Chapter 31
Gene Cloning

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ABSTRACT

The discovery of two naturally occurring biological molecules, plasmid DNA and restriction enzymes, with remarkable properties have made possible the development of methods to isolate and manipulate specific DNA fragments. Through this technology, a DNA fragment, even an entire gene and its controlling elements, can be isolated and rejoined with a plasmid or phage DNA, and the hybrid DNA molecule can be inserted into a bacterium. The foreign DNA insert can be multiplied inside the bacterial host and induced to express or synthesize the protein product of the foreign DNA. The entire process through which this can be achieved is called recombinant DNA technology or genetic engineering. The recombinant DNA technology has been extended to animal and plant cells. In this chapter, methods for isolation, modification, rejoining and replication of genomic DNA, and production of new or enhanced protein products within a host cell have been described.

INTRODUCTION

During early 1970s several new methodologies were developed which have revolutionized the science of modern genetics. The discovery of two naturally occurring biological molecules with remarkable properties have made possible the development of methods to isolate and manipulate specific DNA fragments. These two molecules are plasmid DNA and restriction enzymes. Through this technology, a DNA fragment, even an entire gene and its controlling elements, can be isolated and rejoined with a plasmid or phage DNA, and the hybrid DNA molecule can be inserted into a bacterium. The foreign DNA insert can be multiplied inside the bacterial host and induced to express or synthesize the protein product of the foreign DNA. The entire process through which this can be achieved is called recombinant DNA technology or genetic engineering. A fragment of DNA, representing a gene, when inserted into a vector, leading to the production of a recombinant DNA molecule, is inserted into a host cell, where it is multiplied and passed to its progeny, the inserted gene in the recombinant molecule is said to be cloned. This chapter describes how genomic DNA can be cut, modified, rejoined and replicated, ultimately to produce new or enhanced protein products, within a host cell.

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TOOLS FOR GENE CLONING

For cloning and manipulation of genes, the genetic material (DNA or RNA) requires to be cut, modify and rejoin in the desired manner. Enzymes play a major role in these activities, which are described below.

Enzymes for Cutting DNA Molecules

In a DNA molecule, the phosphodiester bonds that link the nucleotides can be broken by treatment with nucleases. Two different types of nucleases are found: exonucleases (Figure 1a), which remove nucleotides from the end of a DNA strand, one at a time and endonucleases (Figure 1b), which breaks the phosphodiester bonds within a strand of DNA. Exonuclease like Ba131(derived from the bacterium *Alteromonas espejiana*) removes nucleotides from both strands of double-stranded molecule, in contrast enzymes exonuclease III (derived from *E. coli*) degrade only one strand of a double-stranded DNA molecule.

Similarly, S1 endonuclease (derived from fungus *Aspergillus oryzae*) cleaves single strand, both single and double stranded molecules can be cut by DNAse I (derived from pancreas of cow). DNase I is non-specific *i.e* it attack phosphodiester bond of DNA at any site. However, a special group of enzymes called restriction endonucleases cleave double stranded DNA at a specific recognition sites. Thus, this group of enzymes has been utilized in gene cloning.

Discovery of restriction enzymes played a major role in gene cloning. Restriction enzymes were discovered while investigating the mechanism of host-specific restriction in bacteriophages. The first duplex DNA cutting enzyme, called restriction enzyme, was discovered from *Haemophilus influenza* in 1970. It was observed that the bacteria are protected by restriction enzymes against virus infections and appear to serve a host-defense role. When virus particles are used to infect a strain of *E. coil* lacking a restriction enzyme, infection will always be successful. However, if the same strain contains a restriction enzyme, the probability of successful infection is reduced. The presence of additional restriction enzymes has multiple effects, and could make the bacteria virtually impregnable (Brown, 2015). The question that immediately comes to mind is why the restriction enzyme does not chew up the genomic DNA of their host? The answer lies on the fact that in almost all cases, a bacterium also synthesizes another enzyme called DNA methyltransferase, which protects the DNA target sequence, involved in restriction digestion, by methylation. Such interaction between restriction-endonuclease and DNA-methylase is called restriction-modification systems.

Restriction enzymes are found only in prokaryotes and few viruses. Their presence has been reported in thousands of bacteria and archaea. Many types of restriction endonucleases have been isolated from a wide variety of bacteria. Each enzyme is represented by a three-letter code in italics. For example, *Hin* for *Haemophilus influenza*, *Hae* for *Haemophilus aegypticus*, *Eco* for *Escherichia coli* etc. Sometimes a four-letter code is used when the enzymes are isolated from different serotypes of a species. For example, *Hin*f for *Haemophilus inflenzae* serotype f. In those case where more than one restriction enzyme is isolated from a single source (bacterium), they are denoted by Roman numerals *e.g* I, II, III etc. For example *Hin*dII, for the second enzyme of *Haemophilus influenza* serotype d.

Restriction enzymes vary in size from 157 (diminutive) to 1250 amino acids (giant *Cje*I). Over 3000 restriction enzymes have been purified and characterized, and about 250 of them show different sequence-specificities activities. Restriction enzymes with new specificities are found regularly.