Nucleotide Metabolism

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Nucleotide Metabolism

Introduction

Nucleotides are the Building Blocks of the Nucleic Acids - DNA and RNA.

Cells Make Nucleotides by Two Pathways - *de novo* and Salvage Synthesis.

- Purines are Made Separately from Pyrimidines.
- dNTPs are Made from Ribonucleoside Diphosphates.
- Thymidine Nucleotides are Made from Uridine Nucleotides.

![Diagram of nucleotide structure and metabolites](image)
Nucleotide Metabolism

• Introduction

Nucleotides Made from Very Simple Molecules - Amino Acids, One Carbon Donors, CO₂
Synthesis Very Tightly Regulated - Imbalances Favor Mutation
Purine Synthesis Begins on the Ribose Sugar
Pyrimidine Rings Synthesized Separate from Sugar and Then Attached
Purine Metabolism Overview
Nucleotide Metabolism
- *de novo* Purine Metabolism

Ribose-5-Phosphate is Starting Point
PRPP Made, then Aminated Using Glutamine

Unusual Regulation
Nucleotide Metabolism
• Building a Purine Ring

Phosphoribosylamine → Glycine → Aspartate
**Nucleotide Metabolism**

*Paths to Guanine and Adenine Nucleotides*

- IMP
- GTP + Aspartate
- GDP + P
- Glutamine + ATP + AMP + PP_i
- Glutamate + AMP + PP_i
- IMP Dehydrogenase
- Adenylosuccinate Synthetase
- GDP + P_i
- Adenylate Kinase
- GTP
- ADP
- ATP
- ADP
- ATP
- Fumarate
- NAD + H^+ + H_2O
- NADH + H^+
- GMP
- GMP Kinase
- GDP
- NDPK
- GTP
- NDPK
Nucleotide Metabolism

• Purine de novo Metabolism Regulation

Regulation Focused on Balancing Proper Amounts of All Nucleotides

Four Purine Synthesis Enzymes Involved

  PRPP Synthetase - Inhibited by high phosphate and ADP
  PRPP Amidotransferase - Partly Inhibited by AMP and GMP. Fully by Both
  IMP Dehydrogenase - Inhibited by GMP
  Adenylosuccinate Synthetase - Inhibited by AMP
Nucleotide Metabolism
• Purine de novo Metabolism Regulation

Fully Inhibited by AMP & GMP

Ribose-5-phosphate

GTP + Aspartate

IMP

GDP + Pᵢ

Fully Inhibited by AMP

Adenylosuccinate

XMP

Glutamine

AMP

AMP + PPᵢ

GMP

Glutamate

GTP

GDP

Fully Inhibited by GMP

Only Partly Inhibited by AMP or GMP Alone
Nucleotides are the Building Blocks of Nucleic Acids
Nucleotide Metabolism Proceeds Through *de novo* and Salvage Pathways
Purine Nucleotides are Built *de novo* Starting with Ribose-5-phosphate
PRPP is Made From it and Then it is Aminated
Simple Compounds, Such as Amino Acids and 1-Carbon Donors Make the Bases
IMP is a Branch Point for Synthesis of GMP and AMP
AMP Synthesis Requires GTP Energy and is Self-regulating
GMP Synthesis Requires ATP Energy and is Self-regulating
Nucleotide Metabolism Uses Allosteric Controls to Balance Amounts of Nucleotides
Nucleoside Monophosphate Kinases Turn NMPs to NDPs
NDPK Converts NDPs to NTPs
PRPP Amidotransferase is Partly Inhibited by AMP or GMP and Fully Inhibited by Both
Nucleotide Metabolism
• Pyrimidine de novo Metabolism

Ring Built First, Then Attached to Ribose -5-P of PRPP
Six Steps From Bicarbonate to UMP
Nucleotide Metabolism
- *de novo* Pyrimidine Biosynthesis

6. OMP Decarboxylase

Orotidine Monophosphate (OMP) \( \xrightarrow{\text{OMP Decarboxylase}} \) Uridine Monophosphate (UMP)

One of the Most Efficient Enzymes Known
Speeds Reaction by a Factor of \(10^{17}\) Over Uncatalyzed Reaction
Enzyme Uses no Cofactors/Coenzymes
Nucleotide Metabolism
- Pyrimidine de novo Metabolism

Pyrimidine Nucleotide Regulation
Nucleotide Metabolism
• Pyrimidine de novo Metabolism

2. ATCase Reaction

![Diagram showing the ATCase reaction with Carbamoyl-P activating ATP (Purine) and inhibiting CTP (Pyrimidine).]
Nucleotide Metabolism
• de novo Pyrimidine Biosynthesis

Synthesis of UTP and CTP

UMP → UDP
UMP/CMP Kinase

UDP → UTP
NDPK

Glutamine + ATP

Glutamate + ADP + P_i

CTP Synthetase

CTP
Nucleotide Metabolism
• *de novo* Pyrimidine Biosynthesis

CTP Synthetase
  Inhibited by Phosphorylation at 2 Sites
  GTP Activates
  CTP Inhibits Own Synthesis
  Balances Purines and Pyrimidines
Pyrimidine Ring Synthesis Occurs First and Then it is Attached to Ribose
ATCase is a Major Regulator and Balance of Pyrimidine/Purine Nucleotides
ATP Activates, Favors Pyrimidines. CTP Inhibits, Favors Purines
Feedback Inhibition Occurs with CTP.
OMP Decarboxylase is one of the Most Efficient Enzymes Known and Makes UMP
CTP is Synthesized from UTP by CTP Synthetase
CTP Synthetase Activated by GTP, Inhibited by CTP and Phosphorylation
CTP Synthetase Helps to Balance Purines and Pyrimidines
Nucleotide Metabolism

- Catabolism of Guanine Nucleotides

Nucleotides in Nucleic Acids are Monophosphates
Breakdown of RNA and DNA Taken up by Cell Starts with Nucleases

RNA/DNA → RNase → Nucleoside Monophosphates

RNA/DNA → Nucleases → Nucleoside Monophosphates

GMP

Guanosine

Purine Nucleotidase

Nucleotidases

Various Enzymes

Purine Nucleoside Phosphorylase

Bases + Sugars

Guanine + Ribose-1-P
Nucleotide Metabolism
• Catabolism of Adenine Nucleotides

Breakdown of AMP Similar to GMP, but With One Added Path

RNA/DNA → RNA → Nucleoside Monophosphates → Nucleosides

AMP → AMP Deaminase → Inosinic Acid (IMP) → Nucleotidase → Inosine

Adenosine → Adenosine Deaminase → Hypoxanthine

Purine Nucleotidase

Ribose-1-P
**Nucleotide Metabolism**
- Catabolism of Guanine and Hypoxanthine

High Concentrations of Uric Acid Cause Gout
Gout Sufferers Tend to Have a Lower Incidence of Multiple Sclerosis

Hypoxanthine → Xanthine → Guanine (Guanase) → Xanthine (Oxidase) → Uric Acid (Oxidase) → Excreted

Inhibited by Allopurinol
Nucleotide Metabolism

- Catabolism of Guanine and Hypoxanthine

Xanthine vs Allopurinol
Allopurinol Forces Salvage and Prevents Formation of Uric Acid

![Xanthine and Allopurinol Structures](image.png)
**Nucleotide Metabolism**

- **Purine Nucleotide Salvage**

  - **Adenine + PRPP**
    - Adenine Phosphoribosyltransferase
    - \( \text{PP}_i + \text{AMP} \rightarrow \text{ADP} \rightarrow \text{ATP} \)

  - **Guanine + PRPP**
    - Hypoxanthine/Guanine Phosphoribosyltransferase
    - \( \text{PP}_i + \text{GMP} \rightarrow \text{GDP} \rightarrow \text{GTP} \)

  - **Hypoxanthine + PRPP**
    - Hypoxanthine/Guanine Phosphoribosyltransferase
    - \( \text{PP}_i + \text{IMP} \)
      - AMP \( \rightarrow \text{ADP} \rightarrow \text{ATP} \)
      - GMP \( \rightarrow \text{GDP} \rightarrow \text{GTP} \)

- Deficiency Causes Lesch-Nyhan Syndrome
Catabolic and Salvage Pathways for Pyrimidines Overlap
Easy Interconversion of Uracil- and Cytosine-Containing Nucleotides/Nucleosides
Similar Breakdown Scheme to Purines
  Nucleases Break Nucleic Acids to Nucleoside Monophosphates
  Nucleotidases Convert Nucleoside Monophosphates to Nucleosides
However, Nucleosidases Can Use Water to Hydrolyze Bases from Sugars Instead of Phosphorylases
Many of the Same Enzymes Work on Cytosine and Uracil Nucleosides/Nucleotides
Breakdown Converges on Production of Uracil
Thymidine Nucleotides Handled Separately
Nucleotide Metabolism

- Catabolism of Uracil and Thymine

**Pyrimidine Catabolism**

\[
\text{uracil} \xrightarrow{\text{NADPH + H}^+} \text{dihydouracil} \xrightarrow{\text{H}_2\text{O}} \text{3-ureidopropionate} \xrightarrow{\text{H}_2\text{O}} \text{NH}_4^+ + \text{CO}_2
\]

(Urea synthesis)
Nucleotide Metabolism

• Summary

Nucleotide Catabolism Begins with Nucleases to Release Nucleoside Monophosphates
Nucleotidases Remove the Phosphate to Make Nucleosides
Phosphorylases or Nucleosidases Release Bases and Sugars
Adenine-Containing and Guanine Nucleotide Breakdown Processes are Similar, but Deaminases Convert AMP to IMP and Adenosine to Inosine
Inosine is Converted to Hypoxanthine and Ribose-1P by a Purine Phosphorylase
Hypoxanthine (Xanthine Oxidase) and Guanine (Guanase) are Converted to Xanthine
Xanthine is Converted to Uric Acid by Xanthine Oxidase
Uric Acid Crystals are the Cause of Gout
Gout Treated With the Xanthine Oxidase Inhibitor Allopurinol
Allopurinol Forces Recycling of Hypoxanthine and Guanine
Deficiency of the Guanine/Hypoxanthine Enzyme, HGPRT, Causes Lesch-Nyhan Syndrome
Pyrimidine Nucleotide Salvage and Catabolism Pathways Overlap
Uracil and Cytosine Nucleotides Easily Interconverted
Nucleases Break Nucleic Acids to Nucleoside Monophosphates
Nucleotidases Convert Nucleoside Monophosphates to Nucleosides
Nucleosidases Can Use Water to Hydrolyze Bases from Sugars Instead of Phosphorylases
Catabolism Converges on Production of Uracil
Uracil and Thymine Catabolism Similar - Both Produce Urea
**Nucleotide Metabolism**

- Deoxyribonucleotide Synthesis

Deoxyribonucleotides are Made From Ribonucleoside Diphosphates
ADP, CDP, GDP, and UDP
The Enzyme Responsible is Ribonucleotide Reductase

![Diagram showing the synthesis of deoxyribonucleotides from ribonucleotides]
Nucleotide Metabolism

- Ribonucleotide Reductase

Ribonucleotide Reductase (RNR) Has Two Subunits - Large and Small
Large Subunit (R1) Has Two Allosteric Sites and the Active Site
Small Subunit (R2) Has a Tyrosine That Gets Radicalized in the Reaction Mechanism
RNR Controls Balance of Deoxyribonucleotides With Complex Allosteric Controls
Nucleotide Metabolism

- Ribonucleotide Reductase
Nucleotide Metabolism
• RNR Reaction Mechanism

- Reduction of RNR (RNR Reduced From)
  - Abstraction of Proton
  - Regeneration of Radical

- Oxidation of Sulfhydryl

- Loss of Water

- Tyrosyl Radical

- RNR Oxidized From
Nucleotide Metabolism

- Ribonucleotide Reductase

The diagram illustrates the interactions between various nucleotides and their activation or inactivation. The Allosteric Sites and Active Site show the binding of different nucleotides, such as ATP, dGTP, dATP, dTTP, UDP, CDP, GDP, ADP, and ATP, with arrows indicating activation or inactivation processes.
**Nucleotide Metabolism**

- Thymidine Metabolism

- dNDPs are converted to dNTPs (including dUTP) by NDPK.
- Thymidine nucleotides are made from uridine nucleotides.
- dUTP can be used by DNA polymerase to make DNA.

**Diagram:**

- UDP → dUDP → dUTP → dUMP → dTMP → dTDP → dTTP
  - RNR
  - NDPK
  - dUTPase
  - Thymidylate Synthase
  - dTMP Kinase
  - NDPK

- ATP → ADP → ATP → ADP

- 5,10-methylenetetrahydrofolate → dihydrofolate
Nucleotide Metabolism

- Thymidine Metabolism
Nucleotide Metabolism

- Thymidylate Synthase

5-Fluorouracil
Anticancer Treatment

Suicide Inhibitor

5,10-methylenetetrahydrofolate
dihydrofolate
**Nucleotide Metabolism**
- Recycling of Folates

- 5,10-methylenetetrahydrofolate
- Dihydrofolate
- NADPH + H⁺
- NADP⁺
- Tetrahydrofolate
- Dihydrofolate Reductase
- Serine Hydroxymethyltransferase
- Glycine
- Plasmodium Enzyme Different From Human Enzyme
- Target of Anti-malarial Studies

- Methotrexate
Methotrexate is a Competitive Inhibitor of DHFR
Used for Chemotherapy
Effective Against Rapidly Dividing Cells

Methotrexate

Dihydrofolate
Nucleotide Metabolism

• Deoxyribonucleotide Metabolism Summary

Deoxyribonucleotides are Made from Ribonucleoside Diphosphates
Enzyme = Ribonucleotide Reductase (RNR)
RNR is Oxidized in the Catalysis and Gets Reduced by Thioredoxin
Thioredoxin Ultimately is Reduced by NADPH
RNR’s Large Subunit (R1) Contains Two Allosteric Sites and the Active Site
RNR’s Small Subunit (R2) Contains the Tyrosine That Gets Radicalized
RNR’s Allosteric Sites Control Levels of Production of Deoxyribonucleotides
Oxidation of RNR Converts Two Sulfhydryls to a Disulfide That Must be Reduced
RNR’s Activity Site Activates the Enzyme When Bound to ATP
RNR’s Activity Site Inactivates the Enzyme When Bound to dATP
Binding of Nucleotides Below to Specificity Site Favors Following Nucleotide Binding at the Active Site
  dATP or ATP (UDP or CDP)
  dGTP (ADP)
  dTTP (GDP)
dTMP is Made from dUMP by Thymidylate Synthase
5-Fluorouracil Inhibits Thymidylate Synthase
Methyl Carbon Donated by 5,10-methylenetetrahydrofolate, Forming Dihydrofolate
Dihydrofolate Must be Converted to Tetrahydrofolate by Dihydrofolate Reductase
Methotrexate Inhibits DHFR and is Used in Cancer Treatment
Nucleotide Metabolism
• Nucleoside Analogs

Nucleotides are Hard to Get Into Cells
Nucleosides & Analogs Readily Transported
Analogs Get Phosphorylated Inside Cells by Salvage Systems

AMP - Nucleotide
Adenosine - Nucleoside
Nucleotide Metabolism
• Deoxyadenosine Analogs

Hypoxanthine

Didanosine (ddl)

Can be Phosphorylated and Used by DNA Polymerase
Terminates Any DNA Chain
Anti-HIV

Adenosine

No 3' OH

Vidarabine (Ara-A)

Arabinose

Competitive Inhibitor of dATP for Viral DNA Polymerases
Anti-viral
Nucleotide Metabolism

- Adenosine Analogs

Adenosine

BCX 4430

Competitive Inhibitor of ATP
When Phosphorylated
Anti-viral
Used to Treat Ebola
Nucleotide Metabolism

• Deoxycytidine Analogs

Cytarabine (AraC)
Chemotherapy

Emtricitabine
Anti-viral / Anti HIV

Deoxycytidine

Lamivudine (3TC)
Anti-viral / Anti HIV

Zalcitabine (ddC)
Anti-viral / Anti HIV

Arabinose