V. Biosynthesis of Amino acids, nucleotides and related molecules

1. INTRODUCTION
   A. Most of the nitrogen is bound up in amino acids and nucleotides.
   B. Biosynthetic pathways for amino acids and nucleotides:
      B.1 Both contain nitrogen which arises from common biological sources.
      B.2 Two sets of pathways are extensively intertwined with several key intermediates in common.
      B.3 Certain amino acids or parts of amino acids are incorporated into the structure of purines and pyrimidines, and in one case part of a purine ring is incorporated into histidine.
      B.4 Two sets of pathways share much common chemistry: transfer of nitrogen or one-carbon groups.
   C. Regulation is crucial in the biosynthesis of nitrogen-containing compounds.
   D. The different amino acids and nucleotides must be made in the correct ratios and at the right time for protein and nucleic acid synthesis, their biosynthetic pathways must be accurately regulated and coordinated with each other.

2. OVERVIEW OF NITROGEN METABOLISM
   A. Introduction
      A.1 The biosynthetic pathways leading to amino acids and nucleotides share a requirement for nitrogen.
      A.2 Free amino acids, purines and pyrimidines formed during metabolic turnover of proteins and nucleic acids are often salvaged and reused.
   B. The nitrogen cycle maintains a pool of biologically available nitrogen.
      B.1 The nitrogen cycle
         a. Nitrogen fixation: \( \text{N}_2 \rightarrow \text{ammonia} \)
         b. Ammonia assimilation: \( \text{ammonia} \rightarrow \text{reduced nitrogen-compounds} \)
         c. Nitrification: \( \text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^- \)
         d. Denitrification: \( \text{NO}_3^- \rightarrow \text{N}_2 \)
         e. Nitrate reduction: \( \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{ammonia} \)
   C. Nitrogen is fixed by enzymes of the nitrogenase complex.
      C.1 Nitrogen fixation
         a. Nonbiological: lightning discharges and chemical reactions (Haber process)
         b. Biological: only certain prokaryotes can fix atmospheric nitrogen.
Nonsymbiotic: anaerobic (Clostridium pasteurianum) and aerobic bacteria, cyanobacteria (blue-green algae)

Symbiotic: leguminous plants (bean, alfalfa) and Rhizobium (Diazatrophs, bacteroid)

Leghemoglobin (hemoglobin-like protein) is abundant in the nodule.

Some insects (termites and cockroaches) have symbiotic nitrogen fixing bacteria in their intestines.

### C.2 Nitrogen fixation and photosynthesis

- **a.** Energy and low potential electrons are required.
- **b.** Nitrogen fixation is less common.

### C.3 The stoichiometry of the nitrogen fixation

- **a.** \( \text{N}_2 + 10 \text{H}^+ + 8 \text{e}^- + 16 \text{ATP} \rightarrow 2 \text{NH}_4^+ + 16 \text{ADP} + 16 \text{Pi} + \text{H}_2 \)
- **b.** Hydrogen is a by-product.
- **c.** Low potential carrier: ferredoxin, flavodoxin
- **d.** The \( \text{NH}_3 \) can be incorporated into glutamate, glutamine (and asparagine, carbamoyl phosphate).

### C.4 Nitrogenase complex contains several redox centers.

- **a.** Dinitrogenase reductase and dinitrogenase
- **b.** Dinitrogenase reductase (60 kD, dimer of two identical subunits) contains one 4Fe-4S redox center and 2 ATP/ADP binding sites: transfer electrons to dinitrogenase
- **c.** Dinitrogenase (240 kD, \( \alpha_2\beta_2 \) tetramer)

  - The prosthetic groups are \( \rho \)-cluster (bridged pairs of 4Fe-4S complexes) and Fe-Mo cluster (vanadium in place of Mo).
  - Catalyzes the reduction of nitrogen.
- **d.** The flow of electrons in the nitrogenase-catalyzed reduction of nitrogen
- **e.** The role of ATP in nitrogen fixation
- **f.** Nitrogenase complex is an extreme lability when oxygen is present.

  - Anaerobically or repress nitrogenase synthesis when oxygen is present.
  - Partially uncouple electron transport from ATP synthesis, oxygen is burned off.
  - Symbiotic relationship: energy requirements of the reaction and oxygen lability of the enzymes.
- **N.** Crop rotation methods.

### g. Genetics of nitrogen fixation: \( nif \) gene

### h. Nitrogenase activity assay: acetylene \( \rightarrow \) ethylene

### D. Nitrate utilization

#### D.1 The ability to reduce nitrate to ammonia is common to virtually all plants, fungi and bacteria.

#### D.2 Nitrate reductase (800 kD) contains FAD and Mo (molybdopterin) and cytochrome 557.
\[
\text{NO}_3^- + \text{NAD(P)H} + \text{H}^+ \rightarrow \text{NO}_2^- + \text{NAD(P)}^+ + \text{H}_2\text{O}
\]

**a.** Plants : NADH.

**b.** Fungi and bacteria : NADPH.

### D.3 Nitrite reductase contains [2Fe-2S] cluster and siroheme (partially reduced iron porphyrin).

\[
\text{NO}_2^- \rightarrow \text{NO}^- \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NH}_3
\]

Electron donor : ferredoxin.

### E. Ammonia is incorporated into biomolecules through glutamate and glutamine.

**E.1** All organisms share a few common routes for utilization of inorganic nitrogen in the form of ammonia.

**E.2** Ammonia in high concentration is quite toxic, at lower levels it is a central metabolite and serves as substrate for four enzymes. Glu dehydrogenase, Gln synthetase, Asn synthetase and carbamoyl phosphate synthetase.

**E.3** Glutamate and glutamine are the critical entry point in amino acid biosynthesis. One or both of these amino acids are present at higher concentrations than other amino acids.

**E.4** Glutamate : nitrogen requirements and maintain an osmotic balance

**E.5** Glutamine synthetase for glutamine synthesis in all organisms

**E.6** Glutamate : glutamate synthase

**a.** Reaction

\[
\alpha\text{-KG} + \text{glutamine} + \text{NADPH} + \text{H}^+ \rightarrow 2 \text{glutamate} + \text{NADP}^+
\]

**b.** *E. coli* enzyme (800 kD) contains FAD, FMN and nonheme iron.

**c.** Plants : NADPH, NADH or ferredoxin

Other organisms : NADH

**E.7** Glutamate dehydrogenase

**a.** In eukaryotes, the enzyme is located in the mitochondrial matrix.

**b.** High Km for NH\textsubscript{4}\textsuperscript{+} (~ 1mM)

### F. Glutamine synthetase is a primary regulatory point in nitrogen metabolism : generation of biologically active amide nitrogen.

**F.1** Reaction mechanism : acyl phosphate intermediate

**F.2** In animals, glutamine synthetase (GS) is a key participant in detoxifying ammonia formed from amino acid catabolism, particulary in brain. Mammalian GS is activated by \(\alpha\text{-KG}\).

**F.3** *E. coli* enzyme : dodecamer (12 identical subunits with 469 amino acids, 50 \times 12 = 600 kD)

**F.4** Regulation of glutamine synthetase

**a.** Cumulative feedback inhibition : Nine allosteric feedback inhibitors, each with its own binding site : His, Trp, carbamoyl phosphate, AMP, CTP, glucosamine 6-phosphate, Ala, Ser and Gly.
b. Covalent modification (adenylylation) of the enzyme.
Adenylylation site : Tyr 397.
Adenylylation inactivates the catalytic site. An enzyme molecule with all 12 sites adenylylated is completely inactive, while partial adenylylation yields partial inactivation, and increases the enzyme’s susceptibility to cumulative feedback inhibitors.
Regulatory cascades : adenylyltransferase (AT), regulatory protein (P_{II}, tetramer), uridylyltransferase (UT) and uridylyl-removing enzyme.
UT is activated by $\alpha$-KG and ATP, and inhibited by Gln and Pi.
Uridylyl-removing enzyme is activated by glutamine, and inhibited by $\alpha$–KG.
High [Gln] level signals cellular nitrogen sufficiency. GS becomes adenylylated and inactivated. High [$\alpha$-KG] level is an indication of nitrogen limitation and a need for ammonium assimilation by GS.

G. Several classes of reactions play special roles in the biosynthesis of amino acids and nucleotides.
G.1 Transamination and other rearrangements of amino acids : pyridoxal phosphate
G.2 Transfer of one-carbon groups : tetrahydrofolate, S-adenosylmethionine
G.3 Transfer of amino groups from amide nitrogen of Gln
   a. Gln amidotransferase : two structural domains
   b. Gln binding domain
   c. Amino group acceptor domain
   d. Proposed reaction mechanism

3. BIOSYNTHESIS OF AMINO ACIDS

A. Introduction
   A.1 All amino acids are derived from intermediates in glycolysis, citric acid cycle or pentose phosphate pathway.
   A.2 Nitrogen enters these pathways by way of Glu and Gln.
   A.3 Overview of amino acid biosynthesis
   A.4 Nonessential and essential amino acids in humans
   A.5 There is considerable variation in amino acid biosynthetic pathways among different species. In contrast, the basic pathways of carbohydrate and lipid metabolism are all but universal.
   A.6 All the nonessential amino acids except Tyr are synthesized by simple pathways, leading from one of four common metabolic intermediates : pyruvate, oxaloacetate, $\alpha$-ketoglutarate and 3-phosphoglycerate.
   A.7 Sparing effect : Tyr from Phe ; Cys from Met
A.8 Twenty amino acids are organized into families or groups that are related structurally or metabolically.
   a. α-Ketoglutarate
   b. 3-Phosphoglycerate
   c. Oxaloacetate
   d. Pyruvate
   e. Phosphoenolpyruvate and erythrose 4-phosphate
   f. Ribose 5-phosphate and ATP
A.9 5-Phosphoribosyl-1-pyrophosphate (PRPP)
   a. PRPP is an activated ribose 5-phosphate derivative, and is a precursor in the biosynthesis of histidine, tryptophan, purine and pyrimidine nucleotides.
   b. Structure and synthesis of PRPP: ribose phosphate pyrophosphokinase
      \[ \text{ATP} + \text{R5P} \rightarrow \text{PRPP} + \text{AMP} \]
B. α–Ketoglutarate gives rise to Glu, Gln, Pro and Arg.
   B.1 Glu and Gln
   B.2 Biosynthesis of Pro and Arg in bacteria
      a. Glu → glutamate γ–semialdehyde → Pro
      b. Glu → N-acetylglutamate → N-acetylglutamate γ–semialdehyde → ornithine → Arg
   B.3 Biosynthesis of Pro and Arg in mammals
      a. Glu → glutamate γ–semialdehyde → Pro
      b. Arg → ornithine → glutamate γ–semialdehyde → Pro
      c. Glu → glutamate γ–semialdehyde → ornithine → Arg
   B.4 Biosynthesis of hydroxyproline
      a. Hydroxyproline residues are generated by posttranslational modification with procollagen-proline hydroxylase.
      b. The reaction requires ascorbic acid, ferrous iron, molecular oxygen and α–KG.
C. Ser, Gly and Cys are derived from 3-phosphoglycerate.
   C.1 Serine
      a. Ser from 3-phosphoglycerate
      b. Ser from glycine via serine hydroxymethyltransferase reaction.
   C.2 Glycine
      a. Gly from Ser
      b. In the liver of vertebrates, glycine synthase catalyzes the condensation of N⁵,N¹⁰-methylene-THF with CO₂ and NH₄⁺.
   C.3 Cysteine
      a. Plants and microorganisms utilize H₂S for the synthesis of Cys, with Ser providing the carbon skeleton.
Plants and most microorganisms: O-acetylserine as the substrate.

Some bacteria: Ser + H₂S → Cys + H₂O

Met as the source of Cys sulfur in animals:

Cystathionine as an intermediate:
Ser + homocysteine → cystathionine

Cystathionine plays a central role in the pathways leading to both Met and Cys:

\[
\text{Homoserine + succinyl-CoA → O-succinylhomoserine} \quad \xrightarrow{\text{cystathionine}} \quad \text{homocysteine + pyruvate + NH}_3
\]

D. Three nonessential and six essential amino acids are synthesized from OAA and pyruvate.

D.1 Ala and Asp: transamination from pyruvate or OAA

D.2 Asn: Asn synthetase
Asp + Gln (NH₃) + ATP → Asn + Glu + AMP + PPI

D.3 Lys, Met and Thr are derived from Asp:

a. Aspartokinase: a major site for regulation of each biosynthetic pathway:
   E. coli contains 3 distinct forms, one is specifically inhibited by Thr and one by Lys.
   The third enzyme is not feed-back inhibited by Met, but synthesis of this particular enzyme is repressed by Met.

b. Asp → β-aspartate-semialdehyde → Lys:
   i. Diaminopimelate pathway: plants, bacteria and some fungi
      β-aspartate-semialdehyde + pyruvate
      α-Aminoadipate pathway: fungi and protist
      Acetyl-CoA + α-KG
   c. Asp → β-aspartate-semialdehyde → homoserine → Met, Thr and Ile
      Å Homocysteine + N⁵-methyl-THF → Met + THF
      Ç Homoserine → O-phosphohomoserine → Thr → α-ketobutyrate
      É α-ketobutyrate + pyruvate → Ile

D.4 Leucine, isoleucine and valine are synthesized from pyruvate:

a. Pyruvate family: Leu, Ile, Val

b. Biosynthesis of Val and Ile
   Å The last 4 reactions are catalyzed by the same 4 enzymes.
   Ç 2 pyruvate → Val
   É Pyruvate + α-ketobutyrate → Ile

c. Biosynthesis of Leu starts with an intermediate (α-ketoisovalerate) in Val synthesis.
   Acetyl-CoA.

E. Chorismate is a key intermediate in the synthesis of Trp, Phe and Tyr.

E.1 Biosynthesis of aromatic rings: The shikimate pathway
Erythrose 4-phosphate + phosphoenol pyruvate → shikimate → chorismate

E.2 Chorismate → anthranilate → Trp
E.3 Chorismate → prephenate → Phe or Tyr
E.4 In animals: Phe → Tyr (Phe hydroxylase)
E.5 The shikimate pathway is responsible for biosynthesis of nearly all aromatic compounds.
E.6 Regulation of the pathways
   a. Several multifunction enzymes: single polypeptide chain containing 2 or more active sites for catalysis of sequential reactions
   b. EPSP synthase in higher plants: glyphosate (herbicide)

E.7 E. coli tryptophan synthase (αβ2)
   a. α-subunit (29 kD) cleaves indole 3-glycerol phosphate to indole and GAP.
   b. β-subunit (43kD) joins indole with serine to form Trp.
   c. αβ2 tetramer activity

F. Histidine biosynthesis uses precursors of purine biosynthesis.
   F.1 His residue is often components of enzyme active sites where it acts as nucleophiles and/or general acid-base catalysts.
   F.2 ATP + PRPP → histidine.
   F.3 Ten structural genes for the enzymes of His synthesis in enteric bacteria are linked to one another in the same order as the order of the reactions of the pathway. The set of genes (His operon) is coordinately regulated at the transcriptional level, and all 10 genes are transcribed to give one large messenger RNA, which is translated to give the 10 enzymes.
   F.4 His auxotrophic mutations have been useful both for defining the biosynthetic pathway and for analyzing environmental mutagenesis.
   F.5 Ames test for mutagens and suspected carcinogens in the environment.

G. Amino acid biosynthesis is under allosteric regulation.
   G.1 Feedback inhibition of the first reaction in a sequence by the end product of the pathway.
      The first reaction is usually irreversible and is catalyzed by an allosteric enzyme.
   G.2 Allosteric regulation of isoleucine biosynthesis
   G.3 Concerted inhibition of glutamine synthesis
   G.4 Interlocking regulatory mechanisms in the biosynthesis of four amino acids derived from aspartate in E. coli.
      a. Enzyme multiplicity: aspartokinase, homoserine dehydrogenase, threonine dehydratase.
      b. Sequential feedback inhibition
4. MOLECULES DERIVED FROM AMINO ACIDS

A. The biological roles of the amino acids

B. Heme biosynthesis

B.1 Four classes of tetrapyrrole compounds in biology: porphyrins (heme), chlorophylls, phycobilins and cobalamins

B.2 Isotopic tracers demonstrate that all of heme’s C and N atoms can be derived from acetate and glycine.

B.3 Heme biosynthesis takes place partly in the mitochondrion and partly in the cytosol.

B.4 Biosynthesis of tetrapyrroles: the succinate-glycine pathway

a. δ-Aminolevulinic acid (ALA): a common precursor
   Α Succinyl CoA + glycine → ALA: in animals and bacteria
   ζ Glutamate → ALA: in plants

b. 2 ALA → porphobilinogen (PBG): PBG synthase (Zn-required)

c. Porphyrin biosynthesis involves:
   Α Formation of a pyrrole ring → cyclic tetrapyrrole
       4 PBG → uroporphyrinogen III
       Porphobilinogen deaminase (uroporphyrinogen synthase) and uroporphyrinogen III cosynthase
   ζ Side chain modifications and ring oxidations
   Ê Protoporphyrin IX + Fe$^{2+}$ → heme: ferrochelatase

B.5 Hereditary deficiency:

a. Congenital erythropoietic porphyria
   Uroporphyrinogen III cosynthase is defective.

b. Erythropoietic protoporphyria
   Ferrochelatase is defective.

c. Acute intermittent porphyria
   PBG deaminase is deficiency.

B.6 Heme degradation in animals

a. Most of heme comes from breakdown of aged erythrocytes, some comes from cytochromes and other heme proteins.

b. Heme protein degradation in animals releases amino acids and iron, which are reused, and biliverdin, which must be solubilized for excretion.

c. Jaundice

C. Biosynthesis of creatine and glutathione

C.1 Creatine is made from Gly, Arg and Met.

C.2 Glutathione

a. Structure and biosynthesis: Glu, Cys, Gly
b. Glutathione peroxidase: selenium-containing enzyme

D. D-Amino acids are found primarily in bacteria.
   D.1 D-Amino acids do some special functions in the structure of bacterial cell walls and peptide antibiotics.
   D.2 Peptidoglycans: D-Ala and D-Glu
   D.3 Amino acid racemases: L-isomers → D-isomers
   D.4 L-Fluoroalanine and cycloserine

E. Aromatic amino acids are precursors of many plant substances.
   E.1 Phe and Tyr serve as precursors to an enormous number of plant substances, ranging from the polymeric lignin to tannins and pigments to many of the flavor components of spices.
   E.2 Phe and Tyr are precursors to many of the nearly 3000 alkaloids (nitrogenous substances synthesized to specific plants).
   E.3 Trp → indole-3-acetate (auxin, plant growth hormone)

F. Amino acids are converted to biological amines by decarboxylation.
   F.1 Many neurotransmitters are primary or secondary amines from amino acids.
   F.2 Amino acid decarboxylases, pyridoxal phosphate-dependent enzymes, decarboxylate corresponding precursor amino acids to active amines.
   F.3 Physiologically active amines play important roles in regulating mammalian metabolism.
   F.4 Catecholamines (dopamine, norepinephrine and epinephrine)
      a. From Tyr in adrenal medulla and central nervous system
      b. DOPA from Tyr catalyzed by tyrosinase (a copper-containing oxygenase) in the melanocytes: DOPA → melanins (red, black)
         Albinism: a genetic deficiency of tyrosinase causes an individual to lack pigmentation (albinos)
      c. DOPA → dopamine → norepinephrine → epinephrine
   F.5 Serotonin and melatonin (O-methyl-N-acetyl serotonin)
      a. From Trp in pineal gland (regulate light-dark cycles in animals), serotonin and melatonin as regulators of sleep and wakefulness
      b. Serotonin plays multiple regulatory roles in the nervous system, including neurotransmission.
      c. Serotonin is also secreted by cells in the small intestine, which it regulates intestinal peristalsis.
      d. Serotonin is a potent vasoconstrictor that helps regulate blood pressure.
   F.6 γ-Aminobutyric acid (GABA)
      a. Glu → GABA + CO₂
      b. An inhibitory neurotransmitter in the brain
F.7 Histamine
   a. His $\rightarrow$ histamine + CO$_2$
   b. Histamine promotes the secretion of HCl and pepsin in the stomach
   c. Histamine is a potent vasodilator, released locally in site of trauma, inflammation or allergic reaction.
   d. Antihistamines: drugs prevent the binding of histamine to its receptors.

F.8 Spermidine and spermine
   a. Stabilizing intracellular conformations of negatively charged nucleic acids.
   b. Biosynthesis from Met and Arg (ornithine)
   c. Curing African sleeping sickness with a biochemical trojan horse
      Â Ornithine decarboxylase
      Ç Difluoromethylornithine (DFMO)

G. Arginine is the precursor for biological synthesis of nitric oxide.
   G.1 Endothelium-derived relaxing factor (EDRF) was synthesized from Arg by vascular endothelial cells.
   G.2 Arg as the precursor to nitric oxide, a novel second messenger and neurotransmitter.
   G.3 Nitroglycerin: angina pectoris
   G.4 Nitric oxide synthase (NOS) catalyzes Arg to NO and citrulline.
   G.5 NOS is homodimeric protein of 125 to 160 kD subunits. Each subunit contains one FMN, one FAD, one tetrahydrobiopterin and one Fe (III)-heme.
   G.6 NOS
      a. Endothelial cells: in response to a wide variety of agents and physiological conditions
      b. Neuronal cells
      c. Leukocytes (white blood cells)

5. BIOSYNTHESIS AND DEGRADATION OF NUCLEOTIDES

A. Introduction
   A.1 Nucleotides serve as building blocks of nucleic acids, as critical elements in energy metabolism (ATP as high-energy compounds), as carriers of activated metabolites for biosynthesis (sugar nucleotides, CDP-choline), as structural moieties of coenzymes and as metabolic regulators and signal molecules (cAMP).
   A.2 Nucleotides and their constituent bases and nucleosides are not required to meet nutritional needs.
   A.3 Outlines of pathways in nucleotide metabolism
      a. Biosynthetic routes: de novo and salvage pathways
      Â De novo pathways: most organisms can synthesize purine and pyrimidine nucleotides from low-molecular-weight precursors in amounts sufficient for their
needs.
(a) PRPP: whole structure of ribose is retained.
(b) Glycine for purines and aspartate for pyrimidines
(c) Aspartate and glutamine

Salvage pathways: most organisms can synthesize nucleotides from nucleosides or
nucleobases that become available either in the diet or through enzymatic
breakdown of nucleic acids. They involve the utilization of performed purine and
pyrimidine compounds that would otherwise be lost to biodegradation. Salvage
pathways represent important targets for therapy of microbial or parasitic diseases,
as sites for manipulation of biological systems and as biological processes in which
genetic alterations have severe and far-reaching consequences.

Overview of nucleotide metabolism
b. Nucleic acid degradation and the importance of nucleotide salvage

Degradation can occur intracellularly, as a result of cell death, or, in animals, through of nucleic acid ingested in the diet.

 Relationships between nucleic acid catabolism and resynthesis of nucleotides by salvage pathways.

※ Reutilization of purine and pyrimidine bases
Enzymes involves:
(a) Endonucleases (pancreatic DNase or RNase)
(b) Phosphodiesterases
(c) Nucleotidases
(d) Nucleoside phosphorylase (animal cells do not contain uridine phosphorylase)
   \[ \text{Nucleoside} + \text{Pi} \rightleftharpoons \text{Nucleobase} + \text{Ribose 1-phosphate} \]
(e) Nucleoside kinase (animal cells do not contain guanosine kinase)
(f) Phosphoribosyltransferase
   \[ \text{PRPP} + \text{Guanine} \rightleftharpoons \text{GMP} + \text{PPi} \]

B. De novo purine nucleotide synthesis begins with PRPP

B.1 Early studies on de novo purine synthesis
a. Uric acid and pigeon
b. Low-molecular-weight precursors to the purine ring
c. Antibiotics: glutamine analogs (azaserine, 6-diazo-5-oxonorleucine) inhibit purine synthesis
d. Purines are synthesized at the nucleotide level, starting with PRPP conversion to \( \beta \)-5-phosphoribosylamine

B.2 Synthesis of inosine monophosphate (IMP)
a. PRPP: a central metabolite in de novo and salvage pathways
b. Reaction pathway
   Æ The metabolic pathway for the de novo biosynthesis of IMP from \( \alpha \)-D-ribose 5-phosphate. The purine residue is built up on a ribose ring in 11-catalyzed reactions.
   Ç Five ATP are required in the purine biosynthetic pathway from \( \alpha \)-D-ribose 5-phosphate to IMP. Six high-energy phosphates are consumed.
c. The multienzyme complexes
   Æ In eukaryotic cells: single polypeptide with several function.
      (a) Steps 1, 3 and 5 catalyzed by three enzymatic activities localized on a single multifunctional polypeptide.
      (b) Steps 7 and 8, steps 10 and 11 by respective bifunctional polypeptides.
   Ç In bacteria: enzyme activities are found on separate proteins, and a large noncovalent complex may exist.

B.2 Synthesis of adenine and guanine ribonucleotides
a. IMP is a branch point between adenine and guanine biosynthesis.
b. Pathways from IMP to AMP and GMP
c. Nucleoside monophosphates \( \rightarrow \) nucleoside diphosphates
   Æ Nucleoside monophosphate kinases do not discriminate between ribose and deoxyribose in the substrate.
C. Purine nucleotide biosynthesis is regulated by feedback inhibition.

C.1 IMP pathway is regulated at its first two reactions
a. Ribose phosphate pyrophosphokinase is inhibited by various purine nucleotides, particularly IMP, AMP and GMP.
b. Amidophosphoribosyl transferase
   Ä The enzyme binds ATP, ADP or AMP at one inhibitory site, and GTP, GDP or GMP at another.
   Ç The rate of IMP production is independently but synergistically controlled by the levels of adenine nucleotides and guanine nucleotides.
   É The enzyme is allosterically stimulated by PRPP (feedforward activation).

C.2 Pathways from IMP to AMP and GMP
a. AMP inhibits the synthesis of adenylosuccinate.
b. GMP inhibits the conversion of IMP to XMP.
c. GTP is required in the conversion of IMP to AMP, and ATP is required to form GMP from IMP.
d. The rate of synthesis of GMP increases with [ATP], whereas that of AMP increases with [GTP].

D. Pyrimidine nucleotides are made from aspartate, carbamoyl phosphate and PRPP.

D.1 De novo biosynthesis of the pyrimidine ring
a. Pyrimidine ring from aspartate and carbamoyl phosphate
b. Pyrimidine ring is assembled as a free base.
c. Pyrimidine biosynthetic pathway is unbranched.

D.2 Synthesis of UMP
a. Carbamoyl phosphate (CP)
   Ä Carbamoyl phosphate synthetase I (CPS I) in mitochondria
   \[2 \text{ATP} + \text{HCO}_3^- + \text{NH}_3 \rightarrow \text{CP} + 2 \text{ADP} + \text{Pi}\]
   Ç Carbamoyl phosphate synthetase II (CPS II) in cytosol
   \[2 \text{ATP} + \text{HCO}_3^- + \text{Gln} + \text{H}_2\text{O} \rightarrow \text{CP} + 2 \text{ADP} + \text{Pi} + \text{Glu}\]
   É In bacteria, a single enzyme (three separate active sites) supplies CP for the synthesis of Arg and pyrimidines.
b. Reaction pathway for UMP synthesis
   Ä The metabolic pathway consists of six enzyme-catalyzed reactions.
In bacteria, six enzymes occur as independent proteins. ATCase contains 6 each of 2 types of subunits and 6 Zn atoms, arranged as 2 catalytic trimers and 3 regulating dimers.

In animals
(a) A single protein (CAD protein) contains 3 identical polypeptide chains, each of MS about 230 kD, that catalyzes the first three enzymatic reactions: CPS II, ATCase and dihydroorotase.
(b) UMP synthase (orotate phosphoribosyltransferase and OMP decarboxylase)
c. The intermediate products of these multifunctional enzymes are not readily released to the medium but are channeled to the succeeding enzymatic activities of the pathway. This channeling increases the overall rate of these multistep processes and protects intermediates from degradation by other cellular enzymes.

D.3 Synthesis of UTP and CTP
a. UMP → UDP → UTP
   Nucleoside monophosphate kinase and nucleoside diphosphate kinase
b. CTP is formed by amination of UTP by CTP synthetase.
   Â In animals: the amino group is donated by glutamine
   Ç In bacteria: ammonia

E. Pyrimidine nucleotide biosynthesis is regulated by feedback inhibition.
E.1 In bacteria:
   a. ATCase is allosterically inhibited by the end product (CTP in E. coli; UTP in many bacteria) and activated by ATP, the latter possibly representing a mechanism to keep purine and pyrimidine biosynthesis in balance.
   b. Bacteria also regulate pyrimidine metabolism through control of the synthesis of ATCase and the other enzymes.
E.2 In animals:
   a. CPS II is inhibited by UDP and UTP and activated by ATP and PRPP.
   b. OMP decarboxylase is inhibited by UMP and CMP.
E.3 Orotic aciduria results from an inherited enzyme deficiency.
   a. Orotic aciduria
   b. UMP synthase deficiency

F. Ribonucleotides are the precursors of deoxyribonucleotides.
F.1 Most cells contain 5 to 10 times as much RNA as DNA.
F.2 DNA differs chemically from RNA in two major respects:
   a. 2’-Deoxyribose ↔ ribose
   b. Thymine (5-methyluracil) ↔ uracil
F.3 Overview of deoxyribonucleoside triphosphate (dNTP) biosynthesis.
Ribonucleoside diphosphate reductase (ribonucleotide reductase) and nucleoside diphosphate kinase

**F.4** Deoxyribonucleotides are synthesized from their corresponding ribonucleotides by the reduction of their C2’ position rather than by their *de novo* synthesis from deoxyribose-containing precursors.

※ Overview of deoxyribonucleoside triphosphate (dNTP) biosynthesis
F.5 Reduction of ribonucleotides to deoxyribonucleotides.

a. In cyanobacteria, some bacteria and Euglena, the substrates for reduction are the ribonucleoside triphosphates.

b. Most organisms carry out the reduction at the nucleoside diphosphate level. All 4 common ribonucleoside diphosphates (rNDPs) are reduced by the same enzyme, ribonucleotide reductase.

F.6 Structure of ribonucleotide reductase

a. There are 4 classes of ribonucleotide reductases, which differ in their prosthetic groups. Iron-containing enzyme occurs in most eukaryotes and some prokaryotes.

b. The reductase is an $\alpha_2\beta_2$ tetramer.

c. E. coli enzyme contains two proteins: R$_1$ ($\alpha_2$) and R$_2$ ($\beta_2$).
   Molecular mass: $\alpha$ polypeptide: 87 kDa; $\beta$ polypeptide: 43 kDa

d. R$_1$ ($\alpha_2$) subunit
   - Catalytic site (substrate-binding site): ADP, GDP, UDP, CDP.
   - Allosteric sites:
     - Specificity site: ATP, dATP, dGTP, dTTP.
     - Activity site: ATP, dATP.
   - Redoxactive sites: thioredoxin, glutaredoxin.

e. R$_2$ ($\beta_2$) subunit contains active site tyrosine residues (Tyr122) and binuclear Fe$^{3+}$ complex.

F.7 Thioredoxin and glutaredoxin reduce ribonucleotide reductase.

a. The final step in the ribonucleotide reductase catalytic cycle is reduction of the enzyme’s newly formed disulfide bond to reform its redox-active sulfhydryl pair.

b. Thioredoxin (108 amino acid residues, Mr: ~12,000) and thioredoxin reductase (FAD-containing)

c. Glutaredoxin (85 amino acid residues), glutathione and glutathione reductase

d. NADPH

F.8 Proposed mechanism for ribonucleotide reductase

F.9 Ribonucleotide reductase is regulated by a complex feedback network.

a. Maintaining the proper intracellular ratios of dNTPs is essential for normal growth.

b. The activity and specificity of ribonucleotide reductase are essential to control to maintain balanced pools of DNA precursors. This is achieved through binding of NTP effectors to two classes of allosteric sites on the R$_1$ subunit.

c. The activity sites bind either ATP (to increase) or dATP (to inhibit), with relatively low affinity.

d. The specificity sites bind either ATP, dATP, dTTP or dGTP, all with relatively high affinity. Binding of nucleotides at this site modulates the activities toward different substrates, so as to maintain balanced rates of production of 4 dNTPs.

e. Regulation of ribonucleotide reductase by deoxynucleoside triphosphates
f. ATP is a general activator for biosynthesis and ribonucleotide reduction.
g. The presence of dATP in small amounts increases the reduction of pyrimidine nucleotide.

F.10 dNTPs are produced through phosphorylation of dNDPs by nucleoside diphosphate kinase.

G. Thymidylate is derived from dCDP and dUMP.
G.1 dTMP component of DNA is synthesized from dUMP.
G.2 Biosynthesis of dTTP occurs partly from dUDP produced via the reductase and partly from deoxycytidine nucleotides; the ratio varies in different cells and organisms.
G.3 The pathways lead to dUMP.
   a. dUDP $\rightarrow$ dUTP $\rightarrow$ dUMP (dUTPase)
   b. dCDP $\rightarrow$ dCMP $\rightarrow$ dUMP (dCMP deaminase, dCTP as an allosteric activator and dTTP as an inhibitor)
   c. dCDP $\rightarrow$ dCTP $\rightarrow$ dUTP $\rightarrow$ dUMP (*E. coli* and some other bacteria)
G.4 dUTP : cells must minimize dUTP concentration in order to prevent incorporation of uracil into DNA.
G.5 Human dUTPase : a homotrimer of 141-residue subunits
G.6 Thymidylate synthase
   a. Highly conserved 70-kD dimeric protein among mammalian, bacterial, viral, fungal and protozoal sequences.
   b. The catalytic mechanism of thymidylate synthase
   c. Regeneration of $N^5,N^{10}$-methylene tetrahydrofolate : The thymidylate synthase reaction is biochemically unique in that it oxidizes THF to DHF, no other enzymatic reaction employing a THF cofactor alters this coenzyme’s net oxidation state.
   " Dihydrofolate reductase (DHFR) : monomeric, monofunctional enzyme in most organisms. In protozoa and some plants, DHFR and thymidylate synthase occur on the same polypeptide chain to form a bifunction enzyme.
   Ç Serine hydroxymethyltransferase
   d. Inhibition of thymidylate synthase or DHFR blocks dTMP synthesis and is the basis of cancer chemotherapies.

H. Degradation of purines and pyrimidines produces uric acid and urea, respectively.
H.1 The *de novo* pathways of nucleotide biosynthesis largely satisfy an organism’s need for nucleotides. Ingested nucleic acids are mostly degraded and excreted.
H.2 Cellular nucleic acids are also subject to degradation as part of the continual turnover of nearly all cellular components.
H.3 The major pathways of purine catabolism in animals :
   a. The various purine nucleotides are all degraded to uric acid.
b. AMP deaminase (muscles) and adenosine deaminase  
c. Nucleotidase  
d. Purine nucleoside phosphorylase (adenosine and deoxyadenosine are not substrate)  
e. Guanine deaminase  
f. Xanthine oxidase (flavoenzyme, contain Mo and 4Fe-4S centers)  
   It is abundant in milk, liver and small intestinal mucosa.

H.4 Severe combined immunodeficiency disease (SCID)  
a. Adenosine deaminase (ADA) : Zn\(^{2+}\)-dependent enzyme  
b. In the absence of ADA, deoxyadenosine is not degraded.  
c. The concentration of dATP (a strong negative effector of ribonucleotide reductase) increases to a 50-100 fold.  
d. The increase in dATP leads to the general deficiency in levels of other dNTPs that is observed in B and T lymphocytes. Without lymphocytes, no antibodies against antigenic challenge.  
e. Purine nucleoside phosphorylase deficiency kills the T lymphocytes but not the B lymphocytes.

H.5 Xanthine oxidase is a mini-electron-transport system.  
a. In mammals, xanthine oxidase occurs almost exclusively in the liver and the small intestinal mucosa.  
b. A dimeric protein of identical 130 kD subunit  
c. Each subunit contains FAD, Mo and two different Fe-S clusters.  
d. The enzyme catalyzes: hypoxanthine → xanthine → uric acid

H.6 The degradation of uric acid to ammonia  
a. Uric acid : animals lack urate oxidase (Cu-containing enzyme)  
   Á Primates (in urine)  
   Ç Birds, terrestrial reptiles, insects (conserve water)  
b. Allantoin (mammals other than primates)  
c. Allantoic acid (teleost fish)  
d. Urea (cartilaginous fish, amphibia)  
e. Ammonia (marine invertebrates)

H.7 Gout is caused by an excess of uric acid  
a. Uric acid and its urate salts are quite insoluble, this is advantageous to egg-laying animals. However, the insolubility of urates can present difficulties in mammalian metabolism.  
b. Hyperuricemia (gout) : ~3/1000 (predominantly males)  
c. Enzymatic abnormalities that lead to hyperuricemia and gout by elevating the rate of \textit{de novo} purine nucleotide biosynthesis.  
   Á Ribose phosphate pyrophosphokinase : elevated levels.  
   Ç Amidophosphoribosyl transferase : loss of feedback inhibition.
HGPRT: decreased levels.
d. Antimetabolite allopurinol
   A structural analog of hypoxanthine.
\[ \text{Xanthine oxidase hydroxylates allopurinol yielding alloxanthine, alloxanthine remains tightly bound to the enzyme and inhibits xanthine oxidase.} \]

H.8 Catabolism of pyrimidines
a. The major pathways of pyrimidine catabolism in animals
b. \( \beta \)-Alanine and \( \beta \)-aminoisobutyrate are the end products of pyrimidine catabolism.

I. Purine and pyrimidine bases are recycled by salvage pathways.
I.1 Salvage pathways are diverse in character and distribution. In mammals, purines are mostly salvaged by two different enzymes.
a. Adenine phosphoribosyltransferase (APRT):
   \[ \text{Adenine + PRPP} \rightleftharpoons \text{AMP + PPi} \]
b. Hypoxanthine-guanine phosphoribosyltransferase (HGPRT):
   \[ \text{Hypoxanthine + PRPP} \rightleftharpoons \text{IMP + PPi} \]
   \[ \text{Guanine + PRPP} \rightleftharpoons \text{GMP + PPi} \]

I.2 Lesch-Nyhan syndrome results from HGPRT deficiency
a. Lesch-Nyhan syndrome
b. HGPRT deficiency
c. Symptoms for male children: structural gene for HGPRT is located on the X chromosome.
d. A lack of HGPRT results in a rise in PRPP levels, which leads to a general increase in \textit{de novo} purine synthesis (200-fold). Overproduction of purines leads to high levels of uric acid production and goutlike damage to tissue occurs. The brain is especially dependent on the salvage pathways.

I.3 Salvage routes to deoxyribonucleotide synthesis.
a. Thymidine kinase (TK)
   A TK is allosterically inhibited by dTTP.
   Activity of TK in a given cell is closely related to the proliferative state of that cell.
   During the cell cycle, activity of TK rises dramatically as cells enter S phase, and in general rapidly dividing cells have high levels of TK.
   Radiolabeled thymidine is widely used for isotopic labeling of DNA in radioautographic investigations or to estimate rates of intracellular DNA synthesis.
\[ \text{TK also phosphorylates deoxyuridine to dUMP.} \]
b. Deoxycytidine kinase
   It is feedback inhibited by dCTP.
※ Salvage and De novo synthetic pathways to thymine nucleotides
J. Many chemotherapeutic agents target enzymes in the nucleotide biosynthetic pathways.

J.1 Cancer cells grow more rapidly than the cells of most normal tissues, have greater requirements for nucleotides, and are generally more sensitive than normal cells to inhibitors of nucleotide biosynthesis.

J.2 Azaserine and acivicin: analogs of glutamine
   a. Inhibitors of glutamine amidotransferase
   b. Inhibit a number of amino acid and nucleotide biosynthetic pathways.

J.3 Inhibition of thymidylate synthesis in cancer therapy
   a. Thymidylate synthase: a target enzyme for chemotherapy (treatment of diseases with chemical agents)
   b. Thymidylate synthase participates in the synthesis of a deoxyribonucleotide.
   c. The inhibitors of the enzyme block the production of an essential DNA precursor should inhibit DNA replication.
   d. Certain tumor cells take up and metabolize uracil much more rapidly than normal cells.
   e. 5-Fluorouracil and 5-fluorodeoxyuridine are found to be potent inhibitors of DNA synthesis. Their action as inhibitors involves their intracellular conversion to 5-fluorodeoxyuridine monophosphate (FdUMP), a dUMP analog that acts as an irreversible inhibitor of thymidylate synthase. Both have found use in cancer treatment.
   f. FdUMP is a true mechanism-based inhibitor, in that irreversible binding occurs only in the presence of $\text{N}^5,\text{N}^{10}$-methylenetetrahydrofolate. Presumably, binding of the coenzyme induces a conformational change in the active site.
   g. Antifolates: substances interfere with the action of folate cofactors are effective anticancer agents.
      Å Aminopterin
      Ç Methotrexate (amethopterin)
      É Trimethoprim

J.4 All fast-growing cells (including bacteria and protists) are also potential targets.