In continuation of previous lecture

Formation of purine nucleoside diphosphates and triphosphates

The nucleoside monophosphates (AMP and GMP) have to be converted to the corresponding di- and triphosphates to participate in most of the metabolic reactions. This is achieved by the transfer of phosphate group from ATP, catalysed by nucleoside monophosphate (NMP) kinases (**Figure 3**).

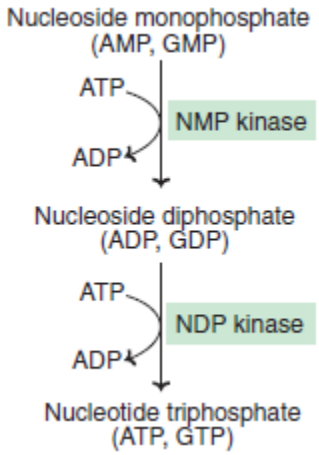

Figure 3: Conversion of nucleoside monophosphates to di- and triphosphates (NMP–Nucleoside monophosphate; NDP–Nucleoside diphosphate).

Salvage pathway for purines

The free purines (adenine, guanine and hypoxanthine) are formed in the normal turnover of nucleic acids (particularly RNA), and also obtained from the dietary sources. The **purines** can be **directly converted to the corresponding nucleotides**, and this process is known as 'salvage pathway' (**Figure 4**). Adenine phosphoribosyl transferase catalyses the formation of AMP from adenine. Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) converts guanine and hypoxanthine, respectively, to GMP and IMP. Phosphoribosyl pyrophosphate (PRPP) is the donor of ribose 5-phosphate in the salvage pathway. The salvage pathway is particularly important in certain tissues such as erythrocytes and brain where de novo (a new) synthesis of purine nucleotides is not operative.

Lesch-Nyhan syndrome

This disorder is due to the deficiency of **hypoxanthine-guanine phosphoribosyl transferase** (HGPRT), an enzyme of purine salvage pathway. It was first described in 1964 by

Michael Lesch (a medical student) and William L. Nyhan (his teacher). Lesch-Nyhan syndrome is a **sex-linked metabolic disorder** since the structural gene for HGPRT is located on the X-chromosome. It **affects** only the **males** and is characterized by excessive uric acid production (often gouty arthritis), and **neurological abnormalities** such as mental retardation, aggressive behavior, learning disability etc. The patients of this disorder have an irresistible urge to bite their fingers and lips, often causing self-mutilation. The overproduction of uric acid in Lesch-Nyhan syndrome is explained. HGPRT deficiency results in the accumulation of PRPP and decrease in GMP and IMP, ultimately leading to increased synthesis and degradation of purines. The biochemical basis for the neurological symptoms observed in Lesch-Nyhan syndrome is not clearly understood. This may be related to the dependence of brain on the salvage pathway for de novo synthesis of purine nucleotides.

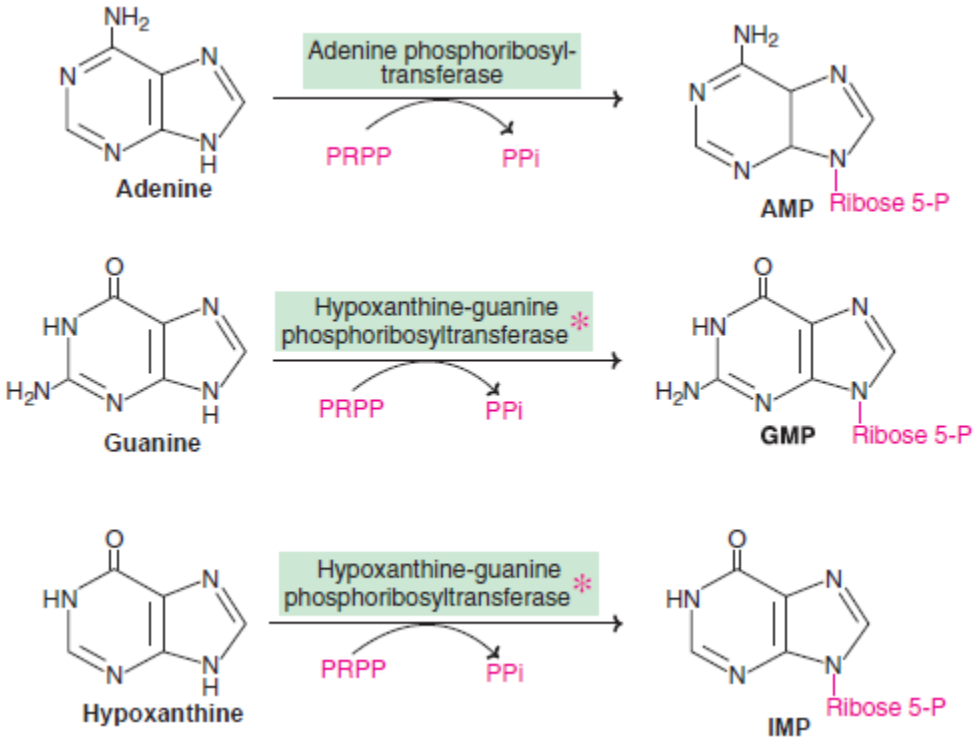

Figure 4: Salvage pathways of purine nucleotide synthesis (PRPP–Phosphoribosyl pyrophosphate; PPi–Inorganic pyrophosphate; AMP–Adenosine monophosphate; GMP–Guanosine monophosphate; IMP–Inosine monophosphate; * Deficiency of HGPRT causes Lesch-Nyhan syndrome).

Regulation of purine nucleotide biosynthesis

The purine nucleotide synthesis is well coordinated to meet the cellular demands. The intracellular concentration of **PRPP** regulates purine synthesis to a large extent. This, in turn, is dependent on the availability of ribose 5-phosphate and the enzyme PRPP synthetase. PRPP glutamyl amidotransferase is controlled by a **feedback mechanism** by purine nucleotides. That is, if AMP and GMP are available in adequate amounts to meet the cellular requirements, their synthesis is turned off at the **amidotransferase** reaction (Reaction 2 of figure 2). Another important stage of regulation is in the conversion of IMP to AMP and GMP. AMP inhibits adenylsuccinate synthetase while GMP inhibits IMP dehydrogenase. Thus, AMP and GMP control their respective synthesis from IMP by a feedback mechanism.

Conversion of ribonucleotides to deoxyribonucleotides

The synthesis of purine and pyrimidine deoxyribonucleotides occurs from ribonucleotides by a reduction at the C2 of ribose moiety (**Figure 5**). This reaction is catalysed by a multisubunit (two B1 and two B2 subunits) enzyme, **ribonucleotide reductase**.

Supply of reducing equivalents : The enzyme ribonucleotide reductase itself provides the hydrogen atoms needed for reduction from its sulfhydryl groups. The reducing equivalents, in turn, are supplied by **thioredoxin**, a monomeric protein with two cysteine residues. NADPH dependent thioredoxin reductase converts the oxidized thioredoxin to reduced form which can be recycled again and again.

Regulation of deoxyribonucleotide synthesis :

Deoxyribonucleotides are mostly required for the synthesis of DNA. The activity of the enzyme ribonucleotide reductase maintains the adequate supply of deoxyribonucleotides. The drug **hydroxyurea** inhibits ribonucleotide reductase by destroying free radicals required by this enzyme. Hydroxyurea is used in the **treatment of cancers** such as chronic myologenous leukemia.

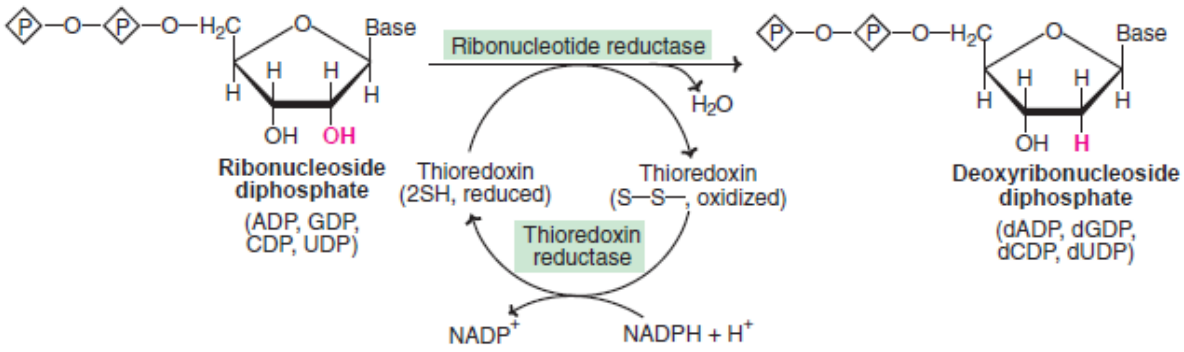

Figure 5: Formation of deoxyribonucleotides from ribonucleotides.

Degradation of Purine nucleotides:

The **end product** of purine metabolism in humans is **uric acid**. The sequence of reactions in purine nucleotide degradation is given in **Figure 6.**

1. The nucleotide monophosphates (AMP, IMP and GMP) are converted to their respective nucleoside forms (adenosine, inosine and guanosine) by the action of **nucleotidase**.
2. The amino group, either from AMP or adenosine, can be removed to produce IMP or inosine, respectively.
3. Inosine and guanosine are, respectively, converted to hypoxanthine and guanine (purine bases) by purine nucleoside phosphorylase. Adenosine is not degraded by this enzyme, hence it has to be converted to inosine.
4. Guanine undergoes deamination by guanase to form **xanthine**.
5. **Xanthine oxidase** is an important enzyme that converts hypoxanthine to xanthine, and xanthine to uric acid. This enzyme contains FAD, molybdenum and iron, and is exclusively found in liver and small intestine. Xanthine oxidase liberates H_2O_2 which is harmful to the tissues. Catalase cleaves H_2O_2 to H_2O and O_2.

Uric acid (2,6,8-trioxypurine) is the final excretory product of purine metabolism in humans. Uric acid can serve as an important **antioxidant** by getting itself converted (nonenzymatically) to allantoin. It is believed that the antioxidant role of ascorbic acid in primates is replaced by uric acid, since these animals have lost the ability to synthesize ascorbic acid. Most animals (other than primates) however, oxidize uric acid by the enzyme uricase to allantoin, where the purine ring is cleaved. Allantoin is then converted to allantoic acid and

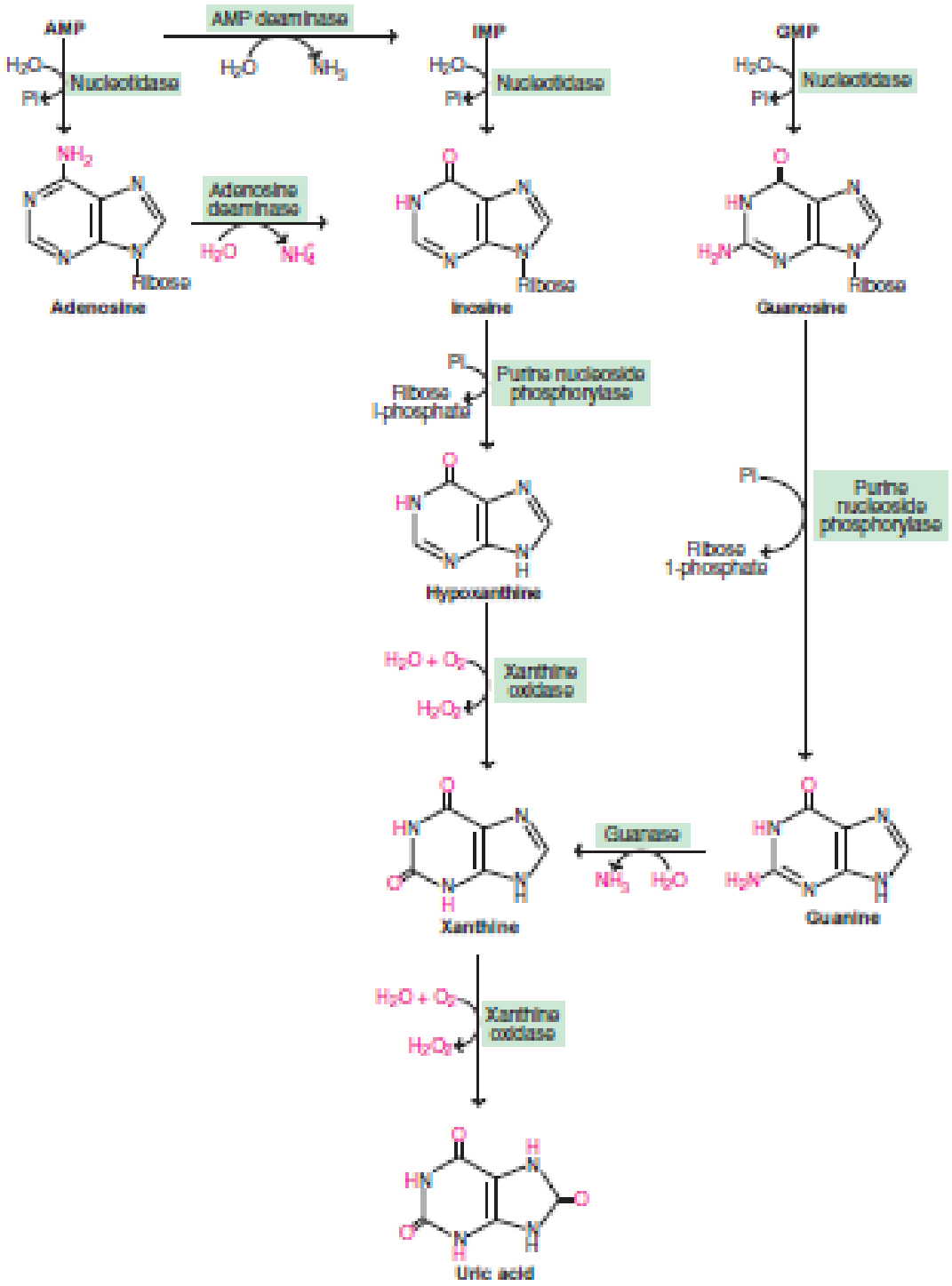

Figure 6: Degradation of purine nucleotides to uric acid (AMP–Adenosine monophosphate; IMP–Inosine monophosphate; GMP–Guanosine monophosphate).

excreted in some fishes (**Figure 7**). Further degradation of allantoic acid may occur to produce urea (in amphibians, most fishes and some molluscs) and, later, to ammonia (in marine invertebrates).

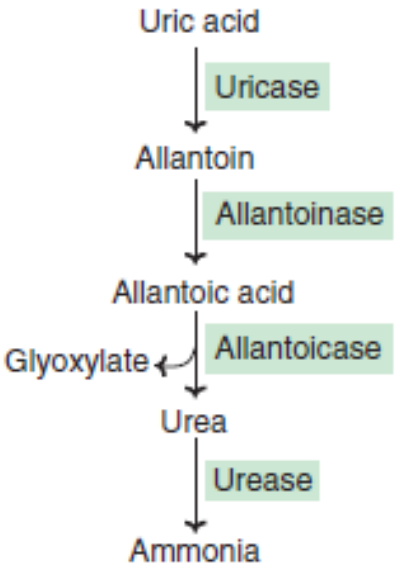

Figure7: Degradation of uric acid in animals other than man.