Quadrant II – Transcript and Related Materials

Programme: Bachelor of Science (Third Year)

Subject: Botany

Course Code: BOC 109

Course Title: Molecular Biology and Genetic Engineering

Unit: Recombinant DNA Technology

Module Name: Enzymes used in DNA recombinant technology: Restriction enzymes, DNA ligases & DNA modifying enzymes

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Notes

Introduction

In Recombinant DNA technology recombinant DNA (rDNA) is produced using a set of enzymes which are an important biological tools. These include Restriction enzymes, DNA ligases & DNA modifying enzymes.

Restriction enzymes

Gene cloning requires that DNA molecules be cut in a very precise and reproducible fashion to insert the new DNA. The verb restricts means 'cut'. Nucleases are enzymes that degrade DNA molecules by breaking the phosphodiester bonds. Nucleases that break RNA and DNA are called as RNase and DNase respectively. Nucleases are two types:

1. Exonuclease: These enzymes digest the base pairs on 5' or 3' ends of a single stranded DNA or at single nicks or gaps in double stranded DNA.

2. Endonuclease: Cleaves the double stranded DNA at any point except the ends, but they act on only one strand.

Restriction Endonucleases

They occur naturally in bacteria and provide a type of defence mechanism called the restriction modification system against the invading viruses and cut both

strands of DNA when certain foreign nucleotides are introduced in the cell. But the host cell own DNA is protected from its endonuclease by methylation of bases at restriction site. Restriction endonuclease enzyme recognize and cut DNA strand at specific sequence called restriction site.

Based on the mode of action there are three different types of restriction endonucleases: Type I, Type II, and Type III.

Type I Restriction Enzyme: They are complex nucleases which function as an endonuclease and a methylase. They move along the DNA in a reaction and require Mg^{++}, S-adenosyl methionine and ATP as co-factor. Mg2+ is required for the catalytic activity of the enzyme. It holds the water molecule in a position where it can attack the phosphoryl group.
They are single multifunctional enzymes and consists of three different subunits such as: restrictive sub-unit, modification sub-unit and specificity sub-unit. The third sub-unit specifies the recognition sites. The enzyme show specificity for recognition site but not for cleavage site, i.e. they recognise specific sites within the DNA but do not cleave at these sites. Therefore, the heterogenous population of DNA fragments is produced.

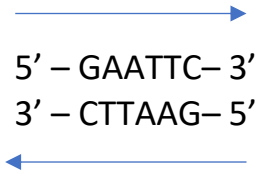
Type I enzyme recognises 15 bp long and cleavage site is about 1000 bp away from the 5'end of the recognition sequence TCA which is present within 15 bp recognition site. The adenine position in the recognition site may be methylated. Such DNA molecules are resistant to Type I endonuclease. When the recognition sequence is unmethylated, then DNA is cleaved as:

*Eco*KI 5'- A A A ·C N N N N G T C G C -3'
3'- T T T G N' N' N' N' C A ·G C G -5'

Here A shows methylation and adenine position, N is unspecified bases and N' is complementary bases to N.

Type II Restriction Enzymes: They consists of single polypeptide chain and do not require ATP for degradation of DNA. They have separate activities for cleaving and methylation of DNA molecules. They are most stable enzymes and require MG^{++} as co-factor. They recognize and cleave in both the strands of DNA and therefore, produce the DNA fragments of a defined length. Their recognition sites are usually undivided and palindromic and 4-8 nucleotides in length. Due to their action the broken nucleotides form a DNA duplex which exhibit two fold symmetry around a given point. In some cases, cleavage in two strands are staggered to produce single stranded short projections opposite to each other

with blunt or mutually cohesive sticky ends which are identical and complementary to each other. These complementary sequences are also known as palindrome sequences or palindromes. Therefore, when read from 5' to 3' both strands have the same sequence.



Nomenclature of Restriction Enzyme

Each enzyme is named after the organism from which it is isolated and is identified by the first letter of genus name and the first two letters of the species name to form the three-letter abbreviation in the italic. E.g *E. Coli* = *Eco*

A strain or type identified is written as subscript E.g. *Eco* K for *E. coli* strain K

When the restriction and modification systems are genetically specified by a virus or plasmid, the extra chromosomal element is identified by a subscript. E.g *Eco* RI

When a strain has several restriction and modification systems, these are identified by Roman numerals. For example *Hin*dI, *Hin*dII *Hin*dIII

For e.g *Eco*RI name: Abbreviation Meaning - *E Escherichia* genus, *co coli* species, R strain, I First identified order of identification in the bacterium

Type III Restriction endonuclease: It is made up of two sub-units, one for site recognition and modification and the other for cleavage. It requires ATP as energy source to cut DNA and Mg^{++} as co-factor. They have symmetrical recognition sites and cleave DNA at specific non- palindromic sequences. It cuts DNA about 25-27 bp away from restriction site. Eg. *Hpa*I, *Mbo*II

Since the cleavage products of these enzymes are homogenous population of DNA fragments, they cannot be used for genetic engineering experiments.

DNA Ligase

Ligases are enzymes that join the nucleic acid molecules together. They can join both DNA (DNA ligase) and RNA (RNA ligase).

DNA ligase is an important cellular enzyme, as it catalyses the formation of a phosphodiester bond that may occur at random or as a consequence of DNA replication or recombination between the 5' phosphate of one strand and the 3' hydroxyl group of another. In genetic engineering it is used to seal discontinuities in the sugar-phosphate chains that arise when recombinant DNA is made by joining DNA molecules from different sources. It is therefore called as molecular glue, which is used to stick pieces of DNA together.

They seal the single strand nicks in DNA which has 5'-3' -OH termini. There are two enzymes which are used for covalently joining restriction fragments:
The ligase from *E. coli* and that encoded by T4 phage. The most widely used DNA ligase is derived from the T4 phage, hence the enzyme is known as T4 DNA ligase. The enzyme is most efficient in sealing gaps in fragments that are held together by cohesive ends, it also joins blunt-ended DNA molecules together under appropriate conditions. However, cohesive end ligation takes place 100 times faster than the blunt end ligation.

E. coli DNA ligase uses nicotinamide adenine dinucleotide (NAD^+) as a cofactor and T4 DNA ligase requires ATP.

DNA modifying enzymes

The enzymes such as alkaline phosphatase, polynucleotide kinase (T4 polynucleotide kinase), and terminal transferase act on the termini of DNA molecules and provide important functions that are used in a variety of ways.

a. Alkaline Phosphatase: It functions to remove the phosphate group from the 5'-end of a DNA and RNA molecule. It also removes phosphates from nucleotides and proteins. They are most active at alkaline pH therefore known as alkaline phosphatase.

Bacterial alkaline phosphatase is the most active of the enzymes, but also most difficult to destroy at the end of the dephosphorylation reaction.

They are used in DNA manipulations for the removal of 5' phosphates from plasmid and bacteriophage vectors and removal of 5' phosphates from fragments of DNA prior to labelling with radioactive phosphate.

b. Polynucleotide Kinase: It catalyzes the transfer of phosphate group from ATP molecule to the 5'-terminus of DNA or RNA molecule after dephosphorylation by alkaline phosphatase. The enzymatic activity of Polynucleotide kinase is used in two types of reactions:

i) In the "forward reaction", PNK transfers the gamma phosphate from ATP to the 5' end of a polynucleotide. The target nucleotide lacks 5' phosphate either because it has been dephosphorylated or it has been chemically synthesized.

ii) In the "exchange reaction", target DNA or RNA that has a 5' phosphate is incubated with an excess of ADP, PNK first transfer the phosphate from the nucleic acid onto an ADP forming ATP leaving a dephosphorylated target. PNK then perform a forward reaction and transfer a phosphate from ATP into the target nucleic acid.

c. Terminal Transferase: It is also known as deoxynucleotidyl transferase enzyme. Adds single stranded sequences to 3'-terminus of the DNA molecule. One or more deoxyribonucleotides (dATP, dGTP, dTTP, dCTP) are added onto the 3'- OH end of the blunt-ended double stranded DNA fragments. Thus, it extends homopolymer tails and this phenomenon is called homopolymer tailing. It requires an oligonucleotide of at least three nucleotides to serve as a primer.

If the restriction enzyme cuts DNA forming blunt ends, then efficiency of ligation is very low. Terminal transferase enzyme synthesize short sequence of complementary nucleotide at free ends of DNA, so that blunt end is converted into sticky end.