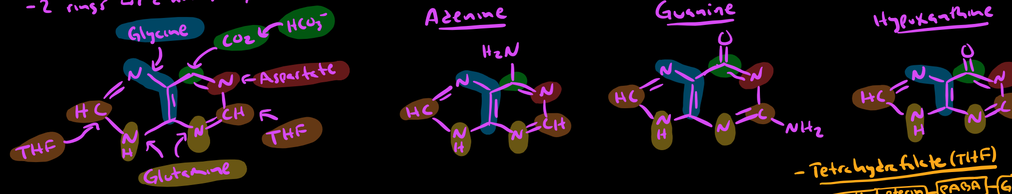
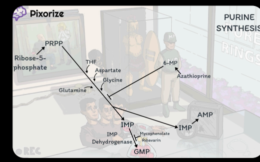
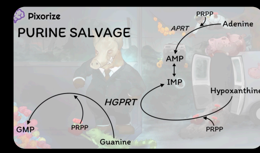
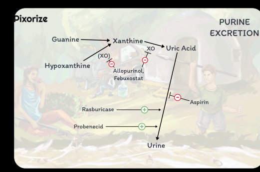
Purines

- 2 rings w/ 2 nitrogens, 4 carbons

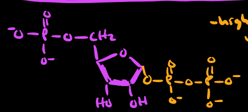








- Purine biosynthetic enzymes = Cytosolic
- 5-phosphoribosylamine Pyrophosphate (PRPP)



- high energy PP group used to drive transfer rxns. After transfer, PP —Pyrophosphatase→ 2 P_i
  - ↳ makes preceding transfer irreversible

Ribose-5-P —(ATP → AMP; PRPP Synthetase (X-linked))→ PRPP

Regulation - reciprocal control for maintaining 20 ATP : 1 GTP synthesis in Animals

1. End products, ATP & GTP, provide energy for each other's reactions
2. Feedback Inhibition

- Tetrahydrofolate (THF)
- Folate = 6-methyl-pterin — PABA — Glutamate
  - (6-methyl-pterin + PABA = Pteroic acid)
- Humans must obtain folate from diet b/c don't have enzymes for PABA synthesis or to link pteric acid to Glutamate
- Bacteria synthesize folate de novo
  - ↳ Sulfonamides = PABA analogues → competitive inhibitors of Dihydropteroate Synthase → ↓ bacterial purine synthesis ONLY
    - ↳ used to kill bacteria b/c

PABA + pteridine —Dihydropteroate Synthase (⊣ Sulfonamides, dapsone)→ Dihydropteroate —(Glu)→ DHF

- Trimethoprim also used, inhibits bacterial Dihydrofolate Reductase
- Synthesized from folate (B_9) via Dihydrofolate Reductase
  - DHF reductase catalyzes both NADPH-dependent rxns

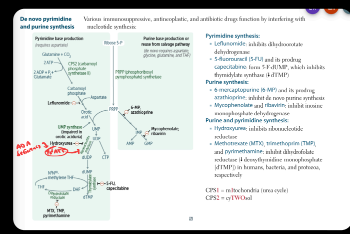
- folate —(NADPH → NADP+)→ Dihydrofolate —(NADPH → NADP+)→ THF

Dihydrofolate Reductase

- Eukaryotes ⊣ Methotrexate, Aminopterin
- Bacteria ⊣ Trimethoprim, Sulfonamides
- Protozoa ⊣ Pyrimethamine

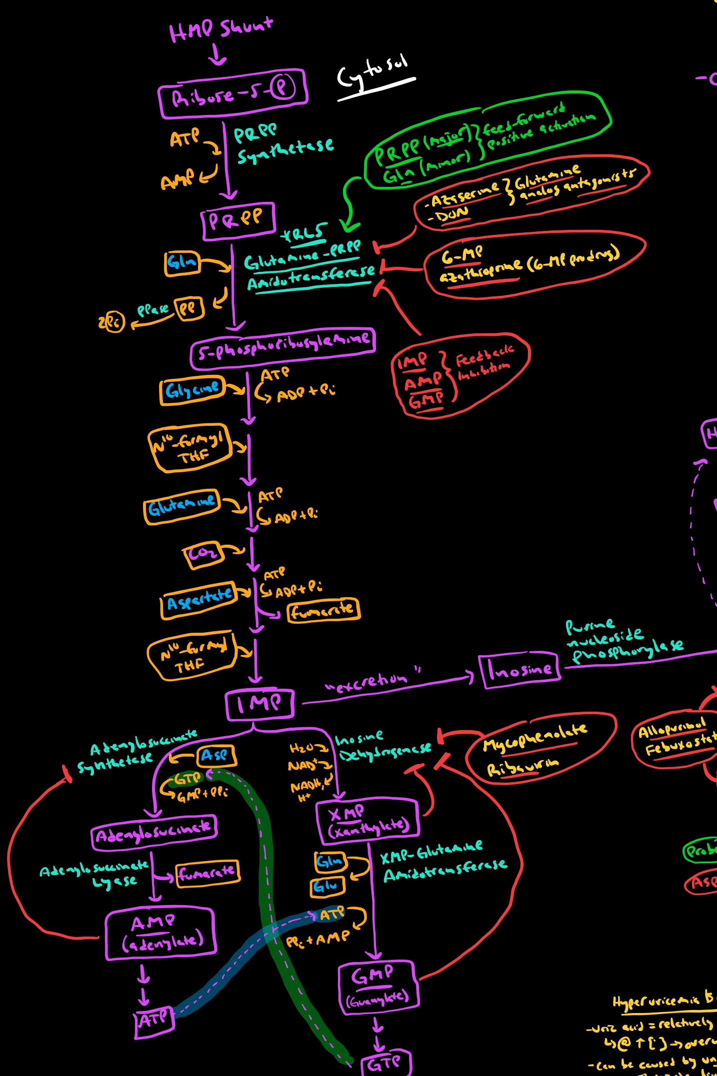
Methotrexate used over aminopterin b/c has better therapeutic index

Aminopterin & **Methotrexate** = immunosuppressant used for Cancer Tx
- ↳ sometimes used in conjunction w/ Leucovorin (folinic acid)
  - ↳ reduces toxicity of methotrexate (severe bone marrow & GI toxicity)



De novo pyrimidine and purine synthesis
Various immunosuppressive, antineoplastic, and antibiotic drugs function by interfering with nucleotide synthesis:
Pyrimidine base production (requires aspartate)
Glutamine + CO_2
CPS2 (carbamoyl phosphate synthetase II)
Carbamoyl phosphate
Aspartate
Leflunomide
Orotic acid
UMP synthase (impaired in orotic aciduria)
UMP
UDP
Hydroxyurea
dUDP
CTP
dUMP
N^5N^{10}-methylene THF
THF
DHF
Dihydrofolate reductase
dTMP
Thymidylate synthase
5-FU, capecitabine
MTX, TMP, pyrimethamine
Ribose 5-P
PRPP
IMP
Mycophenolate, ribavirin
AMP
GMP
ADA deficiency ↑ dATP
Purine and pyrimidine synthesis:
Hydroxyurea: inhibits ribonucleotide reductase
Methotrexate (MTX), trimethoprim (TMP), and pyrimethamine: inhibit dihydrofolate reductase (↓ deoxythymidine monophosphate [dTMP]) in humans, bacteria, and protozoa, respectively
CPS1 = m1tochondria (urea cycle)
CPS2 = cyTWOsol

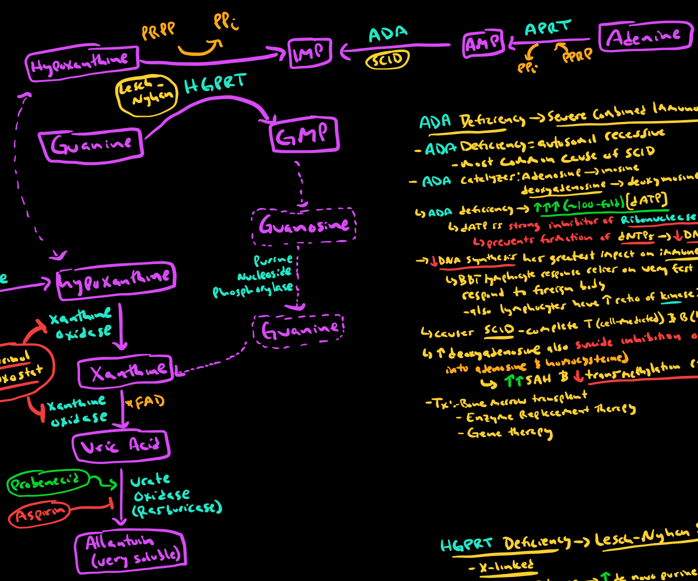
Purine Synthesis



HMP Shunt
Ribose-5-P
Cytosol
ATP
AMP
PRPP Synthetase
PRPP
PRPP (major), Gln (minor) } feed-forward positive activation
-Azaserine, -DON } Glutamine analog antagonists
6-MP, azathioprine (6-MP prodrug)
IMP, AMP, GMP } Feedback Inhibition
RLS
Glutamine-PRPP Amidotransferase
Gln
PP
PPase
2 Pi
5-phosphoribosylamine
Glycine
ATP
ADP + Pi
N^{10}-formyl THF
Glutamine
ATP
ADP + Pi
CO_2
ATP
ADP + Pi
Aspartate
fumarate
N^{10}-formyl THF
IMP
"excretion"
Inosine
Purine nucleoside phosphorylase
Adenylosuccinate Synthetase
Asp
GTP
GMP + PPi
Adenylosuccinate
Adenylosuccinate Lyase
fumarate
AMP (adenylate)
ATP
Inosine Dehydrogenase
H_2O
NAD^+
NADH, H^+
Mycophenolate
Ribavirin
XMP (xanthylate)
Gln
Glu
XMP-Glutamine Amidotransferase
ATP
PPi + AMP
GMP (Guanylate)
GTP

Purine Salvage Pathway

- Catabolism of nucleotides → purines/pyrimidines → 2 possible paths
  1. Excretion as uric acid → ↑ uric acid → uric acidemia → Gout
     - Uric aciduria / Gout Tx: - Probenecid → ↓ Proximal Tubule reabsorption of uric acid
       - To treat symptoms (pain) - NSAIDs
         - ↳ NOT aspirin b/c low-dose aspirin can ↑ uric acid absorption in PT
  2. Salvage - recycled to create AMP & GMP
     - uses PRPP → PPi to drive rxn
     - preferred over de novo synthesis b/c No ATP required
     - very important in RBC's b/c can't synthesize 5-phosphoribosylamine
     - Guanine & Hypoxanthine (inosine precursor) use Hypoxanthine-guanine phosphoribosyl transferase (HGPRT)
     - Adenine → AMP uses Adenosine phosphoribosyl transferase (APRT)
       - ↳ AMP → IMP uses Adenosine Deaminase (ADA)



PRPP
PPi
Hypoxanthine
IMP
ADA
SCID
AMP
APRT
PPi
PRPP
Adenine
Lesch-Nyhan
HGPRT
Guanine
GMP
Guanosine
Purine Nucleoside Phosphorylase
Guanine
Hypoxanthine
Xanthine oxidase
Allopurinol
Febuxostat
Xanthine
Xanthine oxidase
FAD
Uric Acid
Probenecid
Aspirin
Urate Oxidase (Rasburicase)
Allantoin (very soluble)

ADA Deficiency → Severe Combined Immunodeficiency (SCID)

- ADA Deficiency = autosomal recessive
  - most common cause of SCID
- ADA catalyzes: Adenosine → inosine
  - deoxyadenosine → deoxyinosine
- ↳ ADA deficiency → ↑↑↑ (~100-fold) [dATP]
  - ↳ dATP is strong inhibitor of Ribonucleotide Reductase
    - ↳ prevents formation of dNTPs → ↓ DNA synthesis
- → ↓ DNA synthesis has greatest impact on immune cells (B & T)
  - ↳ B & T lymphocyte response relies on very fast synthesis to respond to foreign body
  - also lymphocytes have ↑ ratio of kinase : 5'-nucleotidase
- ↳ causes SCID - complete T (cell-mediated) & B (humoral) cell dysfunction
- ↳ ↑ deoxyadenosine also suicide inhibition of SAH (that usually hydrolyzes into adenosine & homocysteine)
  - ↳ ↑↑ SAH & ↓ transmethylation rxns
- Tx: - Bone marrow transplant
  - Enzyme Replacement Therapy
  - Gene therapy

Hyperuricemia & Gout

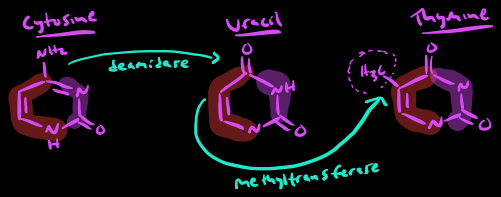
- uric acid = relatively insoluble
  - ↳ @ ↑ [ ] → overwhelms kidney → precipitates out → Stones
- can be caused by under-excretion
  - Thiazide diuretics
  - lactic acidosis
- can be caused by overexcretion
  - Lesch-Nyhan Syndrome
    - ↳ PRPP not used in salvage pathway
      - ↳ ↑ PRPP for purine biosynthesis → ↑ purines for degradation
        - ↳ ↑ uric acid
  - Von Gierke's disease
    - ↳ G6PD deficiency → ↑ [G6P] → ↑ HMP shunt → ↑ purine synthesis
      - ↳ ↑ uric acid
- Tx: Allopurinol → inhibits Xanthine oxidase

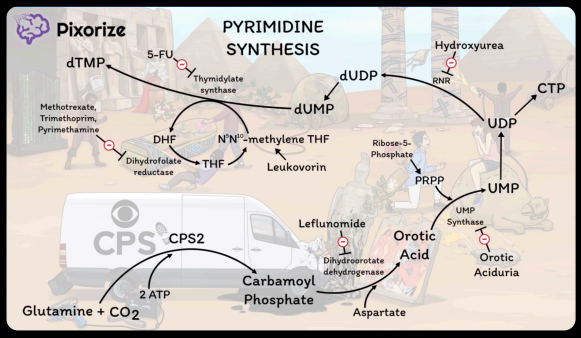
HGPRT Deficiency → Lesch-Nyhan Syndrome

- X-linked
- ↓ purine salvage → ↑ de novo purine synthesis → ↑ uric acid
- Symptoms mnemonic: H G P R T
  - Hyperuricemia
  - Gout
  - Pissed off (aggression, self-mutilation)
  - Retardation (intellectual disability)
  - dysTonia (muscle weakness)
- Tx: Allopurinol (first line)
  - Febuxostat (second line)

Pyrimidines

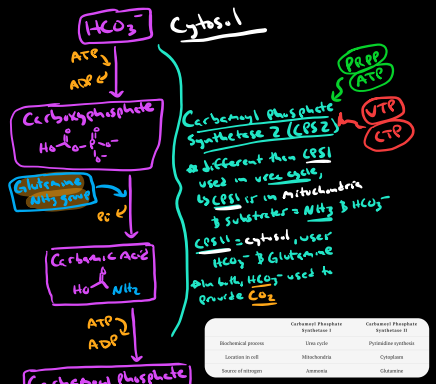
- 1 ring, 2 nitrogens, 4 carbons





de novo Biosynthesis

- beginning substrates = HCO_3^- & Glutamine
- First set of reactions accomplished via CAD
  - CAD = Multifunctional cytosolic protein that converts HCO_3^- & Gln → orotic acid
  - CAD = - CPS 2 (RLS)
    - ATCase
    - DHODH
      - when DHODH functional, CAD complex translocates to outer surface of inner mitochondrial membrane
      - b/c Orotic acid synthesis donates e^- to CoQ → $CoQH_2$ → ETC



	Carbamoyl Phosphate Synthetase 1	Carbamoyl Phosphate Synthetase II
Biochemical process	Urea cycle	Pyrimidine synthesis
Location in cell	Mitochondria	Cytoplasm
Source of nitrogen	Ammonia	Glutamine

- different than CPS1 used in urea cycle,
  - b/c CPS1 is in mitochondria & substrates = NH_3 & HCO_3^-
- CPS II = cytosol, uses HCO_3^- & Glutamine
  - also b/c NH_3, HCO_3^- used to provide CO_2

Orotic Aciduria

- Autosomal recessive
- Deficiency in UMP Synthase
  - ↑ orotic Acid
  - -Stunted growth
  - -Megaloblastic Anemia
- Different than ornithine transcarbamylase deficiency, which causes accumulation of Carbamoyl Phosphate in cytosol
  - b/c of impaired urea cycle → Shunted to Pyrimidine Synthesis
  - b/c since OTC used in urea cycle (function is to clear NH_3) → ↑NH_3
    - ↓ Hyperammonemia
    - ↓ Hepatic encephalopathy
    - ↓ NOT seen w/ orotic aciduria

	Ornithine Transcarbamylase Deficiency	Orotic Aciduria
Biochemical process	Urea cycle	Pyrimidine synthesis
Orotic acid elevated?	Yes	Yes
Ammonia elevated?	Yes	No

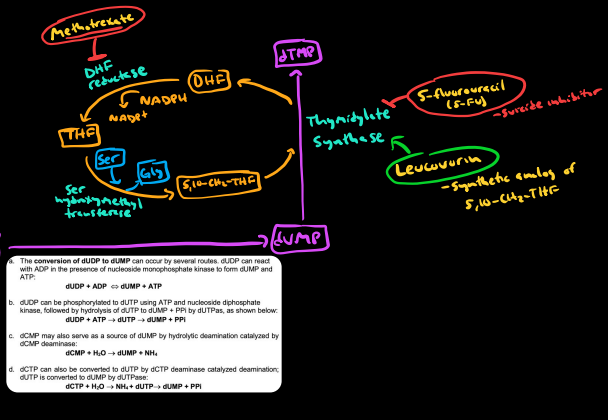
deoxynucleoside triphosphates (dNTPs)

-NDP —ribonucleotide reductase→ dNDP —nucleotide kinase→ dNTP

- Ribonucleotide (rNDP) Reductase
  - -acts on all dNDPs EXCEPT dTNP
  - -NADPH = final reductant
  - -reduces 2'-OH
  - ① rNDP reductase
    - -oxidation of -SH groups → S-S bond @ active site
      - b/c occurs between B1 & B2 dimer Subunits
    - -B1 subunit regulation:
      - ① "S" site -substrate specificity
      - ② "A" site -catalytic
  - ② Thioredoxin or Glutaredoxin
  - ③ Thioredoxin reductase or Glutaredoxin reductase

Ligand bound to "A" site	Ligand bound to "S" site	
		Activity of catalytic site
dATP		Enzyme inactive
ATP	ATP/dATP	Specific for CDP or UDP
ATP	dTTP	Specific for GDP
ATP	dGTP	Specific for ADP

To ensure that the enzyme produces a desired optimized ratio of dNTPs required for DNA synthesis.
- ↑ATP → ↑ reduction of CDP and UDP
- ↑dTTP → ↑ reduction of GDP; ↓ reduction of CDP; ↓ reduction of UDP
- ↑dGTP → ↑ reduction ADP
- ↑Reduction of CDP or UDP also ↓ by dATP or dGTP



a. The **conversion of dUDP to dUMP** can occur by several routes. dUDP can react with ADP in the presence of nucleoside monophosphate kinase to form dUMP and ATP:
dUDP + ADP ⇔ dUMP + ATP

b. dUDP can be phosphorylated to dUTP using ATP and nucleoside diphosphate kinase, followed by hydrolysis of dUTP to dUMP + PPi by dUTPas, as shown below:
dUDP + ATP → dUTP → dUMP + PPi

c. dCMP may also serve as a source of dUMP by hydrolytic deamination catalyzed by dCMP deaminase:
dCMP + H_2O → dUMP + NH_4

d. dCTP can also be converted to dUTP by dCTP deaminase catalyzed deamination; dUTP is converted to dUMP by dUTPase:
dCTP + H_2O → NH_4 + dUTP → dUMP + PPi

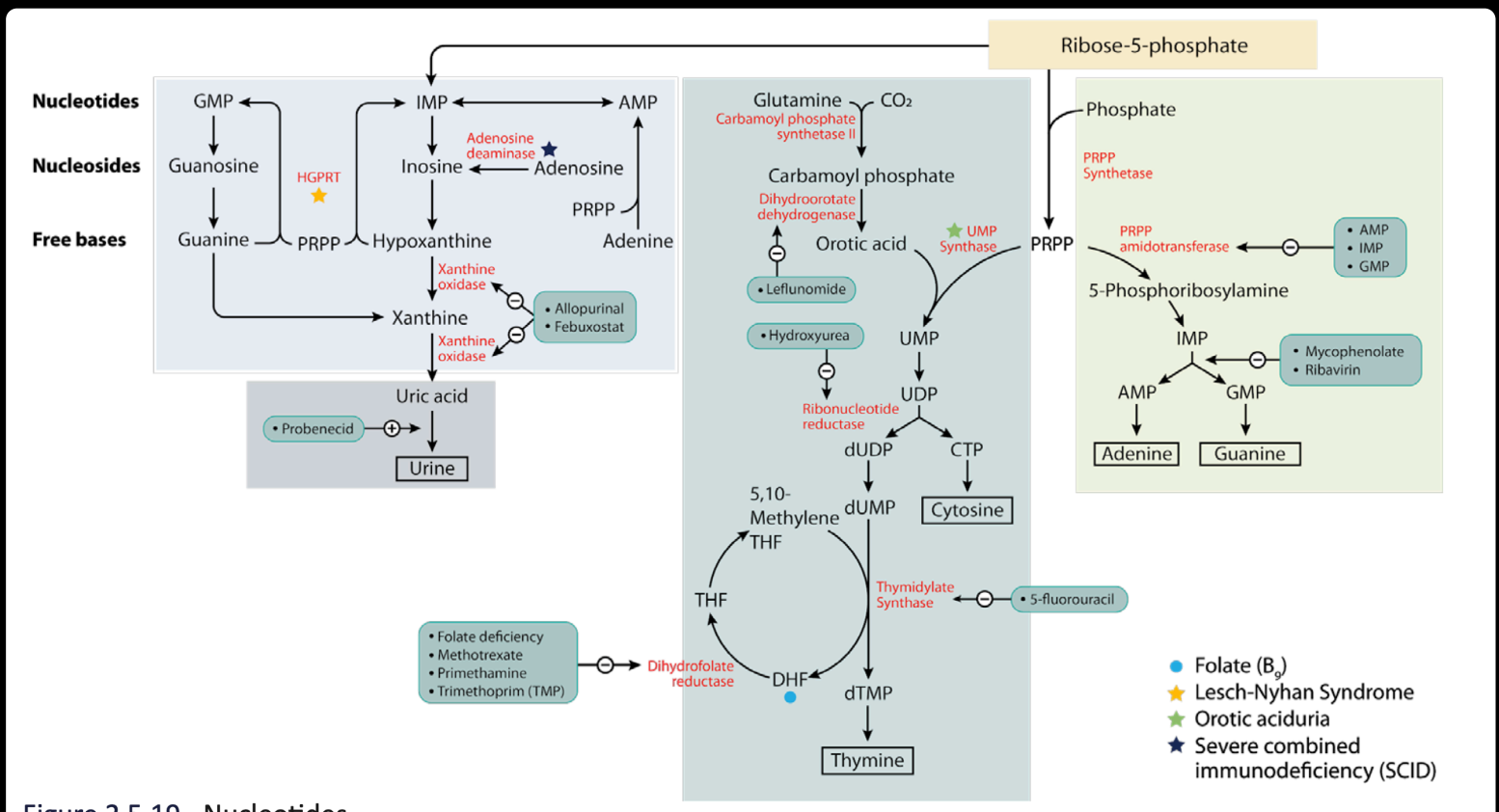


Figure 2.5.19 - Nucleotides