cells, oligodendrocytes)	Astrocytoma, Glioblastoma
Neurofilaments	Neurons	Neuronal tumors (eg, neuroblastoma)

Microtubule

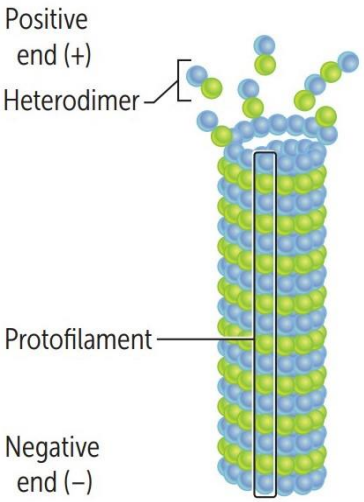
- Cylindrical outer structure composed of a helical array of polymerized heterodimers of α- and β-tubulin. Each dimer has 2 GTP bound.
- Incorporated into flagella, cilia, mitotic spindles.
- Grows slowly, collapses quickly.
- Also involved in slow axoplasmic transport in neurons.
- <u>Molecular motor proteins: transport cellular cargo toward opposite ends of microtubule tracks.</u>

- Dynein: retrograde to microtubule (+ → −).
- Kinesin: anterograde to microtubule (− → +).

- Clostridium tetani toxin, herpes simplex virus, poliovirus, and rabies virus use dynein for retrograde transport to the neuronal cell body.

- Drugs that act on microtubules (Microtubules Get Constructed Very Poorly):

- Mebendazole (antihelminthic).
- Griseofulvin (antifungal).
- Colchicine (antigout).
- Vincristine/Vinblastine (anticancer).
- Paclitaxel (anticancer).



Cilia structure

- 9 doublet + 2 singlet arrangement of microtubules.

- Basal body (base of cilium below cell membrane) consists of 9 microtubule triplets with no central microtubules.

- Axonemal dynein: ATPase that links peripheral 9 doublets and causes bending of cilium by differential sliding of doublets.

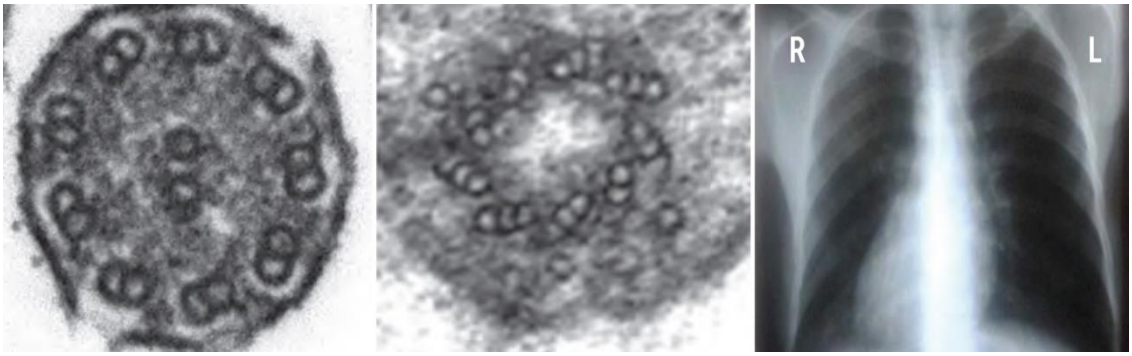
- Kartagener syndrome (1° ciliary dyskinesia):

- Immotile cilia due to a dynein arm defect. This result in impaired cilial function, poor mucociliary clearance of secretions → chronic infections.

- Results in ↓ male and female fertility due to immotile sperm and dysfunctional fallopian tube cilia, respectively; ↑ risk of ectopic pregnancy.

- Can cause bronchiectasis, recurrent sinusitis, chronic ear infections, conductive hearing loss, and situs inversus (dextrocardia on CXR).

- ↓ nasal nitric oxide (used as screening test).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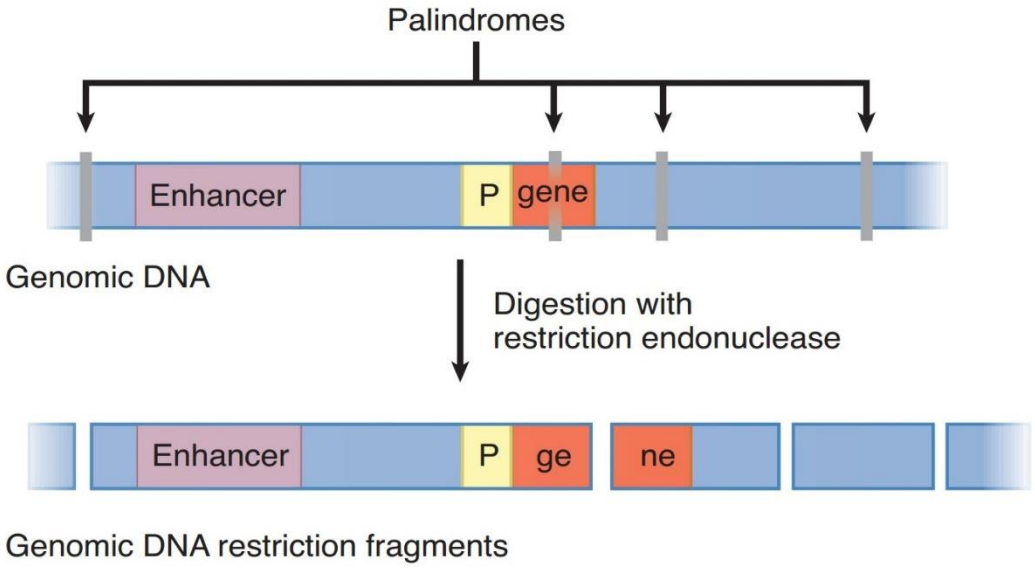
CHAPTER 3

Laboratory Techniques

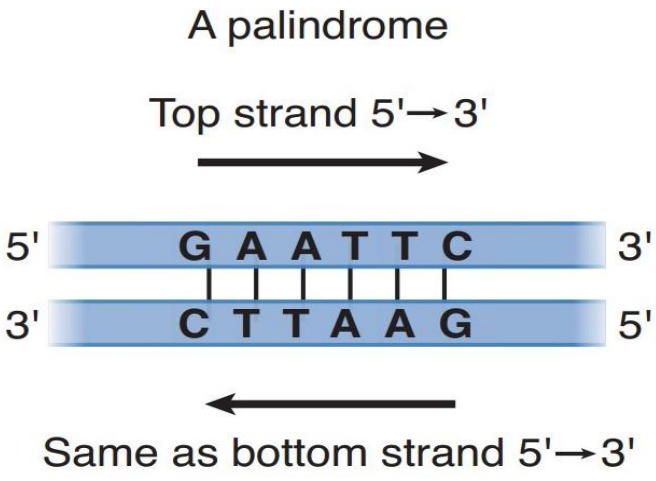
Producing Restriction Fragments: Restriction Endonucleases

- Chromosomes obtained from the DNA donors were cut with restriction endonucleases to produce restriction fragments. These enzymes are isolated from bacteria, their natural source. There are many different restriction endonucleases isolated from a variety of bacteria that are now readily available commercially.



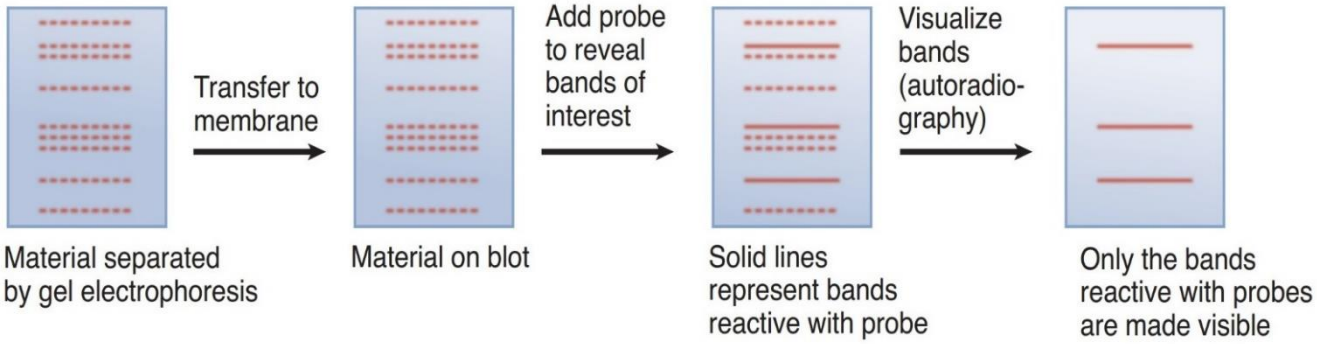
- Restriction endonucleases recognize double-stranded DNA sequences called palindromes (inverted repeats) usually of four to eight base pairs in length. For example, the figure below shows the recognition site for EcoRI, a restriction endonuclease isolated from Escherichia coli.

- A palindrome can be identified by examining the sequence of only one strand. Draw a line through the center of the sequence (through the central base for palindromes with an odd number of nucleotides). If the sequence is folded along this line, the bases should pair.



Blotting techniques

- Blotting techniques have been developed to detect and visualize specific DNA, RNA, and protein among complex mixtures of contaminating molecules. These techniques have allowed the identification and characterization of the genes involved in numerous inherited diseases. The most common types of blots are:



A. Southern blot:

1. DNA sample is enzymatically cleaved into smaller pieces, which are separated on a gel by electrophoresis, the smaller molecules travel faster and appear nearer the bottom of the gel, and then transferred to a filter.

2. Filter is exposed to radiolabeled DNA probe that recognizes and anneals to its complementary strand.

3. Resulting double-stranded, labeled piece of DNA is visualized when filter is exposed to film.

B. Northern blot:

- Similar to Southern blot, except that an RNA sample is electrophoresed.

- Useful for studying mRNA levels, which are reflective of gene expression.

C. Western blot:

- Sample protein is separated via gel electrophoresis and transferred to a membrane.

- Labeled antibody is used to bind to relevant protein.

D. Southwestern blot: Identifies DNA-binding proteins (transcription factors like c-Juna nd c-Fos, Histones) using labeled oligonucleotide probes.

❖ SNoW DRoP:

- Southern = DNA.
- Northern = RNA.
- Western = Protein.

Blotting technique	Substance detected	Type of probe
Northern	RNA	Single-stranded DNA or RNA (hybridization probe)
Southern	DNA	
Western	Protein	Antibody
Southwestern	DNA-binding protein	Double-stranded DNA

Polymerase chain reaction

- Molecular biology lab procedure used to amplify a desired fragment of DNA.
- Useful as a diagnostic tool (neonatal HIV, herpes encephalitis).
- Procedure:

A. Denaturation: DNA is heated to 95°C to separate the strands.

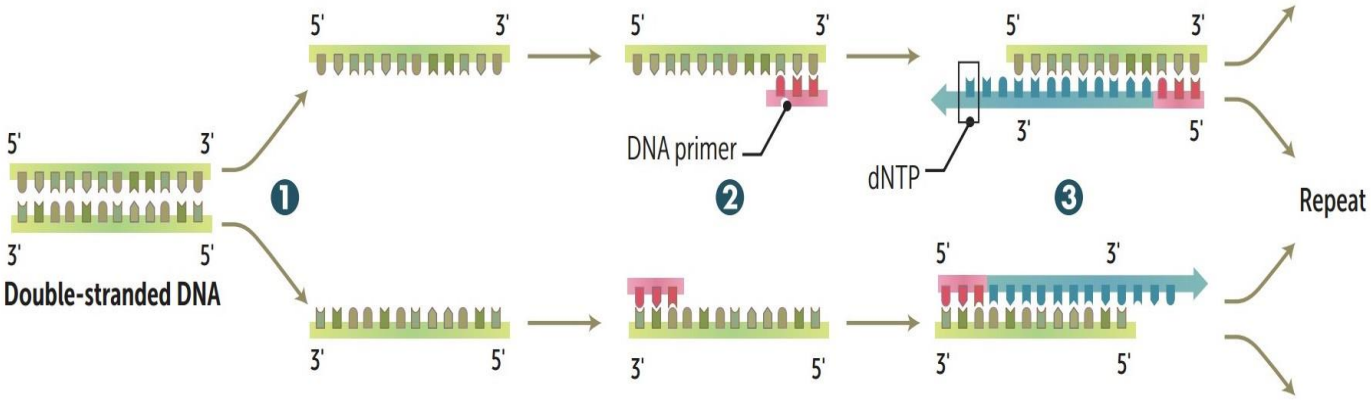
B. Annealing:

- Sample is cooled to 55°C.
- DNA primers, a heat-stable DNA polymerase (Taq), and deoxynucleotide triphosphates (dNTPs) are added.
- It requires primers that are complementary to the regions of DNA flanking the segment of interest.

C. Elongation:

- Temperature is increased to 72°C.
- DNA polymerase attaches dNTPs to the strand to replicate the sequence after each primer.

- Heating and cooling cycles continue until the DNA sample size is sufficient.
- Reverse transcription polymerase chain reaction (RT-PCR): A sample mRNA is converted to complementary DNA (cDNA) by reverse transcriptase → cDNA is amplified by the standard PCR procedure (see above). RT-PCR can be used to diagnose chronic myelogenous leukemia by identifying an mRNA transcript containing both BCR and ABL exons in affected cells.



Molecular cloning

- Production of a recombinant DNA molecule in a bacterial host.

- Steps:

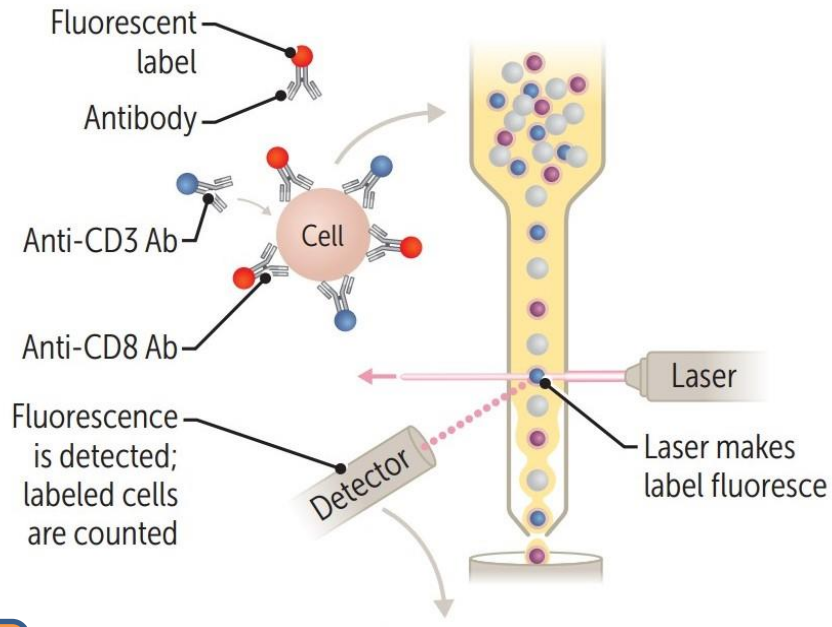
1. Isolate eukaryotic mRNA (post-RNA processing) of interest.
2. Add reverse transcriptase (an RNA-dependent DNA polymerase) to produce complementary DNA (cDNA, lacks introns).
3. Insert cDNA fragments into bacterial plasmids containing antibiotic resistance genes.
4. Transform (insert) recombinant plasmid into bacteria.
5. Surviving bacteria on antibiotic medium produce cloned DNA (copies of cDNA).

Flow cytometry

- Laboratory technique to assess size, granularity, and protein expression (immunophenotype) of individual cells in a sample.

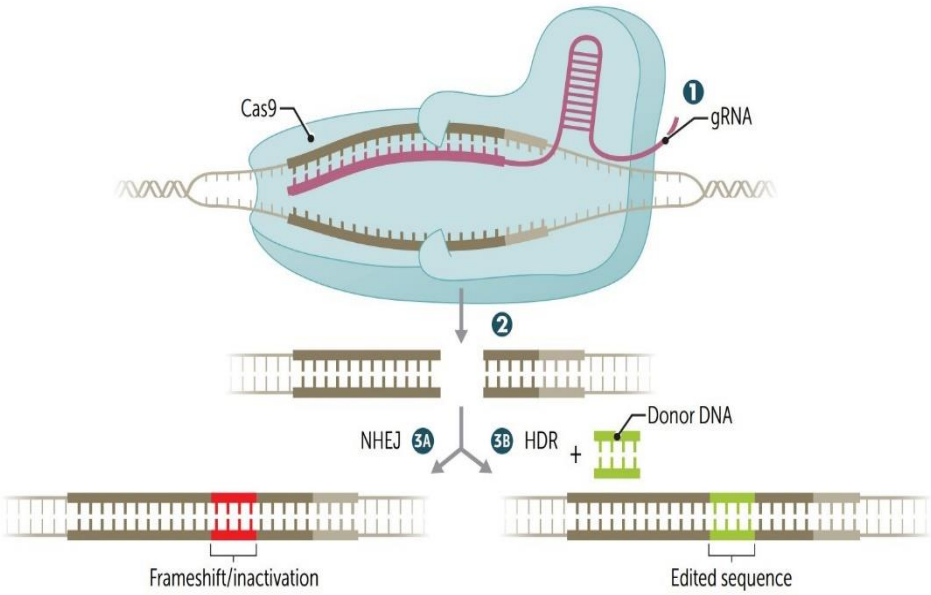
- Cells are tagged with antibodies specific to surface or intracellular proteins. Antibodies are then tagged with a unique fluorescent dye. Sample is analyzed one cell at a time by focusing a laser on the cell and measuring light scatter and intensity of fluorescence.

- Commonly used in workup of hematologic abnormalities (paroxysmal nocturnal hemoglobinuria, fetal RBCs in mother's blood) and immunodeficiencies (CD4 cell count in HIV).



CRISPR/Cas9

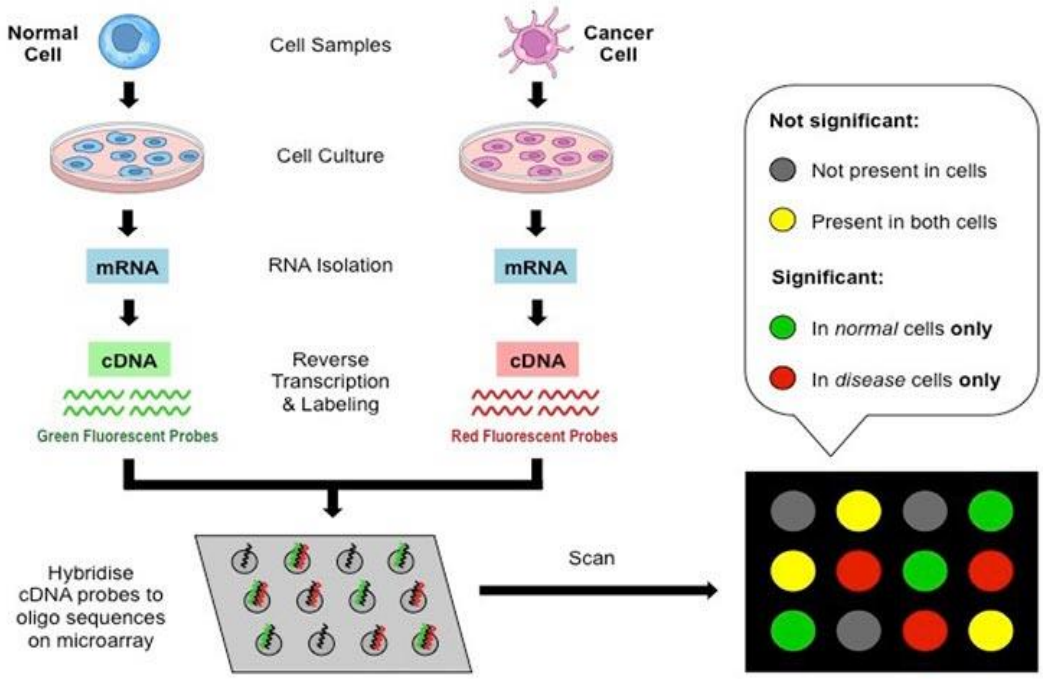
- A genome editing tool derived from bacteria.
- Consists of a guide RNA (gRNA), which is complementary to a target DNA sequence, and an endonuclease (Cas9), which makes a single or double-strand break at the target site.
- Break imperfectly repaired by nonhomologous end joining (NHEJ) → accidental frameshift mutations ("knock-out"), or a donor DNA sequence can be added to fill in the gap using homology-directed repair (HDR).
- Not used clinically. Potential applications include removing virulence factors from pathogens, replacing disease-causing alleles of genes with healthy variants, and specifically targeting tumor cells.



Microarrays

- Thousands of nucleic acid sequences are arranged in grids on glass or silicon. DNA or RNA probes are hybridized to the chip, and a scanner detects the relative amounts of complementary binding.

- Used to profile gene expression levels of thousands of genes simultaneously to study certain diseases and treatments. Able to detect single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) for a variety of applications including genotyping, clinical genetic testing, forensic analysis, cancer mutations, and genetic linkage analysis.

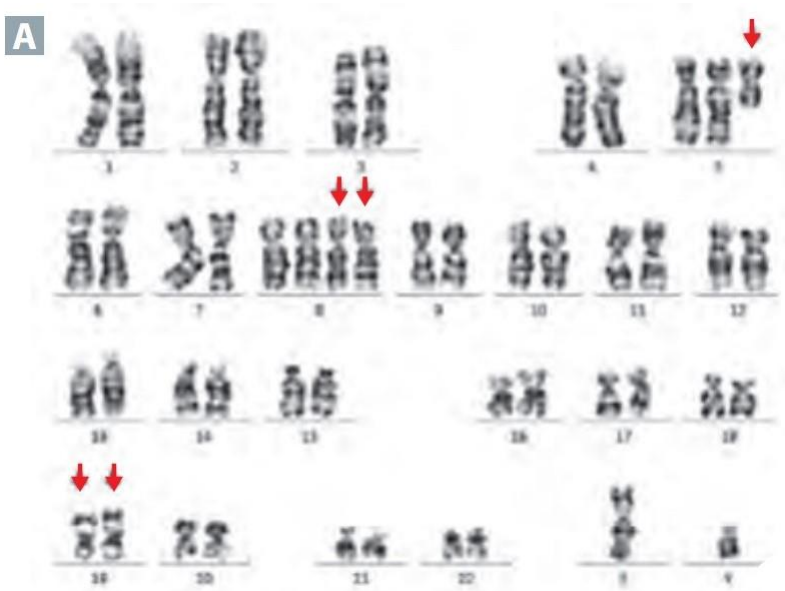


Karyotyping

- A process in which metaphase chromosomes are stained, ordered, and numbered according to morphology, size, arm-length ratio, and banding pattern (arrows in A point to extensive abnormalities in a cancer cell).

- Can be performed on a sample of blood, bone marrow, amniotic fluid, or placental tissue.

- Used to diagnose chromosomal imbalances (autosomal trisomies, sex chromosome disorders).

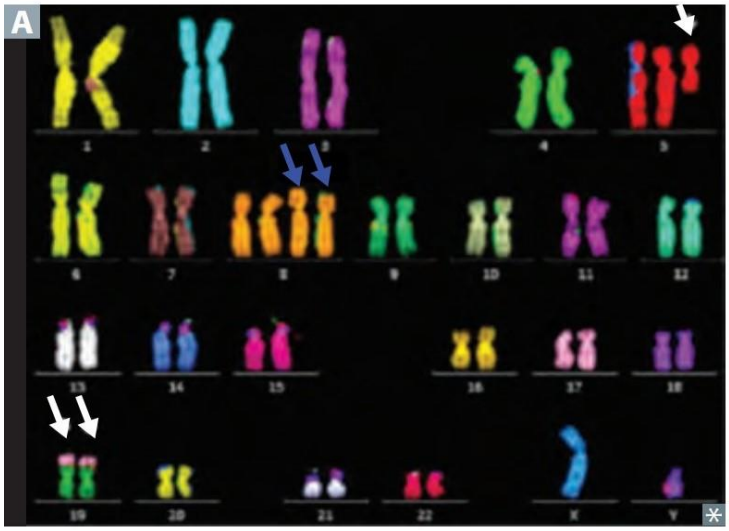


A Fluorescence in situ hybridization

- Fluorescent DNA or RNA probe binds to specific gene site of interest on chromosomes (arrows in A point to abnormalities in a cancer cell, whose karyotype is seen above; each fluorescent color represents a chromosome specific probe).

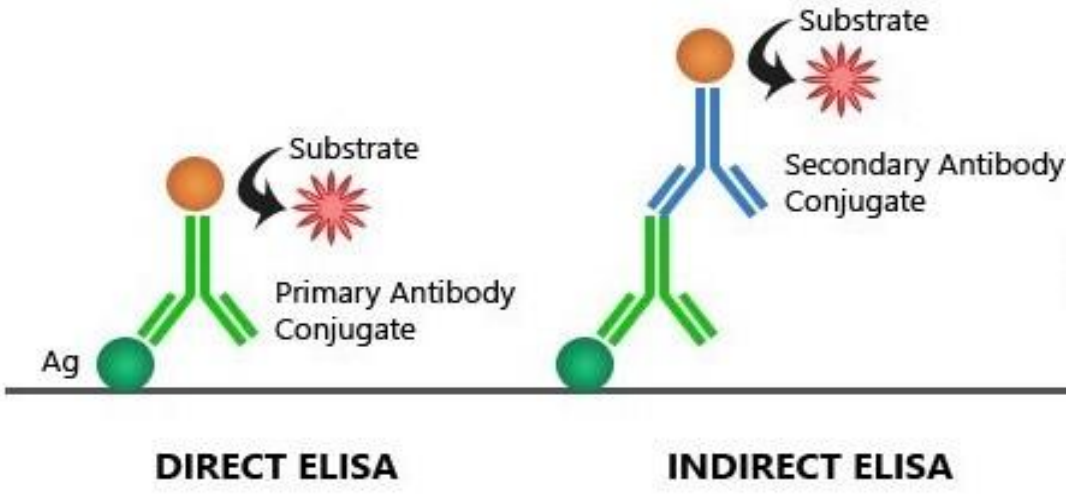
- Used for specific localization of genes and direct visualization of chromosomal anomalies at the molecular level:

A. Microdeletion: no fluorescence on a chromosome compared to fluorescence at the same locus on the second copy of that chromosome
B. Translocation: fluorescence signal that corresponds to one chromosome is found in a different chromosome (two white arrows in A show fragments of chromosome 17 that have translocated to chromosome 19).
C. Duplication: a second copy of a chromosome, resulting in a trisomy or tetrasomy (two blue arrows show duplicated chromosomes 8, resulting in a tetrasomy)



Enzyme-linked immunosorbent assay

- Immunologic test used to detect the presence of either a specific antigen (HBsAg) or antibody (anti-HBs) in a patient's blood sample.
- Detection involves the use of an antibody linked to an enzyme. Added substrate reacts with enzyme, producing a detectable signal. Can have high sensitivity and specificity, but is less specific than Western blot.
- Direct ELISA tests for the antigen directly, while indirect ELISA tests for the antibody (thus indirectly testing for the antigen).



Gene expression modifications

- <u>Transgenic strategies in mice involve:</u>
  - Random insertion of gene into mouse genome.
  - Targeted insertion or deletion of gene through homologous recombination with mouse gene.
  - Knock-out = removing a gene, taking it out.
  - Knock-in = inserting a gene.
- <u>Cre-lox system:</u> Can inducibly manipulate genes at specific developmental points (to study a gene whose deletion causes embryonic death).

CHAPTER 4

Genetics

Genetic terms

- Humans are composed of two groups of cells:

- Gametes (Ova and sperm cells): which are haploid, have one copy of each type of chromosome (1-22, X or Y). This DNA is transmitted to offspring.

- Somatic cells (cells other than gametes): Nearly all somatic cells are diploid, having two copies of each type of autosome (1-22) and either XX or XY.

- Gene: basic unit of inheritance.

- Locus: location of a gene on a chromosome.

- Allele: different forms of a gene.

- Genotype: alleles found at a locus.

- Phenotype: physically observable features.

- Homozygote: alleles at a locus are the same.

- Heterozygote: alleles at a locus are different.

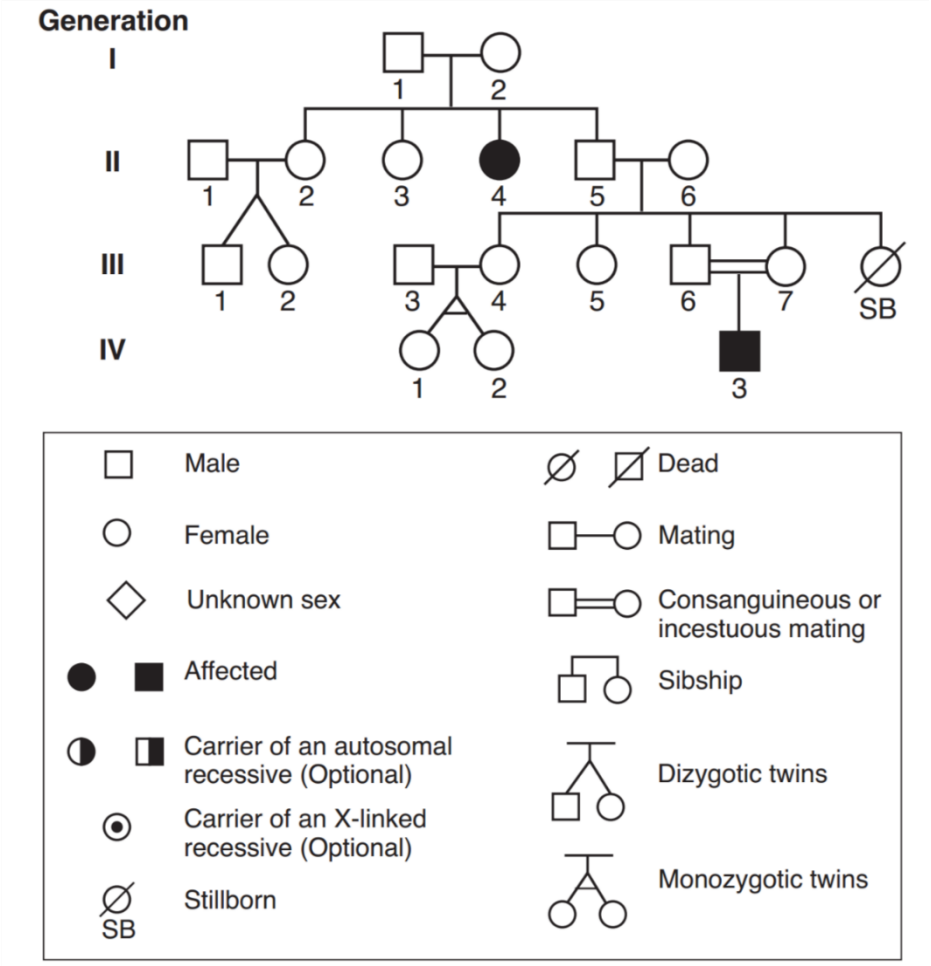
- Dominant: requires only one copy of the mutation to produce disease.

- Recessive: requires 2 copies of the mutation to produce disease.

- Codominance: Both alleles contribute to the phenotype of the heterozygote.
  - EX: Blood groups A, B, AB.

- Recurrence risk: The recurrence risk is the probability that the offpring of a couple will express a genetic disease.

Modes of inheritance



Autosomal dominant

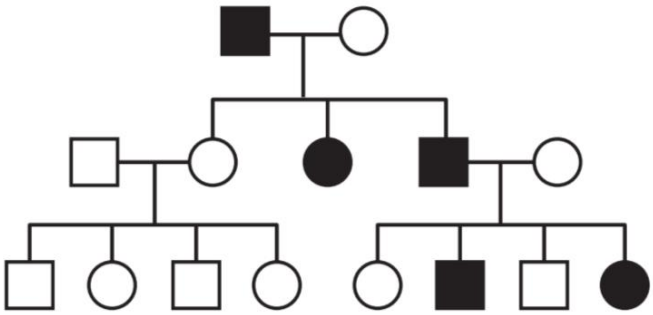
- <u>A number of features in a pedigree help identify autosomal dominant inheritance:</u>

- Because affected individuals must receive a disease-causing gene from an affected parent, the disease is typically observed in multiple generations of a pedigree.

- Because these genes are located on autosomes, males and females are affected in roughly equal frequencies.

- Autosomal dominant alleles are relatively rare in populations, so the typical mating pattern is a heterozygous affected individual (Aa genotype) mating with a homozygous normal individual (aa genotype). Note that, by convention, the dominant allele is shown in uppercase (A) and the recessive allele is shown in lowercase (a). The recurrence risk is thus 50%, and half the children, on average, will be affected with the disease.

- Common Examples: Familial hypercholesterolemia (LDL receptor deficiency), Huntington disease, Neurofibromatosis type 1, Marfan syndrome and Acute intermittent porphyria.



Autosomal Dominant Inheritance

	A	a
a	Aa	aa
a	Aa	aa

A Punnett square: Affected offspring (Aa) are shaded.

Recurrence Risk for the Mating of Affected Individual (Aa) with a Homozygous Unaffected Individual (aa) using a Punnett Square

Autosomal recessive

- Important features that distinguish autosomal recessive inheritance:

- Because autosomal recessive alleles are clinically expressed only in the homozygous state, the offspring must inherit one copy of the disease-causing allele from each parent.

- In contrast to autosomal dominant diseases, autosomal recessive diseases are typically seen in only one generation of a pedigree (skipped generation).

- Because these genes are located on autosomes, males and females are affected in roughly equal frequencies.

- Most commonly, a homozygote is produced by the union of two heterozygous (carrier) parents. The recurrence risk for offspring of such matings is 25%.

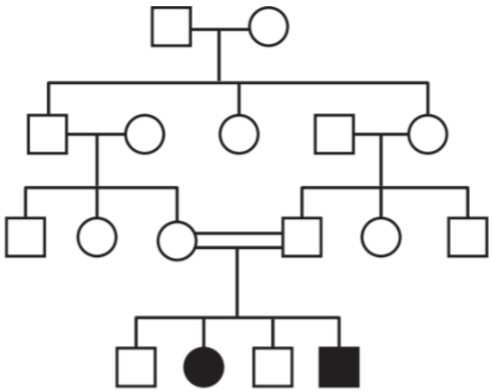
- Often due to enzyme deficiencies.

- Commonly more severe than dominant disorders; patients often present in childhood.

- ↑ risk in consanguineous families.

- With 2 carrier (heterozygous) parents, on average: 1/4 of children will be affected (homozygous), 1/2 of children will be carriers, and ¼ of children will be neither affected nor carriers.

- Common Examples: Sickle cell anemia, Cystic fibrosis, Phenylketonuria (PKU), Tay-Sachs disease (hexosaminidase A deficiency).



A consanguineous mating has produced two affected offspring.

Pedigree for an Autosomal Recessive Disease

	A	a
A	AA	Aa
a	Aa	aa

The affected genotype (aa) is shaded.

Recurrence Risk for the Mating of
Two Heterozygous Carriers (Aa) of a Recessive Mutation

❖ Determining the Recurrence Risk for an Individual Whose Phenotype Is Known. Individual IV-1 may wish to know his risk of being a carrier. Because his phenotype is known, there are only three possible genotypes he can have, assuming complete penetrance of the disease-producing allele. He cannot be homozygous for the recessive allele (aa). Two of the remaining three possibilities are carriers (Aa and aA), and one is homozygous normal (AA). Thus, his risk of being a carrier is 2/3, or 0.67 (67%).

X-linked recessive

- Properties of X-linked recessive inheritance:

- Because males have only one copy of the X chromosome, they are said to be hemizygous (hemi = "half") for the X chromosome. If a recessive disease-causing mutation occurs on the X chromosome, a male will be affected with the disease.

- Because males require only one copy of the mutation to express the disease and females require two copies, X-linked recessive diseases are seen much more commonly in males than in females.

- Skipped generations are seen.

- Male-to-male transmission is not seen in X-linked inheritance; this helps distinguish it from autosomal inheritance.

- Figure below shows the recurrence risks for X-linked recessive diseases:

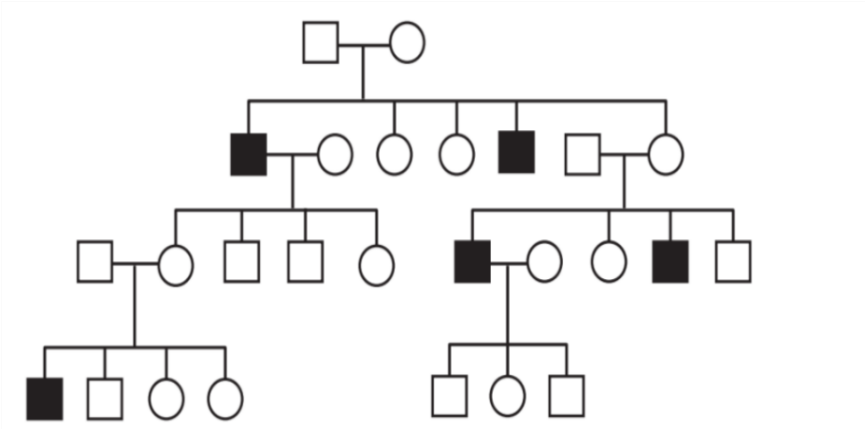
A. Affected male & homozygous normal female: All of the daughters will be heterozygous carriers; all of the sons will be homozygous normal.

B. Normal male & carrier female:

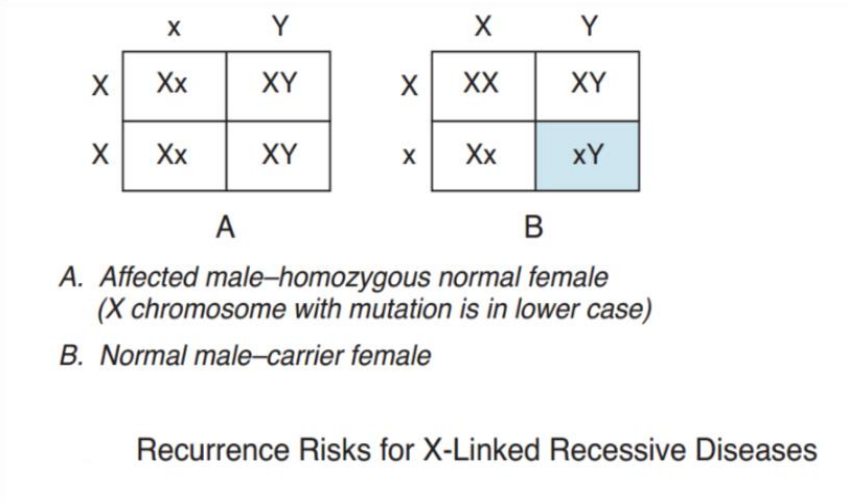
- On average, half of the sons will be affected, and half of the daughters will be carriers. Note that in this case, the recurrence rate is different depending on the sex of the child. If the fetal sex is known, the recurrence rate for a daughter is 0, and that for a son is 50%. If the sex of the fetus is not known, then the recurrence rate is multiplied by 1/2, the probability that the fetus is a male versus a female. Therefore, if the sex is unknown, the recurrence risk is 25%.

- Common examples: Duchenne muscular dystrophy, Lesch-Nyhan syndrome (hypoxanthine-guanine phosphoribosyltransferase [HGPRT] deficiency) Glucose-6-phosphate dehydrogenase deficiency, Hemophilia A and B.

- Females with Turner syndrome (45, XO) are more likely to have an X-linked recessive disorder.



X-Linked Recessive Inheritance



A. Affected male–homozygous normal female (X chromosome with mutation is in lower case)

B. Normal male–carrier female

Recurrence Risks for X-Linked Recessive Diseases

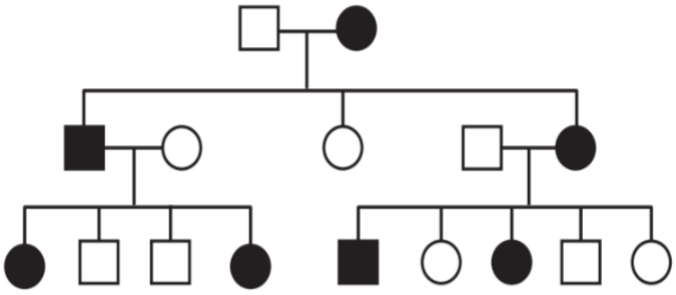
X-linked dominant

- There are relatively few diseases whose inheritance is classified as X-linked dominant. In this condition, females are differently affected than males.
- As in X-linked recessive inheritance, male-male transmission of the disease-causing mutation is not seen.
- Heterozygous females are affected.
- As in autosomal dominant inheritance, the disease phenotype is seen in multiple generations of a pedigree; skipped generations are relatively unusual.
- Transmitted through both parents. Mothers transmit to 50% of daughters and sons; fathers transmit to all daughters but no sons.
- Figure below shows the recurrence risks for X-linked dominant inheritance:

- Affected male & homozygous normal female:
  - None of the sons are affected; all of the daughters are affected. Note that in this case, the recurrence rate is different depending on the sex of the child. If the fetal sex is known, the recurrence rate for a daughter is 100%, and that for a son is 0 %. If the sex of the fetus is not known, then the recurrence rate is multiplied by 1/2, the probability that the fetus is a male versus a female. Therefore, if the sex is unknown, the recurrence risk is 50%.

- Normal male & heterozygous affected female: On average, 50% of sons are affected and 50% of daughters are affected.

- Common examples: Hypophosphatemic rickets, Fragile X syndrome.



X-Linked Dominant Inheritance

Affected male–homozygous normal female (the mutation-carrying chromosome is upper case)			Normal male–heterozygous affected female		
	X	Y		x	Y
x	Xx	xY	X	Xx	XY
x	Xx	xY	x	xx	xY

Recurrence Risks for X-Linked Dominant Inheritance

Mitochondrial inheritance

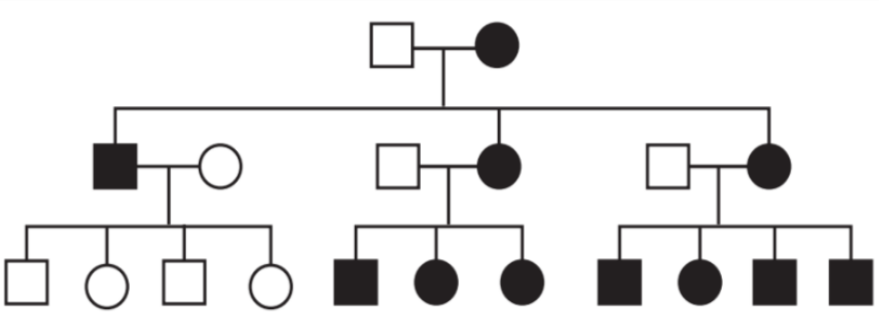
- Mitochondria, which are cytoplasmic organelles involved in cellular respiration, have their own chromosomes, each of which contains DNA base pairs arranged in a circular molecule.

- This DNA encodes 13 proteins that are subunits of complexes in the electron transport and oxidative phosphorylation processes. In addition, mitochondrial DNA encodes 22 transfer RNAs and 2 ribosomal RNAs.

- Because a sperm cell contributes no mitochondria to the egg cell during fertilization, mitochondrial DNA is inherited exclusively through females.

- <u>Pedigrees for mitochondrial diseases thus display a distinct mode of inheritance:</u>

- Transmission of the disease is only from a female.
- All offspring of an affected female are affected.
- None of the offspring of an affected male is affected.
- Diseases are typically neuropathies and/or myopathies.



Pedigree for a Mitochondrial Disease

❖ N.B:

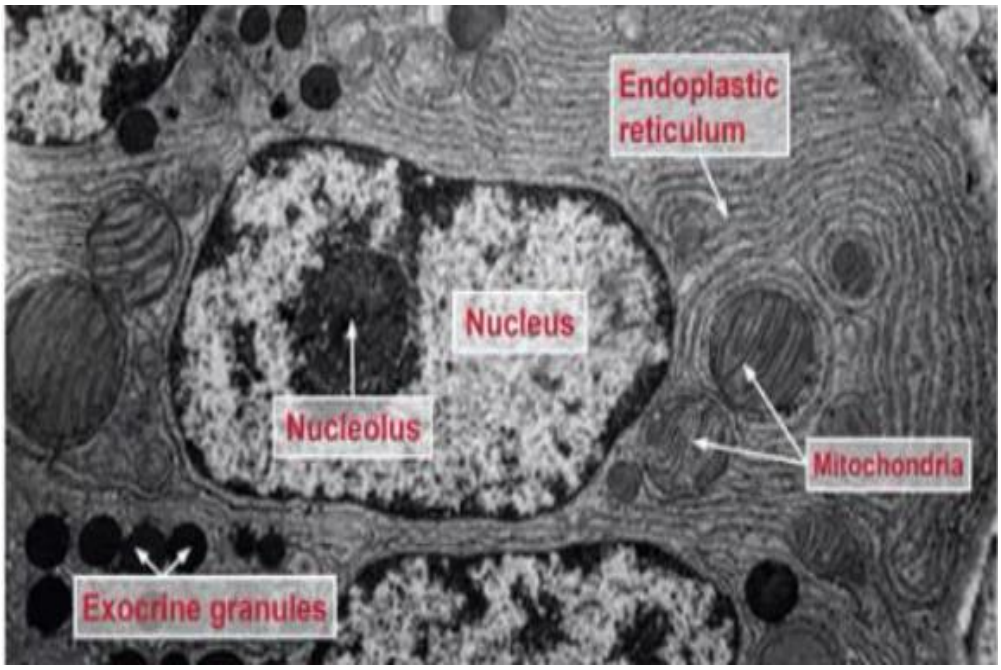
- Mitochondrial DNA (mtDNA) is the most common non-nuclear DNA found in eukaryotic cells. It resembles prokaryotic DNA and is maternally derived. Recall that the ovum is relatively large and has many copies of mtDNA; whereas, the few copies of mtDNA present in sperm are lost during fertilization.
- Mutations involving mtDNA or nuclear DNA that codes for mitochondrial proteins can cause a variety of mitochondrial disorders, including Leigh syndrome and MELAS.
- Mitochondria are responsible for ATP production via oxidative phosphorylation, which is why mitochondrial defects tend to cause lactic acidosis and primarily affect tissues with the highest metabolic rates (neural tissue, muscular tissue). "Red ragged" muscle fibers are seen in mitochondrial diseases. Muscle fibers have this appearance because abnormal mitochondria accumulate under the sarcolemma.
- Though many mitochondrial proteins are coded for in the nuclear genome, mitochondria also contain their own genome, which is also vulnerable to mutations. Defects in the mitochondrial genome may occur in any number of the mitochondria within a cell, and the severity of mitochondrial diseases is often related to the proportion of abnormal to normal mitochondria within a patient's cells. Heteroplasmy describes the condition of having different organellar genomes (mutated and wild-type) within a single cell. For mitochondrial diseases, patients with more severe disease are those with a higher proportion of defective mitochondrial genomes within their cells.
- Mitochondrial diseases affect both male and female offspring with equal frequency (100%), but there are variable degrees of severity.
- This variability occurs because, during mitosis, mitochondria are randomly distributed between daughter cells. As a result, some cells contain mitochondria with mostly damaged mtDNA, while some contain mostly normal mitochondrial genomes. This mixture of two types of genetic material is called heteroplasmy and is responsible for the clinical variability of mitochondrial diseases. The following mitochondrial syndromes are important:

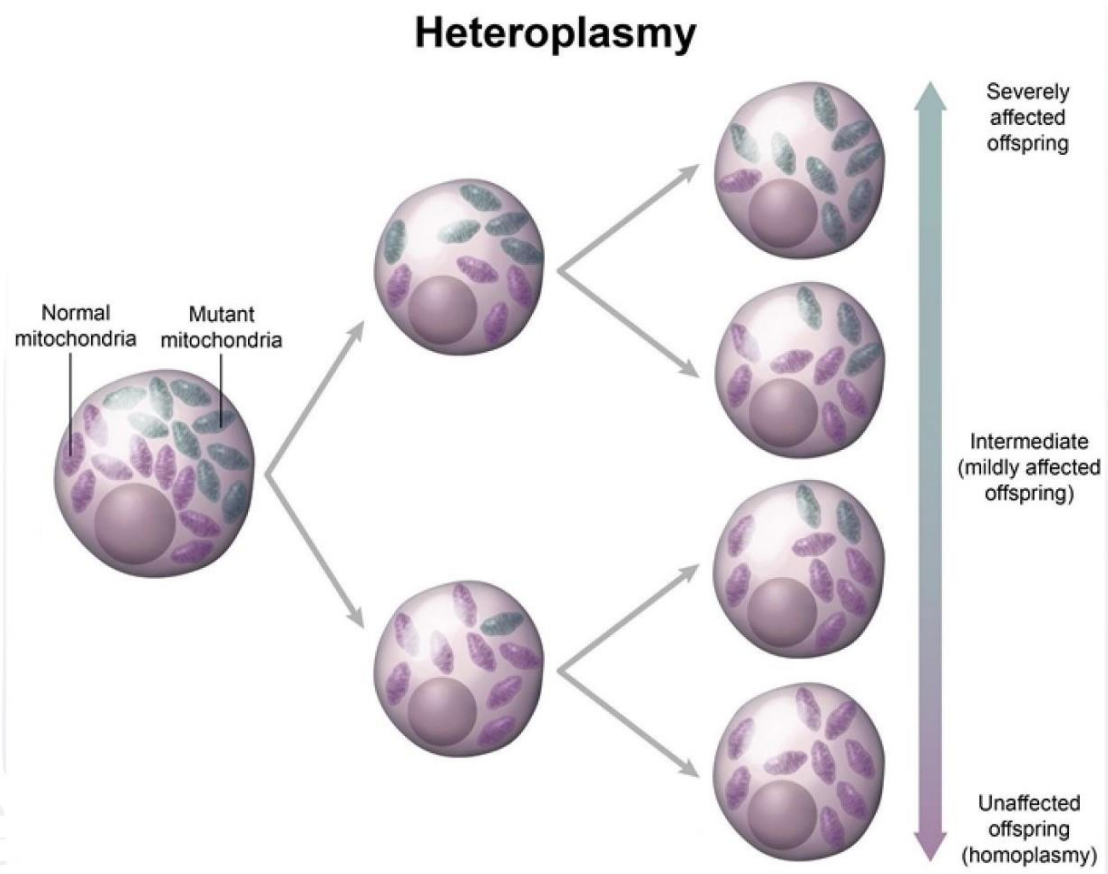
a) Leber hereditary optic neuropathy: cell death in optic nerve neurons → subacute bilateral vision loss in teens/young adults, 90% males. Usually permanent.

b) Myoclonic epilepsy with ragged-red fibers: myoclonic seizures and myopathy associated with exercise. Skeletal muscle biopsy shows irregularly shaped muscle fibers (ragged red fibers).

c) Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS): The clinical presentation of MELAS is described in this vignette.

- Mitochondria can be identified on electron microscopy by their characteristic double membrane and wavy cristae.


Endoplastic reticulum
Nucleus
Nucleolus
Mitochondria
Exocrine granules


Heteroplasmy
Normal mitochondria
Mutant mitochondria
Severely affected offspring
Intermediate (mildly affected offspring)
Unaffected offspring (homoplasmy)

X inactivation

- Normal males inherit an X chromosome from their mother and a Y chromosome from their father, whereas normal females inherit an X chromosome from each parent.

- Because the Y chromosome carries only about 50 protein-coding genes and the X chromosome carries hundreds of protein-coding genes, a mechanism must exist to equalize the amount of protein encoded by X chromosomes in males and females.

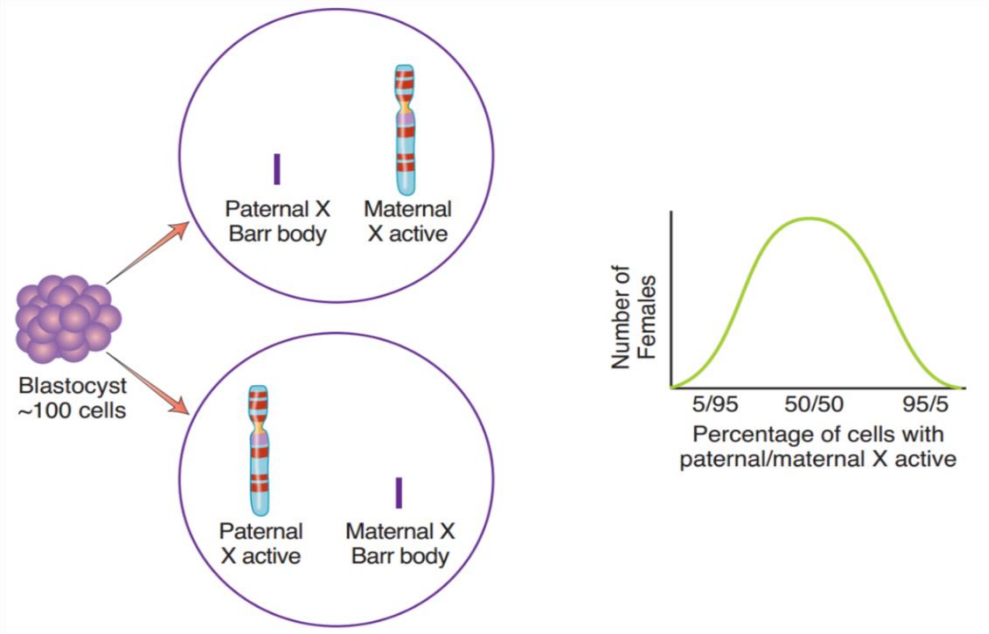
- This mechanism, termed X inactivation, occurs in the blastocyst (~100 cells) during the development of female embryos. When an X chromosome is inactivated, its DNA is not transcribed into mRNA, and the chromosome is visualized under the microscope as a highly condensed Barr body in the nuclei of interphase cells.

- <u>X inactivation has several important characteristics:</u>

- X inactivation is random and fixed: in some cells of the female embryo, the X chromosome inherited from the father is inactivated, and in others the X chromosome inherited from the mother is inactivated.

- Like coin tossing, this is a random process. As shown in Figure below, most women have their paternal X chromosome active in approximately 50% of their cells and the maternal X chromosome active in approximately 50% of their cells.

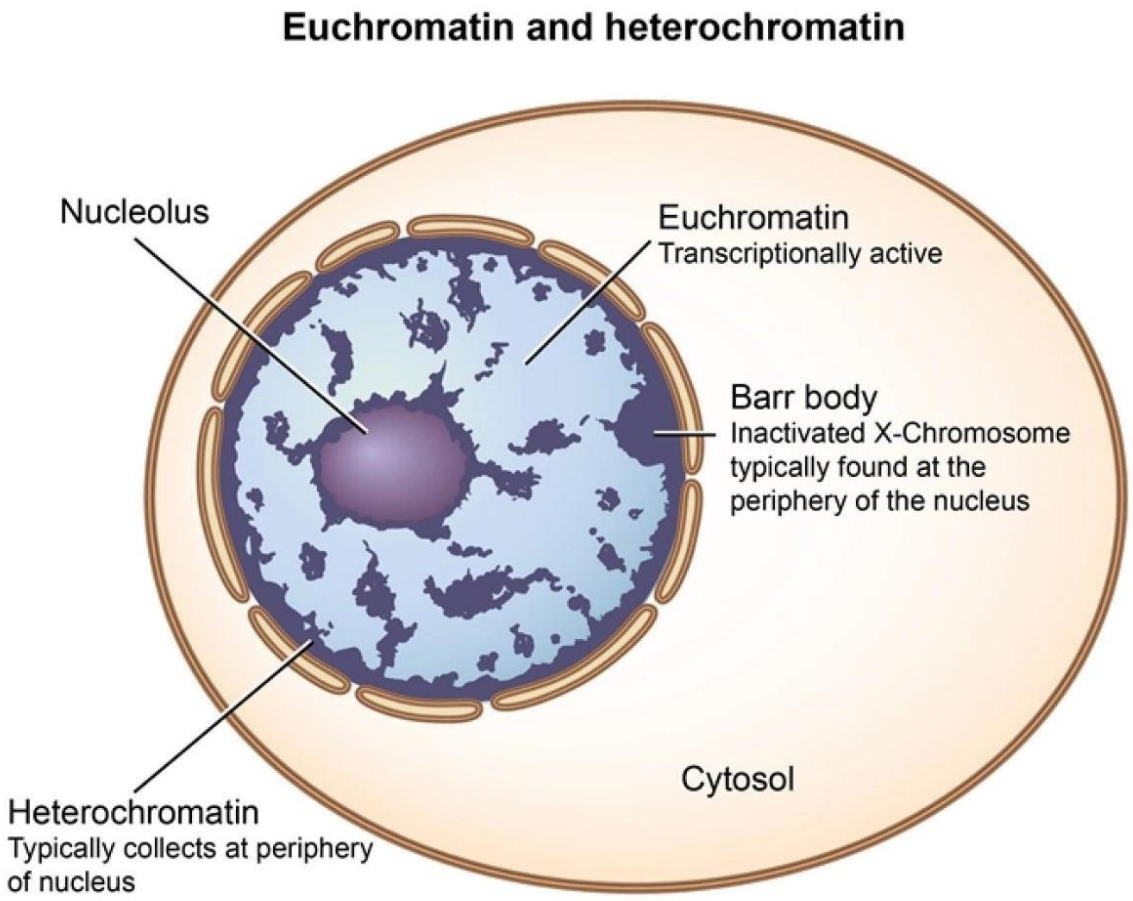
- All X chromosomes in a cell are inactivated except one. For example, females with three X chromosomes in each cell have two X chromosomes inactivated in each cell (thus, two Barr bodies can be visualized in an interphase cell).



Inactivation of the X Chromosome during Embryogenesis Is a Random Process

❖ Manifesting (female) heterozygotes:

- Normal females have two copies of the X chromosome, so they usually require two copies of the mutation to express the disease. However, because X inactivation is a random process, a heterozygous female will occasionally express an X-linked recessive mutation because, by random chance, most of the X chromosomes carrying the normal allele have been inactivated. Such females are termed manifesting heterozygotes. Because they usually have at least a small population of active X chromosomes carrying the normal allele, their disease expression is typically milder than that of hemizygous males.

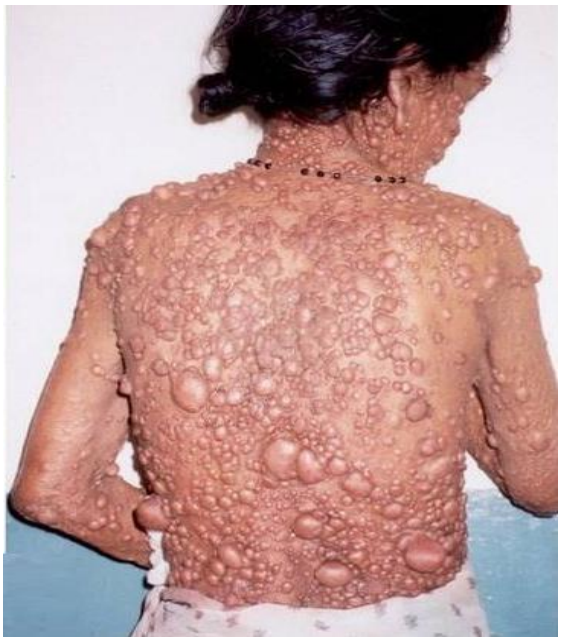


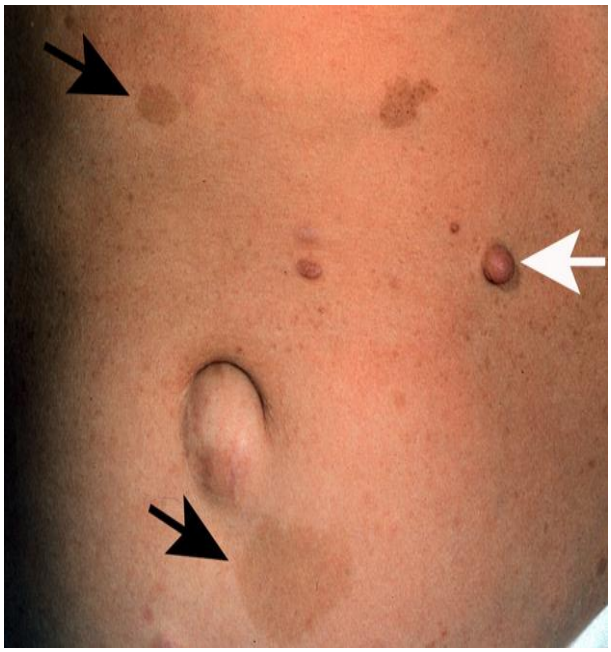
Important principles that can characterize single gene diseases

- Variable expressivity:

- Patients with the same genotype have varying phenotypes.

- 2 patients with neurofibromatosis type 1 (NF1) may have varying disease severity.





- Incomplete penetrance:

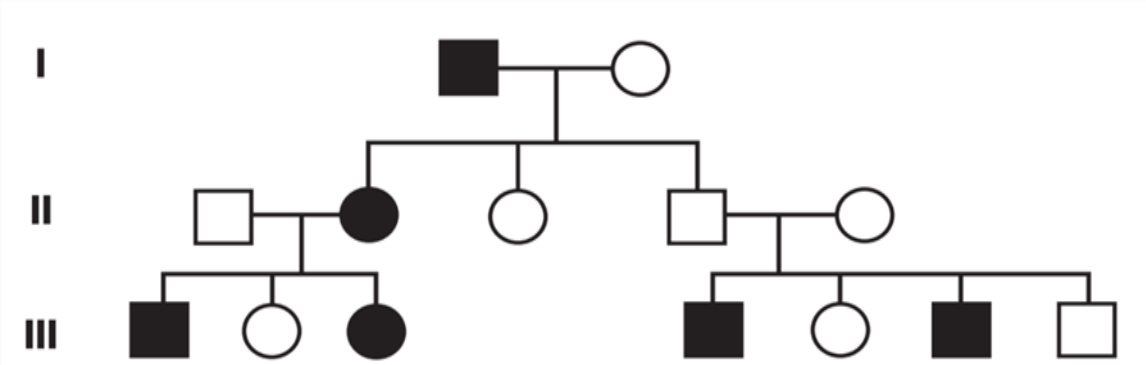
- Not all individuals with a mutant genotype show the mutant phenotype.

- Incomplete penetrance is distinguished from variable expression in that the nonpenetrant gene has no phenotypic expression at all.

- % penetrance × probability of inheriting genotype = risk of expressing phenotype.

- BRCA1 gene mutations do not always result in breast or ovarian cancer.

- Retinoblastoma is an autosomal dominant condition caused by an inherited loss-of-function mutation in the Rb tumor suppressor gene. In 10% of individuals who inherit this mutation, there is no additional somatic mutation in the normal copy and retinoblastoma does not develop, although they can pass the mutation to their offspring. Penetrance of retinoblastoma is therefore 90%.

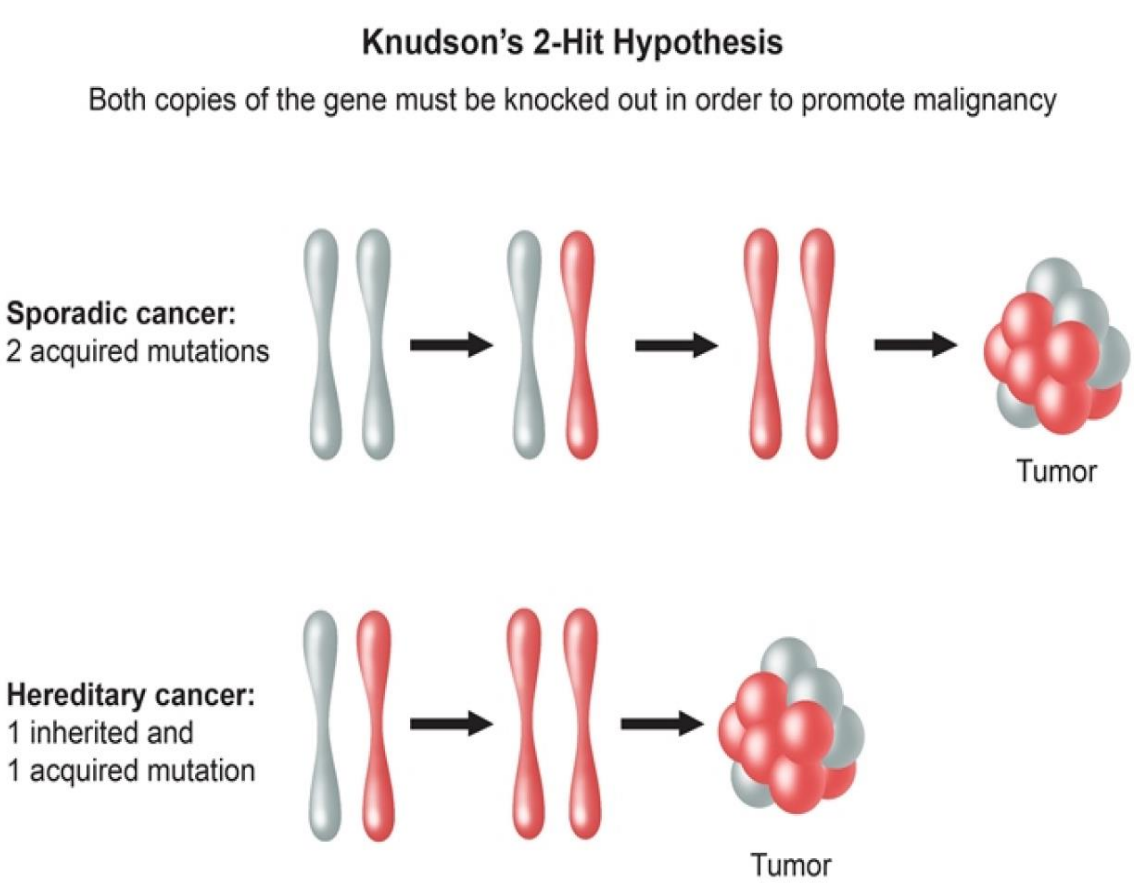


The unaffected male in generation II (II-4) has an affected father and two affected sons. He must have the disease-causing mutation, although it shows incomplete penetrance.

- Loss of heterozygosity:

- If a patient inherits or develops a mutation in a tumor suppressor gene, the complementary allele must be deleted/mutated before cancer develops. This is not true of oncogenes.

- Retinoblastoma and the "two-hit hypothesis," Lynch syndrome (HNPCC), Li-Fraumeni syndrome.



- Pleiotropy:

- The occurrence of multiple phenotypic manifestations, often in different organ systems, because of a single genetic defect.

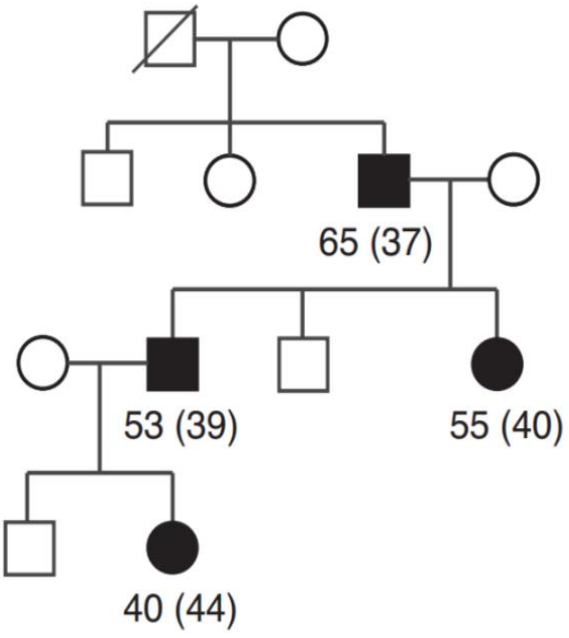
- Most syndromic genetic diseases exhibit pleiotropy (homocystinuria).

- Pleiotropy in Marfan Syndrome:
  - An autosomal dominant disease.
  - It is characterized by skeletal abnormalities (thin, elongated limbs; pectus excavatum; pectus carinatum), hypermobile joints, ocular abnormalities (frequent myopia and detached lens), and most importantly, cardiovascular disease (mitral valve prolapse and aortic aneurysm). Although the features of this disease seem rather disparate, they are all caused by a mutation in the gene that encodes fibrillin, a key component of connective tissue.
  - Fibrillin is expressed in the periosteum and perichondrium, the suspensory ligament of the eye, and the aorta. Defective fibrillin causes the connective tissue to be "stretchy" and leads to all of the observed disease features. Marfan syndrome thus provides a good example of the principle of pleiotropy.

- Anticipation:

- Increased severity or earlier onset of disease in succeeding generations.

- Trinucleotide repeat diseases (Huntington disease).



Numbers under pedigree symbols identify age of onset (CAG repeats).

- Dominant negative mutation:
- Exerts a dominant effect.

- A heterozygote produces a nonfunctional altered protein that also prevents the normal gene product from functioning (the effect of this mutation is antagonistic to the original "normal" gene).

- Mutation of a transcription factor in its allosteric site. Nonfunctioning mutant can still bind DNA, preventing wild-type transcription factor from binding.

- Locus heterogeneity:
- Mutations at different loci can produce a similar phenotype.

- Locus Heterogeneity in Osteogenesis Imperfecta Type 2:
  - Osteogenesis imperfecta (OI) is a disease of bone development. It results from a defect in the collagen protein, a major component of the bone matrix.
  - Four major forms of OI have been identified. The severe perinatal form (type 2) is the result of a defect in type 1 collagen, a trimeric molecule that has a triple helix structure. Two members of the trimer are encoded by a gene on chromosome 17, and the third is encoded by a gene on chromosome 7.
  - Mutations in either of these genes give rise to a faulty collagen molecule, causing type 2 OI.

- Allelic heterogeneity:
- Different mutations in the same locus produce the same phenotype.

- EX: β-thalassemia.

- Heteroplasmy:
- Presence of both normal and mutated mtDNA, resulting in variable expression in mitochondrially inherited disease.

- EX: mtDNA passed from mother to all children.

- Imprinting:
- One gene copy is silenced by methylation, and only the other copy is expressed → parent-of-origin effects.

- DNA methylation is carried out by DNA methyltransferases that transfer methyl groups from methyl group donors (such as S-adenosyl-methionine) to cytosine residues in the DNA molecule.

A. Prader-Willi syndrome:
  - Maternally derived genes are silenced (imprinted).

  - Disease occurs when the Paternal allele is deleted or mutated.

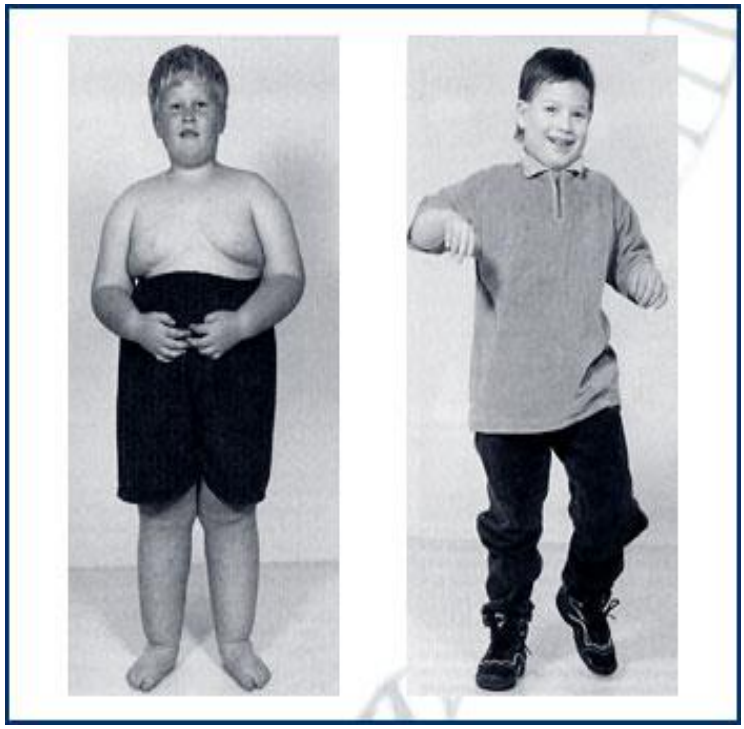
  - Results in hyperphagia, obesity, intellectual disability, hypogonadism, and hypotonia.

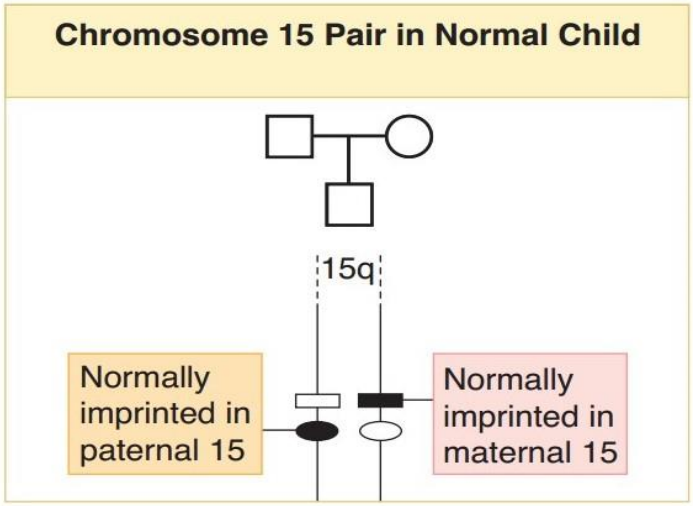
  - Associated with a mutation or deletion of chromosome 15 of paternal origin.

- 25% of cases due to maternal uniparental disomy.

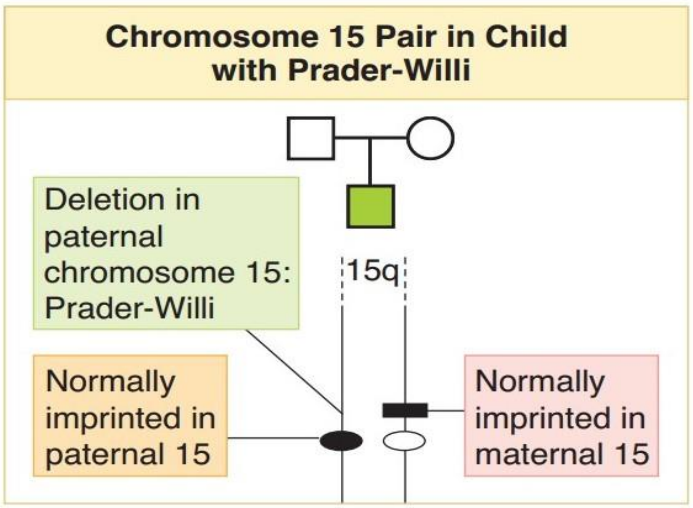
B. AngelMan syndrome:

- Paternally derived UBE3A gene is silenced (imprinted).
- Disease occurs when the Maternal allele is deleted or mutated.
- Results in inappropriate laughter ("happy puppet"), seizures, ataxia, and severe intellectual disability.
- Associated with mutation or deletion of the UBE3A gene on the maternal copy of chromosome 15.
- 5% of cases due to paternal uniparental disomy.

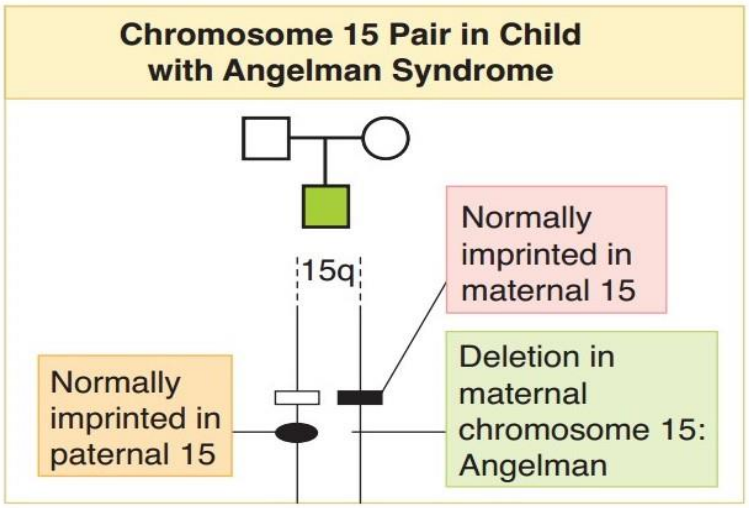



Chromosome 15 Pair in Normal Child
15q
Normally imprinted in paternal 15
Normally imprinted in maternal 15

A.


Chromosome 15 Pair in Child with Prader-Willi
Deletion in paternal chromosome 15: Prader-Willi
15q
Normally imprinted in paternal 15
Normally imprinted in maternal 15

B.


Chromosome 15 Pair in Child with Angelman Syndrome
Normally imprinted in maternal 15
15q
Deletion in maternal chromosome 15: Angelman
Normally imprinted in paternal 15

C.

- Uniparental disomy:

- Offspring receives 2 copies of a chromosome from 1 parent and no copies from the other parent.

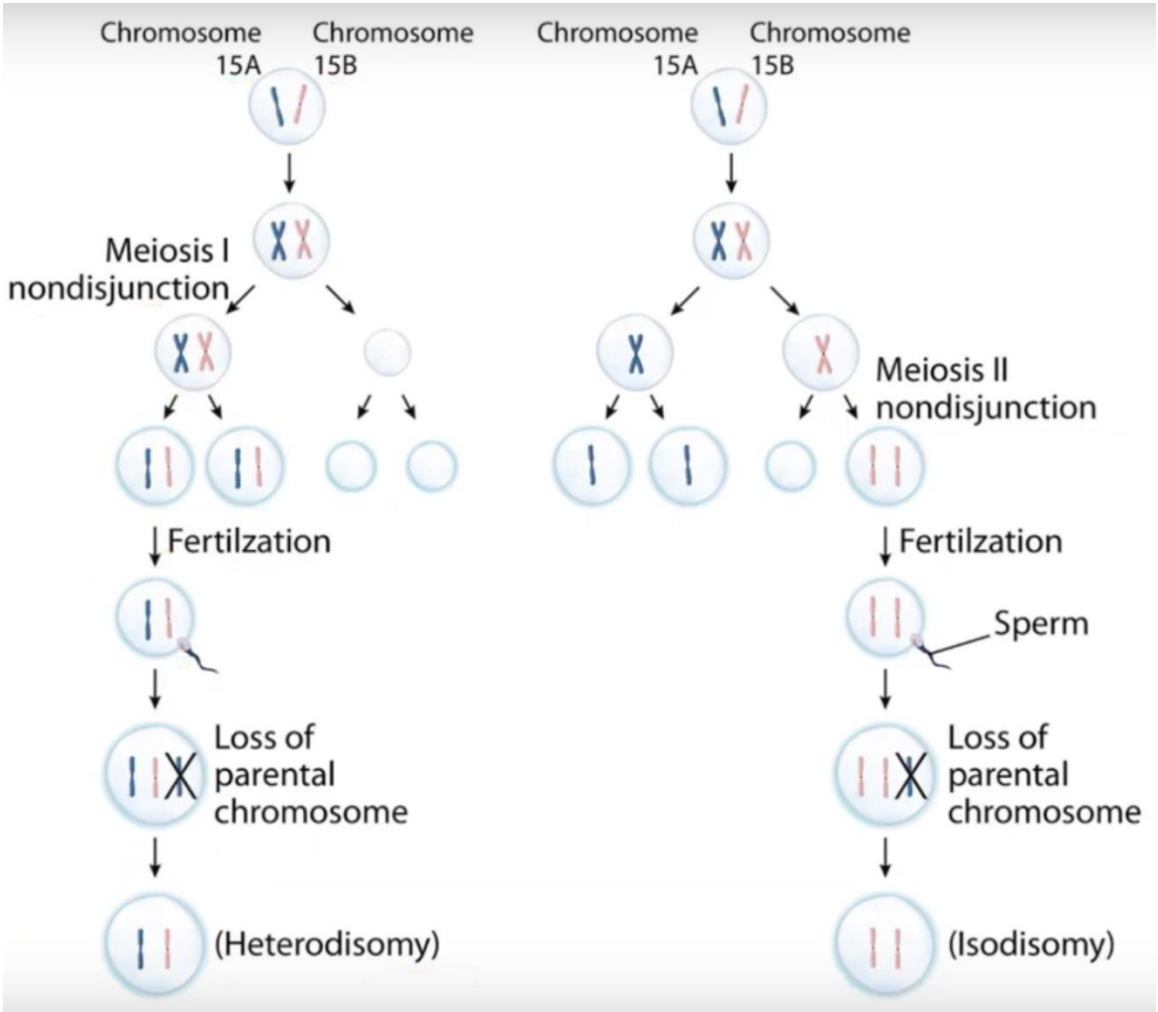
- HeterodIsomy (heterozygous) indicates a meiosis I error.

- IsodIsomy (homozygous) indicates a meiosis II error or postzygotic chromosomal duplication of one of a pair of chromosomes, and loss of the other of the original pair.

- Uniparental is euploid (correct number of chromosomes). Most occurrences of uniparental disomy (UPD) → normal phenotype.

- Consider UPD in an individual manifesting a recessive disorder when only one parent is a carrier.

- Examples: Prader-Willi and Angelman syndromes.



- Mosaicism:

- A Presence of genetically distinct cell lines in the same individual:

A. Somatic mosaicism:

- Somatic mosaicism affects the cells forming the body, causing disease manifestations to develop in affected individuals.

- Mutation arises from mitotic errors after fertilization and propagates through multiple tissues or organs.

- 45, X/46, XX is the most commonly diagnosed mosaicism affecting sex chromosomes. These patients typically have a milder form of Turner syndrome or can be asymptomatic, depending on the ratio of abnormal to normal cells.

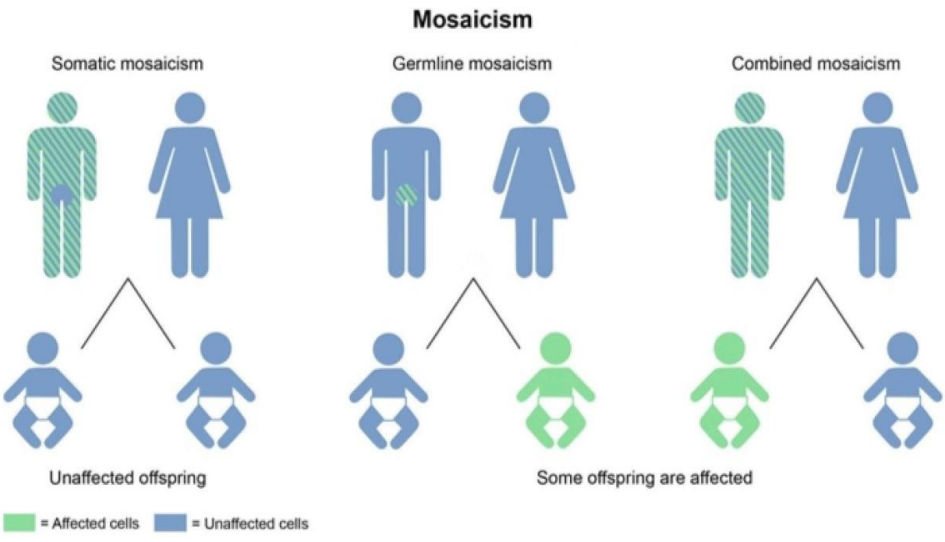
B. Gonadal mosaicism:

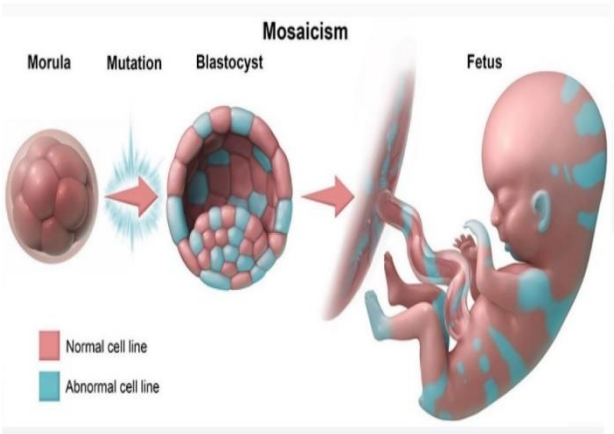
- Affects the cells that give rise to gametes (egg or sperm cells), allowing the affected genes to pass to the offspring.

- The chance of a child being affected depends on the proportion of gametes that carry the mutation.

- When mosaicism is limited to the germline, the affected parent does not develop clinical manifestations.

- If parents and relatives do not have the disease, suspect gonadal (or germline) mosaicism.





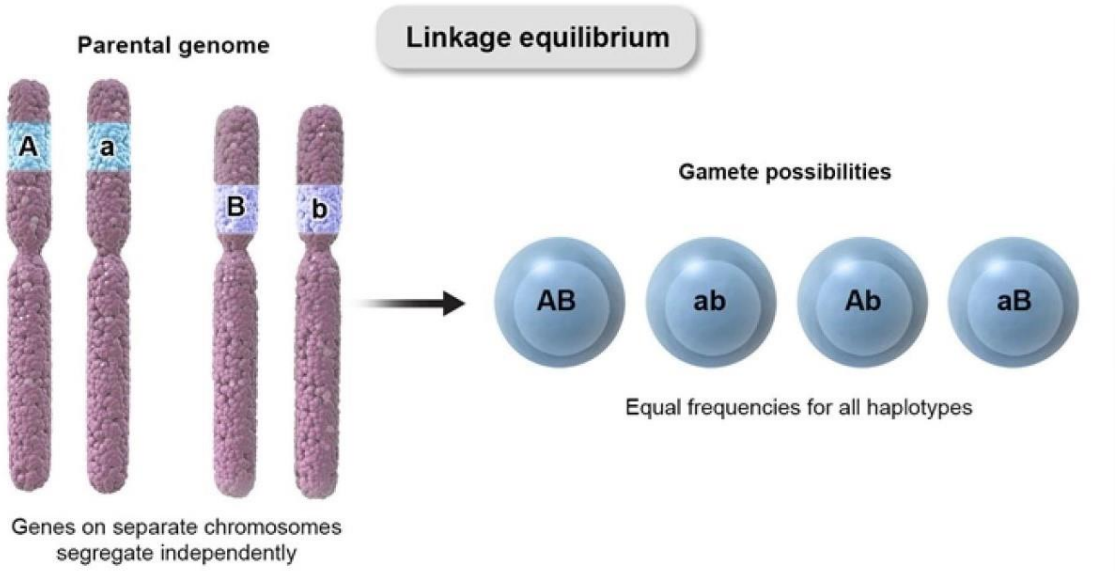
- Linkage disequilibrium:

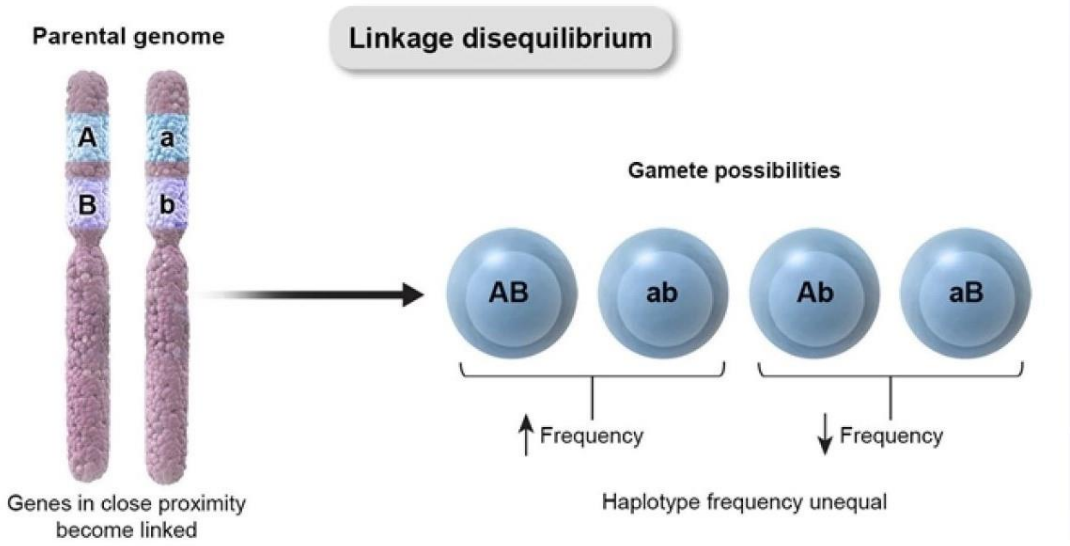
- Tendency for certain alleles at 2 linked loci to occur together more or less often than expected by chance.

- Measured in a population, not in a family, and often varies in different populations.

- However, it is important to realize that linkage disequilibrium does not always imply physical proximity between the allelic loci. Although linkage disequilibrium can be the result of physical linkage of genes (on the same chromosome), it can also occur even if the genes are on different chromosomes due to mutations, genetic drift, migration, selection pressure, and non-random mating.

- To estimate the probability of two alleles appearing together, multiply their occurrence rates.





Autosomal dominant diseases

- Achondroplasia.
- Autosomal dominant polycystic kidney disease.
- Familial adenomatous polyposis.
- Familial hypercholesterolemia.
- Hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu syndrome).
- Hereditary spherocytosis.
- Huntington disease.
- Li-Fraumeni syndrome.
- Marfan syndrome.
- Multiple endocrine neoplasias.
- Myotonic muscular dystrophy.
- Neurofibromatosis type 1 (von Recklinghausen disease).
- Neurofibromatosis type 2.
- Tuberous sclerosis.
- Von Hippel-Lindau disease.

Autosomal recessive diseases

- Albinism.
- Autosomal recessive polycystic kidney disease (ARPKD).
- Cystic fibrosis.
- Friedreich ataxia.
- Glycogen storage diseases.
- Hemochromatosis.

- Kartagener syndrome.
- Mucopolysaccharidoses (except Hunter syndrome).
- Phenylketonuria.
- Sickle cell anemia.
- Sphingolipidoses (except Fabry disease).
- Thalassemia.
- Wilson disease.

Cystic fibrosis

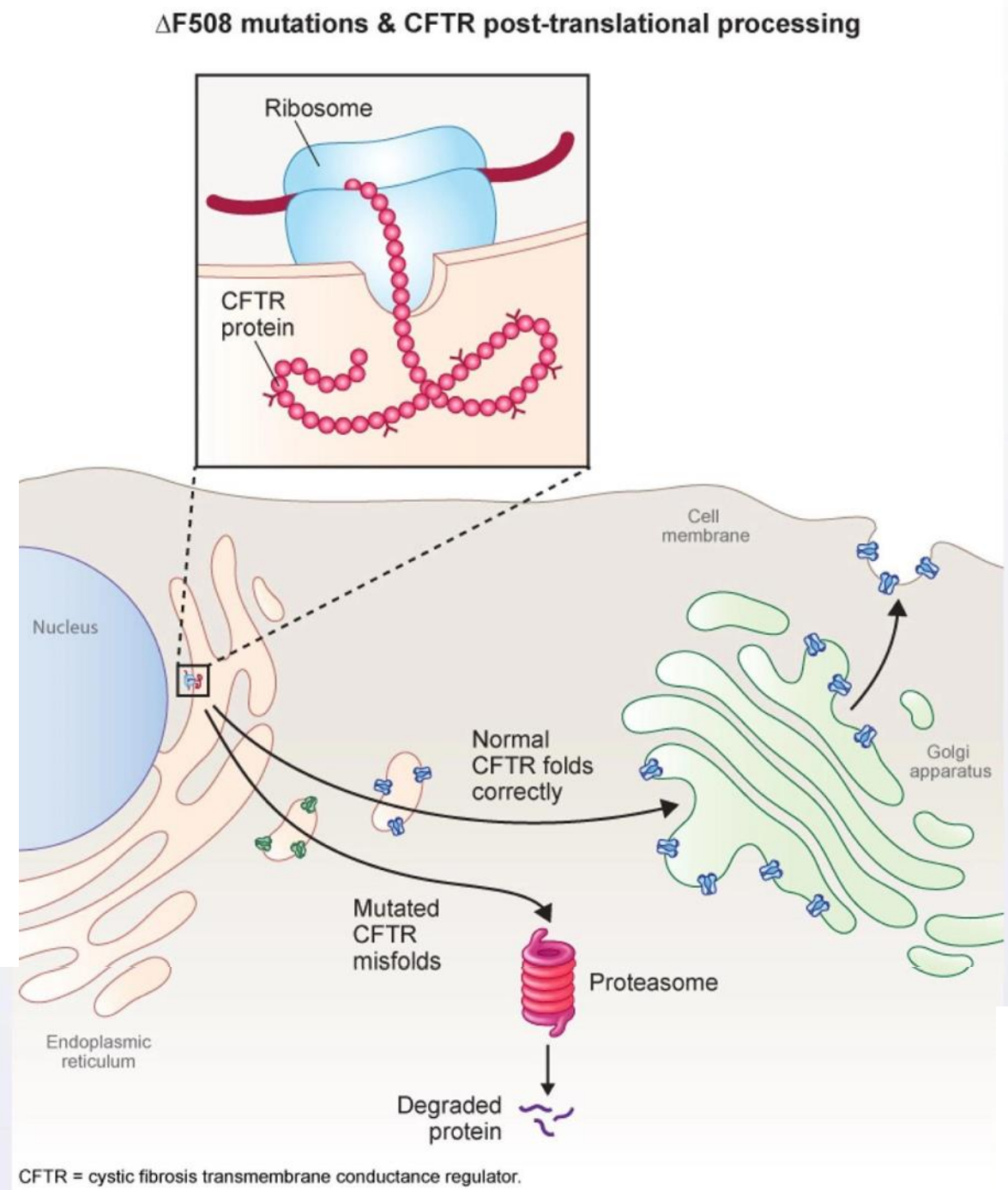
- Genetics:

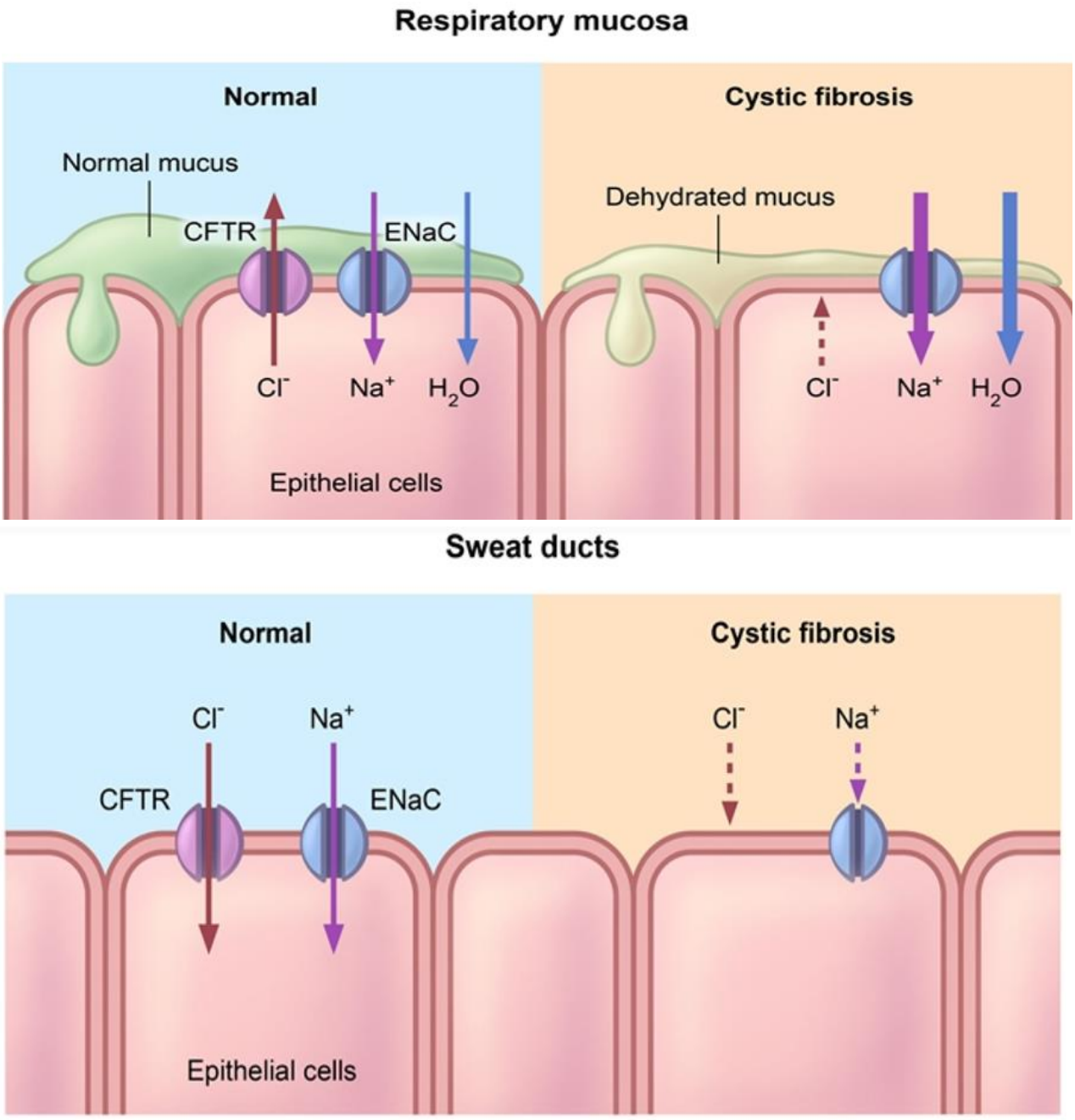
- Autosomal recessive; defect in CFTR gene on chromosome 7; commonly a deletion of Phe508.
- Most common lethal genetic disease in Caucasian population.
- ΔF508 is the most common CFTR gene mutation in patients with cystic fibrosis (70% of CF cases). This mutation deletes the three nucleotides that code for phenylalanine at amino acid position 508.
- The CFTR gene codes for the cystic fibrosis transmembrane regulator (CFTR) protein, an integral membrane protein. The ΔF508 mutation causes abnormal protein folding and failure of glycosylation. The CFTR protein is degraded before it reaches the cell surface, causing its complete absence from the apical membrane of exocrine ductal epithelial cells.

- Pathophysiology:

- CFTR encodes an ATP-gated Cl channel that secretes Cl in lungs and GI tract and reabsorbs Cl in sweat glands against a concentration gradient using ATP hydrolysis for energy.
- By pumping NaCl and water across epithelial membranes, the CFTR hydrates mucosal surfaces like the airways and bowel.
- Finally, the CFTR plays a role in the formation of hypotonic sweat. In the eccrine gland, sweat is initially isotonic with the plasma. During transport through the eccrine ducts, salt is normally removed from the ductal lumen by the action of the CFTR. Patients with CF, however, have an elevated sweat chloride level due to the CFTR defect (Salty taste of skin).

- Note that CFTR mutations affect sweat glands differently than other exocrine glands, since sweat glands normally use CFTR channels to absorb luminal sodium chloride (not secrete it).





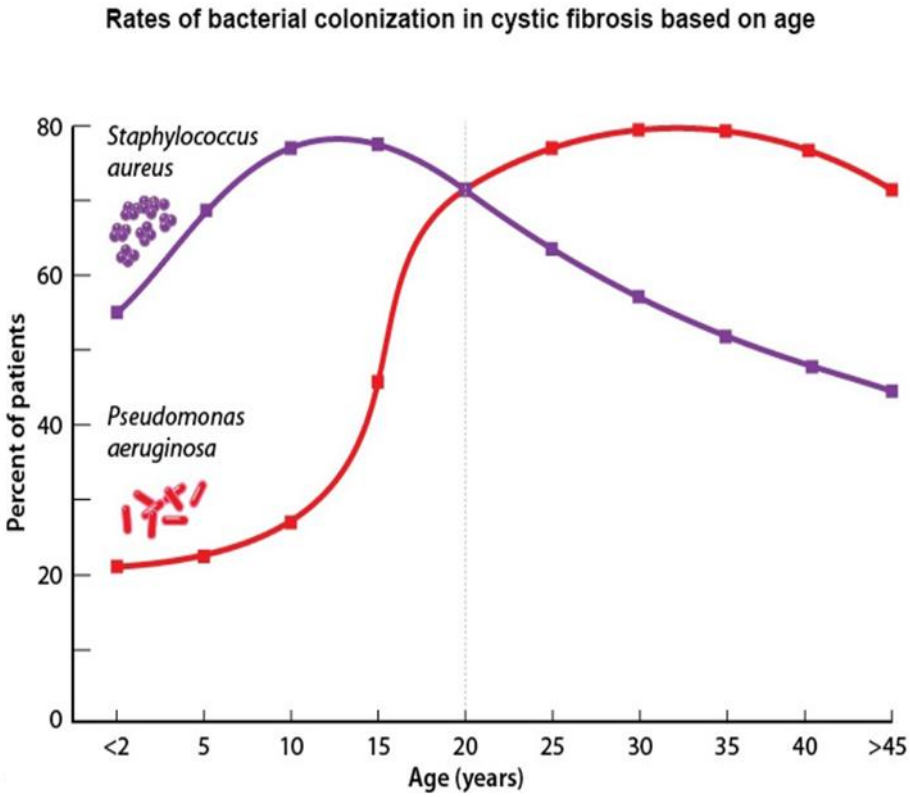
- Complications:

- Recurrent pulmonary infections (S aureus [early infancy], P. aeruginosa [adolescence]), chronic bronchitis and bronchiectasis → reticulonodular pattern on CXR, opacification of sinuses.

- Recurrent sinusitis in a young Caucasian should raise suspicion for cystic fibrosis, as recurrent infections can be caused by the secretion of abnormally thick mucus in the paranasal sinuses.

- Nasal polyps, clubbing of nails.

- Persistent, treatment-resistant infectious pneumonias, bronchiectasis, bronchitic obstructive pulmonary disease and associated cor pulmonale account for 80% of eventual deaths due to CF.



- Pancreatic insufficiency, malabsorption with steatorrhea, fat-soluble vitamin deficiencies (A, D, E, K), biliary cirrhosis, liver disease.

- Meconium ileus in newborns: Meconium ileus is distal small bowel obstruction in a neonate due to abnormally dehydrated meconium. Meconium ileus is quite specific for cystic fibrosis (CF).

- Genitourinary Involvement:
  - Almost all male patients with cystic fibrosis have obstructive azoospermia from congenital bilateral absence of the vas deferens. The vas deferens fails to develop due to accumulation of inspissated mucus in the fetal genital tract, resulting in infertility.
  - Women are infertile because chronic lung disease alters the menstrual cycle and thick cervical mucus blocks sperm entry.

▪ Diagnosis:

- ↑ Cl concentration in pilocarpine-induced sweat test is diagnostic.

- Sweat tests showing chloride levels above 60 mM/L is diagnostic.

- Can present with contraction alkalosis and hypokalemia (ECF effects analogous to a patient taking a loop diuretic) because of ECF H_2O/Na losses and concomitant renal K/H wasting.

- ↑ immunoreactive trypsinogen (newborn screening).

- Treatment:

- Multifactorial: chest physiotherapy, albuterol, aerosolized dornase alfa (DNase), and hypertonic saline facilitate mucus clearance. Azithromycin used as anti-inflammatory agent. Ibuprofen slows disease progression.

- In patients with Phe508 deletion: combination of lumacaftor (corrects misfolded proteins and improves their transport to cell surface) and ivacaftor (opens Cl channels → improved chloride transport).

Features of cystic fibrosis	
Pathogenesis	• Autosomal recessive mutation (**Δ508**) impairs CFTR function • Decreased water content causes **thick, viscous mucus**: ◦ Chronic airway obstruction ◦ Gastrointestinal malabsorption
Clinical manifestations	• Chronic, productive cough • Recurrent **sinopulmonary infections** (especially *Staphylococcus aureus* & *Pseudomonas aeruginosa*) • **Pancreatic insufficiency** • Male infertility (bilateral absence of vas deferens)
Diagnosis	• Elevated sweat chloride levels • Nasal potential difference measurements • Genetic testing for *CFTR* mutations

❖ N.B:

- In the pancreas, severe CF may cause total obstruction followed by complete fibrotic atrophy of the exocrine glands.
- The resulting pancreatic insufficiency can cause a deficiency of fat soluble vitamins.
- Avitaminosis A in particular may contribute to squamous metaplasia of the epithelial lining of pancreatic exocrine ducts, which are already injured and predisposed to squamous metaplasia by inspissated mucus.
- Normal levels of vitamin A and its metabolite, retinoic acid, are required to maintain orderly differentiation of specialized epithelia, including mucus-secreting columnar epithelium.
- When a deficiency state exists, the epithelium undergoes squamous metaplasia to a keratinizing epithelium.

Primary ciliary dyskinesia versus cystic fibrosis		
	Primary ciliary dyskinesia	**Cystic fibrosis**
Pathogenesis	• **Dynein arm defect** → abnormal ciliary motion & impaired mucociliary clearance	• Mutation in the ***CFTR* gene** → impaired ion transport
Respiratory tract features	• Chronic sinopulmonary infections • Nasal polyps • Bronchiectasis • Digital clubbing	• Chronic sinopulmonary infections • Nasal polyps • Bronchiectasis • Digital clubbing
Extrapulmonary features	• Situs inversus (50% of cases) • Infertility due to immotile spermatozoa • Normal growth	• Pancreatic insufficiency • Infertility due to absent vas deferens (azoospermia) • Failure to thrive
Diagnosis	• Low nasal nitric oxide levels • Bronchoscopy & electron microscopic visualization of ciliary abnormalities • Genetic testing	• Elevated sweat chloride levels • Abnormal nasal transepithelial potential difference • Genetic testing

X-linked recessive disorders

- Ornithine transcarbamylase deficiency.
- Fabry disease.
- Wiskott-Aldrich syndrome.
- Ocular albinism.
- G6PD deficiency.
- Hunter syndrome.
- Bruton agammaglobulinemia.
- Hemophilia A and B.
- Lesch-Nyhan syndrome.
- Duchenne (and Becker) muscular dystrophy.
- Oblivious Female Will Often Give Her Boys Her x-Linked Disorders.
- X-inactivation (lyonization): female carriers variably affected depending on the pattern of inactivation of the X chromosome carrying the mutant vs normal gene.
- Females with Turner syndrome (45, XO) are more likely to have an X-linked recessive disorder.

Muscular dystrophies

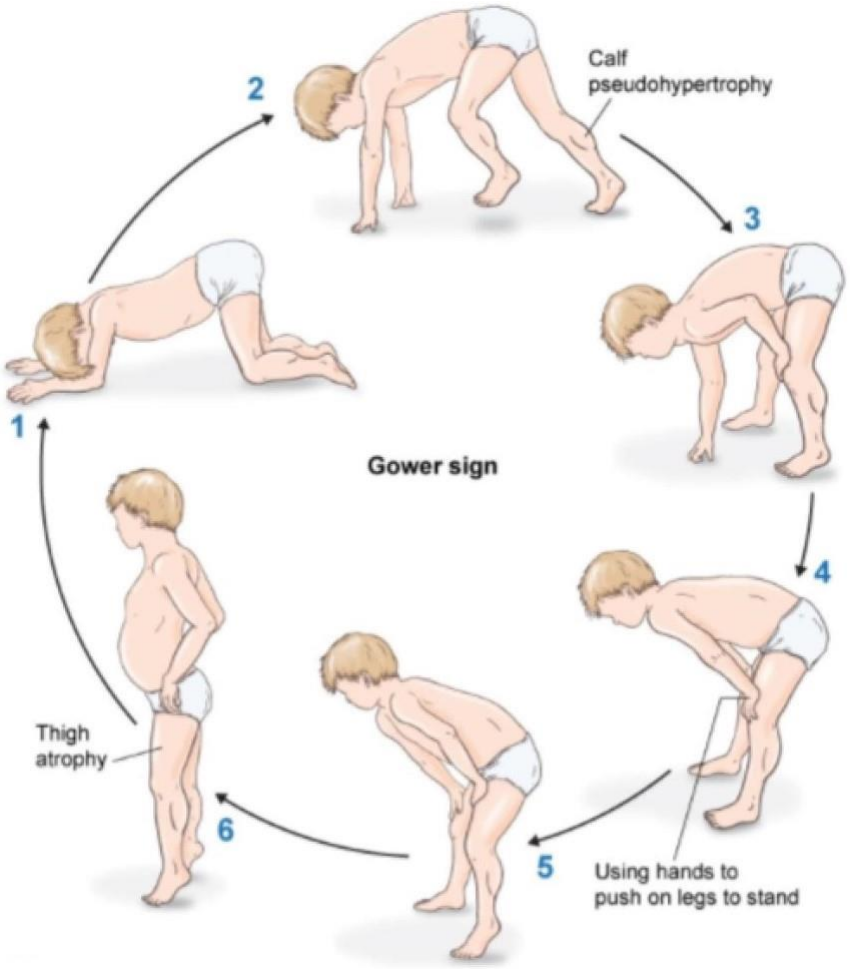
- Muscular dystrophy is a term that applies to the various diseases that manifest with progressive muscular weakness.
- Deletions of the dystrophin gene that encodes the dystrophin protein on X chromosome p21 are the most common mutation in Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD).

Duchenne muscular dystrophy (DMD)

- X-linked disorder and therefore affects primarily boys.
- Typically, due to frameshift or nonsense mutations → truncated or absent dystrophin protein → progressive myofiber damage.
- Duchenne = deleted dystrophin.
- Onset before 5 years of age.
- Dystrophin gene (DMD) is the largest protein-coding human gene → ↑ chance of spontaneous mutation. Dystrophin helps anchor muscle fibers, primarily in skeletal and cardiac muscle. It connects the intracellular cytoskeleton (actin) to the transmembrane proteins α- and β-dystroglycan, which are connected to the extracellular matrix (ECM).
- Loss of dystrophin → myonecrosis.
- Weakness begins in pelvic girdle muscles and progresses superiorly.
- Dilated cardiomyopathy is common cause of death.
- Symptoms of DMD include the following:

1. Walking difficulties: Clumsy, slow, waddling gait; cannot keep up with peers.
2. Gower sign: Progressive weakness in proximal musculature, resulting in use of the hands to support weight on standing. Classically seen in Duchenne muscular dystrophy, but also seen in other muscular dystrophies and inflammatory myopathies (polymyositis).
3. Calf pseudohypertrophy: Calf hypertrophy allows affected children to overcome proximal muscle weakness, but it is later replaced by fat and connective tissue (pseudohypertrophy).
4. Asymmetric weakening of the paraspinal muscles leading to kyphoscoliosis.

- ↑ CK and aldolase; genetic testing confirms diagnosis.
- Duchenne muscular dystrophy should be suspected in a boy age <5 with proximal muscle weakness, Gower sign, and bilateral calf pseudohypertrophy.
- It progressively worsens to involve respiratory and cardiac muscles, eventually causing death by respiratory or heart failure.



Becker muscular dystrophy

- X-linked disorder typically due to non-frameshift deletions in dystrophin gene (partially functional instead of truncated).
- Less severe than Duchenne.
- Onset in adolescence or early adulthood.
- Deletions can cause both Duchenne and Becker muscular dystrophies. 2⁄3 of cases have large deletions spanning one or more exons.

Myotonic type 1

- Autosomal dominant.
- CTG trinucleotide repeat expansion in the dystrophia myotonica protein kinase gene (DMPK) gene → abnormal expression of myotonin protein kinase → Myotonia (delayed muscle relaxation) is most notable when the patient is unable to release the hand after a handshake (grip myotonia), muscle wasting, cataracts, testicular atrophy, frontal balding, arrhythmia.
- Cataracts, Toupee (early balding in men), Gonadal atrophy.

Muscular dystrophies			
Diagnosis	**Duchenne**	**Becker**	**Myotonic**
Genetics	X-linked recessive deletion of dystrophin gene on chromosome Xp21		Autosomal dominant expansion of a CTG trinucleotide repeat in DMPK gene on chromosome 19q 13.3
Clinical presentation	• Onset: age 2-3 • Progressive weakness, Gower maneuver, calf pseudohypertrophy	• Onset: age 5-15 • Milder weakness compared to Duchenne muscular dystrophy	• Onset: age 12-30 • Facial weakness, hand grip myotonia, dysphagia
Comorbidities	• Scoliosis Cardiomyopathy	• Cardiomyopathy	• Arrhythmias • Cataracts • Balding • Testicular atrophy/ infertility
Prognosis	• Wheelchair-dependent by adolescence • Death by age 20-30 from respiratory or heart failure	Death by age 40-50 from heart failure	Death from respiratory or heart failure depending on age of onset

Fragile X syndrome

- X-linked dominant inheritance.
- Fragile X syndrome is caused by mutation of the fragile X mental retardation 1 (FMR1) gene on the long arm of the X chromosome.
- Most common cause of inherited intellectual disability and 2nd most common cause of genetically associated mental deficiency (after Down syndrome).
- Trinucleotide repeat expansion [(CGG)n] occurs during oogenesis.
- The FMR1 gene product is required for normal neural development. Normally this gene has between 5 and 55 CGG trinucleotide repeats. Individuals with an increased number of repeats are said to have a "premutation" as long as the number of repeats remains less than 200. Patients with the premutation are usually phenotypically normal, although the increased number of CGG repeats leads to transcriptional instability which promotes further repeat expansion. "Full mutation" occurs when there are more than 200 CGG repeats on the FMR1 gene. At this point, the increased number of trinucleotide repeats causes hypermethylation of the FMR1 gene leading to gene inactivation.
- Fragile X syndrome is so named because when lymphocytes from affected individuals are cultured in a folate- and thymidine-depleted medium and the resulting karyotype is analyzed, this region of the X-chromosome appears constricted and thin ("fragile").
- Patients with fragile X syndrome display the following features:

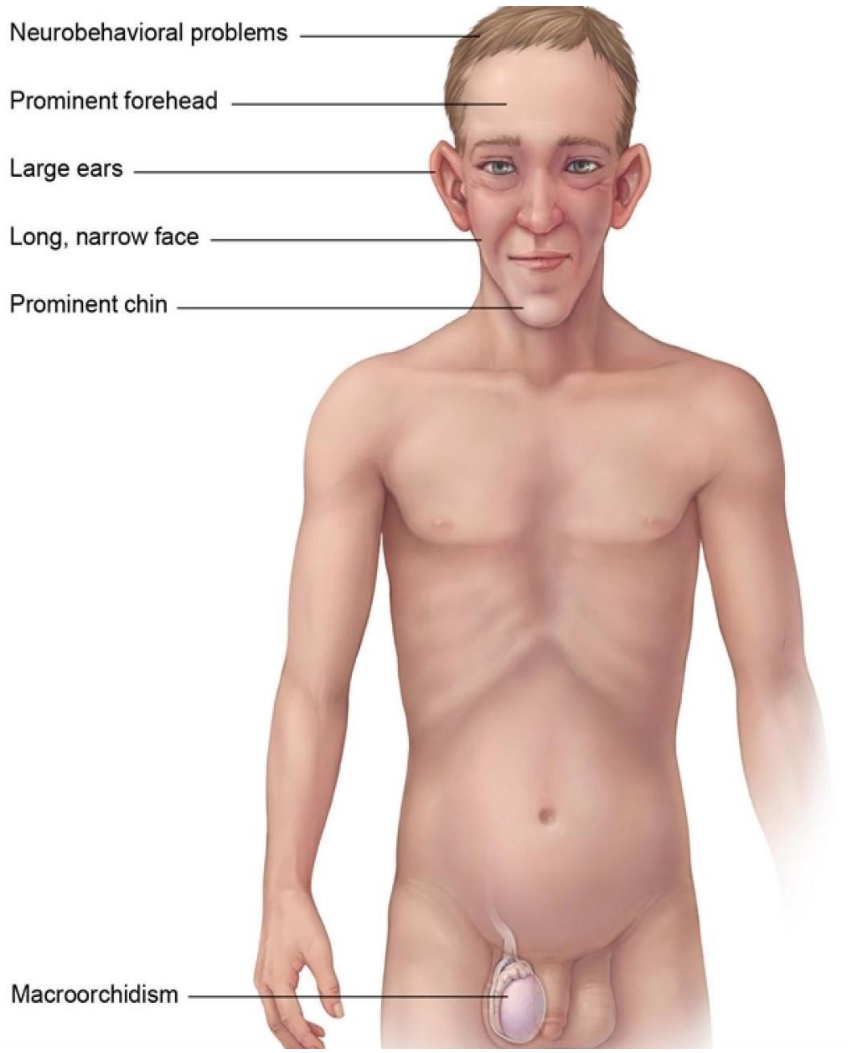
1. Body habitus:

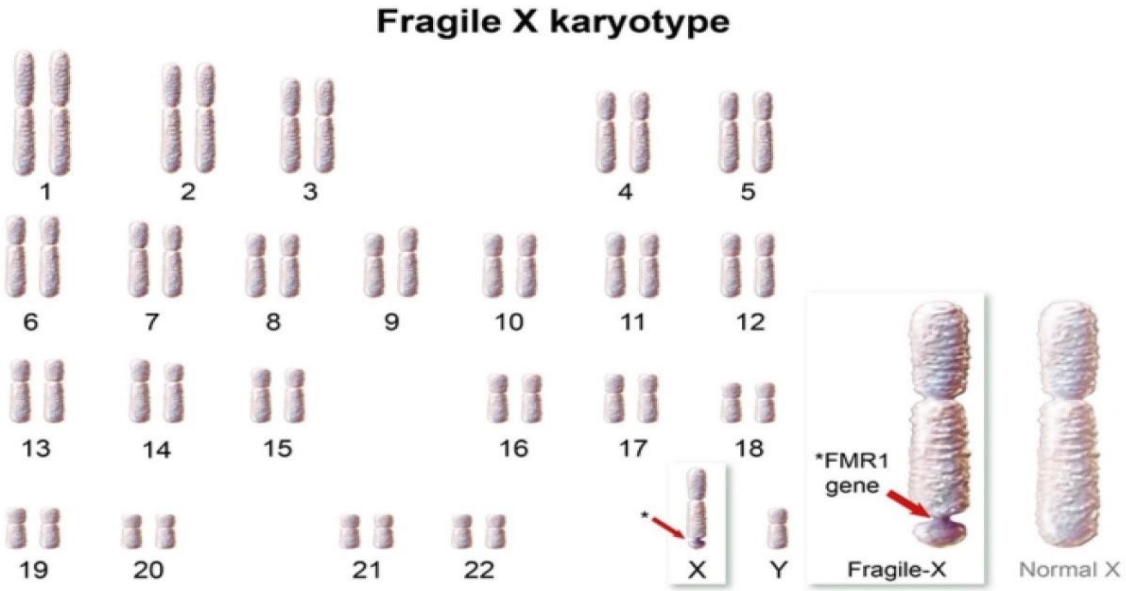
- Macrosomia with increased head circumference may be present at birth.
- Older patients have dysmorphic facial features including large jaw, large protruding ears, long thin face and prominent forehead.
- Postpubertal males invariably have macroorchidism (enlarged testes).

2. Cognitive impairment:

- Becomes evident after the 1st year of life.
- Patients demonstrate mild-to-moderate mental retardation, severe language delay and behavioral abnormalities (such as aggressiveness).
- Autistic features are more common in children with fragile X syndrome than in the general population.

3. Mitral valve prolapse.

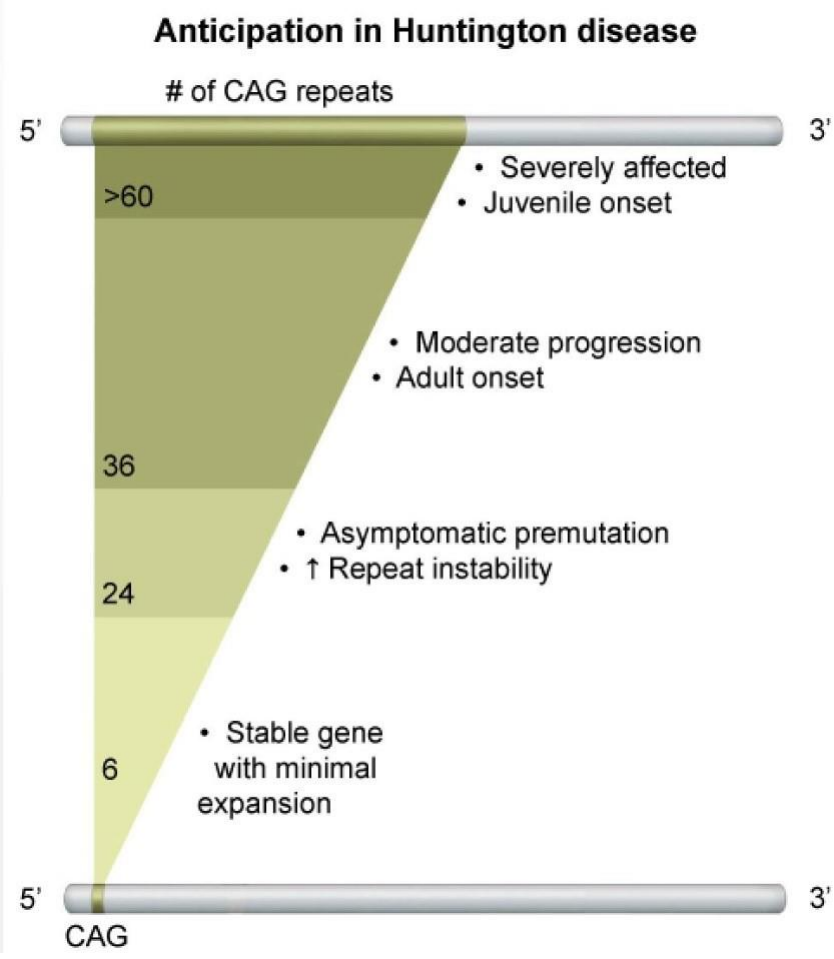




Trinucleotide repeat expansion diseases

- Huntington disease, myotonic dystrophy, fragile X syndrome, and Friedreich ataxia.
- May show genetic anticipation (disease severity ↑ and age of onset ↓ in successive generations).
- Try (trinucleotide) hunting for my fragile cage free eggs (X).

Disease	Trinucleotide Repeat	Mode of inheritance	Mnemonic
Huntington disease	(CAG)n	AD	Caudate has ↓ ACh and GABA
Myotonic dystrophy	(CTG)n	AD	Cataracts, Toupee (early balding in men), Gonadal atrophy
Fragile X syndrome	(CGG)n	XD	Chin (protruding), Giant Gonads
Friedreich ataxia	(GAA)n	AR	Ataxic GAAit



Numerical chromosome abnormalities

A. Euploidy:

- When a cell has a multiple of 23 chromosomes, it is said to be euploid.
- Gametes (sperm and egg cells) are euploid cells that have 23 chromosomes (one member of each pair); they are said to be haploid.
- Most somatic cells are diploid, containing both members of each pair, or 46 chromosomes.
- Two types of euploid cells with abnormal numbers of chromosomes are seen in humans: triploidy and tetraploidy but the vast majority of these conceptions are lost prenatally.

B. Aneuploidy:

- Aneuploidy, a deviation from the euploid number, represents the gain (+) or loss (-) of a specific chromosome. Two major forms of aneuploidy are observed:
  - Monosomy (loss of a chromosome).
  - Trisomy (gain of a chromosome).
- Generally caused by nondisjunction.
- All autosomal monosomies are inconsistent with a live birth.
- Only 3 autosomal trisomies (trisomy 13, 18, and 21) are consistent with a live birth.
- Aneuploidy involving the sex chromosomes is relatively common and tends to have less severe consequences than does autosomal aneuploidy.
- The two important sex chromosome aneuploidies are Turner syndrome and Klinefelter syndrome.

Autosomal trisomies

Down syndrome (trisomy 21)

- The multi-system abnormalities found in Down syndrome are the result of extra genetic material from chromosome 21. Three cytogenetic abnormalities can produce Down syndrome:

1. Trisomy 21 accounts for nearly 95% of Down syndrome cases:
- Meiotic nondisjunction (failure of homologous chromosomes to separate during meiosis) of chromosome 21 occurs in the ovum, resulting in the inheritance of three copies of this chromosome in one daughter cell (trisomy) and one copy in the other daughter cell (monosomy).
- Nondisjunction is almost always of maternal origin; increased maternal age is a risk factor.

2. Unbalanced Robertsonian translocations:
- Account for 2-3% of Down syndrome cases.
- These individuals have 46 chromosomes, but an extra arm of chromosome 21 is attached to another chromosome (translocation).

3. Mosaicism can also cause Down syndrome:
- Patients have two cell lines: one with a normal genotype, and one with trisomy 21.

- Most common viable chromosomal disorder and most common cause of genetic intellectual disability.

- Findings:
- Intellectual disability, flat facies, small mouth, slanted palpebral fissures, prominent epicanthal folds, single palmar crease, gap between 1st 2 toes, Brushfield spots.

- Cardiac defects are found in approximately half of all infants with Down syndrome, with the endocardial cushion defect (atrioventricular septal defect) and ventricular septal defect most often seen.

- Gastrointestinal tract abnormalities are also identified in 10-15% of this patient population, and can include duodenal atresia, Hirschsprung's disease, and tracheoesophageal fistula.

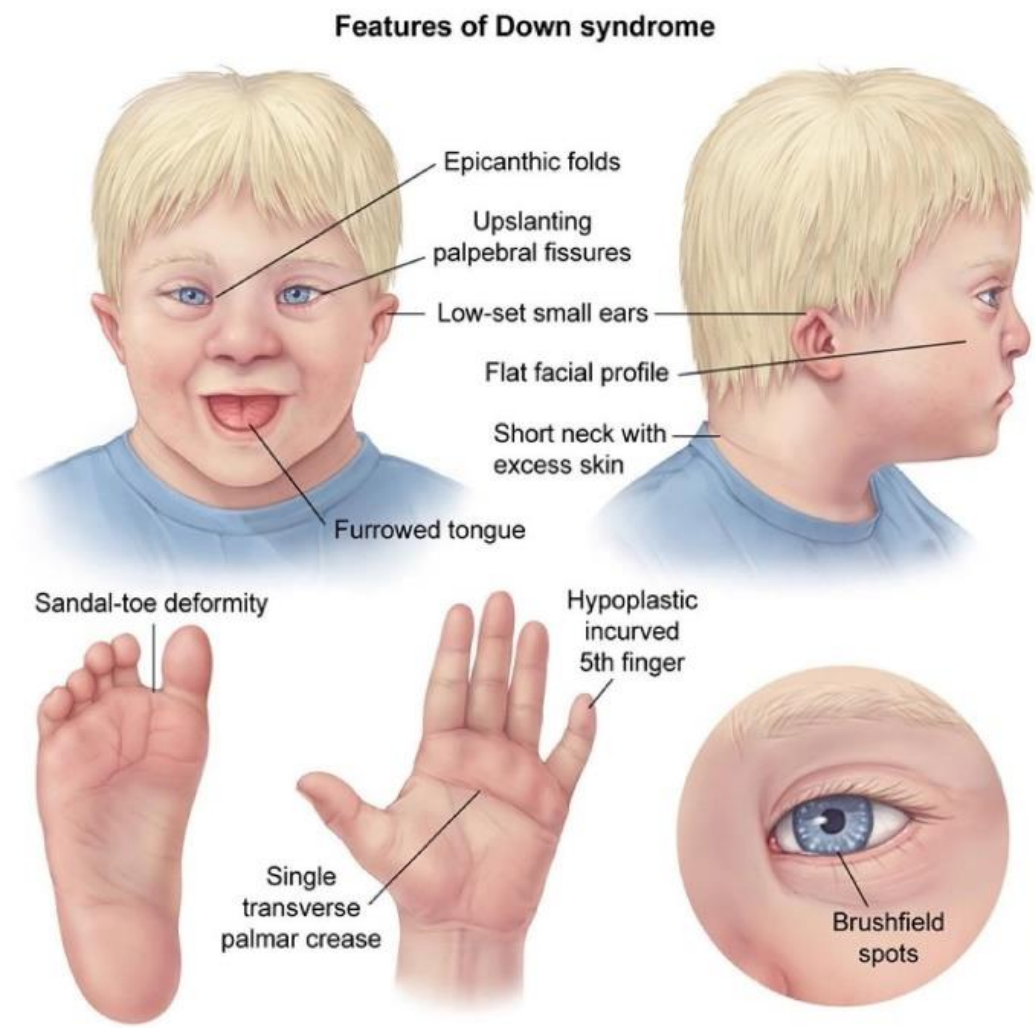
- Alzheimer disease (chromosome 21 codes for amyloid precursor protein) and ↑ risk of ALL and AML. The extra copy of APP present in Down syndrome is thought to accelerate amyloid accumulation and lead to early-onset Alzheimer's dementia.

- Individuals with Down syndrome have a 10- to 20-fold increased risk of developing acute lymphoblastic leukemia, and their risk for developing acute myelogenous leukemia is also increased.

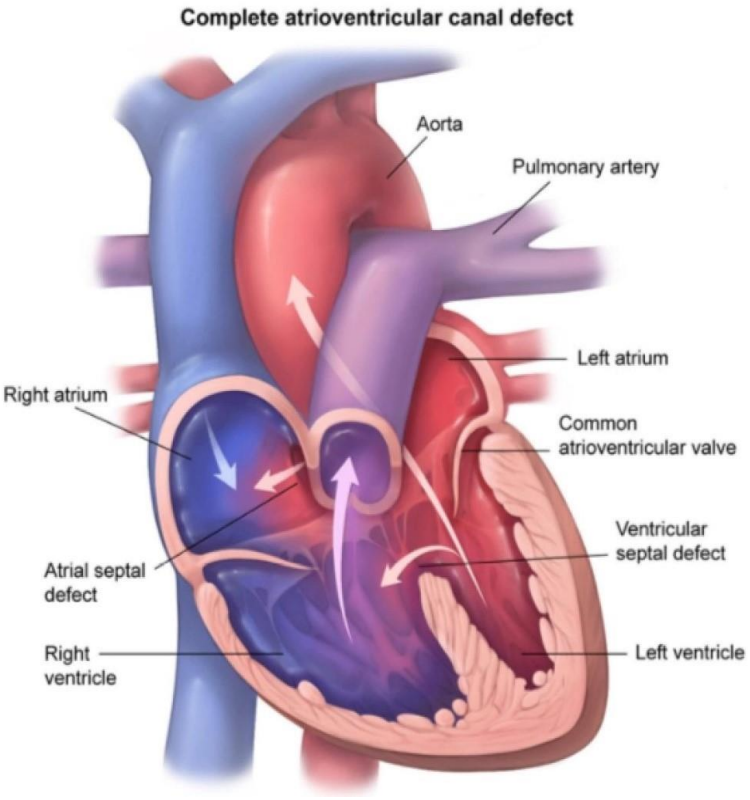
- Incidence 1:700.

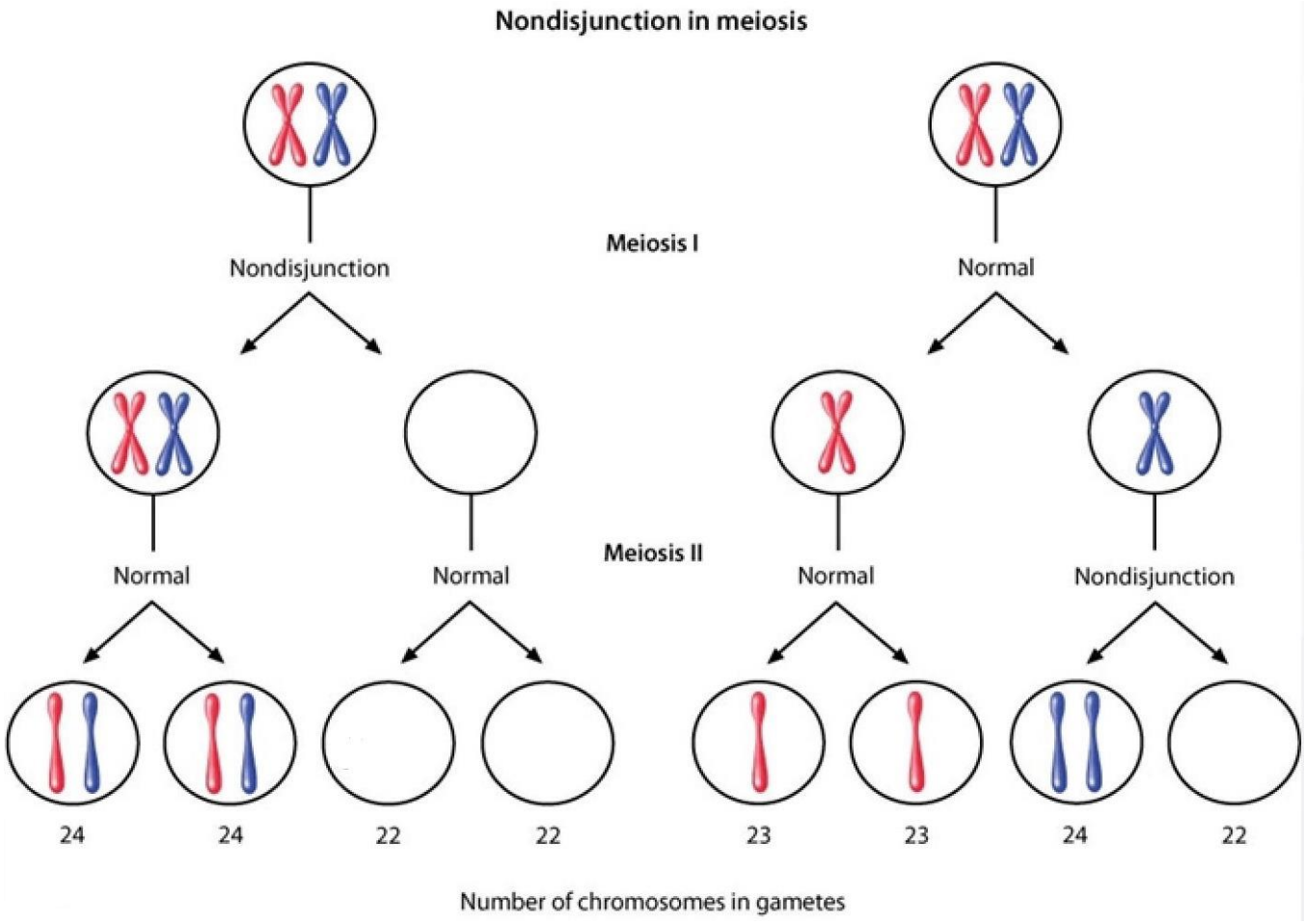
- Drinking age (21).
- First-trimester ultrasound commonly shows ↑ nuchal translucency and hypoplastic nasal bone.
- Triple test, performed at weeks 16-18 of gestation, detects low alpha-fetoprotein (AFP) levels. A finding of low AFP on triple test is associated with a diagnosis of Down syndrome and is therefore an indication for amniocentesis. Karyotyping of fetal cells contained in amniotic fluid can diagnose Down syndrome.
- <u>The 5 A's of Down syndrome:</u>

- Advanced maternal age.
- Atresia (duodenal).
- Atrioventricular septal defect.
- Alzheimer disease (early onset).
- AML/ALL.



Down syndrome comorbidities	
Neurology	• Intellectual disability • Early-onset Alzheimer disease
Cardiology	• Complete atrioventricular septal defect • Ventricular septal defect • Atrial septal defect
Gastroenterology	• Duodenal atresia • Hirschsprung disease
Endocrinology	• Hypothyroidism • Type 1 diabetes mellitus • Obesity
Hematology	• Acute leukemia
Rheumatology	• Atlantoaxial instability



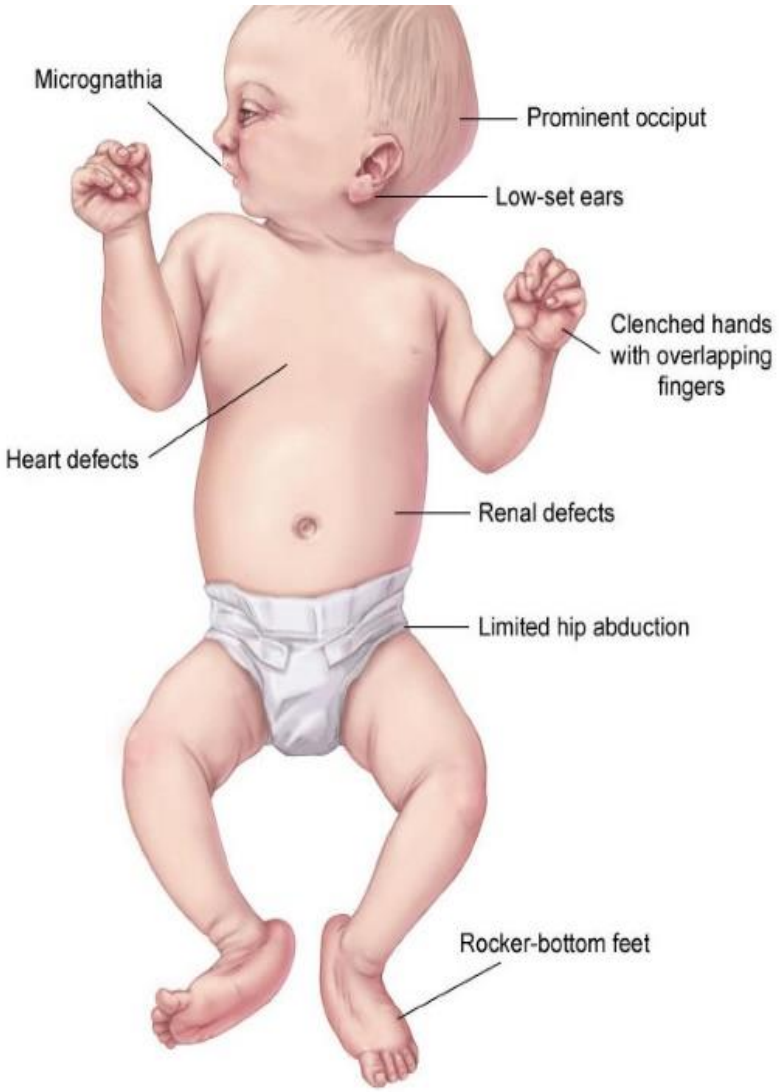


Edwards syndrome (trisomy 18)

- Incidence 1:8000.
- Election age (18).
- 2nd most common autosomal trisomy resulting in live birth (most common is Down syndrome).
- Findings:

- PRINCE Edward: Prominent occiput, Rocker-bottom feet, Intellectual disability, Nondisjunction, Clenched fists (with overlapping fingers), low-set Ears, micrognathia (small jaw), congenital heart disease, omphalocele.

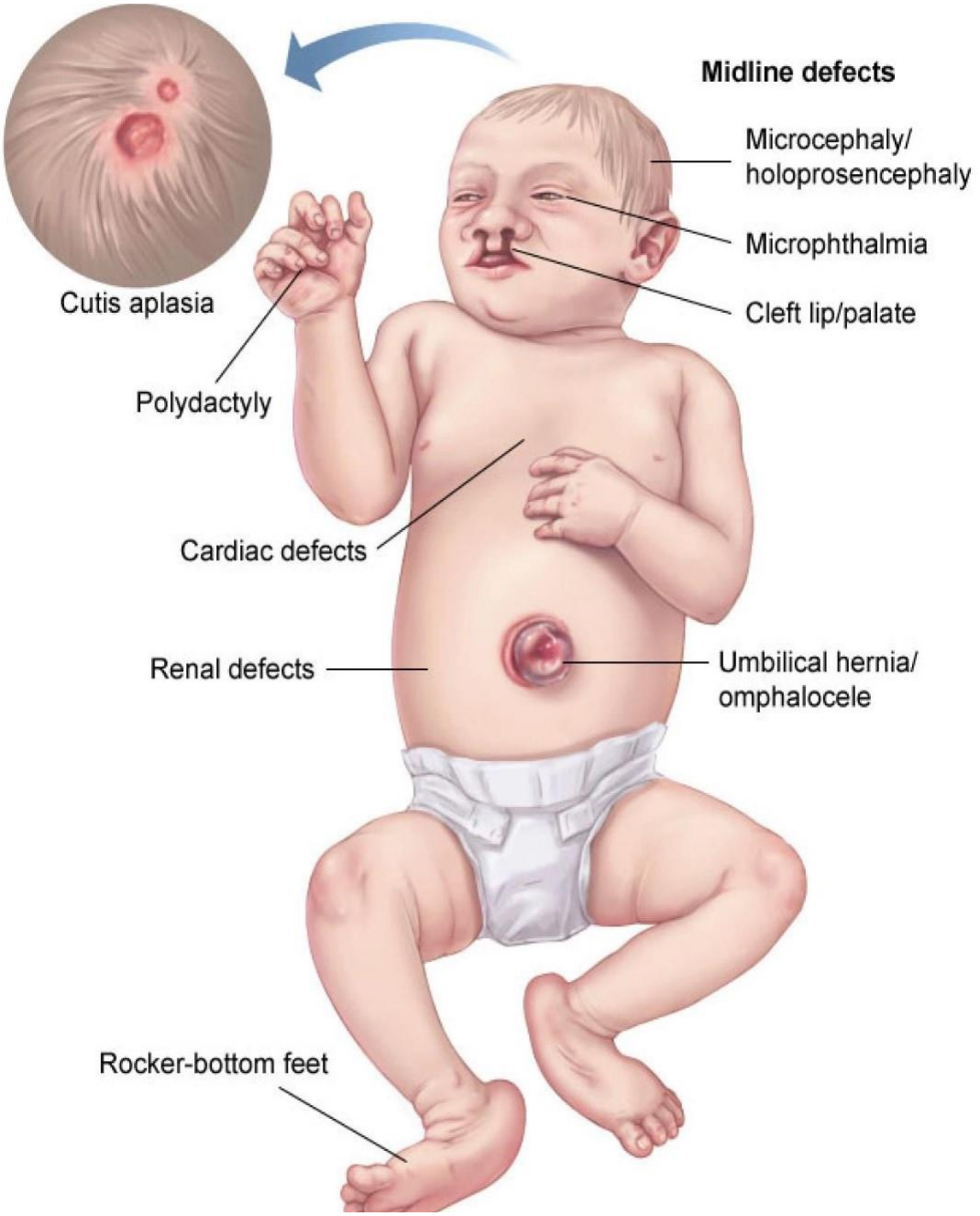
- Death usually occurs by age 1.



Patau syndrome (trisomy 13)

- Incidence 1:15,000.
- Puberty (13).
- Findings:

- Severe intellectual disability, rocker-bottom feet, microphthalmia, microcephaly, cleft liP/Palate, holoProsencephaly, Polydactyly, cutis aPlasia (congenital abscense of the skin), congenital heart disease, Polycystic kidney disease, omphalocele. Death usually occurs by age 1.



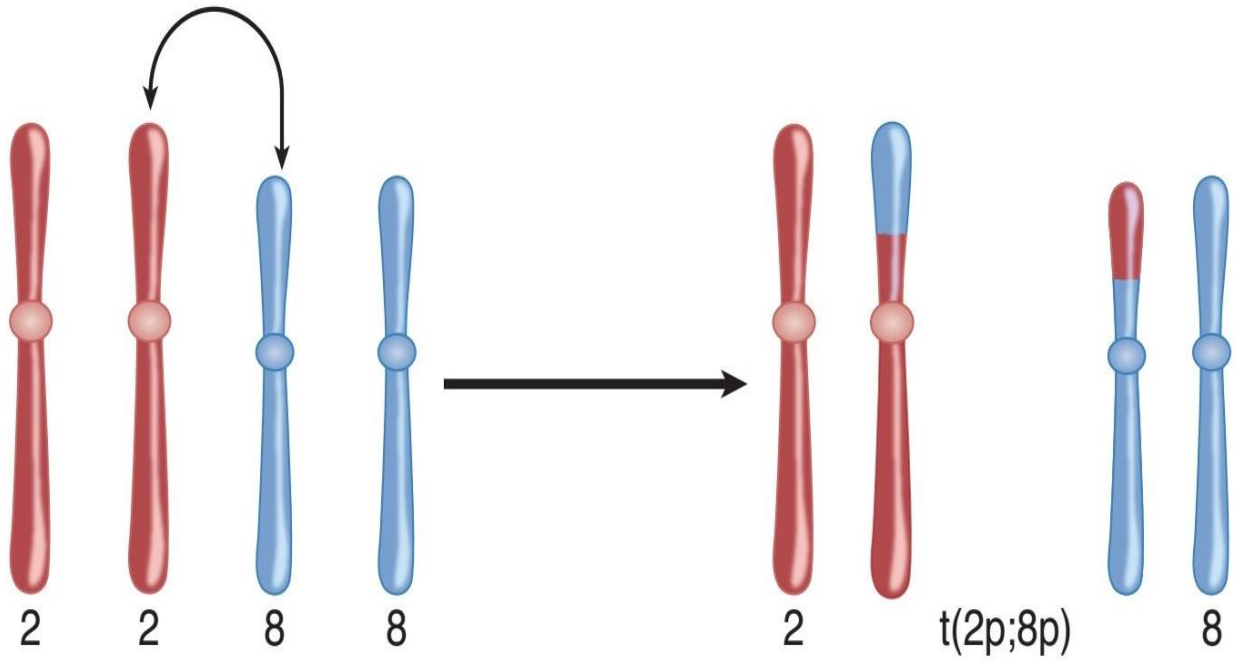
Structural chromosome abnormalities

- Balanced alterations result in no loss or gain of genetic material.
- Unbalanced alterations result in loss or gain of genetic material.
- Those that happen in germline can be transmitted to offspring.
- Those that happen in somatic cells can cause cancer.

Translocation

A. Reciprocal translocation:

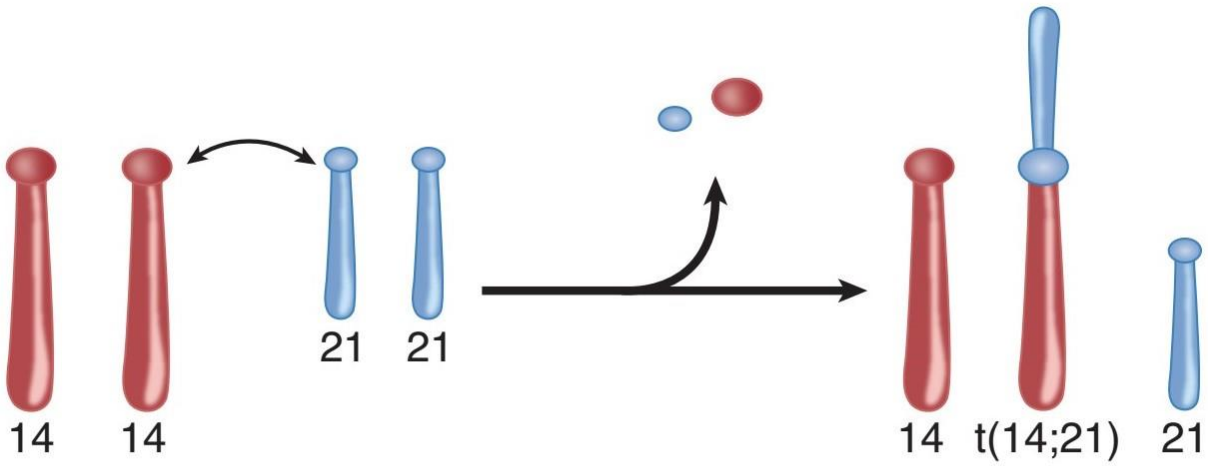
- Reciprocal translocations occur when genetic material is exchanged between nonhomologous chromosomes.



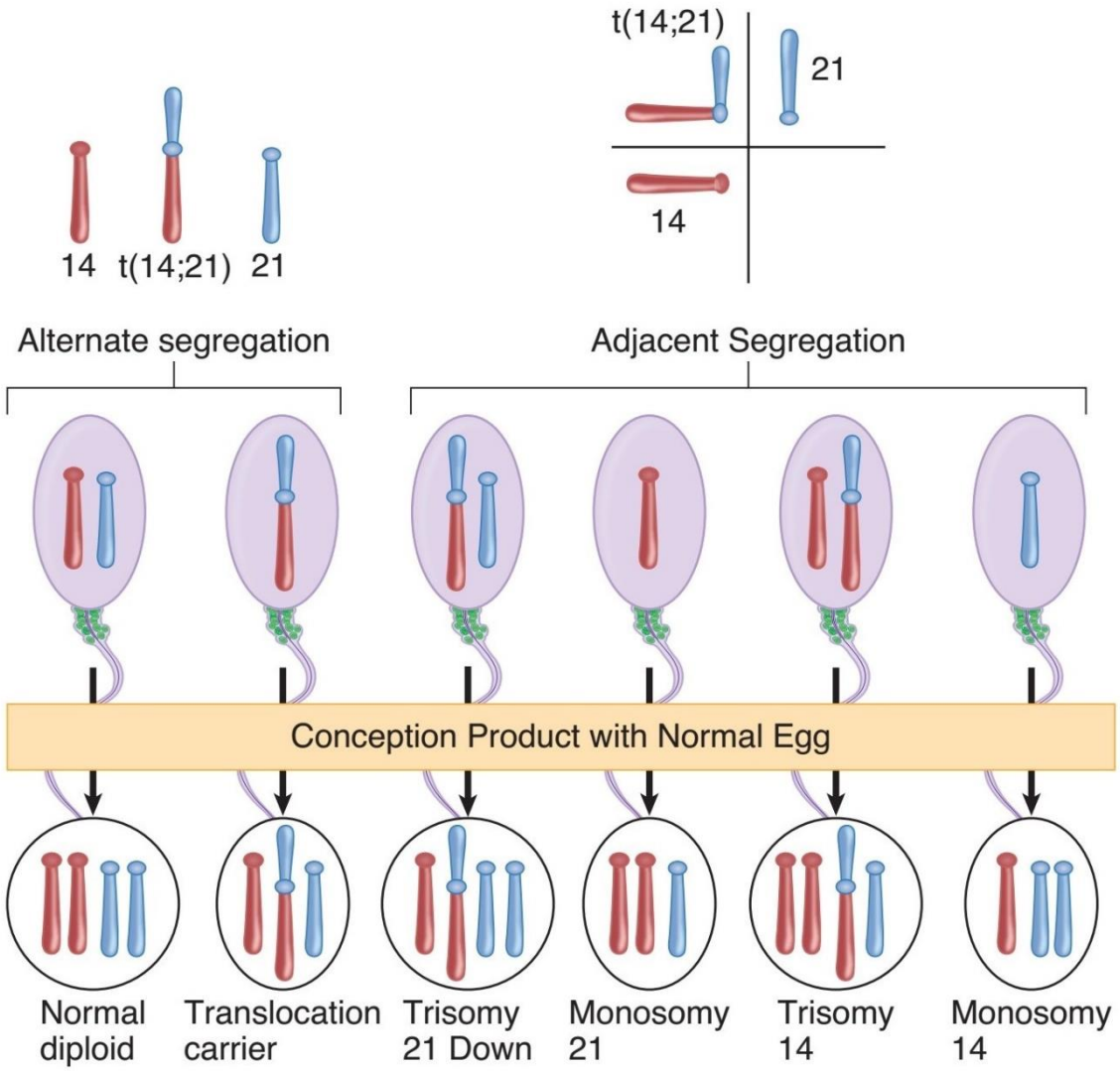
- A classic example is a reciprocal translocation of the long arms of chromosomes 9 and 22, termed the Philadelphia chromosome. This translocation alters the activity of the abl proto-oncogene (proto-oncogenes can lead to cancer). When this alteration occurs in hematopoietic cells, it can result in chronic myelogenous leukemia.

B. Robertsonian translocation:

- One of the most common types of translocation.
- Occurs when the long arms of 2 acrocentric chromosomes (chromosomes with centromeres near their ends) fuse at the centromere and the 2 short arms are lost.
- Because the short arms of the acrocentric chromosomes contain no essential genetic material, their loss produces no clinical consequences, and the translocation carrier is not clinically affected.
- Chromosomal translocation that commonly involves chromosome pairs 13, 14, 15, 21, and 22.
- Balanced translocations normally do not cause any abnormal phenotype.
- Unbalanced translocations can result in miscarriage, stillbirth, and chromosomal imbalance (Down syndrome, Patau syndrome).



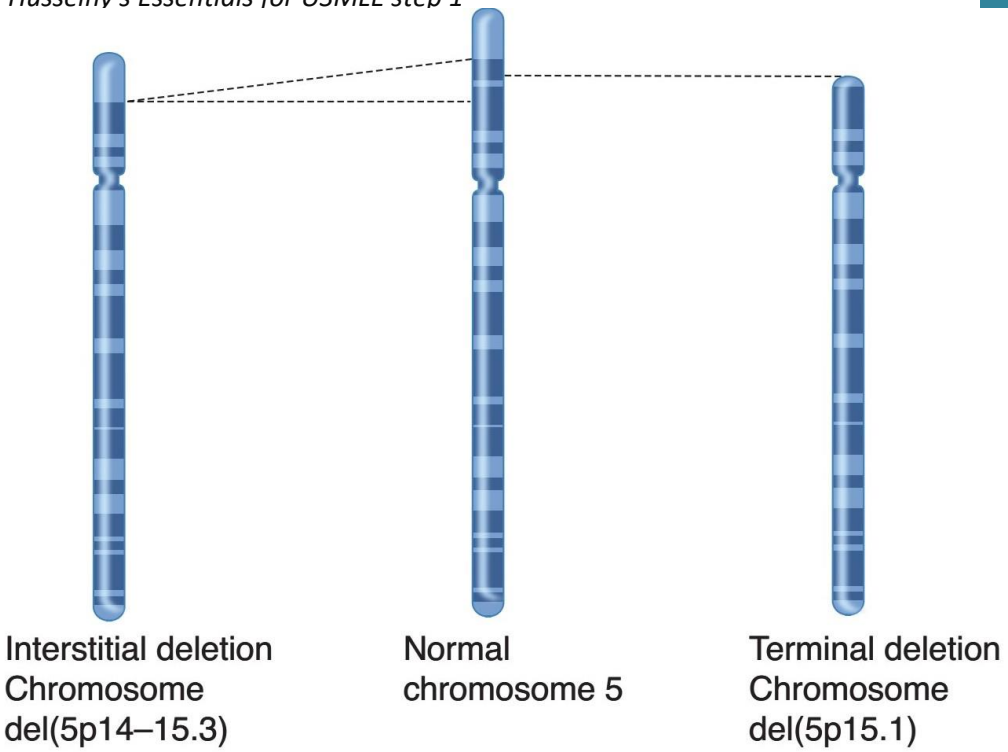
A Robertsonian Translocation



Consequences of a Robertsonian Translocaton in One Parent (Illustrated with Male)

Deletions

- A deletion occurs when a chromosome loses some of its genetic information.
- Terminal deletions (the end of the chromosome is lost) and interstitial deletions (material within the chromosome is lost) may be caused by agents that cause chromosome breaks and by unequal crossover during meiosis.



Terminal and Interstitial Deletions of Chromosome 5p

A. Cri-du-chat syndrome:

- Congenital deletion on short arm of chromosome 5 (46, XX or XY, 5p−).
- Findings: Microcephaly, moderate to severe intellectual disability, high-pitched crying/ meowing, epicanthal folds, cardiac abnormalities (VSD).
- Cri du chat = cry of the cat.

B. Williams syndrome:

- Congenital microdeletion of long arm of chromosome 7 (deleted region includes elastin gene).
- Findings: Distinctive "elfin" facies, intellectual disability, hypercalcemia (↑ sensitivity to vitamin D), well-developed verbal skills, extreme friendliness with strangers, cardiovascular problems (supravalvular aortic stenosis, renal artery stenosis).
- Think Will Ferrell in Elf.

C. 22q11 deletion syndromes:

- Microdeletion at chromosome 22q11 → variable presentations including Cleft palate, Abnormal facies, Thymic aplasia → T-cell deficiency, Cardiac defects (truncus arteriosus, tetralogy of Fallot), and Hypocalcemia 2° to parathyroid aplasia.
- DiGeorge syndrome: thymic, parathyroid, and cardiac defects.
- Velocardiofacial syndrome: palate, facial, and cardiac defects.

- CATCH-22.
- Due to aberrant development of 3rd and 4th branchial (pharyngeal) pouches.

Genetic disorders by chromosome

CHROMOSOME	SELECTED EXAMPLES
3	von Hippel-Lindau disease, renal cell carcinoma
4	ADPKD (*PKD2*), achondroplasia, Huntington disease
5	Cri-du-chat syndrome, familial adenomatous polyposis
6	Hemochromatosis (*HFE*)
7	Williams syndrome, cystic fibrosis
9	Friedreich ataxia, tuberous sclerosis (*TSC1*)
11	Wilms tumor, β-globin gene defects (eg, sickle cell disease, β-thalassemia), MEN1
13	Patau syndrome, Wilson disease, retinoblastoma (*RB1*), *BRCA2*
15	Prader-Willi syndrome, Angelman syndrome, Marfan syndrome
16	ADPKD (*PKD1*), α-globin gene defects (eg, α-thalassemia), tuberous sclerosis (*TSC2*)
17	Neurofibromatosis type 1, *BRCA1*, *TP53*
18	Edwards syndrome
21	Down syndrome
22	Neurofibromatosis type 2, DiGeorge syndrome (22q11)
X	Fragile X syndrome, X-linked agammaglobulinemia, Klinefelter syndrome (XXY)

Hardy-Weinberg population genetics

- <u>If a given gene has 2 possible allels (A and a):</u>

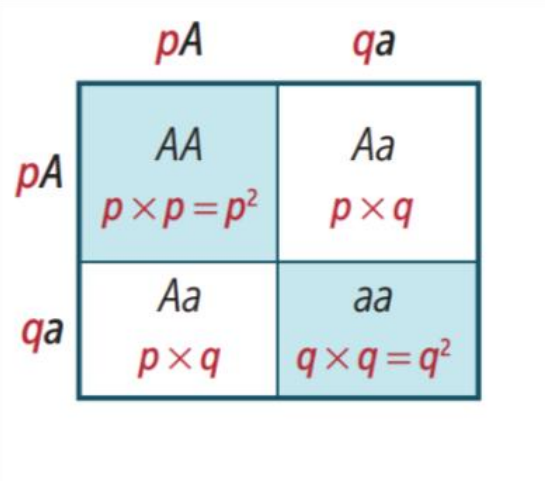
- Allel A found in 40% of genes (p = .4).
- Allel a found in 60% of genes (q = .6).

$$P + q = 1$$

- If a population is large and individuals' mate at random, there should be a constant and predictable relationship between genotype frequencies and allel frequencies:

$$p^2 + 2pq + q^2 = 1$$

- p = frequency of allele A (conventionally the most common, normal allele).
- q = frequency of allele a (conventionally a minor, disease-producing allele).
- p^2 = frequency of genotype A-A (conventionally homozygous normal).
- 2pq = frequency of genotype A-a (conventionally heterozygous).
- q^2 = frequency of genotype a-a (conventionally homozygous affected).



- <u>Hardy-Weinberg law assumptions include:</u>

- No mutation occurring at the locus.
- Natural selection is not occurring.
- Completely random mating.
- No net migration.

- In most cases where this equation is used, a simplification is possible. Generally p, the normal allele frequency in the population, is very close to 1 (most of the alleles of this gene are normal). In this case, we may assume that p ~ 1, and the equation simplifies to:

$$1 + 2q + q^2 \sim 1$$

- Practical Application of Hardy-Weinberg:

1. Autosomal recessive diseases:

A. Prevalence of Phenylketonuria (PKU) is 1/10,000 live births. What is the carrier frequency of this population?

- Disease prevalence = q^2 = 1/10,000 live births → q = 1/100.
- Carrier frequency = 2pq.
- Generally, p the normal allele frequency in the population is very close to 1 (most of the alleles of this gene are normal). In this case, we may assume that p ~ 1, and the carrier frequency is simplified to 2q.
- Carrier frequency = 2q = 2 (1/100) = 1/50 = .02.

B. 1 in every 30 ashkenazi Jews is a carrier for tay-sachs disease. What is the prevalence of tay-sachs in the Ashkenazi Jews population?

- 2q = 1/30 → q = 1/60.
- Prevalence of the disease = $q^2 = (1/60)^2$ = 1/3600.

❖ In a nutshell:

- There are 3 major terms one usually works with in the Hardy-Weinberg equation applied to autosomal recessive conditions:

- q^2 = the disease prevalence.
- 2q = the carrier frequency.
- q = the frequency of the disease-causing allele.

2. Autosomal dominant diseases:

$$p^2 + 2pq + q^2 = 1$$

- q^2 and 2pq are affected (no carriers in autosomal dominant diseases).

3. X linked recessive diseases:

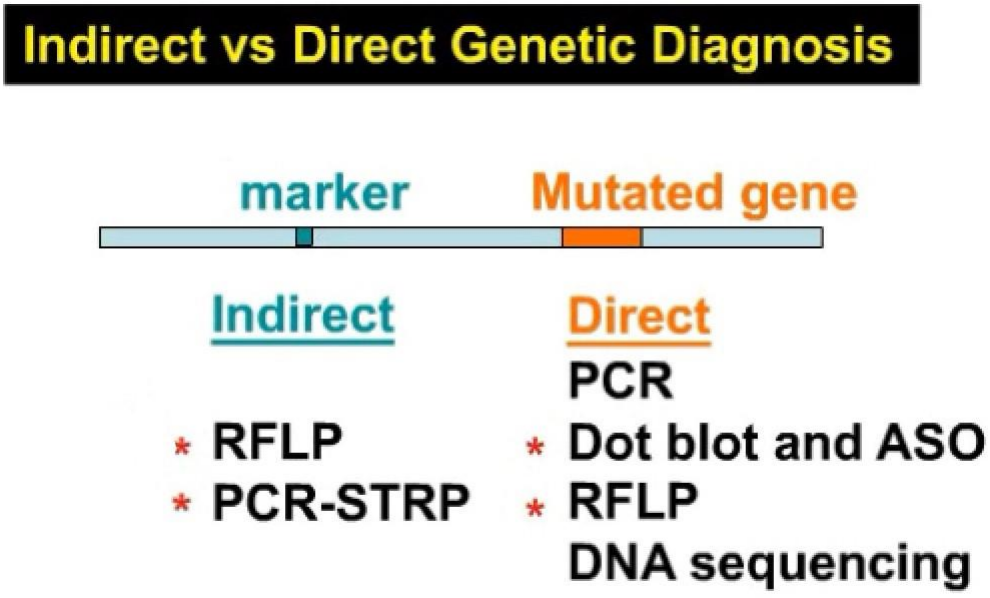
- The allele frequency for X-linked diseases is obtained by counting the number of affected males in the population.
- The frequency of an X-linked recessive disease in males = q and in females = q^2.
- If 1/10000 males have hemophilia A, therefore q = 1/10000. The number of affected females will be $q^2 = 1/10^8$.

Genetic diagnosis

- Once a gene is identified, the associated genetic disease in at-risk individuals can be diagnosed.

- The goal of genetic diagnosis is to determine whether an at-risk individual has inherited a disease-causing gene. Genetic diagnosis can be distinguished into 2 types:

- Direct diagnosis: the mutation itself is examined.
- Indirect diagnosis: linked markers are used to infer whether the individual has inherited the chromosome segment containing the disease-causing mutation.



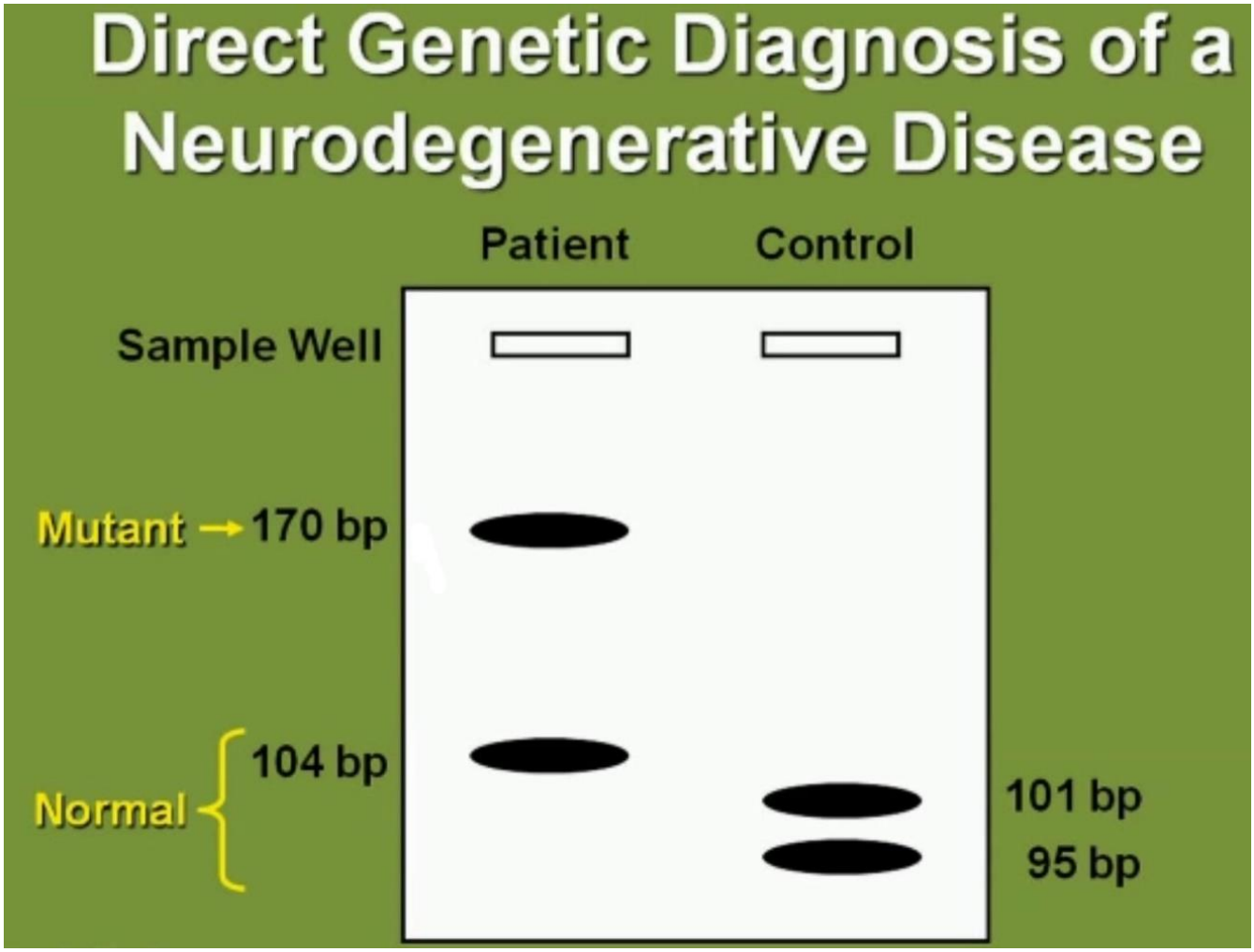
Direct diagnosis

1. Gel electrophoresis of PCR Products:

- Patient suspected of having Huntington disease (AD).

- Amplify portion of gene that may have a trinucleotide repeat expansion by PCR.

- Measure size of products by electrophoresis.



2. Allel-specific oligonucleotide (ASO) probes:

- Patient being tested for hereditary hemochromatosis (AR).

- Prepare two ASO probes:

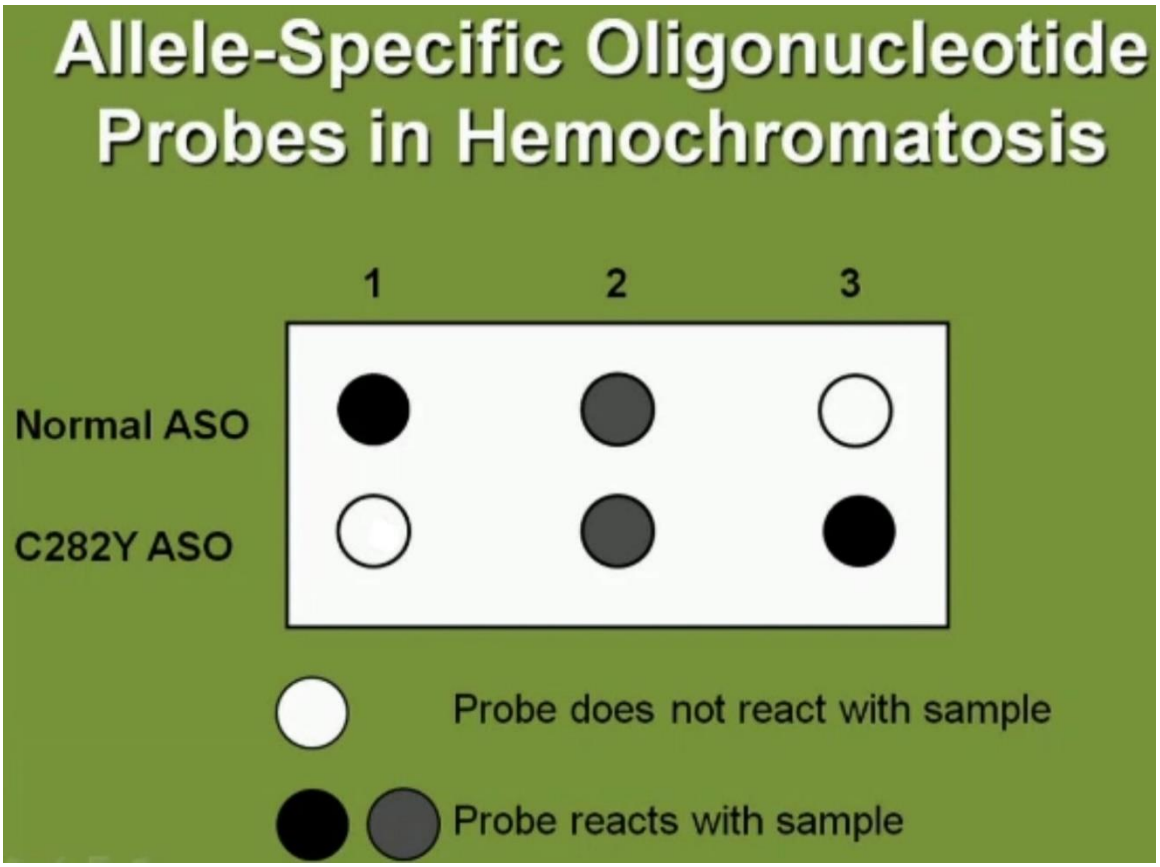
- Normal.
- Mutant.

- Amplify patient's DNA with PCR.

- Probe with normal and mutant ASO probes:

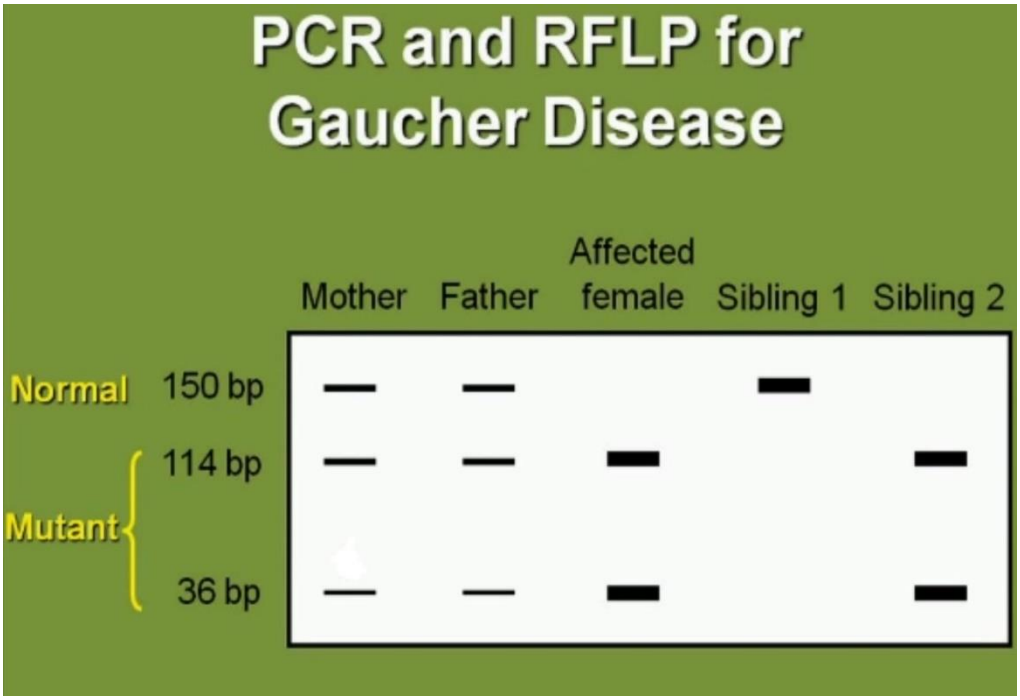
- Patient number 1 is homozygous normal.
- Patient number 2 is heterozygous carrier.
- Patient number 3 is homozygous mutant.

- The reason why patient number 1 has black spot compared to the grey sopt in patient number 2 is that the homozygous has twice the amount of DNA in the same spot.



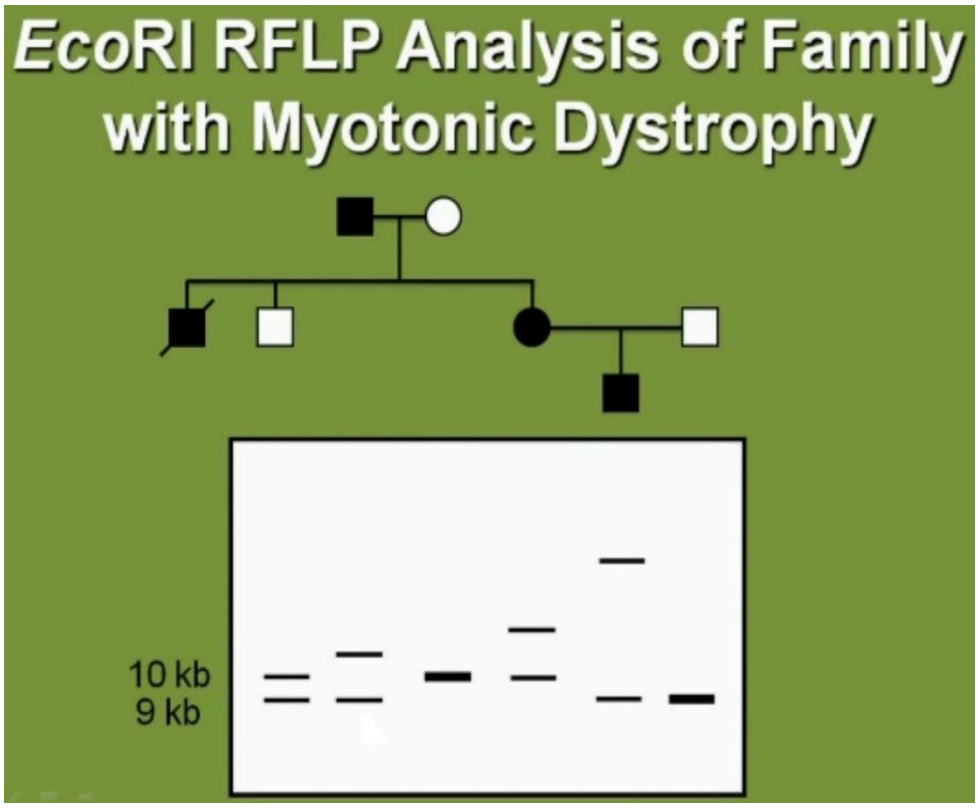
3. RFLP analysis of PCR Products:

- Patient tested for gaucher disease (AR).
- Amplify DNA with PCR.
- Cut PCR products with a restriction endonuclease.
- Normal sequence is not cut.
- Mutant sequence is cut into 2 smaller pieces.
- The mother and the father are heterozygous carriers.
- The affected daughter is homozygous mutant and is drawn thicker because it hase 2 copies of the mutant allel.
- Sibling 1 must be homozygous normal and will not be affected with the disease.
- Sibling 2 will be homozygous mutant and will be affected with the disease.



4. RFLP diagnosis of myotonic dystrophy (AD):

- Caused by trinucleotide repeat expansion.
- Test using southern blotting.
- Size of DNA band increases with each generation (Anticipation).



Indirect genetic diagnosis

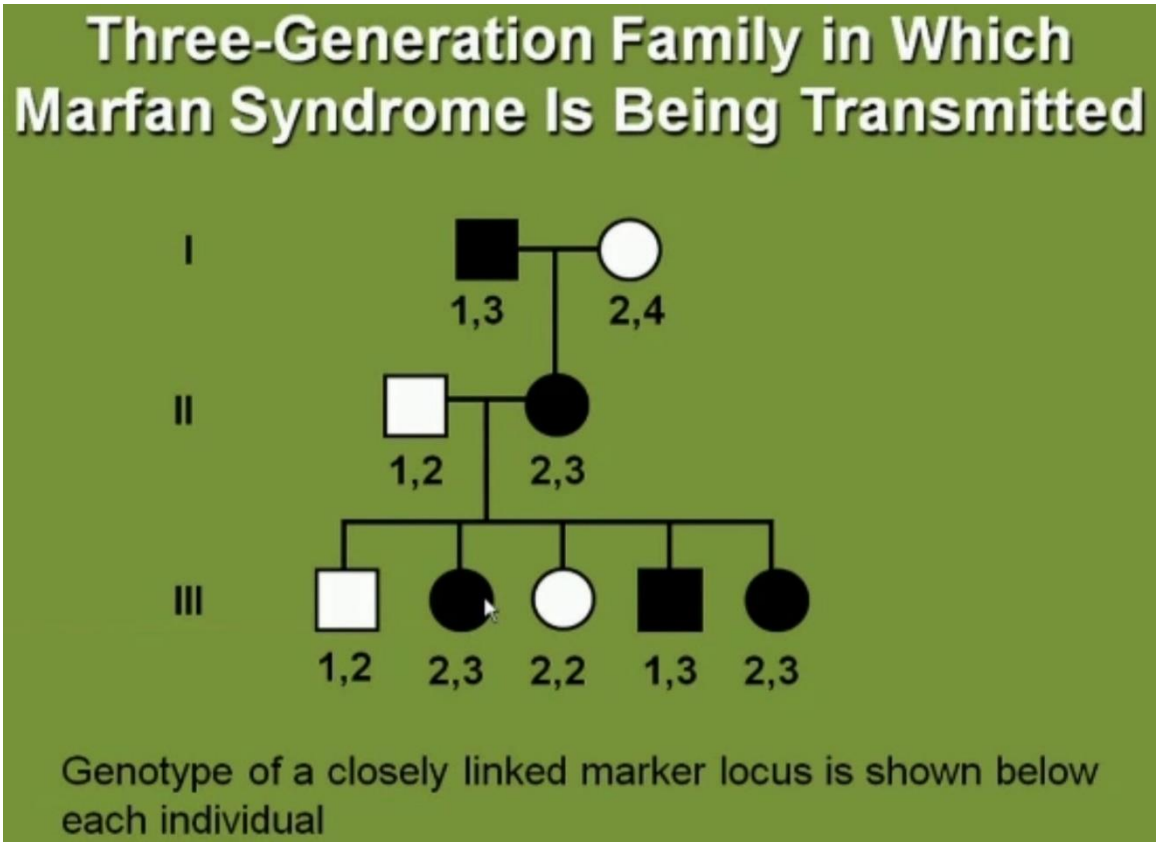
1. Indirect genetic diagnosis using STRs:

- Indirect testing for marfan syndrome using STRs.

- Suppose there is a 3-generation family in which Marfan syndrome (AD) is being transmitted. Each family member has been typed for a 4-allele STRP that is closely linked to the disease locus. The affected father in generation I transmitted the disease-causing mutation to his daughter, and he also transmitted allele 3 of the marker. This allows us to establish linkage phase in this family.

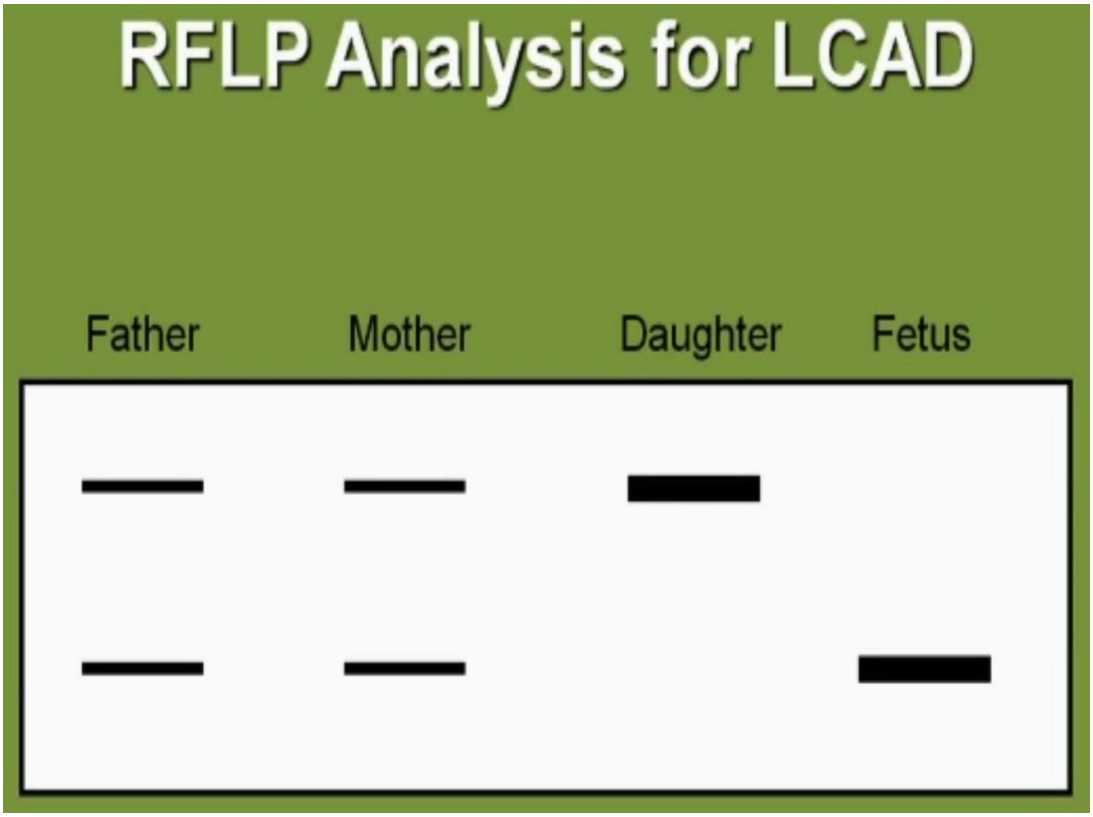
- Because of the close linkage between the marker and the disease locus, we can predict accurately that the offspring in generation III who receive allele 3 from their mother will also receive the disease-causing mutation. Thus, the risk for each child, instead of being the standard 50% recurrence risk for an autosomal dominant disease, is much more definitive: nearly 100% or nearly 0%.

- Recurrence risks may have to take into account the small chance of recombination between the marker allele and the disease-causing gene. If the STR and the disease-causing gene used in this case show 1% recombination, then the recurrence risk for a fetus in generation III whose marker genotype is 2,2 would be 1% rather than 0%. If a fetus in generation III had the marker genotype 2,3, the recurrence risk for that child would be 99%.



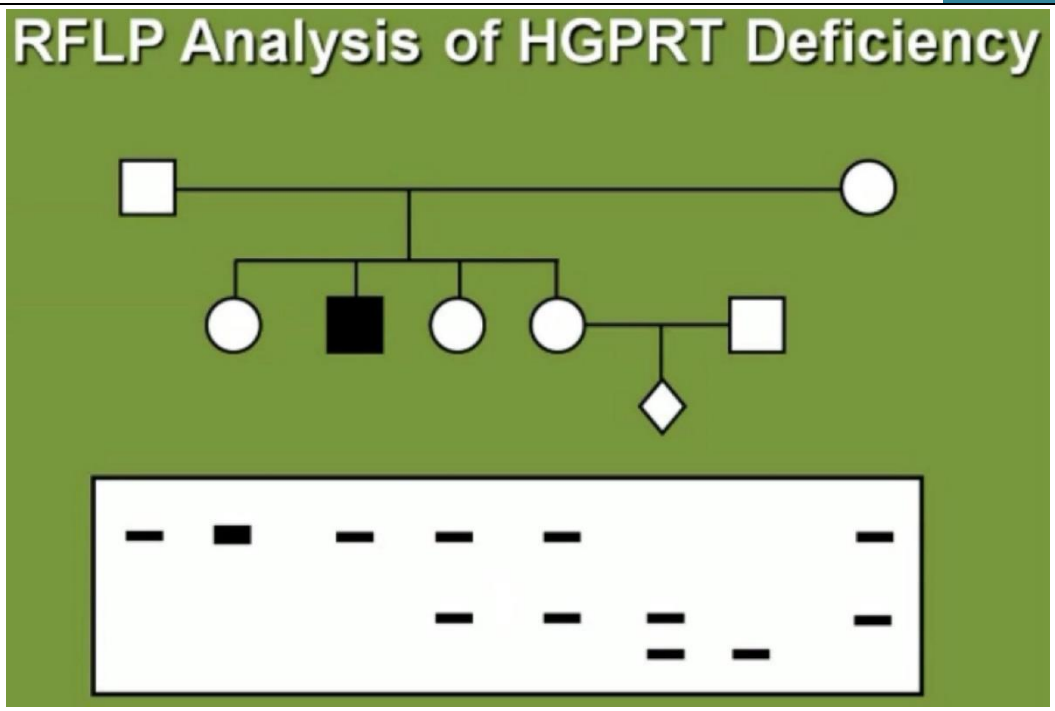
2. Indirect testing using RFLPs:

- Testing for long-chain acyl-CoA dehydrogenase (LCAD) deficiency (AR).
- Analyze southern blots.
- Deduce linkage phase.
- Both father and mother are heterozygous carriers for the disease. Daughter is homozygous normal for the disease (thicker band). Make prediction about fetus?
- Answer: The fetus is homozygous mutant and will be affected by LCAD deficiency.



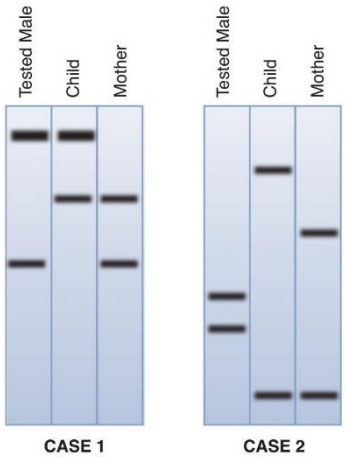
3. RFLP analysis for x-linked gene:

- Testing for HGPRT deficiency (Lesch Nyhan disease).
- Analyze southern blots.
- Determine linkage phase.
- Make prediction about fetus?
- Answer: Fetus (a girl) will not be affected; nor will she be a carrier because her mother, II-4, is not a carrier.



Paternity testing

- Although microsatellite sequences (single-locus sequences, which are highly polymorphic within the population) are distributed throughout the DNA, a single region may be selectively amplified.
- Because humans have pairs of chromosomes, each individual will have a maximum of 2 bands, one from the father and one from the mother.
- For instance, in the figure below, are the tested males in case 1 and case 2 the fathers of the children?



- Case 1: The tested male in case 1 may be the father, as he shares a band with the child. We cannot be certain, however, because many other men in the population could have this same band. Matches are required at several different loci to indicate with high probability that a tested male is the father.
- Case 2: The tested male in case 2 cannot be the father, as neither of his bands is shared with the child.

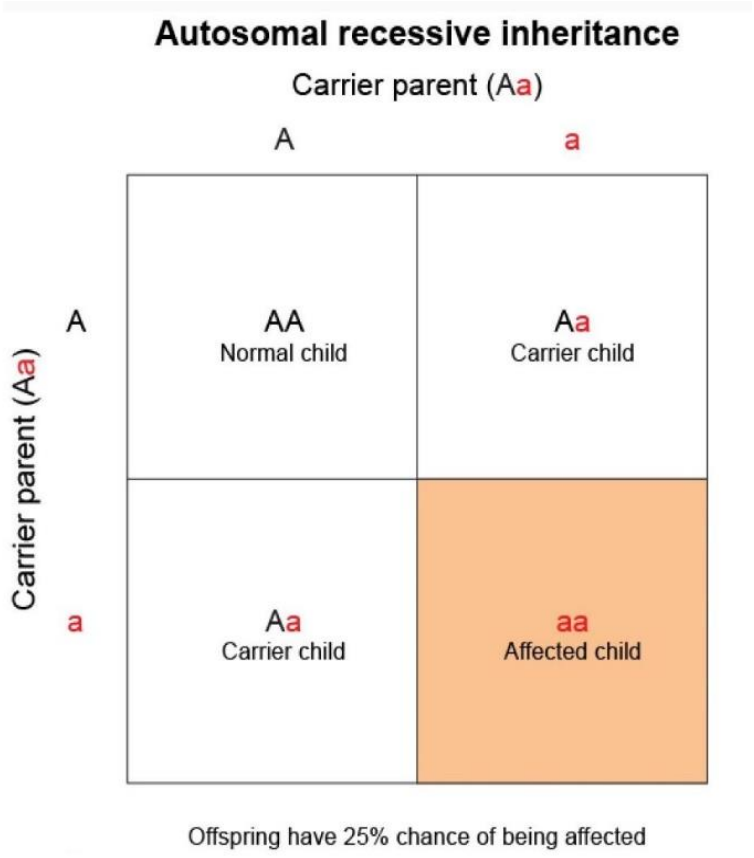
Practicing on Genetics

1. A healthy couple, who recently emigrated from Eastern Europe, bring their 3-year-old son to the office for evaluation of an eczematous rash. On examination, the child also shows signs of intellectual disability and gait abnormality and has a musty body odor. What is the likelihood that this couple's next child will be affected with the same disease?

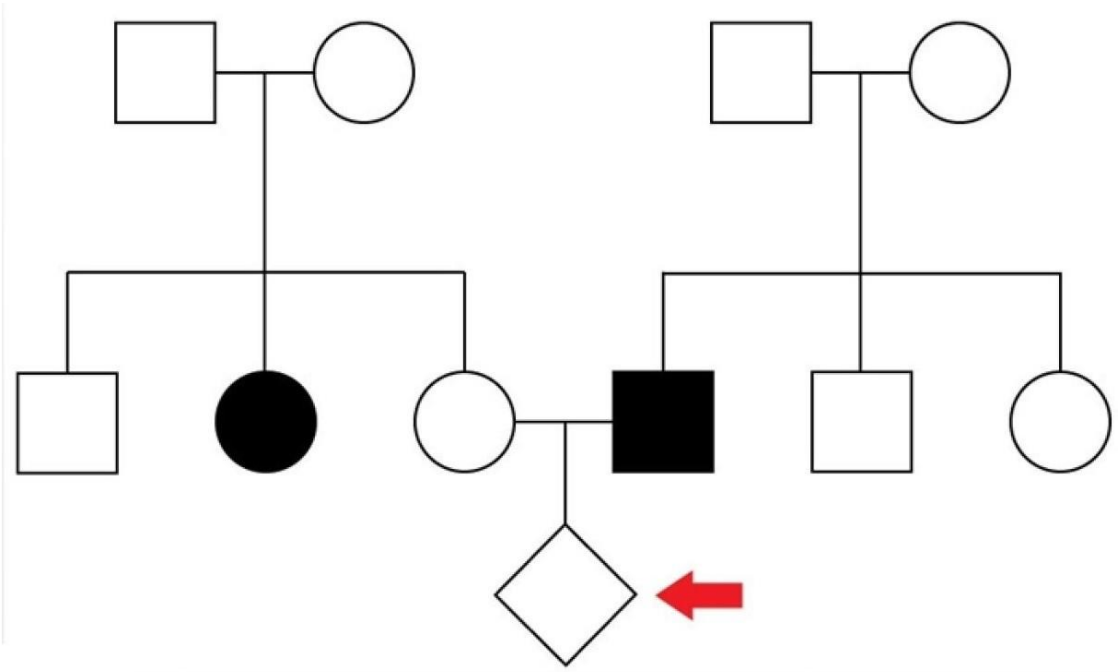
- Intellectual disability, gait or posture abnormality, eczema, and a musty body odor are signs of phenylketonuria (PKU). PKU is an autosomal recessive disease caused by mutation of the gene that codes for phenylalanine hydroxylase.

- Because PKU is inherited in an autosomal recessive fashion, both of the healthy parents must be heterozygous carriers of the mutation. The probability that their next child will inherit the disease is:
  - p_1 = probability that the mother transmits the mutant allele = ½.
  - p_2 = probability that the father transmits the mutant allele = ½.

- The probability that a child will inherit a mutant allele from each carrier parent is equal to $p_1 \times p_2$ = 1/4, as these are independent events.



2. A young couple has undergone a successful in vitro fertilization procedure. The father has cystic fibrosis and the mother has a sister with cystic fibrosis. The father and the mother's sister are both known to have AF508 mutations. Before making the decision to conceive, the couple underwent extensive genetic counseling regarding the potential risks of having a child with cystic fibrosis. However, the mother refused prenatal screening for CF as it would not affect the decision to raise their child. The family pedigree is diagrammed below with the unborn child marked by the red arrow. What is the chance that this child will have cystic fibrosis?



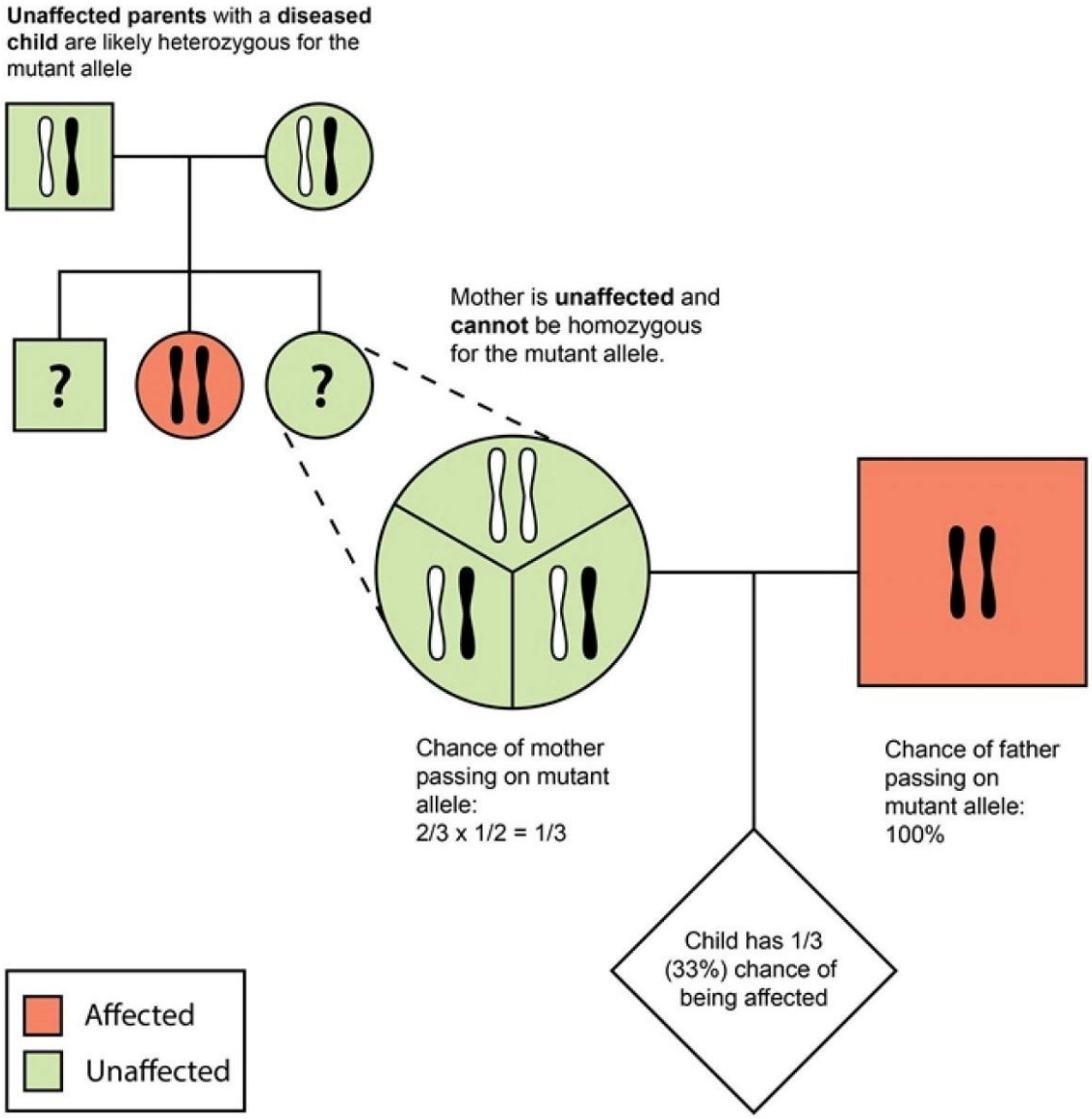
- Cystic fibrosis (CF) results from an autosomal recessive defect in the CF transmembrane conductance regulator (CFTR) gene. Although most men with CF are infertile due to congenital absence of the vas deferens, they are not sterile and can have children via assisted reproductive technology.

- Calculating the probability that the unborn child will have CF can be done by analyzing the above pedigree as follows:

A. Because the father is homozygous for the mutant CFTR allele, he will always transmit the mutant allele to his offspring.

B. Because the mother has an affected sibling and neither of her parents is affected, she most likely had heterozygous carrier parents. Therefore, the mother's 4 possible genotypes are: homozygous for the normal allele, heterozygous with her mother's mutant allele, heterozygous with her father's mutant allele, and homozygous for the mutant allele. However, the mother does not have CF and therefore is not homozygous for the mutant allele. This leaves 3 possible genotypes for the mother. Two of the remaining genotypes result in her being a carrier for the mutant CFTR allele, while the last one results in her being homozygous normal. Therefore, the mother's probability of being a carrier equals 2/3.

C. If the mother is a carrier (2/3 chance), the probability that she will transmit the mutant allele to the child is 1 in 2. As a result, the probability that the child will inherit a mutant allele from the mother (and therefore have CF as the father will always contribute a mutant allele) is: 2/3 x 1/2 = 1/3.



3. A 14-year-old boy experiences severe, prolonged bleeding following a tooth extraction. He also has a history of multiple episodes of painful joint swelling following minor trauma. His parents have no bleeding problems. Evaluation shows that the patient has an inherited disorder and that one of his parents is a genetic carrier. His older sister, who does not have this condition, is pregnant. She does not know the sex of her child. She asks about the risk that her child will be affected. Which of the following is the best estimate that this child will have the disease?

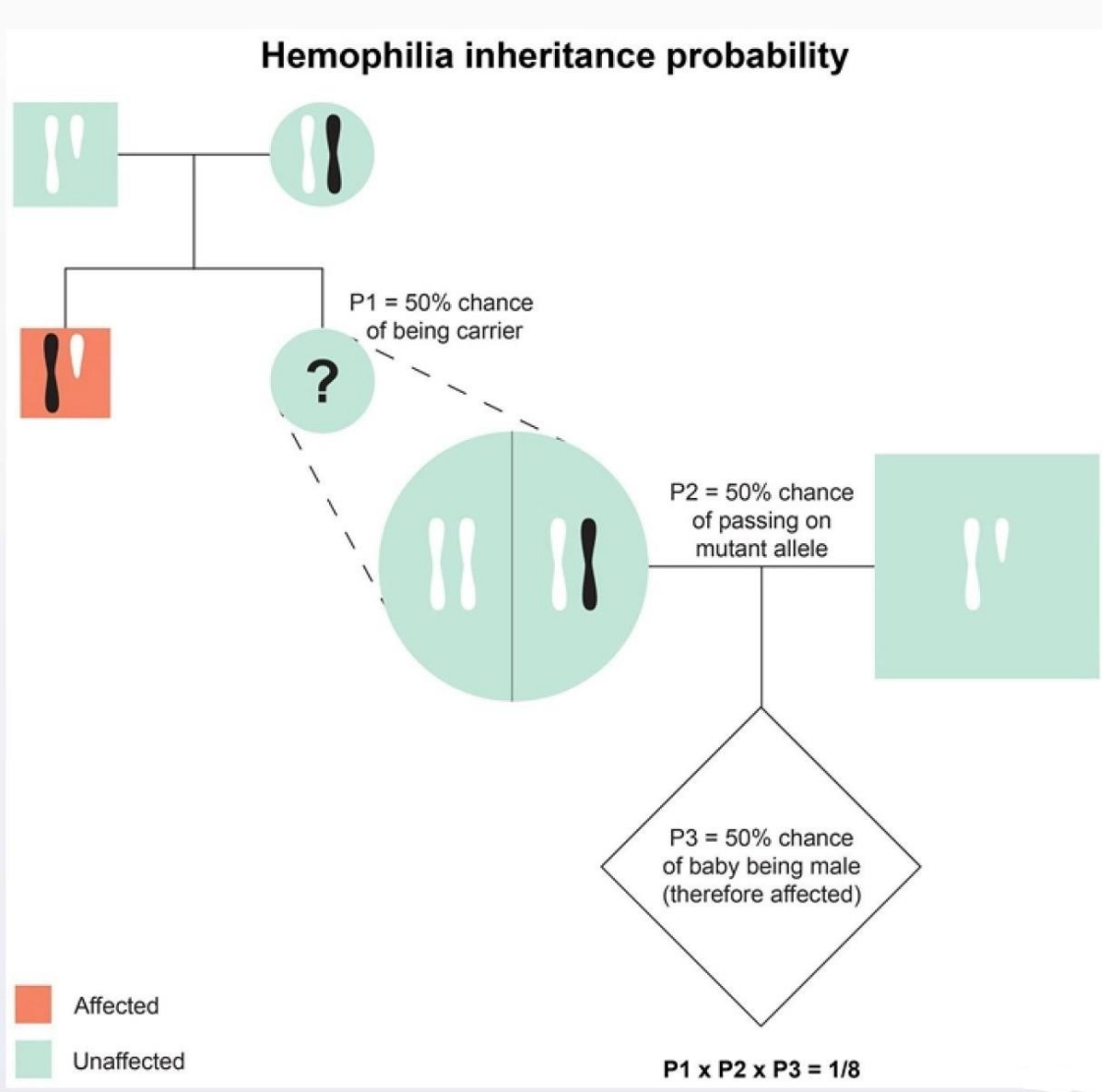
- This patient is a boy with excessive bleeding and hemarthroses, suggesting a diagnosis of hemophilia A or B. Both diseases are X-linked recessive coagulation factor deficiencies. The probability that his sister will give birth to an affected child can be calculated by multiplying the following probabilities:

A. The probability (p_1) that the sister is a carrier = 0.5. The patient's father does not carry the mutation on his X chromosome because he would be affected by the disease if he did. That means the mother carries the mutation on 1 of her 2 X chromosomes. This gives the daughter a 50% chance of having inherited the mutated X chromosome and therefore being a carrier.

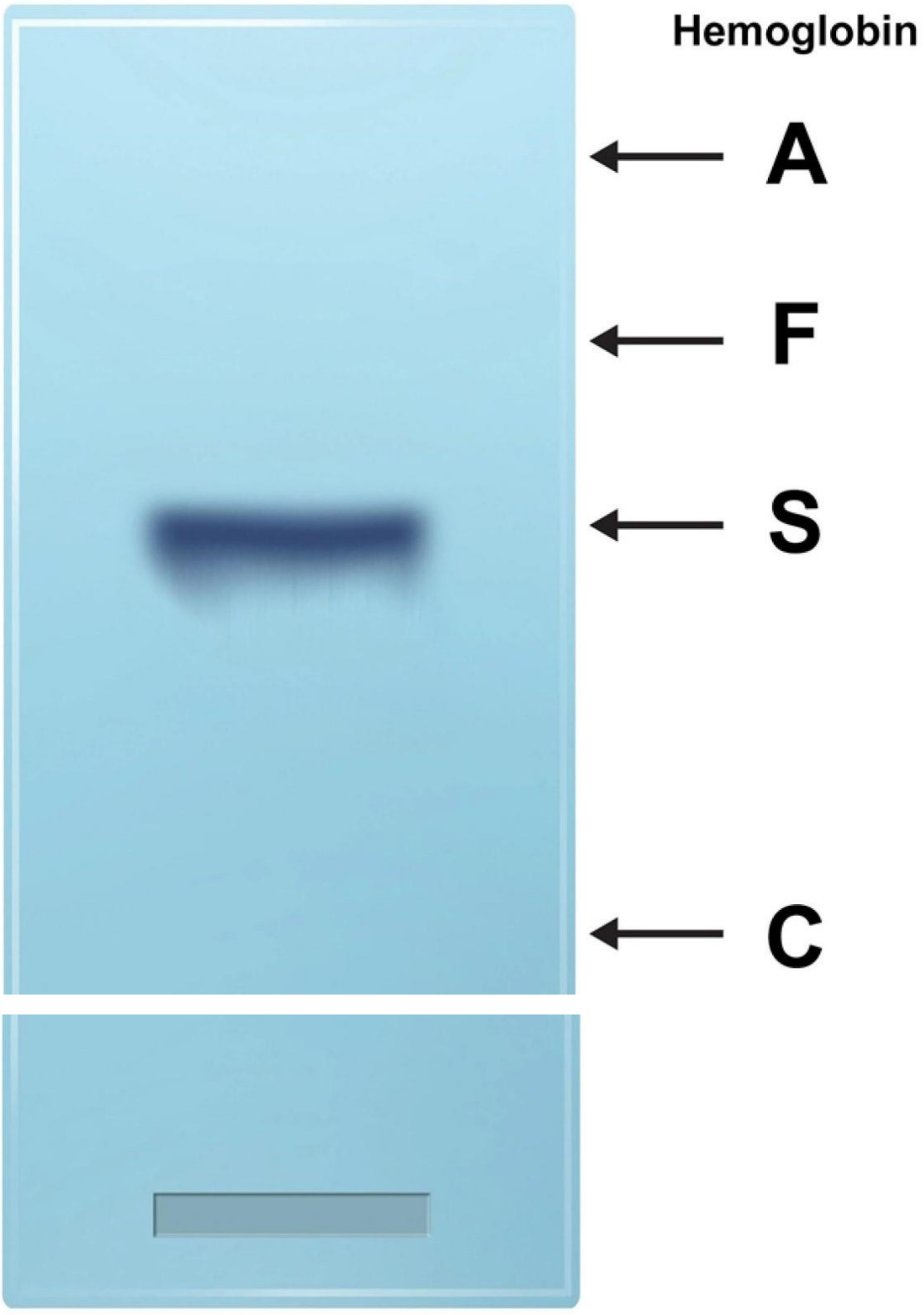
B. The probability (p_2) that the offspring of a female carrier will inherit the X chromosome with the hemophilia gene = 0.5. Assuming the daughter is a carrier, the probability of passing on the mutant allele is 50% as only 1 of her 2 X chromosomes is passed to her offspring.

C. The probability (p_3) that his sister will have a boy = 0.5. If the sister's child is female, the child could be a carrier of the disease but would not be affected by it. If a male child inherits the mutated X chromosome, he will have the disease.

- The probability that the sister will have an affected son is the probability that all 3 of the above events will take place (ie, the product of their individual probabilities): p1 x p2 x p3 = 1/2 x 1/2 x 1/2 = 1/8.



4. A 26-year-old woman comes to the office with her husband for genetic counseling. She is pregnant with their second child, whose gender is unknown. Both parents are asymptomatic, but their firstborn 3-year-old son has recurrent episodes of anemia, jaundice, and painful swelling of the hands and feet. A blood sample is obtained from the boy, and hemoglobin electrophoresis is performed at alkaline pH. The results are shown in the image below. What is the probability that the unborn child will inherit one or more mutant alleles from the parents?

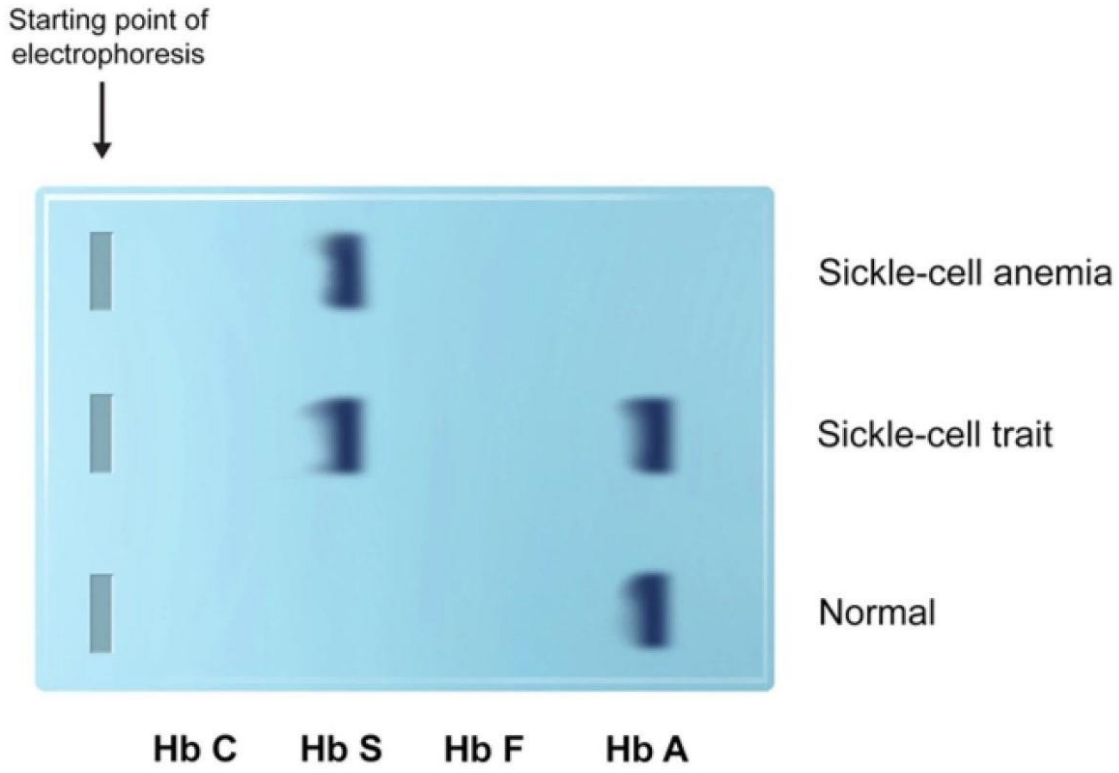


- The couple's 3-year-old son has sickle cell anemia, an autosomal recessive disorder. The disease can be diagnosed using hemoglobin electrophoresis, which is able to determine the different types of hemoglobin in a blood sample based on their electrical charge and the speed by which they move

through the medium. The patient's electrophoresis results show a predominance of hemoglobin S (HbS), which is diagnostic for sickle cell disease.

- In this case, both parents are unaffected and must be heterozygous carriers (Aa) of the disorder because they have an affected child. This means that each parent has a 50% chance of passing either a normal allele (A) or a mutant allele (a) to the offspring. The possible genotypes of the unborn child can be calculated using a Punnett square and are as follows:
  - 25% chance of inheriting two normal alleles (AA, child is unaffected)
  - 50% chance of inheriting one normal and one mutant allele (Aa or aA, child is a carrier)
  - 25% chance of inheriting two mutant alleles (aa, child is affected)

- Only the last 2 options result in the child inheriting a mutant allele, so the probability that the unborn child is carrying one or more mutant alleles is 75%.



5. A 26-year-old woman comes to the office for follow-up. The patient and her husband want to have a child, and she inquires about the risk of certain genetic conditions, including cystic fibrosis (CF). The patient is from a small city with a stable Caucasian population, where the carrier frequency for CF is 1/30 Caucasian individuals. Her husband is from a nearby community, where CF carrier frequency in individuals of Asian descent is 1/100. Both the patient, who is Caucasian, and her husband, who is of Asian descent, are healthy. What is the probability that a child born to a mother from the Caucasian community and a father from the Asian community will have the disease?

- Cystic fibrosis (CF) is an autosomal recessive disease. The patient, who is Caucasian, has a 1/30 probability of carrying the mutant CFTR allele and her husband, who is of Asian descent, has a 1/100 probability of carrying the same allele. If a parent is a carrier, the probability that the child will inherit the mutant allele from that parent is 1/2. However, to develop CF, the child must independently inherit a mutant allele from each parent (1/2 x 1/2 = 1/4 probability). Therefore:

P (affected child) = 1/4 x P (carrier mother) x P (carrier father) = 1/4 x 1/30 x 1/100 = 1/12,000.

6. A healthy 31-year-old woman comes to the office as she and her husband desire a second child. The husband is infertile and the patient's son, who was conceived via donor insemination, was recently diagnosed with glycogen storage disease type II (Pompe disease). This rare autosomal recessive disease is known to affect 1 in 40,000 of the general population. Genetic testing confirms that the patient is a carrier for the disease. A different sperm donor is selected with no personal or family history of Pompe disease; however, his carrier status is unknown. What is the probability of the patient having an affected child with the new sperm donor?

- To be affected by an autosomal recessive disorder, an offspring must inherit 2 copies of the mutant allele (a), a copy from the mother and another copy from the father (in this case, the sperm donor).

- In this example, genetic analysis confirms that the mother is a carrier (Aa), which gives her a 50% chance of passing 1 mutant allele (a) to her child.

- The sperm donor does not have Pompe disease, but his carrier status is unknown (he has either an AA or Aa genotype). Given that he has no family history of the disease, his risk of being a carrier is the same as that of the general population.

- Hardy-Weinberg analysis can be used to estimate the frequency of alleles and genotypes in the general population:

A. The total gene pool is given by (p + q) = 1. By convention, p = normal allele (A) frequency and q = mutant allele (a) frequency in the population of interest.

B. Disease frequency is equivalent to the proportion of homozygous recessive individuals (q^2).

C. Carrier frequency is equal to the proportion of heterozygous individuals. For rare autosomal recessive disorders, p ~ 1; therefore, the probability approximates to 2 times the frequency of the mutant allele, or 2q.

- In this case, 1 in 40,000 individuals is affected by the condition in the general population, so q^2 = 1/40,000 and q → 1/200.

- Thus, the probability of the sperm donor being a carrier = 2q = 2 X (1/200) = 1/100.

- Given that a carrier sperm donor would have a 50% chance of passing on the mutant allele (a), the probability of the child being affected is:

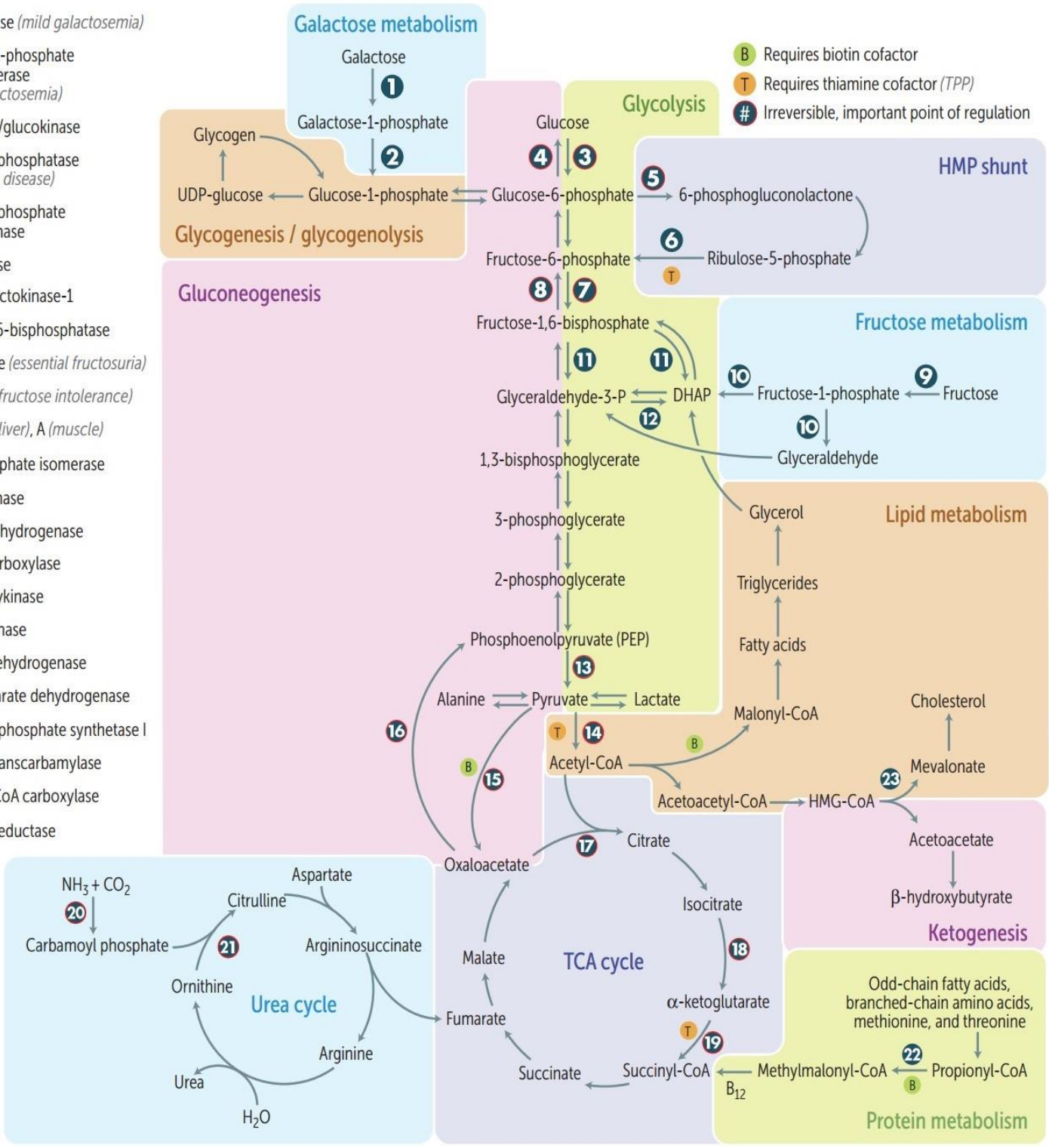
  P (mother gives recessive allele) X P (donor is a carrier) X P (donor gives recessive allele) = (1/2 X 1/100 X 1/2) = 1/400

CHAPTER 5

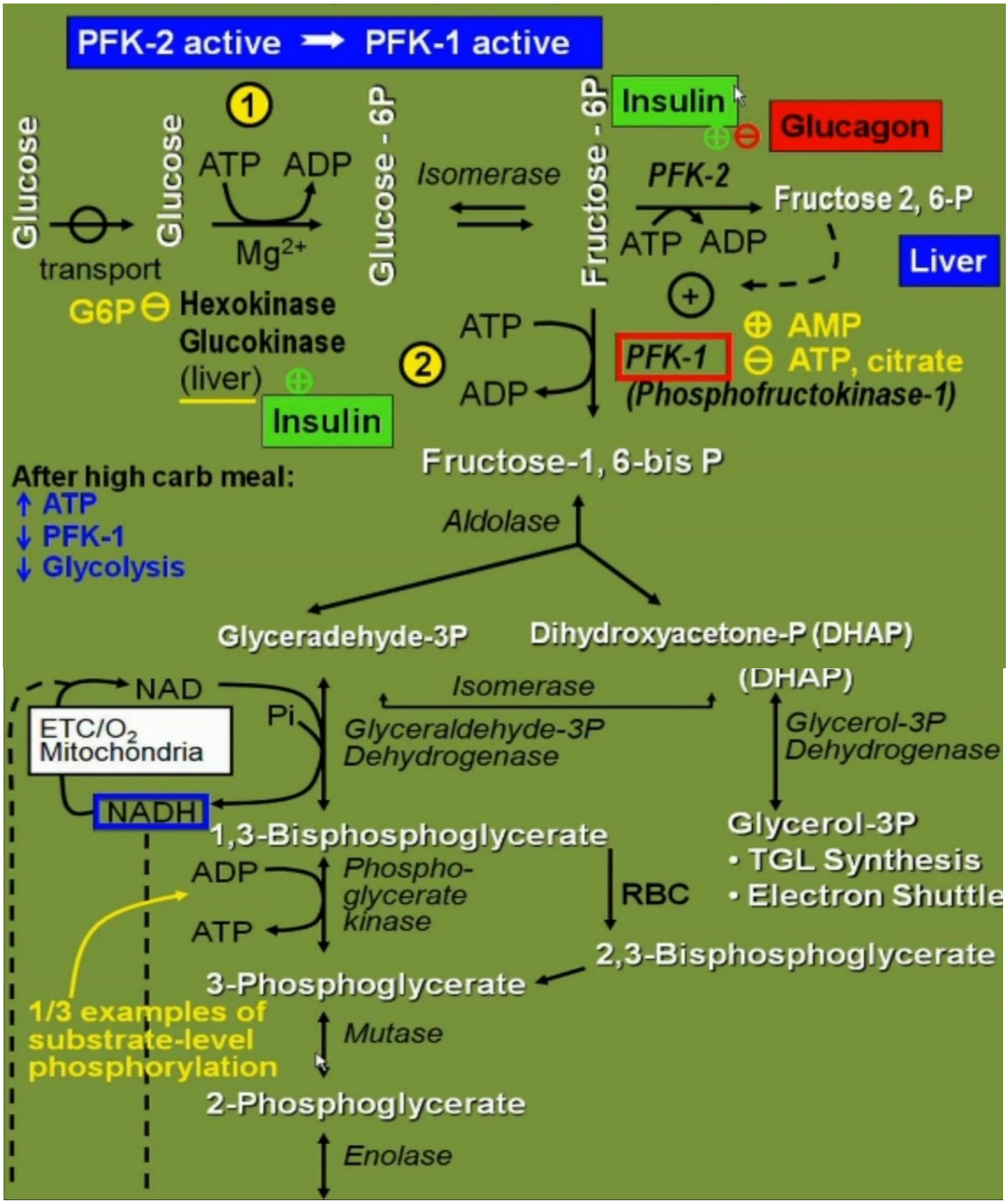
Metabolism

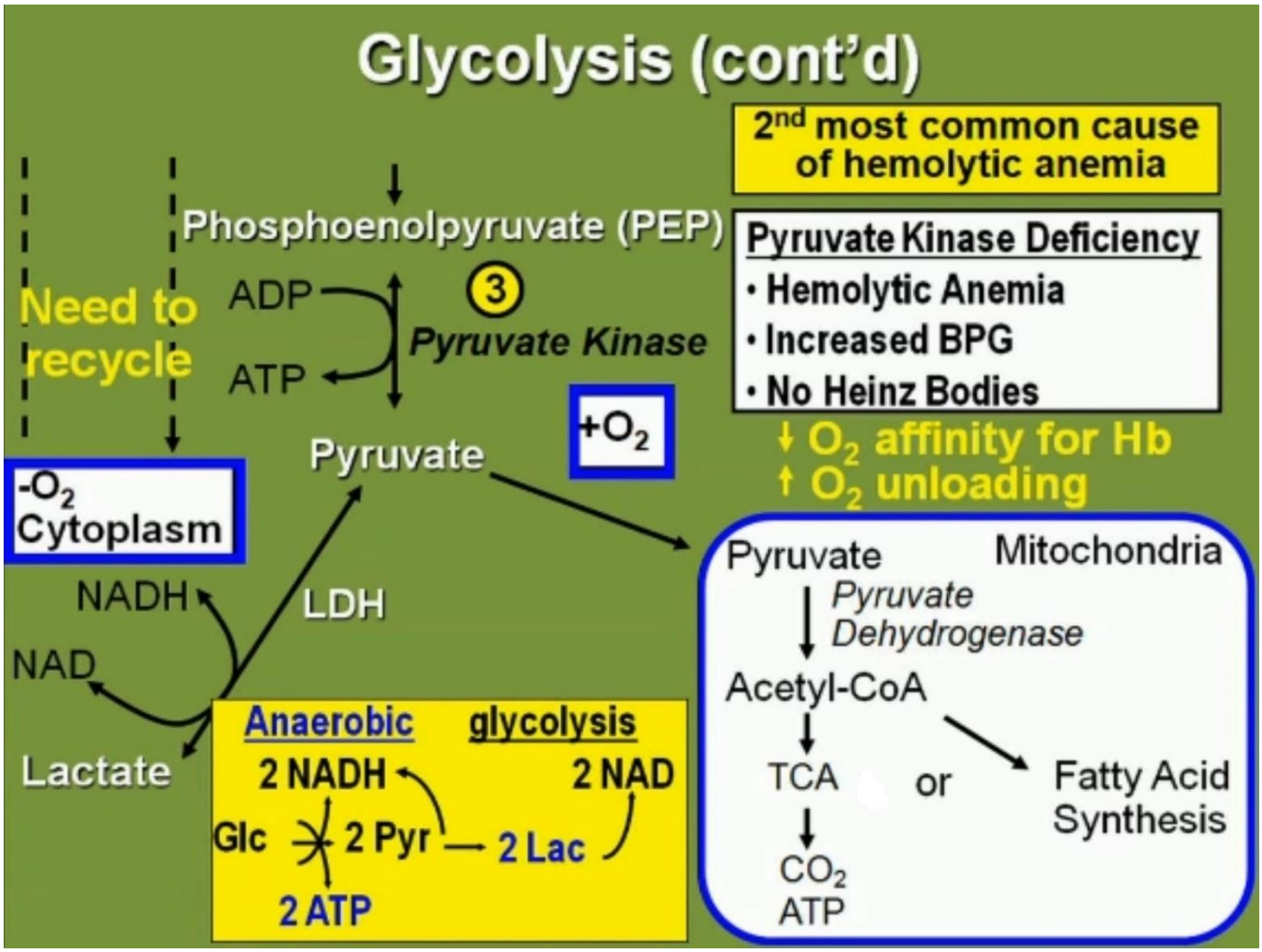
Summary of pathways

1. Galactokinase *(mild galactosemia)*
2. Galactose-1-phosphate uridyltransferase *(severe galactosemia)*
3. Hexokinase/glucokinase
4. Glucose-6-phosphatase *(von Gierke disease)*
5. Glucose-6-phosphate dehydrogenase
6. Transketolase
7. Phosphofructokinase-1
8. Fructose-1,6-bisphosphatase
9. Fructokinase *(essential fructosuria)*
10. Aldolase B *(fructose intolerance)*
11. Aldolase B *(liver)*, A *(muscle)*
12. Triose phosphate isomerase
13. Pyruvate kinase
14. Pyruvate dehydrogenase
15. Pyruvate carboxylase
16. PEP carboxykinase
17. Citrate synthase
18. Isocitrate dehydrogenase
19. α-ketoglutarate dehydrogenase
20. Carbamoyl phosphate synthetase I
21. Ornithine transcarbamylase
22. Propionyl-CoA carboxylase
23. HMG-CoA reductase



Glycolysis


PFK-2 active → PFK-1 active
Glucose
transport
Glucose
1
ATP ADP
Mg2+
Glucose - 6P
Isomerase
Fructose - 6P
Insulin
Glucagon
PFK-2
Fructose 2, 6-P
ATP ADP
Liver
G6P
Hexokinase
Glucokinase
(liver)
Insulin
2
ATP
ADP
PFK-1
(Phosphofructokinase-1)
AMP
ATP, citrate
After high carb meal:
↑ ATP
↓ PFK-1
↓ Glycolysis
Fructose-1, 6-bis P
Aldolase
Glyceradehyde-3P
Dihydroxyacetone-P (DHAP)
NAD
ETC/O2
Mitochondria
NADH
Pi
Isomerase
Glyceraldehyde-3P
Dehydrogenase
(DHAP)
Glycerol-3P
Dehydrogenase
1,3-Bisphosphoglycerate
Glycerol-3P
• TGL Synthesis
• Electron Shuttle
ADP
ATP
Phospho-
glycerate
kinase
RBC
2,3-Bisphosphoglycerate
3-Phosphoglycerate
1/3 examples of
substrate-level
phosphorylation
Mutase
2-Phosphoglycerate
Enolase



Glycolysis regulation, key enzymes

Net glycolysis (cytoplasm):

$$\text{Glucose} + 2\ P_i + 2\ \text{ADP} + 2\ \text{NAD}^+ \rightarrow 2\ \text{pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\ \text{H}^+ + 2\ \text{H}_2\text{O}.$$

Equation not balanced chemically, and exact balanced equation depends on ionization state of reactants and products.

REQUIRE ATP

Glucose —(Hexokinase/glucokinase[a])→ Glucose-6-P

Glucose-6-P ⊖ hexokinase.
Fructose-6-P ⊖ glucokinase.

Fructose-6-P —(Phosphofructokinase-1 (rate-limiting step))→ Fructose-1,6-BP

AMP ⊕, fructose-2,6-bisphosphate ⊕.
ATP ⊖, citrate ⊖.

[a]Glucokinase in liver and β cells of pancreas; hexokinase in all other tissues.

PRODUCE ATP

1,3-BPG ⇌ 3-PG (Phosphoglycerate kinase)

Phosphoenolpyruvate —(Pyruvate kinase)→ Pyruvate

Fructose-1,6-bisphosphate ⊕.
ATP ⊖, alanine ⊖.

- Glycolysis is a cytoplasmic pathway that converts glucose into 2 pyruvates, releasing a modest amount of energy captured in 2 substrate-level phosphorylations and 1 oxidation reaction.

- If a cell has mitochondria and oxygen, glycolysis is aerobic.

- If either mitochondria or oxygen is lacking, glycolysis may occur anaerobically (erythrocytes, exercising skeletal muscle), although some of the available energy is lost.

- The first steps in glucose metabolism in any cell are transport across the membrane and phosphorylation by kinase enzymes inside the cell to prevent it from leaving via the transporter.

- Glycolysis also provides intermediates for other pathways. In the liver, it is part of the process by which excess glucose is converted to fatty acids for storage.

- Important enzymes in glycolysis include:

A. Hexokinase/glucokinase:

- Glucose entering the cell is trapped by phosphorylation using ATP.

- Hexokinase is widely distributed in tissues, whereas glucokinase is found only in hepatocytes and pancreatic β-islet cells.

Hexokinase vs glucokinase

Phosphorylation of glucose to yield glucose-6-phosphate is catalyzed by glucokinase in the liver and hexokinase in other tissues. Hexokinase sequesters glucose in tissues, where it is used even when glucose concentrations are low. At high glucose concentrations, glucokinase helps to store glucose in liver.

	Hexokinase	Glucokinase
Location	Most tissues, except liver and pancreatic β cells	Liver, β cells of pancreas
K_m	Lower (↑ affinity)	Higher (↓ affinity)
V_{max}	Lower (↓ capacity)	Higher (↑ capacity)
Induced by insulin	No	Yes
Feedback-inhibited by glucose-6-phosphate	Yes	No

B. Phosphofructokinases (PFK-1 and PFK-2):

- PFK-1 is the rate-limiting enzyme and main control point in glycolysis.

- In this reaction, fructose 6-phosphate is phosphorylated to fructose 1,6-bisphosphate using ATP.

- PFK-1 is inhibited by ATP and citrate, and activated by AMP.

- Insulin stimulates and glucagon inhibits PFK-1 in hepatocytes by an indirect mechanism involving PFK-2 and fructose 2,6-bisphosphate:
  - Insulin activates PFK-2 (via the tyrosine kinase receptor and activation of protein phosphatases), which converts a tiny amount of fructose 6-phosphate to fructose 2,6-bisphosphate (F2,6-BP). F2,6-BP activates PFK-1.
  - Glucagon inhibits PFK-2 (via cAMP-dependent protein kinase A), lowering F2,6-BP and thereby inhibiting PFK-1.

C. Glyceraldehyde 3-phosphate dehydrogenase:

- Glyceraldehyde 3-phosphate dehydrogenase catalyzes an oxidation and addition of inorganic phosphate (Pi) to its substrate.
- This results in the production of a high-energy intermediate 1,3-bisphosphoglycerate and the reduction of NAD to NADH.
- If glycolysis is aerobic, the NADH can be reoxidized (indirectly) by the mitochondrial electron transport chain, providing energy for ATP synthesis by oxidative phosphorylation.

D. Phosphoglycerate kinase:

- Phosphoglycerate kinase transfers the high-energy phosphate from 1,3-bisphosphoglycerate to ADP, forming ATP and 3-phosphoglycerate.
- This type of reaction, in which ADP is directly phosphorylated to ATP using a high-energy intermediate, is referred to as a substrate-level phosphorylation.
- Unlike oxidative phosphorylation in mitochondria, substrate-level phosphorylations are not dependent on oxygen, and are the only means of ATP generation in an anaerobic tissue.

E. Pyruvate kinase: The last enzyme in aerobic glycolysis, pyruvate kinase catalyzes a substrate-level phosphorylation of ADP using the high-energy substrate phosphoenolpyruvate (PEP).

F. Lactate dehydrogenase:

- Lactate dehydrogenase is used only in anaerobic glycolysis.
- It reoxidizes NADH to NAD, replenishing the oxidized coenzyme for glyceraldehyde 3-phosphate dehydrogenase by reducing pyruvate to lactate and oxidizing NADH to NAD.
- In aerobic tissues, lactate does not normally form in significant amounts. However, when oxygenation is poor (skeletal muscle during strenuous exercise, myocardial infarction), most cellular ATP is generated by anaerobic glycolysis, and lactate production increases.

- Important intermediates of glycolysis include the following:

- Dihydroxyacetone phosphate (DHAP) is used in liver and adipose tissue for triglyceride synthesis.

- 1,3-bisphosphoglycerate and phosphoenolpyruvate (PEP) are high-energy intermediates used to generate ATP by substrate-level phosphorylation.

- Three enzymes in the pathway catalyze reactions that are irreversible. When the liver produces glucose, different reactions and thus different enzymes must be used at these 3 points:

- Glucokinase/hexokinase.
- PFK-1.
- Pyruvate kinase

- ATP Production:

- Anaerobic glycolysis yields 2 ATP/glucose by substrate-level phosphorylation.

- Aerobic glycolysis yields these 2 ATP/glucose plus 2 NADH/glucose that can be utilized for ATP production in the mitochondria; however, the inner membrane is impermeable to NADH. Cytoplasmic NADH is reoxidized to NAD and delivers its electrons to to the electron transport chain.

- Glycolysis in the Erythrocyte:

- In red blood cells, anaerobic glycolysis represents the only pathway for ATP production, yielding a net 2 ATP/glucose.

- Erythrocytes have bisphosphoglycerate mutase, which produces 2,3-bisphosphoglycerate (BPG) from 1,3-BPG in glycolysis. This reaction bypasses an ATP-generating step of glycolysis, causing no net gain in ATP.

- 2,3-BPG binds to the β-chains of hemoglobin A (HbA) and decreases its affinity for oxygen.

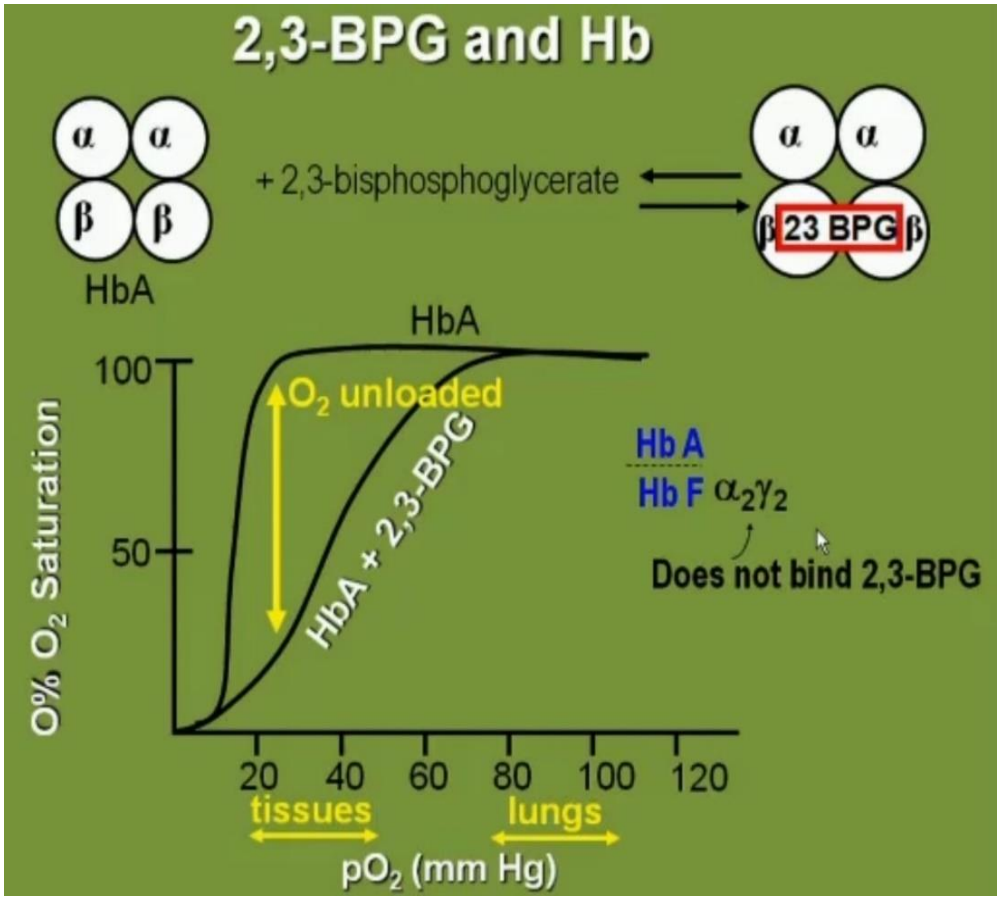
- This effect of 2,3-BPG is seen in the oxygen dissociation curve for HbA. The rightward shift in the curve is sufficient to allow unloading of oxygen in tissues, but still allows 100% saturation in the lungs.

- Although 2,3-BPG binds to HbA, it does not bind well to HbF (α2γ2), with the result that HbF has a higher affinity for oxygen than maternal HbA, allowing transplacental passage of oxygen from mother to fetus.

- Pyruvate kinase deficiency is the second most common genetic deficiency that causes a hemolytic anemia (glucose 6-phosphate dehydrogenase, G6PDH, is the most common). Characteristics include:
  - Chronic hemolysis.
  - Increased 2,3-BPG and therefore a lower-than-normal oxygen affinity of HbA.
  - Absence of Heinz bodies (Heinz bodies are more characteristic of G6PDH deficiency).

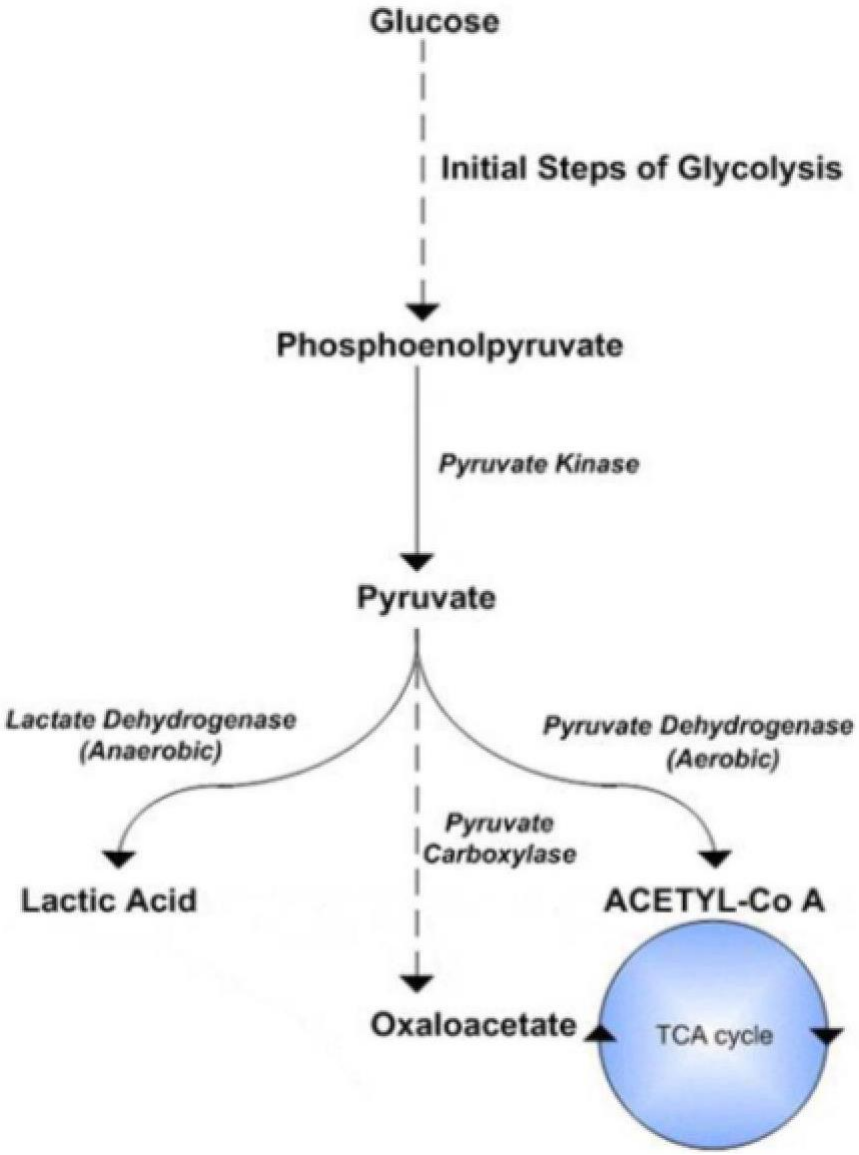
- The red blood cell has no mitochondria and is totally dependent on anaerobic glycolysis for ATP. In pyruvate kinase deficiency, the decrease in ATP causes the erythrocyte to lose its characteristic biconcave shape and signals its destruction in the spleen. In addition, decreased ion pumping by Na/K-ATPase results in loss of ion balance and causes osmotic fragility, leading to swelling and lysis.

- Excessive erythrocyte destruction by the spleen causes splenomegaly due to work hypertrophy (red pulp hyperplesia). Work hypertrophy results from hypertrophy of the reticuloendothelial cells of the splenic parenchyma as these cells are involved in the removal of damaged RBCs.

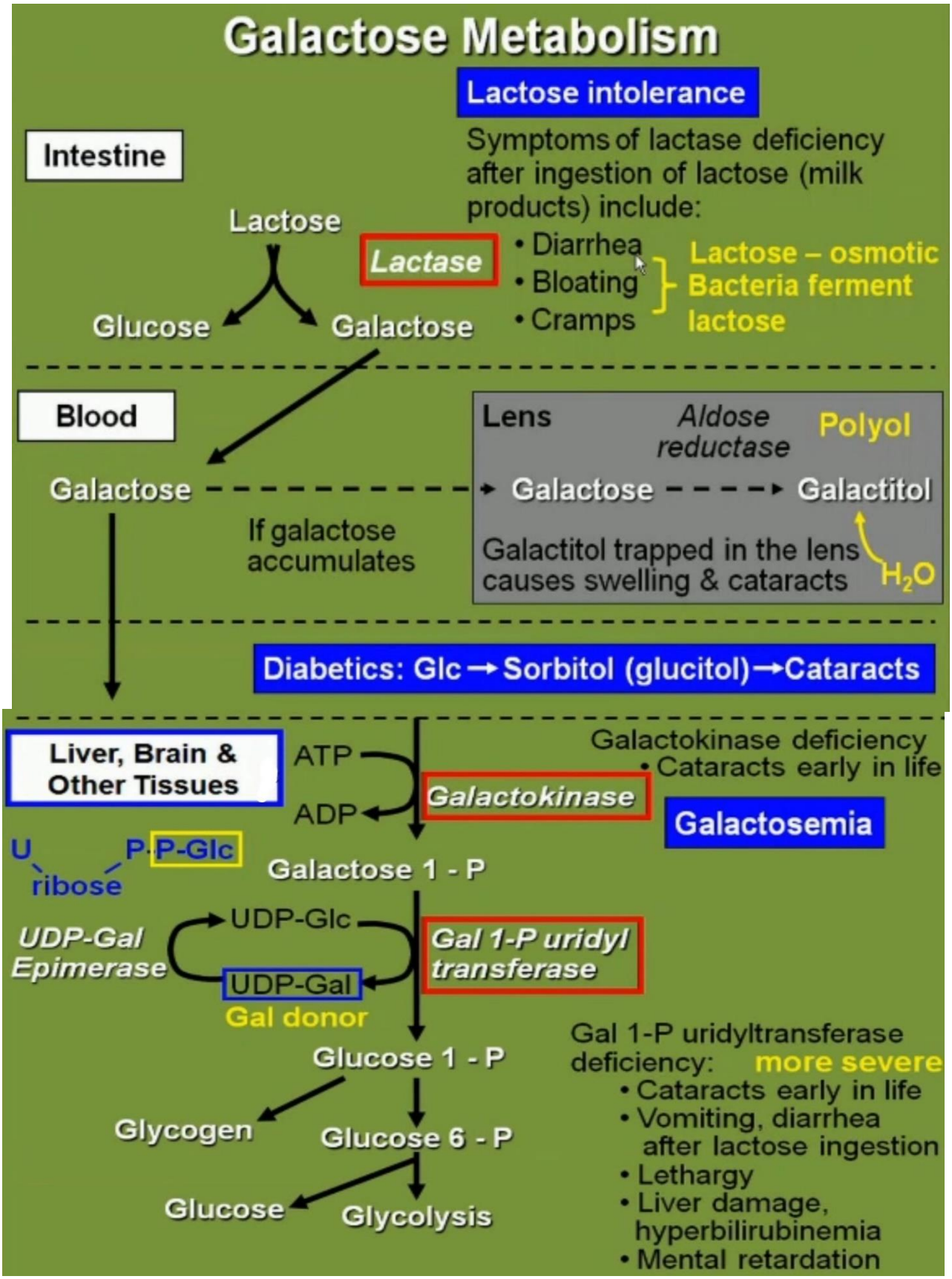


❖ N.B:

- NAD is present in limited amounts in most cells, and it must be regenerated from NADH for glycolysis to continue.
- Under aerobic conditions, NAD is converted to NADH in the TCA cycle. NADH is then reconverted to NAD+ in the electron transport chain as the energy in NADH is utilized to synthesize ATP.
- In anaerobic glycolysis, NAD is regenerated from NADH when pyruvate is converted to lactate via lactate dehydrogenase.
- In patients with lactate dehydrogenase deficiency, glycolysis is inhibited in strenuously exercising muscle as muscle cells cannot regenerate NAD. Consequently, high-intensity physical activity leads to muscle breakdown, pain, and fatigue as insufficient amounts of energy are being produced in the exercising muscle.


Glucose
Initial Steps of Glycolysis
Phosphoenolpyruvate
Pyruvate Kinase
Pyruvate
Lactate Dehydrogenase
(Anaerobic)
Pyruvate Dehydrogenase
(Aerobic)
Pyruvate
Carboxylase
Lactic Acid
ACETYL-Co A
Oxaloacetate
TCA cycle

Galactose metabolism

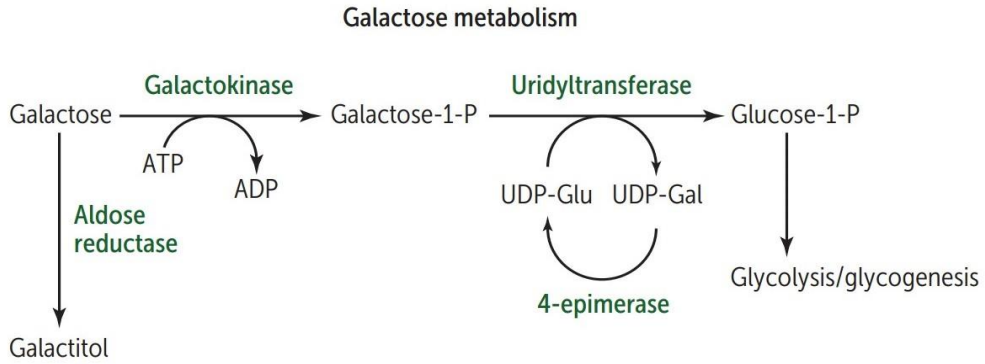


- Once transported into tissues, galactose is phosphorylated (galactokinase), trapping it in the cell.
- Galactose 1-phosphate is converted to glucose 1-phosphate by galactose 1-P uridyl-transferase and an epimerase.
- Important enzymes to remember are galactokinase and galactose 1-phosphate uridyltransferase.
- Genetic deficiencies of these enzymes produce galactosemia.
- Cataracts, a characteristic finding in patients with galactosemia, result from conversion of the excess galactose in peripheral blood to galactitol in the lens of the eye, which has aldose reductase.
- Accumulation of galactitol in the lens causes osmotic damage and cataracts.
- The same mechanism accounts for the cataracts in diabetics because aldose reductase also converts glucose to sorbitol, which causes osmotic damage.

Galactokinase deficiency

- Hereditary deficiency of galactokinase. Autosomal recessive.
- Galactitol accumulates if galactose is present in diet.
- Relatively mild condition. Galactokinase deficiency is kinder (benign condition).
- Symptoms:

- Galactose appears in blood (galactosemia) and urine (galactosuria); infantile cataracts.
- May present as failure to track objects or to develop a social smile.



Classic galactosemia

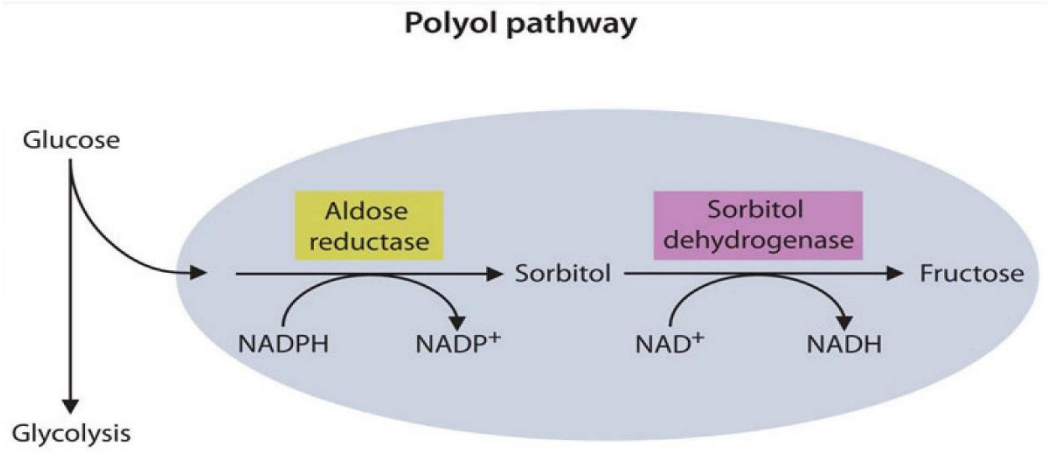
- Classic galactosemia results from deficiency of galactose-1-phosphate uridyl transferase. Autosomal recessive.
- Deficiency of galactose 1-phosphate uridyltransferase produces a more severe disease because, in addition to galactosemia, galactose 1-P accumulates in the liver, brain, kidney and other tissues.
- Damage is caused by accumulation of toxic substances (including galactitol, which accumulates in the lens of the eye).
- Symptoms develop when infant begins feeding (lactose present in breast milk and routine formula) and include failure to thrive, jaundice, hepatomegaly, and renal dysfunction (hyperchloremic metabolic acidosis, aminoaciduria), infantile cataracts, intellectual disability.
- Patients are also predisposed to Escherichia coli (gram-negative rod) sepsis.
- Treatment:

- Exclude galactose and lactose (galactose + glucose) from diet.
- Initiation of soy-milk-based formula can result in regression of cataracts and improvement in renal and liver function. Soy-milk consists of sucrose, which is metabolized to glucose and fructose.

Sorbitol

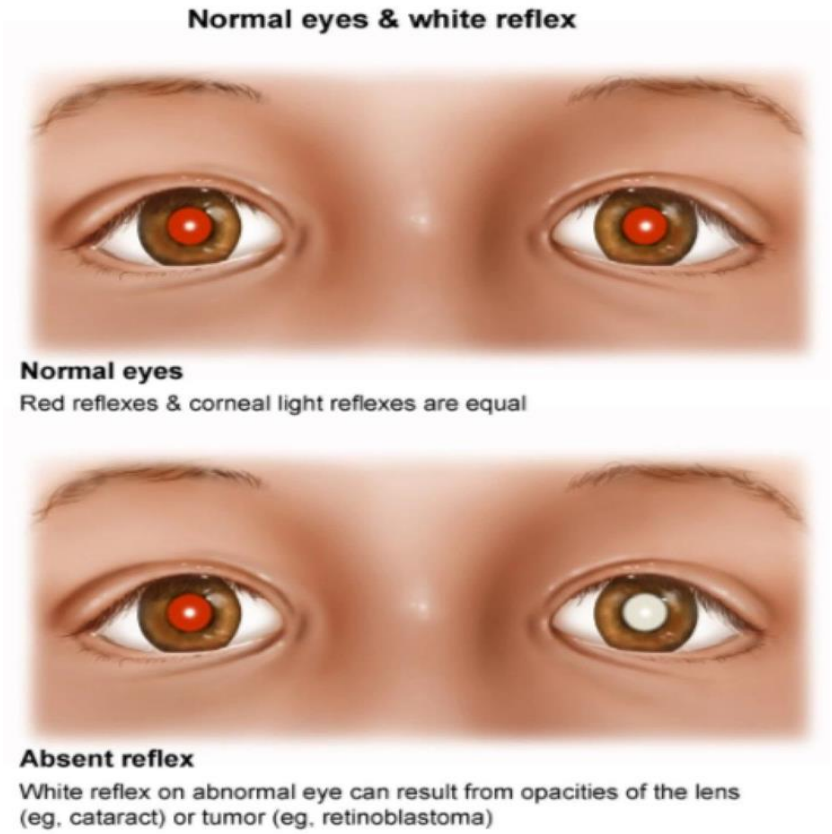
- An alternative method of trapping glucose in the cell is to convert it to its alcohol counterpart, sorbitol, via aldose reductase.
- Sorbitol cannot readily cross cell membranes and is therefore trapped inside the cells within which it is formed.
- If the enzyme sorbitol dehydrogenase (sometimes referred to as polyol dehydrogenase) is also present in the cell, it can convert sorbitol into fructose.
- This pathway, known as the polyol pathway, is especially active in the seminal vesicles, as sperm use fructose as their primary energy source. Other tissues, such as the retina, renal papilla, and Schwann cells, have much less sorbitol dehydrogenase activity.
- Tissues with an insufficient amount/activity of this enzyme are at risk of intracellular sorbitol accumulation, causing osmotic damage (cataracts, retinopathy, and peripheral neuropathy seen with chronic hyperglycemia in diabetes).
- Liver, Ovaries, and Seminal vesicles have both enzymes (they LOSe sorbitol).

- Lens has primarily aldose reductase. Retina, Kidneys, and Schwann cells have only aldose reductase (LuRKS).

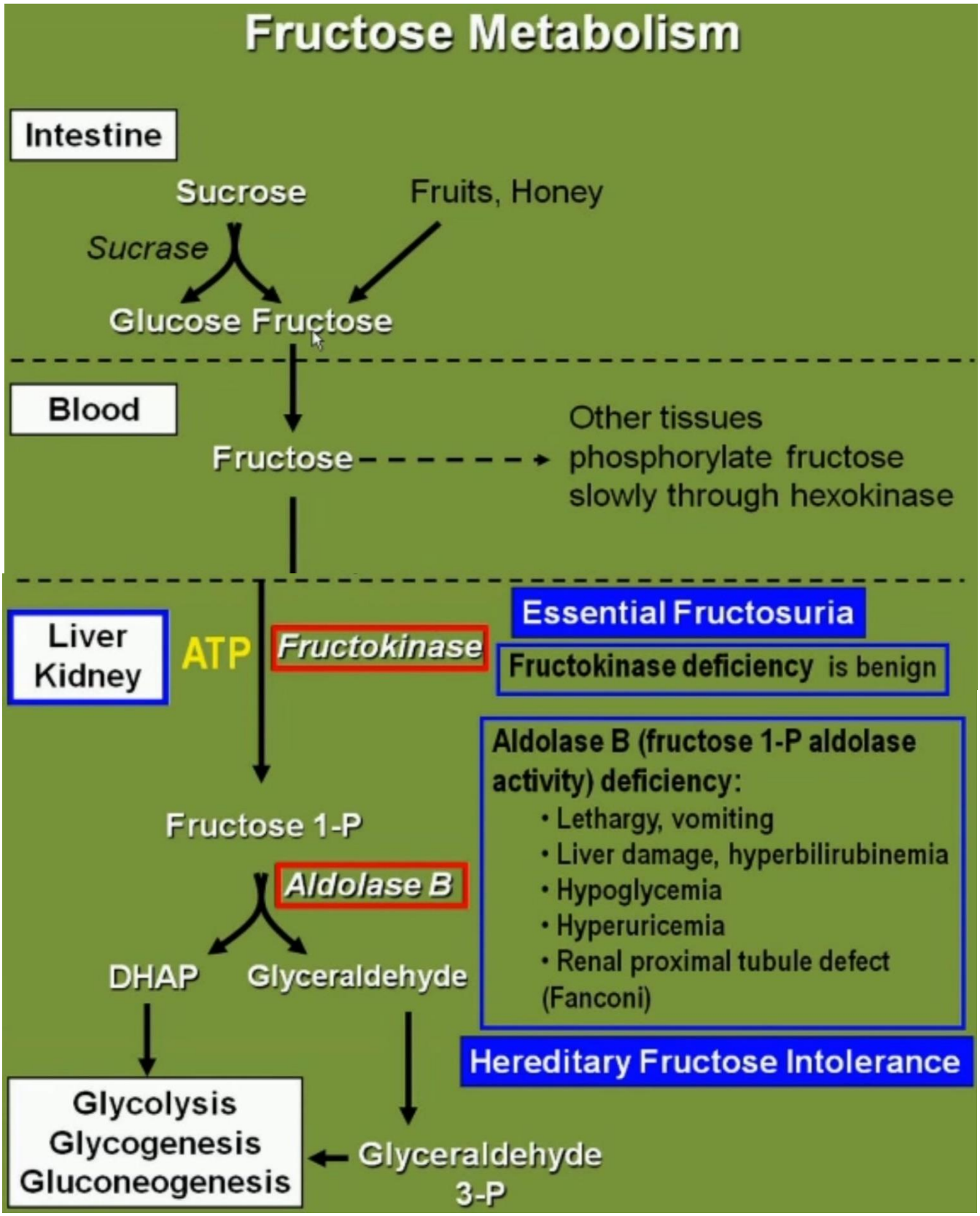


❖ N.B:

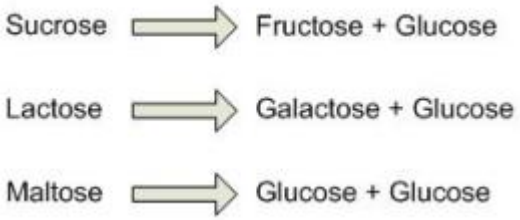
- Cataracts is a vision-impairing opacification of the lens that causes loss of the red reflex with decreased visualization of retinal details on ophthalmoscopic evaluation.
- The incidence of cataracts increases with age; other risk factors include smoking, excessive sunlight exposure, diabetes mellitus, and glucocorticoid use.
- In the polyol pathway, aldose reductase converts glucose into sorbitol, which is slowly metabolized into fructose by sorbitol dehydrogenase.
- Chronic hyperglycemia overwhelms this pathway, causing intracellular sorbitol accumulation and increased osmotic/oxidative stress. This accelerates cataract development in patients with diabetes, and contributes to the pathogenesis of diabetic retinopathy, neuropathy, and nephropathy.



Fructose metabolism



- Fructose is found in honey and fruit and as part of the disaccharide sucrose (common table sugar).
- Sucrose is hydrolyzed by intestinal brush border sucrase, and the resulting monosaccharides, glucose and fructose, are absorbed into the portal blood.
- Fructose is phosphorylated by fructokinase in the liver yielding fructose-1-phosphate (F1P), and metabolism of fructose-1-phosphate by aldolase B generates dihydroxyacetone phosphate (DHAP) and glyceraldehyde.
- Glyceraldehyde and DHAP can be converted to glyceraldehyde-3-phosphate (G3P), which can then be metabolized in the glycolytic pathway.
- Important enzymes to remember are fructokinase and fructose 1-P aldolase (aldolase B).
- Disorders of fructose metabolism cause milder symptoms than analogous disorders of galactose metabolism.
- Cataracts are not a feature of this disease because fructose is not an aldose sugar and therefore not a substrate for aldose reductase in the lens.
- Genetic deficiency of fructokinase is benign and often detected incidentally when urine is checked for glucose with a dipstick.
- Fructose 1-phosphate aldolase deficiency is severe because of accumulation of fructose 1-phosphate in the liver and renal proximal tubules.
- Symptoms are reversed after removing fructose and sucrose from the diet.



Essential fructosuria

- Involves a defect in fructokinase. A rare autosomal recessive asymptomatic disorder (fructokinase deficiency is kinder since fructose is not trapped in cells).
- Fructose from the diet is absorbed in the gut and secreted in the urine unchanged due to defective metabolism.
- In patients with essential fructosuria, some of the dietary fructose load is converted by hexokinase to fructose- 6-phosphate, which can then enter glycolysis; this pathway is not significant in normal individuals.

- Symptoms: fructose appears in blood and urine.

- Fructose, similar to glucose and galactose, is a reducing sugar and can be detected by clinitest tablets, which test nonspecifically for the presence of reducing sugar. A urine dipstick, however, utilizes glucose oxidase for determination of the presence of urine glucose and will not test positive in the presence of fructose or galactose.

Hereditary fructose intolerance

- Hereditary deficiency of aldolase B. Autosomal recessive.

- Symptoms:

- Patients typically present when fructose-containing foods are introduced into the diet.

- Hypoglycemia, vomiting, jaundice, cirrhosis.

- The primary manifestations are vomiting and hypoglycemia about 20-30 minutes after fructose ingestion.

- Hypoglycemia results from intracellular accumulation of fructose-1-phosphate and depletion of inorganic phosphate, which inhibit glycogenolysis and gluconeogenesis.

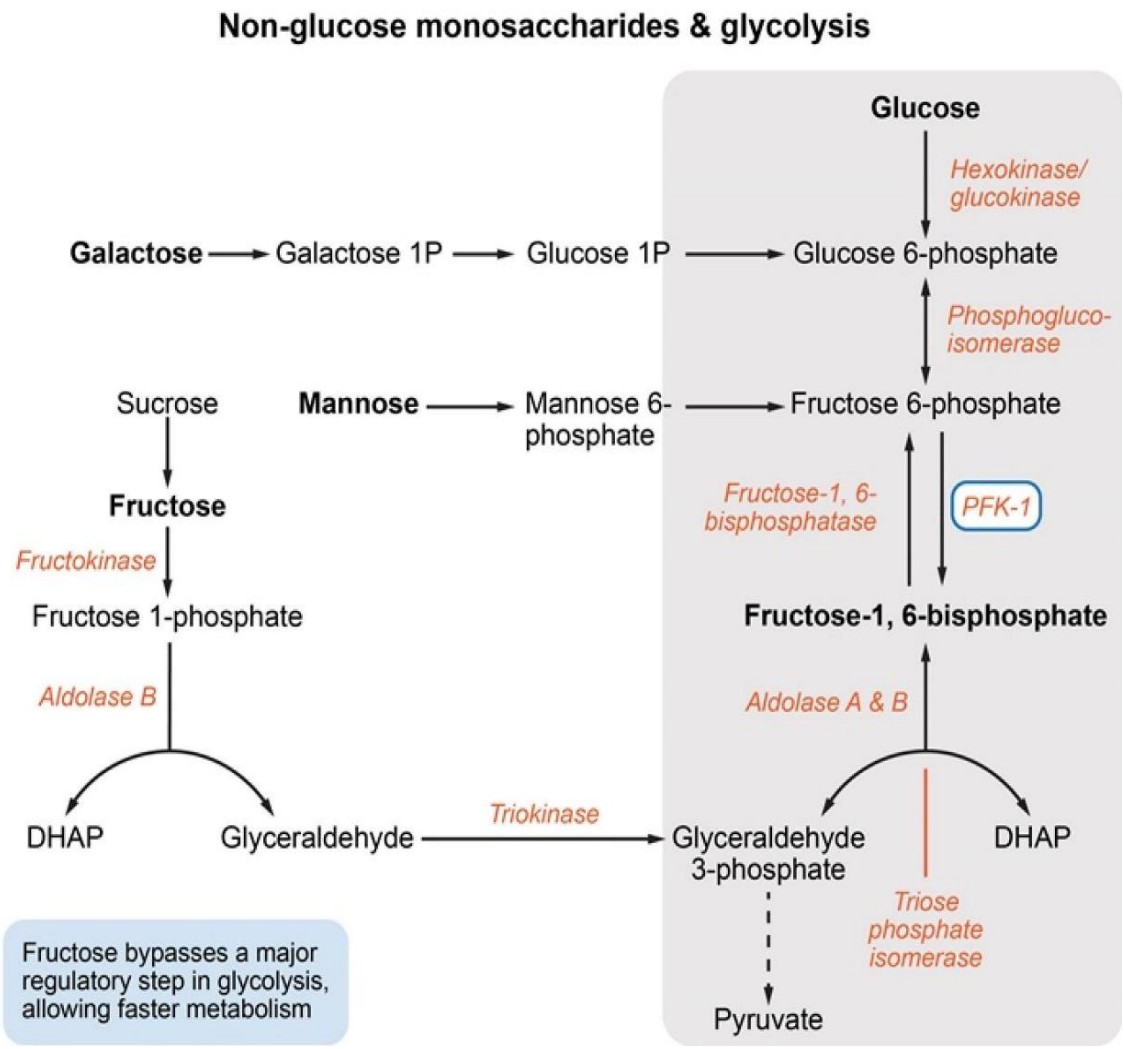
- Failure to thrive, hepatomegaly, and jaundice can also occur. Undiagnosed individuals may eventually develop liver and renal failure.

- Urine dipstick will be ⊖ (tests for glucose only); reducing sugar can be detected in the urine (nonspecific test for inborn errors of carbohydrate metabolism).

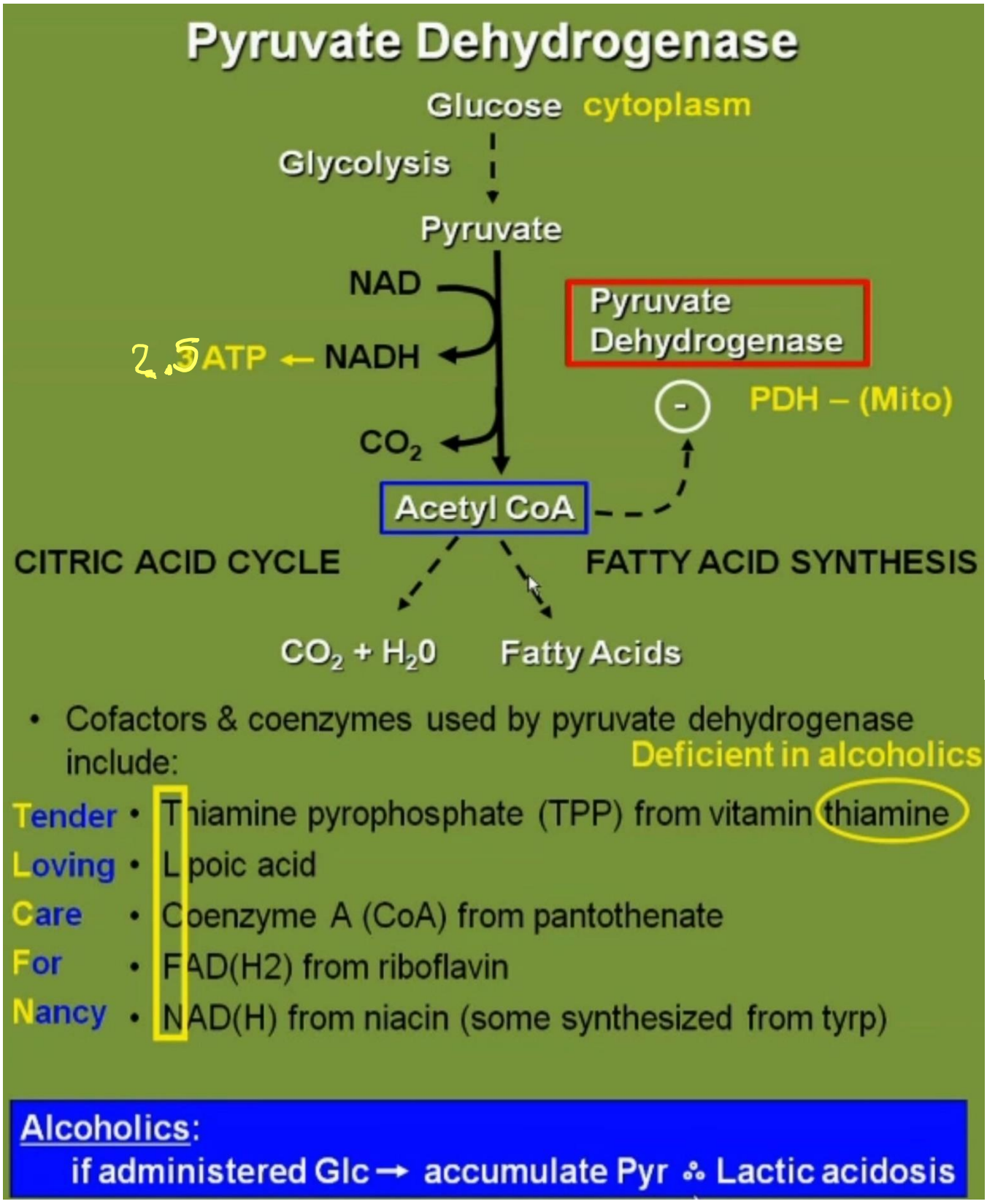
- Treatment: ↓ intake of both fructose and sucrose (glucose + fructose) and results in symptom improvement with a good long-term prognosis.

❖ N.B:

- Dietary fructose is phosphorylated in the liver to F-1-P and is rapidly metabolized because it bypasses PFK-1, the rate-limiting enzyme of glycolysis.
- Other sugars enter glycolysis before this rate-limiting step and are therefore metabolized more slowly due to regulation of PFK-1.
- Non-glucose monosaccharides such as fructose, mannose and galactose enter the glycolytic pathway after initial metabolism to intermediates of glycolysis.
- Glucose-6-phosphate, mannose-6-phosphate, galactose-6-phosphate and glucose-1-phosphate must be metabolized via the rate-limiting PFK-1 step, which slows down their metabolism.
- Fructose-1-phosphate, however, bypasses this step and therefore has the highest rate of metabolism.


Non-glucose monosaccharides & glycolysis
Glucose
Hexokinase/ glucokinase
Galactose
Galactose 1P
Glucose 1P
Glucose 6-phosphate
Phosphogluco-isomerase
Sucrose
Mannose
Mannose 6-phosphate
Fructose 6-phosphate
Fructose
Fructose-1, 6-bisphosphatase
PFK-1
Fructokinase
Fructose 1-phosphate
Fructose-1, 6-bisphosphate
Aldolase B
Aldolase A & B
DHAP
Glyceraldehyde
Triokinase
Glyceraldehyde 3-phosphate
DHAP
Triose phosphate isomerase
Pyruvate
Fructose bypasses a major regulatory step in glycolysis, allowing faster metabolism

Pyruvate dehydrogenase



- Pyruvate from aerobic glycolysis enters mitochondria, where it may be converted to acetyl-CoA for entry into the citric acid cycle if ATP is needed, or for fatty acid synthesis if sufficient ATP is present.

- The pyruvate dehydrogenase (PDH) reaction is irreversible and cannot be used to convert acetyl-CoA to pyruvate or to glucose.

- PDH in the liver is activated by insulin, whereas in the brain and nerves the enzyme is not responsive to hormones.

- Cofactors and coenzymes used by pyruvate dehydrogenase include:

- Thiamine pyrophosphate (TPP) from the vitamin thiamine.
- Lipoic acid.
- Coenzyme A (CoA) from pantothenate.
- FAD(H2) from riboflavin.
- NAD(H) from niacin (some may be synthesized from tryptophan).

- Pyruvate dehydrogenase is inhibited by its product acetyl-CoA. This control is important in several contexts and should be considered along with pyruvate carboxylase, the other mitochondrial enzyme that uses pyruvate (in gluconeogenesis).

- Similar to pyruvate dehydrogenase, 2 enzyme complexes use thiamine:

- α-ketoglutarate dehydrogenase (citric acid cycle).
- Branched-chain ketoacid dehydrogenase (metabolism of branched-chain amino acids).

Pyruvate dehydrogenase complex deficiency

- Causes a buildup of pyruvate that gets shunted to lactate (via LDH) and alanine (via ALT).

- Pyruvate dehydrogenase deficiency is a disease with multiple possible presentations ranging from neonatal death to mild episodic symptoms in adulthood.

- X-linked.

- Findings:

- Neurologic defects, lactic acidosis, ↑ serum alanine starting in infancy.

- Pyruvate dehydrogenase (PDH) is an allosteric enzyme that converts pyruvate into acetyl-CoA in the presence of oxygen (during aerobic metabolism). With a deficiency of PDH, pyruvate is alternatively converted to lactate by the enzyme lactate dehydrogenase in an effort to regenerate NAD. Excessive lactate production in these states results in lactic acidosis.

- Treatment:

- ↑ intake of ketogenic nutrients (high fat & low carbohydrate diet with ↑ lysine and leucine).

- Because carbohydrates may aggravate lactic acidosis, a ketogenic diet is recommended in these patients.

- This diet forces the production of ketone bodies from fat and amino acid catabolism to fuel the body in the place of glucose. The near absence of glucose in the diet decreases the amount of pyruvate generated, thereby decreasing lactate levels.

- Amino acid catabolism following removal of the amino group results in formation of intermediates that are either glucogenic (producing intermediates of the citric acid cycle or pyruvate) or ketogenic (producing acetoacetate or its precursors).

- Leucine and lysine are exclusively ketogenic and would not lead to increased formation of lactic acid. Lysine is an essential amino acid that is totally ketogenic.

Pyruvate metabolism

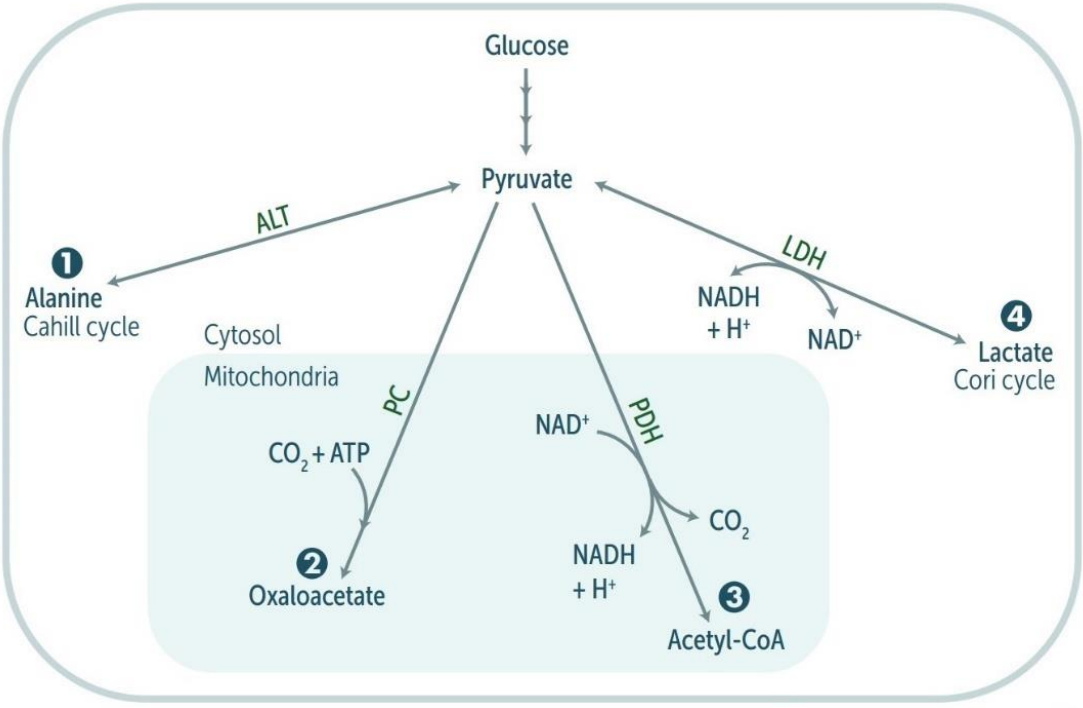
- Functions of different pyruvate metabolic pathways (and their associated cofactors):

A. Alanine aminotransferase (B6): alanine carries amino groups to the liver from muscle.

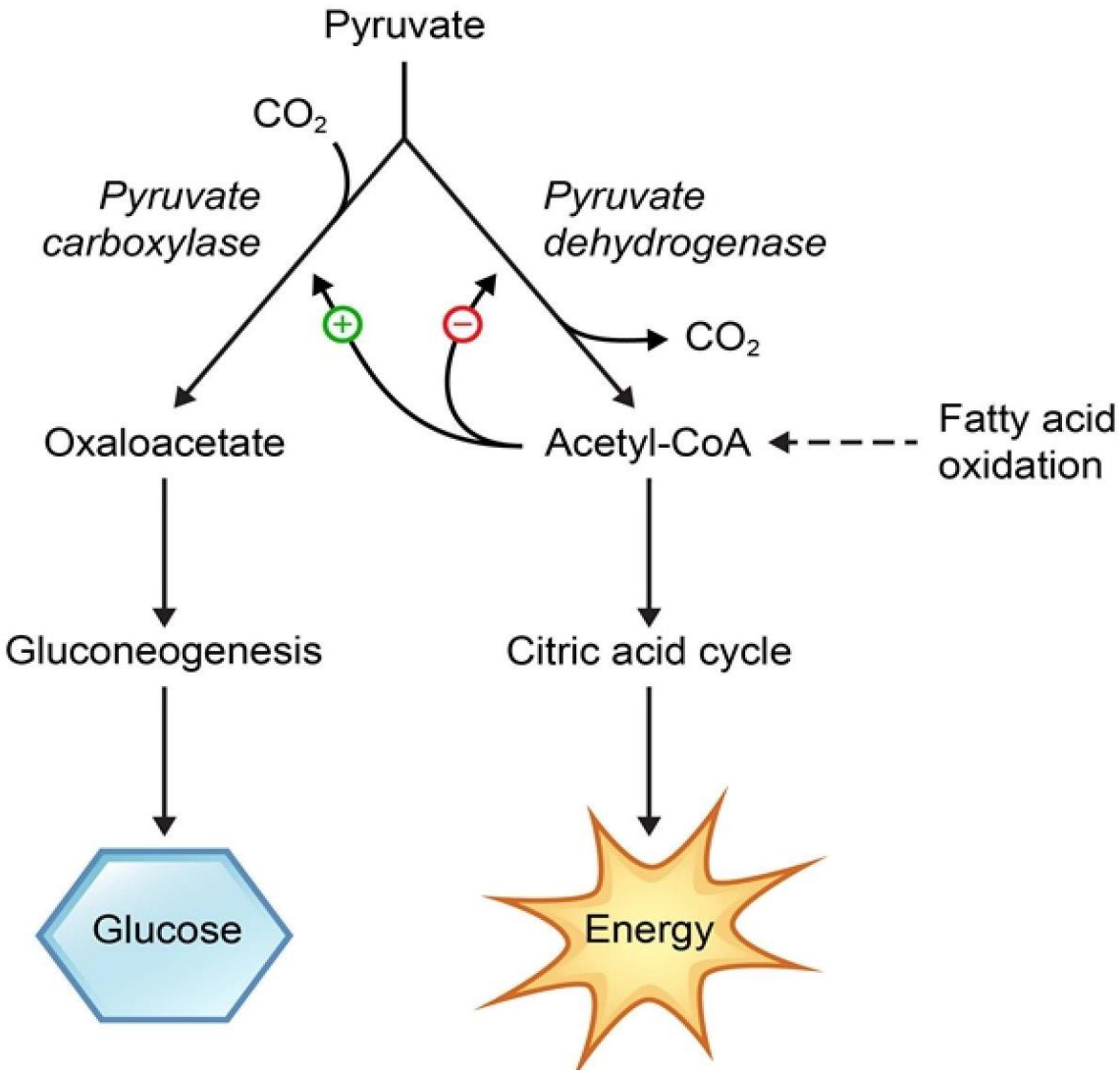
B. Pyruvate carboxylase (biotin): oxaloacetate can replenish TCA cycle or be used in gluconeogenesis.

C. Pyruvate dehydrogenase (B1, B2, B3, B5, lipoic acid): transition from glycolysis to the TCA cycle.

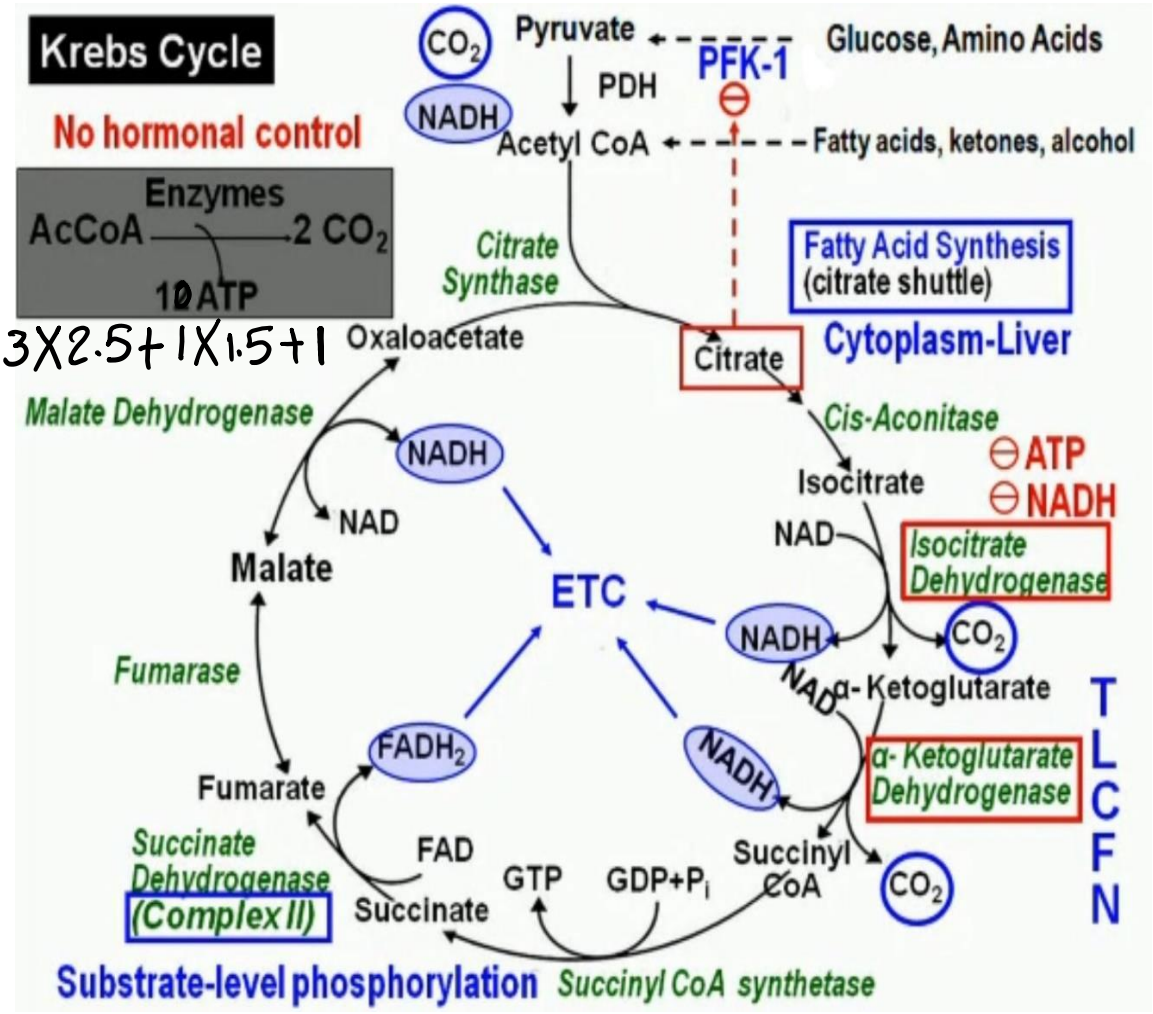
D. Lactic acid dehydrogenase (B3): end of anaerobic glycolysis (major pathway in RBCs, WBCs, kidney medulla, lens, testes, and cornea).



Metabolic fate of pyruvate



Citric acid cycle



$$\text{Acetyl-CoA} \longrightarrow 2\,CO_2$$
$$3\,NAD + FAD + GDP + P_i \longrightarrow 3\,NADH + FADH_2 + GTP$$

- The citric acid cycle (also called Krebs cycle or tricarboxylic acid cycle) occurs in the mitochondria.
- Although oxygen is not directly required in the cycle, the pathway will not occur anaerobically because NADH and FADH2 will accumulate if oxygen is not available for the electron transport chain.
- The primary function of the cycle is oxidation of acetyl-CoA to carbon dioxide.
- The energy released from this oxidation is saved as NADH, $FADH_2$, and guanosine triphosphate (GTP).
- It does not represent a pathway for the net conversion of acetyl-CoA to citrate, to malate, or to any other intermediate of the cycle.

- The only fate of acetyl-CoA in this pathway is its oxidation to CO_2.

- The cycle is central to the oxidation of any fuel that yields acetyl-CoA, including glucose, fatty acids, ketone bodies, ketogenic amino acids, and alcohol.

- There is no hormonal control of the cycle, as activity is necessary irrespective of the fed or fasting state.

- <u>Key points:</u>

- Isocitrate dehydrogenase, the major control enzyme, is inhibited by NADH and ATP and activated by ADP.

- α-ketoglutarate dehydrogenase, like pyruvate dehydrogenase, is a multienzyme complex. It requires thiamine, lipoic acid, CoA, FAD, and NAD. Lack of thiamine slows oxidation of acetyl-CoA in the citric acid cycle.

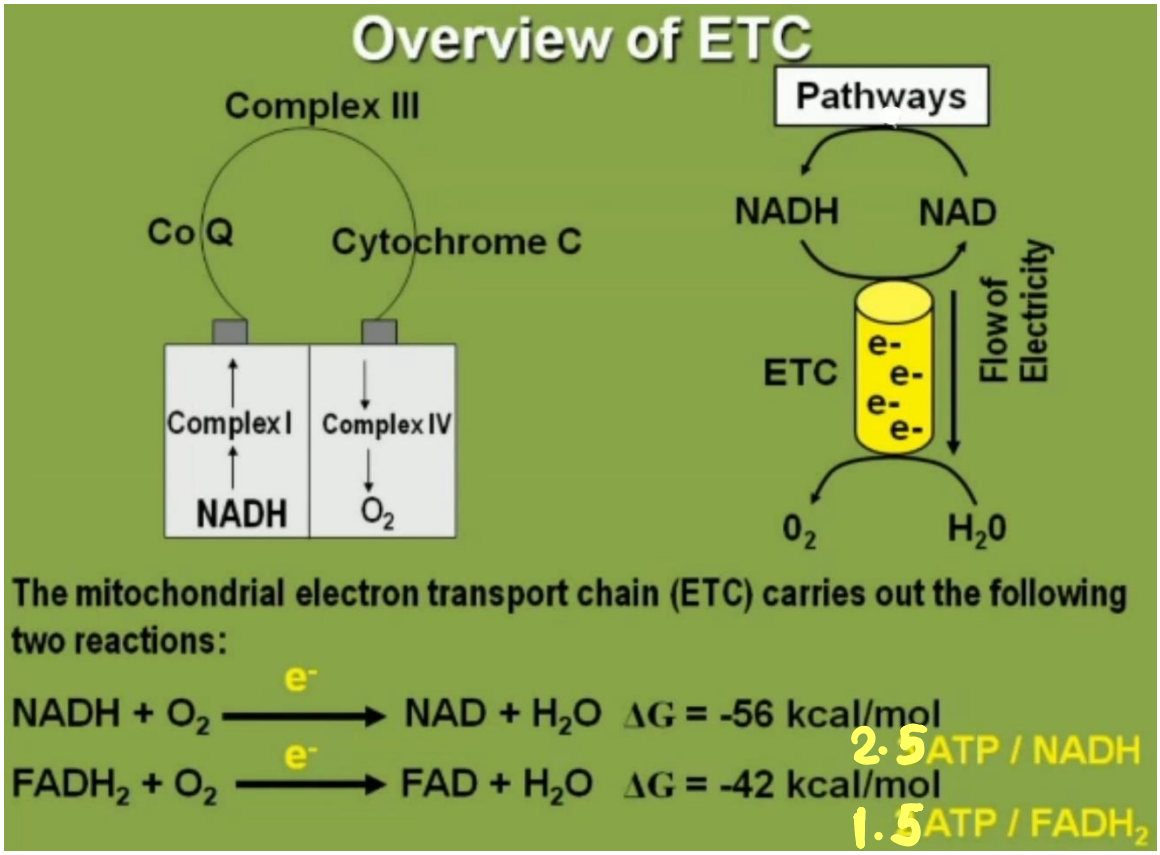
- Succinyl-CoA synthetase (succinate thiokinase) catalyzes a substrate-level phosphorylation of GDP to GTP.

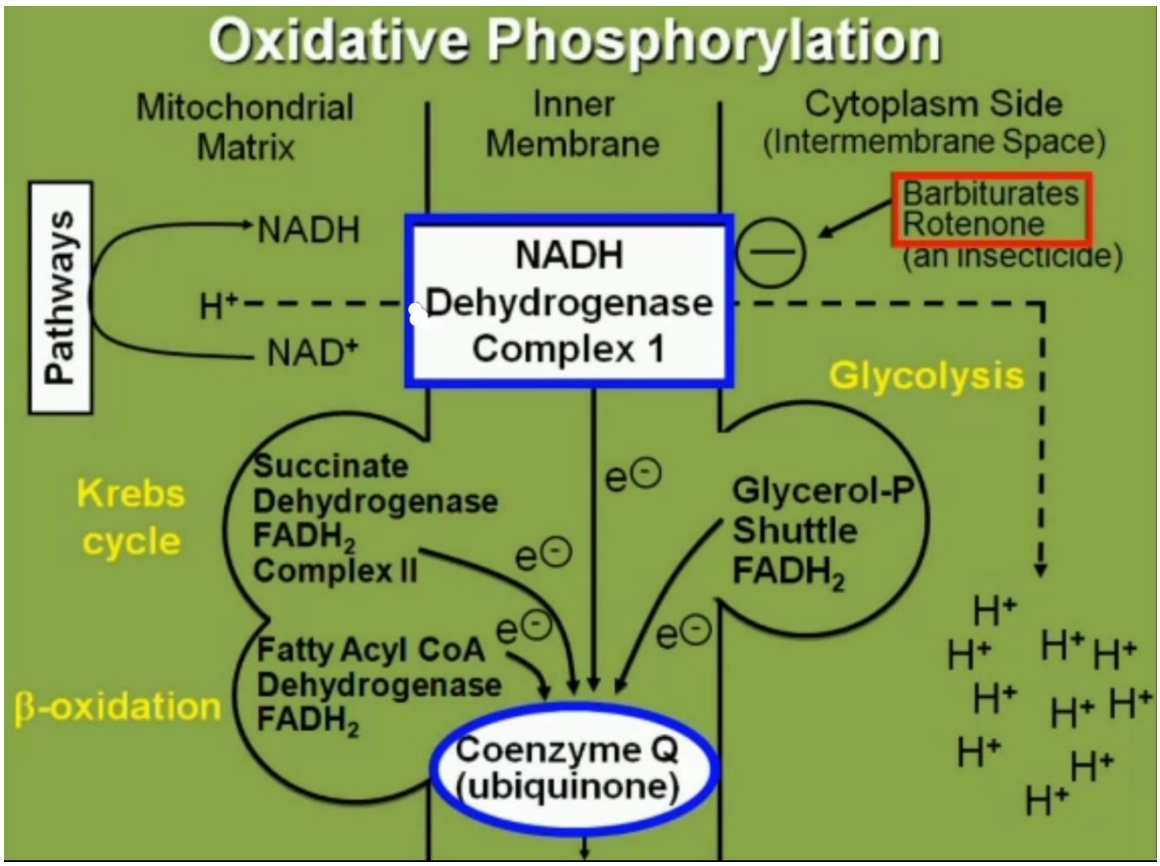
- Succinate dehydrogenase is on the inner mitochondrial membrane, where it also functions as complex II of the electron transport chain.

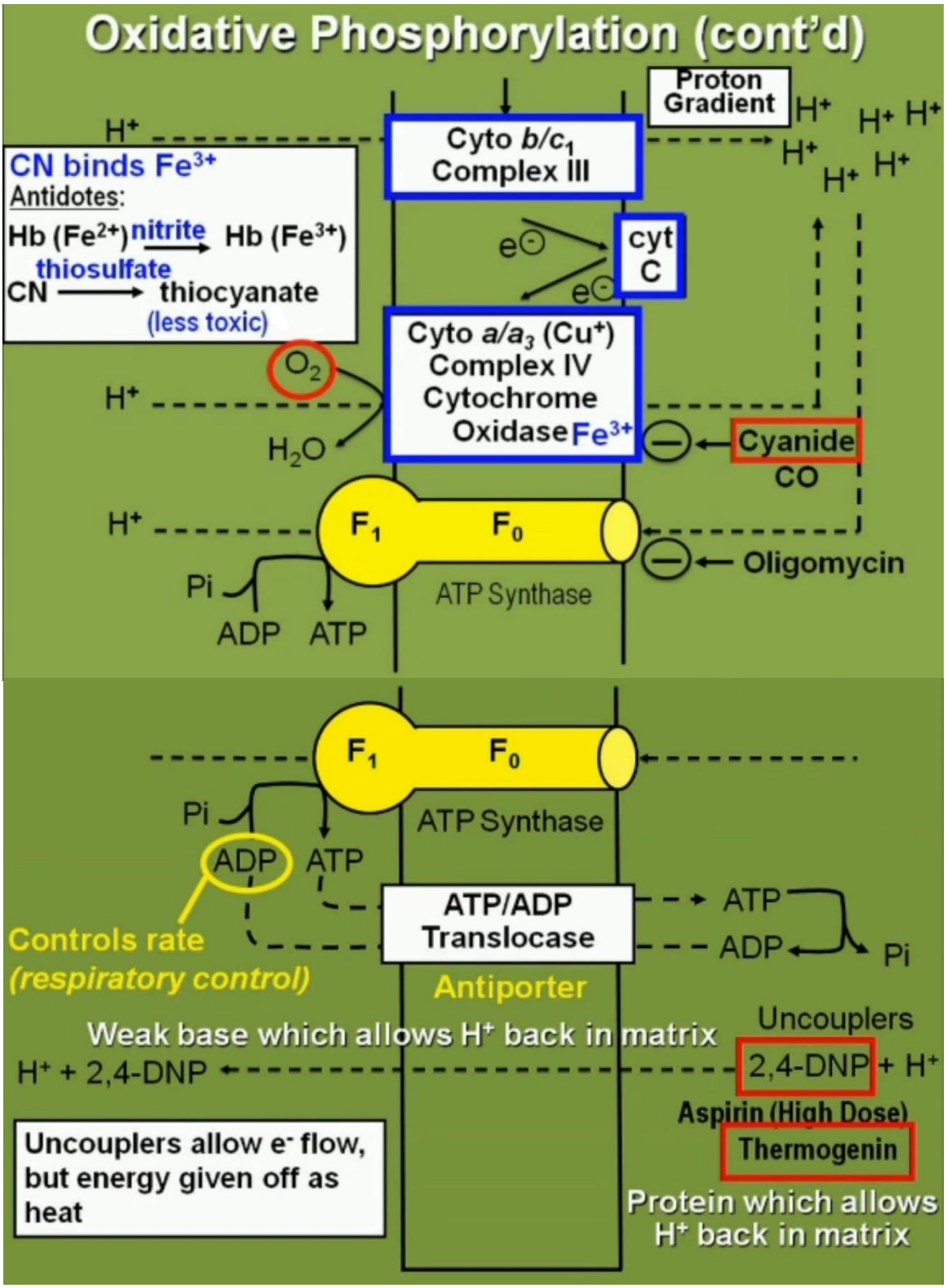
- During the TCA cycle, reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH) are generated. These molecules drive the process of oxidative phosphorylation, which is responsible for converting the reducing potential of these molecules into high-energy ATP via the electron transport chain.

- ATP can also be generated by substrate level phosphorylation, a process which involves the direct transfer of a phosphate group to ADP from a reactive intermediate. Substrate level phosphorylation can occur in both the cytoplasm and the mitochondrial matrix.

Electron transport chain and oxidative phosphorylation






Oxidative Phosphorylation (cont'd)
Proton Gradient
H+
H+ H+
H+
H+ H+
Cyto b/c1 Complex III
CN binds Fe3+
Antidotes:
Hb (Fe2+) nitrite Hb (Fe3+)
thiosulfate
CN thiocyanate
(less toxic)
e-
e-
cyt C
Cyto a/a3 (Cu+) Complex IV Cytochrome Oxidase Fe3+
O2
H2O
Cyanide
CO
F1
F0
Oligomycin
Pi
ADP
ATP
ATP Synthase
ATP/ADP Translocase
Antiporter
Controls rate
(respiratory control)
Weak base which allows H+ back in matrix
H+ + 2,4-DNP
Uncouplers
2,4-DNP + H+
Aspirin (High Dose)
Thermogenin
Uncouplers allow e- flow, but energy given off as heat
Protein which allows H+ back in matrix

- Capturing Chemical Energy as Electricity:

- The mitochondrial electron transport chain works like a chemical battery. In one location, an oxidation reaction is poised to release electrons at very high energy; in another location, a potential electron acceptor waits to be reduced.

- Because the 2 components are physically separated, nothing happens. Once the 2 terminals of the battery are connected by a wire, electrons flow from one compartment to the other through the wire, producing an electrical current or electricity. A light bulb or an electrical pump inserted into the circuit will run on the electricity generated. If no electrical device is in the circuit, all the energy is released as heat. The mitochondrial electron transport chain operates according to the same principle.

- Electron Transport Chain:

- Many enzymes in the mitochondria including those of the citric acid cycle and pyruvate dehydrogenase produce NADH, all of which can be oxidized in the electron transport chain and in the process, capture energy for ATP synthesis by oxidative phosphorylation.

- NADH is oxidized by NADH dehydrogenase (complex I), delivering its electrons into the chain and returning as NAD to enzymes that require it.

- The electrons are passed along a series of protein and lipid carriers that serve as the wire.

- These include, in order:
  - NADH dehydrogenase (complex I) accepts electrons from NADH.
  - Coenzyme Q (a lipid).
  - Cytochrome b/c1 (an Fe/heme protein; complex III).
  - Cytochrome c (an Fe/heme protein).
  - Cytochrome a/a3 (a Cu/heme protein; cytochrome oxidase, complex IV) transfers electrons to oxygen.

- All these components are in the inner membrane of the mitochondria. Succinate dehydrogenase, fatty acyl coA dehydrogynase and the glycerol 3-phosphate shuttle enzymes reoxidize their $FADH_2$ and pass electrons directly to CoQ.

- O_2 is delivered to tissues by hemoglobin. The majority of oxygen required in a tissue is consumed in the ETC. Its function is to accept electrons at the end of the chain, and the water formed is added to the cellular water.

- Proton Gradient:

- The electricity generated by the ETC is used to run proton pumps (translocators), which drive protons from the matrix space across the inner membrane into the intermembrane space, creating a small proton (or pH) gradient.

- The 3 major complexes I, III, and IV (NADH dehydrogenase, cytochrome b/c1, and cytochrome a/a3) all translocate protons in this way as the electricity passes through them.

- ATP synthesis by oxidative phosphorylation uses the energy of the proton gradient and is carried out by the F0/F1 ATP synthase complex. As protons flow into the mitochondria through the F0 component, their energy is used by the F1 component (ATP synthase) to phosphorylate ADP using Pi.

- On average, when an NADH is oxidized in the ETC, sufficient energy is contributed to the proton gradient for the phosphorylation of 2.5 ATP by F0/F1 ATP synthase. FADH2 oxidation provides enough energy for approximately 1.5 ATP.

▪ ETC inhibitors include cyanide and carbon monoxide:

- Directly inhibit electron transport, causing a ↓ proton gradient and block of ATP synthesis.

A. Cyanide:

- Cyanide is a deadly poison because it binds irreversibly to cytochrome a/a3 (complex IV), preventing electron transfer to oxygen.

- Nitrites may be used as an antidote for cyanide poisoning if given rapidly. They convert hemoglobin to methemoglobin, which binds cyanide in the blood before reaching the tissues.

- Cyanide toxicity can be treated with an antidote such as hydroxocobalamin or sodium thiosulfate, which directly binds cyanide molecules generating relatively nontoxic metabolites that are easily excreted in the urine.

B. Carbon monoxide:

- It binds to cytochrome a/a3 but less tightly than cyanide. It also binds to hemoglobin, displacing oxygen.

- Symptoms include headache, nausea, tachycardia, and tachypnea. Lips and cheeks turn a cherry-red color.

- Respiratory depression and coma result in death if not treated by giving oxygen.

▪ ATP synthase inhibitors:

- Directly inhibit mitochondrial ATP synthase, causing an ↑ proton gradient.

- No ATP is produced because electron transport stops.

- Oligomycin.

▪ Uncouping agents:

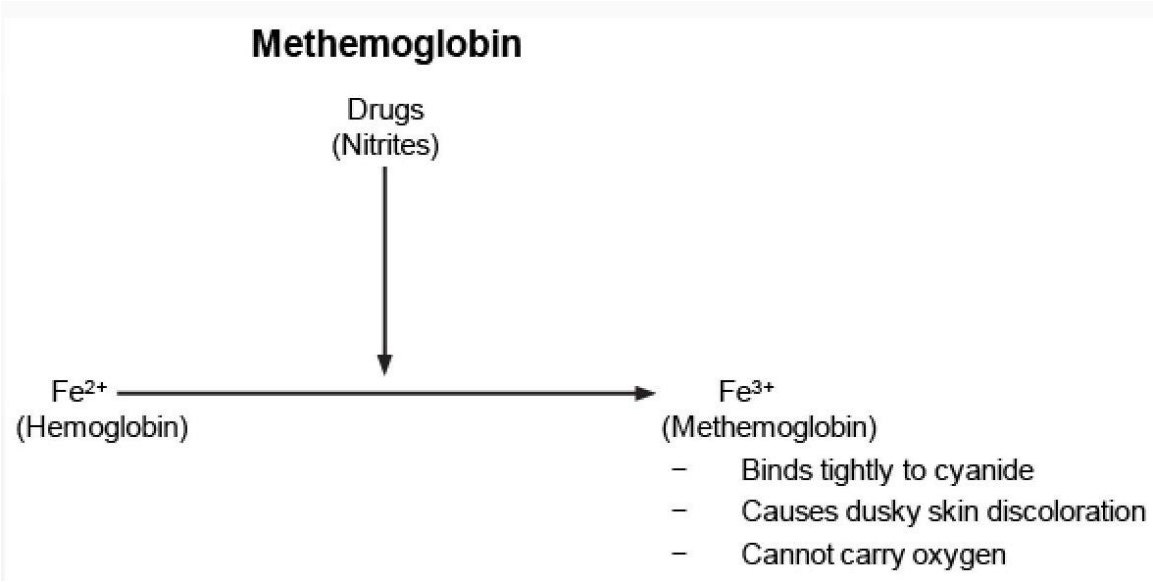
- ↑ permeability of membrane, causing a ↓ proton gradient and ↑ O_2 consumption.

- ATP synthesis stops, but electron transport continues.

- Because the rate of the ETC increases, with no ATP synthesis, energy is released as heat.

- Important uncouplers include 2,4-dinitrophenol (2,4-DNP) and aspirin (and other salicylates).

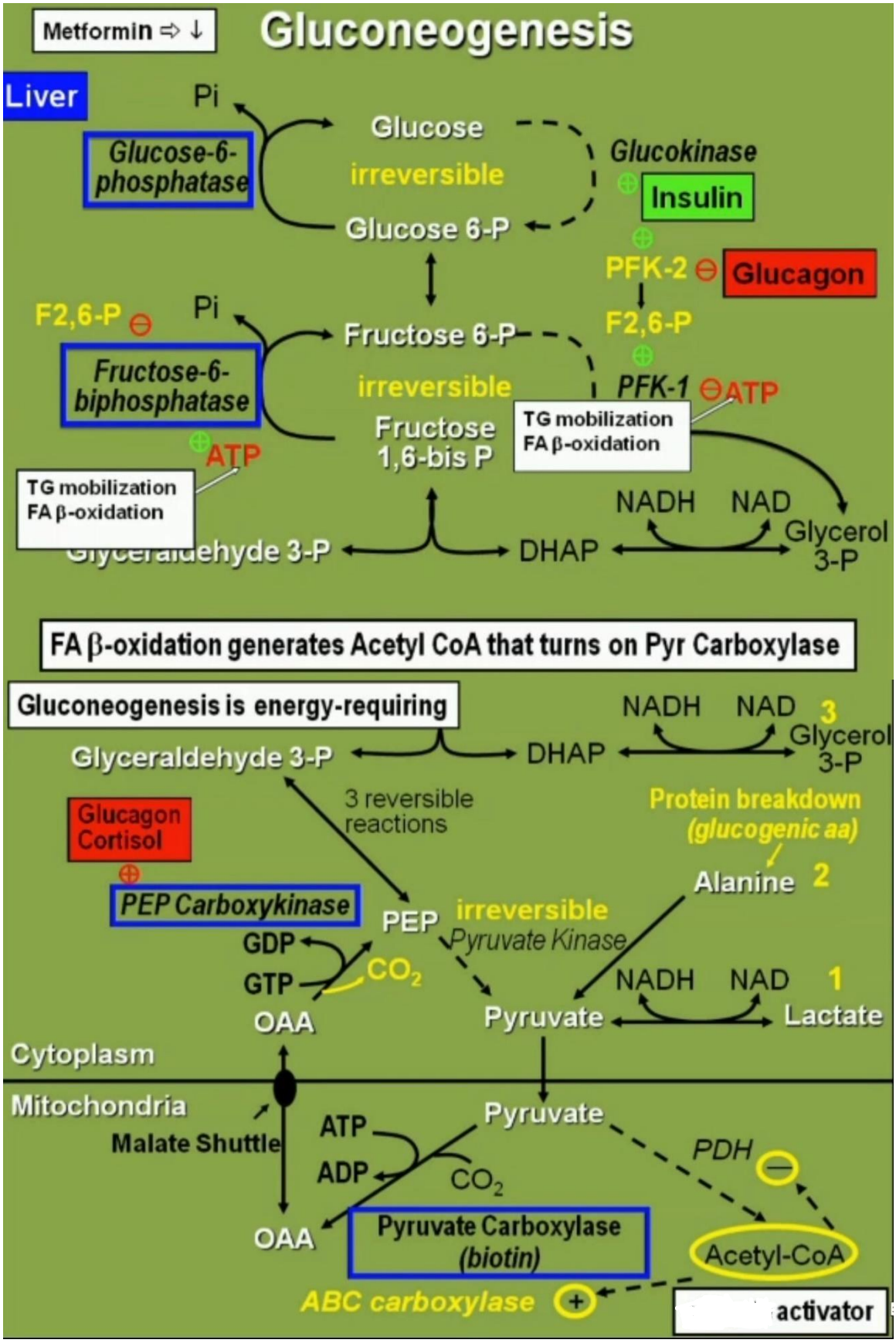
- Brown adipose tissue contains a natural uncoupling protein (UCP, formerly called thermogenin), which allows energy loss as heat to maintain a basal temperature around the kidneys, neck, breastplate, and scapulae in newborns.



❖ N.B:

- The toxicity of cyanide is dependent upon its ability to bind ferric iron (Fe3) with high affinity, inhibiting complex IV in the mitochondria.
- This electron transport chain enzyme is essential for oxidative phosphorylation; inhibition results in severe lactic acidosis and death as a result of cells switching to anaerobic metabolism.
- Cyanide poisoning presents with reddish skin discoloration, tachypnea, headache, and tachycardia, often accompanied by nausea/vomiting, confusion, and weakness. Symptoms develop rapidly and can quickly progress to seizures and cardiovascular collapse. Laboratory studies indicate severe lactic acidosis in conjunction with a narrowing of the venous-arterial PO_2 gradient, resulting from the inability of tissue to extract arterial oxygen.
- Iron bound to heme is normally in the reduced ferrous (Fe2) state. Administration of inhaled amyl nitrite oxidizes ferrous iron (Fe2) present in hemoglobin to ferric iron (Fe3), generating methemoglobin. Methemoglobin is incapable of carrying oxygen but has a high affinity for cyanide; it binds and sequesters cyanide in the blood, freeing it from cytochrome oxidase and limiting its toxic effects.
- Hydroxycobalamin, a vitamin B12 precursor, and sodium thiosulfate are also antidotes for cyanide poisoning. Their interactions with cyanide generate relatively nontoxic metabolites that are easily excreted in the urine.

Gluconeogenesis



- During fasting, the liver maintains glucose levels in blood through glycogenolysis or gluconeogenesis.

- These pathways are promoted by glucagon and epinephrine and inhibited by insulin.

- In fasting, glycogen reserves drop dramatically in the first 12 hours, during which time gluconeogenesis increases. After 24 hours, it represents the sole source of glucose.

- Important substrates for gluconeogenesis are:

- Glycerol 3-phosphate (from triacylglycerol in adipose).
- Lactate (from anaerobic glycolysis).
- Gluconeogenic amino acids (protein from muscle).

- Dietary fructose and galactose can also be converted to glucose in the liver.

- In humans, it is not possible to convert acetyl-CoA to glucose. In as much as most fatty acids are metabolized solely to acetyl-CoA, they are not a major source of glucose either. One minor exception is odd-number carbon fatty acids (C17), which yield a small amount of propionyl-CoA that is gluconeogenic.

- In the pathway of gluconeogenesis:

- lactate is oxidized to pyruvate by lactate dehydrogenase.

- The important gluconeogenic amino acid alanine is converted to pyruvate by alanine aminotransferase (ALT or GPT).

- Glycerol 3-phosphate is oxidized to dihydroxyacetone phosphate (DHAP) by glycerol 3-phosphate dehydrogenase.

- Gluconeogenesis is not simply the reverse of glycolysis, as three of the ten enzymes in glycolysis are unidirectional.

- These enzymes are hexokinase (glucokinase), phosphofructokinase-1 (PFK-1), and pyruvate kinase.

- To reverse glycolysis (to form glucose from pyruvate), four different enzymes are required to overcome these unidirectional roadblocks.

- These four gluconeogenic enzymes are:

1. Pyruvate carboxylase:

- Pyruvate carboxylase is a mitochondrial enzyme requiring biotin.

- It is activated by acetyl-CoA (from β-oxidation). Acetyl-CoA is an important allosteric activator of gluconeogenesis that acts by increasing the activity of pyruvate carboxylase.

- Pyruvate cannot be converted to phosphoenolpyruvate directly because pyruvate kinase is unidirectional. To convert pyruvate to phosphoenolpyruvate, pyruvate first undergoes biotin-

dependent carboxylation to oxaloacetate in the mitochondria. This reaction is catalyzed by pyruvate carboxylase, the activity of which is increased by acetyl- CoA.

- Oxaloacetate is then shuttled out of the mitochondria to be converted back to oxaloacetate.

2. Phosphoenolpyruvate carboxykinase (PEPCK):

- Phosphoenolpyruvate carboxykinase (PEPCK) in the cytoplasm is induced by glucagon and cortisol.

- It converts OAA to phosphoenolpyruvate (PEP) in a reaction that requires GTP.

3. Fructose-1,6-bisphosphatase:

- Fructose-1,6-bisphosphatase in the cytoplasm is a key control point of gluconeogenesis.

- It hydrolyzes phosphate from fructose 1,6-bisphosphate.

- Fructose 2,6-bisphosphate, produced by PFK-2 helps control the balance between gluconeogenesis and glycolysis through inverse regulation of phosphofructokinase-1 (PFK-1) and fructose 1,6-bisphosphatase.

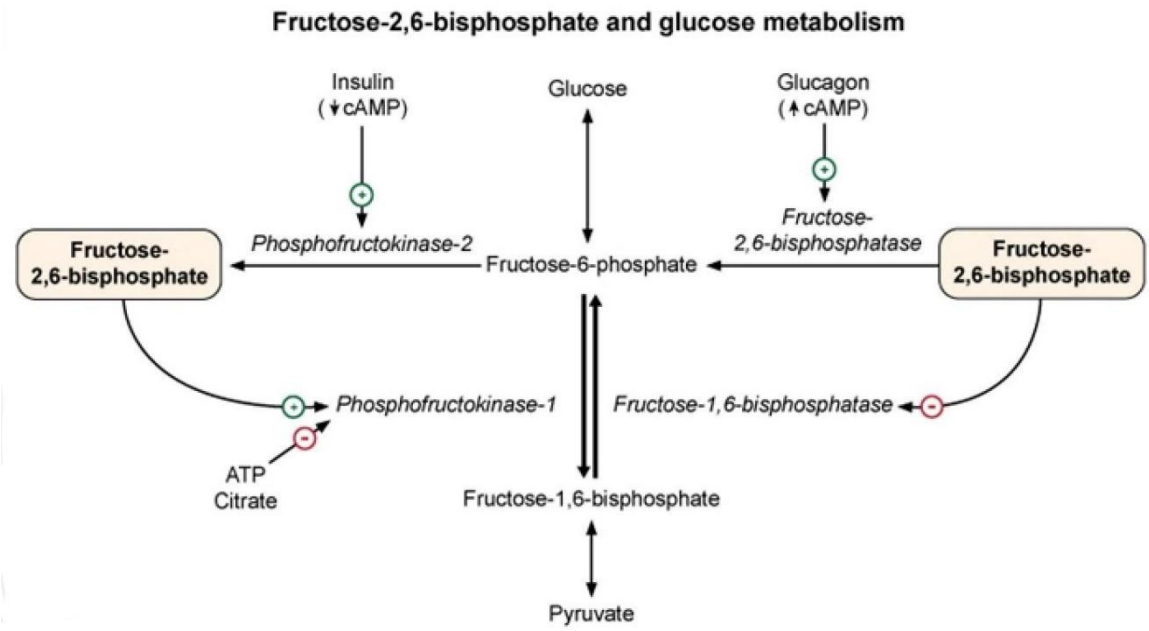
- Fructose 2,6- bisphosphate activates PFK-1, the main regulatory enzyme involved in glycolysis, which converts fructose 6-phosphate to fructose 1, 6-bisphosphate. The opposite reaction (fructose 1,6-bisphosphate to fructose-6- phosphate) occurs in gluconeogenesis and is catalyzed by the enzyme fructose-1,6-bisphosphatase (inhibited by fructose 2,6-bisphosphate).

- Recall that PFK-2 is activated by insulin and inhibited by glucagon. Thus, glucagon will lower F 2,6-BP and stimulate gluconeogenesis, whereas insulin will increase F-2,6-BP and inhibit gluconeogenesis.

- The interconversion of fructose-6-phosphate and fructose 2,6-bisphosphate is achieved by a bifunctional enzyme complex composed of PFK-2 (increases fructose 2,6-bisphosphate levels) and fructose 2,6- bisphosphophosphatase (decreases fructose 2,6-bisphosphate levels).

- Insulin in fed state causes ↑ PFK-2, ↓ 2,6- bisphosphophosphatase activity leading to increased fructose 2,6-bisphosphate levels and augmented glycolysis. High concentrations of fructose 2,6-bisphosphate also inhibit gluconeogenesis, leading to decreased conversion of alanine and other gluconeogenic substrates to glucose.

- Glucagon in fasting state causes ↑ 2,6- bisphosphophosphatase activity & ↓ PFK-2, leading to decreased fructose 2,6-bisphosphate levels and augmented gluconeogenesis.



4. Glucose-6-phosphatase:

- Glucose-6-phosphatase is only in the liver.

- The absence of glucose- 6-phosphatase in skeletal muscle accounts for the fact that muscle glycogen cannot serve as a source of blood glucose.

- Although alanine is the major gluconeogenic amino acid, 18 of the 20 (all but leucine and lysine) are also gluconeogenic.

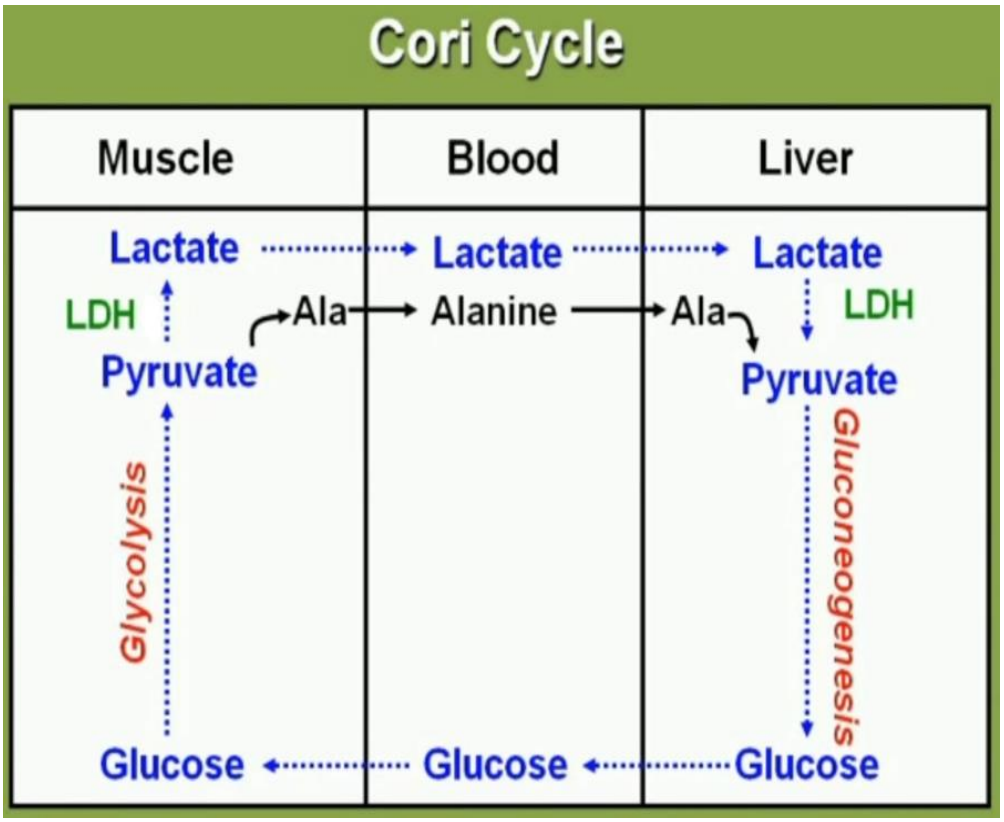
- It is important to note that glucose produced by hepatic gluconeogenesis does not represent an energy source for the liver. Gluconeogenesis requires expenditure of ATP that is provided by β-oxidation of fatty acids.

- Therefore, hepatic gluconeogenesis is always dependent on β-oxidation of fatty acids in the liver. During hypoglycemia, adipose tissue releases these fatty acids by breaking down triglyceride.

- Cori Cycle and Alanine Cycle:

- During fasting, lactate from red blood cells (and possibly exercising skeletal muscle) is converted in the liver to glucose that can be returned to the red blood cell or muscle. This is called the Cori cycle.

- The alanine cycle is a slightly different version of the Cori cycle, in which muscle releases alanine, delivering both a gluconeogenic substrate (pyruvate) and an amino group for urea synthesis.


Cori Cycle
Muscle
Blood
Liver
Lactate
Lactate
Lactate
LDH
Ala
Alanine
Ala
LDH
Pyruvate
Pyruvate
Glycolysis
Gluconeogenesis
Glucose
Glucose
Glucose

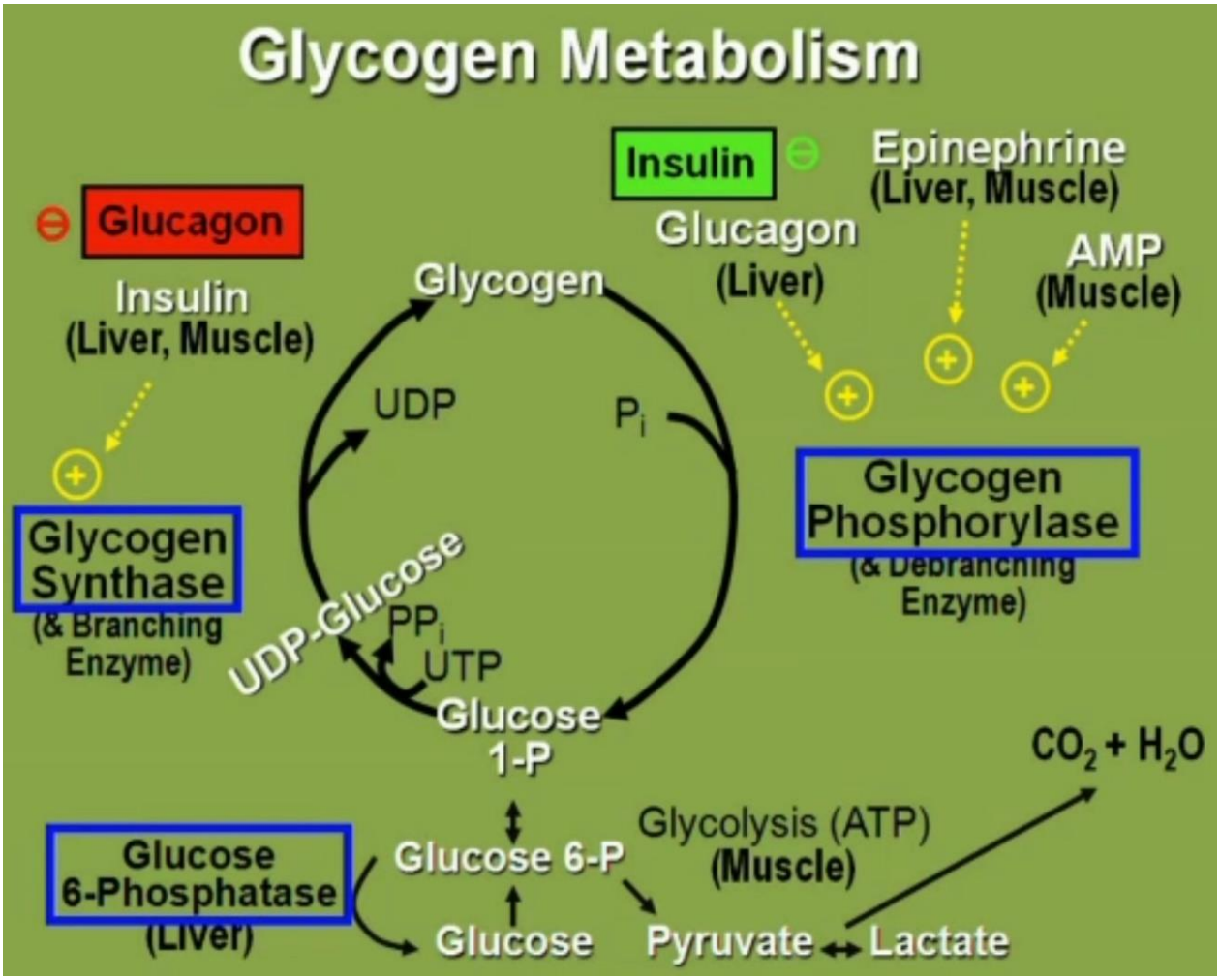
Glycogen metabolism

- Important metabolic pathways for glucose include the following:

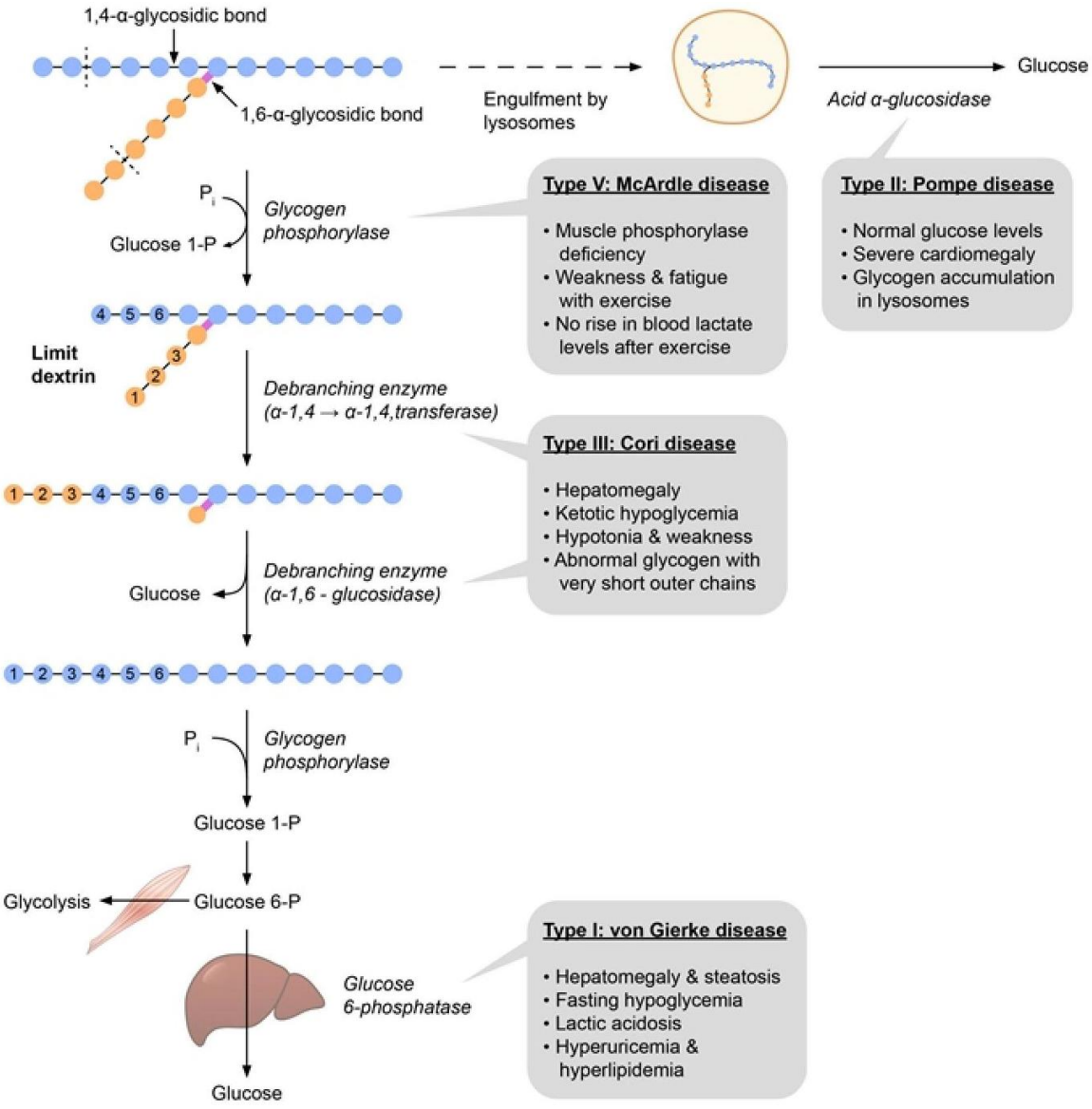
A. Glycolysis and the Krebs cycle (TCA cycle) are used to generate ATP. Glucose is first metabolized to pyruvate during glycolysis, and then pyruvate is converted to acetyl CoA, which enters the TCA cycle.

B. Glycogenesis stores glucose for later use via formation of the glucose polymer glycogen from glucose-1-phosphate (storage form of glucose).

C. The HMP shunt (pentose phosphate pathway) generates pentose sugars and NADPH.



Glycogenesis



- Glycogen, a branched polymer of glucose, represents a storage form of glucose.
- Glycogen synthesis and degradation occur primarily in liver and skeletal muscle, although other tissues such as cardiac muscle and the kidney store smaller quantities.
- Glycogen stored in the liver is a source of glucose mobilized during hypoglycemia.
- Muscle glycogen is stored as an energy reserve for muscle contraction.
- Glucose addition to a granule begins with glucose 6-phosphate, which is converted to glucose 1-phosphate and activated to UDP-glucose for addition to the glycogen chain by glycogen synthase.

- Glycogen synthase is the rate-limiting enzyme of glycogen synthesis.

- Branches have α-(1,6) bonds; linkages have α-(1,4) bonds.

A. Glycogen Synthase: Glycogen synthase forms the α1,4 glycosidic bond found in the linear glucose chains of the granule.

B. Branching Enzyme (Glycosyl α1,4: α1,6 Transferase):

- Branching enzyme is responsible for introducing α1,6-linked branches into the granule as it grows.

- Branching enzyme Hydrolyzes one of the α1,4 bonds to release a block of oligoglucose, which is then moved and added in a slightly different location Forms an α1,6 bond to create a branch.

Glycogenolysis

- The breakdown of glycogen requires three main enzymes: 1) glycogen phosphorylase, 2) a debranching enzyme, and 3) phosphoglucomutase.

- The rate-limiting enzyme of glycogenolysis is glycogen phosphorylase (in contrast to a hydrolase, a phosphorylase breaks bonds using Pi rather than H_2O).

A. Glycogen Phosphorylase:

- Glycogenolysis begins with glycogen phosphorylase shortening the glycogen chain by cleaving the alpha-1,4- glycosidic linkages between glucose residues through simple phosphorylation.

- This occurs until only four residues are remaining at the end of both the main and side chains before a 1,6-glycosidic branch point.

- These remaining glucose residues before a branch point are referred to as "limit dextrins". The debrancher enzyme acts on the glucose polymer at this point.

B. Debranching Enzyme (Glucosyl α1,4: α1,4 Transferase and α1,6 Glucosidase):

- The debrancher enzyme provides two enzymatic activities.
  - The first enzymatic action is performed by alpha- 1,4 glucan transferase. This enzyme removes the outer three residues of the four-glucose residues left by glycogen phosphorylase on the alpha-1,6 side chain and transfers them to the main alpha-1,4 chain.

  - The second action is performed by amylo-alpha-1,6-glucosidase, an enzyme that cleaves the alpha- 1,6-glycosidic bond at the branch point to liberate a free glucose molecule.

- Glycogen phosphorylase can then resume cleaving alpha-1,4-glycosidic linkages leading to the formation of glucose-1-phosphate, which is then converted to glucose-6-phosphate by the enzyme phosphoglucomutase.

- A fourth enzyme, which is only present in the liver, is responsible for the conversion of glucose-6-phosphate to glucose. This enzyme is glucose-6-phosphatase.

- Muscle tissue does not participate in maintaining glucose levels, as it lacks glucose-6-phosphatase. Instead, muscles utilize glucose-6-phosphate liberated from glycogen strictly for their own energy needs during muscle contraction.

❖ Glycogen regulation by insulin and glucagon/epinephrine:

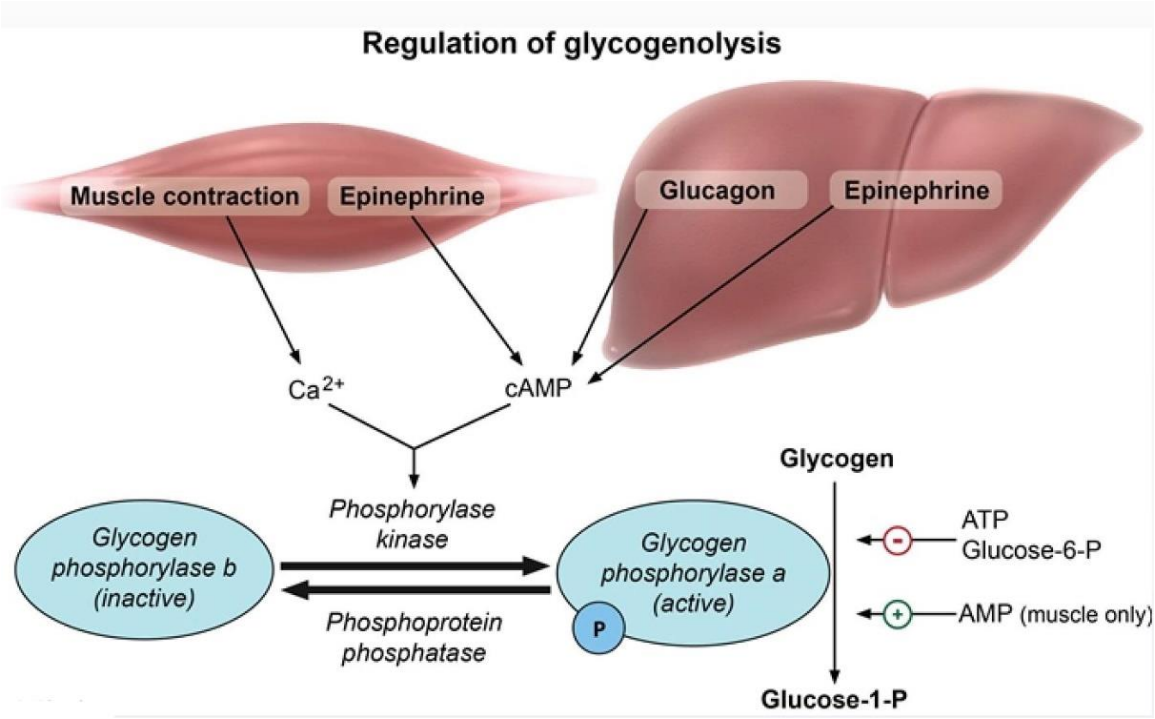
- Glycogen is broken down by the enzyme glycogen phosphorylase, which is regulated through phosphorylation (active state) and dephosphorylation (inactive state).

- Phosphorylase kinase (PK) is the enzyme responsible for the phosphorylation of glycogen phosphorylase, whereas phosphoprotein phosphatase catalyzes its dephosphorylation.

- PK is regulated differently in liver than in muscles. Glycogen stored in the liver is used to maintain blood glucose levels during the fasting state, whereas glycogen in the muscles is used to provide energy for muscle contraction.

- In the liver, PK is activated primarily through the binding of epinephrine and glucagon to Gs protein-coupled receptors, which increases cAMP concentrations and causes phosphorylation of PK (via protein kinase A).

- Skeletal muscle lacks glucagon receptors, but muscle PK can still be phosphorylated in response to an epinephrine-induced increase in cAMP concentrations. However, increased intracellular calcium is a more powerful activator of muscle PK. Release of sarcoplasmic calcium stores following neuromuscular acetylcholine stimulation allows for synchronization of skeletal muscle contraction and glycogen breakdown, providing the energy necessary for anaerobic muscle contraction.



Glycogen storage diseases

- At least 15 types have been identified, all resulting in abnormal glycogen metabolism and an accumulation of glycogen within cells.
- Periodic acid–Schiff stain identifies glycogen and is useful in identifying these diseases.
- Types I, II, III, and V are autosomal recessive.

1. Von Gierke disease (type I):

- Deficient enzyme: Glucose-6-phosphatase.
- Findings:

- Deficiency of hepatic glucose-6-phosphatase produces a profound fasting hypoglycemia, lactic acidosis, and hepatomegaly.
- Liver does not regulate blood glucose (Impaired gluconeogenesis and glycogenolysis).
- ↑ blood lactate.
- ↑↑ Glycogen in liver and kidneys (hepatomegaly and renomegally): Glycogen deposits in the liver (glucose 6-P stimulates glycogen synthesis, and glycogenolysis is inhibited).
- Hyperuricemia predisposing to gout. Decreased Pi causes increased AMP, which is degraded to uric acid. Lactate slows uric acid excretion in the kidney.
- Hyperlipidemia with skin xanthomas.
- Fatty liver.

- Treatment:

- Frequent oral glucose/cornstarch; avoidance of galactose and fructose.
- In a person with glucose-6-phosphatase deficiency, ingestion of galactose or fructose causes no increase in blood glucose, nor does administration of glucagon or epinephrine.

2. Pompe disease (type II):

- Deficient enzyme:

- Deficiency of lysosomal enzyme alpha 1,4-glucosidase (acid maltase), an enzyme responsible for breaking down glycogen within the acidic environment of lysosomes.
- Although most glycogen is degraded in the cytoplasm, a small amount is inadvertently engulfed by lysosomes, especially in cells containing high amounts of glycogen such as hepatocytes and myocytes.

- As such, deficiency of acid maltase results in pathologic accumulation of glycogen within liver and muscle lysosomes. Cardiac and skeletal muscle are particularly susceptible, as the ballooning lysosomes interfere with contractile function.

- Pompe disease is different from the other diseases described here because the enzyme missing is not one in the normal process of glycogenolysis.

▪ Findings:
- The classic form of the disease presents in early infancy with marked cardiomegaly, severe generalized hypotonia, exercise intolerance, macroglossia, and hepatomegaly.

- With infantile onset, massive cardiomegaly is usually the cause of death, typically age <2.

- Blood glucose levels are normal, unlike with glycogen storage diseases that primarily affect the liver (von Gierke).

- A key distinguishing feature is that muscle biopsy will show accumulation of glycogen in lysosomes.

- PomPe trashes the PumP (1st and 4th letter; heart, liver, and muscle)

3. Cori disease (type III):

▪ Deficient enzyme: Debranching enzyme (α-1,6-glucosidase).

▪ Findings:
- Milder form of von Gierke (type I) with normal blood lactate levels.

- Gluconeogenesis is intact.

- Patients with this illness present with the non-specific findings of hypoglycemia, hypertriglyceridemia, ketoacidosis, and hepatomegaly.

- These manifestations are also common with other glycogen storage diseases; however, debranching enzyme deficiency can be differentiated from other glycogen storage diseases by demonstrating the accumulation of abnormally short outer dextrin-like structures in the cytosol of hepatocytes with an absence of histopathological fatty infiltration of the liver (Debranching enzyme deficiency leads to incomplete glycogen degradation.

- Alpha-1,6- glucosidic branch points cannot be degraded, so small chain dextrin-like material accumulates within the cytosol of hepatocytes).

4. McArdle disease (type V):

- Deficient enzyme:

- Skeletal muscle glycogen phosphorylase (Myophosphorylase).

- McArdle = Muscle.

- Findings:

- Deficiency of this enzyme leads to decreased breakdown of glycogen during exercise → ↑ glycogen in muscle → poor exercise tolerance, painful Muscle cramps, Myoglobinuria (red urine) with strenuous exercise, and arrhythmia from electrolyte abnormalities.

- Without an adequate supply of glucose, sufficient energy via glycolysis for carrying out muscle contraction cannot be obtained, explaining why the muscles are not functioning well (weakness and cramps).

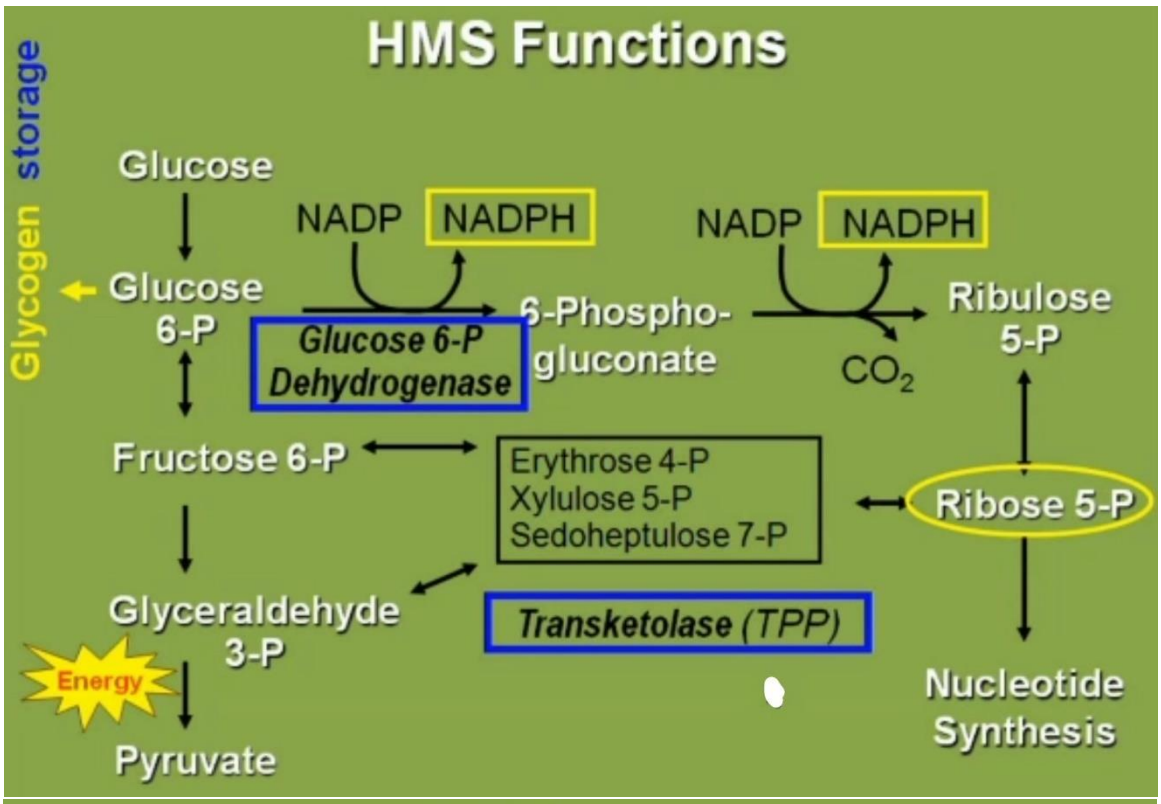
- Second-wind phenomenon (recovery) noted during exercise due to ↑ muscular blood flow after 10-15 minutes of exercise.

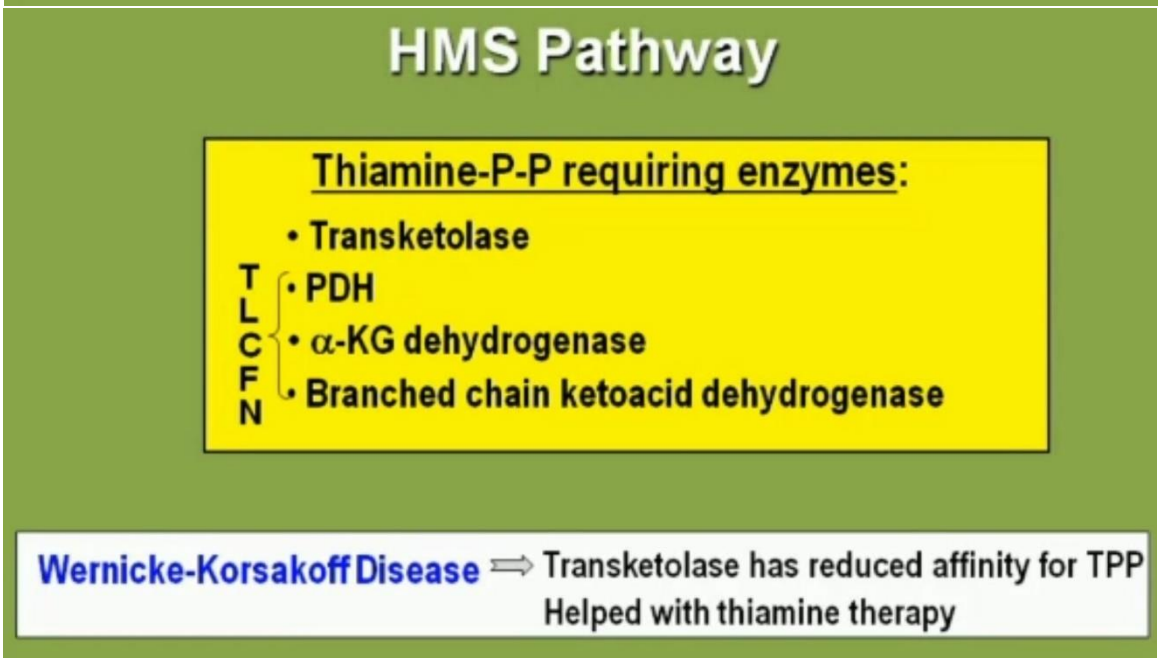
- The prognosis is generally good, and symptoms can be improved by consuming simple sugars before beginning physical activity.

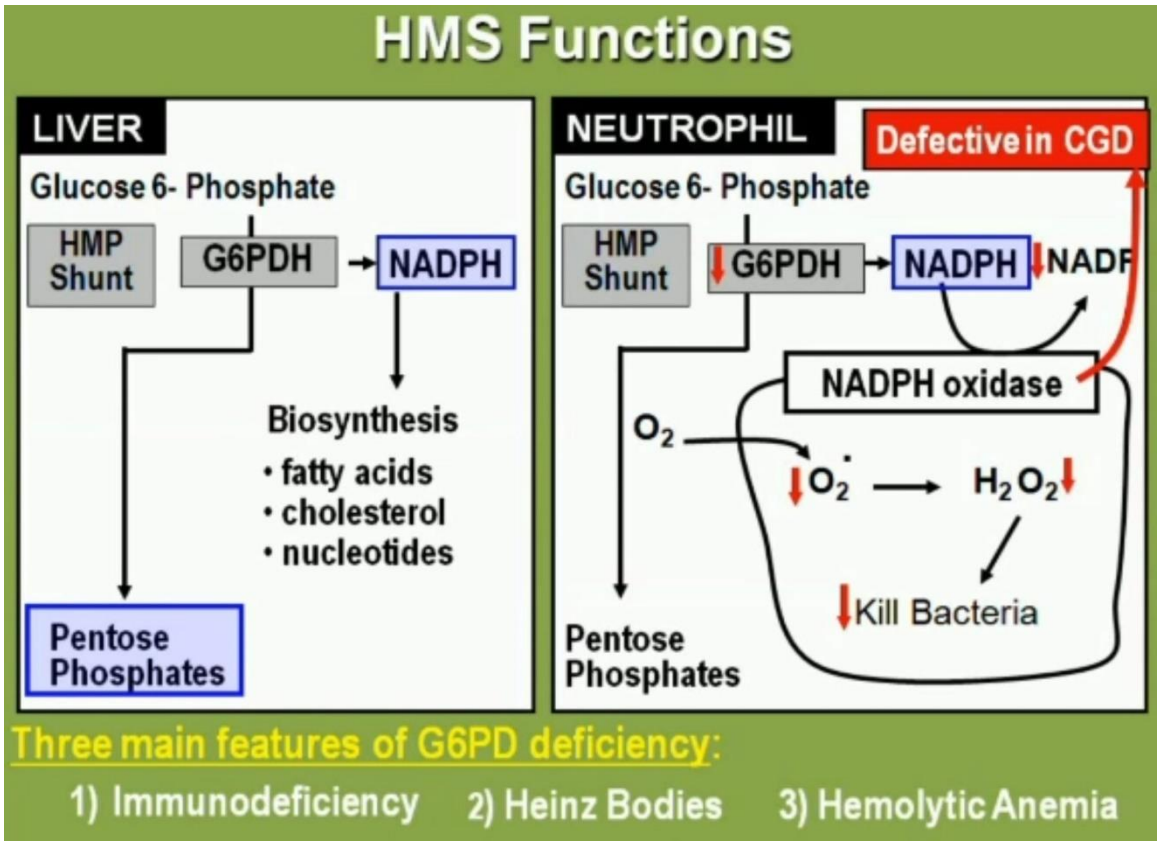
- Hallmark is a flat venous lactate curve with normal rise in ammonia levels during exercise.

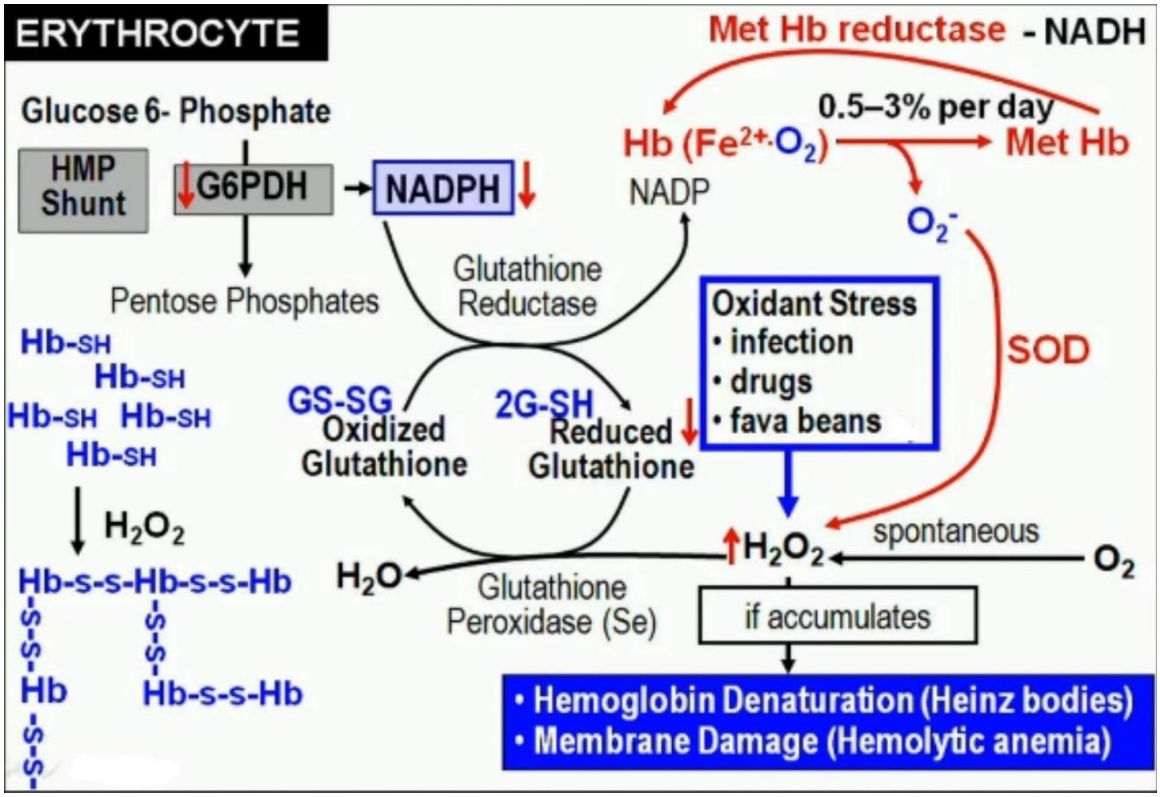
- Blood glucose levels typically unaffected.

Hexose monophosphate shunt






HMS Functions
LIVER
Glucose 6- Phosphate
HMP Shunt
G6PDH
NADPH
Biosynthesis
• fatty acids
• cholesterol
• nucleotides
Pentose Phosphates
NEUTROPHIL
Defective in CGD
Glucose 6- Phosphate
HMP Shunt
G6PDH
NADPH
NADP
NADPH oxidase
O2
O2·
H2O2
Kill Bacteria
Pentose Phosphates
Three main features of G6PD deficiency:
1) Immunodeficiency
2) Heinz Bodies
3) Hemolytic Anemia


ERYTHROCYTE
Met Hb reductase - NADH
Glucose 6- Phosphate
HMP Shunt
G6PDH
NADPH
NADP
0.5–3% per day
Hb (Fe2+.O2)
Met Hb
O2-
Glutathione Reductase
Pentose Phosphates
Oxidant Stress
• infection
• drugs
• fava beans
SOD
Hb-SH
Hb-SH
Hb-SH Hb-SH
Hb-SH
GS-SG
Oxidized Glutathione
2G-SH
Reduced Glutathione
H2O2
Hb-s-s-Hb-s-s-Hb
Hb
Hb-s-s-Hb
H2O
H2O2
spontaneous
O2
Glutathione Peroxidase (Se)
if accumulates
• Hemoglobin Denaturation (Heinz bodies)
• Membrane Damage (Hemolytic anemia)

- The hexose monophosphate (HMP) shunt (pentose phosphate pathway) occurs in the cytoplasm of all cells, where it serves 2 major functions:

- NADPH production.
- Source of ribose 5-phosphate for nucleotide synthesis.

- All reactions of the HMP shunt occur exclusively in the cytoplasm.

- The HMP shunt consists of two different types of reactions: oxidative (irreversible) and nonoxidative (reversible).

A. In the oxidative portion of HMP shunt:

- Glucose 6-phosphate is first converted to 6-phosphogluconolactone producing one molecule of NADPH.

- This reaction is catalyzed by glucose 6-phosphate dehydrogenase, the rate limiting enzyme of the HMP shunt.

- G6PDH is induced by insulin, inhibited by NADPH, and activated by NADP.

- In the second reaction of the oxidative portion of HMP shunt, 6 phosphogluconolactone is hydrolyzed to ribulose 5-phosphate by 6-phosphogluconate dehydrogenase producing a second molecule of NADPH.

- The oxidative portion of the HMP shunt is most active in tissues responsible for reductive biosynthesis such as the liver and adrenals because certain enzymes involved in fatty acid, cholesterol, and steroid synthesis (and also drug metabolism) require NADPH as a cofactor.

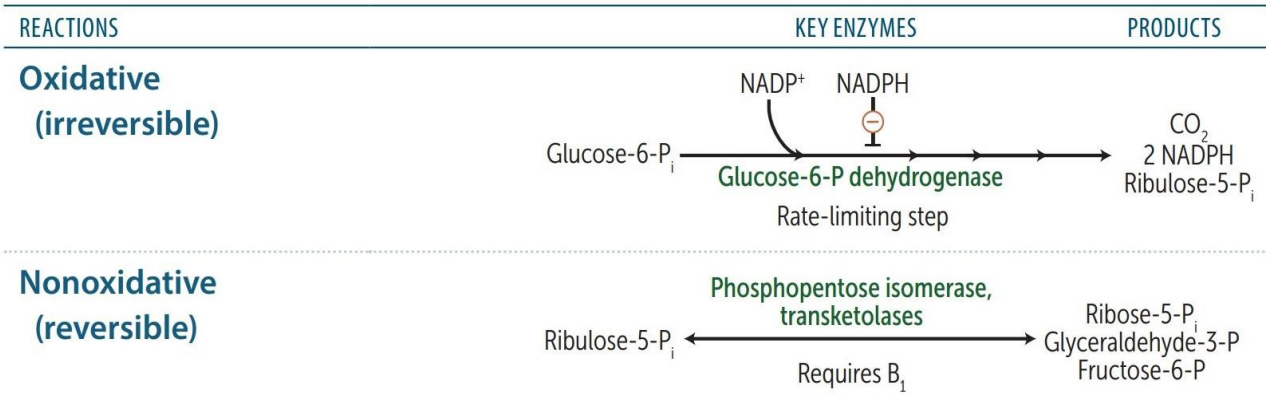
B. The non-oxidative reactions of the HMP:

- They are primarily designed to generate ribose 5-phosphate from intermediates of glycolysis.

- The primary enzymes involved in the non-oxidative steps of the HMP shunt are transaldolase and transketolase.

- Transketolase transfers two-carbon groups between substrates of the HMP shunt and requires thiamine pyrophosphate as a cofactor, and transaldolase transfers three-carbon groups between substrates of the HMP shunt.

- All cells can synthesize ribose from the glycolysis intermediates fructose 6-phosphate and glyceraldehyde 3-phosphate with the help of transketolase and transaldolase even if the oxidative reactions of the HMP pathway are not active in those cells.



- Some cells do not use the oxidative phase reactions to produce cytosolic NADPH, but all cells can synthesize ribose from fructose-6-phosphate using the nonoxidative reactions.

- Functions of NADPH:

- Biosynthesis.
- Maintenance of a supply of reduced glutathione to protect against reactive oxygen species (ROS) in erythrocytes.
- Bactericidal activity in polymorphonuclear leukocytes (Respiratory burst).
- Cytochrome P-450 system.

Glucose-6-phosphate dehydrogenase deficiency

- X-linked recessive disorder; most common human enzyme deficiency; more prevalent among African Americans.

- Red blood cells do not have mitochondria or a nucleus; therefore, metabolism of glucose in these cells occurs via glycolysis and the hexose monophosphate (HMP) shunt.

- Glycolysis provides energy for erythrocyte survival; whereas, the HMP shunt provides the reducing agent NADPH to prevent oxidant damage.

- In erythrocytes, hydrogen peroxide produced by partial reduction of molecular oxygen is detoxified by glutathione peroxidase.

- Glutathione is oxidized during this reaction. The regeneration of reduced glutathione is carried out by the enzyme glutathione reductase using NADPH as an electron donor. NADPH in red blood cells is produced solely by the HMP shunt, and this is how the HMP shunt contributes to protecting red blood cells against free radicals, hydrogen peroxide and other forms of oxidant stress.

- Defective generation of NADPH due to defects in the oxidative portion of the HMP shunt will increase the susceptibility of the RBCs to oxidative damage, and glutathione reductase deficiency will lead to similar clinical picture.

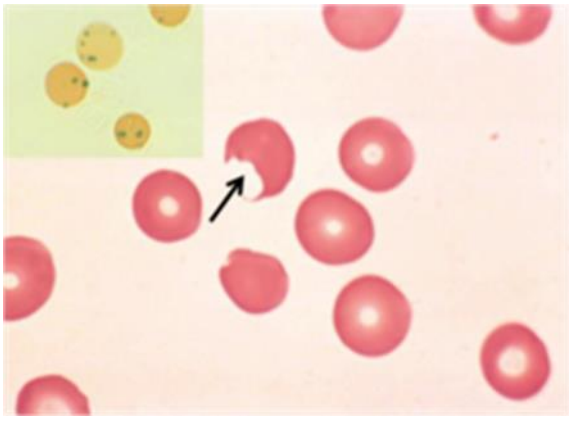
- Oxidative damage to red cells causes denatured hemoglobin to form insoluble Heinz bodies resulting in erythrocyte destruction in the spleen. Additionally, oxidative stress results in stiffening of the erythrocyte membrane and hemolysis in the microvasculature due to an inability of the erythrocyte to deform and fit through capillary beds.

- Examples of oxidizing agents (fava beans, sulfonamides, nitrofurantoin, primaquine/ chloroquine, antituberculosis drugs). Infection (most common cause) can also precipitate hemolysis; inflammatory response produces free radicals that diffuse into RBCs, causing oxidative damage.

- Heinz bodies: denatured globin chains precipitate within RBCs due to oxidative stress.

- Bite cells: result from the phagocytic removal of Heinz bodies by splenic macrophages.

- Think, "Bite into some Heinz ketchup."



ATP production

- Aerobic metabolism of one glucose molecule produces 32 net ATP via malate-aspartate shuttle (heart and liver), 30 net ATP via glycerol-3-phosphate shuttle (muscle).

- Anaerobic glycolysis produces only 2 net ATP per glucose molecule.

- ATP hydrolysis can be coupled to energetically unfavorable reactions.

- Arsenic causes glycolysis to produce zero net ATP.

Universal electron acceptors

- Nicotinamides (NAD, NADP from vitamin B_3) and flavin nucleotides (FAD from vitamin B_2).
- NAD is generally used in catabolic processes to carry reducing equivalents away as NADH.
- NADPH is used in anabolic processes (steroid and fatty acid synthesis) as a supply of reducing equivalents.

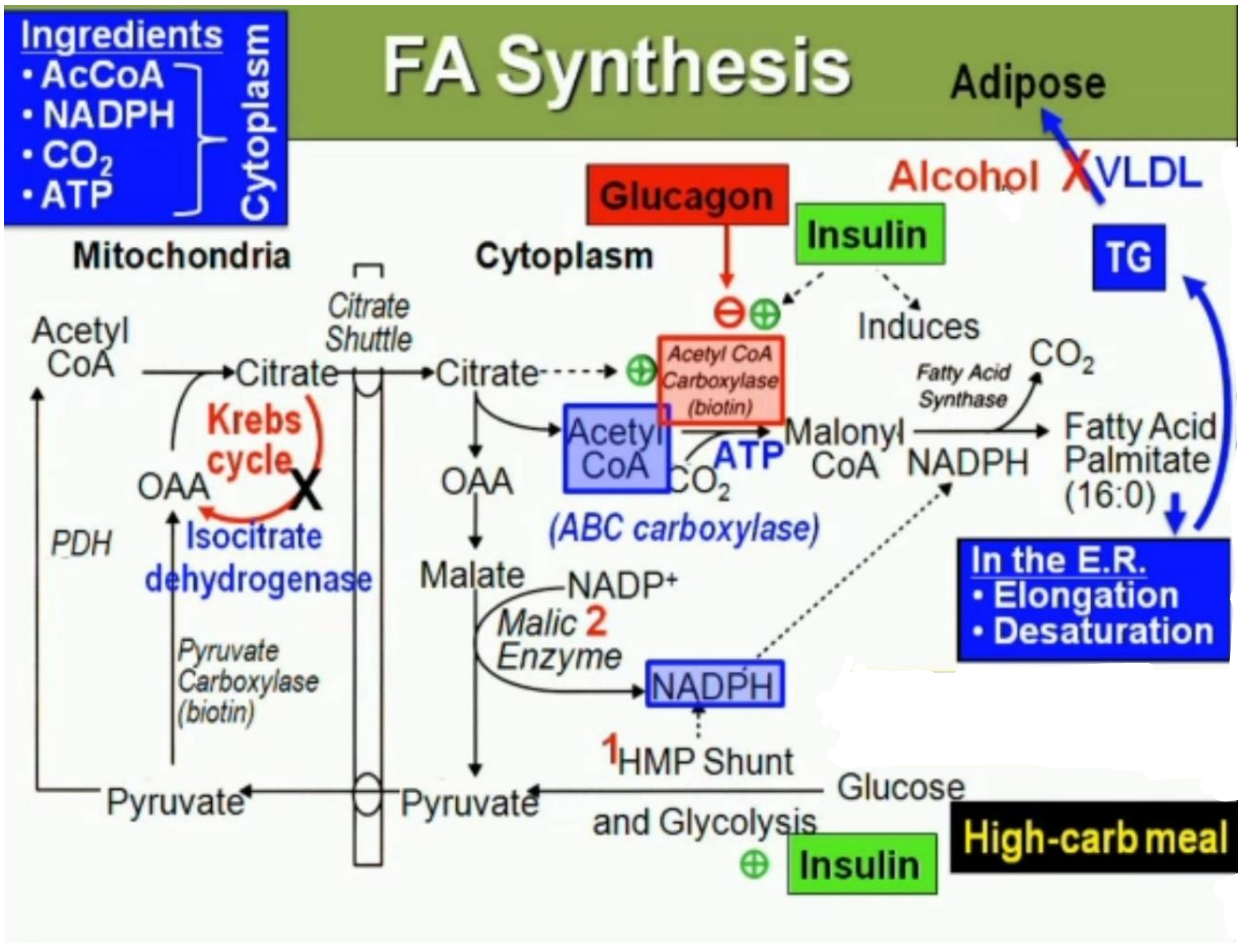
❖ G6P can be metabolized in the following pathways:

A. Conversion to G1P by phosphoglucomutase (G1P can be used for glycogen synthesis).

B. Conversion to glucose by glucose-6-phosphatase (this enzyme is absent in muscles).

C. Conversion to 6-phosphogluconolactone by glucose-6-phosphate dehydrogenase in the first step of the pentose phosphate pathway.

Fatty acid synthesis



- Excess dietary glucose can be converted to fatty acids in the liver and subsequently sent to the adipose tissue for storage.

- Insulin promotes many steps in the conversion of glucose to acetyl CoA in the liver:
  - Glucokinase.
  - PFK-2/PFK-1.
  - Pyruvate dehydrogenase.

- Both of the major enzymes of fatty acid synthesis are also affected by insulin:
  - Acetyl CoA carboxylase.
  - Fatty acid synthase.

- The citrate shuttle transports acetyl CoA groups from the mitochondria to the cytoplasm for fatty acid synthesis. Acetyl CoA combines with oxaloacetate in the mitochondria to form citrate, but rather than continuing in the citric acid cycle, citrate is transported into the cytoplasm. Factors that indirectly promote this process include insulin and high-energy status.

- In the cytoplasm, citrate lyase splits citrate back into acetyl CoA and oxaloacetate. The oxaloacetate returns to the mitochondria to transport additional acetyl CoA.

- This reaction represents an additional source of cytoplasmic NADPH in liver and adipose tissue, supplementing that from the HMP shunt.

A. Acetyl CoA Carboxylase:

- Acetyl CoA is activated in the cytoplasm for incorporation into fatty acids by acetyl CoA carboxylase, the rate-limiting enzyme of fatty acid biosynthesis.

- Acetyl CoA carboxylase requires ATP, Biotin, and CO_2 (ABC Carboxylase).

B. Fatty Acid Synthase:

- Fatty acid synthase is more appropriately called palmitate synthase because palmitate is the only fatty acid that humans can synthesize de novo.

- Although malonyl CoA is the substrate used by fatty acid synthase, only the carbons from the acetyl CoA portion are actually incorporated into the fatty acid produced. Therefore, the fatty acid is derived entirely from acetyl CoA.

- NADPH is required to reduce the acetyl groups added to the fatty acid.

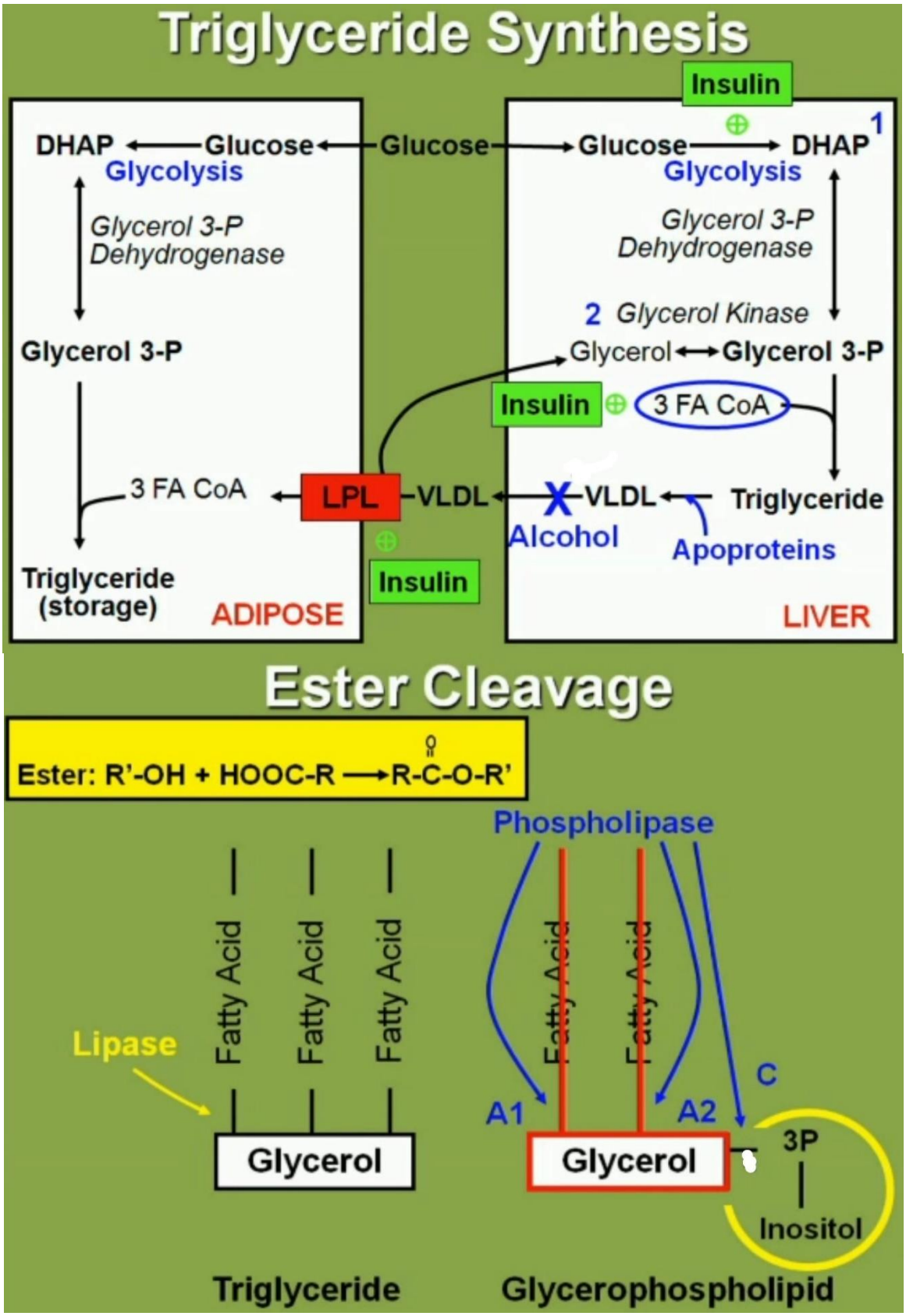
- Eight acetyl CoA groups are required to produce palmitate (16:0).

- Fatty acyl CoA may be elongated and desaturated (to a limited extent in humans) using enzymes associated with the smooth endoplasmic reticulum (SER).

❖ N.B:

- Cytosolic acetyl-CoA carboxylase converts acetyl-CoA to malonyl-CoA during the rate-limiting step of de novo fatty acid synthesis.
- Malonyl-CoA also inhibits the action of mitochondrial carnitine acyltransferase, thereby inhibiting beta-oxidation of newly formed fatty acids.

Triglyceride synthesis



- Triglycerides, the storage form of fatty acids, are formed by attaching 3 fatty acids (as fatty acyl CoA) to glycerol.

- Triglyceride formation from fatty acids and glycerol 3-phosphate occurs primarily in liver and adipose tissue.

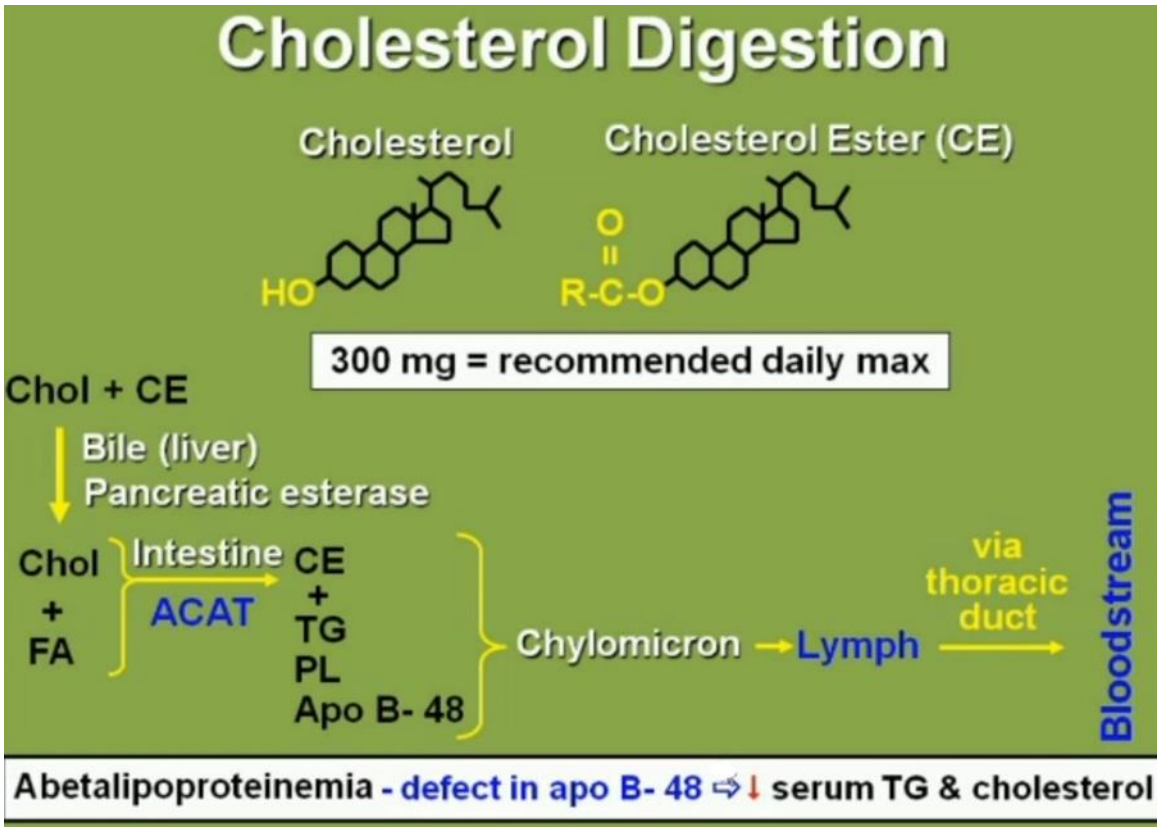
- The liver sends triglycerides to adipose tissue packaged as very low-density lipoproteins (VLDL).

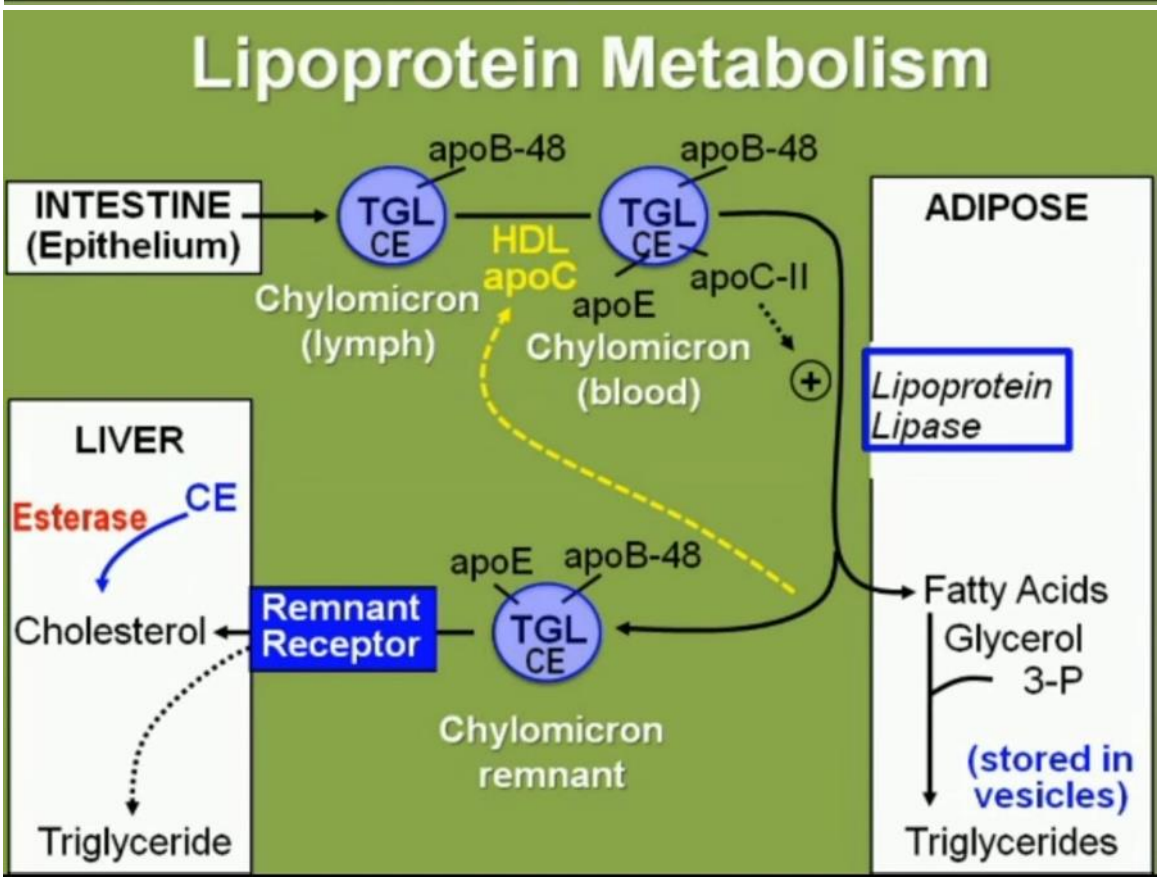
- There are 2 sources of glycerol 3-P for triglyceride synthesis:

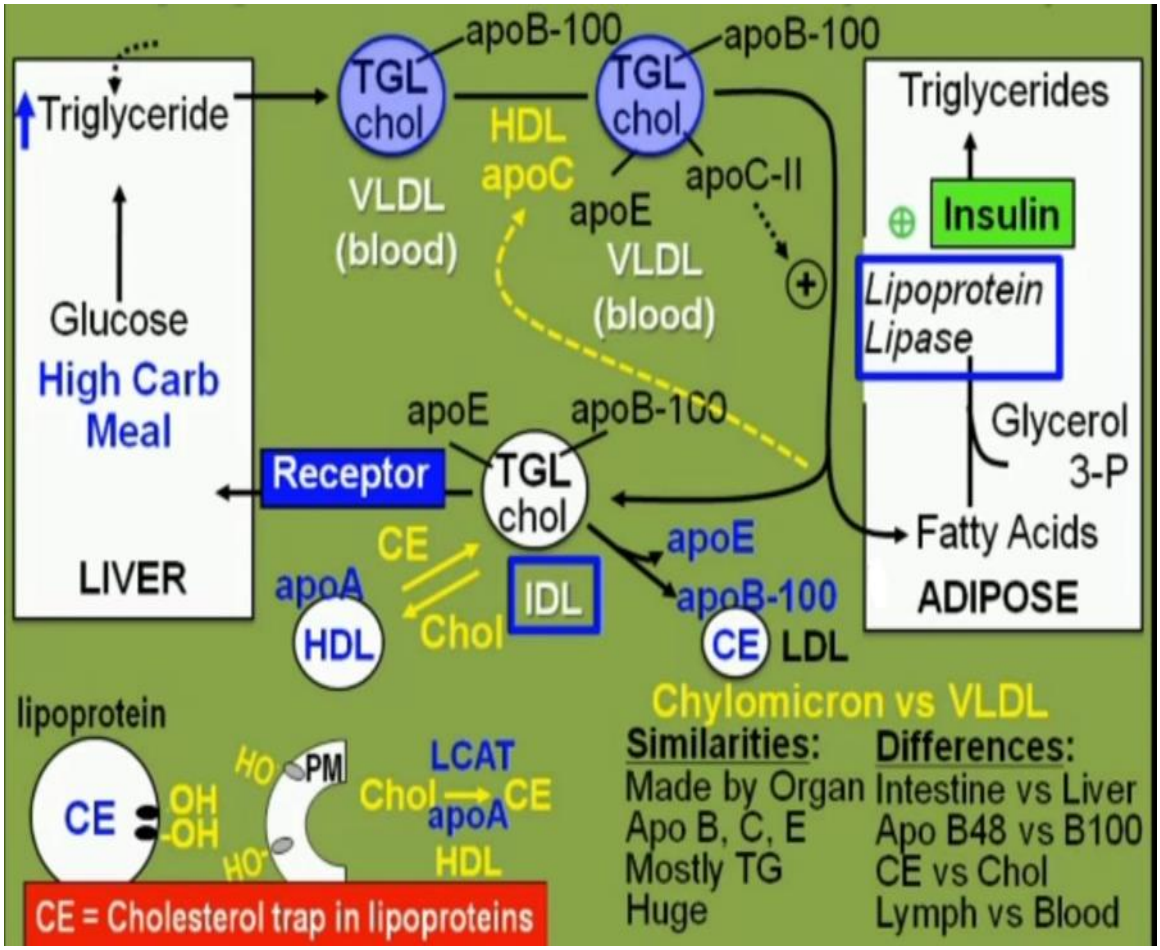
- Reduction of dihydroxyacetone phosphate (DHAP) from glycolysis by glycerol 3-P dehydrogenase, an enzyme in both adipose tissue and liver.

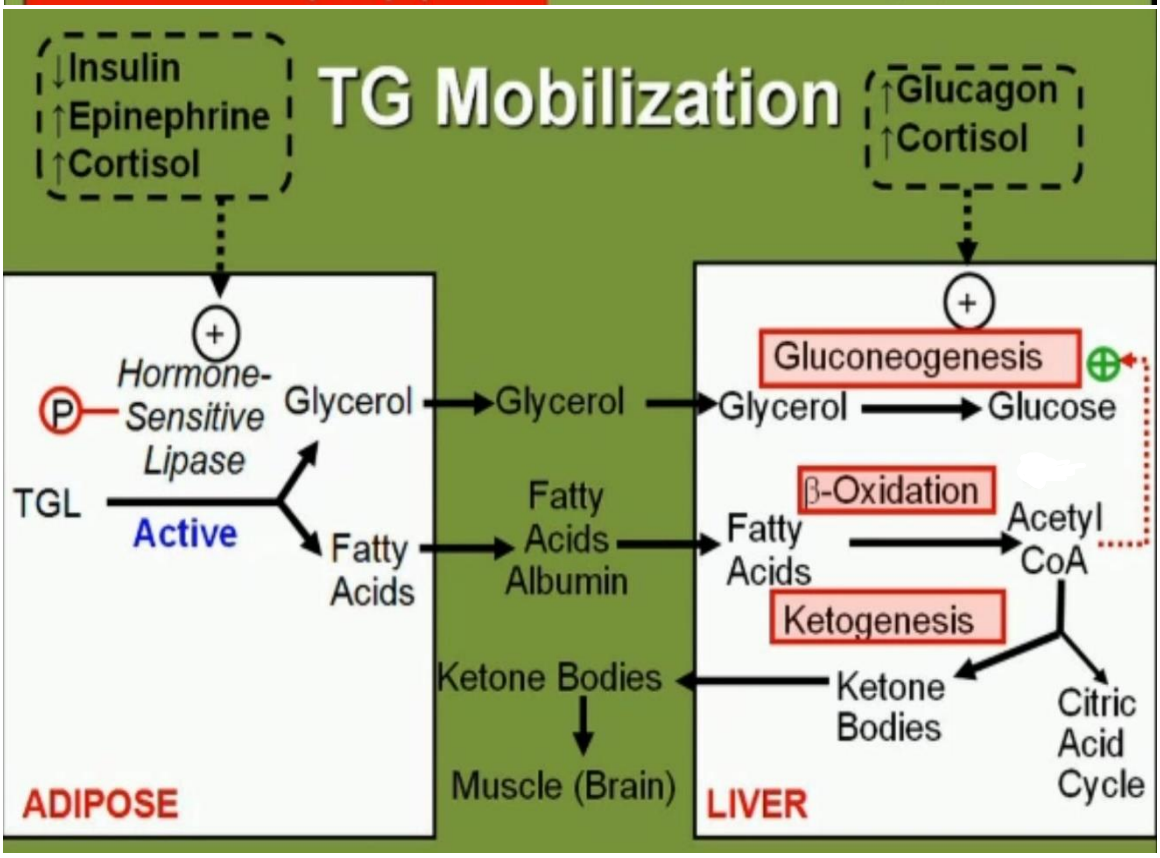
- Phosphorylation of free glycerol by glycerol kinase, an enzyme found in liver but not in adipose tissue. Adipose tissue lacks glycerol kinase and is strictly dependent on glucose uptake to produce DHAP for triglyceride synthesis.

Lipid mobilization

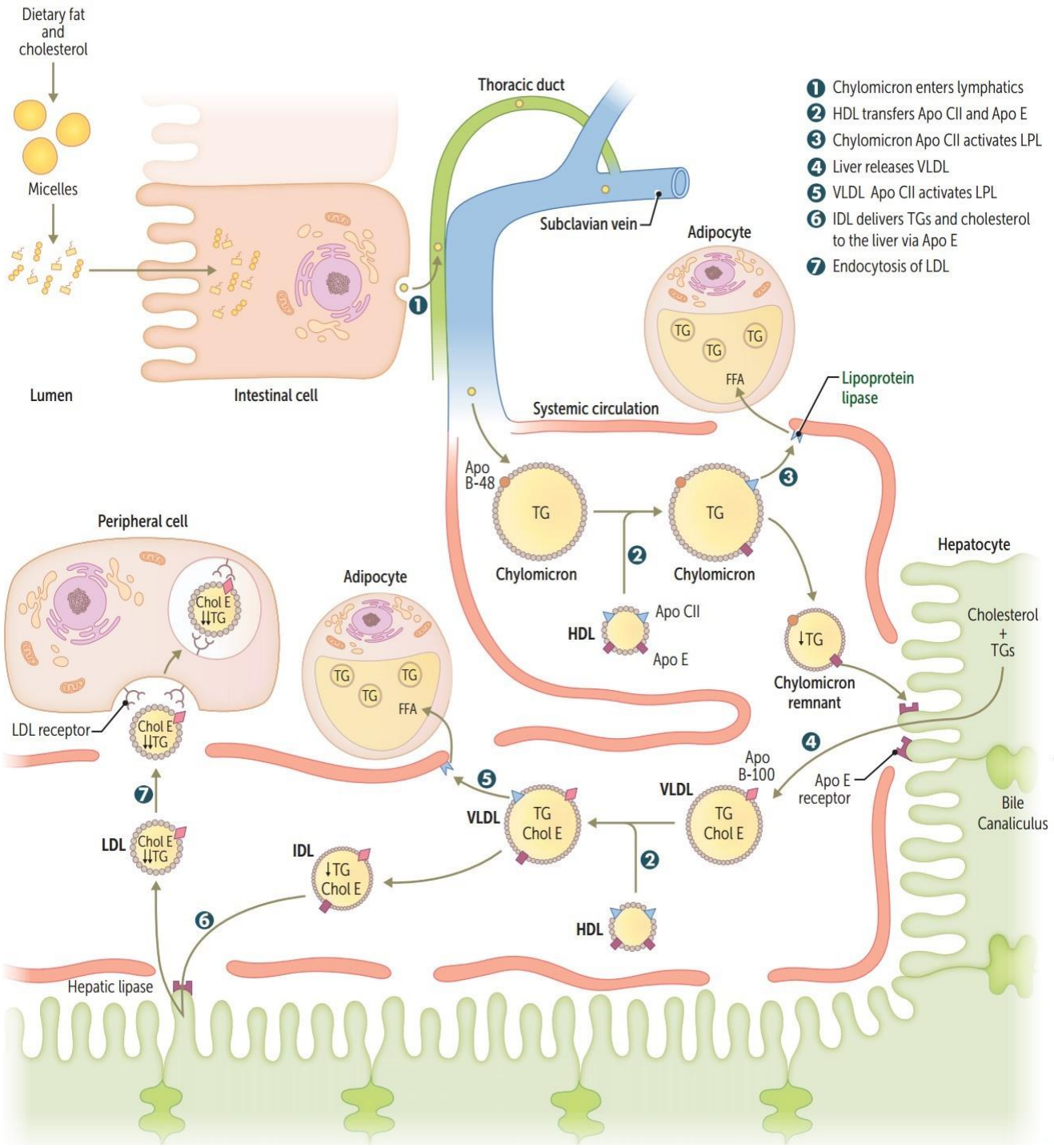


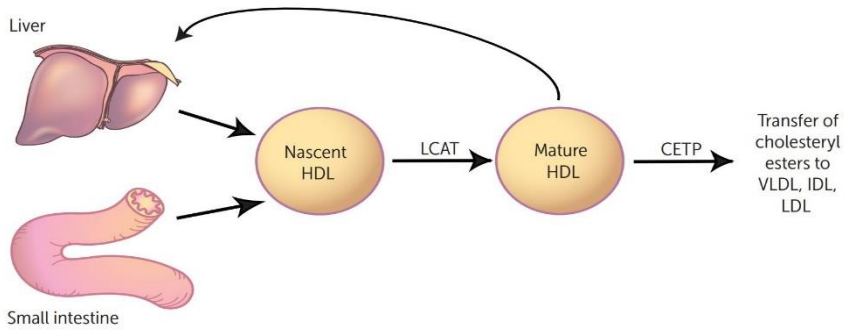



Triglyceride
Glucose
High Carb Meal
LIVER
TGL chol
apoB-100
HDL apoC
apoE
apoC-II
VLDL (blood)
Triglycerides
Insulin
Lipoprotein Lipase
Glycerol 3-P
Fatty Acids
ADIPOSE
Receptor
CE
apoA
HDL
Chol
IDL
apoE
apoB-100
LDL
lipoprotein
PM
LCAT
CE = Cholesterol trap in lipoproteins
Chylomicron vs VLDL
Similarities:
Made by Organ
Apo B, C, E
Mostly TG
Huge
Differences:
Intestine vs Liver
Apo B48 vs B100
CE vs Chol
Lymph vs Blood


TG Mobilization
↓Insulin
↑Epinephrine
↑Cortisol
↑Glucagon
↑Cortisol
Hormone-Sensitive Lipase
Active
TGL
Glycerol
Fatty Acids
Albumin
Gluconeogenesis
Glucose
β-Oxidation
Acetyl CoA
Ketogenesis
Ketone Bodies
Citric Acid Cycle
Muscle (Brain)
ADIPOSE
LIVER

Lipid transport





Key enzymes in lipid transport

- Cholesterol ester transfer protein: mediates transfer of cholesterol esters to other lipoprotein particles.
- Hepatic lipase: Degrades TGs remaining in IDL.
- Hormone-sensitive lipase: Degrades TGs stored in adipocytes.
- Lecithin-cholesterol acyltransferase (LCAT): Catalyzes esterification of 2⁄3 of plasma cholesterol.
- Lipoprotein lipase: Degrades TGs in circulating chylomicrons and VLDLs. Found on vascular endothelial surface.
- Pancreatic lipase: Degrades dietary TGs in small intestine.
- PCSK9: Degrades LDL receptor → ↑ serum LDL. PCSK9 inhibitors → ↑ recycling of LDL receptor → ↓ serum LDL.

❖ N.B:

- Hormone-sensitive lipase is found in adipose tissue, where it functions to drive the breakdown of stored triglycerides into free fatty acids and glycerol.
- During times of starvation, this enzyme provides substrates for hepatic gluconeogenesis and ketone body formation.

Major apolipoproteins

Apolipoprotein	Function	Chylomicron	Chylomicron remnant	VLDL	IDL	LDL	HDL
E	Mediates remnant uptake (Everything Except LDL)	✓	✓	✓	✓		✓
A-I	Activates LCAT						✓
C-II	Lipoprotein lipase Cofactor that Catalyzes Cleavage	✓		✓			✓
B-48	Mediates chylomicron secretion into lymphatics Only on particles originating from the intestines	✓	✓				
B-100	Binds LDL receptor Only on particles originating from the liver			✓	✓	✓	

Lipoprotein functions

- Lipoproteins are composed of varying proportions of TGs, cholesterol, and phospholipids.
- LDL and HDL carry the most cholesterol.
- Cholesterol is needed to maintain cell membrane integrity and synthesize bile acid, steroids, and vitamin D.

A. Chylomicron:

- Secreted by intestinal epithelial cells.
- Chylomicrons leave the lymph and enter the peripheral blood, where the thoracic duct joins the left subclavian vein, thus initially bypassing the liver.
- Chylomicrons are assembled from dietary triglycerides, cholesterol esters, and the 4 lipid-soluble vitamins.
- Delivers dietary TGs to peripheral tissues (adipose tissue).
- Delivers cholesterol to liver in the form of chylomicron remnants, which are mostly depleted of their TGs.

B. VLDL:

- Secreted by liver.
- Delivers hepatic TGs to peripheral tissue (adipose tissue).
- Chylomicrons and VLDL are primarily triglyceride particles, although they each have small quantities of cholesterol esters.
- Lipoprotein lipase hydrolyzes the fatty acids from triglycerides carried by chylomicrons and VLDL and is activated by apoC-II.
- Both chylomicrons and VLDL have apoC-II, apoE, and apoB (apoB-48 on chylomicrons and apoB-100 on VLDL).

C. VLDL remnants (IDL):

- Formed from the degradation of VLDL.
- Delivers TGs and cholesterol to liver.

D. LDL:

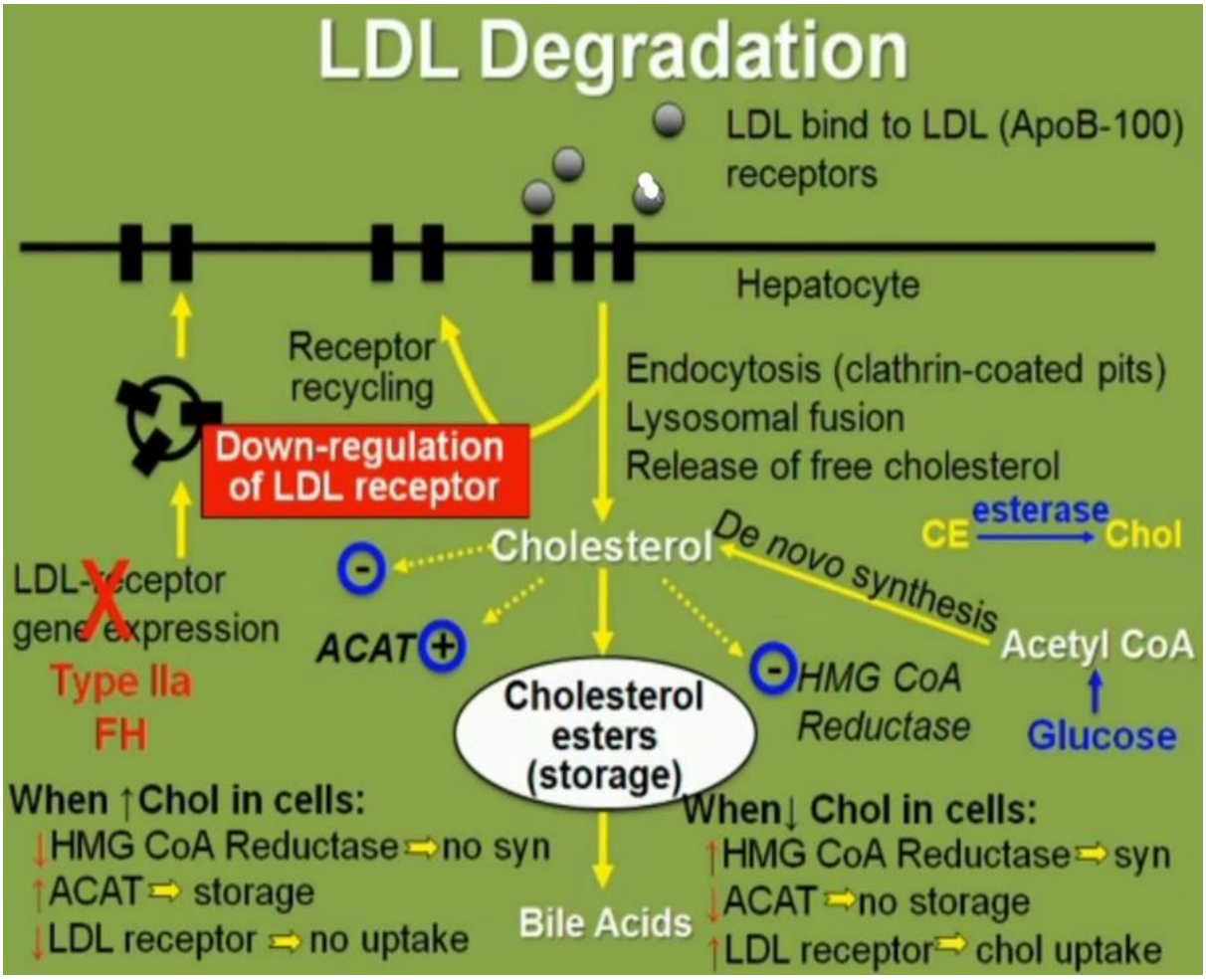
- Delivers hepatic cholesterol to peripheral tissues. LDL is Lousy.
- Formed by hepatic lipase modification of IDL in the liver.
- Taken up by target cells via receptor-mediated endocytosis (LDL Recptors).

E. HDL:

- Secreted from both liver and intestine.
- Mediates reverse cholesterol transport from periphery to liver. HDL is Healthy.
- Acts as a repository for apolipoproteins C and E (which are needed for chylomicron and VLDL metabolism).
- It contains apoA-1 used for cholesterol recovery from fatty streaks in the blood vessels.
- Lecithin-cholesterol acyltransferase (LCAT) is an enzyme in the blood that is activated by apoA-1 on HDL.
- LCAT adds a fatty acid to cholesterol, producing cholesterol esters, which dissolve in the core of the HDL.

- The normal role of LDL is to deliver cholesterol to tissues for biosynthesis. When a cell is repairing membrane or dividing, the cholesterol is required for membrane synthesis. Bile acids and salts are made from cholesterol in the liver, and many other tissues require some cholesterol for steroid synthesis. About 80% of LDL are picked up by hepatocytes, the remainder by peripheral tissues.
- ApoB-100 is the only apoprotein on LDL, and endocytosis of LDL is mediated by apoB-100 receptors (LDL receptors) clustered in areas of cell membranes lined with the protein clathrin.
- The liver has multiple pathways for acquiring cholesterol, including:

- De novo synthesis.
- Endocytosis of LDL.
- Endocytosis of chylomicron remnants with residual dietary cholesterol.


LDL Degradation
LDL bind to LDL (ApoB-100) receptors
Hepatocyte
Receptor recycling
Endocytosis (clathrin-coated pits)
Lysosomal fusion
Release of free cholesterol
Down-regulation of LDL receptor
CE esterase Chol
Cholesterol
De novo synthesis
LDL-receptor gene expression
Type IIa FH
ACAT
HMG CoA Reductase
Acetyl CoA
Glucose
Cholesterol esters (storage)
When ↑Chol in cells:
↓HMG CoA Reductase ➡ no syn
↑ACAT ➡ storage
↓LDL receptor ➡ no uptake
When↓ Chol in cells:
↑HMG CoA Reductase ➡ syn
↓ACAT ➡ no storage
↑LDL receptor ➡ chol uptake
Bile Acids

Abetalipoproteinemia

- Inheritance: Autosomal recessive.

- Pathogenesis:

- Deficiency in ApoB-48, ApoB-100.

- Abetalipoproteinemia is an inherited inability to synthesize apolipoprotein B, an important component of chylomicrons and very low-density lipoprotein.

- ApoB-100 is found in VLDL, and apoB-48 is present in chylomicrons.

- During the synthesis of apoB-containing lipoproteins, microsomal triglyceride transfer protein (MTP) functions as a chaperone protein necessary for proper folding of apoB and also participates in the transfer of lipids to newly formed chylomicrons and VLDL particles.

- Clinical:

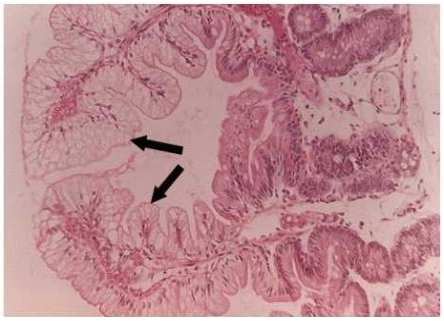
- It manifests during the first year of life with symptoms of malabsorption (abdominal distention, foul-smelling stool), failure to thrive.

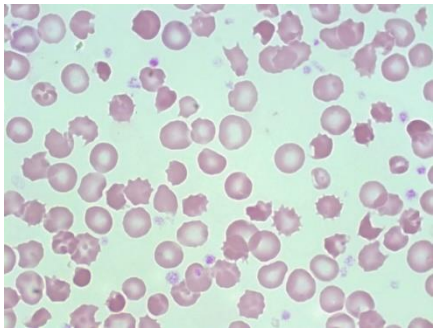
- Poor lipid absorption causes deficiency of fat-soluble vitamins (particularly vitamin E) and essential fatty acids.

- This results in red blood cells with abnormal membranes and thorny projections called acanthocytes as well as multiple neurologic abnormalities (spinocerebellar degeneration, progressive ataxia, retinitis pigmentosa) due to vitamin E and A deficiency.

- Lipids absorbed by the small intestine cannot be transported into the blood and accumulate in the intestinal epithelium, resulting in enterocytes with clear or foamy cytoplasm. The slide below shows normal intestinal mucosal architecture, but the enterocytes contain clear or foamy cytoplasm (arrows) which is more prominent at the tips of the villi.

- Labs: Laboratory studies show very low plasma triglyceride and cholesterol levels, and chylomicrons, VLDLs, and apoB are entirely absent from the blood.





- Treatment: Restriction of long-chain fatty acids, large doses of oral vitamin E.

Familial dyslipidemias

A. Hyperchylomicronemia:

- Inheritance: AR.

- Pathogenesis:

- Lipoprotein lipase or apolipoprotein C-II deficiency.

- Lipoprotein lipase deficiency is a rare disorder that results in increased concentrations of serum chylomicrons.

- Dietary lipids are transported to the peripheral tissues as chylomicrons, at which point they are hydrolyzed by lipoprotein lipase, releasing triglycerides.

- The body is unable to clear dietary lipid loads due to the defective hydrolysis of triglycerides in chylomicrons.

- ↑ blood level: Chylomicrons, TG, cholesterol.

- Clinical:

- Due to deposition of excess triglyceride in the blood in several tissues, including liver, skin, and pancreas.

- Patients present in childhood with marked hyperlipidemia, pancreatitis (abdominal pain), eruptive skin xanthomas (small yellowish papules surrounded by erythema that occur mainly on extensor surfaces of extremities) and hepatosplenomegaly.

- Abdominal pain due to acute pancreatitis is the most likely presentation for hyperchylomicronemia.

- The risk of pancreatitis is significantly increased with serum triglyceride concentrations above 1000 mg/dl.

- Patients with this disorder are not usually at increased risk for premature coronary artery disease.

- Skin xanthomas may be present in hypertriglyceridemia, but tubular/tendon xanthomas and xanthelasmas are present with hypercholesterolemia (high LDL).

- Chylomicronemia produces a milky turbidity in the serum or plasma.

B. Familial hypercholesterolemia:

- Inheritance: one of the most common autosomal dominant disorders.

- Pathogenesis:

- Absent or defective LDL receptors, or defective ApoB-100.

- It is the result of heterozygous or homozygous LDL receptor gene mutations, which cause hepatocyte under-expression of functional LDL receptors.

- ↑ blood level:

- IIa: LDL, cholesterol.

- IIb: LDL, cholesterol, VLDL.

- Clinical:

- Heterozygotes with one mutant LDL receptor gene have a 2- to 3-fold elevation of plasma cholesterol from birth, due to reduced hepatic LDL uptake.

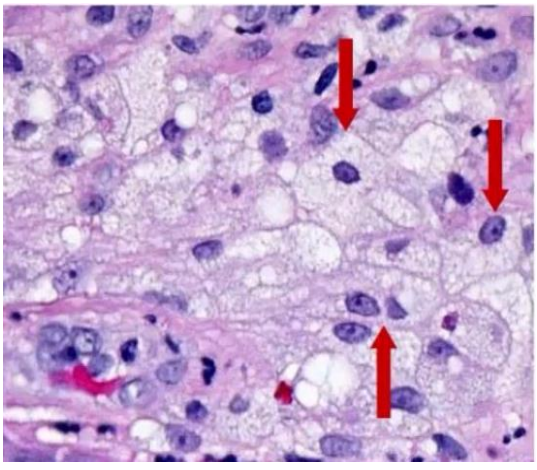
- Homozygotes may have a 5- to 6-fold elevation.

- Heterozygotes (1:500) have cholesterol ≈ 300mg/dL; homozygotes (very rare) have cholesterol ≈ 700+ mg/dL.

- Accelerated atherosclerosis (may have MI before age 20), tendon (Achilles) xanthomas, Xanthelasma (a type of xanthoma usually found on the medial eyelids), and corneal arcus.

- Xanthelasmas are dermal accumulations of benign-appearing macrophages with abundant, finely vacuolated (foamy) cytoplasm containing cholesterol (free and esterified), phospholipids, and triglycerides on the medial eyelids.

- Histologically, xanthomas are composed of benign macrophages packed with finely vacuolated, "foamy cytoplasm". This cytoplasm contains high levels of cholesterol, phospholipids, and triglycerides.



C. Dysbetalipoproteinemia:

- Inheritance: AR.

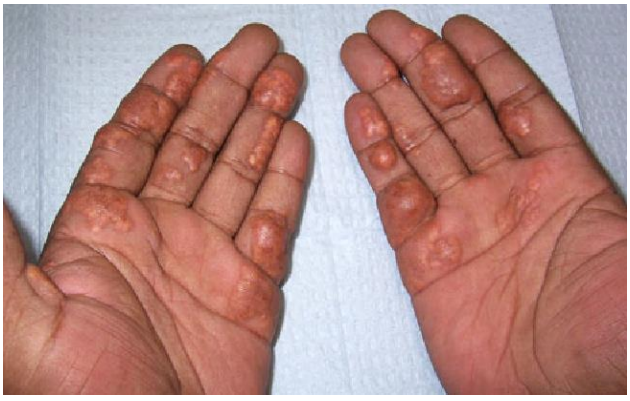
- Pathogenesis:

- The primary defects in familial dysbetalipoproteinemia are in ApoE-3 and ApoE-4, apolipoproteins found on chylomicrons and VLDL that are responsible for binding hepatic apolipoprotein receptors.

- Without ApoE3 and ApoE4, the liver cannot efficiently remove chylomicrons and VLDL remnants from the circulation, causing their accumulation in the serum and resultant elevations in cholesterol and triglyceride levels.

- ↑ blood level: Chylomicrons, VLDL.

- Clinical: Premature atherosclerosis, tubero-eruptive xanthomas, palmar xanthomas.



D. Hypertriglyceridemia:

- Inheritance: AD.

- Pathogenesis: Hepatic overproduction of VLDL.

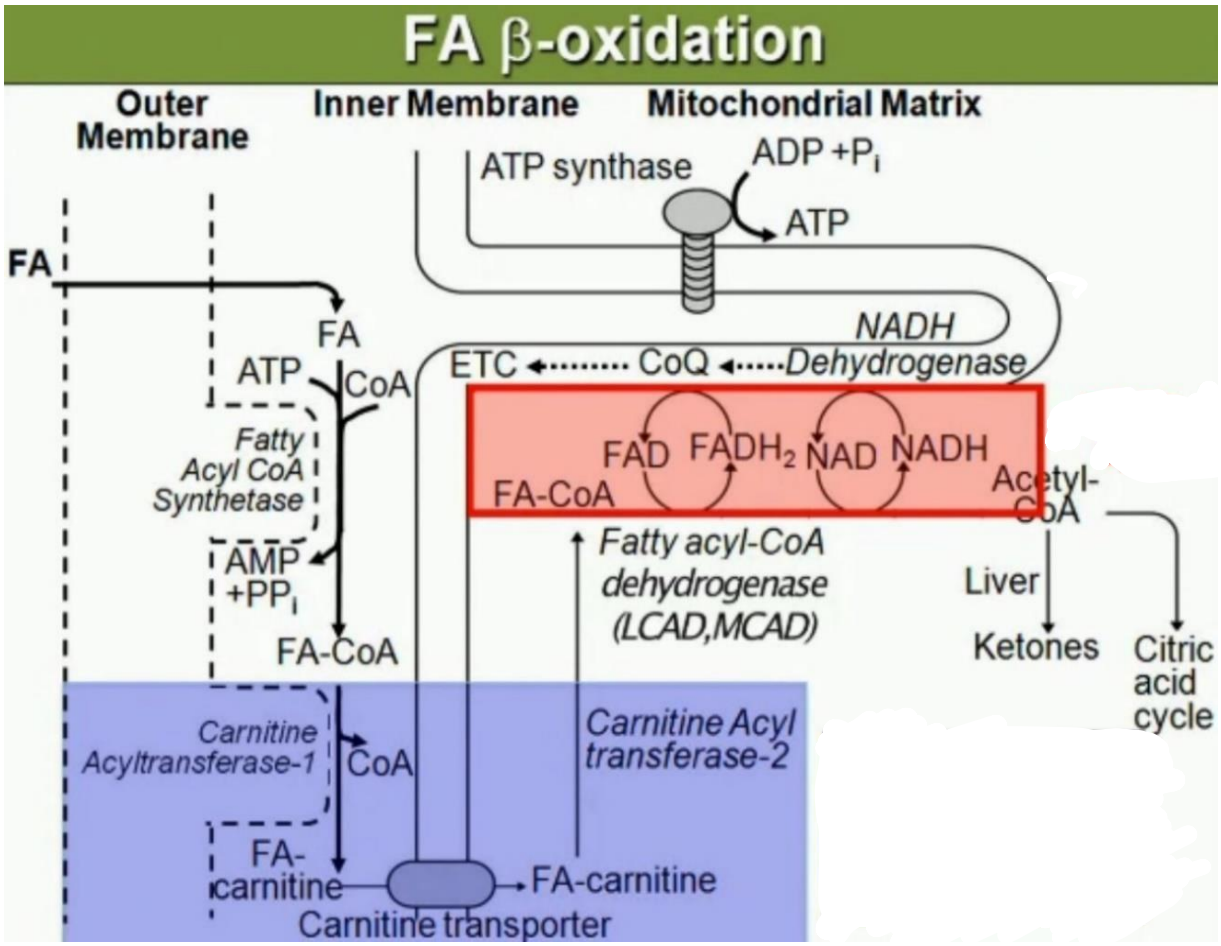
- ↑ blood level: VLDL, TG.

- Clinical:

- Hypertriglyceridemia (> 1000 mg/dL) can cause acute pancreatitis.

- Related to insulin resistance.

Fatty acid oxidation



- Fatty acids are oxidized in several tissues, including liver, muscle, and adipose tissue, by the pathway of β-oxidation.

- This process occurs within mitochondria.

- Neither erythrocytes nor brain can use fatty acids and continue to rely on glucose during normal periods of fasting. Erythrocytes lack mitochondria, and fatty acids do not cross the blood-brain barrier efficiently.

- <u>Fatty Acid Entry into Mitochondria:</u>

- Long-chain fatty acids must be activated and transported into the mitochondria.

- Fatty acyl-CoA synthetase, on the outer mitochondrial membrane, activates the fatty acids by attaching CoA.

- To transport a fatty acyl-CoA from the cytosol into mitochondria, the cell must first form the fatty acyl-carnitine intermediate. This reaction is catalyzed by carnitine acyltransferase I on the outer surface of the inner mitochondrial membrane.

- After the fatty acyl-carnitine molecule is transported into the mitochondria, carnitine acyltransferase II on the inner surface of the inner mitochondrial membrane catalyzes the regeneration of the fatty acyl-CoA molecule and free carnitine.

- This series of reactions is known as the carnitine shuttle.

- β-oxidation of fatty acids involves the sequential removal of two-carbon units from the fatty acid chain by oxidation at the β-carbon position.

- <u>β-Oxidation in Mitochondria:</u>

- β-oxidation reverses the process of fatty acid synthesis by oxidizing (rather than reducing) and releasing (rather than linking) units of acetyl-CoA.

- Each round of fatty acid β-oxidation produces 1 NADH, 1 FADH2, and 1 acetyl-CoA. Acetyl-CoA, the end product of each round of β-oxidation, is further oxidized to CO_2 in the tricarboxylic acid (TCA) cycle, generating 3 NADH, 1 $FADH_2$, and 1 GTP.

- In muscle and adipose tissue, the acetyl-CoA enters the citric acid cycle.

- In liver, the ATP may be used for gluconeogenesis, and the acetyl-CoA (which cannot be converted to glucose) stimulates gluconeogenesis by activating pyruvate carboxylase.

- Much of the acetyl-CoA is used to synthesize ketone bodies (essentially 2 acetyl-CoA groups linked together) that are released into the blood for other tissues.

❖ N.B:

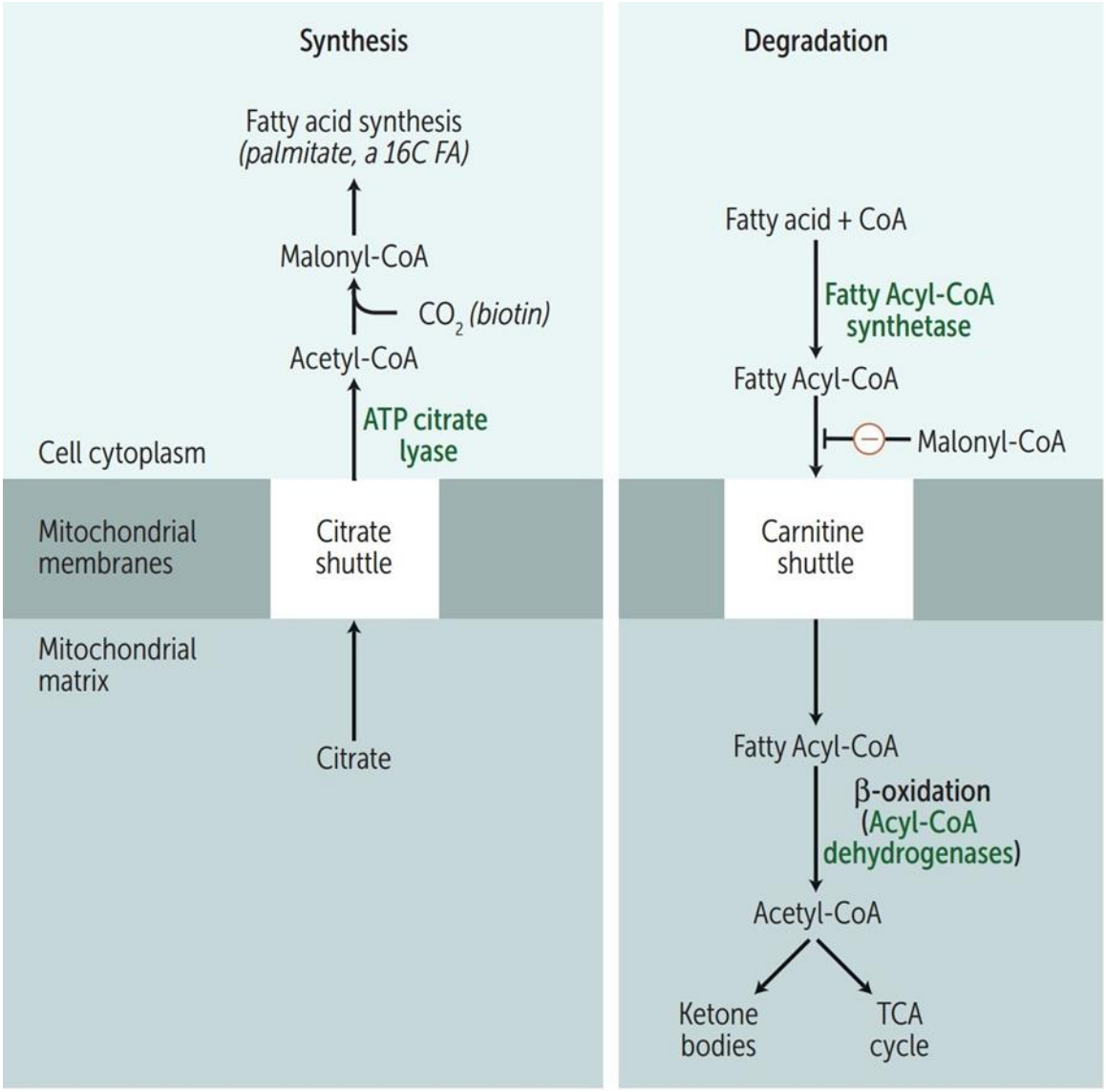
- After a period of starvation lasting 16-24 hours, peripheral tissues shift to rely on lipid-derived fuels such as free fatty acids and ketone bodies instead of glucose for energy production.
- This spares glucose use for tissues that always require some amount of glucose to function, such as the brain. It also limits the need for gluconeogenesis, helping to conserve the body's protein stores.
- Starvation induces the breakdown of triglycerides stored within adipocytes, releasing free fatty acids that can be metabolized to ketone bodies via β-oxidation within mitochondria in the liver. Oxidation of fatty acids yields significantly more energy per carbon atom than carbohydrate oxidation.

Systemic primary carnitine deficiency

- The condition is caused by a defect in the protein responsible for carnitine transport across the mitochondrial membrane.
- Without sufficient carnitine, fatty acids cannot be transported from the cytoplasm into the mitochondria as acyl-carnitine (carnitine shuttle) → toxic accumulation of LCFAs in the cytosol.
- The mitochondria therefore cannot β-oxidize the fatty acids into acetyl CoA, the carbon substrate for the citric acid cycle.
- As a result, cardiac and skeletal myocytes cannot generate ATP from fatty acids (leading to muscle weakness, cardiomyopathy) and the liver is unable to synthesize glucose (gluconeogenesis) or ketone bodies (manifests as hypoketotic hypoglycemia).

Medium chain acyl-CoA dehydrogenase (MCAD) deficiency

- ↓ ability to break down fatty acids into acetyl-CoA → accumulation of fatty acyl carnitines in the blood with hypoketotic hypoglycemia.
- Hypoketotic hypoglycemia should be strongly associated with a block in hepatic β-oxidation.
- Decreased acetyl-CoA lowers pyruvate carboxylase activity and also limits ketogenesis.
- During fasting, hypoglycemia can become profound due to lack of ATP to support gluconeogenesis.
- Causes vomiting, lethargy, seizures, coma, liver dysfunction, hyperammonemia. Can lead to sudden death in infants or children.
- Treatment: Avoid fasting with frequent feeding, high-carbohydrate, low-fat diet. Most patients lead reasonable lives if they take frequent carbohydrate meals to avoid periods of hypoglycemia.



- Propionic Acid Pathway:

- Fatty acids with an odd number of carbon atoms are oxidized by β-oxidation identically to even-carbon fatty acids.

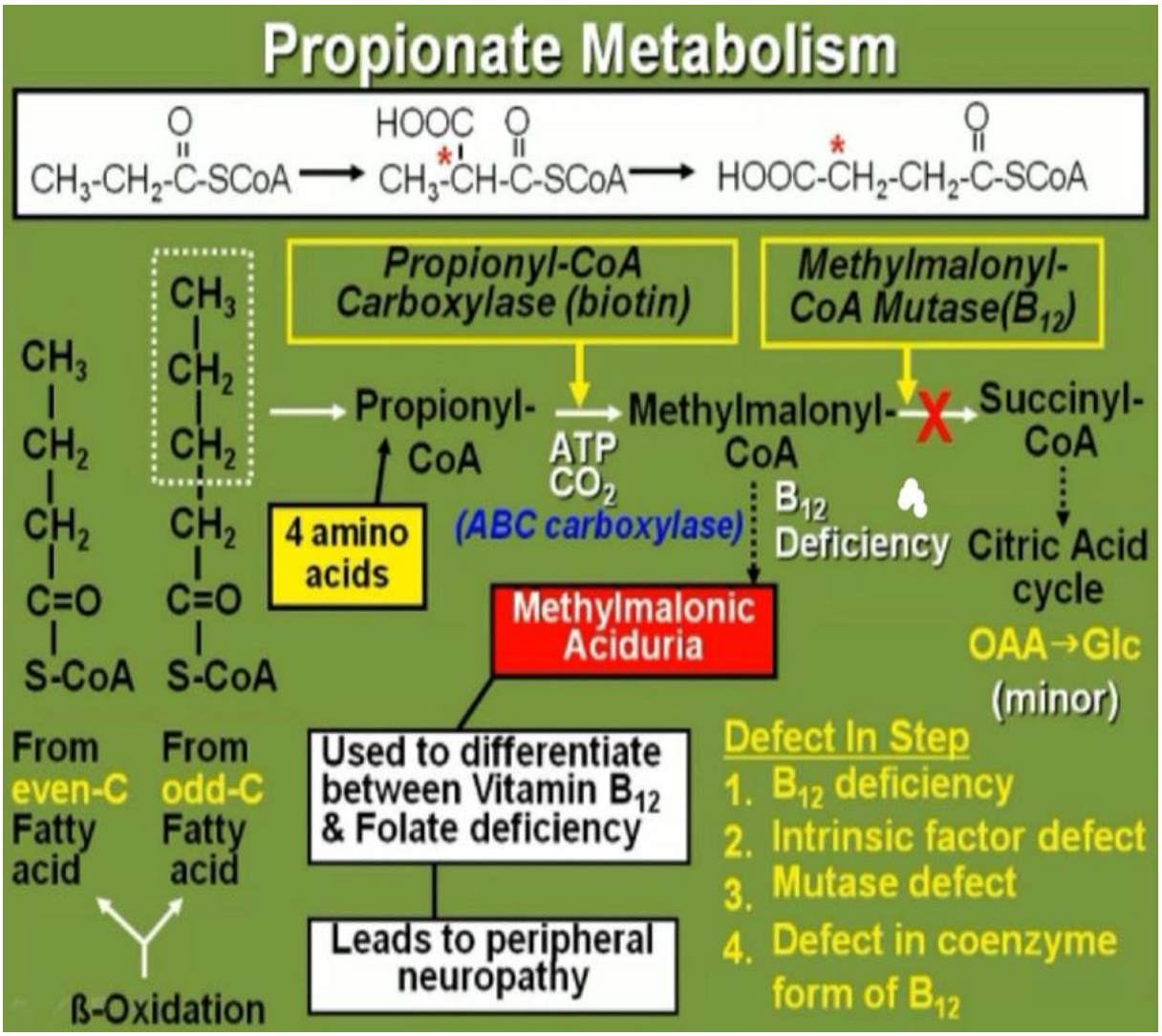
- The difference results only from the final cycle, in which even-carbon fatty acids yield 2 acetyl-CoA (from the 4-carbon fragment remaining) but odd-carbon fatty acids yield one acetyl-CoA and one propionyl-CoA (from the 5-carbon fragment remaining).

- Propionyl-CoA is converted to succinyl-CoA, a citric acid cycle intermediate, in the 2-step propionic acid pathway.

- Because this extra succinyl-CoA can form malate and enter the cytoplasm and gluconeogenesis, odd-carbon fatty acids represent an exception to the rule that fatty acids cannot be converted to glucose in humans.

- The propionic acid pathway includes 2 important enzymes, both in the mitochondria:
  - Propionyl-CoA carboxylase requires ATP, biotin, CO_2 (ABC carboxylase).
  - Methylmalonyl-CoA mutase requires vitamin B12.

  - In a patient with megaloblastic anemia, it is important to determine the underlying cause because B12 deficiency, if not corrected, produces a peripheral neuropathy owing to aberrant fatty acid incorporation into the myelin sheets associated with inadequate methylmalonyl-CoA mutase activity. Excretion of methylmalonic acid indicates a vitamin B12 deficiency rather than folate.



Peroxisomal diseases

- Peroxisome are Membrane-enclosed organelle involved in:

- β-oxidation of very-long-chain fatty acids (VLCFA).
- α-oxidation (strictly peroxisomal process).
- Catabolism of branched-chain fatty acids, amino acids, and ethanol.
- Synthesis of cholesterol, bile acids, and plasmalogens (important membrane phospholipid, especially in white matter of brain).

- Peroxisomal diseases are rare inborn errors of metabolism where peroxisomes are either absent or nonfunctional.

- These fatty acids build up in various tissues. Accumulation in neuronal cell membranes leads to neurologic defects due to improper neuronal migration, myelination, and degeneration.

A. Zellweger syndrome:

- Autosomal recessive disorder of peroxisome biogenesis due to mutated PEX genes.

- In this condition, infants are unable to properly form myelin in the CNS.

- Symptoms of this disease include craniofacial abnormalities (widened sutures, large anterior fontanelles), generalized hypotonia and seizures as well as hepatomegaly, mental retardation, and early death within months of initial presentation.

B. Refsum disease:

- Results from a defect in peroxisomal alpha oxidation and leads to neurologic disturbances in response to accumulation of phytanic acid within the body.

- Scaly skin, ataxia, cataracts/night blindness, shortening of 4th toe, epiphyseal dysplasia.

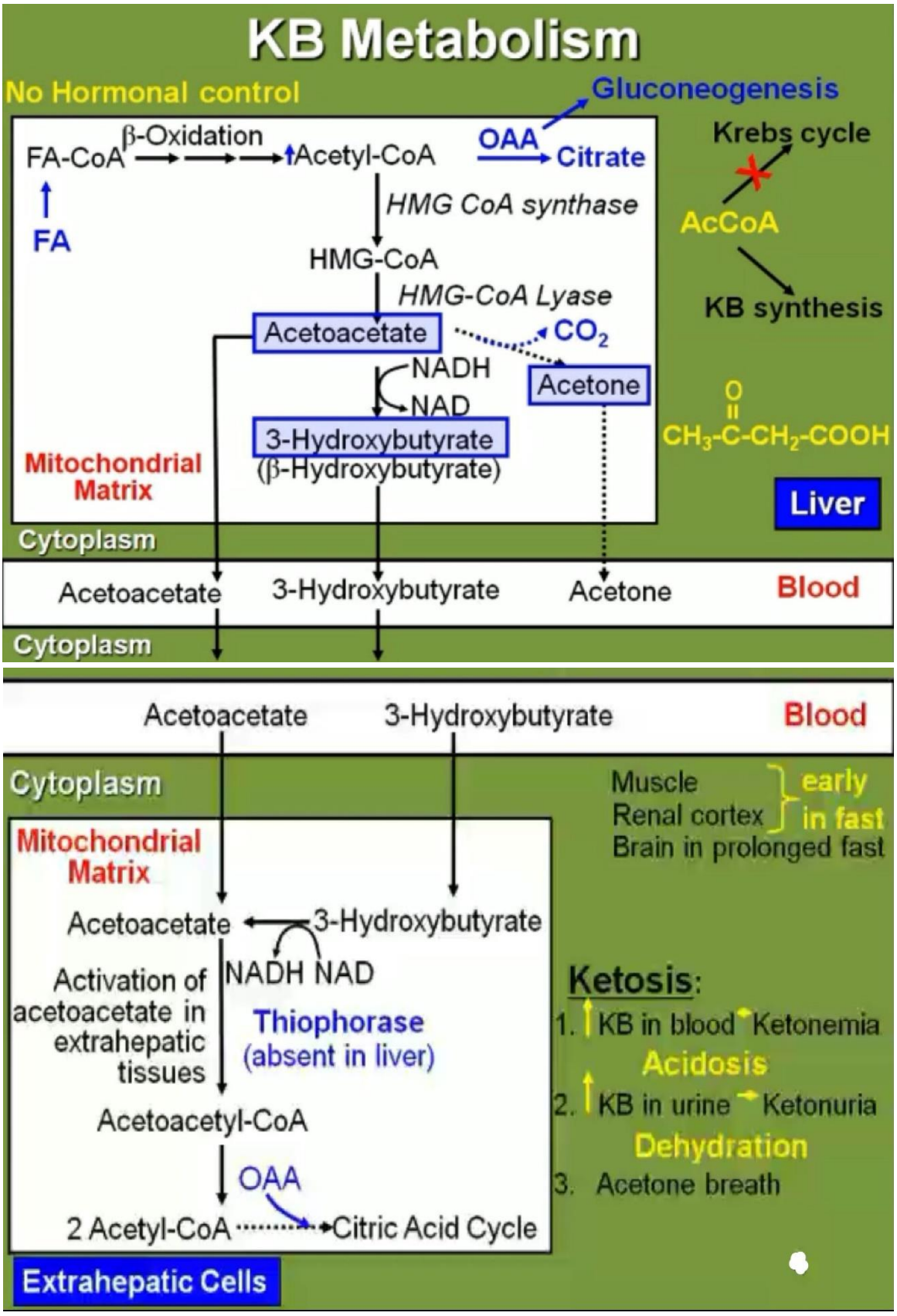
- Treatment of this disease is by strict avoidance of chlorophyll in the diet, plasmapheresis.

C. Adrenoleukodystrophy:

- X-linked recessive disorder of β-oxidation due to mutation in ABCD1 gene → VLCFA buildup in adrenal glands, white (leuko) matter of brain, testes.

- Progressive disease that can lead to adrenal gland crisis, coma, and death.

Ketone bodies metabolism



- Ketone bodies: acetone, acetoacetate, β-hydroxybutyrate.

- Breath smells like acetone (fruity odor).

- Urine test for ketones can detect acetoacetate, but not β-hydroxybutyrate.

- RBCs cannot utilize ketones; they strictly use glucose.

- Ketogenesis:

- Ketogenesis occurs in mitochondria of hepatocytes when excess acetyl-CoA accumulates in the fasting state.

- HMG-CoA synthase forms HMG-CoA, and HMG-CoA lyase breaks HMG-CoA into acetoacetate, which can subsequently be reduced to β-hydroxybutyrate.

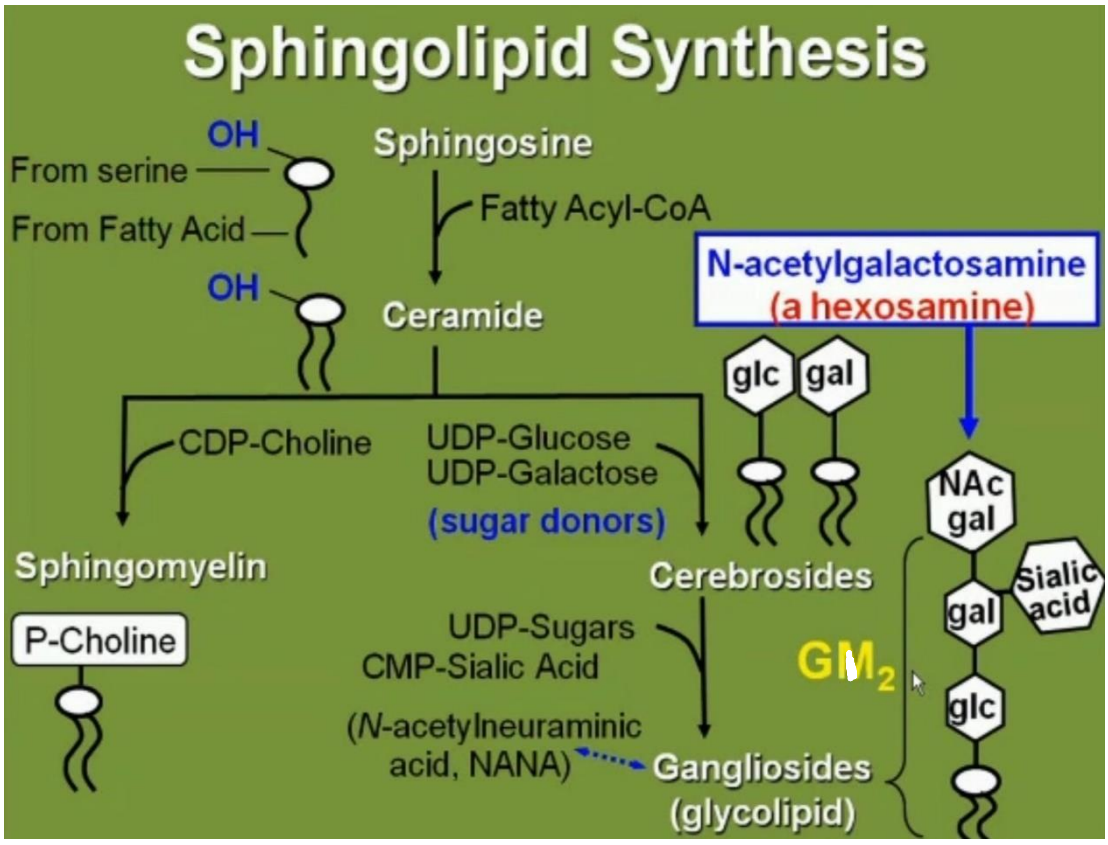
- Acetone is a minor side product formed nonenzymatically but is not used as a fuel in tissues. It does, however, impart a strong odor (sweet or fruity) to the breath, which is almost diagnostic for ketoacidosis.

- Ketogenolysis:

- Acetoacetate picked up from the blood is activated in the mitochondria by succinyl-CoA acetoacetyl-CoA transferase (common name thiophorase), an enzyme present only in extrahepatic tissues; β-hydroxybutyrate is first oxidized to acetoacetate.

- Because the liver lacks this enzyme, it cannot metabolize the ketone bodies.

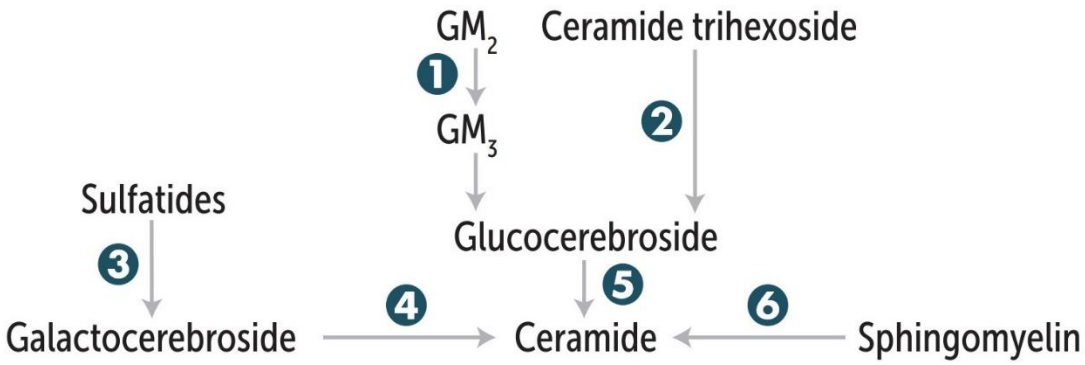
Sphingolipids



- Sphingolipids are important constituents of cell membranes. They have a hydrophilic region and 2 fatty acid-derived hydrophobic tails.

- The various classes of sphingolipids differ primarily in the nature of the hydrophilic region.

- Sphingolipids released when membrane is degraded are digested in endosomes after fusion with lysosomes. Lysosomes contain many enzymes, each of which removes specific groups from individual sphingolipids. Genetic deficiencies of many of these enzymes are known, and the diseases share some of the characteristics of I-cell disease.



Lysosomal storage diseases

- Each is caused by a deficiency in one of the many lysosomal enzymes.
- Results in an accumulation of abnormal metabolic products.
- ↑ incidence of Tay-Sachs, Niemann-Pick, and some forms of Gaucher disease in Ashkenazi Jews due to loss of genetic variability within a group that historically conceived within their own community.

Sphingolipidoses

A. Tay-Sachs disease:

- Inheritance:

- AR.
- It is commonly seen in the Ashkenazi Jewish population.

- Deficient enzyme: HeXosaminidase A ("TAy-SaX").
- Accumulated substrate: Accumulation of the cell membrane glycolipid GM2 ganglioside within cell lysosomes.
- Findings:

- A Progressive neurodegeneration, developmental delay, "cherry-red" spot on macula, lysosomes with onion skin (lamellar lipid rings on electron microscopy), no hepatosplenomegaly (vs Niemann-Pick).
- The center of the fovea (blue arrow) appears bright red (cherry-red macula spot) as it is surrounded by white macula appearing as a halo. The halo results from a loss of retinal transparency due to ganglioside buildup in ganglion cells. The center of the fovea lacks ganglion cells, so the underlying choroid transmits its red color.
- Infants also develop macrocephaly due to accumulation of glycolipid material in the brain.
- Patients eventually develop seizures, blindness, and spasticity.
- Life expectancy is 2-5 years.

B. Niemann-Pick disease:

- Inheritance: AR.

- Deficient enzyme: Sphingomyelinase.

- Accumulated substrate: Sphingomyelin.

- Findings:

- Progressive neurodegeneration (Progressive sphingomyelin accumulation in the central nervous system).

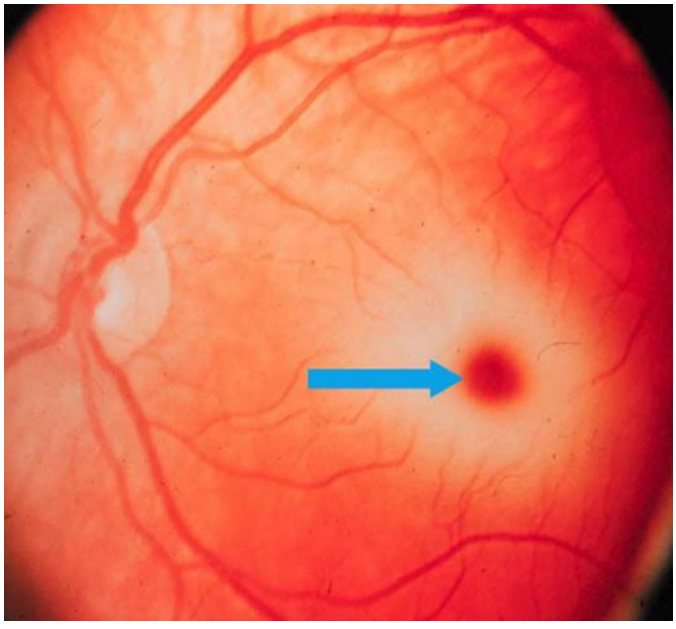
- Following a period of normal development, infants fail to attain new skills and lose previously acquired milestones (sitting with support, head control, social smile).

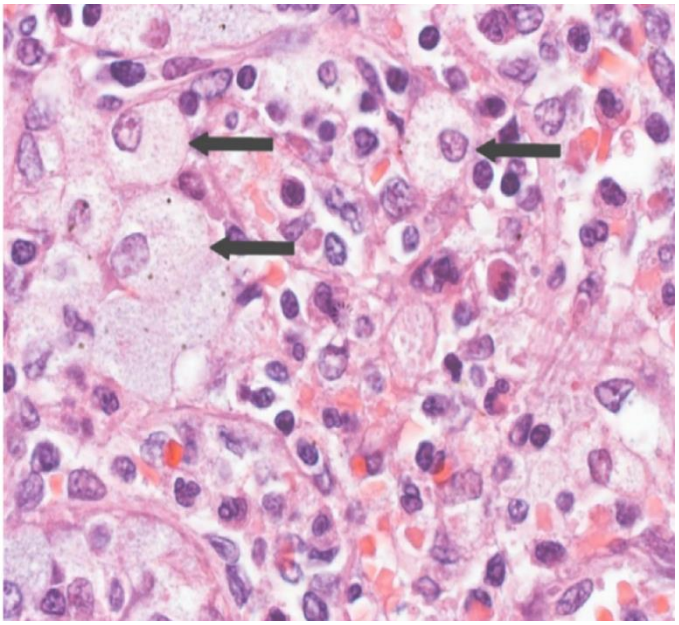
- Sphingomyelin accumulates within phagocytes producing characteristic "foamy histiocytes" → These foamy-appearing, sphingomyelin-laden histiocytes accumulate in the liver and spleen causing massive hepatosplenomegaly.

- Sphingomyelin deposition in the retina causes blindness as well. A cherry-red macular spot, similar to that seen in Tay-Sachs disease, is also often found.

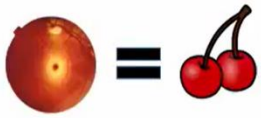
- Death usually occurs before age three.

- No man picks (Niemann-Pick) his nose with his sphinger (sphingomyelinase).





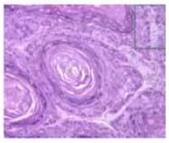
Cherry Red Macula



NO Hepatosplenomegaly

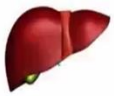
Tay Sachs

- Neurodegeneration, developmental delay
- ONION SKINNING



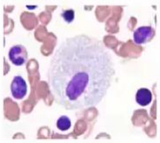
- Absent Hexosaminidase → accumulation of Ganglioside GM2

Hepatosplenomegaly
"palpable abdominal mass"



Niemann Pick

- Neurodegeneration
- FOAM cells



- "No man **Picks** his nose with his **sphinger**" = Sphingomyelinase

Cherry Red Macula

Hepatosplenomegaly

- "Palpable abdominal mass" – They are talking about HSM!
- "Abdominal exam unremarkable" – look elsewhere!

Bone Issues:

- **Osteoporosis**
- **Asepetic necrosis of femur**
- **Bone Crisis**

Niemann-Pick

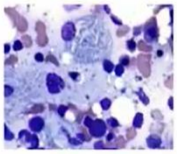
- Neurodegeneration
- FOAM cells

- "No man **Picks** his nose with his **sphinger**" = Sphingomyelinase

Gaucher

Most common

- TISSUE PAPER cells



- Use tissues for mucus, **muco** rhymes with **Glucocerebrosidase**

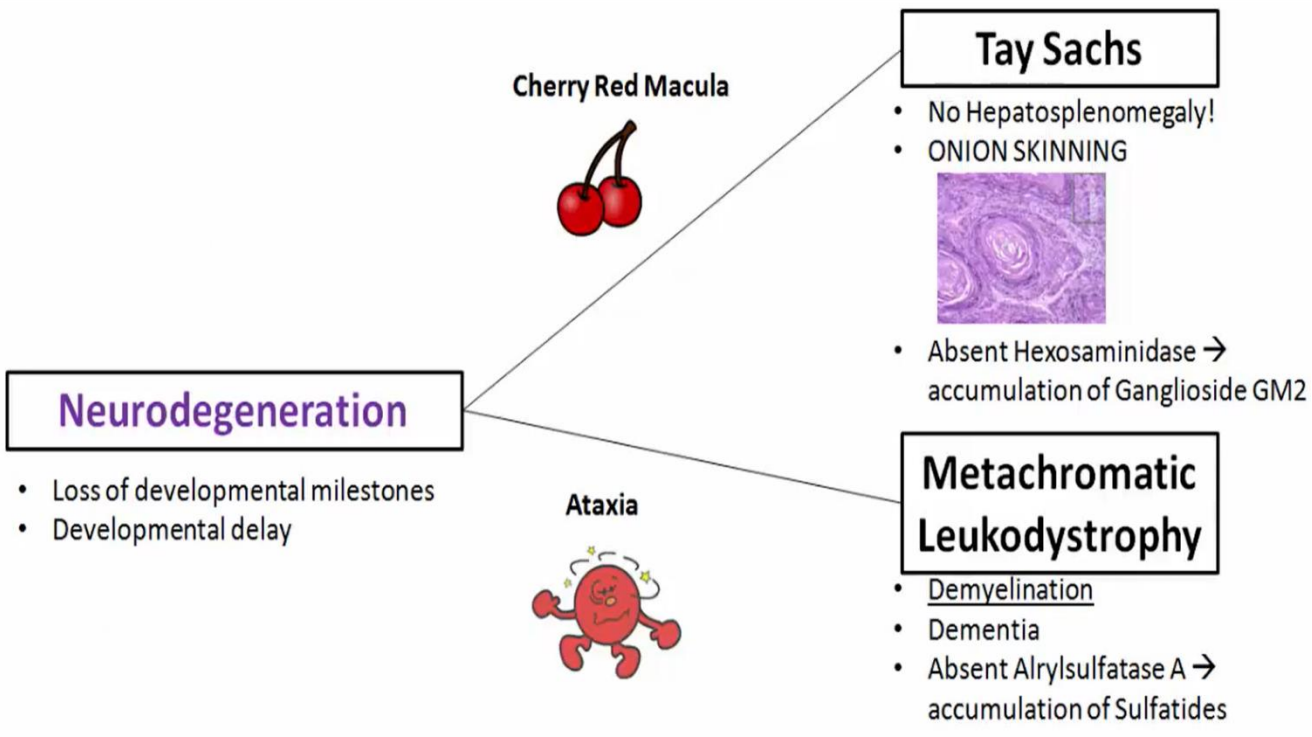
C. Gaucher disease:

- Inheritance: AR.
- Deficient enzyme: Glucocerebrosidase.
- Accumulated substrate: Glucocerebroside (β-glucosidase); treat with recombinant glucocerebrosidase.
- Findings:

- Most common.
- Hepatosplenomegaly, pancytopenia, osteoporosis, avascular necrosis of femur, bone crises, Gaucher cells (lipid-laden macrophages resembling crumpled tissue paper).

D. Metachromatic leukodystrophy:

- Inheritance: AR.
- Deficient enzyme: Arylsulfatase A.
- Accumulated substrate: Cerebroside sulfate.
- Findings: Central and peripheral demyelination with ataxia, dementia.



E. Fabry disease (angiokeratoma corporis diffusum):

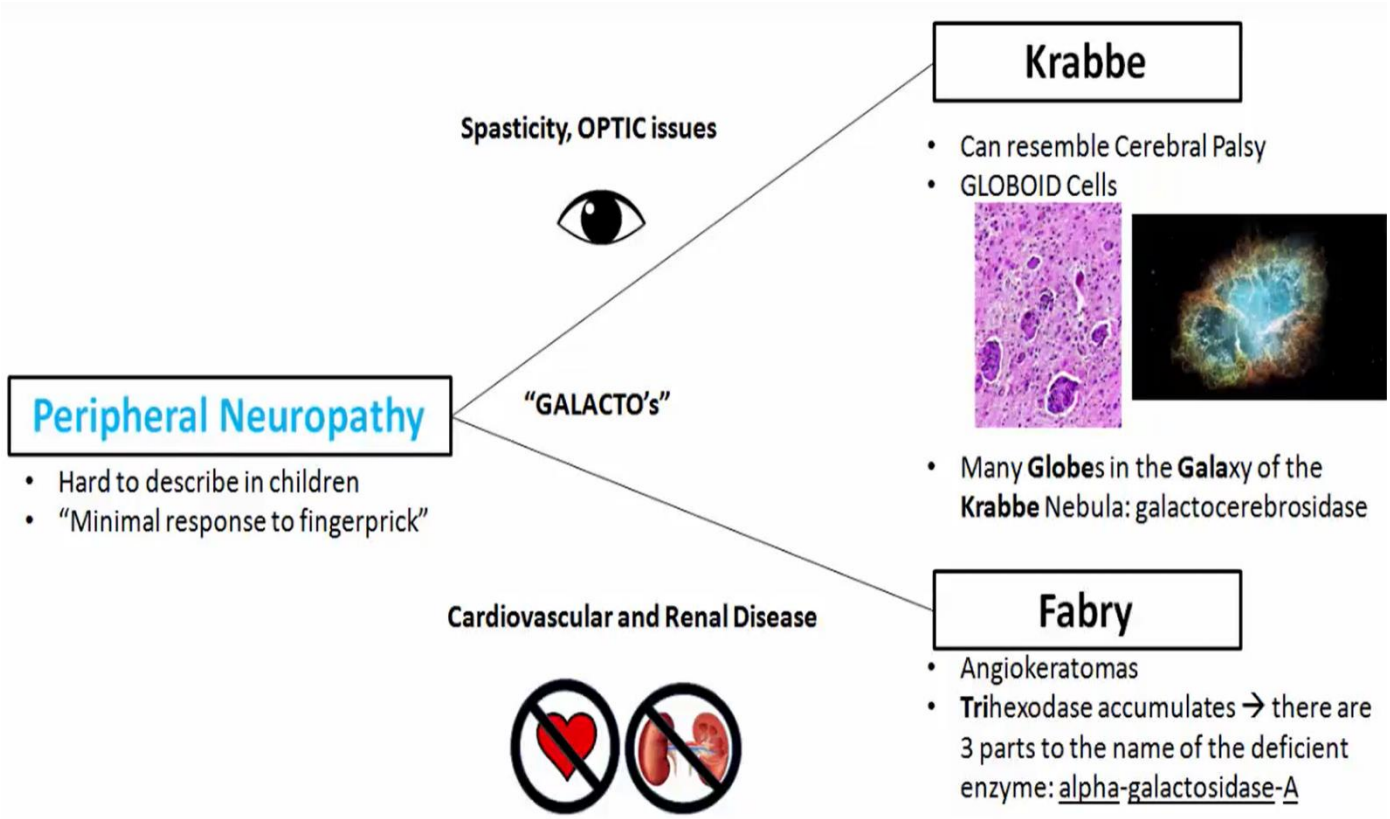
- Inheritance: XR.
- Deficient enzyme: α-galactosidase A.
- Accumulated substrate: the globoside ceramide trihexoside accumulates in tissues.
- Findings:

- Ceramide trihexoside accumulation in vascular smooth muscle cells, glomerular/distal tubule cells, cardiac myocytes, and dorsal root and autonomic ganglia accounts for the adverse manifestations of Fabry disease.

- Early: Triad of episodic acroparesthesia (debilitating, burning neuropathic pain in the extremities), angiokeratomas (punctuate, dark red, non-blanching macules and papules that classically occur between the umbilicus and the knees), hypohidrosis.

- Late: progressive renal failure, cardiovascular disease.

- Without enzyme replacement therapy, progressive renal insufficiency leading to renal failure and death may occur.



F. Krabbe disease:

- Inheritance: AR.
- Deficient enzyme: Galactocerebrosidase.
- Accumulated substrate: Galactocerebroside, psychosine.
- Findings: Peripheral neuropathy, destruction of oligodendrocytes, developmental delay, optic atrophy, globoid cells (giant, multinucleated cells).

Mucopolysaccharidoses

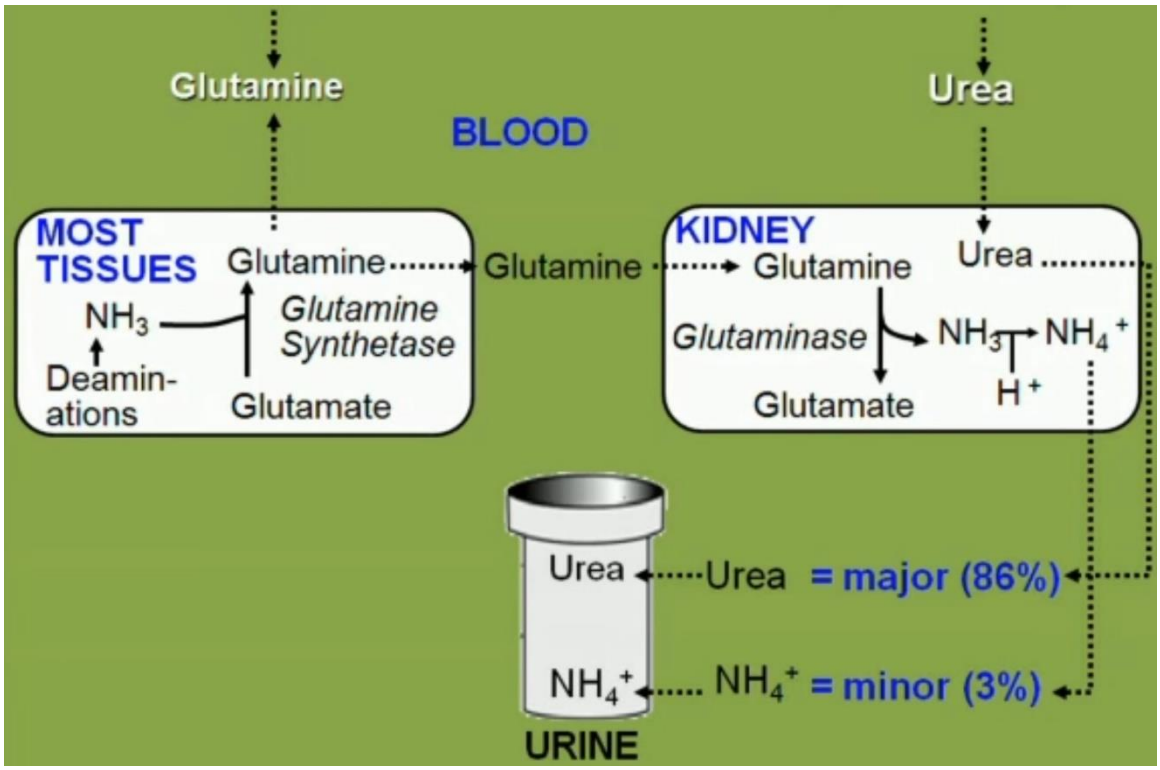
A. Hurler syndrome:

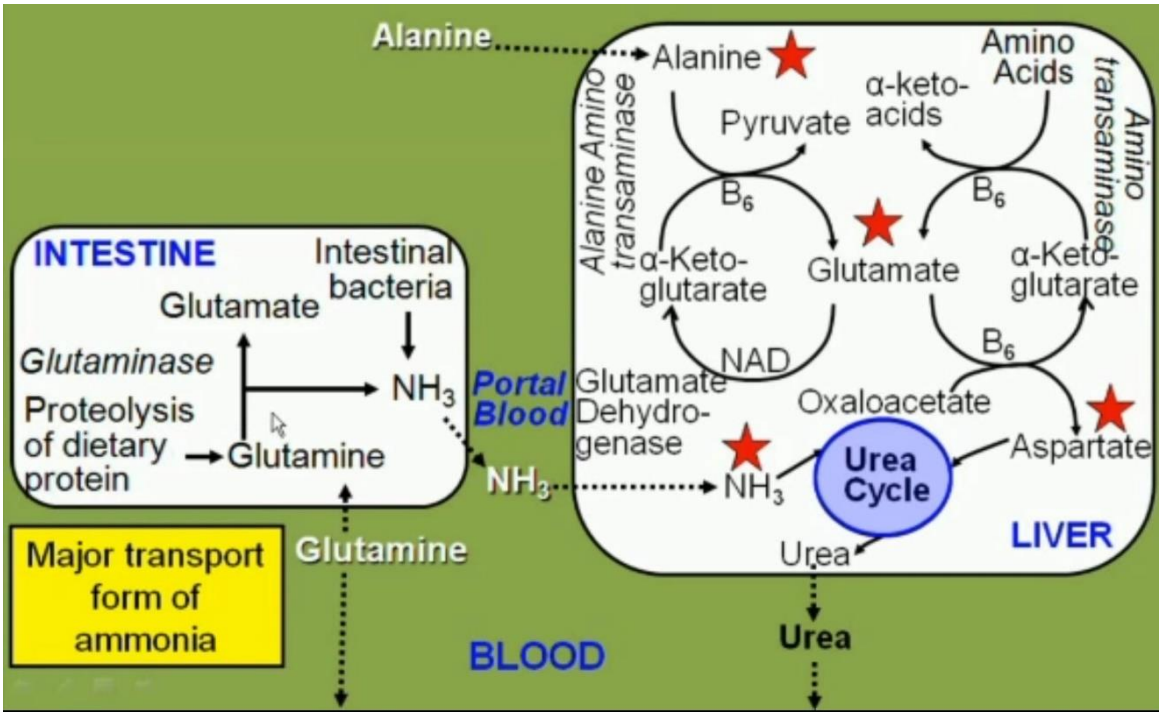
- Inheritance: AR.
- Deficient enzyme: α-L-iduronidase.
- Accumulated substrate: Heparan sulfate, dermatan sulfate.
- Findings: Developmental delay, gargoylism, airway obstruction, corneal clouding, hepatosplenomegaly.

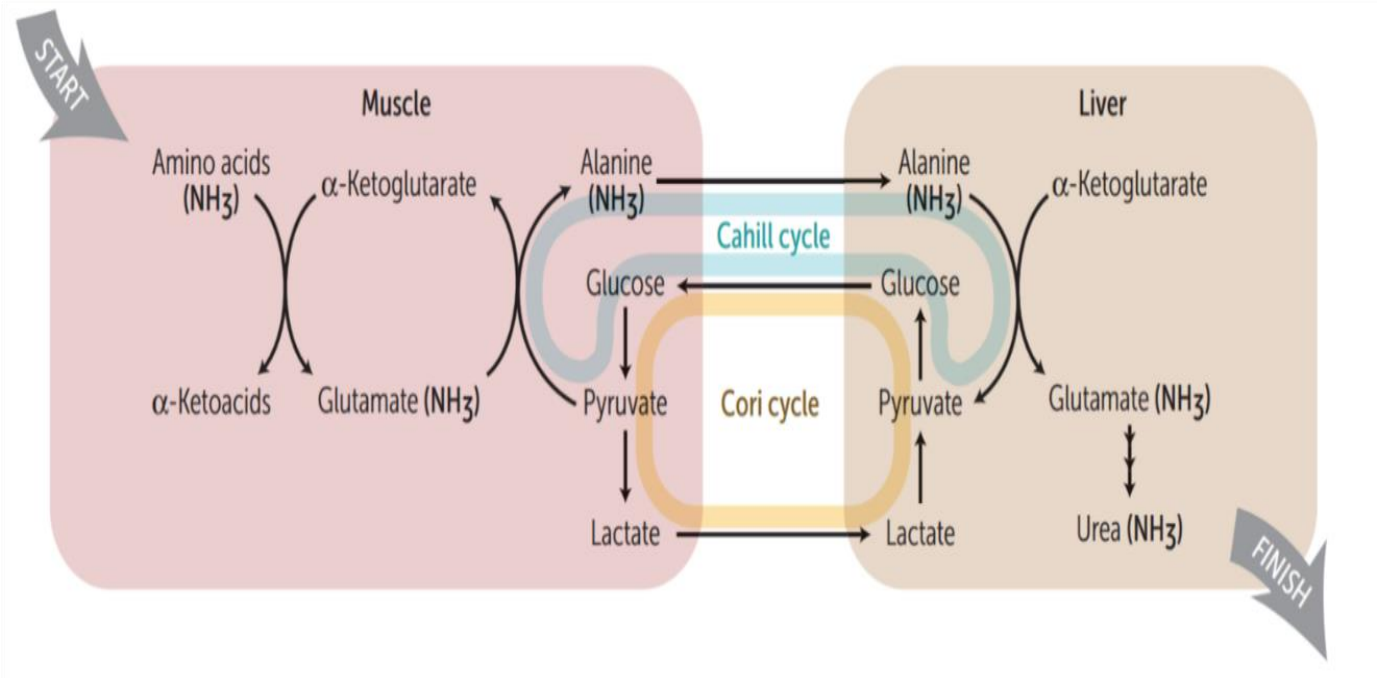
B. Hunter syndrome:

- Inheritance: XR.
- Deficient enzyme: Iduronate-2-sulfatase.
- Accumulated substrate: Heparan sulfate, dermatan sulfate.
- Findings: Mild Hurler + aggressive behavior, no corneal clouding.
- Hunters see clearly (no corneal clouding) and aggressively aim for the X (X-linked recessive).

Amino acid metabolism







- Glutamine Synthetase:

- Most tissues, including muscle, have glutamine synthetase, which captures excess nitrogen by aminating glutamate to form glutamine. The reaction is irreversible.

- Glutamine, a relatively nontoxic substance, is the major carrier of excess nitrogen from tissues.

- Glutaminase:

- The kidney contains glutaminase, allowing it to deaminate glutamine arriving in the blood and to eliminate the amino group as ammonium ion in urine. The reaction is irreversible.

- Kidney glutaminase is induced by chronic acidosis, in which excretion of ammonium may become the major defense mechanism.

- Levels of the glutaminase are high in the intestine where the ammonium ion from deamination can be sent directly to the liver via the portal blood and used for urea synthesis.

- Transport of ammonia by alanine:

- Alanine and glutamine play an important role in transporting nitrogen throughout the body. Glutamine is produced by most body tissues and is catabolized primarily by the gut and kidney for maintenance of cellular metabolism and acid-base regulation, respectively.

- Alanine is also released by skeletal muscle tissue during protein catabolism as part of the glucose-alanine cycle that helps remove excess nitrogen. Alanine is then transported to the liver, where it serves as a vehicle for nitrogen disposal and as a source of carbon skeletons for gluconeogenesis.

- In the liver, alanine is transaminated by alanine aminotransferase to pyruvate with the amino group being transferred to α-ketoglutarate to form glutamate. Almost all aminotransferase enzymes use a-ketoglutarate as the amino group acceptor.

- Glutamate is further metabolized by the enzyme glutamate dehydrogenase, which liberates free ammonia and regenerates α-ketoglutarate. Ammonia then enters the urea cycle to form urea, the primary disposal form of nitrogen in humans. Urea subsequently enters the blood and is excreted in the urine.

▪ Aminotransferases (Transaminases):

- Both muscle and liver have aminotransferases, which, unlike deaminases, do not release the amino groups as free ammonium ion.

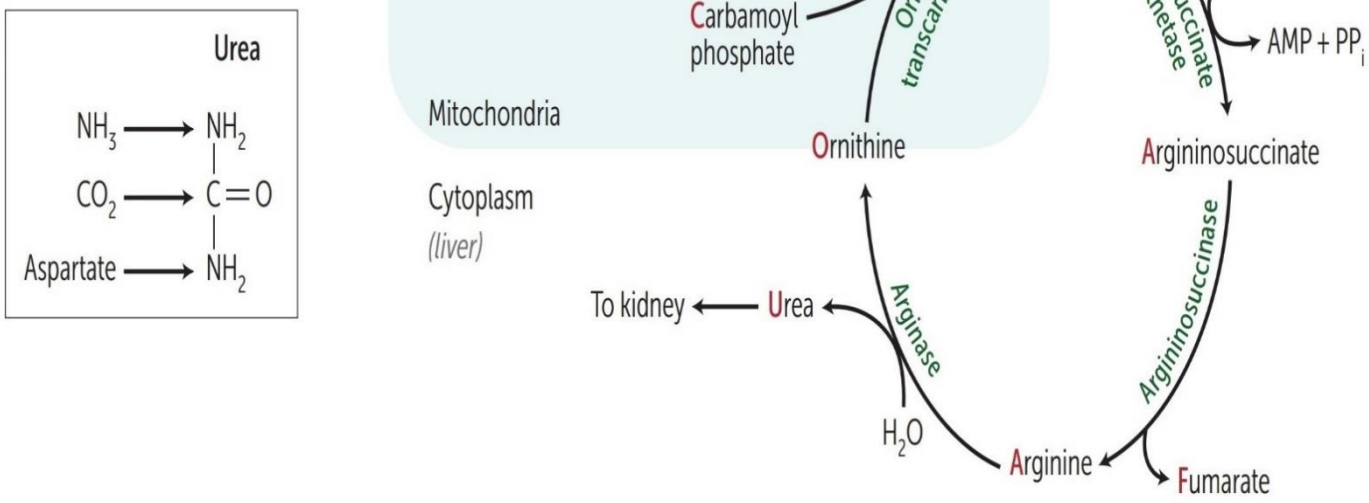
- This class of enzymes transfers the amino group from one carbon skeleton (an amino acid) to another (usually α-ketoglutarate, a citric acid cycle intermediate).

- Pyridoxal phosphate (PLP) derived from vitamin B6 is required to mediate the transfer.

- Aminotransferases are named according to the amino acid donating the amino group to α-ketoglutarate. Two important examples are alanine aminotransferase (ALT, formerly GPT) and aspartate aminotransferase (AST, formerly GOT).

- Although the aminotransferases are in liver and muscle, in pathologic conditions these enzymes may leak into the blood, where they are useful clinical indicators of damage to liver or muscle.

Urea cycle



- The urea cycle involves five enzymatic steps (two in the mitochondrial matrix and three in the cytosol):

- The first step of urea cycle combines CO_2, ammonia, and ATP to form carbamoyl phosphate in a reaction catalyzed by the enzyme carbamoyl phosphate synthetase I, the rate-limiting step in the urea cycle.

- Carbamoyl phosphate synthetase I (CPS) require the presence of N-acetylglutamate (NAG), a molecule formed by NAGS, as this molecule acts as an allosteric activator of CPS.

- Carbamoyl phosphate then combines with ornithine to form citrulline in a reaction catalyzed by ornithine transcarbamoylase in the mitochondrial matrix.

- Citrulline then enters the cytosol and is converted to argininosuccinate, which is then converted to arginine.

- The conversion of arginine to ornithine by the cytosolic enzyme arginase completes the urea cycle by releasing a urea molecule.

- Genetic Deficiencies of the Urea Cycle:

- A combination of hyperammonemia, elevated blood glutamine, and decreased blood urea nitrogen (BUN) suggests a defect in the urea cycle.

- There are 2 deficiencies of the 2 mitochondrial enzymes in the urea cycle: carbamoyl phosphate synthetase and ornithine transcarbamoylase.

- They can be distinguished by an increase in orotic acid and uracil, which occurs in ornithine transcarbamoylase deficiency, but not in the deficiency of carbamoyl phosphate synthetase.

- Orotic acid and uracil are intermediates in pyrimidine synthesis. This pathway is stimulated by the accumulation of carbamoyl phosphate, the substrate for ornithine transcarbamoylase in the urea cycle and for aspartate transcarbamoylase in pyrimidine synthesis.

Hyperammonemia

- Asterixis Can be acquired (liver disease) or hereditary (urea cycle enzyme deficiencies).

- Excess NH_3 depletes glutamate in the CNS and α-ketoglutarate → inhibition of TCA cycle → Flapping tremor (asterixis), slurring of speech, somnolence, vomiting, cerebral edema, blurring of vision.

- Treatment: These conditions can be treated with a low protein diet and administration of sodium benzoate or phenylpyruvate to provide an alternative route for capturing and excreting excess nitrogen.

- May be given to ↓ ammonia levels:

- Lactulose to acidify the GI tract and trap NH_4 for excretion.

- Antibiotics (rifaximin, neomycin) to colonic ammoniagenic bacteria.

Ornithine transcarbamylase deficiency

- Ornithine transcarbamoylase deficiency is the most common disorder of the urea cycle, resulting in severe neurological abnormalities due to high blood and tissue ammonia levels.

- X-linked recessive (vs other urea cycle enzyme deficiencies, which are autosomal recessive).

- Interferes with the body's ability to eliminate ammonia.

- Often evident in the first few days of life but may present later.

- Findings:

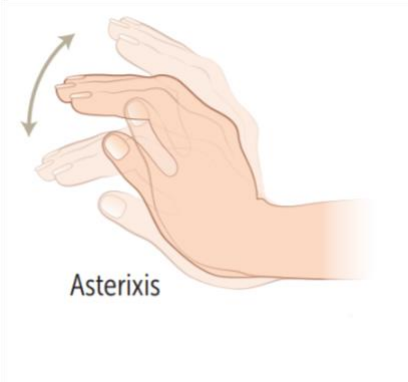
- ↑ orotic acid in blood and urine, ↓ BUN, symptoms of hyperammonemia.

- OTC deficiency results in excess carbamoyl phosphate, which stimulates pyrimidine synthesis. As an intermediate product in this pathway, orotic acid accumulates and results in increased urinary orotic acid.

- Patients also have hyperammonemia due to impaired ammonia excretion, which is a metabolic emergency. Ammonia is neurotoxic and causes episodes of vomiting and confusion/coma. Tachypnea also occurs due to cerebral edema from ammonia buildup, resulting in central hyperventilation and respiratory alkalosis. Metabolic decompensation is often triggered by illness (viral upper respiratory infection, acute otitis media), fasting, or increased protein intake.

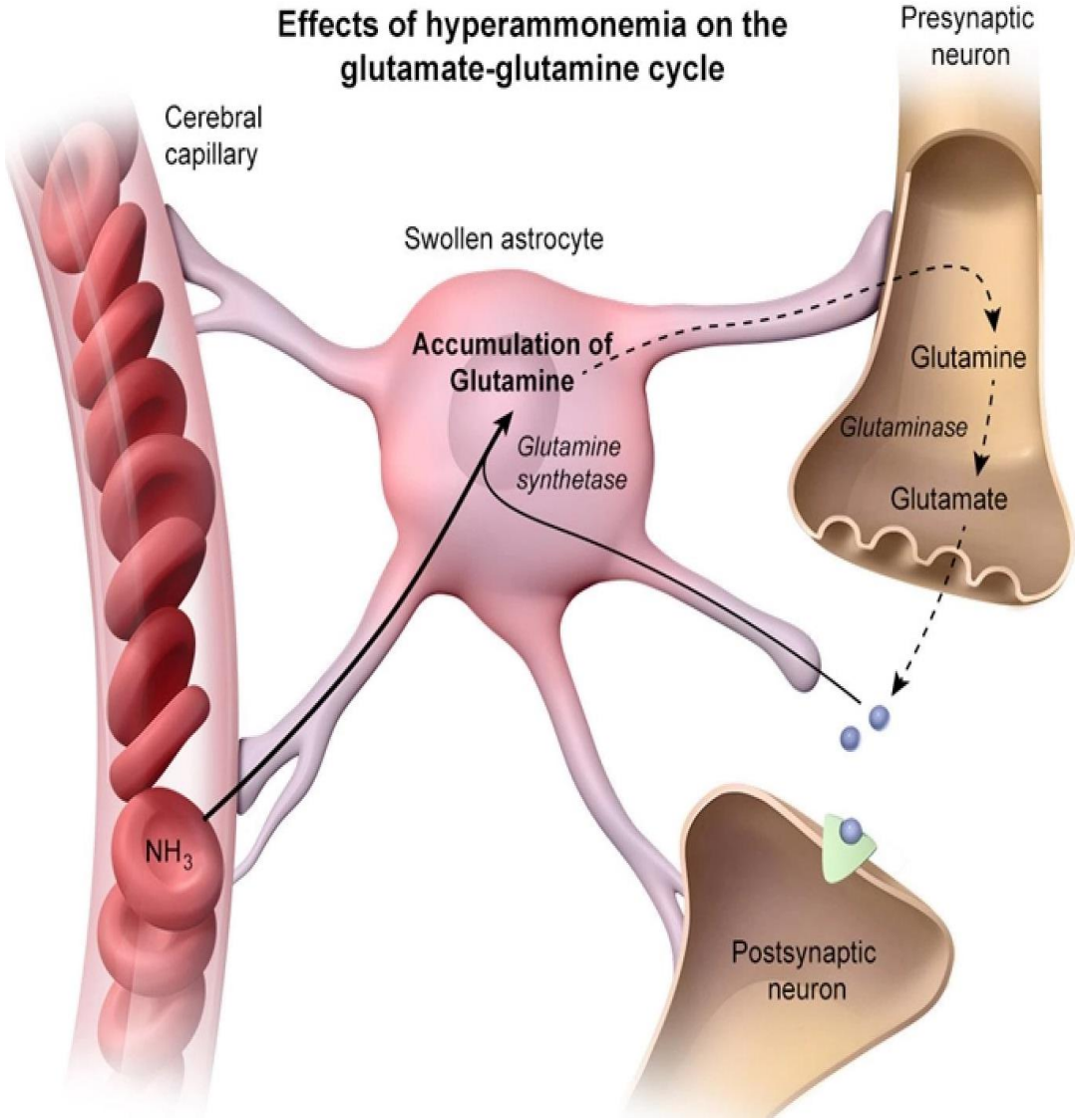
- No megaloblastic anemia (Vs orotic aciduria).

- The treatment of urea cycle disorders consists of balancing dietary protein intake and protein output, such that the body receives the essential amino acids needed for growth and development but not in excess such that excessive ammonia is formed. Thus, protein restriction is the main form of therapy for urea cycle disorders.



❖ N.B:

- Hyperammonemia in hepatic encephalopathy results in depletion of α-ketoglutarate, causing inhibition of the Krebs cycle. Excess ammonia also depletes glutamate, an excitatory neurotransmitter, and causes accumulation of glutamine, resulting in astrocyte swelling and dysfunction.
- Hyperammonemia increases the conversion of glutamate into glutamine by glutamine synthetase within astrocytes. The resulting increase in glutamine leads to hyperosmolarity and mitochondrial dysfunction, causing astrocytic swelling and impairment.
- Increased glutamine formation also decreases total brain glutamate stores, impairing excitatory neurotransmission and neuronal energy production.


Effects of hyperammonemia on the glutamate-glutamine cycle
Presynaptic neuron
Cerebral capillary
Swollen astrocyte
Accumulation of Glutamine
Glutamine synthetase
Glutamine
Glutaminase
Glutamate
NH3
Postsynaptic neuron

Amino acids

A. Essential:

- PVT TIM HaLL: Phenylalanine, Valine, Tryptophan, Threonine, Isoleucine, Methionine, Histidine, Leucine, Lysine.

- Glucogenic: Methionine, histidine, valine. I met his valentine, she is so sweet (glucogenic).

- Glucogenic/ketogenic: Isoleucine, phenylalanine, threonine, tyrosine.

- Ketogenic: Leucine, Lysine. The onLy pureLy ketogenic amino acids.

B. Acidic:

- Aspartic acid, glutamic acid.

- Negatively charged at body pH.

C. Basic:

- Arginine, histidine, lysine.

- Arginine is most basic. Histidine has no charge at body pH.

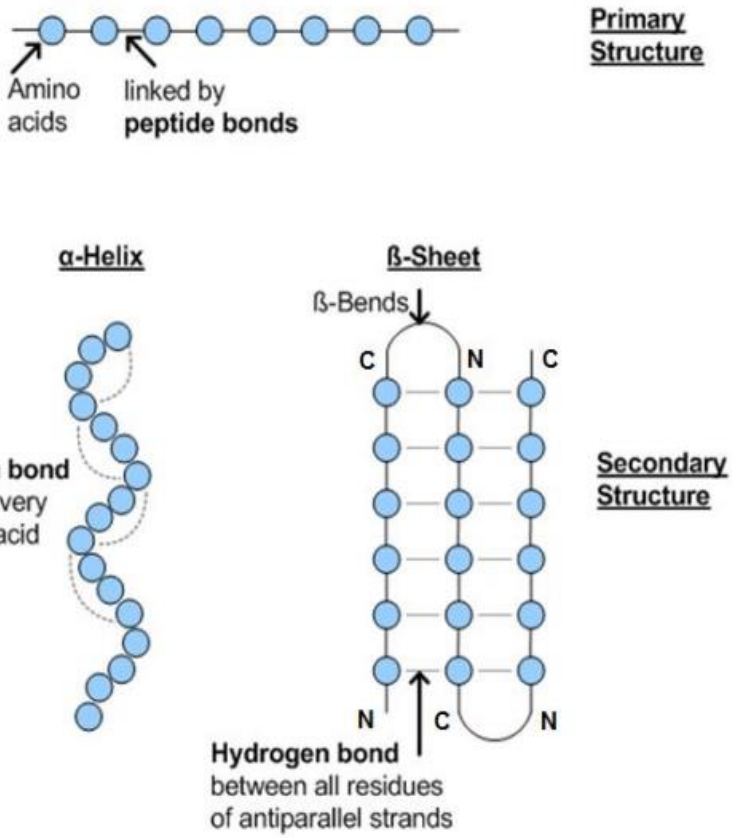
- Arginine and histidine are required during periods of growth.

- Arginine and lysine are ↑ in histones which bind negatively charged DNA.

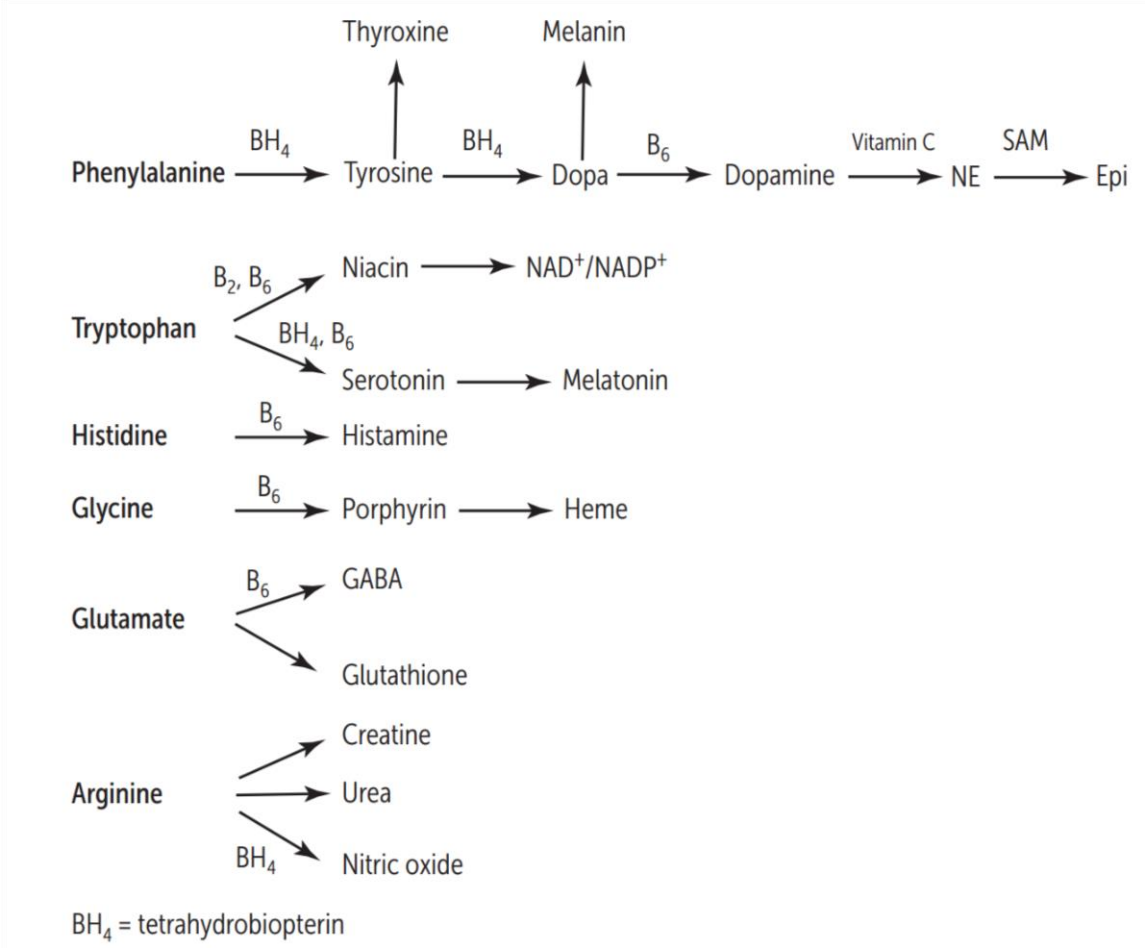
- His lys (lies) are basic.

Protein structure

- A protein's primary structure is the sequence of amino acids linked by covalent peptide bonds.
- Proteins may also assume a secondary structure, such as the alpha-helix or beta-sheet, due to subsequent hydrogen bonding. Hydrogen bonds are the principal stabilizing force for the secondary structure of proteins.
- Tertiary structure is the overall shape that a single polypeptide chain assumes following compact folding of the secondary structure. Many forces combine to stabilize the tertiary structure, including ionic bonds, hydrophobic interactions, hydrogen bonds, and disulfide bonds.



Amino acid derivatives



Catecholamine synthesis/tyrosine catabolism

- The three main circulating catecholamines are dopamine, norepinephrine, and epinephrine.
- In contrast to norepinephrine and dopamine, epinephrine is chiefly produced by the adrenal glands.
- Norepinephrine and dopamine are produced by both the central and peripheral nervous system.
- Dopamine is norepinephrine's precursor.
- In the adrenal medulla, the first step in the synthesis of catecholamine is conversion of tyrosine to dihydroxyphenylalanine, more commonly called "dopa," by the enzyme tyrosine hydroxylase.
- Tyrosine is derived either from ingested food or from phenylalanine synthesis in the liver. The conversion of phenylalanine to tyrosine is accomplished by phenylalanine hydroxylase.

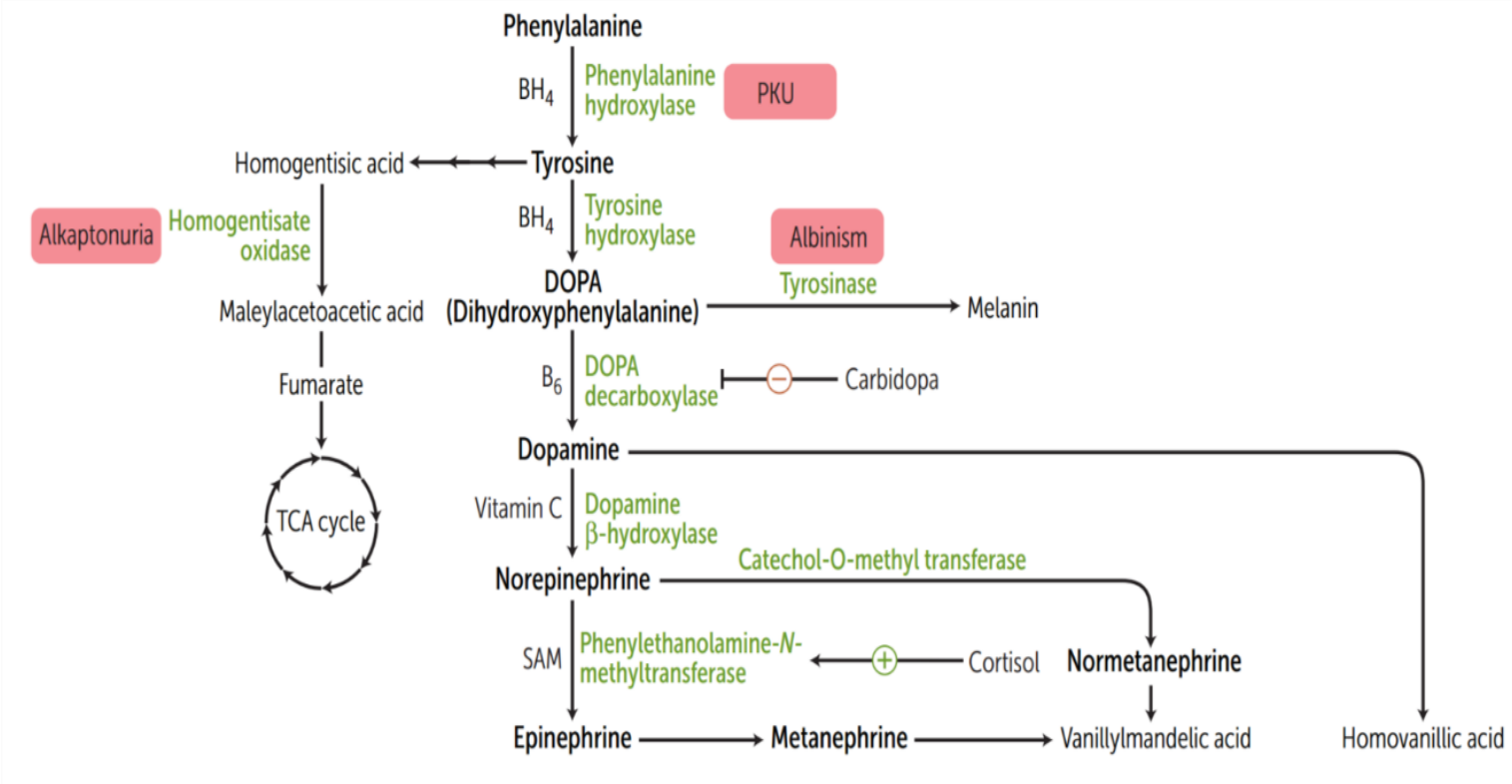
- Dopa, then, is converted to dopamine by dopa decarboxylase, which is then converted to norepinephrine by dopamine β- hydroxylase.

- The conversion of norepinephrine to epinephrine occurs mostly in the adrenal medulla by an enzyme called phenylethanolamine-N-methyltransferase (PNMT).

- Because venous drainage of the adrenal cortex goes through the adrenal medulla, the concentration of cortisol is very high in the adrenal medulla.

- Cortisol increases expression of the gene encoding PNMT. The catecholamine contents of the normal human adrenal medulla are approximately 80% epinephrine and 20% as norepinephrine.

- This epinephrine-heavy ratio is due to the positive effect of cortisol on the expression of the enzyme PNMT, which converts norepinephrine to epinephrine.

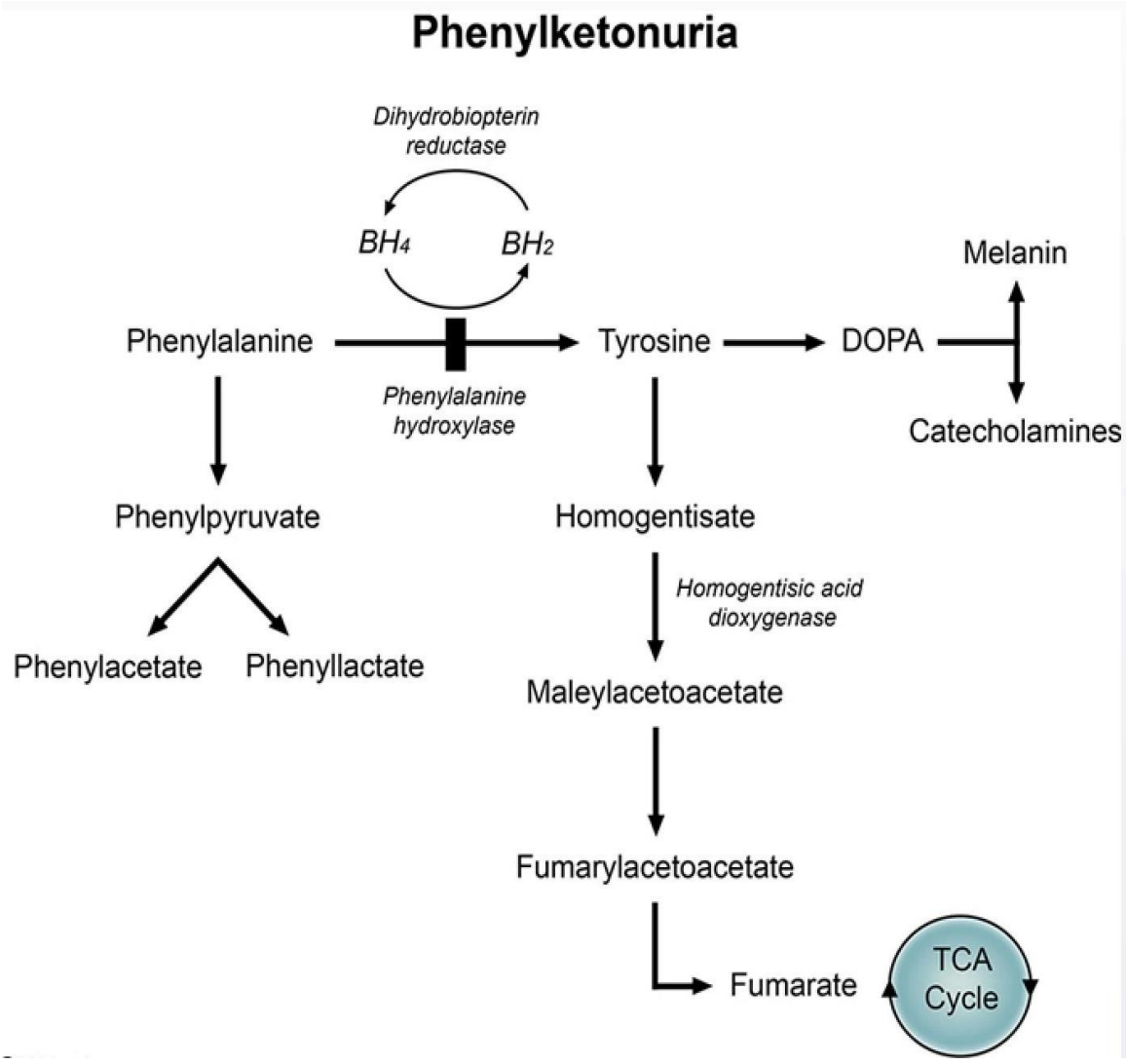


Phenylketonuria

- Due to ↓ phenylalanine hydroxylase or ↓ tetrahydrobiopterin (BH_4) cofactor (atypical or malignant phenylketonuria).

- PKU results from the inability to convert phenylalanine into tyrosine, a reaction which is normally catalyzed by phenylalanine hydroxylase. This enzyme requires the cofactor tetrahydrobiopterin (BH_4), which is regenerated from dihydrobiopterin (BH_2) by the enzyme dihydropteridine reductase.

- Although neonatal hyperphenylalaninemia can be caused by deficiency of either enzyme, most cases are attributable to abnormalities in phenylalanine hydroxylase.
- Tyrosine becomes essential.
- ↑ phenylalanine → excess phenyl ketones in urine.
- Autosomal recessive. Incidence ≈ 1:10,000.
- Screening occurs 2-3 days after birth (normal at birth because of maternal enzyme during fetal life).



- Findings:

- Intellectual disability, growth retardation, seizures, blue eyes, fair complexion, eczema, musty body odor.

- It is believed that excess phenylalanine and the presence of large concentrations of phenylalanine metabolites (phenyllactate & phenylacetate) contribute to the brain damage seen in PKU.

- Hypopigmentation involving the skin, hair, eyes, and catecholaminergic brain nuclei (which produce a dark pigment known as neuromelanin) results from the inhibitory effect of excess phenylalanine on melanin synthesis (the excess phenylalanine present inhibits tyrosinase, the enzyme responsible for the synthesis of melanin from tyrosine).

- Tyrosine is a non-essential amino acid that becomes essential in the setting of phenylketonuria (PKU).

- The classic musty or mousy body odor is due to the accumulation of abnormal phenylalanine metabolites.

- Treatment:
  - ↓ phenylalanine and ↑ tyrosine in diet, tetrahydrobiopterin supplementation.
  - PKU patients must avoid the artificial sweetener aspartame, which contains phenylalanine.

❖ N.B:

- Deficiency of dihydrobiopterin reductase, the enzyme responsible for reduction of dihydrobiopterin (BH_2) to BH_4 is the most common cause for a deficiency of BH4. This results in what is known as atypical or malignant phenylketonuria (account for 2% of hyperphenylalanemia cases).
- Tetrahydrobiopterin is a cofactor for enzymes that participate in the synthesis of tyrosine (a precursor of DOPA), DOPA (the antecedent of the neurotransmitters dopamine, norepinephrine and epinephrine), serotonin (a major neurotransmitter), and nitric oxide.
- Although phenylalanine levels can be controlled by dietary restriction, downstream deficiencies of neurotransmitters (dopamine, norepinephrine, epinephrine, serotonin) lead to progressive neurologic deterioration in these patients.
- Normally, dopamine from the tuberoinfundibular system tonically inhibits prolactin release. Decreased BH_4 causes lower levels of dopamine, which lead to increased prolactin levels.
- The combination of high phenylalanine levels, which may disrupt neuronal and glial development, and low serotonin and other neurotransmitters results in progressive neurologic deterioration in untreated patients.
- Manifestations include developmental delay, hypotonia, dystonia, and seizures.
- Treatment includes both a low phenylalanine diet and BH_4 supplementation.

❖ Maternal PKU:

- The maternal phenylketonuria (PKU) syndrome refers to the teratogenic effects of PKU during pregnancy.

- Lack of proper dietary therapy during pregnancy.

- Findings in infant: Microcephaly, intellectual disability, growth retardation, congenital heart defects.

Maple syrup urine disease

- Blocked degradation of branched amino acids (Isoleucine, Leucine, Valine) due to ↓ branched-chain α-ketoacid dehydrogenase (B1). Autosomal recessive.

- Branched-chain α-ketoacid dehydrogenase, pyruvate dehydrogenase, and α-ketoglutarate dehydrogenase all require five cofactors: Thiamine pyrophosphate, Lipoate, Coenzyme A, FAD, NAD (mnemonic: Tender Loving Care For Nancy).

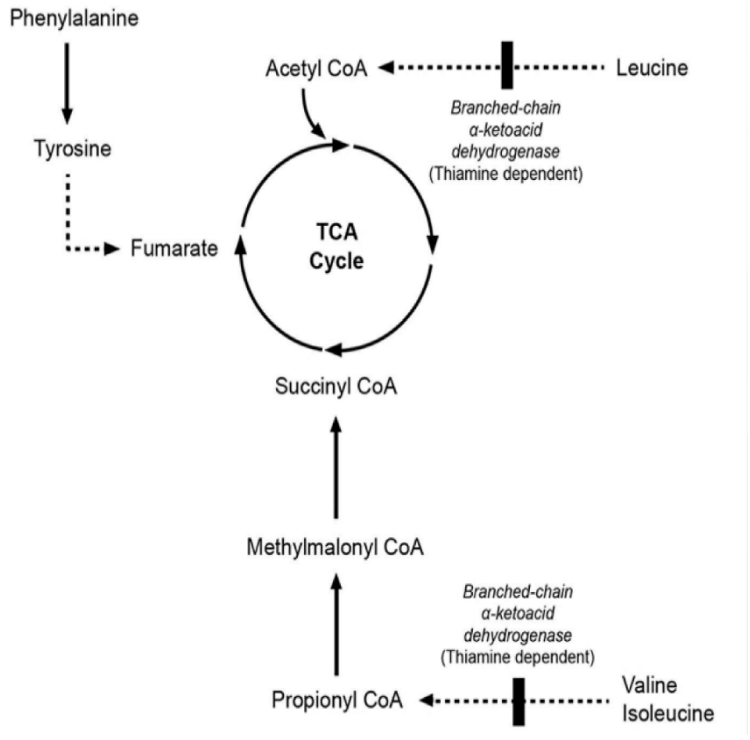
- Causes ↑ α-ketoacids in the blood, especially those of leucine.

- Presentation:

- Vomiting, poor feeding, urine smells like maple syrup/burnt sugar.

- Because their degradation is inhibited at the α-keto acid stage, tissue and serum levels of these branched chain a-keto acids increase, which leads to neurotoxicity (Causes severe CNS defects, intellectual disability, and death).

- Maple syrup urine disease usually manifests within the first few days of life, and classically, the urine of affected infants has a distinctive sweet odor, much like burned caramel "maple syrup scent".



- Treatment:

- Restriction of isoleucine, leucine, valine in diet, and thiamine supplementation.

- Some patients with MSUD improve with high-dose thiamine treatment (thiamine- responsive), but most still require lifelong dietary restrictions.

Alkaptonuria

- Alkaptonuria is a relatively benign disorder of tyrosine metabolism.

- Alkaptonuria is an autosomal-recessive disorder in which deficiency of homogentisate oxidase blocks the metabolism of phenylalanine and tyrosine at the level of homogentisic acid, thereby preventing the conversion of tyrosine to fumarate.

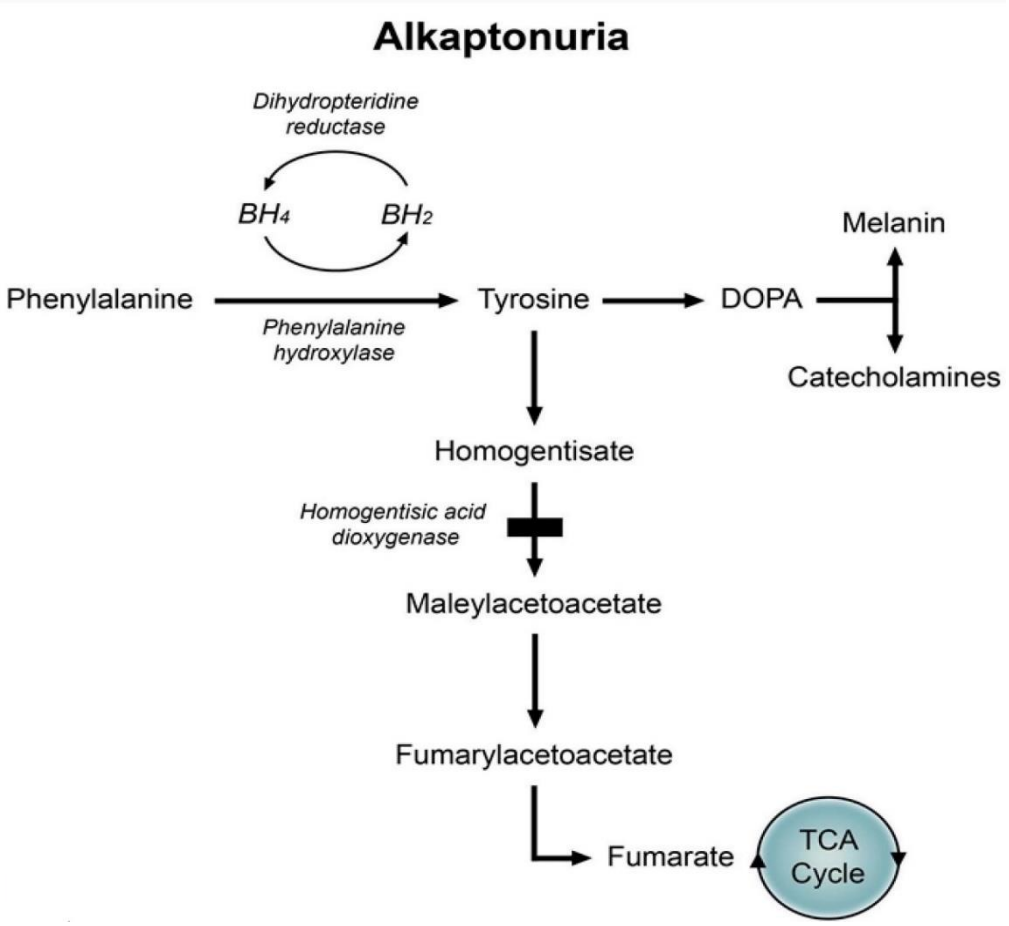
- Findings:

- Bluish-black connective tissue, ear cartilage, and sclerae (ochronosis); urine turns black on prolonged exposure to air. May have debilitating arthralgias (homogentisic acid toxic to cartilage).

- Homogentisic acid accumulates in the body and is excreted in the urine, imparting a black color to the urine if allowed to stand and undergo oxidation.

- In patients with alkaptonuria, the retained homogentisic acid selectively binds to collagen in connective tissues, tendons, and cartilage, causing "ochronosis," a blue-black pigmentation most evident in the ears, nose, and cheeks.

- Deposits also occur in the large joints and spine, causing ankylosis, motion restriction, and significant pain.



Homocystinuria

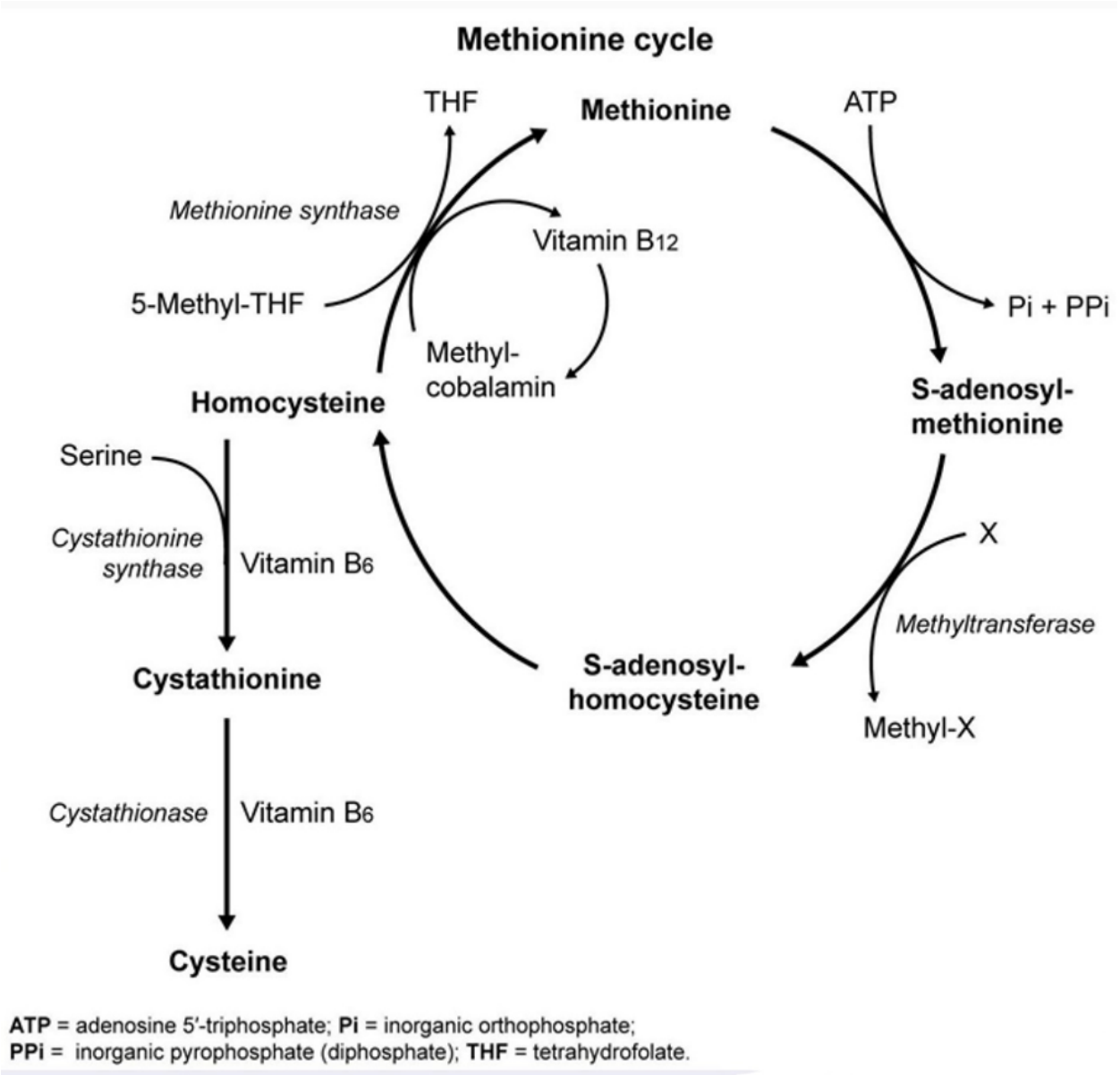
- Types (all autosomal recessive):

- Cystathionine synthase deficiency (treatment: ↓ methionine, ↑ cysteine, ↑ B6, B12, and folate in diet). The most common cause of homocystinuria.

- ↓ affinity of cystathionine synthase for pyridoxal phosphate (treatment: ↑↑ B6 and ↑ cysteine in diet). Many patients respond dramatically to pyridoxine (B6) supplementation, which improves residual enzymatic activity and reduces plasma homocysteine levels.

- Methionine synthase (homocysteine methyltransferase) deficiency (treatment:↑ methionine in diet)

- All forms result in excess homocysteine.



ATP = adenosine 5'-triphosphate; **Pi** = inorganic orthophosphate;
PPi = inorganic pyrophosphate (diphosphate); **THF** = tetrahydrofolate.

- Cysteine becomes an essential amino acid in patients with homocystinuria, as the defective enzyme cystathionine synthetase produces the substrate used by cystathionase for the endogenous production of cysteine.

- HOMOCYstinuria:

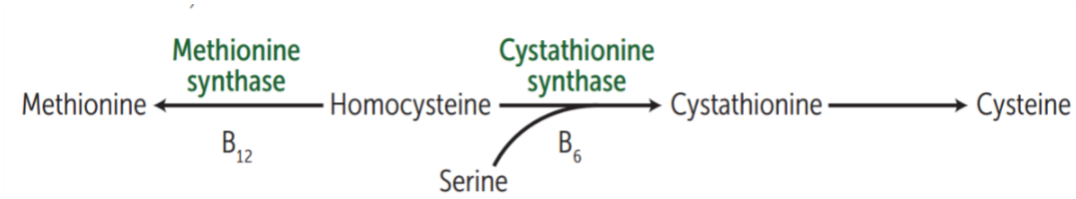
- ↑↑ Homocysteine in urine, Osteoporosis, Marfanoid habitus, Ocular changes (downward and inward lens subluxation), Cardiovascular effects (thrombosis and atherosclerosis → stroke and MI), kYphosis, intellectual disability.

- Most patients present at age 3-10 with ectopia lentis (dislocated lens). About half of patients have intellectual disability.

- Other clinical manifestations include a Marfanoid habitus (elongated limbs, arachnodactyly, scoliosis).

- Patients are at high risk for thromboembolic occlusion of both large and small vessels, especially those of the brain, heart, and kidneys. Thromboembolic complications are the major cause of morbidity and mortality in these patients.

- In homocystinuria, lens subluxes "down and in" (vs Marfan, "up and fans out").



❖ N.B:

- Methionine is a unique amino acid that can be degraded to succinyl-CoA (an intermediate of the citric acid cycle) and S-adenosyl-methionine (SAM) (a major methyl donor in single carbon metabolism).
- SAM has an activated methyl group that can be transferred to a variety of acceptor molecules. After the transfer of a methyl group, S-adenosyl-methionine is converted into S-adenosyl-homocystine, and S-adenosyl-homocystine is then broken down to form adenosine and homocysteine.
- Subsequently, the conversion of homocysteine to cystathionine requires an enzyme called cystathionine synthetase, the amino acid serine, and the presence of the cofactor Vitamin B6. Cystathionine is then converted to cysteine by the enzyme cystathionase, which also requires Vitamin B6 as a cofactor.
- Alternatively, using vitamin B12 as a cofactor, homocysteine can combine with N-5-methyl-tetrahydrofolate to form tetrahydrofolate, a substance needed for purine and thymine synthesis.

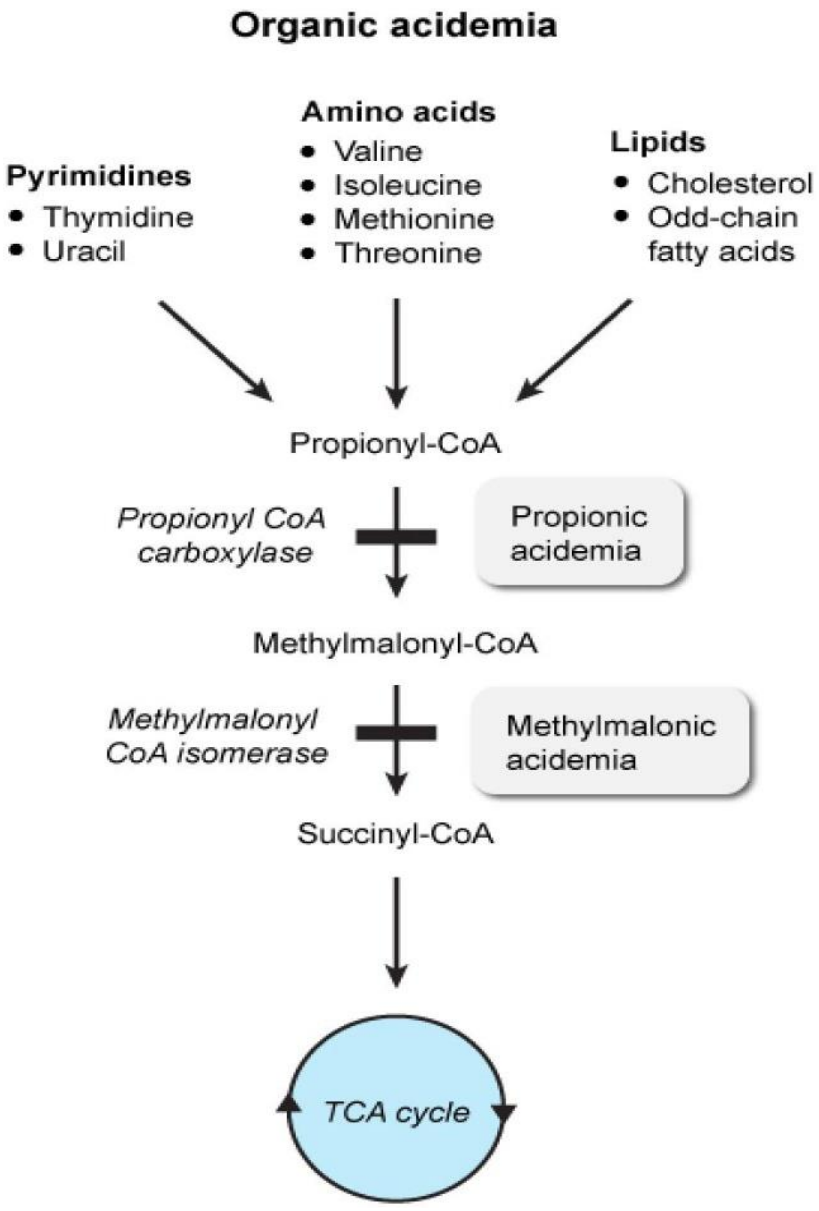
Differential diagnosis of Marfanoid body habitus		
Diagnosis	**Overlapping features**	**Distinguishing features**

Marfan syndrome	• Pectus deformity
	• Tall stature
	  ◦ ↑ Arm : height ratio
	  ◦ ↓ Upper : lower segment ratio
	• Arachnodactyly
	• Joint hyperlaxity
	• Skin hyperelasticity
	• Scoliosis
		• Autosomal dominant
		• Normal intellect
		• **Aortic root dilation**
		• Upward lens dislocation

Homocystinuria		• Autosomal recessive • **Intellectual disability** • **Thrombosis** • Downward lens dislocation • Megaloblastic anemia • Fair complexion

❖ N.B:

- Catabolism of isoleucine, valine, threonine, methionine, cholesterol, and odd-chain fatty acids leads to the formation of propionic acid, which is then converted to methylmalonic acid by biotin-dependent carboxylation.
- A congenital deficiency of propionyl CoA carboxylase, the enzyme responsible for the conversion of propionyl CoA to methylmalonyl CoA, leads to the development of propionic acidemia, as propionyl CoA accumulates. Propionic acidemia is clinically characterized by poor feeding, vomiting, hypotonia, lethargy, dehydration, and an anion gap acidosis.
- Isomerization of methylmalonyl CoA forms succinyl CoA, which then enters the TCA cycle.
- Defects in this isomerization reaction lead to the development of methylmalonic acidemia. Methylmalonic acidemia (also known as methylmalonic aciduria) results from a defect in the isomerization reaction that transforms methylmalonyl CoA to succinyl CoA, prior to succinyl CoA entering the TCA Cycle.



Metabolic fuel use

A. Fed state (after a meal):

- Glycolysis and aerobic respiration.
- Insulin stimulates storage of lipids, proteins, and glycogen.

B. Fasting (between meals):

- The two major processes that maintain plasma glucose between meals are glycogenolysis and gluconeogenesis.
- The breakdown of glycogen by the process of glycogenolysis can maintain blood glucose levels only until liver glycogen stores become depleted. This usually happens after 12 to 18 hours of fasting.
- After liver glycogen is depleted, gluconeogenesis is the primary process used by the body to keep blood glucose levels within the normal range.
- In the process of gluconeogenesis, glucose is formed from lactate, glycerol, and glucogenic amino acids.
- Glucagon and epinephrine stimulate use of fuel reserves.

C. Starvation days 1-3:

- Glycogen reserves depleted after day 1.
- Blood glucose levels maintained by:

- Hepatic gluconeogenesis from peripheral tissue lactate and alanine, and from adipose tissue glycerol and propionyl CoA (from odd-chain FFA).
- Adipose release of FFA (Erythrocytes and brain can't use FFA).
- The brain, kidneys, cardiac muscle, and skeletal muscle can all utilize glucose and/or ketones for energy (Initially, the heart and skeletal muscle consume primarily ketone bodies to preserve glucose for the brain. After prolonged starvation, however, even the brain utilizes ketone bodies for energy).
- During starvation, the continuous supply of energy that ketone bodies provide is especially important to brain functioning, because the brain has no glycogen or triglyceride stores.

D. Starvation after day 3:

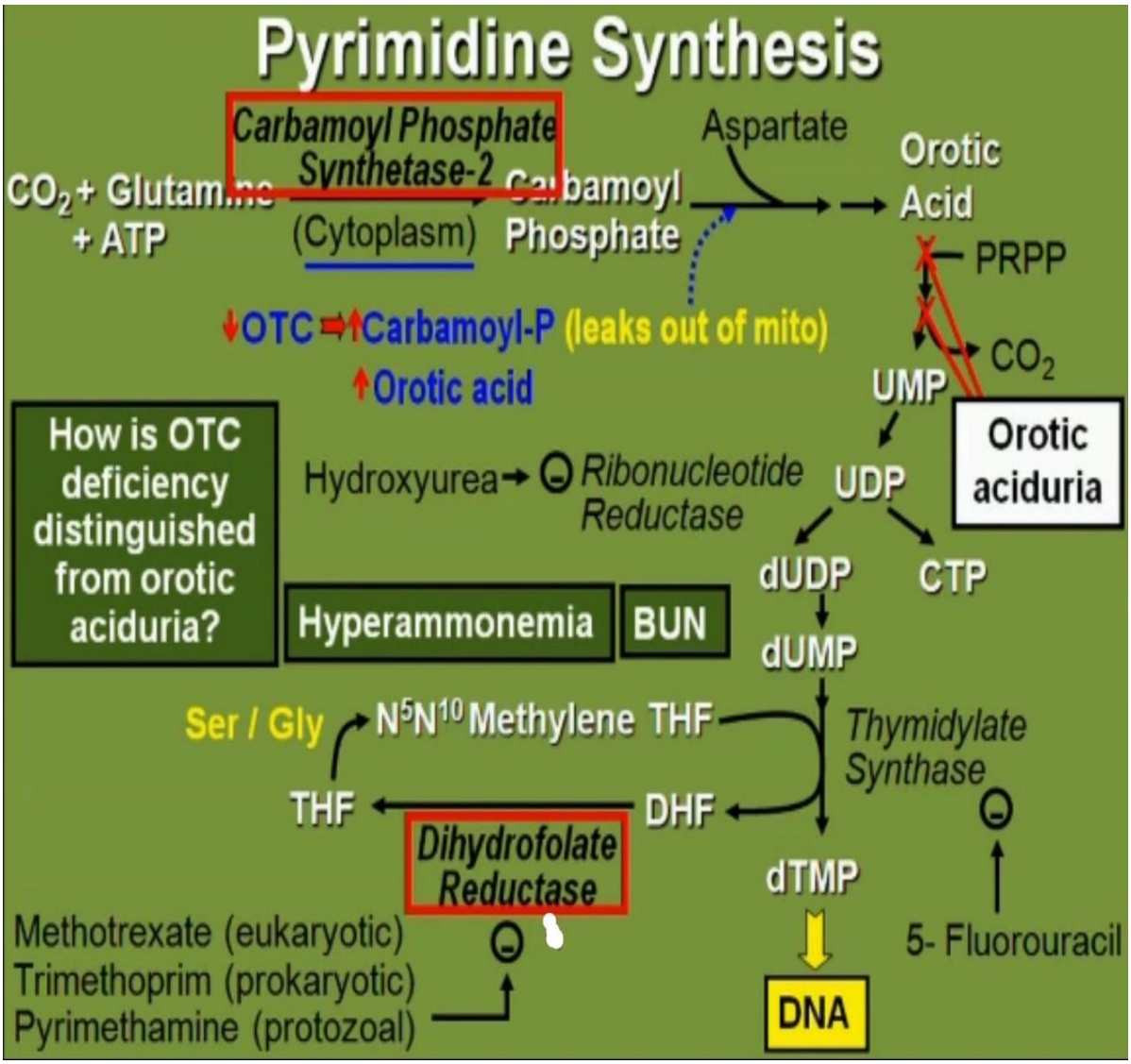
- With prolonged starvation, the body limits its reliance on gluconeogenesis in an effort to conserve protein, and resorts instead to hepatic ketone body synthesis.
- Adipose stores (ketone bodies become the main source of energy for the brain).
- After these are depleted, vital protein degradation accelerates, leading to organ failure and death.

- Amount of excess stores determines survival time.

❖ N.B:

- Dietary energy comes predominantly from protein, carbohydrate, and fat.
- Metabolism yields 4 Calories (Cal) per gram of protein or carbohydrate and 9 Cal per gram of fat. Ethanol yields 7 Cal per gram.
- If a patient is instructed to consume 3000 Cal per day, 900 (30%) of which are to be from protein. Because 1 g of protein yields 4 Cal of energy, this patient should consume (900 Cal /4 Cal) = 225 g/day of protein.

Pyrimidine synthesis



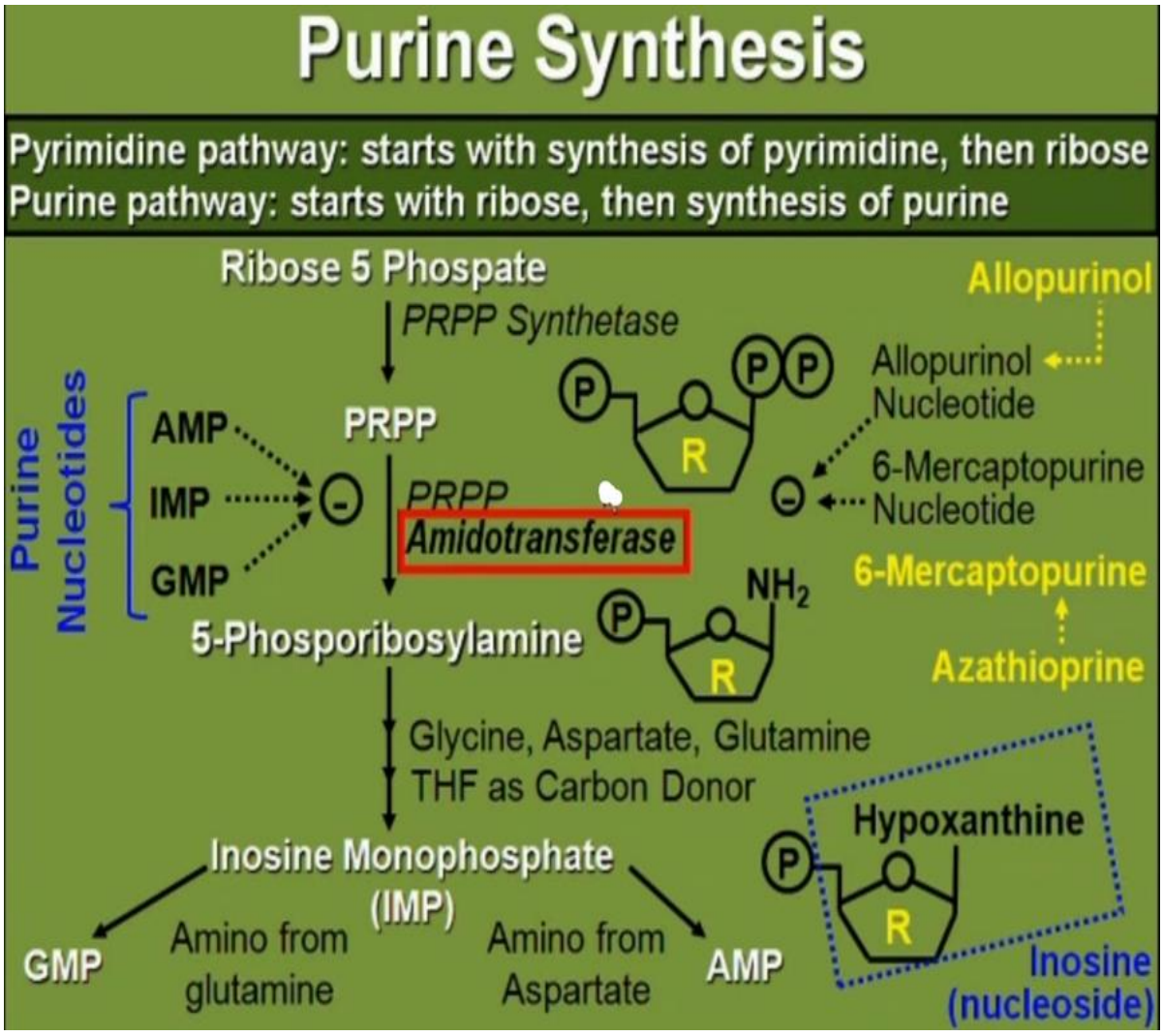
- Nucleotides are needed for DNA and RNA synthesis (DNA replication and transcription) and for energy transfer.
- Ribose 5-phosphate for nucleotide synthesis is derived from the hexose monophosphate shunt and is activated by the addition of pyrophosphate from ATP, forming phosphoribosyl pyrophosphate (PRPP) using PRPP synthetase.
- Cells synthesize nucleotides in 2 ways: de novo synthesis and salvage pathways.
- In de novo synthesis, which occurs predominantly in the liver, purines and pyrimidines are synthesized from smaller precursors, and PRPP is added to the pathway at some point.

- In the salvage pathways, preformed purine and pyrimidine bases can be converted into nucleotides by salvage enzymes distinct from those of de novo synthesis.
- Pyrimidines are synthesized de novo in the cytoplasm from aspartate, CO_2, and glutamine.
- Synthesis involves a cytoplasmic carbamoyl phosphate synthetase-2 that differs from the mitochondrial enzyme with the same name used in the urea cycle.
- CPS1 = m1tochondria (urea cycle). CPS2 = cyTWOsol.
- The primary end product of pyrimidine synthesis is UMP.
- In the conversion of UMP to dTMP, 3 important enzymes are ribonucleotide reductase, thymidylate synthase, and dihydrofolate reductase; all are targets of antineoplastic drugs.
- Ribonucleotide reductase is required for the formation of the deoxyribonucleotides for DNA synthesis. Hydroxyurea, an anticancer drug, blocks DNA synthesis indirectly by inhibiting ribonucleotide reductase.

Orotic aciduria

- Pyrimidine synthesis begins with formation of carbamoyl phosphate from ATP, CO_2 and glutamine by carbamoyl phosphate synthetase II (CPS-II). This is the regulatory step for pyrimidine synthesis. Carbamoyl phosphate is subsequently converted to orotate by a series of reactions.
- The defective enzymes in orotic aciduria are orotate phosphoribosyl transferase and OMP decarboxylase. These two enzymes represent separate active domains on a single polypeptide; this is why a single mutation causes dysfunction of both enzymes.
- These enzymes catalyze the final conversion of orotate to uridine 5'-monophosphate (UMP).
- Presentation: Orotic aciduria is characterized by hypochromic megaloblastic anemia (not responsive to treatment with vitamin B12, folic acid, or iron), neurologic abnormalities, growth retardation and excretion of high amounts of orotic acid in the urine.
- Treatment: This disorder is treated with uridine supplementation. The supplemental uridine is converted to UMP by the action of nucleoside kinases, and UMP in turn inhibits CPS-II, thus attenuating orotic acid production.

Purine synthesis



- Purines are synthesized de novo beginning with PRPP.
- The most important enzyme is PRPP amidotransferase, which catalyzes the first and rate-limiting reaction of the pathway. It is inhibited by the 3-purine nucleotide end products AMP, GMP, and IMP.
- The drugs allopurinol (used for gout) and 6-mercaptopurine (antineoplastic) also inhibit PRPP amidotransferase. These drugs are purine analogs which must be converted to their respective nucleotides by HGPRT within cells.
- Tetrahydrofolate is required for synthesis of all the purines.
- Inosine monophosphate (contains the purine base hypoxanthine) is the precursor for AMP and GMP.

- Various immunosuppressive, antineoplastic, and antibiotic drugs function by interfering with nucleotide synthesis:

1. Pyrimidine synthesis:

- Leflunomide: inhibits dihydroorotate dehydrogenase.

- Methotrexate (MTX), trimethoprim (TMP), and pyrimethamine: inhibit dihydrofolate reductase (↓ deoxythymidine monophosphate [dTMP]) in humans, bacteria, and protozoa, respectively.

- 5-fluorouracil (5-FU) and its prodrug capecitabine: form 5-F-dUMP, which inhibits thymidylate synthase (↓ dTMP).

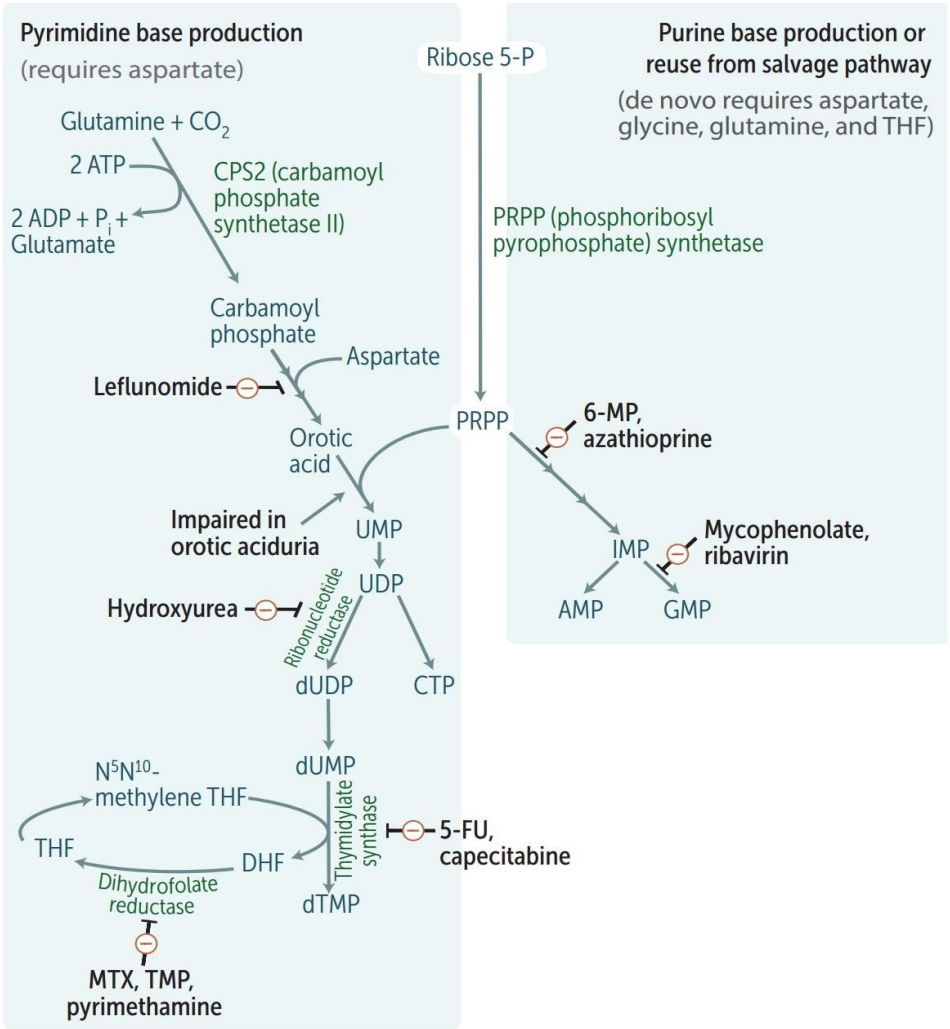
2. Purine synthesis:

- 6-mercaptopurine (6-MP) and its prodrug azathioprine: inhibit de novo purine synthesis (inhibit PRPP amidotransferase).

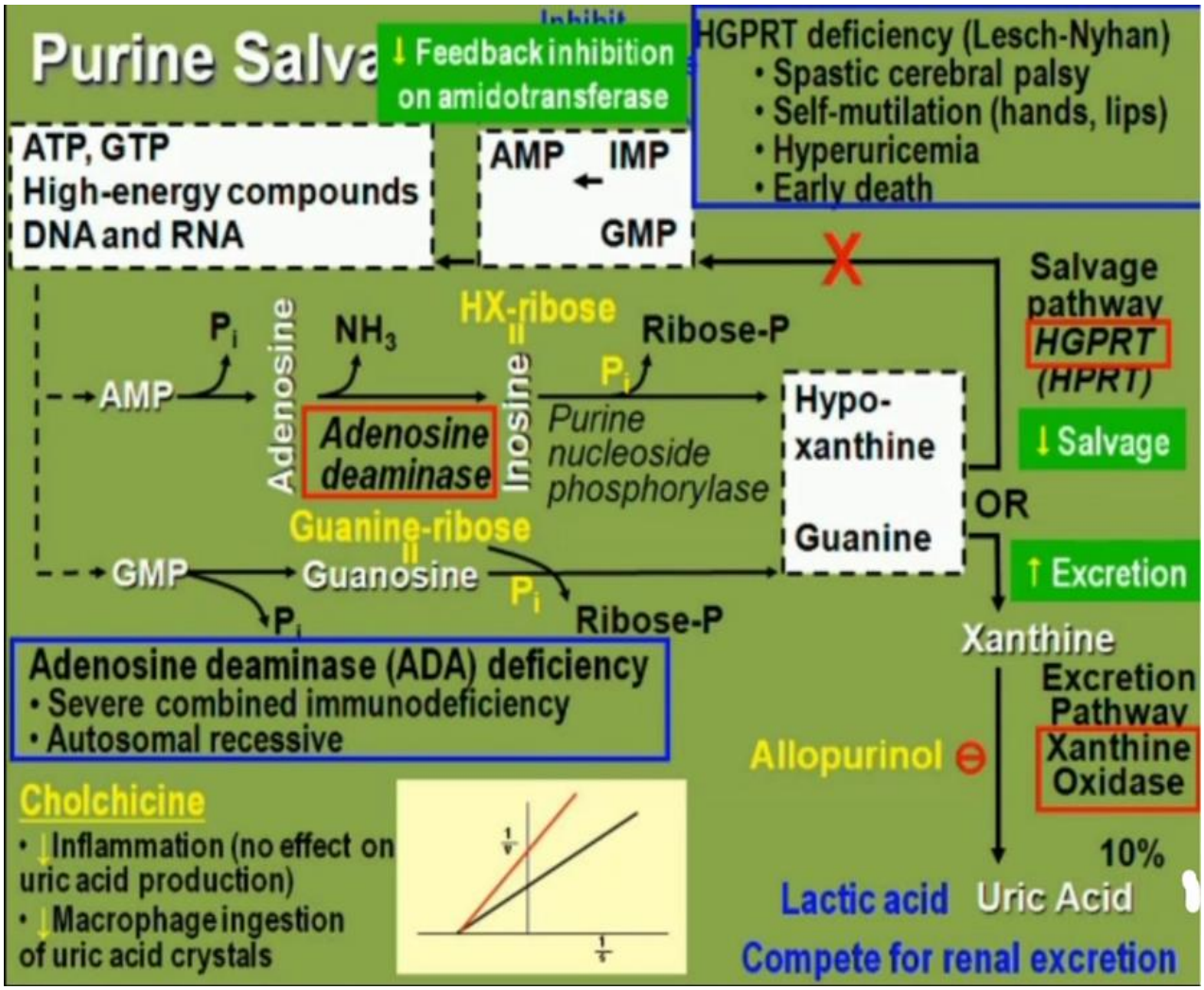
- Mycophenolate and ribavirin: inhibit inosine monophosphate dehydrogenase.

3. Purine and pyrimidine synthesis:

- Hydroxyurea: inhibits ribonucleotide reductase.



Purine salvage pathway



- Normally, the free purine bases (adenine, guanine, and hypoxanthine) generated during cellular metabolism of nucleic acids are reconverted into their corresponding nucleotides by phosphoribosylation in the purine salvage pathway.
- Salvage enzymes recycle normally about 90% of these purines, and 10% are converted to uric acid and excreted in urine.
- When purine catabolism is increased significantly, a person is at risk for developing hyperuricemia and potentially gout.
- Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) is the main enzyme of this pathway, converting hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate.

Adenosine deaminase deficiency

- Adenosine deaminase (ADA) is required for degradation of adenosine and deoxyadenosine.
- In ADA deficiency, ↑ dATP → lymphotoxicity.
- One of the major causes of autosomal recessive SCID.

Lesch-Nyhan syndrome

- Defective purine salvage due to absent HGPRT, which converts hypoxanthine to IMP and guanine to GMP. X-linked recessive.
- Results in excess uric acid production and de novo purine synthesis.
- PRPP is created by PRPP synthetase during the first step of de novo purine synthesis. The increased PRPP concentrations present in Lesch-Nyhan syndrome increase the activity of the downstream enzymes involved in de novo purine biosynthesis.
- Phosphoribosyl pyrophosphate amidotransferase acts upon PRPP to form phosphoribosylamine in the first committed step of de novo purine synthesis; therefore, its activity will be increased in Lesch-Nyhan syndrome secondary to the increased PRPP concentrations.
- Findings:

- Intellectual disability, compulsive self-mutilation, aggression, hyperuricemia (orange "sand" [sodium urate crystals] in diaper), gout, dystonia.
- Cells in the basal ganglia of the brain (fine motor control) normally have very high HPRT activity. Patients also all have hyperuricemia because purines cannot be salvaged, causing gout.

- Treatment: allopurinol or febuxostat (2nd line).
- HGPRT:

- Hyperuricemia, Gout.
- Pissed off (aggression, self-mutilation).
- Retardation (intellectual disability).
- DysTonia.

Metabolism sites

Mitochondria	Fatty acid oxidation (β-oxidation), acetyl-CoA production, TCA cycle, oxidative phosphorylation, ketogenesis.	
Cytoplasm	Glycolysis, HMP shunt, and synthesis of cholesterol (SER), proteins (ribosomes, RER), fatty acids, and nucleotides.	
Both	Heme synthesis, Urea cycle, Gluconeogenesis.	HUGs take two (both).

Rate-determining enzymes of metabolic processes

PROCESS	ENZYME	REGULATORS
Glycolysis	Phosphofructokinase-1 (PFK-1)	AMP ⊕, fructose-2,6-bisphosphate ⊕ ATP ⊖, citrate ⊖
Gluconeogenesis	Fructose-1,6-bisphosphatase	Citrate ⊕ AMP ⊖, fructose-2,6-bisphosphate ⊖
TCA cycle	Isocitrate dehydrogenase	ADP ⊕ ATP ⊖, NADH ⊖
Glycogenesis	Glycogen synthase	Glucose-6-phosphate ⊕, insulin ⊕, cortisol ⊕ Epinephrine ⊖, glucagon ⊖
Glycogenolysis	Glycogen phosphorylase	Epinephrine ⊕, glucagon ⊕, AMP ⊕ Glucose-6-phosphate ⊖, insulin ⊖, ATP ⊖
HMP shunt	Glucose-6-phosphate dehydrogenase (G6PD)	$NADP^+$ ⊕ NADPH ⊖
De novo pyrimidine synthesis	Carbamoyl phosphate synthetase II	ATP ⊕, PRPP ⊕ UTP ⊖
De novo purine synthesis	Glutamine-phosphoribosylpyrophosphate (PRPP) amidotransferase	AMP ⊖, inosine monophosphate (IMP) ⊖, GMP ⊖
Urea cycle	Carbamoyl phosphate synthetase I	N-acetylglutamate ⊕
Fatty acid synthesis	Acetyl-CoA carboxylase (ACC)	Insulin ⊕, citrate ⊕ Glucagon ⊖, palmitoyl-CoA ⊖
Fatty acid oxidation	Carnitine acyltransferase I	Malonyl-CoA ⊖
Ketogenesis	HMG-CoA synthase	
Cholesterol synthesis	HMG-CoA reductase	Insulin ⊕, thyroxine ⊕, estrogen ⊕ Glucagon ⊖, cholesterol ⊖

Enzyme terminology	An enzyme's name often describes its function. For example, glucokinase is an enzyme that catalyzes the phosphorylation of glucose using a molecule of ATP. The following are commonly used enzyme descriptors.
Kinase	Catalyzes transfer of a phosphate group from a high-energy molecule (usually ATP) to a substrate (eg, phosphofructokinase).
Phosphorylase	Adds inorganic phosphate onto substrate without using ATP (eg, glycogen phosphorylase).
Phosphatase	Removes phosphate group from substrate (eg, fructose-1,6-bisphosphatase).
Dehydrogenase	Catalyzes oxidation-reduction reactions (eg, pyruvate dehydrogenase).
Hydroxylase	Adds hydroxyl group (–OH) onto substrate (eg, tyrosine hydroxylase).
Carboxylase	Transfers CO_2 groups with the help of biotin (eg, pyruvate carboxylase).
Mutase	Relocates a functional group within a molecule (eg, vitamin B_{12}–dependent methylmalonyl-CoA mutase).
Synthase/synthetase	Joins two molecules together using a source of energy (eg, ATP, acetyl-CoA, nucleotide sugar).

Water soluble vitamins

- B1 (thiamine: TPP).
- B2 (riboflavin: FAD, FMN).
- B3 (niacin: NAD+).
- B5 (pantothenic acid: CoA).
- B6 (pyridoxine: PLP).
- B7 (biotin).
- B9 (folate).
- B12 (cobalamin).
- C (ascorbic acid).
- All wash out easily from body except B12 and B9 (folate).
- B12 stored in liver for ~ 3-4 years.
- B9 (Folic acid) stored in liver for ~ 3-4 months.
- B-complex deficiencies often result in dermatitis, glossitis, cheilosis, and diarrhea.
- Can be coenzymes (ascorbic acid) or precursors to organic cofactors (FAD, NAD).

Vitamin B1

- Also called thiamine.

- Function:

- In thiamine pyrophosphate (TPP), a cofactor for several dehydrogenase enzyme reactions:
  - Pyruvate dehydrogenase (links glycolysis to TCA cycle).
  - α-ketoglutarate dehydrogenase (TCA cycle).
  - Branched-chain α-ketoacid dehydrogenase.
  - Transketolase (HMP shunt).

- Think ATP: α-ketoglutarate dehydrogenase, Transketolase, and Pyruvate dehydrogenase.

- Deficiency:

- Thiamine deficiency is commonly seen in alcoholics, since alcohol interferes with thiamine absorption from the intestine.

- Impaired glucose breakdown → ATP depletion worsened by glucose infusion.

- Thiamine deficiency significantly impairs glucose oxidation, causing highly aerobic tissues (brain and cardiac muscle) to fail first. In addition, branched chain amino acids are sources of energy in brain and muscle.

- Thiamine deficiency is associated with infantile and adult beriberi, as well as Wernicke-Korsakoff syndrome in alcoholics.

- Manifestations of infantile beriberi appear between the ages of two and three months and include a fulminant cardiac syndrome with cardiomegaly, tachycardia, cyanosis, dyspnea, and vomiting.

- Adult beriberi is categorized as dry or wet:
  - Dry beriberi describes a symmetrical peripheral neuropathy accompanied by sensory and motor impairments, especially of the distal extremities.
  - Wet beriberi includes this neuropathy as well as cardiac involvement (cardiomegaly, cardiomyopathy, congestive heart failure, peripheral edema, tachycardia) owing to inadequate ATP and accumulation of ketoacids in the cardiac muscle. High-output congestive heart failure and neurological symptoms are strongly suggestive of wet beriberi (thiamine deficiency).
  - Spell beriberi as Ber1Ber1 to remember vitamin B1.

- Wernicke-Korsakoff syndrome (due to defective binding of transketolase with thiamine): confusion, ophthalmoplegia, ataxia (classic triad) + memory loss (permanent), confabulation, personality change. Damage to medial dorsal nucleus of thalamus, mammillary bodies.

- An increase in erythrocyte transketolase levels after thiamine infusion is diagnostic for thiamine deficiency (In actual practice, if a patient might be an alcoholic or appears to be very malnourished, presume that the patient is thiamine deficient and give thiamine supplementation with glucose infusion).

- Alcoholics are classically deficient in thiamine. Administration of glucose to these patients without first administering a thiamine supplement can result in acute neurological complications that are characteristic for Wernicke encephalopathy, including confusion, ophthalmoplegia, and ataxia. Treatment with thiamine usually results in prompt resolution of symptoms.

Wernicke encephalopathy	
Associated conditions	• Chronic alcoholism (most common) • Malnutrition (eg, anorexia nervosa) • Hyperemesis gravidarum
Pathophysiology	• Thiamine deficiency
Clinical features	• **Encephalopathy** • **Oculomotor dysfunction** (eg, horizontal nystagmus, bilateral abducens palsy) • Postural & gait **ataxia**
Treatment	• Intravenous thiamine followed by glucose infusion

Vitamin B2

- Also called riboflavin.
- FAD and FMN are derived from riboflavin.
- Function:

- The riboflavin-containing coenzymes are key constituents of the electron transport chain: FMN is a component of complex I, while FAD is a component of complex II.
- FAD is an electron carrier in the tricarboxylic acid cycle (TCA) and serves as a cofactor for succinate dehydrogenase, which is an enzyme that mediates the conversion of succinate into fumarate.

- Deficiency: Dermatitis, glossitis (swelling and redness of the tongue), and Cheilosis (inflammation of lips, scaling and fissures at the corners of the mouth), Corneal vascularization (new blood vessels growth in cornea that can cause vision problems).

Vitamin B3

- Also called niacin.
- Niacin is either obtained through dietary consumption (grains, fruits, vegetables, meats) or is synthesized endogenously from tryptophan.
- Synthesis requires vitamins B2 and B6.

- Function:

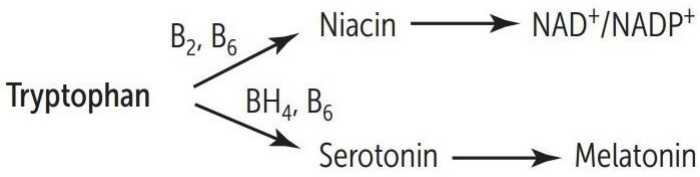
- Constituent of NAD, NADP (used in redox reactions).

- Used to treat dyslipidemia; lowers levels of VLDL and raises levels of HDL.

- Deficiency:

- Glossitis.

- Severe deficiency leads to pellagra, which can also be caused by Hartnup disease, malignant carcinoid syndrome (↑ tryptophan metabolism), and isoniazid (↓ vitamin B6).



- Tryptophan is an essential amino acid and a precursor for nicotinic acid, serotonin, and melatonin.

- Symptoms of pellagra:

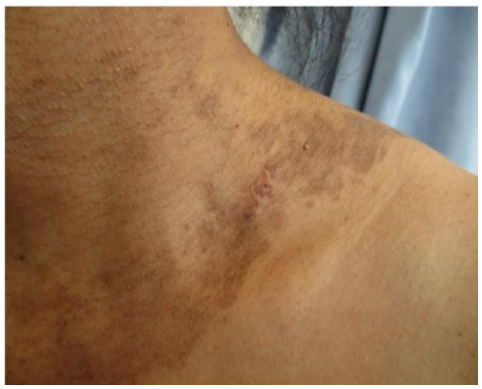
- Pellagra (which means "rough skin" in Italian vernacular) is a clinical syndrome arising secondary to niacin deficiency that is characterized by the "three Ds": dermatitis, diarrhea, and dementia.

- The 3 D's of B3.

- The dermatitis is usually bilateral and symmetric on the sun-exposed areas of the body, consisting of roughened, thickened, and scaly skin.

- The diarrhea arises as a result of columnar epithelium atrophy (and occasionally ulceration) of the gastrointestinal tract.

- The dementia develops secondary to neuronal degeneration in the brain and spinal cord, with lesions similar in appearance to those associated with pernicious anemia.



- Excess: Facial flushing (induced by prostaglandin, not histamine; can avoid by taking aspirin with niacin), hyperglycemia, hyperuricemia.

❖ Hartnup disease:

- Deficiency of neutral amino acid (tryptophan) transporters in proximal renal tubular cells and on enterocytes → neutral aminoaciduria and ↓ absorption from the gut → ↓ tryptophan for conversion to niacin→ pellagra-like symptoms. Autosomal recessive.

- The clinical manifestations of Hartnup disease are primarily due to the malabsorption of tryptophan, resulting in niacin (Vitamin B3) deficiency, because niacin is synthesized from tryptophan.

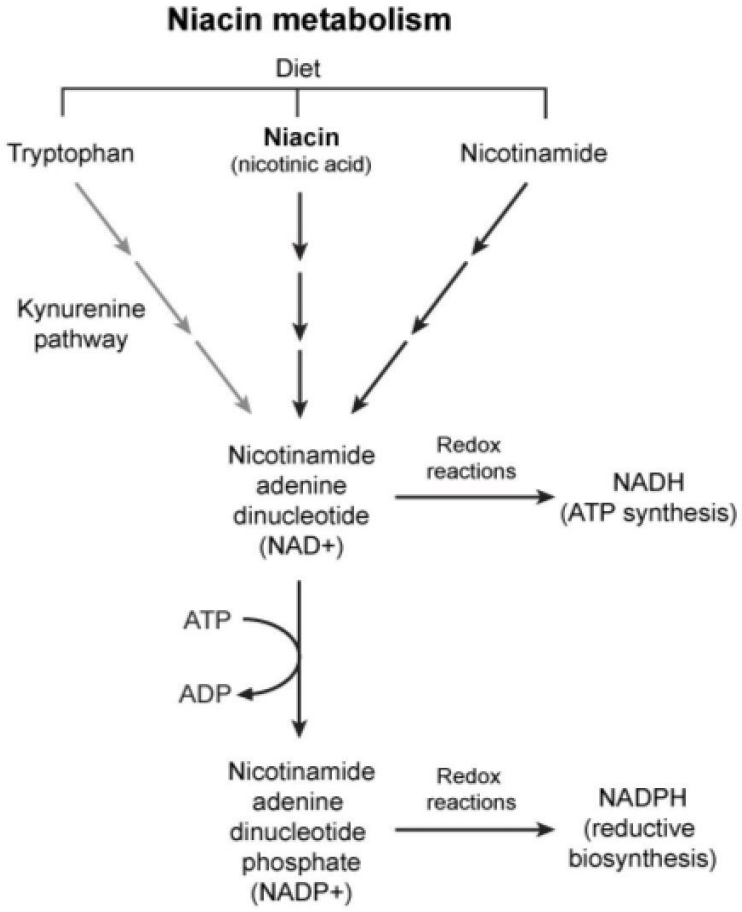
- Most children with Hartnup disease are asymptomatic, but some children experience photosensitivity and pellagra-like skin rashes.

- Neurologic involvement can occur most commonly leading to ataxia. Neurologic and skin symptoms typically wax and wane during the course of this disease.

- The main laboratory finding in Hartnup disease is aminoaciduria, restricted to the neutral amino acids (alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, and histidine).

- The urinary excretion of proline, hydroxyproline, and arginine remains unchanged, and this important finding differentiates Hartnup disease from other causes of generalized aminoaciduria such as Fanconi syndrome.

- Treatment with nicotinic acid or nicotinamide and a high-protein diet generally results in significant improvement of symptoms.



Vitamin B5

- Also called pantothenic acid.
- B5 is "pento"thenic acid.
- Function:

- Essential component of coenzyme A (CoA, a cofactor for acyl transfers) and fatty acid synthase.
- The biologically active form of pantothenic acid is coenzyme A, an essential cofactor in numerous acetylation reactions, including those associated with the tricarboxylic acid (TCA) cycle.
- Coenzyme A is particularly important in the first step of the TCA cycle, as it binds with oxaloacetate to form citrate and then succinyl-CoA.
- Coenzyme A is also important in the synthesis of vitamin A, vitamin D, cholesterol, steroids, heme, fatty acids, amino acids, and proteins.

- Deficiency: Dermatitis, enteritis, alopecia, adrenal insufficiency.

Vitamin B6

- Also called pyridoxine.
- Function:

- Converted to pyridoxal phosphate (PLP), a cofactor used in transamination (ALT and AST), decarboxylation reactions, glycogen phosphorylase.
- Synthesis of cystathionine, heme, niacin, histamine, and neurotransmitters including serotonin, epinephrine, norepinephrine (NE), dopamine, and GABA.

- Deficiency: Convulsions, hyperirritability, peripheral neuropathy (deficiency inducible by isoniazid and oral contraceptives), sideroblastic anemias (due to impaired hemoglobin synthesis and iron excess).

Vitamin B7

- Also called biotin.

- Function:
- Cofactor for carboxylation enzymes (which add a 1-carbon group):
  - Pyruvate carboxylase: pyruvate (3C) → oxaloacetate (4C).

  - Acetyl-CoA carboxylase: acetyl-CoA (2C) → malonyl-CoA (3C).

  - Propionyl-CoA carboxylase: propionyl-CoA (3C) → methylmalonyl-CoA (4C).

- Deficiency:
- Relatively rare.

- Dermatitis, enteritis, alopecia.

- Caused by antibiotic use or excessive ingestion of raw egg whites (due to the high levels of biotin-binding avidin in egg whites preventing its absorption).

- "Avidin in egg whites avidly binds biotin".

Vitamin B9

- Also called folate.

- Found in leafy green vegetables. Absorbed in jejunum.

- Small reserve pool stored primarily in the liver.

- Function:
- Converted to tetrahydrofolic acid (THF), a coenzyme for 1-carbon transfer/methylation reactions.

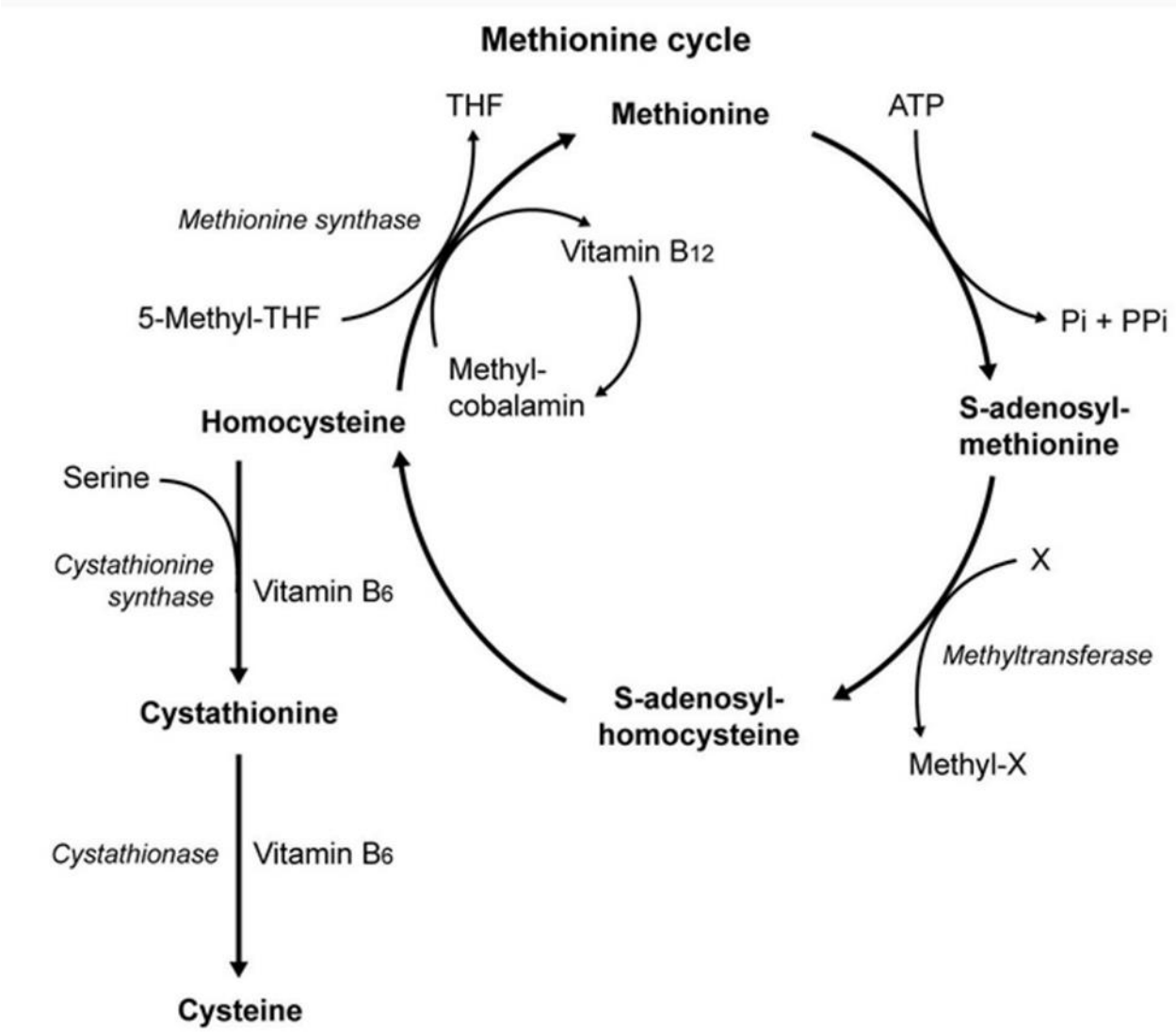
- Important for the synthesis of nitrogenous bases in DNA and RNA.

- Deficiency:
- Deficiency can be caused by several drugs (phenytoin, sulfonamides, methotrexate).

- Macrocytic, megaloblastic anemia; hypersegmented polymorphonuclear cells (PMNs); glossitis; no neurologic symptoms (as opposed to vitamin B12 deficiency).

- Labs: ↑ homocysteine, normal methylmalonic acid levels.

- Supplemental maternal folic acid at least 1 month prior to conception and during early pregnancy to ↓ risk of neural tube defects.
- Give vitamin B9 for the 9 months of pregnancy.

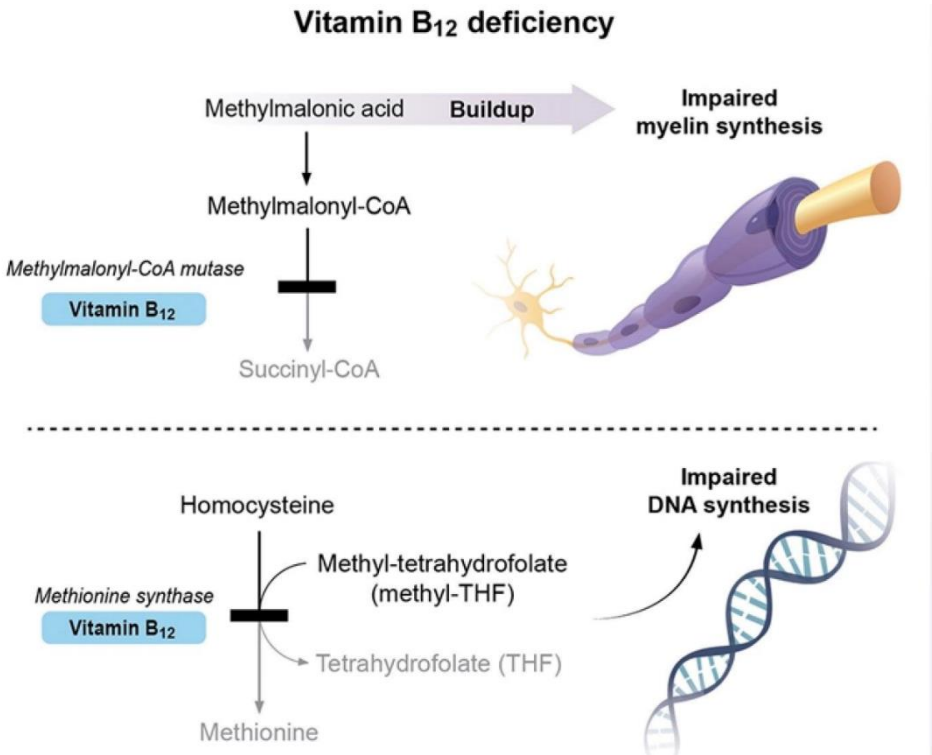


ATP = adenosine 5'-triphosphate; **Pi** = inorganic orthophosphate;
PPi = inorganic pyrophosphate (diphosphate); **THF** = tetrahydrofolate.

Vitamin B12

- Also called cobalamin.
- With the exception of vitamin B12, the body's stores of most water-soluble vitamins are rapidly depleted without dietary intake. In contrast, hepatic stores of vitamin B12 may last up to several years.
- Found in animal products.

- Synthesized only by microorganisms (gut flora).
- Very large reserve pool (several years) stored primarily in the liver.
- Absorption in terminal ileum (need intrinsic factor).
- Deficiency caused by malabsorption (sprue, enteritis, Diphyllobothrium latum, achlorhydria, bacterial overgrowth, alcohol excess), lack of intrinsic factor (pernicious anemia, gastric bypass surgery), absence of terminal ileum (surgical resection, for Crohn disease), or insufficient intake (strict vegetarian for years).
- <u>Function:</u>
  - Cofactor for methionine synthase (transfers CH_3 groups as methylcobalamin) and methylmalonyl-CoA mutase.
  - Important for DNA synthesis.
- <u>Deficiency:</u>
  - Macrocytic, megaloblastic anemia; hypersegmented PMNs; paresthesias and subacute combined degeneration (degeneration of dorsal columns, lateral corticospinal tracts, and spinocerebellar tracts) due to abnormal myelin.
  - Associated with ↑ serum homocysteine and methylmalonic acid levels, along with 2° folate deficiency.
- Prolonged deficiency → irreversible nerve damage.
- Folate supplementation can mask the hematologic symptoms of B12 deficiency, but not the neurologic symptoms.



Vitamin C

- Also called ascorbic acid.

- Vitamin C cannot be synthesized endogenously and therefore must be consumed in the human diet. This is typically not a problem, as ascorbic acid is abundantly found in fruits and vegetables (while also being present to a lesser extent in milk, liver, and fish).

- Deficiencies of vitamin C are therefore rare in developed countries but continue to be a concern in those with inconsistent eating patterns - including the elderly, alcoholics, and persons who live alone.

- Function:

- Antioxidant; also facilitates iron absorption by reducing it to Fe^2 state.

- Necessary for dopamine β-hydroxylase, which converts dopamine to NE.

- Vitamin C is also necessary for the hydroxylation of the proline and lysine residues of pro-collagen. This reaction is executed by prolyl and lysyl hydroxylases, with vitamin C serving as a reducing agent.

- Hydroxyproline and hydroxylysine are essential for cross-linking collagen molecules. In scurvy, collagen cross-linking is compromised, thus greatly reducing its strength.

- Deficiency:

- Scurvy:

  - Gum disease (gum swelling, loosening of the teeth, and periodontal infection), bruising, petechiae, hemarthrosis, anemia, poor wound healing, perifollicular and subperiosteal hemorrhages, "corkscrew hair".

  - The symptoms of vitamin C deficiency are the result of decreased connective tissue strength. The capillary walls are especially fragile, causing easy bruising and a propensity to bleed.

  - Vitamin C deficiency is even more severe in children. Here, hemorrhages may cause subperiosteal and joint hematomas.

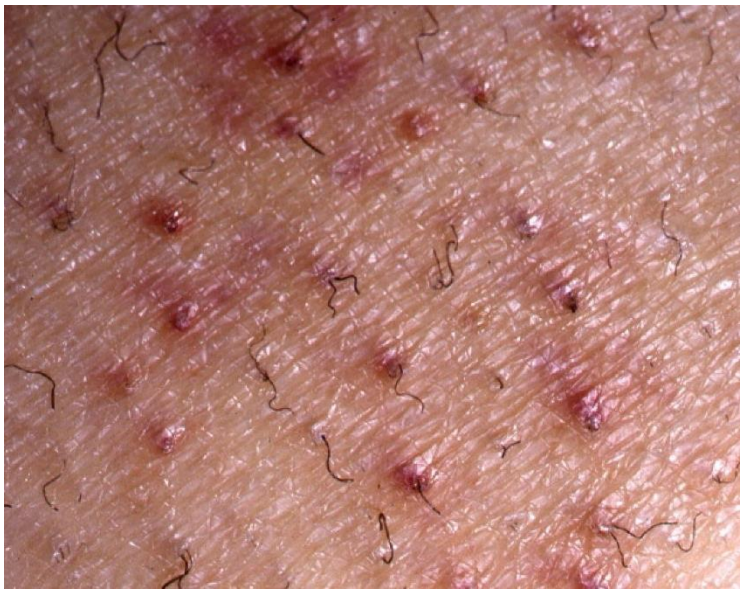
  - Weakened immune response.

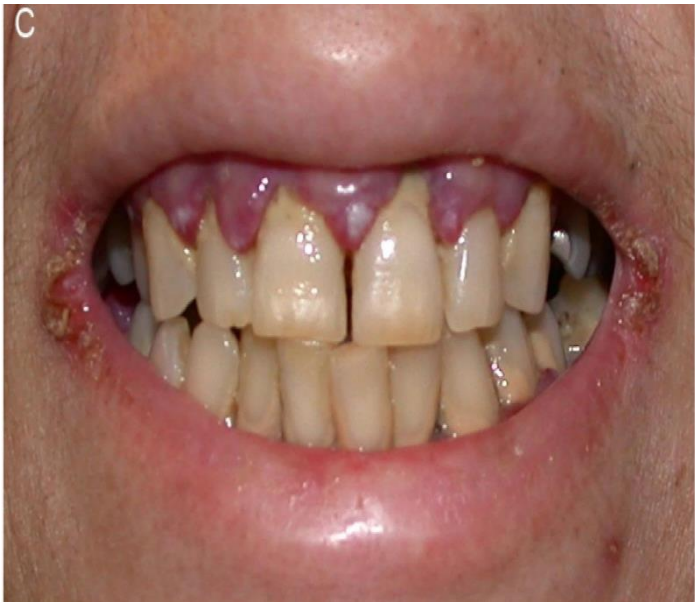
  - Vitamin C deficiency causes sCurvy due to a Collagen synthesis defect.

- Excess:

- Nausea, vomiting, diarrhea, fatigue, calcium oxalate nephrolithiasis.

- Can ↑ iron toxicity in predisposed individuals by increasing dietary iron absorption (can worsen hereditary hemochromatosis or transfusion-related iron overload).

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Fat soluble vitamins

- A, D, E, K.
- Absorption dependent on gut and pancreas.
- Toxicity more common than for water-soluble vitamins because fat-soluble vitamins accumulate in fat.
- Malabsorption syndromes with steatorrhea (cystic fibrosis and celiac disease) or mineral oil intake can cause fat-soluble vitamin deficiencies.

Vitamin A

- Also called retinol.
- Found in liver and leafy vegetables.
- Function:

- Antioxidant.
- Constituent of visual pigments (retinal).
- Essential for normal differentiation of epithelial cells into specialized tissue and prevents squamous metaplasia (retinol).
- Control and limit keratin production.
- Used to treat measles and acute promyelocytic leukemia (all-trans retinoic acid to treat acute promyelocytic leukemia).
- The WHO recommends that vitamin A be administered to all children with measles in areas with widespread vitamin A deficiency or in areas with a measles mortality rate in excess of one percent.

- Deficiency:

- Although rare in the United States and other industrialized nations, vitamin A deficiency is relatively common in Asia, Africa, and South America.
- Malnourishment and fat malabsorption (cystic fibrosis, cholestatic liver disease) are the most significant causes of vitamin A deficiency.
- Clinical manifestations of the condition include night blindness, complete blindness, and xerophthalmia.

- More unusual findings include Bitot's spots (abnormal squamous cell proliferation and keratinization of the conjunctiva), keratomalacia, nonspecific dermatologic abnormalities, and humoral and cell-mediated immune system inhibition via damage done to phagocytes and T cell lymphocytes.

- Death can result if the condition is untreated.

▪ Excess:

- Individuals who consume more than 10 times the Daily Value (Recommended Dietary Allowance) of vitamin A are prone to developing toxicity and may suffer hepatic injury so severe as to cause cirrhosis.

- Vitamin A toxicity has been subdivided into three syndromes (acute, chronic, and teratogenic):

A. The signs and symptoms of acute toxicity occur after the ingestion of a single high dose of vitamin A and include nausea, vomiting, vertigo, and blurred vision.

B. The signs and symptoms of chronic toxicity occur after the long-term ingestion of high doses of vitamin A, and include alopecia, dry skin, hyperlipidemia, hepatotoxicity, hepatosplenomegaly, and visual difficulties. Papilledema, when present, is suggestive of cerebral edema in the setting of benign intracranial hypertension (pseudotumor cerebri).

C. Teratogenic: (cleft palate, cardiac abnormalities), therefore a ⊖ pregnancy test and two forms of contraception are required before isotretinoin (vitamin A derivative) is prescribed.

- Isotretinoin is teratogenic.

▪ Use oral isotretinoin to treat severe cystic acne.

▪ Retinol is vitamin A, so think retin-A (used topically for wrinkles and Acne).

Vitamin D

▪ D_3 (cholecalciferol) from exposure of skin to sun, ingestion of fish, milk, plants.

▪ D_2 (ergocalciferol) from ingestion of plants, fungi, yeasts.

▪ Both converted to 25-OH D3 (storage form) in liver and to the active form 1,25-(OH)2 D_3 (calcitriol) in kidney.

▪ Function:

- ↑ intestinal absorption of Ca and PO_4.

- ↑ bone mineralization at low levels.

- ↑ bone resorption at higher levels.

- Regulation:
- ↑ PTH, ↓ Ca, ↓ PO4 →↑ 1,25-$(OH)_2$ D_3 production.
- 1,25-$(OH)_2$ D_3 feedback inhibits its own production.
- ↑ PTH → ↑ Ca reabsorption and ↓ PO4 reabsorption in the kidney.

- Deficiency:
- Caused by malabsorption, ↓ sun exposure, poor diet, chronic kidney disease.
- Rickets in children (deformity, such as genu varum "bow legs"), osteomalacia in adults (bone pain and muscle weakness), hypocalcemic tetany.
- Give oral vitamin D to breastfed infants.
- Deficiency is exacerbated by pigmented skin, premature birth.

- Excess:
- Hypercalcemia, hypercalciuria, loss of appetite, stupor.
- Seen in granulomatous disease (↑ activation of vitamin D by epithelioid macrophages).

Vitamin E

- Includes tocopherol, tocotrienol.

- Function:
- Antioxidant (protects RBCs and membranes from free radical damage).
- High-dose supplementation may alter metabolism of vitamin K → enhanced anticoagulant effects of warfarin.

- Deficiency:
- Hemolytic anemia, acanthocytosis, muscle weakness, posterior column and spinocerebellar tract demyelination.
- Neurologic presentation may appear similar to vitamin B12 deficiency, but without megaloblastic anemia, hypersegmented neutrophils, or ↑ serum methylmalonic acid levels.

- Excess: Risk of enterocolitis in infants.

Vitamin K

- Includes phytomenadione, phylloquinone, phytonadione, menaquinone.
- In a healthy liver, vitamin K is efficiently recycled such that the daily dietary requirement is minimal.
- The endogenous colonic bacterial flora also generates vitamin K, providing a generous supplement to the dietary intake.
- Foods that contain significant amounts of this fat-soluble vitamin include the dark green vegetables, green tea, and beef liver.
- Four patient populations are most likely to develop vitamin K deficiency: those suffering from malabsorption syndromes; those who have taken broad-spectrum antibiotics that destroy intestinal flora; neonates (secondary to their limited hepatic reserves, unestablished intestinal flora, and the limited bioavailability of vitamin K in breast milk); and those afflicted with generalized liver disease.
- Function:
  - Activated by epoxide reductase to the reduced form, which is a cofactor for the γ-carboxylation of glutamic acid residues on clotting factors II, VII, IX, X, and proteins C and S.
  - Warfarin inhibits vitamin K-dependent synthesis of these factors and proteins.
- Deficiency:
  - Neonatal hemorrhage with ↑ PT and ↑ aPTT but normal bleeding time (neonates have sterile intestines and are unable to synthesize vitamin K).
  - Not in breast milk; neonates are given vitamin K injection at birth to prevent hemorrhagic disease of the newborn.
  - Can also occur after prolonged use of broad-spectrum antibiotics.
  - Vitamin K deficiency eventually leads to a bleeding diathesis in which patients can develop hemorrhage, hematomas, hematuria, melena, ecchymoses, and gingival bleeding. The bleeding may arise at any site but is particularly common in the skin, umbilicus, viscera, and brain.

Zinc

- Function:
  - Mineral essential for the activity of 100+ enzymes.
  - Important in the formation of zinc fingers (transcription factor motif).

- Deficiency:

- A Delayed wound healing, suppressed immunity, hypogonadism, ↓ adult hair (axillary, facial, pubic), dysgeusia, anosmia, acrodermatitis enteropathica.

- May predispose to alcoholic cirrhosis.

Protein-energy malnutrition

Kwashiorkor

- Protein malnutrition resulting in skin lesions (hyperkeratosis, dyspigmentation), edema due to ↓ plasma oncotic pressure, liver malfunction (fatty change due to ↓ apolipoprotein synthesis).

- Clinical picture is small child with swollen abdomen (A).

- Kwashiorkor results from protein-deficient MEALS:

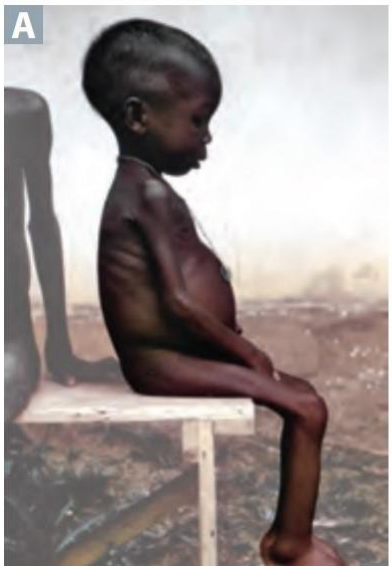
- Malnutrition.
- Edema.
- Anemia.
- Liver (fatty).
- Skin lesions (hyperkeratosis, dyspigmentation).

Marasmus

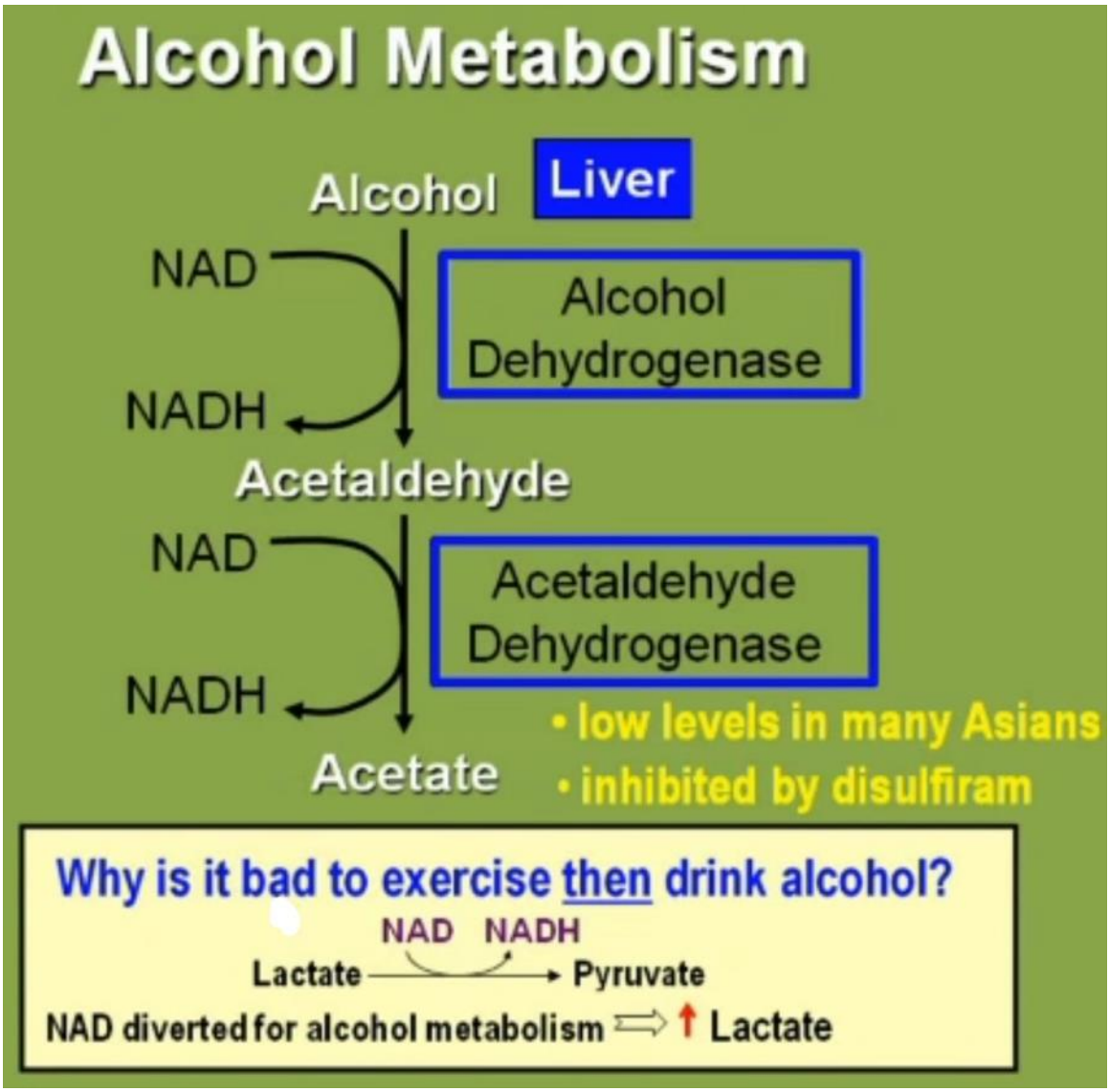
- Diet is deficient in calories but no nutrients are entirely absent.

- Malnutrition not causing edema.

- Marasmus results in Muscle wasting (B).



Ethanol metabolism



- For every equivalent of ethanol metabolized to acetaldehyde by alcohol dehydrogenase and then to acetate by aldehyde dehydrogenase, two equivalents of NAD are reduced to NADH.
- The resulting high intracellular ratio of NADH to NAD favors the conversion of pyruvate to lactate and oxaloacetate to malate. Pyruvate and oxaloacetate are intermediates in gluconeogenesis; conversion of these molecules to lactate and malate, respectively, inhibits gluconeogenesis.
- Because alcoholics tend to have poor nutrition, their glucose intake is generally low. Low glucose intake coupled with alcohol-induced inhibition of gluconeogenesis and baseline low glycogen stores all contribute to alcohol-induced hypoglycemia in alcoholics and binge drinkers.
- Additionally, ↑ NADH/NAD ratio disfavors TCA production of NADH → ↑ utilization of acetyl-CoA for ketogenesis → ketoacidosis and lipogenesis→ hepatosteatosis.

- FOMEpizole: inhibits alcohol dehydrogenase and is an antidote For Overdoses of Methanol or Ethylene glycol.

- Disulfiram: inhibits acetaldehyde dehydrogenase (acetaldehyde accumulates, contributing to hangover symptoms), discouraging drinking.

- Alcohol dehydrogenase operates via zero-order kinetics.

- Ethanol metabolism ↑ NADH/NAD ratio in liver, causing:

1. Lactic acidosis: ↑ Pyruvate → lactate.

2. Fasting hypoglycemia: ↑ Oxaloacetate → malate (prevents gluconeogenesis).

3. Ketoacidosis: diversion of acetyl-CoA into ketogenesis rather than TCA cycle

4. Hepatosteatosis: ↑ Dihydroxyacetone phosphate → glycerol- 3-phosphate (combines with fatty acids to make triglycerides → hepatosteatosis).

- Accumulation of cytoplasmic NADH and glycerol 3-P may also contribute to lipid accumulation in alcoholic liver disease. Free fatty acids released from adipose in part enter the liver where β-oxidation is very slow (high NADH). In the presence of high glycerol 3-P, fatty acids are inappropriately stored in the liver as triglyceride.

Ethanol metabolism