# Lecture 12 - Nucleotide Biosynthesis

Chem 454: Regulatory Mechanisms in Biochemistry
University of Wisconsin-Eau Claire

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## Introduction

Nucleotides perform a wide variety of functions

- Building blocks for nucleic acids
- Universal energy carriers (ATP, GTP)
- Activators (*e.g.* UDP-glucose)
- Components of signal transduction pathways (cAMP, cGMP)

Nucleotides contain

- Ribose or deoxyribose sugar
- One to three phosphate groups
- purine or pyrimidine hetercyclic nitrogen base.

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## Introductions

We will focus on the nucleotide bases

- Glycine and aspartate will provide a carbon scaffold.
- Aspartate and glutamine will provide the nitrogen.

We will look at

- *de novo* synthesis of pyrimidine bases
- *de novo* synthesis of purines bases
- Synthesis of deoxyribonucleotides
- Regulation of nucleotide synthesis

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## Introduction

*de Novo versus* salvage pathways

SALVAGE PATHWAY

Activated ribose (PRPP) + base

↓

Nucleotide

DE NOVO PATHWAY

Activated ribose (PRPP) + amino acids + ATP + $CO_2$ + . . .

↓

Nucleotide

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# Introduction

## Nomenclature

**TABLE 25.1 Nomenclature of bases, nucleosides, and nucleotides**

| Base | Ribonucleoside | Ribonucleotide (5′-monophosphate) |
|---|---|---|
| | RNA | |
| Adenine (A) | Adenosine | Adenylate (AMP) |
| Guanine (G) | Guanosine | Guanylate (GMP) |
| Uracil (U) | Uridine | Uridylate (UMP) |
| Cytosine (C) | Cytidine | Cytidylate (CMP) |
| | DNA | |
| Base | Deoxyribonucleoside | Deoxyribonucleotide (5′-monophosphate) |
| Adenine (A) | Deoxyadenosine | Deoxyadenylate (dAMP) |
| Guanine (G) | Deoxyguanosine | Deoxyguanylate (dGMP) |
| Thymine (T) | Thymidine | Thymidylate (TMP) |
| Cytosine (C) | Deoxycytidine | Deoxycytidylate (dCMP) |

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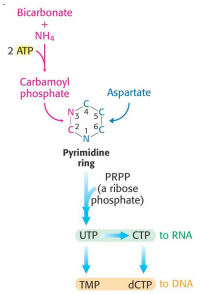
# 1. *de Novo* Synthesis of Pyrimidines

The ring is assembled from bicarbonate, aspartate and glutamate.

- The ring is synthesized first and then added to the ribose.
- The ammonia is produced from the hydrolysis of glutamine

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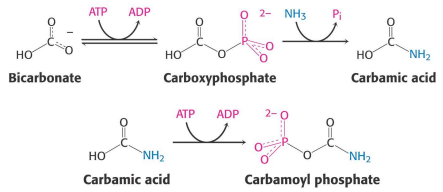
# 1.1 Pyrimidine Synthesis, First Step

Carbarmoyl phosphate is synthesized from bacarbonate and ammonia







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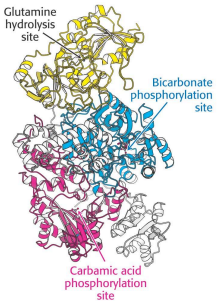
# 1.1 Pyrimidine Synthesis, First Step

Carbamoyl phosphate synthetate

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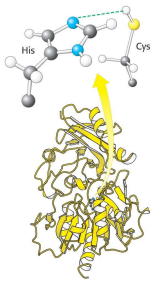
## 1.2 Glutamine Hydrolysis

Carbamoyl phosphate synthetase also contains a glutamine hydrolysis domain



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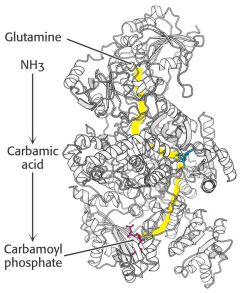
## 1.3 Substrate Channeling

The ammonia is channeled 45Å to the carboxyphosphate

- The carbamic acid is channeled another 35Å to the site where it is phosphorylated



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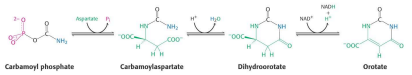
## 1.4 Pyrimidine Synthesis, Second Step

Synthesis of Orotate and attachment to ribose ring.

- The first reaction is catalyzed by aspartate transcarbamylase



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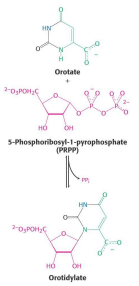
## 1.4 Pyrimidine Synthesis, Second Step

Synthesis of Orotate and attachment to ribose ring.

- Reaction is driven by the hydorlysis of pyrophosphate



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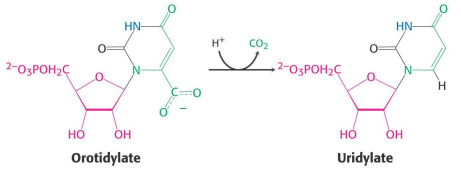
## 1.4 Pyrimidine Synthesis, Second Step

Decarboxylation of orotidylate produces uridylate

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## 1.5 Nucleotides

Nucleotide mono-, di-, and triphospahtes are interconvertible

- Nucleoside monophosphate kinases
- UMP is converted to UTP before going on to produce CTP

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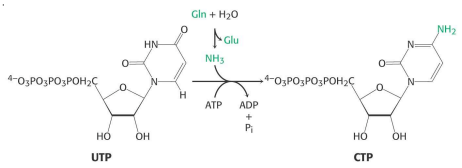
## 1.6 CTP

CTP is formed by amination (not animation!) of UTP.

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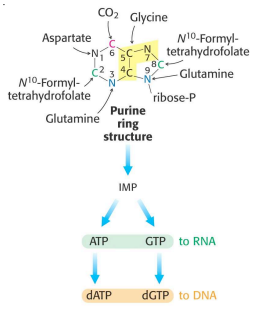
## 2. *de Novo* Synthesis of Purines

Salvage *versus de Novo* synthesis

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## 2.2 Purines Synthesis, Step One

The purine ring system is assembled on a ribose phosphate.

- glutamine phosphoribosyl amidotransferase





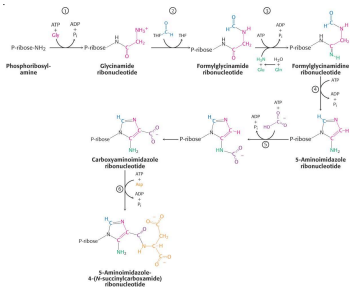
## 2.3 Purine Ring Synthesis

The purine ring is assembled by successive steps of activation by phosphorylation, followed by displacement.





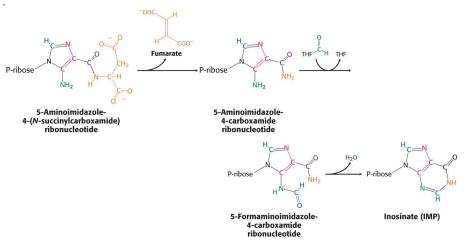
## 2.3 Purine Ring Synthesis





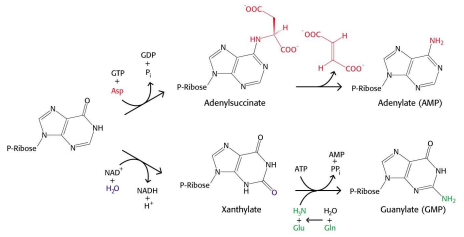



## 2.3 Purine Ring Synthesis



## 2.4 AMP and GMP

AMP and GMP are formed from IMP

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## 3. Deoxyribonucleotides

Deoxyribonucleotides are produced form either ribonucleotide di- or triphophosphates

- The 2'-OH on the ribose sugar is reduced to a hydrogen.
- NADPH + $H^+$ is the reducing agent.
- The enzyme is called *ribonucleotide reductase*

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## 3. Deoxyribonucleotides

Ribonucleotide reductase

- R1 (87 kD dimer)
  - active site
  - allosteric sites
- R2 (43 kD dimer)

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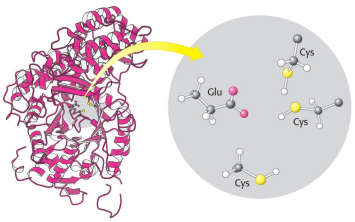
## 3. Deoxyribonucleotides

Ribonucleotide reductase: R1 subunit

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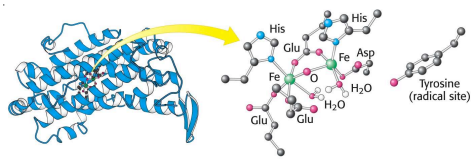
## 3. Deoxyribonucleotides

Ribonucleotide reductase: R2 subunit

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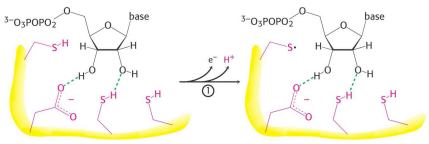
## 3. Deoxyribonucleotides

Ribonucleotide reductase

- 1. Transfer of a electron from a cysteine on R1 to the tyrosyl radical on R2

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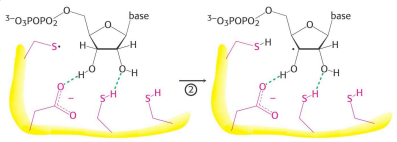
## 3. Deoxyribonucleotides

Ribonucleotide reductase

- 2. The cysteine thiyl radical produced on R1 abstracts a hydrogen from the C-3' of the ribose unit.

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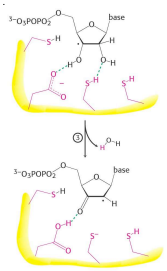
## 3. Deoxyribonucleotides

Ribonucleotide reductase

- 3. The carbon radical at C-3' promotes the release of a hydorxide ion on carbon-2.

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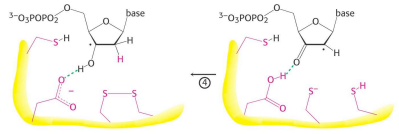
## 3. Deoxyribonucleotides

Ribonucleotide reductase

- 4. Hydride is transfered from a third cysteine residue to complete the reduction of the C-2' position.



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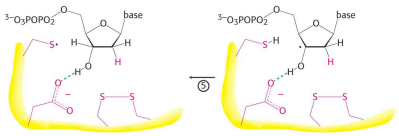
## 3. Deoxyribonucleotides

Ribonucleotide reductase

- 5. The C-3' radical recaptures the hydrogen that was abstracted by the first cysteine residue.



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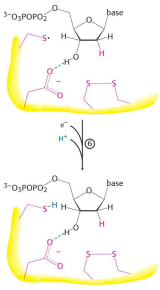
## 3. Deoxyribonucleotides

Ribonucleotide reductase

- 6. The tyrosyl free radical is regenerated



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## 3. Deoxyribonucleotides

Ribonucleotide reductase

- 7. The disulfide is reduced by thioredoxin.
- 8. Thioredoxin is reduced by *thioredoxin reductase* using NADH + H+

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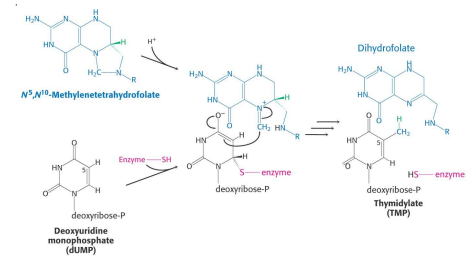
## 3.1 Thymidylated Formed by Methylation

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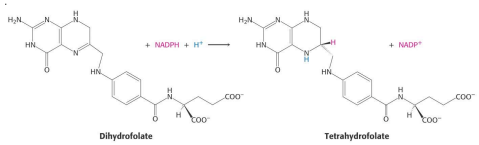
## 3.2 Dihydrofolate Reductase

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## 3.3 Anticancer Drugs

Inhibition of the synthesis of dexoyribonucleotides or thymidylate will selectively inhibit fast growing cells.

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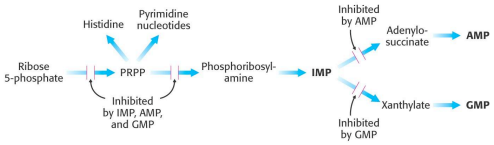
## 4. Regulation of Nucleotide Biosynthesis

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5. $NAD^+$, FAD and Coenzyme A (Skip)

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6. Metabolic Diseases (Skip)

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6.1 Purine Degradation (Skip)

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6.2 Lesch-Nyhan Syndrome (Skip)

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