Transposition

Transposition is a widespread spontaneous process in living organisms by which a DNA sequence inserts itself at a new location in the genome. This type of DNA sequence is called a transposon or transposable element.

It does not have any sequence relationship with the target site. Transposons are a major source of genetic variation.

They play an important role in the evolution of genomes. Tranposition utilizes recombination, but does not result in an exchange. Instead, a transposon moves directly from one site of the genome to another without an intermediary such as plasmid or phage DNA .This results in rearrangements that create new sequences and change the functions of target sequences. In some cases, they cause disease, when inserted into a functioning gene.

Insertion sequences

Insertion of a DNA fragment into a gene will usually result in the inactivation of that gene, and it is by the loss of that function that such events were initially recognized. A number of genetic elements, including some phages and plasmids, can be inserted into the bacterial chromosome. The simplest of these genetic elements are known as Insertion Sequences (IS).

Structure of insertion sequences

There are many IS elements known. They differ in size and other details, but the overall structure of most such elements is similar. One example (IS1) is shown in Figure 1; IS1 is 768 bases long but many other IS elements are longer (usually1300–1500 bases).

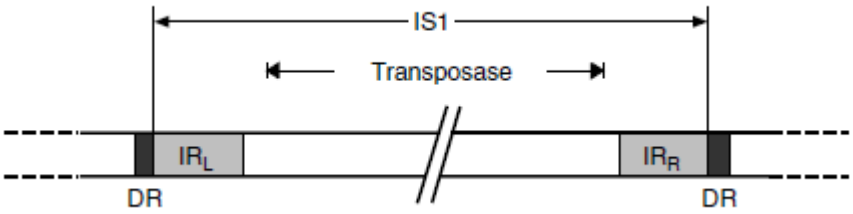

Figure 1. Structure of the insertion sequence ISl. DR, direct repeat (duplicated target sequence); IR, inverted repeats

The central region of an IS element codes for a protein (known as a transposase) which is necessary for the movement of the element from one site to another. At the ends of the insertion sequence are almost perfect inverted repeat (IR) sequences, which in IS1 consist of 23 nucleotides.

Structure of transposons

The structure of a simple transposon, Tn3, is shown in Figure 2; it consists of about 5000 base pairs and has a short (38 bp) inverted repeat sequence at each end. It is therefore analogous to an insertion sequence, the distinction being that a transposon carries an identifiable genetic marker – in this case the ampicillin **resistance gene (*bla*, *β*-lactamase).** Tn3 codes for two other proteins as well: a **transposase (Tnp A), and TnpR**, a bifunctional protein that acts as a repressor and is also responsible for one stage of transposition known as resolution .

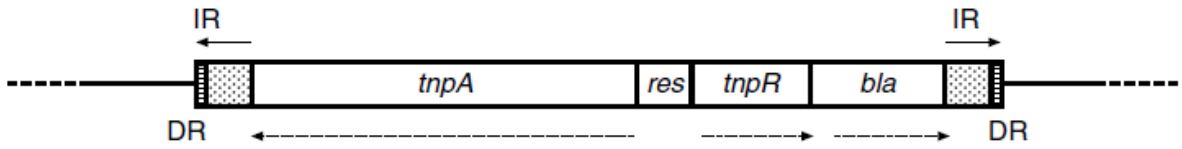

Figure .2 Structure of the transposon Tn3. DR, five-base pair direct repeat (target duplication); IR, 38-base pair inverted repeats; res, resolution site; tnpA, transposase; tnpR, resolvase; *bla*, *β*-lactamase (ampicillin resistance)

Integrons

Extremely complex and large transposons can also be built up by insertion of additional genes within an existing transposon. Many large transposons have been identified which are related to Tn21 which has a structure analogous to class II transposons . it has inverted repeats (38 bp) at each end and carries genes for transposition functions. Tn21 may have developed from a smaller transposon (such as Tn2613) by acquisition of additional genes. Tn2603 and Tn1696 (and a family of other transposons) are also very similar to Tn21 but **contain additional resistance genes**. It is now known that the **transposons in the Tn21 family** have acquired resistance genes by a specific mechanism. Each individual gene has been inserted separately, as a gene cassette, which contains a single gene and a recombination site.

Transposition

Transposons or **transposable elements** are small DNA sequences that can move to virtually any position in a cells genome. Transposition has also been called **illegitimate recombination** because it requires no homology between sequences nor is it site-specific. The transposase makes a staggered cut in the chromosomal DNA and, in a replicative process, a copy of the transposon inserts at the target site (*Fig. 3*). The gaps are filled and sealed by DNA

polymerase I and DNA ligase, resulting in a duplication of the target site and formation of a new **direct** repeat sequence. The gene into which the transposon inserts is usually inactivated, and genes between two copies of a transposon can be deleted by recombination between them. Inversions and other rearrangements of host DNA sequences can also occur.

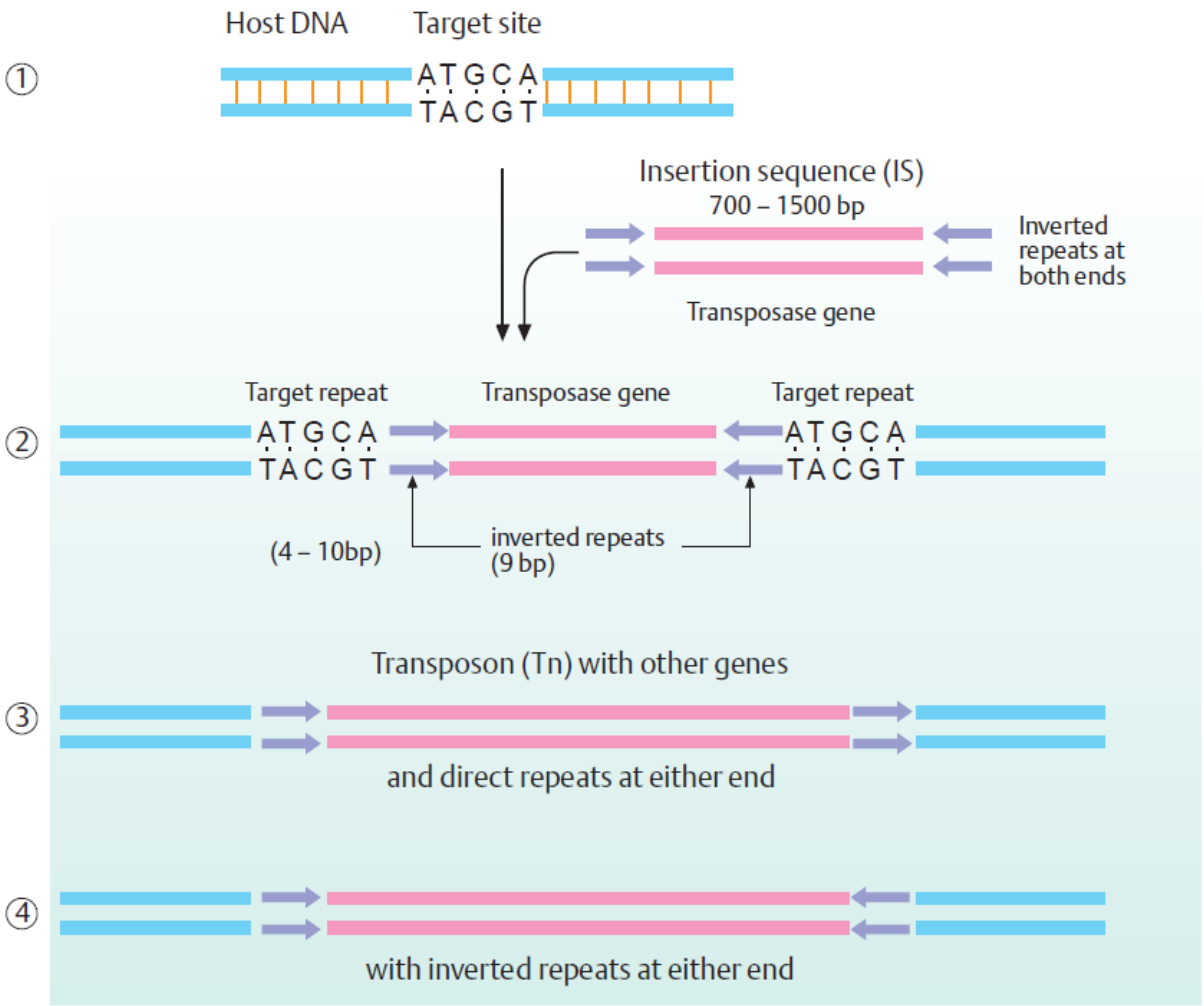

Fig 3.Insertion sequences (IS) and transposons (Tn)

Mutation

Mutations are permanent, heritable a**lterations** in the **base sequence** of the **DNA.** They arise either through **spontaneous errors** in **DNA replication** or (**meiotic recombination** or as a consequence of the **damaging effects** of **physical or chemical agents** on the DNA.

Mutagen: is a physical or chemical agent that causes mutations to occur (or increase the frequency of their occurrence.

Mutagenesis: The process of producing a mutation

Point mutation: involves a single changed base pairs (Base substitution ; Base deletion ; Base addition)
Multiple mutation: involves alteration in two or more base pairs.

Types Of Mutations

Point Mutations

The **simplest mutation** is a **point mutation** – a single base change. This can be either a **transition**, in which one **purine (or pyrimidine**) is replaced by the other, or a **transversion**, where a **purine** replaces a **pyrimidine** or **vice versa**. The **phenotypic effects** of such a mutation can be various.

If it is in a **noncoding** or **nonregulatory piece** of DNA or in the **third position of a codon**, which often has **no effect** on the **amino acid** incorporated into a **protein** , then it may be **silent**.

If it results in an altered amino acid in a **gene product** then it is a **missense mutation** whose effect can vary from none to lethality, depending on the amino acid affected.

Mutations which generate **new stop codons** are **nonsense** mutations and give rise to **truncated protein products**.

Insertions or deletions involve the addition or loss of one or more bases. These can produce **frameshift mutations** in genes, where the **translated protein** sequence to the **C-terminal side** of the mutation is completely changed. **Mutations t**hat affect the processes of cell growth and cell death can result in tumorigenesis .

The accumulation of many silent and other nonlethal mutations in populations produces **genetic polymorphisms** – acceptable variations in the '**normal'** DNA and protein sequences .

There are many ways in which the structure of the genetic material may **change**. Much of the basis of genetics has been established using simple mutations **(point mutations)** in which the sequence of the DNA has been altered at a **single position**. Where this change consists of replacing one **nucleotide by another**, it is known as a base **substitution**.

Conditional Mutants

Many genes do not affect **resistance to antibiotics or bacteriophages, biosynthesis of essential metabolites or utilization of carbon sources.** Some of these genes are indispensable and any mutants defective in those activities would die (or fail to grow). This can be done by using **conditional mutants**. This means that the gene functions normally under certain conditions while the defect is only apparent when the conditions are changed. One very useful type

of conditional mutation confers temperature sensitivity on the relevant function. So, for example, a strain with a **temperature-sensitive mutation** in a gene needed for DNA replication would be able to grow normally at, say 30 ^{0}C (**the permissive temperature**) but would be unable to grow at a higher temperature, such as 42 ^{0}C.

Replication fidelity

The high accuracy of DNA replication (one error per 10^{10} bases incorporated) depends on a combination of proper base pairing of template strand and incoming nucleotide in the active site of the DNA polymerase, proofreading of the incorporated base by 3`→5` exonuclease and mismatch repair.

Induced mutations

Physical mutagens

Ionizing (e.g. X- and Y-rays) and nonionizing (e.g. UV) radiation produce a variety of DNA lesions. Pyrimidine dimers are the commonest product of UV irradiation.

Chemical mutagens

Base analogs can mispair during DNA replication to cause mutations. Nitrous acid deaminates cytosine and adenine. Alkylating and arylating agents generate a variety of adducts that can block transcription and replication and cause mutations by direct or, more commonly, indirect mutagenesis. Most chemical mutagens are carcinogenic.

Direct mutagenesis

If a base analog or modified base whose base pairing properties are different from the parent base is not removed by a DNA repair mechanism before passage of a replication fork, then an incorrect base will be incorporated. A second round of replication fixes the mutation permanently in the DNA.

Indirect mutagenesis

Most lesions in DNA are repaired by error-free direct reversal or excision repair mechanisms before passage of a replication fork. If this is not possible, an error-prone form of translesion DNA synthesis may take place involving specialized DNA polymerases and one or more incorrect bases become incorporated opposite the lesion.

DNA DAMAGE

DNA lesions

The chemical reactivity of DNA with exogenous chemicals or radiation can give rise to changes in its chemical or physical structure. These may block replication or transcription and so be lethal, or they may generate mutations through direct or indirect mutagenesis. The chemical instability of DNA can generate spontaneous lesions such as deamination and depurination.

Oxidative damage

Reactive oxygen species such as superoxide and hydroxyl radicals produce a variety of lesions including 8-oxoguanine and 5-formyluracil. Such damage occurs spontaneously but is increased by some exogenous agents including Y-rays.

Alkylation

Electrophilic alkylating agents such as methylmethane sulfonate and ethylnitrosourea can modify nucleotides in a variety of positions. Most lesions are indirectly mutagenic, but *O*6-alkylguanine is directly mutagenic

Bulky adducts

Bulky lesions such as pyrimidine dimers and arylating agent adducts distort the double helix and cause localized denaturation. This disrupts the normal functioning of the DNA.