

# Nucleotide Metabolism: The Creation

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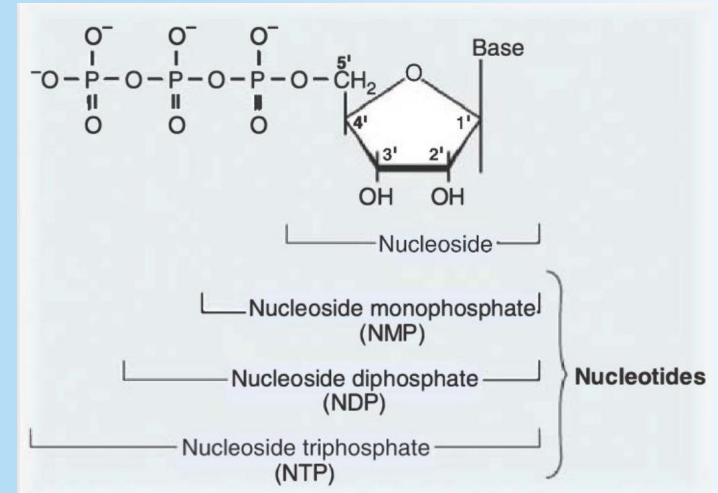
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# What's a nucleotide?

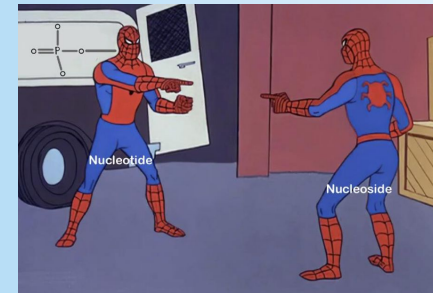
## A molecule composed of...

- **Nitrogenous base:** purine or pyrimidine
  - Purines: Adenine, Guanosine
  - Pyrimidines: Cytosine, Uracil, Thymine
  - “Name-tag” of a nucleotide
- **Pentose sugar:** ribose or deoxyribose
  - Deoxyribose sugar: for DNA nucleotides
  - Ribose sugar: for RNA nucleotides
- **Phosphate group(s)**

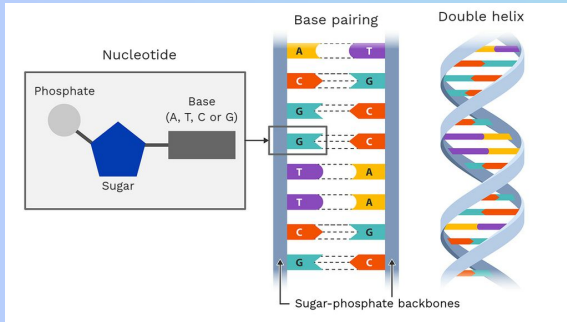


## Difference between nucleotide and nucleoside:

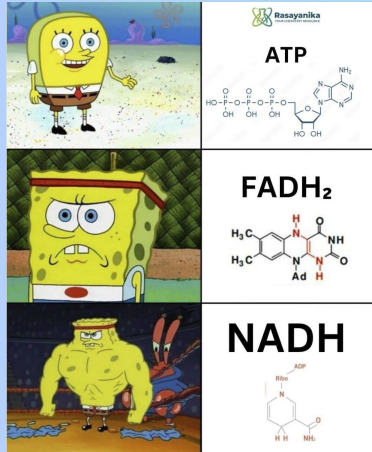
- *Nucleotide*: contains phosphate groups
- *Nucleoside*: does not contain phosphate groups



# Why are nucleotides important?



- **Building blocks for DNA & RNA**
  - DNA: dAMP, dGMP, dCMP, dTMP
  - RNA: AMP, GMP, CMP, UMP
- **Sources of energy:**
  - ATP, GTP
- **Enzyme regulators: ATP, ADP, AMP**
- **Cellular signaling: cyclic AMP**
- **Components of coenzymes: NAD<sup>+</sup>, FAD**



# How do we make nucleotides?

It's kind of integrated with making those nitrogen bases (the “name-tags” of nucleotides) via...

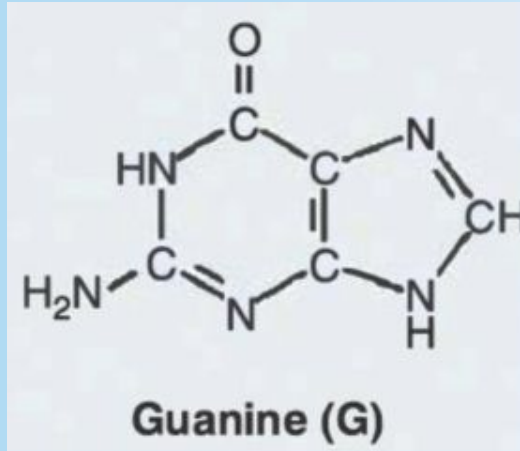
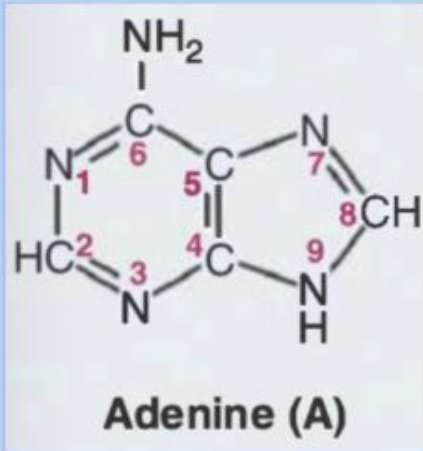
- Purine synthesis
- Pyrimidine synthesis



# Purine synthesis (De novo & salvage)

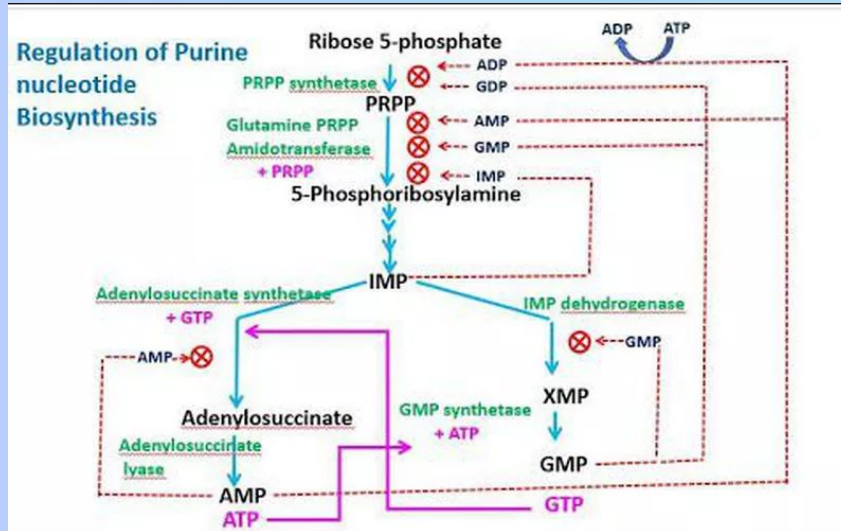
# What are our purines?

“Pure As Gold”

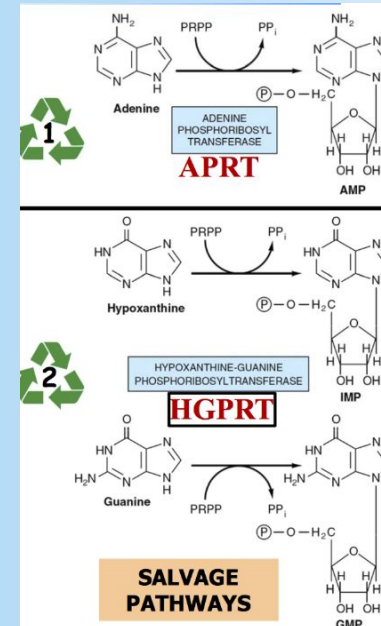


# How do we make purines-based nucleotides?

## 1. De novo pathway

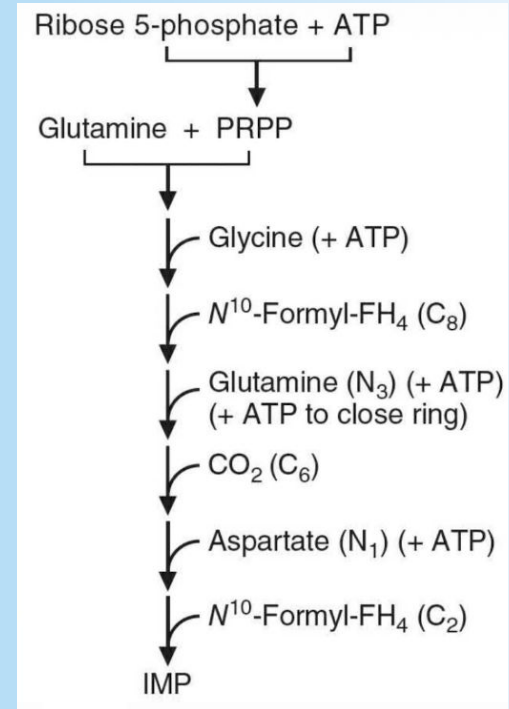


## 2. Salvage pathway

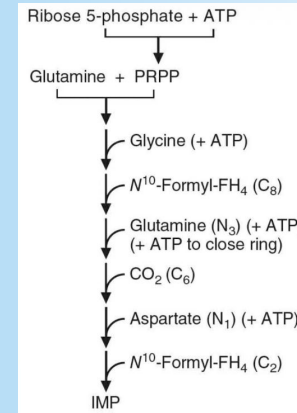
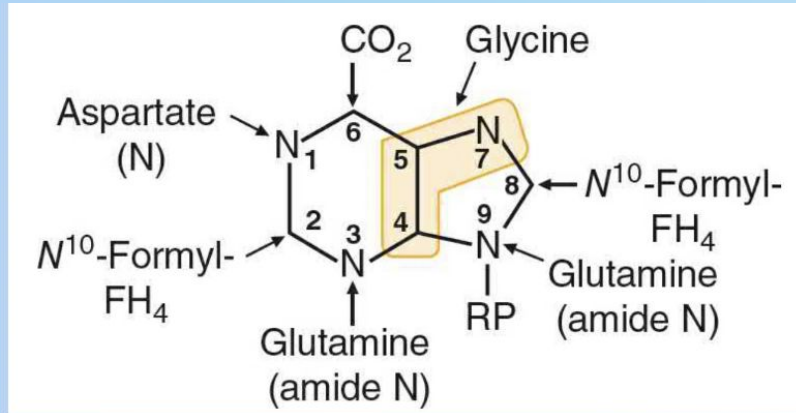


# De Novo Purine Synthesis

- Making purines from scratch
- Occurs in almost all human cells
- Main gist:
  - Start with a ribose sugar, then build up using some amino acids and other molecules
  - Goal: to make IMP (a purine-based nucleotide) which can then turn into AMP or GMP (other purine-based nucleotides)
- Requires ATP to run



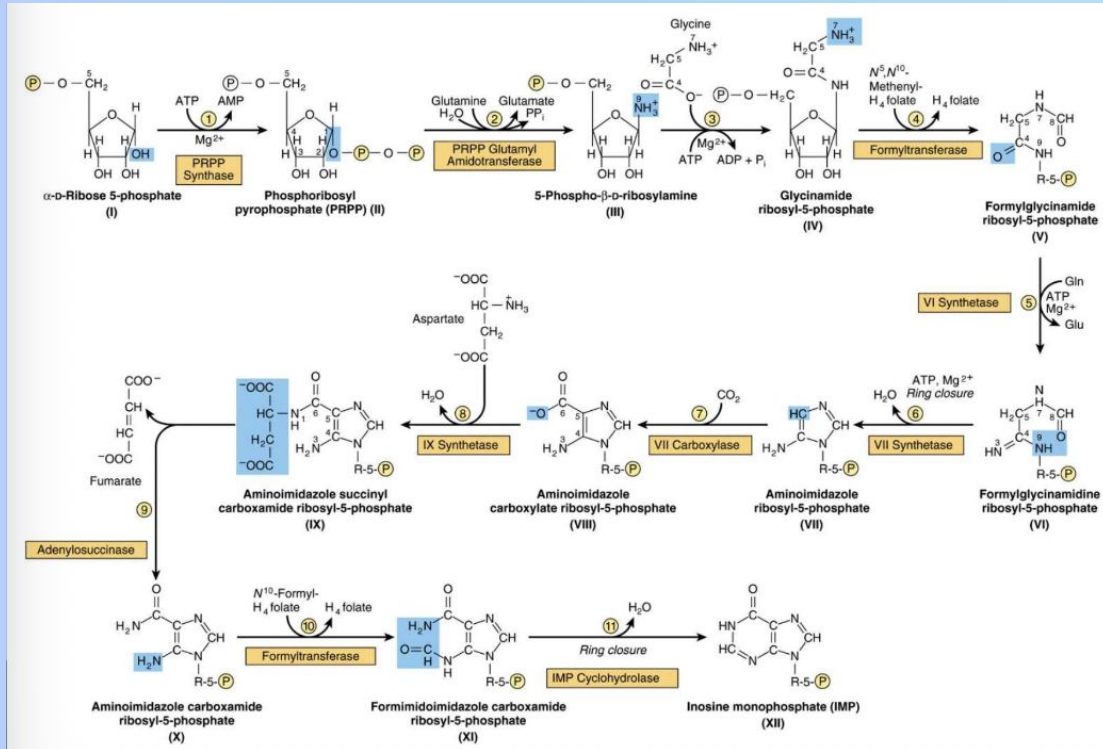
# What atoms are needed to build a basic purine?



- Glycine
- Aspartate
- Glutamine
- $CO_2$
- Tetrahydrofolate (Donates one-carbon units in the form of  $N^{10}$ -Formyl- $FH_4$ )

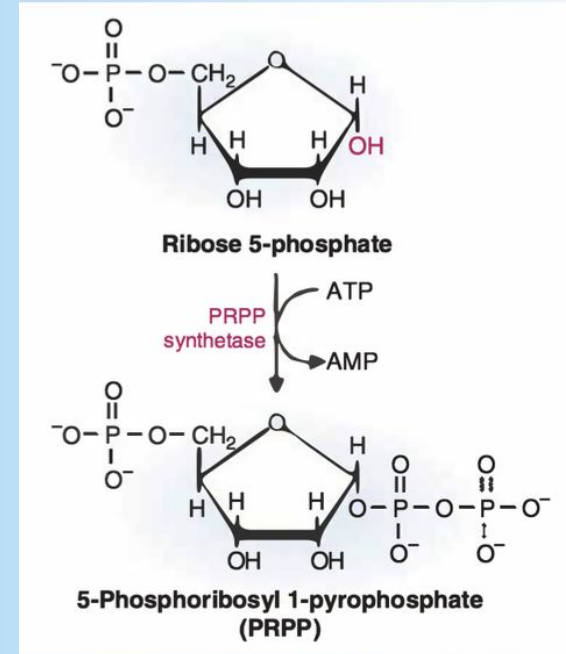
“Glycine And Glutamine Contribute To PURINES”

# The Steps of De Novo Purine Synthesis



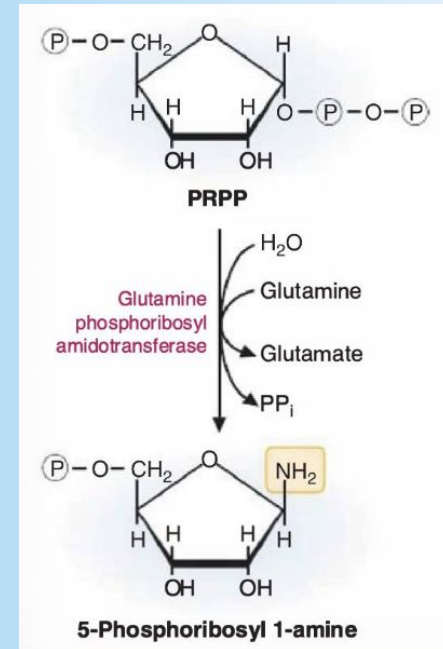
# Step 1: Creating PRPP

- Ribose-5-phosphate → 5-phosphoribosyl-1-pyrophosphate (PRPP)
  - Ribose-5-P: Produced from glucose via pentose phosphate pathway
  - PRPP: high energy form of ribose that's used to initiate purine synthesis
- Enzyme: PRPP synthetase
  - Uses ATP

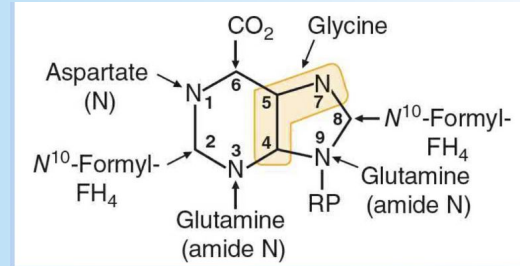
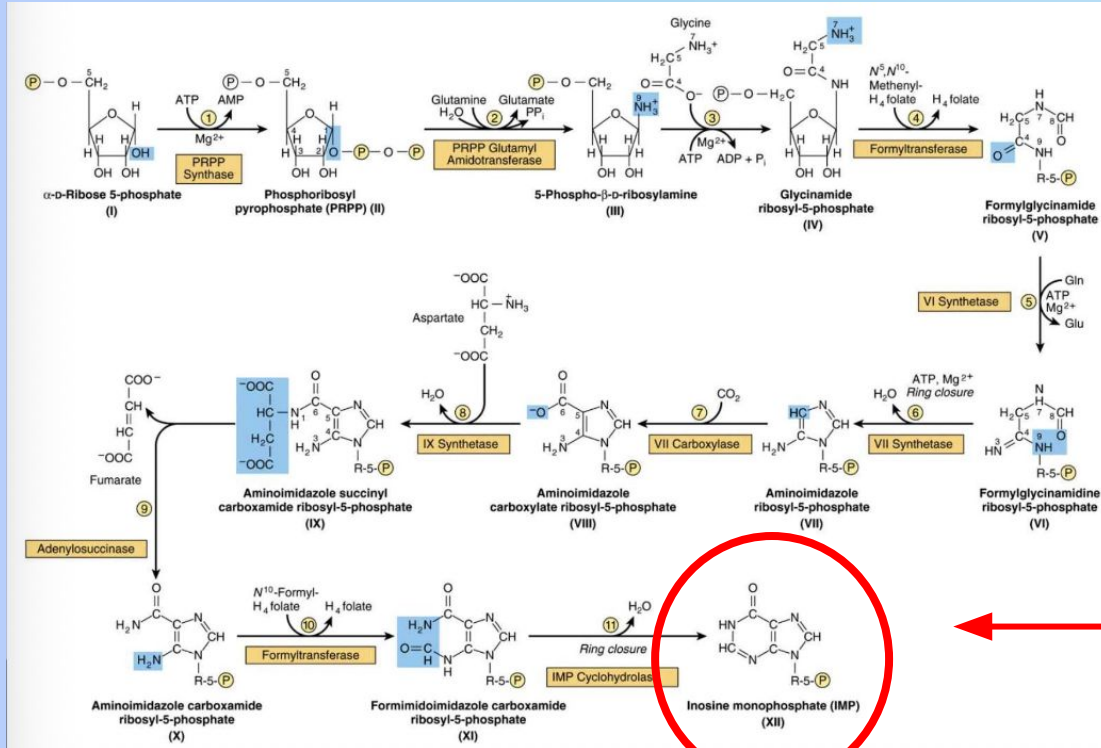


# Step 2: Rate Limiting Step via GPAT

- PRPP → 5-phosphoribosyl 1-amine
- Enzyme: Glutamine phosphoribosyl amidotransferase (GPAT)
  - Uses glutamine & H<sub>2</sub>O
  - Highly regulated enzyme, making this the rate-limiting (committed) step of purine synthesis

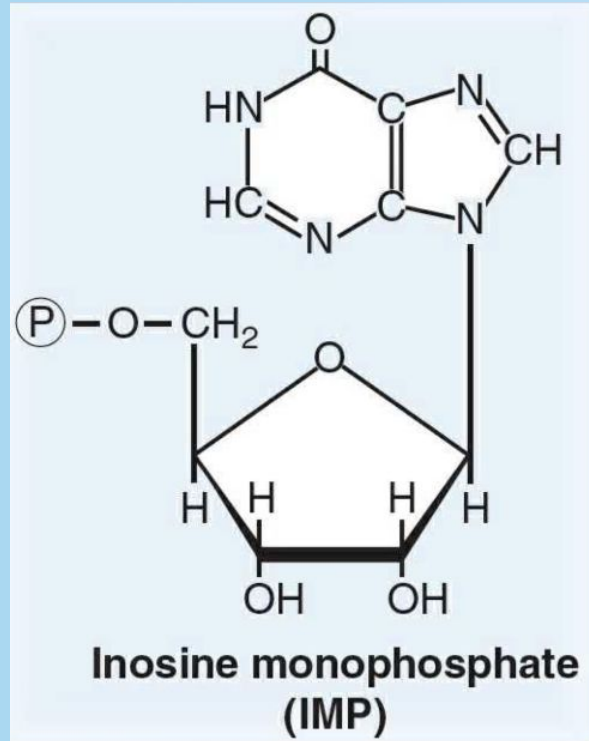


# 9 more steps to get to IMP!

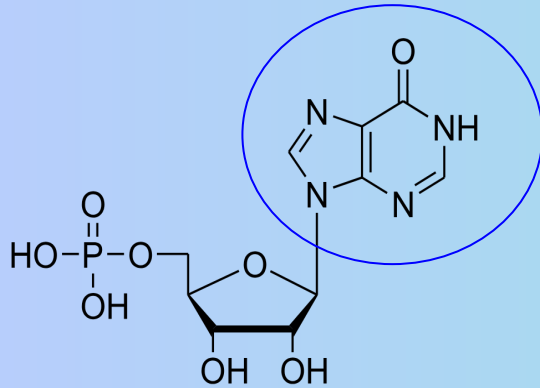


But you just have to know that we eventually get to our lovely nucleotide, IMP, by adding those 5 key atoms along the way 🥳

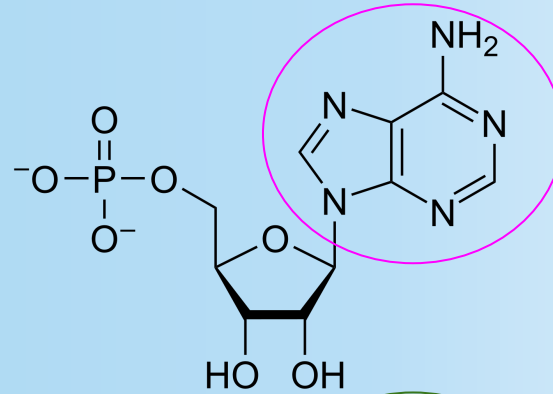
# IMP: our intermediate purine-based nucleotide



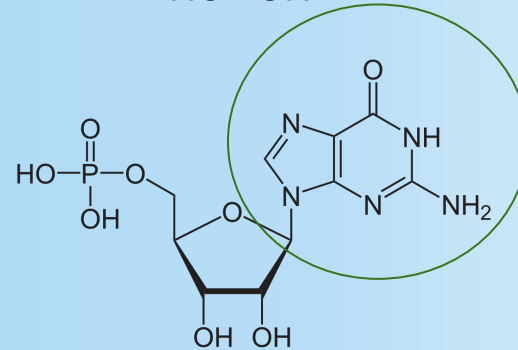
# What can IMP turn into?



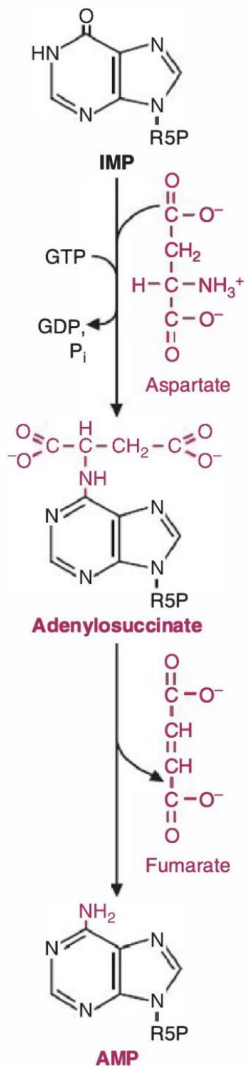
**Inosine**  
Monophosphate (IMP)



**Adenosine**  
Monophosphate (AMP)



**Guanosine**  
Monophosphate (GMP)



# IMP to AMP

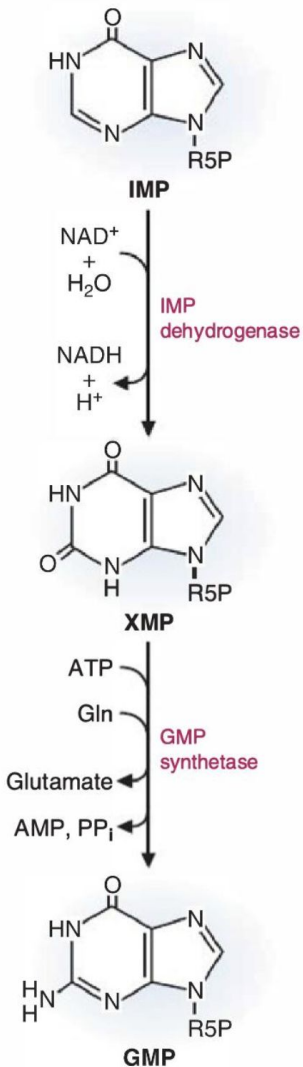
## 1. Formation of adenylosuccinate:

- a. Enzyme: Adenylosuccinate synthetase
  - i. Requires GTP for energy
  - ii. Aspartate is added to IMP
  - iii. Regulation:
    1. Activated by GTP (energy)
    2. Inhibited by AMP (the product)

## 2. Formation of adenosine monophosphate:

- a. Enzyme: Adenylosuccinate lyase
  - i. Fumarate is removed
  - ii.  $NH_2$  stays on IMP

# IMP to GMP



## 1. Formation of Xanthosine monophosphate (XMP):

a. Enzyme: IMP dehydrogenase

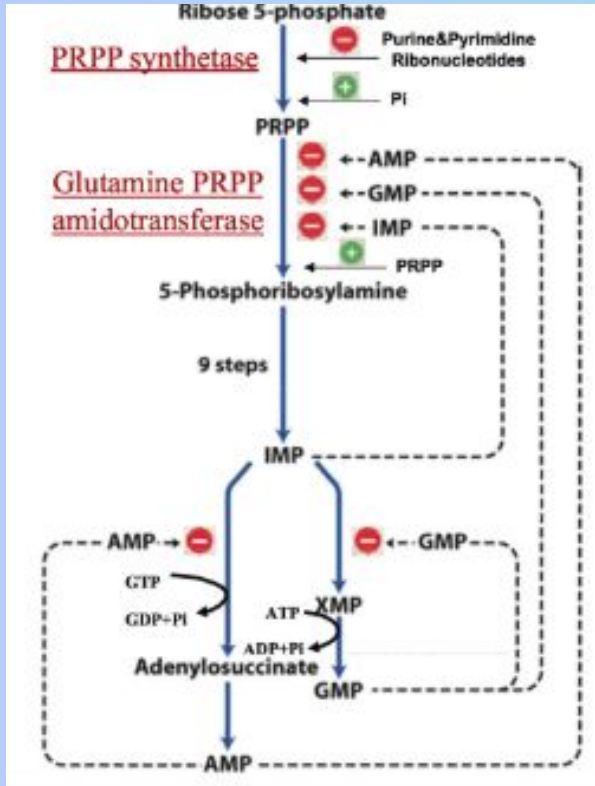
## 2. Formation of guanosine monophosphate (GMP):

a. Enzyme: GMP synthetase

i. Requires ATP for energy

ii. Glutamine donates amide nitrogen to XMP

# De Novo purine synthesis regulation



- **Activation:** via respective precursor or sources of energy
  - GPAT: PRPP
  - Adenylosuccinate synthetase: GTP
  - IMP dehydrogenase: ATP
- **Inhibition:** via respective “product”
  - PRPP synthetase: ADP, GDP
  - GPAT: IMP, AMP, GMP
  - Adenylosuccinate synthetase: AMP
  - IMP dehydrogenase: GMP

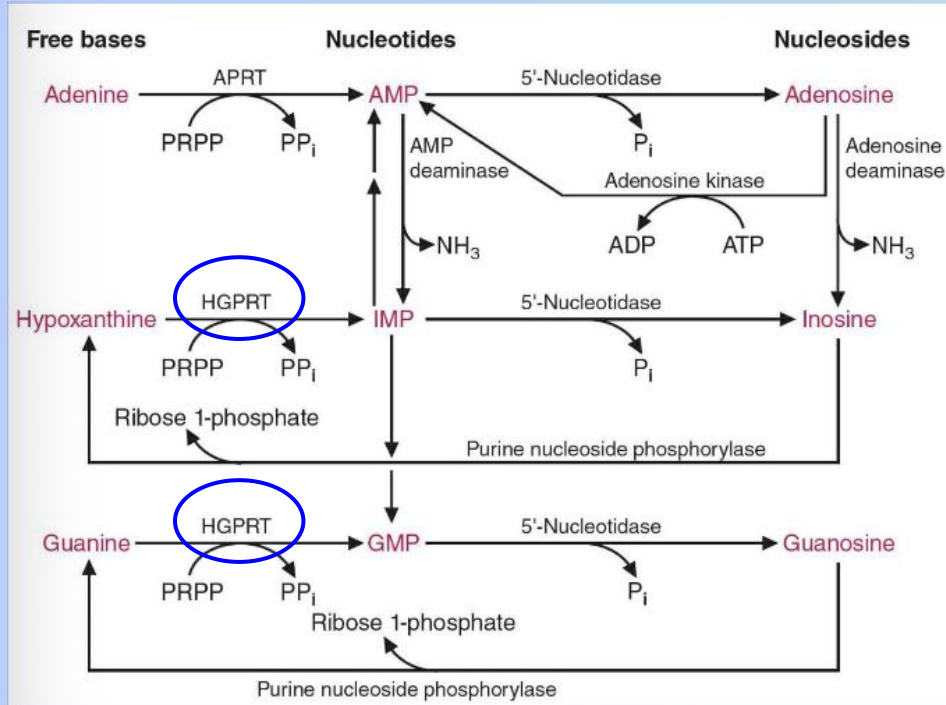
# De Novo Purine Synthesis Summary

- **Goal:**
  - Make IMP (nucleotide with "precursor" purine/name-tag)
  - → make AMP or GMP (purine-based nucleotide)
- **Atoms needed to make a purine:**
  - Glycine
  - Aspartate
  - Glutamine
  - CO<sub>2</sub>
  - THF

# De Novo Purine Synthesis Summary

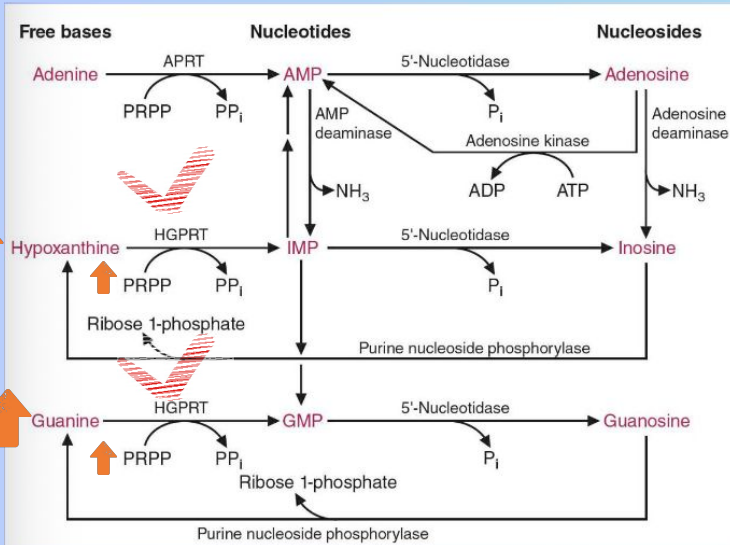
- **Key enzymes:**
  - PRPP synthetase
  - GPAT: **rate-limiting enzyme**
  - IMP dehydrogenase
  - Adenylosuccinate synthetase
- **Purine synthesis regulation:**
  - **Enzyme activation: via their precursors or source of energy**
    - GPAT: PRPP
    - IMP dehydrogenase: ATP
    - Adenylosuccinate synthetase: GTP
  - **Enzyme inhibition: via their “products”**
    - PRPP synthetase: GDP & ADP
    - GPAT: GMP & AMP
    - IMP dehydrogenase: GMP
    - Adenylosuccinate synthetase: AMP

# Purine salvage pathway



- Purpose: To interconvert purine bases, nucleosides & nucleotides
- Energy required: less than De Novo synthesis
- Key enzyme: HGPRT
  - Clinical correlation!

# Clinical Correlation: Lesch-Nyhan Syndrome

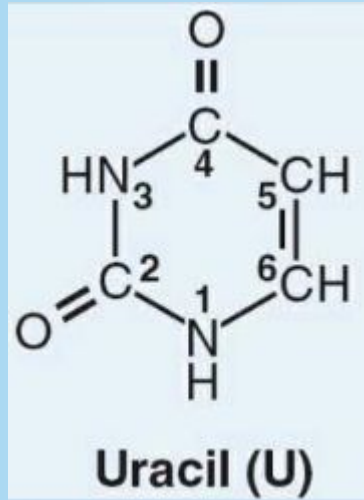
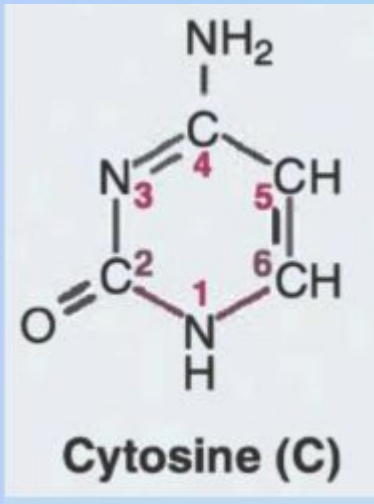


- HGPRT deficiency
- Biochemical effect:
  - Hypoxanthine & guanine aren't salvaged, so instead are degraded into uric acid
  - Overall result: uric acid overproduction
- Clinical picture:
  - Hyperuricemia
  - Gout
  - Aggression & self mutilation
  - Crystals in urine
  - Dystonia: abnormal muscle tone

# Pyrimidine synthesis

# What are our pyrimidines?

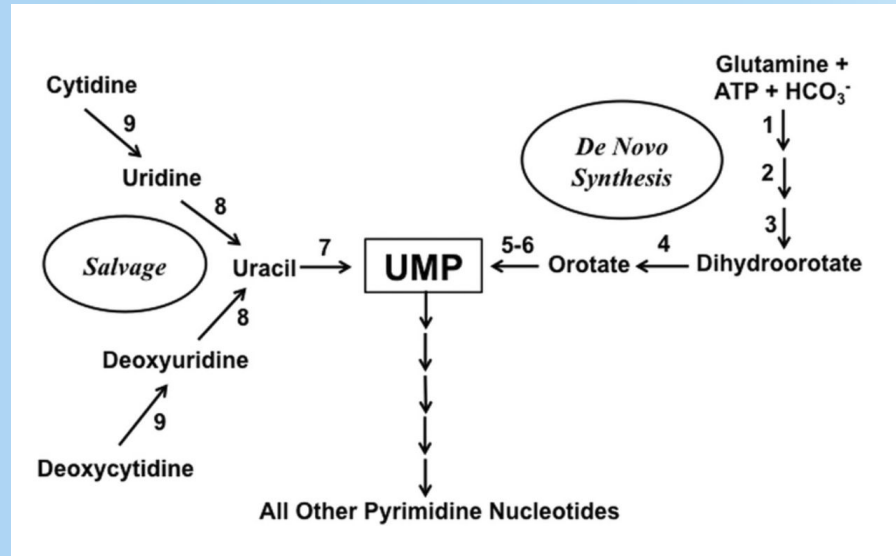
“CUT the pyramid”



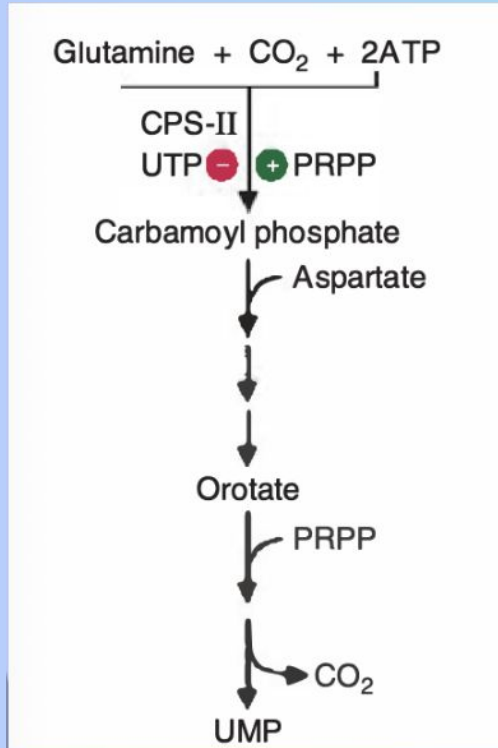
# How do we make pyrimidine-based nucleotides?

1. De Novo Pyrimidine synthesis

2. Salvage pathway

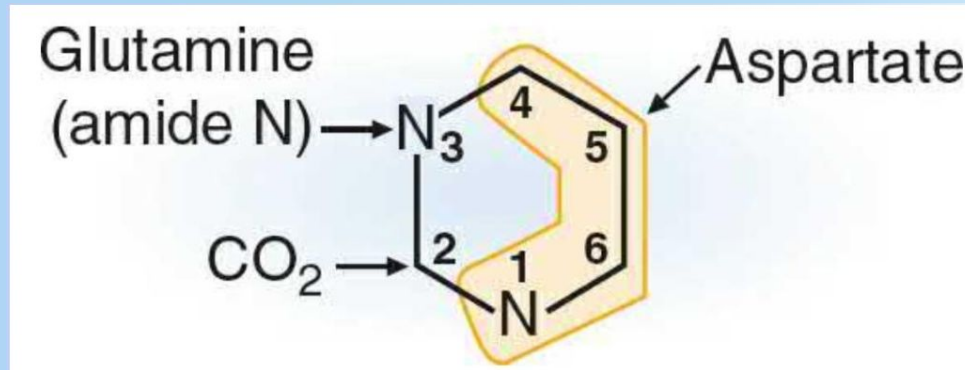


# De Novo Pyrimidine Synthesis



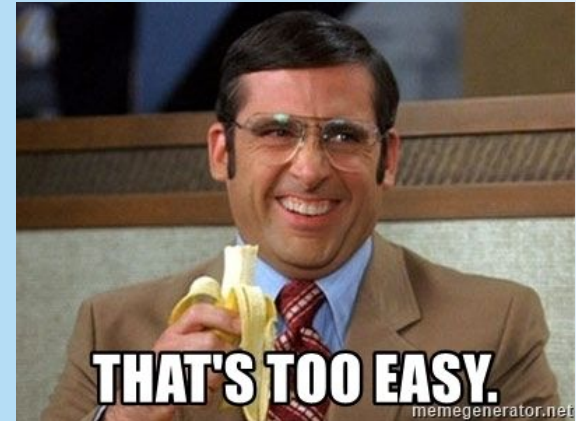
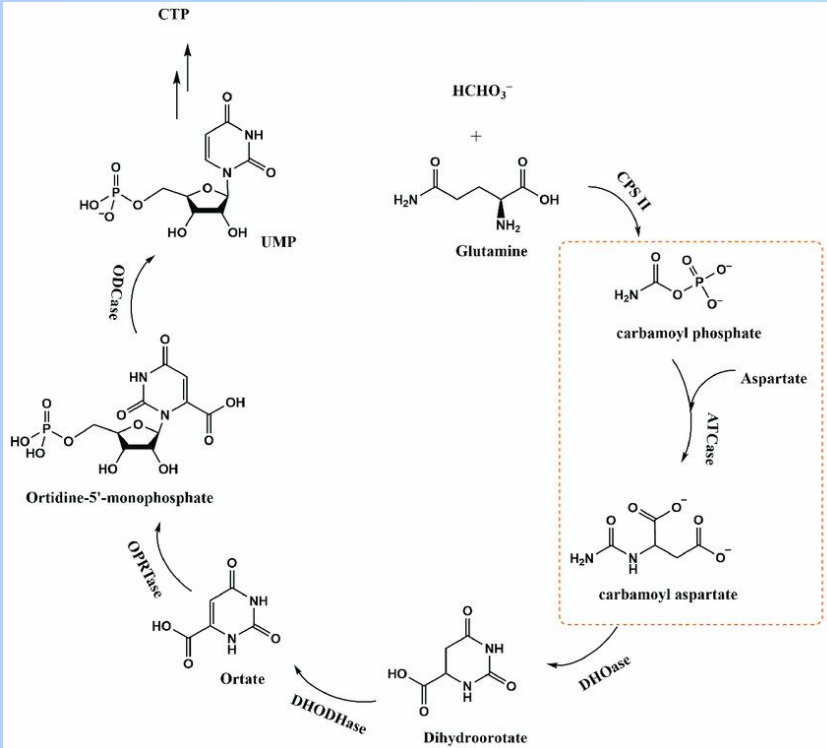
- Making pyrimidines from scratch
- Occurs in almost all human cells
- Main gist:
  - The pyrimidine base is made first, and then ribose-5-phosphate is given from PRPP
  - Key enzyme: CPS II
  - All steps occur in the cytosol (with one exception)...
  - Goal: to make UMP (a pyrimidine-based nucleotide), which then can make any other pyrimidine nucleotide

# What atoms are needed to build a basic pyrimidine?



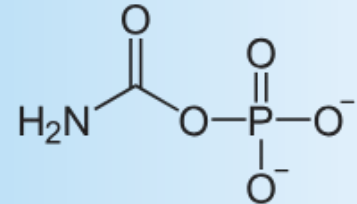
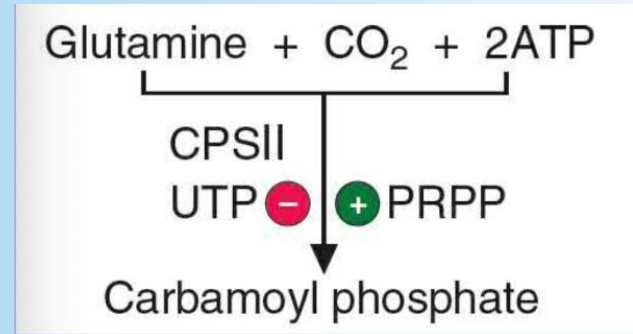
- Glutamine: used in first step
- Aspartate: added in second step
- CO<sub>2</sub>

# The Steps of De Novo Pyrimidine Synthesis

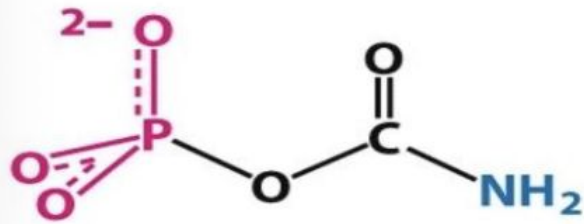


# Step 1: Rate limiting Step via CPS II

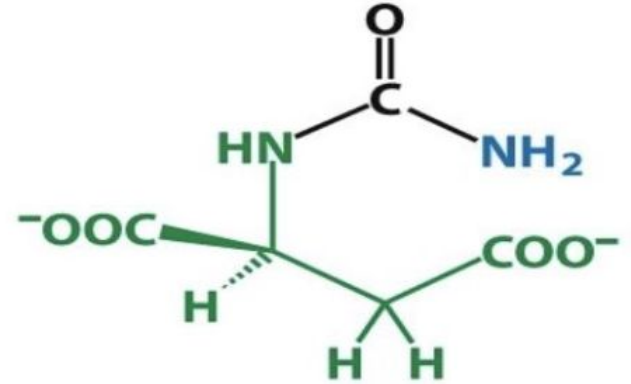
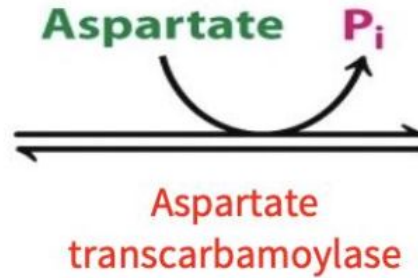
- Glutamine + CO<sub>2</sub> + 2 ATP → Carbamoyl phosphate
- Enzyme: CPS II
  - Different enzyme than CPS I!!! (Which is from urea cycle)
- Rate-limiting step
  - Activated by PRPP (signals low pyrimidine levels)
  - Inhibited by UTP (“product”)



# Step 2: Addition of Aspartate

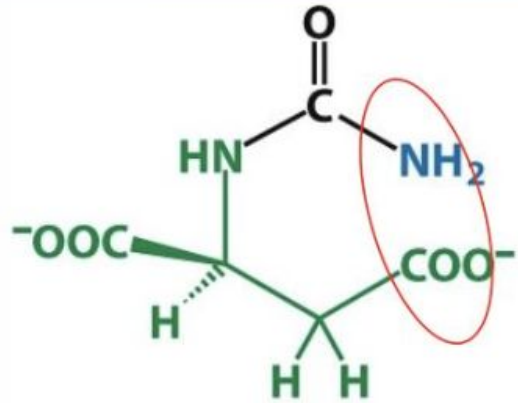


**Carbamoyl phosphate**

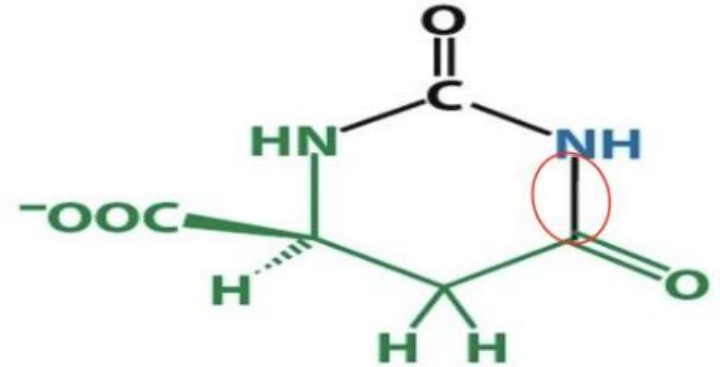
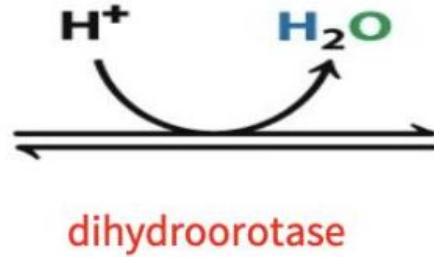


**Carbamoylaspartate**

# Step 3: Ring Closure via Dihydroorotase

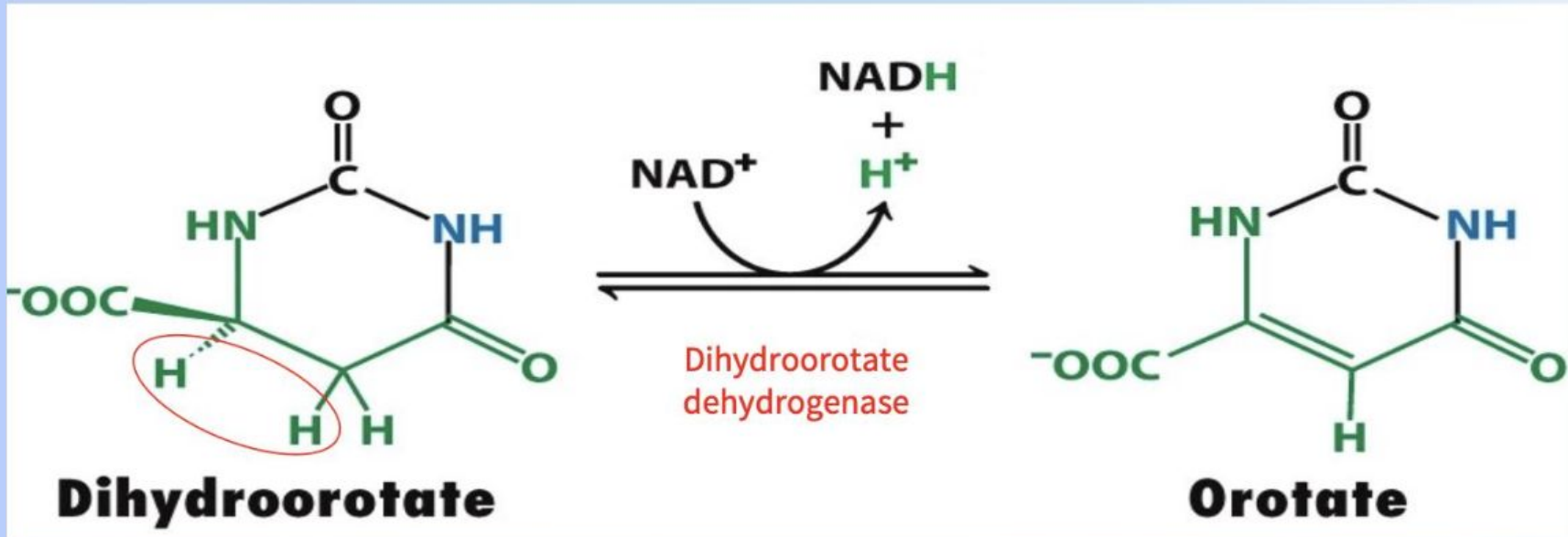


**Carbamoylaspartate**



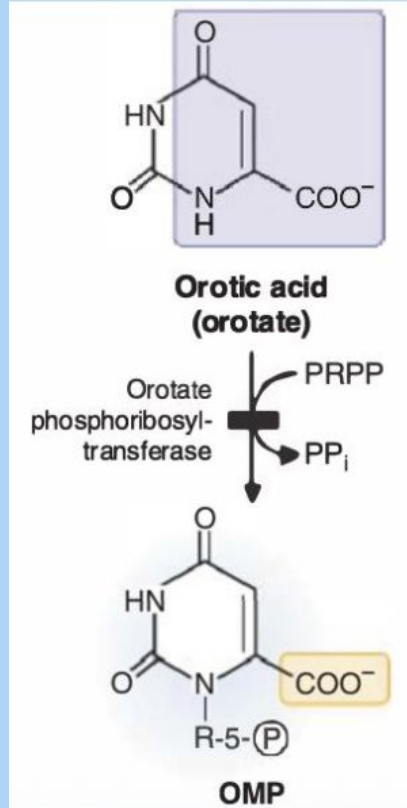
**Dihydroorotate**

# Step 4: Oxidation to Orotic Acid



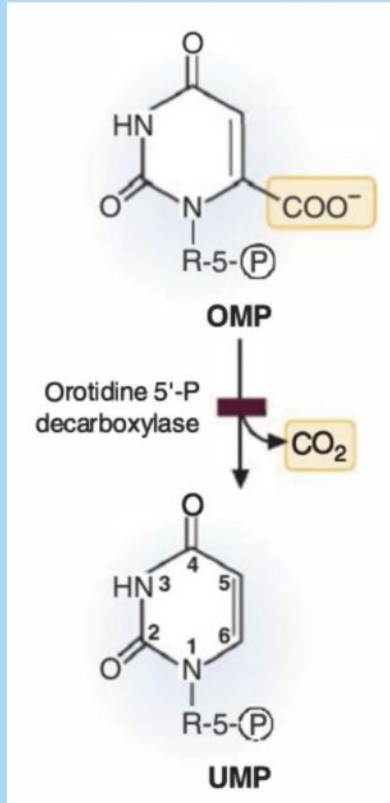
This is the only step that takes place in the mitochondria!

# Step 5: Addition of PRPP



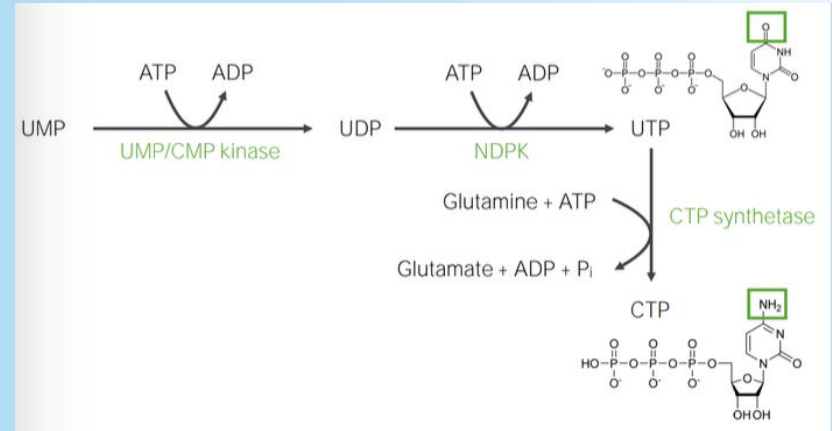
This is when the ribose sugar is added to the forming nucleotide!

# Step 6: Formation of UMP

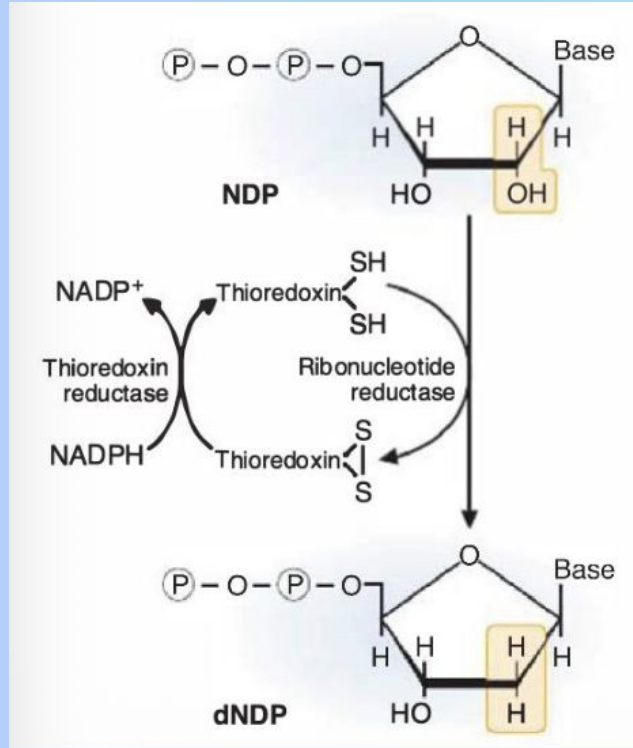


# Now to make some other nucleotides...

- UMP → UTP:
  - Phosphorylation: addition of phosphates (in this case 2 of them)
  - Enzyme: nucleotide kinases
- UTP → CTP
  - An amine group from glutamine is donated to UTP to make CTP
  - Enzyme: CTP synthetase
- These actions require ATP!



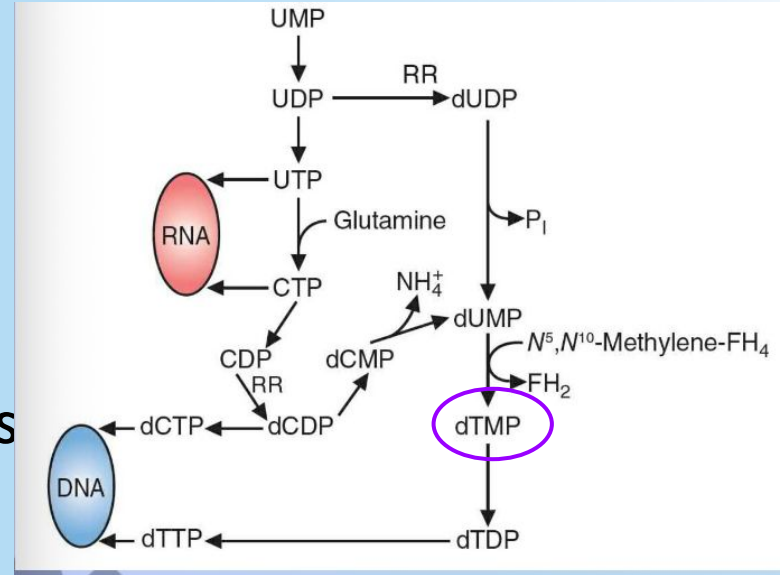
# What about deoxynucleotides?



- To make DNA, nucleotides must have a deoxyribose (not ribose) sugar
- Enzyme: Ribonucleotide reductase
  - Cofactor: Thioredoxin
  - Activation of enzyme: ATP
  - Inhibition of enzyme: dATP
- dNDPs can be phosphorylated into dNTPs for DNA synthesis 😊

# What about dTMP?

- To make dTMP, it must be converted from dUMP!
  - $dUDP \rightarrow dUMP$  or  $dCMP \rightarrow dUMP$  (via deamination: removal of amine group)
- Then,  $dUMP \rightarrow dTMP$ 
  - Enzyme: Thymidylate synthase
  - Cofactor:  $N^5, N^{10}$ -methylene-FH<sub>4</sub>



# De Novo Pyrimidine Synthesis Summary

- **Goal:**
  - Make UMP (nucleotide with "precursor" pyrimidine/name-tag)
  - → can make UTP, CTP, dUMP, dTMP
- **Atoms needed to make a purine:**
  - Glutamine
  - Aspartate
  - CO<sub>2</sub>
- **Rate-limiting enzyme:** Carbamoyl phosphate synthetase II (CPS II)
  - Activated by PRPP (signals low pyrimidine levels)
  - Inhibited by UTP ("product")

# Purine synthesis vs pyrimidine synthesis

	Purine synthesis	Pyrimidine synthesis
What are they?	Adenine, Guanine	Cytosine, Uracil, Thymine
Sources of atoms in the ring(s)	Glutamine, aspartate, CO <sub>2</sub> , glycine, THF	Glutamine, aspartate, CO <sub>2</sub>
Ribose sugar	Is incorporated at the start of synthesis	Is incorporated later in the synthesis
Is PRPP used?	Yes	Yes
Rate-limiting enzyme	Glutamine phosphoribosyl amidotransferase (GPAT)	Carbamoyl phosphate synthetase II (CPS II)

