Clinical Biochemistry department/ College of medicine

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Dr. Ali al-bayati **NUCLEOTIDE METABOLISM** **Lec. 2**

Purine and pyrimidine biosynthesis

The nucleic acids basic structure are derived from the aromatic heterocyclic compounds ***purine*** and ***pyrimidine*** .The biosynthesis of these molecules is complex, but is vital for almost all cells.

The demand for nucleotide biosynthesis can vary greatly. It is high during the S phase of the cell cycle, when cells are about to divide. The process is very active in rapidly growing tissues, actively proliferating cells like blood cells and cancer cells, and when tissues are regenerating. Purine and pyrimidine biosynthesis processes need high amount of energy and are subjected to cellular control regarding the availability of intermediates.

The importance of nucleotides in cellular metabolism is indicated by the observation that nearly all cells can synthesize them both ***de novo (anew)*** and from the degradation products of nucleic acids ***(salvage pathways).*** However, unlike carbohydrates, amino acids, and fatty acids, nucleotides do not provide a significant source of metabolic energy.

De novo **synthesis** refers to the synthesis of complex molecules from simple molecules such as sugars or amino acids which is required in growing cells.

Salvage pathways that recycle preformed bases and nucleosides and provide an adequate supply of nucleotides for cells at rest.

Purines are made separately from Pyrimidines. In case of purine synthesis, purine ring is assembled on ribose sugar while in the Pyrimidines the ring is synthesized first and then added to the ribose.

The de novo synthesis of purine ribonucleotides:

The major site of purine synthesis is in the liver. Synthesis of the purine molecule is complex. The raw materials for purine synthesis are: CO2, nonessential amino acids (Asp, Glu, Gly), and folic acid derivatives which act as **single carbon donors**. Five molecules of

ATP are needed for the synthesis of IMP, the first purine produced and it is the common precursor of AMP and GMP.

The raw materials for purine synthesis come from as the following

The starting material for synthesis of purine is **ribose 5-phosphate** (pentose sugar), comes from the pentose phosphate pathway of glucose metabolism.

Origin of the atoms in purine ring

Three of the ring atoms of the purines are derived from glycine, which donates C-4, C-5, and N- 7. All of the other atoms in the ring are incorporated individually. C-6 comes from HCO_3. Amide groups from *glutamine (yielding glutamate)* provide the atoms N-3 and N-9. The amino group donor for N-1 is *aspartate*, which is converted into fumarate in the process. One carbon in the purine ring comes from carbon dioxide .Finally; the two carbon atoms in the purine structure (C-2 and C-8) are derived from formyl groups in N10- formyl-tetrahydrofolate, which is one reason why tetrahydrofolate (THF) is so important for nucleotide biosynthesis.

In 1948 "John Buchanor" obtains the first clues as to how this process occurs ***de novo*** by feeding a variety of isotopically labeled compounds to pigeons and chemically determining the position of the labeled atoms in their excreted Uric acid. The results of the studies are Purine synthesis.

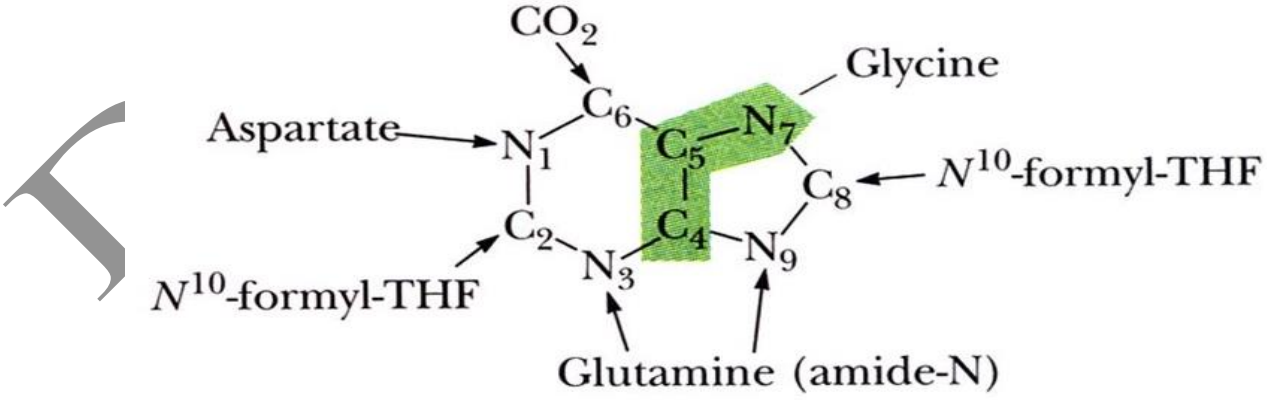

Figure 1: the metabolic origin of the atoms in purine ring.

In this De novo synthesis of purines, each atom in the purine nucleotide came from different sources as mentioned above structure and data. There are 3 major steps are involved in this Purine synthesis pathway.

1. **Ribose-5-Phosphate to IMP synthesis**
2. **Synthesis of AMP from IMP**
3. **Synthesis of GMP from IMP**

Ribose-5-Phosphate to IMP synthesis

Step 1

The first step in synthesis of purine is a common step between both de novo and salvage pathways, achieved by generating an activated form of the pentose phosphate by transferring a pyrophosphate group from ATP to form 5-phosphoribosyl-pyrophosphate (PRPP) and this reaction is catalyzed by the enzyme PRPP synthase.

This enzyme shown to be extremely controled and feedback inhibited byavailability of PRPP concentration, and is also inhibited by each of IMP or AMP or GMP, as well as by combinations of these nucleotides.

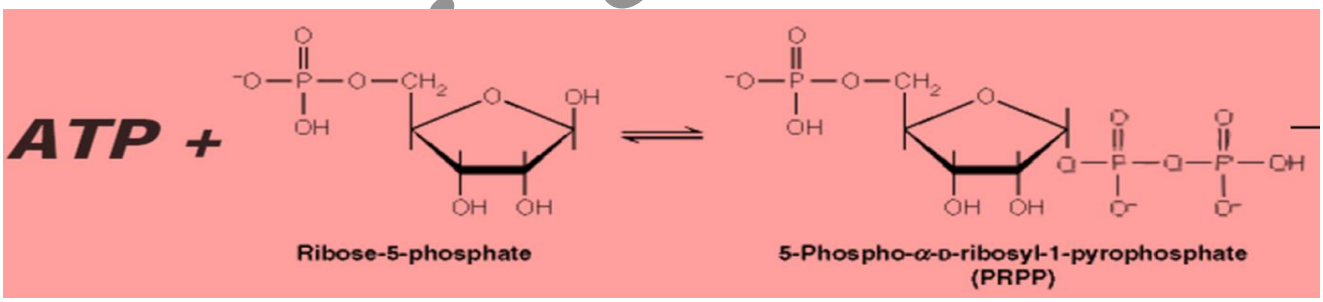

Figure 2: first step of purine synthesis by producing phosphoribosyl-pyrophosphate (PRPP).

The pathway for the de novo synthesis of purine ribonucleotides is started by donates of amide group from glutamine to carbon 1 of PRPP, forming the first nitrogen of the purine ring. The remaining atoms of the purine ring are added stepwise. Additional nitrogen atoms are derived from glycine, glutamate, and aspartate. Carbon atoms are donated by CO2

formyl-Folate. Completion of the base results in a purine nucleoside monophosphate, called inosine -monophosphate **(IMP)**.

IMP is the purine nucleoside monophosphate from which both adenosine monophosphate (AMP) and guanosine monophosphate (GMP) are formed.

1-In the first step of the pathway, an amino group donated by glutamine is attached at C-1 of PRPP. The resulting 5-phosphoribosyl amine is highly unstable. The purine ring is subsequently built up on this structure.

2-The second step is the addition of three atoms from glycine. An ATP is consumed to activate the glycine carboxyl group(C-4, C-5 and N-7).

3-Then formylated by N10- formyl tetrahydrofolate(C-8).

4-Nitrogen is contributed by glutamine (N-9).

5-Dehydration and ring closure yield the five-member **imidazole ring** of the purine nucleus, as 5-aminoimidazole ribonucleotide.

6- Three of the six atoms needed for the second ring in the purine structure are in imidazole ring. To complete the process, a carboxyl group is first added.

7-Aspartate now donates its amino group in two steps formation of an amide bond (N-1), followed by elimination of the carbon skeleton of aspartate (as fumarate).

8-The final carbon(C-6) is contributed by N10-formyltetrahydrofolate and a second ring closure takes place to yield the second fused ring of the purine nucleus.

The first intermediate with a complete purine ring is **inosine monophosphate (IMP).** . This nucleotide does not accumulate, but is rapidly converted into AMP and GMP and contains the base hypoxanthine.

Notes:

1- In biosynthetic reactions, free ammonium is not used as a nitrogen donor in the pathway; the organic nitrogen is obtained from the amino acid glutamine, glycine, and aspartate.

2- Folate cofactors utilized as 1-carbon carriers in two parts of nucleotide biosynthesis, while water-soluble **vitamin B12 (cobalamin)** plays a key role in the formation of active folate. The biologically active form of folate is a reduced derivative of folic acid **tetrahydrofolate** (H4F).

Factors effect purine production

Purine deficiencies in humans are due to:

- ❖ Inhibitors of enzymes in the pathway.
- ❖ Deficiencies in folic acid or Vitamin B12 (cobalamin), which is necessary for removing the methyl from N5-methyl tetrahydrofolate.
- ❖ -Inhibiting synthesis of folate by intestinal microorganisms then convert it to tetrahydrofolate. The antibiotics (sulfonamide drugs) work by inhibiting folate biosynthesis by killing the bacteria without affecting the host. Sulfonamides are analogs of p-aminobenzoic acid, a component of folic acid.

AMP and GMP synthesis

Conversion of inosine monophosphate (IMP) to adenosine monophosphate (AMP) requires the insertion of an **amino group** derived from **aspartate**; this takes place in two reactions similar to those used to introduce N-1 of the purine ring.

Guanosine monophosphate (GMP) is formed by the oxidation of inosine monophosphate at C-2, followed by addition of an **amino group** derived from **glutamine**.

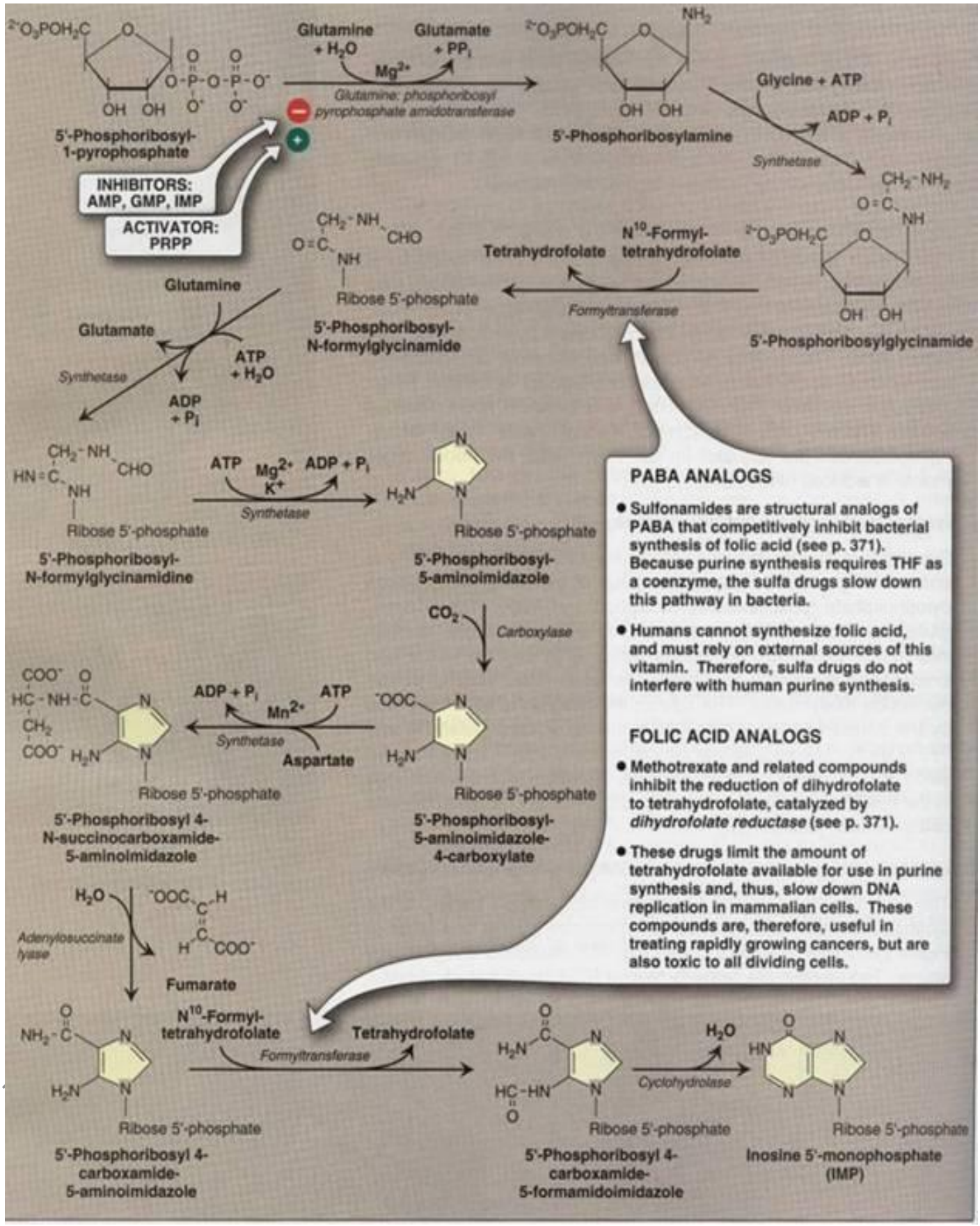

Figure 3: Scheme of de novo pathway of purine synthesis.

Control of purine biosynthesis

Purine Nucleotide Biosynthesis Is Regulated by Feedback Control

Nucleotide Metabolism Uses Allosteric Controls to Balance Amounts of Nucleotides. Three major feedback mechanisms cooperate in regulating the overall rate of de novo purine nucleotide synthesis and the relative rates of formation of the two end products, adenylate and guanylate (Figure 4).

The first of these control mechanisms is exerted on the first reaction that is unique to purine synthesis-the transfer of an amino group to PRPP to form 5-phosphoribosylamine. This reaction is catalyzed by the allosteric enzyme glutamine-PRPP amidotransferase, which is inhibited by the end products IMP, AMP, and GMP. These same nucleotides inhibit the synthesis of PRPP from ribose phosphate by ribose phosphate pyrophosphokinase. AMP and GMP act synergistically in this inhibition. Thus, whenever either AMP or GMP accumulates to excess, the first step in its biosynthesis from PRPP is partially inhibited.

In the second control mechanism, exerted at a later stage, an excess of GMP in the cell inhibits formation of xanthine from inosinate by IMP dehydrogenase, without affecting the formation of AMP. Conversely, an accumulation of adenylate results in inhibition of formation of adenylosuccinate by adenylosuccinate synthetase, without affecting the biosynthesis of GMP.

In the third mechanism, GTP is required in the conversion of IMP to AMP, whereas ATP is required to form GMP from IMP, a reciprocal arrangement that tends to balance synthesis of the two ribonucleotides.

- Feedback inhibition in purine nucleotide biosynthesis

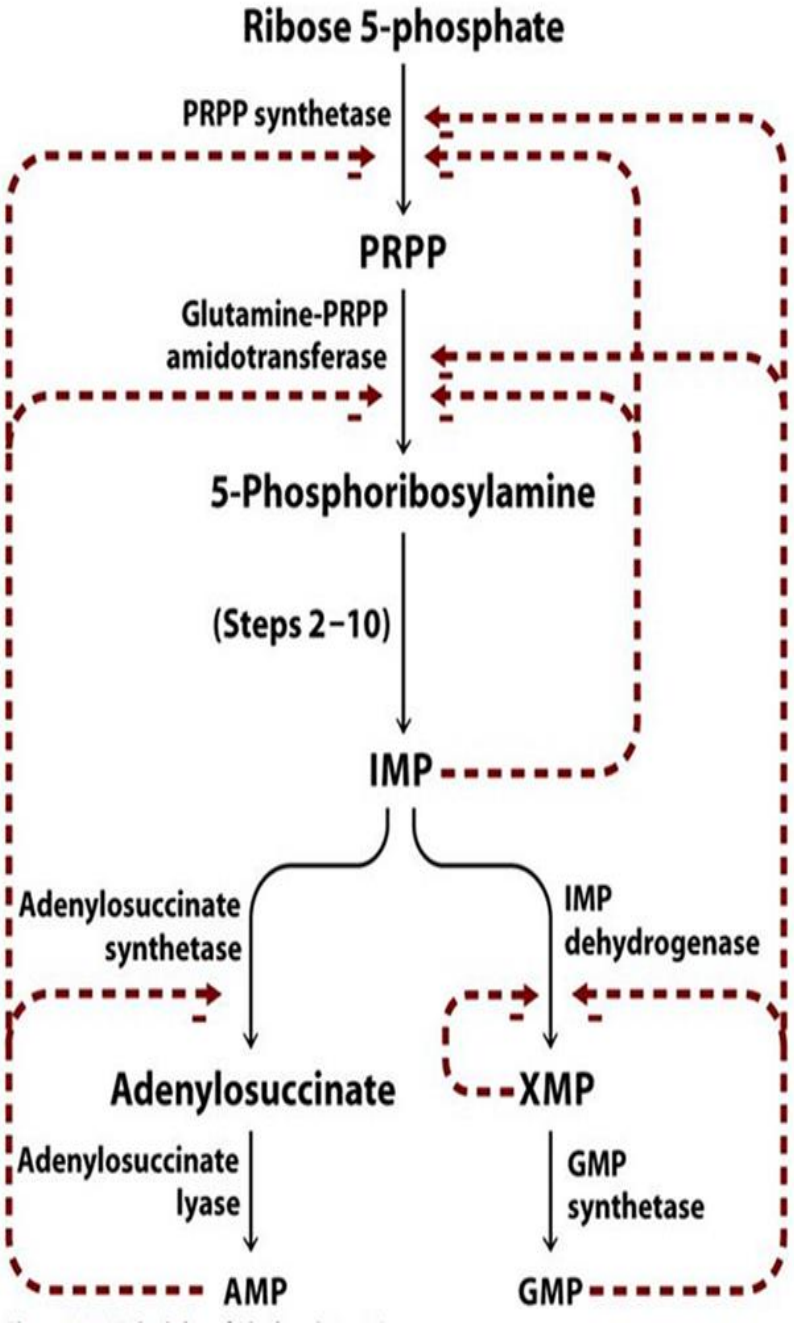

Figure 4: Allosteric Controls feedback inhibition of purine nucleotide iosynthesis.