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A comprehensive exploration of restriction enzymes and their applications in molecular biology: A review

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Abstract

Restriction enzymes, derived from bacteria, serve as indispensable tools in molecular biology, enabling precise manipulation of DNA with specificity. These enzymes recognize specific DNA sequences, cleaving the DNA strand at those designated sites. Two types, blunt-end cutters, and sticky-end producers, offer distinct advantages and disadvantages. In molecular biology, restriction enzymes find diverse applications, prominently in gene cloning, facilitating the insertion of foreign DNA into host organisms. Additionally, they play a vital role in DNA sequencing, DNA fingerprinting, and other techniques crucial for studying gene function and regulation. A groundbreaking development is witnessed in gene editing, where engineered enzymes recognize specific DNA sequences, allowing scientists to target and modify genes with unprecedented precision. This breakthrough holds the potential to revolutionize medicine, paving the way for treating genetic diseases and creating personalized therapies. Research efforts are focused on discovering new restriction enzymes with novel specificities, expanding the range of manipulable DNA sequences. This opens avenues for innovative applications in synthetic biology and biotechnology, further advancing the field. Despite their numerous applications, challenges persist, including the potential for off-target effects and the quest for enzymes with specific recognition sequences. Ongoing research and development continue to push the boundaries of what is achievable with restriction enzymes. In conclusion, restriction enzymes have significantly impacted molecular biology and biotechnology, facilitating the precise manipulation and study of genetic material. Ongoing research promises to unveil new applications and discoveries in this dynamic and promising field.

Keywords: Restriction enzyme; RM system; Blunt end; Sticky end

1. Introduction

Restriction enzymes, also known as restriction endonucleases, are proteins produced by bacteria that can recognize specific base sequences in DNA and cut (restrict) the DNA at that site known as the restriction site (Roberts *et al.*, 2003). It cleaves the sugar-phosphate backbone of both strands of a double-stranded DNA molecule at or near the recognition site. These recognition sites are usually 4 to 8 base pairs in length and palindromic. Palindromic sequences are of two types. One is a mirror-like palindrome in which the same forward and backward sequences are on a single strand of DNA strand, such as GTAATG. The inverted repeat palindrome is a sequence that reads the same forward and backward, but the forward and backward sequences are found in the complementary DNA strand. Restriction enzymes are inverted repeat palindromic sequences and have greater biological importance than mirror-like palindromic sequences. The majority of these enzymes have been isolated from bacteria and archaea, where they carry out a host-defense

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mechanism for the cell. These enzymes have become an indispensable tool in molecular biology, allowing researchers to manipulate DNA by cutting it into precise fragments for various applications, such as gene cloning, gene mapping, DNA sequencing, etc. (Roberts *et al*., 2003). Restriction enzymes recognize and cut DNA at specific palindromic sequences, creating blunt or sticky ends that can be used to join DNA fragments from different sources (Roberts *et al*., 2003).

Restriction enzymes have revolutionized molecular biology by enabling the manipulation of DNA. They are widely used in DNA cloning, genetic engineering, and other molecular biology techniques (Loenen & Raleigh, 2014). For example, restriction enzymes can be used to cut DNA at specific sites, which allows for the insertion of foreign DNA fragments into the cut sites, resulting in the creation of recombinant DNA molecules (Doudna & Charpentier, 2014). The discovery and development of restriction enzymes have a profound impact on molecular biology, enabling the manipulation of DNA and the creation of recombinant DNA molecules for a wide range of applications.

2. History

The concept of Restriction enzymes originated from research on bacteriophage and its host-controlled restriction and modification phenomenon (Winnacker, 1987). It was found that when a phage become established in one strain of *E. coli*, its capacity to multiply in other strains was restricted. In the 1960s, It was established by Werner Arber and Matthew Meselson that the restriction of growth of phage in the host cell was caused by enzymatic degradation of the phage DNA and the enzyme that cleaves the phage DNA was termed a restriction enzyme (Meselson and Yuan, 1968). The enzymes discovered by Arber and Meselson were type I restriction enzymes. In 1970, Hind II, the first type II restriction enzyme, was discovered by Hamilton O. Smith, Thomas Kelly, and Kent Wilcox in the Rd strain of *Haemophilus influenzae* bacteria (Kelly and Smith, 1970). After that, Daniel Nathans and Kathleen Danna reported that the specific fragments that are produced from the restricted digestion of DNA can be separated by using the gel electrophoresis technique. Restriction enzymes cut DNA at specific recognition sequences, which are typically palindromic sequences of four to eight nucleotides. Hamilton O. Smith, Werner Arber, and Daniel Nathans were later awarded the Nobel Prize in Physiology or Medicine in 1978 for the discovery of restriction enzymes (Roberts, 2005).

2.1. Types of Restriction Enzymes

There are four main types of restriction enzymes - Type I, Type II, Type III, and Type IV - each with their unique properties and mechanisms of action. Type II restriction enzymes are the most commonly used enzymes in molecular biology, as they cleave DNA at specific sites and produce ends that are compatible with each other, allowing for the creation of recombinant DNA molecules (Pingoud & Jeltsch, 2001).

Type I restriction enzymes: are large, multi-subunit, both endonuclease and methylase activities, recognize specific DNA sequences but cut at variable distances (up to 1000 bp) from the recognition site (Loenen & Raleigh, 2014). Type I endonucleases have a molecular weight of around 300 kDa are composed of non-identical sub-units and require $Mg2+$, ATP (adenosine triphosphate), and SAM (S-adenosyl-methionine) as cofactors for activity.

Type II restriction enzymes: are the most commonly used in molecular biology and are composed of single subunit proteins that recognize specific DNA sequences and cut within or near recognition sequences (Loenen & Raleigh, 2014). Molecular weights of Type II enzymes in the range of 20 to 100 kDa. They possess identical sub-units and require only $Mg2+$ as a cofactor. These enzymes produce ends that are compatible with each other, allowing for the creation of recombinant DNA molecules (Pingoud & Jeltsch, 2001).

Type III restriction enzymes: are not commonly used because their specificity of cutting the DNA is low. Their size is around 200 kDa, which is smaller than Type I enzymes and they cleave DNA randomly. They have both endonuclease and methylase activity. They consist of non-identical multi-subunit, and more specifically cut at the recognition sequence about 25 bp away from it (Loenen & Raleigh, 2014). The activity of this type of enzyme is dependent on both $Mg2+$ and ATP but does not require SAM as co-factors (Pingoud & Jeltsch, 2001).

Type IV restriction enzymes: recognize the modified DNA such as methylated, glycosyl-hydroxymethylated, and hydroxy methylated DNA.

2.2. The bacterial restriction-modification (RM) system

The RM system is a defensive mechanism of bacteria that protects it from infecting foreign DNA, such as viral DNA or plasmids, by selectively degrading its DNA (Loenen & Raleigh, 2014). The RM system consists of two components: a restriction enzyme and a modification enzyme. The restriction enzyme recognizes and cleaves specific DNA sequences, while the modification enzyme protects the bacterial DNA by methylating the same sequence, preventing it from being recognized and cleaved by the restriction enzyme (Deng & Li, 2014; Loenen & Raleigh, 2014; Roberts *et al*., 2015).

The RM system is highly specific and can distinguish between self and non-self-DNA based on the presence or absence of methyl groups (Loenen & Raleigh, 2014). Bacteria protect their DNA by methylating it with the modification enzyme, whereas foreign DNA lacks the appropriate methyl groups and is targeted for degradation by the restriction enzyme. The RM system is a highly effective defence mechanism against invading foreign DNA, but it can also have unintended consequences. For example, the RM system can prevent the use of restriction enzymes for molecular biology applications, such as cloning or DNA sequencing, if the DNA being studied contains the same sequence recognized by the bacterial RM system (Loenen & Raleigh, 2014).

2.3. Mechanism of action of Restriction Enzymes

The mechanism of action of restriction enzymes involves three steps; recognition, cleavage, and cutting the specific DNA sequences. The most used restriction enzymes are type II enzymes, which cut the DNA within or near their recognition sites (Loenen & Raleigh, 2014). When a restriction enzyme encounters its specific recognition site, it binds to the DNA and makes sequence-specific contacts with the bases, recognizing the symmetry of the sequence (Pingoud & Jeltsch, 2001). The binding of the restriction enzyme to the DNA causes a conformational change in the enzyme that activates its catalytic center, which then cleaves the DNA backbone at a specific position within or near the recognition site and releases the enzyme (Fig: 1). The exact position of the cleavage depends on the specific restriction enzyme and the sequence of the recognition site (Pingoud & Jeltsch, 2001).

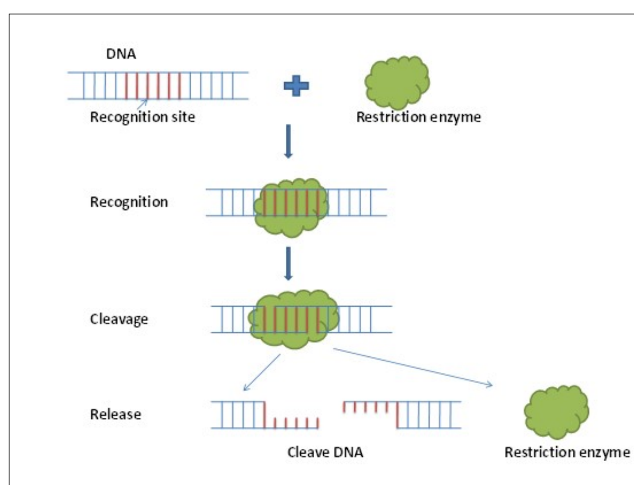

Figure 1 Describing the mechanism of action of restriction enzymes

The cleavage by a restriction enzyme can result in different types of DNA ends, including blunt ends or sticky ends, depending on the specific enzyme and the position of the cleavage (Loenen & Raleigh, 2014). Blunt ends are produced when the enzyme cuts the DNA at the same position on both strands, resulting in straight ends. Sticky ends are created when the enzyme cuts the DNA at slightly different positions on the two strands, resulting in overhanging ends that can base pair with complementary overhangs on other DNA molecules. The mechanism of action of restriction enzymes involves the specific recognition and cleavage of DNA at their recognition sites, resulting in different types of DNA ends.

2.4. Blunt end cutters and sticky end producers

Restriction enzymes produces Blunt end and sticky end depending on the way they cleave DNA (Table 1).

Blunt cutters, also known as type II restriction enzymes with symmetrical recognition sites, cleave the DNA at a specific site within the recognition sequence, producing two straight or blunt-ended fragments (Pingoud & Jeltsch, 2001). Examples of blunt cutters include SmaI (Figure 2a) and EcoRV.

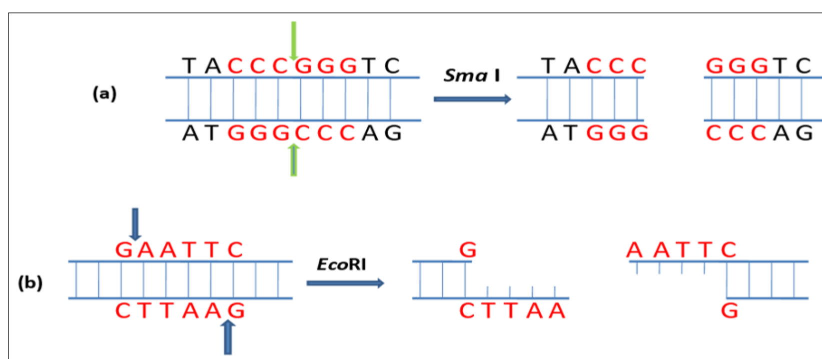

Figure 2 RE that produces (a) Blunt end cutters (b) sticky end cutters

Table 1 Examples of few blunt end and sticky end producing restriction enzymes, along with their origin of isolation

Enzyme Name	Type	Recognition Sequence	Origin of Isolation
EcoRV	Blunt Cutter	5'-GATATC-3'	*E. coli*
SmaI	Blunt Cutter	5'-CCCGGG-3'	*Serratia marcescens*
EcoRI	Sticky End Producer	5'-GAATTC-3'	*E. coli*
BamHI	Sticky End Producer	5'-GGATCC-3'	*Bacillus amyloliquefaciens*
AluI	Blunt Cutter	5'-AGCT-3'	*Arthrobacter luteus*
AseI	Blunt Cutter	5'-ATTAAT-3'	*Arthrobacter species*
PvuII	Blunt Cutter	5'-CAGCTG-3'	*Proteus vulgaris*
DraI	Blunt Cutter	5'-TTTAAA-3'	*Deinococcusradiodurans*
EcoRV	Blunt Cutter	5'-GATATC-3'	*E. coli*
SmaI	Blunt Cutter	5'-CCCGGG-3'	*Serratia marcescens*
EcoRI	Sticky End Producer	5'-GAATTC-3'	*E. coli*
BamHI	Sticky End Producer	5'-GGATCC-3'	*Bacillus amyloliquefaciens*
HindIII	Sticky End Producer	5'-AAGCTT-3'	*Haemophilusinfluenzae*
XhoI	Sticky End Producer	5'-CTCGAG-3'	*Xanthomonas homology*

Pingoud & Jeltsch, 2001; Roberts *et al.*, 2003)

Sticky end producers, also known as type II restriction enzymes with asymmetrical recognition sites, cleave the DNA at different positions on the two complementary strands within the recognition sequence, producing fragments with single-stranded overhangs or "sticky ends" that can form base pairs with complementary overhangs on other DNA molecules (Pingoud & Jeltsch, 2001). Examples of sticky end producers include EcoRI (Figure 2b) and BamHI.

The choice of a blunt cutter or a sticky end producer depends on the specific application. Blunt cutters are useful for certain applications such as subcloning, whereas sticky end producers are useful for other applications such as directional cloning and DNA sequencing (Roberts *et al.*, 2003).

2.5. Applications of RE in Molecular Biology

Restriction enzymes have a wide range of applications in molecular biology and biotechnology. Some of the applications of restriction enzymes are mentioned below:

DNA fingerprinting: Restriction enzymes are used in DNA fingerprinting to create a unique banding pattern of DNA fragments from different individuals, which can be used to identify individuals or determine familial relationships (Budowle *et al.*, 2009). Restriction enzymes are also used for tracking RestrictionFragment Length Polymorphisms, which allows the mutationlocations, to generate human linkage maps, to identify some diseases like sickle cell trait, and Huntington's disease (Wexler, 2012). DNA fingerprinting allows for the identification of criminals and victims.

Restriction enzymes are useful for identifying pathogenic bacteria, such as *S. aureus* spp with antibiotic-resistance and virulence factors mediated by methicillin-resistant *S. aureus* (MRSA) which pose a great threat to humans and animals also (Lindsay, 2010).

Gene cloning: Restriction enzymes are used in gene cloning to cut out a gene of interest from a larger piece of DNA and insert it into a vector for further study or manipulation (Sambrook *et al*., 1989).

Site-directed mutagenesis: Restriction enzymes can be used in site-directed mutagenesis to introduce specific mutations into a gene of interest (Kunkel *et al*., 1987).

DNA sequencing: Restriction enzymes can be used in DNA sequencing to generate a series of overlapping fragments that can be sequenced to determine the sequence of the original DNA molecule (Sanger *et al*., 1977).

Southern blotting: Restriction enzymes are used in Southern blotting to create fragments of DNA that can be separated by gel electrophoresis and transferred onto a membrane for further analysis (Southern, 1975).

RFLP analysis: Restriction enzymes are used in restriction fragment length polymorphism (RFLP) analysis to identify genetic variations in DNA samples by cutting the DNA at specific restriction sites and comparing the resulting fragments (Botstein *et al*., 1980).

Genetic engineering: Type II restriction enzymes have many practical uses as *E. coli* K12, its genes, and its vectors are the warhorses of molecular biology for cloning, DNA libraries, DNA sequencing, detection; production of enzymes and hormones, etc (Murray and Murray, 1974; Midgley and Murray, 1985). After the discovery of synthetic DNA, the applications of Type II restriction enzymes become distended, such as in vitro packaging of DNA in phage particles and developed bacterial hosts and vectors for overexpression and stabilization of proteins (Wil *et al*., 2014).

3. Conclusion

In conclusion, restriction enzymes are an essential tool in molecular biology and biotechnology, providing a simple, specific, and precise way to manipulate DNA. They are produced by bacteria and recognize specific DNA sequences, cutting the DNA at those sites. The applications of restriction enzymes have helped to revolutionize the field of genetics and have made a significant impact on the understanding of genetic diseases, genetic engineering, and biotechnology. The discovery of restriction enzymes and their application in biotechnology has had a tremendous impact on molecular biology and has allowed for the advancement of genetic research, gene therapy, and genetic engineering. The versatility and specificity of restriction enzymes make them a valuable tool for scientists in a wide range of fields and will continue to be an essential component of molecular biology research in the future.

Future prospect

The future prospects of restriction enzymes in biotechnology hold immense promise for advancing molecular research and applications. With ongoing advancements in gene editing technologies, the precision and versatility of restriction enzymes make them indispensable tools. The development of more sophisticated and tailored restriction enzymes, engineered for specific genomic sequences, can revolutionize targeted gene manipulation, paving the way for precise therapeutic interventions in genetic disorders. Furthermore, as biotechnology continues to explore synthetic biology, the integration of restriction enzymes in the construction of custom genetic circuits and synthetic organisms presents exciting possibilities. Collaborations with other cutting-edge technologies, such as CRISPR-Cas systems, may enhance the efficiency and precision of genome editing, opening new avenues for personalized medicine, agriculture, and environmental applications. With ongoing research and development, we can expect to see even more innovative and exciting applications for restriction enzymes in the years to come.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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