Term 1

- Course outline and structure, objectives, test dates…
- Revision: purine & pyrimidine structures, nucleic acid terminology.
- Chemical and Physical Properties of Nucleic acids

- Nucleotide biosynthesis (salvage and de novo): Purines and Pyrimidines

- Conversion between NTP and dNTP
- Similarities vs differences: Purine vs Pyrimidine metabolism
- Nucleotide degradation: Purine and Pyrimidine

- Defects in purine and pyrimidine metabolism – diseases
- Inhibitors of purine and pyrimidine metabolism: treatment of Cancer and HIV

- Additional role of nucleotides
- Experimental Applications of purine and pyrimidine metabolism

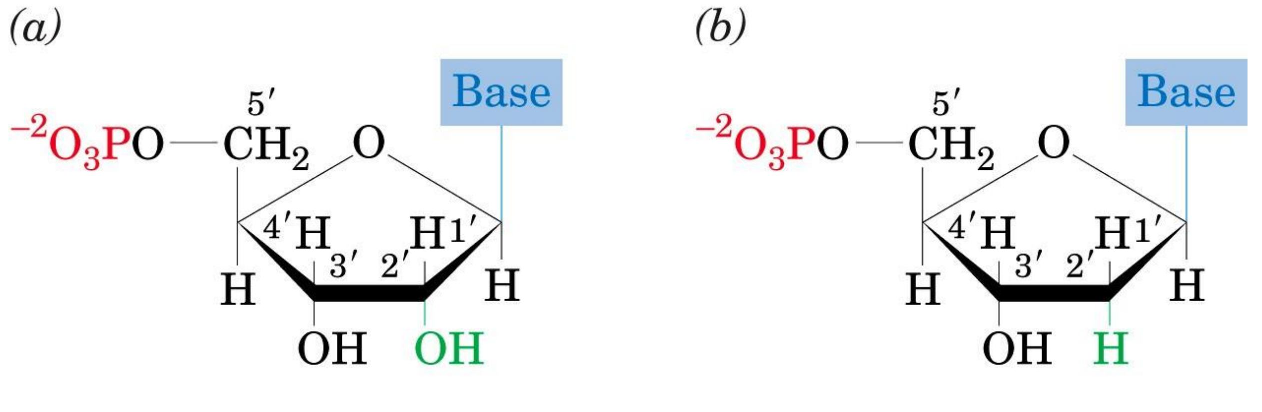
Deoxyribonucleotide metabolism

- There is 5x as much RNA as DNA in a cell
- Most of the nucleotides in a cell are required for RNA (in the form of ribonucleotides)
- **Deoxy**ribonucleoside triphosphates are required for DNA biosynthesis

- Ribonucleotides are the precursors of deoxyribonucleotides

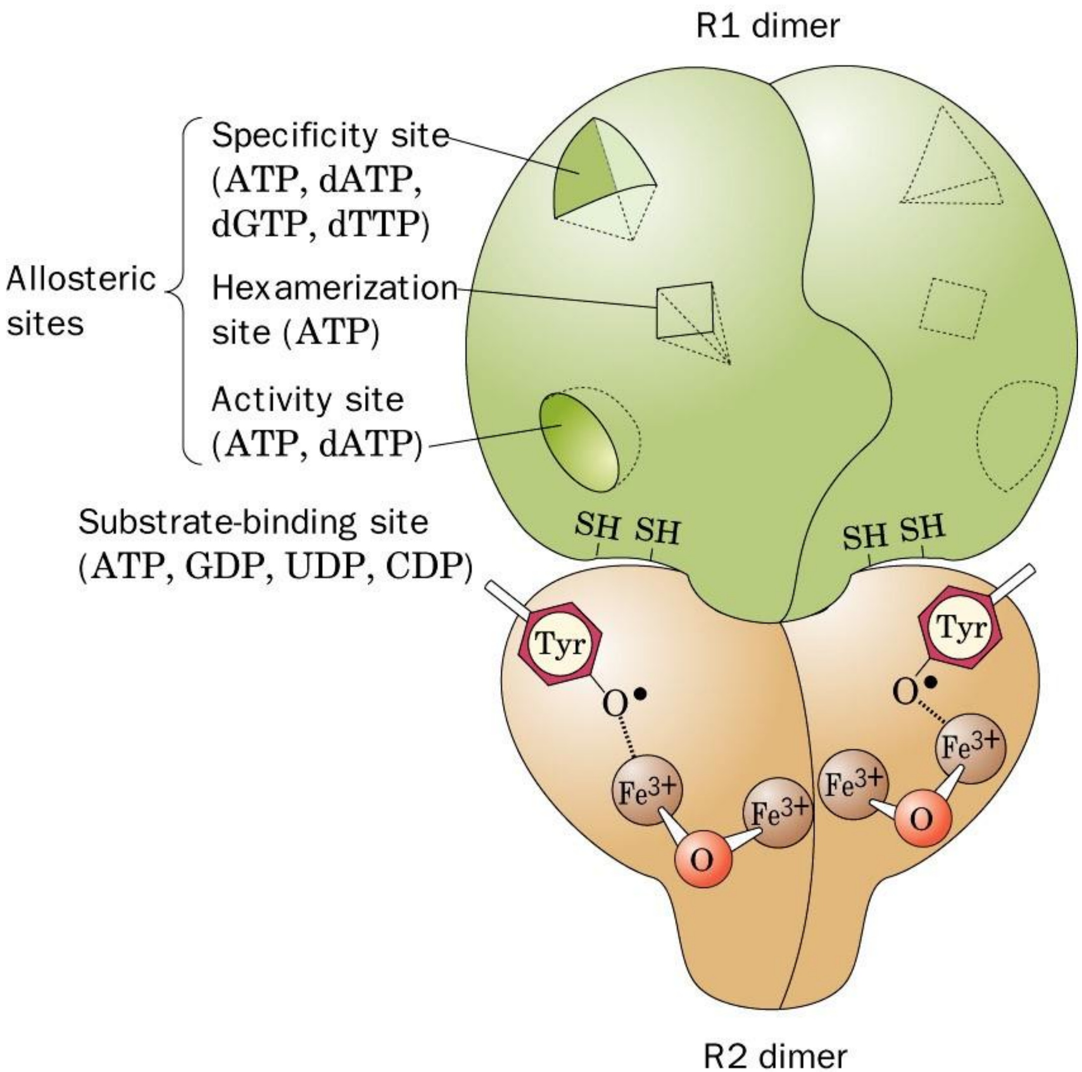
Ribonucleotides → Deoxyribonucleotides

- 2’ carbon on ribose is reduced to 2’deoxy derivative
- NDP → dNDP
- Enzyme: ribonucleotide reductase (has 2 non-identical subunits: R1 and R2)



Ribonucleotides

Deoxyribonucleotides



R1 dimer
Allosteric sites
Specificity site (ATP, dATP, dGTP, dTTP)
Hexamerization site (ATP)
Activity site (ATP, dATP)
Substrate-binding site (ATP, GDP, UDP, CDP)
SH SH
SH SH
Tyr
O•
Fe3+
Fe3+
O
Tyr
O•
Fe3+
Fe3+
O
R2 dimer

Ribonucleotides → Deoxyribonucleotides

- Overall reaction eg. For dADP synthesis

$$ADP + NADPH + H^+ \rightarrow dADP + NADP^+ + H_2O$$

- Ribonucleotide reductase has thioredoxin as a co-substrate (hydrogen carrying protein)
- As the ribosyl is reduced, the enzyme is oxidised. The enzyme is in turn reduced in an NADPH dependent reaction

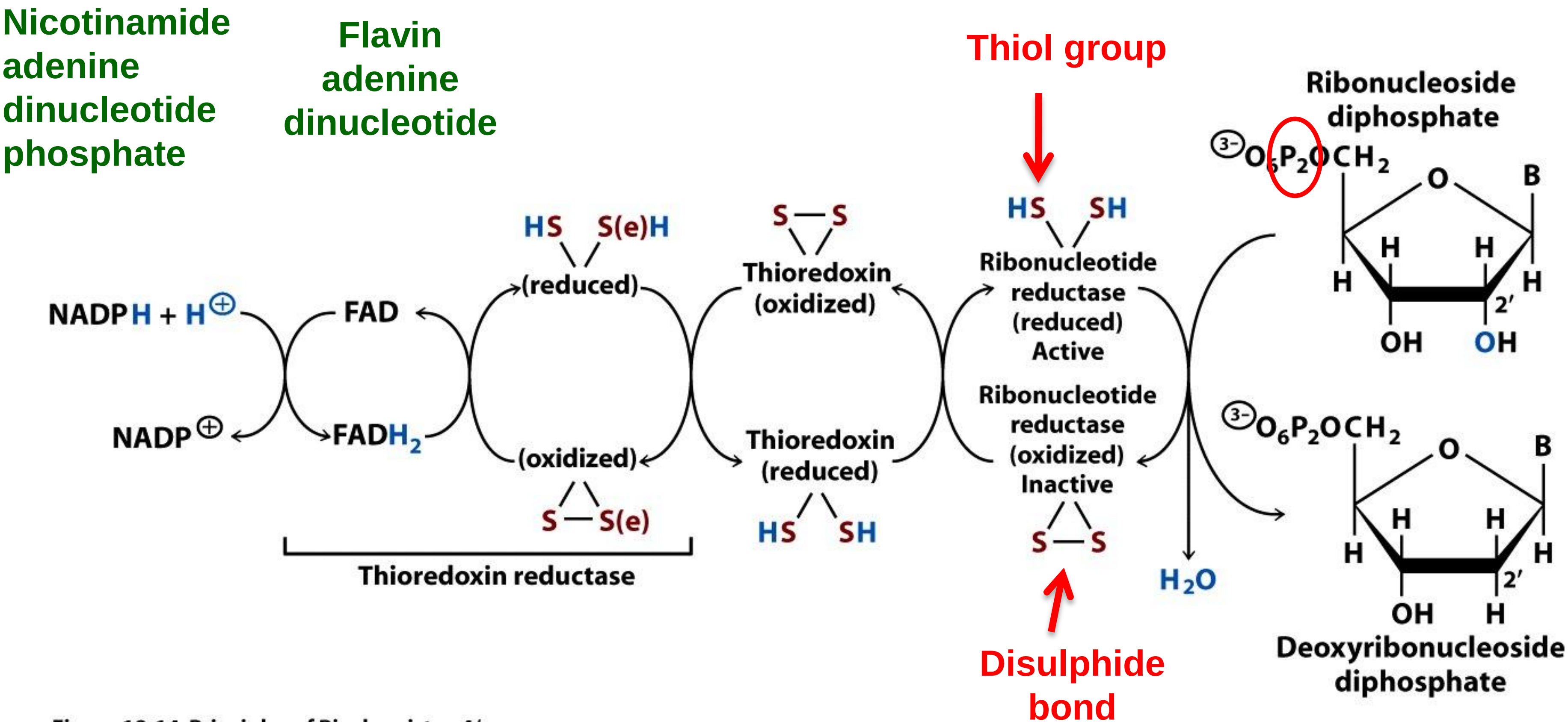


Figure 18-14 Principles of Biochemistry, 4/e

Ribonucleotide reductase.

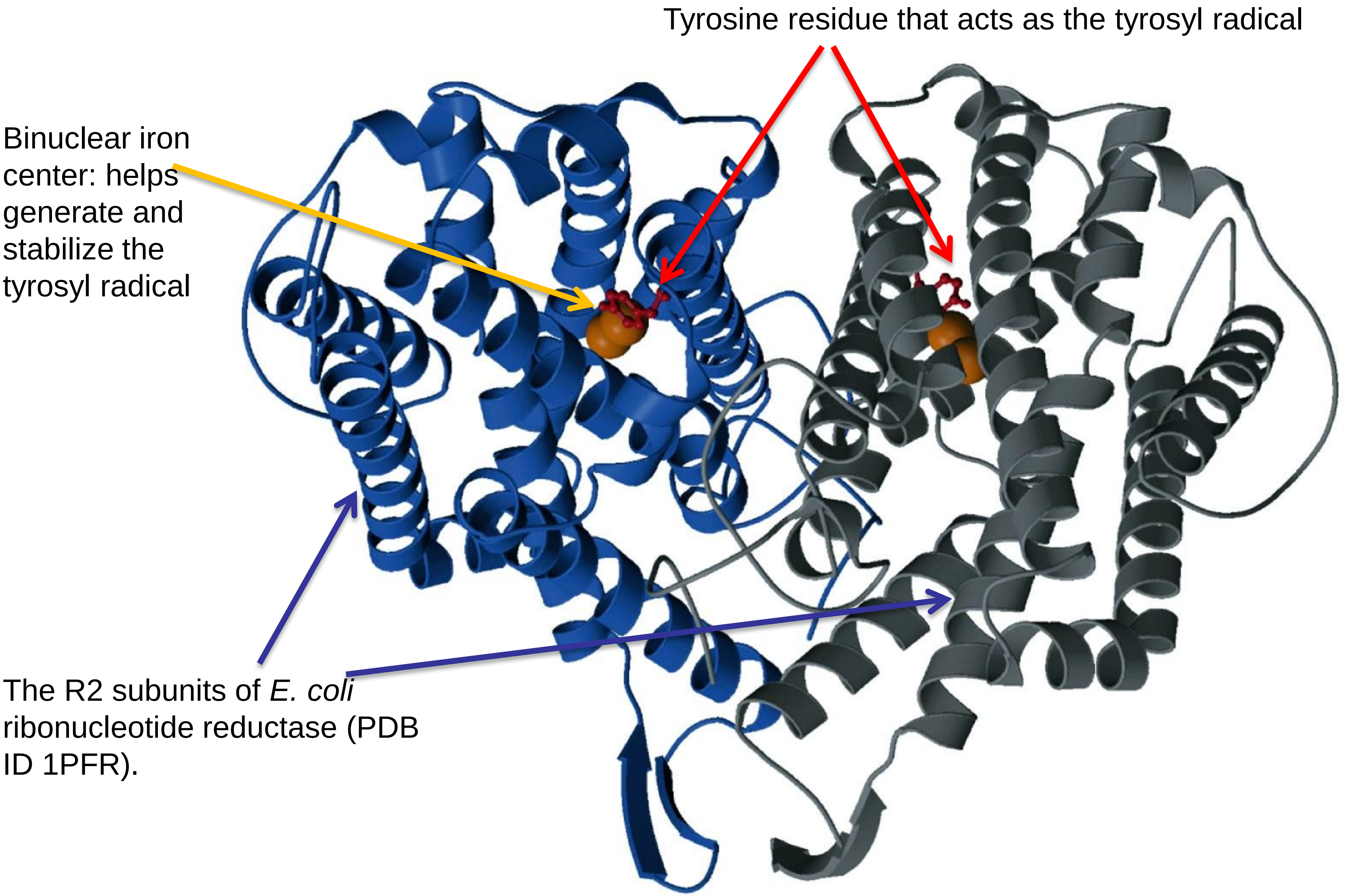


Figure 22-40b
Lehninger Principles of Biochemistry, Fifth Edition

Radicals are atoms, molecules or ions with unpaired electrons and are therefore very chemically reactive.

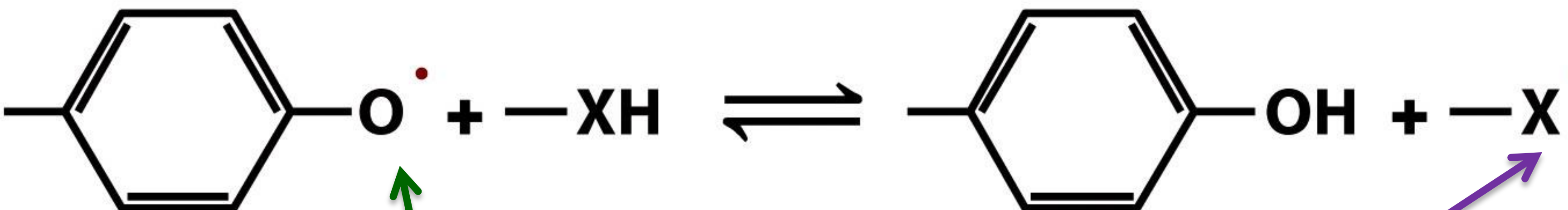
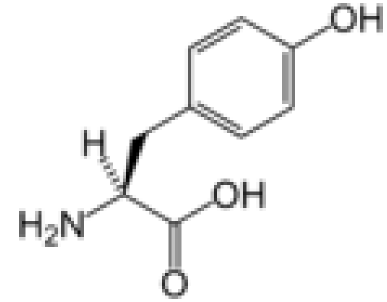


Figure 22-40c
Lehninger Principles of Biochemistry, Fifth Edition

The **tyrosyl radical** functions to generate the **active-site radical (—X·),** which is used by ribonucleotide reductase to reduce the ribosyl group

Tyrosine



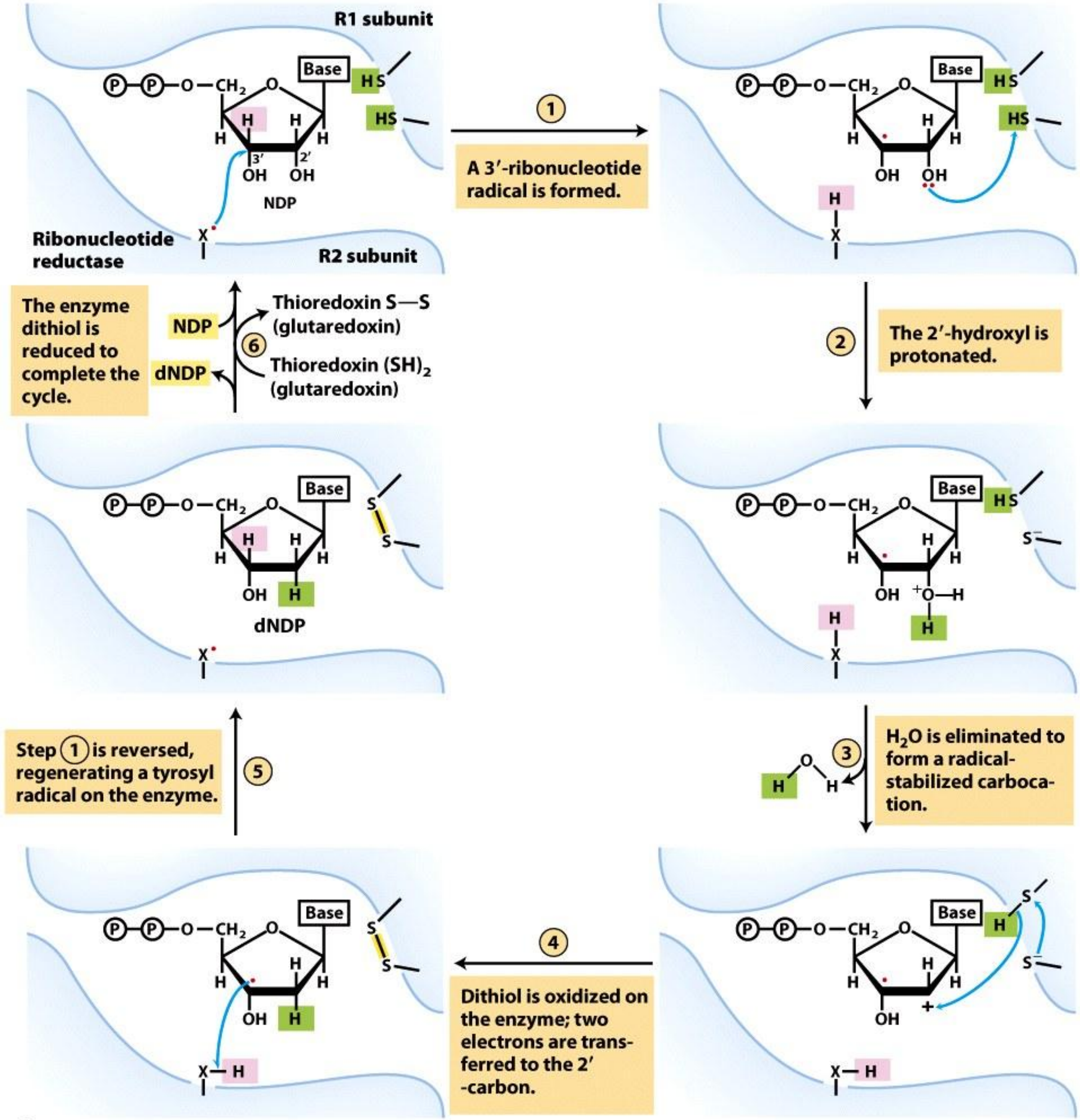
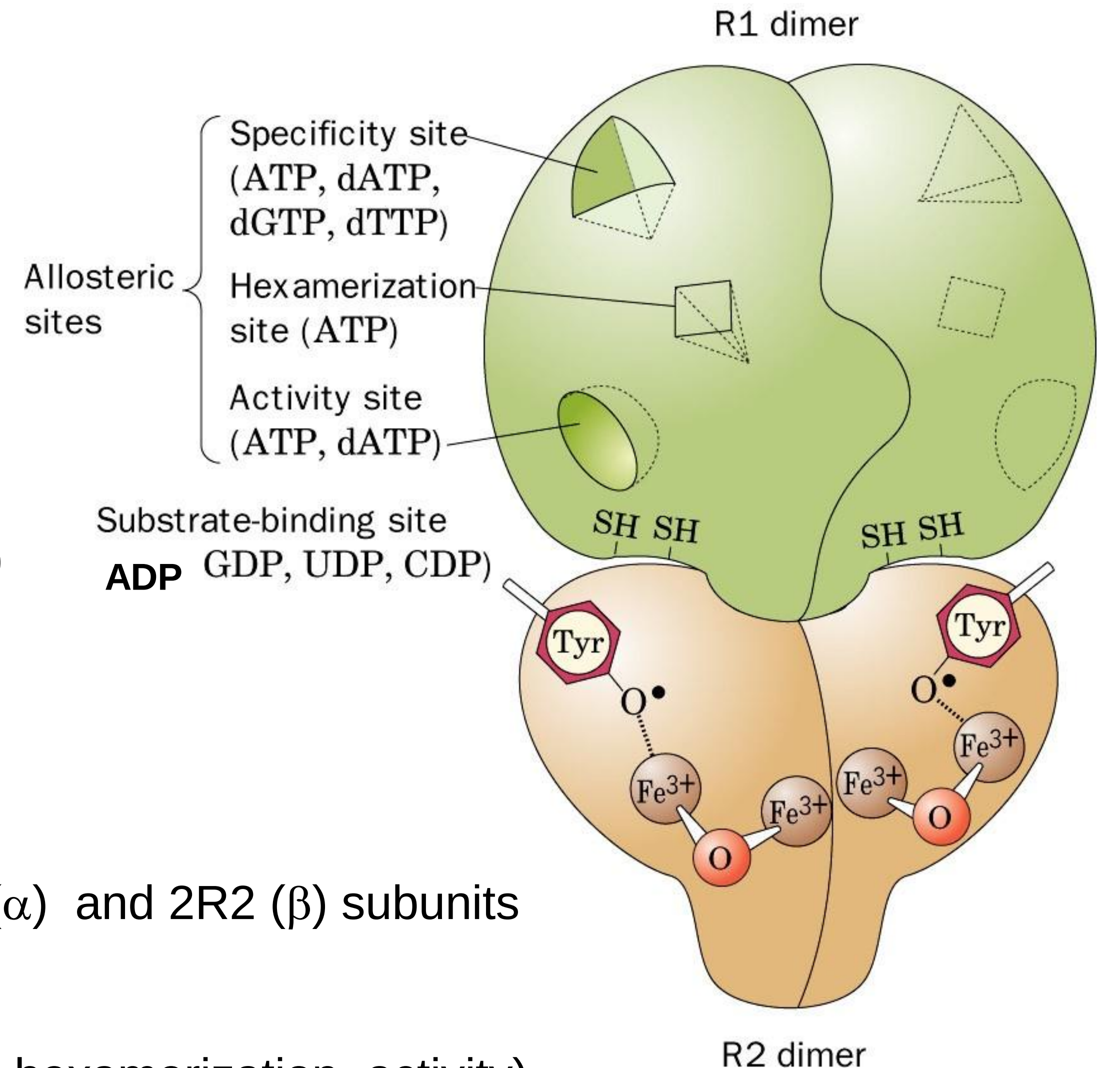


Figure 22-41

Lehninger Principles of Biochemistry, Fifth Edition

Class I ribonucleotide reductase

(Catalytic site)

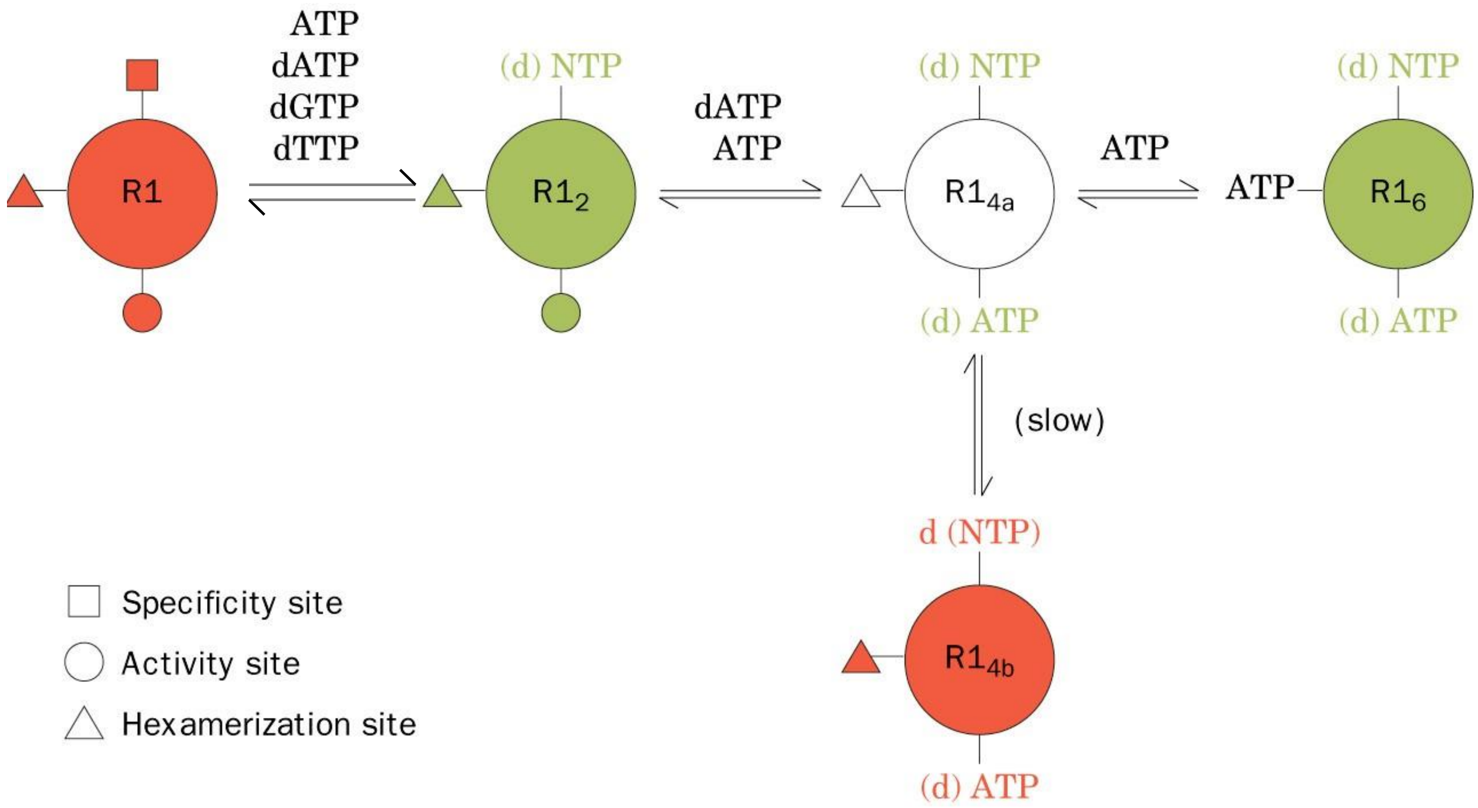


2 sets of 2 identical subunits. 2R1 (α) and 2R2 (β) subunits

R1: Substrate binding site
Allosteric sites (specificity, hexamerization, activity)
R2: Substrate binding site

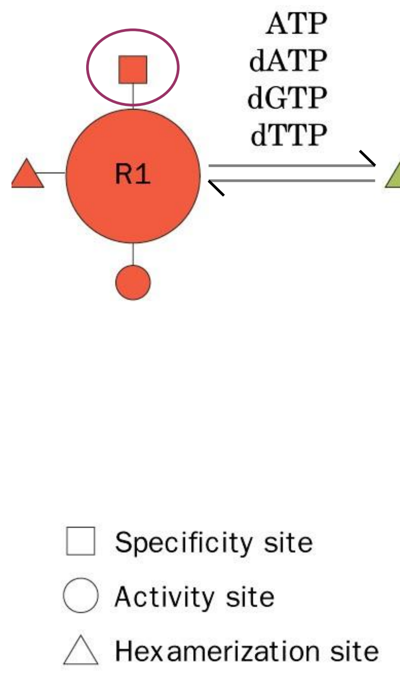
for activity

Allosteric regulation of activity



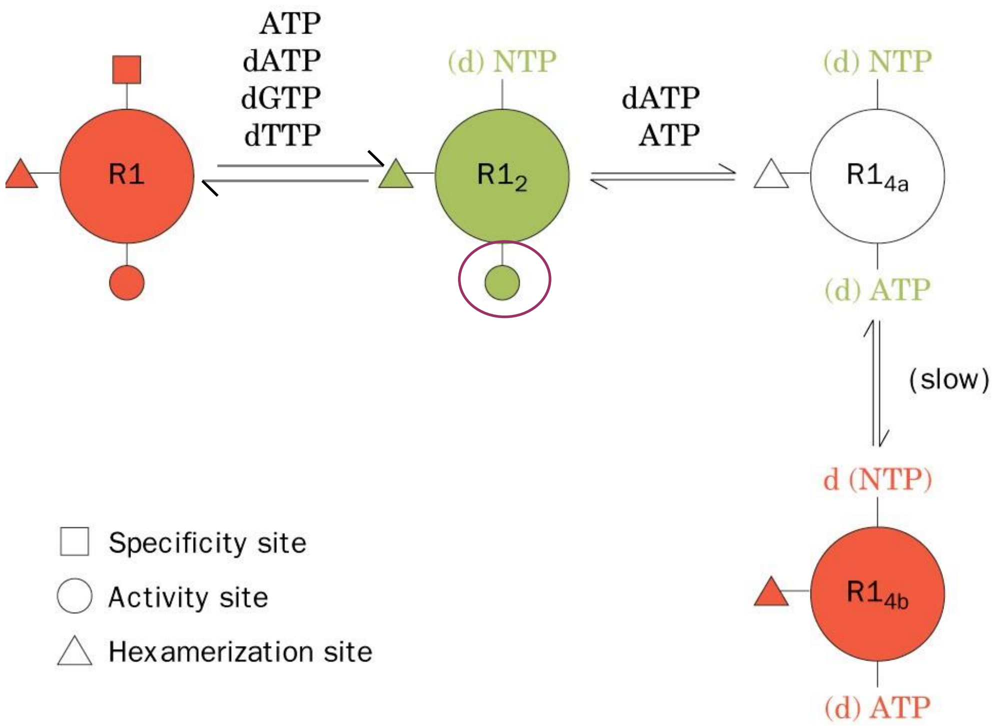
Unliganded R1 is preferentially a monomer

Red: inactive
Green: active



Specificity site:
Binding of ATP/dTTP/dGTP/dATP induces catalytically inactive R1 monomers to form catalytically active dimers $R1_2$

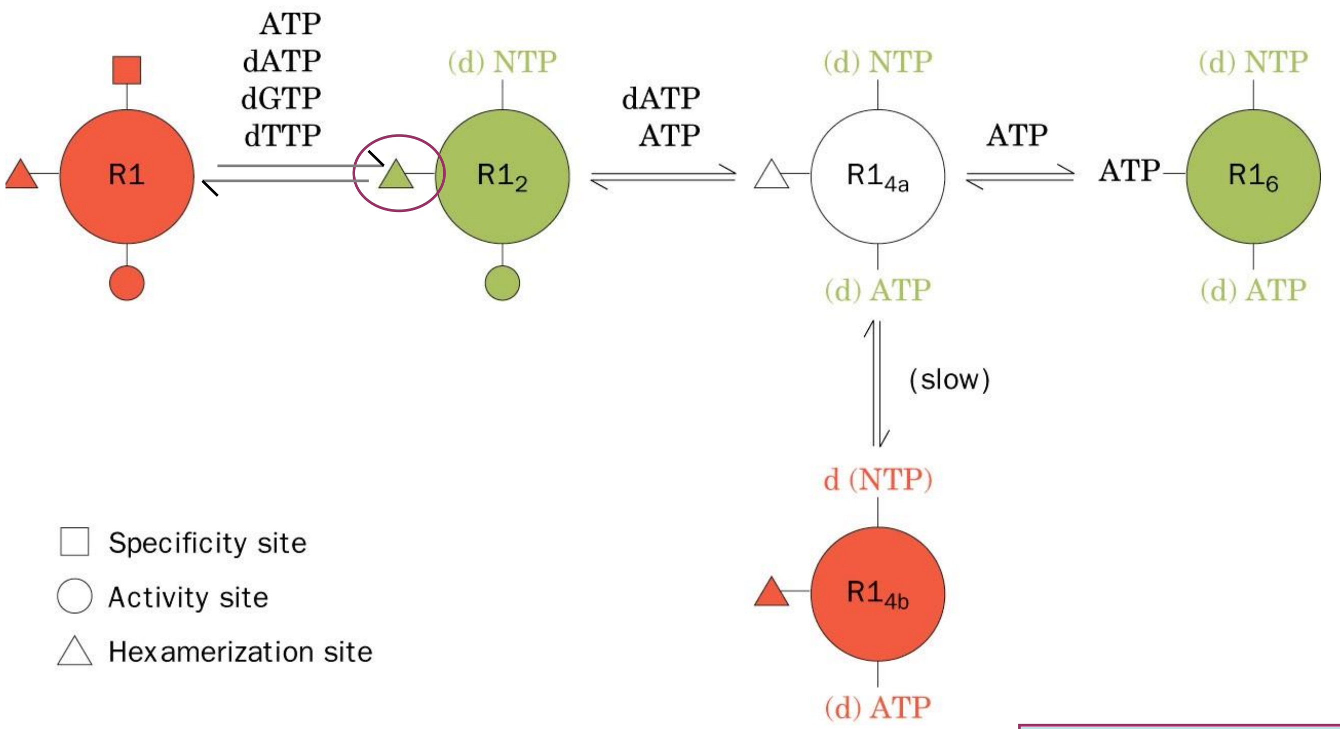
- ATP/dATP induces reduction of CDP and UDP
- dTTP induces reduction of GDP; inhibits reduction of CDP and UDP
- dGTP induces reduction of ADP; inhibits CDP and UDP reduction (in mammals)



Activity site:

Binding of dATP/ATP causes dimers to form catalytically active tetramers $R1_{4a}$, which slowly, but reversibly, change conformation to inactive $R1_{4b}$

- If no/small amounts ATP present (and mostly dATP), then enzyme will be inactive
- dATP: OFF
- ATP: ON



- Only ATP can bind:
- ATP: fully active

<u>Hexamerization site:</u>
Binding of ATP causes tetramers to form catalytically active hexamers $R1_6$, the major active form of RNR

Summary

- A healthy cell produces lots of ATP – occupies the activity and hexamerization sites and enzyme is "ON"
- If [ATP] is high :

A site	S site	Catalyses reduction of	Inhibits reduction of	Result
ATP	**ATP/ dATP**	**CDP/UDP**	**None**	**↑[dCDP, dUDP]***
ATP	**dTTP**	**GDP**	**CDP/UDP**	**↑[dGDP] ↓[dCDP, dUDP]**
ATP	**dGTP**	**ADP**	**CDP/UDP**	**↑[dADP] ↓[dCDP, dUDP]**
dATP	**Any**	**None**	**ADP/GDP/CDP/ UDP**	**OFF**

* **dCDP/dUDP → dUMP → dTMP → dTTP**

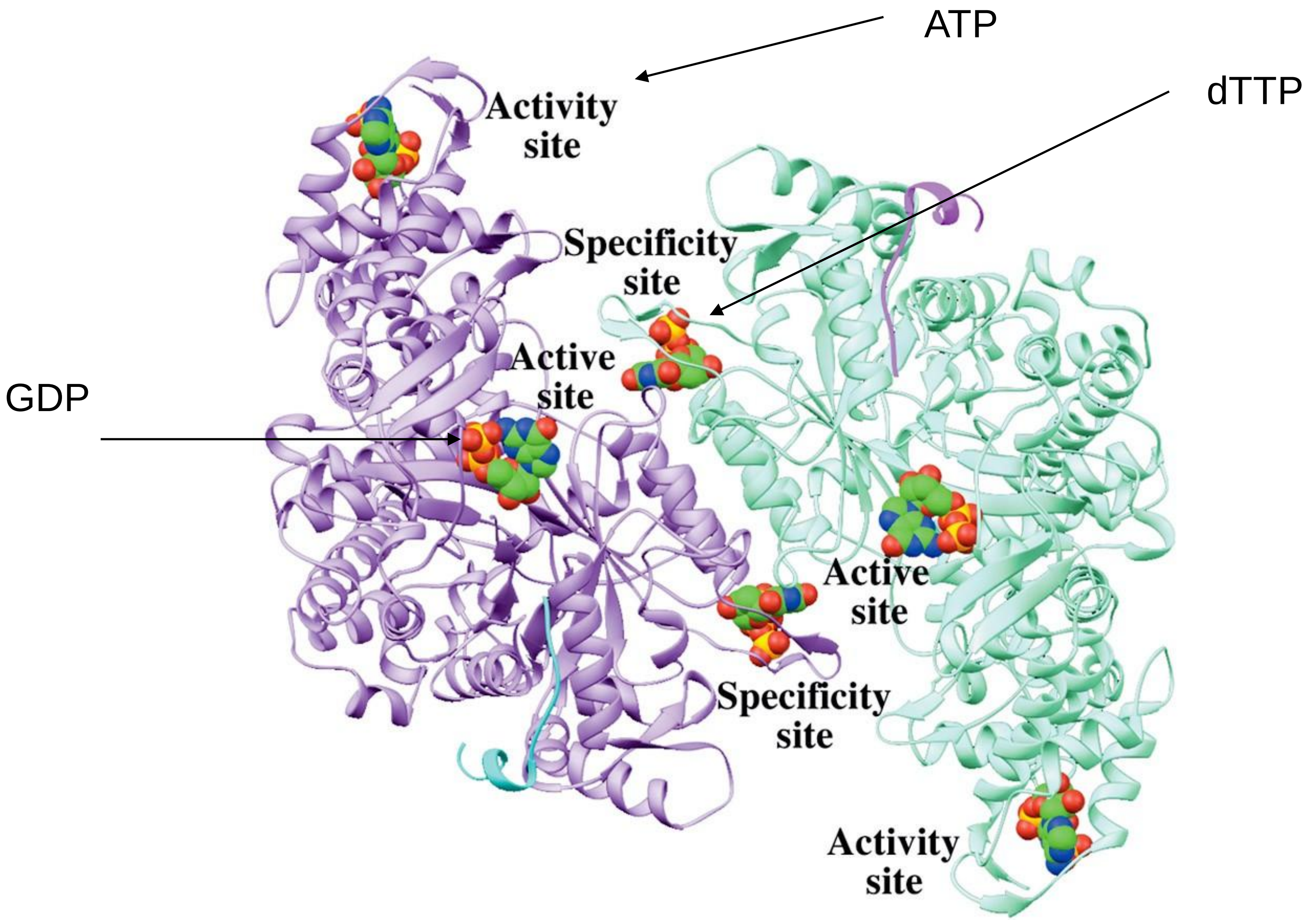
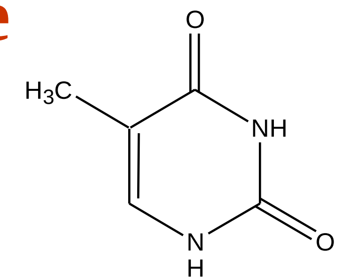


Figure 28-12d Class I ribonucleotide reductase from *E. coli*. The X-ray structure of the R1 dimer.

Past exam question

- **4a).** Write the overall reaction for the synthesis of dADP from ADP (no structures are required). [1]
- b) The formation of a specific radical is central to the catalytic mechanism of ribonucleotide reductase. Which amino acid facilitates this radical formation and how is the radical stabilised? On which subunit is it found? [2]
- c) Describe how the ribonucleotide reductase is able to ensure that the balance of deoxynucleotides is maintained? [6]

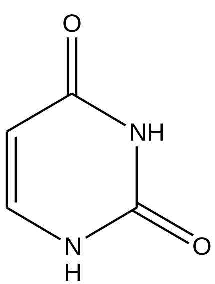
Biosynthesis of Thymidylate



Thymine (T)

Ribonucleotide reductase

UMP → UDP → dUDP → dUMP → dTMP

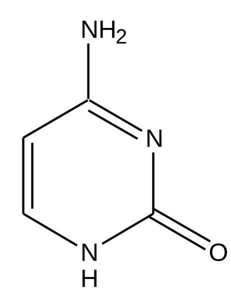


Uracil (U)

dCMP deaminase

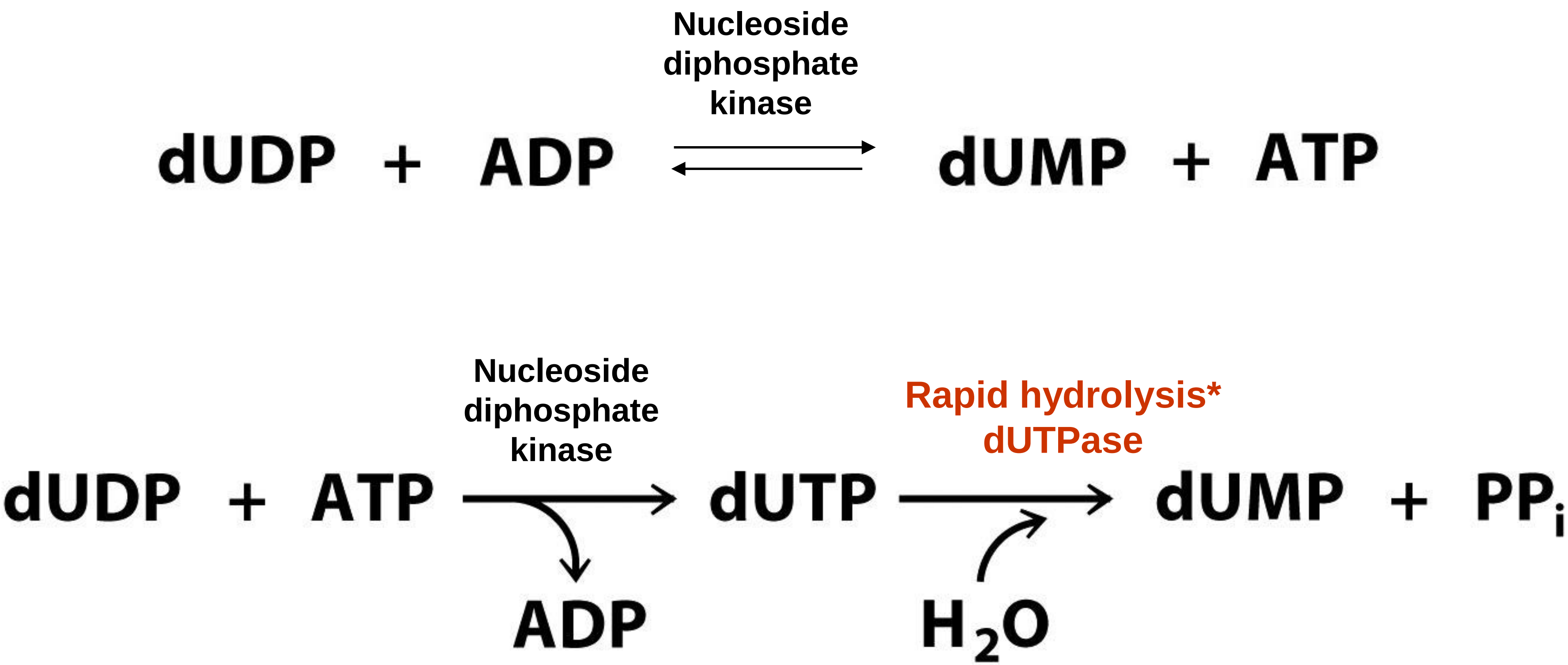
dTTP
dCTP

dCMP



Cytosine (C)

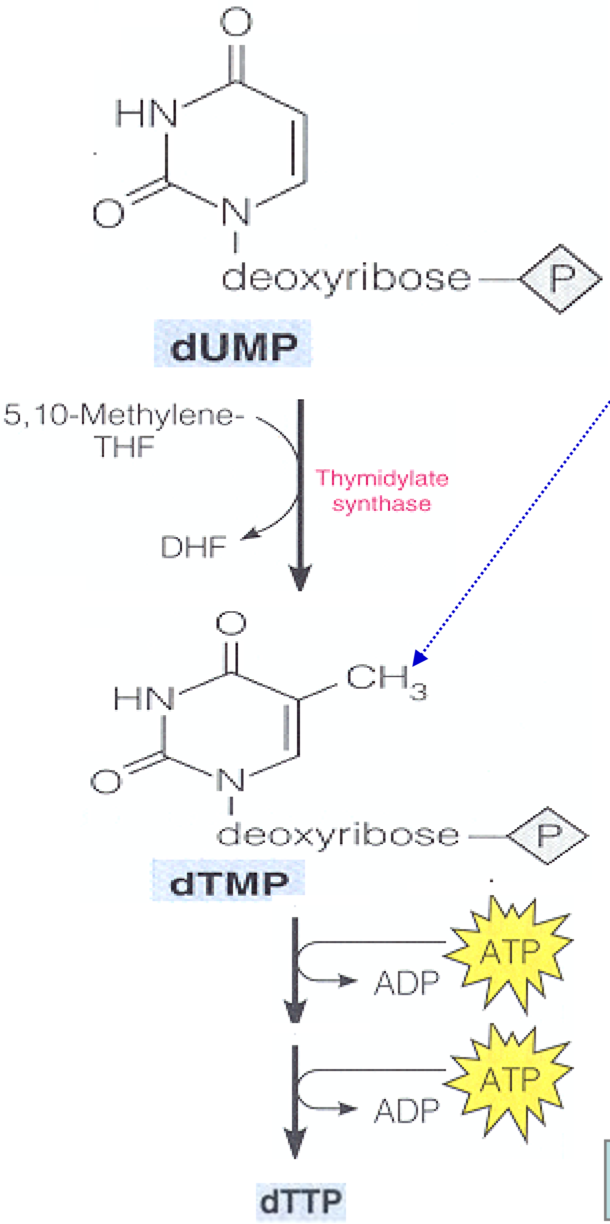
Conversion of dUDP to dUMP



Unnumbered figure pg 571 Principles of Biochemistry, 4/e

*Prevents incorporation into DNA instead of dTTP

dUMP → dTMP



dTMP differs from dUMP by a methyl group at C5.

5,10-methylene-THF is used as methyl donor to transfer a methyl group to dUMP by *thymidylate synthase.*

FOLIC ACID

THF oxidized to DHF

dTMP is then phosphrylated to dTTP.

Due to having only a function in DNA synthesis, thymidylate synthase is an ideal target for the design of anticancer drugs.

Why does nature uses dTTP instead of dUTP in DNA?

Why does nature uses dTTP instead of dUTP in DNA?

dTTP is methylated dUTP.

Methylation protects the DNA and

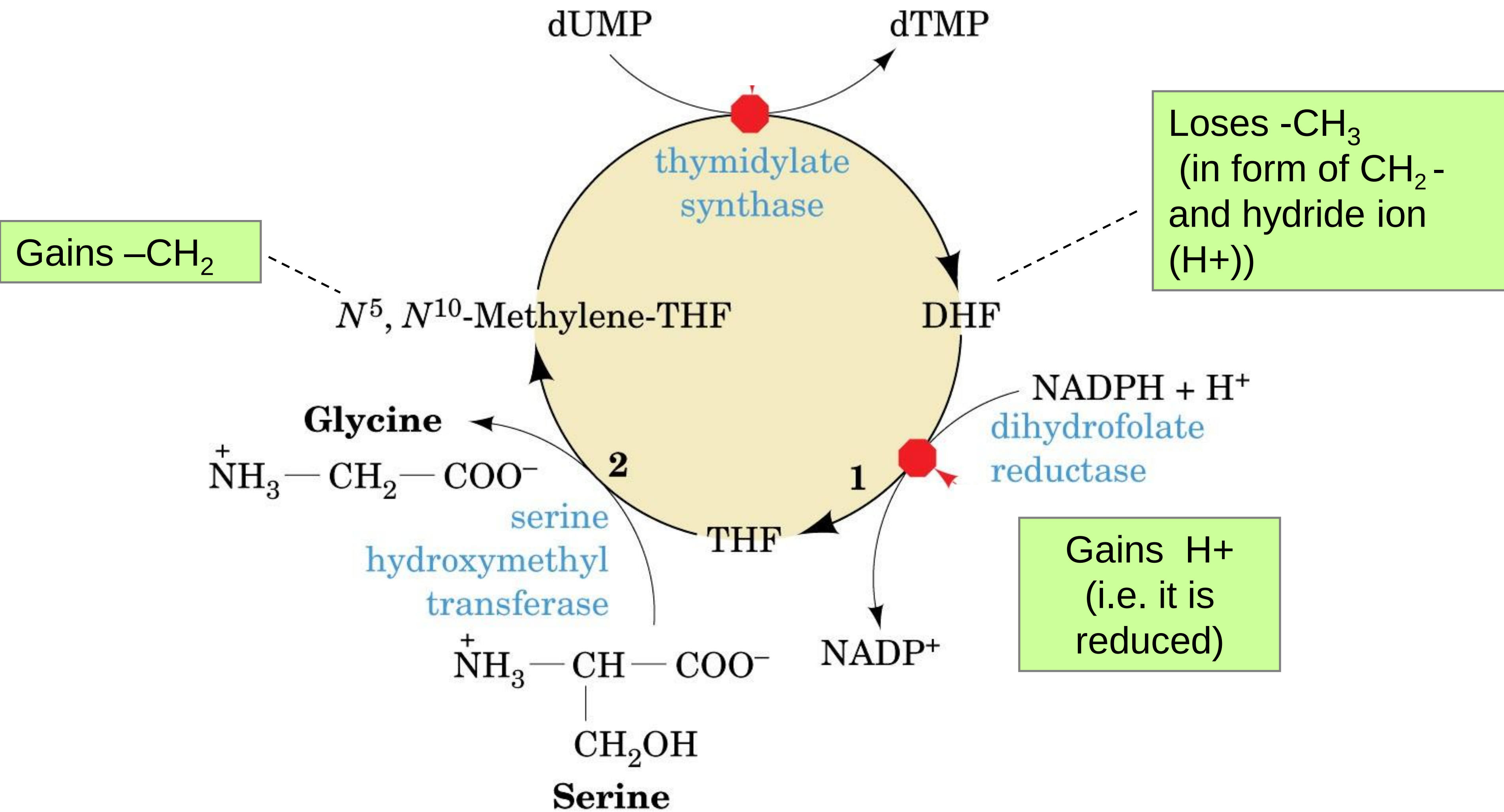
makes DNA unrecognizable to Nucleases (enzymes that break down DNA/RNA

so that it cannot be easily attacked by invaders, like viruses or certain bacteria

ALSO,

Spontaneous deamination of cytosine to uracil occurs naturally
As uracil is not normal part of DNA, it is recognised as foreign
Therefore, if deamination from C to U occurs in DNA, then it will be removed by repair mmechanisms

Thymidylate synthase reaction oxidizes THF to DHF (THF loses methylene group and hydride ion) – no other reaction using THF as cofactor alters the co-enzymes net oxidation state



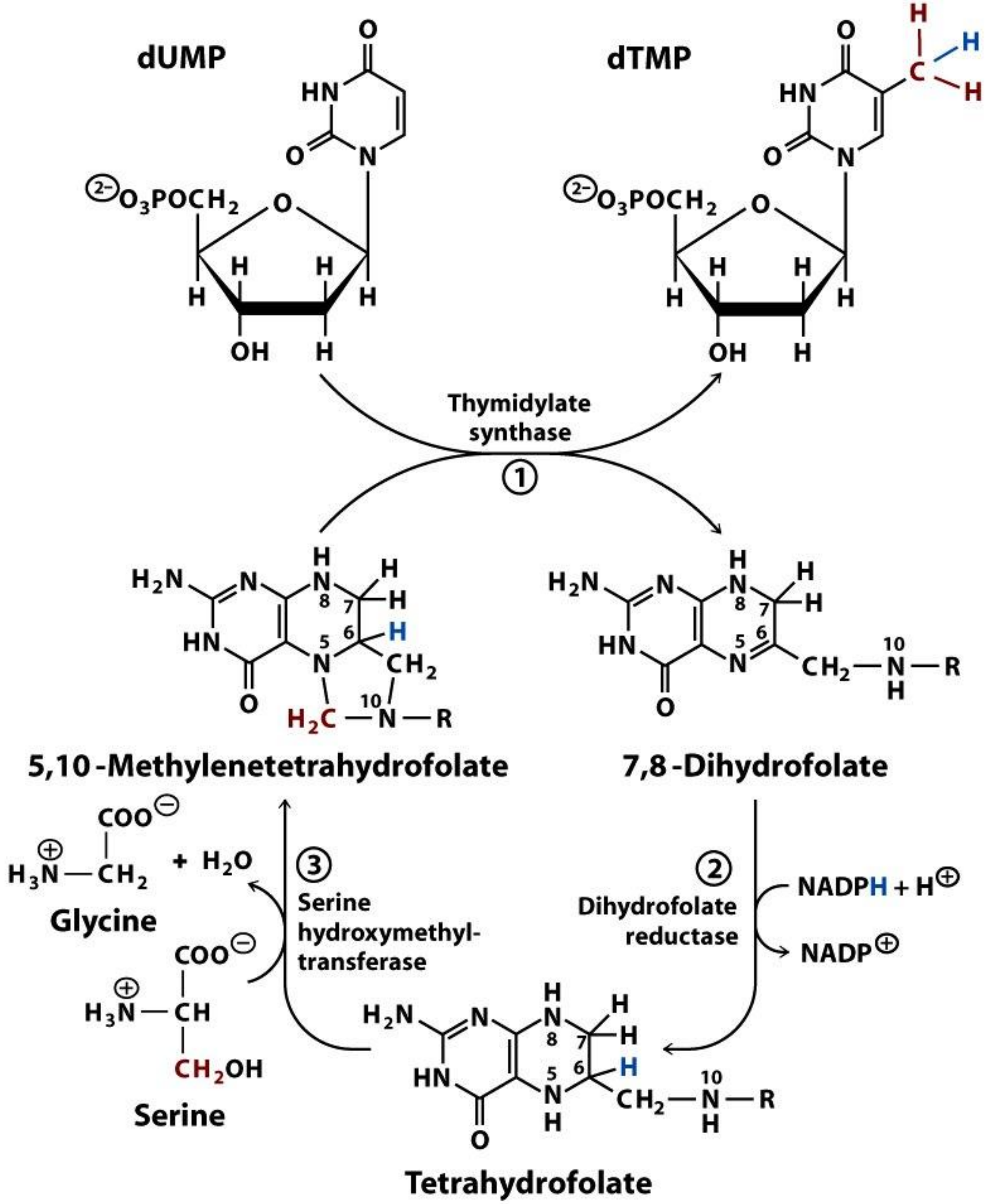


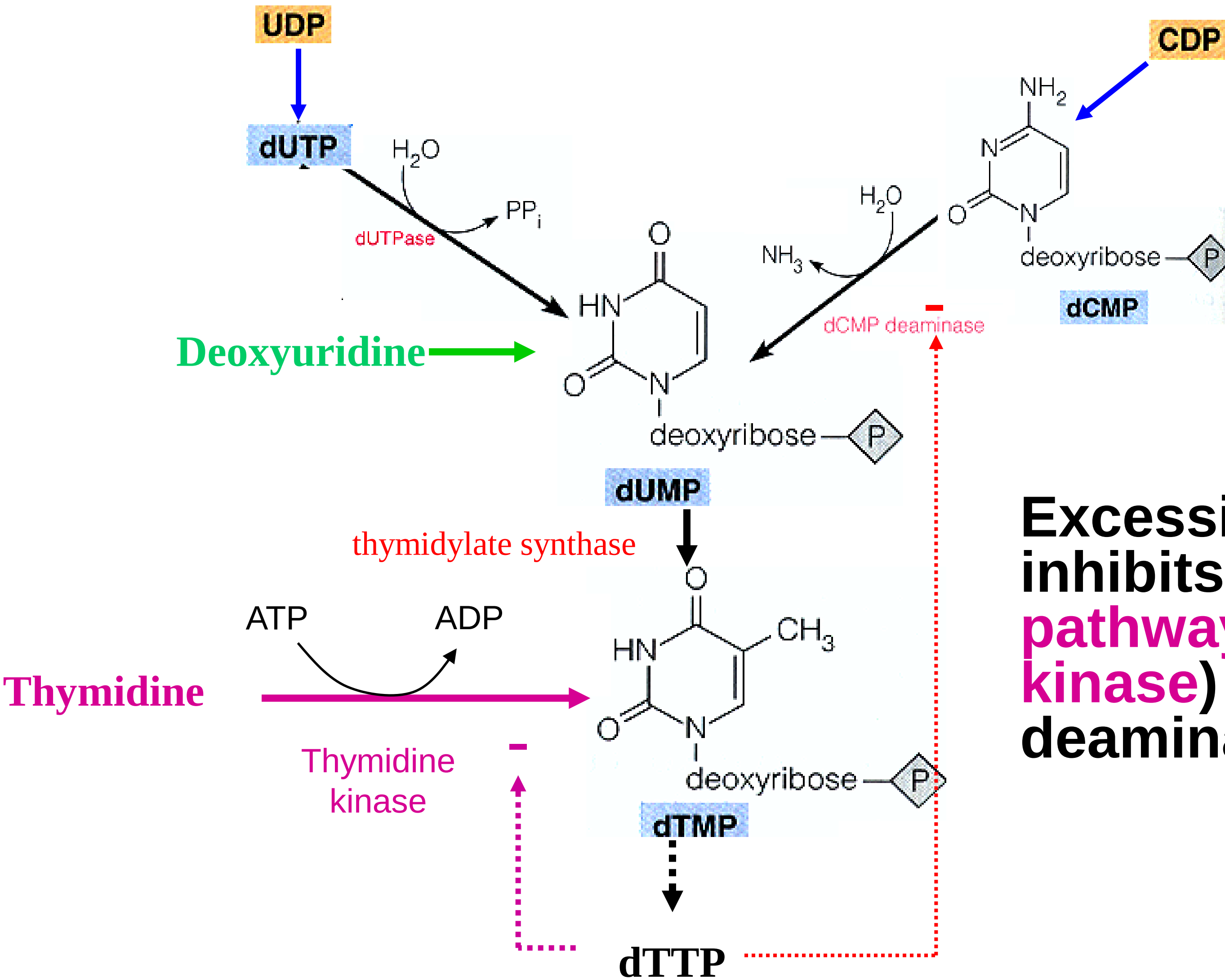
Figure 18-15 Principles of Biochemistry, 4/e

Hydride ion

Methylene group

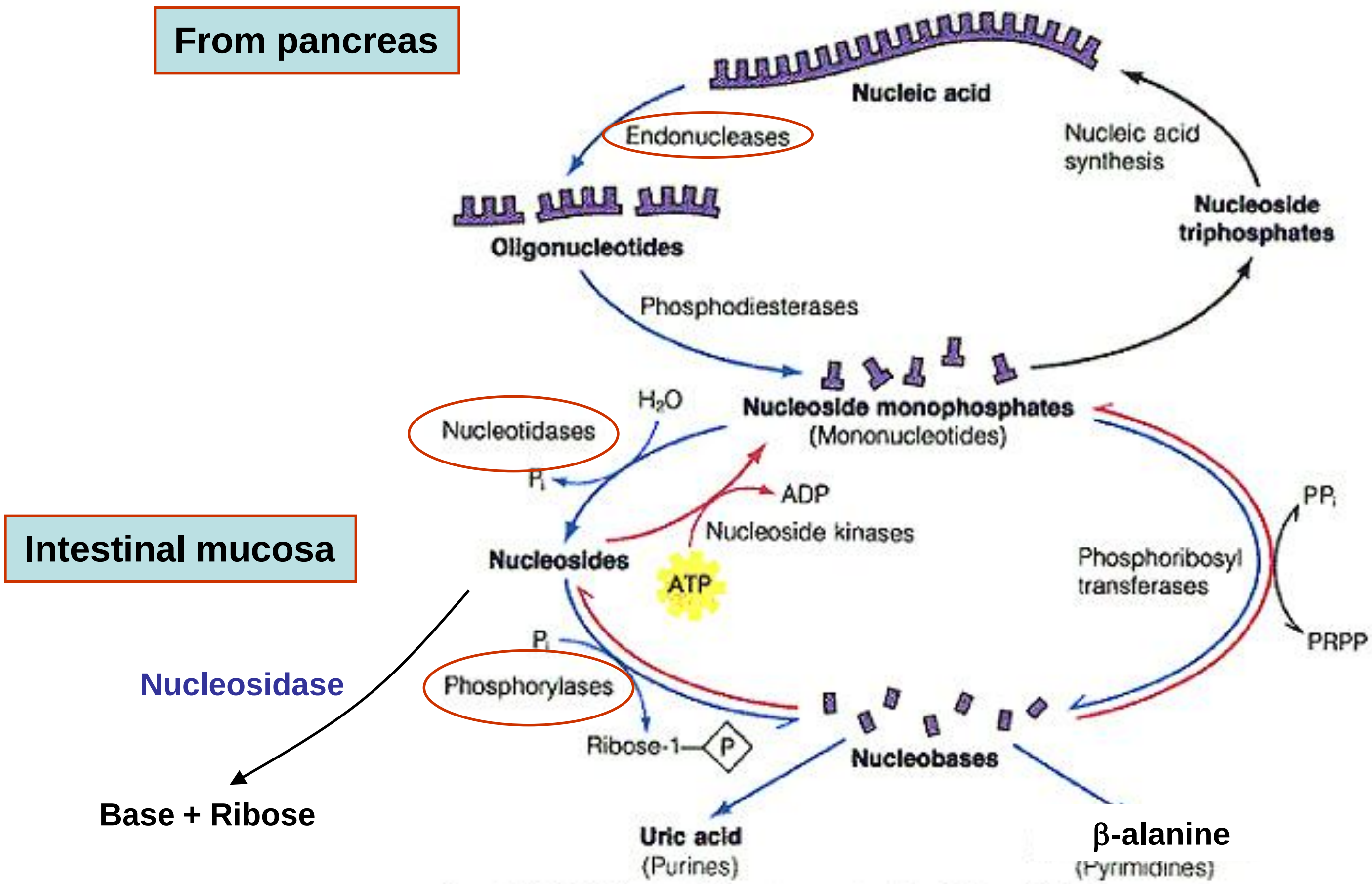
dTMP can be synthesized via SALVAGE pathway

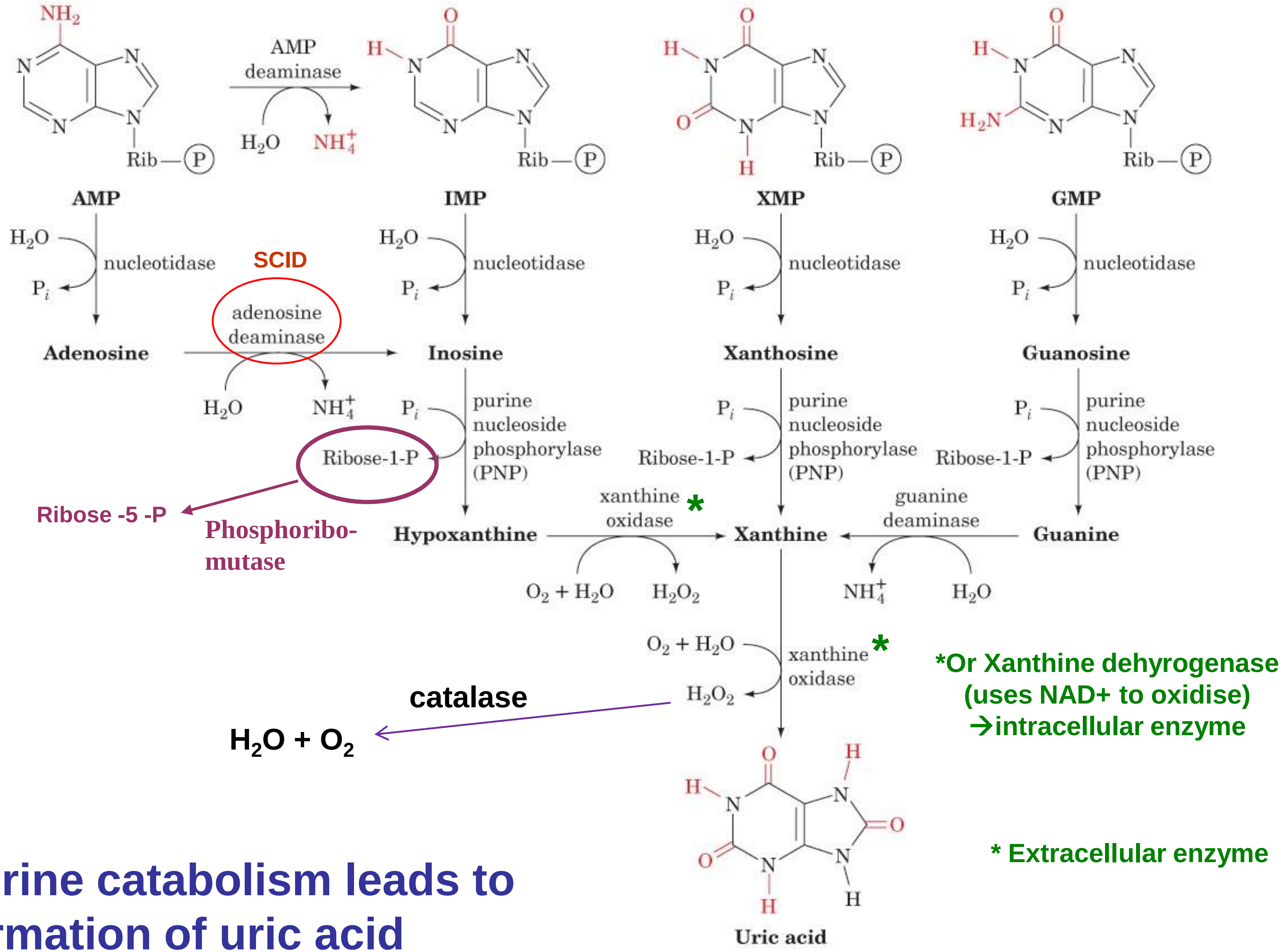
dTMP synthesized a) from dUMP via thymidylate synthase or b) from the salvage pathway via thymidine kinase.



Excessive dTTP inhibits the salvage pathway (thymidine kinase) and dCMP deaminase

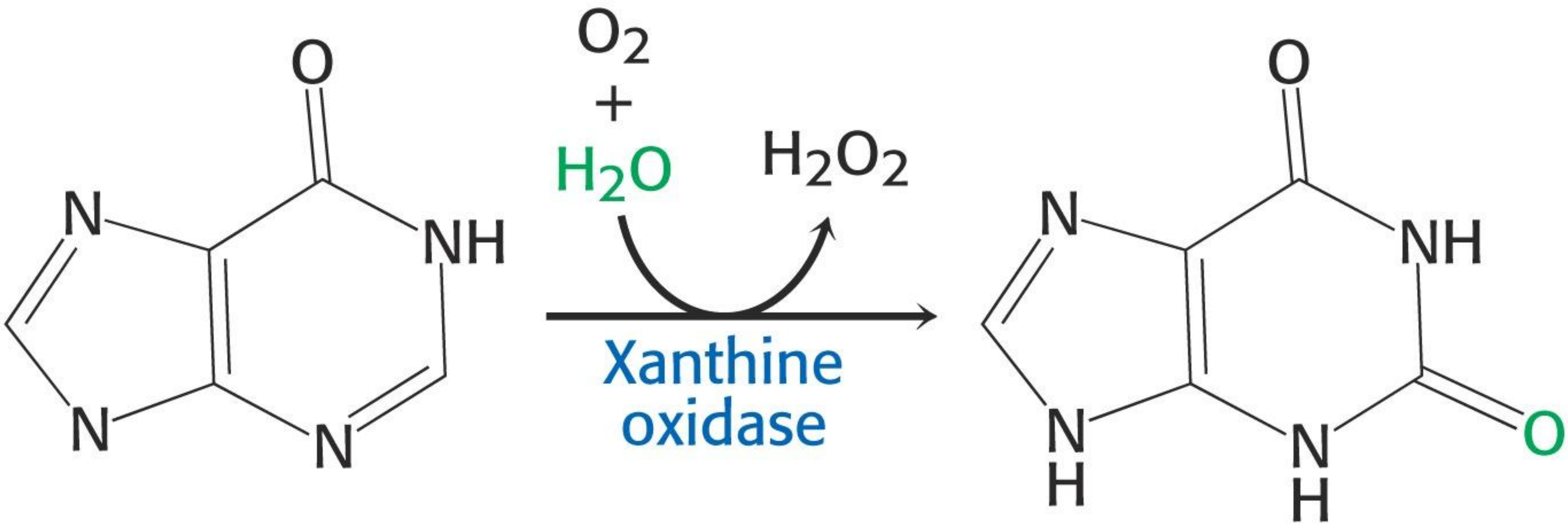
Purine and Pyrimidine catabolism





Purine catabolism leads to formation of uric acid

Hypoxanthine is oxidized to xanthine

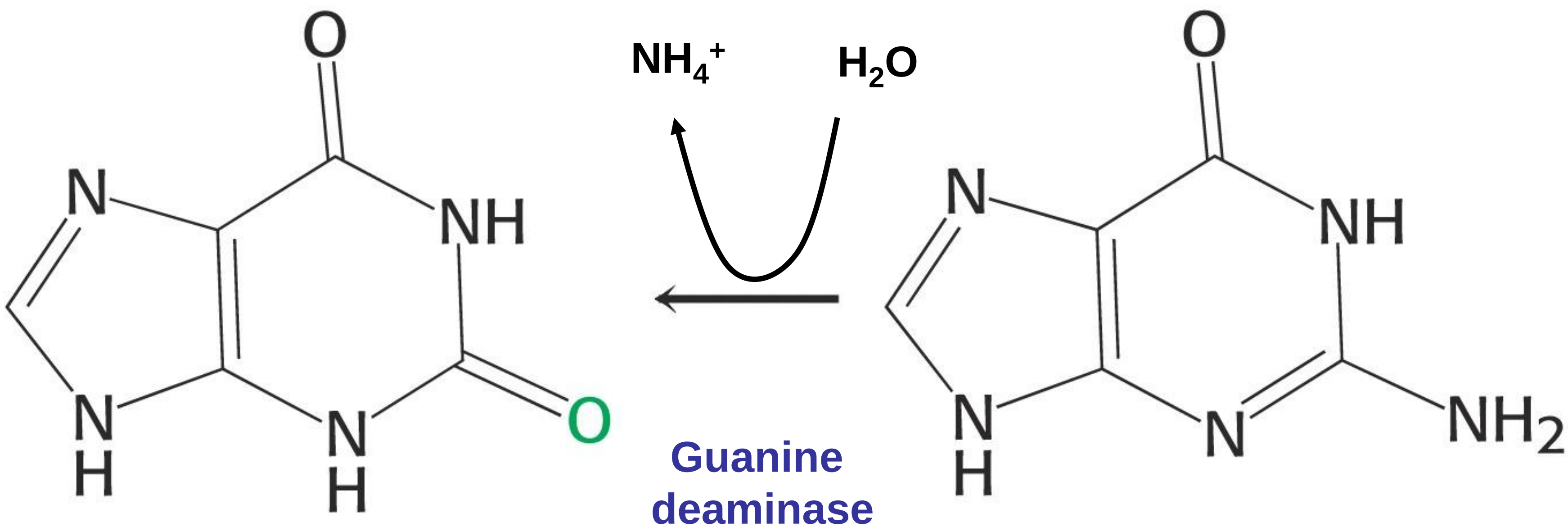


Hypoxanthine **Xanthine**

OR
Xanthine dehyrogenase

$NAD^+ + H_2O \rightarrow NADH + H^+$

Guanine can be deaminated to give xanthine



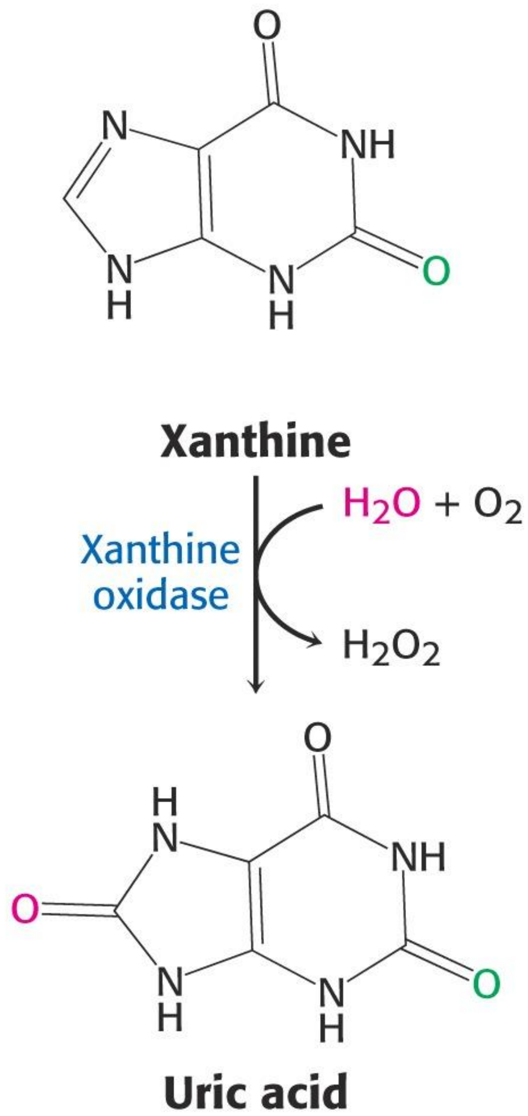
Xanthine

Guanine

Same as Guanase

Uric acid is the final product of purine degradation in mammals

OXIDATION

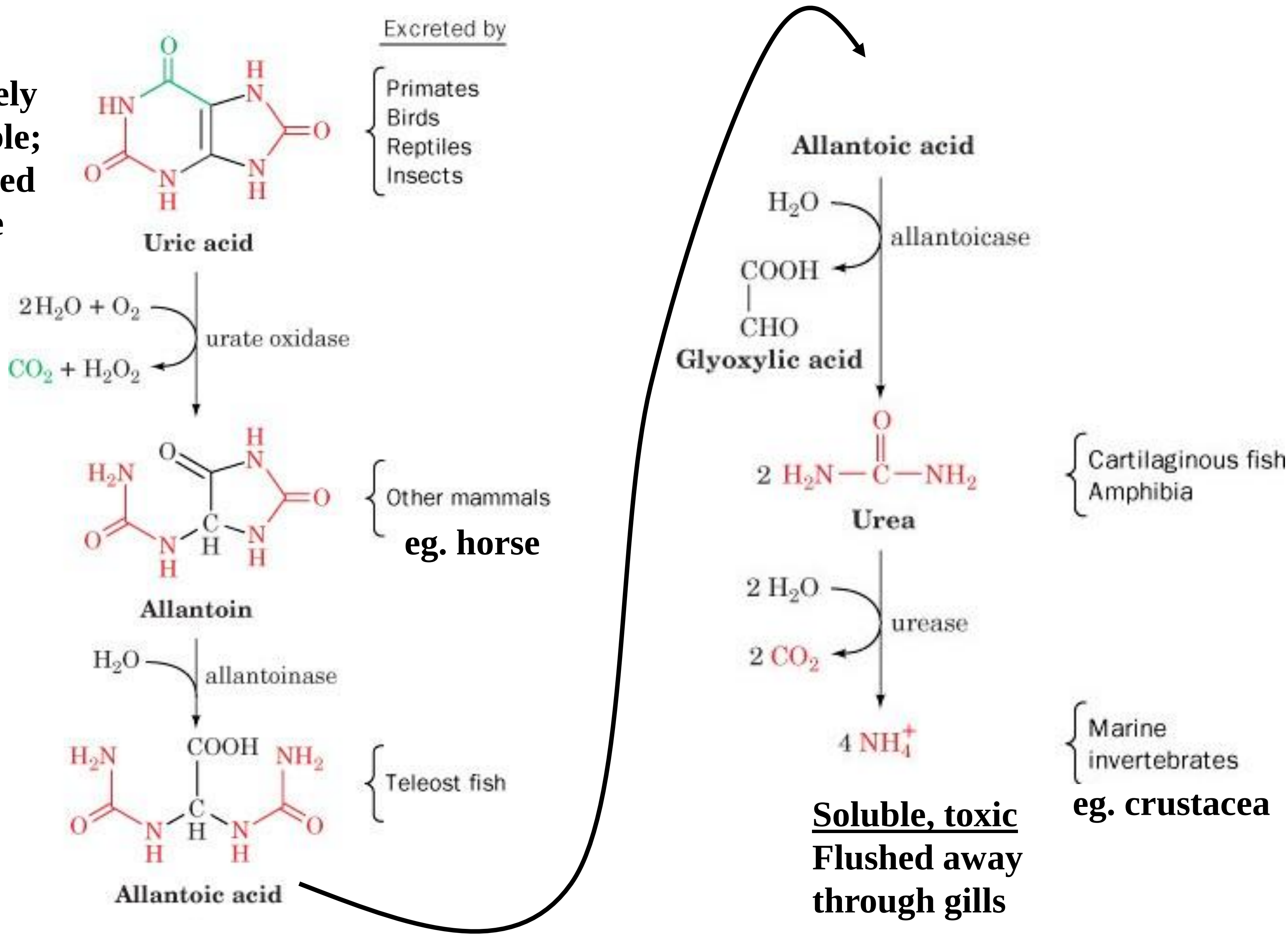


OR
Xanthine dehyrogenase

$NAD^+ + H_2O \rightarrow NADH + H^+$

Organisms have different ways in excreting products of purine catabolism

Relatively insoluble; converted to urate



The purine nucleotide cycle.

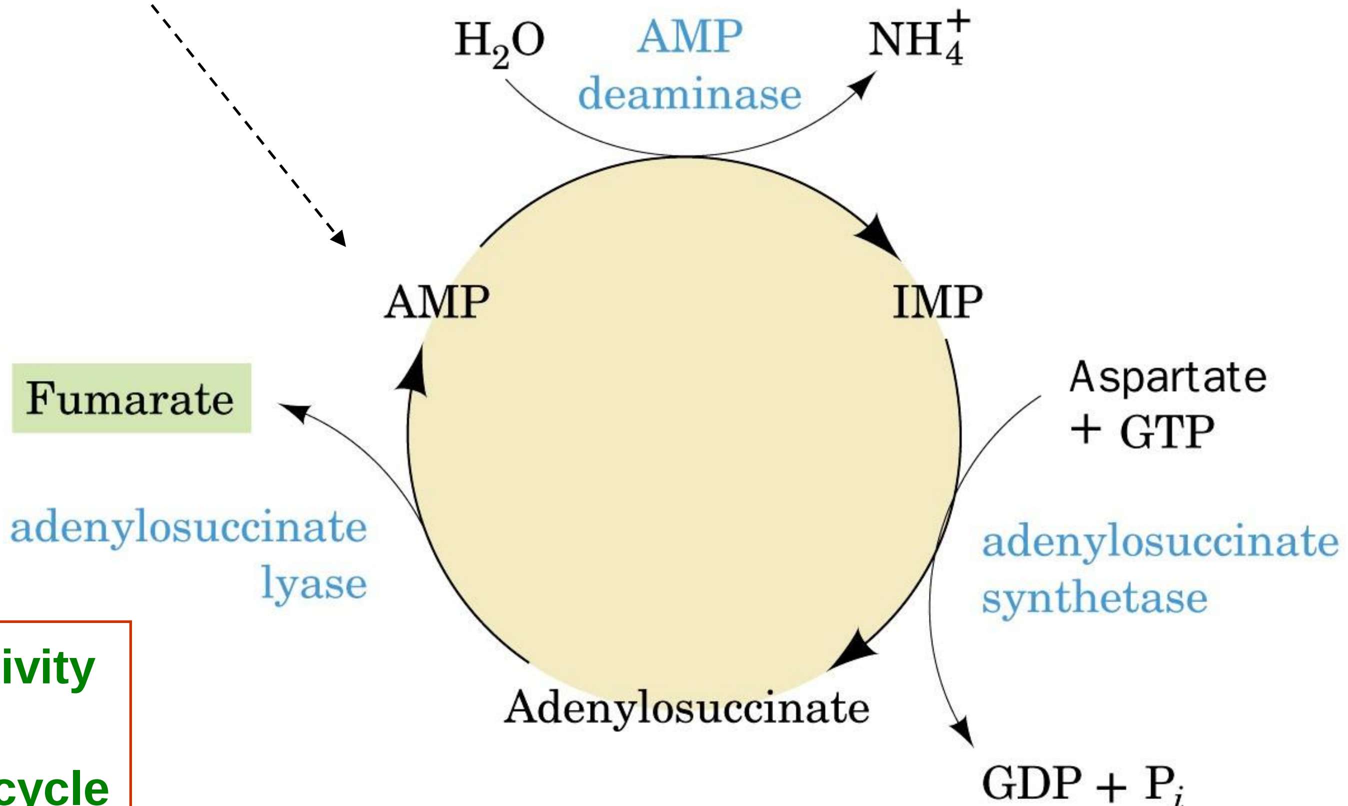
- Skeletal muscle relies on purine nucleotide cycle to support increased activity – purine nucleotide cycle generates citric acid cycle intermediate fumarate (**Figure 18-22):**

- **AMP (generated in active muscle by adenylate kinase) is converted to IMP in the purine nucleotide cycle**
- **Consumption of AMP alters the equilibrium position of adenylate kinase to increase ATP production.**
- **IMP is recycled to AMP via the elimination of fumarate**
- **Fumarate is supplied to the citric acid cycle**

Adenylate kinase

$2ADP \rightarrow \underline{AMP} + ATP$

Specific to muscle (not same as in catabolism; also called **myoadenylate deaminase**



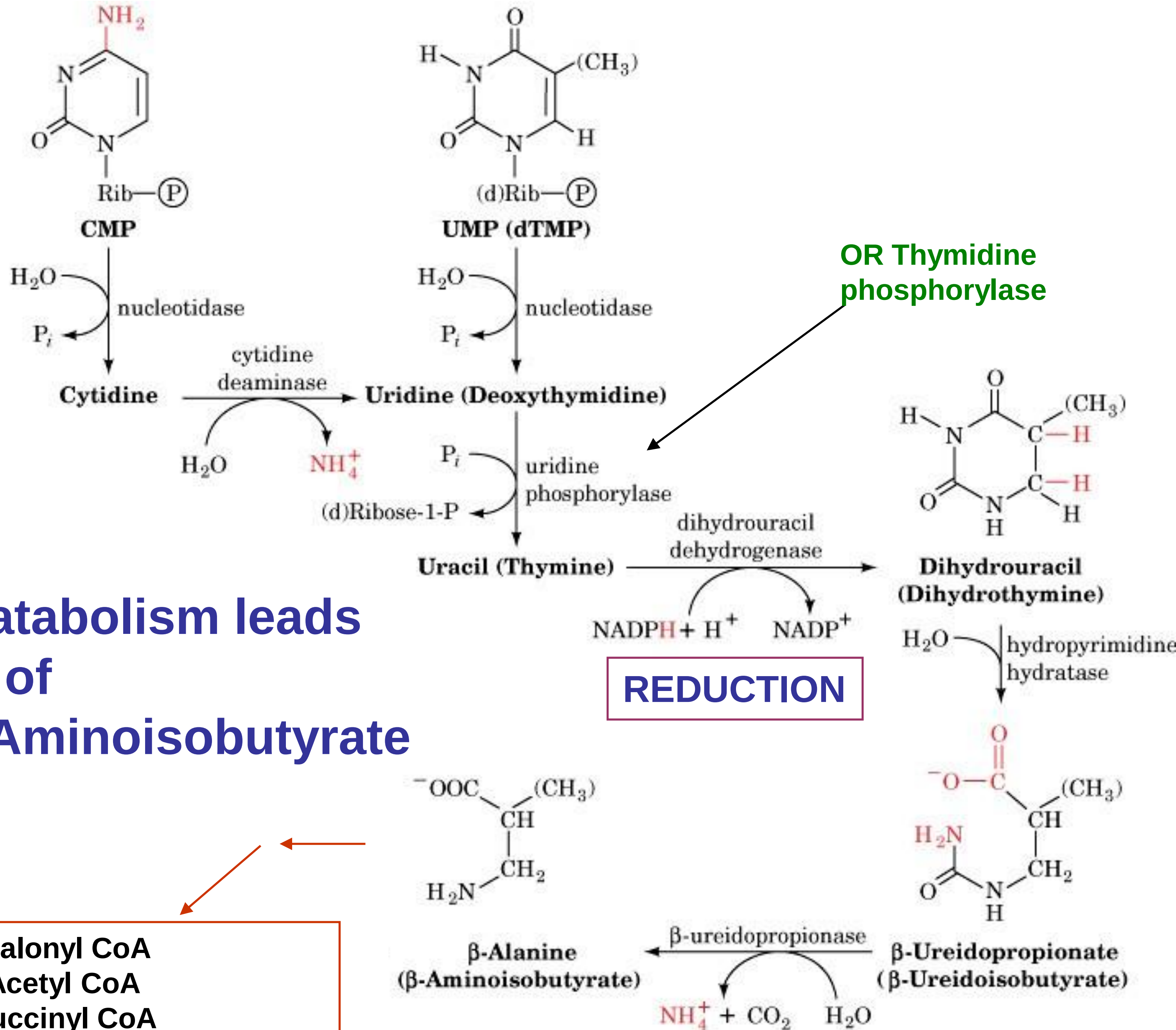
↑muscle activity

=

↑citric acid cycle

Net: H_2O + Aspartate + GTP → NH_4^+ + GDP + P_i + fumarate

Pyrimidine catabolism leads To formation of β-Alanine/ β-Aminoisobutyrate



Malonyl CoA
Acetyl CoA
Succinyl CoA
(citric acid cycle, fatty acid synthesis)