7.2: Nucleotide synthesis

Nucleotides are the fundamental building blocks essential for the synthesis of DNA and RNA. Each nucleotide contains three functional groups: a sugar, a base, and phosphate (figure 7.4).

Nucleotides can be divided into two groups: pyrimidines and purines. The family of pyrimidines includes thymine (T), cytosine (C), and uracil (U), which is only incorporated into RNA. These compounds contain a single-ringed nitrogenous base that pairs with a purine nucleotide counterpart. Thymine pairs with adenine forming two hydrogen bonds, in contrast to cytosine, which pairs with guanine to form three hydrogen bonds. Purines, both guanine (G) and adenine (A), are double-ringed structures and more difficult to break down in the body. As such, the salvage pathway for purine metabolism is of importance (figure 7.5).
Nucleotide synthesis will be described below, but one of the fundamental requirements of the synthesis of either purines or pyrimidines is the need for a five-carbon sugar (ribose). This sugar is generated through glucose oxidation via the pentose phosphate pathway.

For purines synthesis, the base is synthesized and attached to the sugar, while for pyrimidine synthesis, the sugar group is added after the base is produced. In either case, ribose is the added sugar, and this must be converted to the deoxyribose form before the bases can be used for DNA synthesis.

Conversion of ribose to deoxyribose nucleotides

All bases are synthesized in the ribose form and used directly for transcription. They can be converted to the deoxy form, which is needed for DNA replication. The enzyme, ribonucleotide reductase, converts the diphosphate form of a ribose base to the deoxybase form. The enzyme has two sites for regulation: an enzyme activity site and a substrate specificity site. The enzyme activity site must have ATP/ADP bound for the enzyme to be active, while the substrate specificity site will bind different nucleotides influencing the enzyme substrate preference, therefore altering which base is being acted upon depending on cellular needs.

Generation of 5-phosphoribosyl-1-phosphate (PRPP)

Ribose 5-phosphate is not used directly for either purine or pyrimidine synthesis, rather it is used to synthesize the "active pentose" — 5-phosphoribosyl-1-pyrophosphate (PRPP). The conversion is catalyzed by the enzyme phosphoribosyl-1-pyrophosphate (PRPP) synthase. PRPP is the activated five-carbon sugar used for nucleotide synthesis and provides both the sugar and phosphate group to nucleotides (figure 7.6).
Regulation of PRPP synthase

The enzyme, PRPP synthetase, is activated by Pi (inorganic phosphate) and inhibited by the purine bases adenine and guanine.

Synthesis of purines

Purines are composed of a bicyclic structure that is synthesized from carbon and nitrogen donated from various compounds such as carbon dioxide, glycine, glutamine, aspartate, and tetrahydrofolate (TH4). The synthesis of purines starts with the synthesis of 5′phosphoribosylamine from PRPP and glutamine. The enzyme glutamine phosphoribosylpyrophosphate amidotransferase (GPAT) catalyzes this reaction and is the committed step in purine synthesis (figure 7.7). Synthesis continues for nine additional steps culminating in the synthesis of inosine monophosphate (IMP), which contains the base hypoxanthine. IMP is used to generate both AMP and GMP. The synthesis of both AMP and GMP requires energy in the form of the alternative base (i.e., the synthesis of GMP requires ATP while AMP synthesis requires energy in the form of GTP). The synthesis of AMP and GMP is regulated by feedback inhibition (figures 7.7 and 7.8). This allows for the maintenance of nucleotides in a relative ratio that is required for cellular processes. The generated nucleotide monophosphates can be converted to the di and triphosphate forms by nucleotide specific kinases, which will transfer phosphate groups to maintain a balance of the mono, di, and triphosphate forms.
Regulation of purine synthesis

The regulatory enzyme GPAT is allosterically activated by PRPP and inhibited by IMP, AMP, and GMP. All three must be present to inhibit activity of this enzyme.

Degradation of purines

Like amino acids, nucleotides contain nitrogen and must be degraded in a manner that allows for proper nitrogen disposal either through the urea cycle or by the synthesis of a nontoxic compound.

Degradation of dietary nucleotides occurs in the gut, while nucleotides from de novo synthesis are degraded in the liver. The fundamental process involves the dismantling of the sugar, phosphate, and base structure into their own respective units (figure 7.9). In the case of purine degradation, the base is excreted in the form of uric acid. Purine nucleoside
phosphorylase converts inosine and guanosine to their respective bases (hypoxanthine and guanine). Finally, xanthine oxidase will oxidize hypoxanthine to xanthine (guanine can be deaminated to xanthine), and xanthine can be further oxidized to uric acid by the same enzyme. Uric acid is excreted in the urine.

Figure 7.9: Breakdown of nucleotides.

Excess uric acid, hyperuricemia, can cause the precipitation of uric acid crystals in the joints eliciting an inflammatory reaction causing acute pain or gout. The majority of individuals diagnosed with gout present due to underexcretion of uric acid. And this can be caused by the presence of other pathologies, such as lactic acidosis or the use of diuretics. Less common presentations of gout are associated with overproduction of uric acid, which can be caused by increased activity of PRPP synthetase or deficiency in purine recycling enzyme HGPRT caused by Lesch-Nyhan syndrome (figure 7.10).

Figure 7.10: Nucleotide base salvage. Reaction catalyzed by HGPRT is clinically relevant as deficiencies can cause accumulation of uric acid.

Secondary hyperuricemia is also seen in individuals with myeloproliferative disorders undergoing therapy where there is excess cellular turnover (cell lysis leads to an accumulation of nucleotides) or in cases of Von Gierke disease or fructose intolerance, which increases substrate for PRPP synthesis. Xanthine oxidase inhibitors, such as allopurinol, are used as part of the management of gout.
Salvage of purines

The ability to recycle nucleotides is specifically important in the case of purines as de novo synthesis uses much more ATP than salvage. The degradation product of purine bases is uric acid, which is an insoluble compound, and accumulation can result in several clinical disorders as previously discussed. As such, purine bases can also undergo salvage reaction where bases are recycled and used in a new process. To reduce the amount of uric acid production, purines can be salvaged and reconverted back to their triphosphate form to be reused. There are two primary enzymes involved in the salvage pathway: adenine phosphoribosyltransferase (APRT) and xanthine-guanine phosphoribosyltransferase (HGPRT) (figure 7.10). These enzymes will recombine the base (either adenine, guanine, or hypoxanthine) with PRPP to generate AMP, GMP, or IMP respectively. Adenosine is the only nucleoside that can be rephosphorylated to its monophosphate form using adenosine kinase (figure 7.11). All other nucleosides must be degraded to their free base before they can be salvaged.

Figure 7.11: Nucleotide specific pathways for base salvage.
Synthesis of pyrimidines
Glutamine + 2 ATP → Carbamoyl phosphate synthetase II (CPSII)

2 ADP + P_i → Carboxamoyl phosphate

Aspartate transcarbamoylase

N-carbamoyl aspartate

Dihydroorotase

Dihydroorotate

NAD^+ / NADH → Dihydroorotate dehydrogenase (in mitochondria)

Orotate

PRPP + P_P, → Orotate phosphoribosyltransferase

Orotidine-5'-monophosphate (OMP)

CO_2 → OMP decarboxylase (UMP synthase)

Uridine-5'-monophosphate (UMP)

ATP/ADP → UMP kinase

UDP

ATP/ADP → Nucleoside diphosphate kinase

CTP synthetase

CTP + ATP + ADP + P_i → Glutamine + Glutamate

https://med.libretexts.org/Bookshelves/Basic_Science/Cell_Biology_Genetics_and_Biochemistry_for_Pre-Clinical_Students/0...
Figure 7.12: Overview of pyrimidine synthesis. The reaction catalyzed by carbamoyl phosphate synthetase I is the regulatory enzyme of the pathway.

In contrast to purine synthesis, the pyrimidine bases are synthesized before the ribose sugar and phosphate groups are added in the form of PRPP (figure 7.12). The initial step of the pathways involves the synthesis of carbamoyl phosphate from glutamine, carbon dioxide, and 2 ATP. Carbamoyl phosphate synthetase II (CSPII) catalyzes this reaction. (Note there is an analogous enzyme in the mitochondria for the urea cycle termed carbamoyl phosphate synthetase I, which also generates carbamoyl phosphate.) Of clinical importance is the intermediate orotate. Elevations of orotate (orotic acid) are consistent with enzymatic deficiencies in this pathway or urea cycle deficiencies such as a defect in ornithine transcarbamoylase. In the case of a urea cycle deficiency, an excess carbamoyl phosphate can enter pyrimidine synthesis leading to a build up of orotate. Following the synthesis of carbamoyl phosphate, a series of subsequent reactions yield uracil monophosphate, which is the intermediate of pyrimidine synthesis.

UMP, much like IMP, serves as the intermediate to pyrimidine synthesis and can undergo sequential phosphorylation to form UTP, which can be converted to cytidine (CTP). Alternatively, UMP can be converted to a deoxy form (dUDP) to be used as substrate for the synthesis of thymidine. The conversion of dUDP to dTMP is catalyzed by thymidylate synthase, which requires folate (N5,N10 methylene tetrahydrofolate) as a methyl and hydrogen donor to complete this conversion (figure 7.13).

Regulation of pyrimidine synthesis

The reaction catalyzed by CSPII is the regulatory step in the pathway and is activated by PRPP and ATP and inhibited by UTP.

Clinical importance of folate cycle inhibitors and synthesis of dTMP

Synthesis of dTMP for DNA synthesis is the rate-limiting step for the replication process, and therefore disruption of this conversion is very effective at reducing cellular proliferation. Inhibition of thymidylate synthase by 5-fluorouracil (5-FU) is a common anticancer treatment. 5-FU functions as a thymine analog and will irreversibly bind the enzyme. Similarly,
methotrexate is an inhibitor of dihydrofolate reductase (DHFR), which is part of the folate cycle needed to reduce dihydrofolate to tetrahydrofolate. Inhibition of this process reduces substrate needed for the thymidylate synthase reaction and has a similar effect as inhibition of by 5-FU (figure 7.13).

### Summary of pathway regulation

<table>
<thead>
<tr>
<th>Metabolic pathway</th>
<th>Major regulatory enzyme</th>
<th>Allosteric effectors</th>
<th>Hormonal effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrimidine synthesis</td>
<td>CPSII</td>
<td>PRPP, ATP (+), UTP (-)</td>
<td>None</td>
</tr>
<tr>
<td>Purine synthesis</td>
<td>PRPP synthetase</td>
<td>Pi (+), Purine bases (-)</td>
<td>Note: PRPP synthetase is required for both purine and pyrimidine synthesis</td>
</tr>
<tr>
<td></td>
<td>GPAT</td>
<td>PRPP (+), IMP, AMP, GMP (-)</td>
<td></td>
</tr>
</tbody>
</table>

Table 7.2: Summary of pathway regulation.

### References and resources

**Text**


**Figures**

Grey, Kindred, Figure 7.5 Overview of purine and pyrimidine bases. 2021. Chemical structure by Henry Jakubowski. [https://archive.org/details/7.5_20210926, CC BY 4.0](https://archive.org/details/7.5_20210926).

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Grey, Kindred, Figure 7.7 Overview of purine synthesis. The reaction catalyzed by GPAT is the regulatory enzyme of the pathway. 2021. [https://archive.org/details/7.7_20210926, CC BY 4.0](https://archive.org/details/7.7_20210926).

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Grey, Kindred, Figure 7.12 Overview of pyrimidine synthesis. The reaction catalyzed by carbamoyl phosphate synthetase I is the regulatory enzyme of the pathway. 2021. https://archive.org/details/7.12_20210926, CC BY 4.0.


Lieberman M, Peet A. Figure 7.4 Basic structure of nucleotides. Adapted under Fair Use from Marks' Basic Medical Biochemistry. 5th Ed. pp 216. Figure 12.3 Nucleoside and nucleotide structures displayed with ribose as the sugar. 2017. Chemical structure by Henry Jakubowski.