Purine Metabolism

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Nucleotides play key roles in many, many cellular processes

1. Activated precursors of RNA and DNA

2. Adenine nucleotides are components of three major co-enzymes, NAD, FAD, and CoA

3. Nucleotide derivatives are activated intermediates in biosynthetic processes (UDP-glucose, SAM)

4. Serve as metabolic regulators (e.g. cAMP and the activation of cell signaling).

5. Serve as major currency of energy in all cells (ATP and GTP).

6. Many metabolic diseases have their etiology in nucleotide metabolism.
The nomenclature of purines depends on their linkage to a pentose

Adenosine Monophosphate
Adenine Adenosine

The nomenclature of purines depends on their linkage to a pentose

Adenine Adenosine Adenosine Monophosphate
Base Nucleoside* Nucleotide Base (P04 ester)

* when the base is purine, then the nucleoside ends in OSINE (AdenOSINE, GuanOSINE, InOSINE)
The active forms of nucleotides in biosynthesis and energy conversions are di- and tri-phosphates

(i) \[ \text{Nucleoside Monophosphate Kinase} \]
\[ \text{CMP} + \text{ATP} \rightleftharpoons \text{CDP} + \text{ADP} \]

(ii) \[ \text{Nucleoside Diphosphate Kinase} \]
\[ \text{XDP} + \text{YTP} \rightleftharpoons \text{XTP} + \text{YDP} \]
What do nucleosides and nucleotides do?

Purine binding proteins (“the purine proteome”) comprise a family of 3-4,000 proteins and as much as 50% of all druggable targets in biology.

- Kinases
- Helicases
- Reductases
- Transferases
- Synthetases
- Dehydrogenases
- Chaperones
- Metabolic Enzymes
- DNA and RNA processing
  
  Etc
The nomenclature of purines depends on their linkage to a pentose

Adenine

Base

Adenosine

Nucleoside*

Base

Adenosine Monophosphate

Nucleotide

Base (P04 ester)

* when the base is purine, then the nucleoside ends in OSINE (AdenOSINE, GuanOSINE, InOSINE)
Nucleoside Function in extracellular signal transduction

Adenosine nucleoside-increased during ATP degradation.

Released in cells when there is low $O_2$ concentration.

Binds to purinogenic receptors $A_1$, $A_{2A}$, $A_{2B}$, $A_3$.

Slows the heart down, at the same time increases capillary dilation.

Caffeine is a adenine derivative, and antagonizes the effects of adenine.
Cyclic nucleotides are important mediators for Intracellular signal transduction.
Modified nucleotide mono and di phosphates have important in electron transfer and Redox control.
RIBONUCLEOTIDE REDUCTASE

1. Complex enzymatic reaction whereby electrons are transferred from NADPH through a series of sulfhydryl groups at the catalytic site of Ribonucleotide Reductase.

2. Active site of RR contains thioredoxin, a 12 kD protein with two exposed cysteines, which become oxidized.

3. This ultimately allows for the reduction of ribose.

REGULATION

1. Based on the response to cellular need for dATPs.
   dATP is general inhibitor
   ATP is a general activator
Nucleotides are linked by 5’ to 3’ phosphodiester bonds to generate DNA and RNA.

* In DNA, this atom would be H instead of OH.
Structures of Common Purine Bases.

Hypoxanthine

Adenine

H = 6 oxy purine

Xanithine

X = 2,6 dioxy purine

Xanthine

Guanine

A = 6 amino purine

NH_2

G = 2 amino, 6-oxy purine

NH_2

NH_2
Hypoxanthine is an intermediate for Adenine and Guanine

The common mechanistic theme for the conversion of A and G is the conversion of a carbonyl oxygen to an amino group.
There are two basic mechanisms to generate purines and pyrimidines

1. **DE NOVO BIOSYNTHETIC PATHWAYS**
   (building the bases from simple building blocks)

2. **SALVAGE PATHWAYS**
   (the reutilization of bases from dietary or catabolic sources)
The biosynthesis of purine (A and G) begins with the synthesis of the ribose-phosphate.

**Pentose phosphate pathway** → Ribose 5- phosphate + ATP

Ribose phosphate pyrophospho-KINASE

5-Phosphoribosyl-1-pyrophosphate (PRPP)
Oxidative Stage of Pentose Phosphate Pathway

\[ \text{glucose-6-phosphate} \xrightarrow{\text{NADP}^+} \text{glucose-6-phosphate dehydrogenase} \xrightarrow{\text{NADPH}} \text{6-phosphogluconolactone} \]
Oxidative Stages of Pentose Phosphate Pathway

Glucose

\[ \text{Hexokinase} \]

Glucose 6 phosphate

\[ \text{G6P-DH} \]

6-Phosphogluconolactone

\[ \text{H}_{2}\text{O} \]

6-Phosphogluconate

\[ \text{6PG-DH} \]

Ribulose-5-phosphate
The major regulatory step in purine biosynthesis is the conversion of PRPP to 5-Phosphoribosyl-1-amine.

Amidophosphoribosyl transferase is an important regulatory enzyme in purine biosynthesis. It is strongly inhibited by the end products IMP, AMP, and GMP. This type of inhibition is called FEEDBACK INHIBITION.
Several amino acids are utilized in purine biosynthesis,

IMP is the precursor for both AMP and GMP, the base is also called hypoxanthine.
Figure 22.8
Conversion of IMP to AMP and GMP showing feedback inhibition.

MYCOPHENOLIC 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.
Conversion of Hypoxanthine to Adenine/Guanine.

The common mechanistic theme for the conversion of A and G is the conversion of a carbonyl oxygen to an amino group.
Purines: where do the atoms come from?

Purine intermediates include:

1. Glycine
2. 1 C units of 5,10 mTHF
3. Glutamine
4. Asparate
The regulation of purine biosynthesis is a classic example of negative feedback.

- Ribose 5-phosphate → PRPP → Phosphoribosylamine → IMP
- Inhibited by IMP, AMP, and GMP
- IMP → AMP → GMP
  - Inhibited by AMP
  - Inhibited by GMP
Stages of nucleotide metabolism

- Endonuclease
- Phosphodiesterase
- Nucleoside monophosphates (Mononucleosides)
- Nucleoside triphosphate
- Nucleic Acid Synthesis
Endonuclease

Phosphodiesterase

Nucleotidases

Nucleosidases

Nucleoside monophosphates (Mononucleosides)

H₂O

Nucleoside kinase

ATP

ADP

Pi

Phosphorylases

Ribose-1-P

PPi

Phosphoribosyl transferases

Nucleobases

Uric Acid (purines)

Nucleic Acid Synthesis

Nucleoside triphosphate

PRPP
Cytidine Monophosphate

Phosphorylase

Cytosine

Cytidine

Nucleoside

Nucleotidase

Cytidine Monophosphate

Nucleotide Base (P\textsubscript{04} ester)
Gout-Gouty Arthritis
(a metabolic condition of abnormal purine metabolism)
Salvage pathways for the re-utilization of purines;

There are 2 salvage enzymes with different specificity;

1. **Adenine** phosphoribosyl transferase
2. **Hypoxanthine-guanine** phosphoribosyl transferase

\[
PRPP + \text{Adenine} \xrightarrow{A-PRT} \text{Adenylate} \\
PRPP + \text{Guanine} \xrightarrow{HG-PRT} \text{Guanylate}
\]
What happens in gout?

1. Negative regulation of PRPP Synthase & PRPP Amidotransferase is lost
2. PRPP levels are increased because of defects in salvage pathways

Therefore, there is net increase in biosynthetic/degradation pathways!!
Purines in humans are degraded to Urate

Important points:

1. Nucleotides are constantly undergoing turnover!

2. There are many enzymes involved; Nucleotidases Nucleoside phosphorylases Deaminases Xanthine oxidases

3. the final common intermediate in humans is Urate, which is excreted.

4. there are several metabolic disorders resulting from defects in purine catabolism.
Catabolism of Adenosine and Guanosine to Uric acid
**GOUT (Gouty Arthritis): A defect of purine metabolism**

<table>
<thead>
<tr>
<th>Serum Uric Acid Levels (mg/dl)</th>
<th>Incidence of Gout (% of cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;9.0</td>
<td>~10%</td>
</tr>
<tr>
<td>7-9</td>
<td>0.5-3.5%</td>
</tr>
<tr>
<td>&lt;7.0</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

- **Guanine** → **Hypoxanthine**
  - **xanthine oxidase**
  - **Xanthine** → **Urate**
    - **xanthine oxidase**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effect</th>
</tr>
</thead>
</table>
| Allopurinol | a. decrease urate  
  b. increase xanthine & hypoxanthine  
  c. decrease PRPP |
**SCID-Severe Combined Immunodeficiency Syndrome**

- **AMP**
  - $\text{H}_2\text{O}$
  - $\text{Pi}$
  - **Nucleotidase**

- **Adenosine**
  - $\text{H}_2\text{O}$
  - **Adenine deaminase***

- **Inosine** → **Hypoxanthine**

**Autosomal recessive disorder**

**Mutations in ADA**

**Infants subject to bacterial, candidiasis, viral, protozoal infections**

**Both T and B cells are significantly reduced (dATP is toxic)**

**1995-AdV expressing ADA was successfully employed as gene therapy strategy**
## Disorders of Purine Metabolism:

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Defect</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>Gout</td>
<td>PRPP synthase/HGPRT</td>
<td>Hyperuricemia</td>
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<tr>
<td>Lesch Nyhan syndrome</td>
<td>lack of HGPRT</td>
<td>Hyperuricemia</td>
</tr>
<tr>
<td>SCID</td>
<td>ADA</td>
<td>High levels of dAMP</td>
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<tr>
<td>von Gierke’s disease</td>
<td>glucose -6-PTPase</td>
<td>Hyperuricemia</td>
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