DNA repair.

Sources of DNA damage in the cell. Enzymology of DNA repair. Repair of single-strand damage: excisional repair of nucleotides, excisional repair of bases, repair of mismatched bases. Repair of double-stranded damage: homologous recombination, non-homologous end joining.

1. List and describe the sources of DNA damage in the cell.
2. Explain the significance of DNA repair.
3. Explain the mechanisms of base excision, nucleotide excision, homologous recombination, non-homologous end joining modes of repair.

Damage to cellular DNA is involved in mutagenesis and the development of cancer. The DNA in a human cell undergoes several thousand to a million damaging events per day, generated by both external (exogenous) and internal metabolic (endogenous) processes. Changes to the cellular genome can generate errors in the transcription of DNA and ensuing translation into proteins necessary for signaling and cellular function. Genomic mutations can also be carried over into daughter generations of cells if the mutation is not repaired prior to mitosis.

Damage to DNA may occur as a result of normal cellular metabolism (endogenous damage) or under influences of origin external to the cell (exogenous damage). Therefore, DNA damaging agents and the associated damage mechanisms may be broadly classified as agents/mechanisms of endogenous or of exogenous origin.

DNA damage can also result from endogenous metabolic and biochemical reactions, some of which are not well understood

Hydrolysis reactions can partially or completely cleave the nucleotide base from the DNA strand. The chemical bond connecting a purine base (adenine or guanine) to the deoxyribosyl phosphate chain can spontaneously break in the process known as depurination. An estimated 10,000 depurination events occur per day in a mammalian cell.[7] Depyrimidination (loss of pyrimidine base from thymine or cytosine) also occurs, but at a rate 20 to 100-fold lower than depurination.

Deamination occurs within the cell with the loss of amine groups from adenine, guanine, and cytosine rings, resulting in hypoxanthine, xanthine, and uracil, respectively. DNA repair enzymes are able to recognize and correct these unnatural bases. However, an uncorrected uracil base may be misread as a thymine during subsequent DNA replication and generate a C→T point mutation.

DNA methylation, a specific form of alkylation, occurs within the cell due to a reaction with S-adenosyl methionine (SAM). SAM is an intracellular metabolic intermediate that contains a highly reactive methyl group. In mammalian cells, methylation occurs at the 5-position of the cytosine ring of a cytidine base (C) that is 5’ to a guanosine base (G), i.e., sequence CpG. A significant source of mutation error is the spontaneous deamination of the 5-methylcytosine product of methyl-ation. Loss of the amine group results in a thymine base, which is not detected by DNA repair enzymes as an unnatural base. The resulting substitution is retained in DNA replication, creating a C→T point mutation

Normal metabolic processes generate reactive oxygen species (ROS), which modify bases by oxidation. Both purine and pyrimidine bases are subject to oxidation. The most common mutation is guanine oxidized to 8-oxo-7,8-dihydroguanine, resulting in the nucleotide 8-oxo-deoxy guano sine (8-oxo-dG). The 8-oxo-dG is capable of base pairing with deoxyadenosine, instead of pairing with deoxycytotidine as expected. If this error is not detected and corrected by mismatch repair enzymes, the DNA subsequently replicated will contain a C→A point mutation. ROS may also cause depurination, depyrimidination, and single-strand or double strand breaks in the DNA.

Other genomic mutations may be introduced during DNA replication in the S phase of the cell cycle. Polymerases that duplicate template DNA have a small but significant error rate, and may incorporate an incorrect nucleotide based on Watson-Crick pairing versus the template DNA. Chemically altered nucleotide precursors may be incorporated into the generated DNA by

the polymerase, instead of normal bases. In addition, polymerases are prone to "stuttering" when copying sections of DNA that contain a large number of repeating nucleotides or repeating sequences (microsatellite regions). This enzymatic "stuttering" is due to a strand slippage, when the template and replicated strands of DNA slip out of proper alignment. As a result, the polymerase fails to insert the correct number of nucleotides indicated by the template DNA, resulting in too few or too many nucleotides in the daughter strand.

Single strand and double strand cleavage of the DNA may occur. Single strand breaks may result from damage to the deoxyribose moiety of the DNA deoxyribosylphosphate chain. Breaks also result as an intermediate step of the base excision repair pathway after the removal of deoxyribose phosphate by AP-endonuclease 1.[8] When a single strand break occurs, both the nucleotide base and the deoxyribose backbone are lost from the DNA structure. Double strand cleavage most often occurs when the cell is passing through S-phase, as the DNA may be more susceptible to breakage while it is unraveling for use as a template for replication.

Because DNA uniquely serves as a permanent copy of the cell genome, however, changes in its structure are of much greater consequence than are alterations in other cell components, such as RNAs or proteins. Such damage to DNA can block replication or transcription, and can result in a high frequency of mutations—consequences that are unacceptable from the standpoint of cell reproduction. Because DNA is the repository of genetic information in each living cell, its integrity and stability are essential to life. To maintain the integrity of their genomes, cells have therefore had to evolve mechanisms to repair damaged DNA. DNA, is not inert; rather, it is a chemical entity subject to assault from the environment, and any resulting damage, if not repaired, will lead to mutation and possibly disease

DNA repair processes exist in both prokaryotic and eukaryotic organisms, and many of the proteins involved have been highly conserved throughout evolution. In fact, cells have evolved a number of mechanisms to detect and repair the various types of damage that can occur to DNA, no matter whether this damage is caused by the environment or by errors in replication. Because DNA is a molecule that plays an active and critical role in cell division, control of DNA repair is closely tied to regulation of the cell cycle.

Defects in DNA repair underlie a number of human genetic diseases that affect a wide variety of body systems but share a constellation of common traits, most notably a predisposition to cancer (Table 2). These disorders include ataxia-telangiectasia (AT), a degenerative motor condition caused by failure to repair oxidative damage in the cerebellum, and xeroderma pigmentosum (XP), a condition characterized by sensitivity to sunlight and linked to a defect in an important ultraviolet (UV) damage repair pathway

Most damage to DNA is repaired by removal of the damaged bases followed by resynthesis of the excised region. Some lesions in DNA, however, can be repaired by direct reversal of the damage, which may be a more efficient way of dealing with specific types of DNA damage that occur frequently. Only a few types of DNA damage are repaired in this way, particularly pyrimidine dimers resulting from exposure to ultraviolet (UV) light and alkylated guanine residues that have been modified by the addition of methyl or ethyl groups at the O^6 position of the purine ring.

UV light is one of the major sources of damage to DNA and is also the most thoroughly studied form of DNA damage in terms of repair mechanisms. Its importance is illustrated by the fact that exposure to solar UV irradiation is the cause of almost all skin cancer in humans. The major type of damage induced by UV light is the formation of pyrimidine dimers, in which adjacent pyrimidines on the same strand of DNA are joined by the formation of a cyclobutane ring resulting from saturation of the double bonds between carbons 5 and 6.

The formation of such dimers distorts the structure of the DNA chain and blocks transcription or replication past the site of damage, so their repair is closely correlated with the ability of cells to survive UV irradiation. One mechanism of repairing UV-induced pyrimidine dimers is direct reversal of the dimerization reaction. The process is

called photoreactivation because energy derived from visible light is utilized to break the cyclobutane ring structure. The original pyrimidine bases remain in DNA, now restored to their normal state. As might be expected from the fact that solar UV irradiation is a major source of DNA damage for diverse cell types, the repair of pyrimidine dimers by photoreactivation is common to a variety of prokaryotic and eukaryotic cells, including *E. coli*, yeasts, and some species of plants and animals. Curiously, however, photoreactivation is not universal; many species (including humans) lack this mechanism of DNA repair.

Another form of direct repair deals with damage resulting from the reaction between alkylating agents and DNA. Alkylating agents are reactive compounds that can transfer methyl or ethyl groups to a DNA base, thereby chemically modifying the base. A particularly important type of damage is methylation of the O^6 position of guanine, because the product, O^6-methylguanine, forms complementary base pairs with thymine instead of cytosine. This lesion can be repaired by an enzyme (called O^6-methylguanine methyltransferase) that transfers the methyl group from O^6-methylguanine to a cysteine residue in its active site . The potentially mutagenic chemical modification is thus removed, and the original guanine is restored. Enzymes that catalyze this direct repair reaction are widespread in both prokaryotes and eukaryotes, including humans.

Excision Repair

Although direct repair is an efficient way of dealing with particular types of DNA damage, excision repair is a more general means of repairing a wide variety of chemical alterations to DNA. Consequently, the various types of excision repair are the most important DNA repair mechanisms in both prokaryotic and eukaryotic cells. In excision repair, the damaged DNA is recognized and removed, either as free bases or as nucleotides. The resulting gap is then filled in by synthesis of a new DNA strand, using the undamaged complementary strand as a template. Three types of excision repair—base-excision repair, nucleotide-excision repair, and mismatch repair—enable cells to cope with a variety of different kinds of DNA damage.

The repair of uracil-containing DNA is a good example of base-excision repair, in which single damaged bases are recognized and removed from the DNA molecule. Uracil can arise in DNA by two mechanisms: (1) Uracil (as dUTP [deoxyuridine triphosphate]) is occasionally incorporated in place of thymine during DNA synthesis, and (2) uracil can be formed in DNA by the deamination of cytosine . The second mechanism is of much greater biological significance because it alters the normal pattern of complementary base pairing and thus represents a mutagenic event. The excision of uracil in DNA is catalyzed by DNA glycosylase, an enzyme that cleaves the bond linking the base (uracil) to the deoxyribose of the DNA backbone. This reaction yields free uracil and an apyrimidinic site—a sugar with no base attached. DNA glycosylases also recognize and remove other abnormal bases, including hypoxanthine formed by the deamination of adenine, pyrimidine dimers, alkylated purines other than O^6-alkylguanine, and bases damaged by oxidation or ionizing radiation

Nucleotide-excision repair systems have also been studied extensively in eukaryotes, particularly in yeasts and in humans. In yeasts, as in *E. coli*, several genes involved in DNA repair (called *RAD* genes for *rad*iation sensitivity) have been identified by the isolation of mutants with increased sensitivity to UV light. In humans, DNA repair genes have been identified largely by studies of individuals suffering from inherited diseases resulting from deficiencies in the ability to repair DNA damage. The most extensively studied of these diseases is xeroderma pigmentosum (XP), a rare genetic disorder that affects approximately one in 250,000 people. Individuals with this disease are extremely sensitive to UV light and develop multiple skin cancers on the regions of their bodies that are exposed to sunlight. In 1968 James Cleaver made the key discovery that cultured cells from XP patients were deficient in the ability to carry out nucleotide-excision repair. This observation not only provided the first link between DNA repair and cancer, but also suggested the use of XP cells as an experimental system to identify human DNA repair genes. The identification of human DNA repair genes has been accomplished by studies not only of XP cells, but also of two other human diseases resulting from DNA repair defects (Cockayne's syndrome and trichothiodystrophy) and of UV-sensitive mutants of rodent cell lines. The availability of mammalian cells with defects in DNA repair has

allowed the cloning of repair genes based on the ability of wild-type alleles to restore normal UV sensitivity to mutant cells in gene transfer assays, thereby opening the door to experimental analysis of nucleotide-excision repair in mammalian cells.

The direct reversal and excision repair systems act to correct DNA damage before replication, so that replicative DNA synthesis can proceed using an undamaged DNA strand as a template. Should these systems fail, however, the cell has alternative mechanisms for dealing with damaged DNA at the replication fork. Pyrimidine dimers and many other types of lesions cannot be copied by the normal action of DNA polymerases, so replication is blocked at the sites of such damage. Downstream of the damaged site, however, replication can be initiated again by the synthesis of an Okazaki fragment and can proceed along the damaged template strand (Figure 5.27). The result is a daughter strand that has a gap opposite the site of damage to the parental strand. One of two types of mechanisms may be used to repair such gaps in newly synthesized DNA: recombinational repair or error-prone repair.

Recombinational repair depends on the fact that one strand of the parental DNA was undamaged and therefore was copied during replication to yield a normal daughter molecule (see Figure 5.27). The undamaged parental strand can be used to fill the gap opposite the site of damage in the other daughter molecule by recombination between homologous DNA sequences (see the next section). Because the resulting gap in the previously intact parental strand is opposite an undamaged strand, it can be filled in by DNA polymerase. Although the other parent molecule still retains the original damage (e.g., a pyrimidine dimer), the damage now lies opposite a normal strand and can be dealt with later by excision repair. By a similar mechanism, recombination with an intact DNA molecule can be used to repair double strand breaks, which are frequently introduced into DNA by radiation and other damaging agents.

In **error-prone repair**, a gap opposite a site of DNA damage is filled by newly synthesized DNA. Since the new DNA is synthesized from a damaged template strand, this form of DNA synthesis is very inaccurate and leads to frequent mutations. It is used only in bacteria that have been subjected to potentially lethal conditions, such as extensive UV irradiation. Such treatments induce the SOS response, which may be viewed as a mechanism for dealing with extreme environmental stress. The SOS response includes inhibition of cell division and induction of repair systems to cope with a high level of DNA damage. Under these conditions, error-prone repair mechanisms are used, presumably as a way of dealing with damage so extensive that cell death is the only alternative.

Literature:

1. Alberts et al., pp. 266-287;
2. Karp, pp. 552-557.