Biochemistry (Renal module)

❑ Nucleoprotein metabolism

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Nucleoprotein metabolism

Diet contains nucleic acids in the form of nucleoproteins

Type: nucleoproteins are conjugated proteins

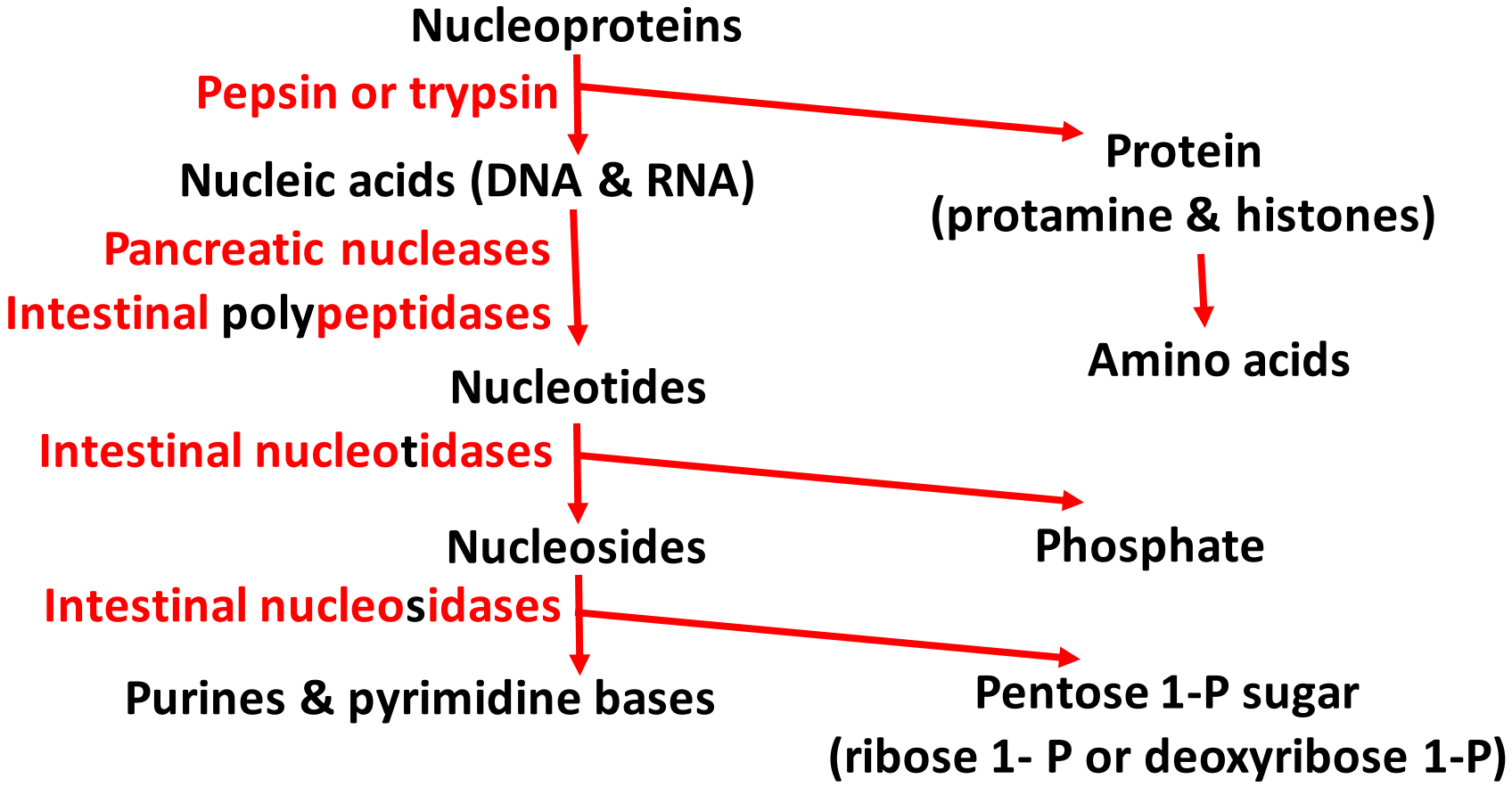
[non-protein prosthetic group (nucleic acid) attached to one or more molecules of a simple protein]

- Simple protein is usually basic protein (histone or protamine)
- Nucleoproteins are found in all animals & plants (in all cell nuclei & protoplasm)

Nucleoprotein metabolism

Nucleoprotein digestion

In the small intestine mainly & to some extent in the stomach)



Fate of absorbed nucleic acids:

1. Absorbed purine & pyrimidine: mainly catabolized in the liver
2. Absorbed nucleosides: may be incorporated in the body nucleic acids

Nucleoprotein metabolism

Purine metabolism

Synthesis of purine nucleotides:

Site: they are synthesized by most tissues, however, the major site is the liver (cytoplasm)

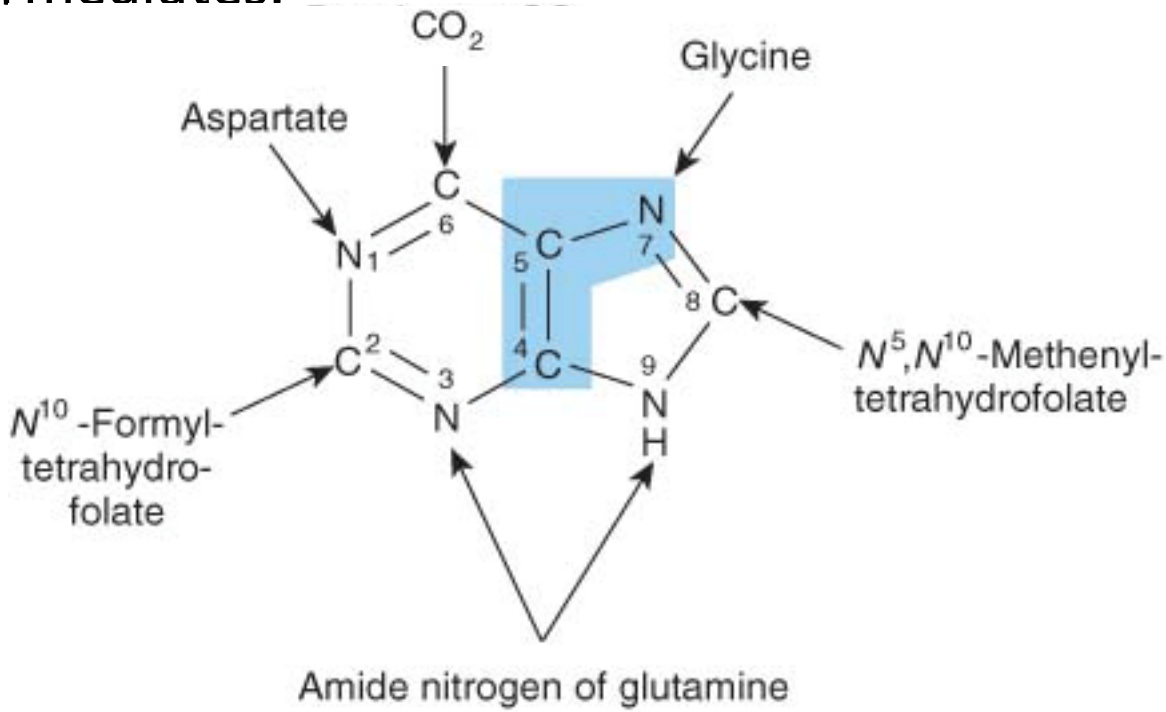
Pathways: major (De novo) and minor (Salvage)

De novo synthesis

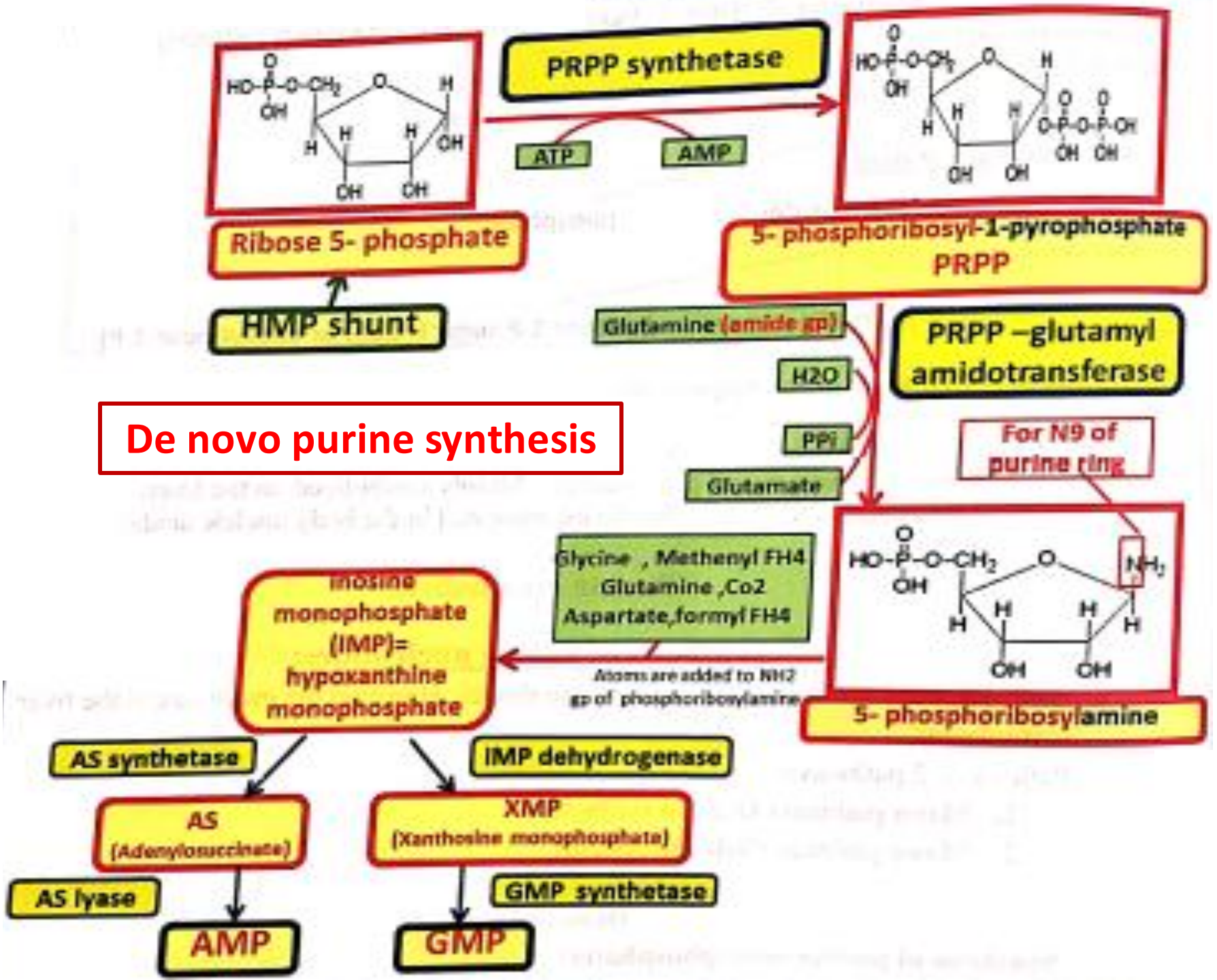
The purine ring is formed (de novo) in the body from different metabolic intermediates.

N.B:

Folate is important for formation of C2 & C8 of purine, so folate antagonists (methotrexate) inhibits purine synthesis and cell division so they are used in treating cancers.



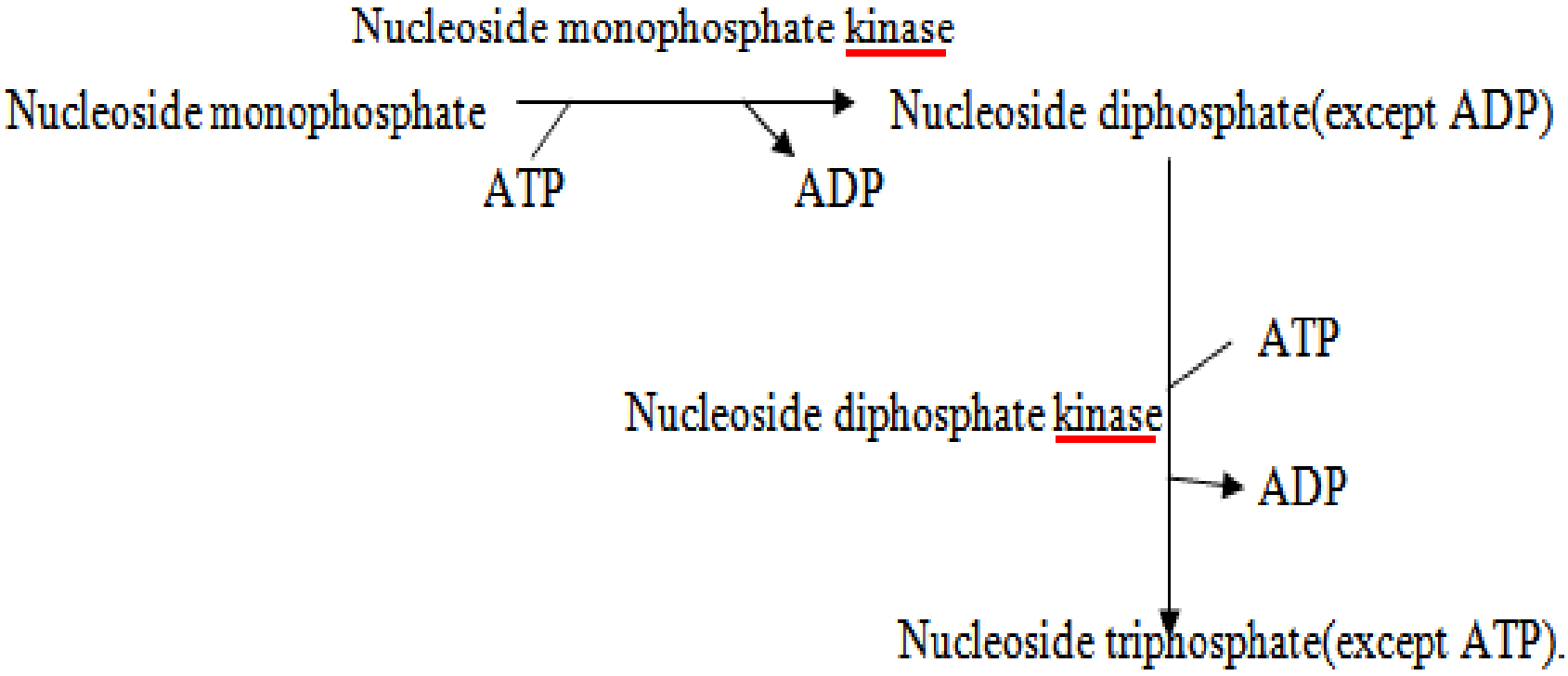
De novo purine synthesis



Nucleoprotein metabolism

Synthesis of nucleoside di- and tri-phosphates:

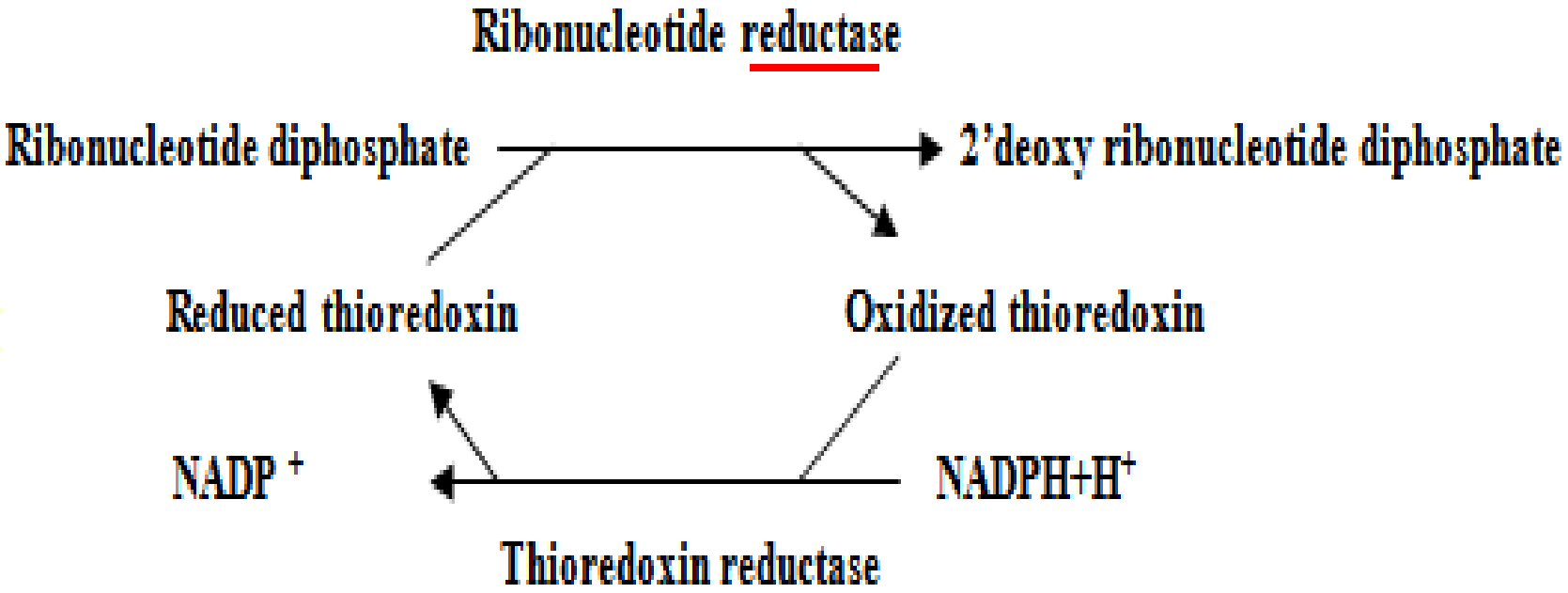
This mechanism is for synthesis of both purine and pyrimidine nucleotide di- and tri-phosphates, which require the corresponding kinase enzyme and ATP.



Nucleoprotein metabolism

Synthesis of deoxyribonucleosides:

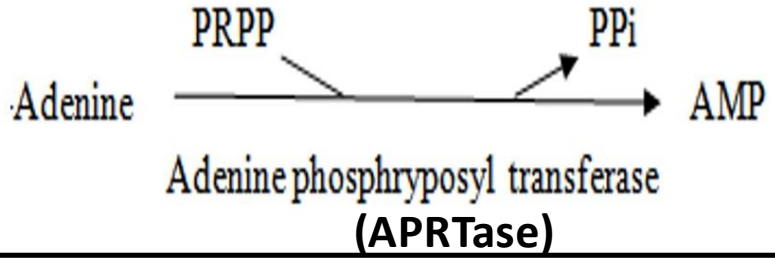
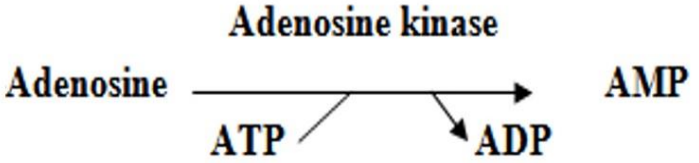
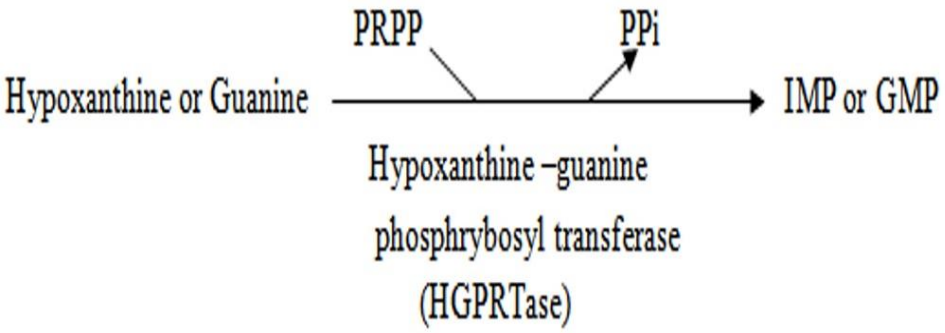
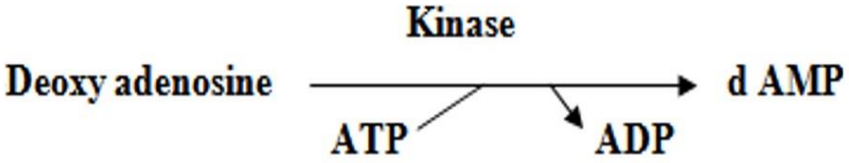
This applies for synthesis of purine & pyrimidine deoxyribonucleotides. The enzyme ribonucleotide reductase is active only during DNA synthesis



Nucleoprotein metabolism

Purine salvage pathway

Importance: Supply purine nucleotides to certain tissue or cell where the de novo synthesis is not active e.g. **brain, red cells and lymphocytes**.

Salvage of free purines	Salvage of purine nucleosides
Salvage of adenine  	**Salvage of adenosine** 
Salvage of guanine  	**Salvage of deoxyadenosine** 

Nucleoprotein metabolism

*Regulation of biosynthesis of purine nucleotides:

1. Ribose-5-P **stimulates** PRPP synthetase so ↑ R-5-P as in ***Von Gierke's disease*** (glucose-6-phosphatase deficiency) leading to ↑ de novo synthesis of purines.
2. Normally, more PRPP is used for purine salvage by HGPRTase than for de novo synthesis, so in ***Lesch-Nyhan syndrome*** **(HGPRTase deficiency)** there is ↑ in de novo synthesis of purines.
3. High concentration of **AMP, ADP, GMP and GDP** produce inhibition of conversion of ribose-5-P to IMP at two sites, **PRPP synthetase and PRPP-glutamyl amidotransferase**.

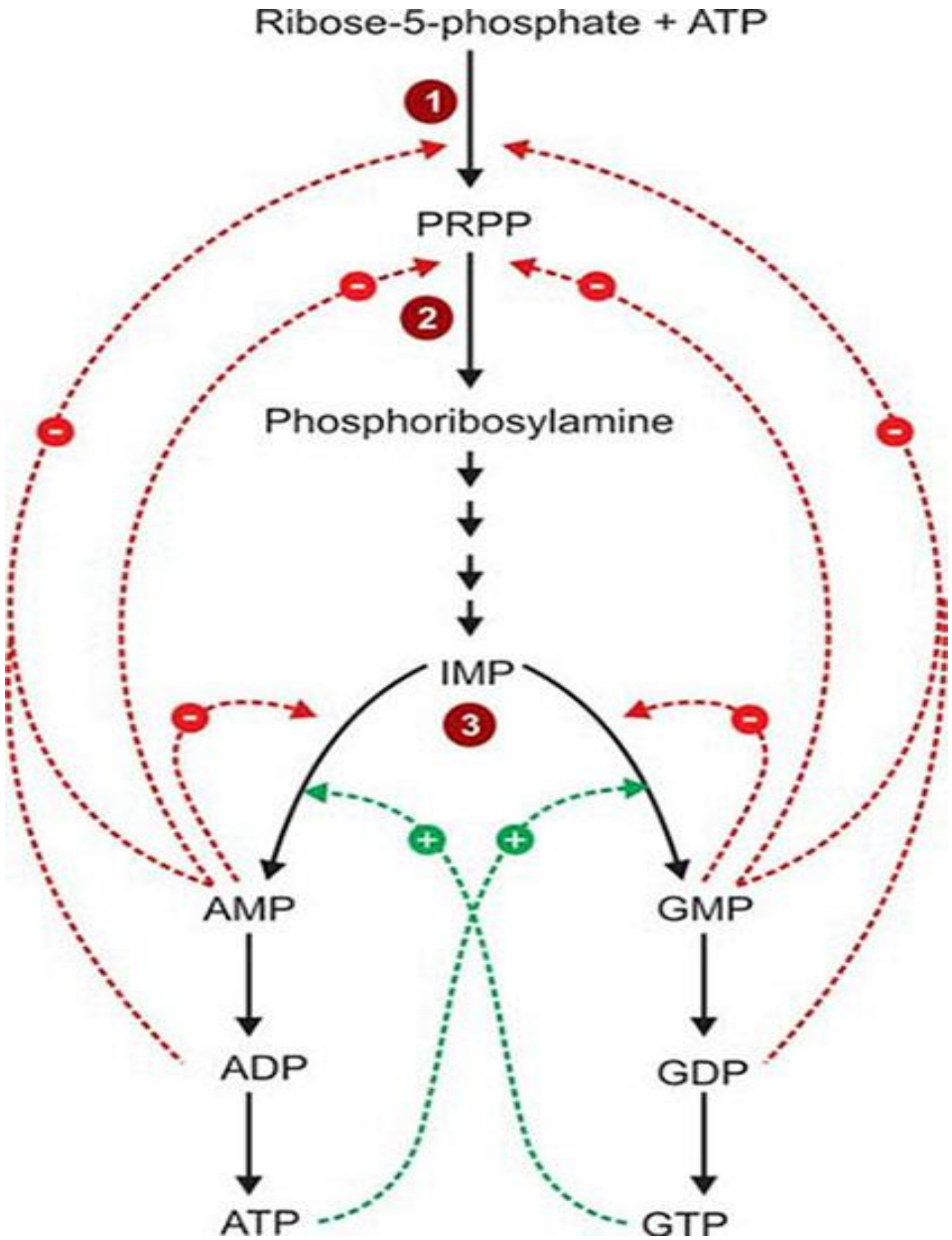
Nucleoprotein metabolism

*Regulation of biosynthesis of purine nucleotides:

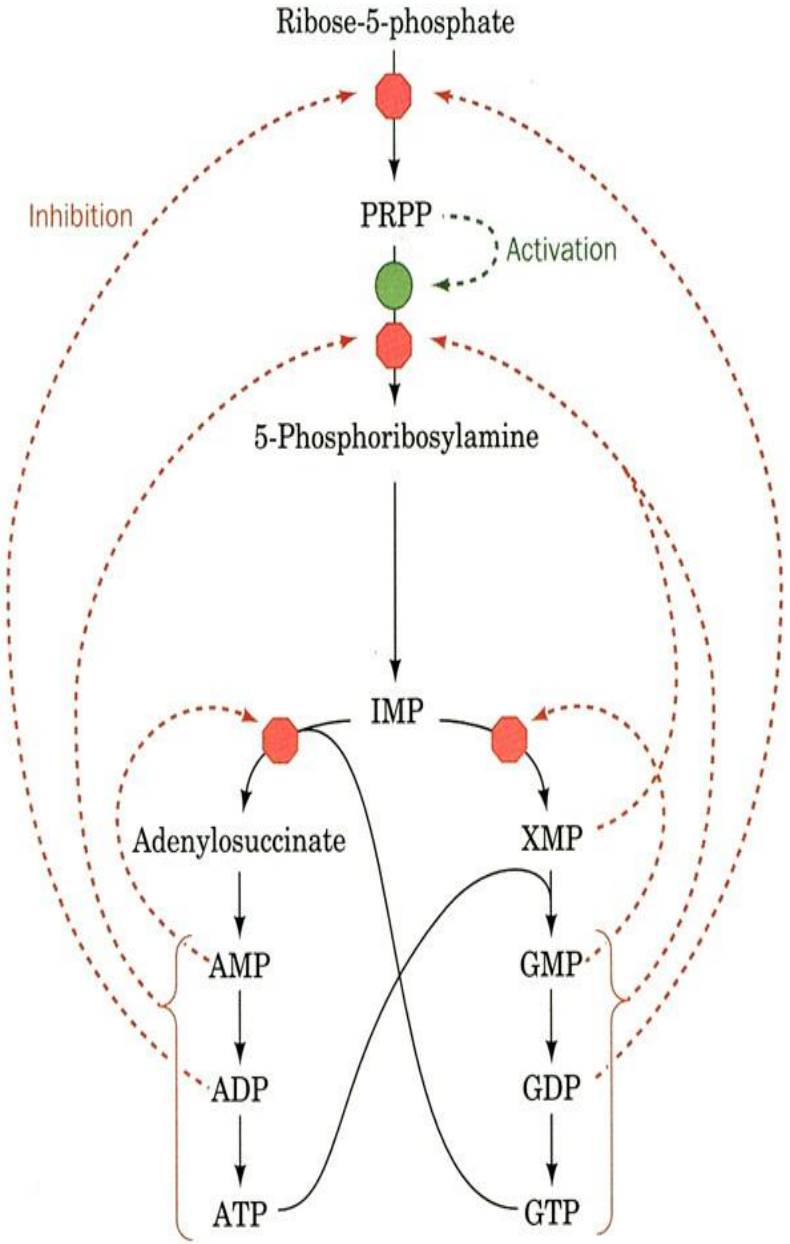
4. Regulation of conversion of IMP to ATP and GTP:

- Both AMP & GMP inhibit their own formation by **feedback inhibition of adenylosuccinate synthetase & IMP dehydrogenase**, respectively.
- ATP produces allosteric activation of **GMP synthetase** that converts XMP to GMP. On the other hand, GTP produces allosteric activation of **AS synthetase** that converts IMP to AS.

*Regulation of purine nucleotides biosynthesis



Nucleoprotein metabolism



Nucleoprotein metabolism

Analogue of purine synthesis inhibitors:

They act as competitive inhibitors of the naturally occurring nucleotides that are used to synthesize DNA. When wrong bases are incorporated, the DNA becomes functionally inactive. Thereby cell division is arrested. So, they are useful as anticancer drugs. A few examples are:

1. **Mercaptopurine** inhibits conversion of IMP to GMP & AMP
2. **Folate antagonists** (methotrexate) would affect the reactions involving one carbon group transfer
3. **Azaserine** (diazo acetyl-L-serine) is a **glutamine antagonist** and therefore inhibits reactions involving glutamine.
4. **Other** synthetic nucleotide analogues used as anticancer agents are 6-thio guanine and 8-aza guanine.

Nucleoprotein metabolism

Purine catabolism

Uric acid is the end product of purine catabolism in humans.

In the tissues:

- Nucleic acids are hydrolyzed to nucleosides
- Adenosine is catabolized to hypoxanthine
- Guanosine is catabolized to xanthine.

In the liver:

- Hypoxanthine gives xanthine by **xanthine oxidase** which is oxidized to uric acid.
- Uric acid goes via blood to be excreted by kidneys in urine.

In lower animals (not in humans) uric acid is further oxidized to allantoin by uricase enzyme.

Serum uric acid level:

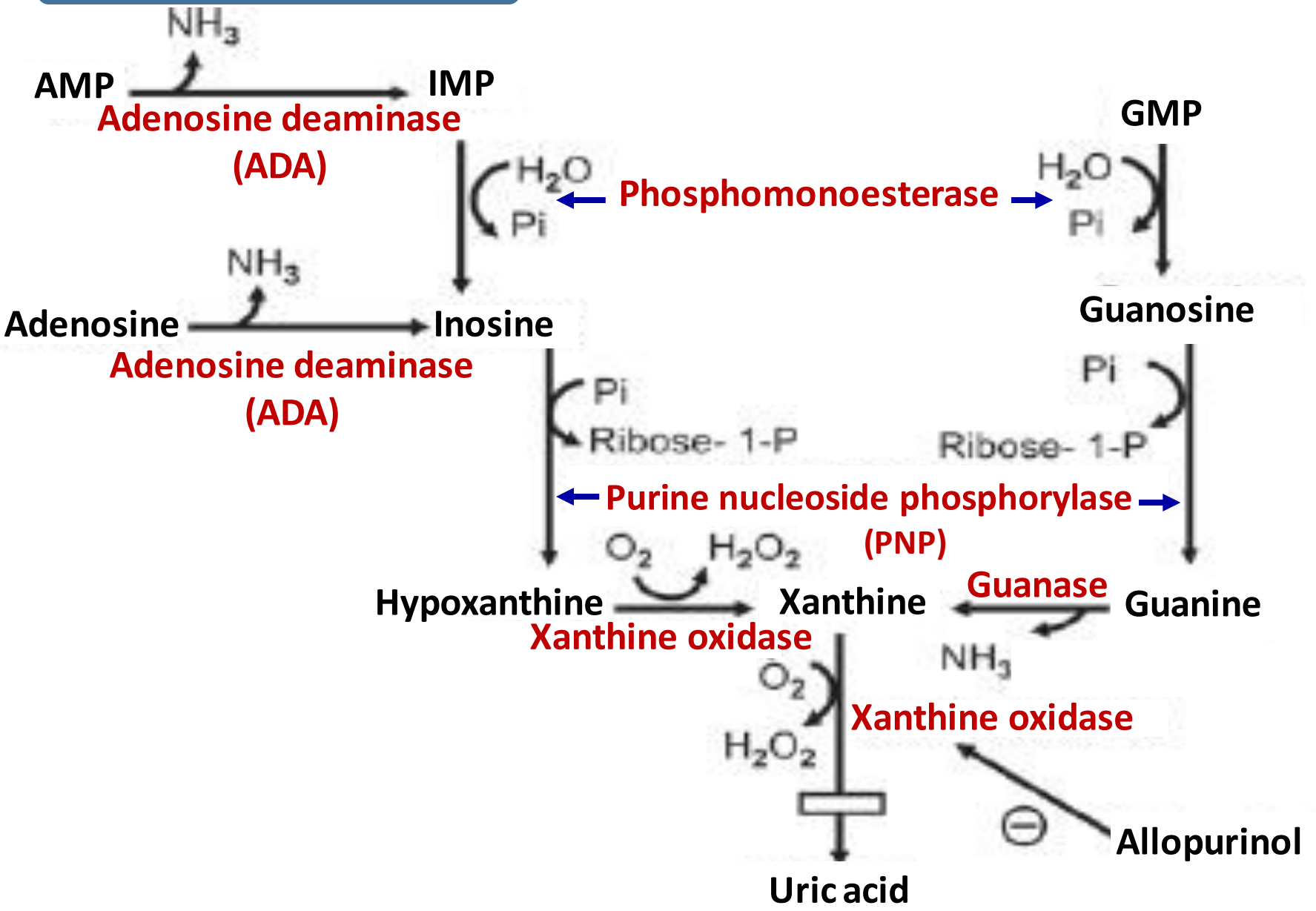
male: 3-7 mg/dl, female: 2-6 mg/dl

Urinary uric acid level: 0.3 – 0.7 g / d

Uric acid is sparingly soluble in water

Nucleoprotein metabolism

Purine catabolism



Nucleoprotein metabolism

Gout

Definition, causes & characters:

- Gout is a form of arthritis caused by excess uric acid in the blood stream (hyperuricemia).
- Urate crystals accumulate in synovial fluid resulting in inflammation leading to acute arthritis.
- At 30^{o}C, uric acid solubility is lowered, so uric acid is deposited in cooler areas of the body to cause tophi seen in distal joints of foot.
- Increased excretion of uric acid (**uricosuria**) may cause deposition of uric acid cystals in the urinary tract leading to calculi or stone formation with renal damage.

Nucleoprotein metabolism

Types of Gout: 1ry & 2ry

Primary gout: about 1:500 of the total population

- About 10% of 1ry gout are idiopathic.
- 1ry gout may show familial incidence

Causes of 1ry gout:

1. **Abnormal phosphoribosyl amido transferase**: The abnormal enzyme is active, but not sensitive to feedback regulation by inhibitory nucleotides →↑ purine synthesis
2. **Abnormal PRPP synthetase (X-linked recessive):** not subject to normal allosteric mechanisms →↑ purine synthesis.
3. **Deficient purine salavage pathway enzymes** e.g. HGPRTase deficiency (Lesch-Nyhan syndrome): X-linked; 1:10.000 males. ↓ HGPRTase → ↓ salvage →↑ PRPP & ↓ inhibitory purines (self mutilation, mental retardation, ↑ urate & nephrolithiasis. Gout develops later in life. (i.e. brain is dependent on salvage pathway for IMP & GMP needs).

Nucleoprotein metabolism

Causes of 1ry gout (continued):

4. **G-6-Pase deficiency (Von Gierke's disease or GSD type I):**
   more glucose enters HMP shunt → ↑ ribose-5-P → ↑ PRPP

5. **Glutathione reductase variant:** this enzyme depends on NADPH from HMP shunt. The abnormality → ↑ ribose-5-P → ↑ PRPP. Dysregulation of the rate limiting step of purine nucleotide synthesis → ↑ synthesis & degradation of uric acid.

Nucleoprotein metabolism

Secondary gout: causes

1. ↑ production of uric acid: ↑ turnover of nucleic acids as in:

a) **Rapidly growing malignant tissues:** leukemia, lymphoma & polycythemia.

b) **↑ tissue breakdown after treatment of large malignant tumors**

c) **↑ tissue damage by trauma & ↑ catabolism (starvation)**

2. Reduced excretion rate of uric acid:

a) **Renal failure**

b) **Treatment with thiazide diuretics** (inhibit tubular secretion of uric acid)

c) **Lactic acidosis and ketoacidosis** (interference with tubular secretion)

Nucleoprotein metabolism

Treatment policies in gout

1. **Reduce the dietary purine intake**
2. **Increase renal excretion by uricosuric drugs,** to decrease urate reabsorption from the renal tubules .e.g. **probenecid**
3. **Reduce urate production by allopurinol**, an analogue of hypoxanthine so it is a competitive inhibitor of xanthine oxidase → ↓ urate formation. Xanthine & hypoxanthine are more soluble & easily excreted. Xanthine oxidase converts **allopurinol** to alloxanthine. (more effective inhibitor of xanthine oxidase (suicide inhibition)
4. **Colchicine;** anti-infalammatory very useful to arrest arthritis in gout

Nucleoprotein metabolism

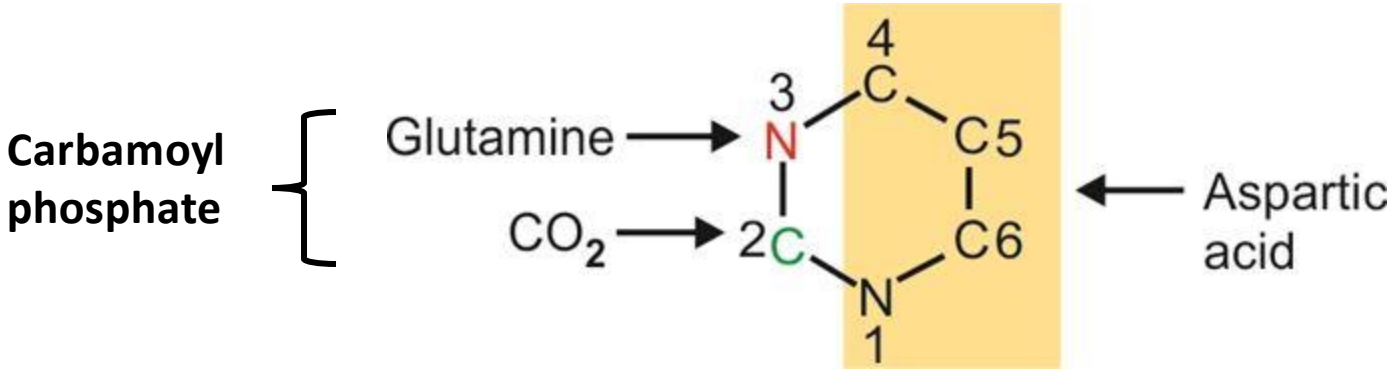
Pyrimidine metabolism

2 pathways; de novo & salvage

Pathways: major (De novo) and minor (Salvage)

De novo synthesis

- The pyrimidine ring (unlike purine) is synthesized as free pyrimidine, then incorporated into nucleotides.
- The origin of atoms of pyrimidine nucleus:



Nucleoprotein metabolism

Steps of De novo Pyrimidine synthesis:

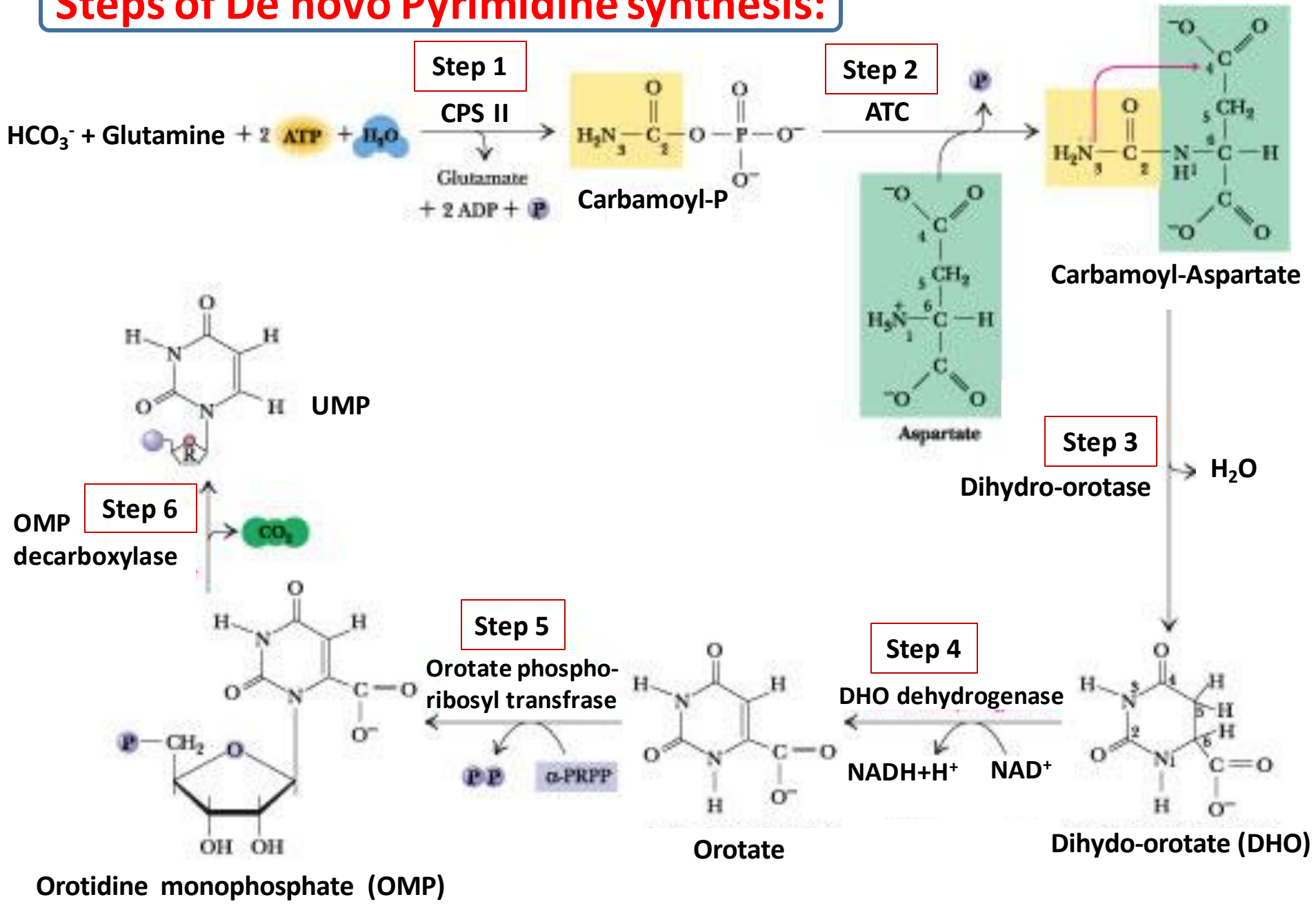
1. **Carbamoyl phosphate synthesis:** cytoplasmic reaction
   It is synthesized from nitrogen of glutamine & bicarbonate **by carbamoyl phosphate synthetase II (CPS II).**
2. **Rate limiting step:** Carbamoyl phosphate & aspartate combine to form carbamoyl aspartate by **aspartyl transcarbamoylase (ATC)**, allosteric regulated. C2 & N3 are derived from carbamoyl phosphate & the rest from aspartate.
3. **Formation of pyrimidine ring:** the 3rd nitrogen & the 4th carbon are joined by a covalent bond. Carbamoyl aspartate is cyclized. Dihydro-orotic acid is produced by **dihydro orotase (DHOase)**
4. **Formation of orotic acid by oxidation:** hydrogen atoms are removed so that orotic acid is produced by dihydro orotate dehydrogenase **(DHODHase)**. It requires NAD co-enzyme.

Nucleoprotein metabolism

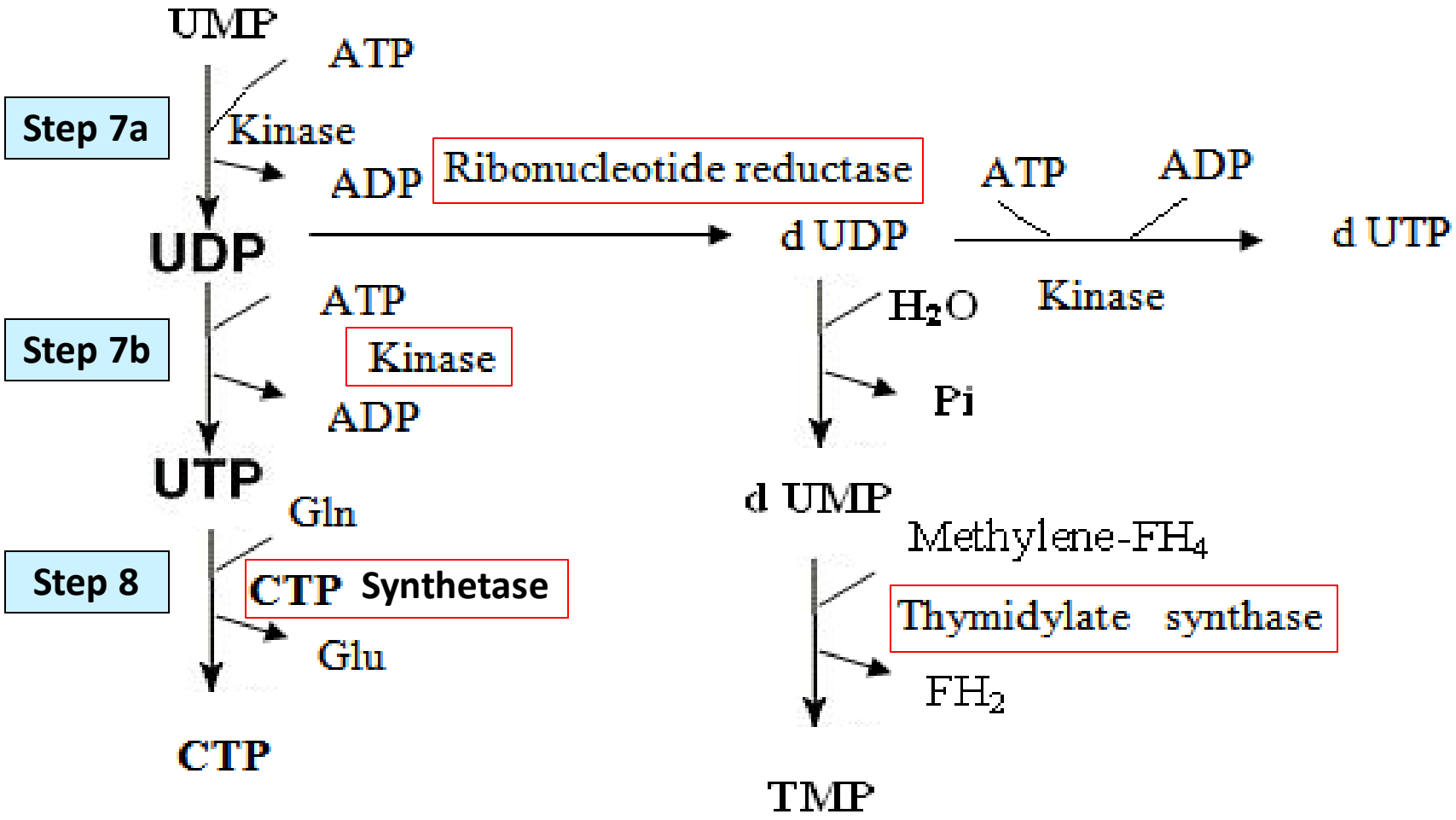
Steps of De novo Pyrimidine synthesis (continued):

5. **Formation of OMP:** Ribose-5-P (from PRPP) is added to orotic acid to form orotidylic acid or orotidine monophosphate (OMP) by orotate phosphoribosyl transferase (OPRT ase)
6. **Formation of uridine monophosphate (UMP):** by OMP decarboxylase (OMPDC). the enzyme is inhibited by 6-aza-uridine (anticancer). UMP is the 1st purine formed.
7. **Synthesis of triphosphates:** UMP is phosphorylated to UDP with the help of ATP by nucleoside monophosphate **kinase** (UMP kinase). UDP is phosphorylated to UTP by nucleoside diphosphate **kinase** (UDP kinase) with ATP help.
8. **Formation of CTP** from UTP by **CTP synthetase** (add amino group from glutamine. It needs ATP)

Steps of De novo Pyrimidine synthesis:



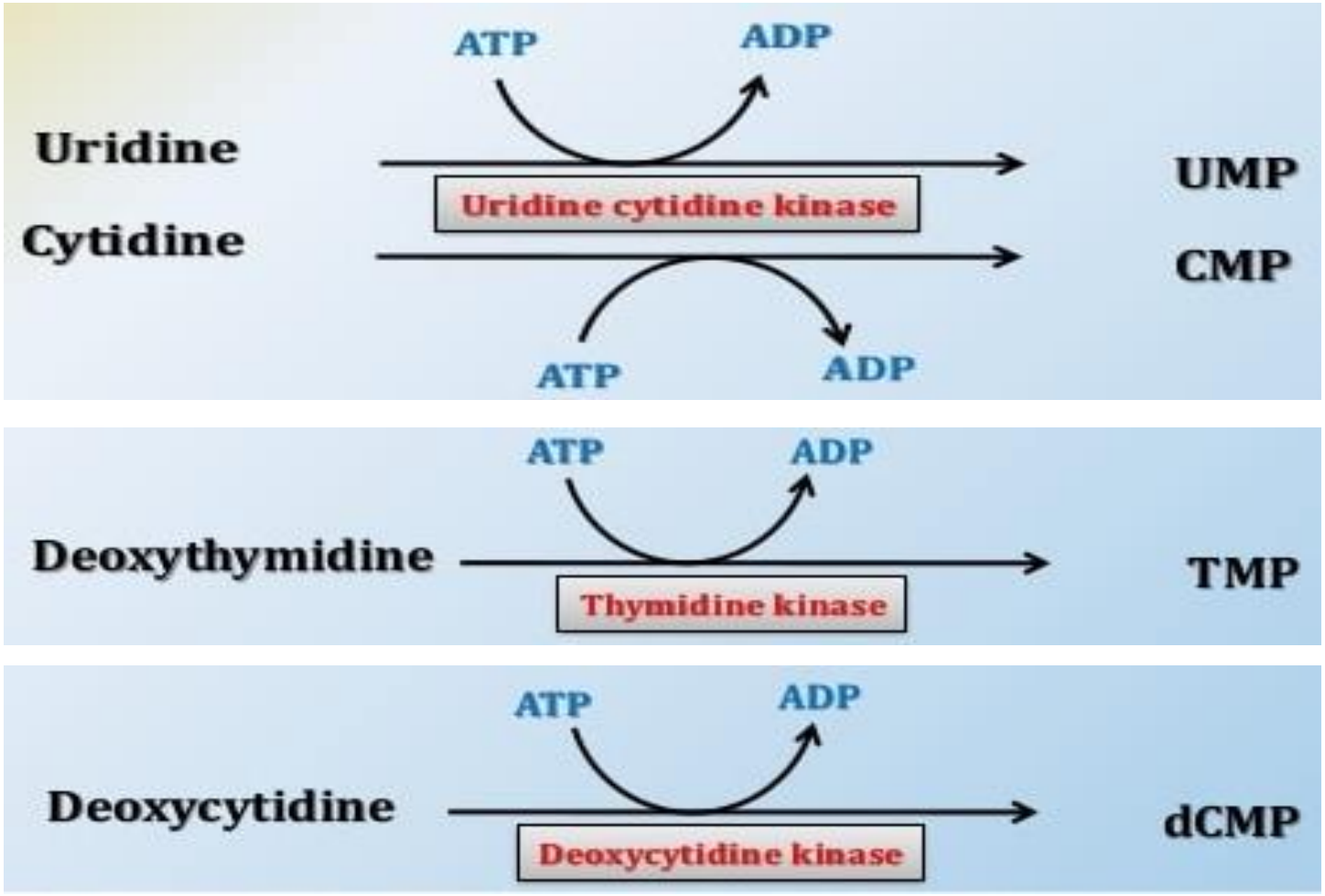
Steps of De novo Pyrimidine synthesis (continued):



Nucleoprotein metabolism

Pyrimidine salvage pathway

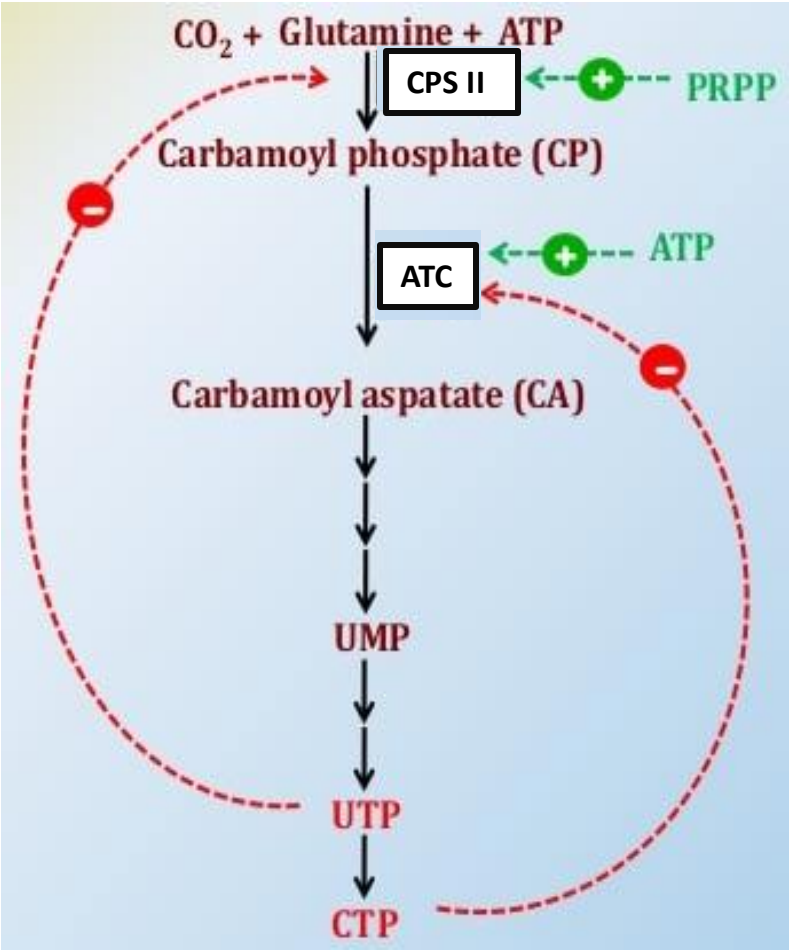
Pyrimidine can also be savaged like purines using PRPP and phosphoribosoyl transferase and nucleoside phosphorylase



Nucleoprotein metabolism

Regulation of pyrimidine synthesis:

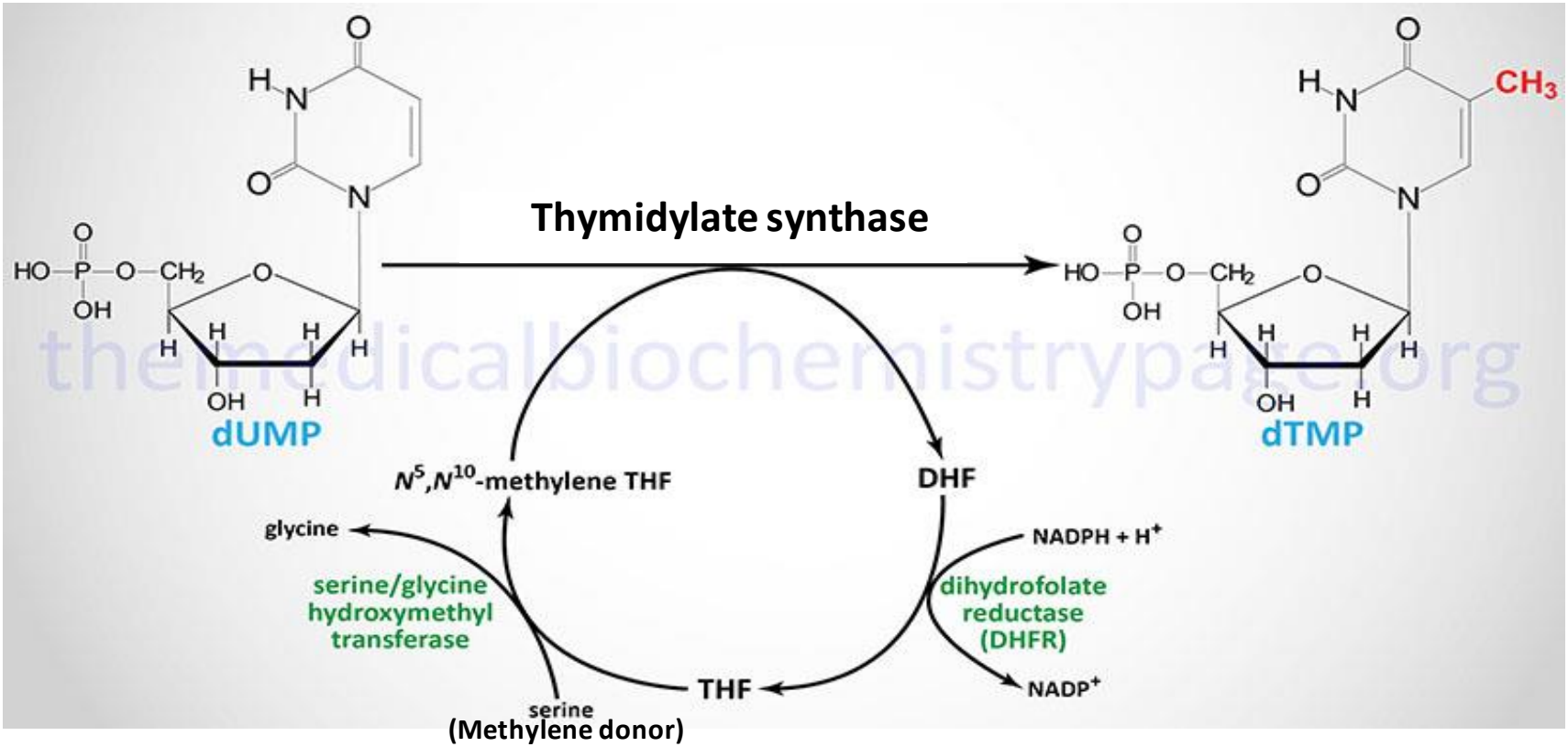
1. The major regulatory step in prokaryotes is the reaction catalyzed by aspartate transcarbamoylase (ATC) which is allosterically inhibited by CTP.
2. In the mammalian cells, the regulation occurs at the level of CPS II which is inhibited by UTP and activated by PRPP. Aspartate transcarbamoylase is inhibited by CTP and is activated by ATP.
3. Further, OMP decarboxylase is inhibited by UMP



Nucleoprotein metabolism

Synthesis of deoxythymine nucleotides:

- The thymine nucleotide is formed by thymidylate synthetase by methylation of dUMP.
- The methyl group is denoted by N5, N10 – methylene – THFA. Later THFA is regenerated by dihydrofolate reductase using NADPH as the reductant.



Nucleoprotein metabolism

Anticancer agents acting on pyrimidines:

1. Methotrexate inhibits the enzyme dihydrofolate reductase → ↓ the regeneration of THFA → -- dTMP synthesis → -- DNA
   (methotrexate is a powerful anticancer agent)
2. 5-fluoro-uracil, 5-iodo uracil, 3-deoxy uridine, 6 aza uridine, 6-aza cytidine and 5-iodo-2-deoxyuridine are anticancer drugs, which competitively inhibit thymidylate synthase. Cytosine arabinoside where ribose is replaced by arabinose is another anticancer agent.

Nucleoprotein metabolism

Orotic aciduria

- It results from absence of one or both enzymes; **OPRT ase and OMP decarboxylase** → ↑ orotic acid production → ↑ excretion of urine
- It is **autosomal recessive**, with retarded growth & megaloblastic anemia. The rapidly growing cells are more affected (anemia). Crystals are excreted in urine which may cause urinary tract obstruction.

Due to lack of feedback inhibition, orotic acid production is excessive

- It can be successfully treated by feeding cytidine or uridine. They may be converted to UTP which can act as feedback inhibitor
- Orotic aciduria may also occur in **ornithine transcarbamoylase deficiency** (urea cycle enzyme) as carbamoyl phosphate accumulates due to defective conversion to citrulline. **Allopurinol** compete with orotic acid for the enzyme orotate phosphoribosoyl transferase, leading to orotic aciduria & orotidinuria.

Nucleoprotein metabolism

Catabolism of pyrimidine nucleotides:

- Uracil & thymine are degraded by analogous reactions. The phosphate is removed from nucleotide → corresponding nucleoside
- In the next step, free base is released, the ring is open
- Finally, β amino isobutyric acid or β alanine are formed.
- These are the end product of pyrimidines. Other products are carbon dioxide and ammonia. Pseudouridine is not metabolized further, and is excreted as such in urine.