Highlights - Nucleotide Metabolism II

1. Ribonucleotide reductase (RNR) catalyzes the formation of deoxyribonucleotides from ribonucleotides. The substrates are ribonucleoside diphosphates (ADP, GDP, CDP, or UDP) and the products are deoxyribonucleoside diphosphates (dADP, dGDP, dCDP, or dUDP).

2. RNR has two pairs of two identical subunits - R1 (large subunit) and R2 (small subunit). R1 (I mistakenly said it was R2 earlier in the lecture) has two allosteric binding sites and the active site of the enzyme. R2 forms a tyrosine radical necessary for the reaction mechanism of the enzyme.

3. Ribonucleotide reductase is allosterically regulated via two binding sites - a specificity binding site (binds dNTPs and controls which substrates the enzyme binds and which deoxyribonucleotides are made) and an activity binding site (controls whether or not enzyme is active - ATP activates, dATP inactivates). Specificity sites act in a generally complementary fashion. Binding of deoxypyrimidine triphosphates to the specificity site tends to inhibit binding and reduction of pyrimidine diphosphates at the enzyme's active site and stimulates binding and reduction of purine diphosphates at the active site. Binding of deoxypurine triphosphates tends to inhibit reduction of purine diphosphates and stimulates reduction of pyrimidine diphosphates. Don't confuse the active site with the activity site. The ACTIVE SITE is where the reaction is catalyzed, whereas the ACTIVITY SITE is the allosteric binding site for ATP or dATP.

4. Synthesis of dTTP by the *de novo* pathway takes a convoluted pathway from dUDP to dUTP to dUMP. The last reaction here is catalyzed dUTPase.

5. The *de novo* pathway for thymidine synthesis converts dUMP to dTMP, using a tetrahydrofolate derivative and the enyzme thymidylate synthase. In the process, dihydrofolate is produced and must be converted back to tetrahyrdolate in order to keep nucleotide synthesis occurring.

6. The enzyme involved in the conversion of dihydrofolate to tetrahydrofolate, dihydrofolate reductase (DHFR), is a target of anticancer drugs which inhibit the enzyme. An inhibitor of DHFR is methotrexate or aminopterin.

7. ATCase is regulated allosterically by ATP (activates) and CTP (inactivates). It is the most important regulatory enzyme in de novo pyrimidine biosynthesis and it helps to balance the relative amounts of purines and pyrimidines. Another important regulatory enzyme in the pathway is CTP synthase, which is inhibited by CTP. This enzyme helps balance the relative amounts of CTP and UTP.

8. PRPP amidotransferase is an important regulatory enzyme for purine biosynthesis. It is inhibited by AMP, GMP, and IMP. If AMP is low and GMP is high (or vice-versa), the enzyme is reduced in activity, but still can function. This is important to help increase the amount of the other one, thus helping to balance AMP and GMP.

9. Salvage of purine nucleotides is important metabolically - perhaps more so than salvage of

pyrimidines. The enzyme HGPRT is involved in the direct salvage of guanine nucleotides and indirectly involved in salvage of adenine nucleotides through IMP and hypoxanthine.

10. Breakdown of purines results in production of xanthine. Oxidation of xanthine yields uric acid. This compound serves an excretory role in birds and dalmations (among other organisms). Uric acid is not very water soluble and can precipitate out, cause the painful condition known as gout. Gout often strikes in the big toe. Uric acid acts as an antioxidant and may have protective roles against diseases, such as multiple sclerosis. The disease is successfully treated with allopurinol, which acts as a suicide inhibitor of the xanthine oxidase enzyme.

11. Severe combined immune deficiency arises from a deficiency of adenosine deaminase. In immune cells of patients with this disease, dATP accumulates, shutting off RNR and stopping cell division.