Nucleotides, Purine and Pyrimididine Biosynthesis and Catabolism
LEARNING POINTS

1. Understand nucleosides*, nucleotides, and their function in DNA and RNA
2. Understand the structure and function of purines
3. Understand the origin of atoms in the purine ring
4. Understand the essential features of purine metabolism and catabolism
5. Understand clinical aspects of purine metabolism and deficiencies

*Keywords are highlighted in yellow
Nucleotides

Chemical compound composed of three components: (1) heterocyclic base; (2) sugar (usually a pentose); and (3) one or more phosphate groups.

Adenosine monophosphate (AMP)
Building blocks for DNA and RNA
Energy Currency

Phosphoric anhydride linkages

ATP (adenosine-5'-triphosphate)
Carriers for Activated Intermediates

**UDP-Galactose**

**Galactose**

**UDP**
Structural Components of:

- Coenzyme A
- Flavin adenine dinucleotide (FAD)
- NAD(P)⁺
Signaling Molecules

3',5'-Cyclic AMP

3',5'-Cyclic GMP
Overview of nucleotide metabolism.
Structure of purines and pyrimidines in a nutshell

**SUGARS**
- Deoxyribose
- Ribose

**PURINE**
- Adenine
- Guanine

**PYRIMIDINE**
- Thymine
- Cytosine
- Uracil

**PENTOSE**
- Base
- Nucleoside
- Nucleoside diphosphate
- Nucleoside triphosphate

**NMP, NDP, NTP**
- dNMP, dNDP, dNTP

**PURINES**
- Adenine
- Adenosine
- Deoxyadenosine
- AMP
- dAMP
- ADP
- dADP
- ATP
- dATP

**PYRIMIDINES**
- Cytosine
- Cytidine
- Deoxycytidine
- CMP
- dCMP
- CDP
- dCDP
- CTP
- dCTP
- Thymine
- Thymidine
- Deoxythymidine
- TMP
- dTMP
- TDP
- dTDP
- TTP
- dTTP
- Uracil
- Uridine
- Deoxyuridine
- UMP
- dUMP
- UDP
- dUDP
- UTP
- dUTP
The Nitrogenous Bases

In DNA:
- Adenine
- Guanine
- *Thymine*
- Cytosine

In RNA:
- Adenine
- Guanine
- *Uracil*
- Cytosine

Figure 22.1
Purines and pyrimidines commonly found in DNA and RNA.
Hypoxanthine

Xanthine

Not typically found in DNA or RNA, but are important metabolic intermediates.
RNA is sensitive to alkaline degradation

<table>
<thead>
<tr>
<th>Base</th>
<th>Ribonucleoside</th>
<th>Ribonucleotide</th>
<th>Deoxyribonucleotide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>Adenosine</td>
<td>Adenylate</td>
<td>Deoxyadenylate</td>
</tr>
<tr>
<td>Guanine</td>
<td>Guanosine</td>
<td>Guanylate</td>
<td>Deoxyguanylate</td>
</tr>
<tr>
<td>Cytosine</td>
<td>Cytidine</td>
<td>Cytidylate</td>
<td>Deoxycytidylate</td>
</tr>
<tr>
<td>Thymine</td>
<td>Thymidine</td>
<td>Ribothymidylate</td>
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</tr>
<tr>
<td>Uracil</td>
<td>Uridine</td>
<td>Uridylate</td>
<td>Deoxyuridylate</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>Inosine</td>
<td>Inosinate</td>
<td>Deoxyinosinate</td>
</tr>
<tr>
<td>Xanthine</td>
<td>Xanthosine</td>
<td>Xanthylate</td>
<td>Deoxanthylate</td>
</tr>
</tbody>
</table>
Mechanism of RNA Hydrolysis

Hydrolysis occurs by nucleophilic attack of the 2’-hydroxyl group on the polarized phosphate to yield a 2’-3’ cyclic phosphodiester intermediate (circled) that subsequently spontaneously hydrolyzes to a mix of 2’- and 3’-phosphomonoesters.
Two Important Points

1. The phosphate groups are responsible for the net negative charge associated with DNA and RNA.
2. The hydroxyl group at the 2’-position accounts for the greater ease with which RNA is degraded by alkali.
Ribonucleotide reductase (RR) produces dNDPs

Nucleotide diphosphate (NDP) kinase uses ATP to phosphorylate dNTP to dNTP triphosphate.
De novo purine synthesis
De novo purine synthesis

- The purine ring is synthesized by a series of reactions that add the carbon and nitrogen atoms to a pre-formed ribose-5-phosphate.
- The ribose-5-phosphate is synthesized as part of the Pentose Phosphate Pathway (or Hexose MonoPhosphate pathway). PPP is a metabolic pathway that runs parallel to glycolysis.
- In humans, all necessary enzymes are found in the cytoplasm of the cell.
The purine synthesis pathway
Purine nucleotide production
Source For Ribose-5-Phosphate

Glucose-6-P
   ↓
  Fructose-6-P
      ↓
     ATP
    Fructose-1,6-bisP
       ↓
      DHAP
       ↓
      Glyceraldehyde-3-P
         ↓
         TK
        Xylulose-5-P
          ↓
          Ru-5-P Isomerase
            ↓
            Ribose-5-P
              ↓
              Ru-5-P Epimerase
                ↓
                Xylulose-5-P
                  ↓
                  Xylulose-5-P
                    ↓
                    Ribose-5-P
                      ↓
                      Ribose-5-P
                        ↓
                        Ribose-5-P
                          ↓
                           Erythrose-4-P
                              ↓
                              TA
                             Sedoheptulose-7-P
                               ↓
                               TK
                              Glyceraldehyde-3-P
                                ↓
                                Ribose-5-P
• The pentose sugar is always a ribose, which may be reduced to deoxyribose after nucleotide synthesis is complete.

• 5-Phosphoribosyl-1-pyrophosphate (PRPP) is also involved in synthesis of pyrimidine nucleotides, NAD\(^+\), and histidine biosynthesis.

Conversion of Ribose-5-phosphate to PRPP
1. First step of purine synthesis is committed step and rate limiting step

2. Intracellular concentrations of glutamine and PRPP control the reaction rate

3. Inhibited by AMP, GMP, and IMP

4. Requires 4 ATP molecules
Sulfonamide
(PABA analogue)
Can synthesize folate

Cannot synthesize folate
Methotrexate and Cancer

• Affects rapidly growing cells
• Adverse events include anemia, scaly skin, GI tract disturbances (diarrhea), and baldness
• Resistance to MTX is caused by amplification of dihydrofolate reductase gene
• Also used for treatment of rheumatoid arthritis and psoriasis at lower doses, though site of action is not through DHFR but inhibition of salvage pathways that lead to increased adenosine that inhibits T cell activation.
1. That sulfonamides inhibit purine synthesis in bacteria by interfering with folate synthesis.

2. That methotrexate inhibits purine synthesis by inhibiting dihydrofolate reductase.

3. That IMP is the end product of de novo purine synthesis.

4. AMP, GMP, and IMP inhibit the reaction. PRPPP is an activator.

5. Rate limiting step of the pathway and source of atoms for the purine ring.
Regulation of purine biosynthesis
High levels shut down de novo purine synthesis.

Mycophenolic acid

Mycophtenolic acid

The drug is a reversible, uncompetitive inhibitor of inosine monophosphate dehydrogenase.

The drug deprives rapidly proliferating T and B cells of key components of nucleic acids.

The drug is used to prevent graft rejection.
Purine Salvage Pathway

• Purines from normal turnover of cellular nucleic acids
• Purines obtained from the diet
• Nucleotides can also be synthesized from the purine bases and purine nucleosides in a series of steps referred to as salvage pathways.
Purine salvage pathway.
Lesch-Nyhan Syndrome (deficiency of HGPRT)

- Build up of hypoxanthine and guanine
- Degradation of hypoxanthine and guanine results in increased uric acid
- Excess uric acid in urine often results in orange crystals in the diaper of affected children
- Severe mental retardation
- Self-mutilation
- Involuntary movements
- Gout

**LESCH-NYHAN SYNDROME**

- This is an X-linked, recessive, inherited disorder associated with a virtually complete deficiency of hypoxanthine-guanine phosphoribosyltransferase and, therefore, the inability to salvage hypoxanthine or guanine.
- The enzyme deficiency results in increased levels of PRPP and decreased IMP and GMP, causing increased de novo purine synthesis.
- This results in the excessive production of uric acid, plus characteristic neurologic features, including self-mutilation and involuntary movements.
Catabolism of purines

AMP (Ribose-5'-P) → IMP (Ribose-5'-P) → XMP (Ribose-5'-P) → GMP (Ribose-5'-P)

- AMP → IMP: AMP Deaminase
- IMP → XMP: Nucleotidase
- XMP → GMP: Nucleotidase

- Adenosine → Inosine: Adenosine Deaminase
- Inosine → Xanthosine: Nucleotidase
- Xanthosine → Guanosine: Nucleotidase

- Adenosine → Hypoxanthine: Purine Nucleotide Phosphorylase
- Hypoxanthine → Xanthine: Xanthine Oxidase
- Xanthine → Guanine: Guanine Deaminase
- Guanine → Uric Acid: Uric Acid Oxidase

- Allopurinol inhibits Xanthine Oxidase
Degradation of Purines
Clinical aspects of purine metabolism and deficiencies
Gout

- Characterized by hyperuricemia and acute arthritic joint inflammation by deposition of uric acid crystals
- **Primary gout** is genetic and mainly affects men over 30
- **Secondary gout** is associated with leukemia, polycythemia, HGPRT deficiency, renal insufficiency, lifestyle (rich foods)
Distribution of Serum Urate Values

Serum urate levels in 1515 men and 1670 women aged ≥30 in Taiwan 1991-1992

Hyperuricemia defined at 7 mg/dL

Urate crystallizes at a level of 6.8 mg/dL

Higher Prevalence of Gout and Clinically Significant Hyperuricemia in Higher Age Groups

<table>
<thead>
<tr>
<th>Very high</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brewer’s yeast</td>
<td>Bacon</td>
</tr>
<tr>
<td>Anchovies</td>
<td>Beer</td>
</tr>
<tr>
<td>Herring</td>
<td>Liver</td>
</tr>
<tr>
<td>Sardines</td>
<td>Lobster</td>
</tr>
<tr>
<td>Mussels</td>
<td>Salmon</td>
</tr>
<tr>
<td>Clams</td>
<td>Sweetbreads (pancreas)</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td></td>
<td>Veal</td>
</tr>
</tbody>
</table>
Differences in Serum Urate Among Alcoholic Beverages

## Drugs That Promote Gout

<table>
<thead>
<tr>
<th>Diuretics</th>
<th>Leads to increased uric acid reabsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-dose aspirin</td>
<td>Over 6% increase in mean serum urate and 23% decrease in uric acid clearance(^1)</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>Gout observed at higher incidence</td>
</tr>
<tr>
<td>Ethambutol</td>
<td></td>
</tr>
<tr>
<td>Niacin</td>
<td></td>
</tr>
</tbody>
</table>

Treatments for gout:

Acute attacks are treated with colchicine and indomethacin for 3 weeks.

Long-term treatment with allopurinol reduces the amount of uric acid in circulation.
Pyrimidine ring is fully synthesized before being attached to the ribose sugar.
Uridine nucleotides are also the precursors for de novo synthesis of the thymine nucleotides.

Potent anticancer/antiproliferative drug (chemotherapy)
Catabolism of pyrimidine rings

Cytosine + H₂O → Uric acid

Cytosine deaminase

NADPH + H⁺

NADP⁺

Dihydropyrimidinase

Ureidopropionate + NH₄⁺ + HCO₃⁻ → β-Alanine

β-Alanine → Acetyl-CoA

TCA cycle
Nucleotide signaling

MAPK kinase activation (Erk/p38)

Ion flux through PM
Take home message

- Nucleosides have either a ribose or 2-deoxyribose bound to purine or pyrimidine. The addition of one or more phosphates to a nucleoside results in a nucleotide.
- Purines (adenine and guanine) are comprised of attached six-membered and five-membered nitrogen-containing rings.
- Pyrimidines (uracil, thymine, and cytosine) have only a six-membered nitrogen-containing ring.
- Ribonucleotide reductase (RR) generates deoxynucleoside diphosphate (dNDP) from ribonucleoside diphosphate (rNDPs). Nucleoside diphosphate (NDP) kinases use ATP to phosphorylate dNDP to produce deoxynucleoside triphosphates (dNTPs).
- Purine nucleotides synthesis begins with 5-phosphoribosyl-1-pyrophosphate (PRPP), which, through a series of reactions, generates the nucleotide inosine 50-monophosphate (IMP). Subsequently, IMP can be converted into either AMP or GMP through distinct reactions. AMP or GMP can be converted to ADP or GDP, respectively.
- Pyrimidine nucleotides synthesis begins with carbamoyl phosphate and aspartate generating the pyrimidine base orotate. Succeeding steps attach PRPP to orotate to generate orotate monophosphate (OMP), which is then decarboxylated to UMP. UMP generates UDP and UTP, which can generate CTP.
- Humans cannot break down the purine ring. The catabolism of purine nucleotides results in a uric acid. In contrast, the pyrimidine ring can be completely degraded. Catabolism of the pyrimidine nucleotides leads, ultimately, to β-alanine or β-aminoisobutyrate production, as well as NH3 and CO2.
- Nucleotides are signaling molecules that regulate multiple physiological processes, including neurotransmission and inflammation.
- ATP activates a family of ionotropic receptors (P2X) and metabotropic receptors (P2Y). Extracellular adenosine activates G-protein-coupled cell-surface receptors, which are divided into four subtypes: A1, A2A, A2B, and A3.