Mutation, Mutagens, and DNA Repair Outline

I. Introduction: Definitions and mutation rates

We have been using the term 'mutation' pretty loosely up to this point in the course...now we need to define it more precisely: **mutation-- a change in the genetic material (ie. DNA)**. We are going to spend some time talking about how mutations can occur and what their consequences may be to cells; we will also be looking at the ways in which cells avoid mutations by repairing DNA damage.

Why this focus? Why are mutations important? There are several reasons: 1) they may have deleterious or (rarely) advantageous consequences to an organism (or its descendants); 2) they are important to geneticists: the most common way we study something is to break it--ie., we search for or make a variant (mutant) lacking the ability to perform a process which we want to study. These genetic variants possess mutant alleles of the genes we are interested in studying. 3) Mutations are important as the major source of genetic variation which fuels evolutionary change (as we will see later when we talk about population genetics and evolution).

Let's further define mutation as a **heritable** change in the genetic material. This point becomes important in multicellular organisms where we must distinguish between changes in gametes (germline mutations) and changes in body cells (somatic mutations). The former are passed on to one's offspring; the latter are not but we will see they can be very important in causing cancer.

In detection of germline mutations in humans and measurement of human mutation rates we have the problem of diploidy. Most forward mutations (normal gene to mutant form) are recessive and so won't be detected unless a zygote gets two copies of the mutant allele. [Reversion or reverse mutation (mutant back to normal) is generally much less frequent because there are a lot more ways to "break" a gene than there are to reverse an existing mutation.] So how can we detect and measure rates of new mutations? We can look at dominant mutations on occuring on the autosomes and at both recessive and dominant mutations on the X chromosome, since males are hemizygous for X-linked genes. Example: achondroplasia occurs sporadically (in families with no previous history) as a result of new mutations in the gene for the fibroblast growth factor receptor. One study detected seven infants born with sporadic achondroplasia in one year among 242,257 total births recorded. So the rate (actually a frequency but we won't be concerned about the difference for the purposes of thinking about rates in this course) is 7/242,257 x 1/2 (2 alleles per zygote) = 1.4 x 10e-5.

This rate is roughly in the middle of the range reported for various human genes: those with high mutation rates like NF1 (neurofibromatosis type 1) and DMD (Duchenne muscular dystrophy) (ca. 1 x 10e-4) and those with low rates

of new mutation like the Huntington's Disease gene (1 x 10e-6). This hundred-fold range shows that mutation rates per gene can be intrinsically different.
Why might this be? Two possible explanations are: 1) target size and 2) hot spots. Some genes are large, meaning that there are many bases at which mutations could alter or disrupt their function. The large target argument could well be responsible for the high rates of mutation of the NF and DMD genes, as these are known to have very large protein coding regions. Alternatively, some genes may be in regions of chromosomes which are more susceptible to genetic damage/change or may contain sequences which are more likely to be altered by spontaneous mutations; the achondroplasia gene is known to contain a hot spot of the latter type (a CpG sequence, discussed below).
From studies like these in vivo and others using human cells in vitro, the overall human mutation rate is estimated to be about 1 x 10e-6 per gene per generation. (Therefore the HD gene rate is probably more typical than the other genes mentioned above.) This rate is similar to those measured in various prokaryotic and eukaryotic microorganisms. We can use the estimated human mutation rate to determine its impact on the likelihood of changes occurring in each generation: a rate of 1 x 10e-6 mutations/gene x 5 x 10e4 genes/haploid genome = 5 x 10e-2 mutations per gamete (=5/100 or 1/20). 1/20 x 2 gametes per zygote = 1/10 chance that each zygote carries a new mutation somewhere in the genome. This seems like a very high number but we need to remember that most mutations are recessive and thus will not be expressed in the heterozygous condition.

II. Types of Mutations

Mutations, or heritable alterations in the genetic material, may be gross (at the level of the chromosome, which we have already discussed) or point alterations (this technically means mutations not visible as cytological abnormalities and/or those which map to a single "point" in experimental crosses). The latter can involve just a single nucleotide pair in DNA. In this section, we will be considering small changes in DNA, of the point mutation type.

A. Base pair (nucleotide pair) substitutions

These are of two types: **transitions** (purine to purine or pyrimidine to pyrimidine) and **transversions** (purine to pyrimidine or pyrimidine to purine). We break these down into the two categories because they can occur in different ways.
The consequences of base substitution mutations in protein coding regions of a gene depend on the substitution and its location. They may be *silent*, not resulting in a new amino acid in the protein sequence, eg. GCA or GCG codons in mRNA both mean arginine [this is often true in the third position of a codon, especially with transitions because of "wobble" base pairing]. A base substitution could also result in an amino acid substitution; this is referred to as a *missense* mutation. For example, CTC in the DNA sense strand [GAG in mRNA] will specify a glutamate residue in the protein; this is altered to CAC in the DNA or GUG in the mRNA, resulting in a valine residue in the beta-globin protein chain causing sickle-cell anemia. Missense mutations may have very serious consquences, as in the case of sickle-cell anemia, mild consequences as in the case of hemoglobin C (a different amino acid substitution in position 6 of beta-globin) or no phenotype as in the case of two known amino acid substitutions at position 7 of beta-globin. Finally, base substitutions in a protein coding region may mutate an amino acid codon to a termination codon or vice versa. The former type, which results in a prematurely shortened protein is referred to as a *nonsense* mutation. The effects of nonsense mutations are variable depending upon how much of the truncated protein is present and is required for its function.
Base substitution mutations may also occur in promoters or 5' regulatory regions of genes or in introns and may affect their transcription, translation, or splicing. Many of the beta-thalassemias are the result of these types of non-structural mutations that affect the level of expression of the globin genes. All of the types of mutation described above have been observed in human globin genes. Their consequences depend on what they do to the level of expression of the gene product and/or on what amino acid substitution may have occurred and where it is in the protein.

B. Frameshift mutations

These result from the insertion or deletion of one or more (not in multiples of three) nucleotides in the coding region of a gene. This causes an alteration of the reading frame: since codons are groups of three nucleotides, there are three possible reading frames for each gene although only one is used.
eg. mRNA with sequence AUG CAG AUA AAC GCU GCA UAA
amino acid sequence from the first reading frame: met gln ile asn ala ala stop
the second reading frame gives: cys arg stop

A mutation of this sort changes all the amino acids downstream and is very likely to create a nonfunctional product since it may differ greatly from the normal protein. Further, reading frames other than the correct one often contain stop codons which will truncate the mutant protein prematurely.

III. Origins of spontaneous mutation

A. Definition and sources

A spontaneous mutation is one that occurs as a result of natural processes in cells. We can distinguish these from induced mutations; those that occur as a result of interaction of DNA with an outside agent or mutagen. Since some of the same mechanisms are involved in producing spontaneous and induced mutations, we will consider them together. Some so-called "spontaneous mutations" probably are the result of naturally occurring mutagens in the environment; nevertheless there are others that definitely arise spontaneously, for example, DNA replication errors.

B. DNA replication errors and polymerase accuracy

Mistakes in DNA replication where an incorrect nucleotide is added will lead to a mutation in the next round of DNA replication of the strand with the incorrect nucleotide.The frequency at which a DNA polymerase makes mistakes (inserts an incorrect base) will influence the spontaneous mutation frequency and it has been observed that different polymerases vary in their accuracy. One major factor affecting polymerase accuracy is the presence of a "proofreading" 3'-5' exonuclease which will remove incorrectly paired bases inserted by the polymerase. This was shown *in vitro* with purified DNA polymerases (those with 3'-5' exonucleases make fewer mistakes) and genetically by Drake with bacteriophage T4 mutants: T4 has its own polymerase with a 3'-5' exo. Drake isolated *mutator* mutants (which had a higher spontaneous mutation rate than normal) and *antimutator* mutants (lower mutation rate than normal) in the polymerase gene and showed that the mutators had a higher ratio of polymerizing to exonuclease activity than normal and that the antimutators had a lower ratio. These studies showed that the function of the 3'-5' exonuclease is to prevent misincorporation during DNA replication and to prevent mutations. Mutator mutants have since been isolated in other organisms and have been shown to affect various components of the DNA replication complex; alterations in a number of these proteins are likely to affect the accuracy of the system.

C. Base alterations and base damage

The bases of DNA are subject to spontaneous structural alterations called **tautomerization**: they are capable of existing in two forms between which they interconvert. For example, guanine can exist in keto or enol forms. The keto form is favored but the enol form can occur by shifting a proton and some electrons; these forms are called tautomers or structural isomers. The various tautomer forms of the bases have different pairing properties. Thymine can also have an enol form; adenine and cytosine exist in amino or imino forms. If during DNA replication, G is in the enol form, the polymerase will add a T across from it instead of the normal C because the base pairing rules are changed (not a polymerase error). The result is a G:C to A:T transition; tautomerization causes transition mutations only.

Another mutatgenic process occurring in cells is spontaneous base degradation. The **deamination** of cytosine to uracil happens at a significant rate in cells.

Deamination can be repaired by a specific repair process which detects uracil, not normally present in DNA; otherwise the U will cause A to be inserted opposite it and cause a C:G to T:A transition when the DNA is replicated.

Deamination of methylcytosine to thymine can also occur. Methylcytosine occurs in the human genome at the sequence 5' CpG3' , which is normally avoided in the coding regions of genes. If the meC is deaminated to T, there is no repair system which can recognize and remove it (because T is a normal base in DNA). This means that wherever CpG occurs in genes it is a "hot spot" for mutation. Such a hot spot has recently been found in the achondroplasia gene.

A third type of spontaneous DNA damage that occurs frequently is damage to the bases by free radicals of oxygen. These arise in cells as a result of oxidative metabolism and also are formed by physical agents such as radiation. An important **oxidation** product is 8-hydroxyguanine, which mispairs with adenine, resulting in G:C to T:A transversions.

Still another type of spontaneous DNA damage is **alkylation**, the addition of alkyl (methyl, ethyl, occasionally propyl) groups to the bases or backbone of DNA. Alkylation can occur through reaction of compounds such as S-adenosyl methionine with DNA. Alkylated bases may be subject to spontaneous breakdown or mispairing.

D. Spontaneous frameshift mutations

Streisinger observed in the 1960' s that frameshift mutations in bacteriophages tended to occur in areas with "runs" of repeats of one nucleotide.

Example: 5' AGTCAATCCATGAAAAAATCAG 3'
3' TCAGTTAGGTACTTTTTTAGTC 5'

He proposed that these frameshifts are the result of "slipped mispairing" between the template DNA strand and the newly synthesized strand during DNA replication. In the sequence above, a likely spot for frameshift mutations to occur would be in the stretch of 6 A:T base pairs. Subsequent studies with genes from other organisms, including humans, have shown that runs of repeated nucleotides are indeed hotspots for frameshift mutations.

IV. Mutagens

A mutagen is a natural or human-made agent (physical or chemical) which can alter the structure or sequence of DNA.

A. Chemical mutagens

The first report of mutagenic action of a chemical was in 1942 by Charlotte Auerbach, who showed that nitrogen mustard (component of poisonous mustard gas used in World Wars I and II) could cause mutations in cells. Since that time, many other mutagenic chemicals have been identified and there is a huge industry and government bureaucracy dedicated to finding them in food additives, industrial wastes, etc.
It is possible to distinguish chemical mutagens by their modes of action; some of these cause mutations by mechanisms similar to those which arise spontaneously while others are more like radiation (to be considered next) in their effects.

1. Base analogs

These chemicals structurally resemble purines and pyrimidines and may be incorporated into DNA in place of the normal bases during DNA replication:

- **bromouracil** (BU)--artificially created compound extensively used in research. Resembles thymine (has Br atom instead of methyl group) and will be incorporated into DNA and pair with A like thymine. It has a higher likelihood for tautomerization to the enol form (BU*)
- **aminopurine** --adenine analog which can pair with T or (less well) with C; causes A:T to G:C or G:C to A:T transitions. Base analogs cause transitions, as do spontaneous tautomerization events.

2. Chemicals which alter structure and pairing properties of bases

There are many such mutagens; some well-known examples are:

- **nitrous acid**--formed by digestion of nitrites (preservatives) in foods. It causes C to U, meC to T, and A to hypoxanthine deaminations. [See above for the consequences of the first two events; hypoxanthine in DNA pairs with C and causes transitions. Deamination by nitrous acid, like spontaneous deamination, causes transitions.
- **nitrosoguanidine, methyl methanesulfonate, ethyl methanesulfonate**--chemical mutagens that react with bases and add methyl or ethyl groups. Depending on the affected atom, the alkylated base may then degrade to yield a baseless site, which is mutagenic and recombinogenic, or mispair to result in mutations upon DNA replication.

3. Intercalating agents

acridine orange, proflavin, ethidium bromide (used in labs as dyes and mutagens)
All are flat, multiple ring molecules which interact with bases of DNA and insert between them. This insertion causes a "stretching" of the DNA duplex and the DNA polymerase is "fooled" into inserting an extra base opposite an intercalated molecule. The result is that intercalating agents cause frameshifts.

4. Agents altering DNA structure

We are using this as a "catch-all" category which includes a variety of different kinds of agents. These may be:

- --large molecules which bind to bases in DNA and cause them to be noncoding--we refer to these as "bulky" lesions (eg. **NAAAF**)
- --agents causing intra- and inter-strand crosslinks (eg. **psoralens**--found in some vegetables and used in treatments of some skin conditions)
- --chemicals causing DNA strand breaks (eg. **peroxides**)

What these agents have in common is that they probably cause mutations not directly but by induction of mutagenic repair processes (to be described later).

B. Radiation

Radiation was the first mutagenic agent known; its effects on genes were first reported in the 1920's. Radiation itself was discovered in 1890's: Roentgen discovered X-rays in 1895, Becquerel discovered radioactivity in 1896, and Marie and Pierre Curie discovered radioactive elements in 1898. These three discoveries and others led to the birth of atomic physics and our understanding of electromagnetic radiation.

1. EM spectrum

Visible light and other forms of radiation are all types of electromagnetic radiation (consists of electric and magnetic waves). The length of EM waves (wavelength) varies widely and is inversely proportional to the energy they contain: this is the basis of the so-called EM spectrum.

The longest waves (AM radio) have the least energy while successively shorter waves and increasing energy are seen with FM radio, TV, microwaves, infrared, visible, ultraviolet (UV), X and gamma radiation. The portion which is biologically significant is UV and higher energy radiation.

2. Ionizing radiation

X- and gamma-rays are energetic enough that they produce reactive ions (charged atoms or molecules) when they react with biological molecules; thus they are referred to as ionizing radiation. This term also includes corpuscular radiation--streams of atomic and subatomic particles emitted by radioactive elements: these are of two types, alpha- and beta-particles [alpha are helium nuclei, 2 protons and 2 neutrons; beta are electrons].

UV radiation is not ionizing but can react with DNA and other biological molecules and is also important as a mutagen.

The units now used for ionizing radiation of all types are rems (roentgen equivalent man): 1 rem of any ionizing radiation produces similar biological effects. The unit used previously was the rad (radiation absorbed dose). However, the effects of different types of radiation differ for one rad unit: one rad of alpha particles has a much greater damaging effect than one rad of gamma rays; alpha particles have a greater RBE (relative biological effectiveness) than gamma rays. The relationship between these units is that:

rads x RBE = # rems

In addition to the energy type and total dose of radiation the dose rate should be considered: the same number of rems given in a brief, intense exposure (high dose rate) causes burns and skin damage versus a long-term weak exposure (low dose rate) which would only increase risk of mutation and cancer.

3. Sources of radiation

Natural sources of radiation produce so-called background radiation. These include cosmic rays from the sun and outer space, radioactive elements in soil and terrestrial products (wood, stone) and in the atmosphere (radon). One's exposure due to background radiation varies with geographic location.

In addition, humans have created artificial sources of radiation which contribute to our radiation exposure. Among these are medical testing (diagnostic X-rays and other procedures), nuclear testing and power plants, and various other products (TV's, smoke detectors, airport X-rays).

Taken together, our overall total average exposure from all sources is about 350 mrem/year; the major contributor of which is from radon exposure. See the graph on page 281 of your text for the breakdown.

4. Biological effects of radiation

Ionizing radiation produces a range of damage to cells and organisms primarily due to the production of free radicals of water (the hydroxyl or OH radical). Free radicals possess unpaired electrons and are chemically very reactive and will interact with DNA, proteins, lipids in cell membranes, etc. Thus X-rays can cause DNA and protein damage which may result in organelle failure, block cell division, or cause cell death. The rapidly dividing cell types (blood cell-forming areas of bone marrow, gastrointestinal tract lining) are the most affected by ionizing radiation and the

severity of the effects depends upon the dose received. The information below is based upon accidental exposures of nuclear plant workers and victims of atomic bomb explosions such as those in Hiroshima and Nagasaki:
sublethal dose (100-250 rems): nausea and vomiting early; 1-2 wk. latent period followed by malaise, anorexia, diarrhea, hair loss, recovery (latency due to time it takes hematopoetic or other damage to show up)
lethal dose (350-450 rems): nausea and vomiting early; 1 wk. latent period followed by above with more severe symptoms including internal bleeding; a 50% chance of death [LD50 : dose at which half of exposed individuals will die; ca. 400 rems for humans]. Death is due to blood cell or gastrointestinal failure.
supralethal dose (>650 rems): nausea and vomiting early, followed by shock, abdominal pain, diarrhea, fever and death within hours or days. Death is due to heart or CNS damage.
For the affected tissues and organs, the number of destroyed cells and the likelihood of their replacement determines the survival chances. The long term effects include increased cancer risk and increased risk of mutations in one' s offspring.

5. Genetic effects of radiation

Ionizing radiation produces a range of effects on DNA both through free radical effects and direct action:

- -breaks in one or both strands (can lead to rearrangements, deletions, chromosome loss, death if unrepaired; this is from stimulation of recombination)
- -damage to/loss of bases (mutations)
- -crosslinking of DNA to itself or proteins

The genetic effects of radiation were reported in 1927 in Drosophila by Muller and in 1928 in plants (barley) by Stadler; both showed that the frequency of induced mutations is a function of X-ray dose. Their experiments revealed that there was a linear relationship between X-ray dose and induced mutation level, that there was no threshold or "safe" dose of radiation and that all doses are significant, and finally, that "split dose" experiments showed that the genetic effects of radiation are cumulative.

6. UV (ultraviolet)

UV radiation is less energetic, and therefore non-ionizing, but its wavelengths are preferentially absorbed by bases of DNA and by aromatic amino acids of proteins, so it, too, has important biological and genetic effects.
UV is normally classified in terms of its wavelength: **UV-C** (180-290 nm)--"germicidal"--most energetic and lethal, it is not found in sunlight because it is absorbed by the ozone layer; **UV-B** (290-320 nm)--major lethal/mutagenic fraction of sunlight; **UV-A** (320 nm--visible)--"near UV"--also has deleterious effects (primarily because it creates oxygen radicals) but it produces very few pyrimidine dimers. Tanning beds will have UV-A and UV-B. To see a graphic representation of the wavelengths of UV and ozone absorption, click here.
The major lethal lesions are pyrimidine dimers in DNA (produced by UV-B and UV-C)--these are the result of a covalent attachment between adjacent pyrimidines in one strand. This is shown here for a thymine-thymine dimer and here for a thymine-cytosine dimer. These dimers, like bulky lesions from chemicals, block transcription and DNA replication and are lethal if unrepaired. They can stimulate mutation and chromosome rearrangement as well.

V. DNA repair systems

Because DNA damage occurs spontaneously and as a result to ubiquitous environmental agents, most organisms possess some capacity to repair their DNA and DNA is the only macromolecule which IS repaired by cells. We can divide "repair" mechanisms into 3 categories:
damage reversal--simplest; enzymatic action restores normal structure without breaking backbone
damage removal--involves cutting out and replacing a damaged or inappropriate base or section of nucleotides
damage tolerance--not truly repair but a way of coping with damage so that life can go on
We will look at examples of each type of repair, the mechanisms, the consequences of mutations in each, in both model organisms and in humans.

A. Damage reversal

1. Photoreactivation

This is one of the simplest and perhaps oldest repair systems: it consists of a single enzyme which can split pyrimidine dimers (break the covalent bond) in presence of light. Click here to see the photoreactivation reaction.

The photolyase enzyme catalyzes this reaction; it is found in many bacteria, lower eukaryotes, insects, and plants. It seems to be absent in mammals (including humans). The gene is present in mammals but may code for a protein with an accessory function in another type of repair.

2. Ligation of single strand breaks

X-rays and some chemicals like peroxides can cause breaks in backbone of DNA. Simple breaks in one strand are rapidly repaired by DNA ligase. Microbial mutants lacking ligase tend to have high levels of recombination since DNA ends are recombinogenic (very reactive). A human known only by the code name of 46BR was found to have mutations in both of her DNA ligase I genes; she had poor growth, immunodeficiency, and sun sensitivity and died at a young age of lymphoma. Fibroblast cells from 46BR are sensitive to killing by DNA damaging agents including ionizing radiation. In addition, the rare hereditary disease **Bloom syndrome** also somehow is involved with DNA ligase deficiency (although the Bloom syndrome protein is a DNA helicase); patients' cultured cells have high levels of chromosome aberrations and spontaneous mutation.

B. Damage removal

1. Base excision repair

The damaged or inappropriate base is removed from its sugar linkage and replaced. These are glycosylase enzymes which cut the base-sugar bond. example: **uracil glycosylase**--enzyme which removes uracil from DNA. Uracil is not supposed to be in DNA--can occur if RNA primers not removed in DNA replication or (more likely) if cytosine is deaminated (this is potentially mutagenic). The enzyme recognizes uracil and cuts the glyscosyl linkage to deoxyribose. The sugar is then cleaved and a new base put in by DNA polymerase using the other strand as a template. Mutants lacking uracil glycosylase have elevated spontaneous mutation levels (C to U is not fixed, which leads to transitions) and are hyper-sensitive to killing and mutation by nitrous acid (which causes C to U deamination).
There are other specific glycosylases for particular types of DNA damage caused by radiation and chemicals.

2. Mismatch repair

This process occurs after DNA replication as a last "spellcheck" on its accuracy. In E. coli, it adds another 100-1000-fold accuracy to replication. It is carried out by a group of proteins which can scan DNA and look for incorrectly paired bases (or unpaired bases) which will have aberrant dimensions in the double helix. The incorrect nucleotide is removed as part of a short stretch and then the DNA polymerase gets a second try to get the right sequence.
Human mismatch repair proteins have recently been identified and are very similar to those of the prokaryote *E. coli* and the simple eukaryote yeast (this is an old invention of cells); mutations are found to be passed in the germline of families with some types of inherited colon cancer (**HPNCC**).

3. Nucleotide excision repair

This system works on DNA damage which is "bulky" and creates a block to DNA replication and transcription (so--UV-induced dimers and some kinds of chemical adducts). It probably recognizes not a specific structure but a distortion in the double helix. The mechanism consists of cleavage of the DNA strand containing the damage by endonucleases on either side of damage followed by exonuclease removal of a short segment containing the damaged region. DNA polymerase can fill in the gap that results. Excision repair is shown here .
Mutants that are defective in NER have been isolated in many organisms and are sensitive to killing and mutagenesis by UV and chemicals which act like UV. Humans with the hereditary disease **xeroderma pigmentosum** are sunlight-sensitive, they have very high risks of skin cancers on sun-exposed areas of the body and have defects in genes homologous to those required for NER in simple eukaryotes. NER mutants in lower organisms are UV-sensitive and have elevated levels of mutation and recombination induced by UV (because they are unable to use the accurate NER method to remove pyrimidine dimers and must use mutagenic or recombinogenic systems).

C. DNA damage tolerance

Not all DNA damage is or can be removed immediately; some of it may persist for a while. If a DNA replication fork encounters DNA damage such as a pyrimidine dimer it will normally act as a block to further replication.
However, in eukaryotes, DNA replication initiates at multiple sites and it may be able to resume downstream of a dimer, leaving a "gap" of single-stranded unreplicated DNA. The gap is potentially just as dangerous if not more so

than the dimer if the cell divides. So there is a way to repair the gap by recombination with either the other homolog or the sister chromatid--this yields two intact daughter molecules, one of which still contains the dimer.

1. Recombinational (daughter-strand gap) repair

This is a repair mechanism which promotes recombination to fix the daughter-strand gap--not the dimer--and is a way to cope with the problems of a non-coding lesion persisting in DNA. The events of recombinational repair are shown here . This type of recombinational repair is generally accurate (although it can cause homozygosis of deleterious recessive alleles) and requires a homolog or sister chromatid. The products of the human breast cancer susceptibility genes *BRCA1* and *BRCA2* may be involved in recombinational repair together with homologs of the yeast *RAD51* and *RAD52* genes.

A second type of recombinational repair which is used primarily to repair broken DNA ends such as are caused by ionizing radiation and chemical mutagens with similar action is the non-homologous end-joining reaction. This repair system is also employed by B and T cells of the immune system for genetic rearrangements needed for their function. The Ku70, Ku80, and DNA-dependent protein kinase proteins are needed for non-homologous end-joining. Rodent cell lines with mutations in these genes are very sensitive to killing by ionizing radiation and defective in immune system rearrangement.

2. Mutagenic repair (trans-lesion synthesis)

An alternative scenario for a DNA polymerase blocked at a dimer is to change its specificity so that it can insert any nucleotide opposite the dimer and continue replication ("mutate or die" scenario). See the figure . We know that this can happen in bacteria and think that it probably happens in eukaryotes, though the mechanism is not well understood. This is a reason why repair may sometimes cause mutations.

VI. Checkpoints

Ataxia telangiectasia is a human autosomal recessive hereditary disease which causes several defects including about a hundred-fold increase in cancer susceptibility. AT patients' cells in culture show abnormalities including spontaneous and radiation-induced chromosome breaks and sensitivity to killing by X-rays. (Ironically, the patients also show extreme sensitivity to killing by X-ray doses intended to be therapeutic for their cancers.) However, AT cultured cells do not show a defect in repair of X-ray damage to their DNA; instead, unlike normal cells, they continue to replicate their DNA even when it has been damaged by X-rays. It is the failure to recognize DNA damage and respond appropriately by halting the cell cycle until repair can occur that leads to chromosome aberrations and death after X-ray in the AT patients.

The defect in AT is one in a cell cycle checkpoint, a decision point that governs progression through the next phase of the cell cycle. There are genetically controlled checkpoints that decide entry into a new cell cycle (G0 to G1 point), the decision to replicate the DNA (G1 to S point), and the decision to divide (G2 to M point). Mutations in the checkpoint genes can lead to uncontrolled cell growth, ie. cancer.

Although AT itself is a rare condition, it has been estimated that the frequency of heterozygotes with one AT mutation is about 1% in the population. These individuals also have a higher cancer risk and intermediate radiation sensitivity. Thus, screening by X-ray methods (eg. mammography) may increase the chances of an AT heterozygote developing cancer.

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