METABOLISM

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❖ Nucleotide

- **Nucleotides:** Nitrogen containing compounds, function as:
  - Carriers of *activated intermediates* in the synthesis of some carbohydrates, lipids, and proteins
  - Structural components of several essential *coenzymes* (such as CoA, FAD, and $NADP^+$)
  - *Second messengers* in signal transduction pathways (such as cAMP and cGMP)
  - *Energy currency* in the cell (ATP, GTP...)
  - *Regulatory compounds* for many metabolic pathways by inhibiting or activating key enzymes
- Nitrogenous bases can be purines (2 rings '6+5 membered) and pyrimidine (1 ring '6 membered')
  - Purines are A and G, pyrimidines are C, U, T
- Nucleo***side*** = *Pentose sugar* + *Base (on C1')*, where Ribonucleoside is Ribose + base
  - deoxyribonucleoside is 2-deoxyribose + base
- Nucleoside + one or more *phosphate (on C5')* = Nucleo***tide***
  - The second and third phosphates are each connected to the nucleotide by a "high-energy" bond
  - The phosphate groups are negatively charged causing DNA and RNA to be nucleic acids
- Base modifications affect **gene expression** by activating (acetylation) or inhibiting (methylation) it
  - These modifications include reduction, acetylation, methylation and glycosylation
- Purine and pyrimidine sources: synthesis de novo, salvage pathways (reuse) of preformed bases resulted from cell turnover, and a little of the bases supplied by diet are utilized
- Atoms forming N.Bs are contributed from multiple sources including *amino acids* (aspartic acid, glycine [added as a whole], and glutamine [donate amide], where all N atoms come from Amino acids), CO_2, *N10–formyltetrahydrofolate* (donate formyl group)
  - These atoms are added to a preformed ribose 5-phosphate which is synthesized by PPP
- Synthesis of purine (A, G) nucleotides, steps:
  - Synthesis of 5-phosphoribosyl-1-pyrophosphate (*PRPP*) by *PRPP synthetase* (*Ribose phosphate pyrophosphokinase*) which requires $\mathbf{Mg^{+2}}$ and **ATP** hydrolysis into AMP
    - PRPP is considered as an **activated pentose**
    - It is activated by $\boldsymbol{P_i}$ and inhibited by ***purines*** and ***ribonucleotides***
    - The sugar moiety of PRPP is ribose producing ribonucleotides
    - When deoxyribonucleotides are required for DNA synthesis, the ribose sugar moiety is **reduced**
  - Synthesis of *5'-phosphoribosylamine* where pyrophosphate is replaced by amino group from **glutamine** (become glutamate) by *glutamine PRPP aminotransferase*
    - It is the committed step, where inhibited by ***AMP*** and ***GMP***, and activated by ***PRPP***
  - Synthesis of *inosine monophosphate (IMP)*, the **parent** purine nucleotide
    - The next nine steps lead to the synthesis of IMP, which require ATP as an energy source
    - Its base is **hypoxanthine**
  - Conversion of *IMP to AMP or GMP*
    - For **GMP**, *IMP dehydrogenase* converts IMP into XMP producing NADH, then glutamine donates amine group for XMP forming GMP which inhibits IMP dehydrogenase
    - For **AMP**, *adenylosuccinate synthetase* produces adenylosuccinate with the use of aspartic acid and GTP hydrolysis, then adenylosuccinate is converted into AMP which inhibits synthetase
  - Conversion of nucleoside monophosphates to nucleoside diphosphates and triphosphates
    - *Base-specific nucleoside monophosphate kinases* do not discriminate between ribose or deoxyribose in the substrate, but discriminate between types of bases, where we have Adenylate kinase (AK) and Guanylate kinase (GK)

- ✓ ATP is the general source of the phosphate (present in higher concentrations than the others)
- ✓ AK is active in liver and muscle and maintains equilibrium among AMP, ADP, ATP
- ✓ *Nucleoside diphosphate kinase* has a broad specificity

- Synthetic inhibitors of purine synthesis:
  - ***Sulfonamides:*** inhibit the growth of rapidly dividing microorganisms without interfering human cell
  - ***Methotrexate:*** structural ***analog of folic acid*** (control the spread of **cancer**)
    - ✓ Anticancer drugs result in adverse effects, including anemia, scaly skin, GI tract disturbance, immunodeficiencies, and hair loss

- Salvage pathway for purines is done by synthesis of purines from the normal turnover of cellular nucleic acids and diet purines that are not degraded (small amount)
- Conversion of purine bases into nucleotides
  - *APRT* and *HGPRT* use PRPP as the source of ***ribose 5-phosphate***
  - Pyrophosphate is hydrolyzed by pyrophosphatase which makes the reaction irreversible
- Adenosine is the only purine nucleoside to be salvaged, by phosphorylation to AMP by *adenosine kinase*

- ***Lesch-Nyhan syndrome:*** A rare, X-linked, recessive disorder associated with **HGPRT deficiency**
  - ***Hyperuricemia:*** ***High amounts of uric acid*** (the end product of purine degradation)
  - Increased PRPP levels and decreased IMP and GMP levels, the committed step in purine synthesis has excess substrate and decreased inhibitors available, and de ***novo purine synthesis is increased***
  - Hyperuricemia results in: *Uric acid stones in the kidneys* (*urolithiasis*), the *deposition of urate crystals in the joints* (*gouty arthritis*) and soft tissues
  - The syndrome is characterized by: *motor dysfunction*, *cognitive deficits*, *behavioral disturbances* that include self-mutilation

- Synthesis of Deoxyribonucleotides:
  - 2'-deoxyribonucleotides are produced from ribonucleoside diphosphates by the enzyme ***ribonucleotide reductase (RR)*** during the S-phase of the cell cycle
  - The only reason to synthesis 2'-deoxyribonucleotides is for *DNA replication*
  - RR is specific for the reduction of purine nucleoside **diphosphates** (ADP and GDP) to their deoxyforms (dADP and dGDP) and pyrimidine nucleoside diphosphates, cytidine diphosphate (CDP) and uridine diphosphate (UDP) to their deoxyforms (dCDP, and dUDP)
  - During the reduction of ribonucleotide into deoxyribonucleotide, *thioredoxin is oxidized*
    - ✓ Thioredoxin is recycled by ***thioredoxin reductase*** consuming NADPH

- Ribonucleotide reductase is composed of two non-identical dimeric subunits, R1 and R2
  - RR maintain balanced supply of deoxyribonucleotides required for DNA synthesis
  - **Activity sites (allosteric sites):** ***dATP*** inhibits the enzyme and prevents the reduction of any of the four nucleoside diphosphates resulting in preventing DNA synthesis, while ***ATP*** activates it
  - **Substrate specificity sites (allosteric sites):** Nucleoside triphosphates regulate substrate specificity, causing an increase in the conversion of different species of ribonucleotides to deoxyribonucleotides. **dTTP** binding **activates** the reduction of GDP to **dGDP** at the catalytic site

- The drug **hydroxyurea** destroys the free radical required for the activity of ribonucleotide reductase, so ***inhibiting the generation*** of substrates for DNA synthesis
  - Hydroxyurea has been used in the treatment of cancers such as CML

- Dietary nucleic acids degradation occurs in the **small intestine**
  - ***Ribonucleases*** and ***deoxyribonucleases***, secreted by the pancreas, hydrolyze dietary RNA and DNA to oligonucleotides which are further hydrolyzed by pancreatic ***phosphodiesterases***, producing a mixture of 3'- and 5'-mononucleotides

- In the intestinal mucosal cells, ***nucleotidases*** remove the phosphate groups hydrolytically, releasing nucleosides that are further degraded to free bases
- Dietary purine bases are not an appreciable source for the synthesis of tissue nucleic acids, so they are converted to **uric acid** (excreted in urine) in intestinal mucosal cells
- Purine nucleotides from de novo synthesis are degraded in the **liver** primarily, then free bases are sent out from liver and salvaged by peripheral tissues

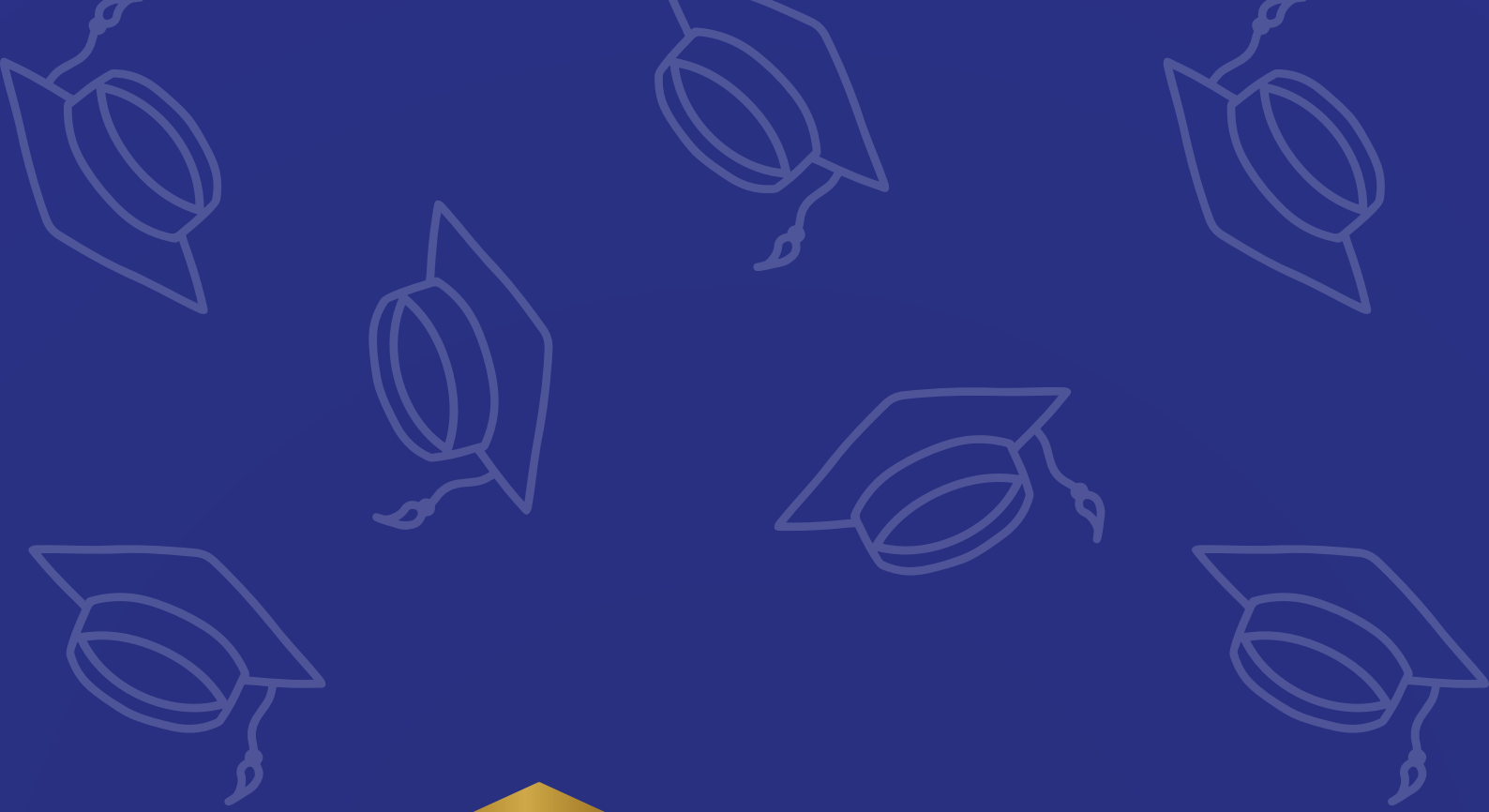
- Formation of uric acid:
  - An amino group is removed from AMP to produce IMP by ***AMP deaminase***, or from adenosine to produce inosine (hypoxanthine ribose) by ***adenosine deaminase***
  - IMP and GMP are converted into their nucleoside forms (inosine and guanosine), by the action of ***5'-nucleotidase***, inosine & guanosine are converted into their respective purine bases (hypoxanthine and guanine) by ***purine nucleoside phosphorylase,*** then mutase interconverts ribose 1-P into 5-P
  - Guanine is deaminated to form xanthine, and hypoxanthine is oxidized by ***xanthine oxidase*** to xanthine, which is further oxidized by xanthine oxidase to uric acid
- Diseases associated with purine degradation = **Gout**
- High levels of uric acid in blood (hyperuricemia) due to overproduction or underexcretion of uric acid
- Hyperuricemia leads to the deposition of monosodium urate crystals in the joints, and an inflammatory response to the crystals, causing first acute and then chronic gouty arthritis
- Nodular masses of monosodium urate crystals (tophi) may be deposited in the soft tissues, resulting in chronic tophaceous gout
- Formation of kidney stones (Uric acid stones) in the kidney (urolithiasis)
  - ***Underexcretion*** of uric acid: Most gout patients In the vast majority of patients, Underexcretion can be primary (due to unidentified inherent excretory defects) Or secondary to known disease that affects the kidney function in handling urate, such as lactic acidosis (lactate and urate compete for the same renal transporter) and environmental factors such as drugs (**thiazide diuretics**) or exposure to lead (saturnine gout)
  - ***Overproduction*** of uric acid: less common, several identified mutations in the X-linked PRPP synthetase gene that increase PRPP production
- Diagnosis of gout requires aspiration and examination of synovial fluid from an affected joint (tophus) using polarized light microscopy to confirm the presence of needle-shaped monosodium urate crystals

- Pyrimidine Synthesis:
  - The pyrimidine ring is synthesized before being attached to ribose 5-phosphate
  - We start the pathway by producing *Carbamoyl phosphate* from CO_2 and glutamine with the consumption of 2 ATP by enzyme ***CPS II***
    - ✓ CPS II is activated by PRPP and inhibited by UTP
  - Carbamoyl phosphate converted into carbamoyl aspartate by aspartate transcarbamoylase
  - Dihydroorotase converts carbamoyl aspartate into dihydroorotate
  - The enzyme that produces **orotate**, ***dihydroorotate dehydrogenase***, is associated with the ***inner mitochondrial*** membrane
    - ✓ Produces **$FADH_2$**
    - ✓ All other enzymes in pyrimidine biosynthesis are ***cytosolic***
  - The completed pyrimidine ring is converted to the nucleotide ***orotidine 5'-monophosphate (OMP),*** (PRPP is added) or the *parent pyrimidine mononucleotide*
    - ✓ The reaction releases pyrophosphate, thus, it is irreversible
  - OMP is decarboxylated by OMP decarboxylase forming UMP

CPS II, aspartate transcarbamoylase, and dihydroorotase are 3 different catalytic domains of a single polypeptide chain (CAD)

- Thymine can be produced by ***methylation*** (adding methyl)
- Cytosine can be produced by ***removing O*** and adding ***NH_2*** group by **CTP synthetase**
  - ✓ To produce CTP, UTP is required not UMP

- Both purine and pyrimidine synthesis require Gln, Asp, and PRPP as essential precursors
- Orotate phosphoribosyl transferase and orotidylate decarboxylase are catalytic domains of a single polypeptide chain called ***UMP synthase***
- **Orotic aciduria**, a rare genetic defect, caused by a deficiency of one or both activities of the bifunctional UMP synthase resulting in orotic acid in the urine
- UMP is phosphorylated to UDP and then UTP
  - UDP is a substrate for ribonucleotide reductase, which generates dUDP
  - dUDP is phosphorylated to dUTP, which is rapidly hydrolyzed to dUMP by ***UTP diphosphatase*** (dUTPase) reducing the available dUTP for DNA synthesis, prevent incorporation of uracil to DNA
- Thymidylate synthase converts dUMP into dTMP
- Thymidylate synthase inhibitors include thymine analogs such as 5-fluorouracil (antitumor agents)
  - 5-Fluorouracil and Methotrexate are anti cancerous agents
  - 5-Fluorouracil (suicide inhibitor) is converted to 5-FdUMP that permanently binds to the inactivated *thymidylate synthase*
  - Methotrexate inhibits dihydrofolate reductase reducing THF, inhibits purine synthesis and prevents methylation of dUMP to *dTMP*, resulting in DNA synthesis inhibition and cell growth slow down
- Pyrimidine nucleosides uses nucleoside kinase to produce nucleotides
  - No nitrogenous base salvage
- The pyrimidine ring is opened and degraded to highly soluble products (*β-alanine*, *β-aminoisobutyrate*) with the production of NH_3 and CO_2



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