Enzymes in Genetic Engineering

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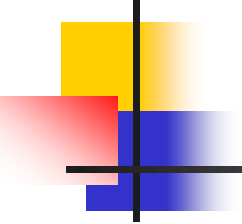
Enzymes

- Enzymes used in plant biotechnology/ genetic engineering can be grouped into four broad classes, depending on the type of reaction that they catalyze:

- **Nucleases** are enzymes that cut, shorten, or degrade nucleic acid molecules.

- **Ligases** join nucleic acid molecules together.

- **Polymerases** make copies of molecules.

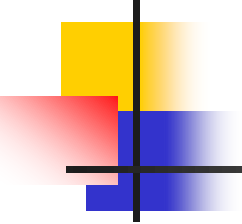- **Modifying enzymes** remove or add chemical groups.

Nucleases

- 'Nucleases degrade DNA molecules by breaking the phosphodiester bonds that link one nucleotide to the next in a DNA strand. In addition to their important biological role, nucleases have emerged as useful tools in laboratory studies, and have led to the development of such fields as recombinant DNA technology, molecular cloning, and genomics'.

- "Processes under control of nucleases are for example protective mechanisms against "foreign" (invading) DNA, degradation of host cell DNA after virus infections, DNA repair, DNA recombination, DNA synthesis DNA packaging in chromosomes and viral compartments, maturation of RNAs or RNA splicing."

- Nucleases are phosphoidesterases with a tremendous variability in their substrate requirements. There are two different kinds of nuclease

- **Exonucleases** remove nucleotides one at a time from the end of a DNA molecule.
- **Endonucleases** are able to break internal phosphodiester bonds within a DNA molecule.

Classification

- They are classified by their specificity of their requirement for either a free end (exo) to start working or they start from anywhere within a molecule (endo) even when no free ends are available as for example in a covalently closed circle

Exonucleases

- The main distinction between different exonucleases lies in the number of strands that are degraded when a double-stranded molecule is attacked.

- For example Bal31 degrades both strand and *E. coli* exonuclease III degrades only one strand and only from the 3' terminus.
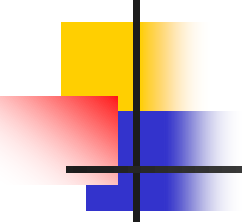
Endonucleases

- The same criterion can be used to classify endonucleases

- 'S1 endonuclease cleaves single strand where as DNase I cuts both single and double-stranded molecules'

- Restriction enzymes are the special group of endonucleases that cleaves double stranded DNA only at a limited number of specific recognition sites

Restriction endonucleases the enzymes for cutting DNA

- The discovery of these enzymes, led to Nobel Prizes for W. Arber, H. Smith, and D. Nathans in 1978

- Restriction endonucleases are synthesized by many, perhaps all, species of bacteria: over 2500 different ones have been isolated and more than 300 are available for use in the laboratory.
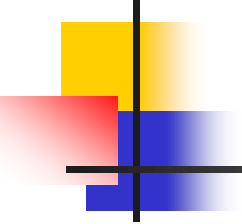
- Five different classes of restriction endonuclease are recognized, each distinguished by a slightly different mode of action.

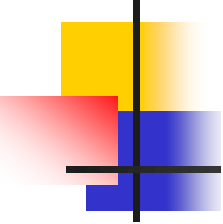- Types I and III are rather complex and have only a limited role in genetic engineering.

Type I

- Type I restriction enzymes were the first to be identified and were first identified in two different strains (K-12 and B) of *E. coli* . For example EcoK . These enzymes cut at a site that differs, and is a random distance (at least 1000 bp) away, from their recognition site. Cleavage at these random sites follows a process of DNA translocation, which shows that these enzymes are also molecular motors.

- 'The cofactors S-Adenosyl methionine (AdoMet), hydrolyzed adenosine triphosphate (ATP), and magnesium (Mg^{2+}) ions, are required for their full activity. '
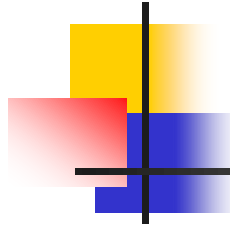
- The recognition site is asymmetrical and is composed of two specific portions—one containing 3–4 nucleotides, and another containing 4–5 nucleotides—separated by a non-specific spacer of about 6–8 nucleotides.

- These enzymes are multifunctional and are capable of both restriction and modification activities, depending upon the methylation status of the target DNA.

Type III

- Type III restriction enzymes (e.g. EcoP15 and *Bsm* FI) recognize two separate non-palindromic sequences that are inversely oriented. They cut DNA about 20-30 base pairs after the recognition site
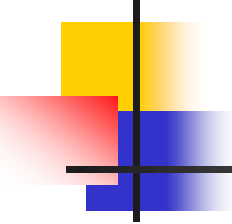
- Type II restriction endonucleases, on the other hand, are the cutting enzymes that are important in gene cloning.

- The central feature of type II restriction endonucleasesis that each enzyme has a specific recognition sequence at which it cuts a DNA molecule.

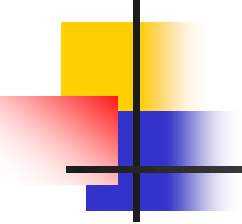# Recognition sequences for some restriction endonucleases.

- ENZYME   ORGANISM   RECOGNITION SEQUENCE*   BLUNT OR STICKY END

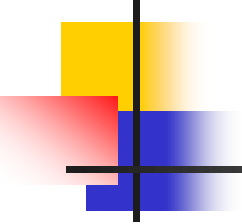| ENZYME	ORGANISM	RECOGNITION SEQUENCE*	BLUNT OR STICKY END
*Eco*RI	*Escherichia coli*	GAATTC	Sticky
*Bam*HI	*Bacillus amyloliquefaciens*	GGATCC	Sticky
*Bgl*II	*Bacillus globigii*	AGATCT	Sticky
*Pvu*I	*Proteus vulgaris*	CGATCG	Sticky
***Pvu*II**	***Proteus vulgaris***	**CAGCTG**	**Blunt**
*Hin*dIII	*Haemophilus influenzae*	AAGCTT	Sticky
***Alu*I**	***Arthrobacter luteus***	**AGCT**	**Blunt**
*Taq*I	*Thermus aquaticus*	TCGA	Sticky

G A A T T C
C T T A A G

C C C G G G
G G G C C C

- The exact nature of the cut produced by a restriction endonuclease is of considerable importance in the design of a gene cloning experiment.

- Many restriction endonucleases make a simple double-stranded cut in the middle of the recognition sequence, resulting in a **blunt end** or **flush end**.
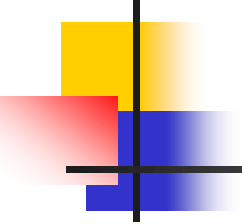
- Other restriction endonucleases cut DNA in a slightly different way. With these enzymes the two DNA strands are not cut at exactly the same position.

- Instead the cleavage is staggered, usually by two or four nucleotides, so that the resulting DNA fragments have short single-stranded overhangs at each end

- **Type IV**

- Type IV enzymes recognize modified, typically methylated DNA and are exemplified by the McrBC and Mrr systems of *E. coli*

- It requires GTP for DNA cleavage

- It has methyltransferase (MTase) and endonuclease (ENase) activity, and are combined together in one polypeptide chain and the ENase activity is positively affected by S-adenosine-L-methionine (AdoMet) but ATP has no influence on activity of the enzymes.

Type V

- Type V restriction enzymes (e.g., the cas9-gRNA complex from CRISPRs) utilize guide RNAs to target specific non-palindromic sequences found on invading organisms. They can cut DNA of variable length, provided that a suitable guide RNA is provided. The flexibility and ease of use of these enzymes make them promising for future genetic engineering applications
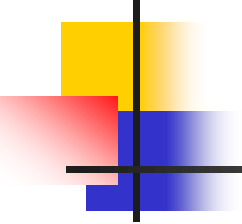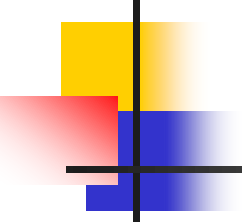
Ligases

- The function of DNA ligase is to repair single-stranded breaks ("discontinuities") that arise in double-stranded DNA molecules during, for example, DNA replication.

- DNA ligases from most organisms can also join together two individual fragments of double-stranded DNA

- The final step in construction of a recombinant DNA molecule is the joining together of the vector molecule and the DNA to be cloned.

- All living cells produce DNA ligases, but the enzyme used in genetic engineering is usually purified from *E. coli* bacteria that have been infected with T4 phage.
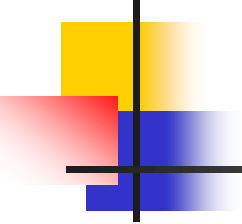
- Within the cell the enzyme carries out the very important function of repairing any discontinuities

- Although discontinuities may arise by chance breakage of the cell's DNA molecules, they are also a natural result of processes such as DNA replication and recombination.
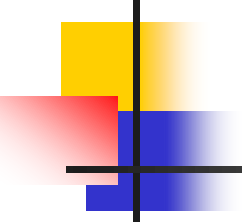
Polymerases

- DNA polymerases are enzymes that synthesize a new strand of DNA complementary to an existing DNA or RNA template.

- Most polymerases can function only if the **template** possesses a double-stranded region that acts as a **primer** for initiation of polymerization.

- Four types of DNA polymerase are used routinely in genetic engineering. The first is DNA polymerase I, which is usually prepared from *E. coli*. This enzyme attaches to a short single-stranded region (or **nick**) in a mainly double-stranded DNA molecule, and then synthesizes a completely new strand, degrading the existing strand as it proceeds.

- DNA polymerase I is therefore an example of an enzyme with a dual activity—DNA polymerization and DNA degradation.

- The polymerase and nuclease activities of DNA polymerase I are controlled by different parts of the enzyme molecule.

- The nuclease activity is contained in the first 323 amino acids of the polypeptide, so removal of this segment leaves a modified enzyme that retains the polymerase function but is unable to degrade DNA.

- This modified enzyme, called the **Klenow fragment**, can still synthesize a complementary DNA strand on a single-stranded template, but as it has no nuclease activity it cannot continue the synthesis once the nick is filled in

Taq DNA polymerase is DNA polymerase I

- The *Taq* DNA polymerase used in the polymerase chain reaction (PCR) is the DNA polymerase I enzyme of the bacterium *Thermus aquaticus*.

Reverse transcriptase

- The final type of DNA polymerase that is important in genetic engineering is **reverse transcriptase**, an enzyme involved in the replication of several kinds of virus. Reverse transcriptase is unique in that it uses as a template not DNA but RNA.

- The ability of this enzyme to synthesize a DNA strand complementary to an RNA template is central to the technique called complementary DNA (cDNA) cloning.
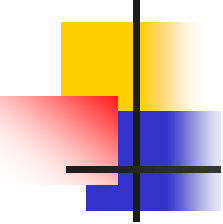
DNA modifying enzymes

- There are numerous enzymes that modify DNA molecules by addition or removal of specific chemical groups. The most important are as follows:
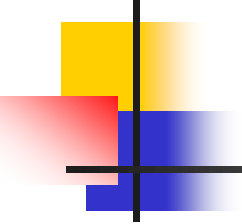
- **Alkaline phosphatase** (from *E. coli*, calf intestinal tissue, or arctic shrimp), which removes the phosphate group present at the **5′ terminus** of a DNA molecule.

- **Polynucleotide kinase** (from *E. coli* infected with T4 phage), which has the reverse effect to alkaline phosphatase, adding phosphate groups onto free 5′ termini.

- **Terminal deoxynucleotidyl transferase** (from calf thymus tissue), which adds one or more deoxyribonucleotides onto the **3′ terminus** of a DNA molecule.
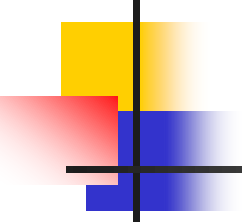
Homopolymer OR T/A Tailing

- **Homopolymer OR T/A Tailing**- The important component in this method is terminal deoxynucleotidyl transferase. This enzyme adds nucleotides at the 3 -OH end of DNA without any complementary sequence. It can add up to 10-40 nucleotide which can be a single type nucleotide (homopolymer) residue at the end. This method can be applied to both the vector and insert simultaneously.

- This method uses the ability of annealing of complementary strands or sequences. Suppose a vector has an oligo(dA) sequence at the 3 -OH end and the insert has an oligo(dT) sequence at its 3 -OH end. Then when both the molecules are mixed, the molecules are held by hydrogen bond or can anneal until the ligase joins them by phosphodiester bond.

- Brown, T. A. (2016). *Gene cloning and DNA analysis: an introduction*. John Wiley & Sons.
- Free web resources