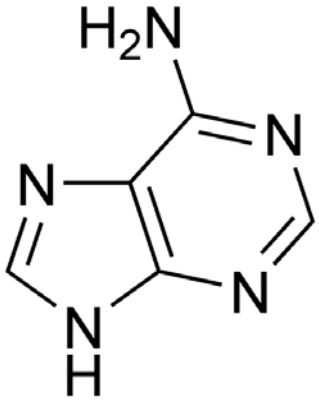
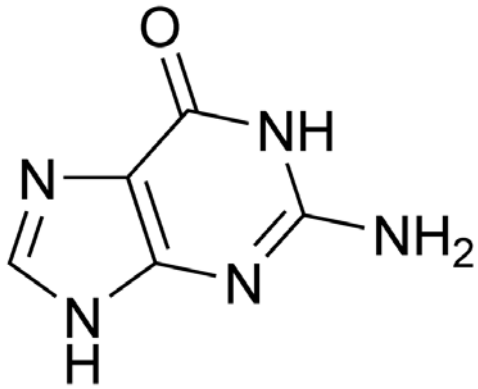
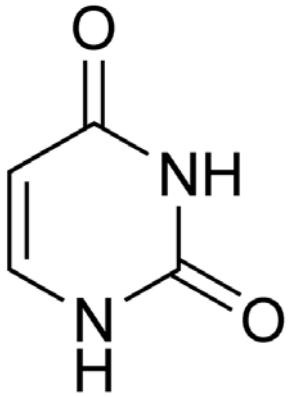
Nucleotide Metabolism



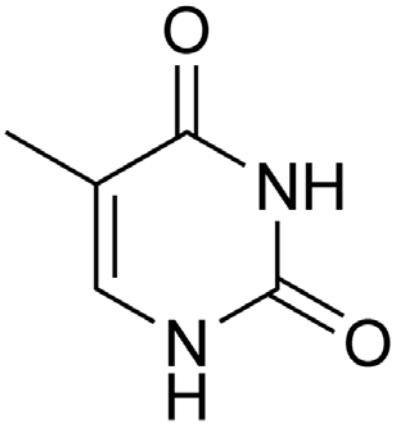
Adenine



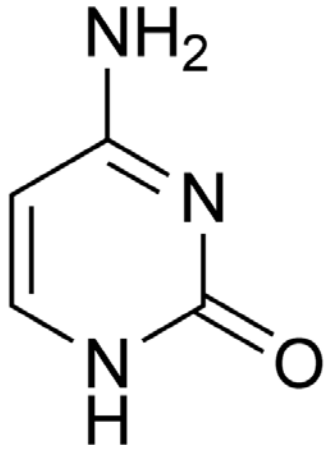
Guanidine



Uracil

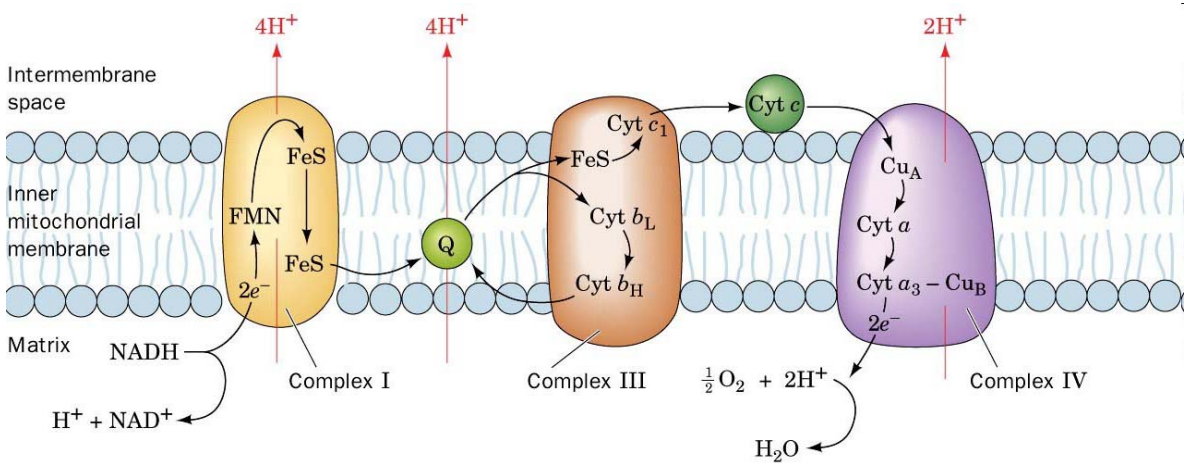


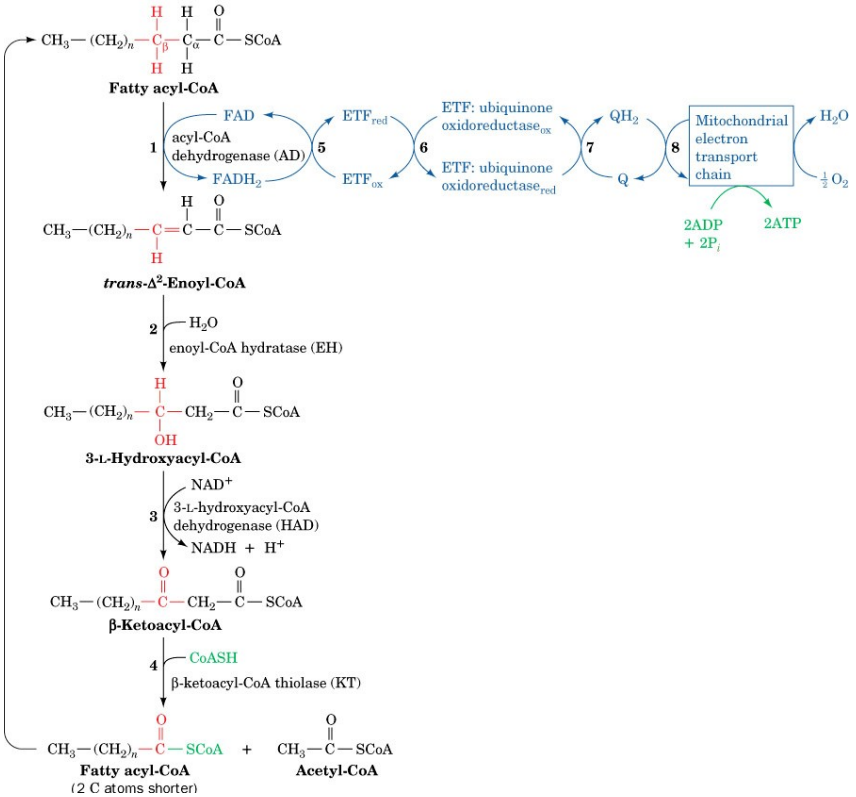
Thymine

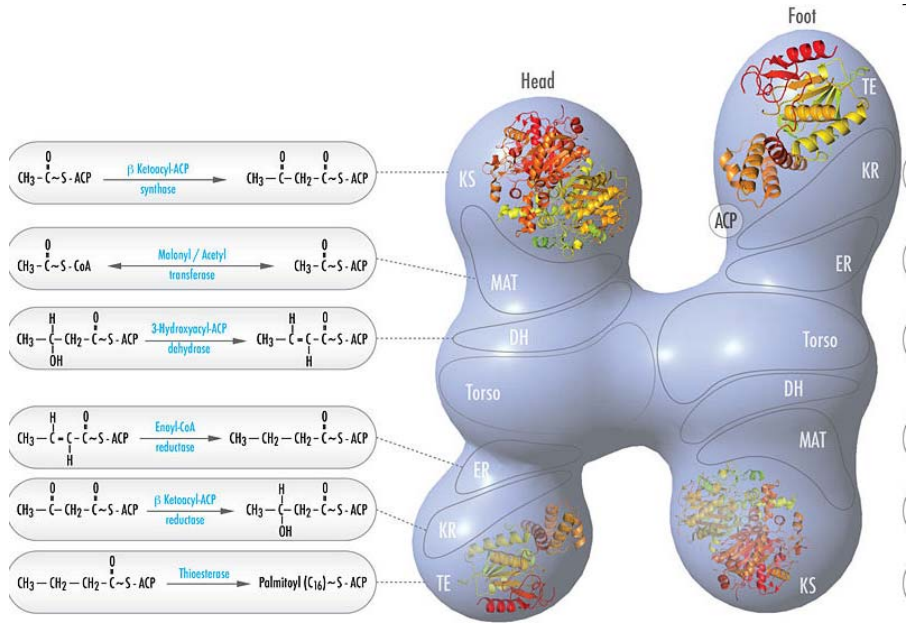


Cytosine

Last Week...

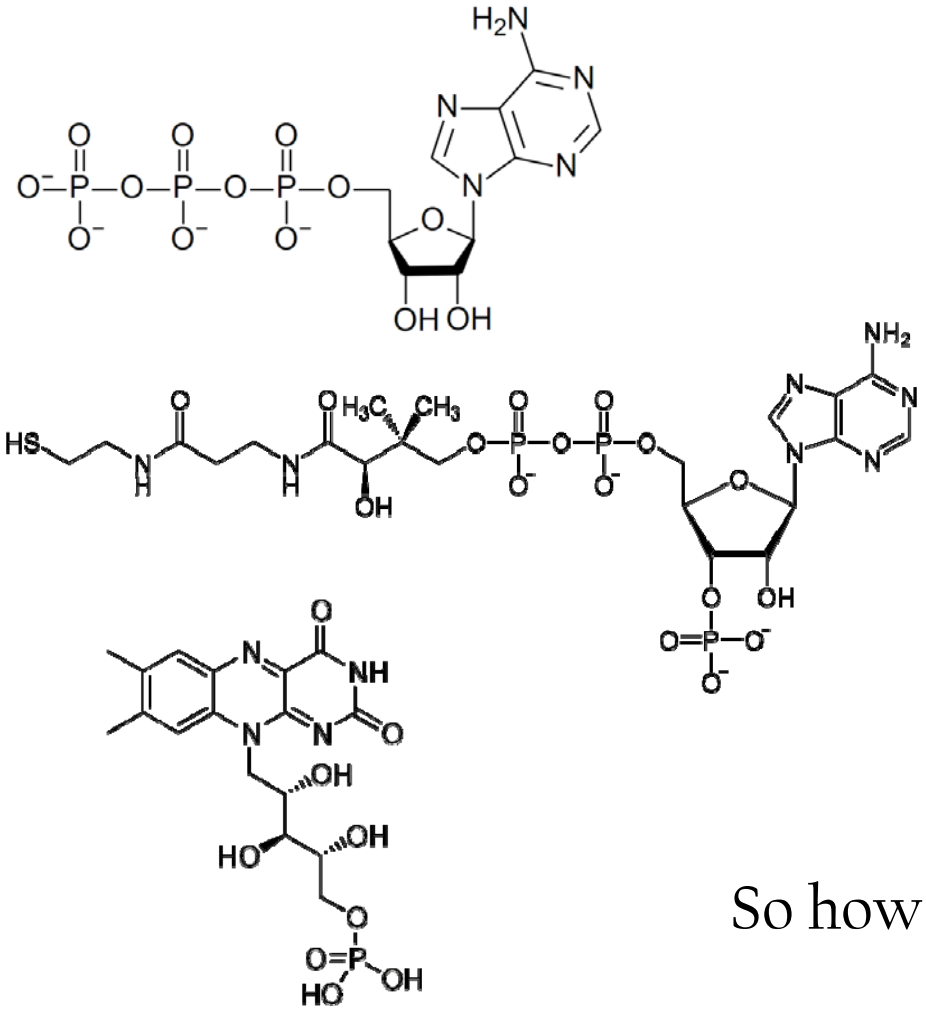

4H+
4H+
2H+
Intermembrane space
Inner mitochondrial membrane
Matrix
FMN
FeS
FeS
FeS
Q
Cyt c1
Cyt bL
Cyt bH
Cyt c
CuA
Cyt a
Cyt a3 – CuB
2e−
2e−
NADH
H+ + NAD+
Complex I
Complex III
½ O2 + 2H+
H2O
Complex IV

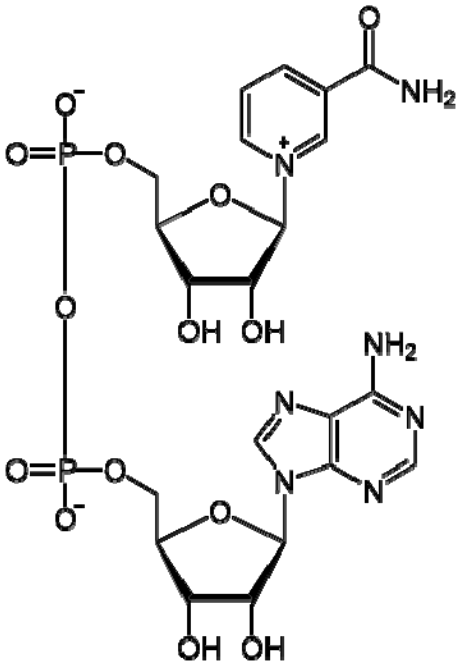

Fatty acyl-CoA
FAD
acyl-CoA dehydrogenase (AD)
FADH2
ETFred
ETFox
ETF: ubiquinone oxidoreductaseox
ETF: ubiquinone oxidoreductasered
QH2
Q
Mitochondrial electron transport chain
H2O
½ O2
2ADP + 2Pi
2ATP
trans-Δ2-Enoyl-CoA
H2O
enoyl-CoA hydratase (EH)
3-L-Hydroxyacyl-CoA
NAD+
3-L-hydroxyacyl-CoA dehydrogenase (HAD)
NADH + H+
β-Ketoacyl-CoA
CoASH
β-ketoacyl-CoA thiolase (KT)
Fatty acyl-CoA (2 C atoms shorter)
Acetyl-CoA


β Ketoacyl-ACP synthase
Malonyl / Acetyl transferase
3-Hydroxyacyl-ACP dehydrase
Enoyl-CoA reductase
β Ketoacyl-ACP reductase
Thioesterase
Palmitoyl (C16)~S-ACP
Head
Foot
KS
KR
ACP
MAT
ER
DH
Torso
TE

Nucleotide Metabolism

- Mostly, we think of nucleotides as being part of RNA/DNA, but now that we've studied metabolism, we know they're all over!

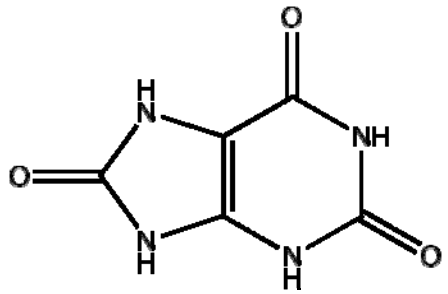




So how do we make these things?

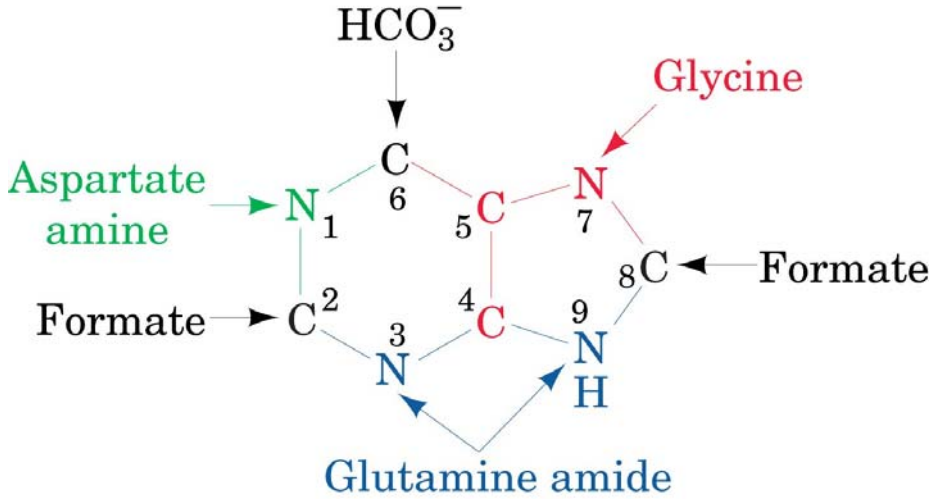
Making Purines

- Purines have a common 6,5 ring structure



Which is based on Uric Acid

- These nitrogenous rings (nitrogenous bases) are cobbled together from a number of sources:

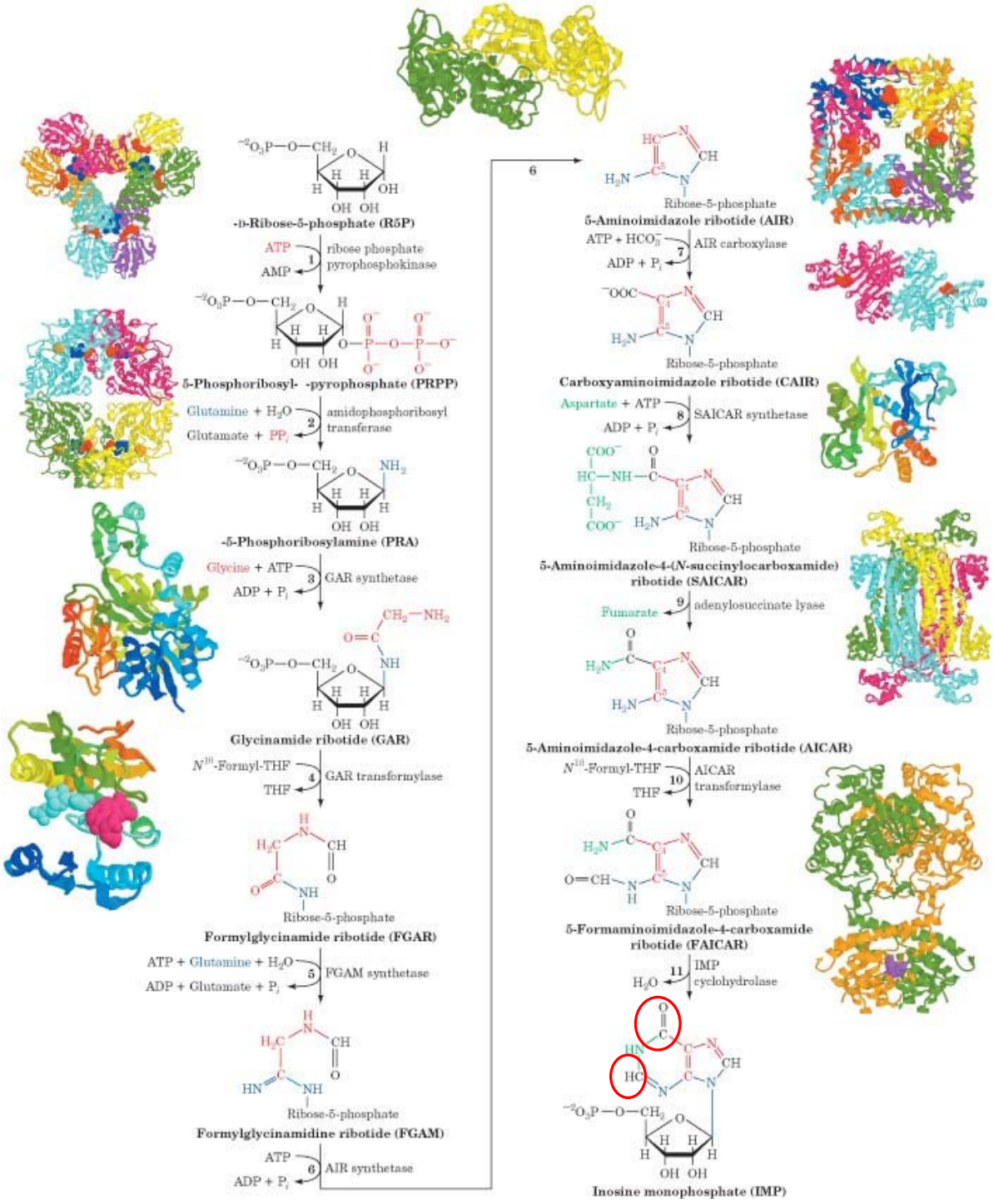


- The starting material is Ribose-5-phosphate

- The purine ring is actually constructed *on* the ribose

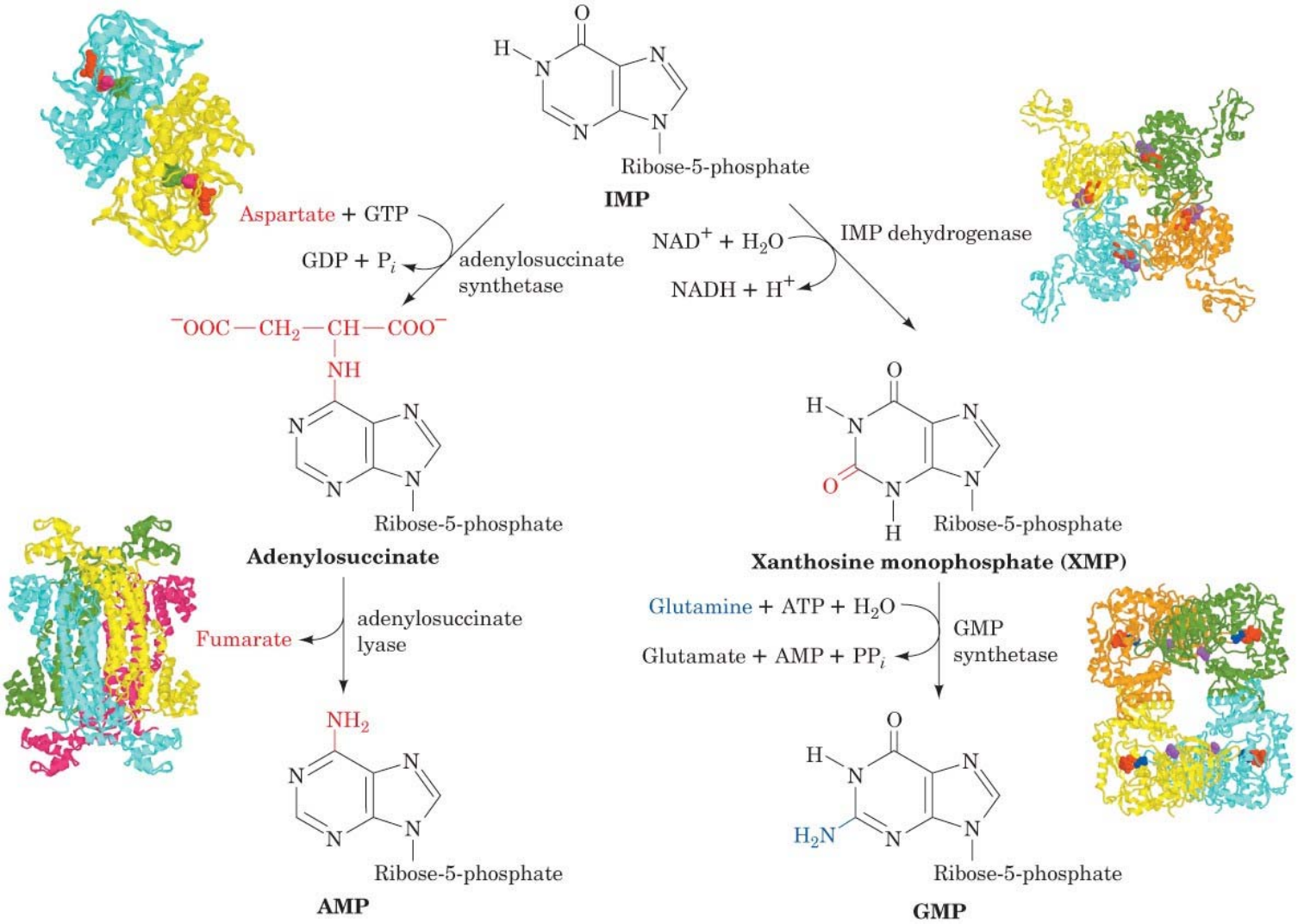
- The end product is inosine monophosphate which is similar to Adenine, except for the carbonyl at the C6 position (should be NH_2)

- For Guanidine we'll need to add an NH_2 group at C2



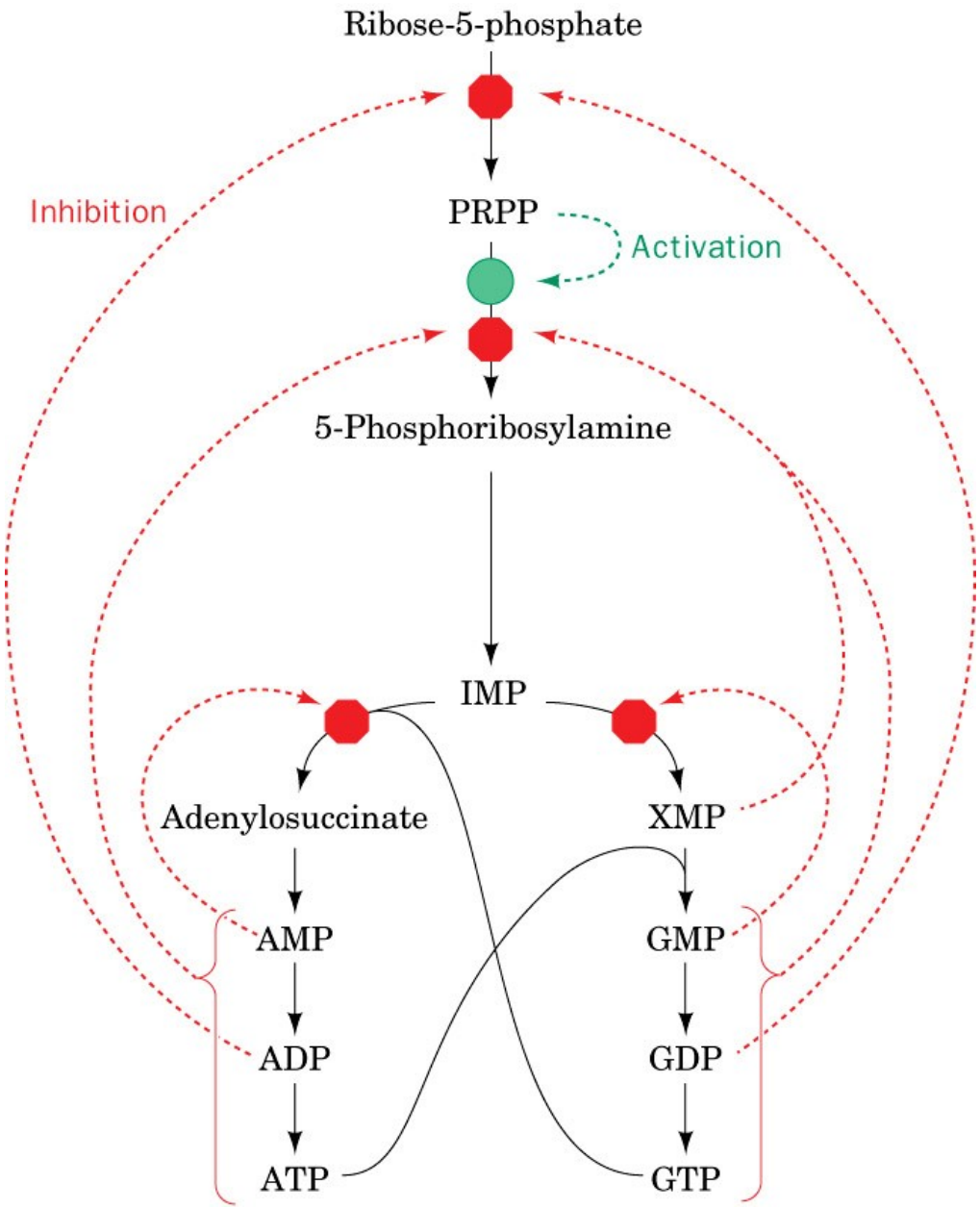
Purine Biosynthesis Part Deux

- Getting from Inosine Monophospahate to AMP and GMP



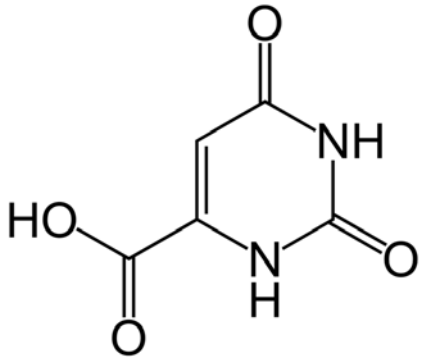
Control of Purine Synthesis

- Purine synthesis is end-product inhibited

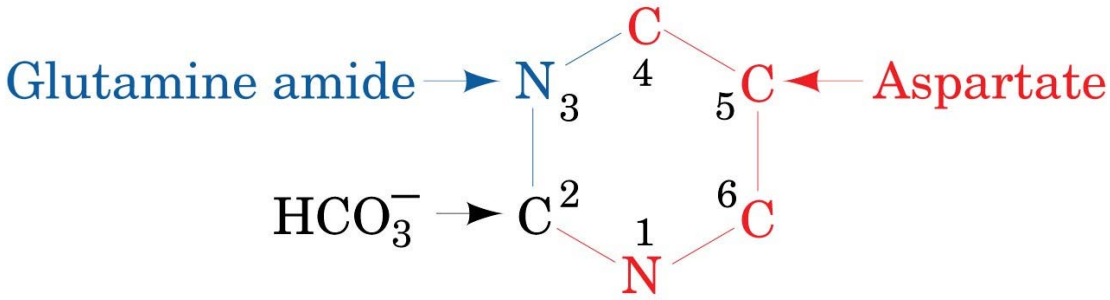


Pyridine Synthesis

- Pyridine Rings have this characteristic 6 membered ring structure typified by orotic acid (minus the COOH):

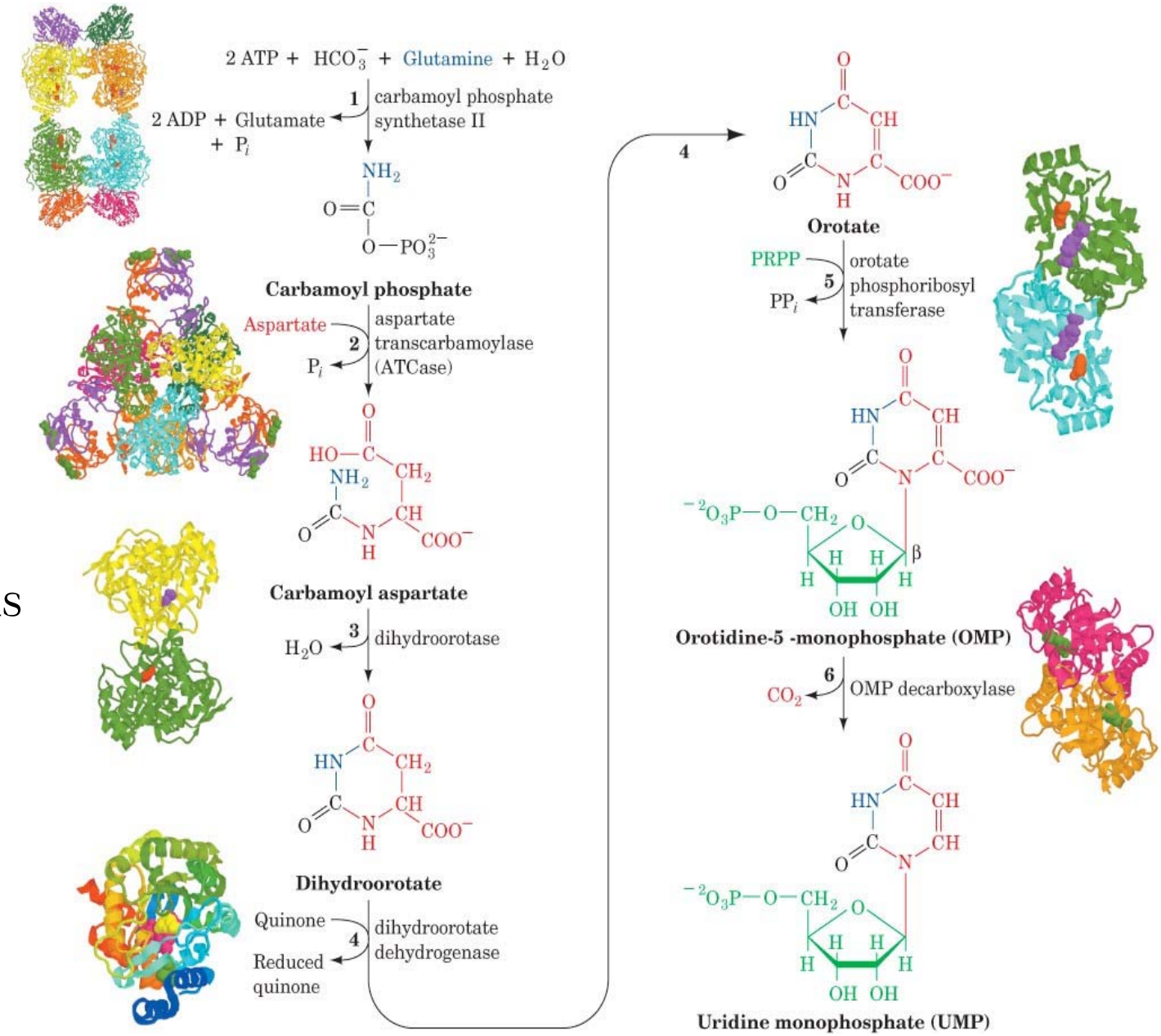


- Most of the ring comes from and Aspartate residue



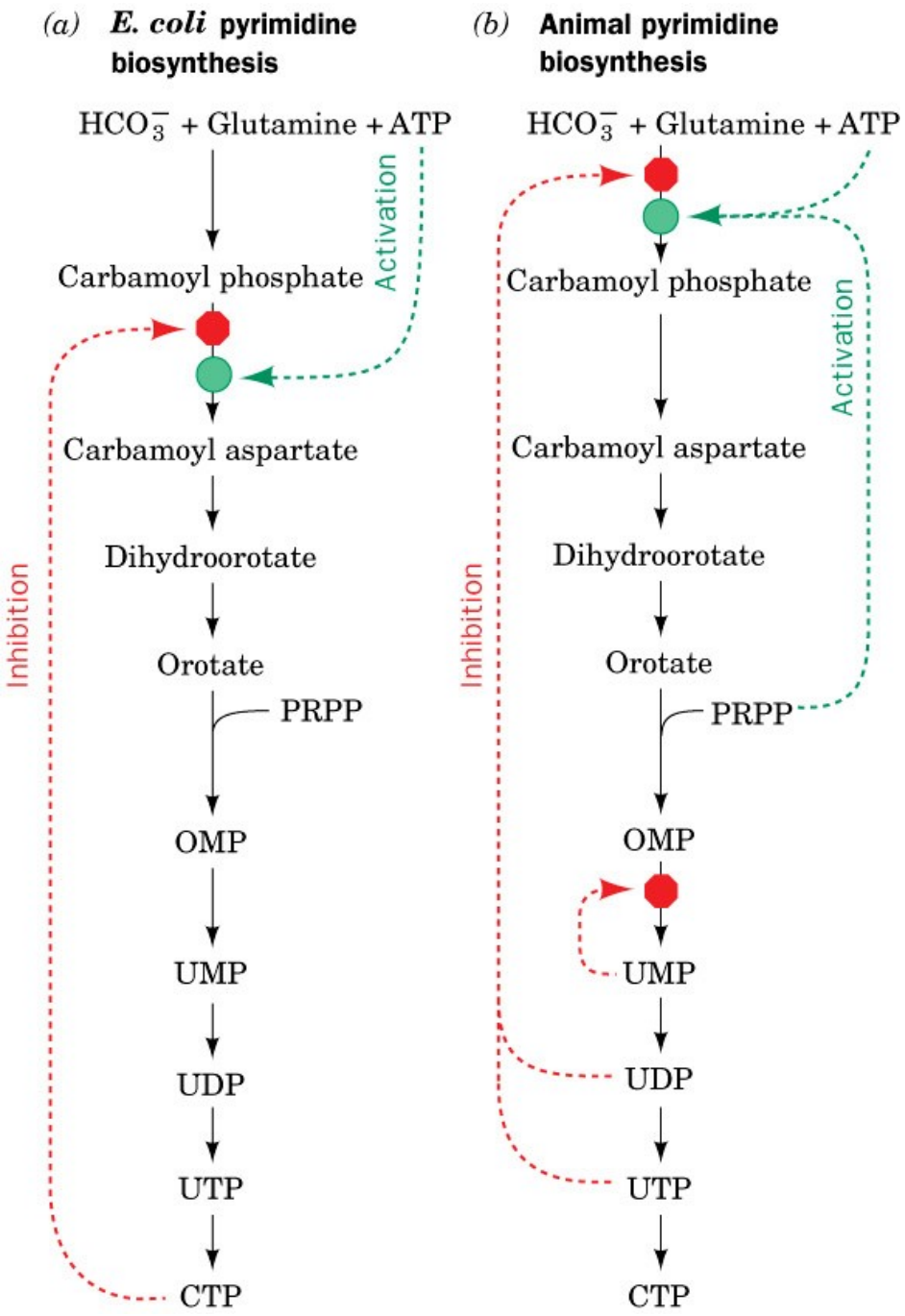
- Unlike with purines, most of the synthesis is not carried out on a ribose sugar

- PRPP (step 5) is an early intermediate in purine synthesis



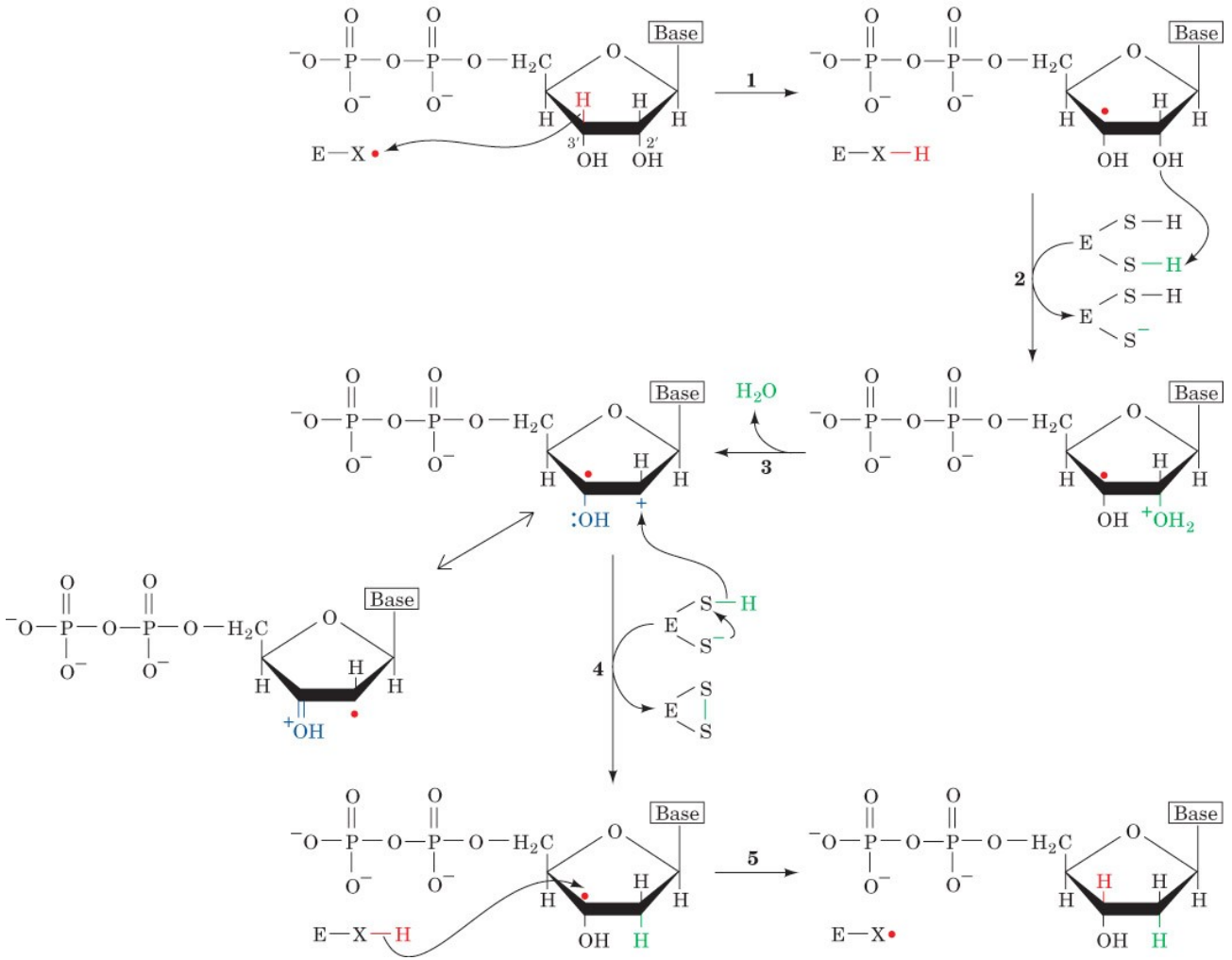
Control of Pyridine Synthesis

- Again, mostly end product inhibited



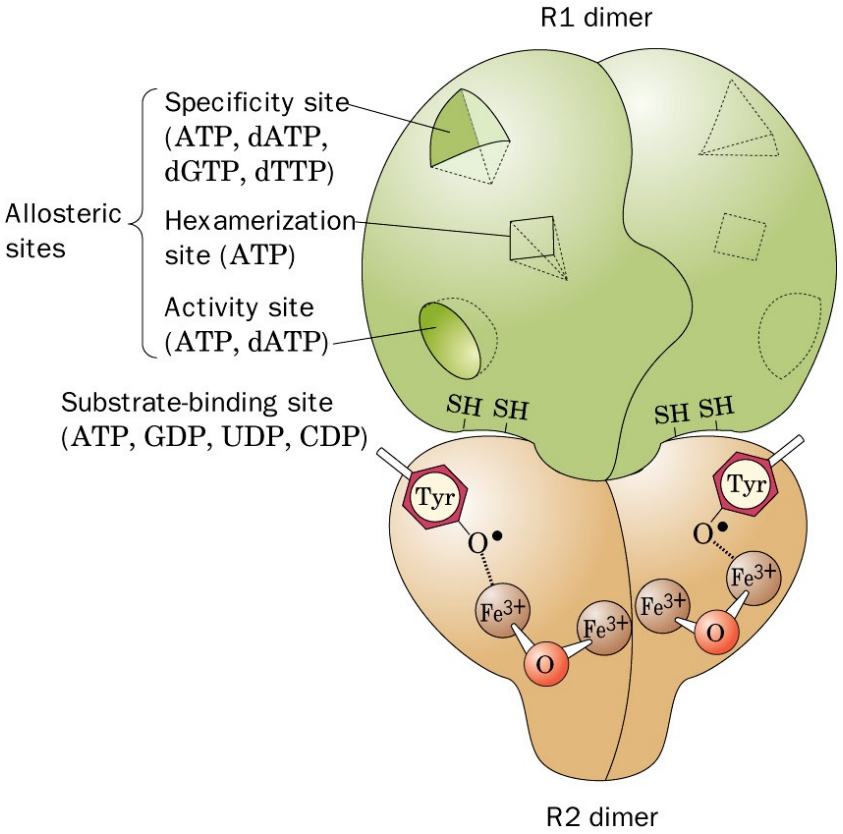
Making Deoxyribonucleotides

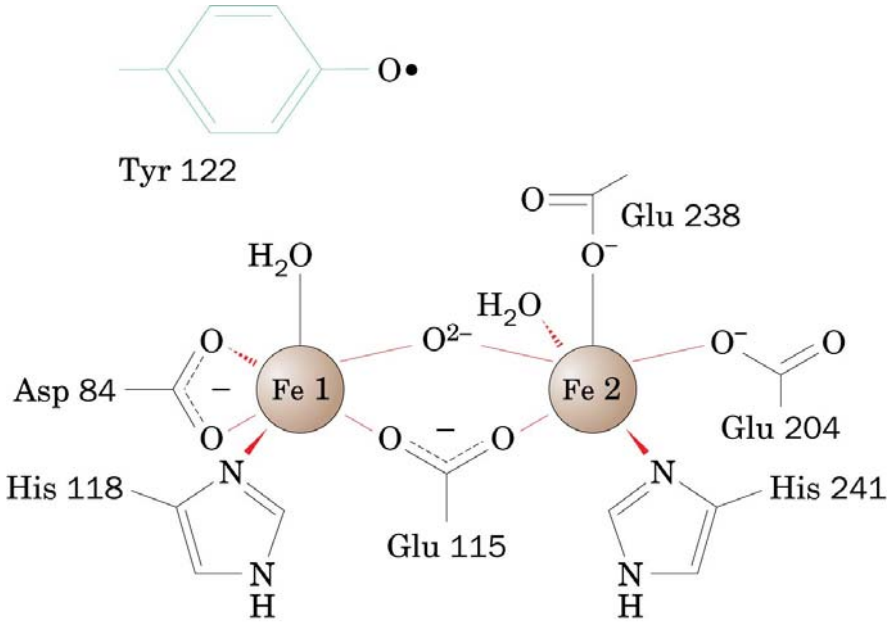
- Deoxynucleotides are made by Ribonucleotide Reductases which operate via a weird free radical mechanism



Ribonucleotide Reductases

- How do we make the E-X•?



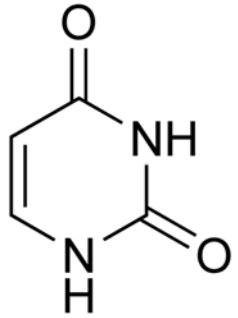


- Complete extraction of an electron from Tyr122 by Fe^{3+}

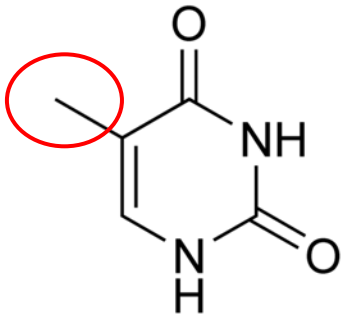
- Activated by oxygen

Thymidilate Synthase: Methylation is Hard to Do

- The difference between Thymine and Uracil is a Methyl Group

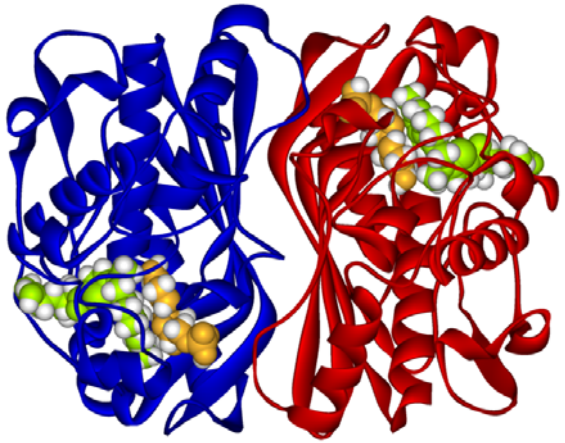


Uracil



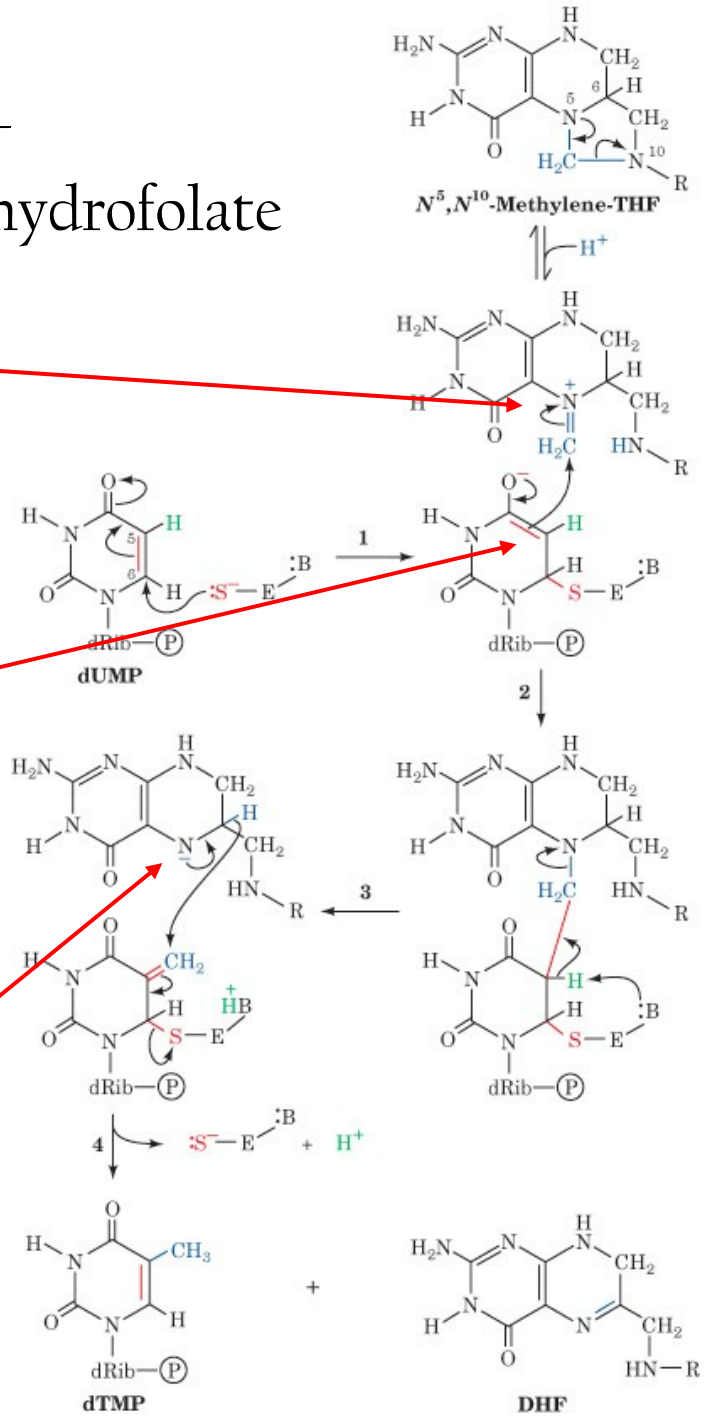
Thymine

- The Enzyme that makes thymine is Thyamidilate Synthase



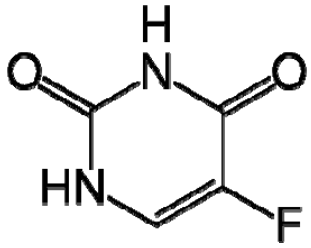
Thymidilate Synthase Mechanism

- This enzyme uses N^5,N^{10}-Methylenetetrahydrofolate
  - Step 0: Generate iminium cation by breaking 5 membered ring, attack C6 of dUMP. Moves double bond to C5/C4
  - Step 1: Schiff-base-like generation of a Carbocation, electrophilic attack by dUMP'
  - Step 2: Extraction of proton, C=C at C5 of dUMP'
  - Step 3: NADH-like hydride transfer to CH_2 on C5 of dUMP', electrons transferred back to cysteine on enzyme



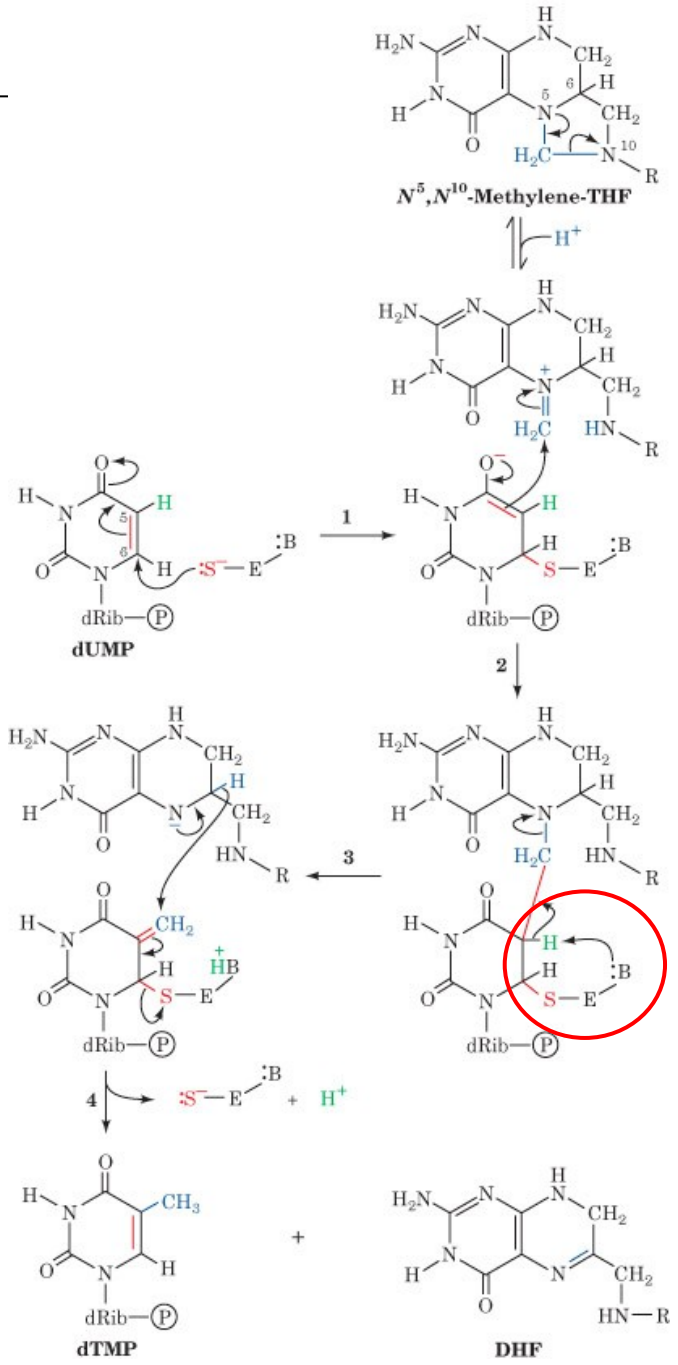
Anticancer Drug

- Fluorouracil is an anti-cancer drug that has been in use for over 40 years



- It is an irreversible inhibitor of Thyamine Synthase

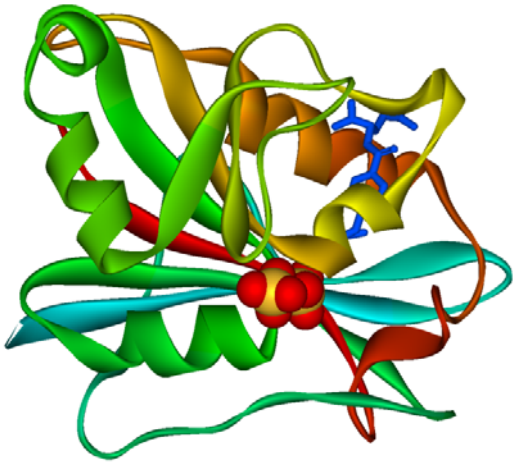
- Interferes with step 3 because F cannot be extracted by the base.



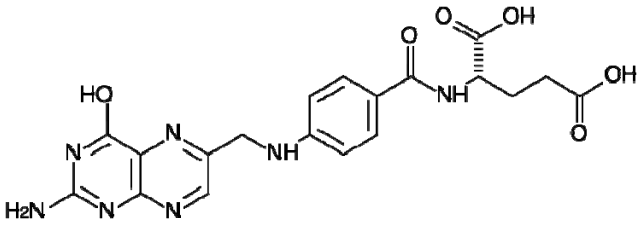
More Anticancer Drugs

- In order to keep generating dTMP, cells need to regenerate N^5,N^{10}-Methylenetetrahydrofolate.

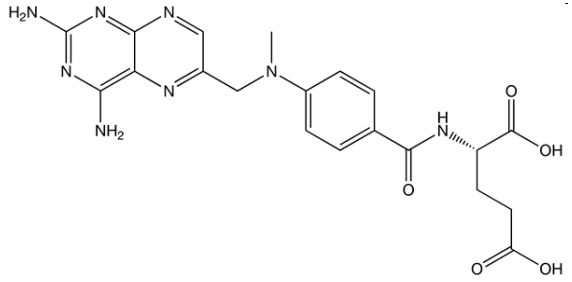
- This is normally done by dihydrofolate reductase (DHFR)



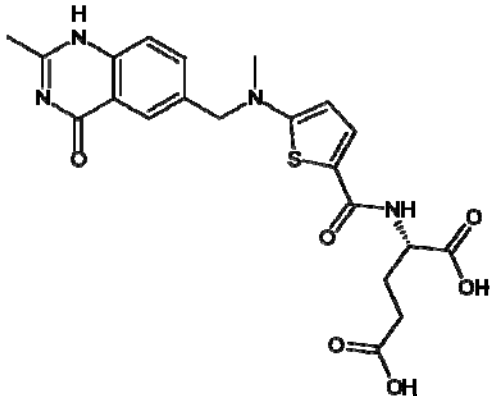
- One anti-cancer strategy is therefore to inhibit DHFR using dihydrofolate mimic inhibitors



Folic acid



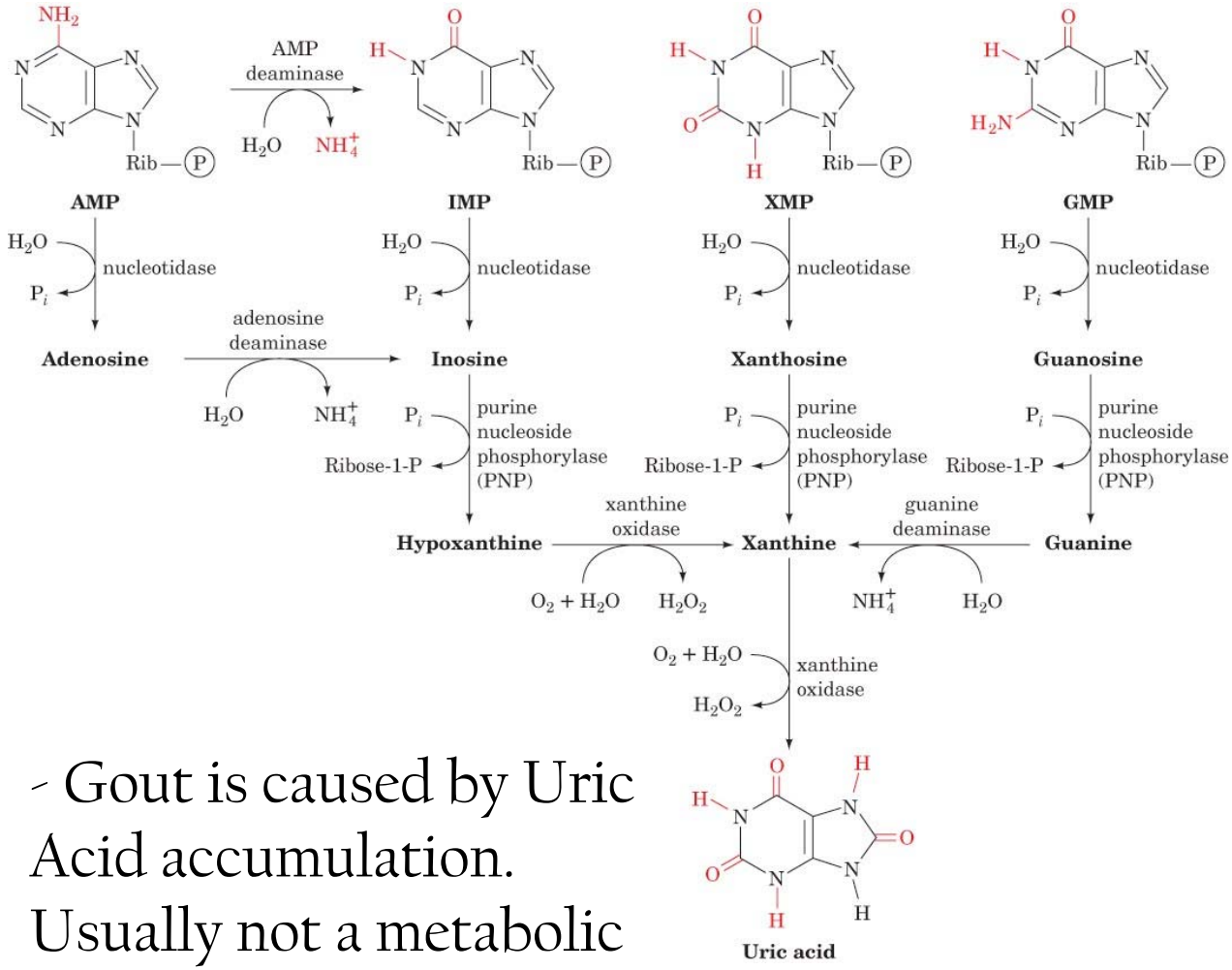
Methotrexate



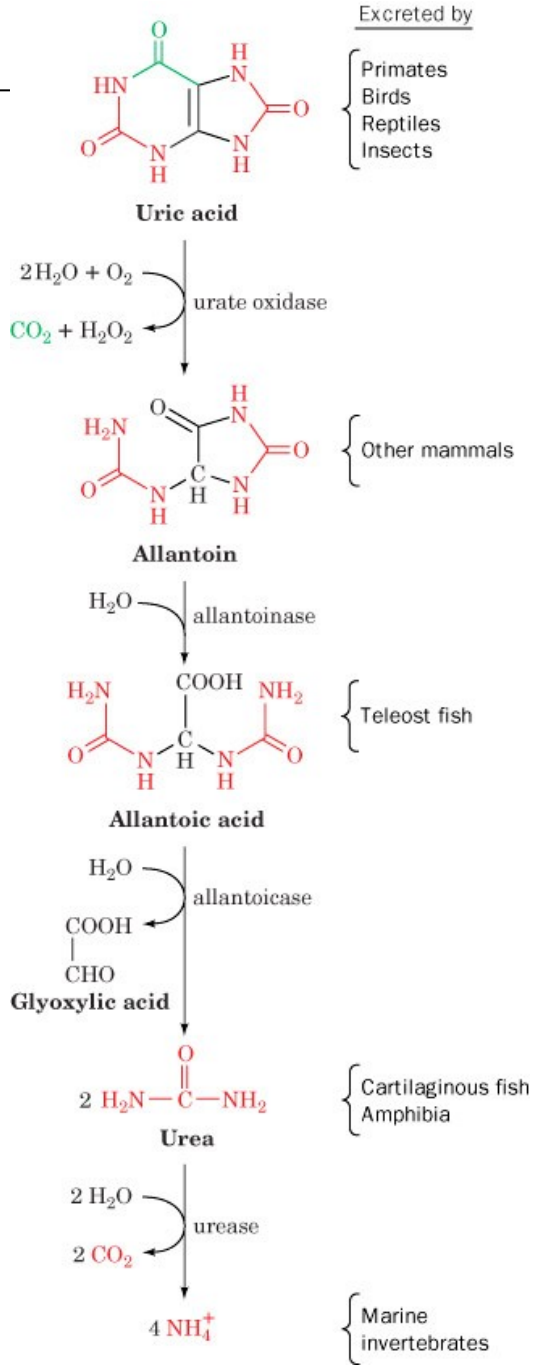
Raltitrexed

Degradation of Purines

- Purines are degraded to Uric Acid



- Gout is caused by Uric Acid accumulation. Usually not a metabolic problem



Biosynthesis of NAD

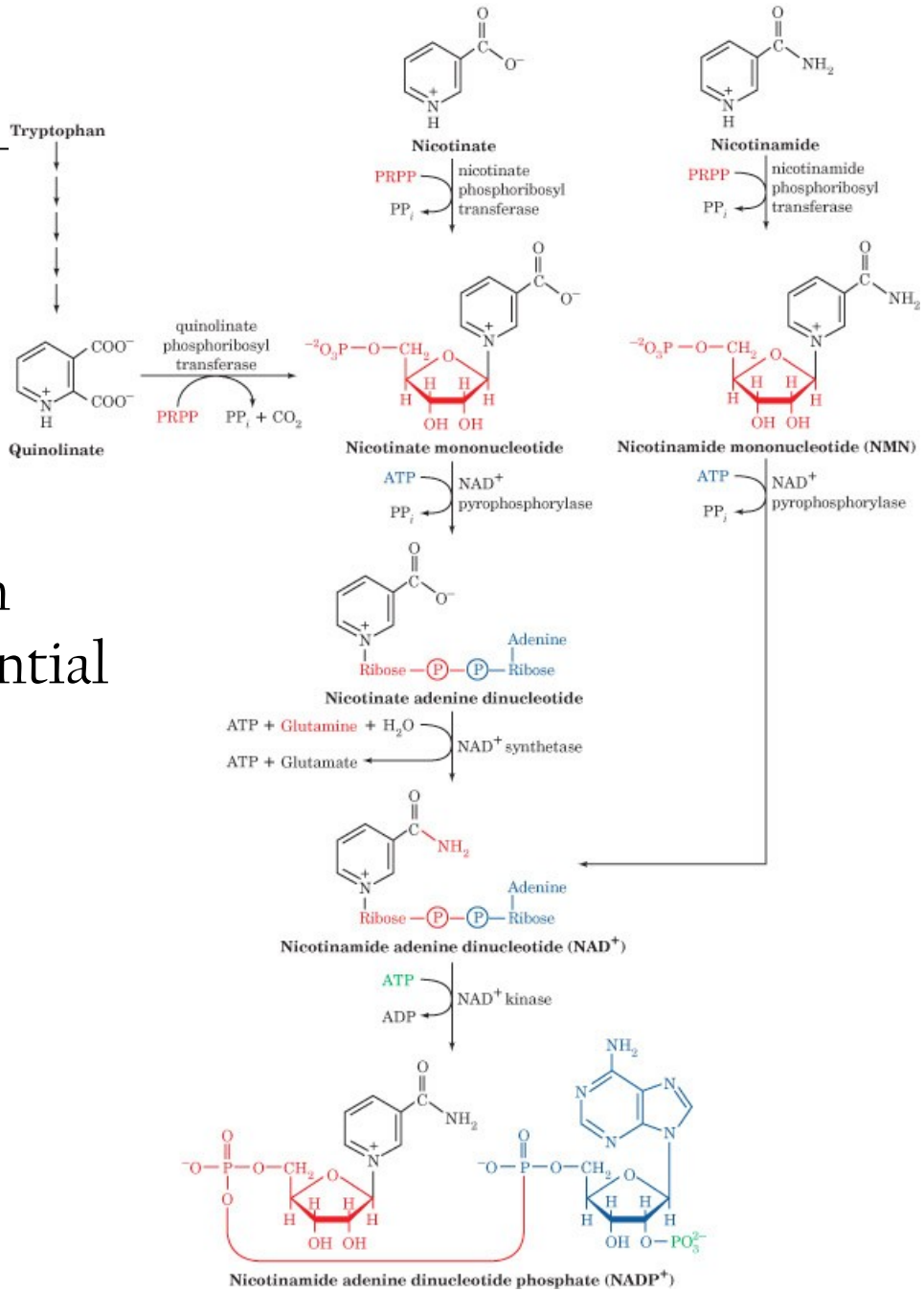
- Nicotinamide is derived from dietary sources. We do not make it!

- We can make nicotinate from tryptophan, but that is an essential amino acid

- We stick on PRPP

- Extra step from nicotinate: Amidation from glutamine

- Add AMP and voila!



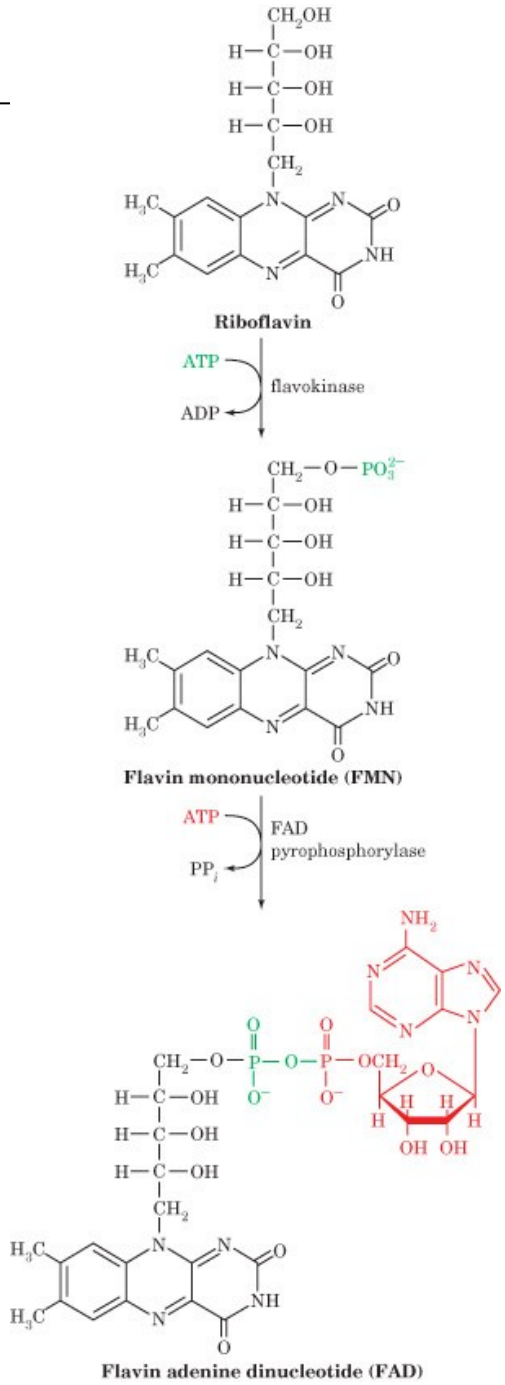
Making Flavin Nucleotides

- Start with Riboflavin (vitamin B2) from dietary sources. We do not make it!

- Acticate the terminal ribose –OH group by phosphorylation (flavokinase)

- Add adenosine monophosphate (FAD pyrophosphorylase)

- Again, we're getting the oxidized form of the molecule.



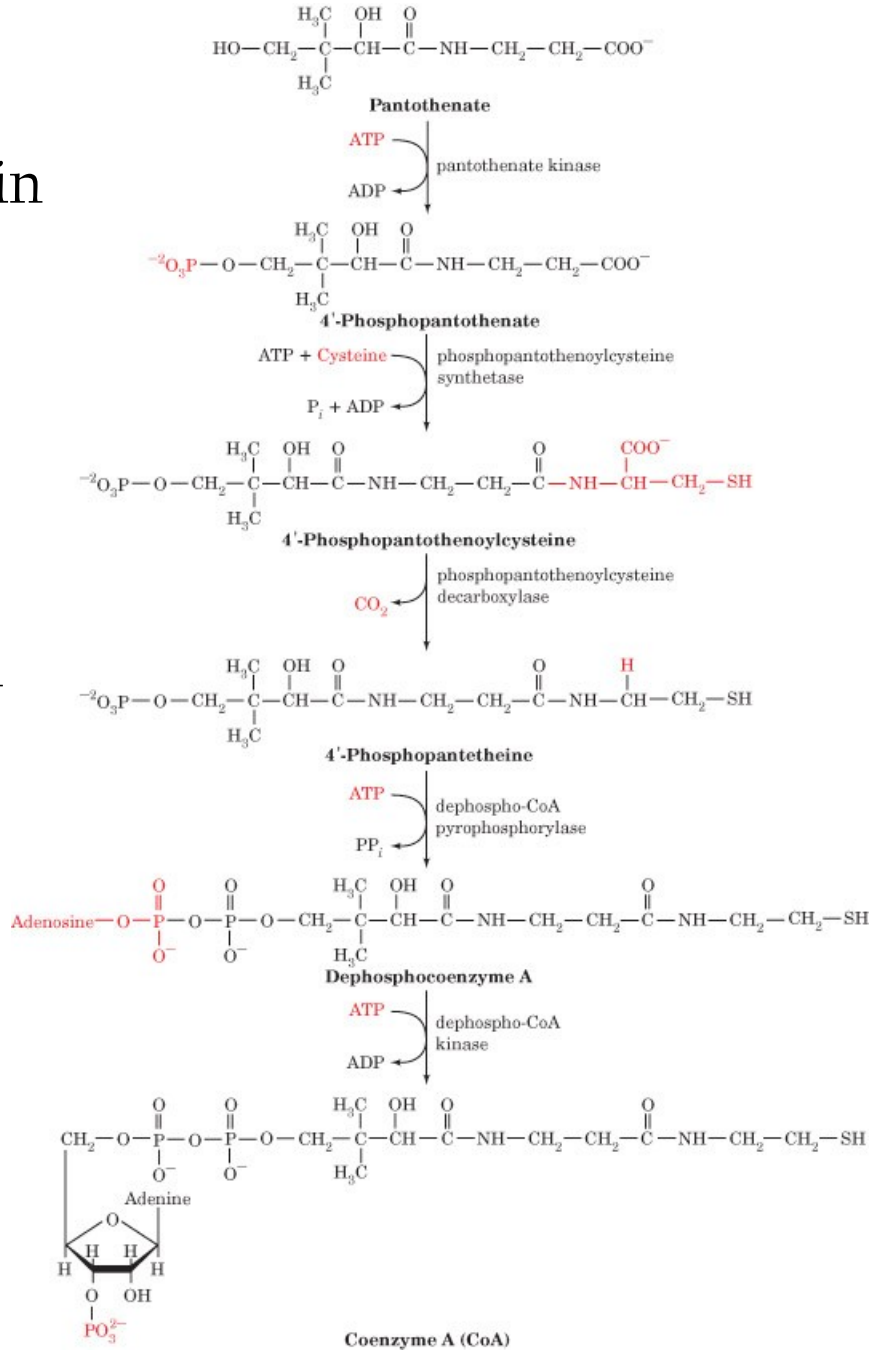
Making CoA

- Start with Pantothenate (vitamin B5) which we do not make for ourselves.

- The reactive sulfur comes from cysteine!

- Removal of CO_2 by phosphopan tothenoylcysteine decarboxylase, uses a flavin cofactor

- Tack on AMP and you're done!



Linearized Michaelis-Menten Kinetics

- But how do we uncover these mechanisms?

Linearized Michaelis-Menten Kinetics

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