

## HIGH-YIELD PRINCIPLES IN

# Biochemistry

*“The nitrogen in our DNA, the calcium in our teeth, the iron in our blood, the carbon in our apple pies were made in the interiors of collapsing stars. We are made of starstuff.”*

—Carl Sagan

*“Biochemistry is the study of carbon compounds that crawl.”*

—Mike Adams

*“The power to control our species’ genetic future is awesome and terrifying.”*

—A Crack in Creation

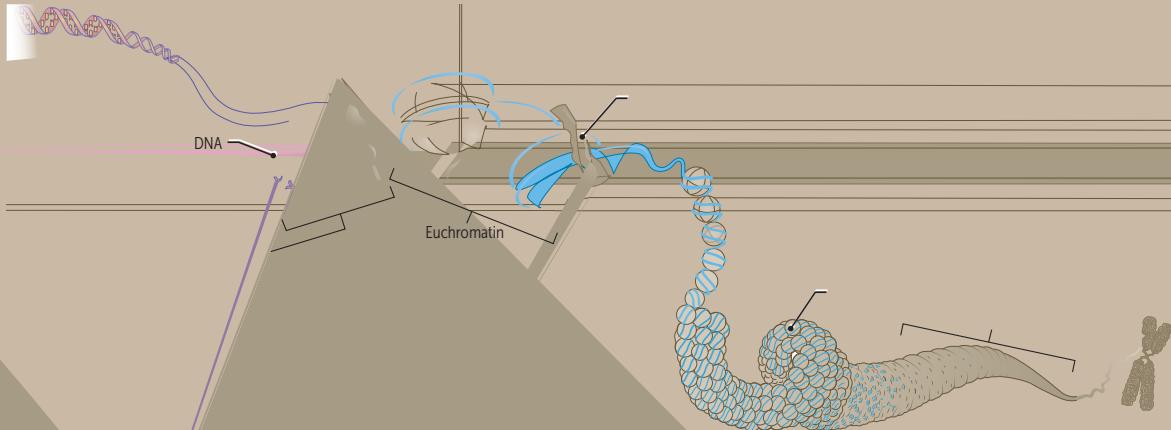
*“Nothing in this world is to be feared, it is only to be understood.”*

—Marie Curie

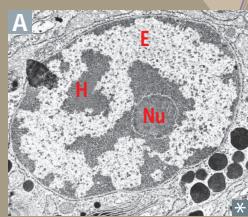
This high-yield material includes molecular biology, genetics, cell biology, and principles of metabolism (especially vitamins, cofactors, minerals, and single-enzyme-deficiency diseases). When studying metabolic pathways, emphasize important regulatory steps and enzyme deficiencies that result in disease, as well as reactions targeted by pharmacologic interventions. For example, understanding the defect in Lesch-Nyhan syndrome and its clinical consequences is higher yield than memorizing every intermediate in the purine salvage pathway.

Do not spend time learning details of organic chemistry, mechanisms, or physical chemistry. Detailed chemical structures are infrequently tested; however, many structures have been included here to help students learn reactions and the important enzymes involved. Familiarity with the biochemical techniques that have medical relevance—such as ELISA, immunoelectrophoresis, Southern blotting, and PCR—is useful. Review the related biochemistry when studying pharmacology or genetic diseases as a way to reinforce and integrate the material.

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### Heterochromatin



Condensed, appears darker on EM (labeled H in **A**; Nu, nucleolus). Sterically inaccessible, thus transcriptionally inactive. ↑ methylation, ↓ acetylation.

**Heterochromatin = highly condensed.**

Barr bodies (inactive X chromosomes) may be visible on the periphery of nucleus.

### Euchromatin

Less condensed, appears lighter on EM (labeled E in **A**). Transcriptionally active, sterically accessible.

*Eu* = true, “truly transcribed.”

Euchromatin is **expressed**.

### DNA methylation

Changes the expression of a DNA segment without changing the sequence. Involved with aging, carcinogenesis, genomic imprinting, transposable element repression, and X chromosome inactivation (lyonization).

DNA is methylated in imprinting.

Methylation within gene promoter (CpG islands) typically represses (silences) gene transcription.

CpG **methylation makes DNA mute.**

Dysregulated DNA methylation is implicated in Fragile X syndrome.

Histone **methylation mostly makes DNA mute.** Lysine and arginine residues of histones can be methylated.

Thyroid hormone synthesis is altered by acetylation of thyroid hormone receptor. Histone **acetylation makes DNA active.**

Histone deacetylation may be responsible for altered gene expression in Huntington disease. Histone **deacetylation deactivates DNA.**

### Histone methylation

Usually causes reversible transcriptional suppression, but can also cause activation depending on location of methyl groups.

Histone **methylation mostly makes DNA mute.** Lysine and arginine residues of histones can be methylated.

### Histone acetylation

Removal of histone's + charge → relaxed DNA coiling → ↑ transcription.

Thyroid hormone synthesis is altered by acetylation of thyroid hormone receptor. Histone **acetylation makes DNA active.**

### Histone deacetylation

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

Histone deacetylation may be responsible for altered gene expression in Huntington disease. Histone **deacetylation deactivates DNA.**

**Nucleotides**

Nucleoside = base + (deoxy)ribose (sugar).

Nucleotide = base + (deoxy)ribose + phosphate;  
linked by 3'-5' phosphodiester bond.

5' end of incoming nucleotide bears the triphosphate (energy source for the bond).  
 $\alpha$ -Phosphate is target of 3' hydroxyl attack.

**Purines (A,G)**—2 rings.

**Pyrimidines (C,U,T)**—1 ring.

Deamination reactions:

Cytosine  $\rightarrow$  uracil

Adenine  $\rightarrow$  hypoxanthine

Guanine  $\rightarrow$  xanthine

5-methylcytosine  $\rightarrow$  thymine

**Pure As Gold.**

**CUT** the pyramid.

**Thymine has a methyl.**

C-G bond (3 H bonds) stronger than A-T bond  
(2 H bonds).  $\uparrow$  C-G content  $\rightarrow$   $\uparrow$  melting temperature of DNA. “**C-G** bonds are like **Crazy Glue**.”

Uracil found in RNA; thymine in DNA.

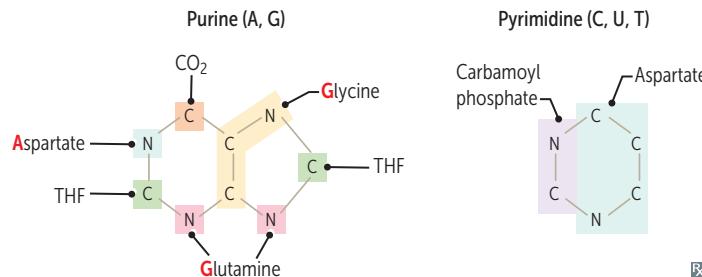
Methylation of uracil makes thymine.

Amino acids necessary for **purine** synthesis (cats **purr** until they **GAG**):

**Glycine**

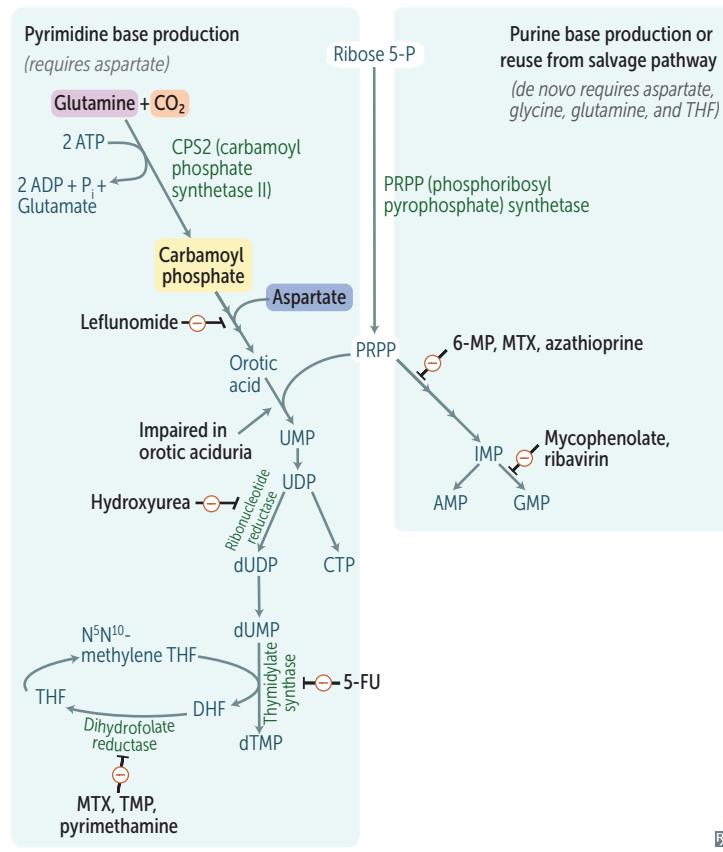
**Aspartate**

**Glutamine**



## De novo pyrimidine and purine synthesis

Various immunosuppressive, antineoplastic, and antibiotic drugs function by interfering with nucleotide synthesis:



### Pyrimidine synthesis:

- **Leflunomide:** inhibits dihydroorotate dehydrogenase
- **5-fluorouracil (5-FU)** and its prodrug **capecitabine:** form 5-F-dUMP, which inhibits thymidylate synthase ( $\downarrow$  dTMP)

### Purine synthesis:

- **6-mercaptopurine (6-MP)** and its prodrug **azathioprine:** inhibit de novo purine synthesis (guanine phosphoribosyltransferase); azathioprine is metabolized via purine degradation pathway and can lead to immunosuppression when administered with xanthine oxidase inhibitor
- **Mycophenolate** and **ribavirin:** inhibit inosine monophosphate dehydrogenase

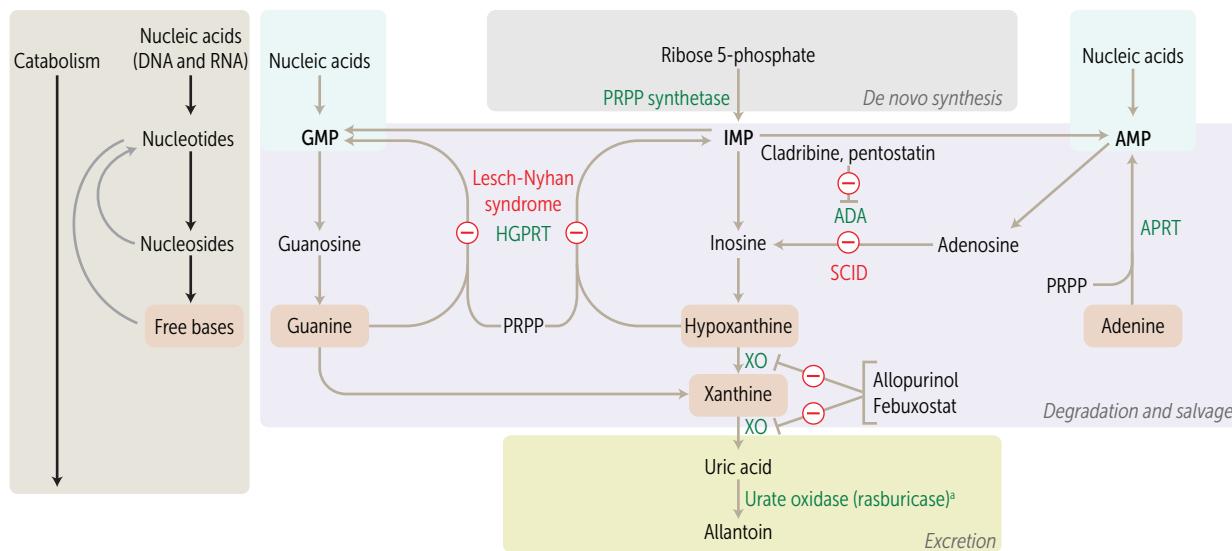
### Purine and pyrimidine synthesis:

- **Hydroxyurea:** inhibits ribonucleotide reductase
- **Methotrexate (MTX)**, **trimethoprim (TMP)**, and **pyrimethamine:** inhibit dihydrofolate reductase ( $\downarrow$  deoxythymidine monophosphate [dTDP]) in humans (methotrexate), bacteria (trimethoprim), and protozoa (pyrimethamine)

CPS1 = m1ochondria, urea cycle, found in liver

CPS2 = cytwoesol, pyrimidine synthesis, found in most cells

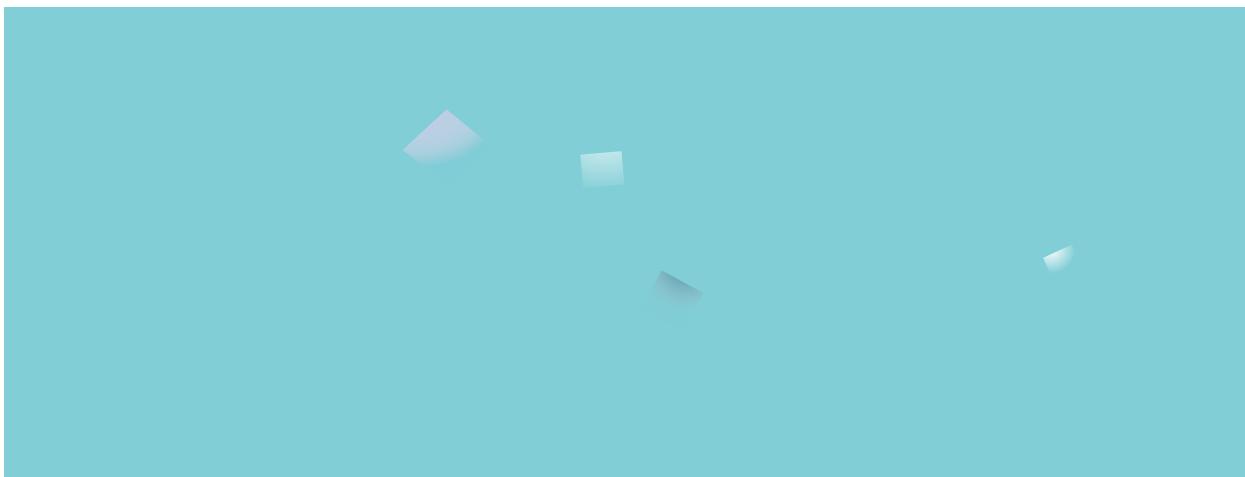
### Purine salvage de ciancias



<sup>a</sup>Absent in humans.

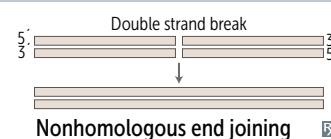
ADA, adenosine deaminase; APRT, adenine phosphoribosyltransferase; HGPRT, hypoxanthine guanine phosphoribosyltransferase; XO, xanthine oxidase; SCID, severe combined immune deficiency (autosomal recessive inheritance)

<b>DNA replication</b>	Occurs in <b>5' → 3'</b> direction ("5ynth3sis") in continuous and discontinuous (Okazaki fragment) fashion. Semiconservative. More complex in eukaryotes than in prokaryotes, but shares analogous enzymes.	
<b>Origin of replication A</b>	Particular consensus sequence in genome where DNA replication begins. May be single (prokaryotes) or multiple (eukaryotes).	AT-rich sequences (eg, TATA box regions) are found in promoters (often upstream) and origins of replication (ori).
<b>Replication fork B</b>	Y-shaped region along DNA template where leading and lagging strands are synthesized.	
<b>Helicase C</b>	Unwinds DNA template at replication fork.	<b>Helicase</b> halves DNA. Deficient in <b>Bloom</b> syndrome ( <b>BLM</b> gene mutation).
<b>Single-stranded binding proteins D</b>	Prevent strands from reannealing or degradation by nucleases.	
<b>DNA topoisomerases E</b>	Creates a <b>single-</b> (topoisomerase I) or <b>double-</b> (topoisomerase II) stranded break in the helix to add or remove supercoils (as needed due to underwinding or overwinding of DNA).	In eukaryotes: irinotecan/topotecan inhibit topoisomerase (TOP) I, etoposide/teniposide inhibit TOP II. In prokaryotes: fluoroquinolones inhibit TOP II (DNA gyrase) and TOP IV.
<b>Primase F</b>	Makes RNA primer for DNA polymerase III to initiate replication.	
<b>DNA polymerase III G</b>	Prokaryotes only. Elongates leading strand by adding deoxynucleotides to the 3' end. Elongates lagging strand until it reaches primer of preceding fragment.	DNA polymerase III has <b>5' → 3'</b> synthesis and proofreads with <b>3' → 5'</b> exonuclease. Drugs blocking DNA replication often have a modified 3' OH, thereby preventing addition of the next nucleotide ("chain termination").
<b>DNA polymerase I H</b>	Prokaryotes only. Degrades RNA primer; replaces it with DNA.	Same functions as DNA polymerase III, also excises RNA primer with <b>5' → 3'</b> exonuclease.
<b>DNA ligase I</b>	Catalyzes the formation of a phosphodiester bond within a strand of double-stranded DNA.	Joins Okazaki fragments. <b>Ligase</b> links DNA.
<b>Telomerase</b>	Eukaryotes only. A reverse transcriptase (RNA-dependent DNA polymerase) that adds DNA ( <b>TTAGGG</b> ) to 3' ends of chromosomes to avoid loss of genetic material with every duplication.	Upregulated in progenitor cells and also often in cancer; downregulated in aging and progeria. <b>Telomerase</b> <b>TAGs</b> for <b>G</b> reatness and <b>G</b> lory.

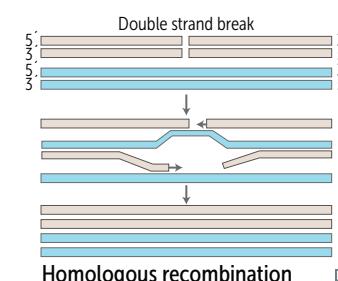


**DNA repair****Double strand****Nonhomologous end joining**

Brings together 2 ends of DNA fragments to repair double-stranded breaks.  
Homology not required. Part of the DNA may be lost or translocated.  
May be dysfunctional in ataxia telangiectasia.

**Homologous recombination**

Requires 2 homologous DNA duplexes. A strand from damaged dsDNA is repaired using a complementary strand from intact homologous dsDNA as a template.  
Defective in breast/ovarian cancers with *BRCA1* or *BRCA2* mutations and in Fanconi anemia.  
Restores duplexes accurately without loss of nucleotides.

**Single strand****Nucleotide excision repair**

Specific endonucleases remove the oligonucleotides containing damaged bases; DNA polymerase and ligase fill and reseal the gap, respectively. Repairs bulky helix-distorting lesions (eg, pyrimidine dimers).

Occurs in G<sub>1</sub> phase of cell cycle.  
Defective in **xeroderma pigmentosum** (inability to repair DNA pyrimidine dimers caused by UV exposure). Presents with dry skin, photosensitivity, skin cancer.

**Base excision repair**

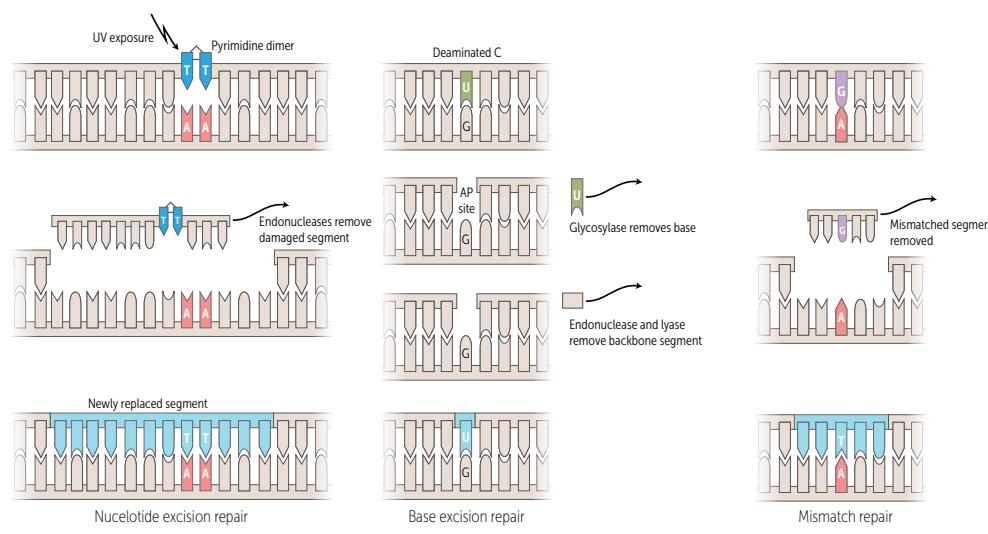
Base-specific **Glycosylase** removes altered base and creates AP site (apurinic/apyrimidinic). One or more nucleotides are removed by **AP-Endonuclease**, which cleaves 5' end. **AP-Lyase** cleaves 3' end. DNA **Polymerase-β** fills the gap and **DNA ligase** seals it.

Occurs throughout cell cycle.  
Important in repair of spontaneous/toxic deamination.  
**“GEL Please.”**

**Mismatch repair**

Mismatched nucleotides in newly synthesized strand are removed and gap is filled and resealed.

Occurs predominantly in S phase of cell cycle.  
Defective in Lynch syndrome (hereditary nonpolyposis colorectal cancer [HNPPC]).



**Mutations in DNA**

Degree of change: silent << missense < nonsense < frameshift. Single nucleotide substitutions are repaired by DNA polymerase and DNA ligase. Types of single nucleotide (point) mutations:

- **Transition**—purine to purine (eg, A to G) or pyrimidine to pyrimidine (eg, C to T).
- **Transversion**—purine to pyrimidine (eg, A to T) or pyrimidine to purine (eg, C to G).

**Single nucleotide substitutions**

**Silent mutation** Codes for same (synonymous) amino acid; often involves 3rd position of codon (tRNA wobble).

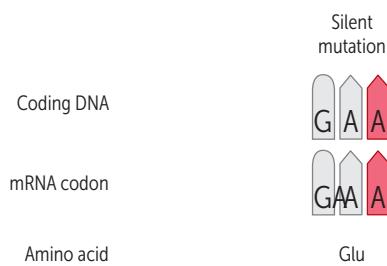
**Missense mutation** Results in changed amino acid (called conservative if new amino acid has similar chemical structure). Examples: sickle cell disease (substitution of glutamic acid with valine).

**Nonsense mutation** Results in early **stop** codon (UGA, UAA, UAG). Usually generates nonfunctional protein. **Stop the nonsense!**

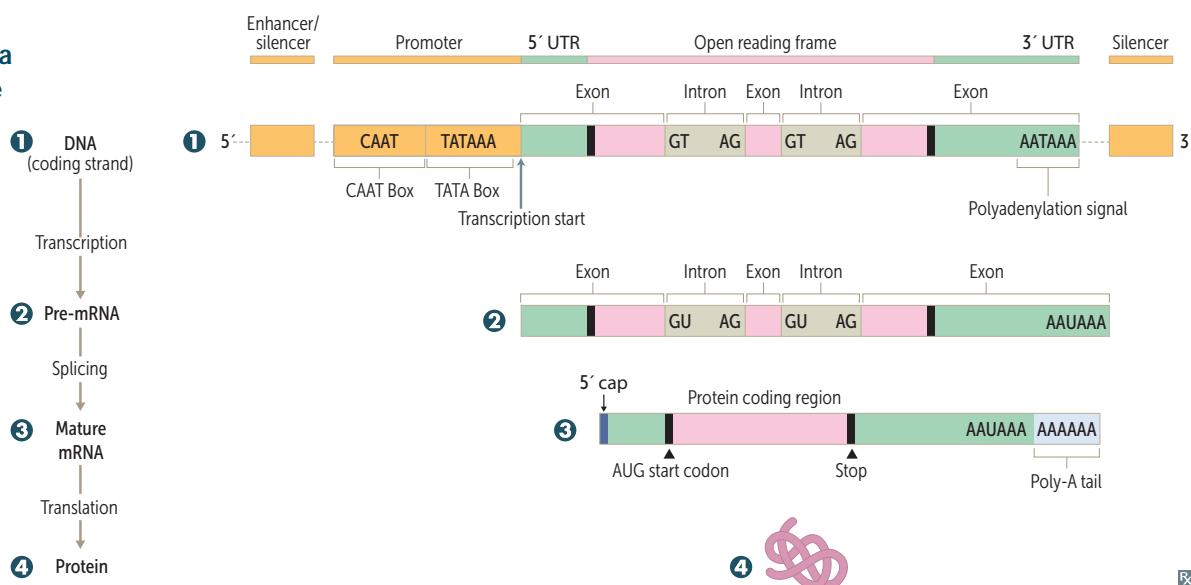
**Other mutations**

**Frameshift mutation** Deletion or insertion of any number of nucleotides not divisible by 3 → misreading of all nucleotides downstream. Protein may be shorter or longer, and its function may be disrupted or altered. Examples: Duchenne muscular dystrophy, Tay-Sachs disease, cystic fibrosis.

**Splice site mutation** Retained intron in mRNA → protein with impaired or altered function. Examples: rare causes of cancers, dementia, epilepsy, some types of β-thalassemia, Gaucher disease, Marfan syndrome.



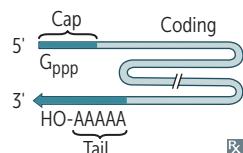
### Functional organization of a eukaryotic gene



### Regulation of gene expression

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, which differ between eukaryotes and prokaryotes). Promoters increase ori activity.	Promoter mutation commonly results in dramatic ↓ in level of gene transcription.
Enhancer	DNA locus where regulatory proteins (“activators”) bind, <b>increasing</b> 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 they regulate.
Silencer	DNA locus where regulatory proteins (“repressors”) bind, <b>decreasing</b> expression of a gene on the same chromosome.	
Epigenetics	Changes made to gene expression (heritable mitotically/meiotically) without a change in underlying DNA sequence.	Primary mechanisms of epigenetic change include DNA methylation, histone modification, and noncoding RNA.

### RNA processing (eukaryotes)



Initial transcript is called heterogeneous nuclear RNA (hnRNA). hnRNA is then modified and becomes mRNA.

The following processes occur in the nucleus:

- Capping of 5' end (addition of 7-methylguanosine cap; cotranscriptional)
- Polyadenylation of 3' end (~200 As → poly-A tail; posttranscriptional)
- Splicing out of introns (posttranscriptional)

Capped, tailed, and spliced transcript is called mRNA.

mRNA is transported out of nucleus to be translated in cytosol.

mRNA quality control occurs at cytoplasmic processing bodies (P-bodies), which contain exonucleases, decapping enzymes, and microRNAs; mRNAs may be degraded or stored in P-bodies for future translation.

Poly-A polymerase does not require a template. AAUAAA = polyadenylation signal. Mutation in polyadenylation signal → early degradation prior to translation.

Kozak sequence—initiation site in most eukaryotic mRNA. Facilitates binding of small subunit of ribosome to mRNA. Mutations in sequence → impairment of initiation of translation → ↓ protein synthesis.

### RNA polymerases

#### Eukaryotes

RNA polymerase I makes rRNA, the most common (**rampant**) type; present only in nucleolus.

RNA polymerase II makes mRNA (**massive**), microRNA (**miRNA**), and small nuclear RNA (**snRNA**).

RNA polymerase III makes 5S rRNA, tRNA (**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.

$\alpha$ -amanitin, found in *Amanita phalloides* (death cap mushrooms), inhibits RNA polymerase II. Causes dysentery and severe hepatotoxicity if ingested.

Dactinomycin inhibits RNA polymerase in both prokaryotes and eukaryotes.

#### Prokaryotes

I RNA polymerase (multisubunit complex) makes all 3 kinds of RNA.

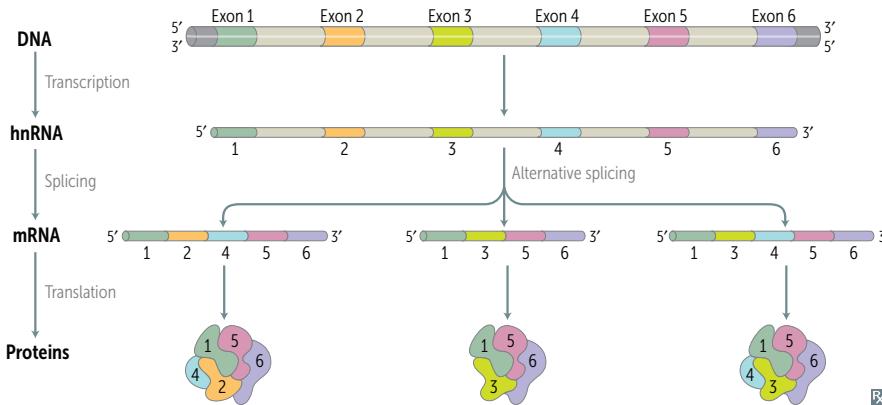
Rifamycins (rifampin, rifabutin) inhibit DNA-dependent RNA polymerase in prokaryotes.

**Introns vs exons**

Exons contain the actual genetic information coding for protein or functional RNA. Introns do not code for protein, but are important in regulation of gene expression. Different exons are frequently combined by alternative splicing to produce a larger number of unique proteins.

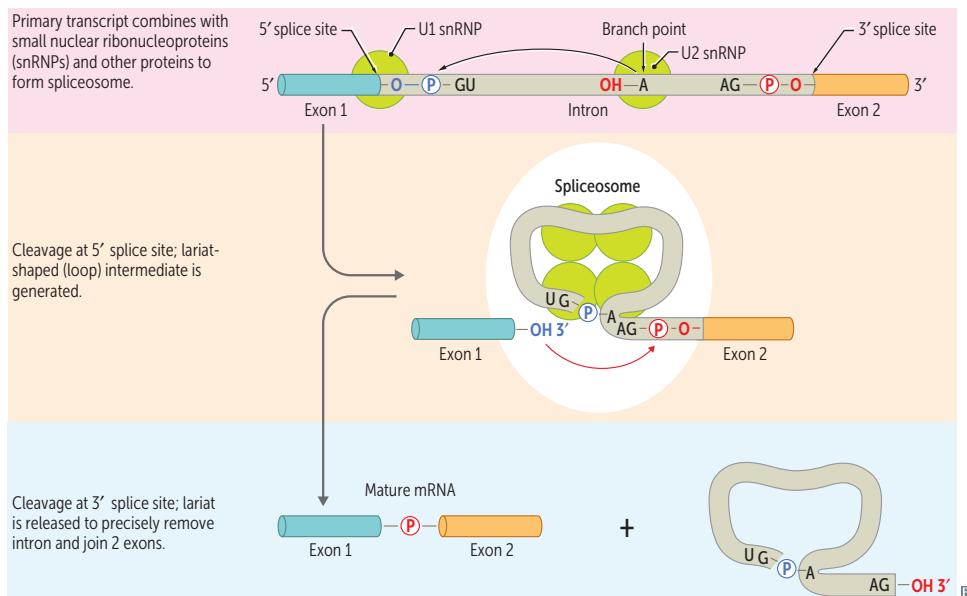
**Introns** are **intervening sequences** and stay **in** the nucleus, whereas **exons** **exit** and are **expressed**.

**Alternative splicing**—can produce a variety of protein products from a single hnRNA (heterogenous nuclear RNA) sequence (eg, transmembrane vs secreted Ig, tropomyosin variants in muscle, dopamine receptors in the brain, host defense evasion by tumor cells).

**Splicing of pre-mRNA**

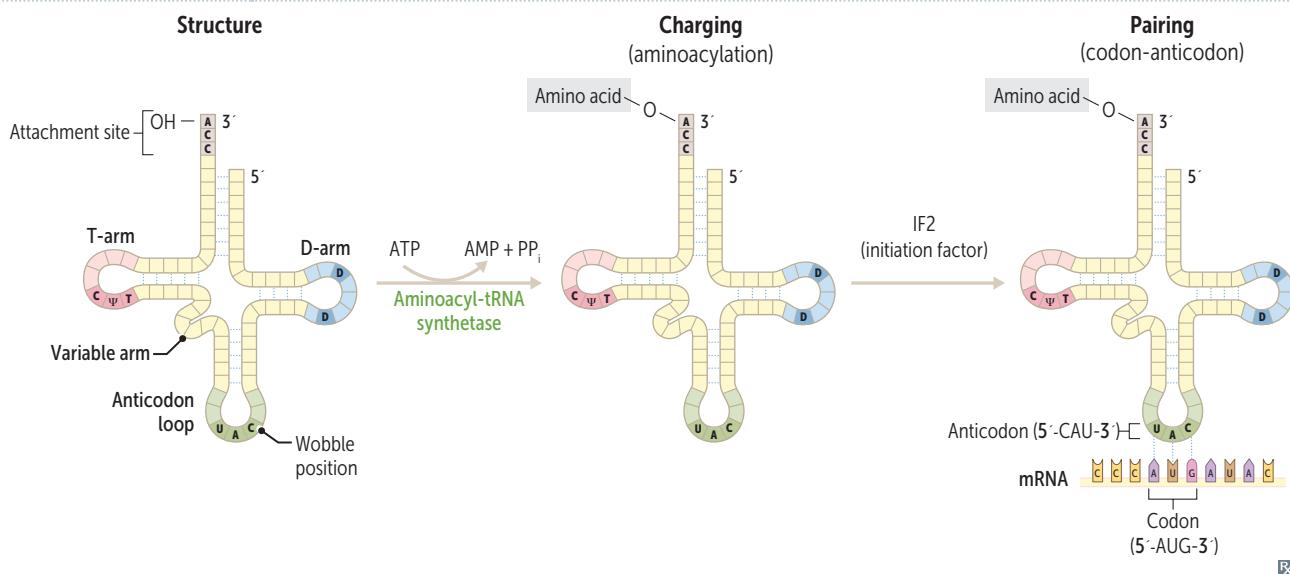
Part of process by which precursor mRNA (pre-mRNA) is transformed into mature mRNA. Introns typically begin with GU and end with AG. Alterations in snRNP assembly can cause clinical disease; eg, in spinal muscular atrophy, snRNP assembly is affected due to ↓ SMN protein → congenital degeneration of anterior horns of spinal cord → symmetric weakness (hypotonia, or “floppy baby syndrome”).

snRNPs are snRNA bound to proteins (eg, Smith [Sm]) to form a spliceosome that cleaves pre-mRNA. Anti-U1 snRNP antibodies are associated with SLE, mixed connective tissue disease, other rheumatic diseases.



**tRNA**

<b>Structure</b>	75–90 nucleotides, 2° 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. <b>CCA Can Carry Amino acids.</b>
T-arm:	contains the TΨC (ribothymidine, pseudouridine, cytidine) sequence necessary for tRNA-ribosome binding. <b>T-arm Tethers</b> tRNA molecule to ribosome.
D-arm:	contains Dihydrouridine residues necessary for tRNA recognition by the correct aminoacyl-tRNA synthetase. <b>D-arm</b> allows <b>Detection</b> of the tRNA by aminoacyl-tRNA synthetase.
Attachment site:	3'- <b>ACC</b> -5' is the amino acid <b>ACCEptor</b> site.
<b>Charging</b>	Aminoacyl-tRNA synthetase (uses ATP; 1 unique enzyme per respective amino acid) and binding of charged tRNA to the codon are responsible for the accuracy of amino acid selection. Aminoacyl-tRNA synthetase matches an amino acid to the tRNA by scrutinizing the amino acid before and after it binds to tRNA. If an incorrect amino acid is attached, the bond is hydrolyzed. A mischarged tRNA reads the usual codon but inserts the wrong amino acid.

**Start and stop codons**

<b>mRNA start codon</b>	AUG.	AUG in AUGurates protein synthesis.
Eukaryotes	Codes for methionine, which may be removed before translation is completed.	
Prokaryotes	Codes for N-formylmethionine (fMet).	fMet stimulates neutrophil chemotaxis.
<b>mRNA stop codons</b>	UGA, UAA, UAG. Recognized by release factors.	UGA = U Go Away. UAA = U Are Away. UAG = U Are Gone.

## Protein synthesis

### Initiation

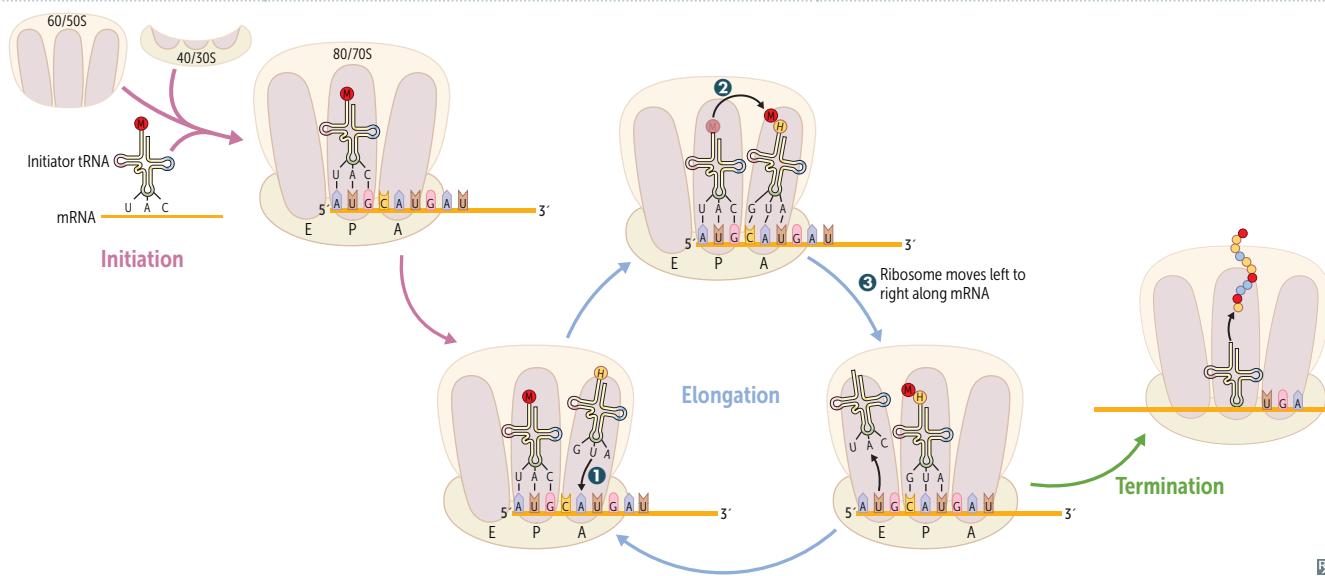
- Eukaryotic initiation factors (eIFs) identify the 5' cap.
- eIFs help assemble the 40S ribosomal subunit with the initiator tRNA.
- eIFs released when the mRNA and the ribosomal 60S subunit assemble with the complex. Requires GTP.

### Elongation

- Aminoacyl-tRNA binds to A site (except for initiator methionine, which binds the P site), requires an elongation factor and GTP.
- 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).

### Termination

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



## Posttranslational modifications

Trimming 0 1 86m 410 0 308.0.201 S Q q 1 06n9Q BT 0009.0.65 RemPPvI - ami5 (i[(Pn-0 m ()-0 m (I p)9m3)-r)2inpeai51 0.8(d)8m

## ► BIOCHEMISTRY—CELLULAR

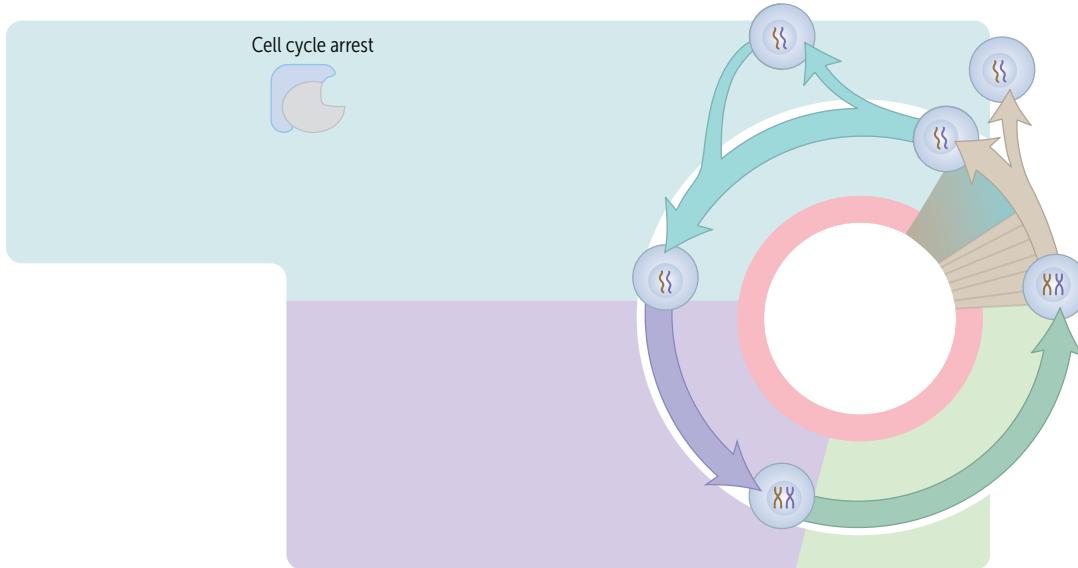
**Cell cycle phases** Checkpoints control transitions between phases of cell cycle. This process is regulated by cyclins, cyclin-dependent kinases (CDKs), and tumor suppressors. M phase (shortest phase of cell cycle) includes mitosis (prophase, prometaphase, metaphase, anaphase, telophase) and cytokinesis (cytoplasm splits in two). G<sub>1</sub> is of variable duration.

## REGULATION OF CELL CYCLE

<b>Cyclin-dependent kinases</b>	Constitutively expressed but inactive when not bound to cyclin.
<b>Cyclin-CDK complexes</b>	Cyclins are phase-specific regulatory proteins that activate CDKs when stimulated by growth factors. The cyclin-CDK complex can then phosphorylate other proteins (eg, Rb) to coordinate cell cycle progression. This complex must be activated/inactivated at appropriate times for cell cycle to progress.
<b>Tumor suppressors</b>	p53 → p21 induction → CDK inhibition → Rb hypophosphorylation (activation) → G <sub>1</sub> -S progression inhibition. Mutations in tumor suppressor genes can result in unrestrained cell division (eg, Li-Fraumeni syndrome). Growth factors (eg, insulin, PDGF, EPO, EGF) bind tyrosine kinase receptors to transition the cell from G <sub>1</sub> to S phase.

## CELL TYPES

<b>Permanent</b>	Remain in G <sub>0</sub> , regenerate from stem cells.	Neurons, skeletal and cardiac muscle, RBCs.
<b>Stable (quiescent)</b>	Enter G <sub>1</sub> from G <sub>0</sub> when stimulated.	Hepatocytes, lymphocytes, PCT, periosteal cells.
<b>Labile</b>	Never go to G <sub>0</sub> , divide rapidly with a short G <sub>1</sub> . Most affected by chemotherapy.	Bone marrow, gut epithelium, skin, hair follicles, germ cells.





**Peroxisome**

Membrane-enclosed organelle involved in:

- $\beta$ -oxidation of very-long-chain fatty acids (VLCFA) (strictly peroxisomal process)
- $\alpha$ -oxidation of branched-chain fatty acids (strictly peroxisomal process)
- Catabolism of amino acids and ethanol
- Synthesis of bile acids and plasmalogens (important membrane phospholipid, especially in white matter of brain)

**Zellweger syndrome**—autosomal recessive disorder of peroxisome biogenesis due to mutated *PEX* genes. Hypotonia, seizures, jaundice, craniofacial dysmorphia, hepatomegaly, early death.

**Refsum disease**—autosomal recessive disorder of  $\alpha$ -oxidation → buildup of phytanic acid due to inability to degrade it. Scaly skin, ataxia, cataracts/night blindness, shortening of 4th toe, epiphyseal dysplasia. Treatment: diet, plasmapheresis.

**Adrenoleukodystrophy**—X-linked recessive disorder of  $\beta$ -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, progressive loss of neurologic function, death.

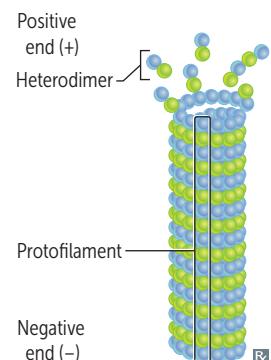
**Proteasome**

Barrel-shaped protein complex that degrades polyubiquitin-tagged proteins. Plays a role in many cellular processes, including immune response (MHC I-mediated). Defects in ubiquitin-proteasome system also implicated in diverse human diseases including neurodegenerative diseases.

**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
<b>Micro filaments</b>	Muscle contraction, cytokinesis	Actin, microvilli.
<b>Intermediate filaments</b>	Maintain cell structure	Vimentin, desmin, cytokeratin, lamins, glial fibrillary acidic protein (GFAP), neurofilaments.
<b>Microtubules</b>	Movement, cell division	Cilia, flagella, mitotic spindle, axonal trafficking, centrioles.

**Microtubule**

Cylindrical outer structure composed of a helical array of polymerized heterodimers of  $\alpha$ - and  $\beta$ -tubulin. Each dimer has 2 GTP bound. Incorporated into flagella, cilia, mitotic spindles. Also involved in slow axoplasmic transport in neurons.

**Molecular motor proteins**—transport cellular cargo toward opposite ends of microtubule.

- Retrograde to microtubule (+ → -)—**dynein**.
- Anterograde to microtubule (- → +)—**kinesin**.

*Clostridium tetani* toxin, poliovirus, rabies virus, and herpes simplex virus (HSV) use dynein for retrograde transport to the neuronal cell body. HSV reactivation occurs via anterograde transport from cell body (kinesin mediated).

Slow anterograde transport rate limiting step of peripheral nerve regeneration after injury.

Drugs that act on microtubules (**microtubules** get constructed **very terribly**):

- **Mebendazole** (antihelminthic)
- **Griseofulvin** (antifungal)
- **Colchicine** (antigout)
- **Vinca alkaloids** (anticancer)
- **Taxanes** (anticancer)

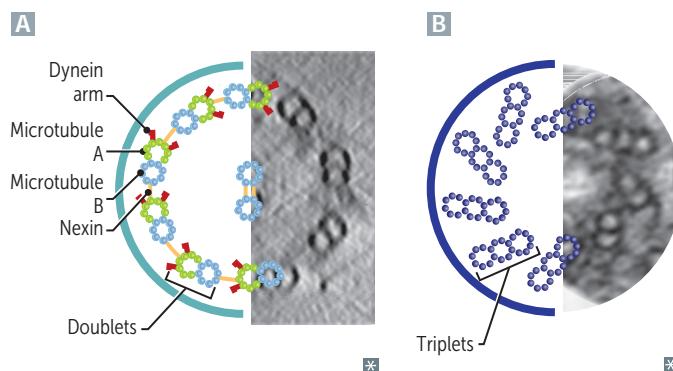
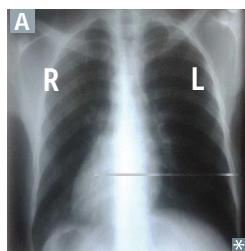
Negative end **near nucleus**.

Positive end **points to periphery**.

**Ready? Attack!**

**Cilia structure**

Motile cilia consist of 9 doublet + 2 singlet arrangement of microtubules (axoneme) **A**. Basal body (base of cilium below cell membrane) consists of 9 microtubule triplets **B** with no central microtubules. Nonmotile (primary) cilia work as chemical signal sensors and have a role in signal transduction and cell growth control. Dysgenesis may lead to polycystic kidney disease, mitral valve prolapse, or retinal degeneration. Axonemal dynein—ATPase that links peripheral 9 doublets and causes bending of cilium by differential sliding of doublets. Gap junctions enable coordinated ciliary movement.

**Primary ciliary dyskinesia**

Autosomal recessive. Dynein arm defect → immotile cilia → dysfunctional ciliated epithelia. Most common type is Kartagener syndrome (PCD with situs inversus).

Developmental abnormalities due to impaired migration and orientation (eg, situs inversus **A**, hearing loss due to dysfunctional eustachian tube cilia); recurrent infections (eg, sinusitis, ear infections, bronchiectasis due to impaired ciliary clearance of debris/pathogens); infertility ( $\uparrow$  risk of ectopic pregnancy due to dysfunctional fallopian tube cilia, immotile spermatozoa).

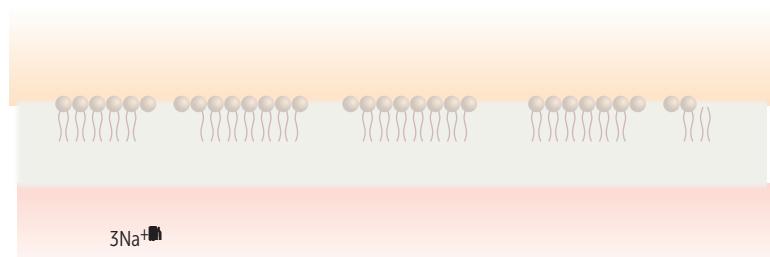
Lab findings:  $\downarrow$  nasal nitric oxide (used as screening test).

**Sodium-potassium pump**

$\text{Na}^+/\text{K}^+$ -ATPase is located in the plasma membrane with ATP site on cytosolic side. For each ATP consumed, **2 K<sup>+</sup>** go **in** to the cell (pump dephosphorylated) and **3 Na<sup>+</sup>** go **out** of the cell (pump phosphorylated).

**2** strikes? **K**, you're still **in**. **3** strikes? **Nah**, you're **out**!

Digoxin directly inhibits  $\text{Na}^+/\text{K}^+$ -ATPase → indirect inhibition of  $\text{Na}^+/\text{Ca}^{2+}$  exchange →  $\uparrow [\text{Ca}^{2+}]_i \rightarrow \uparrow$  cardiac contractility.



**Collagen**

Most abundant protein in the human body.  
Extensively modified by posttranslational modification.  
Organizes and strengthens extracellular matrix.  
Types I to IV are the most common types in humans.

Type I - **Skeleton**  
Type II - **Cartilage**  
Type III - **Arteries**  
Type IV - **Basement membrane**  
**SCAB**

**Type I**

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

Type I: **bone**, **tendone**.  
↓ production in osteogenesis imperfecta type I.

**Type II**

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

Type II: **cartwolage**.

**Type III**

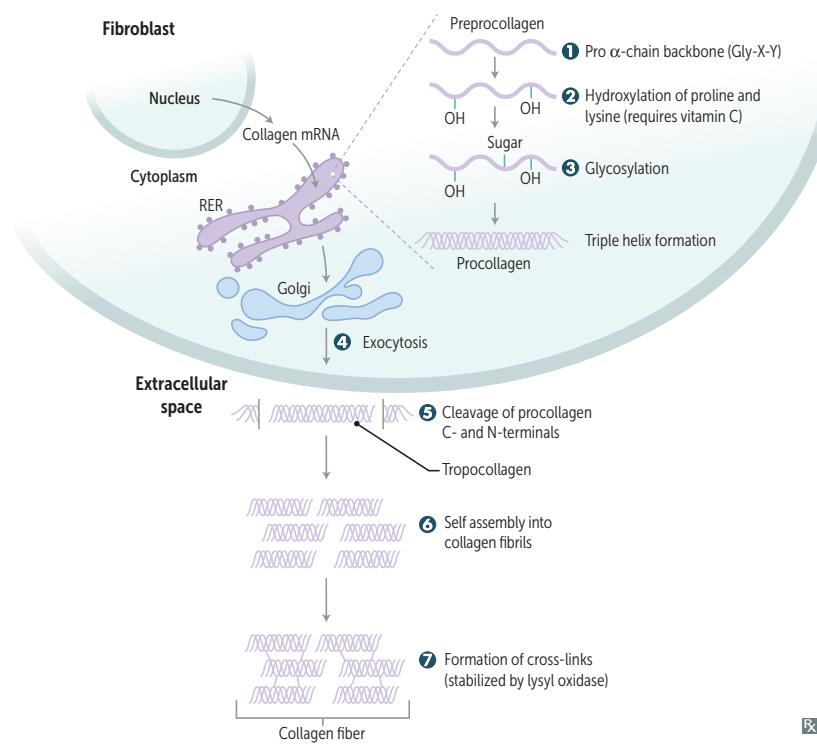
Reticulin—skin, **blood vessels**, uterus, fetal tissue, **early** wound repair.

Type III: deficient in **vascular** type of Ehlers-Danlos syndrome (**threE D**).

**Type IV**

Basement membrane/basal lamina (glomerulus, cochlea), lens.

Type IV: under the **floor** (basement membrane). Defective in Alport syndrome; targeted by autoantibodies in Goodpasture syndrome. Myofibroblasts are responsible for secretion (proliferative stage) and wound contraction.

**Collagen synthesis and structure**

**1 Synthesis**—translation of collagen α chains (preprocollagen)—usually Gly-X-Y (X is often proline or lysine and Y is often hydroxyproline or hydroxylysine). Collagen is 1/3 glycine; glycine content of collagen is less variable than that of lysine and proline.

**2 Hydroxylation**—hydroxylation (“hydroxylCylation”) 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.

**6 Assembly and alignment**—collagen assembles in fibrils and aligns for cross-linking.

**7 Cross-linking**—reinforcement of staggered tropocollagen molecules by covalent lysine-hydroxylysine cross-linkage (by copper-containing lysyl oxidase) to make collagen fibers. Cross-linking of collagen ↑ with age. Problems with cross-linking → Menkes disease.

### Osteogenesis imperfecta



**A**

Upper extremity

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 otherwise normal type I collagen (altered triple helix formation). Manifestations include:

- Multiple fractures and bone deformities (arrows in **A**) after minimal trauma (eg, during birth)
- Blue sclerae **B** 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)
- Conductive hearing loss (abnormal ossicles)

May be confused with child abuse.  
Treat with bisphosphonates to ↓ fracture risk.

Patients can't **BITE**:

**Bones** = multiple fractures

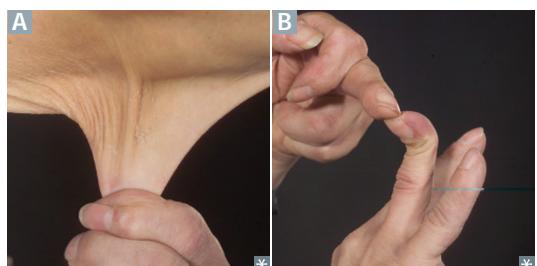
**I** (eye) = blue sclerae

**Teeth** = dental imperfections

**Ear** = hearing loss



**B**



**A**

**B**

### Ehlers-Danlos syndrome

Faulty collagen synthesis causing hyperextensible skin **A**, hypermobile joints **B**, and tendency to bleed (easy bruising).

Multiple types. Inheritance and severity vary. Can be autosomal dominant or recessive. May be associated with joint dislocation, berry and aortic aneurysms, organ rupture.

Hypermobility type (joint instability): most common type.

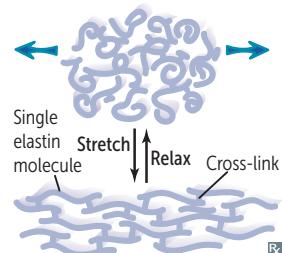
Classical type (joint and skin symptoms): caused by a mutation in type V collagen (eg, COL5A1, COL5A2).

Vascular type (fragile tissues including vessels [eg, aorta], muscles, and organs that are prone to rupture [eg, gravid uterus]): mutations in type III procollagen (eg, COL3A1).

Can be caused by procollagen peptidase deficiency.

### Menkes disease

X-linked recessive co

**Elastin**

Stretchy protein within skin, lungs, large arteries, elastic ligaments, vocal cords, epiglottis, ligamenta flava (connect vertebrae → relaxed and stretched conformations).

Rich in nonhydroxylated proline, glycine, and lysine residues, vs the hydroxylated residues of collagen.

Tropoelastin with fibrillin scaffolding.

Cross-linking occurs extracellularly via lysyl oxidase and gives elastin its elastic properties.

Broken down by elastase, which is normally inhibited by  $\alpha_1$ -antitrypsin.

$\alpha_1$ -Antitrypsin deficiency results in unopposed elastase activity, which can cause COPD.

**Marfan syndrome**—autosomal dominant (with variable expression) connective tissue disorder affecting skeleton, heart, and eyes. *FBN1* gene mutation on chromosome 15 (fifteen) results in defective fibrillin-1, a glycoprotein that forms a sheath around elastin and sequesters TGF- $\beta$ . Findings: tall with long extremities; chest wall deformity (pectus carinatum [pigeon chest] or pectus excavatum **A**); hypermobile joints; long, tapering fingers and toes (arachnodactyly); cystic medial necrosis of aorta; aortic root aneurysm rupture or dissection (most common cause of death); mitral valve prolapse; ↑ risk of spontaneous pneumothorax.

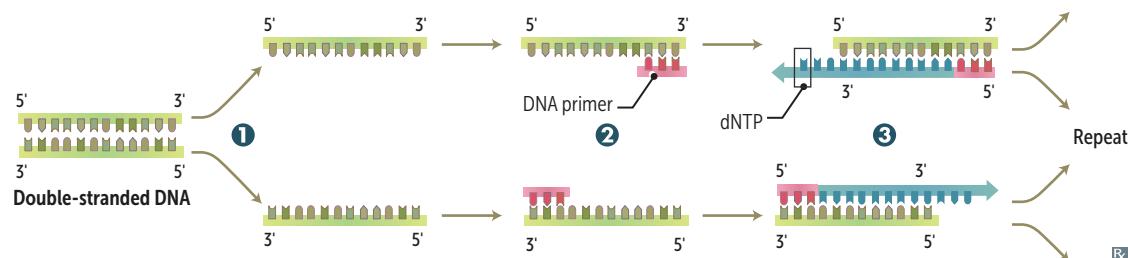
**Homocystinuria**—most commonly due to cystathione synthase deficiency leading to homocysteine buildup. Presentation similar to Marfan syndrome with pectus deformity, tall stature, ↑ arm:height ratio, ↓ upper:lower body segment ratio, arachnodactyly, joint hyperlaxity, skin hyperelasticity, scoliosis, fair complexion (vs Marfan syndrome).

	Marfan syndrome	Homocystinuria
INHERITANCE	Autosomal dominant	Autosomal recessive
INTELLECT	Normal	Decreased
VASCULAR COMPLICATIONS	Aortic root dilatation	Thrombosis
LENS DISLOCATION	Upward/temporal (Marfan fans out)	Downward/nasal

## ► BIOCHEMISTRY—LABORATORY TECHNIQUES

**Polymerase chain reaction**

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



**1 Denaturation**—DNA template, DNA primers, a heat-stable DNA polymerase, and deoxynucleotide triphosphates (dNTPs) are heated to ~95°C to separate the DNA strands.

**2 Annealing**—sample is cooled to ~55°C. DNA primers anneal to the specific sequence to be amplified on the DNA template.

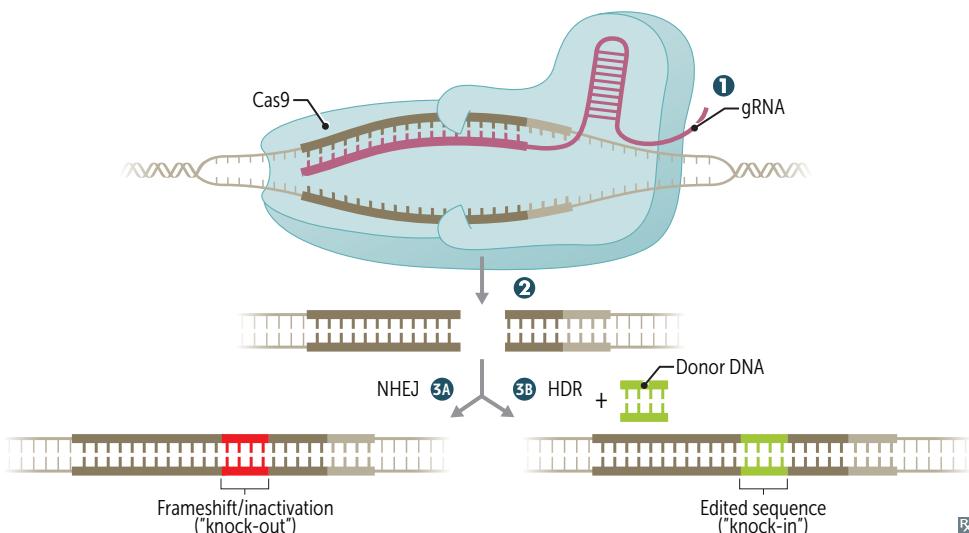
**3 Elongation**—temperature is increased to ~72°C. DNA polymerase adds dNTPs to the strand to replicate the sequence after each primer.

Heating and cooling cycles continue until the amount of DNA is sufficient.

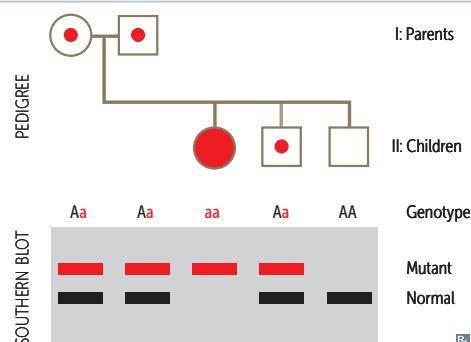
**CRISPR/Cas9**

A genome editing tool derived from bacteria. Consists of a guide RNA (gRNA) ①, which is complementary to a target DNA sequence, and an endonuclease (Cas9), which makes a single- or double-strand break at the target site ②. Imperfectly cut segments are repaired by nonhomologous end joining (NHEJ) → accidental frameshift mutations (“knock-out”) ③A, or a donor DNA sequence can be added to fill in the gap using homology-directed repair (HDR) ③B.

Potential applications include removing virulence factors from pathogens, replacing disease-causing alleles of genes with healthy variants (in clinical trials for sickle cell disease), and specifically targeting tumor cells.

**Blotting procedures****Southern blot**

1. DNA sample is enzymatically cleaved into smaller pieces, which are separated by gel electrophoresis, and then transferred to a membrane.
  2. Membrane is exposed to labeled DNA probe that anneals to its complementary strand.
  3. Resulting double-stranded, labeled piece of DNA is visualized when membrane is exposed to film or digital imager.
- Useful to identify size of specific sequences (eg, determination of heterozygosity [as seen in image], # of CGG repeats in FMR1 to diagnose Fragile X syndrome)

**SNOW DRoP:**

Southern = DNA

Northern = RNA

Western = Protein

**Northern blot**

Similar to Southern blot, except that an RNA sample is electrophoresed. Useful for studying mRNA levels and size, which are reflective of gene expression. Detects splicing errors.

**Western blot**

Sample protein is separated via gel electrophoresis and transferred to a membrane. Labeled antibody is used to bind relevant protein. This helps identify specific protein and determines quantity.

**Southwestern blot**

Identifies DNA-binding proteins (eg, c-Jun, c-Fos [leucine zipper motif]) using labeled double-stranded DNA probes.

Southern (DNA) + Western (protein) = Southwestern (DNA-binding protein).

### Flow cytometry

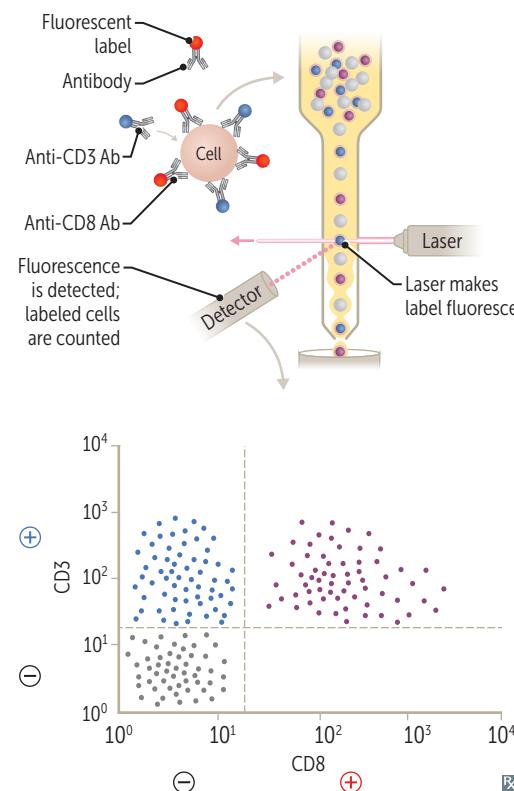
Laboratory technique to assess size, granularity, and protein expression (immunophenotype) of individual cells in a sample.

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.

Data are plotted either as histogram (one measure) or scatter plot (any two measures, as shown). In illustration:

- Cells in left lower quadrant  $\ominus$  for both CD8 and CD3.
- Cells in right lower quadrant  $\oplus$  for CD8 and  $\ominus$  for CD3. In this example, right lower quadrant is empty because all CD8-expressing cells also express CD3.
- Cells in left upper quadrant  $\oplus$  for CD3 and  $\ominus$  for CD8.
- Cells in right upper quadrant  $\oplus$  for both CD8 and CD3.

Commonly used in workup of hematologic abnormalities (eg, leukemia, paroxysmal nocturnal hemoglobinuria, fetal RBCs in pregnant person's blood) and immunodeficiencies (eg, CD4+ cell count in HIV).



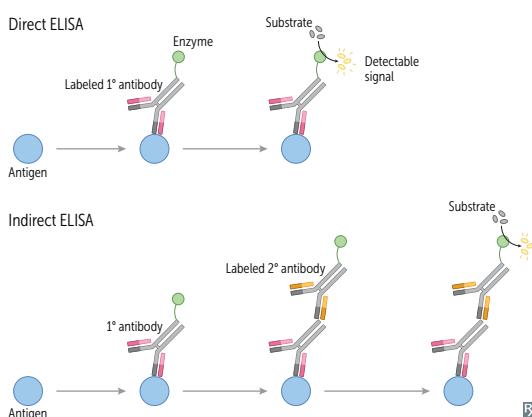
### Microarrays

Array consisting of thousands of DNA oligonucleotides arranged in a grid on a glass or silicon chip. The DNA or RNA samples being compared are attached to different fluorophores and hybridized to the array. The ratio of fluorescence signal at a particular oligonucleotide reflects the relative amount of the hybridizing nucleic acid in the two samples.

Used to compare the relative transcription of genes in two RNA samples. Can detect single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) for genotyping, clinical genetic testing, forensic analysis, and cancer mutation and genetic linkage analysis when DNA is used.

### Enzyme-linked immunosorbent assay

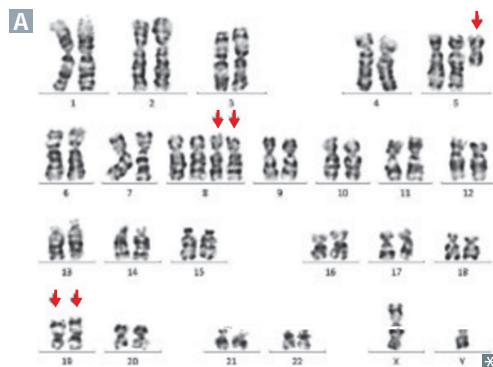
Immunologic test used to detect the presence of either a specific antigen or antibody in a patient's blood sample. Detection involves the use of an antibody linked to an enzyme. Added substrate reacts with the enzyme, producing a detectable signal. Can have high sensitivity and specificity, but is less specific than Western blot. Often used to screen for HIV infection.



**Karyotyping**

Colchicine is added to cultured cells to halt chromosomes in 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).

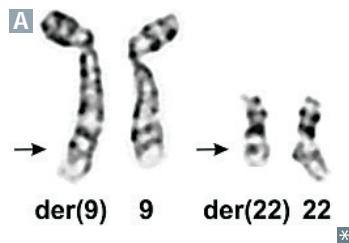
Can be performed on a sample of blood, bone marrow, amniotic fluid, or placental tissue. Used to diagnose chromosomal imbalances (eg, autosomal trisomies, sex chromosome disorders).

**Fluorescence in situ hybridization**

Fluorescent DNA or RNA probe binds to specific gene or other site of interest on chromosomes.

Used for specific localization of genes and direct visualization of chromosomal anomalies.

- Microdeletion—no fluorescence on a chromosome compared to fluorescence at the same locus on the second copy of that chromosome.
- Translocation—A fluorescence signal (from *ABL* gene) that corresponds to one chromosome (chromosome 9) is found in a different chromosome (chromosome 22, next to *BCR* gene).
- Duplication—a second copy of a chromosome, resulting in a trisomy or tetrasomy.

**Molecular cloning**

Production of a recombinant DNA molecule in a bacterial host. Useful for production of human proteins in bacteria (eg, human growth hormone, insulin).

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).

<b>Gene expression modifications</b>	Transgenic strategies in mice involve: <ul style="list-style-type: none"><li>▪ Random insertion of gene into mouse genome</li><li>▪ Targeted insertion or deletion of gene through homologous recombination with mouse gene</li></ul>	Knock-out = removing a gene, taking it out. Knock-in = inserting a gene.  Random insertion—constitutive expression. Targeted insertion—conditional expression.
<b>RNA interference</b>	Process whereby small non-coding RNA molecules target mRNAs to inhibit gene expression.	
<b>MicroRNA</b>	Naturally produced by cell as hairpin structures. Loose nucleotide pairing allows broad targeting of related mRNAs. When miRNA binds to mRNA, it blocks translation of mRNA and sometimes facilitates its degradation.	Abnormal expression of miRNAs contributes to certain malignancies (eg, by silencing an mRNA from a tumor suppressor gene).
<b>Small interfering RNA</b>	Usually derived from exogenous dsRNA source (eg, virus). Once inside a cell, siRNA requires complete nucleotide pairing, leading to highly specific mRNA targeting. Results in mRNA cleavage prior to translation.	Can be produced by transcription or chemically synthesized for gene “knockdown” experiments.

## ► BIOCHEMISTRY—GENETICS

**Genetic terms**

TERM	DEFINITION	EXAMPLE
<b>Codominance</b>	Both alleles contribute to the phenotype of the heterozygote.	Blood groups A, B, AB; $\alpha_1$ -antitrypsin deficiency; HLA groups.
<b>Variable expressivity</b>	Patients with the same genotype have varying phenotypes.	Two patients with neurofibromatosis type 1 (NFI) may have varying disease severity.
<b>Incomplete penetrance</b>	Not all individuals with a disease show the disease. $\% \text{ penetrance} \times \text{probability of inheriting genotype} = \text{risk of expressing phenotype}$ .	BRCA1 gene mutations do not always result in breast or ovarian cancer.
<b>Pleiotropy</b>	One gene contributes to multiple phenotypic effects.	Untreated phenylketonuria (PKU) manifests with light skin, intellectual disability, musty body odor.
<b>Anticipation</b>	Increased severity or earlier onset of disease in succeeding generations.	Trinucleotide repeat diseases (eg, Huntington disease).
<b>Loss of heterozygosity</b>	If a patient inherits or develops a mutation in a tumor suppressor gene, the wild type allele must be deleted/mutated/eliminated before cancer develops. This is not true of oncogenes.	Retinoblastoma and the “two-hit hypothesis,” Lynch syndrome (HNPCC), Li-Fraumeni syndrome.
<b>Epistasis</b>	The allele of one gene affects the phenotypic expression of alleles in another gene.	Albinism, alopecia.
<b>Aneuploidy</b>	An abnormal number of chromosomes; due to chromosomal nondisjunction during mitosis or meiosis.	Down syndrome, Turner syndrome, oncogenesis.

**Dominant negative mutation**

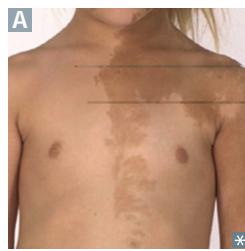
Exerts a dominant effect. A heterozygote produces a nonfunctional altered protein that also prevents the normal gene product from functioning.

A single mutated *p53* tumor suppressor gene results in a protein that is able to bind DNA and block the wild type *p53* from binding to the promoter.

**Linkage disequilibrium**

Tendency for certain alleles to occur in close proximity on the same chromosome more or less often than expected by chance. Measured in a population, not in a family, and often varies in different populations.

d a e d t t . 8

**Mosaicism**

Presence of genetically distinct cell lines in the same individual.

Somatic mosaicism—mutation arises from mitotic errors after fertilization and propagates through multiple tissues or organs.

Germline (gonadal) mosaicism—mutation only in egg or sperm cells. If parents and relatives do not have the disease, suspect gonadal (or germline) mosaicism.

McCune-Albright syndrome—due to G<sub>d</sub>-diyeilec<sub>c</sub>  
dfe1.3a.4/c1-6se70 Tc 0

de35 2f -3(d)20fe

### Hardy-Weinberg principle

A (p)	a (q)
A (p)	AA ( $p^2$ )      Aa ( $pq$ )
a (q)	Aa ( $pq$ )      aa ( $q^2$ )

In a given population where mating is at random, allele and genotype frequencies will be constant. If  $p$  and  $q$  represent the frequencies of alleles A and a in a population, respectively, then  $p + q = 1$ , where:

- $p^2$  = frequency of homozygosity for allele A
- $q^2$  = frequency of homozygosity for allele a
- $2pq$  = frequency of heterozygosity (carrier frequency, if an autosomal recessive disease)

Therefore the sum of the frequencies of these genotypes is  $p^2 + 2pq + q^2 = 1$ .

The frequency of an X-linked recessive disease in males =  $q$  and in females =  $q^2$ .

Hardy-Weinberg law assumptions include:

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

If a population is in Hardy-Weinberg equilibrium, then the values of  $p$  and  $q$  remain constant from generation to generation.

In rare autosomal recessive diseases,  $p \approx 1$ .

Example: The prevalence of cystic fibrosis (an autosomal recessive disease) in the US is approximately 1/3200, which tells us that  $q^2 = 1/3200$ , with  $q \approx 0.017$  or 1.7% of the population. Since  $p + q = 1$ , we know that  $p = 1 - \sqrt{1/3200} \approx 0.982$ , which gives us a heterozygous carrier frequency of  $2pq = 0.035$  or 3.5% of the population. Notice that since the disease is relatively rare, we could have approximated  $p \approx 1$  and obtained a similar result.

### Disorders of imprinting

One gene copy is silenced by methylation, and only the other copy is expressed → parent-of-origin effects. The expressed copy may be mutated, may not be expressed, or may be deleted altogether.

#### Prader-Willi syndrome

##### WHICH GENE IS SILENT?

Maternally derived genes are silenced  
Disease occurs when the paternal allele is deleted or mutated

##### SIGNS AND SYMPTOMS

Hyperphagia, obesity, intellectual disability, hypogonadism, hypotonia

##### CHROMOSOMES INVOLVED

Chromosome 15 of paternal origin

##### NOTES

25% of cases are due to maternal uniparental disomy

**POP:** Prader-Willi, Obesity/overeating, Paternal allele deleted

#### Angelman syndrome

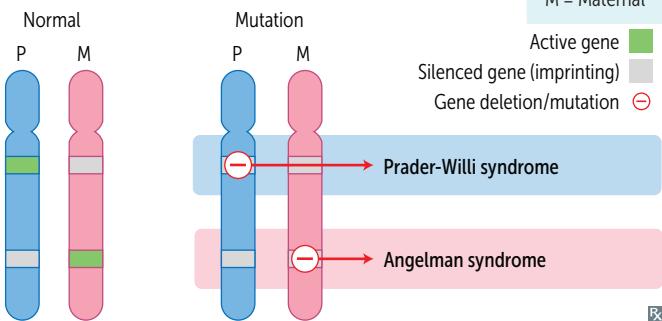
Paternally derived UBE3A is silenced  
Disease occurs when the maternal allele is deleted or mutated

Hand-flapping, Ataxia, severe Intellectual disability, inappropriate Laughter, Seizures.  
**HAILS** the Angels.

UBE3A on maternal copy of chromosome 15

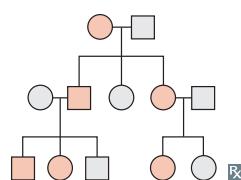
5% of cases are due to paternal uniparental disomy

**MAMAS:** Maternal allele deleted, Angelman syndrome, Mood, Ataxia, Seizures



### Modes of inheritance

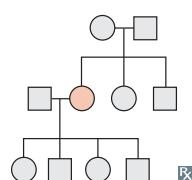
#### Autosomal dominant



Often due to defects in structural genes. Many generations, both males and females are affected.

	A	a
a	Aa	aa
a	Aa	aa

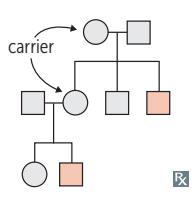
#### Autosomal recessive



With 2 carrier (heterozygous) parents, on average: each child has a 25% chance of being affected, 50% chance of being a carrier, and 25% chance of not being affected nor a carrier.

	A	a
A	AA	Aa
a	Aa	aa

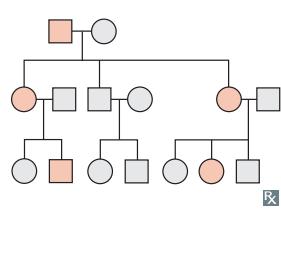
#### X-linked recessive



Sons of heterozygous mothers have a 50% chance of being affected. No male-to-male transmission. Skips generations.

	X	X	X	X
X	XX	XX	XX	XX
Y	XY	XY	XY	XY

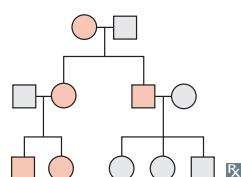
#### X-linked dominant



Transmitted through both parents. Children of affected mothers each have a 50% chance of being affected. 100% of daughters and 0% of sons of affected fathers will be affected.

	X	X	X	X
X	XX	XX	XX	XX
Y	XY	XY	XY	XY

#### Mitochondrial inheritance



Transmitted only through the mother. All offspring of affected females may show signs of disease.

Variable expression in a population or even within a family due to heteroplasmy.

Often pleiotropic (multiple apparently unrelated effects) and variably expressive (different between individuals). Family history crucial to diagnosis. With one affected (heterozygous) parent, each child has a 50% chance of being affected.

Often due to enzyme deficiencies. Usually seen in only 1 generation. Commonly more severe than dominant disorders; patients often present in childhood.

↑ risk in consanguineous families.  
Unaffected individual with affected sibling has 2/3 probability of being a carrier.

Commonly more severe in males. Females usually must be homozygous to be affected.

Examples: fragile X syndrome, Alport syndrome, **hypophosphatemic rickets** (also called X-linked hypophosphatemia)—phosphate wasting at proximal tubule → ricketslike presentation.

Caused by mutations in mtDNA.  
Examples: mitochondrial myopathies, Leber hereditary optic neuropathy.

□ = unaffected male; ■ = affected male; ○ = unaffected female; ▨ = affected female.

**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**

Mostly consist of enzyme defects. **Oculocutaneous albinism**, **phenylketonuria**, **cystic fibrosis**, **sickle cell disease**, **Wilson disease**, **sphingolipidoses** (except Fabry disease), **hemochromatosis**, **glycogen storage diseases**, **thalassemia**, **mucopolysaccharidoses** (except Hunter syndrome), **Friedreich ataxia**, **Kartagener syndrome**, **ARPKD**. Oh, please! Can students who score high grades tell me features of the kidney disorder **Autosomal Recessive Polycystic Kidney Disease?**

**Cystic fibrosis****GENETICS**

Autosomal recessive; defect in CFTR gene on chromosome 7 (deletion; ΔF508). Most common lethal genetic disease in patients with European ancestry.

**PATHOPHYSIOLOGY**

CFTR encodes an ATP-gated Cl<sup>-</sup> channel (secretes Cl<sup>-</sup> in lungs/GI tract, reabsorbs Cl<sup>-</sup> in sweat glands). Phe508 deletion → misfolded protein → improper protein trafficking → protein absent from cell membrane → ↓ Cl<sup>-</sup> (and H<sub>2</sub>O) secretion → compensatory ↑ Na<sup>+</sup> reabsorption via epithelial Na<sup>+</sup> channels (ENaC) → ↑ H<sub>2</sub>O reabsorption → abnormally thick mucus secreted into lungs/GI tract. ↑ Na<sup>+</sup> reabsorption = more negative transepithelial potential difference.

**DIAGNOSIS**

↑ Cl<sup>-</sup> concentration in pilocarpine-induced sweat test. Can present with contraction alkalosis and hypokalemia (ECF effects analogous loop diuretic effect) due to ECF H<sub>2</sub>O/Na<sup>+</sup> losses via sweating and concomitant renal K<sup>+</sup>/H<sup>+</sup> wasting. ↑ immunoreactive trypsinogen (newborn screening) due to clogging of pancreatic duct.

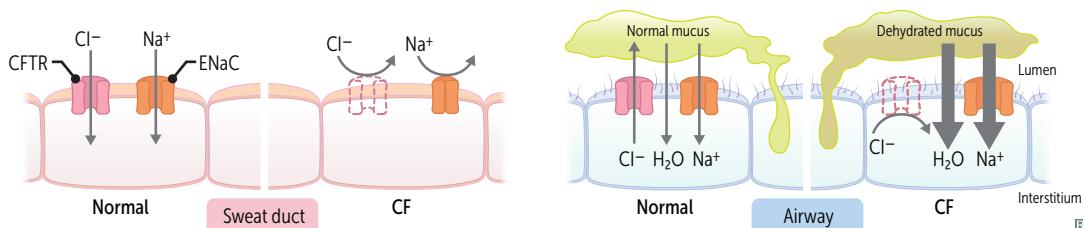
**COMPLICATIONS**

Recurrent pulmonary infections (eg, *S aureus* [infancy and early childhood], *P aeruginosa* [adulthood], allergic bronchopulmonary aspergillosis [ABPA]), chronic bronchitis and bronchiectasis → reticulonodular pattern on CXR, opacification of sinuses. Nasal polyps, nail clubbing. Pancreatic insufficiency, malabsorption with steatorrhea, and fat-soluble vitamin deficiencies (A, D, E, K) progressing to endocrine dysfunction (CF-related diabetes), biliary cirrhosis, liver disease. Meconium ileus in newborns.

Infertility in males (absence of vas deferens, spermatogenesis may be unaffected) and subfertility in females (amenorrhea, abnormally thick cervical mucus).

**TREATMENT**

Multifactorial: chest physiotherapy, aerosolized dornase alfa (DNase), and inhaled hypertonic saline → mucus clearance. Azithromycin prevents acute exacerbations. Ibuprofen for anti-inflammatory effect. Pancreatic enzyme replacement therapy (pancrelipase) for pancreatic insufficiency. CFTR modulators can be used alone or in combination. Efficacy varies by different genetic mutations (pharmacogenomics). Are either potentiators (hold gate of CFTR channel open → Cl<sup>-</sup> flows through cell membrane; eg, ivacaftor) or correctors (help CFTR protein to form right 3-D shape → moves to the cell surface; eg, lumacaftor, tezacaftor).

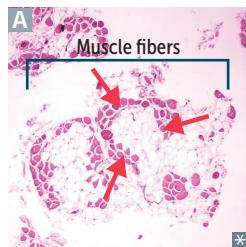


**X-linked recessive diseases**

Bruton agammaglobulinemia, Duchenne and Becker muscular dystrophies, Fabry disease, G6PD deficiency, hemophilia A and B, Hunter syndrome, Lesch-Nyhan syndrome, ocular albinism, ornithine transcarbamylase (OTC) deficiency, Wiskott-Aldrich syndrome.

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

**X-inactivation (lyonization)**—during development, one of the X chromosomes in each XX cell is randomly deactivated and condensed into a Barr body (methylated heterochromatin). If skewed inactivation occurs, XX individuals may express X-linked recessive diseases (eg, G6PD); penetrance and severity of X-linked dominant diseases in XX individuals may also be impacted.

**Muscular dystrophies****Duchenne**

X-linked recessive disorder typically due to **frameshift** deletions or nonsense mutations  
→ truncated or absent dystrophin protein  
→ progressive myofiber damage. Can also result from splicing errors.

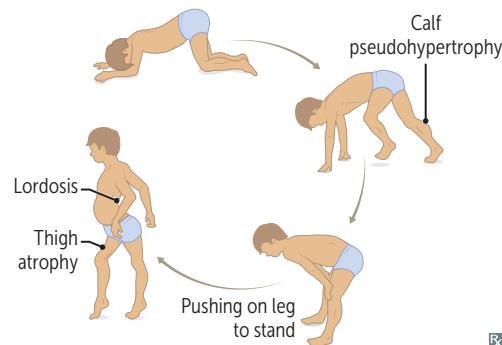
Weakness begins in pelvic girdle muscles and progresses superiorly. Pseudohypertrophy of calf muscles due to fibrofatty replacement of muscle **A**. Waddling gait.

Onset before 5 years of age. Dilated cardiomyopathy is common cause of death.

**Gowers sign**—patient uses upper extremities to help stand up. Classically seen in Duchenne muscular dystrophy, but also seen in other muscular dystrophies and inflammatory myopathies (eg, polymyositis).

Duchenne = **deleted dystrophin**.

Dystrophin gene (*DMD*) is the largest protein-coding human gene → ↑ chance of spontaneous mutation. Dystrophin helps to anchor muscle fibers to the extracellular matrix, primarily in skeletal and cardiac muscles. Loss of dystrophin → myonecrosis.  
↑ CK and aldolase; genetic testing confirms diagnosis.

**Becker**

X-linked recessive disorder typically due to **non-frameshift** deletions in dystrophin gene (partially functional instead of truncated). Less severe than Duchenne (Becker is better). Onset in adolescence or early adulthood.

**Myotonic dystrophy**

Autosomal dominant. Onset 20–30 years. **CTG** trinucleotide repeat expansion in the *DMPK* gene → abnormal expression of myotonin protein kinase → myotonia (eg, difficulty releasing hand from handshake), muscle wasting, cataracts, testicular atrophy, frontal balding, arrhythmia.

Deletions can cause both Duchenne and Becker muscular dystrophies.  $\frac{2}{3}$  of cases have large deletions spanning one or more exons.

Cataracts, Toupee (early balding in males), Gonadal atrophy.

**Mitochondrial diseases** Rare disorders arising 2° to failure in oxidative phosphorylation. Tissues with ↑ energy requirements are preferentially affected (eg, CNS, skeletal muscle).

**Mitochondrial myopathies**—include **MELAS** (mitochondrial encephalomyopathy with lactic acidosis and strokelike episodes) and **MERRF** (myoclonic epilepsy with ragged red fibers). Light microscopy with stain: ragged red fibers due to compensatory proliferation of mitochondria. Electron microscopy: mitochondrial crystalline inclusions.

**Leber hereditary optic neuropathy**—mutations in complex I of ETC → neuronal death in retina and optic nerve → subacute bilateral vision loss in teens/young adults (males > females). Usually permanent. May be accompanied by neurologic dysfunction (eg, tremors, multiple sclerosis-like illness).

### Rett syndrome

Sporadic disorder caused by de novo mutation of *MECP2* on X chromosome. Seen mostly in females. Embryonically lethal in males. Individuals with **Rett** syndrome experience initial normal development (6–18 months) followed by regression (“**return**”) in motor, verbal, and cognitive abilities; ataxia; seizures; scoliosis; and stereotypic hand-wringing.

### Fragile X syndrome

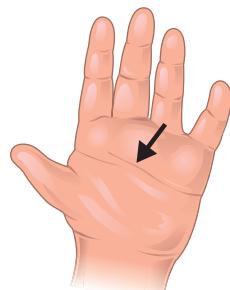
X-linked dominant inheritance. Trinucleotide repeats in *FMRI* → hypermethylation of cytosine residues → ↓ expression. Most common inherited cause of intellectual disability (Down syndrome is most common genetic cause, but most cases occur sporadically).

Trinucleotide repeat expansion [(CGG)<sub>n</sub>] occurs during oogenesis. Premutation (50–200 repeats) → tremor, ataxia, 1° ovarian insufficiency. Full mutation (>200 repeats) → postpubertal macroorchidism (enlarged testes), long face with large jaw, large everted ears, autism, mitral valve prolapse, hypermobile joints. Self-mutilation is common and can be confused with Lesch-Nyhan syndrome.

### Trinucleotide repeat expansion diseases

May show genetic anticipation (disease severity ↑ and age of onset ↓ in successive generations).

DISEASE	TRINUCLEOTIDE REPEAT	MODE OF INHERITANCE	MNEMONIC
Huntington disease	(CAG) <sub>n</sub>	AD	Caudate has ↓ ACh and GABA
Myotonic dystrophy	(CTG) <sub>n</sub>	AD	Cataracts, Toupee (early balding in males), Gonadal atrophy in males, reduced fertility in females
Fragile X syndrome	(CGG) <sub>n</sub>	XD	Chin (protruding), Giant Gonads
Friedreich ataxia	(GAA) <sub>n</sub>	AR	Ataxic GAAit

**Autosomal trisomies****Down syndrome  
(trisomy 21)**

Single palmar crease

Autosomal trisomies are screened in first and second trimesters with noninvasive prenatal tests. Incidence of trisomies: Down (21) > Edwards (18) > Patau (13). Autosomal monosomies are incompatible with life (high chance of recessive trait expression).

**Findings:** intellectual disability, flat facies, prominent epicanthal folds, single palmar crease, incurved 5th finger, gap between 1st 2 toes, duodenal atresia, Hirschsprung disease, congenital heart disease (eg, AVSD), Brushfield spots (whitish spots at the periphery of the iris). Associated with early-onset Alzheimer disease (chromosome 21 codes for amyloid precursor protein), ↑ risk of AML/ALL.

95% of cases due to meiotic nondisjunction, most commonly during meiosis I (↑ with advanced maternal age: from 1:1500 in females < 20 to 1:25 in females > 45). 4% of cases due to unbalanced Robertsonian translocation, most typically between chromosomes 14 and 21. 1% of cases due to postfertilization mitotic error.

**Drinking age (21).**

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

First-trimester ultrasound commonly shows ↑ nuchal translucency and hypoplastic nasal bone. Markers for Down syndrome are **hi up**: ↑ hCG, ↑ inhibin.

↑ risk of umbilical hernia (incomplete closure of umbilical ring).

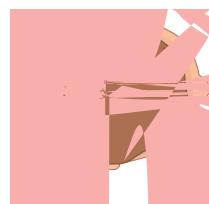
**The 5 A's of Down syndrome:**

- Advanced maternal age
- Atresia (duodenal)
- Atrioventricular septal defect
- Alzheimer disease (early onset)
- AML (<5 years of age)/ALL (>5 years of age)

**Election age (18).**

2nd most common autosomal trisomy resulting in live birth (most common is Down syndrome).

In **Edwards syndrome**, every prenatal screening marker **decreases**.

**Edwards syndrome  
(trisomy 18)****Patau syndrome  
(trisomy 13)**

**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 (eg, VSD), omphalocele, myelomeningocele. Death usually occurs by age 1.

**Genetic disorders by chromosome**

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

**Williams syndrome**

Congenital microdeletion of long arm of chromosome 7 (deleted region includes elastin gene). Findings: distinctive “elfin” facies, intellectual disability, hypercalcemia, well-developed verbal skills, extreme friendliness with strangers, cardiovascular problems (eg, supravalvular aortic stenosis, renal artery stenosis).

## ► BIOCHEMISTRY—NUTRITION

**Essential fatty acids**

Polyunsaturated fatty acids that cannot be synthesized in the body and must be provided in the diet (eg, nuts/seeds, plant oils, seafood). Linoleic acid (omega-6) is metabolized to arachidonic acid, which serves as the precursor to leukotrienes and prostaglandins. Linolenic acid (omega-3) and its metabolites have cardioprotective and antihyperlipidemic effects.

In contrast, consumption of *trans*-unsaturated fatty acids (found in fast food) promotes cardiovascular disease by ↑ LDL and ↓ HDL.

**Vitamins: fat soluble**

A, D, E, K. Absorption dependent on bile emulsification, pancreatic secretions, and intact ileum. Toxicity more common than for water-soluble vitamins because fat-soluble vitamins accumulate in fat.

Malabsorption syndromes with steatorrhea (eg, cystic fibrosis and celiac disease) or mineral oil intake can cause fat-soluble vitamin deficiencies.

**Vitamins: water soluble**

B<sub>1</sub> (thiamine: TPP)  
B<sub>2</sub> (riboflavin: FAD, FMN)  
B<sub>3</sub> (niacin: NAD<sup>+</sup>)  
B<sub>5</sub> (pantothenic acid: CoA)  
B<sub>6</sub> (pyridoxine: PLP)  
B<sub>7</sub> (biotin)  
B<sub>9</sub> (folate)  
B<sub>12</sub> (cobalamin)  
C (ascorbic acid)

Wash out easily from body except B<sub>12</sub> and B<sub>9</sub>. B<sub>12</sub> stored in liver for ~3–4 years. B<sub>9</sub> stored in liver for ~3–4 months. B-complex deficiencies often result in dermatitis, glossitis, and diarrhea. Can be coenzymes (eg, ascorbic acid) or precursors to coenzymes (eg, FAD, NAD<sup>+</sup>).

**Dietary supplementation**

DIET	SUPPLEMENTATION REQUIRED
Vegetarian/vegan	Vitamin B <sub>12</sub> Iron Vitamin B <sub>2</sub> Frequently, vitamin D (although this is commonly deficient in many diets)
High egg white (raw)	Vitamin B <sub>7</sub> (avidin in egg whites binds biotin and prevents absorption)
Untreated corn	Vitamin B <sub>3</sub> (deficiency is common in resource-limited areas)

**Vitamin A**

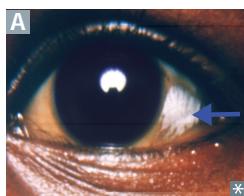
## FUNCTION

Includes retinal, retinol, retinoic acid.

Antioxidant; constituent of visual pigments (**retinal**); essential for normal differentiation of epithelial cells into specialized tissue (pancreatic cells, mucus-secreting cells); prevents squamous metaplasia.

**Retinol** is vitamin **A**, so think **retin-A** (used topically for wrinkles and **Acne**).  
Found in liver and leafy vegetables.  
Supplementation in vitamin A-deficient measles patients may improve outcomes.  
Use oral isotretinoin to treat severe cystic acne.  
Use *all-trans* retinoic acid to treat acute promyelocytic leukemia.

## DEFICIENCY



Night blindness (nyctalopia); dry, scaly skin (xerosis cutis); dry eyes (xerophthalmia); conjunctival squamous metaplasia → Bitot spots (keratin debris; foamy appearance on conjunctiva **A**); corneal degeneration (keratomalacia); immunosuppression.

## EXCESS

Acute toxicity—nausea, vomiting, ↑ ICP (eg, vertigo, blurred vision).  
Chronic toxicity—alopecia, dry skin (eg, scaliness), hepatic toxicity and enlargement, arthralgias, and idiopathic intracranial hypertension.

Teratogenic (interferes with homeobox gene; 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.**

**Vitamin B<sub>1</sub>**

Also called thiamine.

## FUNCTION

In thiamine pyrophosphate (TPP), a cofactor for several dehydrogenase enzyme reactions (**Be APT**):

- Branched-chain ketoacid dehydrogenase
- $\alpha$ -Ketoglutarate dehydrogenase (TCA cycle)
- Pyruvate dehydrogenase (links glycolysis to TCA cycle)
- Transketolase (HMP shunt)

## DEFICIENCY

Impaired glucose breakdown → ATP depletion worsened by glucose infusion; highly aerobic tissues (eg, brain, heart) are affected first. In patients with chronic alcohol overuse or malnutrition, give thiamine before dextrose to ↓ risk of precipitating Wernicke encephalopathy.  
Diagnosis made by ↑ in RBC transketolase activity following vitamin B<sub>1</sub> administration.

## DISORDER

## CHARACTERISTICS

<b>Wernicke encephalopathy</b>	Acute, reversible, life-threatening neurologic condition. Symptoms: <b>Confusion</b> , <b>Ophthalmoplegia</b> / <b>Nystagmus</b> , <b>Ataxia</b> ( <b>CorONA</b> beer).
<b>Korsakoff syndrome</b>	Amnestic disorder due to chronic alcohol overuse; presents with confabulation, personality changes, memory loss (permanent).
<b>Wernicke-Korsakoff syndrome</b>	Damage to medial dorsal nucleus of thalamus, mammillary bodies. Presentation is combination of Wernicke encephalopathy and Korsakoff syndrome.
<b>Dry beriberi</b>	Polyneuropathy, symmetric muscle wasting.
<b>Wet beriberi</b>	High-output cardiac failure (due to systemic vasodilation).

Spell beriberi as **Ber1Ber1** to remember vitamin **B<sub>1</sub>**.

**Vitamin B<sub>2</sub>**

Also called riboflavin.

**FUNCTION**

Component of flavins FAD and FMN, used as cofactors in redox reactions, eg, the succinate dehydrogenase reaction in the TCA cycle.

FAD and FMN are derived from riboFlavin ( $B_2 \approx 2$  ATP).**DEFICIENCY****Cheilosis** (inflammation of lips, scaling and fissures at the corners of the mouth), “magenta” tongue, **corneal vascularization**.The **2 C's** of  $B_2$ .**Vitamin B<sub>3</sub>**

Also called niacin, nicotinic acid.

**FUNCTION**Constituent of NAD<sup>+</sup>, NADP<sup>+</sup> (used in redox reactions and as cofactor by dehydrogenases). Derived from tryptophan. Synthesis requires vitamins B<sub>2</sub> and B<sub>6</sub>. Used to treat dyslipidemia ( $\downarrow$  VLDL,  $\uparrow$  HDL).NAD derived from **Niacin** ( $B_3 \approx 3$  ATP).**DEFICIENCY**

Glossitis. Severe deficiency of  $B_3$  leads to pellagra, which can also be caused by Hartnup disease, malignant carcinoid syndrome ( $\uparrow$  tryptophan metabolism  $\rightarrow$   $\uparrow$  serotonin synthesis), and isoniazid ( $\downarrow$  vitamin B<sub>6</sub>). Symptoms of  $B_3$  deficiency (pellagra) (the **3 D's**): **d**iarrhea, **d**ementia (also hallucinations), **d**ermatitis (C3/C4 dermatome circumferential “broad collar” rash [Casal necklace], hyperpigmentation of sun-exposed limbs **A**).

**Hartnup disease**—autosomal recessive.

Deficiency of neutral amino acid (eg, tryptophan) transporters in proximal renal tubular cells and on enterocytes  $\rightarrow$  neutral aminoaciduria and  $\downarrow$  absorption from the gut  $\rightarrow$   $\downarrow$  tryptophan for conversion to niacin  $\rightarrow$  pellagra-like symptoms. Treat with high-protein diet and nicotinic acid.

Pellagra = vitamin B<sub>3</sub> levels fell.**EXCESS**

Facial flushing (induced by prostaglandin, not histamine; can avoid by taking aspirin before niacin), hyperglycemia, hyperuricemia.

Podagra = vitamin B<sub>3</sub> OD (overdose).**Vitamin B<sub>5</sub>**Also called pantothenic acid. B<sub>5</sub> is “**pento**”thenic acid.**FUNCTION**

Component of coenzyme A (CoA, a cofactor for acyl transfers) and fatty acid synthase.

**DEFICIENCY**

Dermatitis, enteritis, alopecia, adrenal insufficiency may lead to burning sensation of feet (“burning feet syndrome”; distal paresthesias, dysesthesia).

**Vitamin B<sub>6</sub>**

Also called pyridoxine.

**FUNCTION**

Converted to pyridoxal phosphate (PLP), a cofactor used in transamination (eg, ALT and AST), decarboxylation reactions, glycogen phosphorylase. Synthesis of glutathione, 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 anemia (due to impaired hemoglobin synthesis and iron excess).

**Vitamin B<sub>7</sub>**

Also called biotin.

**FUNCTION**

Cofactor for carboxylation enzymes (which add a 1-carbon group):

- Pyruvate carboxylase (gluconeogenesis): pyruvate (3C) → oxaloacetate (4C)
- Acetyl-CoA carboxylase (fatty acid synthesis): acetyl-CoA (2C) → malonyl-CoA (3C)
- Propionyl-CoA carboxylase (fatty acid oxidation and branched-chain amino acid breakdown): propionyl-CoA (3C) → methylmalonyl-CoA (4C)

**DEFICIENCY**

Relatively rare. Dermatitis, enteritis, alopecia. Caused by long-term antibiotic use or excessive ingestion of raw egg whites.

**Avidin** in egg whites **avidly** binds biotin.”

**Vitamin B<sub>9</sub>**

Also called folate.

**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.

Found in leafy green vegetables. Also produced by gut microbiota. **Folate** absorbed in **jejunum** (think **foliage** in the “**jejun**”gle).

Small reserve pool stored primarily in the liver.

**DEFICIENCY**

Macrocytic, megaloblastic anemia; hypersegmented polymorphonuclear cells (PMNs); glossitis; no neurologic symptoms (as opposed to vitamin B<sub>12</sub> deficiency).

Labs: ↑ homocysteine, normal methylmalonic acid levels. Seen in chronic alcohol overuse and in pregnancy.

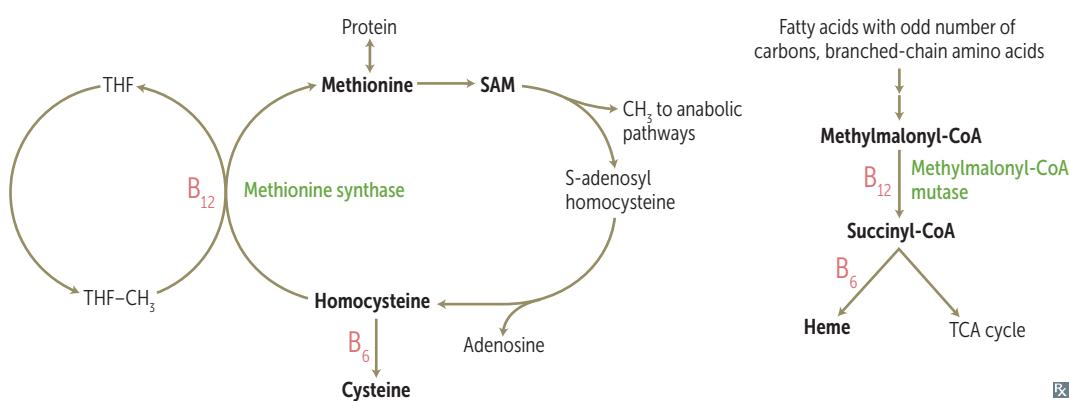
Deficiency can be caused by several drugs (eg, phenytoin, trimethoprim, methotrexate).

Supplemental folic acid at least 1 month prior to conception and during pregnancy to ↓ risk of neural tube defects. Give vitamin B<sub>9</sub> for the **9** months of pregnancy, and 1 month prior to conception.

**Vitamin B<sub>12</sub>**

Also called cobalamin.

FUNCTION	Cofactor for methionine synthase (transfers CH <sub>3</sub> groups as methylcobalamin) and methylmalonyl-CoA mutase. Important for DNA synthesis.	Found in animal products. Synthesized only by intestinal microbiota. Site of synthesis in humans is distal to site of absorption; thus B <sub>12</sub> must be consumed via animal products.
DEFICIENCY	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.	Very large reserve pool (several years) stored primarily in the liver. Deficiency caused by malabsorption (eg, sprue, enteritis, <i>Diphyllobothrium latum</i> , achlorhydria, bacterial overgrowth, alcohol overuse), lack of intrinsic factor (eg, pernicious anemia, gastric bypass surgery), absence of terminal ileum (surgical resection, eg, for Crohn disease), certain drugs (eg, metformin), or insufficient intake (eg, veganism). B <sub>9</sub> (folate) supplementation can mask the hematologic symptoms of B <sub>12</sub> deficiency, but not the neurologic symptoms.

**Vitamin C**

Also called ascorbic acid.

FUNCTION	Antioxidant; also facilitates iron absorption by reducing it to Fe <sup>2+</sup> state. Necessary for hydroxylation of proline and lysine in collagen synthesis. Necessary for dopamine β-hydroxylase (converts dopamine to NE).	Found in fruits and vegetables. Pronounce “ <b>absorbic</b> ” acid. Ancillary treatment for methemoglobinemia by reducing Fe <sup>3+</sup> to Fe <sup>2+</sup> .
DEFICIENCY	<b>Scurvy</b> —swollen gums, easy bruising, petechiae, hemarthrosis, anemia, poor wound healing, perifollicular and subperiosteal hemorrhages, “corkscrew” hair. Weakened immune response.	Deficiency may be precipitated by tea and toast diet. Vitamin C deficiency causes <b>sCurvy</b> due to a <b>Collagen hydroCylation</b> defect.
EXCESS	Nausea, vomiting, diarrhea, fatigue, calcium oxalate nephrolithiasis (excess oxalate from vitamin C metabolism). Can ↑ iron toxicity in predisposed individuals by increasing dietary iron absorption (ie, can worsen hemochromatosis or transfusion-related iron overload).	

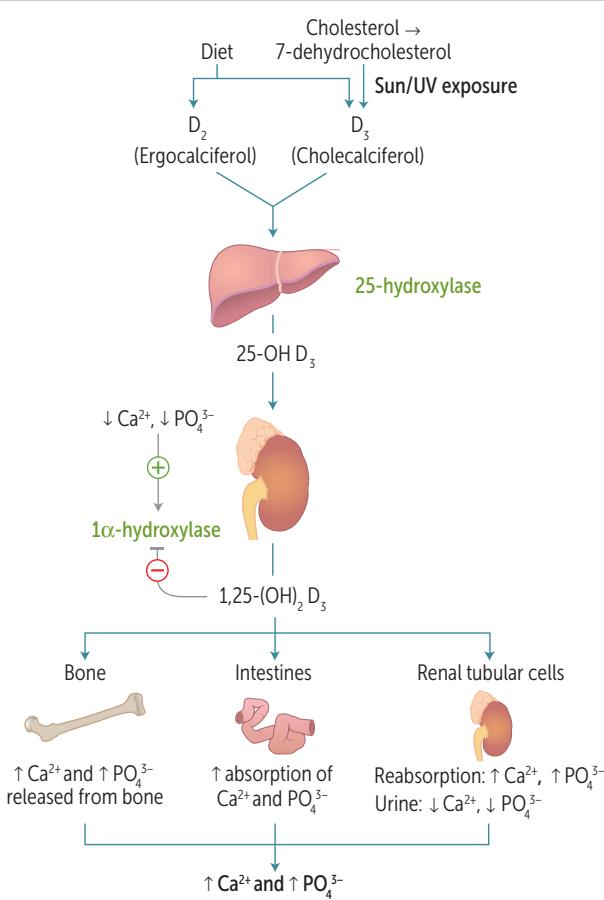
**Vitamin D**

D<sub>3</sub> (cholecalciferol) from exposure of skin (stratum basale) to sun, ingestion of fish, milk, plants.

D<sub>2</sub> (ergocalciferol) from ingestion of plants, fungi, yeasts.

Both converted to 25-OH D<sub>3</sub> (storage form) in liver and to the active form 1,25-(OH)<sub>2</sub> D<sub>3</sub> (calcitriol) in kidney.

<b>FUNCTION</b>	<ul style="list-style-type: none"> <li>↑ intestinal absorption of Ca<sup>2+</sup> and PO<sub>4</sub><sup>3-</sup>.</li> <li>↑ bone mineralization at low levels.</li> <li>↑ bone resorption at higher levels.</li> </ul>
<b>REGULATION</b>	<ul style="list-style-type: none"> <li>↑ PTH, ↓ Ca<sup>2+</sup>, ↓ PO<sub>4</sub><sup>3-</sup> <ul style="list-style-type: none"> <li>→ ↑ 1,25-(OH)<sub>2</sub>D<sub>3</sub> production.</li> </ul> </li> <li>1,25-(OH)<sub>2</sub>D<sub>3</sub> feedback inhibits its own production.</li> <li>↑ PTH → ↑ Ca<sup>2+</sup> reabsorption and ↓ PO<sub>4</sub><sup>3-</sup> reabsorption in the kidney.</li> </ul>
<b>DEFICIENCY</b>	<p>Rickets in children (deformity, such as genu varum “bowlegs” <b>A</b>), osteomalacia in adults (bone pain and muscle weakness), hypocalcemic tetany.</p> <p>Caused by malabsorption, ↓ sun exposure, poor diet, chronic kidney disease (CKD), advanced liver disease. Give oral vitamin D to breastfed infants. Darker skin and prematurity predispose to deficiency.</p>
<b>EXCESS</b>	<p>Hypercalcemia, hypercalciuria, loss of appetite, stupor. Seen in granulomatous diseases (↑ activation of vitamin D by epithelioid macrophages).</p>

**Vitamin E**

Includes tocopherol, tocotrienol.

<b>FUNCTION</b>	Antioxidant (protects RBCs and neuronal membranes from free radical damage).
<b>DEFICIENCY</b>	Hemolytic anemia, acanthocytosis, muscle weakness, demyelination of posterior columns (↓ proprioception and vibration sensation) and spinocerebellar tract (ataxia). Closely mimics Friedreich ataxia.
<b>EXCESS</b>	Risk of enterocolitis in infants with excess of vitamin E.

Neurologic presentation may appear similar to vitamin B<sub>12</sub> deficiency, but without megaloblastic anemia, hypersegmented neutrophils, or ↑ serum methylmalonic acid levels.

High-dose supplementation may alter metabolism of vitamin K → enhanced anticoagulant effects of warfarin.

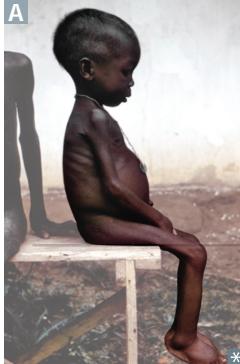
**Vitamin K**

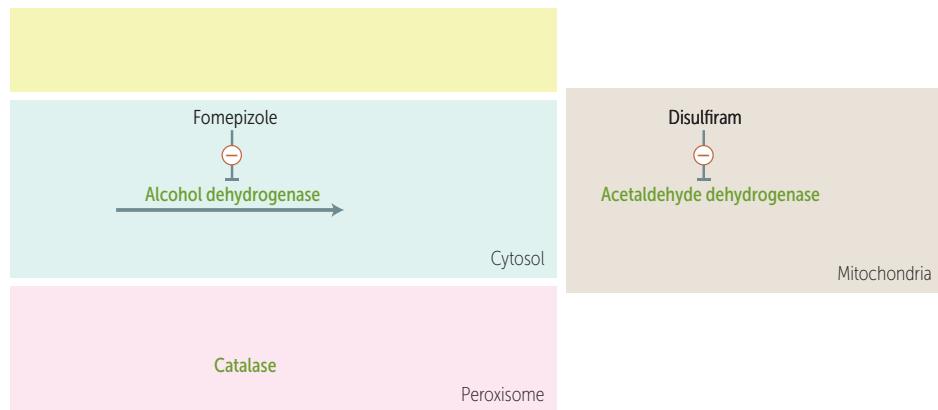
<b>FUNCTION</b>	Includes phytomenadione, phylloquinone, phytonadione, menaquinone.	
<b>DEFICIENCY</b>	Activated by epoxide reductase to the reduced form, which is a cofactor for the $\gamma$ -carboxylation of glutamic acid residues on various proteins required for blood clotting. Synthesized by intestinal microbiota.	<b>K</b> is for <b>Koagulation</b> . Necessary for the maturation of clotting factors II, VII, IX, X, and proteins C and S. Warfarin inhibits vitamin K-dependent synthesis of these factors and proteins.
	Neonatal hemorrhage with $\uparrow$ PT and $\uparrow$ aPTT but normal bleeding time (neonates have sterile intestines and are unable to synthesize vitamin K). Can also occur after prolonged use of broad-spectrum antibiotics or hepatocellular disease.	Not in breast milk; “breast-fed infants <b>Don’t Know</b> about vitamins <b>D</b> and <b>K</b> ”. Neonates are given vitamin K injection at birth to prevent hemorrhagic disease of the newborn.

**Zinc**

<b>FUNCTION</b>	Mineral essential for the activity of 100+ enzymes. Important in the formation of zinc fingers (transcription factor motif).
<b>DEFICIENCY</b>	Delayed wound healing, suppressed immunity, male hypogonadism, $\downarrow$ adult hair (axillary, facial, pubic), dysgeusia, anosmia. Associated with acrodermatitis enteropathica <b>A</b> (congenital defect in intestinal zinc absorption manifesting with triad of hair loss, diarrhea, and inflammatory skin rash around body openings (periorificial) and tips of fingers/toes (acral). May predispose to alcoholic cirrhosis.  

**Protein-energy malnutrition**

<b>Kwashiorkor</b>	Protein malnutrition resulting in skin lesions, edema due to $\downarrow$ plasma oncotic pressure (due to low serum albumin), liver malfunction (fatty change due to $\downarrow$ apolipoprotein synthesis and deposition). Clinical picture is small child with swollen abdomen <b>A</b> . Kwashiorkor results from protein-deficient <b>MEALS</b> : <b>M</b> alnutrition <b>E</b> dema <b>A</b> nemia <b>L</b> iver (fatty) <b>S</b> kin lesions (eg, hyperkeratosis, dyspigmentation)	 
<b>Marasmus</b>	Malnutrition not causing edema. Diet is deficient in calories but no nutrients are entirely absent. Marasmus results in muscle wasting <b>B</b> .	Linear growth maintained in acute protein-energy malnutrition (vs chronic malnutrition).

**Ethanol metabolism**

## ► BIOCHEMISTRY—METABOLISM

<b>Enzyme terminology</b>	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.
<b>Kinase</b>	Catalyzes transfer of a phosphate group from a high-energy molecule (usually ATP) to a substrate (eg, phosphofructokinase).
<b>Phosphorylase</b>	Adds inorganic phosphate onto substrate without using ATP (eg, glycogen phosphorylase).
<b>Phosphatase</b>	Removes phosphate group from substrate (eg, fructose-1,6-bisphosphatase 1).
<b>Dehydrogenase</b>	Catalyzes oxidation-reduction reactions (eg, pyruvate dehydrogenase).
<b>Hydroxylase</b>	Adds hydroxyl group ( $-OH$ ) onto substrate (eg, tyrosine hydroxylase).
<b>Carboxylase</b>	Transfers carboxyl groups ( $-COOH$ ) with the help of biotin (eg, pyruvate carboxylase).
<b>Mutase</b>	Relocates a functional group within a molecule (eg, vitamin $B_{12}$ -dependent methylmalonyl-CoA mutase).
<b>Synthase/synthetase</b>	Joins two molecules together using a source of energy (eg, ATP, acetyl-CoA, nucleotide sugar).

**Rate-determining enzymes of metabolic processes**

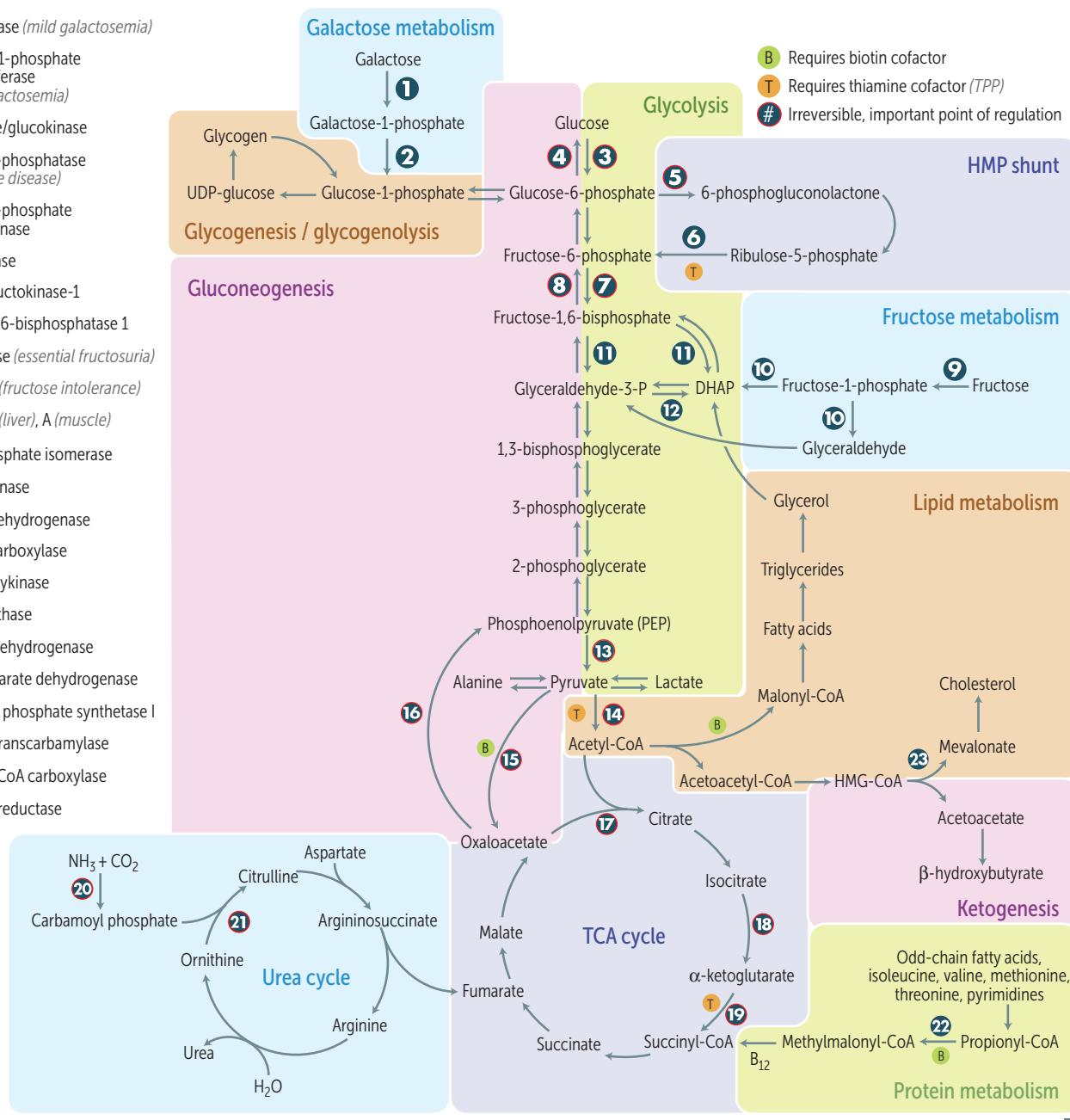
PROCESS	ENZYME	REGULATORS
Glycolysis	Phosphofructokinase-1 (PFK-1)	AMP $\oplus$ , fructose-2,6-bisphosphate $\oplus$ ATP $\ominus$ , citrate $\ominus$
Gluconeogenesis	Fructose-1,6-bisphosphatase 1	AMP $\ominus$ , fructose-2,6-bisphosphate $\ominus$
TCA cycle	Isocitrate dehydrogenase	ADP $\oplus$ ATP $\ominus$ , NADH $\ominus$
Glycogenesis	Glycogen synthase	Glucose-6-phosphate $\oplus$ , insulin $\oplus$ , cortisol $\oplus$ Epinephrine $\ominus$ , glucagon $\ominus$
Glycogenolysis	Glycogen phosphorylase	Epinephrine $\oplus$ , glucagon $\oplus$ , AMP $\oplus$ Glucose-6-phosphate $\ominus$ , insulin $\ominus$ , ATP $\ominus$
HMP shunt	Glucose-6-phosphate dehydrogenase (G6PD)	NADP $^+$ $\oplus$ NADPH $\ominus$
De novo pyrimidine synthesis	Carbamoyl phosphate synthetase II	ATP $\oplus$ , PRPP $\oplus$ UTP $\ominus$
De novo purine synthesis	Glutamine-phosphoribosylpyrophosphate (PRPP) amidotransferase	AMP $\ominus$ , inosine monophosphate (IMP) $\ominus$ , GMP $\ominus$
Urea cycle	Carbamoyl phosphate synthetase I	N-acetylglutamate $\oplus$
Fatty acid synthesis	Acetyl-CoA carboxylase (ACC)	Insulin $\oplus$ , citrate $\oplus$ Glucagon $\ominus$ , palmitoyl-CoA $\ominus$
Fatty acid oxidation	Carnitine acyltransferase I	Malonyl-CoA $\ominus$
Ketogenesis	HMG-CoA synthase (HOMG! I'm starving!)	
Cholesterol synthesis	HMG-CoA reductase	Insulin $\oplus$ , thyroxine $\oplus$ , estrogen $\oplus$ Glucagon $\ominus$ , cholesterol $\ominus$

**Metabolism sites**

<b>Mitochondria</b>	Fatty acid oxidation ( $\beta$ -oxidation), acetyl-CoA production, TCA cycle, oxidative phosphorylation, ketogenesis.
<b>Cytoplasm</b>	Glycolysis, HMP shunt, and synthesis of cholesterol (SER), proteins (ribosomes, RER), fatty acids, and nucleotides.
<b>Both</b>	Heme synthesis, urea cycle, gluconeogenesis. <b>Hugs take two</b> (both).

**Summary of pathways**

- ① Galactokinase (*mild galactosemia*)
- ② Galactose-1-phosphate uridylyltransferase (*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 1
- 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  $\alpha$ -ketoglutarate dehydrogenase
- ⑳ Carbamoyl phosphate synthetase I
- ㉑ Ornithine transcarbamylase
- ㉒ Propionyl-CoA carboxylase
- ㉓ HMG-CoA reductase



**Activated carriers**

CARRIER MOLECULE	CARRIED IN ACTIVATED FORM
ATP	Phosphoryl groups
NADH, NADPH, FADH <sub>2</sub>	Electrons
CoA, lipoamide	Acyl groups
Biotin	CO <sub>2</sub>
Tetrahydrofolates	1-carbon units
S-adenosylmethionine (SAM)	CH <sub>3</sub> groups
TPP	Aldehydes

**Universal electron acceptors**

Nicotinamides (NAD<sup>+</sup>, NADP<sup>+</sup> from vitamin B<sub>3</sub>) and flavin nucleotides (FAD from vitamin B<sub>2</sub>).

NAD<sup>+</sup> is generally used in **catabolic** processes to carry reducing equivalents away as NADH.

NADPH is used in **anabolic** processes to add electrons.

### Glycolysis regulation, key enzymes

Net glycolysis (cytoplasm):

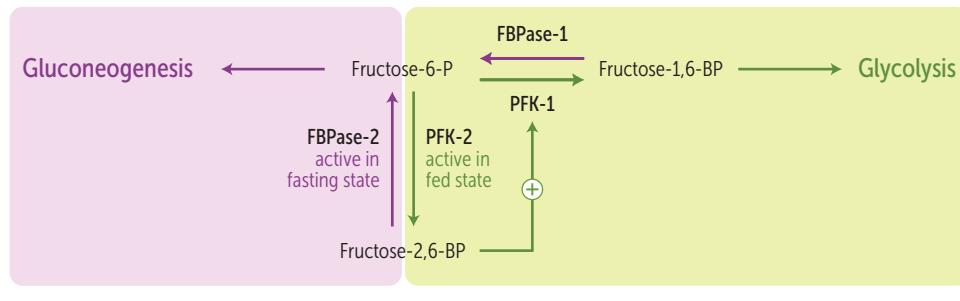


Equation not balanced chemically, and exact balanced equation depends on ionization state of reactants and products.

	REQUIRE ATP	PRODUCE ATP	
	Glucose → Glucose-6-P Hexokinase/glucokinase		Glucose-6-P ⊖ hexokinase. Fructose-6-P ⊖ glucokinase.
	Fructose-6-P → Fructose-1,6-BP Phosphofructokinase-1 (rate-limiting step)		AMP ⊕, fructose-2,6-bisphosphate ⊕. ATP ⊖, citrate ⊖.
		1,3-BPG ←→ 3-PG Phosphoglycerate kinase	
		Phosphoenolpyruvate → Pyruvate Pyruvate kinase	Fructose-1,6-bisphosphate ⊕. ATP ⊖, alanine ⊖, glucagon ⊖.

### Regulation by fructose-2,6-bisphosphate

Fructose bisphosphatase-2 (FBPase-2) and phosphofructokinase-2 (PFK-2) are the same bifunctional enzyme whose function is reversed by phosphorylation by protein kinase A.



Fasting state: ↑ glucagon → ↑ cAMP → ↑ protein kinase A → ↑ FBPase-2, ↓ PFK-2, less glycolysis, more gluconeogenesis.

**FaBian the Peasant (FBP)** has to work hard when starving.

Fed state: ↑ insulin → ↓ cAMP → ↓ protein kinase A → ↓ FBPase-2, ↑ PFK-2, more glycolysis, less gluconeogenesis.

**Prince FredericK (PFK)** works only when fed.

### Pyruvate dehydrogenase complex

Mitochondrial enzyme complex linking glycolysis and TCA cycle. Differentially regulated in fed (active)/fasting (inactive) states. Reaction: pyruvate + NAD<sup>+</sup> + CoA → acetyl-CoA + CO<sub>2</sub> + NADH. Contains 3 enzymes requiring 5 cofactors:

1. Thiamine pyrophosphate (B<sub>1</sub>)
2. Lipoic acid
3. CoA (B<sub>5</sub>, pantothenic acid)
4. FAD (B<sub>2</sub>, riboflavin)
5. NAD<sup>+</sup> (B<sub>3</sub>, niacin)

Activated by: ↑ NAD<sup>+</sup>/NADH ratio, ↑ ADP, ↑ Ca<sup>2+</sup>.

The complex is similar to the α-ketoglutarate dehydrogenase complex (same cofactors, similar substrate and action), which converts α-ketoglutarate → succinyl-CoA (TCA cycle).

**The lovely coenzymes for nerds.**

Arsenic inhibits lipoic acid. Arsenic poisoning clinical findings: imagine a vampire (pigmentary skin changes, skin cancer), vomiting and having diarrhea, running away from a cutie (QT prolongation) with garlic breath.

### Pyruvate dehydrogenase complex deficiency

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

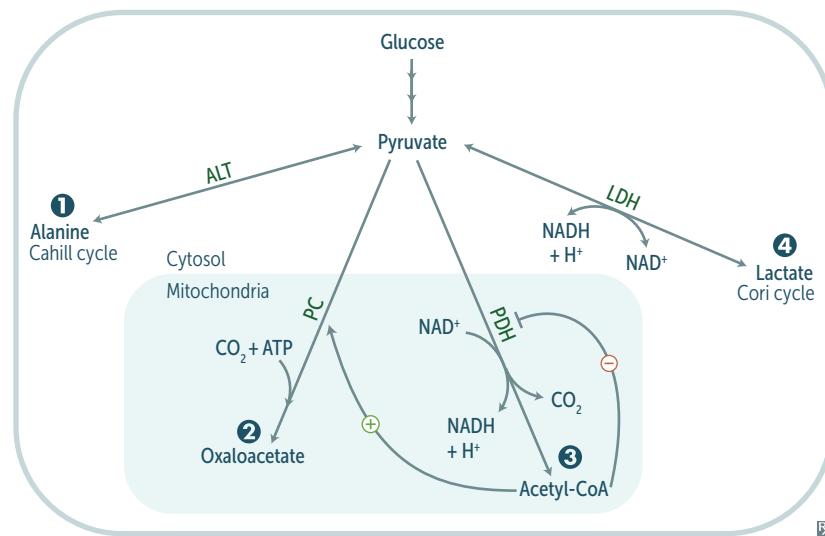
#### FINDINGS

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

#### TREATMENT

↑ intake of ketogenic nutrients (eg, high fat content or ↑ lysine and leucine).

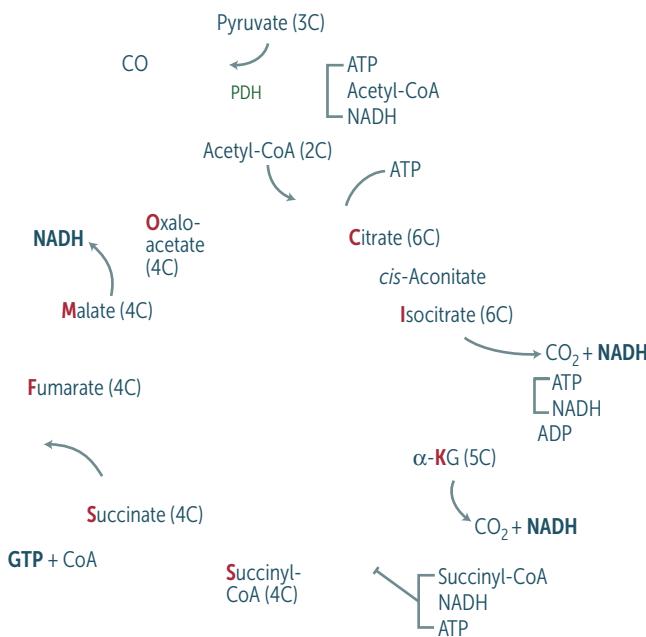
### Pyruvate metabolism

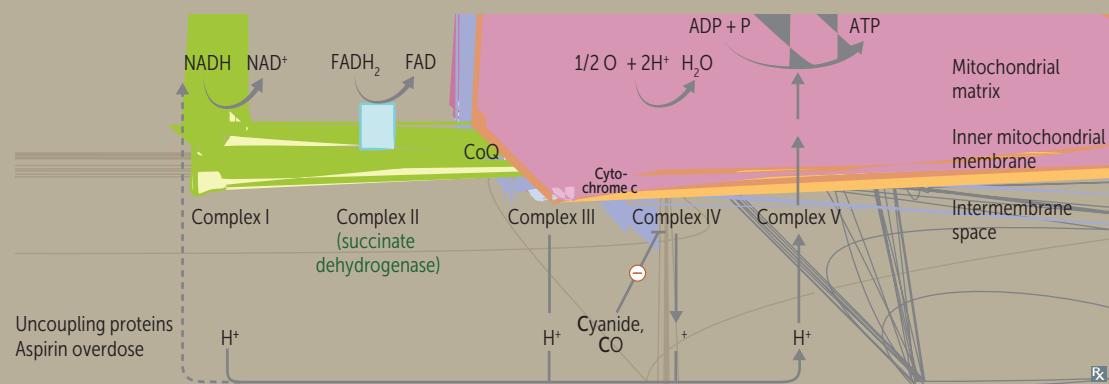


Functions of different pyruvate metabolic pathways (and their associated cofactors):

- ① Alanine aminotransferase (B<sub>6</sub>): alanine carries amino groups to the liver from muscle
- ② Pyruvate carboxylase (B<sub>7</sub>): oxaloacetate can replenish TCA cycle or be used in gluconeogenesis
- ③ Pyruvate dehydrogenase (B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>5</sub>, lipoic acid): transition from glycolysis to the TCA cycle
- ④ Lactic acid dehydrogenase (B<sub>3</sub>): end of anaerobic glycolysis (major pathway in RBCs, WBCs, kidney medulla, lens, testes, and cornea)

### TCA cycle





### Gluconeogenesis, irreversible enzymes

**Pyruvate carboxylase**

**Phosphoenolpyruvate carboxykinase**

**Fructose-1,6-bisphosphatase 1**

**Glucose-6-phosphatase**

All enzymes may be subject to activation by glucagon in fasting state.

In mitochondria. Pyruvate → oxaloacetate.

In cytosol. Oxaloacetate → phosphoenolpyruvate (PEP).

In cytosol. Fructose-1,6-bisphosphate → fructose-6-phosphate.

In ER. Glucose-6-phosphate → glucose.

Pathway produces **fresh glucose**.

Requires biotin, ATP. Activated by acetyl-CoA.

Requires GTP.

Citrate  $\oplus$ , AMP  $\ominus$ , fructose 2,6-bisphosphate  $\ominus$ .

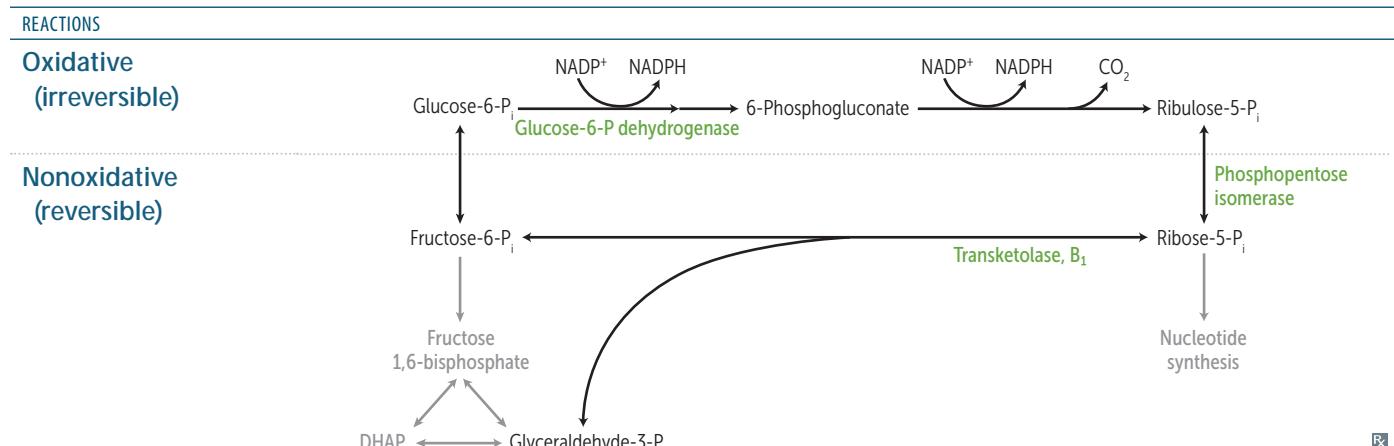
Occurs primarily in liver; serves to maintain euglycemia during fasting. Enzymes also found in kidney, intestinal epithelium. Deficiency of the key gluconeogenic enzymes causes hypoglycemia. (Muscle cannot participate in gluconeogenesis because it lacks glucose-6-phosphatase).

**Odd-chain fatty acids** yield 1 propionyl-CoA during metabolism, which can enter the TCA cycle (as succinyl-CoA), undergo gluconeogenesis, and serve as a **glucose** source (It's **odd** for **fatty acids** to make **glucose**). Even-chain fatty acids cannot produce new glucose, since they yield only acetyl-CoA equivalents.

### Pentose phosphate pathway

Also called HMP shunt. Provides a source of NADPH from abundantly available glucose-6-P (NADPH is required for reductive reactions, eg, glutathione reduction inside RBCs, fatty acid and cholesterol biosynthesis). Additionally, this pathway yields ribose for nucleotide synthesis. Two distinct phases (oxidative and nonoxidative), both of which occur in the cytoplasm. No ATP is used or produced.

Sites: lactating mammary glands, liver, adrenal cortex (sites of fatty acid or steroid synthesis), RBCs.



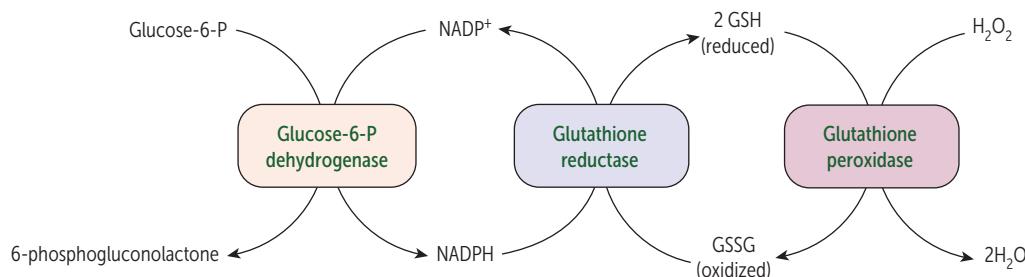
### Glucose-6-phosphate dehydrogenase deficiency

NADPH is necessary to keep glutathione reduced, which in turn detoxifies free radicals and peroxides. ↓ NADPH in RBCs leads to hemolytic anemia due to poor RBC defense against oxidizing agents (eg, fava beans, sulfonamides, nitrofurantoin, primaquine). Infection (most common cause) can also precipitate hemolysis; inflammatory response produces free radicals that diffuse into RBCs, causing oxidative damage.

X-linked recessive disorder; most common human enzyme deficiency; more prevalent among descendants of populations in malaria-endemic regions (eg, sub-Saharan Africa, Southeast Asia).

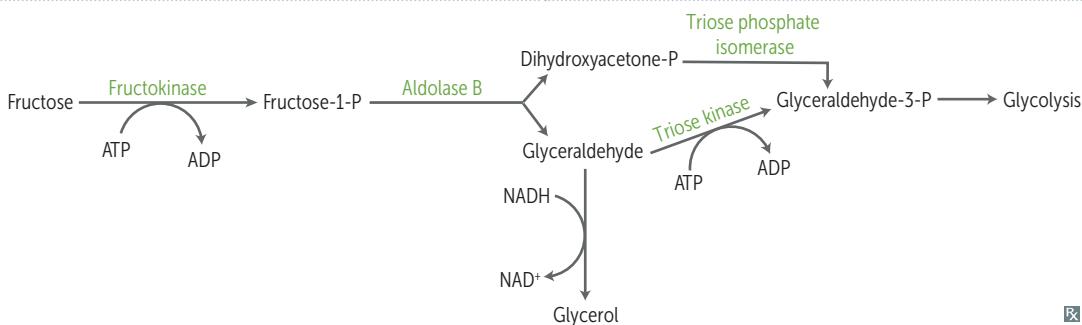
**Heinz bodies**—denatured globin chains precipitate within RBCs due to oxidative stress.

**Bite cells**—result from the phagocytic removal of **Heinz** bodies by splenic macrophages. Think, “**Bite** into some **Heinz** ketchup.”

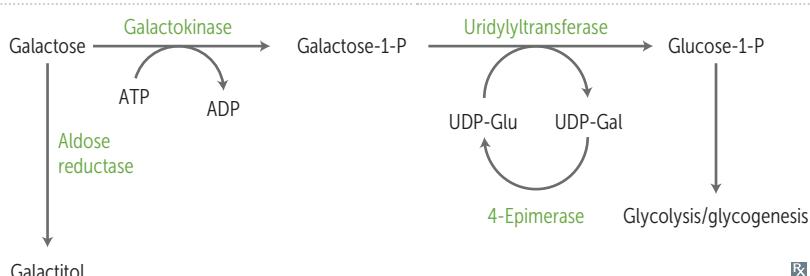


**Disorders of fructose metabolism**

	<b>Essential fructosuria</b>	<b>Hereditary fructose intolerance</b>
<b>ENZYME DEFICIENCY</b>	Fructokinase (autosomal recessive)	Aldolase B (autosomal recessive)
<b>PATHOPHYSIOLOGY</b>	Fructose is not trapped into cells. Hexokinase becomes 1° pathway for converting fructose to fructose-6-phosphate.	Fructose-1-phosphate accumulates → ↓ available phosphate → inhibition of glycogenolysis and gluconeogenesis.
<b>PRESENTATION (SIGNS/SYMPOMTS)</b>	Asymptomatic, benign. Fructose appears in blood and urine (fructokinase deficiency is kinder).	Hypoglycemia, jaundice, cirrhosis, vomiting. Symptoms only present following consumption of fruit, juice, or honey.
<b>ADDITIONAL REMARKS</b>	Urine dipstick will be ⊖ (tests for glucose only); reducing sugar can be detected in the urine (nonspecific test for inborn errors of carbohydrate metabolism).	↓ intake of fructose, sucrose (glucose + fructose), and sorbitol (metabolized to fructose).
<b>TREATMENT</b>	–	–

**Disorders of galactose metabolism**

	<b>Galactokinase deficiency</b>	<b>Classic galactosemia</b>
<b>ENZYME DEFICIENCY</b>	Galactokinase (autosomal recessive).	Galactose-1-phosphate uridylyltransferase (autosomal recessive).
<b>PATHOPHYSIOLOGY</b>	Galactitol accumulates if diet has galactose.	Damage caused by accumulation of toxic substances (eg, galactitol).
<b>PRESENTATION (SIGNS/SYMPOMTS)</b>	Relatively mild/benign condition (galactokinase deficiency is kinder). Galactose appears in blood (galactosemia) and urine (galactosuria); infantile cataracts. May present as failure to track objects or develop social smile.	Symptoms start when infant is fed formula or breast milk → failure to thrive, jaundice, hepatomegaly, infantile cataracts (galactitol deposition in eye lens), intellectual disability. Can predispose neonates to <i>E. coli</i> sepsis.
<b>TREATMENT</b>	–	Exclude galactose and lactose (galactose + glucose) from diet.



**Sorbitol**

An alternative method of trapping glucose in the cell is to convert it to its alcohol counterpart, sorbitol, via aldose reductase. Some tissues then convert sorbitol to fructose using sorbitol dehydrogenase; tissues with an insufficient amount/activity of this enzyme are at risk of intracellular sorbitol accumulation, causing osmotic damage (eg, cataracts, retinopathy, and peripheral neuropathy seen with chronic hyperglycemia in diabetes).

High blood levels of galactose also result in conversion to the osmotically active galactitol via aldose reductase.

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 (**LARKS**).

**Lactase deficiency**

Insufficient lactase enzyme → dietary lactose intolerance. Lactase functions on the intestinal brush border to digest lactose (in milk and milk products) into glucose and galactose.

Primary: age-dependent decline after childhood (absence of lactase-persistent allele), common in people of Asian, African, or Native American descent.

Secondary: loss of intestinal brush border due to gastroenteritis (eg, rotavirus), autoimmune disease. Congenital lactase deficiency: rare, due to defective gene.

Stool demonstrates ↓ pH and breath shows ↑ hydrogen content with lactose hydrogen breath test ( $\text{H}^+$  is produced when colonic bacteria ferment undigested lactose). Intestinal biopsy reveals normal mucosa in patients with hereditary lactose intolerance.

**FINDINGS**

Bloating, cramps, flatulence (all due to fermentation of lactose by colonic bacteria → gas), and osmotic diarrhea (undigested lactose).

**TREATMENT**

Avoid dairy products or add lactase pills to diet; lactose-free milk.

**Amino acids**

Only L-amino acids are found in proteins.

**Essential**

**PVT TIM HALL:** Phenylalanine, Valine, Tryptophan, Threonine, Isoleucine, Methionine, Histidine, Leucine, Lysine.

Glucogenic: **Methionine, histidine, valine**. We **met his valentine**, who is so **sweet** (glucogenic).

Glucogenic/ketogenic: Isoleucine, phenylalanine, threonine, tryptophan.

Ketogenic: leucine, lysine. The only purely ketogenic amino acids.

**Acidic**

Aspartic acid, glutamic acid.

Negatively charged at body pH.

**Basic**

Arginine, histidine, lysine.

Arginine is most **basic**. Histidine has no charge at body pH.

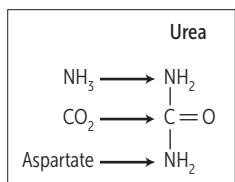
Arginine and histidine are required during periods of growth.

Arginine and lysine are ↑ in histones which bind negatively charged DNA.

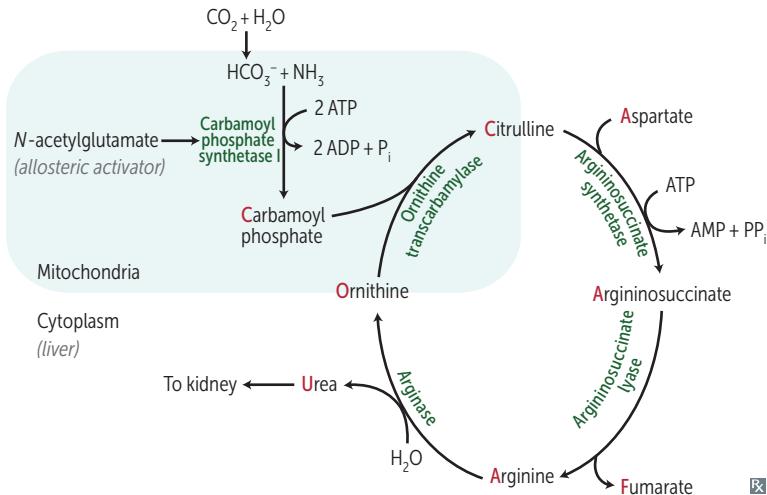
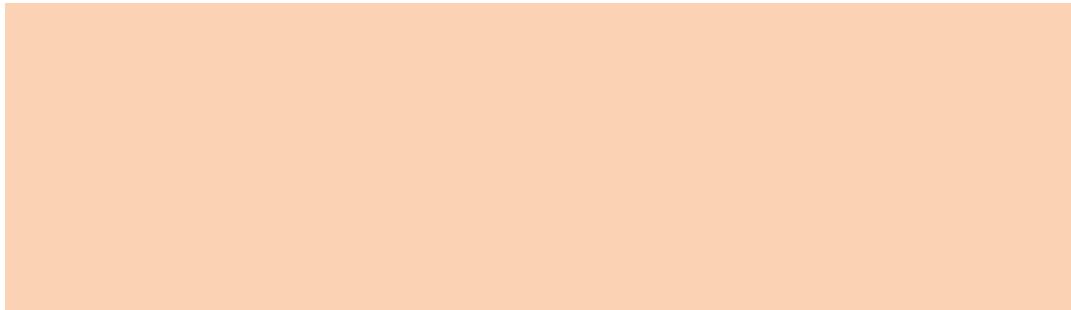
**His lys (lies) are basic.**

**Urea cycle**

Amino acid catabolism generates common metabolites (eg, pyruvate, acetyl-CoA), which serve as metabolic fuels. Excess nitrogen is converted to urea and excreted by the kidneys.



Ordinarily, Careless Crappers Are Also Frivolous About Urination.

**Transport of ammonia by alanine**

**Ornithine  
transcarbamylase  
deficiency**

Most common urea cycle disorder. 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. Excess carbamoyl phosphate is converted to orotic acid (part of the pyrimidine synthesis pathway).

Findings: ↑ orotic acid in blood and urine, ↓ BUN, symptoms of hyperammonemia. No megaloblastic anemia (vs orotic aciduria).

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**Phenylketonuria**

Caused by ↓ phenylalanine hydroxylase (PAH). Tyrosine becomes essential. ↑ phenylalanine → ↑ phenyl ketones in urine.

**Tetrahydrobiopterin ( $BH_4$ ) deficiency**— $BH_4$  is an essential cofactor for PAH.  $BH_4$  deficiency → ↑ phenylalanine. Varying degrees of clinical severity. Untreated patients typically die in infancy.

**Phenylalanine embryopathy**—↑ phenylalanine levels in pregnant patients with untreated PKU can cause fetal growth restriction, microcephaly, intellectual disability, congenital heart defects. Can be prevented with dietary measures.

Autosomal recessive.

Screening occurs 2–3 days after birth (normal at birth because of maternal enzyme during fetal life).

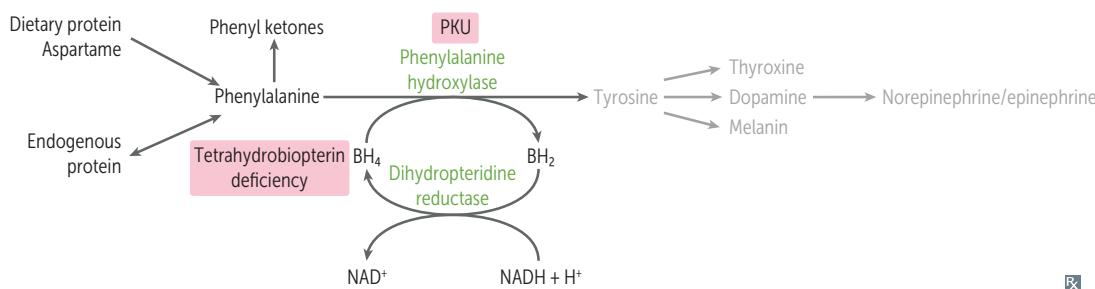
Findings: intellectual disability, microcephaly, seizures, hypopigmented skin, eczema, musty body odor.

Treatment: ↓ phenylalanine and ↑ tyrosine in diet (eg, soy products, chicken, fish, milk), tetrahydrobiopterin supplementation.

Phenyl ketones—phenylacetate, phenyllactate, and phenylpyruvate.

Disorder of **aromatic** amino acid metabolism → musty body **odor**.

Patients with PKU must avoid the artificial sweetener aspartame, which contains phenylalanine.



Rx

**Maple syrup urine disease**

Blocked degradation of **branched** amino acids (Isoleucine, leucine, valine) due to ↓ branched-chain α-ketoacid dehydrogenase ( $B_1$ ). Causes ↑ α-ketoacids in the blood, especially those of leucine.

Treatment: restriction of isoleucine, leucine, valine in diet, and thiamine supplementation.

Autosomal recessive.

Presentation: vomiting, poor feeding, urine smells like maple syrup/burnt sugar. Causes progressive neurological decline.

I love Vermont **maple syrup** from maple trees (with **B**ranches).

**Alkaptonuria**

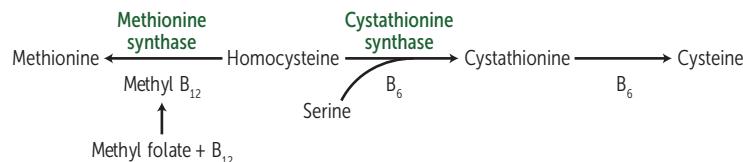
Congenital deficiency of homogentisate oxidase in the degradative pathway of tyrosine to fumarate → pigment-forming homogentisic acid builds up in tissue. Autosomal recessive. Usually benign.

Findings: bluish-black connective tissue, ear cartilage, and sclerae (ochronosis **A**); urine turns black on prolonged exposure to air. May have debilitating arthralgias (homogentisic acid toxic to cartilage).

**Homocystinuria**

Causes (all autosomal recessive):

- Cystathione synthase deficiency  
(treatment: ↓ methionine, ↑ cysteine, ↑ B<sub>6</sub>, B<sub>12</sub>, and folate in diet)
- ↓ affinity of cystathione synthase for pyridoxal phosphate (treatment: ↑↑ B<sub>6</sub> and ↑ cysteine in diet)
- Methionine synthase (homocysteine methyltransferase) deficiency (treatment: ↑ methionine in diet)
- Methylenetetrahydrofolate reductase (MTHFR) deficiency (treatment: ↑ folate in diet)

**Cystinuria**

Hereditary defect of renal PCT and intestinal amino acid transporter that prevents reabsorption of **Cystine**, **Ornithine**, **Lysine**, and **Arginine** (**COLA**).

Cystine is made of 2 cysteines connected by a disulfide bond.

Excess cystine in the urine can lead to recurrent precipitation of hexagonal cystine stones **A**.

Treatment: urinary alkalinization (eg, potassium citrate, acetazolamide) and chelating agents (eg, penicillamine) ↑ solubility of cystine stones; good hydration; diet low in methionine.

All forms result in excess homocysteine.

**HOMOCY**sturia: ↑↑ Homocysteine in urine, **Osteoporosis**, **Marfanoid habitus**, **Ocular changes** (downward and inward lens subluxation), **Cardiovascular effects** (thrombosis and atherosclerosis → stroke and MI), **KYphosis**, intellectual disability, hypopigmented skin. In homocystinuria, lens subluxes “down and in” (vs **Marfan**, “up and **fans** out”).

Autosomal recessive. Common (1:7000).

Cystinuria detected with urinary sodium-cyanide nitroprusside test and proton nuclear magnetic resonance spectroscopy of urine.

**Organic acidemias**

Most commonly present in infancy with poor feeding, vomiting, hypotonia, high anion gap metabolic acidosis, hepatomegaly, seizures. Organic acid accumulation:

- Inhibits gluconeogenesis → ↓ fasting blood glucose levels, ↑ ketoacidosis → high anion gap metabolic acidosis
- Inhibits urea cycle → hyperammonemia

**Propionic acidemia**

Deficiency of propionyl-CoA carboxylase → ↑ propionyl-CoA, ↓ methylmalonic acid.

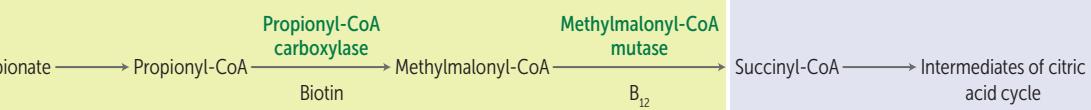
Treatment: low-protein diet limited in substances that metabolize into propionyl-CoA: **Valine**, **Odd-chain fatty acids**, **Methionine**, **Isoleucine**, **Threonine** (**VOMIT**).

**Methylmalonic acidemia**

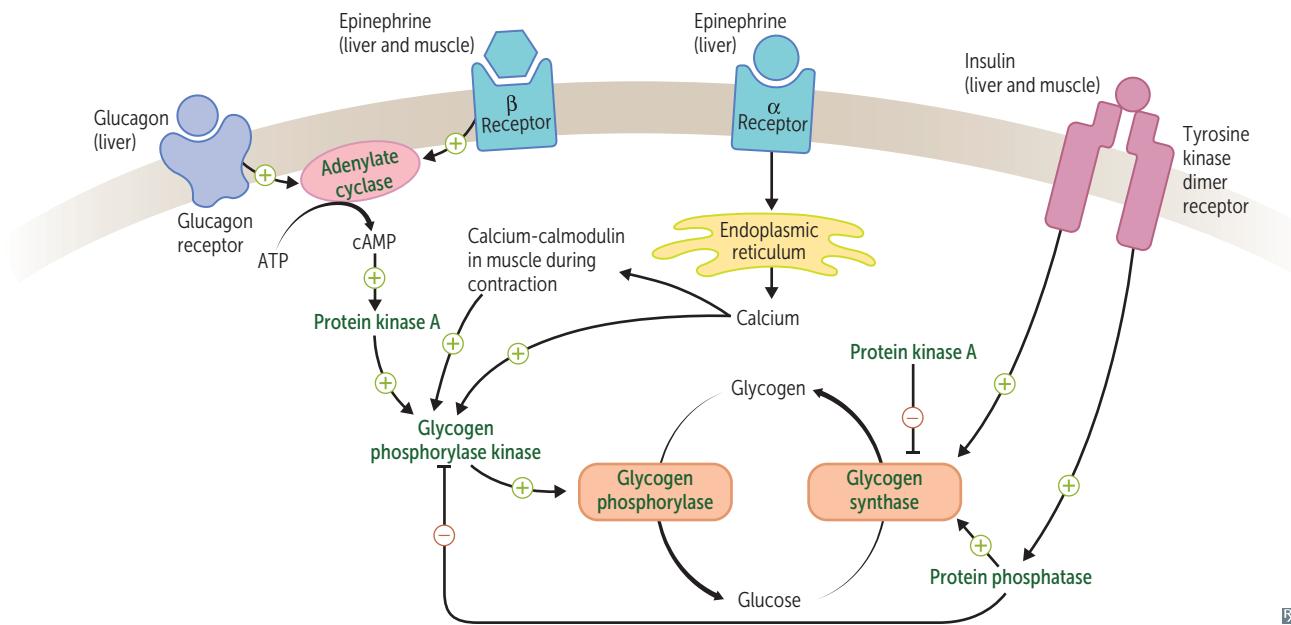
Deficiency of methylmalonyl-CoA mutase or vitamin B<sub>12</sub>.

**Protein metabolism**

Valine  
Odd-chain fatty acids  
Methionine  
Isoleucine  
Threonine



### Glycogen regulation by insulin and glucagon/epinephrine



### Glycogen

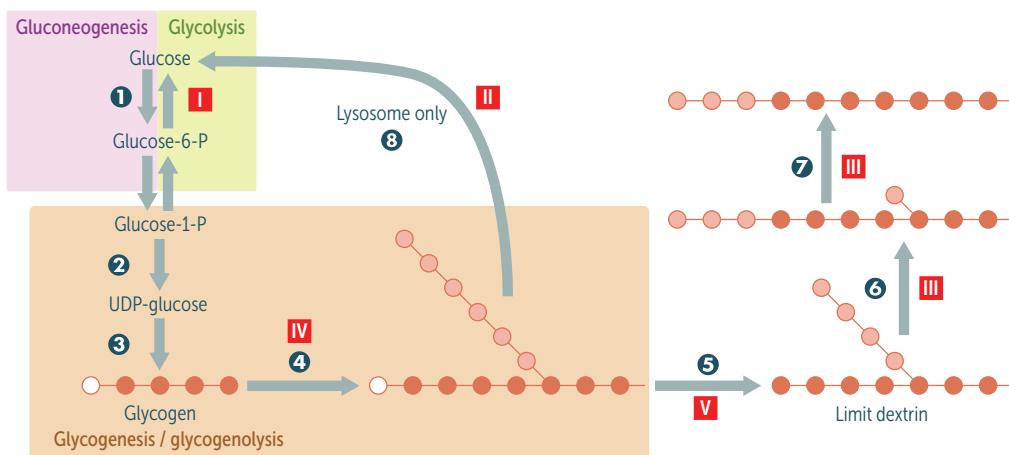
Branches have  $\alpha$ -(1,6) bonds; linear linkages have  $\alpha$ -(1,4) bonds.

### Skeletal muscle

Glycogen undergoes glycogenolysis  $\rightarrow$  glucose-1-phosphate  $\rightarrow$  glucose-6-phosphate, which is rapidly metabolized during exercise.

### Hepatocytes

Glycogen is stored and undergoes glycogenolysis to maintain blood sugar at appropriate levels. Glycogen phosphorylase ④ liberates glucose-1-phosphate residues off branched glycogen until 4 glucose units remain on a branch. Then 4- $\alpha$ -D-glucanotransferase (debranching enzyme ⑤) moves 3 of the 4 glucose units from the branch to the linear linkage. Then  $\alpha$ -1,6-glucosidase (debranching enzyme ⑥) cleaves off the last residue, liberating a free glucose. Limit dextrin—2–4 residues remaining on a branch after glycogen phosphorylase has shortened it.



Note: A small amount of glycogen is degraded in lysosomes by ⑦  $\alpha$ -1,4-glucosidase (acid maltase).

### Glycogen storage disease type

- I Von Gierke disease
- II Pompe disease
- III Cori disease
- IV Anderson disease
- V McArdle disease

### Glycogen enzymes

- ① Glucose-6-phosphatase
- ② UDP-glucose pyrophosphorylase
- ③ Glycogen synthase
- ④ Branching enzyme
- ⑤ Glycogen phosphorylase
- ⑥ Debranching enzyme (4- $\alpha$ -D-glucanotransferase)
- ⑦ Debranching enzyme ( $\alpha$ -1,6-glucosidase)
- ⑧  $\alpha$ -1,4-glucosidase

**Glycogen storage diseases**

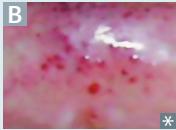
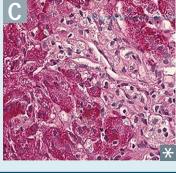
At least 15 types have been identified, all resulting in abnormal glycogen metabolism and an accumulation of glycogen within cells. Periodic acid–Schiff stain identifies glycogen and is useful in identifying these diseases.

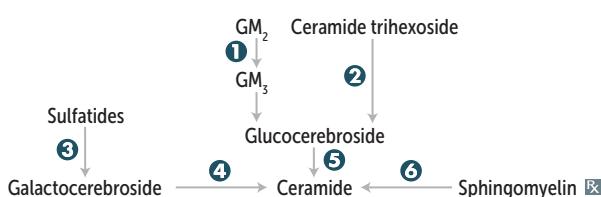
Vice president can't accept money.  
Types I-IV are autosomal recessive.  
**Andersen:** Branching.  
**Cori:** Debranching. (**ABCD**)

DISEASE	FINDINGS	DEFICIENT ENZYME	COMMENTS
<b>Von Gierke disease (type I)</b>	Severe fasting hypoglycemia, ↑↑ Glycogen in liver and kidneys, ↑ blood lactate, ↑ triglycerides, ↑ uric acid (Gout), and hepatomegaly, renomegaly. Liver does not regulate blood glucose.	Glucose-6-phosphatase.	Treatment: frequent oral glucose/cornstarch; avoidance of fructose and galactose. Impaired gluconeogenesis and glycogenolysis.
<b>Pompe disease (type II)</b>	Cardiomyopathy, hypotonia, exercise intolerance, enlarged tongue, and systemic findings lead to early death.	Lysosomal acid $\alpha$ -1,4-glucosidase (acid maltase).	<b>Pompe</b> trashes the <b>pump</b> (1st and 4th letter; heart, liver, and muscle).
<b>Cori disease (type III)</b>	Similar to von Gierke disease, but milder symptoms and normal blood lactate levels. Can lead to cardiomyopathy. Limit dextrin-like structures accumulate in cytosol.	Debranching enzymes ( $\alpha$ -1,6-glucosidase and 4- $\alpha$ -D-glucanotransferase).	Gluconeogenesis is intact.
<b>Andersen disease (type IV)</b>	Most commonly presents with hepatosplenomegaly and failure to thrive in early infancy. Other findings include infantile cirrhosis, muscular weakness, hypotonia, cardiomyopathy early childhood death.	Branching enzyme. Neuromuscular form can present at any age.	Hypoglycemia occurs late in the disease.
<b>McArdle disease (type V)</b>	↑ glycogen in muscle, but muscle cannot break it down → painful muscle cramps, myoglobinuria (red urine) with strenuous exercise, and arrhythmia from electrolyte abnormalities. Second-wind phenomenon noted during exercise due to ↑ muscular blood flow.	Skeletal muscle glycogen phosphorylase (myophosphorylase). Characterized by a flat venous lactate curve with normal rise in ammonia levels during exercise.	Blood glucose levels typically unaffected. <b>McArdle = muscle.</b>

**Lysosomal storage diseases**

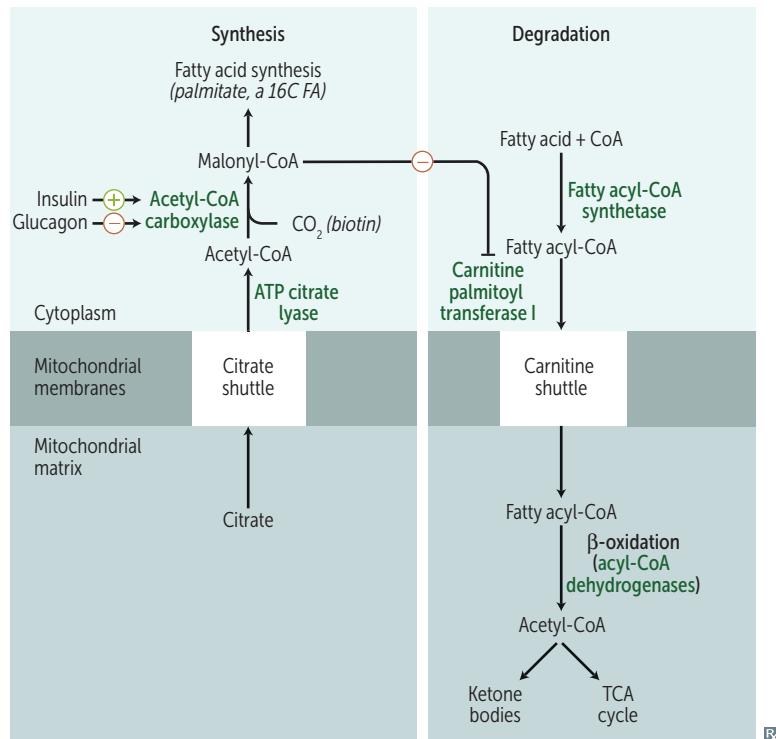
Lysosomal enzyme deficiency → accumulation of abnormal metabolic products. ↑ incidence of Tay-Sachs, Niemann-Pick, and some forms of Gaucher disease in Ashkenazi Jews.

DISEASE	FINDINGS	DEFICIENT ENZYME	ACCUMULATED SUBSTRATE	INHERITANCE
<b>Sphingolipidoses</b>				
Tay-Sachs disease 	Progressive neurodegeneration, developmental delay, hyperreflexia, hyperacusis, “cherry-red” spot on macula <b>A</b> (lipid accumulation in ganglion cell layer), lysosomes with onion skin, no hepatosplenomegaly (vs Niemann-Pick).	➊ Hexosaminidase A (“TAY-Sax”).	GM <sub>2</sub> ganglioside.	AR
Fabry disease 	Early: triad of episodic peripheral neuropathy, angiokeratomas <b>B</b> , hypohidrosis. Late: progressive renal failure, cardiovascular disease.	➋ α-galactosidase A.	Ceramide trihexoside (globotriaosylceramide).	XR
Metachromatic leukodystrophy	Central and peripheral demyelination with ataxia, dementia.	➌ Arylsulfatase A.	Cerebroside sulfate.	AR
Krabbe disease	Peripheral neuropathy, destruction of oligodendrocytes, developmental delay, CN II atrophy, globoid cells.	➍ Galactocerebrosidase (galactosylceramidase).	Galactocerebroside, psychosine.	AR
Gaucher disease	Most common. Hepatosplenomegaly, pancytopenia, osteoporosis, avascular necrosis of femur, bone crises, Gaucher cells (lipid-laden macrophages resembling crumpled tissue paper).	➎ Glucocerebrosidase (β-glucosidase); treat with recombinant glucocerebrosidase.	Glucocerebroside.	AR
Niemann-Pick disease 	Progressive neurodegeneration, hepatosplenomegaly, foam cells (lipid-laden macrophages) <b>C</b> , “cherry-red” spot on macula <b>A</b> .	➏ Sphingomyelinase.	Sphingomyelin.	AR
<b>Mucopolysaccharidoses</b>				
Hurler syndrome	Developmental delay, hirsutism, skeletal anomalies, airway obstruction, clouded cornea, hepatosplenomegaly.	α-L-iduronidase.	Heparan sulfate, dermatan sulfate.	AR
Hunter syndrome	Mild Hurler + aggressive behavior, no corneal clouding.	Iduronate-2 (two)-sulfatase.	Heparan sulfate, dermatan sulfate.	XR



**Hunters** see clearly (no corneal clouding) and aggressively aim for the **X** (X-linked recessive).

### Fatty acid metabolism



Fatty acid synthesis requires transport of citrate from mitochondria to cytosol. Predominantly occurs in liver, lactating mammary glands, and adipose tissue.

Long-chain fatty acid (LCFA) degradation requires carnitine-dependent transport into the mitochondrial matrix.

**“Synthesis” = synthesis.**

**Carnitine = carnage of fatty acids.**

**Systemic 1° carnitine deficiency**—no cellular uptake of carnitine → no transport of LCFAs into mitochondria → toxic accumulation of LCFAs in the cytosol. Causes weakness, hypotonia, hypoketotic hypoglycemia, dilated cardiomyopathy.

#### Medium-chain acyl-CoA dehydrogenase deficiency

↓ ability to break down fatty acids into acetyl-CoA → accumulation of fatty acyl carnitines in the blood with hypoketotic hypoglycemia. Causes vomiting, lethargy, seizures, coma, liver dysfunction, hyperammonemia. Can lead to sudden death in infants or children. Treat by avoiding fasting.

**Ketone bodies**

In the liver, fatty acids and amino acids are metabolized to acetoacetate and  $\beta$ -hydroxybutyrate (to be used in muscle and brain).

In prolonged starvation and diabetic ketoacidosis, oxaloacetate is depleted for gluconeogenesis. With chronic alcohol overuse, high NADH state leads to accumulation of oxaloacetate (downregulated TCA cycle), shunting it to malate.

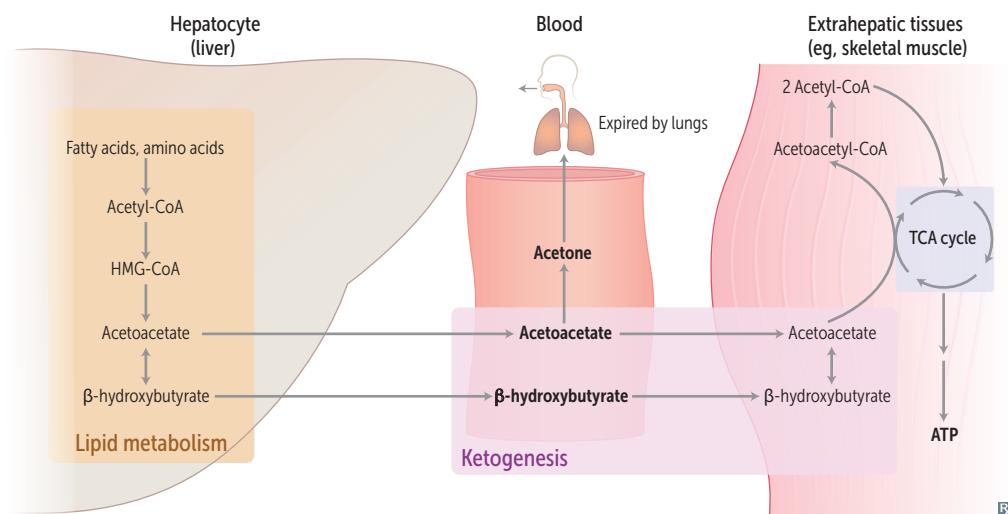
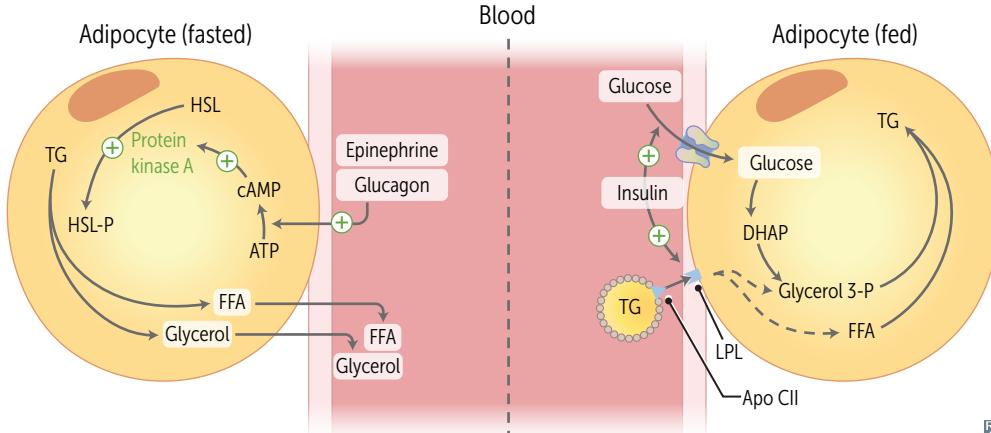
**Ketone bodies:** acetone, (ketone) acetoacetate (ketoacid),  $\beta$ -hydroxybutyrate (ketoacid).

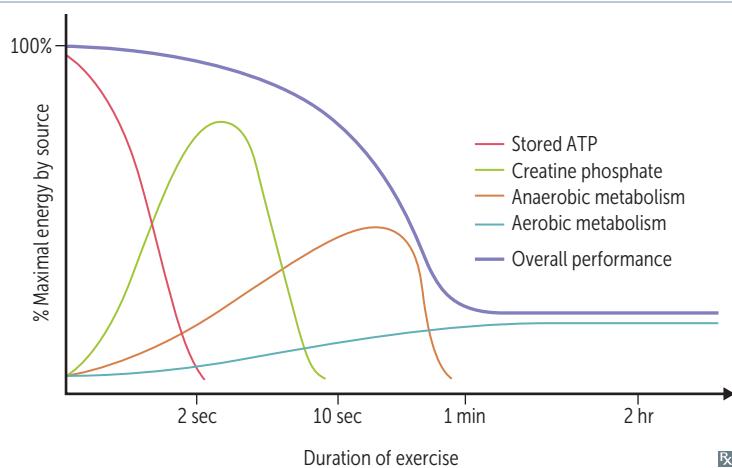
Breath smells like acetone (fruity odor).

Urine test for ketones can detect acetoacetate, but not  $\beta$ -hydroxybutyrate.

RBCs cannot utilize ketone bodies; they strictly use glucose. Liver cells lack  $\beta$  ketoacyl-CoA transferase → cannot use ketone bodies as fuel. HMG-CoA lyase for ketone body production. HMG-CoA reductase for cholesterol synthesis.

	Hyperammonemia	Hypoketosis	Ketosis
KETONE LEVELS	Normal	↓	↑
GLUCOSE LEVELS	Normal	↓	↓
DEFICIENCY	OTC (urea cycle)	MCAD deficiency	Methylmalonic acidemia, propionic acidemia

**Fasted vs fed state**

**Metabolic fuel use**

1g carb/protein = 4 kcal  
 1g alcohol = 7 kcal  
 1g fatty acid = 9 kcal  
 (# letters = # kcal)

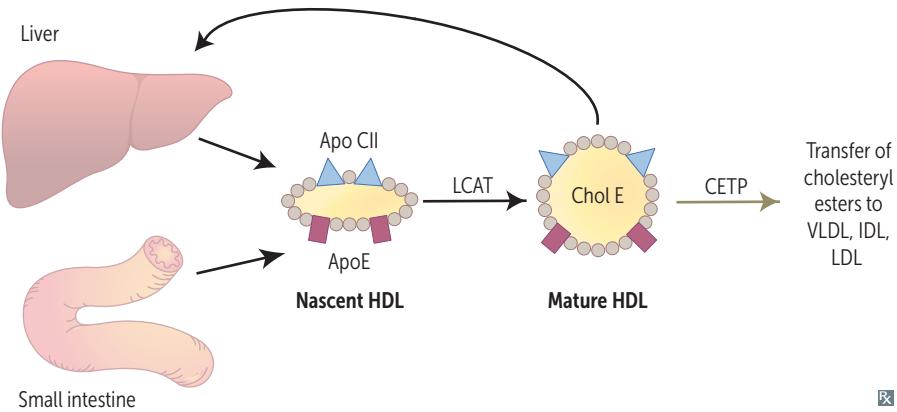
**Fasting and starvation** Priorities are to supply sufficient glucose to the brain and RBCs and to preserve protein.

Fed state (after a meal)	Glycolysis and aerobic respiration.	Insulin stimulates storage of lipids, proteins, and glycogen.
Fasting (between meals)	Hepatic glycogenolysis (major); hepatic gluconeogenesis, adipose release of FFA (minor).	Glucagon and epinephrine stimulate use of fuel reserves.
Starvation days 1–3	Blood glucose levels mt (8 1 Tf -0.017 Tc 0.017 Tw -31.2 -2.95 Td [(St)-23.5 5.2Td [(St)-1 (i)3. u)-7Td [(St)-SO	



**Key enzymes in lipid transport**

<b>Cholesteryl ester transfer protein</b>	Mediates transfer of cholesteryl esters to other lipoprotein particles.
<b>Hepatic lipase</b>	Degradates TGs remaining in IDL and chylomicron remnants.
<b>Hormone-sensitive lipase</b>	Degradates TGs stored in adipocytes. Promotes gluconeogenesis by releasing glycerol.
<b>Lecithin-cholesterol acyltransferase</b>	Catalyzes esterification of $\frac{1}{2}$ of plasma cholesterol (ie, required for HDL maturation).
<b>Lipoprotein lipase</b>	Degradates TGs in circulating chylomicrons and VLDL.
<b>Pancreatic lipase</b>	Degradates dietary TGs in small intestine.
<b>PCSK9</b>	Degrades LDL receptor $\rightarrow$ ↑ serum LDL. Inhibition $\rightarrow$ ↑ LDL receptor recycling $\rightarrow$ ↓ serum LDL.

**Major apolipoproteins**

APOLIPROTEIN	FUNCTION	CHYLOMICRON	CHYLOMICRON REMNANT	VLDL	IDL	LDL	HDL
E	Mediates remnant uptake (everything except LDL)	✓	✓	✓	✓	✓	✓
AI	Found only on alpha-lipoproteins (HDL), activates LCAT						✓
CII	Lipoprotein lipase cofactor that catalyzes cleavage	✓		✓	✓		✓
B <sub>48</sub>	Mediates chylomicron secretion into lymphatics Only on particles originating from the intestines	✓	✓				
B <sub>100</sub>	Binds LDL receptor Only on particles originating from the liver (I hope I live to Be 100)			✓	✓	✓	

**Lipoprotein functions**

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

**Chylomicron**

Delivers dietary TGs to peripheral tissues. Delivers cholesterol to liver in the form of chylomicron remnants, which are mostly depleted of their TGs. Secreted by intestinal epithelial cells.

**VLDL**

Delivers hepatic TGs to peripheral tissue. Secreted by liver.

**IDL**

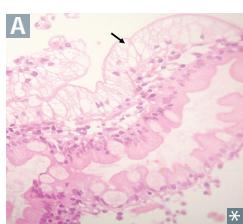
Delivers TGs and cholesterol to liver. Formed from degradation of VLDL.

**LDL**

Delivers hepatic cholesterol to peripheral tissues. Formed by hepatic lipase modification of IDL in the liver and peripheral tissue. Taken up by target cells via receptor-mediated endocytosis. **LDL is Lethal.**

**HDL**

Mediates reverse cholesterol transport from peripheral tissues to liver. Acts as a repository for apoC and apoE (which are needed for chylomicron and VLDL metabolism). Secreted from both liver and intestine. Alcohol ↑ synthesis. **HDL is Healthy.**

**Abetalipoproteinemia**

Autosomal recessive. Mutation in gene that encodes microsomal transfer protein (MTP).

Chylomicrons, VLDL, LDL absent. Deficiency in apo B<sub>48</sub>- and apo B<sub>100</sub>-containing lipoproteins. Affected infants present with severe fat malabsorption, steatorrhea, failure to thrive. Later manifestations include retinitis pigmentosa, spinocerebellar degeneration due to vitamin E deficiency, progressive ataxia, acanthocytosis. Intestinal biopsy shows lipid-laden enterocytes (arrow in **A**).

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

**Familial dyslipidemias**

TYPE	INHERITANCE	PATHOGENESIS	↑ BLOOD LEVEL	CLINICAL
I—Hyper-chylomicronemia	AR	Lipoprotein lipase or apo CII deficiency	Chylomicrons, TG, cholesterol	Pancreatitis, hepatosplenomegaly, and eruptive/pruritic xanthomas (no ↑ risk for atherosclerosis). Creamy layer in supernatant.
II—Hyper-cholesterolemia	AD	Absent or defective LDL receptors, or defective apo B <sub>100</sub>	IIa: LDL, cholesterol IIb: LDL, cholesterol, VLDL	Heterozygotes (1:500) have cholesterol ≈ 300 mg/dL; homozygotes (very rare) have cholesterol ≥ 700 mg/dL. Accelerated atherosclerosis (may have MI before age 20), tendon (Achilles) xanthomas, and corneal arcus.
III—Dysbeta-lipoproteinemia	AR	ApoE (defective in type thr <sup>EE</sup> )	Chylomicrons, VLDL	Premature atherosclerosis, tuberoeruptive and palmar xanthomas.
IV—Hyper-triglyceridemia	AD	Hepatic overproduction of VLDL	VLDL, TG	Hypertriglyceridemia (> 1000 mg/dL) can cause acute pancreatitis. Related to insulin resistance.