METABOLISM OF NUCLEOTIDES

BCH 342

METABOLISM OF MACROMOLECULES

Textbooks

- BIOCHEMISTRY, Garrett & Grisham 4th Ed.
- BIOCHEMISTRY, Campbell & Farrell 7th Ed.
- BIOCHEMISTRY, Berg, Tymoczko & Stryer 7th Ed.
- BIOCHEMISTRY, Donald Voet & Judith Voet 4th Ed.
- HARPER’S ILLUSTRATED BIOCHEMISTRY 26th Ed.
- COLOR ATLAS OF BIOCHEMISTRY, Koolman & Roehm 2nd Ed.

ODUGBEMI A. I.
NUCLEOTIDES

- Nucleotides are the building blocks of nucleic acids, which are necessary for life sustaining cellular activities like storage and expression of genetic information.

- An adenine nucleotide, ATP, is the universal currency of energy.

- A guanine nucleotide, GTP, also serves as an energy source for a more select group of biological processes.

- Nucleotide derivatives such as UDP-glucose participate in biosynthetic processes such as the formation of glycogen.

- Nucleotides are essential components of signal-transduction pathways. Cyclic nucleotides such as cyclic AMP and cyclic GMP are second messengers that transmit signals both within and between cells.
NUCLEOTIDES

Each nucleotide consist of a **nitrogenous base**, **ribose sugar** and a **phosphate residue**.

**Note:** The glycosidic linkage of a nitrogenous base to a ribose sugar (in the absence of a phosphate residue) is a **NUCLEOSIDE**.

Therefore, **Nucleoside + Phosphate residue = Nucleotide**

Then nitrogenous bases are derivatives of two parent compounds, **pyrimidine** and **purine**.

There are 3 pyrimidine compounds (Uracil, Thymine and Cytosine) and 2 common purine compounds (Adenine and Guanine) that exist as components of nucleotides and largely nucleic acid.
NUCLEOTIDE BIOSYNTHESIS

The pathways for the biosynthesis of nucleotides fall into two classes: de novo pathways and salvage pathways.

- In de novo (from scratch) pathways, the nucleotide bases are assembled from simpler compounds. The framework for a pyrimidine base is assembled first and then attached to ribose. In contrast, the framework for a purine base is synthesized piece by piece directly onto a ribose-based structure.

- In salvage pathways, preformed bases are recovered and reconnected to a ribose unit.
DE-NOVO SYNTHESIS OF PURINE NUCLEOTIDE

Purine biosynthesis from ribose 5-phosphate and ATP
DE-NOVO SYNTHESIS OF PURINE NUCLEOTIDE

- **Step 1**: Ribose-5-phosphate is activated via the direct transfer of a pyrophosphoryl group from ATP to C-1 of the ribose, yielding 5-phosphoribosyl-α-pyrophosphate (PRPP). The enzyme is ribose-5-phosphate pyrophosphokinase.

- **Step 2**: This step is catalyzed by glutamine phosphoribosyl pyrophosphate amidotransferase which converts PRPP to Phosphoribosyl-β-amine (a β-glycoside). The anomeric carbon atom of the substrate PRPP is in the α-configuration; the product is a β-glycoside (recall that all the biologically important nucleotides are β-glycosides). Because PRPP serves additional metabolic needs, this step is actually the committed step in the pathway.

- **Step 3** is carried out by glycinamide ribonucleotide synthetase (GAR synthetase) via its ATP-dependent condensation of the glycine carboxyl group with the amine of 5-phosphoribosyl-β-amine.

- **Step 4**: Is the first of two THF-dependent reactions in the purine pathway. GAR transformylase transfers the N10-formyl group of N10-formyl-THF to the free amino group of GAR to yield α-N-formylglycinamide ribonucleotide (FGAR). Thus, C-8 of the purine is from a formyl group.
DE-NOVO SYNTHESIS OF PURINE NUCLEOTIDE

- **Step 5:** Catalyzed by FGAR amidotransferase (also known as FGAM synthetase). ATP-dependent transfer of the glutamine amido group to the C-4-carbonyl of FGAR yields formylglycinamidine ribonucleotide (FGAM).

- **Step 6:** ATP is used to phosphorylate the oxygen atom of the formyl group, activating it for the ring closure step that follows. Because the product is 5-aminoimidazole ribonucleotide, or AIR, this enzyme is called AIR synthetase.

- **Step 7:** Here, carbon dioxide is added at the C-4 position of the imidazole ring by AIR carboxylase in an ATP-dependent reaction; the carbon of CO$_2$ will become C-6 of the purine ring. The product is carboxyaminoimidazole ribonucleotide (CAIR).

- **Step 8:** the amino-N of aspartate provides N-1 through linkage to the C-6 carboxyl function of CAIR. ATP hydrolysis drives the condensation of Asp with CAIR. The product is N-succinylo-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR). SAICAR synthetase catalyzes the reaction.
DE-NOVO SYNTHESIS OF PURINE NUCLEOTIDE

- **Step 9** removes the four carbons of Asp as fumarate in a nonhydrolytic cleavage. The product is 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR); the enzyme is adenylosuccinase (adenylosuccinate lyase). Adenylosuccinase acts again in that part of the purine pathway leading from IMP to AMP.

- **Step 10** adds the formyl carbon of N\(^{10}\)-formyl-THF as the ninth and last atom necessary for forming the purine nucleus. The enzyme is called AICAR transformylase; the products are THF and N-formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR).

- **Step 11** involves dehydration and ring closure to form the purine nucleotide IMP (inosine-5’-monophosphate or inosinic acid); this completes the initial phase of purine biosynthesis. The enzyme is IMP cyclohydrolase (also known as IMP synthase and inosinicase). Unlike step 6, this ring closure does not require ATP.
AMP AND GMP ARE SYNTHESIZED FROM IMP

(a) AMP synthesis: The two reactions of AMP synthesis mimic steps 8 and 9 in the purine pathway leading to IMP.

(b) GMP synthesis.

The synthesis of AMP and GMP from IMP. (a) AMP synthesis: The two reactions of AMP synthesis mimic steps 8 and 9 in the purine pathway leading to IMP. (b) GMP synthesis.
SALVAGE PATH FOR PURINE SYNTHESIS

- Nucleic acid turnover (synthesis and degradation) is an ongoing metabolic process in most cells. Messenger RNA in particular is actively synthesized and degraded. These degradative processes can lead to the release of free purines in the form of adenine, guanine, and hypoxanthine (the base in IMP).

- Purines represent a metabolic investment by cells. So-called salvage pathways exist to recover them in useful form. Salvage reactions involve re-synthesis of nucleotides from bases via phosphoribosyltransferases.

- \[
  \text{Base} + PRPP \xrightarrow{\text{HGPRT or APRT}} \text{nucleoside} - 5' - \text{phosphate} + \text{PPI}
  \]

- The subsequent hydrolysis of PPI to inorganic phosphate by pyrophosphatases renders the phosphoribosyltransferase reaction effectively irreversible.

- Adenine phosphoribosyltransferase (APRT) - mediates AMP formation

- Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) - acts on either hypoxanthine to form IMP or guanine to form GMP
SALVAGE PATH FOR PURINE SYNTHESIS

Hypoxanthine + α-PRPP $\xrightarrow{\text{HGPRT}}$ IMP

Guanine + α-PRPP $\xrightarrow{\text{HGPRT}}$ GMP

Purine Salvage by HGPRT
PYRIMIDINE BIOSYNTHESIS

- In the synthesis of pyrimidines, the ring is synthesized first and then it is attached to a ribose phosphate to form a pyrimidine nucleotide. Pyrimidine rings are assembled from bicarbonate, aspartate, and ammonia. Although an ammonia molecule already present in solution can be used, the ammonia is usually produced from the hydrolysis of the side chain of glutamine.

- **Step 1**: The first step in de novo pyrimidine biosynthesis is the synthesis of carbamoyl phosphate from bicarbonate and ammonia (from glutamine) in a multistep process, requiring the cleavage of two molecules of ATP. This reaction is catalyzed by **carbamoyl phosphate synthetase II (CPS-II)**, a cytosolic enzyme. Because carbamoyl phosphate made by CPS-II in mammals has no fate other than incorporation into pyrimidines, mammalian CPS-II can be viewed as the committed step in the pyrimidine de novo pathway. Bacteria and plants have but one CPS, and its carbamoyl phosphate product is incorporated into arginine as well as pyrimidines. Thus, the committed step in bacterial pyrimidine synthesis is the next reaction, which is mediated by aspartate transcarbamoylase (ATCase).

**Note**: Mammals have two enzymes for carbamoyl phosphate synthesis. Carbamoyl phosphate synthetase I (CPS-I) is a mitochondrial enzyme dedicated to the urea cycle and arginine biosynthesis.
PYRIMIDINE BIOSYNTHESIS

\[
\text{HCO}_3^- + \text{Glutamine} + 2 \text{ATP} + \text{H}_2\text{O} \rightarrow \text{Glutamate} + 2 \text{ADP} + \text{P} + \text{Carbamoyl-} \text{P} \rightarrow \text{Aspartate} \rightarrow \text{Carbamoyl-Asp} \rightarrow \text{Dihydroorotic acid (DHO)} \rightarrow \text{Orotic acid (a pyrimidine)} \rightarrow \text{Orotidine 5'-monophosphate (OMP)}
\]

**Step 1:** CPS-II (Citrulline-ornithine pathway)

**Step 2:** ATCase

**Step 3:** Dihydroorotase

**Step 4:** DHO dehydrogenase

**Step 5:** Orotate phosphoribosyltransferase

**Step 6:** OMP decarboxylase

**FIGURE 26.15** The de novo pyrimidine biosynthetic pathway.
PYRIMIDINE BIOSYNTHESIS

- **Step 2**: Here, aspartate transcarbamoylase (ATCase) catalyzes the condensation of carbamoyl phosphate with aspartate to form carbamoyl aspartate. No ATP input is required at this step because carbamoyl phosphate represents an “activated” carbamoyl group.

- **Step 3**: Involves ring closure and dehydration via linkage of the —NH₂ group introduced by carbamoyl phosphate with the former β-COO⁻ of aspartate; this reaction is mediated by the enzyme dihydroorotase. The product of the reaction is dihydroorotate (DHO).

- **Step 4**: This is where oxidation of DHO to yield Orotate occurs. This oxidation is catalyzed by dihydroorotate dehydrogenase.

- **Step 5**: At this stage, ribose-5-phosphate is joined to N-1 of orotate in appropriate N-β-glycosidic configuration, giving the pyrimidine nucleotide orotidine-5′-monophosphate (OMP). The ribose phosphate donor is PRPP; the enzyme is orotate phosphoribosyltransferase.

- **Step 6**: Decarboxylation of OMP gives UMP (uridine-5′-monophosphate, or uridylic acid) and it is catalyzed by OMP decarboxylase.
UMP Synthesis Leads to Formation of the Ribonucleotides—UTP and CTP

- The two prominent pyrimidine ribonucleotide products are derived from UMP via the same unbranched pathway. First, UDP is formed from UMP via an ATP-dependent nucleoside monophosphate kinase.

\[
UMP + ATP \rightarrow UDP + ADP
\]

- Then, UTP is formed by nucleoside diphosphate kinase

\[
UDP + ATP \rightarrow UTP + ADP
\]

- Amination of UTP at the 6-position gives CTP. It is catalized by enzyme CTP synthetase, a glutamine amidotransferase.

\[
\text{CTP} = \text{UTP} + \text{Glutamine} + \text{Glutamate} + \text{ATP} + \text{ADP} + \text{P} + \text{H}_2\text{O}
\]
Formation of DeoxyRibonucleotide

- The deoxyribonucleotides have only one metabolic purpose: to serve as precursors for DNA synthesis. In most organisms, ribonucleoside diphosphates (NDPs) are the substrates for deoxyribonucleotide formation. Reduction at the 2′-position of the ribose ring in NDPs produces 2′-deoxy forms of these nucleotides. This reaction involves replacement of the 2′-OH by a hydride ion (H:\textsuperscript{−}) and is catalyzed by an enzyme known as ribonucleotide reductase. NADPH is the ultimate source of reducing equivalents for ribonucleotide reduction, but the immediate source is reduced thioredoxin.

Oxidation–reduction cycle involving ribonucleotide reductase, thioredoxin, thioredoxin reductase, and NADPH.
Thymine nucleotide synthesis

\[ \text{dUMP} \xrightarrow{\text{Thymidylate synthase}} \text{dTMP} \]

\[ \text{N}^5\text{N}^10\text{-methylene-THF} \]

\[ \text{Dihydrofolate acid (DHF)} \]
Purines Biosynthesis Regulation

6.6 The regulatory circuit controlling purine synthesis.
Pyrimidine Biosynthesis Regulation

Pyrimidine biosynthesis in bacteria is allosterically regulated at **aspartate transcarbamoylase (ATCase)**. *Escherichia coli* ATCase is feedback-inhibited by the end product, CTP. ATP, which can be viewed as a signal of both energy availability and purine sufficiency, is an allosteric activator of ATCase. CTP and ATP compete for a common allosteric site on the enzyme. In many bacteria, UTP, not CTP, acts as the ATCase feedback inhibitor.

In animals, CPS-II catalyzes the committed step in pyrimidine synthesis and serves as the focal point for allosteric regulation. UDP and UTP are feedback inhibitors of CPS-II, whereas PRPP and ATP are allosteric activators.
DEGRADATION OF NUCLEOTIDES

Purine Nucleotide Degradation

- The major pathways of purine catabolism in animals lead to uric acid formation.
- The various nucleotides are first converted to nucleosides by intracellular nucleotidases.
- Nucleosides are then degraded by the enzyme purine nucleoside phosphorylase (PNP) to release the purine base and ribose-1-P.
- Note that neither adenosine nor deoxyadenosine is a substrate for PNP. Instead, these nucleosides are first converted to inosine by adenosine deaminase.
- The PNP products are merged into xanthine by guanine deaminase and xanthine oxidase, and xanthine is then oxidized to uric acid by this latter enzyme.
Different Animals Oxidize Uric Acid to Form Excretory Products

- In humans and other primates, uric acid is the end product of purine catabolism and is excreted in the urine.
- Birds, terrestrial reptiles, and many insects also excrete uric acid, but in these organisms, uric acid represents the major nitrogen excretory compound, because, unlike mammals, they do not also produce urea.
In some organisms, free pyrimidines, like purines, are salvaged and recycled to form nucleotides via phosphoribosyltransferase reactions similar to those discussed earlier. In humans, however, pyrimidines are recycled from nucleosides, but free pyrimidine bases are not salvaged.

Pyrimidine catabolism results in degradation of the pyrimidine ring to products reminiscent of the original substrates, aspartate, CO2, and ammonia. β-Alanine can be recycled into the synthesis of coenzyme A. Catabolism of the pyrimidine base, thymine (5-methyluracil), yields β-aminoisobutyric acid instead of β-alanine.
GOUT

- Gout is the clinical term describing the physiological consequences accompanying excessive uric acid accumulation in body fluids.
- Uric acid and urate salts are rather insoluble in water and tend to precipitate from solution if produced in excess.
- The most common symptom of gout is arthritic pain in the joints as a result of urate deposition in cartilaginous tissue. The joint of the big toe is particularly susceptible.
- Urate crystals may also appear as kidney stones and lead to painful obstruction of the urinary tract.
- Hyperuricemia, chronic elevation of blood uric acid levels, occurs in about 3% of the population as a consequence of impaired excretion of uric acid or overproduction of purines.
- Purine-rich foods such as fish eggs (rich in nucleic acids) may exacerbate the condition.
- The biochemical causes of gout are varied. However, a common treatment is **allopurinol**.
  - **Allopurinol** is a hypoxanthine analog that binds tightly to xanthine oxidase, thereby inhibiting its activity and preventing uric acid formation.
  - Hypoxanthine and xanthine do not accumulate to harmful concentrations because they are more soluble and thus more easily excreted.
Adenylosuccinase Deficiency

- It is a rare hereditary defect.
- It is an autosomal recessive disorder causing profound intellectual disability, autistic behaviour, and seizures.
- Diagnosis is established by gene sequencing and also by identifying elevated levels of succinylaminoimidazole carboxamide riboside and succinyladenosine in CSF and urine.
- There is no effective treatment for adenylosuccinase deficiency.
Hyperactivity of Phosphoribosyl Pyrophosphate Synthetase

- It is sex linked (X-linked) recessive disorder causes purine overproduction.

- Excess purine is degraded, resulting in hyperuricemia and gout and neurologic and developmental abnormalities.

- Diagnosis of phosphoribosylpyrophosphate synthetase superactivity is by enzyme studies on RBCs and cultured skin fibroblasts. Diagnosis can also be done by gene sequencing

- Treatment is with allopurinol and a low-purine diet.
Severe Combined Immunodeficiency Disease (SCID)

- It is the common name of the hereditary deficit of the enzyme adenosine deaminase.

- The enzyme deficiency results in accumulation of adenosine, which is converted to its ribonucleotide and deoxyribonucleotide (dATP) forms by cellular kinases.

- Pathogenesis is due to the accumulation in the cells of deoxyAdenosine triphosphate (dATP), that inhibits the enzyme ribonucleotide reductase and slows down the biosynthesis of DNA.

- All rapidly regenerating tissues are affected and the precursors of granulocytes and lymphocytes are affected most.

- Immune cells are especially sensitive to this defect. The disease is invariably fatal because of infections.

- Diagnosis is suspected on clinical grounds (severely reduced lymphocyte and granulocyte count) and confirmed by genetic analysis.

- Treatment of adenosine deaminase deficiency is by bone marrow or stem cell transplantation and enzyme replacement therapy.
**Purine Nucleoside Phosphorylase (PNP) Deficiency**

- This is a rare autosomal recessive deficiency characterized by immunodeficiency with severe T-cell dysfunction and often neurologic symptoms.

- Manifestations are lymphopenia, thymic deficiency, recurrent infections, and hypouricemia. Many patients have developmental delay, ataxia, or spasticity.

- The clinical picture is that of an immune deficiency, less severe than SCID.

- Diagnosis of purine nucleoside phosphorylase deficiency is by low enzyme activity in RBCs.

- Treatment is with bone marrow or stem cell transplantation.
Xanthine oxidase deficiency

- It causes buildup of xanthine, which may precipitate in the urine, causing symptomatic stones with hematuria, urinary colic, and UTIs.

- Diagnosis of xanthine oxidase deficiency is by low serum uric acid and high urine and plasma hypoxanthine and xanthine.

- Enzyme determination requires liver or intestinal mucosal biopsy and is rarely indicated.

- Treatment of xanthine oxidase deficiency is high fluid intake to minimize likelihood of stone formation.
Lesch-Nyhan syndrome

- It is an inherited disorder caused by a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT).
- It is produced by mutations in the HPRT gene located on the X chromosome. The degree of deficiency (and hence manifestations) vary with the specific mutation.
- The disease usually manifests between 3 months and 12 months of age with the appearance of orange sandy precipitate (xanthine) in the urine; it progresses to CNS involvement with intellectual disability, spastic cerebral palsy, involuntary movements, and self-mutilating behavior (particularly biting).
- There is failure of the salvage pathway for hypoxanthine and guanine. These purines are instead degraded to uric acid.
- There is manifestation of Hyperuricemia predisposes to gout and its complications.
- Diagnosis of Lesch-Nyhan syndrome is suggested by the combination of dystonia, intellectual disability, and self-mutilation. Serum uric acid levels are usually elevated, but confirmation by HGPRT enzyme assay is usually done.
- CNS dysfunction has no known treatment; management is supportive. Self-mutilation may require physical restraint, dental extraction, and sometimes drug therapy; a variety of drugs has been used. Hyperuricemia is treated with a low-purine diet (e.g, avoiding organ meats, beans, sardines) and allopurinol.
PYRIMIDINE METABOLISM DISORDERS

Orotic Aciduria

- This condition is due to hereditary deficiency of the enzyme uridine monophosphate synthase, a bifunctional enzyme that catalyzes two reactions in the pyrimidine biosynthesis pathway (orotate phosphoribosyl transferase and OMP decarboxylase activity)

- With deficiency, orotic acid accumulates, causing clinical manifestations of megaloblastic anemia, mental retardation, orotic crystalluria and nephropathy, cardiac malformations, and stunted growth.

- Diagnosis may include high amounts of orotic acid in the urine. Gene sequencing is also possible and reveals the mutation.

- Treatment of uridine monophosphate synthase deficiency is with oral uridine supplementation.