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Restriction Endonucleases and Exonucleases

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INTRODUCTION

Restriction enzymes, commonly referred to as restriction endonucleases are bacterial proteins capable of cutting DNA at highly specific nucleotide sequences. Due to their accuracy in recognizing and cleaving DNA, they are popularly known as molecular scissors. In nature, these enzymes function as a protective system in bacteria, helping them defend against foreign genetic material such as bacteriophage DNA. Every bacterial species produces a distinct set of restriction enzymes, and each enzyme shows strict sequence specificity, recognizing and cutting only a particular DNA sequence. This remarkable specificity has made restriction enzymes indispensable tools in molecular biology, biotechnology, and genetic engineering. The specific DNA sequences recognized and cleaved by restriction enzymes are known as recognition sites. These sites are generally palindromic in nature, meaning that the nucleotide sequence reads the same in the 5′→3′ direction on both strands of the DNA molecule. A typical example is the EcoRI recognition site i.e. 5′-GAATTC-3′ and 3′-CTTAAG-5′. Such palindromic sequences possess a central axis of symmetry, usually located at the midpoint of the sequence and restriction enzymes introduce cuts at or near this symmetrical region. When a restriction endonuclease binds to its recognition site, it cleaves the DNA by catalyzing the hydrolysis of the phosphodiester bond between adjacent nucleotides. This reaction involves the addition of a water molecule and results in precise fragmentation of the DNA. Based on their structural organization, cleavage behavior, sequence specificity and cofactor requirements, restriction enzymes are classified into four major types: Type I, Type II, Type III, and Type IV. Type I restriction enzymes cut DNA at positions far removed from the recognition site, often more than 1000 base pairs away.

Their activity requires ATP, Mg^{2+} and S-adenosyl-L-methionine (SAM). These enzymes are large multifunctional complexes that combine both restriction and DNA methylation activities. Due to their random and unpredictable cleavage pattern, they are not suitable for use in genetic engineering; EcoKI is a common example. Type II restriction enzymes cleave DNA at or very close to the recognition site and require only Mg^{2+} ions as a cofactor. In these systems, restriction enzymes and methylases are produced as separate proteins. Because they generate precise and reproducible DNA fragments, Type II enzymes *viz*., EcoRI, HindIII, and BamHI are the most widely used in molecular cloning and genetic engineering. Type III restriction enzymes cleave DNA approximately 20-30 base pairs away from the recognition sequence. Their activity depends on ATP and Mg^{2+}, while SAM enhances the reaction but is not essential. These enzymes function as complexes associated with methylation activity and exhibit a partially predictable cleavage pattern. The examples of Type III RE includes EcoP1, EcoP15I and Hinf III. Type IV restriction enzymes specifically recognize and cleave chemically modified DNA, such as methylated or hydroxymethylated DNA and help restrict foreign DNA that may escape other bacterial defense mechanisms.

Table: Important features of different types of restriction enzymes

Features	Type I	Type II	Type III
Discovery	First discovered	Most widely studied	Discovered after Type I
Recognition site	Specific DNA sequence	Specific palindromic DNA sequence	Specific DNA sequence
Cutting site	Cuts DNA far away (1000+ bp) from recognition site	Cuts at or very near the recognition site	Cuts ~20–30 bp away from recognition site
Cofactors required	ATP, Mg^{2+}, S-adenosyl methionine (SAM)	Mg^{2+} only	ATP, Mg^{2+}, SAM
Enzyme structure	Single large multifunctional enzyme	Separate restriction enzyme and methylase	Two subunits (restriction + methylation)
Cleavage pattern	Random and unpredictable	Predictable and precise	Semi-predictable
Biological role	Host defense	Host defense	Host defense
Use in genetic engineering	Not useful	Highly useful	Limited use
Examples	EcoKI	EcoRI, HindIII, BamHI	EcoP1

Restriction enzymes can be categorized based on similarities in their recognition and cleavage patterns. Isoschizomers are enzymes that recognize identical DNA sequences and cleave them at the same position, such as HpaII and MspI, which cut the sequence 5′C↓CGG3′ on one strand and 3′ GGC↓C5′ on other strand. Neoschizomers recognize the same DNA sequence but cleave at different positions; examples include SmaI and XmaI, which recognize the sequence 5′CCCGGG3′ but SmaI cuts bluntly between the third C and first G as 5′CCC↓ GGG3′ on one strand and 3′GGG ↓CCC5′ on other strand while XmaI cuts asymmetrically, producing sticky ends as 5′C↓ CCGGG3′ on one strand and 3′GGGCC↓C5′ on other strand. Isocaudomers, on the other hand, recognize different DNA sequences but generate identical cohesive or sticky ends, allowing compatible ligation; for example, BamHI and BglII both produce a common 5′GATC3′ sticky end butr have different recognition sequence (table 2).

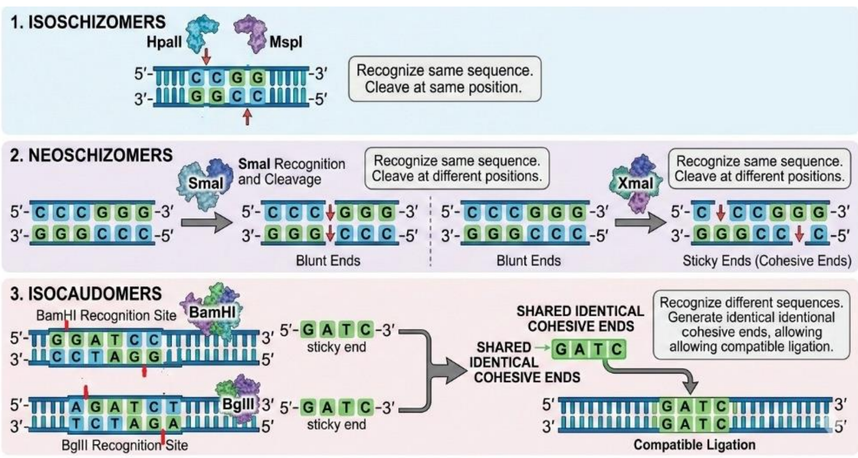

Fig.: Recognition and restriction sites in isoschizomer, neoschizomer and isocaudomer

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Table 2: Sources, recognition and restriction site for some common restriction endonucleases

Enzyme	Obtained from	Recognition Sequence (5’→3’)	Restriction Site
EcoRI	*Escherichia coli*	GAATTC	G↓AATTC
EcoRII	*Escherichia coli*	CCWGG (W = A/T)	C↓CWGG
BamHI	*Bacillus amyloliquefaciens*	GGATCC	G↓GATCC
BglII	*Bacillus globigii*	AGATCT	A↓GATCT
HindIII	*Haemophilus influenzae*	AAGCTT	A↓AGCTT
PstI	*Providencia stuartii*	CTGCAG	C↓TGCAG
SalI	*Streptomyces albus*	GTCGAC	G↓TCGAC
XhoI	*Xanthomonas holcicola*	CTCGAG	C↓TCGAG
NotI	*Nocardia otitidiscaviarum*	GCGGCCGC	GC↓GGCCGC
SmaI	*Serratia marcescens*	CCCGGG	CCC↓GGG (blunt cut)
ApaI	*Acinetobacter calcoaceticus*	GGGCCC	GGG↓CCC

Exonucleases

In addition to restriction endonucleases, exonucleases play an important role in DNA processing. Exonucleases are specialized enzymes that act on nucleic acids by progressively removing nucleotides from the ends of DNA or RNA molecules. Unlike endonucleases that cut internally, exonucleases work in a stepwise fashion, cleaving one nucleotide at a time from either the 5′ or the 3′ end, depending on their directional specificity. This gradual trimming process is essential in several cellular functions including DNA replication in which exonucleases contribute to proofreading by excising incorrectly paired bases. In E. coli, the 5′ - 3′ exonuclease activity of DNA polymerase I is crucial for removing RNA primers during replication. By carefully controlling the ends of nucleic acid molecules, exonucleases maintain genetic stability and ensure the fidelity of information transfer across generations. BAL-31 is an exonuclease that degrades both the 3′ and 5′ termini of double-stranded DNA and can also act at internal nicks that create free DNA ends. Some exonucleases act specifically from the 3′ end; these enzymes remove nucleotides sequentially from the 3′-hydroxyl terminus of duplex DNA and can act on blunt ends, 5′ overhangs, or internal nicks, but they cannot digest DNA containing 3′ overhangs. Exonuclease III is a common example of this feature. Other exonucleases act from the 5′ end of DNA, such as λ exonuclease and T5 exonuclease. Mung bean nuclease is a single-strand-specific endonuclease that degrades single-stranded regions of DNA or RNA. It efficiently removes both 5′ and 3′ overhangs to produce blunt-ended DNA fragments and is therefore widely used in molecular cloning techniques.

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