

SOS in Biochemistry, Jiwaji University, Gwalior

M.Sc. II Semester (2019-20)

Paper BCH 205: Fundamentals of Molecular Biology (Unit 1)

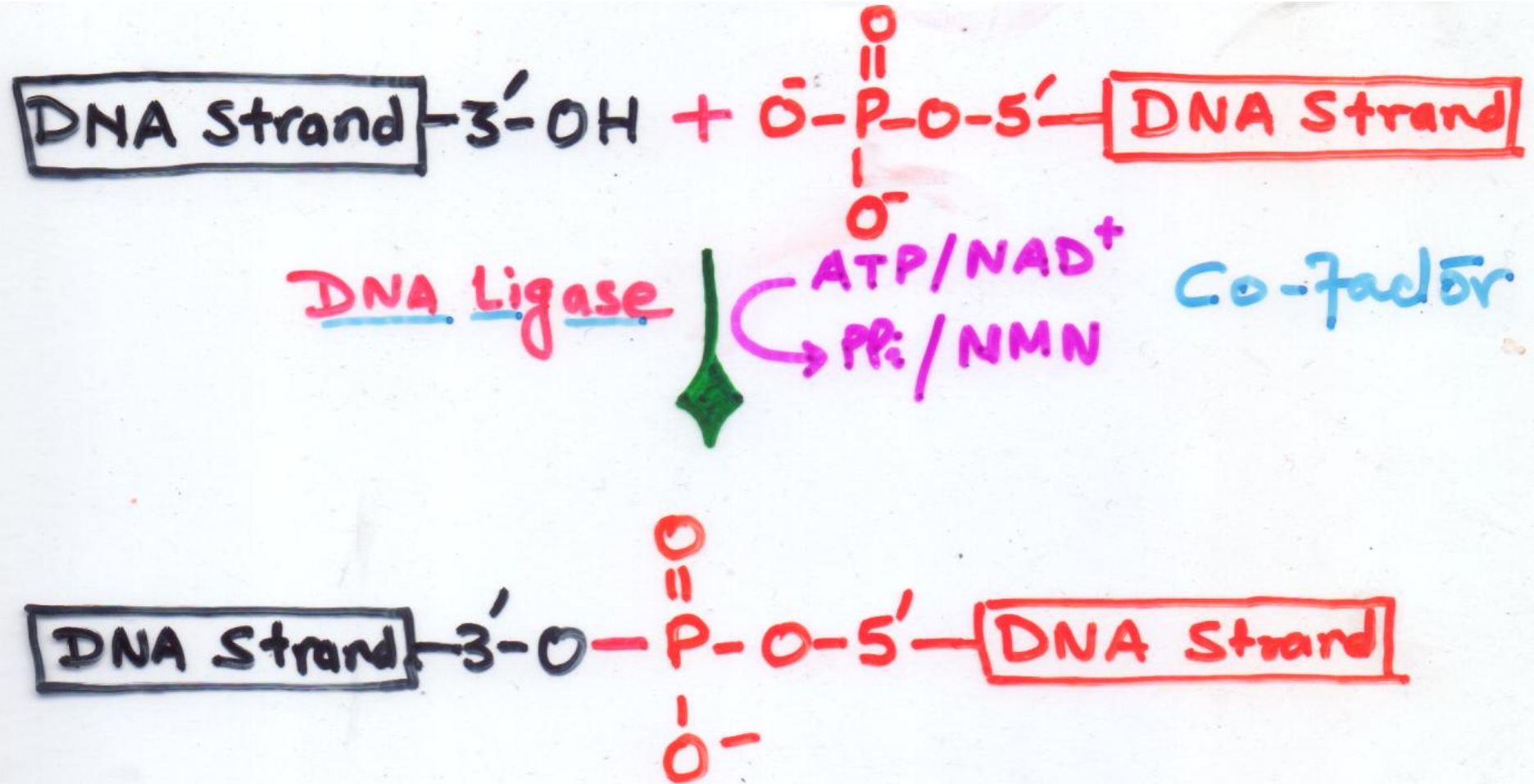
ENZYMOMOLOGY

of

DNA REPLICATION - III

DNA Ligase

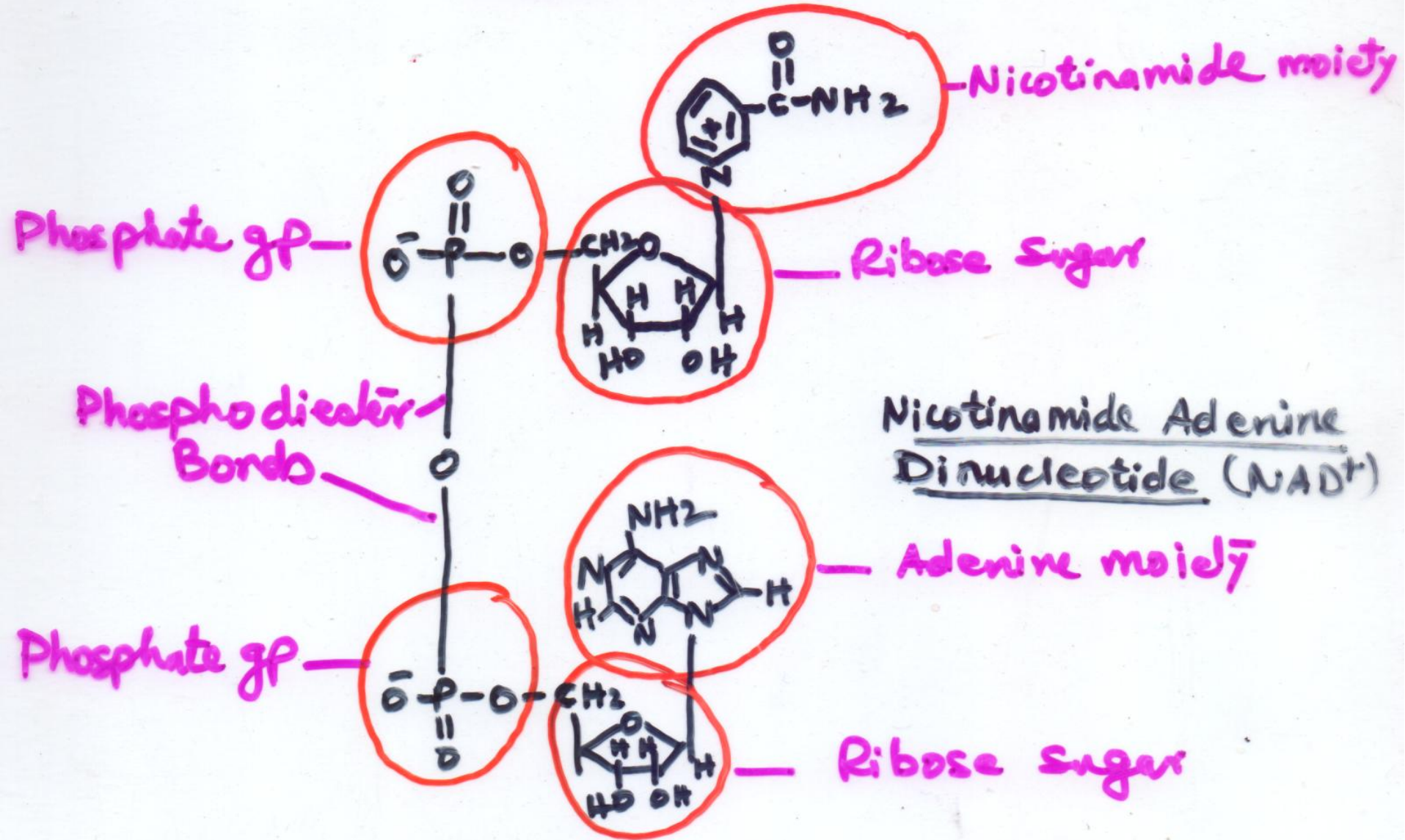
DNA Ligase (EC6.5.1.1)



The first DNA ligase was purified and characterized in 1967 by the Gellert, Lehman, Richardson, and Hurwitz laboratories. It was first purified and characterized by Weiss and Richardson

Co-factor

NAD⁺



Action of DNA Ligase

E. coli Ligase
B. subtilis



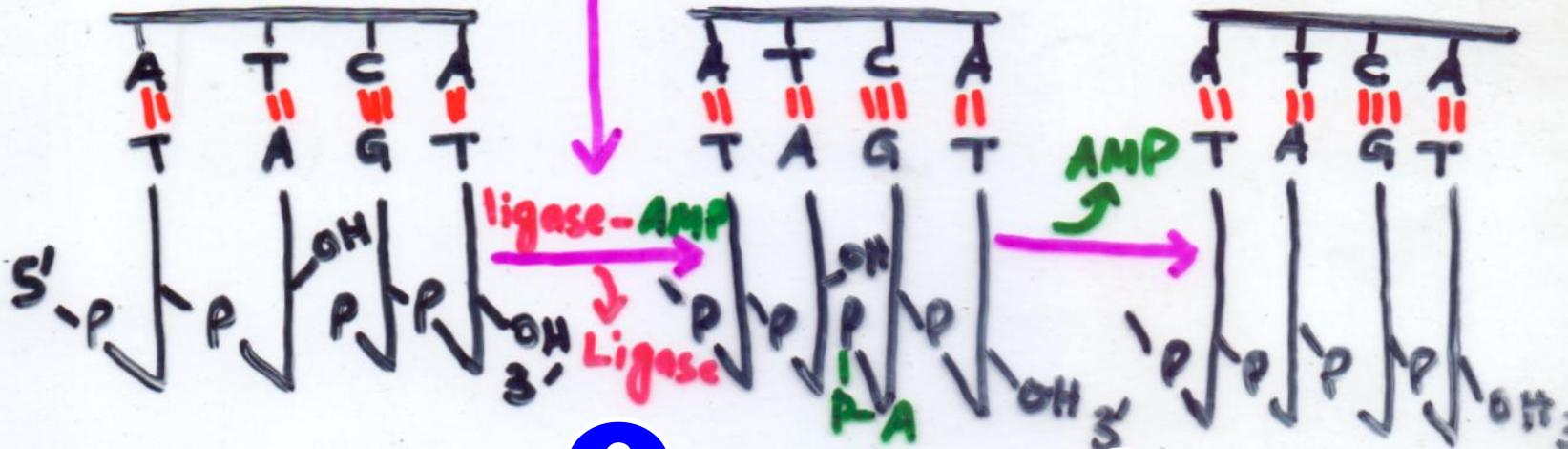
T4 & Mammalian Ligase



Ligase-AMP

1

ϵ -amino group of lys residue in the enzyme via a phosphoric acid amide bond



2

3

DNA-adenylylated Complex

Mechanism of Action of DNA Ligase

✓ **A Three Step Reaction**

A 3-step Reaction:

1. AMP is transferred to Lysine residue on enzyme
2. AMP transferred to open 5' phosphate via temporary pyrophosphate
3. AMP released, phosphodiester linkage made

✓ Uses NAD^+ or ATP for coupled reaction

✓ $\text{NAD}^+ \rightarrow \text{NMN} + \text{AMP}$

✓ $\text{ATP} \rightarrow \text{AMP} + \text{PPi}$

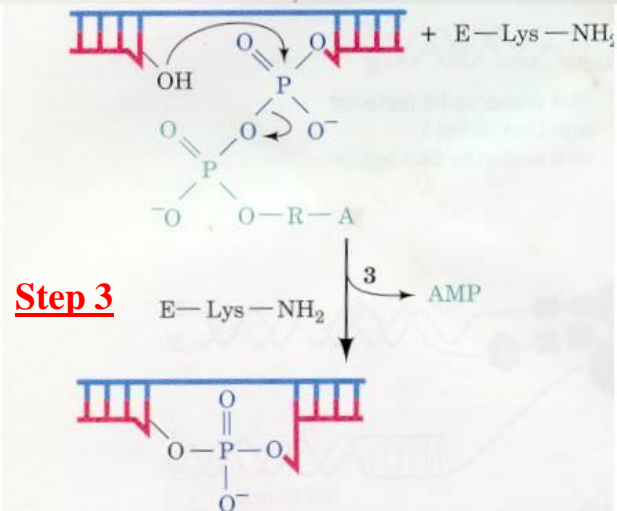
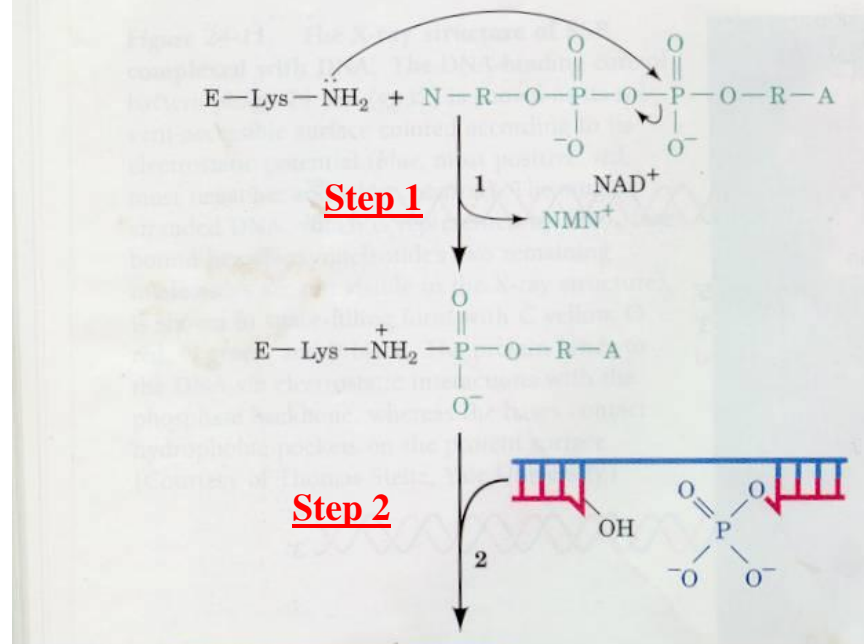
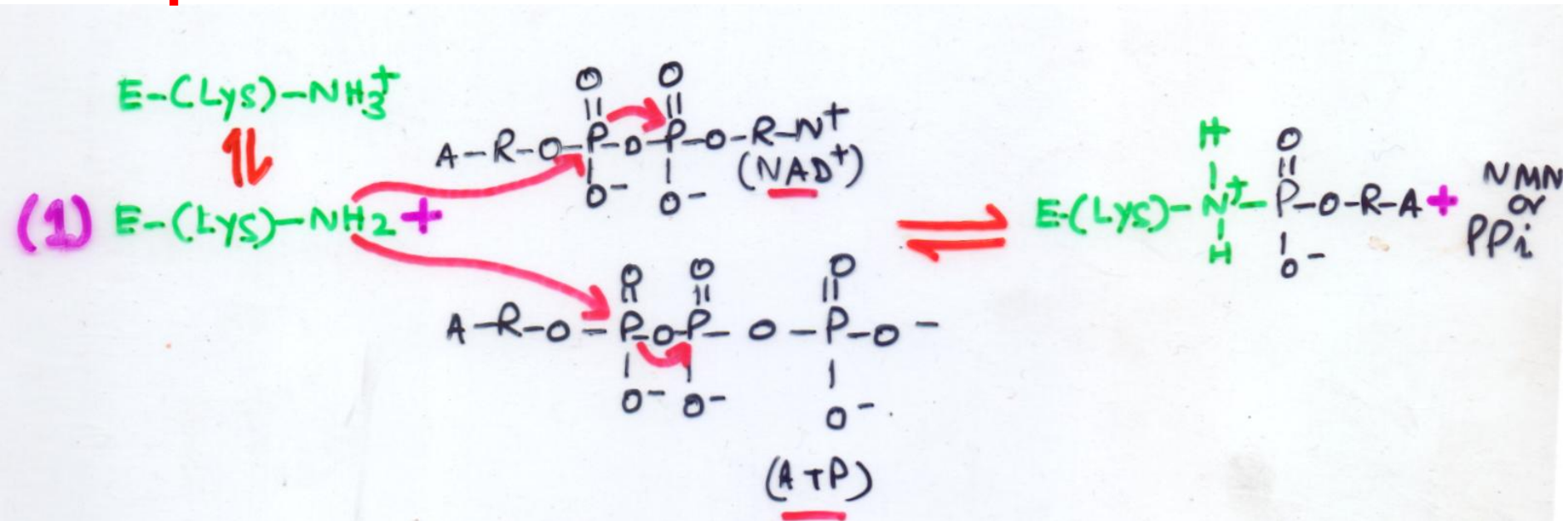


Figure 24-13. The reactions catalyzed by *E. coli* DNA ligase. In eukaryotic and T4 ligases, NAD^+ is replaced by ATP so that PP_i rather than NMN^+ is eliminated in the first reaction step. Here A, R, and N represent the adenine, ribose, and nicotinamide residues, respectively.

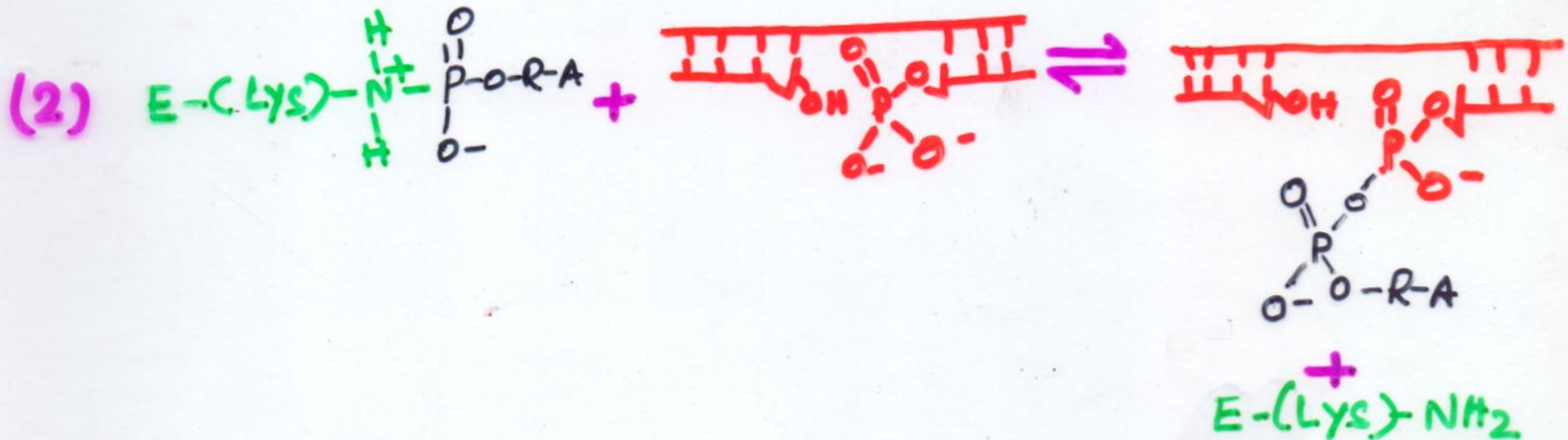
Mechanism of Action of DNA Ligase

Step 1



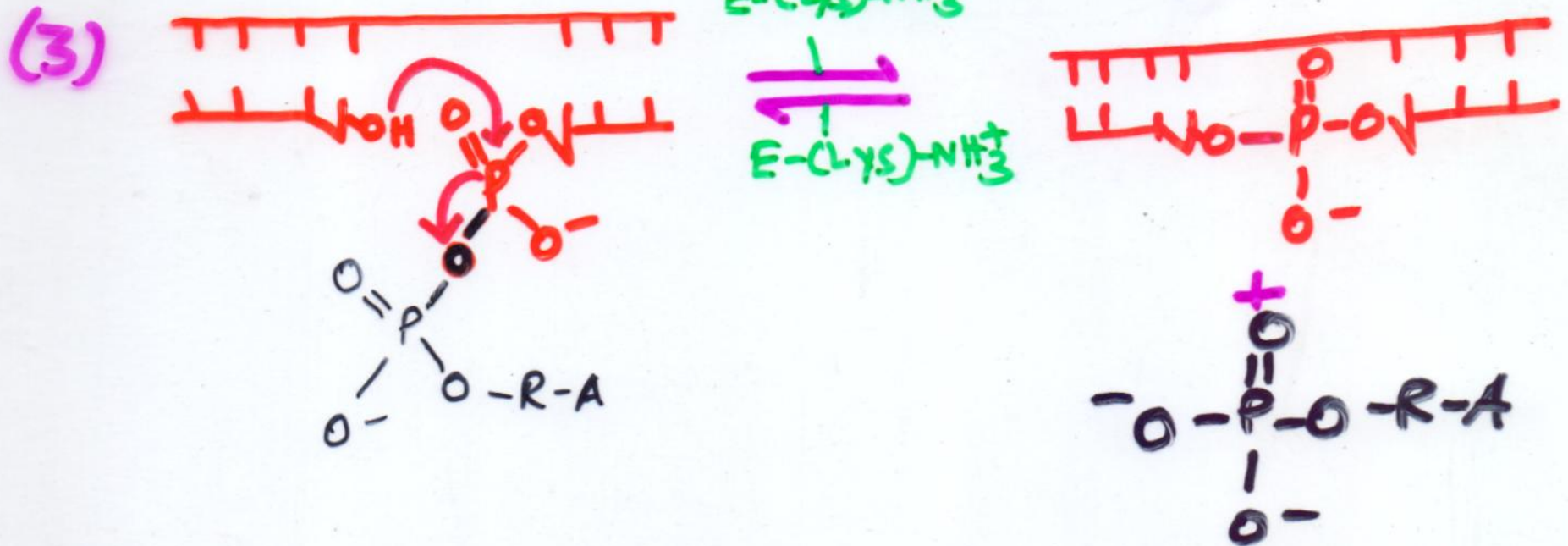
Mechanism of Action of DNA Ligase

Step 2



Mechanism of Action of DNA Ligase

Step 3

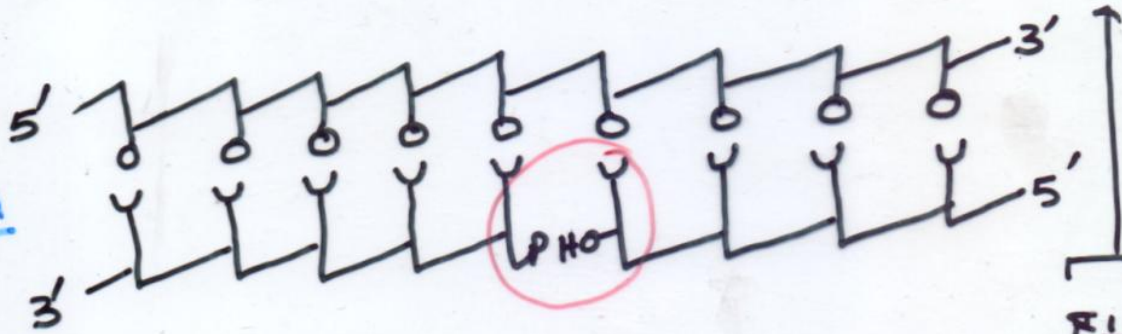


Substrates for DNA Ligase Activity

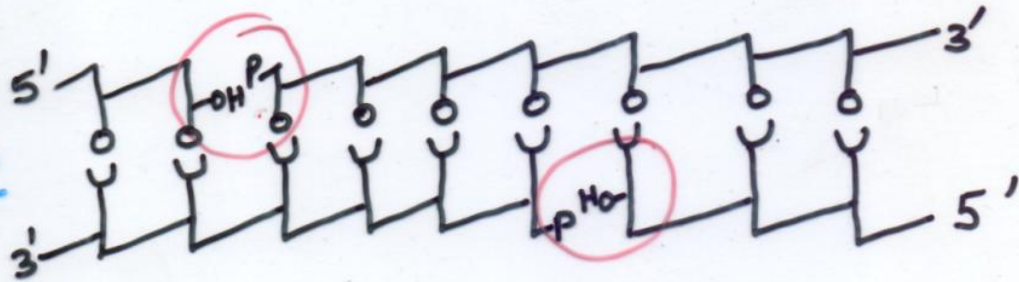
Points to be remembered.....

1. DNA ligase cannot link two molecules of ss DNA.
2. Rather, the DNA chains joined by DNA ligase must belong to double helical molecule.
3. A separate enzyme, RNA ligase is capable of ligating ss polynucleotide chains.

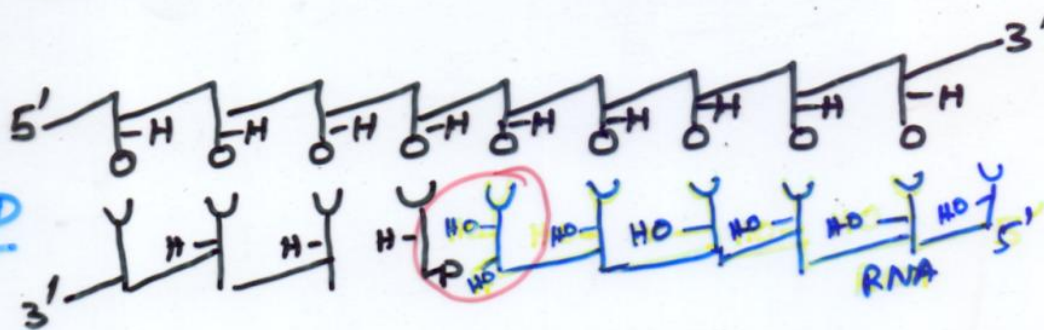
Type A



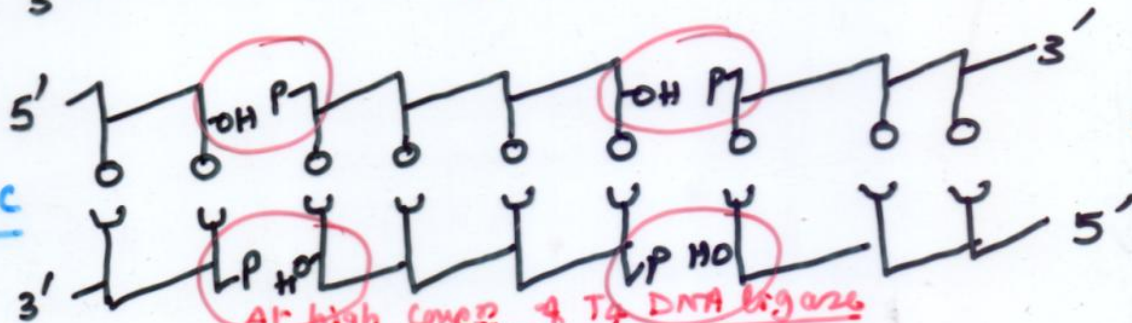
Type B



Type D



Type C



Both E. coli & T4 DNA ligase can carry out -
A, B & D type of ligation

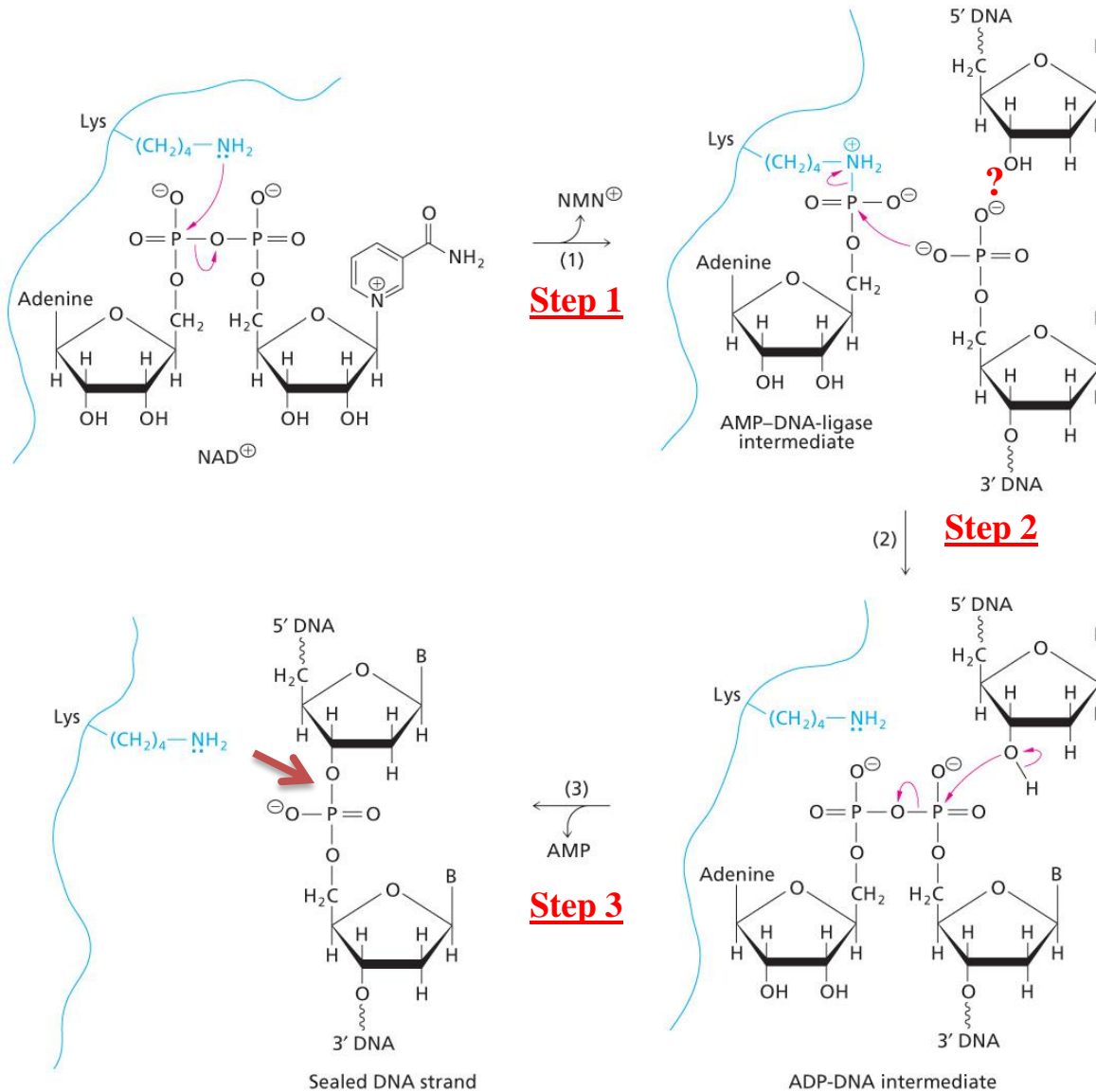
Only T4 DNA ligase can carry out - blunt end ligation reaction at higher conc

All high conc? of T4 DNA ligase

Two more Points to be remembered..

4. The ligation reaction of type A, B & C can be achieved by increasing ATP concentration.
5. A further higher concⁿ of ATP may inhibit all the three types (A, B & C) of ligation reaction.

Mechanism of Action of Ligase



© 2012 Pearson Education, Inc.

