Recombinant DNA Technology

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Abstract

Recombinant DNA or rDNA technology refers to the creation of a hybrid or chimeric DNA by inserting a foreign sequence into the DNA of another species. It starts with extracting the gene of interest by using an appropriate restriction endonuclease enzyme and incorporating this gene into a suitable self-replicating vector such as a plasmid. This is made possible by cleaving the vector's DNA using the same restriction endonuclease and sealing it back using DNA ligase. Finally, the vector is inserted into the host such as *E. coli* or *Saccharomyces cerevisiae*. Now the plasmid will multiply and the translation machinery of the E. coli will synthesize proteins from this plasmid. rDNA technology has been used to synthesize hormones, vaccines, drugs like alpha-interferon, and genetically modified food, and in gene therapy. Two similarly manufactured recombinant DNA technology products are termed *biosimilars* as opposed to generic drugs. In 2012, the draft guidelines were launched on "Similar Biologics: Regulatory Requirements for Marketing Authorization in India."

Keywords

Recombinant DNA (rDNA) technology · Genetic engineering · DNA restriction enzymes

34.1 Introduction

- Recombinant DNA or rDNA technology refers to the creation of a hybrid or chimeric DNA by inserting a foreign sequence into the DNA of another species.

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- Foreign DNA can be inserted into the pre-existing genome of an organism (gene therapy), or the recombinant DNA can be created ex vivo and then inserted inside the host to synthesize proteins of interest.
- The products obtained by rDNA technology are termed as *biologics*, and their generic versions are termed as *biosimilars*. Biologic is a broad term which includes any product obtained from a living system. Other biologics include monoclonal antibodies.

34.2 Steps in the Synthesis of an rDNA (Fig. 34.1)

- **Choosing a DNA of interest**
  - This depends on the protein end product, for example, insulin can be synthesized from the human gene which codes for insulin.
- Getting multiple copies of this DNA using the *polymerase chain reaction (PCR)*
- *Extracting this gene* by using an appropriate *restriction endonuclease enzyme*
- **Choosing a suitable vector such as a plasmid or bacteriophage virus**
  - This vector should be *self-multiplying* after it reaches the host cell. Hence, the vector should have genes like initiation codon and promoter regions.
  - The vector should also have a genetic marker for selection (antibiotic resistance genes) containing restriction sites and minimum nonessential DNA.
- **Cleaving the vector's DNA using the same restriction endonuclease**
  - The sticky ends thus created should be able to accept the previously isolated DNA of interest. The vector's DNA can be sealed back using *DNA ligase*.

34.3 Steps in Synthesis of Protein from the Generated rDNA

- **Inserting the vector into the host**
  - The host can be *E. coli* or *Saccharomyces cerevisiae* as their genome has been studied enormously. *Bacteria are preferred as they are easy to grow and maintain, they multiply easily, and bacterial plasmids can be manipulated easily*.
  - The vector can be inserted using techniques such as transduction (if the vector is a bacteriophage) or conjugation (for plasmid vectors).
- Now the plasmid will multiply, and the translation machinery of the *E. coli* will synthesize proteins from this plasmid. Hence, we will get human insulin from *E. coli*. This will be called as recombinant insulin.

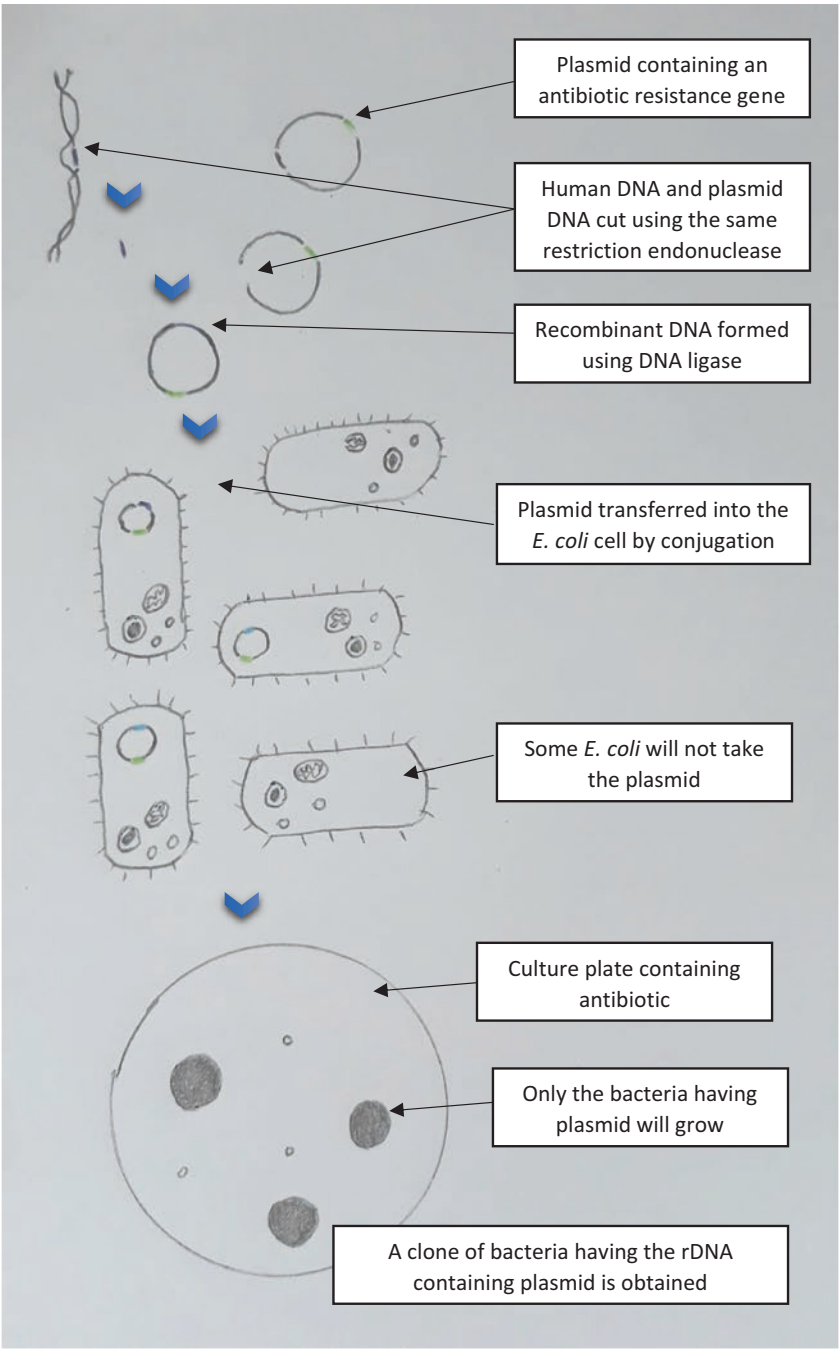

Fig. 34.1 Steps in obtaining bacteria with rDNA plasmid

34.4 Challenges in Recombinant DNA Technology

34.4.1 Manufacturing

- Only some *E. coli* cells will accept the plasmid. The challenge is to identify which ones have accepted. For this, *a plasmid containing antibiotic resistance genes is used*.
- When the *E. coli* cultures are exposed to antibiotics, only those which have incorporated the plasmid survive.

34.4.2 Safety

- *Unexpected adverse events* are more likely to happen with recombinant products, due to chances of impurities in the manufacturing process. For example, minor change in the packaging process of erythropoietin caused pure red cell aplasia.

34.4.3 Dispensing

- Noninterchangeable: *biotechnology products must be prescribed by brand name* and cannot be substituted like other generics.

34.5 Uses of Recombinant DNA Technology

- *Synthesis of hormones* such as insulin, erythropoietin, and growth hormone
  - Recombinant technology allows mass production of safe products, free from foreign antigens, and in case of insulin, recombinant insulins have replaced older products like bovine insulin.
- *Vaccines* such as hepatitis B
- Therapeutic *drugs* like alpha interferon, blood clotting factor VIII, and streptokinase.
- *Genetically modified food* (GM food), which may increase yield, impart resistance to pests or modify quality, e.g., BT cotton which produces a pesticide.
- *Gene therapy*: replacement of disease-causing gene with a normal gene such as cystic fibrosis, hemophilia, muscular dystrophy, and sickle cell anemia (Refer to Chap. 23 on Gene therapy).

34.6 Development of Regulatory Guidelines

Guidelines that control recombinant DNA technology have been developed due to concerns about the following:

- Use of plasmids with various combinations of *antibiotic resistance genes*. The product from the antibiotic resistance genes can enter into humans along with the protein of interest.
- Moving DNA among various species is a *departure from the natural evolution* and selection of species.
- *Health effects* of genetically modified food.
- Regulations are comparatively recently formed: "Draft Guidelines on Similar Biologics: Regulatory Requirements for Marketing Authorization in India" were announced in 2012, by the Department of Biotechnology (DBT) to address the pre-marketing and post-marketing regulatory requirement.
- Getting marketing approval for generic versions of recombinant products is not the same as other drugs. They are considered as new products and therefore require the permission of the Drug Control Authority for both import and local production for marketing purposes.
  - As opposed to simple chemical drugs, where bioequivalence studies can be done to prove similarity, in case of biosimilars, *the safety, potency, and efficacy have to be proven again as no two biosimilar products are exactly similar.* This is because of the complexity in manufacturing these products.

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