The Metabolism (Synthesis and Degradation) of Nucleotides

Objectives

I. Activation of Ribose for Nucleotide Biosynthesis
   A. Describe the synthesis of 5-phosphoribosyl-α1-pyrophosphate.
   B. Describe the importance of this reaction.
   C. Describe the allosteric control of this reaction.

II. Purine Biosynthesis
   A. What molecules serve as the source of the atoms used in the synthesis of a purine nucleotide?
   B. Delineate the sequence of reactions (group additions) involved in the synthesis of a purine nucleotide.
   C. Detail the reactions leading from inosine-5′-monophosphate to adenosine-5′-monophosphate and inosine-5′-monophosphate to guanosine-5′-monophosphate.
   D. Note similarities among the reactions of the urea cycle and the reactions of purine biosynthesis.
   E. Describe the control of purine biosynthesis.
      1. The allosteric enzymes and molecules that control their activity.

III. Pyrimidine Biosynthesis
   A. What molecules serve as the source of the atoms used in the synthesis of a pyrimidine nucleotide?
   B. Delineate the sequence of reactions / group additions involved in the synthesis of a pyrimidine nucleotide.
   C. Detail the reactions leading from uridine-5′-monophosphate to cytidine-5′-triphosphate.
   D. Note similarities among the reactions of the urea cycle and the reactions of pyrimidine biosynthesis.
   E. Describe the control of pyrimidine biosynthesis.
      1. The allosteric enzymes and molecules that control their activity

IV. Formation of Deoxynucleotides
   A. Describe the enzymes and small molecules involved in the reduction of nucleotides to deoxynucleotides.
   B. Electron donor molecule
   C. Delineate the flow of electrons through this “electron transport chain” to the ribose moiety.
   D. Recount the control mechanisms of this enzyme.

V. Deoxythymidine Synthesis
   A. Describe the enzymes and cosubstrates involved in this reaction.
   B. What function(s) does N5, N10-methylenetetrahydrofolate serve during this reaction?
   C. What is the medicinally important inhibitor of this reaction and what is its mechanism?

VI. Salvage Pathways
   A. What are the purine salvage pathways?
   B. Why are they important?
   C. What enzymes are involved in these pathways?
   D. Describe possible reason(s) for a lack of pyrimidine salvage enzymes.

VII. Purine Catabolism
   A. Understand the general principles of the process.
      1. What is the final product of this pathway?
      2. What disease state is associated with excess purine catabolism and/or malfunctioning

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enzymes?

VIII. Pyrimidine Catabolism
A. Understand the general principles of the process.
   1. What are the final products of cytidine or uridine catabolism?
   2. What are the final products of thymidine catabolism?
      a) How does the catabolism of thymidine interrelate with fatty acid and amino acid catabolism?

IX. Integrate Nucleotide Metabolism with Carbohydrate Metabolism, Lipid Metabolism, & Amino Acid Metabolism.

X. Ask yourself “What If Questions”

Nucleotide Biosynthesis

**PURINE BIOSYNTHESIS** starts with the formation of an activated ribose intermediate. Ribose is activated by the enzyme **Ribose-5-phosphate Pyrophosphokinase**. An older name for the enzyme is **5-Phosphoribosyl-1-pyrophosphate Synthase**. This enzyme catalyzes the transfer of **pyrophosphate** from ATP to the anomeric hydroxyl group, the hydroxyl group on carbon one of ribose-5-phosphate. The product of the reaction is **5-phosphoribosyl-α1-pyrophosphate (PRPP)** and AMP. **PYRIMIDINE BIOSYNTHESIS** and the **PURINE SALVAGE PATHWAYS** (see below) also require PRPP, the activated ribose molecule.

Ribose-5-phosphate Pyrophosphokinase is an allosteric enzyme. Its activity is inhibited when the cell contains adequate amounts of the nucleotides.

**Purine Biosynthesis**

Purine nucleotide biosynthesis is a complex 10 step process. This pathway will be very very briefly examined. The source of the atoms that makeup the purine ring and the order in which they are added to form the purine ring is necessary information:

- **N1** is from Aspartate
- **C2 and C8** are donated by **N10-Formyl-Tetrahydrofolate**
- **N3 and N9** are donated by Glutamine
- **C4, C5 and N7** are from Glycine
- **C6** is from CO2 (HCO3⁻)

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The pathway starts with Glutamine-PRPP Amidotransferase transferring the amide nitrogen of glutamine to the anomeric carbon, C1 of 5-phosphoribosyl-1-pyrophosphate (PRPP). When the amide group is transferred, the pyrophosphate on C1 is released and the configuration around the anomeric carbon switches from $\alpha$ to $\beta$. The product is 5-phospho-$\beta$-d-ribosylamine. The transferred amine group is N9 of the purine ring system.

(2) - Glycine (C4, C5, and N7) is added to N9 of the growing purine.

(3) - A formyl group (C8) donated by N10-formyl-tetrahydrofolate is attached to the free amino group.

(4) - Glutamine now donates its amido group (N3) to the carbonyl carbon.

(5) - The five membered ring of the purine nucleus is closed by the enzyme AIR Synthetase.

(6) - CO$_2$ (C6) is added to the growing purine ring system.

(7) - The amino group of aspartate is now linked to the just added carboxyl group.

(8) - The intermediated is cleaved releasing fumarate. The amino group of aspartate is left behind and it becomes N1 of the purine.
(9) - A formyl group (C2) from N\textsuperscript{10}-formyl-tetrahydrofolate is now added.

(10) - In the last step the 6 membered ring of the purine nucleus is closed by the action of IMP Cyclohydrolase. The product is INOSINE-5’-MONOPHOSPHATE (IMP).

**IMP to AMP and GMP**

Inosine-5’-monophosphate (IMP) is the precursor for Adenosine-5’-monophosphate (AMP) and Guanosine-5’-monophosphate (GMP). IMP is converted to AMP in a two step pathway. In the first step aspartate is added to the carbonyl group on C6 of IMP to form adenylosuccinate. This reaction is catalyzed by Adenylosuccinate Synthetase. The energy released by the hydrolysis of GTP to GDP and PO\textsubscript{4} occurs is used to drive the formation of the new chemical bond.
The adenylosuccinate is now cleaved by the enzyme Adenylosuccinate Lyase to form AMP and fumarate. The donation of an amino group by adding an aspartate and removing a fumarate is a repeating theme in biosynthesis and it is one of the two ways in which amino/amide groups are transferred.

IMP is converted to GMP in two steps. First, \( \text{H}_2\text{O} \) is added across the double bond between \( \text{C}2 \) and \( \text{N}3 \) of the purine ring and the resulting hydroxyl group on \( \text{C}2 \) is oxidized to a carbonyl group. This reaction is catalyzed by IMP Dehydrogenase. Xanthosine-5’-monophosphate (XMP) is the product.

Glutamine then donates its amide group to the newly formed carbonyl group to form GMP and glutamate. During this reaction, catalyzed by GMP Synthetase, a molecule of ATP is hydrolyzed to AMP and two phosphates to supply the energy for the formation of the new chemical bond. Glutamine donating its amide group is the second method in which amino/amide groups are transferred.
Control of Purine Biosynthesis

Purine nucleotide biosynthesis is an energy expensive pathway and as such it is tightly regulated. The cell has no need to synthesize more purines than are absolutely necessary. This pathway is controlled at several points.

IMP, AMP, and GMP are allosteric effectors for Ribose-5-phosphate Pyrophosphokinase. When their concentrations are elevated, the activity of this enzyme is inhibited.

The Committed Step for purine biosynthesis is the reaction catalyzed by Glutamine-PRPP Amidotransferase. This enzyme is allosterically inhibited by IMP, AMP, and GMP.

The pathways from IMP to AMP and from IMP to GMP are also allosterically controlled. AMP inhibits the activity of Adenylosuccinate Synthetase. XMP and GMP inhibit the action of IMP Dehydrogenase.

There is cross talk between the pathways from IMP to AMP and from IMP to GMP assuring that AMP and GMP are synthesized in balanced adequate amounts. The need for GTP in AMP synthesis and ATP in GMP synthesis helps to maintain the balance between GMP and AMP concentrations in the cell. The IMP to AMP pathway, the IMP to GMP pathway, and the control points of purine biosynthesis are important to know.

Pyrimidine Biosynthesis

Pyrimidine nucleotide biosynthesis is a much more straight forward process. C2 of the pyrimidine ring comes from HCO₃⁻ (CO₂), N3 comes from glutamine, and the remainder of the pyrimidine molecule (N1, C4, C5, and C6) comes from a molecule of Aspartate. The pyrimidine ring is synthesized in four steps and then joined to PRPP to form the nucleoside-5´-monophosphate. This is different from purine synthesis where the ring is built step by step on ribose-5´-phosphate starting with PRPP.

The pathway for the formation of pyrimidine nucleotides begins with the formation of carbamoylphosphate. This reaction is catalyzed by Carbamoylphosphate Synthetase II. This enzyme takes glutamine as the ammonia donor, HCO₃⁻, and 2 ATP molecules and catalyzes the formation of carbamoylphosphate. Other products include glutamate, 2 ADP and a phosphate. This enzyme is different from Carbamoylphosphate Synthetase I used for urea synthesis. Carbamoylphosphate Synthetase II is a cytosolic enzyme requiring glutamine as the nitrogen donor, whereas Carbamoylphosphate Synthetase I is a mitochondrial enzyme that utilizes ammonia.

Carbamoylphosphate is then condensed with a molecule of aspartate to form carbamoylaspartate. This reaction is catalyzed by the enzyme Aspartate Transcarbamoylase.

Dihydroorotate catalyzes a dehydration reaction that results in closure of the pyrimidine ring. Dihydroorotate is the product of this reaction. The first three enzymes of the pathway; Carbamoyl
phosphate Synthetase II, Aspartate Transcarbamoylase, and Dihydroorotase; are contained on a single multifunctional protein present in the cytoplasm.
The dihydroorotate is oxidized to orotate by Dihydroorotate Dehydrogenase. In bacteria the enzyme is a NAD-linked flavoprotein containing bound FAD, FMN and Fe-S centers. In eukaryotes the enzyme is bound to the inner mitochondrial membrane. The electrons are immediately accepted by a quinone (a CoQ like molecule) and then passed to the ET/OxPhos pathway for ATP generation.

Orotate is now coupled to PRPP to form Orotidine-5’-monophosphate (OMP). This reaction is catalyzed by Orotate phosphoribosyl Transferase.

In the last step of the pathway OMP is decarboxylated by OMP Decarboxylase to form Uridine-5’-monophosphate (UMP). The last two enzymes of the pathway; Orotate phosphoribosyl Transferase and OMP Decarboxylase; are contained on a single multifunctional protein present in the cytoplasm.

Cytidine nucleotides are synthesized from UMP. However, before the uridine base can be converted to cytidine the UMP must be phosphorylated to UTP.

The UTP is then converted to Cytidine-5’-triphosphate (CTP) by CTP Synthetase. This enzyme takes the amide group from glutamine and attaches it to the carbonyl carbon, C4, of UTP. The hydrolysis of ATP drives the reaction to completion. The other product is glutamate.
Control of Pyrimidine Biosynthesis

Pyrimidine nucleotide biosynthesis is controlled at the step catalyzed by Carbamoyl phosphate Synthetase II. This is an allosteric enzyme. PRPP and ATP activate the enzyme and UDP and UTP are allosteric inhibitors of its activity.

Deoxyribonucleotides

The four ribonucleotides obtained from the biosynthesis pathways - AMP, GMP, UMP, and CTP are reduced to the deoxyribonucleotides needed for DNA synthesis. Before they can be reduced to deoxyribonucleotides they must all be converted to the nucleoside diphosphate forms. The addition or removal of phosphate from the various nucleotides is accomplished by the Nucleoside Monophosphate Kinases or Nucleoside Diphosphate Kinase (see below).

The reduction reaction is catalyzed by the enzyme Ribonucleotide Reductase. This enzyme has associated with it one of two possible small electron transport chains composed of two other proteins; one is an enzyme and the other is a small disulfide containing protein. In the first system Thioredoxin Reductase and Thioredoxin make up the electron transport chain and reduction of the ribose moiety of the nucleotides precedes as follows:

\[
\text{NADPH} + \text{H}^+ \rightarrow \text{FAD} \rightarrow \text{FADH}_2 \rightarrow \text{Thioredoxin} \rightarrow \text{Ribonucleotide Reductase} \rightarrow \text{H}_2\text{O}
\]

NADPH passes a pair of electrons to a FAD covalently linked to Thioredoxin Reductase to form NADP and FADH₂. The FADH₂ then reduces a disulfide (–S–S–) group on Thioredoxin Reductase to a pair of thiol (–SH) groups. FADH₂ is oxidized to FAD. Thioredoxin Reductase passes the electrons to a disulfide (–S–S–) on the protein Thioredoxin. The disulfide (–S–S–) on Thioredoxin is reduced to a pair of thiol (–SH) groups and the thiols (–SH) on Thioredoxin Reductase are oxidized to a disulfide (–S–S–). Ribonucleotide
Reductase picks up the elections from Thioredoxin, oxidizing the thiol (–SH) groups on Thioredoxin to a disulfide (–S–S–) and reducing the disulfide (–S–S–) group on Ribonucleotide Reductase to a pair of thiol (–SH) groups. Finally, the electrons are used to reduce the ribose moiety of the ribonucleotides to 2-deoxyribose. During this reduction reaction the thiol (–SH) groups on Ribonucleotide Reductase are oxidized to a disulfide (–S–S–).

In the second system Glutathione (a small molecule), Glutaredoxin Reductase (an enzyme), and Glutaredoxin (a protein) make up the electron transport chain. The flow of electrons to reduce the ribose is for the most part identical, except the NADPH reduces a molecule of Oxidized glutathione in the first step of the electron pathway rather than reducing a molecule of FAD.

Ribonucleotide Reductase has a unique control mechanism to assure that the deoxyribonucleotides are synthesized in adequate and balanced amounts. This enzyme contains an Activity Site, a Specificity Site, and the catalytic site. The Activity Site turns the enzyme “ON” or “OFF”; the Specificity Site controls which nucleotide will be reduced; and the catalytic site performs the reduction.

When the Activity Site is occupied by ATP the enzyme is turned “ON”.
When the Activity Site is occupied by deoxy ATP the enzyme is turned “OFF”.

When the Specificity Site is occupied by ATP or deoxy ATP (dATP) then CDP or UDP is reduced. When the Specificity Site is occupied by deoxyTTP (dTTP) then GDP is reduced. When the Specificity Site is occupied by deoxyGTP (dGTP) then ADP is reduced.

The Specificity Site assures that the deoxyribonucleotides are synthesized in balanced and adequate amounts. Remember that in DNA A pairs with T and G pairs with C.

1. When the concentration of ATP is high, the cell is energy rich, it has the energy to synthesize DNA and divide. ATP binds to the activity site to turn the enzyme “ON”. ATP also binds to the specificity site to stimulate the reduction of the pyrimidines, UDP and CDP. DeoxyUDP is the precursor of deoxythymidine (dTTP), the base pair partner of dATP in DNA. High ATP concentrations stimulate the synthesis of its partner in DNA and the partner of deoxyguanosine.
2. As dTTP concentrations build up it signals that the deoxy pyrimidines are present in adequate
amounts for DNA replication. dTTP binds to the specificity site and stimulates the reduction of one of the purines, GDP to dGDP.

3. As dGTP concentrations increase, it binds to the specificity site and stimulates the reduction of the other purine. ADP is reduced to dADP.

4. As dATP concentrations increase they signal that all four deoxy nucleotide triphosphates are present in adequate amounts for DNA replication. dATP replaces ATP in the activity site and the enzyme is turned “OFF”.

Formation of DeoxyTMP from DeoxyUMP

Deoxyuridylate nucleotides are never incorporated into DNA. Two mechanisms assure that the deoxyuridylate nucleotides are not incorporated into DNA. First, the enzyme Deoxyuridine Triphosphate Diphosphohydrolase rapidly converts any deoxyUTP that is formed to deoxyUMP. Second, the deoxyUMP is rapidly and quantitatively converted to deoxyTMP.

The conversion of deoxyUMP (dUMP) to deoxyTMP (dTMP) is catalyzed by the enzyme Thymidylate Synthase. N5,N10-Methylene-Tetrahydrofolate (N5,N10-Methylene-TH4) serves two functions during the course of this reaction. First it donates a one carbon fragment to the dUMP nucleotide. The one carbon fragment donated by tetrahydrofolate is in the methylene (–CH2–) oxidation state. In the final product the one carbon fragment is in the methyl (–CH3) oxidation state. During the second part of the reaction the tetrahydrofolate molecule acts as a reducing agent. It donates a pair of hydrogen atoms to reduce the one
carbon fragment from the methylene oxidation state to the methyl oxidation state. The enzyme Thymidylate Synthase catalyzes the transfer of the one carbon methylene fragment from TH$_4$ to C5 of uridine and it simultaneously reduces the one carbon fragment to a methyl group. The products of this reaction are dTMP and dihydrofolate.

Dihydrofolate is useless to the cell. It must be reduced to tetrahydrofolate if cellular metabolism is to be maintained. The reduction process is catalyzed by the enzyme Dihydrofolate Reductase. NADPH and a hydrogen ion (H$^+$) donates the hydrogens and electrons necessary for the reaction. Once the TH$_4$ is reformed it accepts a one carbon fragment from serine or glycine and it is ready for the next cycle of reactions.

The enzyme Dihydrofolate Reductase was the first target for cancer chemotherapeutic agents. dTMP (dTTP) is needed for DNA replication, inhibiting the formation of dTTP would inhibit DNA replication.
With DNA synthesis inhibited, cancer cells would cease to divide, and the tumor would stop growing. In fact all rapidly dividing cells cease to multiply. The drug METHOTREXATE is a specific competitive inhibitor of the enzyme Dihydrofolate Reductase. This enzyme inhibitor was the first cancer chemotherapeutic agent. The side effects; hair loss, loss of appetite, etc.; are due to inhibition of normal cell division.

Purine Salvage Pathways

The synthesis of purines is an energy expensive pathway and only a small amount of energy is recovered during their degradation. To save energy the cell recycles as many of the purine nucleotides as possible using the Purine Salvage Pathways.

During the digestion of food stuffs and cellular metabolism, the purine nucleotides are broken down to phosphate, ribose (deoxyribose) and the bases adenine, guanine, and/or hypoxanthine. Hypoxanthine is the purine base present on Inosine-5'-monophosphate, its the base on IMP.

The purine bases are salvaged by the action of two enzymes. Adenine phosphoribosyl Transferase couples the adenine base to 5-phosphoribosyl-α1-pyrophosphate (PRPP) to form AMP. Hypoxanthine-Guanine phosphoribosyl Transferase joins the hypoxanthine base to PRPP to form IMP and/or it attaches guanine to PRPP to form GMP.
Bacteria have a salvage pathway for the pyrimidine bases. Humans use Orotate phosphoribosyl Transferase and OMP Decarboxylase to salvage pyrimidines.

**Catabolism of Purines**

- **AMP**: Deamination → IMP
  - **AMP Deaminase**
  - **5'-Nucleotidase**
  - **Adenosine Deaminase**
  - **Ribose-1-phosphate**
  - **Purine Nucleoside Phosphorylase**
  - **Xanthine Oxidase**
  - **Urate Oxidase**
  - **Allantoin**
- **GMP**: Oxidation → Uric Acid
  - **5'-Nucleotidase**
  - **Purine Nucleoside Phosphorylase**
  - **Guanine**
  - **Guanase**
Excess purines and pyrimidines originating from ingested nucleotides or from routine turnover of cellular nucleic acids are catabolized. Most intracellular purine bases are salvaged and pyrimidine salvage probably occurs. Purine breakdown yields only waste products that must be excreted, whereas the pyrimidines yields molecules that can enter metabolism for energy generation.

During the catabolic process of AMP can be converted to IMP by AMP Deaminase and then the IMP is converted to inosine (a nucleoside) by the enzyme 5’-Nucleotidase, or AMP is first dephosphorylated to adenosine (a nucleoside) by 5’-Nucleotidase and then the adenosine is converted to inosine by Adenosine Deaminase. The net result of these two pairs of reactions is the conversion of AMP (a nucleotide) to inosine (a nucleoside). The inosine is then phosphorolytically cleaved, phosphate is added across the N-glycosidic bond, to yield the base hypoxanthine and ribose-1-phosphate by the enzyme Purine Nucleoside Phosphorylase.

Hypoxanthine is converted to xanthine by the action of the enzyme Xanthine Oxidase. This enzyme uses molecular oxygen (O$_2$) to oxidize hypoxanthine to xanthine and hydrogen peroxide (H$_2$O$_2$). Hydrogen peroxide is a very destructive compound to have within the cell. It is rapidly and quantitatively destroyed by the enzyme Catalase. Xanthine Oxidase resides in lysosomes and peroxisomes.

Xanthine is then converted to uric acid, the final excretory product in mammals, by a second reaction catalyzed by Xanthine Oxidase.

GMP catabolism is similar. GMP is first dephosphorylated to guanosine (a nucleoside) by the action of 5’-Nucleotidase. The guanine base is then released from the nucleoside by Purine Nucleoside Phosphorylase. The guanine base is converted to xanthine by the enzyme Guanase. Once formed, xanthine is converted to uric acid by the action of Xanthine Oxidase.

In Mammals the uric acid is usually oxidized to Allantoin by Urate Oxidase and the allantoin is the major secretory product.

**Pyrimidine Catabolism**

The pyrimidine nucleotides are converted to their respective nucleosides by the action of 5’-Nucleotidase. Cytidine (nucleoside) is converted to uridine (nucleoside) by the action of Cytidine Deaminase.

Ribose is removed from uridine by the enzyme Uridine Phosphorylase to release the free base uracil, and it is removed from thymidine by the action of Thymidine Phosphorylase to release the free base thymine.

The enzyme Dihydouracil Dehydrogenase reduces the bases uracil and thymine to dihydrouracil and dihydrothymine, respectively.

These two compounds are then acted upon by the enzyme Dihydropyrimidinase to form ureidopropionate or ureidoisobutyrate.
The enzyme Ureidopropionase hydrolytically removes $\text{NH}_4^+$ and $\text{HCO}_3^-$ from these compounds to form $\beta$-alanine (from uracil) and $\beta$-aminoisobutyrate (from thymine).

An Aminotransferase (Transaminase) converts $\beta$-alanine into malonic semialdehyde and converts $\beta$-aminoisobutyrate into methylmalonic semialdehyde.

A Dehydrogenase Complex oxidizes malonic semialdehyde and couples it to Coenzyme A to form malonyl-CoA.
The malonyl-CoA can enter fatty acid biosynthesis or more likely it is decarboxylated by Malonyl-CoA Decarboxylase to acetyl-CoA, and the acetyl-CoA oxidized for energy (ATP).

The same or a similar Dehydrogenase Complex oxidizes methylmalonic semialdehyde and couples it to CoA forming D-methylmalonyl-CoA. D-methylmalonyl-CoA is an intermediate in the metabolism of odd chain length fatty acids and the amino acids, Met, Val, Thr, and Ile. D-methylmalonyl-CoA is ultimately converted to succinyl-CoA as described previously.

Deoxyribose Metabolism

After the base is phosphoryllytically released from the deoxyribose by Nucleoside Phosphorylase or Thymidine Phosphorylase the phosphate is moved from C-1 to C-5 by Phosphopentose Mutase to form deoxyribose-5-phosphate. The deoxyribose-5-phosphate is cleaved to ethanal and glyceraldehyde-3-phosphate by 2-Deoxyribose-5-phosphate Aldolase. Carbon 1 & 2 becomes the ethanal and 3, 4, &5 become glyceraldehyde-3-phosphate. The glyceraldehyde-3-phosphate enters glycolysis or gluconeogenesis depending upon the tissue and blood glucose levels. Ethanal is oxidized to acetate by Aldehyde Dehydrogenase and then the acetate is coupled to Coenzyme A by Acetyl-CoA Synthetase. Acetyl-CoA enters any of the pathways that utilizes Acetyl-CoA, most likely the TCA cycle.