Chapter 18 – Nucleotide Metabolism

Roles of nucleotides in the cell:

1) Activated precursors of DNA and RNA
2) Nucleotide derivatives are activated intermediates in many biosynthetic pathways
   e.g. UDP-glucose, CDP-diacylglycerol
3) Universal currency of cell (i.e. ATP)
4) Components of three major coenzymes: NAD^+, FAD, and CoA
5) Metabolic regulators (i.e. cyclic AMP)

Nucleotide synthesis can either by *de novo* or by recycling preformed bases (salvage pathway).

Nomenclature:

Nucleotides are composed of three components:

1) nitrogenous base - pyrimidine (cytosine, uracil, thymine) or purine (adenine or guanine)
2) pentose sugar - ribose (RNA) or deoxyribose (DNA)
3) phosphate group

nucleoside - purine or pyrimidine base linked to pentose sugar
nucleotide - phosphate ester of nucleoside

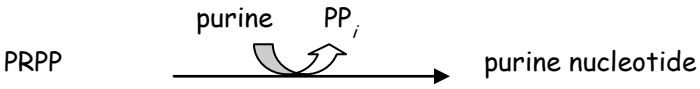
SYNTHESIS OF PURINE NUCLEOTIDES

- Purine ring is synthesized de novo from 5 different precursors: aspartate (N-1 atom), CO_2 (C-6 atom), glycine (C-4, C-5, N-7 atoms), tetrahydrofolate (C-2, C-8 atoms) and glutamine (N-3, N-9).
- Purine ring structure is synthesized from ribose 5-phosphate; PRPP then donates ribose 5-phosphate for purine synthesis.
- Purine ring is built onto the ribose 5-phosphate via a 10 -tep pathway: glutamine, glycine, tetrahydrofolate, and glutamine make contributions to form 5-membered ring; construction of 6-membered ring forms inosine 5'-monophosphate (IMP).
- IMP can be converted into AMP or GMP
  - For AMP synthesis, aspartate amino group condenses with keto-group of IMP; GTP-dependent reaction
  - For GMP synthesis, C-2 is oxidized to form xanthosine monophosphate (XMP)
    Amide nitrogen of glutamine replaces oxygen of C-2 to form GMP
    ATP-dependent reaction

Synthesis of purine bases using the salvage pathway:

- Free purine bases are formed by degradation of nucleic acids and nucleotides.
- Purine nucleotides can be synthesized from preformed bases by salvage reactions (simpler and less costly than *de novo* pathway).

- Ribose phosphate portion of PRPP is transferred to purine to form the corresponding ribonucleotide:



Two salvage enzymes recover purine bases:

1) adenine phosphoribosyl transferase

adenine + PRPP --------> adenylate + PP_i

2) hypoxanthine-guanine phosphoribosyl transferase (HGPRTase)

hypoxanthine + PRPP --------> inosinate + PP_i

guanine + PRPP ---------> guanylate + PP_i

Regulation of purine nucleotide synthesis:

Probably largely by feedback inhibition.
Glutamine-PRPP amidotransferase (in main pathway) is allosterically inhbited by IMP, AMP, GMP.
Those steps leading specifically to AMP or GMP synthesis work primarily by feedback inhibition

XMP and GMP inhibit **IMP dehydrogenase**
AMP inhibits **adenylosuccinate synthetase**

SYNTHESIS OF PYRIMIDINE NUCLEOTIDES

- Pyrimidine ring is assembled first, then linked to ribose phosphate ---> pyrimidine nucleotide.
- Requires fewer ATPs than purine synthesis (2 vs. 4).
- Pyrimidine ring has three metabolic precursors: bicarbonate, amide group of glutamine, aspartate.
- PRPP is also required.
- There is a 6-step pathway for *de novo* synthesis of UMP:
  1) glutamine combines with bicarbonate ion + 2ATPs to yield carbamoyl phosphate + glutamate
  2) carbamoyl phosphate combines with aspartate via aspartate transcarbamolyase to form carbamoyl aspartate (product contains all the atoms necessary for pyrimidine ring).
  3) carbamoyl phosphate is cyclized enzymatically to form L- dihydroorotate.
  4) L-dihydroorotate is oxidized by dihydroorotate dehydrogenase to form orotate; e^- removed from substrate are transferred to ubiquinone ---> O_2 to ETS.
  5) Orotate replaces pyrophosphate group of PRPP to form orotidine 5'-monophosphate(OMP)

6) OMP is decarboxylated by OMP decarboxylase to form uridine 5'-monophosphate (UMP)

Dihydroorotate is produced in the cytosol, then passes through the outer mitochondrial membrane.

Enzyme dihydroorotate DH is on outer surface of inner mitochondrial membrane

Orotate then moves back into cytosol

Regulation of UMP synthesis:

asparate carbamoylase (ATCase)- main regulatory enzyme

Inhibited by UTP and CTP

Activated by ATP

Keeps purines and pyrimidines in equal amounts

Synthesis of CTP

Formation of CTP from UMP in three reactions (see pathway sheet).

Regulation of pathway is via **CTP synthetase**

- allosterically inhibited by CTP

CONVERSION OF RIBONUCLEOTIDES TO DEOXYRIBONUCLEOTIDES

Deoxyribonucleotides are formed from ribonucleotides by **ribonucleoside diphosphate reductase**.

Energy to fuel reduction comes from NADPH.

There are really three proteins involved:

1) thioredoxin reductase
2) thioredoxin
3) ribonucleotide reductase

Once dADP, dGDP, and dCDP are formed, they are phosphorylated by nucleoside diphosphate kinases.

Regulation of ribonucleoside diphosphate reductase is complex because there are 2 regulatory sites:

1) Activity site - a.k.a. allosteric site - controls catalytic site
2) Specificity site - also allosterically regulated- controls substrate specificity

If ATP is bound in activity, enzyme is ACTIVE

If dATP or ATP is bound, reductase is pyrimidine specific

CDP --> dCDP

UDP --> dUDP

Binding of dTTP to specificity site causes enzyme to take GDP --> dGDP.

Binding of dGTP to specificity site causes enzyme to take ADP --> dADP.

Synthesis of Deoxythymidylate (dTMP) by Methylation of dUMP

dTMP is formed from dUMP, which is formed by any of the following:

dUDP + ADP ⟶ dUMP + ATP enzyme is **nucleoside monophosphate kinase**

dUDP + ATP ⟶ dUTP ⟶ dUMP + PP_i

dCMP + H_2O ⟶ dUMP + NH_4^+

dUMP is converted to dTMP by **thymidylate synthase**
Methyl group donor is methylene tetrahydrofolate
Many cancer drugs inhibit the activity of thymidylate synthase and dihydrofolate reductase --> decreased levels of dTMP synthesis --> decreased DNA synthesis

SALVAGE OF PURINES AND PYRIMIDINES

Purine Catabolism

Many organisms convert purine nucleotides to uric acid (see pathway sheet)

AMP ---> IMP ---> hypoxanthine ----->

GMP --> xanthine ----->

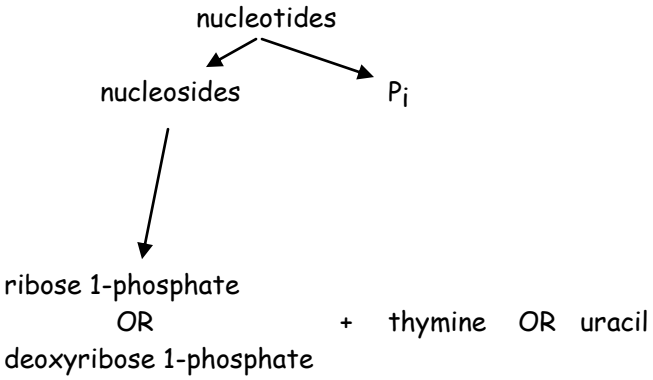
High serum levels of uric acid may lead to **gout**
Inflammation of joints is due to precipitation of sodium urate crystals
Kidneys may also be damaged by deposition of crystals
Gout is thought to be an inherited metabolic disease
Some patients with gout have a partial deficiency of HGPRTase
- leads to reduced synthesis of GMP and IMP by salvage pathway
- causes increase in PRPP levels --> increased purine biosynthesis by *de novo* pathway
Gout can also be caused by increased levels of PRPP caused by a yperactive synthetase
Gout can be treated with allopurinol, an analog of hypoxanthine --> ultimately acts as an inhibitor of xanthine oxidase; called suicide inhibition

Lesch-Nyhan syndrome
Total lack of HGPRTase.
Results in compulsive self-destructive behavior.
Self-mutilation, mental deficiency, spasticity.
Elevated levels of PRPP ---> increased rate of purine biosynthesis by *de novo* pathway ---> overproduction of uric acid.
Possible that brain may rely heavily on salvage pathway for IMP and GMP synthesis.

Shows that abnormal behavior can be caused by absence of a single enzyme.

Pyrimidine Catabolism

Begins with the hydrolysis of nucleosides and Pi from nucleotides.
Successive reactions produce ribose 1-phosphate or deoxyribose 1-phosphate.



Thymine is ultimately broken down to succinyl CoA.

Uracil and cytosine are broken down into alanine, then acetyl CoA.

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