Biosynthesis of Nucleotides
Nucleotides actively participate in many biochemical reactions:

- **ATP and GTP as energy sources**
- **Uridine derivatives of sugars participate in carbohydrate metabolism**
- **Coenzymes (NAD, FAD, CoA) are nucleotide derivatives**
- **[ATP], [ADP], [AMP] act as allosteric regulators of key enzymes**
- **Monomeric units of nucleic acids**
Two Pathways for Nucleotide Biosynthesis:

**de novo pathway (anew; from scratch):**
* nucleotides are constructed from simple precursors

**salvage pathways:** recovery and recycling of nucleotides obtained in the diet
De Novo Biosynthesis of Purines:

*Studied first in pigeons. Birds excrete nitrogen as the purine uric acid:*

![Purine Structure]

*Pigeons were fed isotopically labeled compounds and the distribution of labeled atoms examined in uric acid.*
Origin of ring atoms of purines:

- Aspartate
- $\text{CO}_2$
- Glycine
- $\text{N}^{10}\text{-formyl-THF}$
- Glutamine
- $\text{N}^{10}\text{-formyl-THF}$
Atoms forming the purine ring are successively added to ribose-5-P.

Purines are thus directly synthesized as nucleotide derivatives by assembling the atoms comprising the purine ring directly on ribose.
Phosphoribosylpyrophosphate (PRPP) is formed from ribose-5-P and ATP by PRPP synthetase. PRPP is the donor of the ribose ring of the nucleotides.

PRPP also participates in pyrimidine biosynthesis and in the synthesis of histidine and tryptophan.
$\text{HC-NH-CH}_2\text{COO-}$

$\text{H}_2\text{N}$

$\text{O}$

$\text{N}$

$\text{N}$

$\text{C}$

$\text{C}$

$\text{N}$

$\text{C}$

$\text{C}$

$\text{O}$
Inosine Monophosphate (IMP)

AMP and GMP are synthesized from IMP
Aspartate GTP, Adenylosuccinate Synthetase, GDP, Pi

\(-\text{OOC-CH}_2\text{-CH-COO}^{-}\)

IMP

Adenylosuccinate

\(-\text{OOC-CH}_2\text{-CH-COO}^{-}\)
-OOC-CH$_2$-CH-COO$^-$

**fumarate**

Adenylosuccinase

**AMP**
IMP D’hase

NADH

NAD^+

Xanthosine monophosphate (XMP)
Glutamine + ATP + AMP, PPi → Glutaminate + GMP Synthetase

Glutamine

AMP, PPi

Glutamate

ATP

GMP

Synthetase

XMP

GMP
Regulation of Purine Biosynthesis:

- **PRPP synthetase is feedback inhibited by AMP, GMP and IMP.**
- **Adenylosuccinate synthetase is inhibited by AMP.**
- **IMP d’hase is inhibited by XMP and GMP.**
Purine Salvage:

- During cellular metabolism and during digestion in animals, nucleic acids are degraded to mononucleotides, nucleosides, and free purine bases.

- Some purines are further degraded to uric acid, but a considerable fraction are directly converted back to purine ribonucleotides.
AMP

PPi

Adenine phosphoribosyl transferase

PRPP

AMP

Adenosine

Adenine
Inosine → Hypoxanthine → PRPP → PPI → IMP → Inosine

Hypoxanthine-guanine phosphoribosyltransferase

PRPP → GMP → Guanosine

Hypoxanthine → GUANINE
Lesch-Nyhan Syndrome:


- Hereditary deficiency of hypoxanthine-guanine phosphoribosyltransferase. Disease affects mostly males.

- Hypoxanthine and guanine are degraded to uric acid instead of being converted to IMP and GMP.

- Symptoms: mental retardation; spasticity; bizarre tendency to self-mutilate.
Pyrimidine Biosynthesis
• The common pyrimidine ribonucleotides are cytidine-5’-monophosphate and uridine-5’-monophosphate

• The pyrimidine ring is synthesized first, then attached to ribose-5-phosphate

• Pyrimidine nucleotides are made from aspartate, PRPP and carbamoyl phosphate
Origin of ring atoms of pyrimidines:

Carbamoyl Phosphate

(N$_3$ originally from glutamine; C$_2$ from HCO$_3^-$)

Aspartate
Glutamine + HCO$_3^-$ + 2 ATP

Carbamoyl Phosphate Synthase II
(committed step in mammals)

H$_2$N-C-OPO$_3^{2-}$ + 2 ADP, Pi
Aspartate transcarbamoylase

Aspartate + carbamoyl phosphate

\[
\begin{aligned}
\text{-O} & -\text{C}=\text{O} \\
\text{H}_2\text{N} & \text{CH}_2 \\
\text{O=CO} & \text{CH-COO}^- \\
\text{N} & \text{H}
\end{aligned}
\]

N-carbamoylaspartate

*ATCase was the first allosteric enzyme to be characterized*
Dihydroorotate D’hase

NAD⁺ (Q)

NADH (QH₂)

Orotate

Dihydroorotate D’hase

Orotate

NAD⁺ (Q)

NADH (QH₂)
Orotate
Phosphoribosyl Transferase

Orotidylate

O = C
HN
C=CH
C - COO -

PRPP

PPi
Orotidylate Decarboxylase

O

N

C

H

O=O

N

C

CH

CH

O=O

N

C

H

O=O

N

C

CH

CH

O=O

N

C

H

O=O

N

C

CH

CH

Uridine 5’-monophosphate (UMP)
Kinases

Uridine 5’-Triphosphate (UTP)
Cytidylate Synthetase

*Allosterically inhibited by CTP

Glutamate

Glutamine

Cytidine 5’-Triphosphate (CTP)
Mammalian pyrimidine synthesis is an example of metabolite channeling.

In bacteria, the six enzymes of de novo pyrimidine synthesis are separate proteins.

In mammals, the six activities are contained within three proteins.

CPS-II, asparate transcarbamoylase, dihydroorotase are all contained within a single cytosolic protein. DHO d’hase is localized in the inner mito membrane. Orotate phosphoribosyltransferase and OMP decarboxylase are contained with a single protein called OMP synthase.
Regulation of Pyrimidine Biosynthesis:

- *Carbamoyl phosphate synthetase II* is allosterically activated by PRPP and ATP. Pyrimidine nucleotides (UDP, UTP) inhibit.

- *Aspartate transcarbamoylase (ATCase)* from *E. coli* is inhibited by pyrimidine nucleotides (CTP and UTP). ATP is an allosteric activator.
• Deoxyribonucleotides are synthesized by reduction of ribonucleosides

• All 4 ribonucleoside diphosphates (ADP, GDP, CDP, UDP) are substrates for \textbf{Ribonucleotide Reductase}

• Ribonucleotide Reductase has both a catalytic site and two allosteric sites. One allosteric site (Activity site) controls activity at the catalytic site. The second (Specificity site) determines which nucleoside diphosphate binds the active site.
<table>
<thead>
<tr>
<th>Ligand bound to Activity Site</th>
<th>Ligand bound to Specificity Site</th>
<th>Activity of Catalytic Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>dATP</td>
<td>dATP</td>
<td>Enzyme Inactive</td>
</tr>
<tr>
<td>ATP</td>
<td>ATP or dATP</td>
<td>CDP or UDP</td>
</tr>
<tr>
<td>ATP</td>
<td>dTTP</td>
<td>GDP</td>
</tr>
<tr>
<td>ATP</td>
<td>dGTP</td>
<td>ADP</td>
</tr>
</tbody>
</table>
dTMP is formed from dUMP:

\[
\text{UMP} \rightarrow \text{UDP} \rightarrow \text{dUDP} \rightarrow \text{dUTP} \rightarrow \text{dUMP} \rightarrow \text{dTMP}
\]

- Nucleoside Monophosphate Kinase
- Ribonucleotide Reductase
- Nucleoside Diphosphate Kinase
- dUTPase
- Thymidylate Synthase
dTMP is also formed from dCDP:

\[ \text{dCDP} \rightarrow \text{dCMP} \rightarrow \text{dUMP} \]

Cytidine deaminase

(activated by dCTP, inhibited by dTTP)

Of the 4 dNTPs, only dCTP does not interact with the regulatory sites on ribonucleotide reductase, instead it interacts with dCMP deaminase.
N⁵N¹⁰-Methylene-tetrahydrofolate

Dihydrofolate

Tetrahydrofolate

Dihydrofolate Reductase

methotrexate

N⁵N¹⁰-Methylene-tetrahydrofolate
dTMP can also be synthesized via salvage of thymidine:

Thymidine $\xrightarrow{\text{Thymidine kinase}}$ ATP $\xrightarrow{\text{ADP}}$ dTMP

Radioactive thymidine is used for monitoring intracellular synthesis of DNA because it enters cells easily and its principle metabolic fate is salvage leading to incorporation into DNA.
Many anticancer drugs target DNA synthesis; particularly thymidylate synthesis.

Methotrexate and aminopterin inhibit dihydrofolate reductase; thymidylate cannot be formed thus DNA cannot be replicated.

5-Fluorouracil is converted to 5-Fluorodeoxyuridylate which binds tightly to thymidylase synthase and inhibits the enzyme.
Azaserine and acivicin are glutamine analogues also used as chemotherapeutic agents.