CARBOHYDRATES – glycosidic bond

- Reducing sugar: free aldehyde/ketone group
- Amylose: linear, α1-4, hard to digest
- Glycogen / Amylopectin > glucose storage, linear α1-4 chains, branch at α1-6] α1-4, α1-6 in glycogen
  - * glycogen = more freq. branch points
- Cellulose – β 1-4, not digested in humans
- Blood antigens = N or O glycolipids

LIPIDS – ester bond

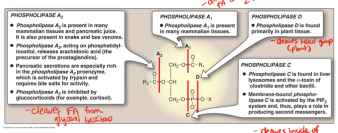
- Unsaturated: kink in chain, more fluid, double bonds
- Saturated: straight chain, more solid, packs tightly

- Glycero-Phospholipids – glycerol + 2 FA
  - Phosphatidylcholine (PC) – most common
  - Phosphatidylserine (PS) – inner leaflet of PM, ⊖, "eat me" signal
  - Phosphatidylethanolamine (PE) – bacterial mem.
  - Phosphatidylinositol (PI) – intracellular signaling → cleavage produces IP_3, Ca^{2+} release
- Sphingolipids
  - Phospho-Sphingolipids: sphingomyelin
  - Glyco-sphingolipids,
- Cholesterol and Cholesterol esters
- Other Lipids
  - CardioLipins, PAF, Plasmalogen etc.

PI + PS = inner leaflet
glycolipids = outside

Cholesterol = mem. fluidity
Sphingolipids → ceramide
Glycolipids → carbs
Plasmalogens → ether bonds

Degradation of Phospholipids



Receptor:
- nuclear/intracellular = hydrophobic ligand
  - * class I = in cytosol pre-bound of HSP90, hormone binds → dimerizes → nucleus
  - * class II = bound to DNA → ligand binds → release co-repressor → recruitment of coactivator protein

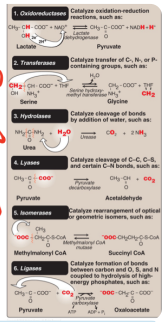
- GPCR = ligand + GPCR → $G_s\alpha$ + βγ → ↑ AC → ↑ cAMP → ↑ PKA → Ⓟ → gene transcription
  - small g-proteins (RAS) need GAP = inactivate (GTP hydrolysis), GEF = activate (GDP → GTP)
  - G_s = stimulates AC (β adrenergic)
  - G_i = inhibits AC (α_2 adrenergic)
  - G_q = Ca^{2+} signalling (α_1 adrenergic – phospholipase C)
  - GPCR inactivation: – ligand dissociates
    - phosph. of GRK → arrestin binding
    - endocytosis
    - Ⓟ removed by phosphatase
    - cAMP → AMP (phosphodiesterase)
    - GTPase (intrinsic / GAPs)

ENZYMOLOGY

CK → creatine phosphate + ADP → creatine + ATP * ATP made fast

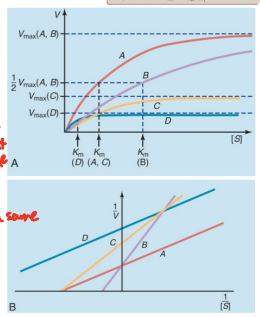
Classification of Enzymes

- Oxido-reductases – redox reactions (NADH/NADPH)
  - HMG-CoA reductase, the rate-limiting step in cholesterol synthesis
- Transferases – transpher phosphate / group to other, or other groups like methyl
  - Protein Kinase A (PKA), Protein kinase G (PKG)
- Hydrolases – water to break bond
  - Amylase, Trypsin, Lipase, RNAase, DNAase, Lactase,
- Lyases – break things apart without water
  - Histidine decarboxylase 1→2 w/o water
- Isomerases – single substrate, single product (rearrangement)
  - Phosphogluco-isomerase
- Ligases – put things back together
  - DNA, RNA ligases, DNA and RNA polymerases, Pyruvate carboxylase 2→1 (opposite of lyase)



Enzyme Kinetics Summary

- Various types of inhibition shown as MM plots and as double reciprocal plots
- A – no inhibitor
- B – Competitive Inhibition – ↑ Km, = Vmax same
- C – Noncompetitive inhibition or suicide inhibitor – Vmax change / Km same (cov.)
- D – Uncompetitive inhibition
  - ↳ only binds ES
  - ↳ changes Km/Vmax



prostaglandins ↑ Cox ← arachidonic acid ← PKC ← phospholipase A_2

Ca^{2+} – calmodulin → MLCK → Ⓟ MLC → contraction

epinephrine → α_1 adrenergic → $G_{q\alpha}$ – GTP + βγ → act. PLC → PIP_2 → DAG, IP_3 → opens Ca^{2+} channels → release Ca^{2+} from SR → contraction

* CAM Kinase = calmodulin act. kinase → Ⓟ protein targets in response to ↑ Ca^{2+}

* NO = smooth m. relaxer

Ach binds muscarinic Ach R (GPCR) → act. PLC → PIP_2 → IP_3 → ↑ Ca^{2+} → act. NOS (NO synthase)

NO binds ← guanylate cyclase (active) ← CGMP ← ↑ PKG ← Vasodilation ← relaxation

– Tyrosine Kinase – Growth Factor Receptor

inactive monomer → ligand binds → dimerization → act. kinase → Ⓟ tyrosine → signals

- MAP Kinase pathway → cell cycle
- JAK / Stat → cytokine
• SH2 domain
- PDGF → act. PLC → ↑ Ca^{2+}
- PI3 Kinases → (P) PIP_2 → PIP_3 → AKT + PDK1 to PM → AKT (P) Bad ↓ Bad diss. from apoptosis inhibitory protein + act. it → apoptosis
  ↳ apoptosis via AKT/Tor pathway

TABLE 17–1 The Major Cyclins and Cdks of Vert

	Vertebrates	
Cyclin–Cdk complex	Cyclin	Cdk partner
G_1-Cdk	Cyclin D*	Cdk4, Cdk6
G_1/S-Cdk	Cyclin E	Cdk2
S-Cdk	Cyclin A	Cdk2, Cdk1**
M-Cdk	Cyclin B	Cdk1

* There are three D cyclins in mammals (cyclins D1, D2,
** The original name of Cdk1 was Cdc2 in both vertebra budding yeast.

Table 17-1 Molecular Biology of the Cell 6e (© Garland Science 2015)

know the pairs!

- Cyclin dep. Kinase (CdK) → (P) substrate to get to next cell cycle stage
- Cyclin-CdK reg. cell cycle → cyclin-CdK (partial activ.) → cyclin-CdK-(P) by CAK = full activation
- Wee1 = inhibitor of CdK by (P)
- Cdc25 = phosphatase (removes (P)) = reactivates] Cdc25/wee1 = G_2 ↓ act. MPF (mitosis)
- p27 = direct inhibition of CdK
- APC/C = act by Cdc20 → cyclin A/B degraded → inactivation of most CdKs → degrades securin - separase cleaves cohesins
  • permits metaphase → anaphase transition
- p53 = ⊖ reg. of cell cycle (↑ Myc = active p53 = apoptosis) Myc = promotes formation of cyclin D + G_1-CdK
- TOR → act S6K → (P) S6 → ↑ translation of rib. proteins → inhibits eIF4E
- MAP K path: Fos dimerizes with Jun → transcriptional enhancer
- E2F + Rb → inhibits
- Rb (P) by G_1-CdK → E2F released
- E2F = TF that promotes G_1-S cyclin

p21 = inhibitor of CdK

* S phase: S-cyclin promotes entry to DNA rep. CdK2 phosph. Cdc6 → Cdc6 dissociates • GINS displaces → GINS opens rep. fork

↑ Myc, p21, wee1 = cell cycle arrest

Rb (P) not bound to E2F in cancer

- restriction endonucleases → palindromes (same forward/back)

Mismatch Repair

BER - loss of base, meth.
gNER - thymine dimers, bulky lesions, distorted helix
NHEJ HR MMEJ - ds breaks single-strand breaks

- Dominant ⊖ = mutant causes normal not to function properly
- Haploinsufficiency = heterozygote does not have enough gene product

BER = Lynch syndrome - cancer
NER = XP (photosensitivity) GNER
TcNER [trichothiodystrophy (rough hair/skin) Cockayne

Mutations or Modifications in Factors of clinical importance

- Ribosome
  - **Antibiotics**
  - **Ricin** removes an Adenine base from the elongation factor binding site preventing elongation.
- Initiation factors
  - eIF2B – is required to deliver the Met-tRNA$_i$ to the "P" site in the preinitiation complex (positions tRNA met find start codon)
    - **Diseases associated with loss of eIF2B**
      - **Vanishing White Matter** disease a type of Leukodystrohy
      - **reticulocyte apoptosis** in response to iron deficiency
  - eIF3 - is required for assembly of the 40S subunit onto a 5' cap or IRES (↳ internal rib. entry site)
    - **Cancers** often have reduced levels or mutated eIF3
  - eIF4G – regulated via phosphorylation to stop translation or modified to allow IRES use
    - **Wasting diseases** occur when too much eIF4G phosphorylation
    - **Polio** (or any picornavirus) proteolysis of eIF4G prevents use of host transcript whereas viral RNA is used for translation preferentially.
- Elongation factors
  - EF2 – translocation of "P" site tRNA to the "E" site after peptide bond formation
    - ADP-ribosylated by **diphtheria toxin** prevents elongation stage of protein synthesis

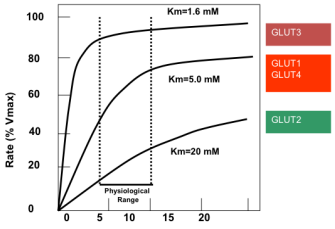
Metagenomic sequencing – sequencing only a small set of genes
Nucleic acid amplification test (NAAT) (based on PCR) – amplifies a few segments of DNA which are diagnostic of disease
Microarrays – use DNA, Proteins or anchored sugars to identify pathogenic organisms
Multilocus sequence test (MLST) – uses multiple genes from pathogenic organisms to differentiate between species and cultivars
Pulsed Field Gel Electrophoresis (PFGE) – uses electrophoresis to analyze DNA sizes of pathogens
Multilocus variable number repeat analysis (MLVA) – uses the repetitive sequences in the pathogens to subtype individual strains of pathogens
Single nucleotide polymorphisms (SNPs) – can be detected by microarrays, Southern blotting or Northern blotting
Whole genome sequencing – looks at whole genome

Glycolysis

- hexokinase = traps glucose, glucose committing step
- PFK1 = committed, rate limiting step
- pyruvate Kinase

* Arsenate poisoning → acts on GAPDH (no ATP made), phosphoglycerate kinase can't make ATP
* 2,3 BPG → red blood cells, reg. hemoglobin, ↑ O_2 delivery to tissues
* Anaerobic: GAPDH limited by NAD^+ (pyruvate → lactate to get NAD^+ from NADH + ATP)
* lactic acidosis = ↓ pH, lactate dehydrogenase
* Cori cycle = recycle lactate in exercise. ↑ lactate - ox. deficit, lactate back to pyruvate → TCA cycle → ox phos when O_2 ↑

Name	Tissue Distribution	K_m	Important features
GLUT1	Erythrocytes Fetal tissue Placenta	5-7mM	Present in most tissues except liver, kidney, intestine, and β-cells
GLUT2	Kidney Liver Intestine Pancreatic β-cell	7-20 mM	High K_m allows glucose to 'equilibrate' across the membrane
GLUT3	Brain	1.6 mM	Low K_m allows relatively constant rate of glucose uptake independent of extracellular concentration over the normal range
GLUT4	Adipose tissue, Muscle	5mM	The insulin-sensitive glucose transporter



PFK Regulation

* Fructose 2,6 Bisphosphate (activates PFK1)

glucose
↓
glucose-6-phosphate ↑ insulin → PFK2 ⊕
↓
fructose-6-phosphate ⇌ F2,6BP (PFK2 / FBP2) → act. PFK1 (inhibits FBP1) → ↑ glycolysis

* glucagon → act. gluconeogenesis, Ⓟ PFK2, ↓ F2,6BP, ↓ PFK1, ↓ glycolysis

TCA cycle

* arsenite poisoning = PDH (binds lipoic acid, prevents transferase activity, kills activity of PDH)

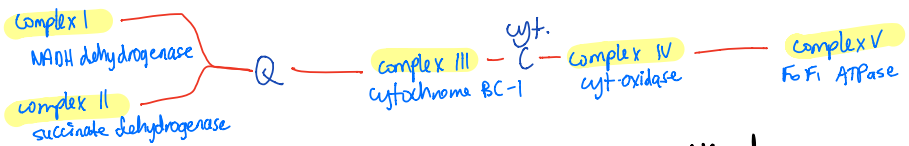
PDH (pyruvate → acetyl coA) - TPP, CoA, FAD, NADH, lipoic acid (not a vitamin)

Coenzyme A - vit B5, pantothenic acid

- citrate synthase → OAA + acetyl coA → citrate
- isocitrate dehydrogenase → NADH produced, alpha ketoglutarate made
- alpha-ketoglutarate dehydrogenase → similar to PDH

] regulated steps

- Succinate dehydrogenase - part of ETC, not soluble, IMM
- Anaplerotic rxns = refill TCA cycle, pyruvate carboxylase (biotin) pyruvate → OAA



Uncouplers
- no ATP
- heat made
* 2,4 dinitrophenol
* UCP1

Inhibitors → upstream = reduced / downstream = oxidized

complex I = rotenone / amytal
complex III = antimycin A
complex IV = azide, cyanide, HCN, CO
ATP synthase: oligomycin, atractyloside, bongkrekate (ATP antiporter)

Hypoxia (HIF)

→ vit C / iron co-factors

Normal = PH hydroxylates HIF α → ubiquinated → proteosome

Hypoxia = HIF α not degraded → binds HIF β → stim. transcr. / glycolysis genes ↑

* Superoxide dismutase = $O_2^{-\bullet} \rightarrow H_2O_2$
* Catalase = $H_2O_2 \rightarrow H_2O + O_2$
* Glutathione peroxidase = $H_2O_2 \rightarrow H_2O + O_2$

$H_2O_2 \rightarrow OH^\bullet$ by free iron * Heiber-weiss = $H_2O_2 + O_2^{\cdot -} \rightarrow HO^\bullet + OH^- + O_2$ → very reactive !!

* Glutathione: GSH needed for antioxidant defense
  - need high NADPH / NADP+ ratio
* Favism: Glucose-6-phosphate dehydrogenase deficiency → impairs RBC from forming NADPH
  - hemolytic anemia
  ↳ HMP shunt enzyme, can't make NADPH = hemolysis
  ↳ ox. stress
* vit E = prevents free radical propagation in lipid tissue chains
* vit C = free radical scavenger → collagen
* NADPH oxidase = phagocytosis (kill bacteria w/ ROS)

KEAP1 NRF2 (reduced) S-H → ubiquinated → proteosome

oxidants: KEAP1 NRF2 S-S → KEAP1 releases NRF2 → transcription (glutathione met. genes + NADPH production)

Hemoglobin

- binds Fe^{2+}
- 2 α, 2 β subunits
- 2,3 BPG releases O_2 to tissues (binds donut hole on T) — stabilizes T, ↓ O_2 affinity
- T state / R-state
- His F8 moves it into heme plane
- (+) cooperativity
- CO competes with O_2 for heme binding (2,3 BPG / CO ↑ in smokers)
  ↳ forms carboxyhemoglobin = treated with hyperbaric therapy
- methemoglobin = Fe^{3+} → can't bind O_2 = use for cyanide poisoning

Ferric ⟷ ferrous

Fe^{3+} — Fe^{2+} - binds O_2

Storage: ferritin / hemosiderin (high iron) Fe^{3+}

Transport: transferrin Fe^{3+}

uptake: $Fe^{3+} \rightarrow Fe^{2+}$ (DMT1)

ferroportin: Fe^{2+} across membrane
↳ iron exporter

* low iron = ↑ transferrin, IRPs → IRE
* ↑ iron, ↑ hepcidin = inhibits ferroportin
* hemochromatosis = genetic iron overload → phlebotomy

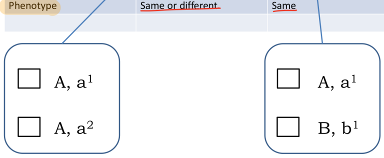
macrocytic anemia — B12 vs folate → methionine synthase = B12 def (methylmalonate) + neurological symptoms

- pernicious anemia: loss of B12 due to loss of IF + poor absorption

GENETICS

Genetic Heterogeneity

	Allelic	Locus
Gene	Only one	Two or more
Variation	Two or more alleles	At least one bad allele per gene
Phenotype	Same or different	Same



Locus = diff. genes

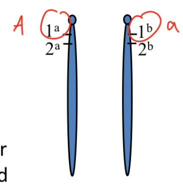
Allelic = same locus/gene

AR → healthy parents
→ sibling affected

2/3

Linked markers

Locus 1: alleles 1^a and 1^b
Locus 2: alleles 2^a and 2^b



- If close together on the same chromosome: linkage, 1^a and 2^a together 100% of the time
- For this section, we want loci being so close together that they are not separated by recombination

Definition of marker: a *polymorphism* (e.g., microsatellite) that is used to identify a chromosomal segment; can for example be used to predict carrier status of a person

Grouping of Non-banded Chromosomes

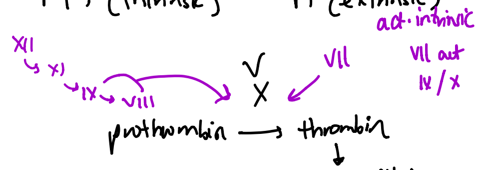
- A (1-3): large, near metacentric
- B (4-5): submetacentric, smaller than A
- C (6-12, X): submetacentric, smaller than B
- D (13-15): larger acrocentric
- E (16-18): submetacentric, smaller than C
- F (19-20): small metacentric
- G (21-22, Y): small acrocentric

D/G are acrocentric! KNOW THIS

Clotting

- aspirin prevents clotting
  → Arachidonic acid →(COX) thromboxane
- vWF → VIII (VIII released = active)

PTT (intrinsic) PT (extrinsic)



prothrombin → thrombin
↓
fibrinogen → fibrin

Plasminogen → plasmin → breaks down

Activated by: tPA, urokinase, streptokinase

Inhibit coagulation
- antithrombin | Heparin
- thrombomodulin (C+S)
- Warfarin (vit K antagonist) ← comp. inhibitor

Vit K = 2, 7, 9, 10