بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Metabolism | FINAL 20

Nucleotide Metabolism

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وَلِلَّهِ الْأَسْمَاءُ الْحُسْنَى فَادْعُوهُ بِهَا

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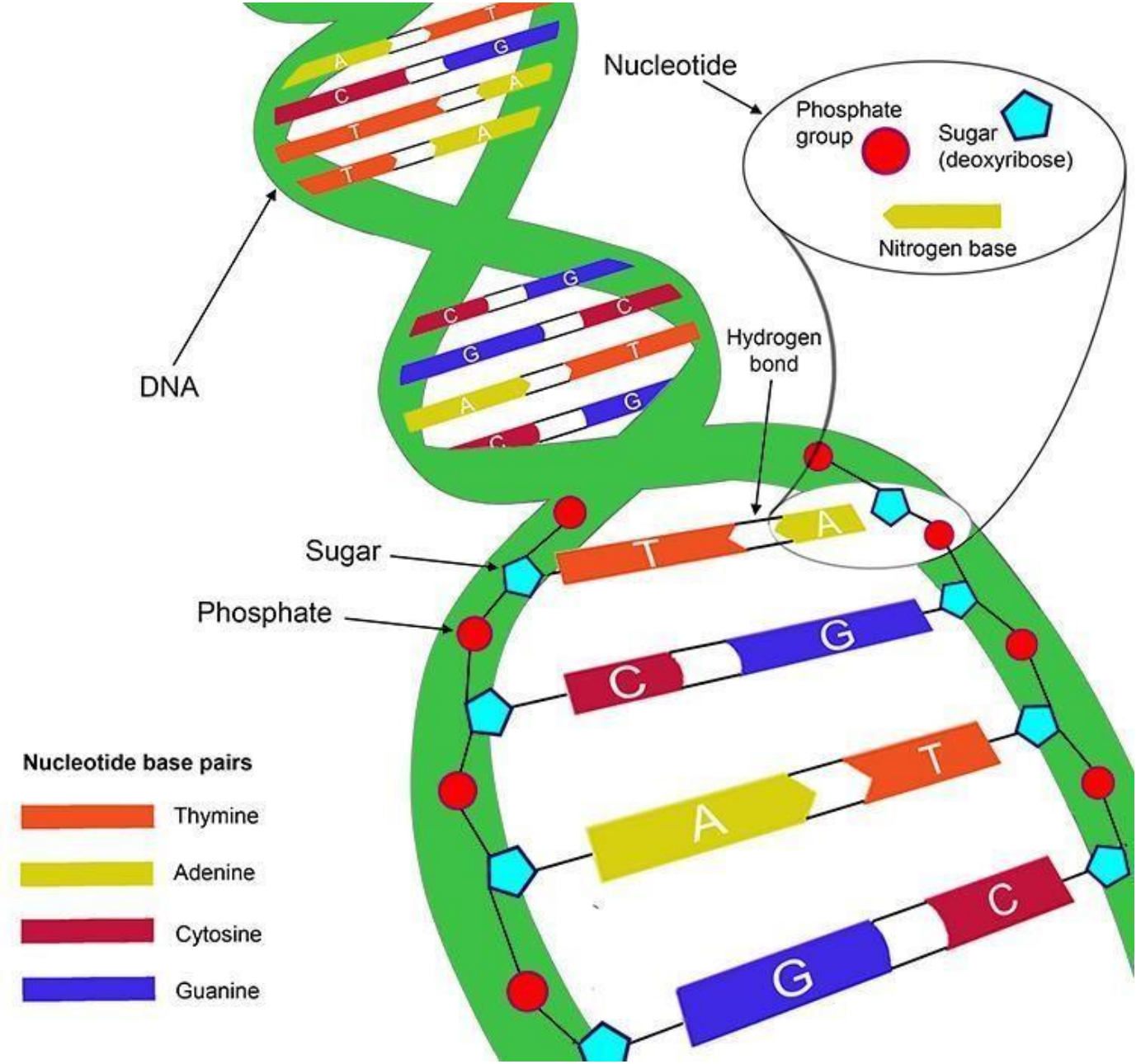
اضغط هنا لشرحٍ أكثر تفصيلًا

Nucleotide Metabolism

Dr. Diala Abu-Hassan, DDS, PhD

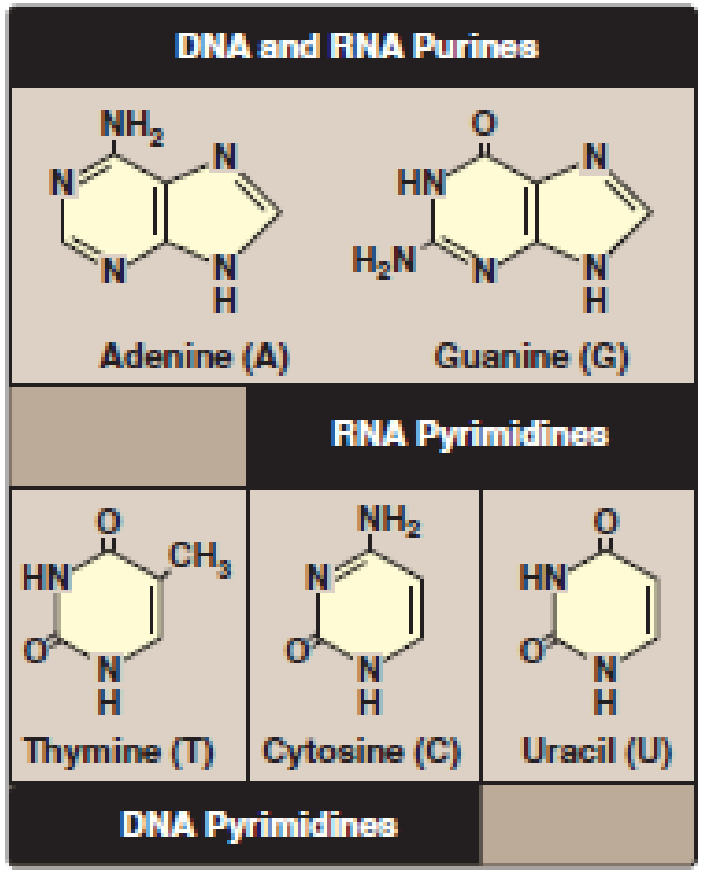


(mammothmemory.net)

All images were taken from Lippincott's Biochemistry textbook except where noted

Purine and pyrimidine structures and roles

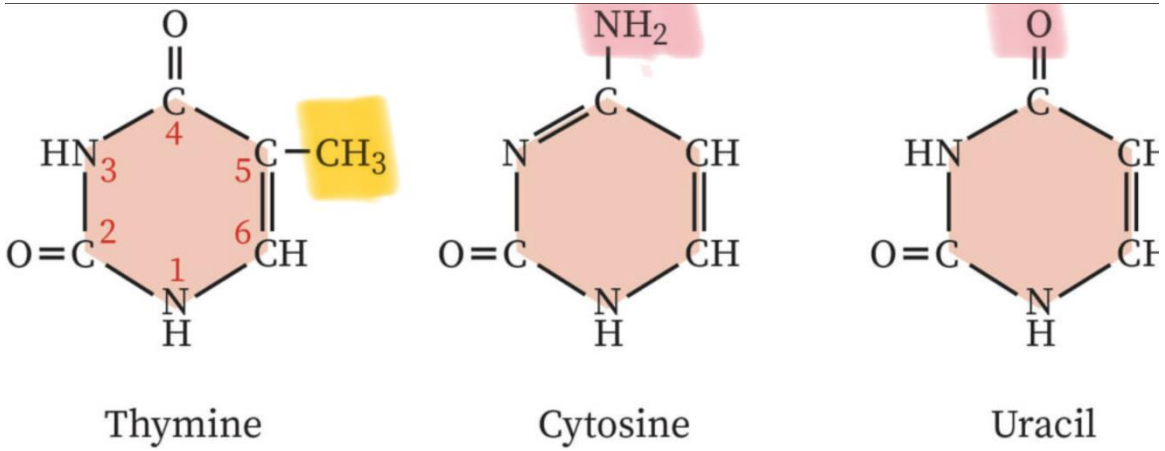
- Essential for RNA and DNA synthesis.
- They serve as carriers of activated intermediates in the synthesis of some carbohydrates, lipids, and conjugated proteins, such as, UDP-glucose and CDP-choline
- They are structural components of several essential coenzymes, such as coenzyme A, FAD, NAD+, and NADP+. Electron carrier
- They serve as second messengers in signal transduction pathways, such as cAMP and cGMP
- They are "energy currency" in the cell.
- They act as regulatory compounds for many metabolic pathways by inhibiting or activating key enzymes.



Notes on the Previous Slide

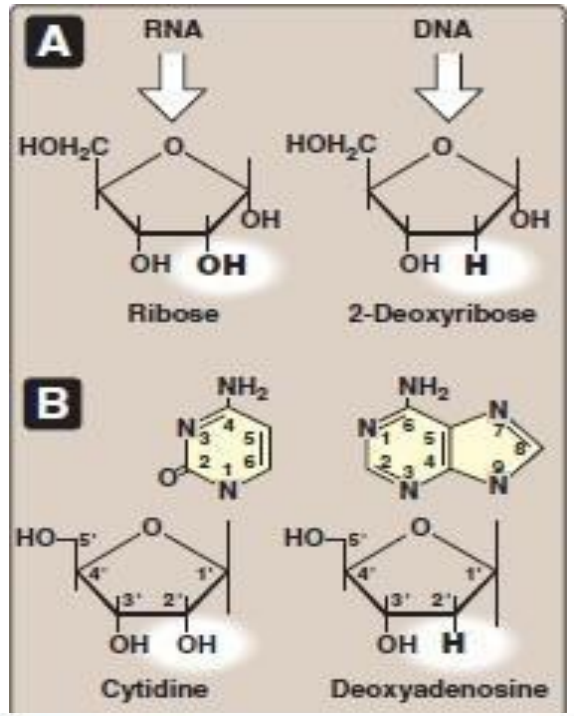
- Nucleotides are distinct molecules but are considered derivatives of amino acids because they are nitrogen-containing compounds and are synthesized predominantly from amino acids.
- They can be classified based on their structure as purines and pyrimidines. Purines have a two-ring structure, consisting of a five-membered ring fused to a six-membered ring, and include adenine and guanine. Pyrimidines have a single six-membered ring and include cytosine, uracil, and thymine.
- Nucleotides perform a wide variety of functions, including DNA and RNA synthesis, serving as energy carriers (e.g., ATP, GTP), and acting as second messengers (e.g., cAMP, cGMP). Electron carriers such as NAD and FAD contain nucleotide components within their structure. Nucleotides also participate in reactions as carriers, such as UDP with glucose and glucuronic acid, and CDP (**C**ytosine **Dip**hosphate) with lipids. They act as allosteric regulators of enzymatic activity, such as ATP and AMP, which modulate specific enzymes by either activating or inhibiting them.
- The uracil is considered a parent (precursor) for pyrimidines and it's the smallest one ,which can be modified to form cytosine and thymine .

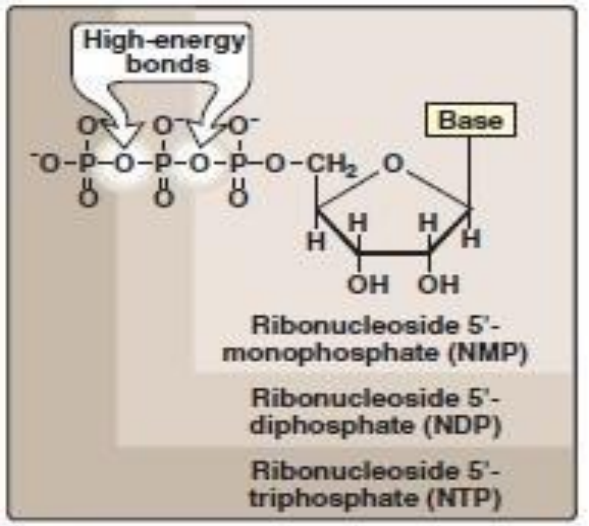
- The difference between uracil and thymine is the presence of a methyl group in thymine.
- The difference between uracil and cytosine is that cytosine has an amino group instead of an oxygen.
- The sugar serves as a attachment point between the nitrogenous base and the phosphate groups.



Nucleosides vs Nucleotides

- ✓ Nucleoside= Pentose sugar + Base
- ✓ Ribose + base = Ribonucleoside (adenosine, guanosine, cytidine, and uridine)
- ✓ 2-deoxyribose + base = deoxyribonucleoside (deoxyadenosine, deoxyguanosine, deoxycytidine, and deoxythymidine)
- ✓ Nucleoside + one or more phosphate groups = Nucleotide
- ✓ The first P group is attached by an ester linkage to the 5'-OH of the pentose forming a nucleoside 5'-phosphate or a 5'-nucleotide.
- ✓ The second and third phosphates are each connected to the nucleotide by a "high-energy" bond.
- ✓ The phosphate groups are negatively charged causing DNA and RNA to be nucleic acids.





Notes on the Previous Slide

- In nucleotide structure, the nitrogenous base is bonded to a pentose sugar, forming a nucleoside. When one, two, or three phosphate groups are added to the nucleoside, it becomes a nucleotide.
- To distinguish between nucleotides with different numbers of phosphate groups, they are named by the nucleoside followed by the number of phosphates, such as **adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP).** These names specify the nucleotide and its phosphate count.
- If the nucleotide is found in RNA or other structures like energy molecules, **a ribose sugar is present. In DNA, a deoxyribose sugar is used ,** Deoxyribonucleotides are found only in DNA and are produced by reduction of ribonucleotides to supply the precursors required for DNA synthesis during replication.
- In a nucleotide, the phosphate group is attached to the **5' carbon of the sugar,** and the nitrogenous base is attached to the **1' carbon.** Each component— nitrogenous base, sugar, and phosphate—plays a critical role in nucleotide function.

Sources of purines and pyrimidines

- The purine and pyrimidine bases can be synthesized de novo
- Or can be obtained through salvage pathways (reuse of the preformed bases resulting from normal cell turnover).
- Little of the purines and pyrimidines supplied by diet are utilized, and are degraded instead

Notes on the Previous Slide

- Nucleotides can be obtained from the diet, but this is not the primary source for most cells. Instead, cells depend mainly on their own synthetic pathways.
- Nucleotide synthesis can occur via the de novo pathway, where nucleotides are synthesized from simple precursor molecules, or through the salvage pathway, where pre- existing nitrogenous bases, nucleosides, or nucleotide fragments are recycled to form nucleotides, eliminating the need to synthesize the bases from scratch.

Purine synthesis- the contributing compounds

The atoms of the purine ring are contributed by a number of compounds:

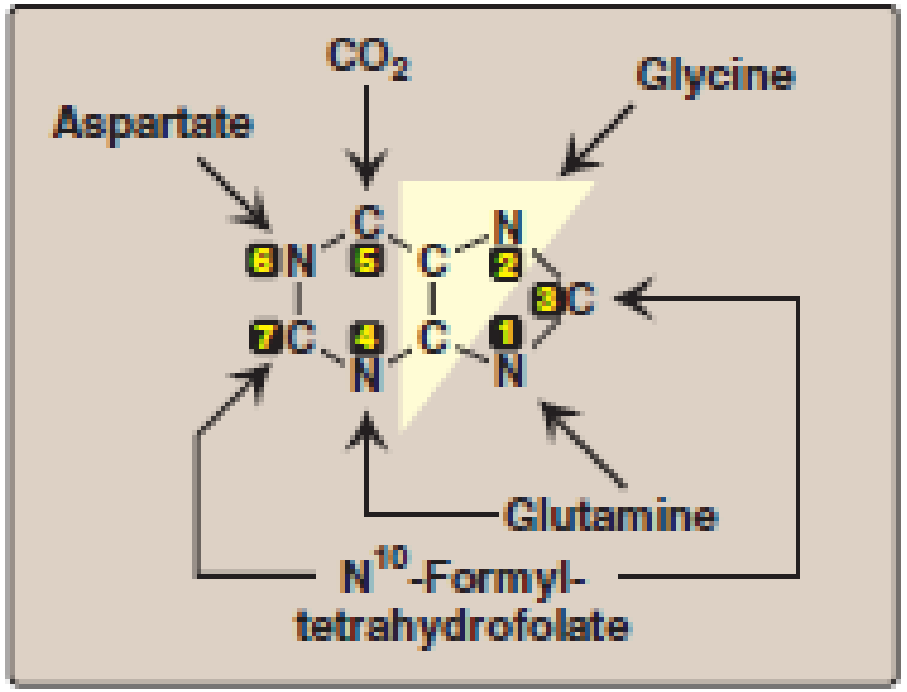
1. Amino acids (aspartic acid, glycine, and glutamine)

2. CO2

3. N10–formyltetrahydrofolate

The purine ring is constructed primarily in the liver by a series of reactions that add the donated carbons and nitrogens to a preformed ribose 5-phosphate.

Ribose 5-phosphate is synthesized by the pentose phosphate pathway



Sources of the individual atoms in the purine ring. The order in which the atoms are added is shown by the numbers in the black boxes.

Further Explanation of the Previous Slide

- Purines are composed of two fused rings: one six-membered and one five-membered. The structure contains a total of **nine atoms**, with **two atoms shared between the rings.** These atoms are derived from various sources: all nitrogens come from amino acids such as **glutamine, glycine , and aspartate**, while carbons are contributed by **CO_2 (respiration process)** and **N10-formyl- tetrahydrofolate (a derivative of folic acid(B9)).**
- The synthesis of purine nitrogenous bases occurs primarily in the **liver**, and the **ribose 5-phosphate** required for nucleotide assembly is derived from the **pentose phosphate pathway (PPP).**

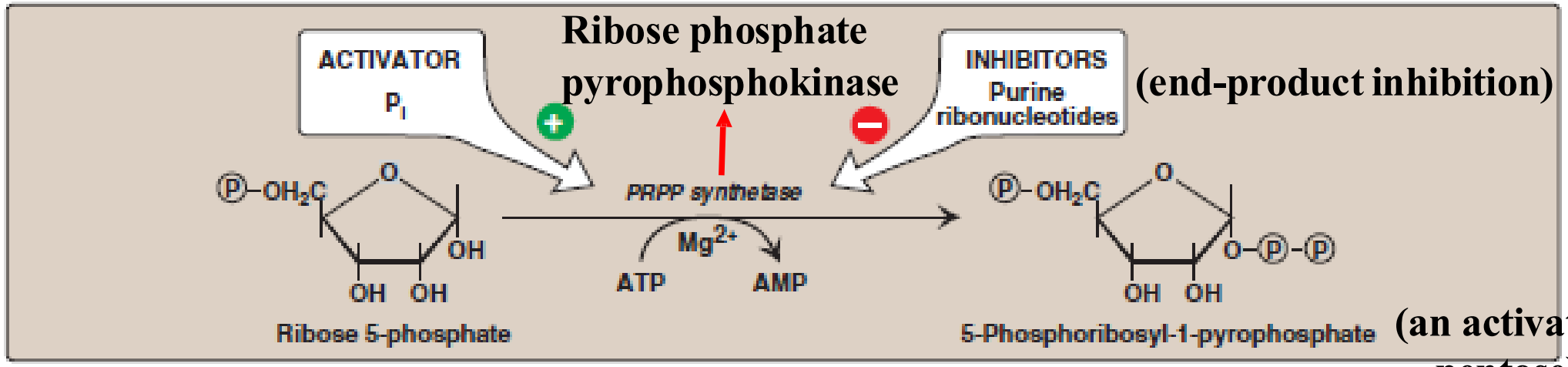
Note about N^{10}-formyl tetrahydrofolate (THF)

- Tetrahydrofolate (THF) is the active form of folic acid (vitamin B9), produced by reduction of folic acid via dihydrofolate reductase.
- THF functions as a single-carbon unit carrier.
- One-carbon units are carried on the N^5 and/or N^{10} positions of THF in different oxidation states, including:
- N^5-methyl-THF
- N^{10}-formyl-THF
- N^5,N^{10}-methylene-THF
- N^5,N^{10}-methenyl-THF
- Although THF is sometimes compared to S-adenosylmethionine (SAM), SAM transfers only methyl groups, whereas THF carries various one-carbon units.

The first step is important. For the remaining steps, we are not required to identify the exact origin of each carbon only a general understanding of the process is needed.

Synthesis of Purine Nucleotides In well-fed state

Steps:



1. Synthesis of 5-phosphoribosyl-1-pyrophosphate (PRPP)

The sugar moiety of PRPP is ribose, therefore, ribonucleotides are the end products of de novo purine synthesis.

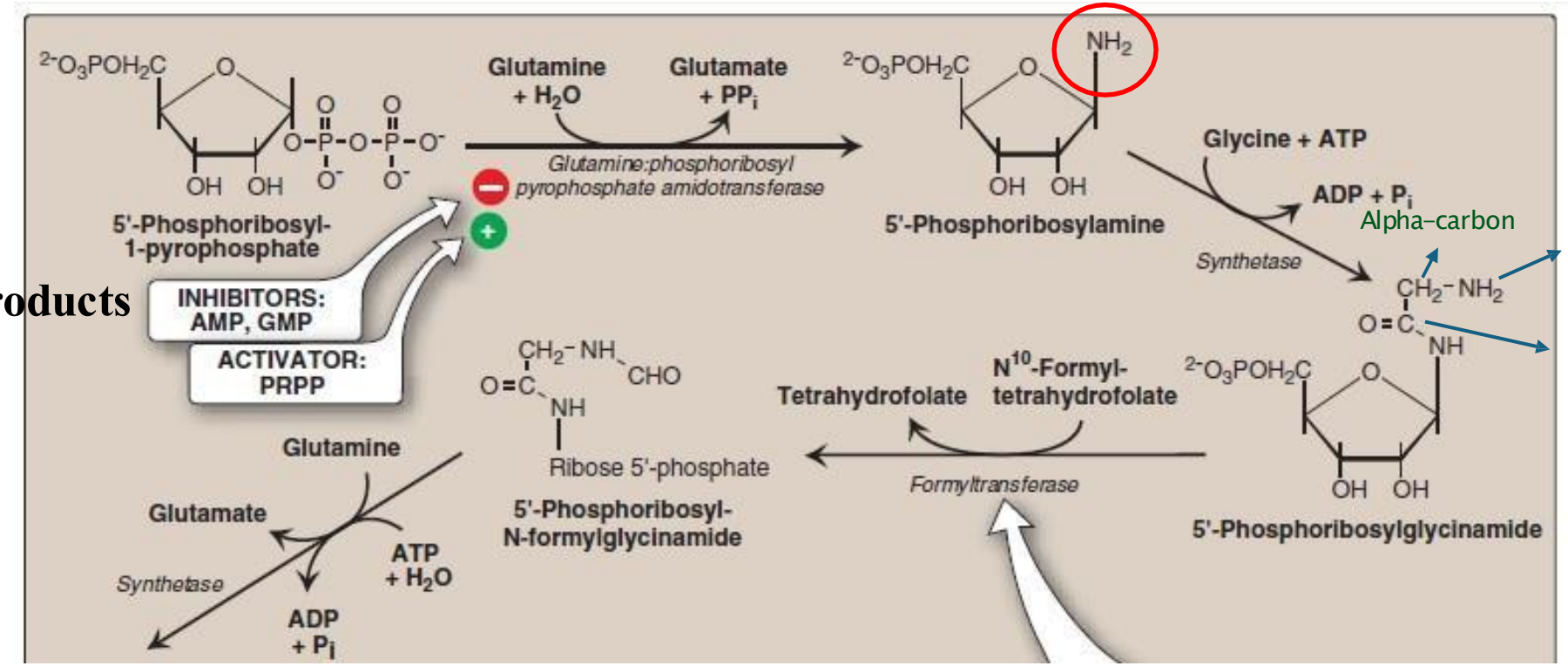
When deoxy ribonucleotides are required for DNA synthesis, the ribose sugar moiety is reduced

Explanation of Step One

- The phosphorylated sugar (ribose 5-phosphate) must be activated to initiate the synthesis of nitrogenous bases. Activation occurs through the addition of **pyrophosphate (PPi)** to **carbon 1** of the ribose, which is the same site where the nitrogenous base will later attach. This pyrophosphate is derived from **ATP, which is converted to AMP** during the reaction.
- The enzyme **PRPP synthetase (phosphoribosyl pyrophosphate synthetase | ribose phosphate pyrophosphokinase)** catalyzes this process, producing **5-phosphoribosyl-1-pyrophosphate (PRPP).** This step is not exclusive to purine synthesis; PRPP serves as a key intermediate in the synthesis of purines, pyrimidines, and other nucleotide-related pathways.

Synthesis of Purine Nucleotides

End products



Steps:

2. Synthesis of 5'-phosphoribosylamine (the committed step in purine nucleotide biosynthesis).

3. Synthesis of inosine monophosphate, the "parent" purine nucleotide

The next nine steps lead to the synthesis of IMP, whose base is hypoxanthine

This pathway requires ATP as an energy source.

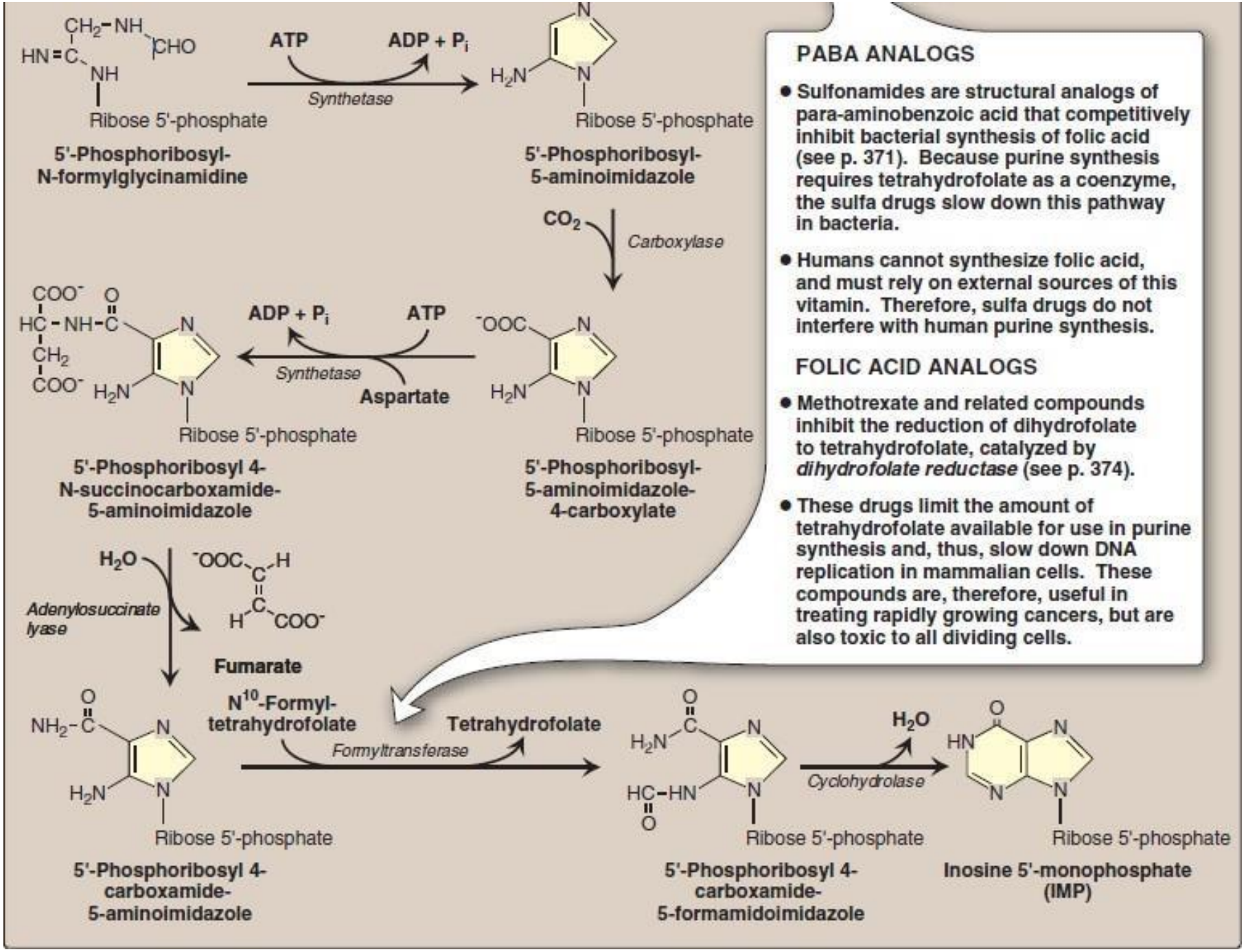
Explanation of the Previous Slide

- With **PRPP** activated, the synthesis of the nitrogenous base begins. The **pyrophosphate (PPi)** is removed during the addition of amine group from glutamine , as it is not part of the final nitrogenous base structure but serves only to activate the sugar (PRPP) for the subsequent reactions.
- After removing PPi, **glutamine** donates an amide group through the enzyme **glutamine:phosphoribosyl pyrophosphate amidotransferase,** forming **5'-phosphoribosylamine.** Next, **glycine** is added by a synthetase enzyme requiring ATP. Since glycine contributes multiple atoms to the purine ring, its entire structure attaches to the previous molecule, resulting in **5'-phosphoribosylglycinamide,** which contains four atoms of the first ring.

Explanation of the Previous Slide

- The next step involves **N10-formyl-tetrahydrofolate,** which donates a formyl group via a **formyl transferase enzyme,** releasing tetrahydrofolate and forming **5'-phosphoribosyl-N- formylglycinamide.** At this stage, the molecule has a five-atom tail attached to the **1' carbon** and is primed to form a five-membered ring. However, before the ring closes, **a second amino group is donated by glutamine** through a synthetase enzyme using ATP, and the five- membered ring is completed.
- Once the **five-membered ring** is closed, **the sixth atom, contributed by the amino group added from glutamine,** remains outside the ring. This step marks the beginning of the **six-membered ring synthesis,** producing the intermediate **5'-phosphoribosyl-5-aminoimidazole.**

Synthesis of Purine Nucleotides



PABA ANALOGS

- **Sulfonamides are structural analogs of para-aminobenzoic acid that competitively inhibit bacterial synthesis of folic acid (see p. 371). Because purine synthesis requires tetrahydrofolate as a coenzyme, the sulfa drugs slow down this pathway in bacteria.**
- **Humans cannot synthesize folic acid, and must rely on external sources of this vitamin. Therefore, sulfa drugs do not interfere with human purine synthesis.**

FOLIC ACID ANALOGS

- **Methotrexate and related compounds inhibit the reduction of dihydrofolate to tetrahydrofolate, catalyzed by *dihydrofolate reductase* (see p. 374).**
- **These drugs limit the amount of tetrahydrofolate available for use in purine synthesis and, thus, slow down DNA replication in mammalian cells. These compounds are, therefore, useful in treating rapidly growing cancers, but are also toxic to all dividing cells.**

Interpretation of the Illustration

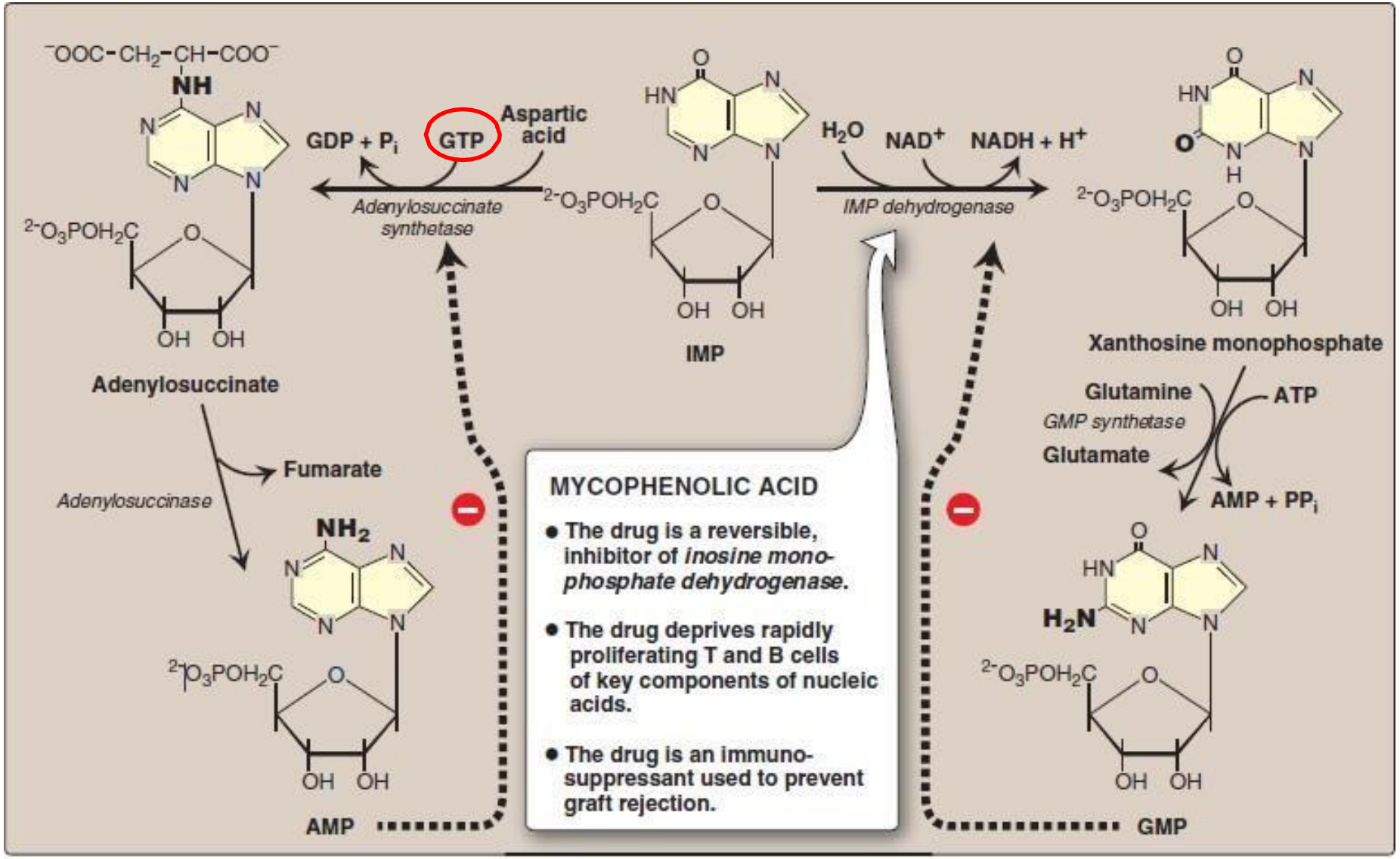
- After **5'-phosphoribosyl-5-aminoimidazole** is synthesized, a **CO_2 molecule** is added by a **carboxylase enzyme,** introducing a carboxyl group. This is the only step in purine synthesis that requires a carboxylase enzyme.
- To complete the six-membered ring, **aspartate** is added to the reaction by a **synthetase enzyme,** utilizing ATP hydrolysis for energy. Aspartate addition follows a distinct pattern: the entire aspartate molecule initially attaches, but only its **amino group** is incorporated into the ring, while the remaining portion is released as **fumarate** in the next step. This reaction produces **5'- phosphoribosyl-4-carboxyamide-5-aminoimidazole.**

Interpretation of the Illustration

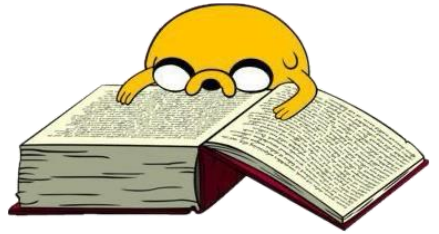
- Next, the last atom required to complete the six-membered ring is contributed by **N10-formyl-tetrahydrofolate.** A **formyl transferase enzyme** catalyzes the addition of a **formyl group (-CHO)** to the molecule. Finally, the ring is closed by a **cyclohydrolase enzyme** through a dehydration reaction, forming the first cyclic purine nitrogenous base, **inosine monophosphate (IMP).**

all the names of the intermediates and enzymes are important

Synthesis of Purine Nucleotides
4. Conversion of IMP to AMP and GMP



Interpretation of the Illustration



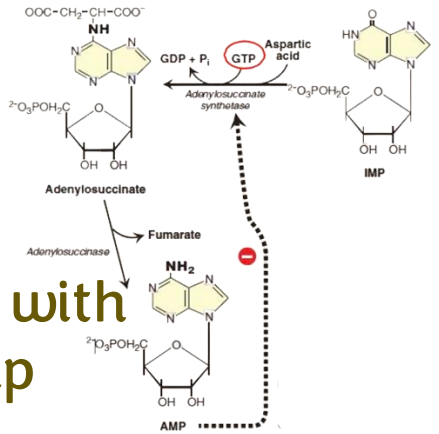
·IMP (inosine monophosphate) is a critical branching point in purine nucleotide synthesis, serving as a precursor for both **GMP (guanosine monophosphate)** and **AMP (adenosine monophosphate).** The key difference between the conversion of IMP to GMP or AMP lies in the addition of an amino group, which occurs at different positions on the nitrogenous base.

To form GMP, **IMP dehydrogenase** catalyze this oxidation reduction add this oxygen atom to form a **carbonyl group,** and reduce **NAD^+ to NADH** ,preducing **xanthosine monophosphate (XMP)** (It's considered as nucleotide,but not for DNA or RNA),

· In the Next step the **carbonyl group is replaced by an amino group,** donated by **glutamine** through the action of **GMP synthetase.** This reaction requires **ATP** as an energy source. The final product is GMP.

The synthesis of GMP is tightly regulated by feedback inhibition. A high concentration of **GMP** inhibits **IMP dehydrogenase,** the enzyme catalyzing the first step of GMP synthesis.

Interpretation of the Illustration



- For AMP synthesis, a different set of enzymes is involved. The process begins with adenylosuccinate synthetase, which uses aspartate as a source for amino group (aspartic acid in the illustration) as a substrate.

In the next step, The whole structure will be added to IMP producing adenylosuccinate, and then remove fumarate by (adenylosuccinate lysis) leaving amino group to producing AMP. This enzyme require energy in the form GTP rather than ATP Why? **Because, adenine is still being synthesized, so the cell needs adenine itself, not ATP as an energy source. Therefore, a different form of energy (GTP) is used)** .

Similarly, ATP is used in GMP synthesis because guanine is still being synthesized.

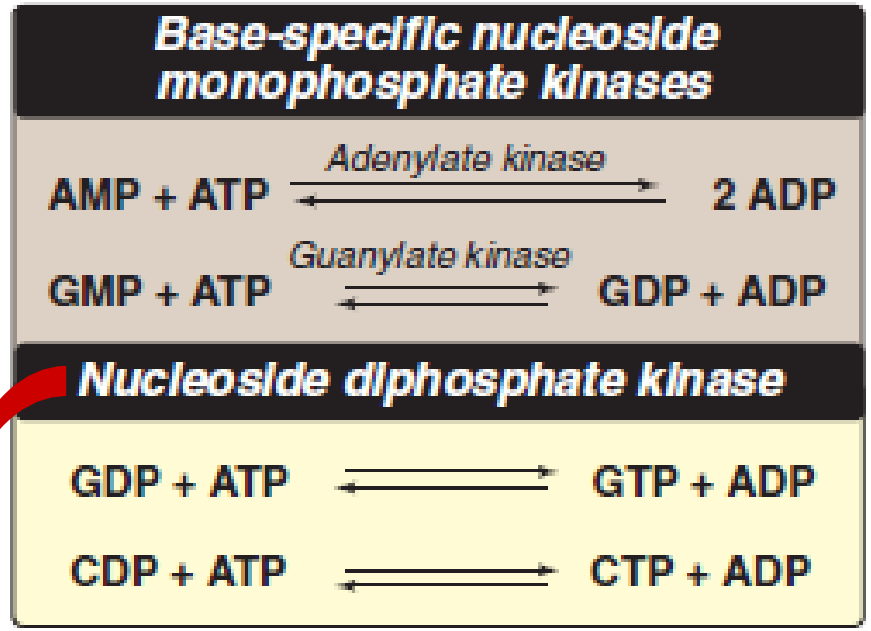
(Remember: The source of amino group in adenine is aspertic acids and the source for guanosine is glutamic acid, Guanine synthesis uses ATP, whereas adenine synthesis uses GTP).

Regulation

- The first step of purine nucleotide synthesis, catalyzed by **PRPP synthetase,** is highly regulated. This enzyme is **activated by inorganic phosphate (Pi),** with high levels of Pi signaling the need for purine nucleotide synthesis. Conversely, when **purine nucleotides (AMP and GMP)** are present in high concentrations, they inhibit **PRPP synthetase** through feedback inhibition, preventing excessive nucleotide production.
- The next key enzyme in the pathway, **glutamine: phosphoribosyl pyrophosphate amidotransferase,** which catalyzes the addition of the first nitrogenous atom to PRPP, is also tightly regulated. This enzyme is **activated by PRPP,** linking its activity to the availability of the activated sugar. However, like PRPP synthetase, amidotransferase is **inhibited by AMP and GMP** through feedback mechanisms, ensuring that purine nucleotide synthesis is balanced with cellular demand.

Synthesis of Purine Nucleotides

5. Conversion of nucleoside monophosphates to nucleoside diphosphates and triphosphates



Broad specificity not like the monophosphate kinases

Base-specific nucleoside monophosphate kinases do not discriminate between ribose or deoxyribose in the substrate

ATPis the general source of the phosphate, since it is present in higher concentrations than the other nucleoside triphosphates.

Adenylate kinase (AK) is particularly active in liver and muscle

AK maintains an equilibrium among AMP,ADP, and ATP

Detailed Explanation of Nucleotide Modifications: Phosphorylation

- Further modifications are required for nucleotides to fulfill various cellular functions. One key modification involves **reducing the sugar (ribose to deoxyribose)** by the enzyme **ribonucleotide reductase,** enabling the nucleotide to be incorporated into the DNA structure. Another critical modification is the **addition of phosphate groups** to produce nucleotides with varying phosphorylation states.
- The addition of a **second phosphate** to a nucleotide is catalyzed by **base-specific nucleoside monophosphate kinases.** For instance, **AMP** is phosphorylated by **adenylate kinase,** while **GMP** is phosphorylated by **guanylate kinase.** In these reactions, ATP donates a phosphate group, producing **two ADP molecules** during AMP phosphorylation, and produce **GDP and ADP** during GMP phosphorylation.
- The **third phosphate** is added by a non-specific enzyme (general enzyme) , **nucleoside diphosphate kinase,** which is not exclusive to purines or pyrimidines. This enzyme can phosphorylate molecules like **CDP or GDP** without distinguishing between them. Again, ATP serves as the phosphate donor, releasing **ADP** as a byproduct.
- These modifications produce nucleotides with different phosphorylation states, which are essential for various functions, including serving as **energy molecules (e.g., ATP, GTP)** or as substrates for specific biochemical processes requiring particular phosphorylated forms.

Application: Synthetic inhibitors of purine synthesis

Synthetic inhibitors of purine synthesis (the sulfonamides1), are designed to inhibit the growth of rapidly dividing microorganisms without interfering with human cell functions

Other purine synthesis inhibitors, such as structural analogs of folic acid (such as, methotrexate2), are used as drugs that control the spread of cancer by interfering with the synthesis of nucleotides and, therefore, of DNA and RNA.

Inhibitors of human purine synthesis are extremely toxic to tissues, especially to developing structures such as in a fetus, or to cell types that normally replicate rapidly, including those of bone marrow, skin, GI tract, immune system, or hair follicles.

Thus, anticancer drugs result in adverse effects, including anemia, scaly skin, GI tract disturbance, immunodeficiencies, and hair loss.

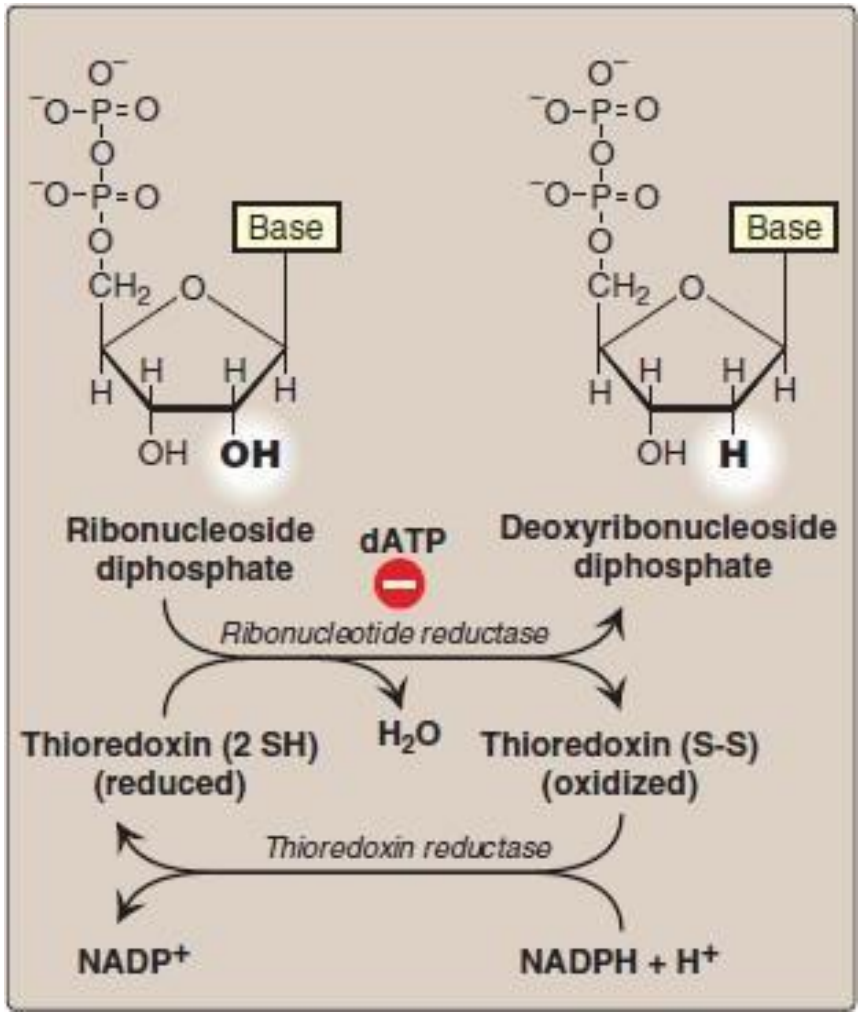
Impact of Purine Synthesis Inhibition on DNA Replication and Cancer Treatment

- Inhibition of **purine de novo synthesis** disrupts the production of two nucleotide types essential for DNA structure. As a result, **cell division is halted**, where DNA replication requires a substantial amount of nucleotides to double the DNA. If an adequate supply of **both purines and pyrimidines** is unavailable, DNA replication is hindered, leading to **cell cycle arrest** and cessation of cell division.
- This concept is central to the development of **anti-cancer drugs**, targeting the hallmark of cancer—**uncontrolled replication and division**—and thereby reducing cancer-associated abnormalities.
- **Methotrexate** is an example of such drugs, inhibiting both purine and pyrimidine synthesis. It acts as a **folic acid analogue**, inhibiting **dihydrofolate reductase**, which reduces the availability of tetrahydrofolate. This affects multiple steps in nucleotide synthesis, including two steps involving **N10- formyl-tetrahydrofolate** in purine synthesis.

Application of Purine Synthesis Inhibitors in Cancer Therapy and Antibiotics

- However, these drugs lack specificity for cancer cells, resulting in the inhibition of division in both cancerous and normal cells. Consequently, rapidly dividing normal cells, such as those in the **intestine, hair follicles, and immune system**, are also affected. For instance, **intestinal cells**, which are replenished approximately every three days, cease to divide, leading to significant side effects. Other side effects also include, **hair loss, immunosuppression, and gastrointestinal problems.** These adverse effects are characteristic of chemotherapy.
- Targeting cancer cells is challenging, because each histological type has unique markers, many of which are also present on normal cells
- A similar concept is employed in the development of **antibiotics** that target purine synthesis in microorganisms. **Sulfonamides** are an example of such antibiotics. These drugs inhibit **dihydropteroate synthase** in bacteria, which disrupts folate synthesis, thereby indirectly affecting purine production. As bacterial purine synthesis differs from that of humans, sulfonamides selectively inhibit bacterial pathways without interfering with human purine synthesis.

Synthesis of Deoxyribonucleotides



2'-deoxyribonucleotides are produced from ribonucleoside diphosphates by the enzyme ribonucleotide reductase during the S-phase of the cell cycle.

The same enzyme acts on pyrimidine ribonucleotides.

1. Ribonucleotide reductase (RR)

RR is specific for the reduction of:

A. Purine nucleoside diphosphates (ADP and GDP) to their deoxyforms (dADP and dGDP).

B. Pyrimidine nucleoside diphosphates, cytidine diphosphate (CDP) and uridine diphosphate (UDP) to their deoxyforms (dCDP, and dUDP).

2. Regeneration of reduced enzyme:

Thioredoxin—a peptide coenzyme of RR

3. Regeneration of reduced thioredoxin:

Thioredoxin must be converted back to its reduced form NADPH + H+ are needed

The reaction is catalyzed by thioredoxin reductase

Synthesis of Deoxyribonucleotides: Role of Ribonucleotide Reductase

- The **reduction of ribose** to deoxyribose is catalyzed by the enzyme **ribonucleotide reductase,** which specifically acts on **ribonucleoside diphosphates form** (e.g., ADP and CDP). This enzyme converts both **purines and pyrimidines** into their corresponding **deoxyribonucleosides.**
- As part of this **oxidation-reduction reaction,** the hydroxyl group (-OH) on the **second carbon** of ribose is removed as a water molecule (H_2O), reducing the sugar to deoxyribose. During this process, the protein **thioredoxin** is oxidized by ribonucleotide reductase, forming a **disulfide bridge.**
- To regenerate **thioredoxin** in its reduced form, the enzyme **thioredoxin reductase** reduces thioredoxin using electrons from **NADPH,** which is oxidized to $NADP^+$ in the process. This ensures the continuous activity of ribonucleotide reductase for deoxyribonucleotide production.
- It is important to note that **NADPH,** a key reducing agent in this pathway, is also widely used in other biosynthetic and antioxidant pathways in the cell (eg: cholesterol & lipids synthesis).

Regulation of deoxyribonucleotide synthesis

Ribonucleotide reductase is composed of two non identical dimeric subunits, R1 and R2

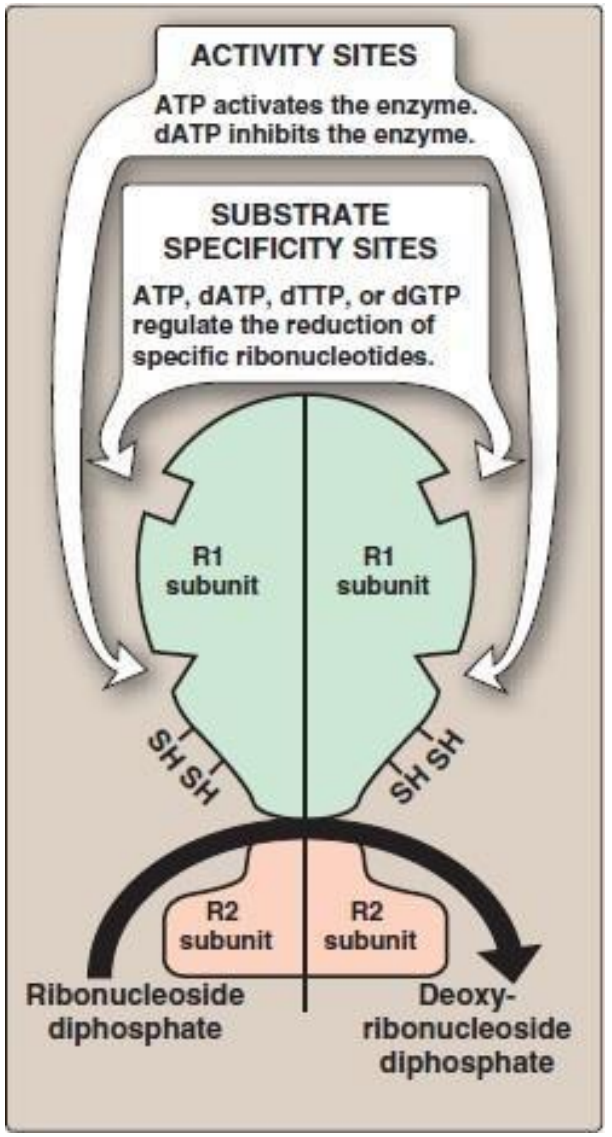
RR is responsible for maintaining a balanced supply of the deoxyribonucleotides required for DNA synthesis.

1. Activity sites (allosteric sites):

A. dATP inhibits the enzyme and prevents the reduction of any of the four nucleoside diphosphates resulting in preventing DNA synthesis.
B. ATP activates the enzyme.

2. Substrate specificity sites (allosteric sites):

Nucleoside triphosphates regulate substrate specificity, causing an increase in the conversion of different species of ribonucleotides to deoxyribonucleotides.
dTTP binding activates the reduction of GDP to dGDP at the catalytic site.



Regulation of Ribonucleotide Reductase: Activity Sites

- Ribonucleotide reductase (RR) is a large enzyme composed of **four subunits:** two identical **R1 subunits** and two identical **R2 subunits.** It is a highly regulated enzyme, ensuring the production of deoxyribonucleotides is tightly controlled based on cellular needs. Regulation is achieved through **multiple allosteric sites** of two main types: **activity sites** and **substrate specificity sites.**

1. **Activity Sites: (simple)**

- These sites regulate the overall activity of the enzyme. When **dATP levels are high,** the enzyme is inhibited, signaling that deoxyribonucleotide levels are sufficient. Conversely, when **ATP levels are high,** the enzyme is activated, indicating an abundance of ribonucleotides and a need for deoxyribonucleotide production.

Regulation of Ribonucleotide Reductase: Substrate Specificity Sites

2. Substrate Specificity Sites:

- These sites regulate the relative production of different deoxyribonucleotides, ensuring a balanced supply of all four types. For example, when **dTTP levels are high,** these sites promote the reduction of **GDP to dGDP,** shifting the enzyme's activity to favor the synthesis of another nucleotide type that why this enzyme considers as drug target . This mechanism ensures that an **equilibrium** is maintained among deoxyribonucleotide concentrations, preventing an overproduction of any single type.
- In Summary, the **activity sites** control when deoxyribonucleotide synthesis occurs, limiting production when not needed. Meanwhile, the **substrate specificity sites** maintain balanced and consistent levels of all four deoxyribonucleotide types, critical for DNA synthesis and repair.

Application: Hydroxyurea and ribonucleotide reductase

The drug hydroxyurea destroys the free radical required for the activity of ribonucleotide reductase

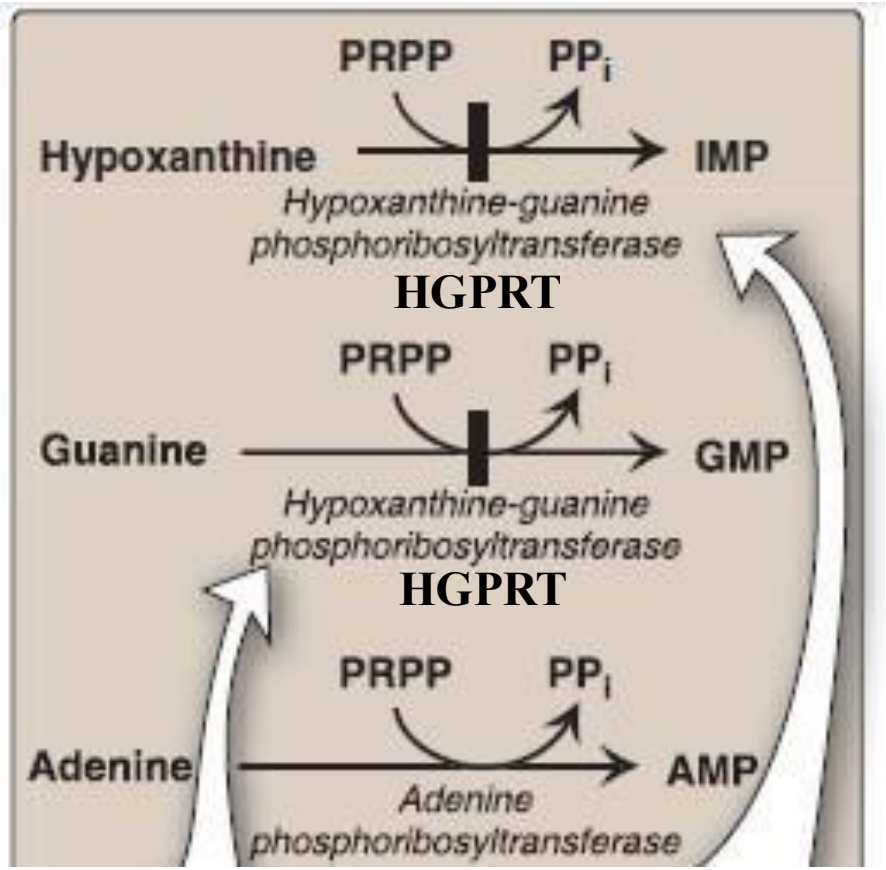
Hydroxyurea inhibits the generation of substrates for DNA synthesis.

Hydroxyurea has been used in the treatment of cancers such as CML **(Chronic Myelogenous Leukemia)**

Application of Hydroxyurea: Inhibition of Ribonucleotide Reductase and Therapeutic Uses

- Ribonucleotide reductase (RR) has been a significant target for drug development. One notable inhibitor, **hydroxyurea,** directly inhibits RR by scavenging its free radicals, effectively halting the production of deoxyribonucleotides. Hydroxyurea has been widely used as an **anti- cancer agent,** particularly for managing certain conditions like **chronic myeloid leukemia (CML),** where it induces **myelosuppression** to control the excessive production of leukemic cells.
- Beyond its anti-cancer applications, hydroxyurea has been repurposed for other therapeutic uses, reflecting a growing trend in the pharmaceutical industry to maximize the utility of existing drugs.
- With this, the explanation of the **de novo synthesis of purine nucleotides** concludes, highlighting its significance in cellular processes and its role as a critical target for therapeutic interventions.

Salvage pathway for purines



Salvage pathway for purines is purine synthesis from:

1. The normal turnover of cellular nucleic acids
2. Diet purines that are not degraded (small amounts)

Conversion of purine bases to nucleotides:

- Both APRT and HGPRT use PRPP as the source of the ribose 5-phosphate group.
- PP is released and hydrolyzed by pyrophosphatase making these reactions irreversible.
- Adenosine is the only purine nucleoside to be salvaged. It is phosphorylated to AMP by adenosine kinase.

Salvage Pathway for Purines: Recycling Preexisting Nitrogenous Bases

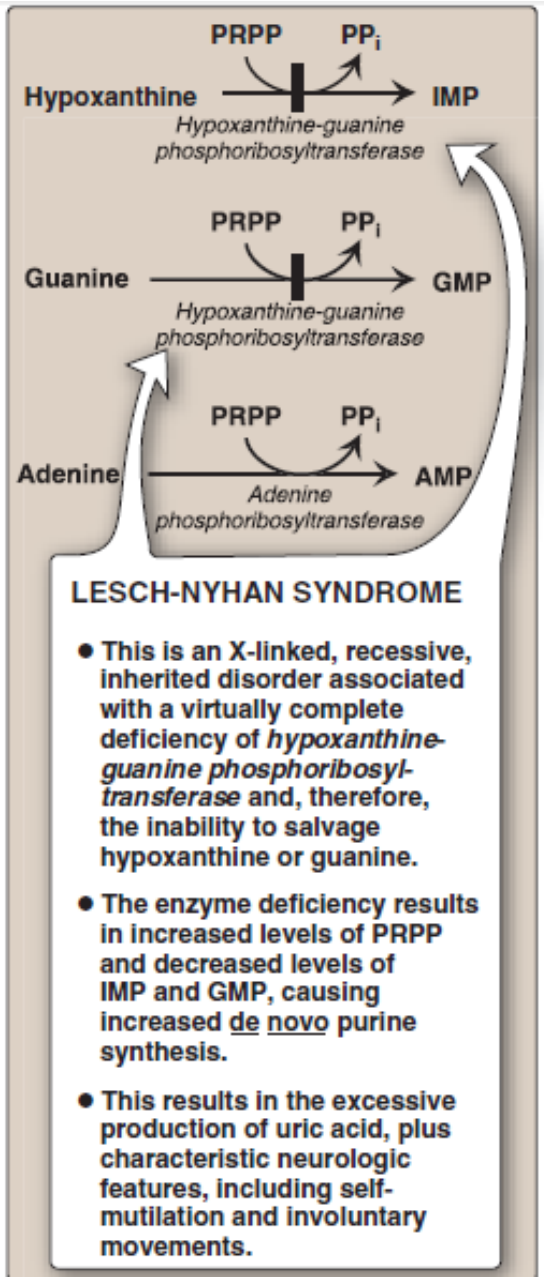
- The **salvage pathway** is a simple and efficient process that recycles preexisting nitrogenous bases and nucleosides to synthesize nucleotides, reducing the need for de novo synthesis.

1. **Salvaging Adenine:**
   - Adenine, a nitrogenous base, can be salvaged by adding a ribose sugar and phosphate group, both provided by **PRPP (5-phosphoribosyl-1-pyrophosphate).** This reaction, catalyzed by **adenine phosphoribosyltransferase (APRT),** produces **AMP (adenosine monophosphate)** while releasing **PPi (pyrophosphate).**
2. **Salvaging Guanine and Hypoxanthine:**
   - Guanine and hypoxanthine are salvaged by the enzyme **hypoxanthine-guanine phosphoribosyltransferase (HGPRT).** Similar to adenine salvage, PRPP donates ribose and phosphate to form **GMP (guanine monophosphate)** from guanine and **IMP (inosine monophosphate)** from hypoxanthine. This enzyme can act on multiple nitrogenous bases, unlike APRT, which is specific to adenine.

Salvage Pathway for Purines: Recycling Preexisting Nitrogenous Bases

3. Recycling IMP:
   - IMP, synthesized during de novo purine synthesis, can re-enter the pathway through salvage after hypoxanthine is recycled, ensuring efficient use of cellular resources.
4. Alternative Salvage of Adenine via Adenosine:
   - Adenine can also be salvaged indirectly by phosphorylating adenosine (a nucleoside) using adenosine kinase. This pathway is exclusive to adenine and converts adenosine into AMP by adding a phosphate group.

Application: Salvage pathway for purines- Lesch-Nyhan syndrome



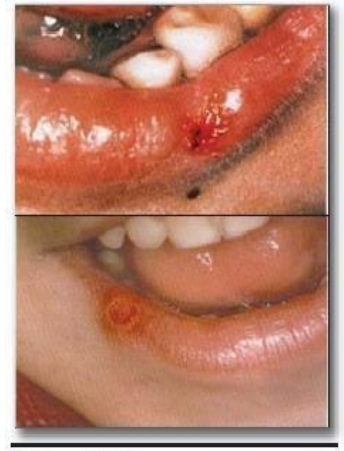


Figure 22.11
Lesions on the lips of Lesch-Nyhar patients caused by self-mutilation.

- A rare, X-linked, recessive
- HGPRT deficiency.
- **Inability to salvage hypoxanthine or guanine** resulting in high
  - amounts of uric acid (the end product of purine degradation)
- Increased PRPP levels and decreased IMP and GMP levels.
- The committed step in purine synthesis has excess substrate and decreased inhibitors available, and **de novo purine synthesis is increased.**
- The decreased purine reutilization and increased purine synthesis results in increased degradation of purines and the production of large amounts of uric acid (hyperuricemia)
- Hyperuricemia results in uric acid stones in the kidneys (urolithiasis) and the deposition of monosodium urate crystals in the joints (gouty arthritis) and soft tissues.
- The syndrome is characterized by motor dysfunction, cognitive deficits and behavioral disturbances that include self-mutilation (biting of lips and fingers)

Lesch-Nyhan Syndrome: HGPRT Deficiency and Clinical Manifestations

- **Lesch-Nyhan syndrome** is a rare, hereditary, **X-linked recessive disorder**, making males more susceptible because they require only one copy of the defective gene to express the disease. This syndrome is caused by a deficiency of **hypoxanthine-guanine phosphoribosyltransferase (HGPRT),** the enzyme responsible for salvaging guanine and hypoxanthine.
- Due to HGPRT deficiency, **guanine salvaging is disrupted,** leading to increased reliance on **de novo purine synthesis.** AMP, however, can still be synthesized through both de novo and salvage pathways, resulting in a higher concentration of AMP compared to GMP. The excess AMP is targeted for degradation, increasing purine breakdown.
- Purine degradation produces **uric acid** as the final product, which is excreted in urine. High levels of uric acid cause **hyperuricemia,** leading to **gout-like symptoms.** Uric acid can form **monosodium urate crystals,** which accumulate in the **synovial fluid of joints,** causing **gouty arthritis.** Additionally, uric acid in high concentrations may exceed its solubility in urine, leading to **precipitation and kidney stone formation.**
- Apart from metabolic symptoms, Lesch-Nyhan syndrome is characterized by **neurological and behavioral abnormalities,** including impaired cognitive skills, self-mutilating behaviors, and other **severe behavioral disturbances.** These symptoms arise from the toxic effects of elevated uric acid and associated metabolic disruptions in the central nervous system.

Degradation of Purine Nucleotides

A. Degradation of dietary nucleic acids in the small intestine

-Ribonucleases and deoxyribonucleases, secreted by the **pancreas**, hydrolyze dietary RNA and DNA to oligonucleotides.

-Oligonucleotides are further hydrolyzed by **pancreatic** phosphodiesterases, producing a mixture of 3'- and 5'-mononucleotides.

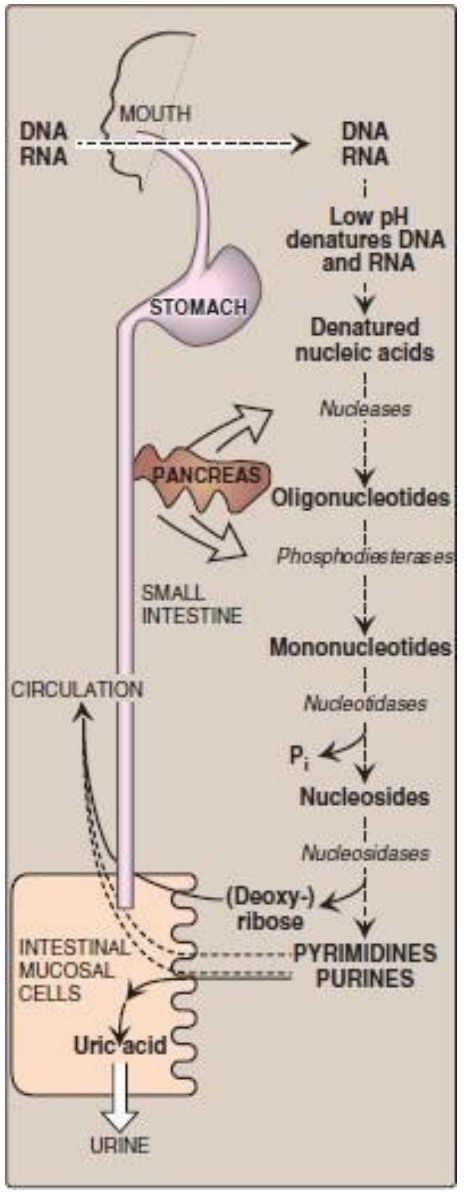
-In the intestinal mucosal cells, nucleotidases remove the phosphate groups hydrolytically, releasing nucleosides that are further degraded to free bases.

-Dietary purine bases are not an appreciable source for the synthesis of tissue nucleic acids.

-Dietary purines are generally converted to uric acid (excreted in urine) in intestinal mucosal cells.

-Purine nucleotides <u>from de novo synthesis</u> are degraded in the <u>liver</u> primarily.

-The free bases are sent out from liver and salvaged by peripheral tissues



PLEASE SEE NEXT SLIDE

Degradation of Purine Nucleotides

Dietary nucleotides serve as a minor source of nucleotides in our bodies. These nucleotides are present in the diet in the form of DNA and RNA , which are nucleic acids. Their digestion begins in the small intestine , where pancreatic enzymes **ribonuclease and deoxyribonuclease** break down RNA and DNA , respectively , into oligonucleotides (smaller fragments)

Then , the pancreas secretes additional enzymes known as **phosphodiesterases** , which further degrade oligonucleotides into mononucleotides. These mononucleotides are then ready for absorption. They cross the brush border of the intestinal cells and enter the cells for further processing

Most of the degradation process occurs within the intestinal cells and the liver , depending on the source of the nucleotides. Dietary nucleotides are primarily degraded in the intestinal cells , while purines synthesized within the body are metabolized in hepatocytes

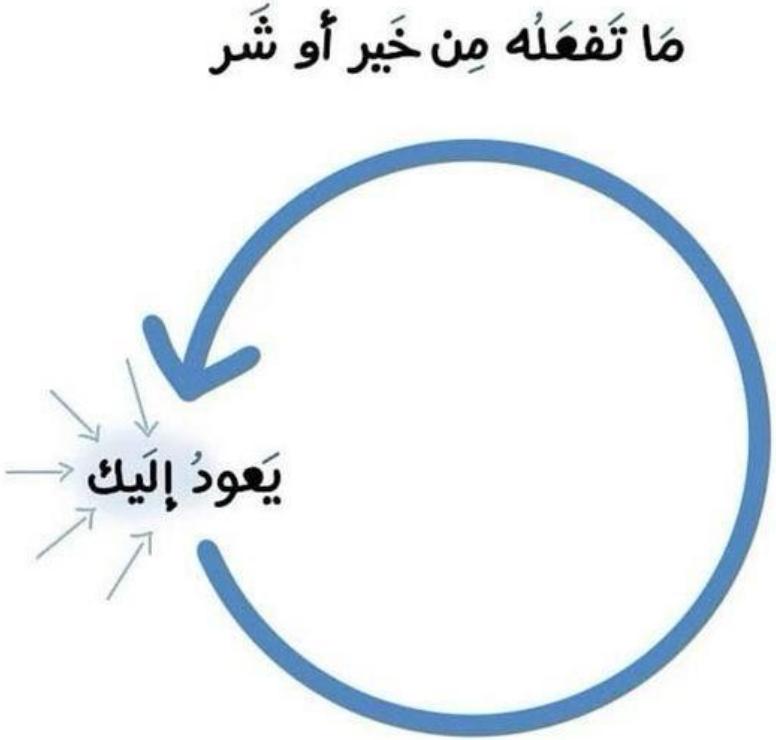
The degradation process involves the removal of the phosphate group from nucleotides by the enzyme nucleotidase , converting them into nucleosides. Next , nucleosidase enzymes remove the sugar , leaving only the nitrogenous base. The fate of the nitrogenous base depends on its type. For instance , purines (adenine and guanine) are generally degraded into uric acid (not urea) , which is excreted through urine

There is no information in this explanation that has not been mentioned in the slide above , it is merely a clarification and organization of the information mentioned

رسالة من الفريق العلمي:

ما من مسلمٍ يَغْرِسُ غَرْسًا أو يَزْرَعُ زَرْعًا فيَأْكُلُ منه طيرٌ ولا إنسانٌ إلا كان له به صدقةً

أي ما من عبد مسلم يقوم بعمل نافع إلا كتب الله به الأجر والثواب.



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Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			