Slide 1

Chapter 27

Nucleotide Metabolism

Slide 2

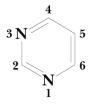
Review Nucleotide Structure and Nomenclature (Chapter 11)

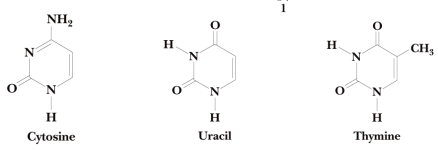
- Composition
  - Heterocyclic Base
  - Pentose
  - Phosphate
- Besides being the building blocks of nucleic acids, nucleotides have many roles in metabolism

Slide 3

Heterocyclic Bases—Pyrimidines

The pyrimidine ring





- DNA and RNA RNA only DNA only

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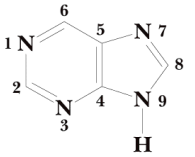
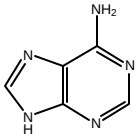
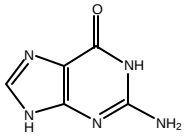
Heterocyclic Bases –Purines

The Purine Ring System:





Adenine



Guanine

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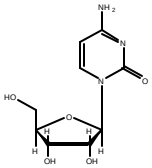
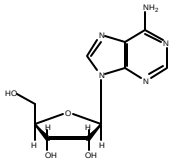
Nucleosides

- β glycosidic linkage at N-1 of pyrimidine and N-9 of purine



Cytidine



Adenosine

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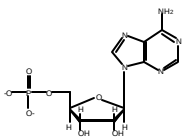
Nucleotides

- Nucleotides are phosphate esters of nucleosides
- Named as "nucleoside-X'-phosphate" where X' is the ribose position to which the phosphate is attached
- Example: adenosine-5'-monophosphate



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"C-1" Metabolism

- Before beginning discussion of purine and pyrimidine biosynthesis, we need to discuss metabolism of one-carbon units.
- Biotin was introduced as a "carrier" of CO_2
- Other C-1 units include **methanol**, **formaldehyde**, and **formic acid**
- These are metabolized while covalently bound to **tetrahydrofolic acid**
  - Review chapter 18, pages 602-603

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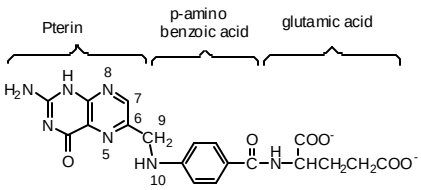

Folic Acid (Pteroyl Glutamic Acid)

Folic acid derivatives with up to 7 glutamate residues are found. The glutamates are attached through amide linkage to the gamma carboxyl group.

Sulfa drugs, among the earliest antibiotics, are structural analogues of p-aminobenzoic acid and inhibit the biosynthesis of folic acid.

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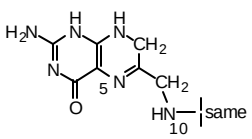
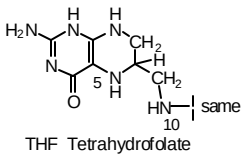
Reduced Forms of Folic Acid



DHF Dihydrofolate



THF Tetrahydrofolate

- Reductions occur with NADPH
  - See Fig 18.35

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"C-1" Derivatives of THF
(See also Table 18.6, p 603)

- Methanol oxidation level (Ox. No. –2)
  - N^5-methyl THF
- Formaldehyde oxidation level (Ox. No. 0)
  - N^5,N^{10}-methylene THF
- Formic acid oxidation level (Ox. No. +2)
  - N^5-formyl THF and N^{10}-formyl THF
  - N^5-formimino THF
  - N^5, N^{10}-methenyl THF

The oxidation number of carbon in CO_2 is +4.

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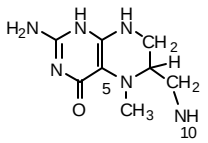

N^5 Methyl THF

- Donor of methyl group in methionine biosynthesis:

homocysteine + N^5-methyl THF → methionine + THF

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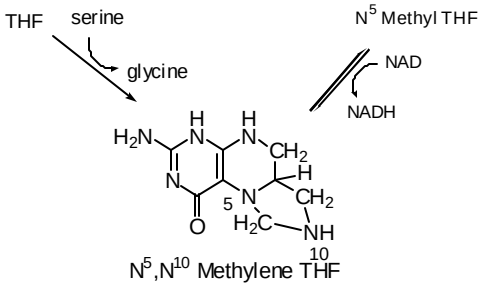

N^5,N^{10} Methylene THF

The pyridoxal phosphate derivative of serine is involved in this reaction. Recall we discussed how that cofactor makes it easier to break the C-C bond of serine. The enzyme is **serine hydroxymethyl transferase**, and serine is the principle source of the one-carbon units.

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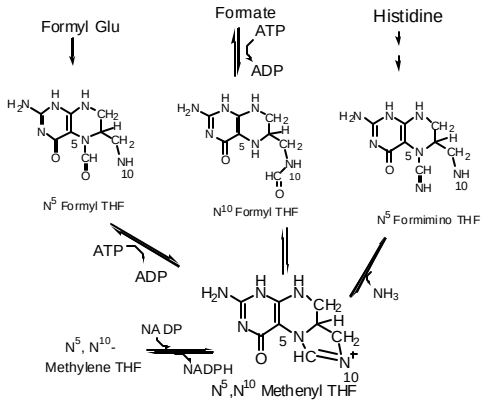

See "A Deeper Look", Page 901, for a summary of these reactions.

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Purine Biosynthesis

- "De Novo" Pathway
  - Ring is built up from precursors.
  - Occurs with all intermediates attached to ribose
  - Pathway subject to feedback regulation
- Salvage Pathway
  - Salvages purine rings by re-attaching them to ribose
- Phosphoribosyl Pyrophosphate (PRPP) is beginning point of both

  Ribose-5-P + ATP $\rightarrow$ PRPP + AMP

Slide 15

Purine Biosynthesis, con't.

- Early labeling studies established origin of each atom in the purine ring:
  - N-1 from aspartic acid
  - N-3, N-9 from glutamine
  - C-4, C-5, N-7 from glycine
  - C-6 from CO_2
  - C-2, C-8 from C-1 source (N^{10} Formyl THF)
    - See Fig 27.2

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Purine De Novo Pathway

- Overall pathway shown in Fig 27.3
- Step 1—formation of PRPP
  - Branch point for both purines and pyrimidines
- Step 2—PRPP + glutamine

  PRPP $\rightarrow$ Phosphoribosylamine
  - First committed step
  - Pyrophosphate is a product
  - Enzyme inhibited by G and A nucleotides

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Purine De Novo Pathway, con't.

- Step 3 (Glycine added, requires ATP)

  Phosphoribosylamine $\rightarrow$ GAR
- Step 4 (N^{10} Formyl THF donates C-8)

  GAR $\rightarrow$ FGAR
- Step 5 (Gln adds N, requires ATP)

  FGAR $\rightarrow$ FGAM
- Step 6 (Ring is closed, requires ATP)

  FGAM $\rightarrow$ AIR

GAR = glycinamide ribonucleotide
FGAR = formylglycinamide ribonucleotide
FGAM = formylglycinamidine ribonucleotide
AIR = 5-aminoimidazole ribonucleotide

Slide 18

Purine De Novo Pathway, con't.

- Step 7 (CO_2 added, requires ATP, but does **not** require biotin)

  AIR $\rightarrow$ CAIR
- Step 8 (Aspartate donates nitrogen)

  CAIR $\rightarrow$ SCAIR
- Step 9 (Fumarate released)

  SCAIR $\rightarrow$ AICAR

CAIR = carboxyaminoimidazole ribonucleotide
SCAIR = N-succinylo-5-amino-4-carboxamide ribonucleotide
AICAR = 5-Aminoimidazole-4-carboxamide ribonucleotide

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Purine De Novo Pathway, con't.

- Step 10 (N^{10} Formyl THF donates C-2)
  AICAR → FAICAR
- Step 11 (Ring closure)
  FAICAR → IMP
- IMP is a branch point in the pathway
- First step inhibited by GDP and ADP
- Second step inhibited by
  - ATP, ADP, AMP at one site,
  - GTP, GDP, GMP at a second site

FAICAR = N-formylaminoimidazole-4-carboxamide ribonucleotide
IMP = inosine monophosphate (the purine is **hypoxanthine**).

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Multifunctional Proteins in Purine Pathway

- In avian liver some enzymes are part of multifunctional polypeptide chains:
  - Enzymes for steps 3, 4, and 6 (110 kD)
  - Enzymes for steps 7 and 8
  - Enzymes for steps 10 and 11 (135 kD dimer)
- THF metabolizing enzymes are complexed with enzymes 4 and 10
  - (The steps using N^{10}-Formyl THF)

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Drugs Affecting Purine Synthesis

- Several compounds inhibiting steps of the purine pathway are used as antitumor agents or immunosuppresive agents.
  - Azaserine inhibits glutamine enzymes (Fig 27.4)
  - Folic acid antagonists also block
    - Amithopterin and methotrexate (Fig 27.30)
    - Sulfonamides were early antibiotics (Fig 27.5)

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IMP to GMP and AMP

- AMP synthesis
  - Aspartate donates N, GTP required
  - Fumarate released in second step
- GMP synthesis
  - NAD oxidizes hypoxanthine ring to xanthine
    - IMP to XMP
  - Glutamine donates N, ATP required
- GMP and AMP inhibit branch point enzymes
- See Fig 27.7 for overall regulation scheme

Note that GTP is required in AMP synthesis, and ATP is required in GMP synthesis.
Note discrepancy in nomenclature. XMP stands for **xanthosine monophosphate**, the nucleotide of xanthine, but the nucleotide of hypoxanthine is **inosine monophosphate** or IMP.

Slide 23

Synthesis of GTP and ATP

- Adenylate kinase (aka **myokinase**)
  AMP + ATP $\rightarrow$ 2 ADP
- Guanylate kinase
  GMP + ATP $\rightarrow$ GDP + ADP
- Nucleoside diphosphate kinase
  GDP + ATP $\rightarrow$ GTP + ADP
- Oxidative phosphorylation
  ADP + P_i $\rightarrow$ ATP (coupled to electron transport)

Slide 24

Salvage Pathway

- Two main enzymes
  - Adenine phosphoribosyltransferase (APRT)
    Adenine + PRPP $\rightarrow$ AMP + PP
  - Hypoxanthine-Guanine phosphoribosyltransfersase (HGPRT)
    Hypoxanthine + PRPP $\rightarrow$ IMP + PP
    Guanine + PRPP $\rightarrow$ GMP + PP
  - Defect in HGPRT causes **Lesch-Nyhan syndrome** (See Fig 27.8)

Lesch-Nyhan syndrome is caused by a crippling gouty arthritis with excess uric acid accumulation. Deficiency in salvage pathway removes inhibitors of de novo pathway, leading to excess purine biosynthesis. Also the lack of use of PRPP in the salvage reaction can lead to its accumulation, and it is a "feed forward" activator of Gln-PRPP amidotransferase.

Slide 25

Catabolism of Purine Nucleotides

- Nucleotidases convert to nucleosidases
  - Adenine deaminated at both AMP and adenosine levels
- Phosphorylase cleaves glycosidic bond, forming ribose-1-phosphate and free base
- Guanine deaminase forms xanthine
- Xanthine oxidase converts hypoxanthine and xanthine to uric acid
  - See Fig 27.9

Gout is caused by accumulation of uric acid, which is relatively insoluble, and forms crystals in joints that are painful. Causes are varied but include overproduction of purines or impaired excretion of uric acid. **Allopurinol**, an inhibitor of xanthine oxidase (Fig 27.13), helps the condition because xanthine and hypoxanthine are more soluble.

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Catabolism of Purine Nucleotides, con't.

- Deficiency of **adenosine deaminase** causes the **severe combined immunodeficiency disease (SCID)**
  - Deoxyadenosine is not deaminated, accumulates, and is converted to dATP
  - dATP inhibits deoxynucleotide synthesis
  - DNA synthesis and cell division blocked
    - See Fig 27.10
  - One of first diseases attacked experimentally by gene therapy

Slide 27

Purine Nucleoside Cycle

- AMP deaminase can combine with two biosynthetic enzymes to convert **aspartate** to **fumarate** as an anaplerotic reaction:

  $AMP + H_2O \rightarrow IMP + NH_3$

  $IMP + aspartate + GTP \rightarrow andenylosuccinate + GDP + P_i$

  $Adenylosuccinate \rightarrow AMP + fumarate$
- Sum:

  $Aspartate + GTP \rightarrow fumarate + NH_3 + GDP + P_i$

  (See Fig 27.11)

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Pyrimidine Biosynthesis

- Pyrimidine ring made before attachment to ribose.
- Six atoms of the pyrimidine ring come from carbamoyl-phosphate and aspartate.
  - See Fig. 27.15
- Carbamoyl phosphate made by **carbamoyl phosphate synthase II**
  - Cytosolic enzyme
  - Glutamine furnishes the nitrogen
  - 2 ATP's required
    - See Fig. 27.16

Bacteria have only one carbamoyl phosphate synthase utilized in making both arginine and pyrimidines.

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Pyrimidine Biosynthesis, con't.

- Aspartate transcarbamoylase transfers carbamoyl group to aspartate.
- Dihydroorotase closes the ring
- Dihydroorotate dehydrogenase oxidizes to orotate, the first pyrimidine
- PRPP adds ribose to form the nucleoside mono phosphate OMP.
- Decarboxylation produces UMP
  - See Fig 27.17

OMP is **orotidine monophosphate**.

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Multifunctional Proteins in the Pyrimidine Pathway

- As in eukaryotic purine synthesis, some of the enzymes are **multifunctional proteins**.
  - First three enzymes are on one polypeptide chain and are coded by one gene
  - Fifth and sixth enzyme are part of a single protein called **UMP synthase**
  - Fourth enzyme is on external face of inner mitochondrial membrane.

Having enzyme sites close together on the same protein is believed to cause **metabolic channeling**, where the product of one reaction is immediately available for the next enzyme without having to diffuse through the solution to find it.

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Pyrimidine Biosynthesis, con't.

- UMP is converted to UDP and then to UTP
- CTP synthase forms CTP from UTP
  - Nitrogen comes from glutamine (See Fig 27.18)

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Regulation of Pyrimidine Biosynthesis in Bacteria

- The **end product**, CTP, inhibits the first step (ATCase)
  - This was the prototype enzyme showing **allosteric** behavior. (See Section 15.5, Chapter 15)
    - CTP is a heterotropic allosteric inhibitor
      - (Binds to T state of regulatory subunit)
    - ATP is a heterotropic allosteric activator
      - (Binds to R state of regulatory subunit)
    - Aspartate is a homotropic allosteric activator
      - (Binds to R state of catalytic subunit)

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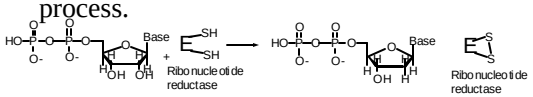
Regulation of Pyrimidine Biosynthesis in Eukaryotes

- UDP and UTP inhibit CPS-II
  - (This is the first committed step in eukaryotes)
- PRPP and ATP activate CPS-II
- See Fig. 27.19 for comparison.

Slide 34

Deoxyribonucleotide Synthesis

- Reduction of ribose takes place at the nucleotide diphosphate level.
- One enzyme, **ribonucleotide reductase**, reduces all four nucleotide diphosphates.
- Two SH groups of **R.R.** are oxidized in the process.



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Ribonucleotide Reductase, Mechanism

- Dimer of two subunits ($\alpha_2\beta_2$)
- Tyrosine and Fe part of β subunit
- Disulfide and regulatory sites part of α subunit.
  - (See Fig.27.22)
- Mechanism involves enzyme free radical removing 3' H, followed by 2'OH elimination, followed by disulfide formation.
  - (See Fig. 27.23)

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Reoxidation of Ribonucleotide Reductase

- Two mechanisms, depending on organism:
  - Reduction by **thioredoxin**, which is reduced by **thioredoxin reductase.**
    - See Fig 27.24
  - In E. coli, reduction by **glutaredoxin**, which is reduced by **glutathione**, which is reduced by **glutathione reductase**.
    - See Fig 27.25
  - Electrons come from NADPH in both cases.

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Regulation of Ribonucleotide Reductase

- Most studied is E. coli enzyme
- Activity site on each α subunit
  - ATP activates, dATP inhibits
- Specificity site on each α subunit
  - dATP favors UDP and CDP reduction
  - dTTP favors GDP and ADP reduction
  - dGTP favors ATP reduction
- Regulation designed to produce a proper balance of the four deoxynucleotides.

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Thymidylate Synthesis

- Methylation of uracil occurs at the **dUMP** level.
- dUMP formed from dUDP and dUTP via an active **dUTPase** (a pyrophosphatase)
- Or by deamination of dCMP.
  - Reaction stimulated by dCTP, inhibited by dTTP (See Fig 27.28)

dUTPase prevents dUTP from serving as a substrate in DNA synthesis.

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Thymidylate Synthesis, con't.

- dUMP accepts CH_3- group from N_5,N_{10}-methylene THF
  - THF derivative at formaldehyde oxidation level
  - Methyl of dTMP at methanol oxidation level
  - Electrons for reduction come from THF oxidation to DHF

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Inhibition of Thymidylate Synthesis

- DHF must be reduced to THF (by dihydrofolate reductase) for cycle to be complete. (See Fig 27.29)
- Inhibitors of dihydrofolate reductase block thymidylate synthesis, in turn blocking DNA synthesis and cell division.
  - See Fig 27.30 for examples of such analogues used as antitumor drugs.

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Inhibition of Thymidylate Synthesis, con't.

- 5-fluoro derivatives of pyrimidines (Fig 27.31) are converted to 5-FdUMP
- Intermediate in the methylation reaction cannot eliminate F as F^+
- Enzyme is irreversibly inhibited
  - **Suicide inhibitor**, **mechanism-based inhibitor**, or **Trojan horse substrate**
  - See Fig Page 924.