BIOTECHNOLOGY
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Lecture: 2

Restriction Enzymes

restriction enzyme, also called **restriction endonuclease**, a protein produced by bacteria that cleaves DNA at specific sites along the molecule. In the bacterial cell, restriction enzymes cleave foreign DNA, thus eliminating infecting organisms. Restriction enzymes can be isolated from bacterial cells and used in the laboratory to manipulate fragments of DNA.

Restriction enzymes were discovered and characterized in the late 1960s and early 1970s by molecular biologists Werner Arber, Hamilton O. Smith, and Daniel Nathans. The ability of the enzymes to cut DNA at precise locations enabled researchers to isolate gene-containing fragments and recombine them with other molecules of DNA—i.e., to clone genes. The names of restriction enzymes are derived from the genus, species, and strain designations of the bacteria that produce them; for example, the enzyme *Eco*RI is produced by *Escherichia coli* strain RY13. It is thought that restriction enzymes originated from a common ancestral protein and evolved to recognize specific sequences through processes such as genetic recombination and gene amplification.

An endonuclease is a group of enzymes that cleave the phosphodiester bond present within a polynucleotide chain.

Endonucleases are capable of breaking the bond from the middle of a chain.

These enzymes are either specific or non-specific to the sequences being cleaved. The endonucleases that are specific to a particular sequence are termed restriction endonucleases.

Restriction endonucleases are obtained from various bacteria and archaea, each of which is specific for different sites in the polynucleotide chain.

Exonucleases are enzymes that cleave DNA sequences in a polynucleotide chain from either the 5’ or 3’ end one at a time.

Exonuclease, like endonuclease, is a hydrolyzing enzyme that cleaves the phosphodiester bond between the nucleotides. Exonulceases are important during replication as one of these enzymes works together with RNA polymerase II degrade the newly formed RNA primer present on the new transcript which is then replaced by DNA nucleotides.

In molecular biology it is used as a restriction enzyme. *Eco*RI creates 4 nucleotide sticky ends with 5' end overhangs of AATT. The nucleic acid recognition sequence where the enzyme cuts is G↓AATTC, which has a palindromic, complementary sequence of CTTAA↓G. Other restriction enzymes, depending on their cut sites, can also leave 3' overhangs or blunt ends with no overhangs.

EcoRI digestion produces "sticky" ends,

whereas SmaI restriction enzyme cleavage produces "blunt" ends:

Recognition sequences in DNA differ for each restriction enzyme, producing differences in the length, sequence and strand orientation (5' end or 3' end) of a sticky-end "overhang" of an enzyme restriction.

FACTORS THAT AFFECT RESTRICTION ENZYME ACTIVITY

The digestion activity of restriction enzymes depends on the following factors:

- Temperature: Most endonucleases digest the target DNA at 37 °C with few exceptions. Some work at lower temperatures (~25 °C, *Sma* 1) while *Taq* I works at 65 °C.
- Cofactors: Restriction endonucleases require certain cofactors or combination of cofactors to digest at the recognition site. All enzymes require Mg^{2+} as a cofactor for the endonuclease activity. In R-M systems with separate proteins having the restriction and methylation activities, S-adenosylmethionine (SAM) and ATP are required for methylation activity.
- Ionic Conditions: As mentioned previously, Mg^{2+} is required for all endonucleases but some enzymes also require ions such as Na^{+} and K^{+}.
- Buffer systems: Most restriction enzymes are active in the pH range of 7.0–8.0. Tris-HCl, a temperature-dependent buffer, is the most commonly used buffer.
- Methylation status of DNA: Methylation of adenine or cytidine residues affects the digestion of DNA.

APPLICATIONS OF RESTRICTION ENZYMES

Restriction endonucleases are widely used in molecular biology research for the following applications:

Genetic Engineering: The most popular application of restriction endonucleases is as a tool for genetic engineering. The endonuclease activity enables manipulation of the genome as well as introduction of sequences of interest in the host organism. This results in the production of the desired gene product by the host. This concept has wide range of applications in biotechnology in the production of antibiotics, antibodies, enzymes, and several secondary metabolites.

DNA mapping: DNA mapping using restriction enzymes (also known as restriction mapping) is a method to obtain structural information of the DNA fragment. In this technique the DNA is digested with a series of restriction enzymes to produce DNA fragments of various sizes. The resultant fragments are separated by agarose gel electrophoresis and the distance between the restriction enzyme sites can be estimated. This can be used to determine the structure of an unknown DNA fragment.

Gene Sequencing: A large DNA molecule is digested using restriction enzymes and the resulting fragments are processed through DNA sequencer to obtain the nucleotide sequence.

The other applications of restriction endonucleases include gene expression and mutation studies and examination of population polymorphisms.