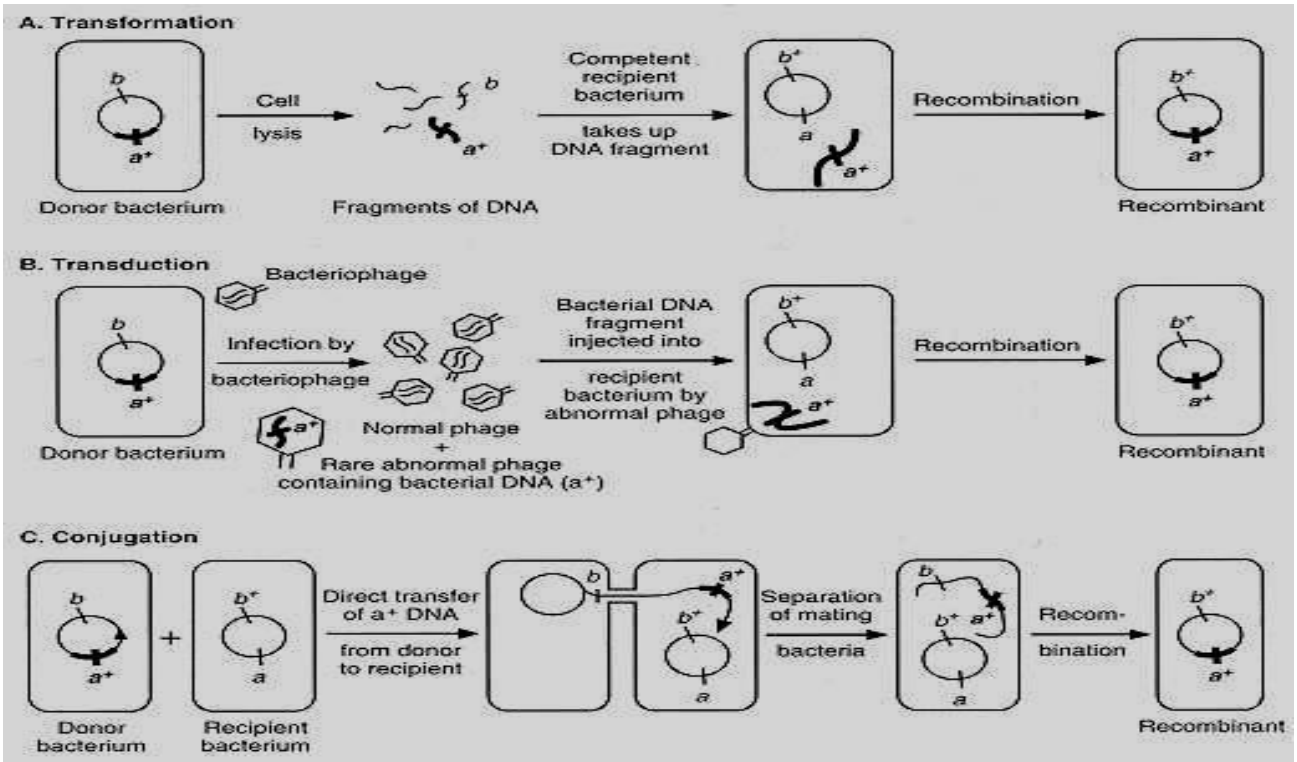
. Sexual reproduction or Genetic recombination in bacteria

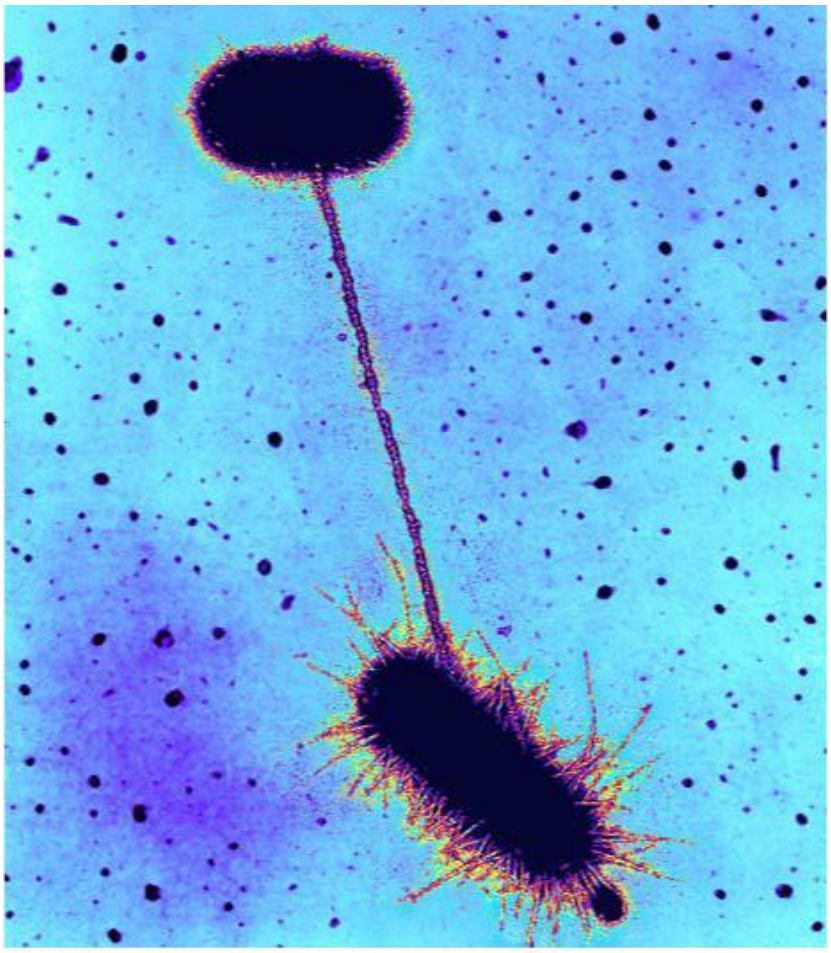
Cytological observations and genetic studies indicate that something like sexual reproduction, involving the fusion of two different cells and a transfer of hereditary factors, occurs in bacteria, although infrequently. But, typical sexual reproduction through the agency of gametes is absent in bacteria. There is no fertilization and meiosis. However, the gene transfer in bacteria occurs by three methods—Conjugation, Transformation and Transduction. (1) **Conjugation** involves transfer of DNA from a donor or male cell to a recipient or female cell through a specialized sex pilus or conjugation tube. (2) **Transduction** involves transfer of bacterial genes from a donor cell to a recipient cell by a bacteriophage. (3) **Transformation** involves the uptake of naked DNA molecules from one bacterium (the donor cell) by another bacterium (the recipient cell). Therefore, conjugation occurs through direct cell-to-cell contact, but transformation and transduction do not involve any such contact. The transfer of genetic information is, thus, a one-way transfer, rather than a reciprocal exchange of genetic material. The phenomenon has been called 'Parasexuality' or 'Genetic Recombination'. However, sexual reproduction or even conjugation is unknown in cyanobacteria.



Types of genetic recombination in bacteria

The three processes are explained as under:

1. Conjugation: A mechanism resembling sexual reproduction was discovered by Joshua Lederberg and Edward Tatum (both from USA) in 1946 in bacterium *E. coli*. It was confirmed by Hayes (London) and Wollman (Paris) in the same bacterium. They found that physical contact was involved in conjugation between the two conjugants and that DNA from one is transferred into another through a conjugation tube. Therefore, bacterial conjugation may be defined as the transfer of genetic material from a donor cell to a recipient cell through a specialized intercellular connection, or conjugation tube, that forms between them. The donor and recipient cells are sometimes referred to as male and female cells, respectively.

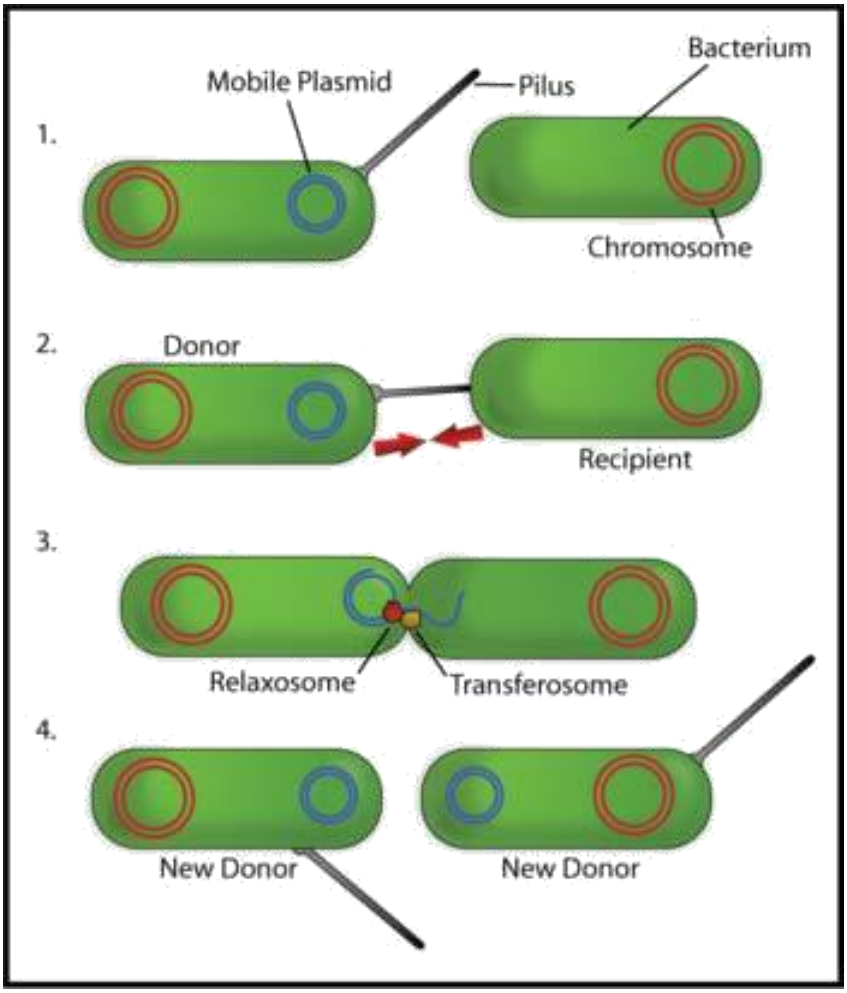


Conjugation in bacteria

Mechanism of conjugation

The cells that have the capacity to serve as donors during conjugation are differentiated by the presence of specialized cell-surface appendages called ***F pili***. The synthesis of these F pili is controlled by several genes that are carried by small circular molecule of DNA (about 100kbp) called an ***F factor*** (for fertility factor) or also called as ***F-plasmid*** or 'sex factor'. The F-plasmid is an **episome**—a plasmid that can exist in two different states: (i) the ***autonomous state***, in which it replicates independently of the host chromosome, and (ii) the ***integrated state***, in which it is covalently inserted into the host chromosome and replicates along with the host chromosome like any other set of chromosomal genes. It carries its own **origin of replication,** the *oriV*, as well as an origin of transfer, or *oriT*. There can only be one copy of the F-plasmid in a given bacterium, either free or integrated (two immediately before cell division). The donor bacteria carrying an F factor form the conjugation tube and are called *F-positive* or *F-plus* (denoted as F^+). Strains that lack F plasmids are called *F-negative* or *F-minus* (denoted as F^-).

When conjugation is initiated via a mating signal, a **relaxase** enzyme creates a nick in one plasmid DNA strand at the origin of transfer, or *oriT*. The relaxase may work alone or in a complex of over a dozen proteins, known collectively as a **relaxosome**. The transferred, or *T-strand*, is unwound from the duplex plasmid and transferred into the recipient bacterium in a 5'-terminus to 3'-terminus direction. The remaining strand is replicated, either independent of conjugative action (vegetative replication, beginning at the *oriV*) or in concert with conjugation (conjugative replication similar to the rolling circle replication of *lambda* phage). Conjugative replication may necessitate a second nick before successful transfer can occur.

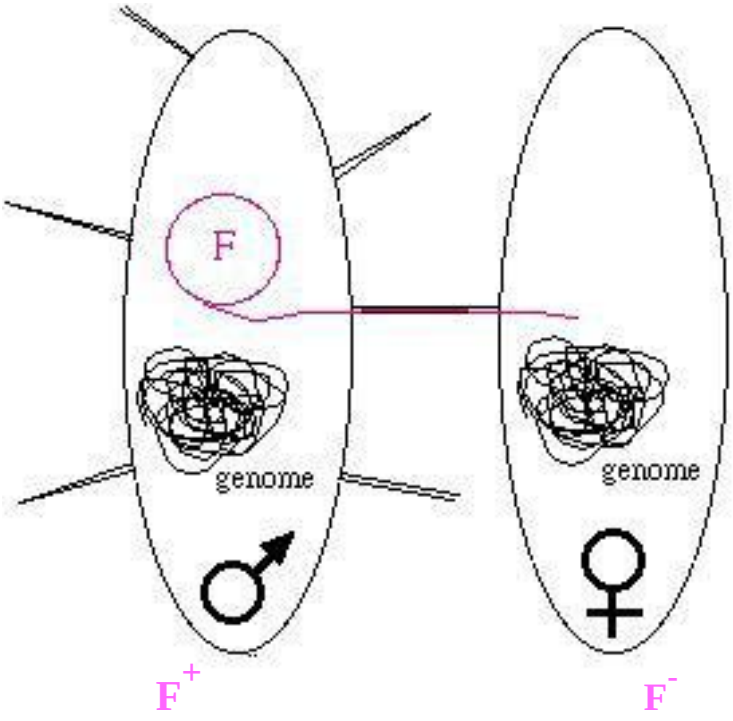


Schematic drawing of bacterial conjugation.
Conjugation diagram 1- Donor cell produces pilus. **2-** Pilus attaches to recipient cell, brings the two cells together. **3-** The mobile plasmid is nicked and a single strand of DNA is then transferred to the recipient cell. **4-** Both cells recircularize their plasmids, synthesize second strands, and reproduce pili; both cells are now viable donors.

In bacteria, conjugation can occur in several ways. Some important examples are as follows:

a. Conjugation between F^+ male and F^- female

E. coli shows two strains, one acting as donor (F^+ male) and other as recipient (F^-female). The donor cell contains the F factor in the autonomous (F^+ cell) conjugates with an F^- recipient cell, only the F factor is transferred. The fertility factor is usually accompanied by the presence of pili. They help the donor cell to get attached to the recipient cell. In the region of contact, a pilus grows in size and produces a conjugation tube. F factor replicates. A copy of it gets transferred to the recipient cell, which also becomes donor. Thus, mixing a population of F^+ cells with a population of F^- cells results virtually in all the cells in the new population becoming F^+. In other words, the sex in *E. coli* can be called infutious.

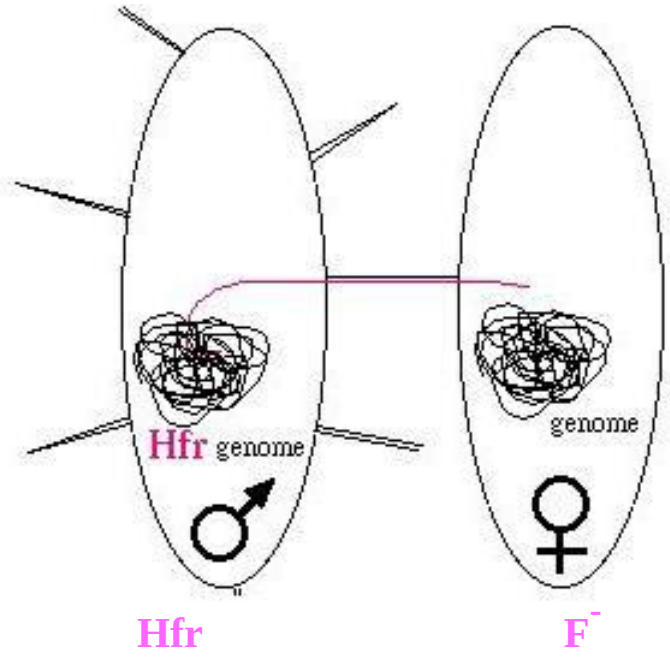


Conjugation in bacteria - transfer of an F factor

b. Conjugation between Hfr male and F^- female

The F factor can integrate into the host chromosome. An F^+ cell carrying an integrated F factor is called an ***Hfr*** (for high-frequency recombination). Therefore, the F+ male becomes ***Hfr*** male. In the integrated state, the F factor mediates the transfer of a chromosome of the Hfr male cell to a recipient (F^-) cell. Usually only a portion of the Hfr chromosome is transferred before the cells separate, thus, breaking the chromosome. Only rarely will an entire Hfr chromosome be transferred.

The mechanism of transfer of DNA from a donor to a recipient cell during conjugation appears to be the same, whether just the F factor is being transferred, as in F^+ by F^- matings, or the chromosome is being transferred, as in Hfr by F^- matings. Transfer is believed to be initiated by an endonucleolytic nick in one strand at a specific site (the "origin" of transfer) on the F factor. The 5′ end of the nicked strand is then transferred through the conjugation tube into the recipient cell. Transfer is believed to be coupled to rolling circle replication with the intact circular strand being replicated in the donor cell and the displaced strand being replicated in the recipient cell as it is transferred. Because the origin of transfer is within the integrated F factor, one portion of the F factor is transferred from an Hfr cell to an F^- cell prior to the sequential transfer of chromosomal genes. The remaining part of the F factor, however, is the last segment of DNA to be transferred. Thus, in Hfr by F^- matings, the recipient F^- cell acquires a complete F factor (thus becoming an Hfr donor) only in those rare cases when an entire Hfr chromosome, with its integrated F factor, is transferred.



Conjugation in Hfr bacteria - transfer of a chromosome fragment

c. Gene transfer by F′ factor

The integration of the F factor into the bacterial chromosome is reversible. The F factors can be excised from the chromosomes, so that the Hfr cell reverts to an F^+ cell. This excision occurs at about the same frequency as integration. Correct excision depends upon a break occuring at the same site on the chromosome as the integration site. In rare cases, however, the break occurs at a neighbouring site, so that on excision a neighbouring segment of the host DNA remains attached to the F factor. Such an F factor, containing a small piece of chromosomal DNA is called an **F′ factor.** The origin of an F′ factor as analogous to the formation of specific transducing phage.

The cell containing an F′ factor is called a primary F′ cell. The DNA integrated into the F′ factor can now be transferred from the F′ donor cell to an F^- recipient cell with the same frequency (100%) as the F factor from F^+ strains to F^- strains. The same piece of chromosomal DNA would be transferred from Hfr cells to F^- cells only with a frequency of 1%. Transfer of the F′ factor from a primary F′ cell in which it originated to normal F^- cell results in a secondary F′ cell. In this cell the segment of the bacterial chromosome is present twice (i.e. in the diploid state) resulting in the formation of ***partial diploids*** or ***merozygotes***. Recombination of this type mediated by F′ factors is called ***Sexduction*** or F-duction. Because of the partial diploidy, resulting from sexduction, it provides an important method for determining dominance relationships between alleles and defining genes by complementation tests in bacteria.

2. Transduction

It was first discovered by N. Zinder and J. Lederberg in 1952, in *Salmonella typhimurium*, a mouse typhoid bacterium. Transduction is the transfer of DNA from a donor cell to a recipient cell by bacteriophages. In most cases only a small segment of the host (i.e. the donor) DNA is transferred.

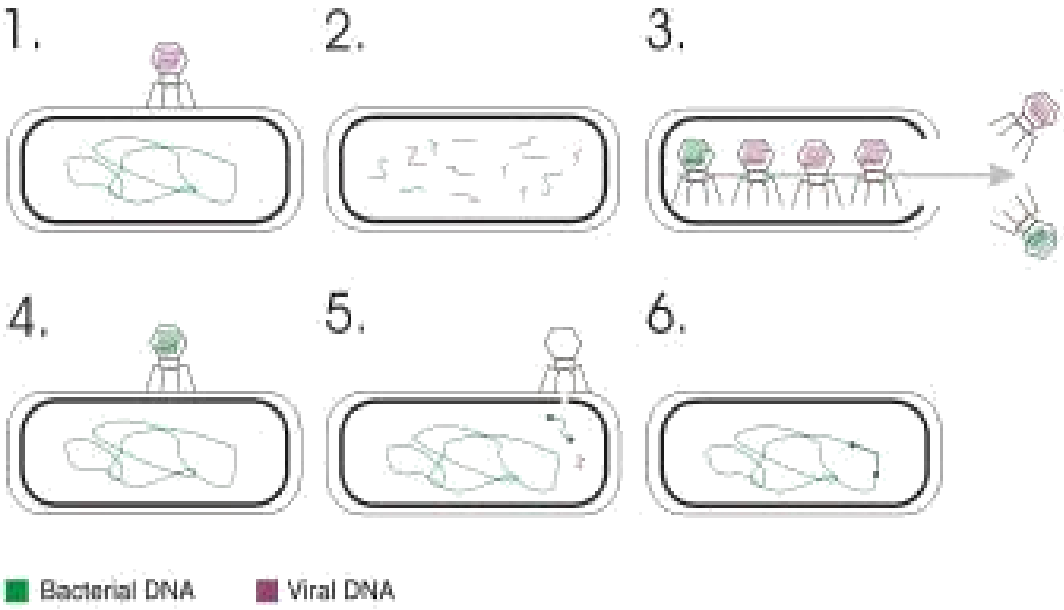
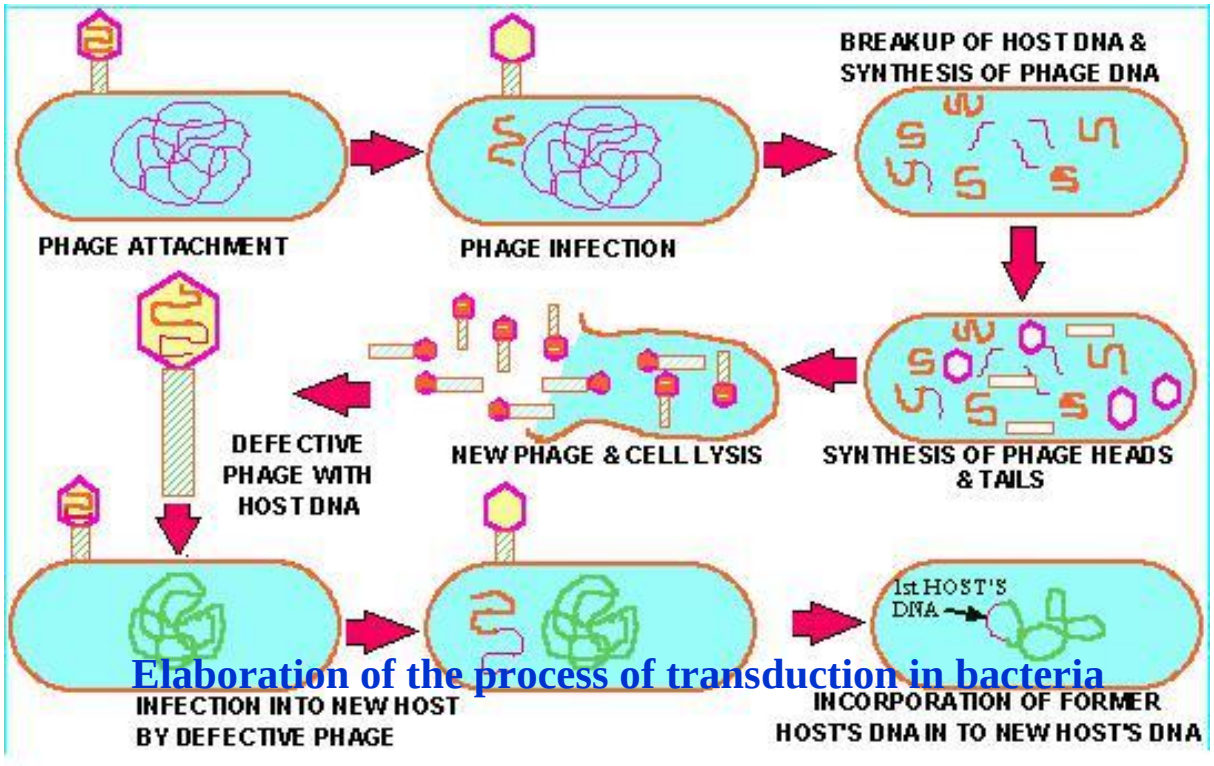


Diagram explaining the process of Transduction in bacteria



Elaboration of the process of transduction in bacteria

Lytic and lysogenic (temperate) cycles

Transduction happens through either the **lytic** cycle or the **lysogenic** cycle. Based on their interactions with the bacterial cell, bacteriophages are classified into two types: **virulent** phages, which always multiply and lyse the host cell; and **temperate** phages, which have a choice between two life-styles after infection. They can either (i) enter the **lytic** cycle, during which they reproduce and lyse their host cells just like virulent phages, or alternatively, they can (ii) enter the lysogenic pathway, during which the phage chromosome is integrated into the bacterial chromosome, where it can remain dormant for thousands of generations, and replicate like any other segment of the host chromosome. If the lysogen is induced (by UV light for example), the phage genome is excised from the bacterial chromosome and initiates the lytic cycle, which culminates in lysis of the cell and the release of phage particles. The lytic cycle leads to the production of new phage particles which are released by lysis of the host. In both cases the transducing phages are usually defective in some respect; for example they often lose the ability to lyse host cells.

Types of transduction

Two kinds of transduction can be distinguished: generalized transduction, which can transfer any part of the host DNA; and specialized transduction, which is restricted to the transfer of specific DNA segments. In certain cases, bacterial DNA is injected by a phage, but it does not replicate. This kind of transduction is referred to as abortive transduction.

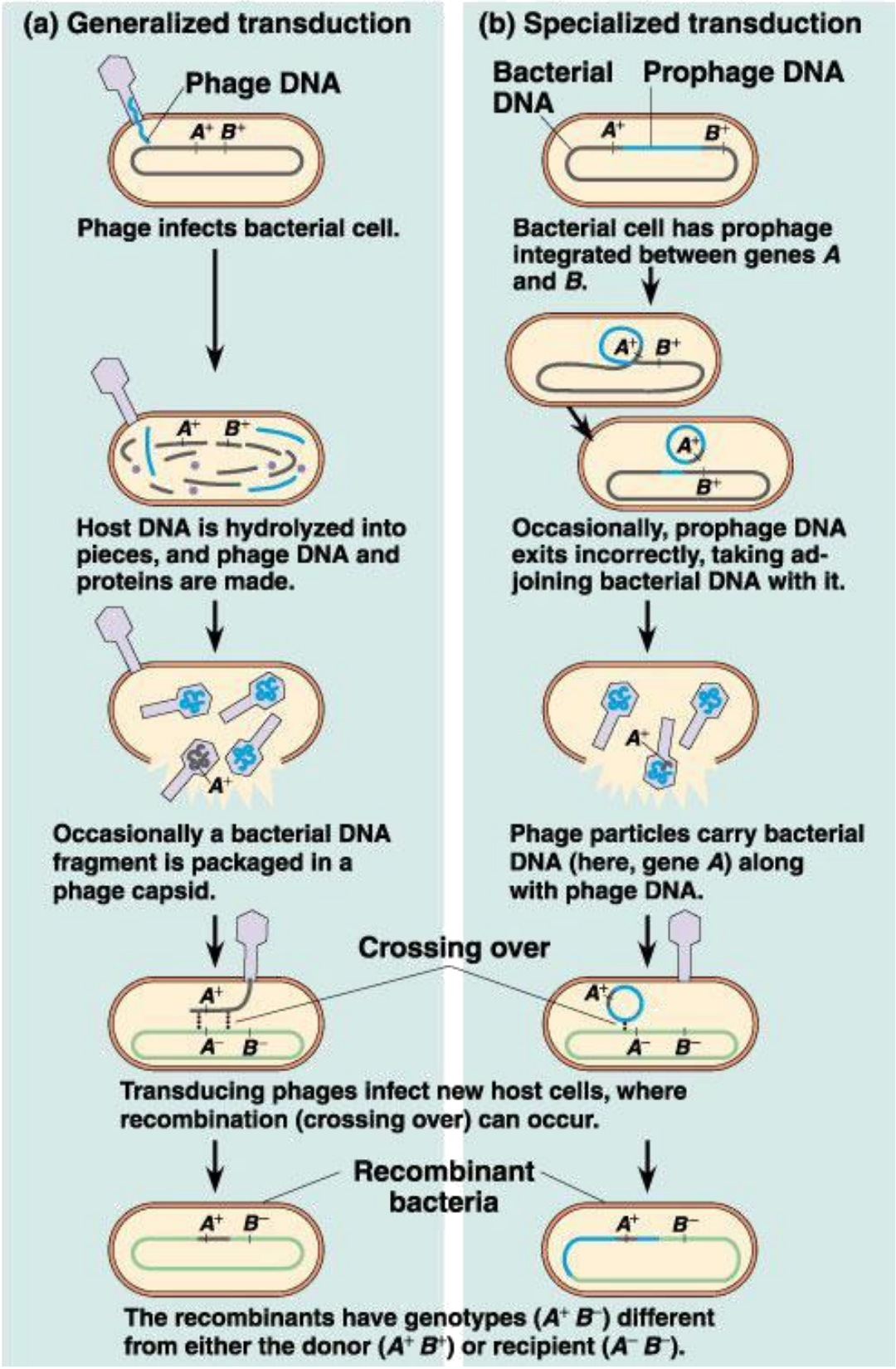
a) Generalized transduction: In generalized transduction, a random or nearly random segment of bacterial DNA is "wrapped up" during phage maturation in place of, or along with, the phage chromosome in a few "progeny" particles, called transducing particles. Generalized transducing phages can, therefore, transport any gene of the donor cell to the recipient cell. Since all the genes of the donor are represented in a population of these transducing particles, this type of transduction was named "generalized transduction". In some cases, generalized transducing particles contain only bacterial DNA and, in other cases, they contain both phage and bacterial DNA.

Generalized transduction is mediated by some virulent bacteriophages and by certain temperate bacteriophages whose chromosomes are not integrated at specified attachment sites on the host

chromosome. Generalized transducing particles are produced during the lytic cycles of these phages.

b) Specialized transduction: In specialized transduction, a recombination event, involving the host chromosome and the phage chromosome, occurs, producing a phage chromosome containing a segment of bacterial DNA. Specialized transducing particles, thus, always contain both phage and bacterial DNA. Specialized transduction is so named because a given virus transduces only genetic markers of the host that are located in one small region of the bacterial chromosome.

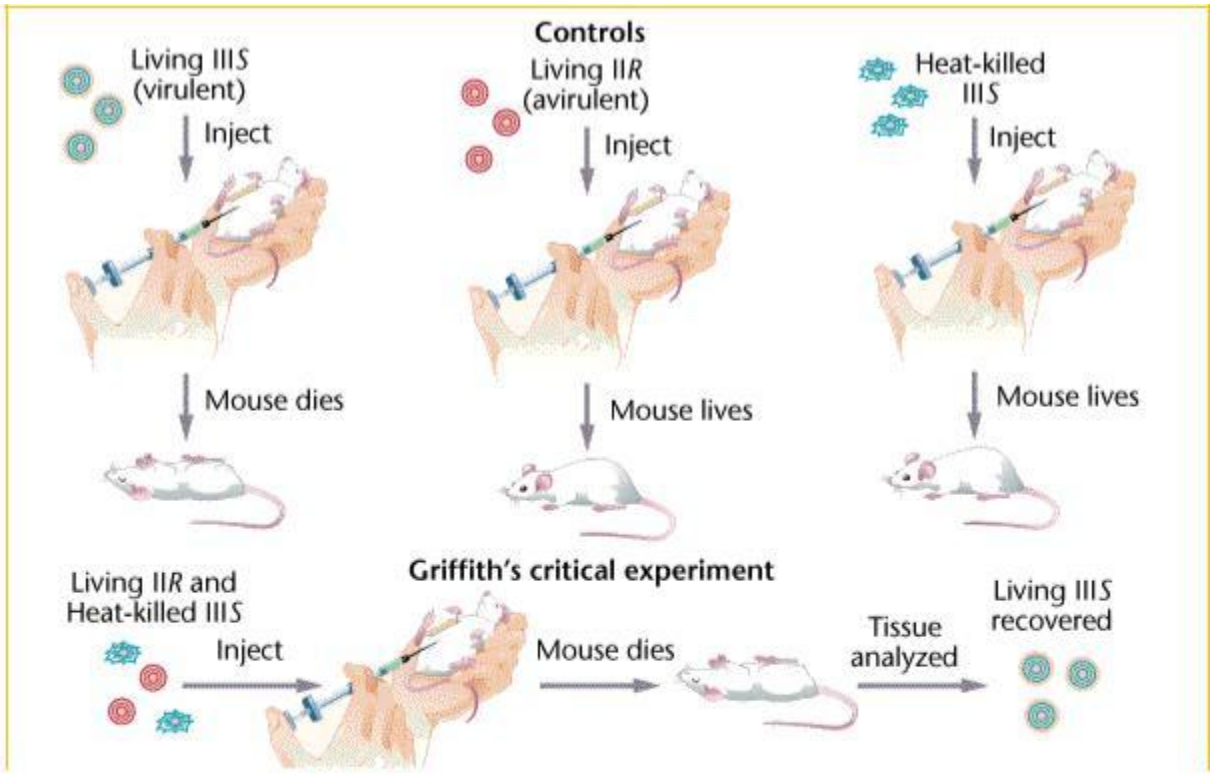
Specialized transduction is mediated by the temperate bacteriophages, whose chromosomes are able to integrate at one, or a few specified attachment sites on the host chromosome. In its integrated state, the phage chromosome is called a **prophage**. The chromosomes of temperate phages of this type are thus capable of both (i) autonomous replication (replication independent of the replication of the host chromosome) and (ii) integrated replication (replication as a segment of the host chromosome). As such, they are examples of genetic elements called episomes.



Differences between generalized and specialized transduction

3. Transformation

Frederick Griffith (1928), an English bacteriologist, accidently found that the heat-killed bacteria of virulent strain (type) of *Pneumococcus pneumoniae* could transfer characteristics of its strain to the non-virulent strain of living bacteria.

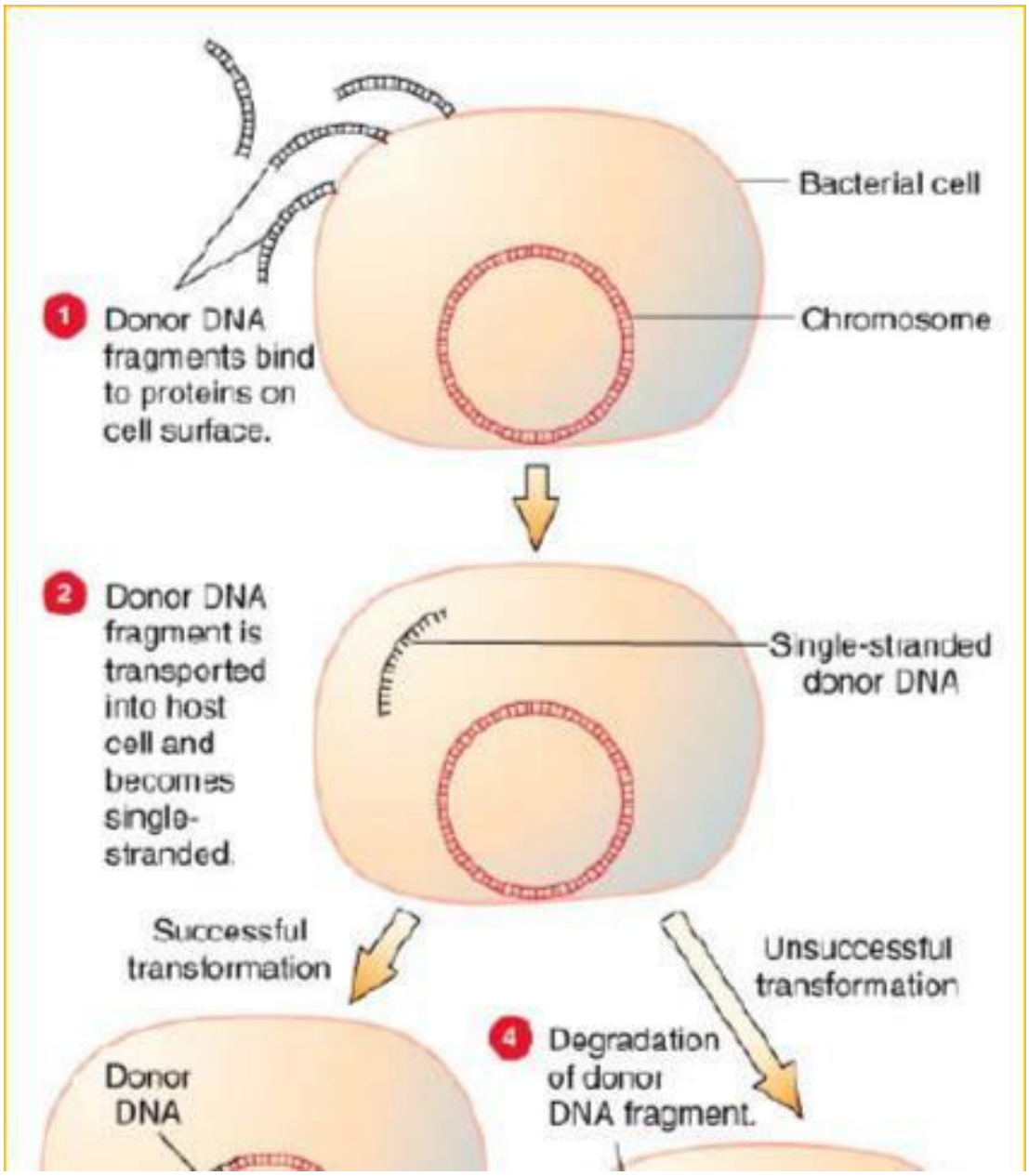


Griffith's Transformation Experiment

***Pneumococcus* bacteria include two strains, a *virulent* S strain with a *S*mooth coat that kills mice (left), and a *non -virulent* R *R*ough strain that does not (middle). Heating destroys the virulence of S (right). When heat-killed S is mixed with live R and injected into mice, the mouse dies. Its tissue contains living bacteria with *smooth coats* like S, *and* these bacteria are subsequently virulent to mice. Something in the heat-killed S bacteria has '*transformed*' the biological and especially the hereditary properties of the R bacteria.**

Avery, Macleod and McCarty (1944) observed that it is due to the transfer of DNA segments from the dead cells to the living cells. They called the uptake and incorporation of DNA by bacteria as "transformation" and the normal ability to take up exogenous DNA from the

environment as “competence”. Therefore, transformation may be defined as the gene transfer by soluble or naked DNA, which has been extracted or otherwise liberated from a donor bacterium to a recipient bacterium. This is the longest-known and historically the most important kind of gene transfer in bacteria.



DNA damage, repair and mutations

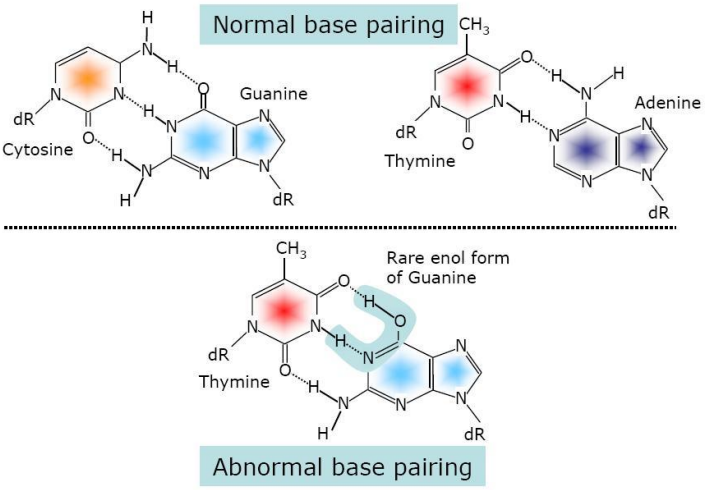
Harry Vrieling

Introduction

DNA contains the blueprint for the proper development, functioning and reproduction of organisms ranging from bacteria, low eukaryotes to vertebrates like man. It is therefore of extreme importance that both replication, i.e. the duplication of DNA, and the segregation of fully replicated DNA molecules (i.e. the chromosomes) over the two daughter cells occur with high accuracy.

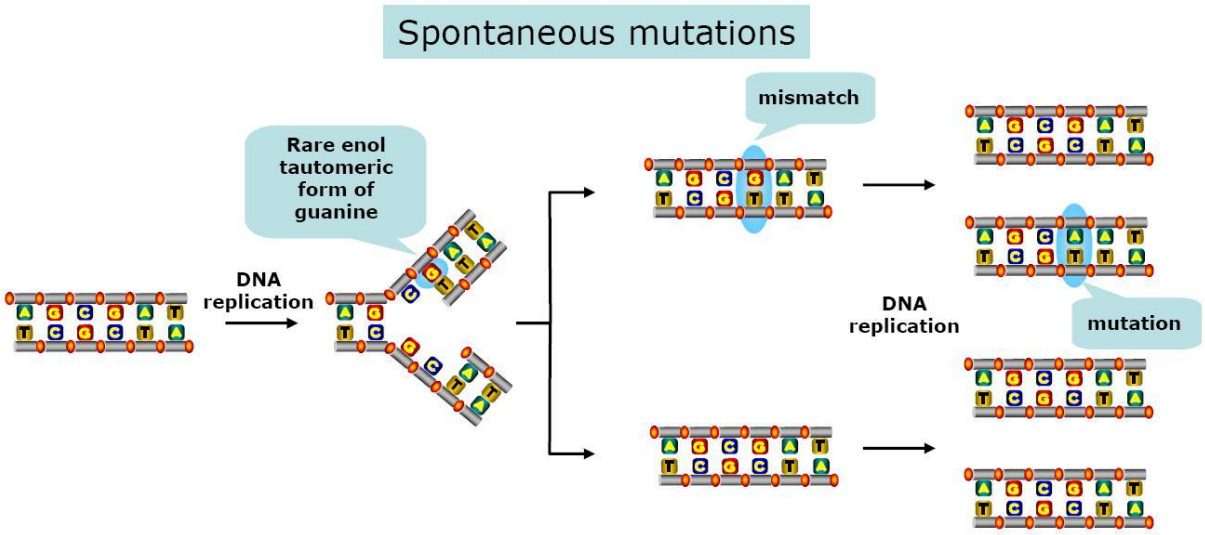
Mutations

Mutations are defined as permanent changes in the nucleotide sequence and comprise various types. Mutations are necessary for adaptation and evolution, but high mutation rates increase



cancer susceptibility and may endanger survival of the species. There are several sources for the formation of spontaneous mutations.

Misincorporations can occur even when the inserted nucleotide basepairs with the template. This is because every nucleotide base can occur as either of 2 alternative tautomers, structural isomers that are in dynamic equilibrium. For instance, while most of the guanines will be in the keto form,

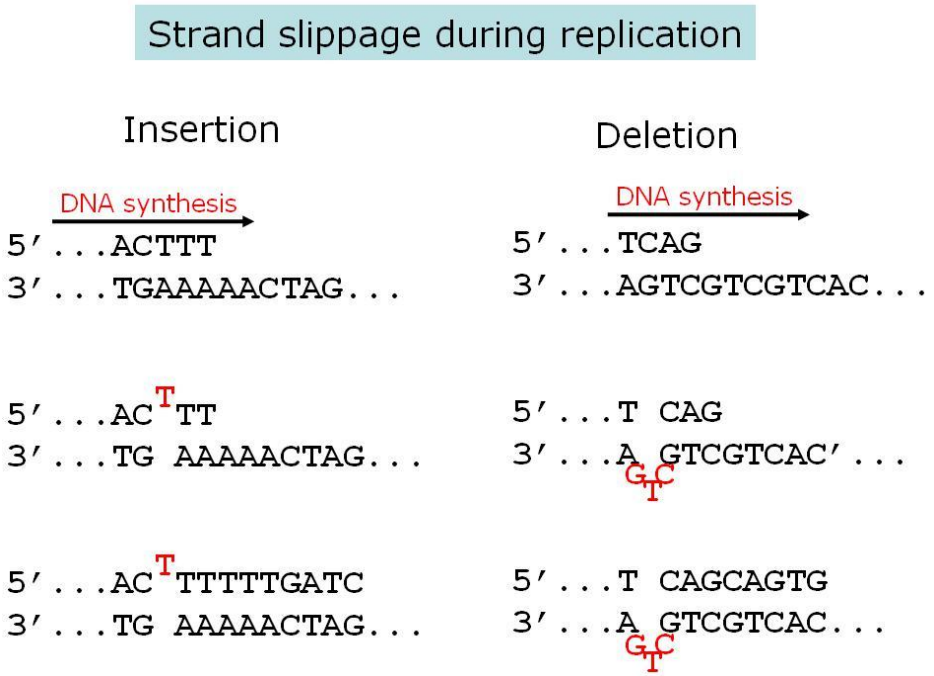


and will base pair with cytosine, the rare tautomeric enol form of guanine will base pair with thymine.

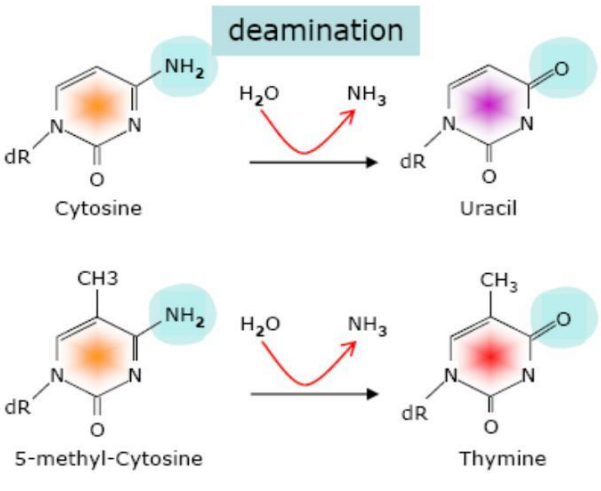
After replication, eventually this rare tautomer will revert back to its more common form, leading to a G-T mismatch in the daughter double helix. When left non-repaired, such a mismatch will in

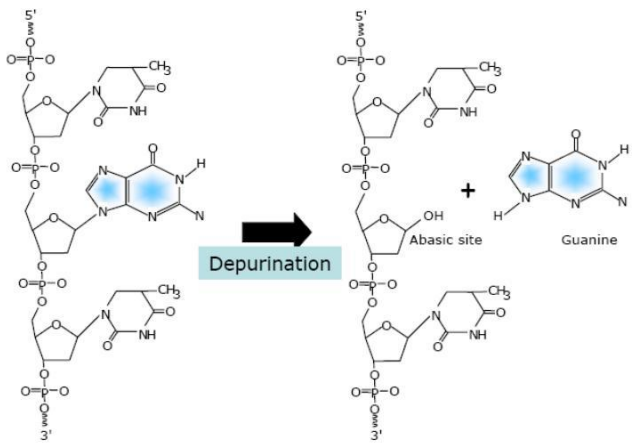
the next round of replication lead to a mutation, where a GC base pair is changed into an AT base pair.

Misincorporations can lead to six different types of base pair substitutions: i.e. **transitions** in which a pyrimidine is changed into the other pyrimidine and the purine into the other purine (AT -> GC and GC -> AT) and **transversions** in which a pyrimidine is changed into a purine and *vice versa* (GC -> TA, GC -> CG, AT -> CG and AT -> TA). Whether these mutations affect gene function depends on where they are located within a gene and whether they affect levels of gene expression, mRNA splicing or protein composition (See the paragraph **Type of mutations** on page 13 for more details).



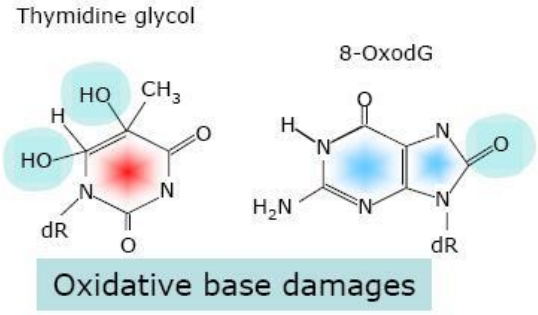
Slippage of either the template or the newly synthesized strand during replication is another source of replication errors. Slippage will result in the omission or addition of extra bases. Slippage happens most frequently on repetitive sequences in DNA, e.g. stretches of a particular nucleotide (e.g. …TTTTTT…) or at dinucleotide (e.g …CTCTCTCT…) or trinucleotide repeats (e.g. …CAGCAGCAGCAG…). It is a consequence of detachment of the polymerase from the template while copying such a repeat, and reattachment at another position in the same repeat. The template strand and its copy therefore shift their relative positions so that part of the template is either copied twice or skipped.
Replication slippage frequently leads to insertion or deletion of one or two bases. These mutations are called **frameshift mutations** when they occur in the coding region of a gene and change the reading frame used for translation. Generally this will result in a non-functional or truncated protein.





DNA damage and its consequences

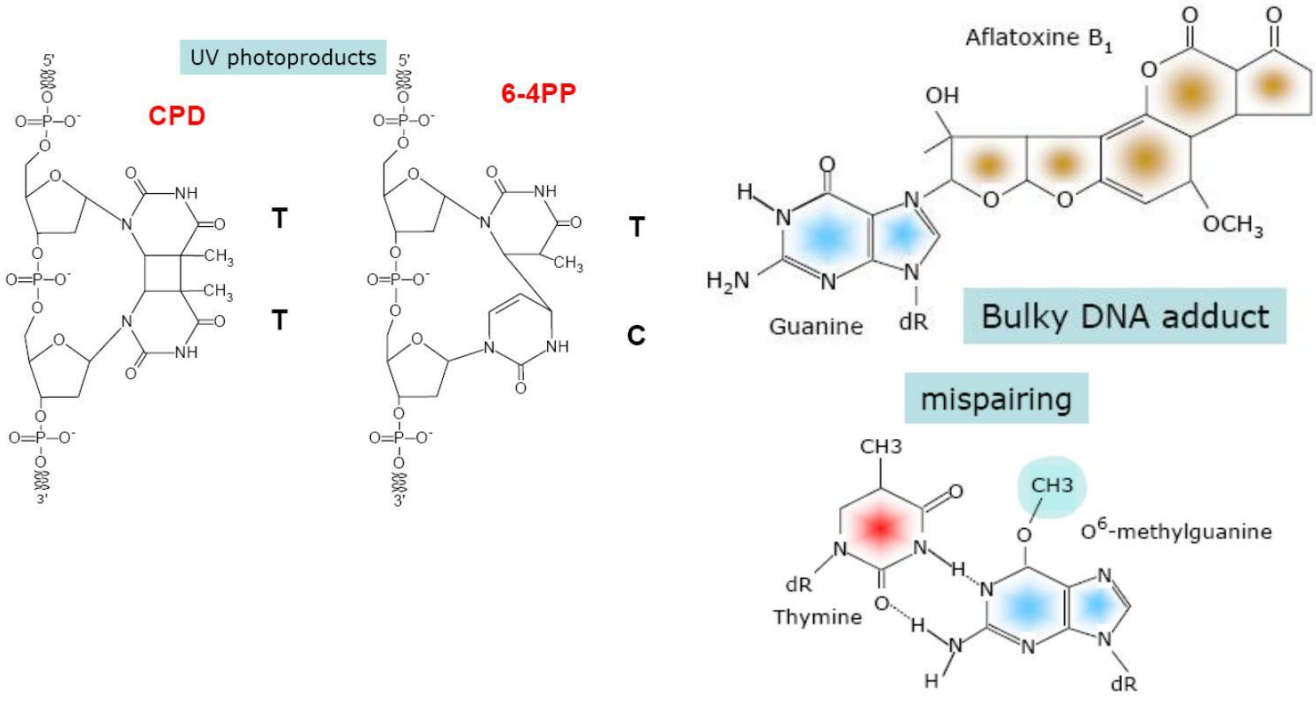
Spontaneous base decay and base damage by endogenous and exogenous sources continuously threaten the structure and integrity of DNA molecules. Spontaneous alterations can occur through intrinsic instability of chemical bonds in DNA (e.g. **deamination**, **depurination**, hydrolysis), or by interaction with endogenous reactive molecules in the cell resulting in e.g. **oxidation** or **methylation** of DNA bases. A frequently occurring type of spontaneous DNA damage is depurination and depyrimidination of bases, which results in apurinic or apyrimidinic **(AP) sites**



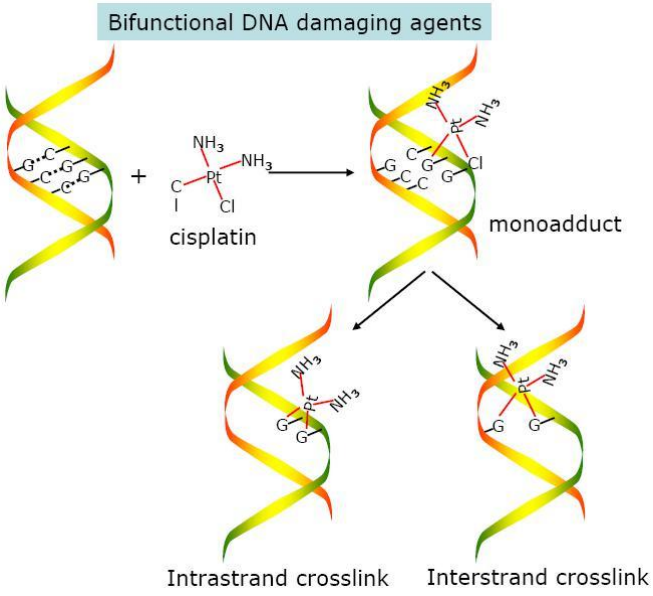
that have lost their base. Deamination of cytosine results in uracil in DNA, which when not removed will base pair with adenine during replication, leading to GC > AT transitions. Methylation of at the 5-position of cytosines that are part of a CpG sequence in promoter regions plays an important role in the regulation of gene expression. Deamination of 5-methyl-cytosine results in a thymidine and a G:T mismatch that needs to be repaired.

Physical and chemical environmental agents can also compromise the integrity of DNA. Major physical genotoxic agents are ionising radiation (e.g. X-rays, γ-rays, α-particles), polycyclic aromatic hydrocarbons (PAHs, e.g. benzo[a]pyrene) present in cigarette smoke or roasted meat and ultraviolet (UV) light. Ionising radiation gives rise to the formation of **single and double strand breaks**, apurinic sites and modified DNA bases. The major-formed DNA lesions by UV light are **cyclobutane pyrimidine dimers** (CPD) and **6-4 photoproducts** (6-4-PP) through covalent linking of neighbouring pyrimidines. In nature, these lesions are mainly caused by UV-B (320-290 nm) light, the most mutagenic part of the UV spectrum in normal daylight. **Bulky DNA adducts** such as UV photoproducts will distort the DNA helix extensively and will loose correct base pairing capacity. Chemical agents can either directly react with DNA or require first metabolic activation to obtain a DNA-damaging metabolite. Chemical agents can cause all kinds of DNA adducts ranging from "small" methylated bases to "bulky" lesions resulting in helical distortions.

Also addition of small alkyl groups to DNA bases, such as methylation of guanine at the O^6 position, might alter base pairing properties resulting in **mispairing** during replication.



Poly-functional agents like cisplatin and mitomycin C have more than one reactive site that interacts with the DNA, leading to mono-adducts when only one of the active group has reacted with DNA and **cross-links** within the same strand or to the opposing DNA strand (intra- or inter-strand crosslinks, respectively) when both reactive groups of the agent have reacted with the DNA molecule.



DNA repair pathways
In order to avoid the mutagenic and toxic effects of DNA damage, most DNA lesions will have to be recognised and removed before DNA replication will fix them into permanent genetic changes. Remaining and erroneously repaired lesions hamper cellular processes like transcription and replication resulting in cell cycle arrest, (programmed) cell death and fixation of mutations. At the level of the organism, mutations in germ cells can result in genetically inherited diseases, while accumulation of mutations in somatic cells is associated the initiation and progression of cancer and possibly ageing. Therefore, a complex network of complementary DNA repair pathways has arisen. Considerable overlap exists in substrate specificity of repair pathways and certain proteins function in more than one pathway.

1-Direct repair
A few highly specialized repair systems exist that are capable of restoring the structure and chemistry of the damaged DNA to its original state.

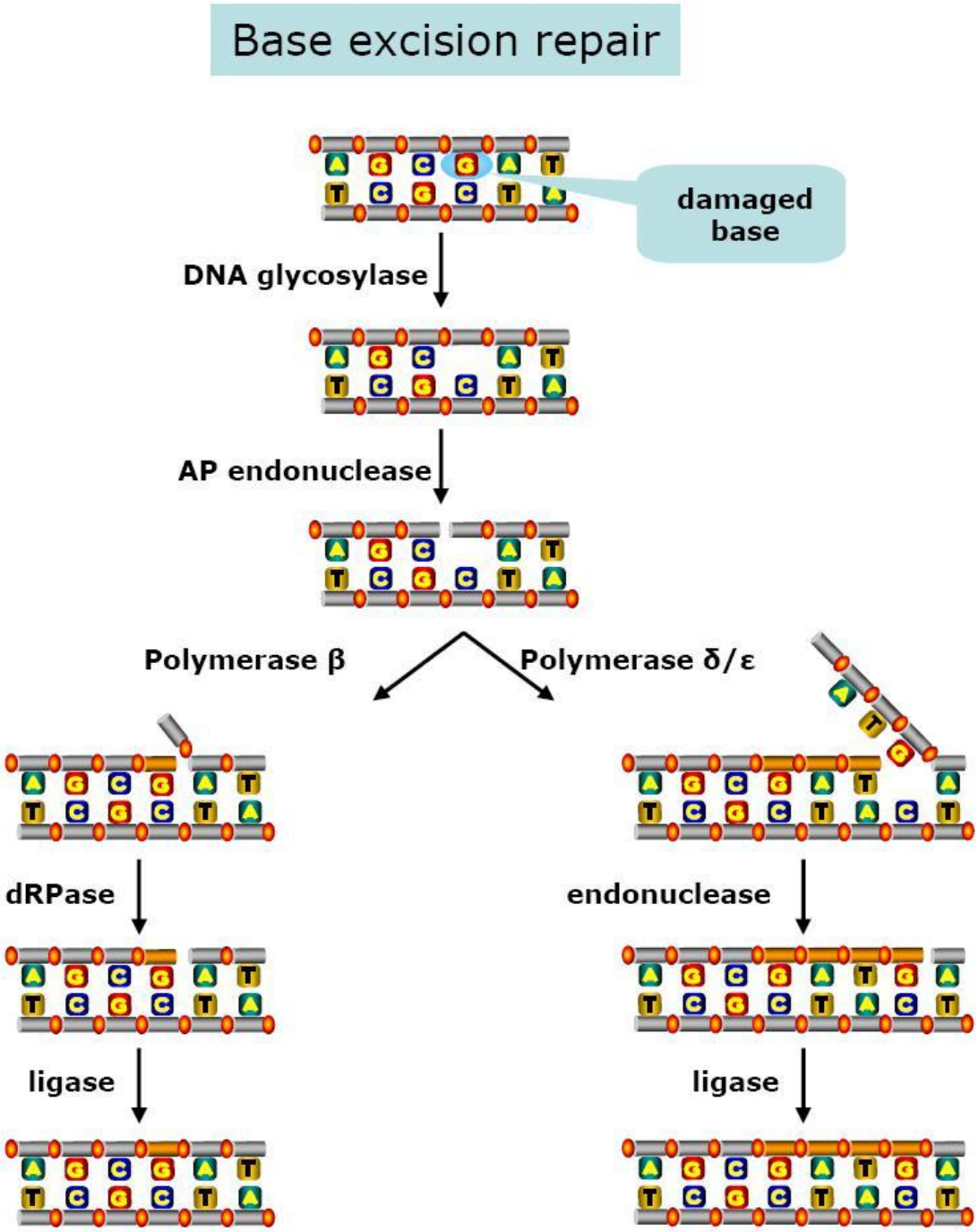
A. Photolyase
Several species possess photoreactivating enzymes (photolyases) that are capable of converting UV-induced photoproducts into their original bases when stimulated by light with a wavelength of 300 - 500 nm. Both CPD as well as (6-4)PP-specific photolyases exist. Although they are present in many bacteria and even in some vertebrates (i.e. marsupials), they are absent in human and other placental mammals.

B. O^6-Methyl-G DNA methyltransferase
Some forms of alkylation damage are directly reversible by enzymes that transfer the alkyl group from the base to their own polypeptide chain. Mammalian O^6-Methyl-guanine-DNA methyltransferase (MGMT) is capable of repairing the highly mutagenic O^6-Me-guanine and O^4-Me-thymine lesions from DNA by transferring the methyl group to a cysteine residue in the protein. Since it thereby inactivates itself, MGMT is a suicide protein.

2-Excision repair

A. Base excision repair
Most lesions that are repaired by base excision repair (BER) are caused by spontaneous hydrolytic deaminations, reactive oxygen species or methylating agents. In the first step of BER, damaged purine or pyrimidine bases are excised from the DNA by lesion-specific **DNA glycosylases** that hydrolyse the base-sugar bond resulting in an apurinic/apyrimidinic (AP) site. Although each

member of this group of enzymes exerts high specificity for specific types of base damage, the fundamental mechanism seems to be universal for all DNA glycosylases. The remaining **AP site** is further processed by an AP endonuclease that cuts the sugar phosphate backbone and creates a single stranded break. Polymerase β incorporates the required undamaged nucleotide and then a dRPase removes the abasic sugar-phosphate (dRP) group before subsequent ligation by DNA ligase. Besides the above described "short-patch" BER, also a "long-patch" BER mechanism exists which replaces 6-13 nucleotides. **Single-strand breaks** in the sugar-phosphate backbone of DNA caused for instance by ionizing radiation are following some processing of the single stranded DNA ends also repaired by the BER pathway.



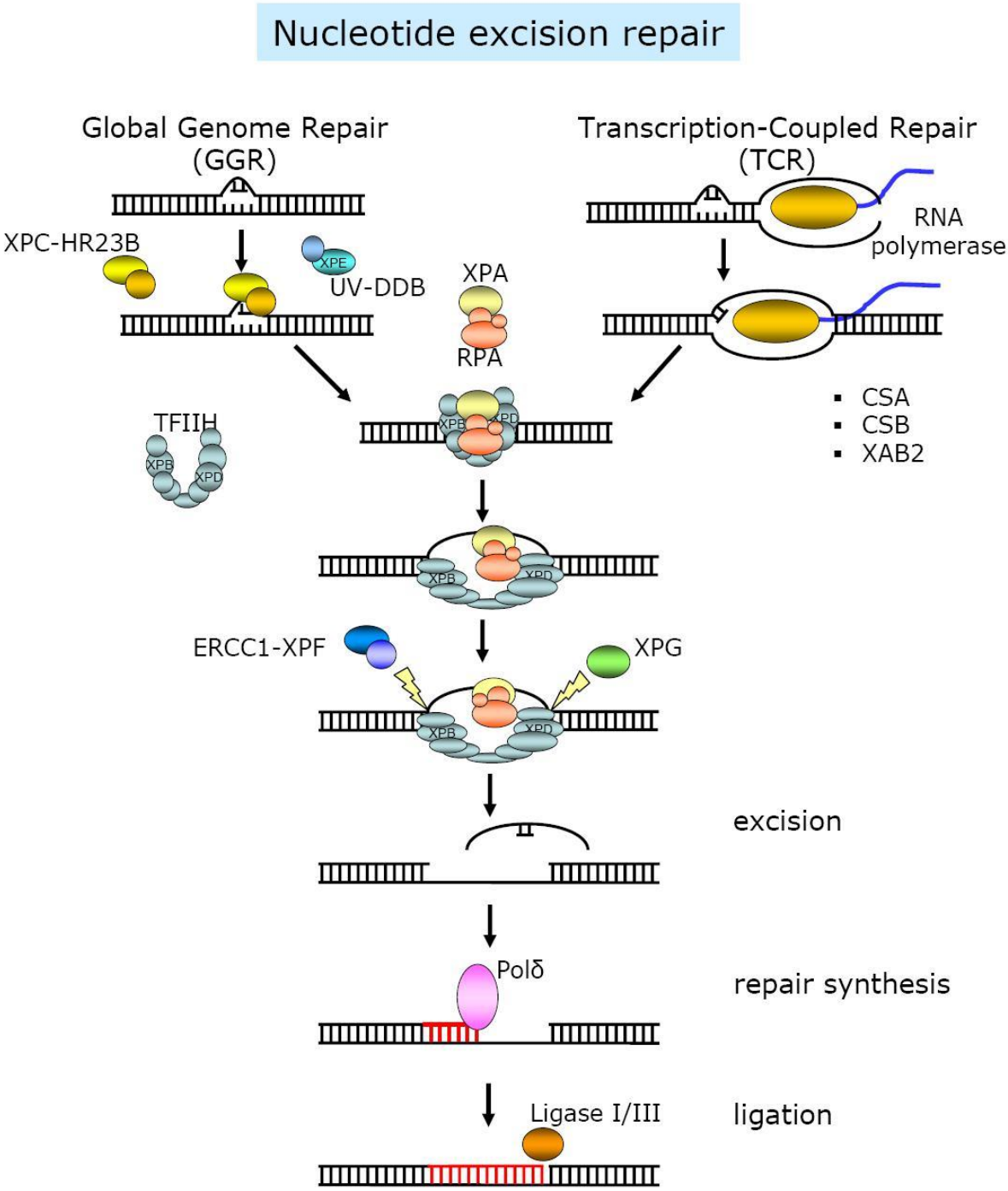
B. Nucleotide excision repair

Nucleotide excision repair (NER) is a versatile repair pathway that can eliminate a broad variety of structurally unrelated DNA lesions and is preferentially recruited to remove potentially mutagenic and toxic DNA lesions that locally destabilise the DNA helix. Therefore, it is not the actual lesion

that is recognised by the NER-system, but rather the damage-induced conformational change in DNA. Clinically relevant NER substrates are PAHs and UV-induced CPDs and (6-4)PPs.

The molecular mechanism of NER and proteins involved

The basic mechanism of NER is conserved from *E.coli* to man and consists of several successive steps: the recognition of the DNA damage, local opening of the DNA double helix around the injury and incision of the damage-containing DNA strand on either side of the lesion. After excision of the oligonucleotide containing the damage, DNA repair synthesis fills the resulting gap and the newly synthesised strand is ligated.



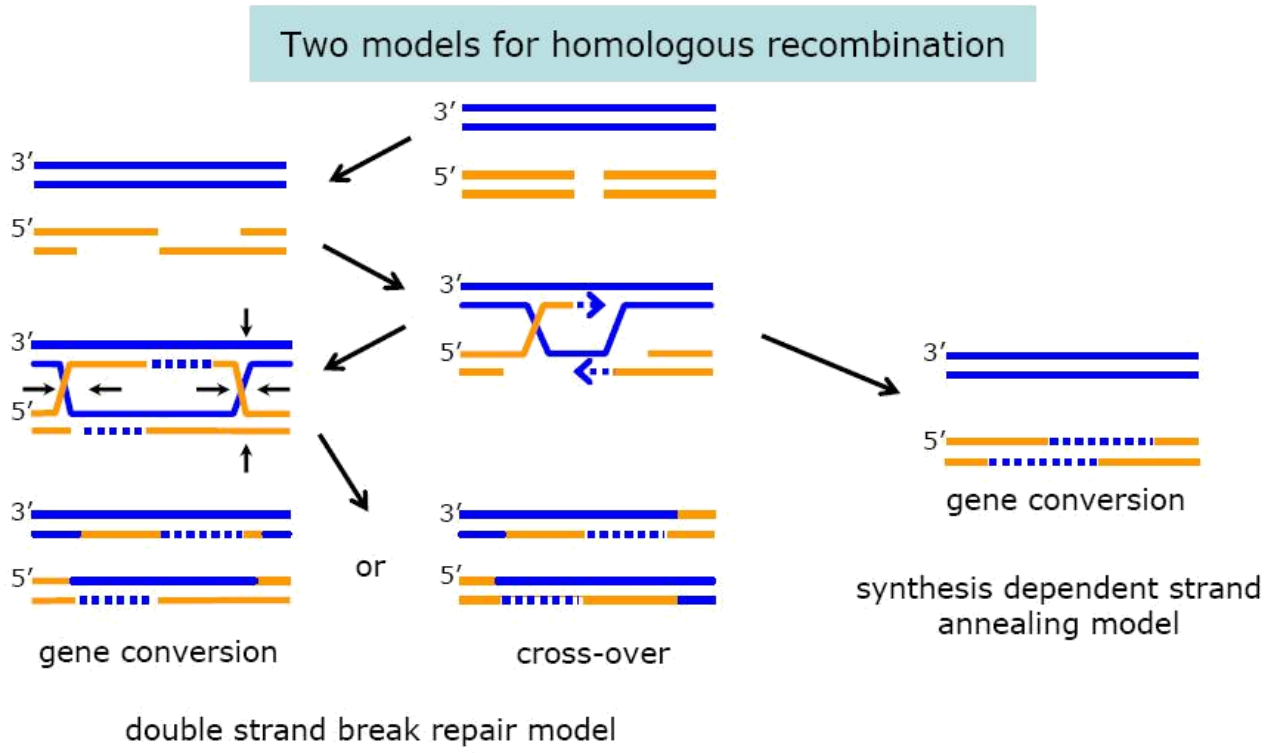
Damage recognition. The NER reaction starts with recognition of the DNA injury. This initial damage-recognising step consists of binding of the XPC/hHR23B complex to the damage, thereby recruiting the repair protein apparatus to the injury. Recently, it has become clear that UV-DDB, a heterodimer of the DDB1 and DDB2 (=XPE) proteins, can accelerate repair of certain types of DNA damage. XPA, the first human NER protein shown to have preferential affinity for DNA lesions,

appears to be involved in the verification of the damage and proper organisation of the repair apparatus with the assistance of the single strand DNA binding protein complex RPA.
Open complex formation and lesion demarcation. XPC/hHR23B and TFIIH are required at the earliest steps of opening of the helix. Full opening of the helix is dependent on the presence of ATP, which suggests that the XPB and XPD helicases of the TFIIH complex that possess opposite polarity (XPB: 3'→5'; XPD: 5'→3'), are actively involved.
Dual incision of the damaged strand. The first incision 3' of the open complex is performed by the structure-specific endonuclease XPG followed by the 5' incision by the ERCC1/XPF complex, to excise an oligonucleotide of 24-32 nucleotides containing the lesion.
Gap filling and ligation. The final step in NER is gap-filling of the excised patch by DNA repair synthesis. For this process the presence of DNA replication factors RPA, RFC, proliferating cell nuclear antigen (PCNA) and the DNA polymerases δ and/or ε are necessary. Ligation of the newly synthesised DNA is most likely performed by ligase I or ligase III, since mutations in the corresponding genes can give rise to a UV-sensitive phenotype.

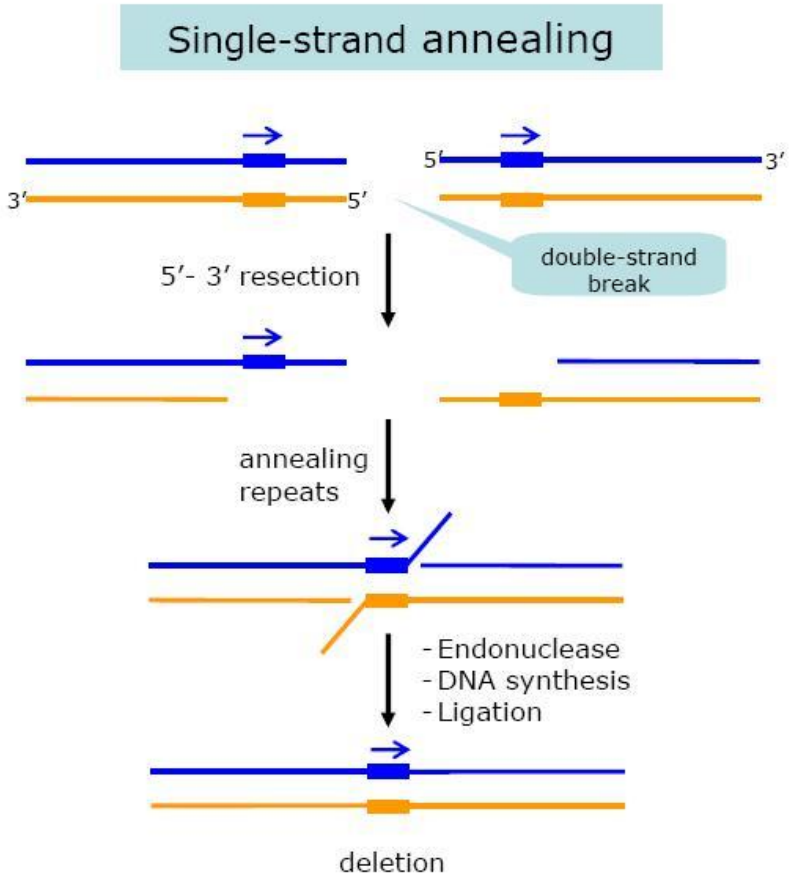
Two subpathways of NER
Two different subpathways of NER are known. The reaction mechanism described above involves the repair of DNA damage from any place in the genome. This (for the majority of lesions relatively slow) process is called **global genome repair** (GGR or GG-NER). In contrast, lesions that are located in the transcribed strand of active genes are repaired more efficiently by **transcription-coupled repair** (TCR or TC-NER), which exclusively removes DNA adducts that block transcription of an active gene. GGR and TCR are mechanistically the same, except for the initial damage recognition step. The XPC/hHR23B complex is not needed in TCR, since damage recognition is performed by a stalled RNA polymerase II (RNAPII), followed by activation of the repair machinery. The CSA and CSB proteins fulfill key roles in attracting NER proteins and chromatin remodeling factors to the stalled RNAPII. Cells lacking either of the two CS proteins are not capable of performing TCR.

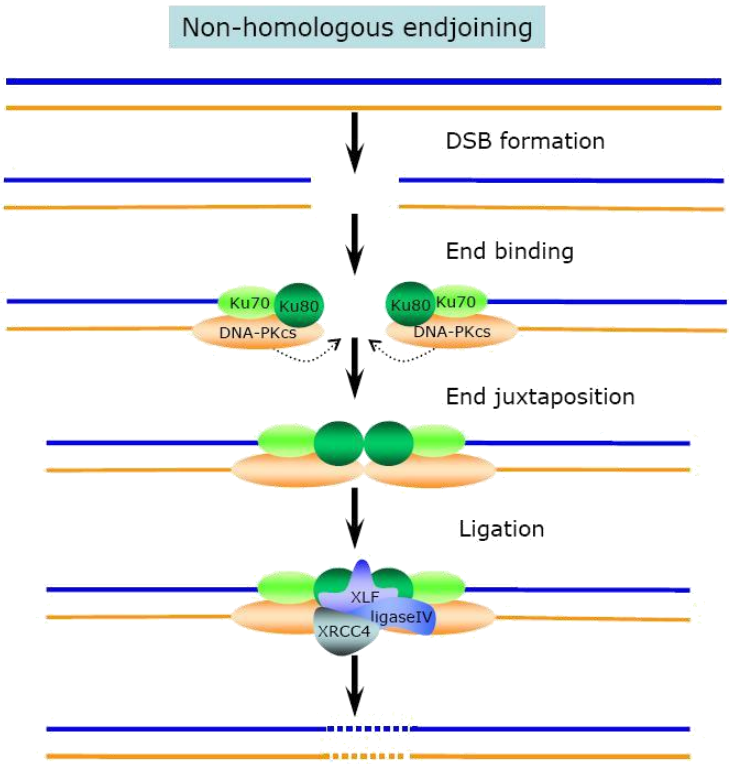
3-Double strand break repair.
DNA double-strand breaks (DSBs) are frequently formed by exogenous and endogenous factors including ionising radiation, oxidative damage of the DNA backbone, mechanical stress, cellular DNA metabolising agents and through the process of DNA replication itself, when the replication fork encounters single stranded nicks in the DNA. Efficient repair of DSBs is necessary since replication and transcription are blocked at the site of a DSB and the exposed ends are susceptible to degradation, possibly leading to the loss of genetic information. A complex consisting of the MRE11, hRAD50 and NBS1 proteins plays an important role in the recognition of DSBs, their exonucleolytic processing and the signalling to downstream repair pathways. DSBs in the DNA are repaired via two main pathways.

A. Homologous recombination (HR). In the process of homologous recombination, genetic information from a highly homologous DNA molecule (often the sister chromatid) is used as a template for repair. Firstly, the broken DNA strands are kept together to allow efficient repair. Then single stranded DNA regions are created with 3'-OH overhanging ends which are coated with a recombinase (Rad51 in human cells) that can invade a homologous DNA molecule. Recently, it has been shown that the breast cancer susceptibility gene, BRCA2, is involved in the loading of Rad51. Subsequently, various additional proteins (e.g. the Rad51 paralogs) are recruited which function in the stabilisation of the complex, branch migration, DNA synthesis or resolution of generated crossover junctions.



Single strand annealing (SSA). SSA is considered to be an error-prone subpathway of HR. It requires short sequence repeats flanking the break. Following a single stranded resection of the 5′ ends, repeat units from each end will base pair in order to align the DNA strands for rejoining. The non-complementary ssDNA tails are trimmed before ligation can take place. In this process the intervening sequence between the repeats is permanently lost.



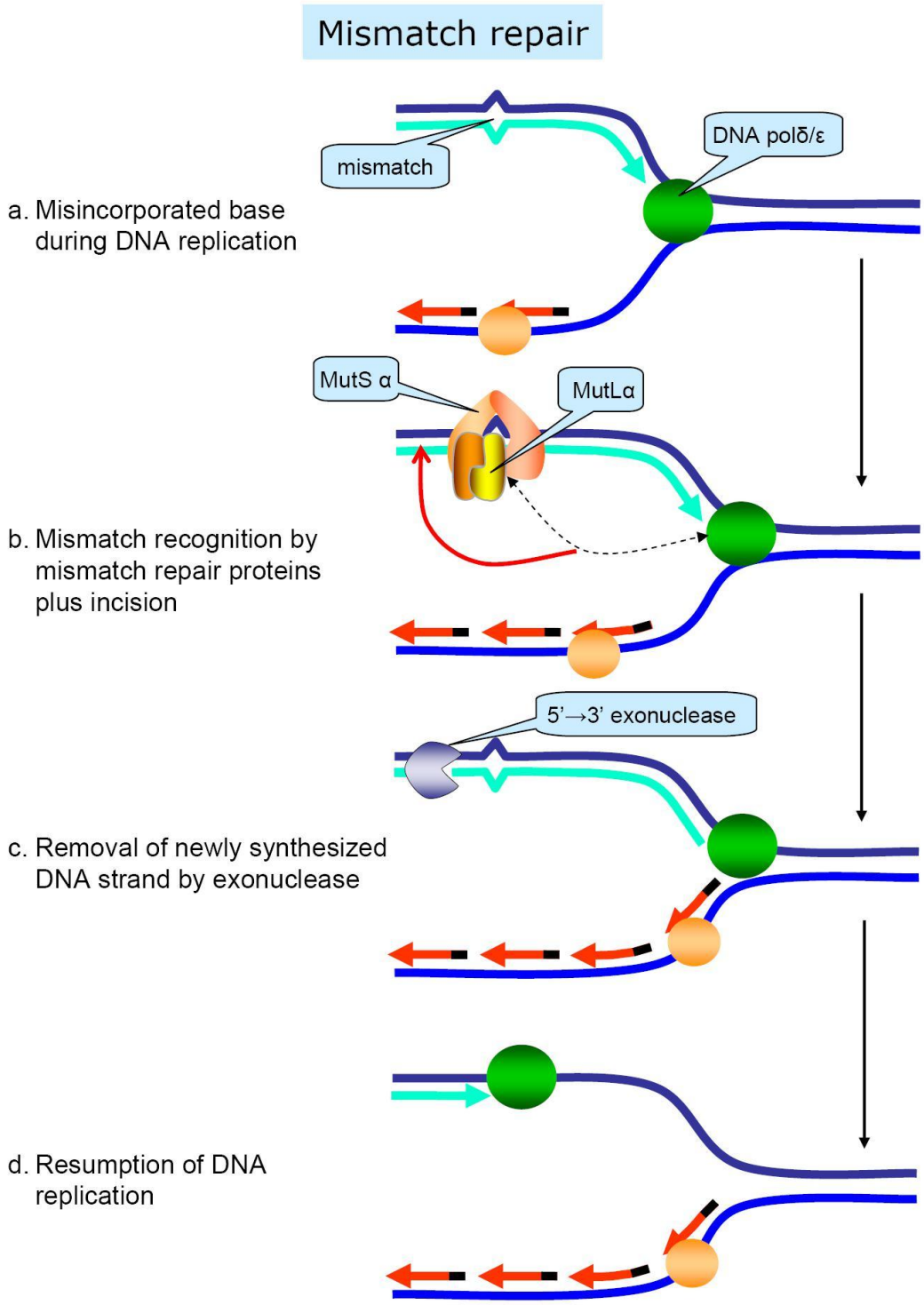


B. Non-homologous end-joining (NHEJ).
NHEJ does not require any sequence homology since the termini of the DSB are joined and ligated independently of the DNA sequence. The heterodimer Ku70/Ku80 binds to the DNA ends, and activates the catalytic subunit of DNA-PK (DNA-PKcs), which brings the ends together. Possible additional damage to DNA bases close to the DSB is removed by the action of the versatile endonuclease Artemis. XRCC4, XLF and DNA ligase IV perform the actual rejoining. This process frequently leads to loss of genetic information.

Choice for HR or NHEJ
Which of the above-described pathways is used for the repair of DSBs is dependent on the species and cell cycle stage. DSBs induced by ionizing radiation are in yeast predominantly repaired by recombinational repair, while in mammals NHEJ appears to play a pronounced role in their repair. However also in mammalian cells, recombinational repair is the most important pathway for DSBs caused by DNA inter-strand crosslinks or DSBs that occur during replication. The phase of the cell cycle thus appears to be important in deciding which pathway is most frequently used; HR (and SSA) is most efficient during the late S- and G2-phase when sister chromatids have been formed that allow error free recombinational repair whereas NHEJ predominates during G0 and G1.

4-Mismatch repair (MMR).
DNA mismatches loops arise not only by deamination of (5-methyl)cytosine but also by incorporation of inappropriate nucleotides during DNA synthesis, and during recombination. In *E. coli*, the MMR (MutHLS) pathway that repairs replication-associated mismatches and insertion/deletion loops has been studied extensively. The **MutS** protein recognises the mismatch and recruits **MutL** to form a MutS/MutL/DNA complex. In bacteria the newly synthesized strand, in which the misincorporation error was made, is recognized because it transiently lacks a specific type of methylation of adenine bases. **MutH** endonuclease will subsequently induce a single

stranded break at the hemi-methylated adenines at either side of the mismatch on the newly replicated strand. The single strand DNA fragment is excised after which a DNA polymerase can fill in the gap. The proteins in the MMR pathway of mammals have been conserved and some components are highly related to the bacterial MMR system. However, the complexity of the pathway has increased during evolution and the mechanism of strand discrimination in mammals is



different. Here, the newly replicated DNA strand containing the replication error likely is recognized through an interaction between replication factors and the MMR proteins. A 5′ → 3′ exonuclease removes the stretch of newly synthesized DNA containing the mismatch, after which the replicative DNA polymerase will resume replication. The mammalian homologues of *E. coli* MMR genes act as heterodimers; the homologue of the MutS homodimer is one of the hMSH2/hMSH6 (MutSα) or hMSH2/hMSH3 (MutSβ) dimers, hMSH2/6 recognizing base-base mismatches and small slipped

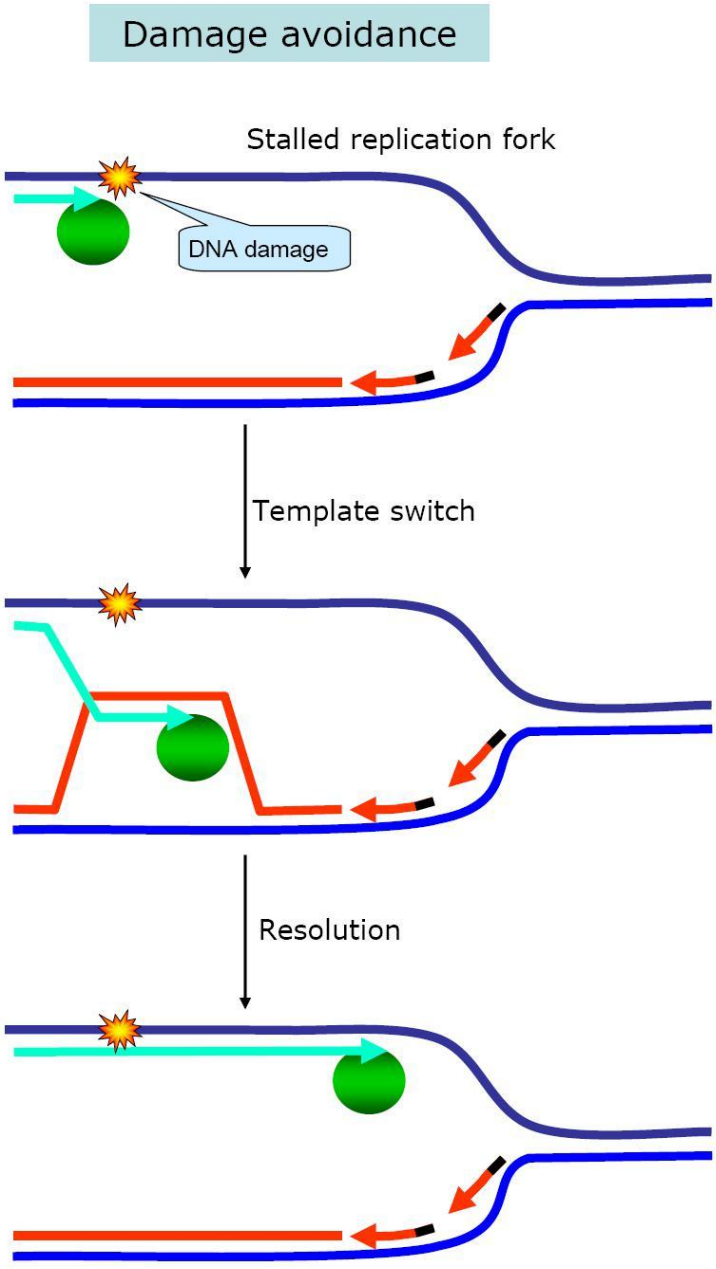
replication intermediates and hMSH2/3 being specialized for the repair of larger slipped replication intermediates. The MutL homologue consists of hMLH1/hPMS2, hMLH1/hPMS1 or hMLH1/hMLH3. Inborn mutations in the MMR genes hMSH2, hMLH1, hPMS1 and hPMS2 in humans are associated with the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome.

Bypass of nucleotide lesions

As described in the previous sections, a combination of different DNA repair systems effectively ensures that mutagenic and cytotoxic lesions are removed from the DNA. However, when this process is not complete, replication forks in the S-phase of the cell cycle may encounter the damage in the DNA template. To avoid cell death, DNA lesions have to be bypassed to complete the replication process. Two major pathways operate in DNA damage bypass.

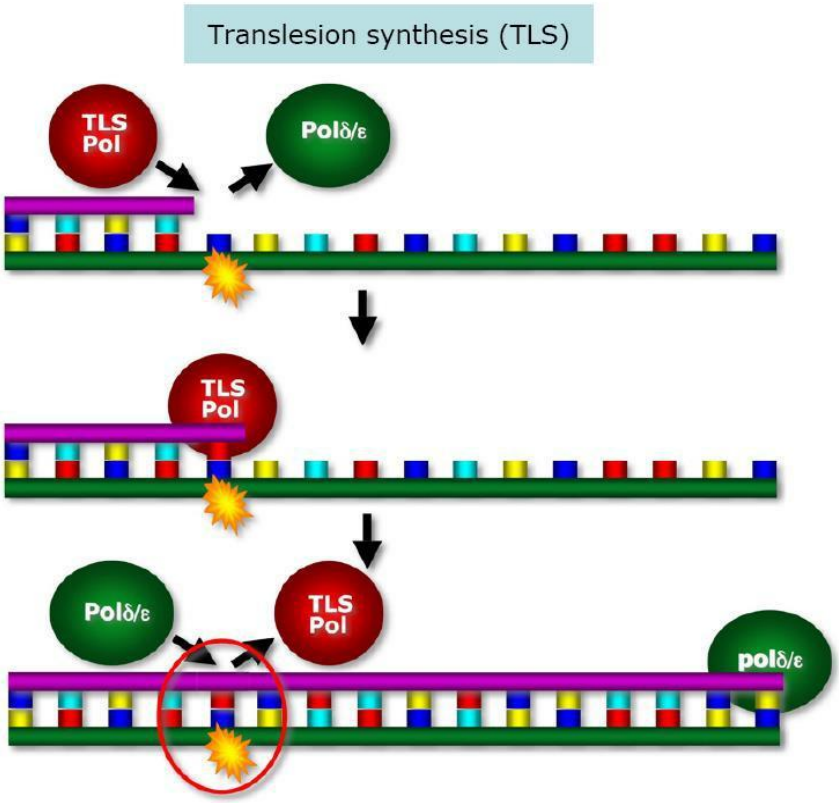
A-Damage avoidance

A replication fork arrest of one of the two nascent DNA strands at a DNA damage results in the formation of a single-stranded DNA region in the strand containing the blocked polymerase. The close presence of the sister chromatid allows pairing between the free 3' end of the arrested DNA strand with the complementary strand from the sister chromatid. Next, the sister chromatid serves as template for DNA synthesis from the invading strand. This choice for an alternative, but identical, template effectively results in (indirect) bypass of the template damage. Finally the nascent DNA reverts to its 'own' template after which normal replication can resume. Damage avoidance is error-free. The major players in this pathway yet have to be identified.

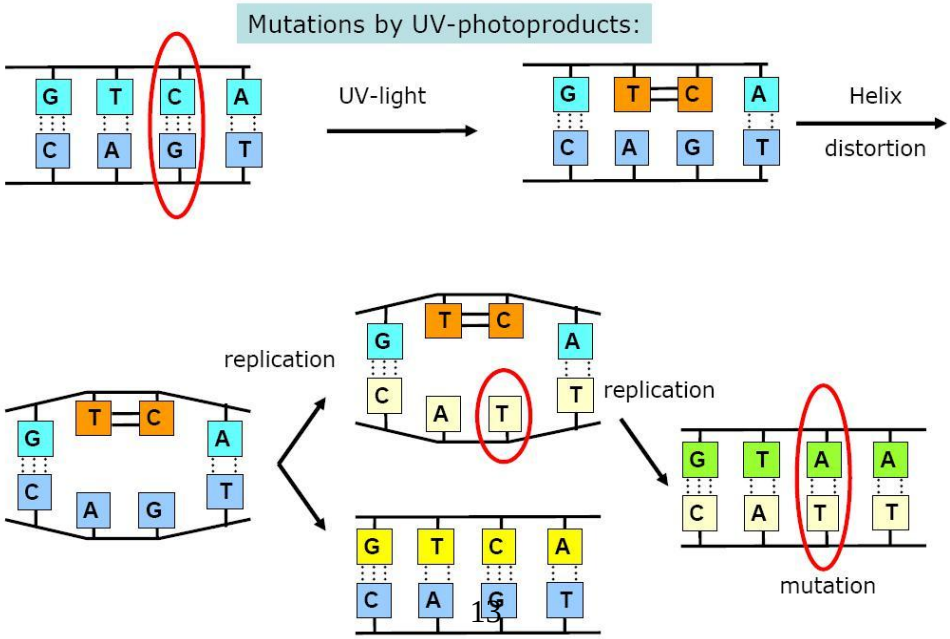


B-Translesion synthesis

The second pathway to bypass a damaged template during replication is called translesion synthesis (TLS). The replicative polymerase δ or ε are unable to replicate the damaged nucleotide leaving the 3' end of the nascent strand opposite the lesion. The replicative polymerase is then replaced by one of a number of specialized DNA TLS polymerases. TLS polymerases have greatly reduced template specificities and are therefore able to replicate across the damage, even if the helix is distorted as a consequence of the damage, and when base pairing and base stacking are not optimal. TLS polymerases, in contrast to the processive replicative polymerases are highly distributive, synthesizing only one or a few nucleotides at a time. After damage bypass the TLS polymerase is removed and normal processive replication can resume. An eventual consequence of



TLS is the incorporation of a wrong nucleotide, resulting in a so-called compound DNA lesion, i.e. a misincorporation opposite a damaged nucleotide. During the next replication cycle the misincorporation will be replicated and therefore be fixed into a mutation. Although TLS of DNA damage helps the cell survive nucleotide damage, it is a major source of mutations induced by DNA nucleotide damage and therefore plays an important role in DNA damage-induced pathologies like cancer or inherited disease.



Types of mutations
Fixation of mutations occurs when the DNA sequence is permanently changed via replication or bypass of the lesion, followed by transmission to daughter cells. On basis of the sequence alterations they cause in the DNA, mutations can be classified.

Point mutations/intragenic mutations. Point mutations result from the substitution of one base pair for another (**transitions** and **transversions**) or from the addition or deletion of a small number of base pairs, resulting in frame shifts. Point mutations within the coding region of a gene can also be referred to as intragenic mutations because they almost exclusively affect a single gene. The consequence of intragenic mutations can be an altered biological function of a protein via amino acid changes or truncation of the protein.

Base pair substitutions

```
                  Lys Gln Val
Wild type:   5' -----AAG CAA GTT -------- 3'
             3' -----TTC GTT CAA -------- 5'

                  Lys Stop
Nonsense     5' -----AAG TAA GTT -------- 3'  Protein truncation:
mutation:    3' -----TTC ATT CAA -------- 5'  (partial) loss of function

                  Lys Glu Val
Missense     5' -----AAG GAA GTT -------- 3'  Amino acid change:
mutation:    3' -----TTC CTT CAA -------- 5'  (partial) loss of function?
```

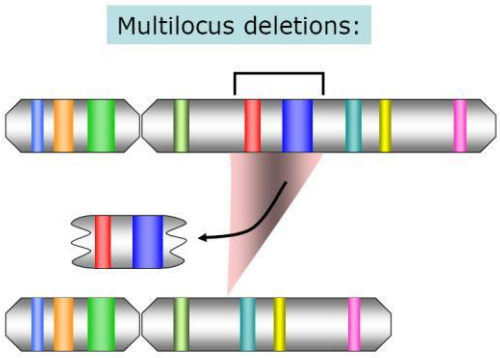
Frameshifts

```
                  Leu Glu Lys Gly Val Ile
Wild type:   5'--- TTA GAA AAG GGG GTA ATC --- 3'
             3'--- AAT CTT TTC CCC CAT TAG --- 5'

                  Leu Glu Lys Gly stop
-1 frameshift 5'--- TTA GAA AAG GGG TAA TC --- 3'
              3'--- AAT CTT TTC CCC ATT AG --- 5'
```

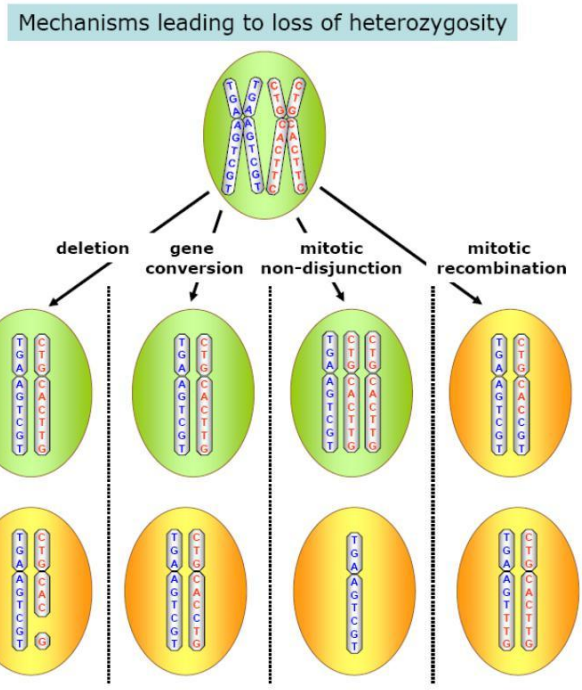
Protein truncation: (partial) loss of function

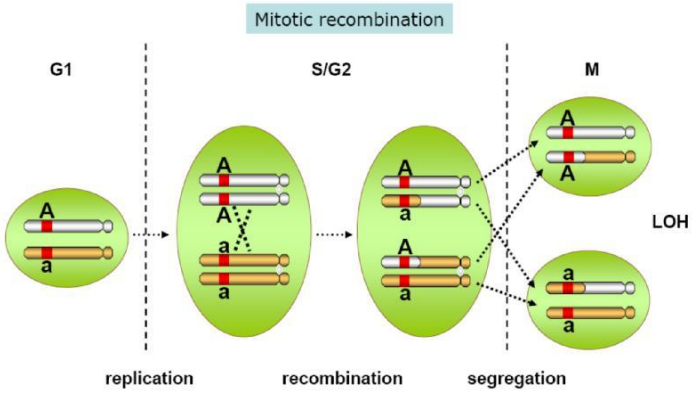
Chromosomal type of mutations. Chromosomal type of mutations are more extensive changes in the DNA sequence, including big deletions, insertions, duplications and inversions and can involve large stretches of DNA, encoding many genes. Chromosomal rearrangements result from mutational events that involve joining of broken chromosomes. In addition, errors during chromosome segregation at mitosis can affect the distribution of chromosomes over daughter cells resulting in the loss or gain of chromosomes.

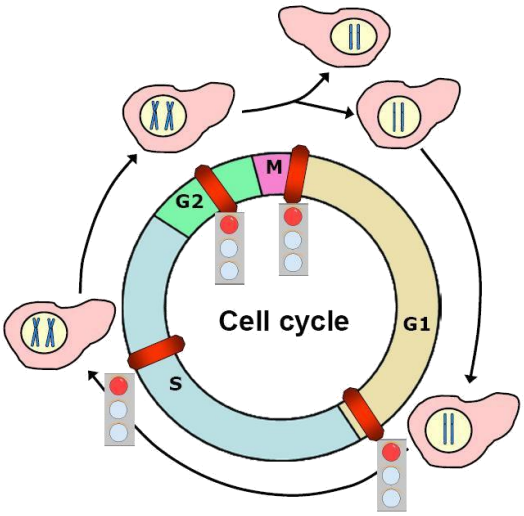


- Deletion on an autosomal chromosome: hemizygosity for multiple genes (only 1 allele remains)
- Deletion on a sex chromosome (X,Y): functional loss of multiple genes

Chromosomal types of mutations can lead to **loss of heterozygosity (LOH)** of large parts of chromosomes containing many genes, e.g. tumour suppressor genes. LOH is an important event in the aetiology of cancer, since up to 50% of the chromosomes in sporadic tumours have undergone LOH events. Several mutagenic events may give rise to LOH; deletion, gene conversion, mitotic non-disjunction and mitotic recombination. In humans the DNA sequence between two homologous chromosomes, one inherited from the father and the other from the mother, differs at a frequency of about 1:1000 base pairs, with the most frequent difference in DNA sequence being single nucleotide polymorphisms (SNPs). Microarray technologies currently allow determination of the status (homozygous or heterozygous) of hundreds of thousands of SNPs along all chromosomes in a single analysis, making it possible to accurately determine the extent and nature of individual LOH events.







Cell cycle arrest and apoptosis

Essential for all defense mechanisms of the cell in response to DNA damage are signal transduction pathways that lead to **cell cycle arrest** and programmed cell death (**apoptosis**). The cell cycle can be arrested temporarily by cell cycle checkpoints that are induced when cells contain a certain amount of DNA damage. The cell cycle is delayed until the lesions are repaired by one of the previously described repair mechanisms. However, if the damage is too severe to be adequately repaired, the cell may undergo apoptosis or enter an irreversible senescence-like state. A family of protein kinases called cyclin dependent kinases (Cdks) regulate, together with the associated family of cyclins that can activate the Cdks, the progression of a cell through the different phases of the cell cycle. Three different cell cycle checkpoints can be discriminated in eukaryotic cells i.e. the G1 to S transition, a checkpoint during S-phase and at the G2 to M transition. The *p53* tumor suppressor gene was the first gene that was discovered to be involved in checkpoint control. Cells with a deficiency in this tumour suppressor gene do not arrest at the G1/S checkpoint after cellular stress, indicating that this protein is important in the DNA damage-induced cell cycle arrest. The same holds for the *ATM* gene, being mutated in ataxia telangiectasia patients. ATM-deficient cells exhibit severely impaired G1, S and G2 checkpoint functions. ATM is an upstream regulator of **p53**; it stabilises and activates p53 in response to ionising radiation. An alternative downstream event mediated by p53 is the induction of apoptosis which is cell type-specific. The multi step process of apoptosis, that is an important mechanism during embryonic development and normal immune cell proliferation, is characterised by membrane instability, cell shrinkage, chromatin condensation and DNA fragmentation. Upon exposure to genotoxic agents, apoptosis is induced in p53-dependent and p53-independent manners in order to prevent the mutagenic consequences of DNA damage.