Nucleotide metabolism

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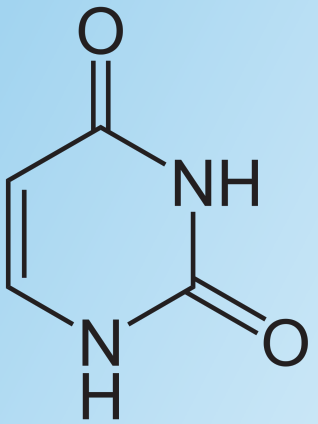
We will cover…

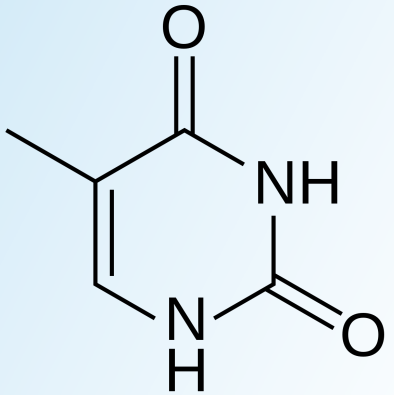
- ❑ Nucleobases and their functions
- ❑ Ribonucleotide synthesis
  - ❑ Purine synthesis: “de novo” & salvage pathway
  - ❑ Pyrimidine synthesis
- ❑ Deoxyribonucleotide synthesis
  - ❑ From ribonucleotides to deoxyribonuclotides

First a quick review!

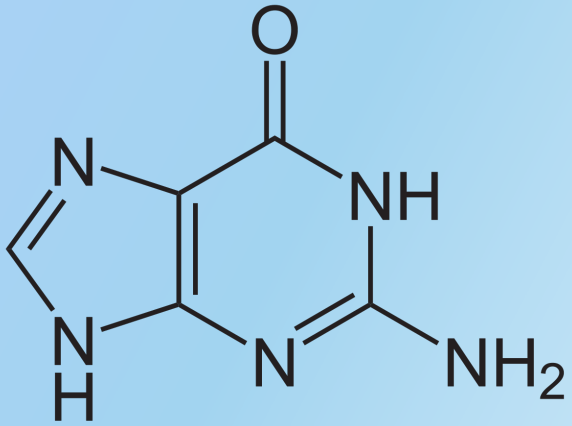
Adenine



Uracil



Thymine



Guanine

Cytosine

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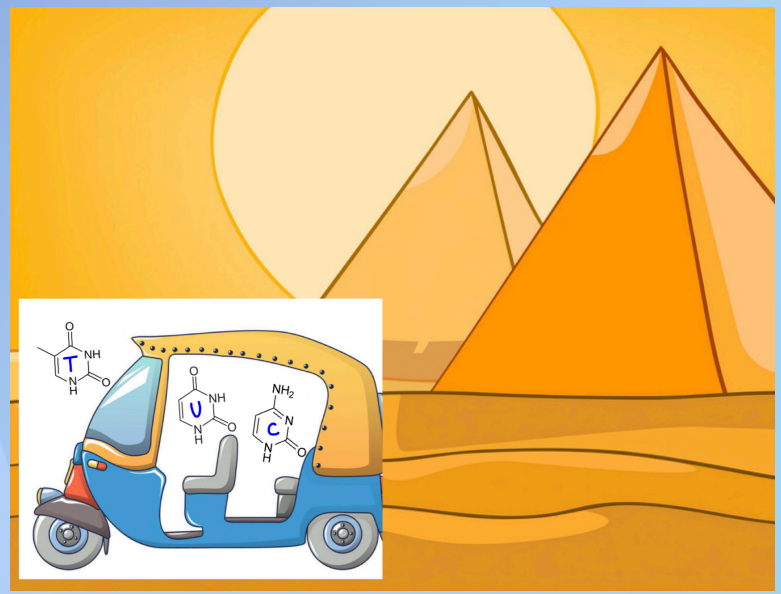
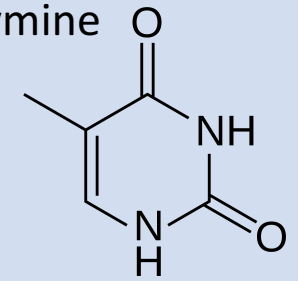
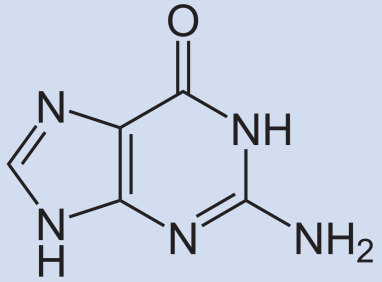
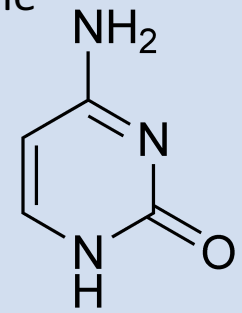
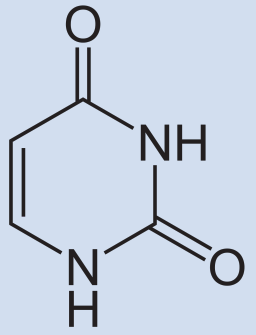
Nucleobases



"TUC-TUC around the pyramids"

PURE AS GOLD

PURINES	PYRIMIDINES
Adenine	Thymine 
Guanine 	Cytosine 
	Uracil (RNA) 

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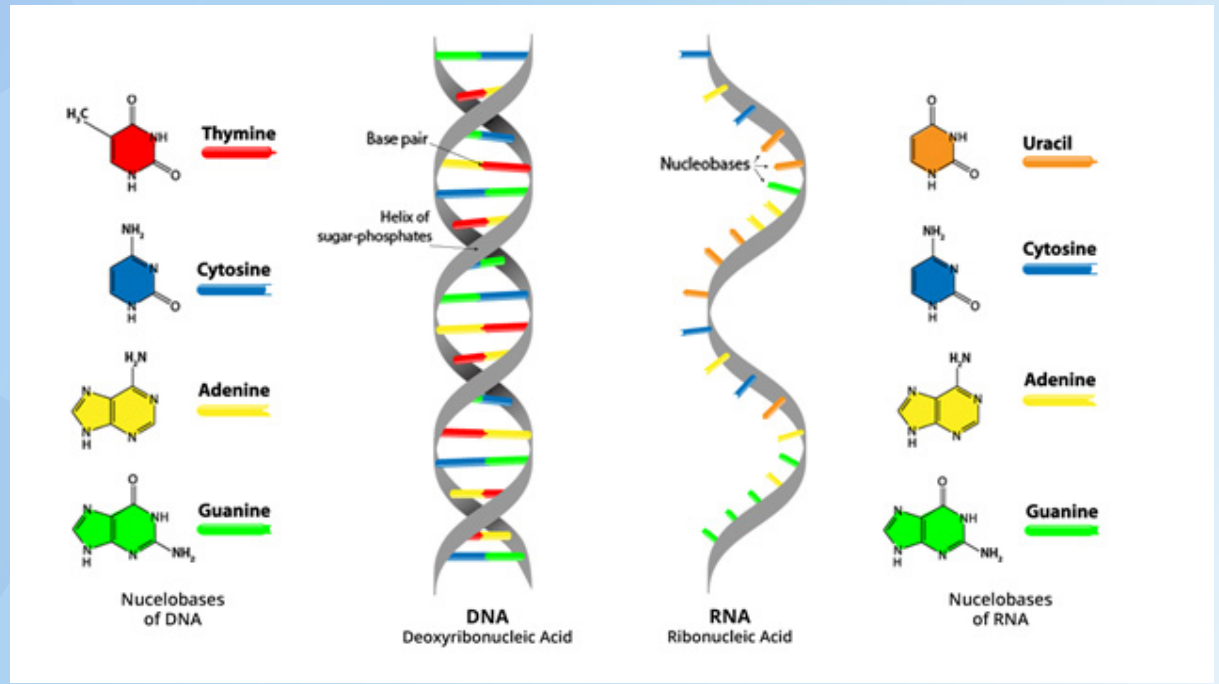
Why do we need nucleotides?

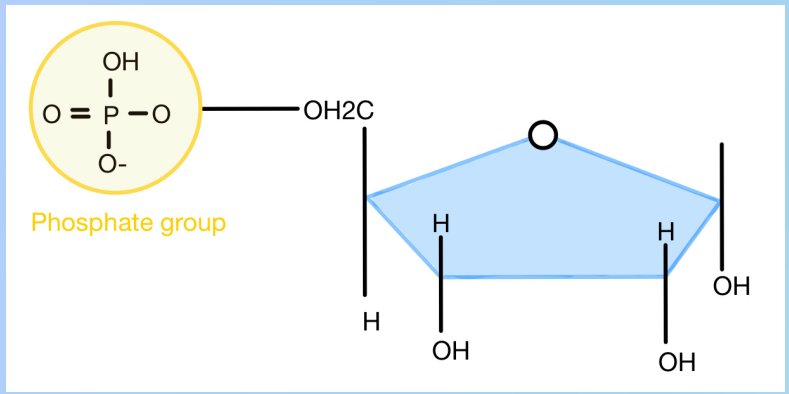


NAD
FAD
CoA

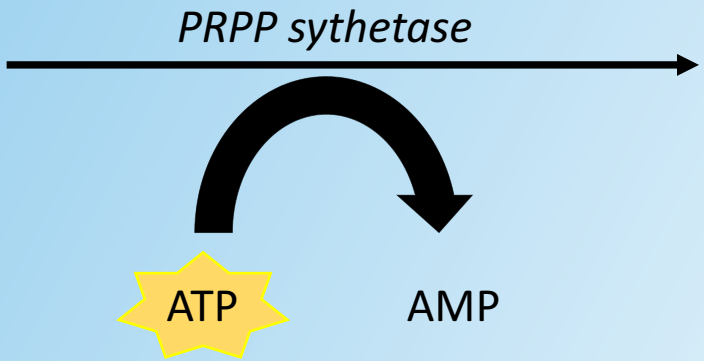
Why do we need to know these pathways?

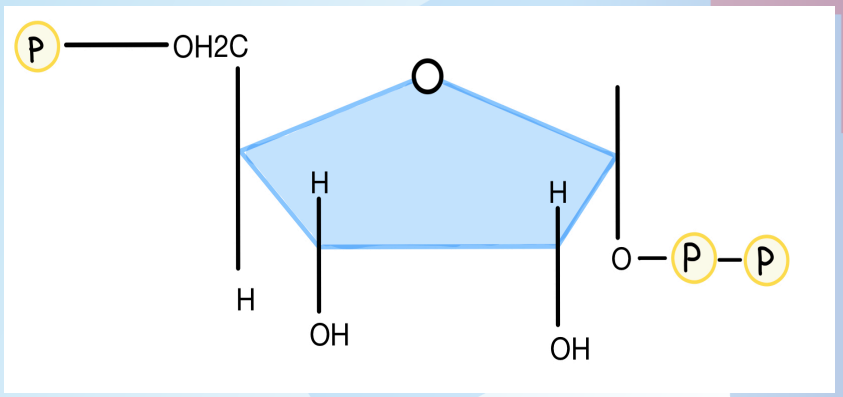
5-phosphoribosyl-1-pyrophosphate (PRPP)

- Synthesized from ATP and ribose 5-phosphate
- Catalyzed by *PRPP synthetase*
- Key substrate in <u>both</u> pyrimidine and purine synthesis



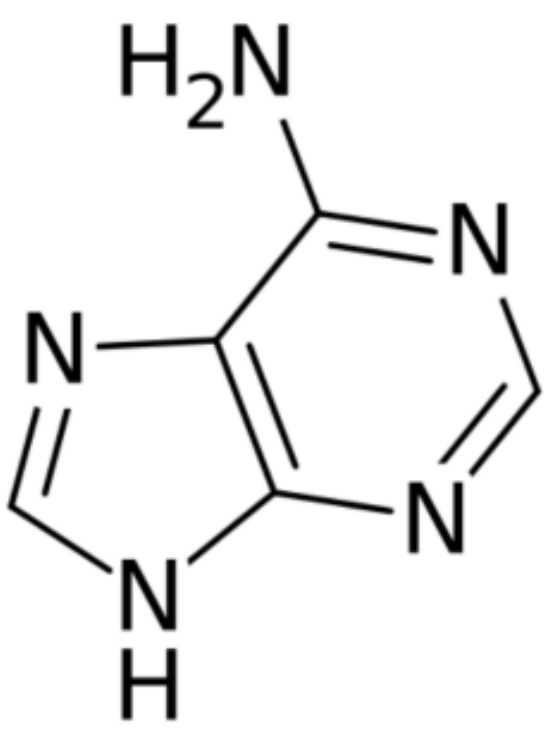
Ribose 5-phosphate



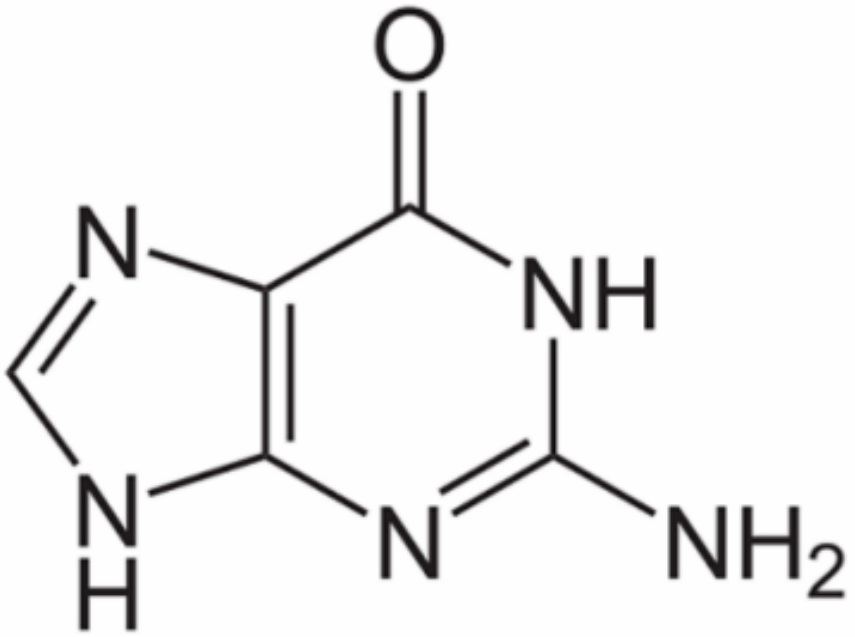


PRPP

Purine synthesis



Adenine



Guanine

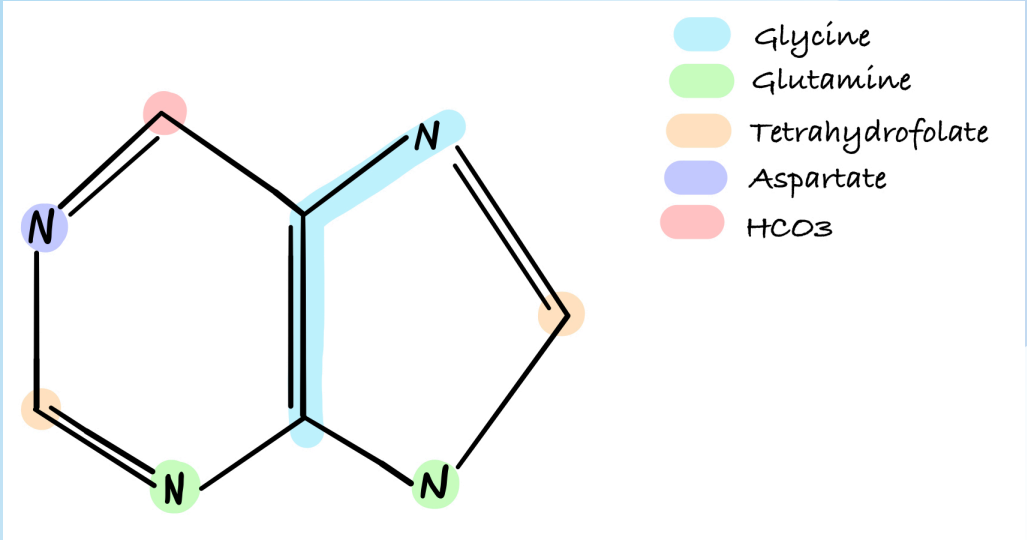
”De novo”

Let’s begin

Ingredients

1 PRPP
2 Glutamine
1 Glycine
1 Aspartate
2 N^{10} formyl-THF
1 HCO_3 (CO_2)
6 ATP

Our goal



”GAG” – Glycine, Aspartate, Glutamine

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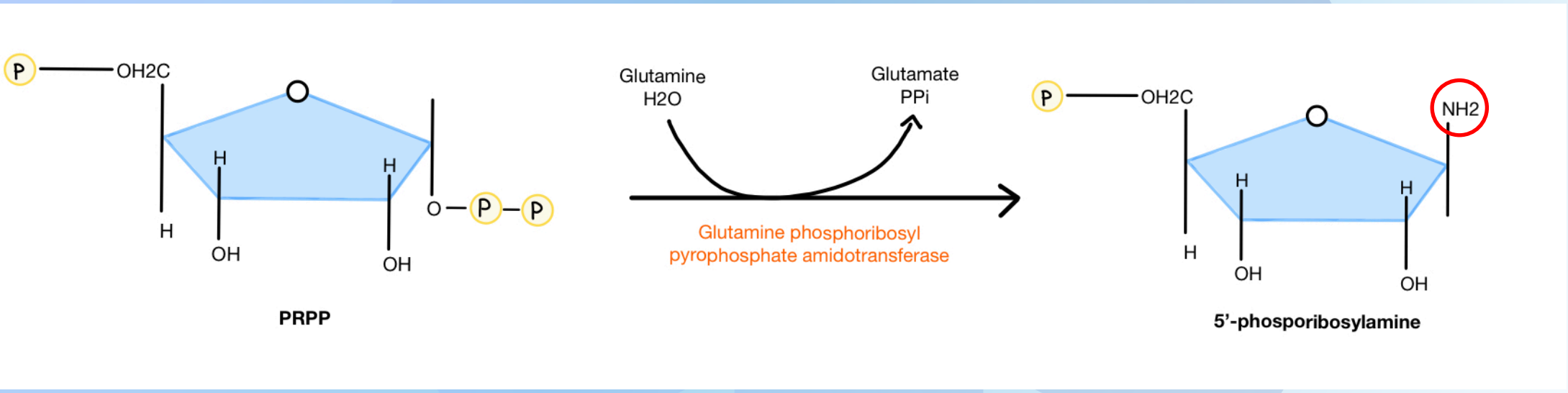
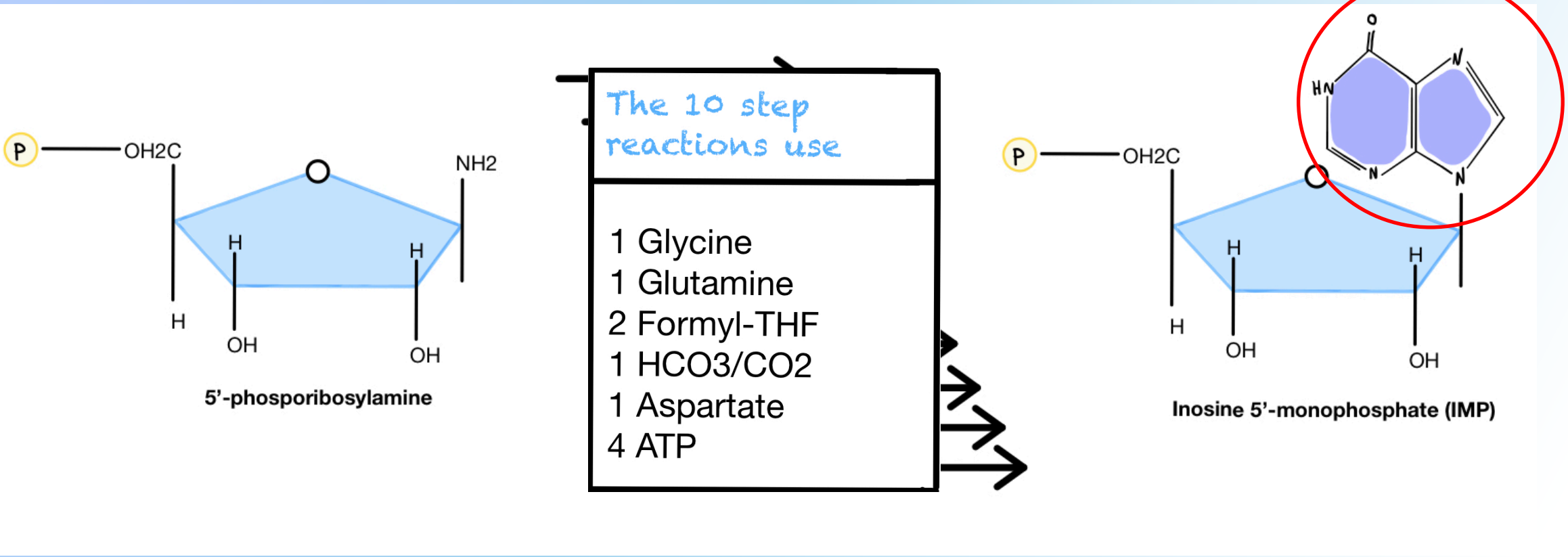
Step 1

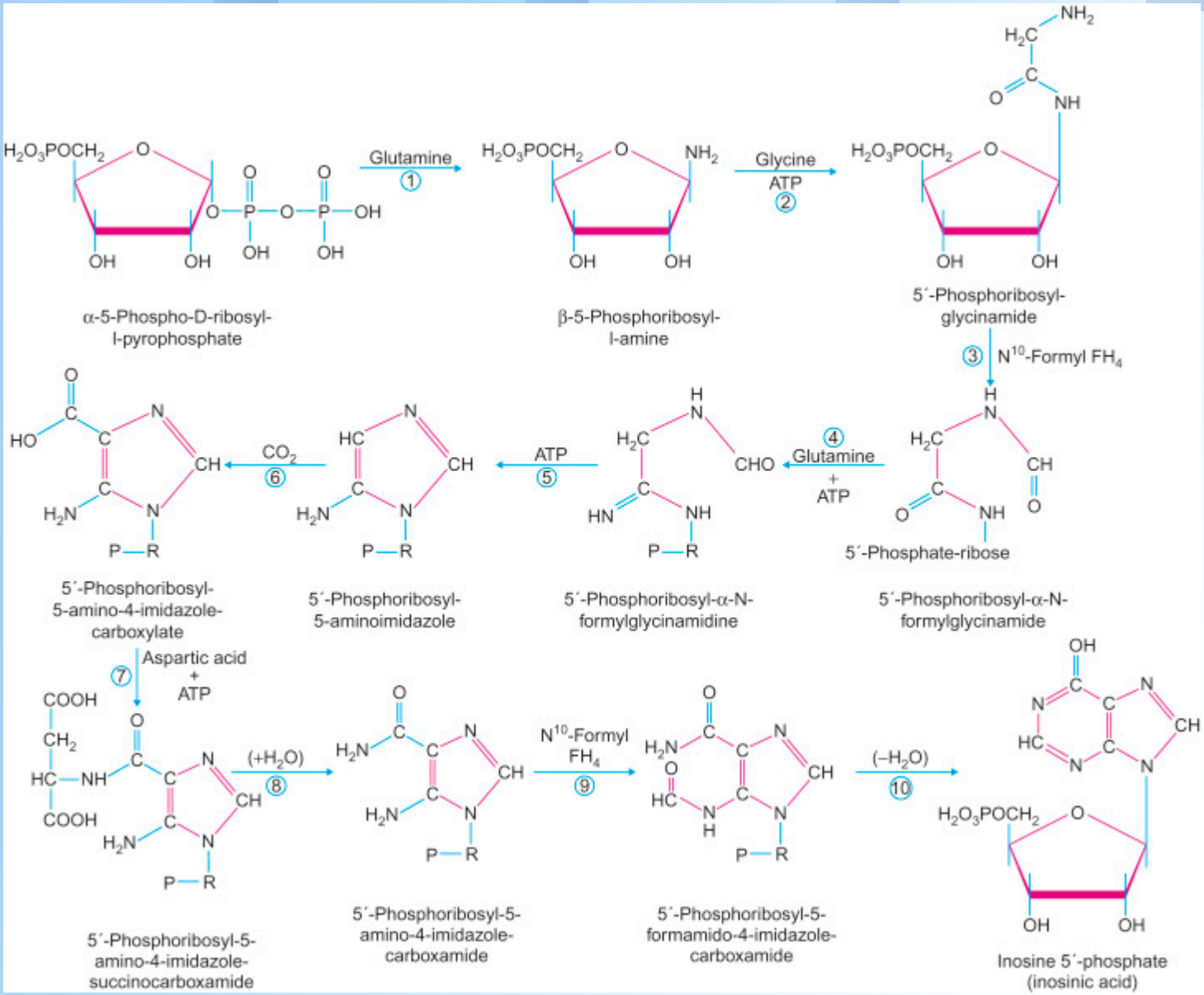

P
OH2C
O
H
H
OH
H
OH
O
P
P
PRPP
Glutamine
H2O
Glutamate
PPi
Glutamine phosphoribosyl
pyrophosphate amidotransferase
P
OH2C
NH2
O
H
H
H
OH
OH
5'-phosporibosylamine

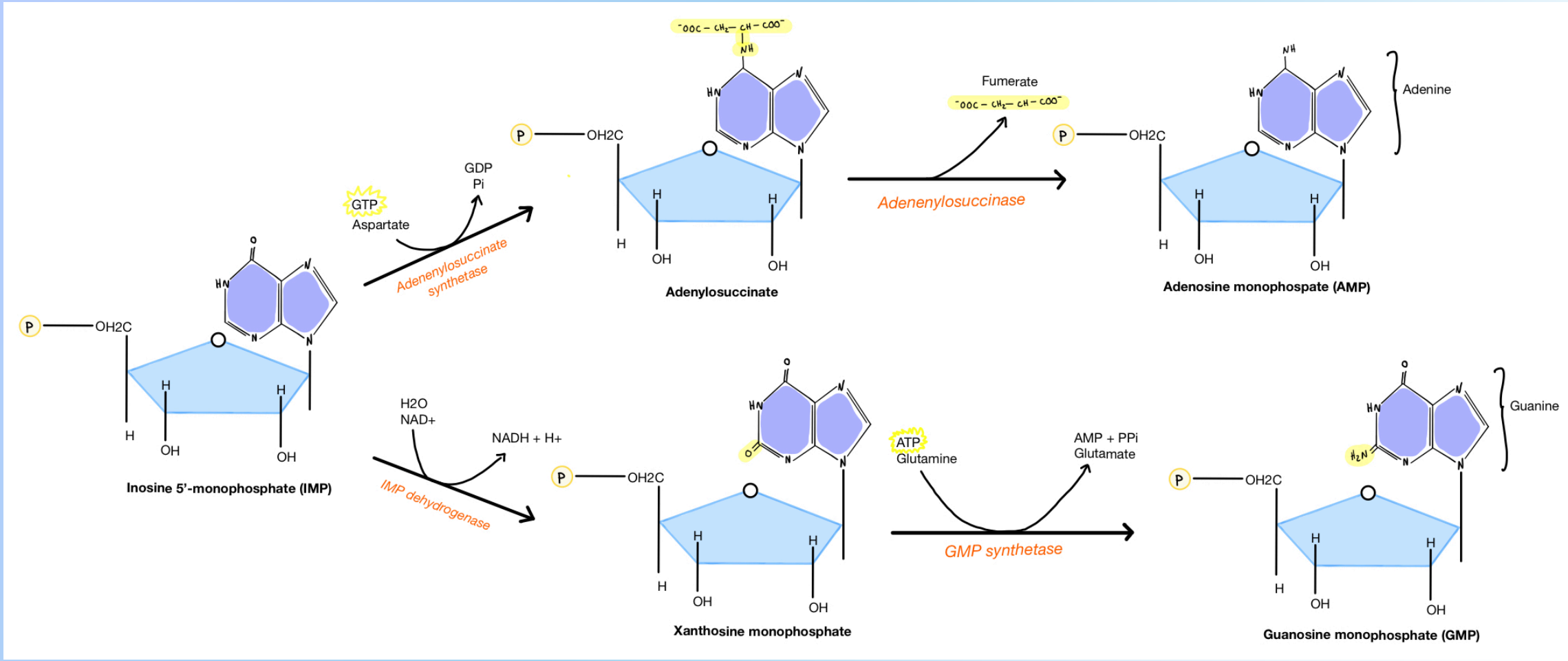
The next 10 steps



The 10 step process

1. Glutamine:phosphoribosyl pyrophosphate amindotransferase
2. GAR synthetase
3. Formyltransferase
4. Synthetase
5. Synthetase
6. Carboxylase
7. Synthetase
8. Adenylsuccinate lyase
9. Fromyltrransferase
10. Synthase




GTP
Aspartate
GDP
Pi
Adenylosuccinate synthetase
Adenylosuccinate
Fumerate
Adenenylosuccinase
Adenine
Adenosine monophospate (AMP)
Inosine 5'-monophosphate (IMP)
H2O
NAD+
NADH + H+
IMP dehydrogenase
Xanthosine monophosphate
ATP
Glutamine
AMP + PPi
Glutamate
GMP synthetase
Guanine
Guanosine monophosphate (GMP)

Regulation

What will be inhibited if we have…		What activates?	
↑ IMP	→ X	*Glutamine phosporibosyl:pyrophosphatate amidotransferase*	← + PRPP
↑ AMP	→ X	*Adenylosuccinate synthetase*	← + ↓ AMP
		Glutamine phosporibosyl:pyrophosphatate amidotransferase	← + PRPP
↑ GMP	→ X	*IMP dehydrogenase*	← + ↓ GMP
		Glutamine phosporibosyl:pyrophosphatate amidotransferase	← + PRPP

*negative feedback inhibition

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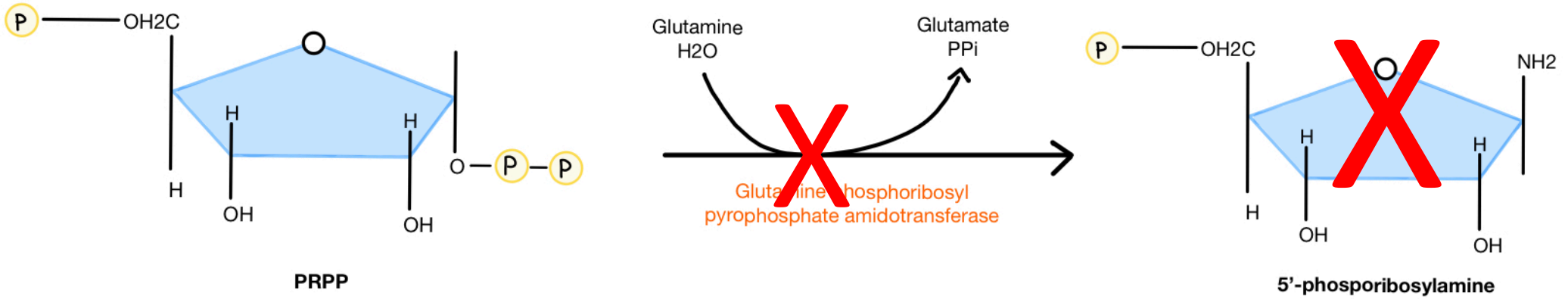
Clinical correlation

6-Mercaptopurine

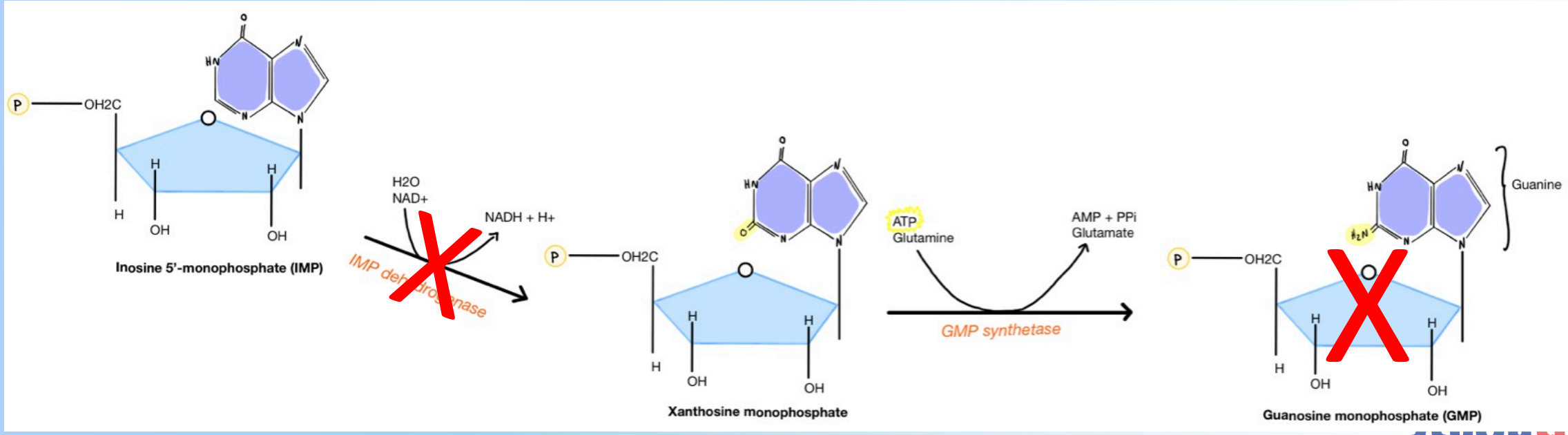
- Immunosuppressive drug
- Inhibits *PRPP amidotransferase*



Clinical correlation

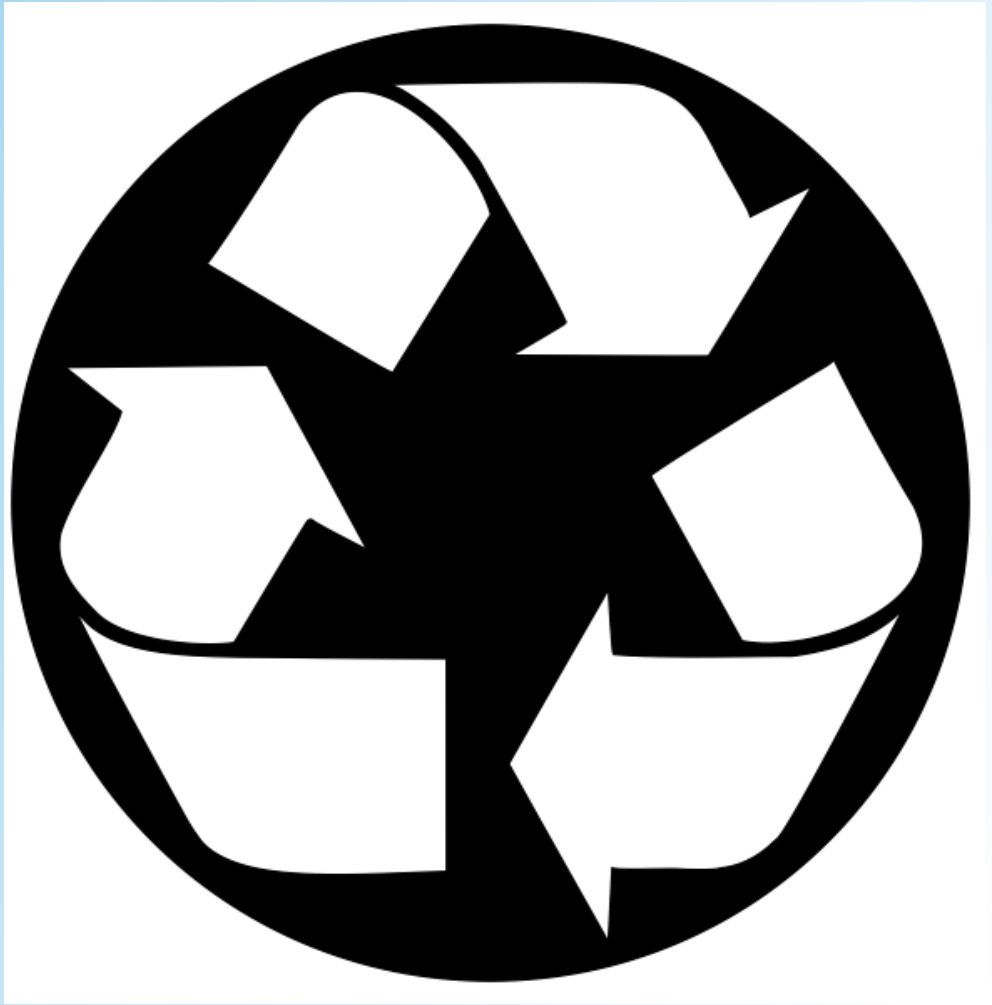
Mycophenolic acid

- Immunosuppressive drug
- Inhibits *IMP dehydogenase*
- Resulting in ↓ GMP production → ↓ production of T and B cells
- Clinical use: prevent graft rejection



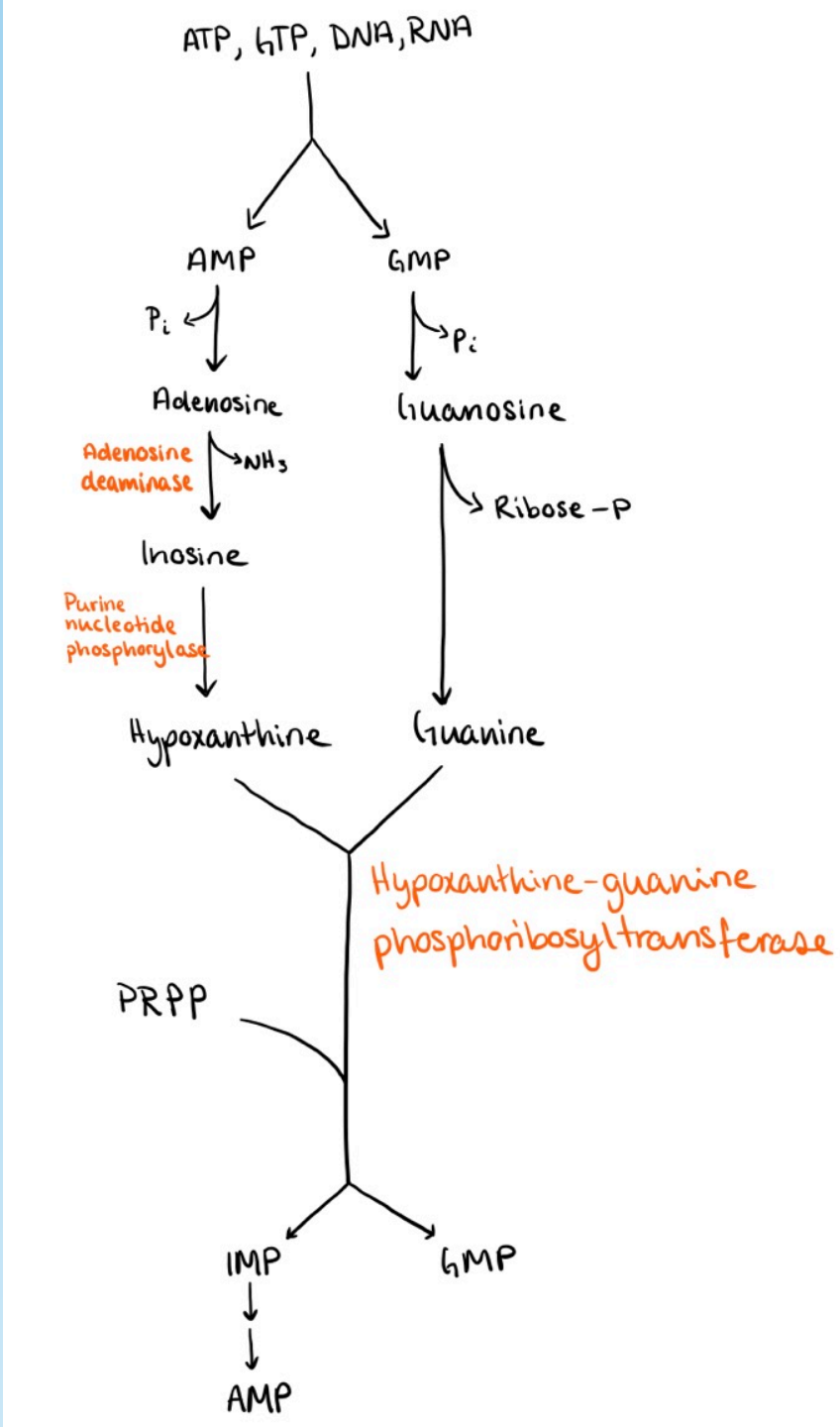
Purine salvage pathway

- Lets draw!



Purine salvage pathway

- High yield enzyme: *Hypoxanthine-guanine phosphoribosyltransferase (HGPRT)*



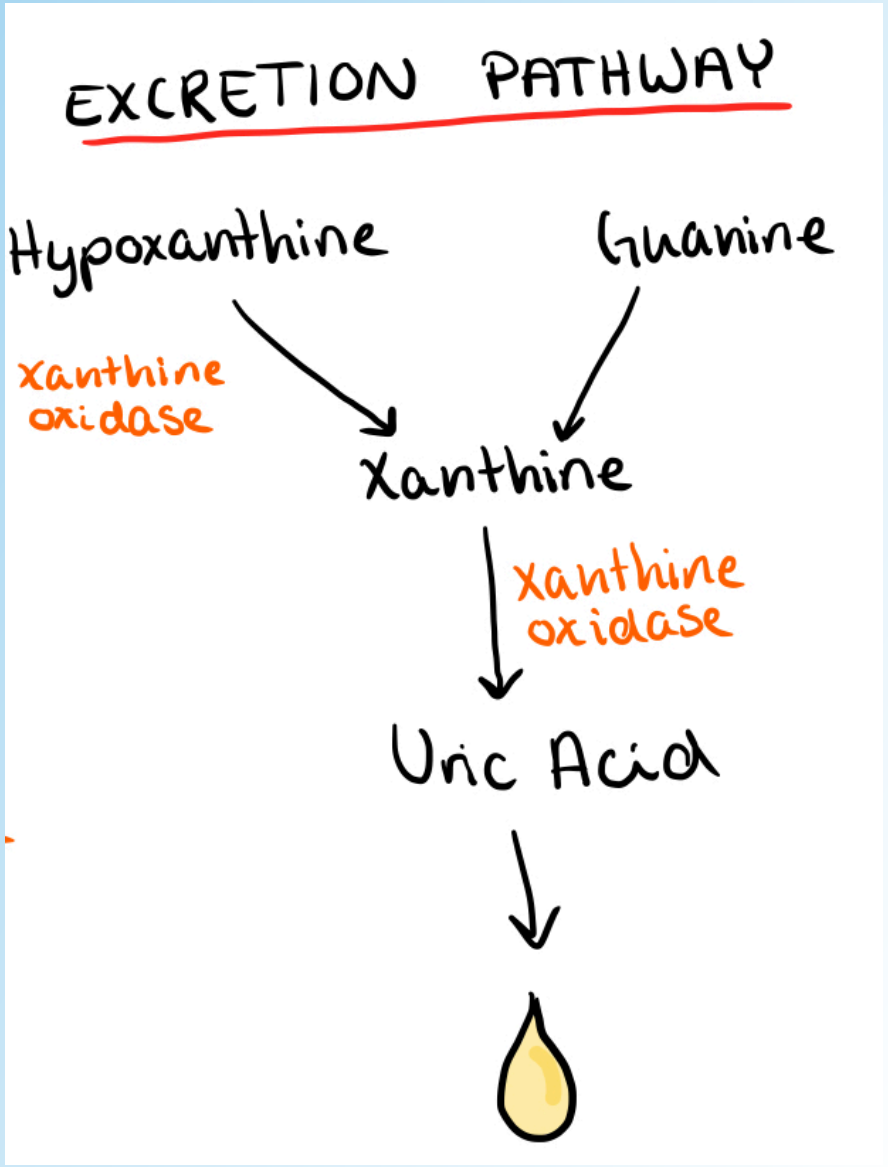
Excretion pathway

- Back to the board

Excretion pathway

Allopurinol

- Inhibits *Xanthine oxidase*
- Gout treatment
- Hypoxanthine analogue



Clinical correlation

Lesch-Nyhan syndrome

Salvage pathway

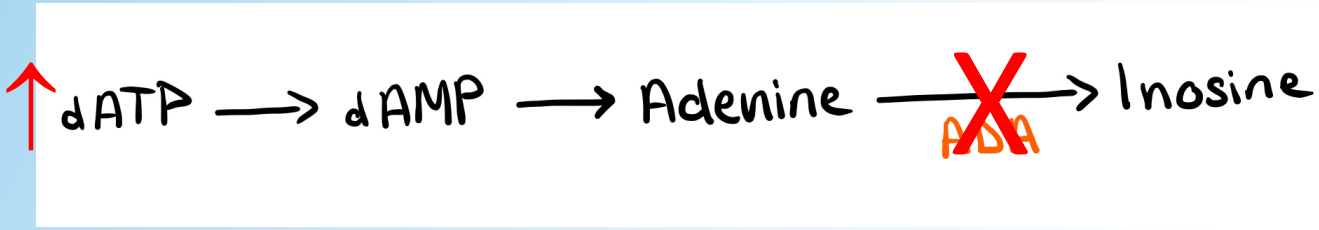
- *HGPRT* deficiency
- Excess uric acid production and de novo purine synthesis

Hyperuricemia
Gout
Pissed off (aggression, self mutilation)
Retardation
DysTonia

Adenosine deaminase deficiency

Degradation pathway

- *ADA* deficiency
- One of the major causes of autosomal recessive SCID
- Excess dATP, resulting in lymphotoxicity



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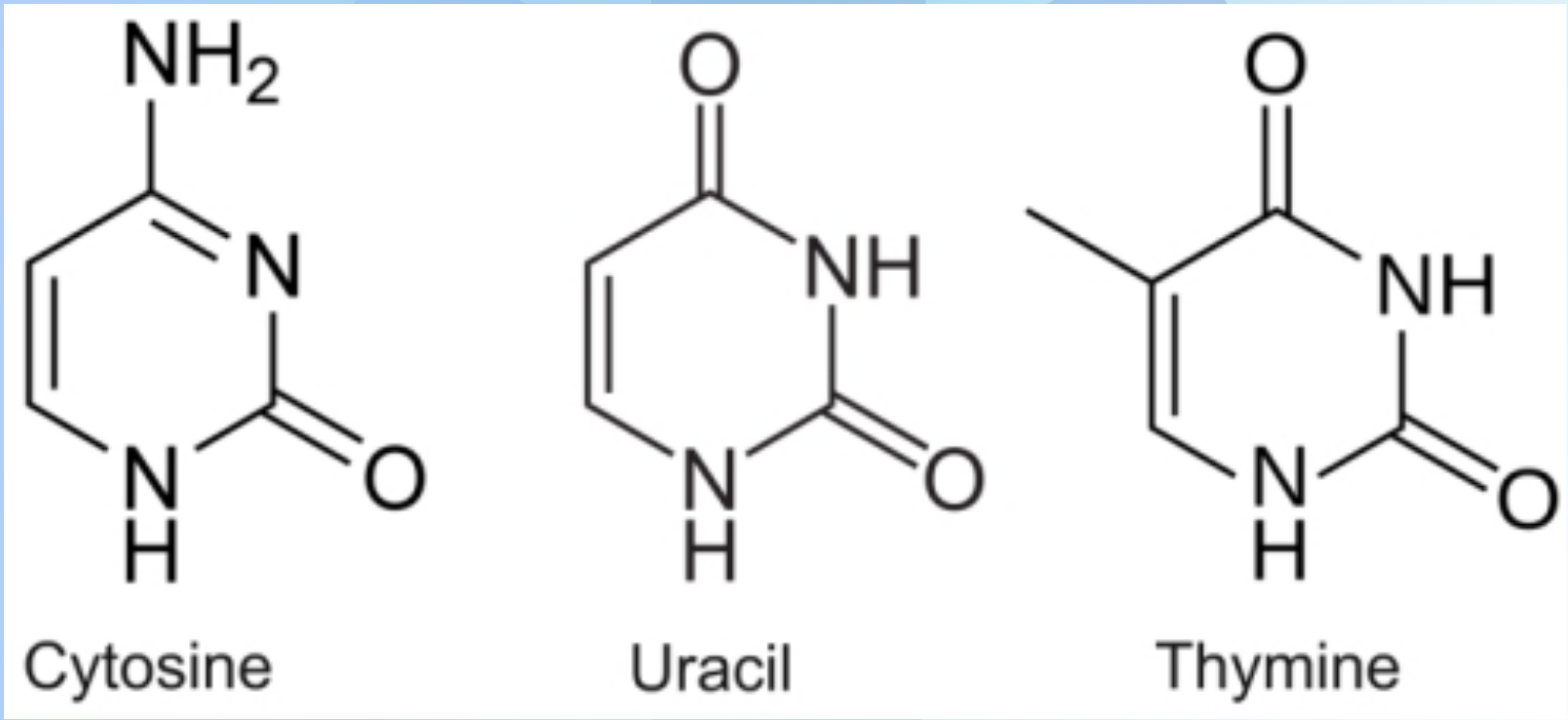
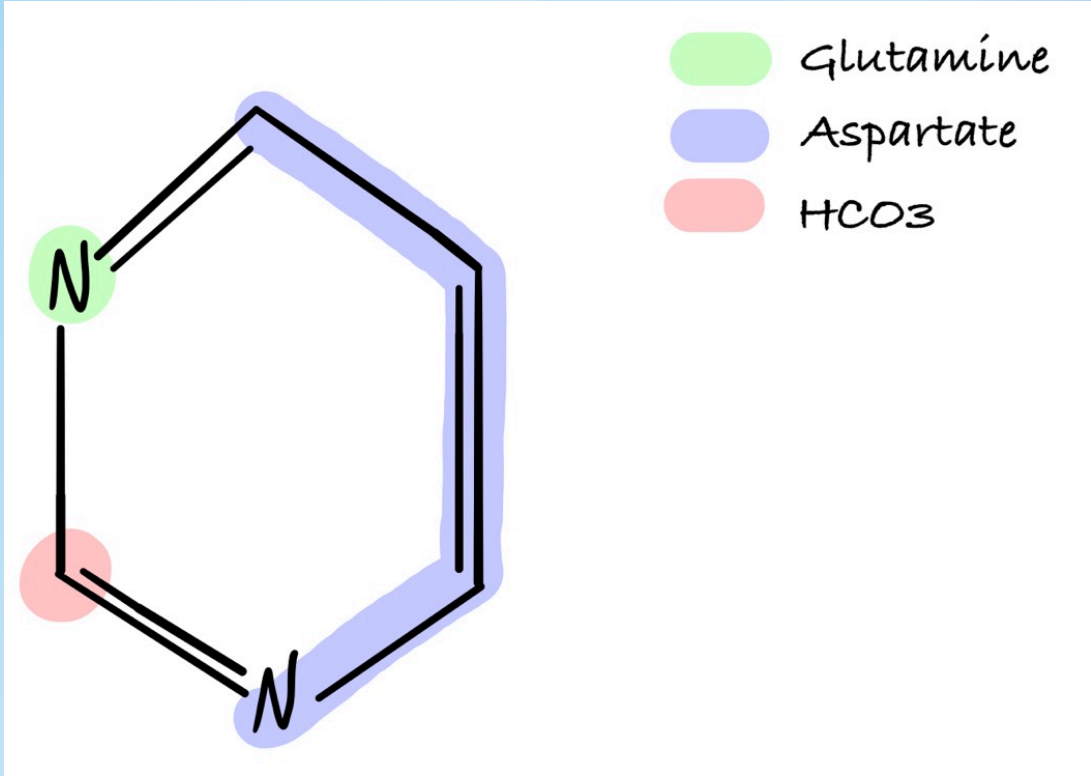
Lesch Nyhan Syndrome

Pyrimidine synthesis - ”De novo”



Let’s start from scratch

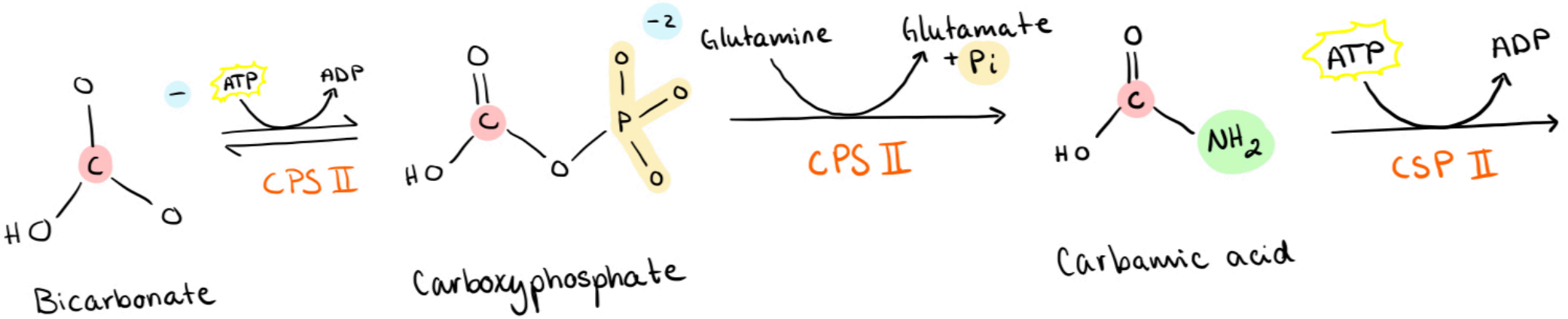
Ingredients
1 PRPP 1 Glutamine 1 Aspartate 1 HCO_3 (CO_2) 1 NAD^+ 4 ATP



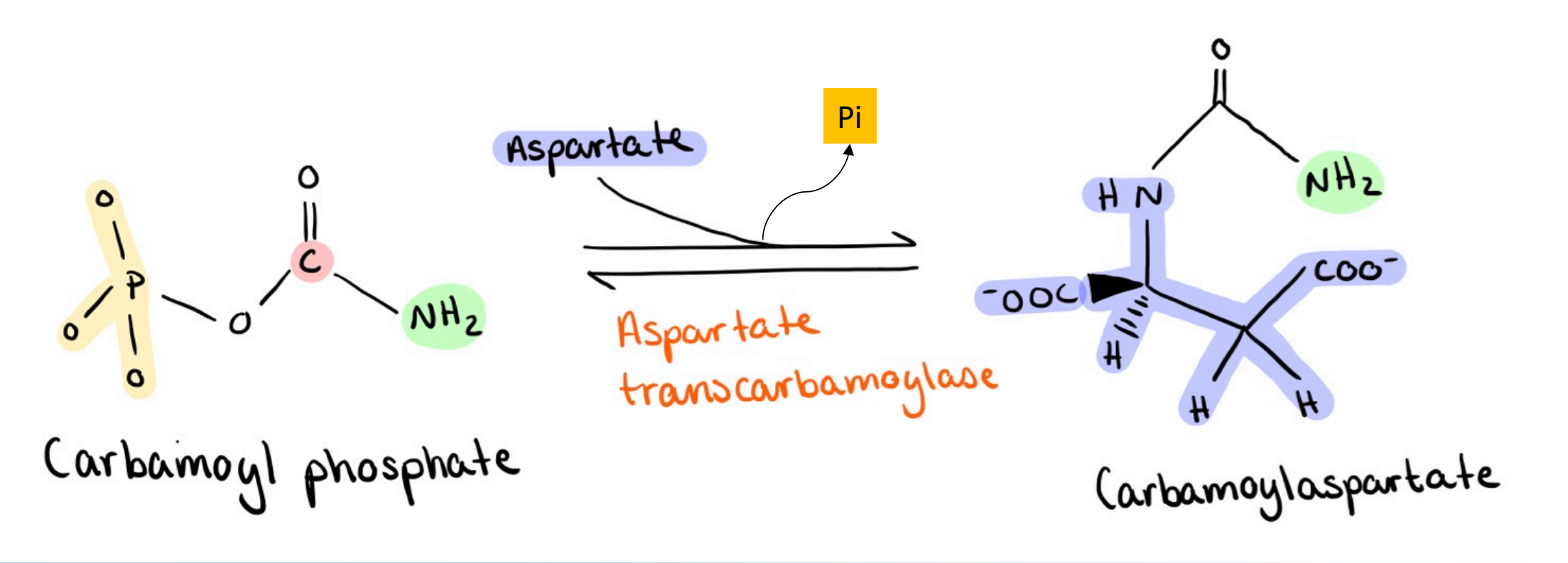
Step 1: Carbamoyl phosphate synthetase II

	CPS-I	CPS-II
Location	Mitochondria	Cytosol
Pathway	Urea cycle	Pyrimidine ”de novo” synthesis
Regulation	+ N-acetylglutamate	+ PRPP X UTP

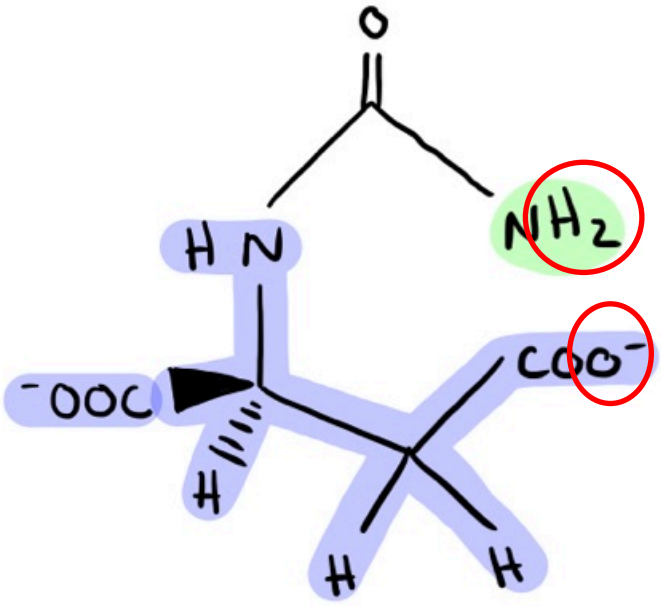
Step 1: 3 step reactions of CPS II



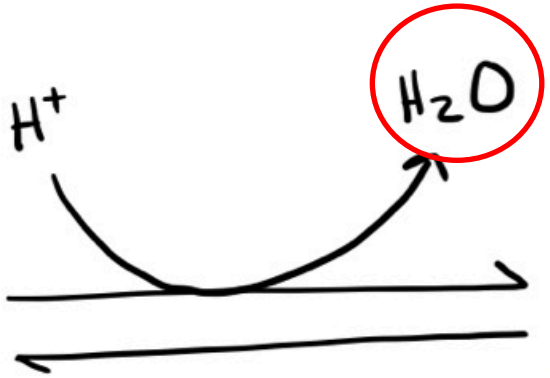
Step 2: Aspartate transcarbamoylase



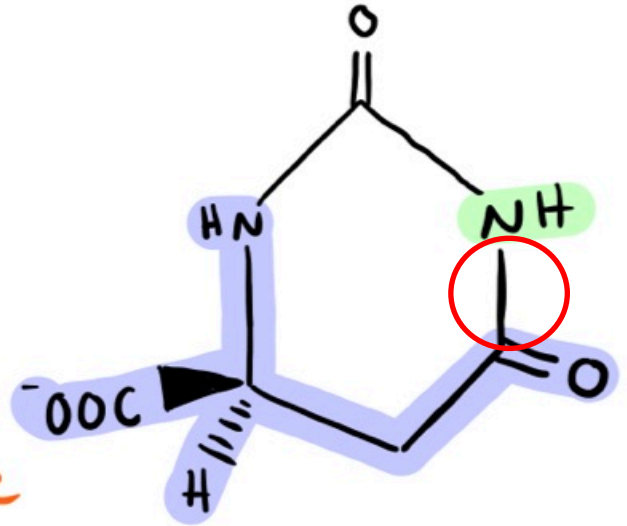
Step 3: Dehydratation



Carbamoylaspartate



Dihydroorotase



Dihydroorotate

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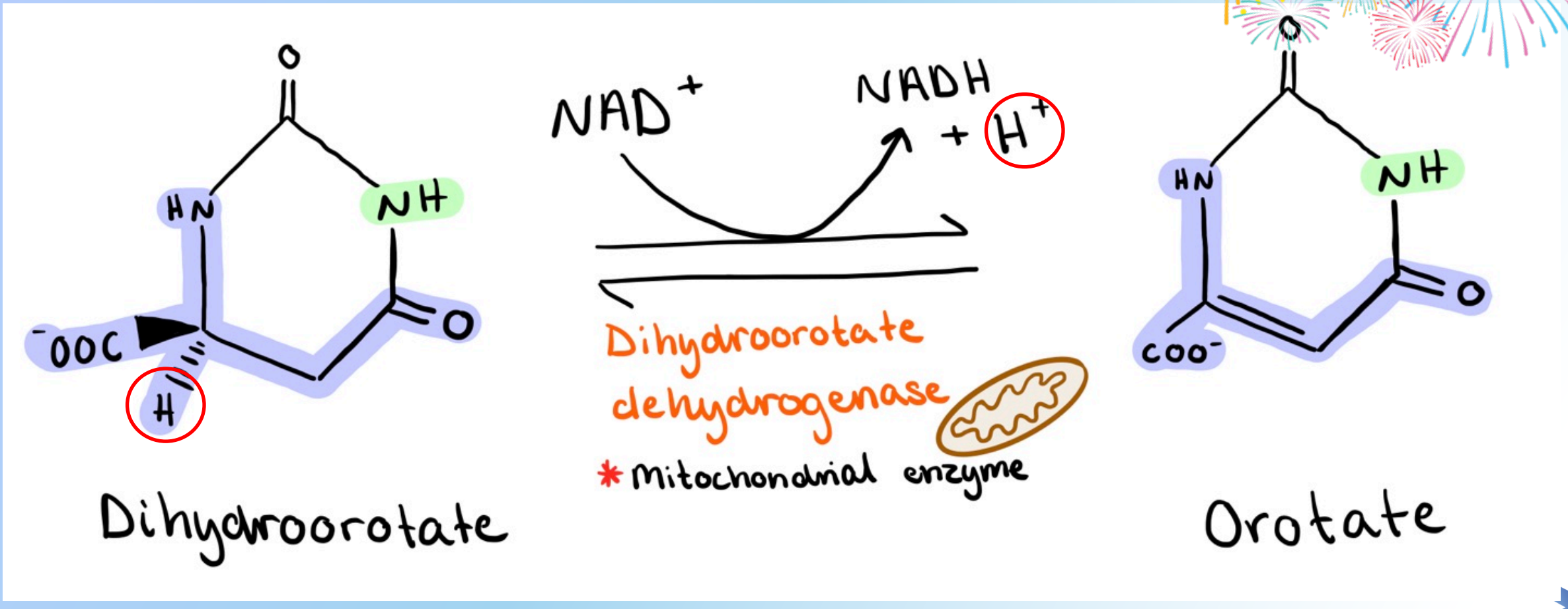
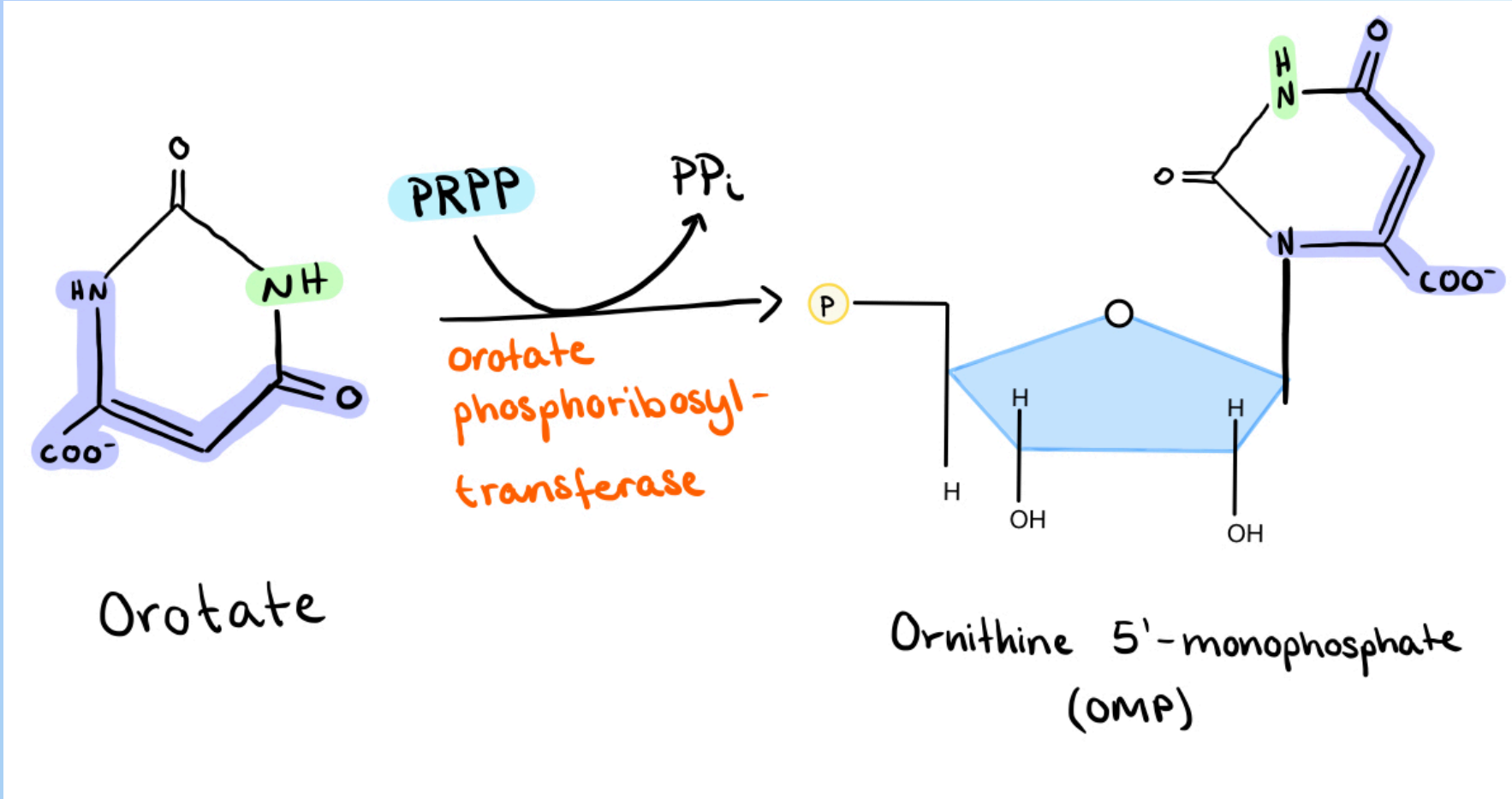
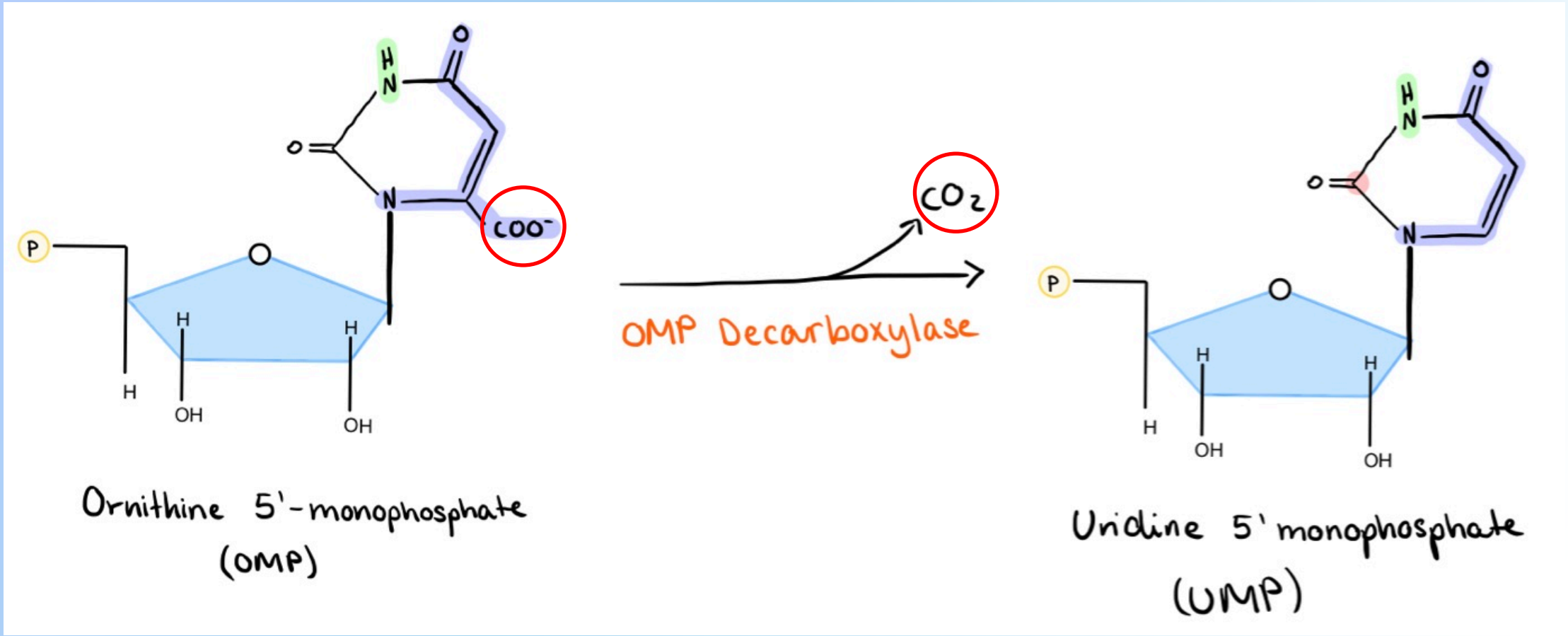
Step 4: Oxidation-reduction reaction



Step 5: adding PRPP



Final and 6th step: formation of UMP



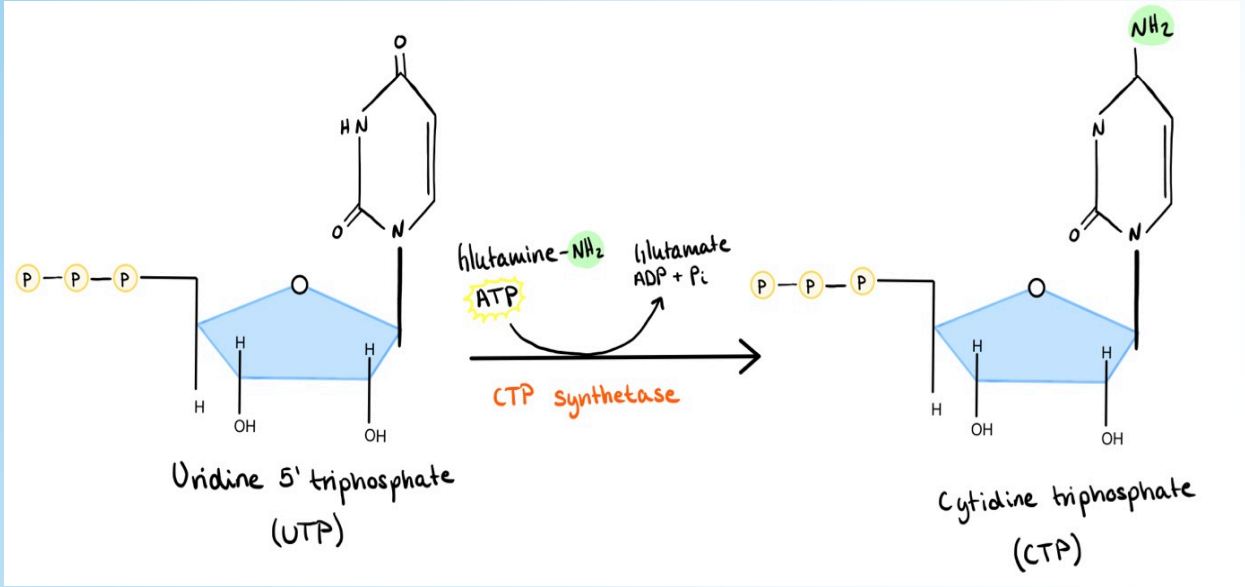
Nucleotide triphosphate formation

NMP → NTP

- Phosphorylation of NMP to NDP then TTP
- *Kinase activity*
- Usage: 2 ATP

- The same goes for both purines and pyrimidines

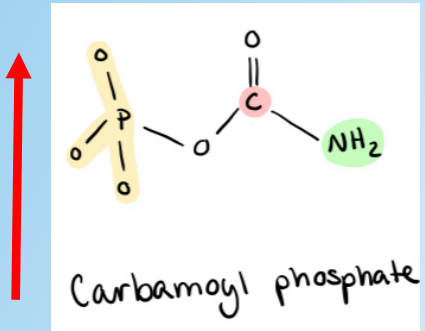
UTP → CTP

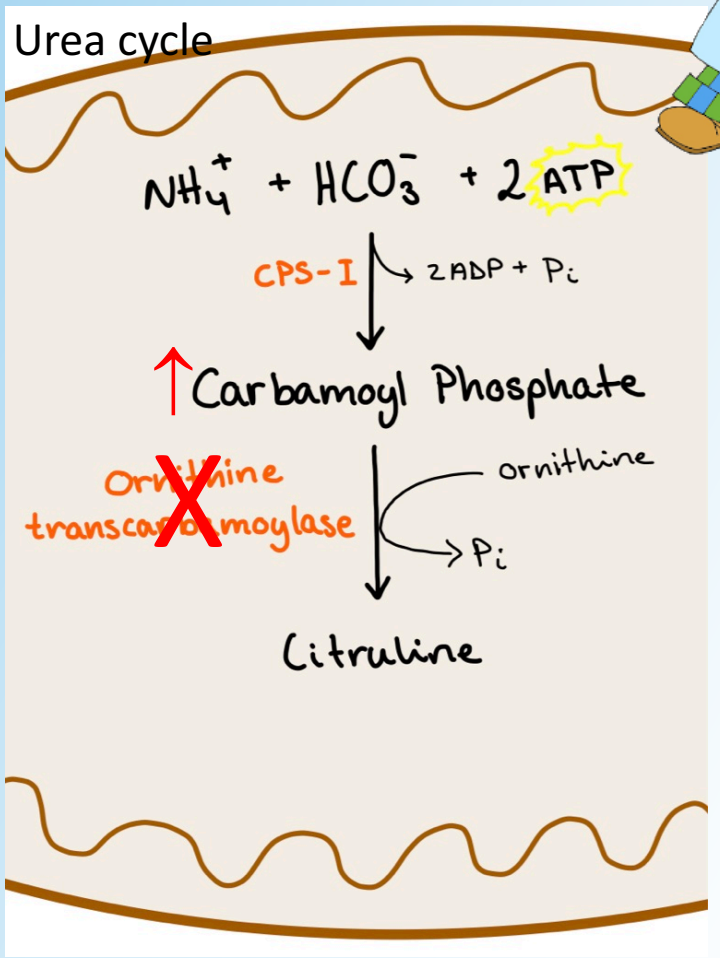


Clinical correlation

Ornithine transcarbamoylase deficiency

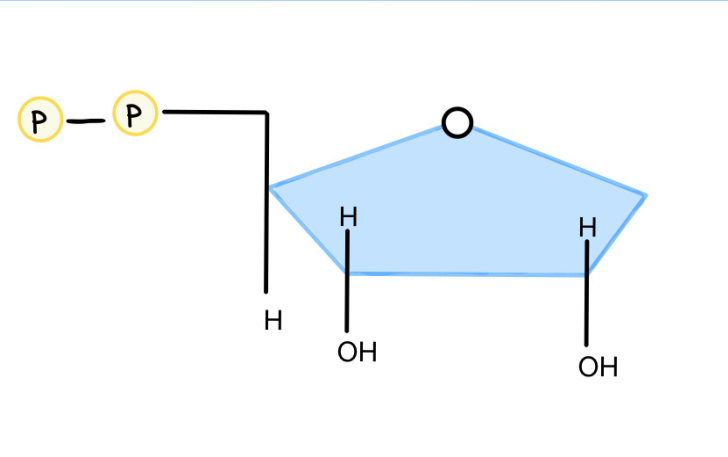
- ↑ Carbamoyl phosphate availability
- Carbamoyl phosphate leaks out into the cytoplasm
- ↑ pyrimidine synthesis
- Result: Orotic aciduria



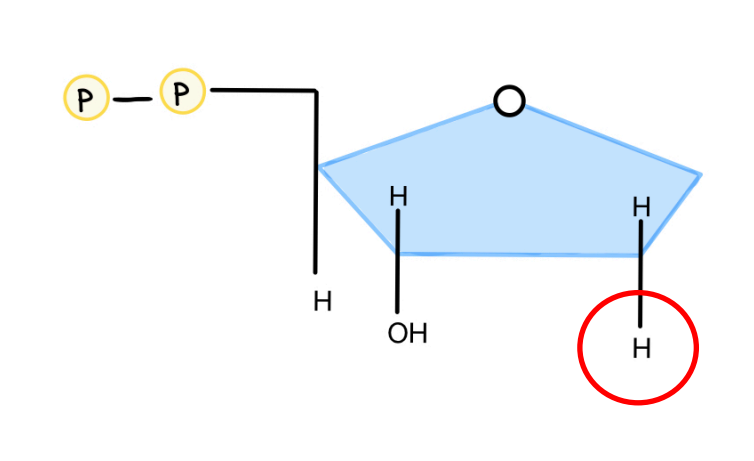


What is the difference between a ribonucleotide to deoxyribonucleotide?

Ribonucleotide

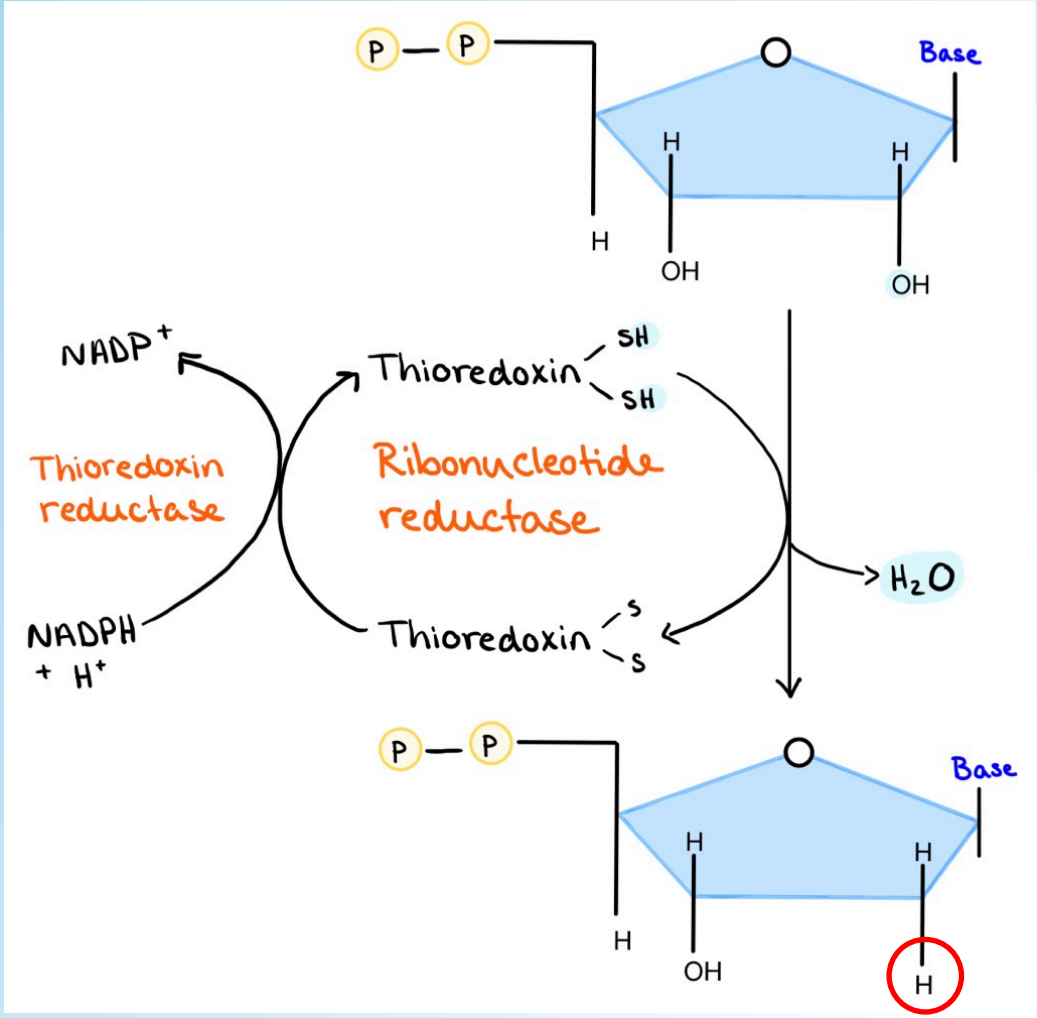


Deoxyribonucleotide

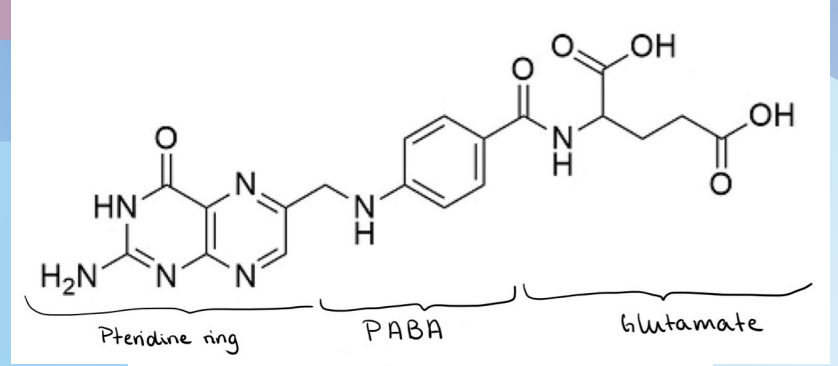


Conversion from ribonucleotide to deoxyribonucleotide

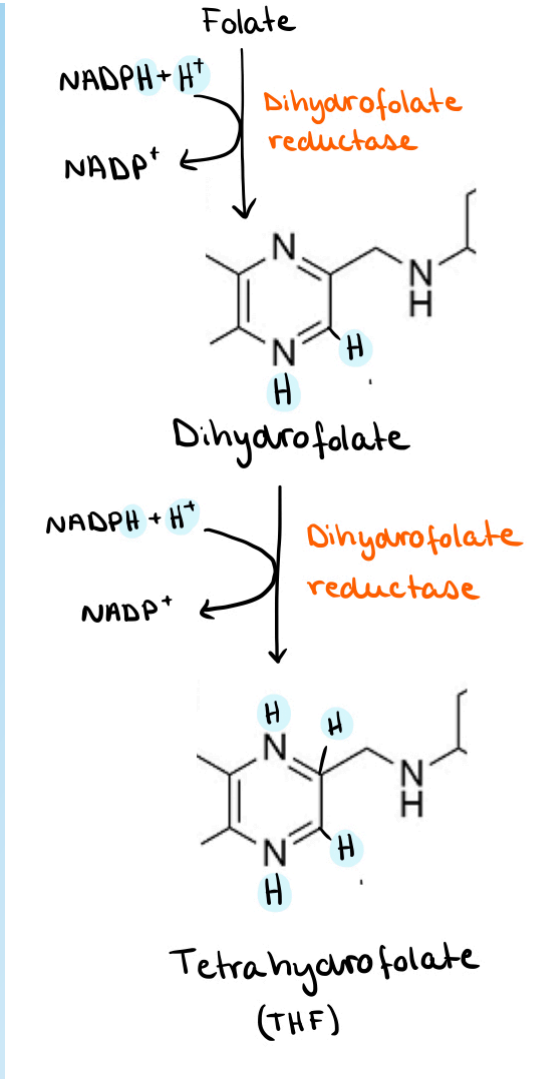
- Essential enzyme:
  Ribonucleotide reductase
- Regulation:
  - \+ ATP
  - x dATP
  - x *Hydroxyurea* – anticancer drug



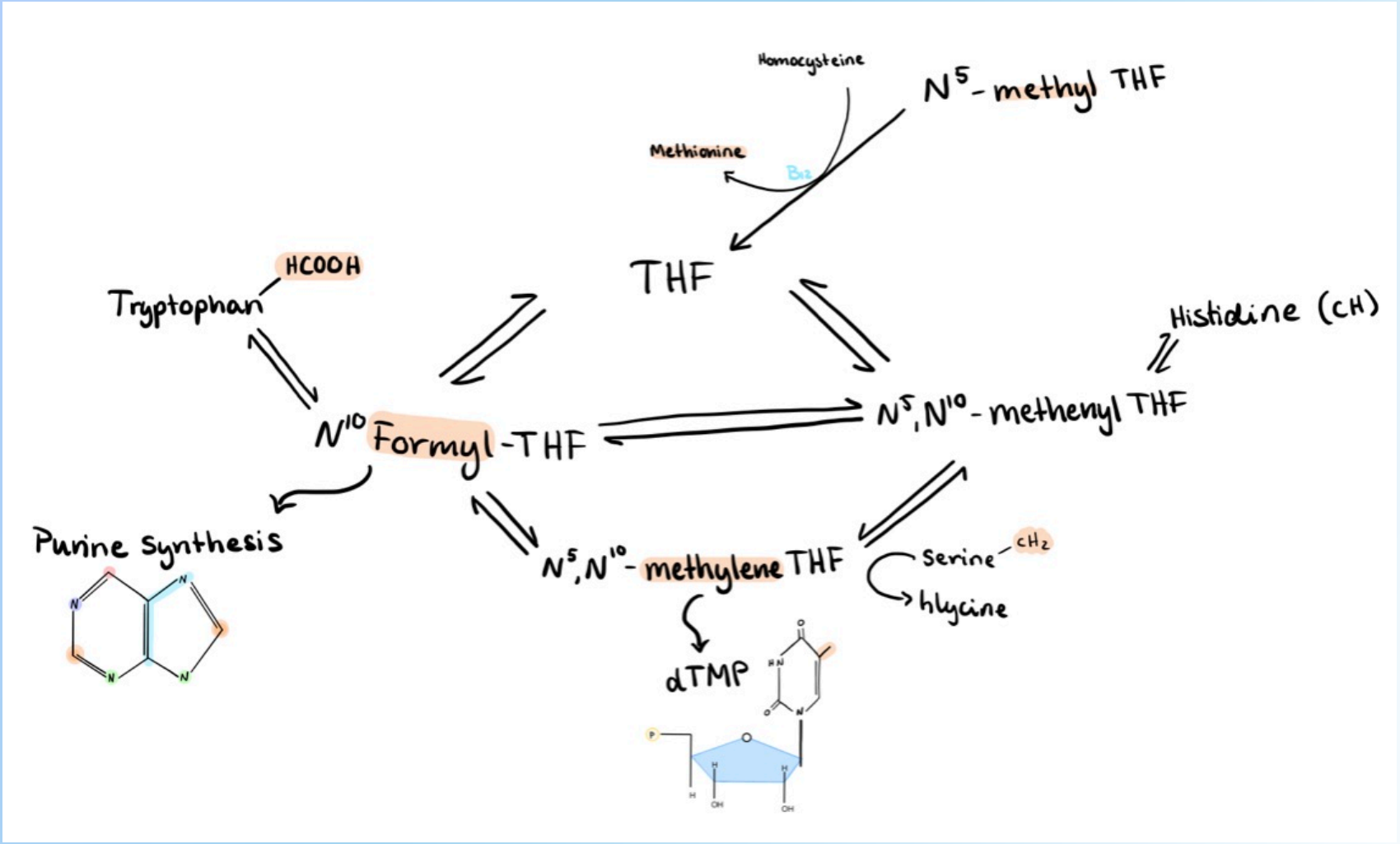
Folic acid (vitamin B_9)



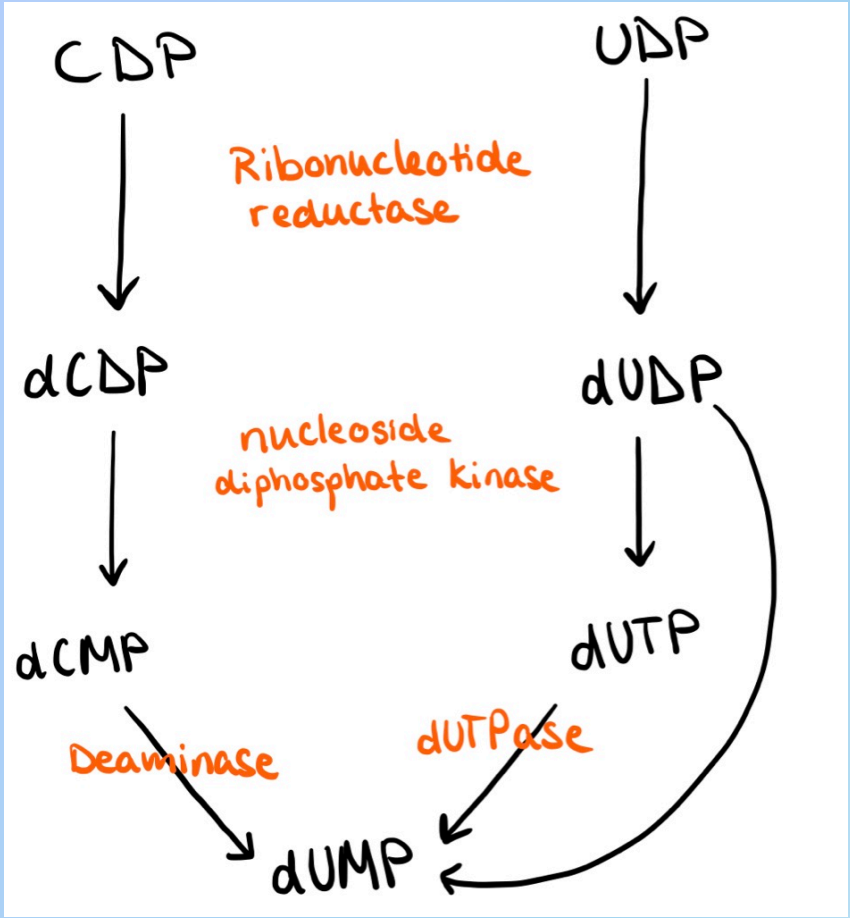
- THF is the active form of folic acid
- Requires 2 NADPH
- Essential enzyme: *Dihydrofolate reductase*
- A carrier of one-carbon units

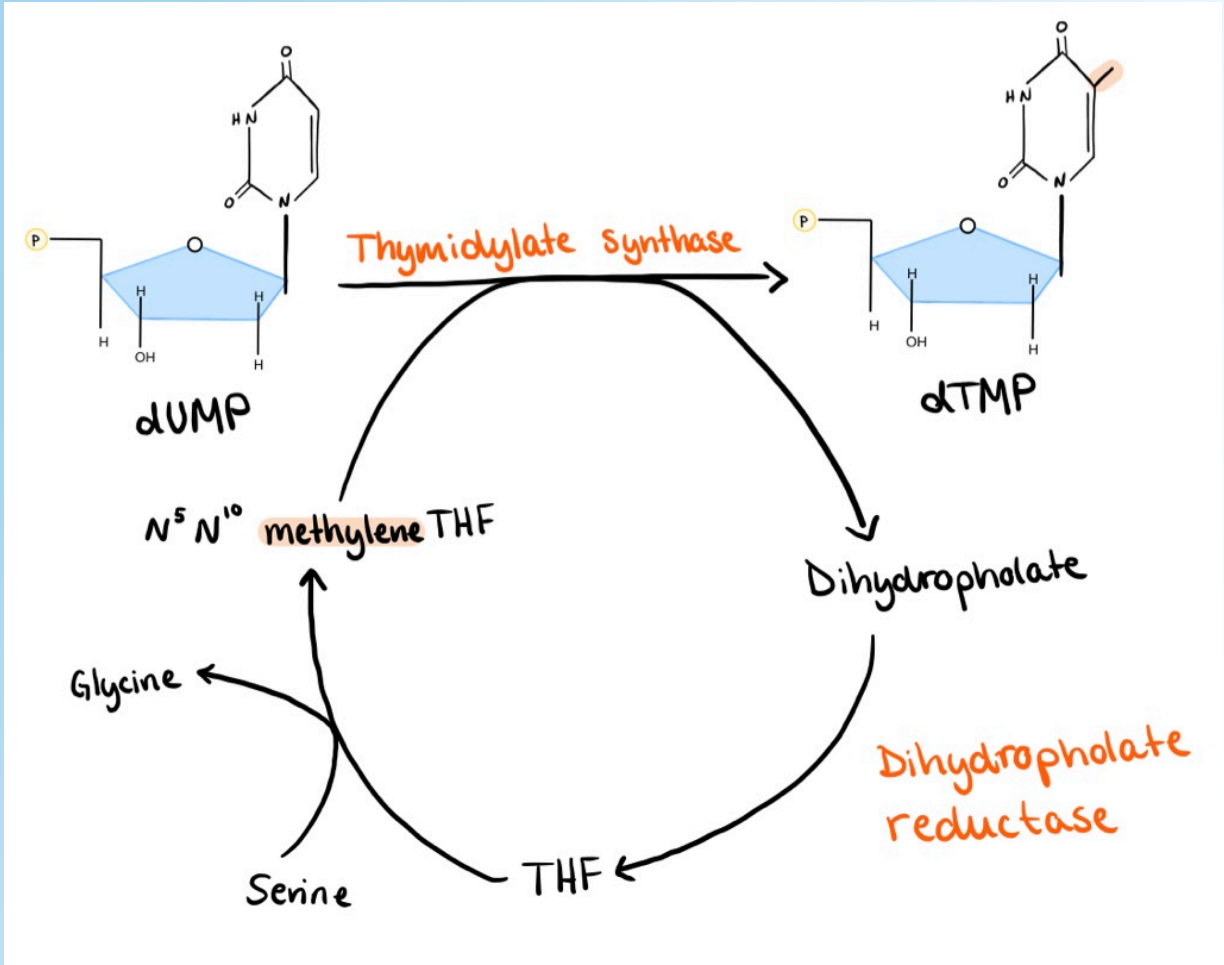


The 1-carbon donor system



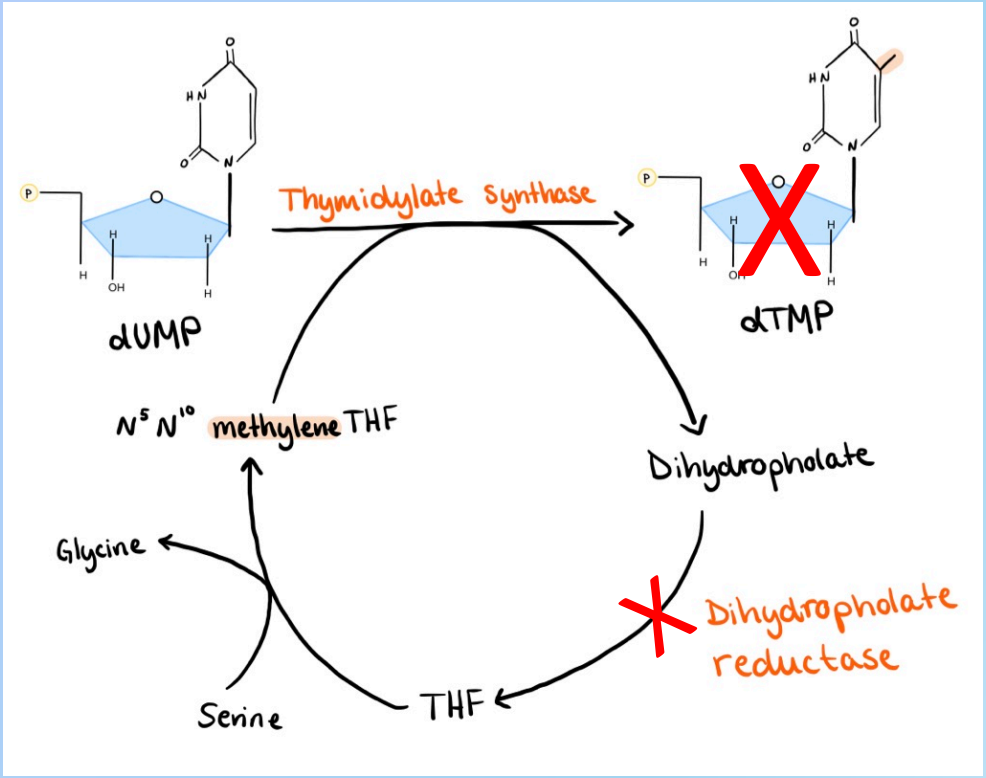
dUMP → dTMP


CDP
UDP
Ribonucleotide reductase
dCDP
dUDP
nucleoside diphosphate kinase
dCMP
dUTP
Deaminase
dUTPase
dUMP

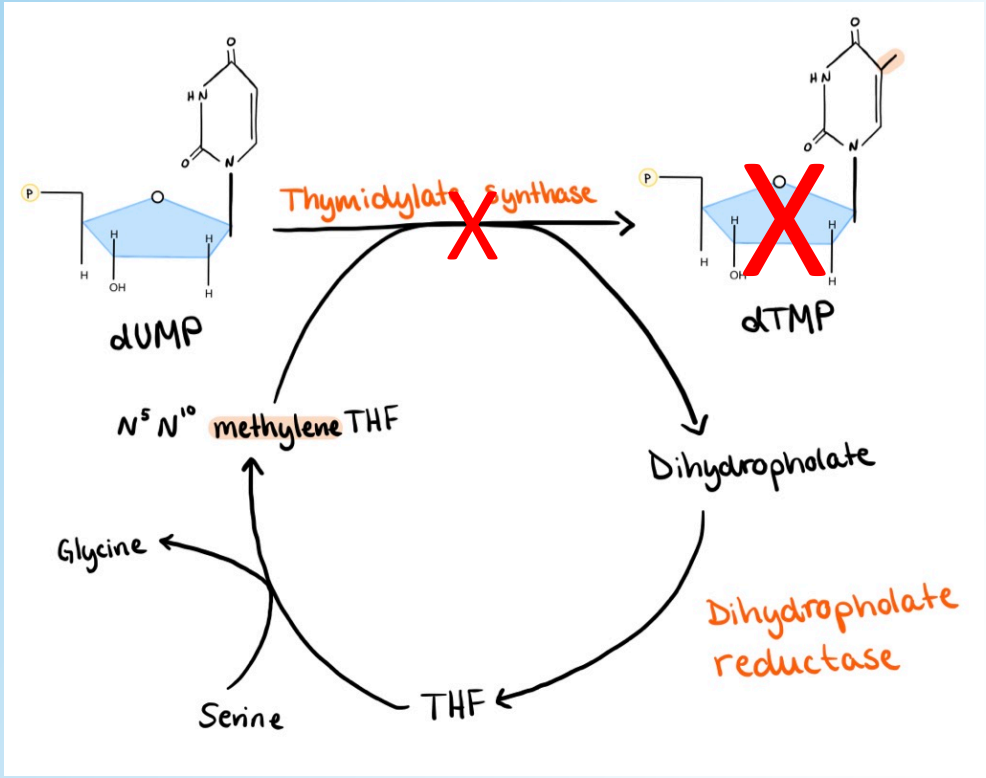

Thymidylate synthase
dUMP
dTMP
N5 N10 methylene THF
Dihydrophalate
Glycine
Dihydrophalate reductase
Serine
THF

Clinical correlation: Anticancer drugs

Methotrexate



5-flurouracil



Good luck ☺