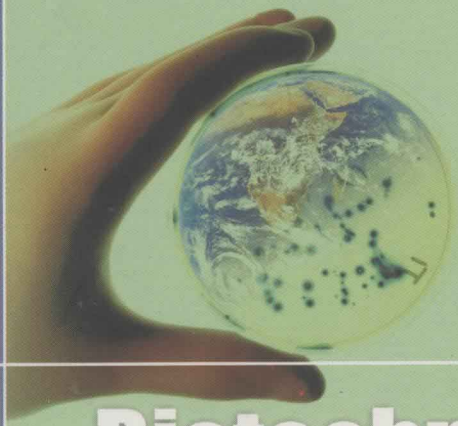

Biotechnology

Fundamentals and Applications

Ashok Ganguli

Biotechnology:

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Preface

Biotechnology is the science which involves the use of biological processes and organisms for medical, industrial or manufacturing purposes. Humans have long used yeast for brewing and bacteria for products such as cheese and yoghurt, but it has only been in the 20th century that a wide-seal application of biotechnological principles has been possible. By growing microorganisms in the laboratory, new drugs and chemicals are produced. Genetic engineering techniques of cloning, splicing and mining genes, facilitate, for example, the growing of crops outside their normal environment, and diseases. Hormones are also produced, such as insulin for treating diabetes.

Biotechnology today is amongst the most relevant, as well as the most exciting sciences to emerge in the last quarter of the last century. Not only dos it widen the scope of scientific intervention in human life, lent also offers prospects to improve upon them. This book has been conceptualised and designed not just as a manual which elucidates upon the basic principles and practices which determines biotechnology, but also as a manual which takes into consideration the issues, dilemmas and concerns which are central to the subject. Taking into account contemporary trends and development the book also incorporates critical perspectives which aid in enhancing the context of the book, in terms of depth.

Editor

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Contents

1

Principles of Genetic Engineering

Genetic engineering is a laboratory technique used by scientists to change the DNA of living organisms. DNA is the blueprint for the individuality of an organism. The organism relies upon the information stored in its DNA for the management of every biochemical process. The life, growth and unique features of the organism depend on its DNA. The segments of DNA which have been associated with specific features or functions of an organism are called **genes**.

Molecular biologists have discovered many enzymes which change the structure of DNA in living organisms. Some of these enzymes can cut and join strands of DNA. Using such enzymes, scientists learned to cut specific genes from DNA and to build customised DNA using these genes. They also learned about **vectors**, strands of DNA such as viruses, which can infect a cell and insert themselves into its DNA.

With this knowledge, scientists started to build vectors which incorporated genes of their choosing and used the new vectors to insert these genes into the DNA of living organisms. Genetic engineers believe they can improve the foods we eat by doing this. For example, tomatoes are sensitive to frost. This shortens their growing season. Fish, on the other hand, survive in very cold water. Scientists identified a particular gene which enables a flounder to

resist cold and used the technology of genetic engineering to insert this 'anti-freeze' gene into a tomato. This makes it possible to extend the growing season of the tomato.

IMPORTANCE OF GENE EXPRESSION

Genes are DNA (deoxyribonucleic acid) sequences that control traits (characteristics) in organisms by coding for the production of individual proteins. This genetic code consists of 4 nucleotides: adenine (A), guanine (G), cytosine (C), and thymine (T).

DNA sequences in all living things use these same 4 nucleotides, but the number and order of them vary among species. This important fact makes genetic engineering possible because this way, DNA sequences can be transferred from one organism to another and still be expressed (or read) to create individual proteins.

For thousands upon thousands of years, the genetics of all living things have changed through natural or human selection of traits to help organisms become better adapted to the natural environment or express characteristics preferred by humans.

The main purpose of intentionally changing the genetics in certain organisms is to remove or add characteristics not present ordinarily. But how do living things know which protein to make and in what part of the organism? This is where DNA and genes become crucial. The 2 main steps in this process are transcription (copying DNA into mRNA, a temporary molecule to transport genetic information) and translation (translating mRNA code into specific proteins).

Effect of Transcription

DNA is too valuable to use in translation in the ribosome so instead, a copy (mRNA) is made during transcription. When a specific protein is needed in a specific cell, the

gene's promoter sequence turns on the gene itself. Attached to this promoter sequence is RNA (ribonucleic acid) polymerase, an enzyme needed for transcription.

RNA polymerase travels along the gene's promoter sequence until it reaches a series of A and T nucleotides called the TATA box. This TATA box signals to RNA polymerase that it has reached the end of the promoter and now has the "green light" to read the coding region of the DNA to make the RNA copy.

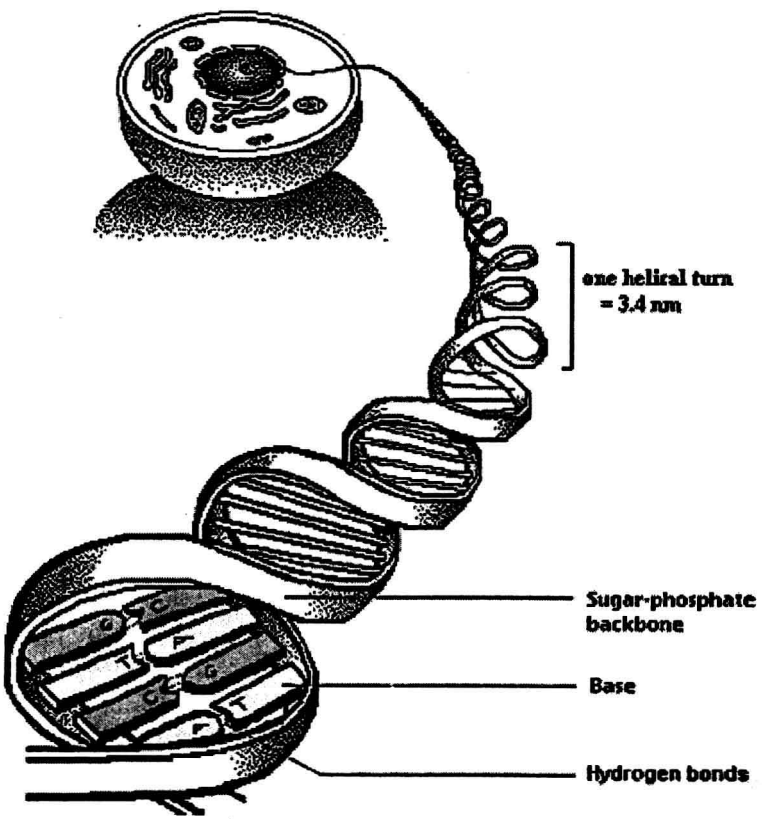

Figure 1: Structure of DNA

If promoter sequences didn't exist, RNA polymerase would keep travelling along the DNA strand meaning that other genes, on the same chromosome, might be expressed in the wrong cells or at the wrong time. Then, the termination region signals that it has reached the end of the gene.

Effect of Translation

Translation involves converting mRNA code into a chain of amino acids, which in turn forms specific proteins. This happens inside the ribosome (site of protein production in cells) along with the help of tRNA. tRNAs are small sections of knob shaped RNA sequences with 3 nucleotides near the middle, forming the anti-codon. This anti-codon becomes one end of the tRNA molecule while the other end (codon) attaches to a specific amino acid coded for by the anit-codon. tRNAs attach amino acids one at a time to eventually produce a full protein molecule.

Ribosomes read mRNA quickly and accurately, but it must know where to start. Ribosomes "look" for the sequence AUG or the start codon to signal where they should begin building the protein sequence. From this point, ribosomes read the continuous chain of nucleotides in groups of three (anti-codons) and continue placing amino acids in the correct position in the growing protein chain according to the instructions in the mRNA sequence.

Typically, proteins are several hundred or more amino acids in length. When a complete protein is built, the ribosome leaves the current mRNA strand and looks for another mRNA strand to begin translation. Because proteins are hundreds of amino acids long, the tRNA molecules must be recycled by the cell so they can contribute to the production of another protein. Each time a ribosome translates a mRNA strand, one copy of a protein is made.

TYPES OF TRADITIONAL PLANT BREEDING

Genetic modification also includes traditional plant breeding, not just genetic engineering. There are 3 types of traditional plant breeding:

Natural Crossbreeding

— Varieties naturally selected by environment
— Natural Breeding

Before humans, genes continually changed (by random mutations) and evolved to form new, better adapted species. Because the natural environment promoted these changes, we called this process natural selection.

Open-pollination

— Varieties selected by humans
— Natural Breeding

Plants pollinate (transfer of pollen from male to female flower parts for the purpose of fertilisation) with the help of insects, birds, wind and water. With open-pollination, plants breed naturally, but humans select which individuals carrying on to the next generation.

Selective Crossbreeding

— Varieties selected by humans
— Artificial Breeding i.e. human intervention in the natural cross-breeding process

Hybrids plants are produced by crossing two or more inbred lines that are genetically quite different from each other. They have extra growth vigour or heterosis. However, hybrids are usually either sterile or produce offspring that do not perform well (much like donkeys).

Before the hybrid is produced, parent plants are made to be inbred for several generations until a consistent plant

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phenotype is seen (more homozygous). Then these inbred plants are cross-bred with other inbred lines so the plants become more heterozygous again. This way a breeder can balance the 4 inbred lines in a way that the desirable traits outweigh the undesirable ones. The majority of the varieties commonly seen today were developed by producing a double hybrid cross using 4 inbred lines that would complement each other (each of which was selected for some particular trait of interest):

Commercial seed companies produce these hybrid seeds for farmers to purchase every year for planting (this is much more common in the developed world where many farmers don't save their seed for the next growing season). With any type of genetic modification, genetic variability (or biodiversity) is needed to provide a source of genetic information from which desirable traits can be selected from by either nature or humans. For example, natural selection is only possible because of random mutations in individuals. The same is true for open-pollinated and selective crossbreeding processes because genetic variability give humans something new to chose when selecting the next generation.

Humans have searched for and created genetic diversity (created random mutations) so they could have new traits to choose from. When looking specifically at genetic engineering, there is even more genetic diversity to choose from because researchers are not limited to only looking for different traits within specific species—one can look at any species because GE allows for transferring genes across natural species boundaries. In other words, is a breeder wants to change a characteristic of corn, they're not limited to looking for different traits in other types of corn, they can look of that gene in any living thing.

Open-Pollination and Selective Cross-Breeding

For example, suppose a farmer owns a field of sweet corn.

With a keen eye, he observes that some of the corn plants grow faster than others and yet others survive a disease called "corn rust," a type of reddish-brown fungus that appears as little spots on the leaves of infected plants. What this farmer would like is corn that grows quickly but is also resistant to corn rust. The farmer gets an idea, what if seeds from the top 10% fastest growing corn plants and the healthiest plants (with the least corn rust) are collected to then be the parents for next generation? Because these 2 groups of plants have been growing in physically separate fields for several years, they are inbred enough to create a valuable single hybrid cross.

If the farmer grows these plants side by side, they will naturally crosspollinate to form hybrid offspring. When these offspring (hybrids) mature, the farmer selects the seeds (kernels) from any plants showing both these desired traits. The farmer then grows these seeds and again selects seeds from any offspring showing both traits to then be planted the following year. This process will continue for several generations until both traits are dominant (homozygous) enough that all plants show both traits. What the farmer has done is use open-pollination to create a high yielding, corn rust resistant hybrid by selecting the breeding population and then allowing natural breeding to take place.

With selective crossbreeding the farmer still selects the population, but also intervenes in the natural breeding process by physically transferring pollen from the male part of 1st plant (high yielding) to the female part of the 2nd plant (corn rust resistant). This ensures that there is always cross-pollination happening between the 2 varieties.

What any corn breeder would do, is tie a plastic bag around the corn tassels to collect the plant's pollen during its 1-2 week pollen shedding period (around July/August). Bags are also tied around the corncobs to prevent fertilisation by the wrong pollen. Then, once all the pollen

is collected, pollen from plant A is sprinkled on the silk of plant B (corn silk is the stringy white material that sticks out the top of corncobs). Each kernel is the result of an individual fertilisation of a corn silk by a single pollen grain.

Selective Crossbreeding and Genetic Engineering

If you were to record the genetic sequence (i.e. the order of A,G,C, & T nucleotides) of a wheat plant, you could fill enough books to create a 20- storey high stack represented by the column on the left in each of the pictures below. Let's say researchers are trying to improve wheat's tolerance to cold temperatures. Let's also say researchers have found that one of wheat's ancient relatives is able to grow in northern Russia because of a random mutation in a single gene 2000 years ago. Researchers decide to introduce this cold tolerance gene into the modern wheat plant. This can be done using either selective crossbreeding or genetic engineering.

In the selective crossbreeding picture, the first 2 stacks represent the genome (all the plant's genetic information) of 2 wheat plants: 1 plant is already widely grown by farmers because of its high yield but unable to grow in the cold while the other is a lower yielding ancient wheat plant with a cold tolerance gene. When researchers selectively cross breed cross these 2 plants, the third stack represents their offspring's genome. The offspring now have a equal mix of genes from each of the plants meaning they grow well in the cold but yield less than modern wheat because of undesirable genes present in the ancient wheat plant. With time, the undesirable trait can be made recessive in order to reduce their impact with several years of selective crossbreeding.

In the second diagram, the researchers identified the gene responsible for cold tolerance in the ancient wheat plants. Instead of selectively crossbreeding these 2 plants,

they removed one gene (for cold tolerance) from the ancient wheat and transferred it into the modern plant. Instead of equally mixing genes, the new wheat plant has all the genetic information from the modern wheat plant and only the desired gene from the ancient wheat plant. This means that the GE wheat plant grows well in the cold and produces a high yield. This example illustrates some of the differences between selective crossbreeding and GE:

- Selective Crossbreeding
  - Increased chance of undesirable traits i.e. varieties become more homozygous for both desired & undesired traits
  - Slower process requiring several generations to obtain results, depending on the organism' reproductive cycle
  - Trial and error process, with no guarantee that desired traits will be obtained
  - Only closely-related species can cross-breed
- Genetic Engineering
  - Decreased chance of undesirable traits
  - Faster process—plants don't need to be planted for several generations to reduce undesirable traits in offspring
  - More precise and controlled process because small DNA piece transferred (not working with whole genome)
  - Allows crossing of natural species barriers so more combinations possible

GENETIC ENGINEERING METHODS

Genetic engineering of plants can take anywhere from 6 to 15+ years before a GE variety is ready to be grown in farmers' fields, depending upon the gene, crop species, and available resources. The steps include:

Identification of Trait and its Source

This step involves some problem solving; knowing what gene needs to be transferred and where to find it. For example, the trait incorporated into Bt corn (for resistance to the European corn borer pest) was discovered about a 100 years ago. When silk worm farmers noticed that silk worms were dying, scientists discovered that a naturally occurring soil bacterium was the cause. Bacillus thuringiensis, or Bt for short, produced a protein (Bt protein) that was toxic to the silk worms. Although scientists didn't know it at the time, they identified the desired trait and source for the Bt protein. Later on, it was found that the Bt protein also killed the European corn borer because it belonged to the same classification order as the silk worm.

DNA Extraction

To be able to work with DNA, scientists need to extract it from its source. DNA extraction involves removing the organism's entire DNA genome meaning the extracted DNA sample contains the gene for the desired trait plus the rest of the organism's DNA. Then, step 3 identifies the specific gene responsible and separates it from the rest of the organism's DNA sequence. But before moving onto step 3, scientists use PCR or Polymerase Chain Reaction to create thousands of exact copies of the extracted DNA. This way, there is plenty of DNA available for further testing instead of only having a small sample to work with.

Gene Isolation

Scientists use gene sequencing and gene mapping to identify and locate the gene responsible for expressing the desired trait. Gene sequencing involves determining the order of all the nucleotides as they appear in the gene. This information is then used to create a gene map showing the location of this gene within the organism's whole genome.

Both public and private research institutions heavily research new technologies to quickly sequence DNA and determine functions of specific genes for important crop species. These efforts result in identification of a large number of genes potentially useful for producing GE varieties.

Once the gene has been identified and located, scientists need to remove it from the rest of the organism's genome. For this task, scientists use restriction enzymes that act like DNA scissors that only cut at specific nucleotide sequences. Restriction enzymes come from bacteria where they're used as a defence mechanism. When viruses (or other bacteria) attack, bacteria kill them by cutting up their DNA with restriction enzymes. Scientists have 100's of restriction enzymes to chose from, each of which cut DNA at different nucleotide sequences. For example, the EcoRI enzyme cuts DNA strands whenever the sequence GAATTC appears while the HpaII cuts wherever the sequence CCGG appears. Once scientists know the sequence of the gene they want to isolate plus the surrounding sequence, they use whichever restriction enzymes are needed to cut around it.

At this point, scientists add the necessary restriction enzymes to the DNA solution containing the organism's entire genome. The only problem is that these restriction enzymes simply cut where they recognise their specific sequence regardless of whether they are cutting around the desired gene or elsewhere in the genome. The scientist is left with a bunch of DNA pieces of varying lengths, one of which is actually the gene meant to be isolated. However, they have one piece of valuable information determined from gene sequencing—they know how many nucleotides make up the gene being isolated.

They need to filter out the sequence that matches the number of nucleotides they are looking for. This "filtration" process is called gel electrophoresis. It involves placing these DNA pieces onto a tray of agarose gel (looks and feels

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