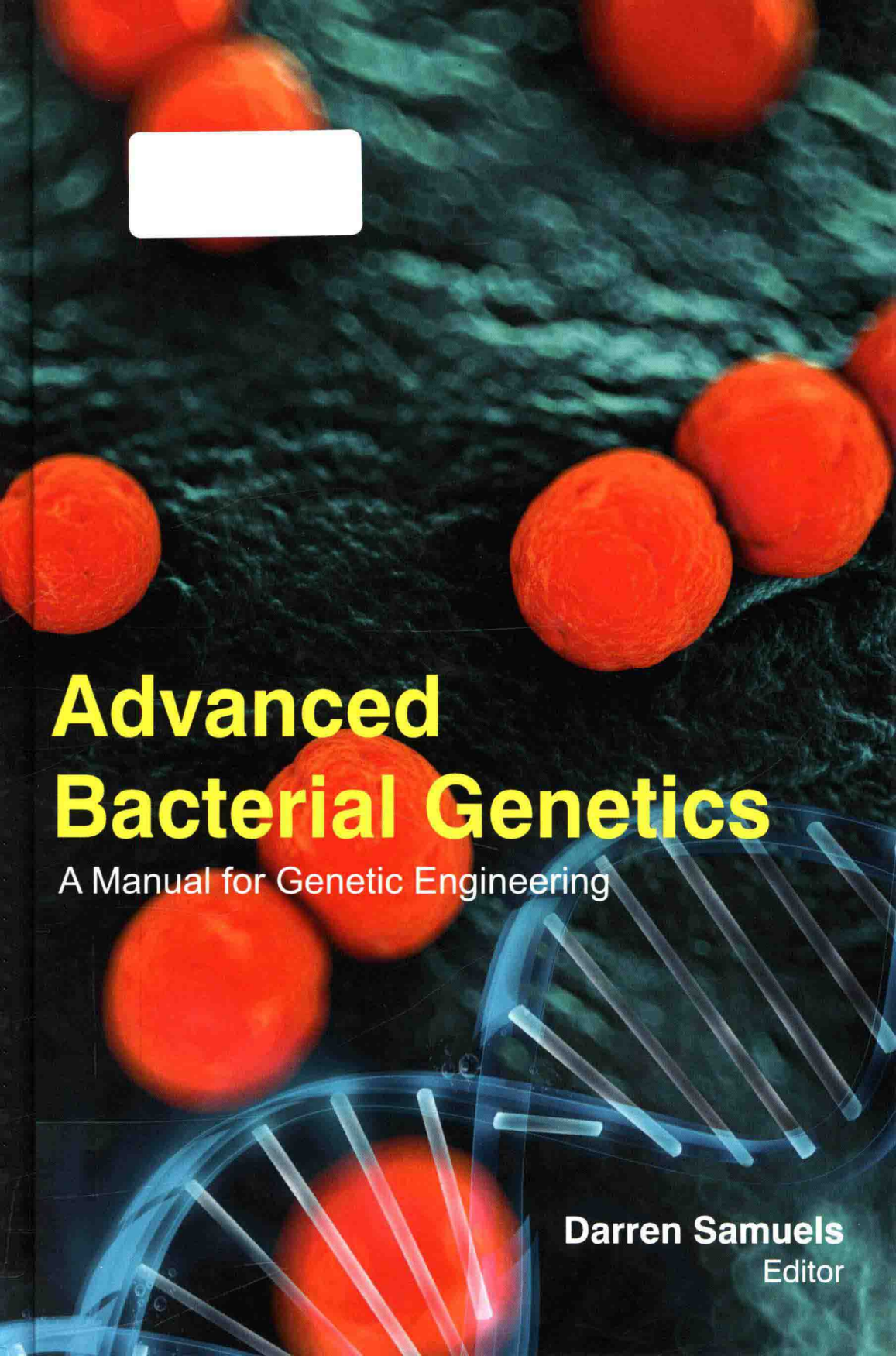
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A Manual for Genetic Engineering
Darren Samuels
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Preface

Genetics is the study of genes including the structure of genetic materials, what information is stored in the genes, how the genes are expressed and how the genetic information is transferred. Genetics is also the study of heredity and variation. The arrangement of genes within organisms is its genotype and the physical characteristics an organism based on its genotype and the interaction with its environment, make up its phenotype. The order of DNA bases constitutes the bacterium's genotype. A particular organism may possess alternate forms of some genes. Such alternate forms of genes are referred to as alleles. The cell's genome is stored in chromosomes, which are chains of double stranded DNA. Genes are sequences of nucleotides within DNA that code for functional proteins.

The genetic material of bacteria and plasmids is DNA. The two essential functions of genetic material are replication and expression. Bacteria utilize a special energy saving system of genetic control called operons. The operon is a sequence of DNA that contains multiple genes used to produce multiple proteins for a single purpose. An example of an operon is the lac operon in E. coli. In order to break down lactose, E. coli must use a series of. The genes for these three enzymes are located in a row on the DNA and share a single promoter. Genes determining structure of a particular protein are called structural genes and the activity of structural genes are controlled by regulator genes, which lie adjacent to them. The genes lacZ, lacY and lacA which code for the three enzymes are the structural genes. lacI gene codes for the repressor protein, hence is the regulator gene. Between the lacI gene and the structural genes lie promoter and operator genes. For transcription of the structural genes, the enzyme RNA polymerase first has to bind to promoter region. The operator region lies in between the promoter and structural genes and the RNA polymerase has to go through the operator region. Under normal

circumstances, when the structural genes are not transcribed, the repressor protein is bound to the operator region thus preventing the passage of RNA polymerase from the operator region towards the operon. When lactose is available in the environment, the repressor protein leaves the operator region and binds to lactose because it has high affinity for lactose. This frees the operator region and the RNA polymerase enzyme moves towards the operon and transcribes the structural genes. The products of structural genes result in the metabolism of lactose. When lactose is no more available, the repressor protein goes back and binds to the operator region, thus stopping further transcription of structural genes. This way lactose acts both as inducer as well as a substrate for beta galactosidase. Sometimes when two pieces of DNA come into contact with each other, sections of each DNA strand will be exchanged. This is usually done through a process called crossing over in which the DNA breaks and is attached on the other DNA strand leading to the transfer of genes and possibly the formation of new genes. Genetic recombination is the transfer of DNA from one organism to another. The transferred donor DNA may then be integrated into the recipient's nucleoid by various mechanisms. In the case of homologous recombination, homologous DNA sequences having nearly the same nucleotide sequences are exchanged by means of breakage and reunion of paired DNA segments. Genetic information can be transferred from organism to organism through vertical transfer (from a parent to offspring) or through horizontal transfer methods such as conjugation, transformation or transduction. Bacterial genes are usually transferred to members of the same species but occasionally transfer to other species can also occur.

The present book deals with all the important dimensions of this subject. It is a valuable reference source for all those concerned with this subject.

—Editor

Contents

Chapter 1

Microbial Genetics

Introduction to Microbial Genetics

Microorganisms have the ability to acquire genes and thereby undergo the process of recombination. In recombination, a new chromosome with a genotype different from that of the parent results from the combination of genetic material from two organisms. This new arrangement of genes is usuall accompanied by new chemical or physical properties. In microorganisms, several kinds of recombination are known to occur. The most common form is general recombination, which sually involves a recirocal exchange of DNA between a pair of DNA sequences. It occurs anywhere on the microbial chromosome and is typified by the exchanges occurring in bacterial transformation, bacterial recombination, and bacterial transduction. A second type of recombination, called site-specific recombination, involves the integration of a viral genome into the bacteial chromosome.

A third type is replicative rcombination, which is due to the movement of genetic elements as they switch position from one place on the chromosome to another. The principles of recombination apply to prokaryotic microorganisms but not to eukaryotic microorganism. Eukaryotes exhibit a complete sexual life cycle, including miosis. In this process, new combinations of a particular gene frm during the process of crossing over.

This process occurs between homologous chromosomes and is not seen in bacteria, whre only a single chromosome exists. Much of the wok in microbial genetics has been performed with bacteria, and the unique features of microbial genetics are usually those associaed with prokaryotes such as bacteria.

The Bacterial Chromosome and Plasmid

While eukaryotes have two or more chromosomes, prokaryotes such as bacteria possess a single hromosome composed f double-stranded DNA in a loop. The DNA is located in the nucleoid of the cell and is not asociated with protein. In *Escherichia coli*, the length of the chromosome, when open, is many times the length of the cel. Many bacteria (and some yeasts or othr fungi) also possess looped bits of DA known as plasmids, which exist and replicate independently of the chromosome. Plasmids have relatively few genes (fewer than 30). The genetic information of the plasmid is usually not essential to survival of the host bacteria. Plasmids can be removed from the host cell in the process of curing. Curing may occur spontaneously or may be induced by treatments such as ultraviolet light. Certain plasmids, called episomes, may be integrated into the bacterial chromosome. Others contain genes for certain types of pili and are able to transfer copies of themselves to other bacteria. Such plasmids are referred to as conjugative plasmds.

A special plasmid called a fertility (F) factor plays an important role in conjugation. The F factor contains genes that encourage cellular attachment during conjugation and accelerate plasmid transfer between conjugating baterial cells. Those cells contributing DNA are called $^{+}$ (onor) cells, while those receiving DNA are the F^{-} (recipient) cells. The F factor can exist outside the bacterial chromosome or ay be integrated into the hromosome.

Plasmids contain genes that impart antibiotic resistance. Up to eight genes for resisting eight different antibiotics may be found on a single plasmid. Genes that enode a series of bacteriocins are also found on plasmids. Bacteriocins are bacterial proteins capable of destroying other bacteria. Still other plasmids increase the pathogenicity of their host bacteria because the plasmid contains genes for toxin synthesis. Transposable elements.

Transposable elements, als known as transposons, are segments of DNA that move about within the chromosome and estblish new genetic sequences. First discovered by Barbara McClintock in the 1940s, transposons behave somewhat like lysogenic viruses except that they cannot exist apart from the chromosome or reproduce themselves.

The simplest transposons, inserion sequences, are short sequences of DNA bounded at both ends by identical sequences of nucleotides in reverse orentation (inverted repeats). Insertion sequences can insert

within a gene and cause a rearrangement mutation of the genetic material. If the sequence carries a stop coon, it may block transcription of the DNA during protein synthesis. Insertion sequences may also encourage the movement of drug-resistance genes between plasmids and chromosomes.

Mutation

In molecular biology and genetics, mutationsare changes in a genomic sequence: the DNA sequence of a cell's genome or the DNA or RNA sequence of a virus. Mutations are caused by radiation, viruses, transposons and mutagenic chemicals, as wellas errors that occur during meiosis or DNA replication. They can also be induced by the organism itself, by cellular processes such as hypermutation. Mutation can result n several different types of change in DNA sequences; these can either have no effect, alter the product of a gene, or prevent the gene from functioning properly or completely. Studies in the fly Drosophila melanogaster suggest that if a mutation changes a protein produced by a gene, this will probably be harmful, with about 70 per cent of these mutations having damaging effects, and the remainder being either neutral or weakly beneficial.

Due to the damaging effects that mutations can have on enes, organisms have mechanisms such as DNA repair to remove mutations. Therefore, the optimal mutation rate for a species is a trade-off between costs of a high mutation rate, such as deleterious mutations, and the metabolic costs of maintaining systms to reduce the mutation rate, such as DNA repair enzymes. Viruses that use RNA as their genetic material have rapid mutation rates, which can be an advantage since these viruses will evolve constantly and rapidly, and thus evade the defensive responses of *e.g.* the human immune system.

Description

Mutations can involve large sections of DNA becoming duplicated, usually through genetic recombination. These duplications are a major source of raw material for evolving new genes, with tens o hundreds of genes duplicated in animal genomes every million years. Most genes belong to larger families of genes of shared ancestry. Novel genes are produced by several methods, commonly through the duplication and mutation of an ancestral gene, or by recombining parts of different genes to form new combinations with new functions. Here, domains act as modules, each with a particular and independent function, that can be mixed together to roduce genes encoding new

proteins with novel properties. For example, the human eye uses four genes to make structures that sense light: three for color vision and one for night vision; all four arose from a single ancestral gene.

Another advantage of duplicating a gene (or even an entire genome) is that this increases redundancy; this allows one gene in the pair to acquire a new function while the other copy performs the original function. Other types of mutation occasioally create new genes from previously noncoding DNA. Changes in chromosome number may invlve even larger mutations, where segments of the DNA within chromosomes break and then rearrange.

For example, two chromosomes in the Homo genus fused to produce human chromosome 2; this fusion did not occur in the lineage of the other apes, and they retain these separate chromosomes. In evolution, the most important role of such chromosomal rearrangements may be to accelerate the divergence of a population into new species by making populations less likely to interbreed, and thereby preserving genetic diferences between these populations.

Sequences of DNA that can move about the genome, such as transposons, make up a major fraction of the genetic material of plants and animals and may have been important in the evolution of genomes. For example, more than a million copies of the Alu sequence re present in the human genome, and these sequences have now been recruited to perform functions such as regulatinggene expression.

Another effect of these mobile DNA sequences is that when they move within a genome, they can mutate or delete existing genes and thereby produce genetic diversity. In multicellular organisms with dedicated reproductive cells, mutations can be subdivided into germ line mutations, which can be passed on to descendants through their reproductive cells, and somatic mutations (also called acquired mutations), which involve cells outside the dedicated reproductive group and which are not usually tansmitted to descendants.

If the organism can reproduce asexually through mechanisms such as cuttings or budding the distinction can become blurred. For example, plants can sometimes transmit somatic mutations to their descendants asexually or sexually where flower buds develop in somatically mutated parts of plants. A new mutation that was not inherited from either parent is called a de novo mutation.

The source of the mutation is unrelated to the consequence, although the consequences are related to which cells were mutated. Nonlethal mutations accumulate within the gene pool and increase

the amount of genetic variation. The abundance of some genetic changes within the gene pool can be reduced by natural selection, while other "more favourable" mutations may accumulate and result in adaptive changes.

For example, a butterfly may produce offspring with new mutations. The majority of these mutations will have no effect; but one might change the color of one of the butterfly's offspring, making it harder (or easier) for predators to see. If this color change is advantageous, the chance of this butterfly surviving and producing its own offspring are a little better, and over time the number of butterflies with this mutation may form a larger percentage of the population. Neutral mutations are defined as mutations whose effects do not influence the fitness of an individual.

These can accumulate over time due to genetic drift. It is believed that the overwhelming majority of mutations have no sinificant effect on an organism's fitness. Also, DNA repair mechanisms are able to mend most changes before they become permanent mutations, and many organisms have mechanisms for eliminating otherwise permanently mutated somatic cells. Mutation is generally accepted by biologists as the mechanism by which natural selection acts, generating advantageous new traits that survive and multiply in offspring as well as disadvantageous traits, in less fit offspring, that tend to die out.

Causes

Two classes of mutations are spontaneous mutations (molecular decay) and induced mutations caused by mutagens.

Spontaneous mutations on the molecular level can be caused by:

- *Tautomerism*: A base is changed by the repositioning of a hydrogen atom, altering the hydrogen boning pattern of that base resulting in incorrect base pairing during replication.
- *Depurination*: Loss of a purine base (A or G) to form an apurinic site (AP site).
- *Deamination*: Hydrolysis changes a normal base to an atypical base containing a keto group in place of the original amine group. Examples include C → U and → HX (hypoxanthine), which can be corrected by DNA repair mechanisms; and 5MeC (5-methylcytosine) → T, which is less likely to be deected as a mutation because thymine is a normal DNA base.

- *Slipped strand mispairing*: Denaturation of the new strand from the template during replication, followed by renaturation in a different spot ("slipping"). This can lead to insertions or deletions.

Induced mutations on the molecular level can be caused by:

- Chemicals
  - Hydroxylamine NH_2OH
  - Base analogs (*e.g.* BrdU)

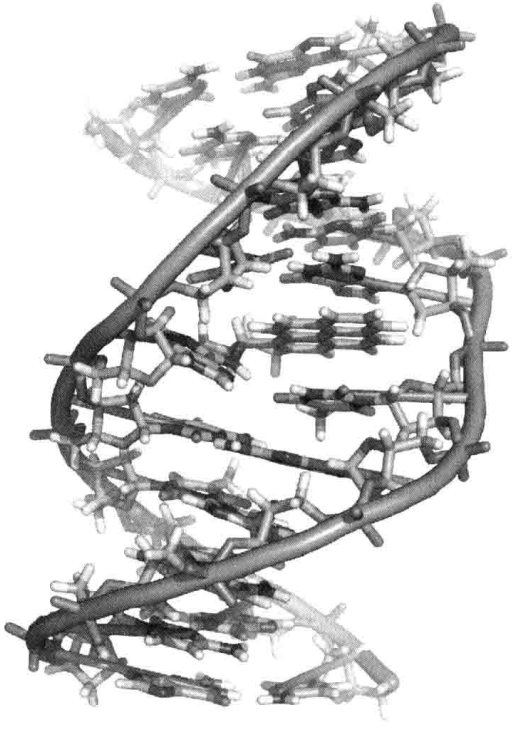

Figure: *A covalent adduct between benzo[a]pyrene, the major mutagen in tobacco smoke, and DNA*

  - Alkylating agents (*e.g.* N-ethyl-N-nitrosourea) These agentscan mutate both replicating and non-replicating DNA In contrast, a base analog can only mutate the DNA whn the analog is incorporated in replicating the DNA. Each of these classes of chemical mutagens has certain effects that then lead to transitions, ransversions, or deletions.
  - Agents that form DNA adducts (*e.g.* ochratoxin A metabolites)
  - DNAintercalating agents (*e.g.* ethidium bromide)
  - DNA crosslinkers

  - Oxidative damage
  - Nitrous acid converts amine groups on A and C to diazo groups, altering their hyrogen bonding patterns which leads to incorrect base pairing during replication.
- Radiation
  - Ultraviolet radiation nonionizing radiation). Two nucleotide bases in DNA–cytosine and thymine–are mot vulnerable to radiation that can change their properties. UVlight can induce adjacent pyrimidine bases in a DNA strand to become covalently joined as a pyrimidine dimer. UV radiation, particularly loner-wave UVA, can also cause oxidative damage to DNA.
  - Ionizing radiation
- Viral infections

DNA has so-called hotspots, where mutations occur up to 100 times more frequently than the normal mutation rate. A hotspot can be at an unusual base, *e.g.*, 5-methylcytosine. Mutation rates also var across species. Evolutionary biologists have theorized that higher mutation rates are beneficial in some situations, because they allow organisms to evolve and therefore adapt more quickly to their environments. For example, repeated exposure of bacteria to antibiotics, and selection of resistnt mutants, can result in the selection of bacteria that have a much higher mutation rate than the original population (mutator strains).

Classification of Mutation Types

By Effect on Structure

The sequence of a gene can be altered in a number of ways. Gene mutations have varying effects on health depending on where they occur and whether they alter the function of essential proteins.

Mutations in the structure of genes can be classified as:

- *Small-scale mutations, such as those affecting a small gene in one or a few nucleotides, including*:
  - Point mutatins, often caused by chemicals or malfunction of DNA replication, exchange a single nucleotide for another. These changes are classified as transitions or transversions. Most common is the ransition that exchanges a purine for a purine (A ↔ G) or a pyrimidine for a pyrimidine, (C ↔ T). A transition can be caused by nitrous acid, base mis-