Summary of Chapter 22

1. Synthesis of purine ribonucleotides
   • Origin of purine ring atoms are:
     ![Diagram of purine ring atoms]
     - Uric acid
     - Asp amine
     - Formate
     - Gln amide
     - Gly

   • The initially synthesized purine derivative is inosine phosphate (IMP), whose base is hypoxanthine.
   • IMP biosynthesis:
     1. Formation of phosphoribosylpyrophosphate (PRPP) from R5P with ATP.
     2. Replace PPi with Gln [N9], attach Gly for [C4,C5,N7], formate for [C8] from THF, Gln for [N3], formation imidazole ring, attach HCO₃⁻ for [C6], Asp for [N1], and formate for [C2] from THF, and then cyclization to form IMP.
   • de novo synthesis: biosynthesis using small molecules, such as IMP with PRPP, amino acids.
   • AMP is synthesized from IMP and Asp for N6.
   • GMP is synthesized from IMP → xanthosine monophosphate (XMP) \( \xrightarrow{\text{Gln for } N2} \) GMP.
   • Di- and tri-phosphate nucleotides are synthesized by nucleotide mono- and di-phosphate kinases.
   • Adenine, guanine, and hypoxanthine produced by nucleotide degradation are salvaged and reused for synthesis of AMP, GMP, IMP --- Salvage synthesis.
     
     (Adenine, Guanine, Hypoxanthine) + PRPP \( \xrightarrow{} \) (AMP, GMP, IMP) + PP₁
   • Regulation of purine nucleotide biosynthesis
     - Regulation sites: PRPP, 5-phosphoribosylamine, adenylosuccinate, and XMP syntheses.
     - Most are feedback inhibitions:
       - ADP and GDP inhibit PRPP synthesis.
       - All purine nucleotides inhibit 5-phosphoribosylamine synthesis.
       - AMP inhibits adenylosuccinate synthesis, whereas GMP inhibits XMP synthesis
     - Activation
       - PRPP activates 5-phosphoribosylamine synthesis.
       - ATP activates GMP synthesis, whereas GTP activates AMP synthesis.

2. Synthesis of pyrimidine ribonucleotides
   • Pyrimidine ring is synthesized from Asp [N1, C6, C5, C4], HCO₃⁻ [C2], and Gln [N3].

   ![Diagram of pyrimidine ring]
   - Orotic acid
   • Orotic acid is the first base.
• Unlike purine synthesis, the base is first synthesized, and then PRPP is attached.
• UMP synthesis:
  1. 2ATP + HCO₃⁻ + Gln → Carbamoyl phosphate + 2ADP + Pi + Glu
  2. Carbamoyl phosphate + Asp → Carbamoyl aspartate + Pi
  3. Cyclization and reduction → Orotate
  4. Orotate + PRPP → Orotidine monophosphate (OMP) + PPᵢ
  5. OMP → UMP + CO₂
• UDP and UTP are synthesized from UMP by kinases.
• UTP + Gln + ATP → CTP + Glu + ADP + Pi
• Regulation of pyrimidine nucleotide biosynthesis
  Animal: Carbamoyl phosphate synthetase II is activated by ATP and PRPP, and is feedback-inhibited by UTP and CTP. UMP inhibits own synthesis from OMP.
  Bacteria: Aspartate transcarbamoylase (ATCase) is activated by ATP and Gln, and is feedback-inhibited by CTP.

3. Formation of deoxyribonucleotides
• Deoxyribonucleotides are synthesized from their corresponding ribonucleotides by reducing the C2’ position with ribonucleotide reductases.
• Class I ribonucleotide reductase is found in all eukaryotes and some prokaryotes, and composes of 2 pairs of dimer, R₁(α₂R₂(β₂).
• α-subunit has two different allosteric sites (1. Activity site; 2. Specificity site), and contains important Cys residues.
• β-subunit contains the tyrosyl radical that is stabilized by Fe-O₂⁻-Fe complex.
• Catalytic site is located interface between α- and β-subunits.
• Reaction mechanism:
  1. The tyrosyl radical abstracts H-atom from C3’, and thus C 3’ radical is generated.
  2. E-SH donates a proton to 2’-OH → 2’-OH⁺H₂, and thus -OH is cleaved.
  3. Another E-SH donates a proton to C⁺2’, and the Tyr gives back the proton to C3’.
Note: Two oxidized E-S make -S-S- bond. The -S-S- bond is reduced indirectly by NADPH.
  Reduction process: [NADPH] → [FADH₂ → 2SH in thiodoxin reductase] → [2SH in thiodoxin] → [2SH in ribonucleotide reductase]
• Regulation of ribonucleotide reductase
  1. Relatively large amount of ATP is present in cells. The ATP stimulates the reduction of pyrimidine nucleotides (CDP & UDP).
  2. As a feedback mechanism, dTTP inhibits the reduction of pyrimidine nucleotides, and stimulates the reduction of GDP.
  3. As a feedback mechanism, dGTP inhibits the reduction of CDP, UDP, and GDP, and stimulates the reduction of ADP.
  4. As a feedback mechanism, dATP inhibits the reduction of all NDPs unless [ATP] is sufficiently high.

4. Thymidylate synthesis
• dTMP is synthesized from dUMP and N⁵,N¹⁰-methylene-THF by thymidylate synthase.
• N⁵,N¹⁰-methylene-THF donates methylene (CH₂) and electron.
• Reaction mechanism
  1. E-S: is attached covalently to C6 of dUMP.
2. The methylene at N5 of THF forms a covalent bond with C5 of dUMP (E-dUMP-THF ternary complex).
3. Base E-B: removes the proton on C5, and thus the methylene is transferred to dUMP and DHF is released.
4. E-S-C5 bond is cleaved and dTMP is formed.
• 5-Fluorouridylate (FdUMP) is an important anti-cancer drug, since the enzyme cannot remove F at C5. Thus the catalytic reaction is completely stopped at the step-3 (dead-end compound).
• Regeneration N5,N10-methylene-THF from DHF
   1. Oxidized dihydrofolate (DHF) is reduced to THF by dihydrofolate reductase using NADPH. Methotrexate, THF analogue, inhibits the enzyme.
   2. THF receives methylene group from Ser by serine hydroxymethyl transferase.

5. Purine nucleotide degradation
   \[\begin{align*}
   \text{AMP} & \rightarrow \text{Adenosine} \\
   \text{IMP} & \rightarrow \text{Inosin} \rightarrow \text{Hypoxanthine} \\
   \text{XMP} & \rightarrow \text{Xanthosine} \rightarrow \text{Xanthine} \rightarrow \text{Uric acid} \\
   \text{GMP} & \rightarrow \text{Guanosine} \rightarrow \text{Guanine}
   \end{align*}\]

6. Pyrimidine nucleotide degradation
   • Pyrimidines are metabolized to malonyl-CoA (methylmalonyl-CoA) which are further metabolized to succinyl-CoA and enter the citric acid cycle.
   \[\begin{align*}
   \text{CMP} & \rightarrow \text{Cytidine} \\
   \downarrow
   \text{UMP(dTMP)} & \rightarrow \text{Uridine (deoxythymine)} \rightarrow \text{Malonyl-CoA (Methylmalonyl-CoA)} \\
   \text{Malonyl-CoA} & \rightarrow \text{Fatty acid synthesis and/or Citric acid cycle.} \\
   \text{Methylmalonyl-CoA} & \rightarrow \text{Vitamin B12} \rightarrow \text{Succinyl-CoA} \rightarrow \text{Citric acid cycle}
   \end{align*}\]