NUCLEOTIDE METABOLISM

DR. A. TARAB
DEPT. OF BIOCHEMISTRY
HKMU
OVERVIEW

• Nucleotides are essential for all cells
• Without them, neither DNA or RNA can be produced and, therefore, proteins cannot be synthesized or cells proliferate
• Nucleotide also serve as carriers of activated intermediates in the synthesis of some carbohydrates, lipids and proteins, and are structural components of several essential coenzymes, e.g, coenzyme A, FAD, NAD$^+$ and NADP$^+$
• Nucleotides, such as cyclic AMP (cAMP) and cyclic GMP (cGMP), serve as second messengers in signal transduction pathways.
• In addition, nucleotides play an important role as “energy currency” in the cell.
• Finally, nucleotides are important regulatory compounds for many of the pathways of intermediary metabolism, inhibiting or activating key enzymes.
• The purine and pyrimidine bases found in nucleotides can be synthesized de novo, or can be obtained through salvage pathways that allow the re-use of the preformed bases resulting from normal cell turnover or from the diet
NUCLEOTIDE STRUCTURE

• Nucleotides are composed of a nitrogenous base, a pentose monosaccharide and one, two or three phosphate groups
• The nitrogen containing bases belong to two families of compounds: the purines and the pyrimidines
• A. Purine and pyrimidine structures
• Both DNA and RNA contain the same purine bases: adenine (A) and guanine (G)
• Both DNA and RNA contain the pyrimidine cytosine (C) but they differ in their second pyrimidine base: DNA contains thymine (T), whereas RNA contains uracil (U)

• T and U differ by only one methyl group, which is present on T but absent on U

• **B. Nucleosides**

• The addition of a pentose sugar to a base produces a nucleoside
• If the sugar is ribose, a ribonucleoside is produced; if the sugar is 2-deoxyribose, a deoxyribonucleoside is produced
• The ribonucleosides of A, G, C and U are named adenosine, guanosine, cytidine and uridine respectively
• The deoxyribonucleoside of A, G, C and T have the added prefix, “deoxy-”, for example deoxyadenosine
* Note: The compound deoxythymidine is often simply called thymidine, with the “deoxy” prefix being understood.

- The carbon and nitrogen atoms in the rings of the base and the sugar are numbered separately.
- Note that the atoms in the ring of the bases are numbered 1 to 6 in pyrimidines and 1 to 9 in purines, whereas the carbons in the pentose are numbered 1’ to 5’.
• Thus, when the 5′-carbon of a nucleoside (or nucleotide) is referred to, a carbon atom in the pentose, rather than an atom in the base, is being specified

• **C. Nucleotides**

• Nucleotides are monophosphate, diphosphate or triphosphate esters of nucleosides

• The first phosphate group is attached by an ester linkage to the 5′-OH of the pentose
• Such a compound is called a **nucleoside 5’-phosphate** or a **5’-nucleotide**
• The type of pentose is denoted by the prefix in the names “5’-ribonucleotide” and “5’-deoxyribonucleotide”
• If one phosphate group is attached to the 5’-carbon of the pentose, the structure is a nucleoside monophosphate (NMP), like AMP or CMP
• If a second or third phosphate is added to the nucleoside, a nucleoside diphosphate (for example, ADP) or triphosphate (for example, ATP) results.
• The second and third phosphates are each connected to the nucleotide by a “high-energy” bond.
• *Note: The phosphate groups are responsible for the negative charges associated with nucleotides, and cause DNA and RNA to be referred to as “nucleic acids”
Ribonucleoside 5'-monophosphate (NMP)

Ribonucleoside 5'-diphosphate (NDP)

Ribonucleoside 5'-triphosphate (NTP)
SYNTHESIS OF PURINE NUCLEOTIDES

• The atoms of the purine ring are contributed by a number of compounds, including amino acids (aspartic acid, glycine and glutamine), CO$_2$ and N$^{10}$-formyltetrahydrofolate

• The purine ring is constructed by a series of reactions that add the donated carbons and nitrogens to a preformed ribose 5-phosphate
Figure 22.5
Sources of the individual atoms in the purine ring.
• A. Synthesis of 5-phosphoribosyl-1-pyrophosphate (PRPP)

• PRPP is an “activated pentose” that participates in the synthesis of purines and pyrimidines, and in the salvage of purine bases

• Synthesis of PRPP from ATP and ribose 5-phosphate is catalyzed by PRPP synthetase (ribose phosphate pyrophosphokinase)

• Ribose 5-phosphate + ATP → 5-PRPP + AMP
• This enzyme is activated by inorganic phosphate (Pi) and inhibited by purine nucleotides (end-product inhibition)

• **B. Synthesis of 5’-phosphoribosylamine**
  • The amide group of glutamine replaces the pyrophosphate group attached to carbon 1 of PRPP
  • The enzyme, *glutamine:phosphoribosyl pyrophosphate amidotransferase*, is inhibited by the purine 5’-nucleotides AMP, GMP and IMP – the end products of the pathways
• This is the committed step in purine nucleotide biosynthesis
• The rate of the reaction is also controlled by the intracellular concentration of the substrates glutamine and PRPP
• **C. Synthesis of inosine monophosphate, the “parent” purine nucleotide**
• The next nine steps in purine nucleotide biosynthesis leading to the synthesis of IMP requires four ATP molecules as an energy source
• Two steps in the pathway require $\text{N}^{10}$-formylTHF
The diagram illustrates the conversion of PRPP into IMP (inosine monophosphate) through two pathways:

A: Glutamine-PRPP amidotransferase
B: Adenylosuccinate lyase

Key reactions include:
- Glutamine + PRPP → Glutamate + N\(^{10}\)-formyl-THF
- Glycine + N\(^{10}\)-formyl-THF → THF + Glutamate
- Fumarate + ADP + P\(_i\) → ADP + P\(_i\) + CO\(_2\)
- N\(^{10}\)-formyl-THF + THF → H\(_2\)O

The diagram also includes the participation of ATP and ADP in various reactions.
Reaction No 2

5-phosphoribosyl amine (PRA) + ATP \rightarrow ADP + Pi + 5'-phosphoribosyl-glycinamide (GAR)

glycinamide ribonucleotide synthetase (GAR synthetase)
Reaction No 3

5′-phosphoribosyl-glycinamide (GAR) → 5′-phosphoribosyl-N-formylglycinamide (FGAR)

phosphoribosylglycinamide formyltransferase (GAR transformylase)

10-formyl tetrahydrofolate → tetrahydrofolate
Reaction No 4

5'-phosphoribosyl-N-formylglycinamide (FGAR) + ATP, H₂O → ADP + Pi + 5'-phosphoribosyl-N-formylglycinaminide (FGAM)

See reaction mechanism
Reaction No 5

5'-phosphoribosyl-N-formylglycinamidine (FGAM)

phosphoribosyl-aminomimidazole synthase (AIR synthase)

ATP

5'-phosphoribosyl-aminomimidazole (AIR)

ADP + Pi
Reaction No 6

5'-phosphoribosyl-aminoimidazole (AIR)

\[ \text{phosphoribosyl-aminoimidazole carboxylase (AIR carboxylase)} \]

\[ \text{5'-phosphoribosyl-aminoimidazole (AIR)} \rightarrow \text{1-(5'-phosphoribosyl)-5-amino-4-carboxyimidazole (CAIR)} \]

\[ \text{CO}_2 \]
1-(5'-phosphoribosyl)-5-amino-4-carboxyimidazole (CAIR)

ATP

aspartate

phosphoribosylaminomimidazole
-succinocarboxamide synthetase
( SAICAR synthetase )

ADP + Pi + (5')phosphoribosyl)
1-(5'-phosphoribosyl)-4-(N-succinocarboxamide)-5-aminoimidazole (SAICAR)

Adenylosuccinatleyase

Fumarate

1-(5'-phosphoribosyl)-5-amino-4-imidazolecarboxamide
1-(5'-phosphoribosyl)
-5-amino
-4-imidazolecarboxamide
(AICAR)

10-formyl tetrahydrofolate

phosphoribosylaminoimidazolecarboxamide
for methyltransferase
(AICAR transformylase)

tetrahydrofolate

1-(5'-phosphoribosyl)
-5-formamido
-4-imidazolecarboxamide
1-(5'-phosphoribosyl) -5-formamido -4-imidazolcarboxamide (FAICAR)

IMP cyclohydrolase (IMP synthetase)

H₂O

inosine 5'-monophosphate (IMP)
• D. Synthetic inhibitors of purine synthesis

• Some synthetic inhibitors of purine synthesis (e.g., the **sulfonamides**) 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 (e.g., **methotrexate**) are used pharmacologically to control the spread of cancer by interfering with the synthesis of nucleotides and, therefore of DNA and RNA.
• Trimethoprim, another folate analog, has potent antibacterial activity because of its selective inhibition of bacterial dihydrofolate reductase

• **E. Conversion of IMP to AMP and GMP**

• The conversion of IMP to either AMP or GMP uses a two-step, energy-requiring pathway

• Note that the synthesis of AMP requires GTP as an energy source, whereas the synthesis of GMP requires ATP
FIGURE 22-34  Biosynthesis of AMP and GMP from IMP.
• Also, the first reaction in each pathway is inhibited by the end product of that pathway
• If both AMP and GMP are present in adequate amounts, the de novo pathway of purine synthesis is turned off at the *amidotransferase* step
FIGURE 22–35 Regulatory mechanisms in the biosynthesis of adenine and guanine nucleotides in E. coli. Regulation of these pathways differs in other organisms.
AMP and GMP are feedback inhibitors of purine nucleotide biosynthesis
• F. Conversion of nucleoside monophosphates to nucleoside diphosphates and triphosphates

• Nucleoside diphosphates (NDP) are synthesized from the corresponding nucleoside monophosphate (NMP) by base-specific nucleoside monophosphate kinases.
Base-specific nucleoside monophosphate kinases

<table>
<thead>
<tr>
<th>Reaction</th>
<th>kinase</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP + ATP</td>
<td>Adenylate kinase</td>
<td>2 ADP</td>
</tr>
<tr>
<td>GMP + ATP</td>
<td>Guanylate kinase</td>
<td>GDP + ADP</td>
</tr>
</tbody>
</table>

Nucleoside diphosphate kinase

<table>
<thead>
<tr>
<th>Reaction</th>
<th>kinase</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDP + ATP</td>
<td></td>
<td>GTP + ADP</td>
</tr>
<tr>
<td>CDP + ATP</td>
<td></td>
<td>CTP + ADP</td>
</tr>
</tbody>
</table>

---

**Figure 22.9**

Conversion of nucleoside monophosphates to nucleoside diphosphates and triphosphates.
• ATP is generally the source of the transferred phosphate, because it is present in higher concentrations than the other nucleoside triphosphates

• *Adenylate kinase* is particularly active in liver and muscle, where the turnover of energy from ATP is high

• Its function is to maintain an equilibrium among AMP, ADP and ATP
• Nucleoside diphosphates and triphosphates are interconverted by *nucleoside diphosphate kinase* – an enzyme that, unlike the *monophosphate kinases*, has **broad specificity**

• **G. Salvage pathway for purines**

• Purines that result from the normal turnover of cellular nucleic acids, or that are obtained from the diet and not degraded, can be reconverted into nucleoside triphosphates and used by the body
• This is referred to as the “salvage pathway” for purines

• 1. Conversion of purine bases to nucleotides:
   Two enzymes are involved: i) adenine phosphoribosyltransferase (APRT)
   • Adenine + PRPP $\rightarrow$ adenylate + PPI
• ii) hypo-xanthine-guanine phosphoribosyltransferase (HPRT)

  • Guanine + PRPP $\rightarrow$ guanylate + PPI
  • Hypoxanthine + PRPP $\rightarrow$ inosinate + PPI
  • Both enzymes use PRPP as the source of the ribose 5-phosphate group
  • The release of pyrophosphate makes these reactions irreversible
2. **Lesch-Nyhan syndrome**: This syndrome is an X-linked, recessive disorder associated with a virtually complete deficiency of *HPRT*. This deficiency results in an inability to salvage hypoxanthine or guanine, from which excessive amounts of uric acid are produced. In addition, the lack of this salvage pathway causes increased PRPP levels and decreased IMP and GMP levels.
• As a result, glutamine:phosphoribosylpyrophosphate amidotransferase (the committed step in purine synthesis) has excess substrate and decreased inhibitors available, and de novo purine synthesis is increased
• The combination of decreased purine reutilization and increased purine synthesis results in the production of large amounts of uric acid, making the Lesch-Nyhan syndrome a severe heritable form of gout.

• Patients with Lesch-Nyhan syndrome tend to produce urate kidney stones.

• In addition, characteristic neurologic features of the disorder include self-mutilation and involuntary movements.
SYNTHESIS OF DEOXYRIBONUCLEOTIDES

• The nucleotides described thus far all contain ribose (ribonucleotides)
• The nucleotides required for DNA synthesis, however, are 2’-deoxyribonucleotides, which are produced from ribonucleoside diphosphates by the enzyme *ribonucleotide reductase*
• **A. Ribonucleotide reductase**

• *Ribonucleotide reductase (ribonucleoside diphosphate reductase)* is a multisubunit enzyme (two identical B1 subunits and two identical B2 subunits) that is specific for the reduction of nucleoside diphosphates (ADP, GDP, CDP and UDP) to their deoxy-forms (dADP, dGDP, dCDP and dUDP)
• The immediate donors of the hydrogen atoms needed for the reduction of the 2’hydroxyl group are two sulfhydryl groups on the enzyme itself, which, during the reaction, form a disulfide bond.

• 1. Regeneration of reduced enzyme: In order for ribonucleotide reductase to continue to produce deoxyribonucleotides, the disulfide bond created during the production of the 2’-deoxy carbon must be reduced.
• The source of the reducing equivalents is **thioredoxin** — a peptide coenzyme of *ribonucleotide reductase*

• Thioredoxin contains two cysteine residues separated by two amino acids in the peptide chain

• The two sulfhydryl groups of thioredoxin donate their hydrogen atoms to *ribonucleotide reductase*, in the process forming a disulfide bond
• 2. Regeneration of reduced thioredoxin: Thioredoxin must be converted back to its reduced form in order to continue to perform its function

• The necessary reducing equivalents are provided by NADPH + H⁺, and the reaction is catalyzed by *thioredoxin reductase*
METHANISM FIGURE 22-41 Proposed mechanism for ribonucleotide reductase. In the enzyme of E. coli and most eukaryotes, the active thiol groups are on the R1 subunit; the active-site radical (—X') is on the R2 subunit and in E. coli is probably a thyl radical of Cys₄²⁹ (see Fig. 22-40). Steps 1 through 6 are described in the text.
• **B. Regulation of deoxyribonucleotide synthesis**
  
  *Ribonucleotide reductase* is responsible for maintaining a balanced supply of the deoxyribonucleotides required for DNA synthesis.

• To achieve this, the regulation of the enzyme is complex

• In addition to the single active site, there are two sites on the enzyme involved in regulating its activity.
• 1. **Activity site**: The binding of dATP to an allosteric site (known as the activity site) on the enzyme inhibits the overall catalytic activity of the enzyme and, therefore, prevents the reduction of any of the four nucleoside diphosphates.

• This effectively prevents DNA synthesis, and explains the toxicity of increased levels of dATP seen in conditions such as *adenosine deaminase* deficiency.
2. **Substrate specificity site**: The binding of nucleoside triphosphates to an additional allosteric site (known as the substrate specificity site) on the enzyme regulates substrate specificity, causing an increase in the conversion of different species of ribonucleotides to deoxyribonucleotides as they are required for DNA synthesis.
DEGRADATION OF PURINE NUCLEOTIDES

• Degradation of dietary nucleic acids occur in the small intestine, where a family of pancreatic enzymes hydrolyzes the nucleotides to nucleosides and free bases.

• Inside cells, purine nucleotides are sequentially degraded by specific enzymes, with the uric acid as the end product of this pathway.
• *Note: Mammals other than primates oxidize uric acid further to allantoin, which, in some animals other than mammals, may be further degraded to urea or ammonia

• A. Degradation of dietary nucleic acids in the small intestine

• Ribonucleases and deoxyribonucleases, secreted by the pancreas, hydrolyze RNA and DNA primarily to oligonucleotides
• Oligonucleotides are further hydrolyzed by pancreatic phosphodiesterases, producing a mixture of 3’- and 5’-mononucleotides
• A family of nucleotidases removes the phosphate groups hydrolytically, releasing nucleosides that may be absorbed by the intestinal mucosal cells, or be further degraded to free bases before uptake
• *Note – dietary purines and pyrimidines are not used to a large extent for the synthesis of tissue nucleic acids
• Instead, the dietary purines are generally converted to uric acid by intestinal mucosal cells
• Most of the uric acid enters the blood, and is eventually excreted in the urine
• For this reason, individuals with a tendency toward gout should be careful about consuming foods such as organ meats, anchovies, sardines, or dried beans, which contain high amounts of nucleic acids.

• The remainder of the dietary purines are metabolized by the intestinal flora.
• **B. Formation of uric acid**

  1. An amino group is removed from AMP to produce IMP, or from adenosine to produce inosine (hypoxanthine-ribose) by AMP or *adenosine deaminase*

  2. IMP and GMP are converted into their nucleoside forms – inosine and guanosine – by the action of 5’-nucleotidase
• 3. *Purine nucleoside phosphorylase* converts inosine and guanosine into their respective purine bases, hypoxanthine and guanine
• 4. Guanine is deaminated to form xanthine
• 5. Hypoxanthine is oxidized by *xanthine oxidase* to xanthine which is further oxidized by *xanthine oxidase* to uric acid, the final product of human purine degradation
• Uric acid is excreted in the urine
FIGURE 22-45 Catabolism of purine nucleotides. Note that primates excrete much more nitrogen as urea via the urea cycle (Chapter 18) than as uric acid from purine degradation. Similarly, fish excrete much more nitrogen as NH₄⁺ than as urea produced by the pathway shown here.

Excreted by:

- Primates, birds, reptiles, insects: Uric acid
- Most mammals: Allantoin
- Bony fishes: Allantoate
- Amphibians, cartilaginous fishes: Glyoxylate
- Marine invertebrates: Urea
Urate is further degraded in some organisms

- Mammals other than primates excrete allantoin, formed by oxidation of urate
- Teleost fish excrete allantoate, formed by hydration of allantoin
- In amphibians and most fish, allantoate is hydrolysed to two molecules of urea and one of glyoxylate
- Some marine invertebrates hydrolyse urea to $\text{NH}_4^+$ and $\text{CO}_2$
C. Diseases associated with purine degradation

1. **Gout**: Gout is a disorder characterized by high levels of uric acid in the blood, as a result of their over-production or under excretion of uric acid.

   Hyperuricemia results in the deposition of crystals of sodium urate – the end product of purine metabolism – in tissues, especially the kidney and joints, causing first **acute** and progressing to **chronic gouty arthritis**
Figure 22.16
Tophaceous gout.

Figure 22.17
Gout can be diagnosed by the presence of negatively birefringent monosodium urate crystals in aspirated synovial fluid examined by polarized-light microscopy. Here, crystals are within polymorphonuclear leukocytes.
a. **Primary gout**: In most patients, gout is caused by the under excretion of uric acid due to defective renal secretion.

- However, overproduction of uric acid may occur because of an inherited abnormality in the enzymes of purine metabolism.
- This is defined as “primary gout”.

- Lesch-Nyhan syndrome also causes hyperuricemia, as a result of the decreased salvage of hypoxanthine and guanine bases.
• **Secondary hyperuricemia**: This form of gout is caused by a variety of disorders and lifestyles, e.g., in patients with chronic renal insufficiency, those undergoing chemotherapy, those with myeloproliferative disorders and those who consume excessive amounts of alcohol or purine-rich foods.

• Gout can also be an adverse effect of seemingly unrelated metabolic diseases, such as von Gierke disease or fructose intolerance.
• **C. Treatment for gout:**
  
  • Acute attacks are treated with colchicine to decrease movement of granulocytes into the effected area, and with anti-inflammatory drugs, such as aspirin, to provide pain relief.
  
  • Most therapeutic strategies for gout involve lowering the uric acid level below the saturation point, thus preventing the deposition of urate crystals.
• C. Treatment for gout:
• Can be effectively treated by a combination of nutritional and drug therapies

**Nutritional therapies**

• Try to cut down or avoid:
  - Red meats which comes from cows or sheep and include steak, chops, corned beef, and larger pieces of meat usually roasted in the oven
• - **Offal foods** such as brains, kidneys, liver, heart and tongue
• - **Shellfish** such as pauas, pipis, mussels, oysters and sea eggs
• - Peas and beans
• - **Alcohol**, especially beer and wine
• - Try to keep your weight down
• Drug therapies:

• **Allopurinol**, analog of hypoxanthine in which N – 7 and C – 8 of hypoxanthine are reversed.

• Allopurinol is first a substrate, then a potent inhibitor of **xanthine oxidase**.

• Allopurinol is converted by xanthine oxidase to **oxipurinol** which promptly forms a **chelate** with a Mo$^{4+}$ of xanthine oxidase.
• The chelate traps the enzyme in its reduced state and renders it inactive.
• Thus, since uric acid cannot be formed from hypoxanthine and xanthine, these bases are excreted instead, and the symptoms of gout can be relieved.
• Allopurinol - an example of a suicide inhibitor.
**FIGURE 22-47** Allopurinol, an inhibitor of xanthine oxidase. Hypoxanthine is the normal substrate of xanthine oxidase. Only a slight alteration in the structure of hypoxanthine (shaded pink) yields the medically effective enzyme inhibitor allopurinol. At the active site, allopurinol is converted to oxypurinol, a strong competitive inhibitor that remains tightly bound to the reduced form of the enzyme.
Gertrude Elion (1918–1999) and George Hitchings (1905–1998)

• Allopurinol was developed by Gertrude Elion and George Hitchings, who also developed acyclovir, used in treating people with AIDS, and other purine analogs used in cancer chemotherapy
2. **Adenosine deaminase deficiency**: Adenosine deaminase (ADA) is expressed in the cytosol of all cells, but, in humans, lymphocytes have the highest activity of this enzyme.

- A deficiency of ADA results in an accumulation of adenosine which, is converted to its ribonucleotide or deoxyribonucleotide forms by cellular kinases.
As dATP levels rise, ribonucleotide reductase is inhibited, thus preventing the production of all deoxyribose-containing nucleotides.

Consequently, cells cannot make DNA and divide.

In its most severe form, this autosomal recessive disorder cases severe combined immunodeficiency disease (SCID), involving of both T cells and B cells.
• Children with this disorder must live in a sterile environment, and usually die by the age of two

• Treatment requires either bone marrow replacement or enzyme replacement therapy
Figure 22.18
Young child born with immune deficiency syndrome plays in the hospital.
PYRIMIDINE SYNTHESIS AND DEGRADATION

• Unlike the synthesis of the purine ring, in which the ring is constructed on a preexisting ribose 5-phosphate, the pyrimidine ring is synthesized before being attached to ribose 5-phosphate, which is donated by PRPP

• The sources of the atoms in the pyrimidine ring are glutamine, CO$_2$ and aspartic acid

• * Note – glutamine and aspartic acid are thus required for both purine and pyrimidine synthesis
Figure 22.19
Sources of the individual atoms in the pyrimidine ring.
A. Synthesis of carbamoyl phosphate

The regulated step of this pathway in mammalian cells is the synthesis of carbamoyl phosphate from glutamine and CO$_2$, catalyzed by carbamoyl phosphate synthetase II (CPS II)

$$2 \text{ ATP} + \text{CO}_2 + \text{Glutamine} \rightarrow \text{carbamoyl phosphate} + 2 \text{ ADP} + \text{Pi} + \text{glutamate}$$
• *CPS II* is inhibited by UTP (the end-product of this pathway, which can be converted into the other pyrimidine nucleotides) and is activated by ATP and PRPP
<table>
<thead>
<tr>
<th></th>
<th>CPS I</th>
<th>CPS II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cellular location</strong></td>
<td>Mitochondria</td>
<td>Cytosol</td>
</tr>
<tr>
<td><strong>Pathway involved</strong></td>
<td>Urea cycle</td>
<td>Pyrimidine synthesis</td>
</tr>
<tr>
<td><strong>Source of nitrogen</strong></td>
<td>Ammonia</td>
<td>γ-Amide group of glutamine</td>
</tr>
<tr>
<td><strong>Regulators</strong></td>
<td>Activator: N-acetyl-glutamate</td>
<td>Inhibitor: UTP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Activator: ATP</td>
</tr>
</tbody>
</table>
B. Synthesis of orotic acid

The second step in pyrimidine synthesis is the formation of carbamoylaspartate, catalyzed by aspartate transcarbamoylase.

The pyrimidine ring is then closed hydrolytically by dihydroorotase.

The resulting dihydroorotatate is oxidized to produce orotic acid.
Reaction No 1

\[
\text{carbamoyl phosphate} \xrightarrow{\text{aspartate transcarbamylase}} \text{carbamoyl aspartate}
\]

\[
\text{aspartate phosphate} \rightarrow \text{aspartate} + \text{Pi}
\]
Reaction No 2

carbamoyl aspartate \rightarrow \text{dihydroorotate} \\
\text{dihydroorotase} \quad H_2O
Reaction No 3

dihydroorotate dehydrogenase

\[
\text{dihydroorotate} \rightarrow Q \rightarrow QH_2 \rightarrow \text{orotate}
\]
• The enzyme that produces orotate, *dihydroorotate dehydrogenase*, is located inside the mitochondria

• All other reactions in pyrimidine biosynthesis are cytosolic

• **C. Formation of a pyrimidine nucleotide**

• The completed pyrimidine ring is converted to the nucleotide orotidine 5’-monophosphate (OMP) in the second stage of pyrimidine nucleotide synthesis
• PRPP is again the ribose 5-phosphate donor
• The enzyme orotate phosphoribosyltransferase produces OMP and releases pyrophosphate, thereby making the reaction biologically irreversible
• OMP, the parent pyrimidine mononucleotide, is converted to uridine monophosphate (UMP) by orotidylate decarboxylase, which removes the acidic carboxyl group
Reaction No 4

orotate phosphoribosyltransferase

orotate

PRPP

PPI

orotidine 5'-phosphate (OMP)
Reaction No 5

orotidine 5'-phosphate (OMP) → orotidine-5'-phosphate decarboxylase \[\text{CO}_2\] → uridine 5'-monophosphate (UMP)
Click on numbers to see reactions

1. Carbamoyl phosphate + aspartate → carbamoyl aspartate
2. Carbamoyl aspartate + H₂O → dihydroorotate
3. Q → orotate
4. Orotidine 5'-phosphate (OMP) + PPI + PRPP → orotate
5. Orotidine 5'-phosphate (OMP) → CO₂ + uridine 5'-monophosphate (UMP)
• Orotate phosphoribosyltransferase and orotidylate decarboxylase are domains of a single polypeptide chain called UMP synthase
• Orotic aciduria – a rare genetic defect – is caused by a deficiency of this bifunctional enzyme, resulting in orotic acid in the urine
• **D. Synthesis of uridine triphosphate and cytidine triphosphate**

• Cytidine triphosphate (CTP) is produced by amination of UTP by *CTP synthetase*

• *Note – the nitrogen is provided by glutamine – another example of a reaction in nucleotide biosynthesis in which this amino acid is required*
• E. Synthesis of thymidine monophosphate from dUMP

• dUMP is converted to dTMP by thymidylate synthase, which uses \( \text{N}^5,\text{N}^{10} \)-methylene tetrahydrofolate as the source of the methyl group

• This is an unusual reaction in that tetrahydrofolate (THF) contributes not only a carbon unit but also two hydrogen atoms from the pteridine ring, resulting in the oxidation of THF to dihydrofolate (DHF)
Inhibitors of *thymidylate synthase* include thymine analogs such as 5-flourouracil, which serve as successful antitumor agents. 5-flourouracil is metabolically converted to 5-FdUMP, which becomes permanently bound to the inactivated *thymidylate synthase*; for this reason the drug is called a “suicide inhibitor.”
• DHF can be reduced to THF by *dihydrofolate reductase*, an enzyme that is inhibited in the presence of drugs such as methotrexate

• By decreasing the supply of THF, these folate analogs not only inhibit purine synthesis, but by preventing methylation of dUMP to dTMP, they also lower the cellular concentration of this essential component of DNA
• DNA synthesis is, therefore, inhibited and cell growth slowed
• Because of their ability to slow the replication of DNA by decreasing the availability of nucleotide precursors, drugs such as those described above are used to decrease the growth rate of cancer cells
Conversion of dUMP to dTMP and its inhibition by FdUMP
• **F. Salvage of pyrimidines**

• Few pyrimidine bases are salvaged in human cells

• However, the pyrimidine nucleosides *uridine* and *cytidine* can be salvaged by *uridine-cytidine kinase*, *deoxycytidine* can be salvaged by *deoxycytidine kinase*

• Each of these enzymes catalyzes the phosphorylation of a nucleoside(s), utilizing ATP and forming UMP, CMP, dCMP and TMP
• *Note – herpes simplex virus encodes a virus-specific thymidine kinase, which phosphorylates the nucleoside analog acyclovir (acycloguanosine) to form acycloguanosine monophosphate

• After further phosphorylation, the resulting acycloguanosine triphosphate is incorporated by the viral DNA polymerase into viral DNA, causing chain termination in virus-infected cells
• **G. Degradation of pyrimidine nucleotides**

  Unlike the purine rings, which are not cleaved in human cells, the pyrimidine ring can be opened and degraded to highly soluble structures, such as β-alanine and β-aminoisobutyrate, which can serve as precursors of acetyl CoA and succinyl CoA, respectively.