MSc III Sem – Biotechnology

Course – Principles of Genetic Engineering

DNA Ligase

DNA Ligase

- **DNA ligase** is a specific type of enzyme, a ligase, (EC 6.5.1.1) that facilitates the joining of DNA strands together by catalyzing the formation of a phosphodiester bond.
- DNA ligase is used in both DNA repair and DNA replication .
- It plays a role in repairing single-strand breaks in duplex DNA in living organisms, but some forms (such as DNA ligase IV) may specifically repair double-strand breaks (i.e. a break in both complementary strands of DNA).
- DNA ligase has extensive use in molecular biology laboratories for recombinant DNA experiments for gene cloning to join DNA molecules together.
- This enzyme catalyzes the formation of phosphodiester bonds at the ends of DNA strands that are already held together by the base pairing of two extensions.
- DNA ligase also joins blunt ends that come in contact when they both bind to the enzyme . Blunt-end ligations require 10 to 100 times more T4 DNA ligase than do ligations of sticky DNA molecules.

DNA Ligase: types

***E. coli* DNA ligase** is encoded by the *lig* gene. It uses energy gained by cleaving nicotinamide adenine dinucleotide (NAD) to create the phosphodiester bond. **It does not ligate blunt-ended DNA** except under conditions of molecular crowding with polyethylene glycol, and cannot join RNA to DNA efficiently.

T4 DNA ligase is obtained from bacteriophage T4 (a bacteriophage that infects *Escherichia coli* bacteria). The T4 ligase is the most-commonly used in laboratory research. It can ligate either cohesive or blunt ends of DNA, oligonucleotides, as well as RNA and RNA-DNA hybrids, **but not single-stranded nucleic acids**. It can also ligate blunt-ended DNA with **much greater efficiency** than *E. coli* DNA ligase.

Mammalian DNA ligase are of four specific types:

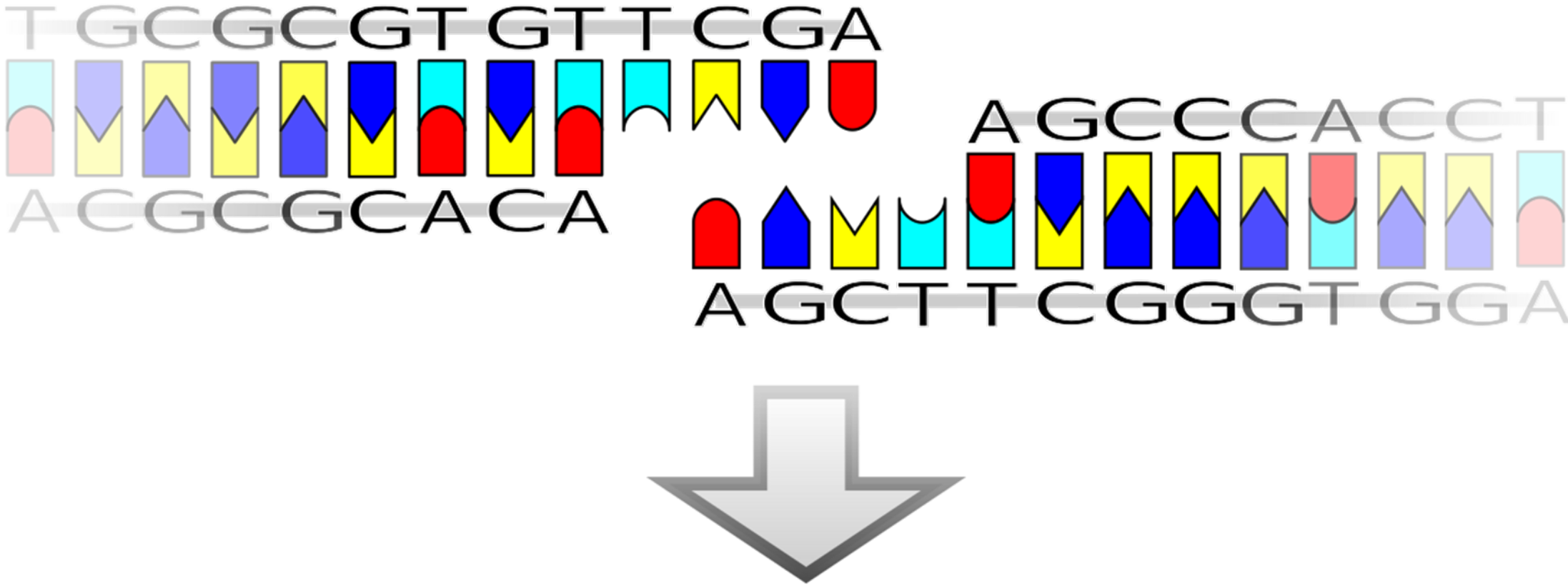
1. DNA ligase I: ligates the nascent DNA of the lagging strand after the Ribonuclease H has removed the RNA primer from the Okazaki fragments.
2. DNA ligase III: complexes with DNA repair protein XRCC1 to aid in sealing DNA during the process of nucleotide excision repair and recombinant fragments.
3. DNA ligase IV: complexes with XRCC4. It catalyzes the final step in the non-homologous end joining DNA double-strand break repair pathway. It is also required for V(D)J recombination, the process that generates diversity in immunoglobulin and T-cell receptor loci during immune system development.
4. DNA ligase II: A purification artifact resulting from proteolytic degradation of DNA ligase III. Initially, it has been recognized as another DNA ligase and it is the reason for the unusual nomenclature of DNA ligases.

Thermostable DNA ligase derived from a thermophilic bacterium, the enzyme is stable and active at much higher temperatures than conventional DNA ligases. Its half-life is 48 hours at 65 °C and greater than 1 hour at 95 °C.

DNA Ligase: enzymatic mechanism

The mechanism of DNA ligase is to form two covalent phosphodiester bonds between 3' hydroxyl ends of one nucleotide ("acceptor"), with the 5' phosphate end of another ("donor"). Two ATP molecules are consumed for each phosphodiester bond formed. AMP is required for the ligase reaction, which proceeds in four steps:

1. Reorganization of activity site such as nicks in DNA segments or Okazaki fragments etc.
2. Adenylylation (addition of AMP) of a lysine residue in the active center of the enzyme, pyrophosphate is released;
3. Transfer of the AMP to the 5' phosphate of the so-called donor, formation of a pyrophosphate bond;
4. Formation of a phosphodiester bond between the 5' phosphate of the donor and the 3' hydroxyl of the acceptor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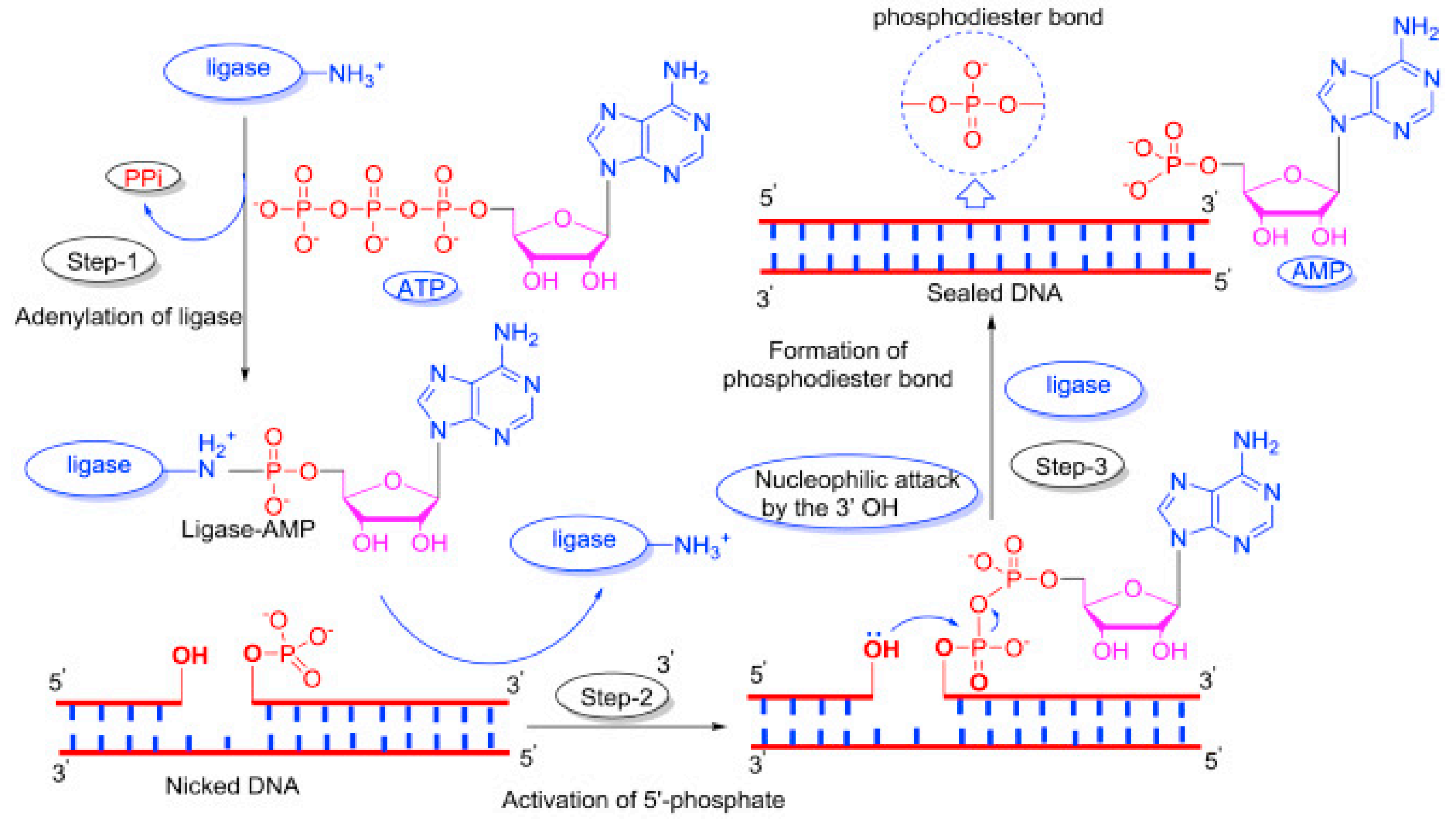
phosphodiester bond
ligase
NH_3^+
PPi
Step-1
Adenylation of ligase
ATP
NH_2
OH OH
Ligase-AMP
H_2^+N
Formation of phosphodiester bond
Sealed DNA
AMP
Step-3
Nucleophilic attack by the 3' OH
Step-2
Nicked DNA
Activation of 5'-phosphate
5'
3'

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