Mutation Due to Different Base Modifications

Mutations can result from chemical or physical events that lead to base modification. When they affect the base-pairing pattern, they interfere with replication or transcription. Chemical
substancees able to induce such changes are called mutagens. Mutagens cause mutations in different ways. Spontaneous oxidation, hydrolysis, uncontrolled methylation, alkylation, and ultraviolet irradiation result in alterations that modify nucleotide bases. DNA-reactive chemicals change nucleotide bases into different chemical structures or remove a base.

A. Deamination and methylation

Cytosine, adenine, and guanine contain an amino group. When this is removed (deamination), a modified base with a different basepairing pattern results. Nitrous acid typically removes the amino group. This also occurs spontaneously at a rate of 100 bases per genome per day (Alberts et al., 1994, p. 245). Deamination of cytosine removes the amino group in position 4 (1). The resulting molecule is uracil (2). This pairs with adenine rather than guanine. Normally this change is efficiently repaired by uracil-DNA glycosylase. Deamination at the RNA level occurs in RNA editing (see Expression of genes). Methylation of the carbon atom in position 5 of cytosine results in 5 methylcytosine, containing a methyl group in position 5 (3). Deamination of 5-methylcytosine will result in a change to thymine, containing an oxygen in position 4 instead of an amino group (4). This mutation will not be corrected because thymine is a natural base. Adenine (5) can be deaminated in position 6 to form hypoxanthine, which contains an oxygen in this position instead of an amino group (6), and which pairs with cytosine instead of thymine.

The resulting change after DNA replication is a cytosine instead of a thymine in the mutant strand.

B. Depurination

About 5000 purine bases (adenine and guanine) are lost per day from DNA in each cell (depurination) owing to thermal fluctuations. Depurination of DNA involves hydrolytic cleavage of the N-glycosyl linkage of deoxyribose to the guanine nitrogen in position 9.This leaves a depurinated sugar. The loss of a base pair will lead to a deletion after the next replication if not repaired in time (see DNA repair).

C. Alkylation

Alkylation is the introduction of a methyl or an ethyl group into a molecule. The alkylation of guanine involves the replacement of the hydrogen bond to the oxygen atom in position 6 by a methyl group, to form 6-methylguanine. This can no longer pair with cytosine. Instead, it will pair with thymine. Thus, after the next replication the opposite cytosine (C) is replaced by a thymine (T) in the mutant daughter molecule. Important alkylating agents are ethylnitroso urea (ENU), ethylmethane sulfonate (EMS), dimethylnitrosamine, and N-methyl-N-nitroN-nitrosoguanidine.

D. Nucleotide base analogue

Base analogs are purines or pyrimidines that are similar enough to the regular nucleotide DNA bases to be incorporated into the new strand during replication. 5-Bromodeoxyuridine (5BrdU) is an analog of thymine. It contains a bromine atom instead of the methyl group in position 5.Thus, it can be incorporated into the new DNA strand during replication. However, the presence of the bromine atom causes ambiguous and often wrong base pairing. E. UV-light-induced thymine dimers

Ultraviolet irradiation at 260 nm wavelength induces covalent bonds between adjacent thymine residues at carbon positions 5 and 6. If located within a gene, this will interfere with replication and transcription unless repaired. Another important type of UV-induced change is a photoproduct consisting of a covalent bond between the carbons in positions 4 and 6 of two adjacent nucleotides, the 4–6 photoproduct.

Changes in DNA (Mutations)

When it was recognized that changes (mutations) in genes occur spontaneously (T. H. Morgan, 1910) and can be induced by X-rays (H. J. Muller, 1927), the mutation theory of heredity became a cornerstone of early genetics. Genes were defined as mutable units, but the question what genes and mutations are remained. Today we know that mutations are changes in the structure of DNA and their functional consequences. The study of mutations is important for several reasons. Mutations cause diseases, including all forms of cancer. They can be induced by chemicals and by irradiation. Thus, they represent a link between heredity and environment. And without mutations, well-organized forms of life would not have evolved.

The following two plates summarize the chemical nature of mutations.

A. Error in replication

The synthesis of a new strand of DNA occurs by semiconservative replication based on complementary base pairing. Errors in replication occur at a rate of about 1 in 10 5 . This rate is reduced to about 1 in 10 7 to 10 9 by proofreading mechanisms. When an error in replication occurs

before the next cell division (here referred to as the first division after the mutation), e.g., a cytosine (C) might be incorporated instead of an adenine (A) at the fifth base pair as shown here, the resulting mismatch will be recognized and eliminated by mismatchre pair in most cases.

However, if the error is undetected and allowed tostand, the next(second) division will result in a mutant molecule containing a CG instead of an AT pair at this position. This mutation will be perpetuated in all daughter cells. Depending on its location within or outside of the coding region of a gene, functional consequences due to a change in a codon could result.

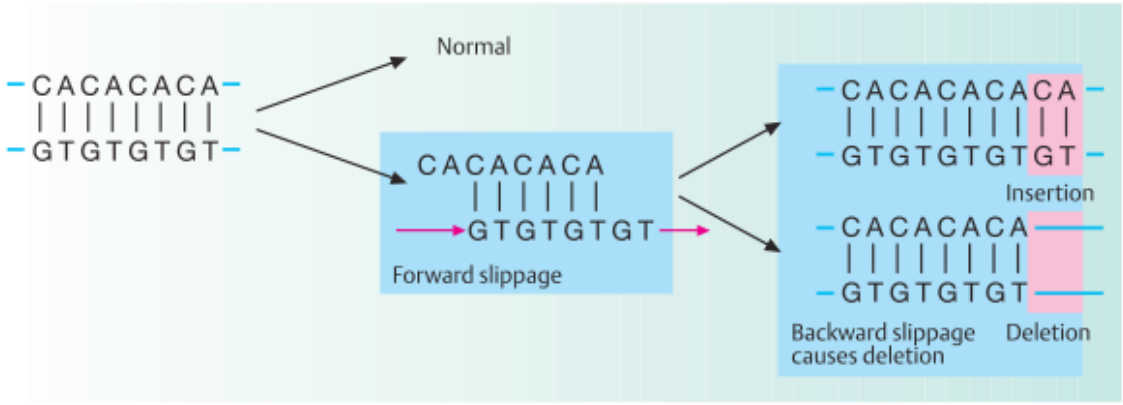
B. Mutagenic alteration of a nucleotide

A mutation may result when a structural change of a nucleotide affects its base-pairing capability. The altered nucleotide is usually present in one strand of the parent molecule. If this leads to incorporation of a wrong base, such as a C instead of a T in the fifth base pair as shown here, the next (second) round of replication will result in two mutant molecules.

C. Replication slippage

A different class of mutations does not involve an alteration of individual nucleotides, but results from incorrect alignment between allelic or nonallelic DNA sequences during replication. When the template strand contains short tandem repeats, e.g., CA repeats as in microsatellites (see DNA polymorphism and Part II, Genomics), the newly replicated strand and the template strand may shift their positions relative to each other. With replication or polymerase slippage, leading to incorrect pairing of repeats, some repeats are copied twice or not at all, depending on the

direction of the shift. One can distinguish forward slippage (shown here) and backward slippage with respect to the newly replicated strand. If the newly synthesized DNA strand slips forward, a region of nonpairing remains in the parental strand. Forward slippage results in an insertion. Backward slippage of the new strand results in deletion. Microsatellite instability is a characteristic feature of hereditary nonpolyposis cancer of the colon (HNPCC). HNPCC genes are localized on human chromosomes at 2p15–22 and 3p21.3. About 15% of all colorectal, gastric, and endometrial carcinomas show microsatellite instability. Replication slippage must be distinguished from unequal crossing-over during meiosis. This is the result of recombination between adjacent, but not allelic, sequences on nonsister chromatids of homologous chromosomes (Figures redrawn from Brown, 1999).



Replication slippage