Genetics and Genomics Chapter 4

Questions & Answers

Multiple Choice Questions

Question 4.1

Which, if any, of the following statements is false?

a) Most of the inherited changes in our DNA arise because of exposure to extracellular mutagens, including radiation sources and chemical mutagens.
b) Most of the inherited changes in our DNA arise because of unavoidable endogenous errors in cellular mechanisms and harmful effects of certain natural molecules and atoms within our cells.
c) Errors in DNA replication and DNA repair are a major source of mutations in our cells.
d) Significant chemical damage is sustained by DNA because of its proximity to water molecules in our cells.

Answer 4.1

a) Most of the inherited changes in our DNA arise because of exposure to extracellular mutagens, including radiation sources and chemical mutagens.

Question 4.2

With reference to base cross-linking, which, if any, of the following statements, is false?

a) Base cross-linking means that covalent bonds form between two bases.
b) The cross-linked bases are on opposing DNA strands.
c) The anti-cancer agent cisplatin causes a type of cross-linking between two guanine residues.
d) Pyrimidine dimers are a type of base cross-linking that is commonly induced by excess exposure to sunlight.

Answer 4.2

b) The cross-linked bases are on opposing DNA strands.

Explanation 4.2

Base cross-linking can involve bases on the same DNA strand, such as pyrimidine dimers, as well as bases on opposing DNA strands. See Figure 4.1D on page 83

Question 4.3

Match individual environmental factors a) to e) to associated types of chemical damage to DNA i) to iv)

Environmental factors	Chemical change to DNA
a) ionizing radiation	i) intrastrand base cross-linking
b) cigarette smoke	ii) interstrand base cross-linking
c) ultraviolet radiation	iii) aromatic hydrocarbon DNA adducts
d) electrophilic alkylating agents	iv) increased reactive oxygen species (ROS)-mediated damage
e) automobile fumes	

Answer 4.3

a) iv)
b) iii)
c) i)
d) ii)
e) iii)

Explanation 4.3

Ionizing radiation (such as X-rays and gamma rays) interacts with cellular molecules to generate additional ROS.

Question 4.4

With reference to DNA repair, which of the following statements, if any, is false?

a) In the great majority of cases, DNA repair is some kind of mechanism that does not directly reverse the molecular steps that cause DNA damage.
b) Most of the time DNA repair involves a mechanism that makes a repair to both DNA strands.
c) When DNA repair involves repairing both DNA strands, the accuracy of the repair is higher in cells where the DNA has replicated than in cells before DNA replication.
d) DNA repair mechanisms are not evolutionarily well-conserved; human repair mechanisms differ significantly from those in the cells of other vertebrates.

Answer 4.4

b) Most of the time DNA repair involves a mechanism that makes a repair to both DNA strands.
d) DNA repair mechanisms are not evolutionarily well-conserved; human repair mechanisms differ significantly from those in the cells of other vertebrates.

Question 4.5

With reference to DNA repair, which of the following statements, if any, is false?

a) Crosslinking of bases on opposing DNA strands is especially problematic for cells because it presents an obstacle to DNA replication (the replication fork stalls).
b) Crosslinking of bases on opposing DNA strands can be problematic for cells because it may present an obstacle to transcription (the RNA polymerase stalls).
c) Double strand DNA breaks are a challenge for cells because if repair is not affected immediately the ends can drift apart quickly making correct repair impossible.
d) Repair of double stranded DNA breaks is easier in cells prior to DNA replication than after DNA replication has occurred.

Answer 4.5

d) Repair of double stranded DNA breaks is easier in cells prior to DNA replication than after DNA replication has occurred.

Question 4.6

With reference to hydrolytic damage to DNA which of the following statements, if any, is false?

a) Hydrolytic attack commonly causes cleavage of the *N*-glycosidic bond, resulting in loss of bases.
b) Loss of pyrimidines is particularly common.
c) Hydrolytic attack also commonly causes amino groups to be stripped from bases (deamination).
d) Cytosines are often deaminated to give thymines.

Answer 4.6

b) Loss of pyrimidines is particularly common.
d) Cytosines are often deaminated to give thymines.

Explanation 4.6

Loss of purines is much more common than loss of pyrimidines. Cytosines are deaminated to give uracils which base pair with adenines, effectively causing a C→T transition.

Question 4.7

With reference to reactive oxygen species (ROS), which of the following statements, if any, is false?

a) ROS are an inevitable consequence of the chemical reactions that occur in cells and are formed by the incomplete one-electron reduction of oxygen.

b) Common examples of ROS include hydrogen peroxide (H_2O_2), superoxide anions (O_2^-) and hydroxyl radicals ($OH^·$).
c) ROS are generated in different intracellular compartments, but notably in mitochondria.
d) ROS are functionally valuable: they play important roles in both intercellular and intracellular signalling.

Answer 4.7
None of them.

Explanation 4.7
All are true.

Question 4.8

With reference to aberrant methylation of bases which of the following statements, if any, is false?

a) *S*-adenosylmethionine donates methyl groups to a range of different molecules in cells and frequently inappropriately methylates bases in DNA.
b) Guanine is occasionally methylated to give *O*-6-methylguanine which base pairs with adenine rather than with cytidine.
c) In each nucleated cell, about 300-600 adenines are converted to 3-methyladenine per day.
d) 3-methyladenine can be a cytotoxic base: it distorts the double helix and that can disrupt crucial DNA-protein interactions.

Answer 4.8

b) Guanine is occasionally methylated to give *O*-6-methylguanine which base pairs with adenine rather than with cytidine.

Explanation 4.8
O-6-methylguanine base pairs with thymine rather than with adenine

Question 4.9

Match the types of DNA damage a) to g) to the most appropriate of the DNA repair mechanisms i) to v) that can be expected to repair the damage.

Type of DNA damage	**DNA repair mechanisms**
a) disintegration of a sugar residue due to oxidative damage.	i) nonhomologous end joining ii) base excision repair

b) a simple base modification, such as 8-oxoguanine
c) a double-stranded DNA break occurring in G_1 phase.
d) a pyrimidine dimer
e) an abasic site due to depurination
f) a double-stranded DNA break occurring in G_2 phase.
g) a bulky aromatic hydrocarbon adduct that distorts the double helix.

i) base excision repair
ii) mismatch repair
iii) nucleotide excision repair
iv) homologous recombination-mediated DNA repair

Answer 4.9

a) iii)
b) ii)
c) i)
d) iii)
e) ii)
f) iv) or ii)
g) iii)

Question 4.10

List three ways in which an unrepaired double-stranded DNA break can be highly dangerous to the cell in which it occurs.

Answer 4.10

1) The break may sever an important gene, inactivating it.
2) The break may sever the intervening DNA between an important gene and an important distant control sequence on the same DNA molecule.
3) The broken ends are liable to recombine with other DNA molecules, causing chromosome rearrangements that are harmful, or lethal to the cell.

Question 4.11

What, approximately, is the fraction of genetic variation in the nuclear genome is that is expected to have a harmful effect on gene function?

a) 50%.
b) 25%.
c) 10%.

d) 1%.

Answer 4.11

d) 1%.

Question 4.12

Which, if any, of the following observations are *consistent* with the effect of purifying selection, and which, if any, are *consistent* with the effect of positive selection?

a) Human populations that are accustomed to high-starch diets have comparatively higher copy numbers of the α-amylase gene.
b) Telomere DNA sequences in vertebrates have tandem TTAGGG repeats
c) Humans show very high levels of heterozygosity at the classical HLA loci.
d) Human populations that live in more northerly latitudes have a high frequency of pale skin color.
e) Human calmodulin and an ortholog in *Drosophila* each have 149 amino acid differences and differ at just four amino acid positions.

Answer 4.12

a) Positive selection
b) Purifying selection
c) Positive selection
d) Positive selection
e) Purifying selection.

Question 4.13

Which, if any, of the following statements is incorrect?

a) Each person makes many millions of different HLA proteins so as to be able to recognize and bind foreign antigens.
b) Classical HLA proteins are highly polymorphic; non-classical HLA proteins show very limited polymorphism.
c) Classical class I HLA proteins are displayed on the surface of very few cell types, notably immune system cells.
d) HLA proteins are the most polymorphic human proteins.

Answer 4.13

a) Each person makes many millions of different HLA proteins so as to be able to recognize and bind foreign antigens.
c) Classical class I HLA proteins are displayed on the surface of very few cell types, notably immune system cells.

Explanation 4.13

a) We each make a very limited set of HLA proteins, unlike the huge numbers of immunoglobulins and T-cell receptors that we make.
c) Classical class I HLA proteins are expressed on the surfaces of almost all human nucleated cells.

Question 4.14

Which, if any, of the following statements, is false?

a) As a result of many post-zygotic changes in the DNA of our cells, each of us is a genetic mosaic.
b) The vast majority of the post-zygotic DNA changes are random mutations.
c) The vast majority of the post-zygotic DNA changes do not affect gene expression.
d) Some post-zygotic DNA changes are programmed to occur in a very limited number of cell types.

Answer 4.14

None.

Explanation 4.14

All are true. The programmed DNA changes in (d) are programmed rearrangements in B and T cells to produce, respectively, immunoglobulins and T-cell receptors.

Question 4.15

Classical class I and class II HLA proteins are both highly polymorphic heterodimers polymorphic heterodimers that help lymphocytes to recognize peptide antigens but they differ in many ways. Which, if any of the following statements, is true?

a) The two chains of any class I HLA protein are made by genes that are located on different chromosomes.
b) Each of the two protein chains of a classical class I HLA protein are highly polymorphic, unlike for classical class II HLA proteins where only one of the protein chains is polymorphic.
c) Class I HLA proteins assist helper T lymphocytes to recognize peptide antigens, whereas class II HLA proteins assist cytotoxic T lymphocytes to recognize peptide antigens.
d) Class II HLA proteins are expressed on the surfaces of almost all nucleated cells, but the expression of class I HLA proteins is confined to just a few types of cell, notably certain immune system cells.

Answer 4.15

a) The two chains of any class I HLA protein are made by genes that are located on different chromosomes.

Fill in the Blanks Questions

Question 4.16

Fill in the blanks below.

In the nuclear genome, a ___1____ means a unique location for A DNA sequence. At each diploid ___1____ a person has inherited two ___2____ , one that is paternally inherited and one that is maternally inherited. If the maternal and paternal ___2____ are identical, the person is said to be ____3____ at that ____1____, but if the maternal and paternal _____2_____ differ by even a single nucleotide, the person is said to be ____4_____ at that locus. Whereas women have 23 pairs of homologous chromosomes, men have 22 pairs of autosomal homologs but very different sex chromosomes. As a result, most of the DNA sequences on the X and on the Y chromosomes do not have a counterpart on the other sex chromosome. A ___1____on the X chromosome in men often, therefore, has just a maternal ____2____ , and most loci on the Y have just a paternal ____2____ allele. At loci like these, a man would be said to be _____5_____.

Answer 4.16

1. locus. 2. allele(s). 3. homozygous. 4. heterozygous. 5. hemizygous.

Question 4.17

Fill in the blanks below.

The DNA in our cells is susceptible to damage as a result of exposure to harmful radiation or to ____1____ mutagens. Excess exposure to ___2____ radiation within sunlight, for example, or to tobacco carcinogens induces harmful changes in our DNA. However, the most frequent changes in the base sequence of our DNA arise from _____3____ sources, arising from proximity to harmful chemicals within our cells, notably ____4____ ____5____ ____6____ , and as a consequence of occasional errors in various ____3____ mechanisms, such as DNA ____7____, DNA ____8____ , and chromosome ____9_____.

Answer 4.17

1. chemical. 2. ultraviolet. 3. endogenous. 4. reactive. 5. oxygen. 6. species. 7. replication. 8. repair. 9. segregation.

Essay, Lists & Diagram Questions

Question 4.18

Some of our DNA polymerases have a proofreading function. What is meant by this and how common is it.

Answer 4.18

The classical DNA polymerases that serve to replicate all the DNA in our cells in preparation for cell division have a proofreading function which allows errors in base insertion by the DNA polymerase to be corrected. (A total of 6×10^9 nucleotides need to be inserted in the correct order to make new DNA strands in diploid human cells, and so it is not surprising that every so often a mistake is made by the DNA polymerase).

The proofreading function resides in an additional intrinsic 3' – 5' exonuclease function that these DNA polymerases have. If, by error, the wrong base is inserted, the 3′→5′ exonuclease is activated and degrades the newly synthesized DNA strand from its 3′ end, removing the wrongly inserted nucleotide and a short stretch before it. Then the DNA polymerase resumes synthesis again.

The proofreading function serves as a back-up system to correct errors made by the classical DNA polymerases (but if mispaired bases are not eliminated by the DNA polymerase, the DNA mismatch repair system is activated). However, several non-classical DNA polymerases in our cells do not have proof-reading functions (and so the fidelity of DNA replication is much lower). They work in DNA repair (where only short DNA sequences need to be synthesized, meaning that base incorporation errors are extremely unlikely to begin with), or during somatic mechanisms that operate in B cells and T cells and are designed to maximize variation in respectively, immunoglobulins and T cell receptors.

Question 4.19

Outline the four broad classes of chemical damage to DNA.

Answer 4.19

1) *DNA strand breakage*. A single strand may be broken by simple cleavage of a phosphodiester bond (a *nick*) or by a more complex single-strand break (leading to damaged ends, sometimes with loss of one or more nucleotides. Double-strand DNA breaks occur when both strands are broken at sites that are in very close proximity, and need to be repaired very quickly if the cell is to survive.
2) *Base deletion*. Hydrolysis cleaves the covalent N-glycosidic bond connecting a base to its sugar.

3) *Base modification*. May involve small chemical modifications to bases (such as changing guanine to 8-oxoguanine or modifying thymine to produce thymidine glycol). Alternatively, complex chemical groups, such as aromatic hydrocarbons can be added to bases and are known as DNA adducts.
4) *Base cross-linking*. This involves the formation of new covalent bonds linking two bases that may be on the same DNA strand or on opposing DNA strands DNA strands. For example, excess exposure to sunlight can lead to pyrimidine dimers (neighboring pyrimidines on the same DNA strand are cross-linked). The anti-cancer agent cisplatin is an example of a chemical that induces interstrand cross-links between guanines on opposite strands.

Question 4.20

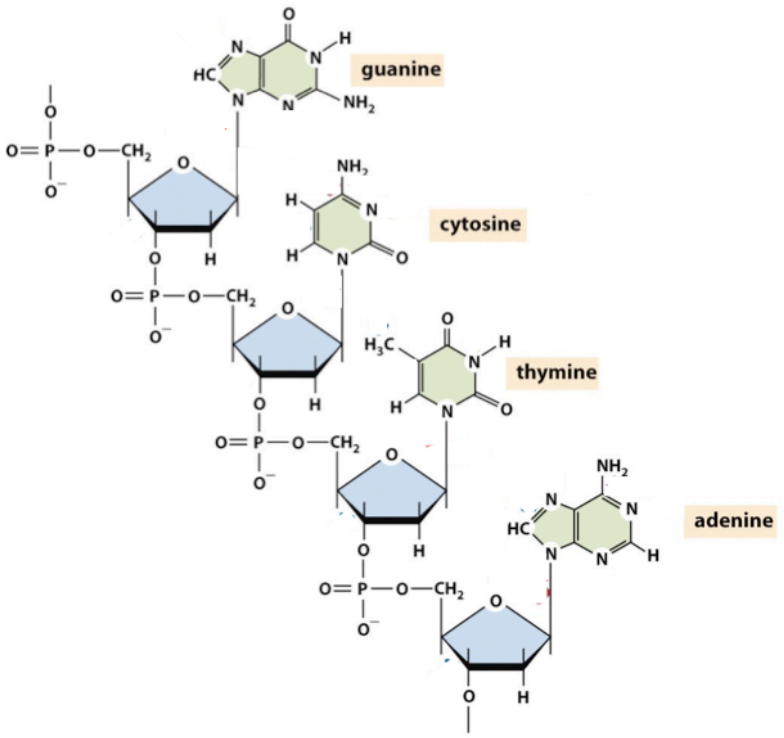
List three types of chemical reaction that cause damage to DNA, and illustrate your answer with examples.

Answer 4.20

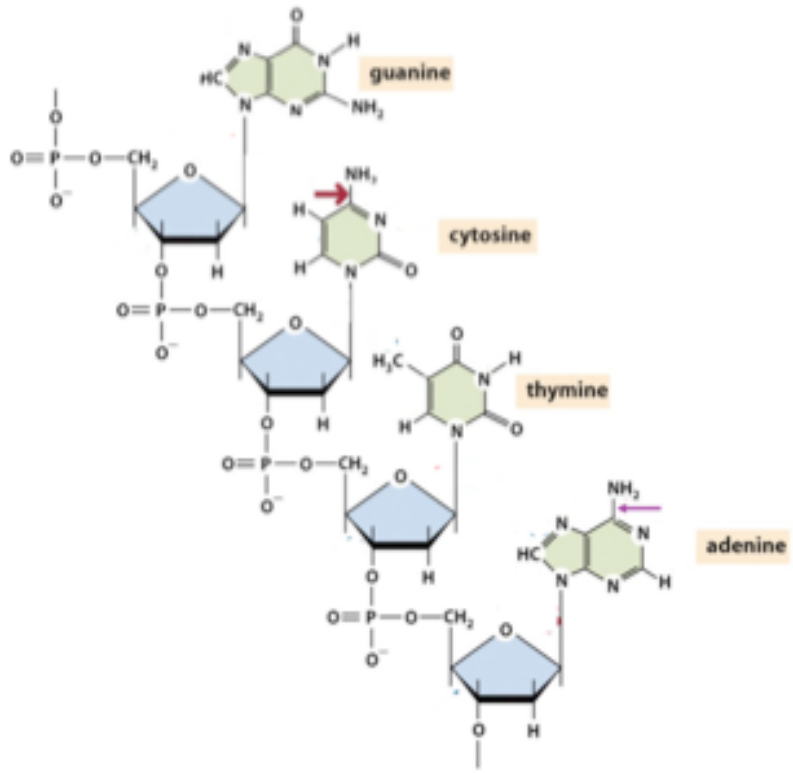
1) *Hydrolytic damage.* Hydrolysis can disrupt bonds that hold bases to sugars, cleaving the base from a sugar (loss of purine bases is particularly common). It also strips amino groups from some bases (deamination), leaving a carbonyl (C=O) group. Cytosines are often deaminated to give uracil, which base pairs with adenine; adenine is occasionally deaminated to produce hypoxanthine, which effectively behaves like guanine by base pairing with cytosine.
2) *Oxidative damage.* Reactive oxygen species (ROS) formed by the incomplete one-electron reduction of oxygen mostly originate in mitochondria and have important roles in certain intercellular and intracellular signaling pathways. They attack covalent bonds in sugars, causing DNA strands to break, and they also attack DNA bases, especially purines. Many derivatives are produced from each base, some of them highly mutagenic, such as 8-oxoguanine which base pairs with adenine.
3) *Aberrant DNA methylation.* Cells use *S*-adenosylmethionine (SAM) as a methyl donor in a non-enzymatic reaction to methylate different types of molecules, but sometimes SAM can inappropriately methylate DNA to produce harmful bases. Each day about 300–600 adenines in each nucleated cell are converted to 3-methyladenine, a cytotoxic base that distorts the double helix, disrupting crucial DNA–protein interactions.

Question 4.21

On the diagram below, identify the covalent bonds that break when hydrolytic attack results in deamination.

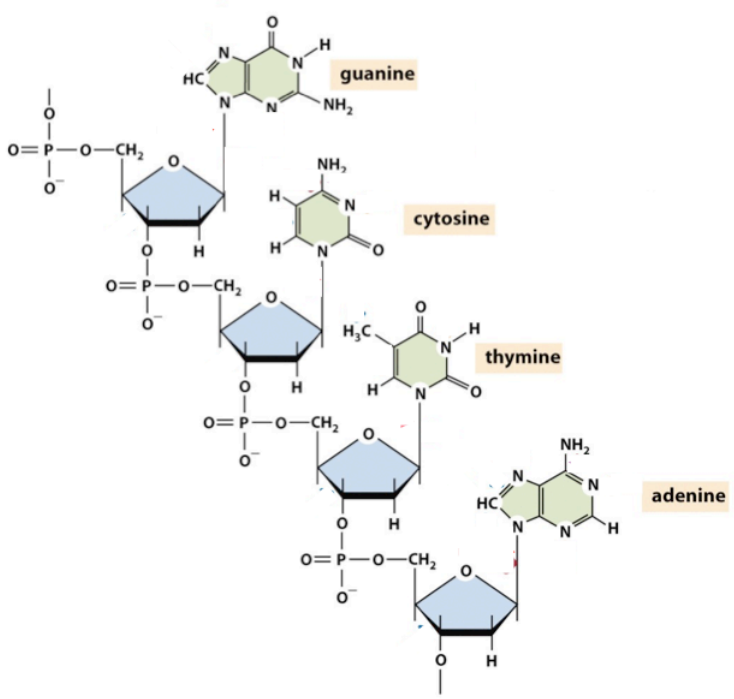

guanine
cytosine
thymine
adenine

Answer 4.21

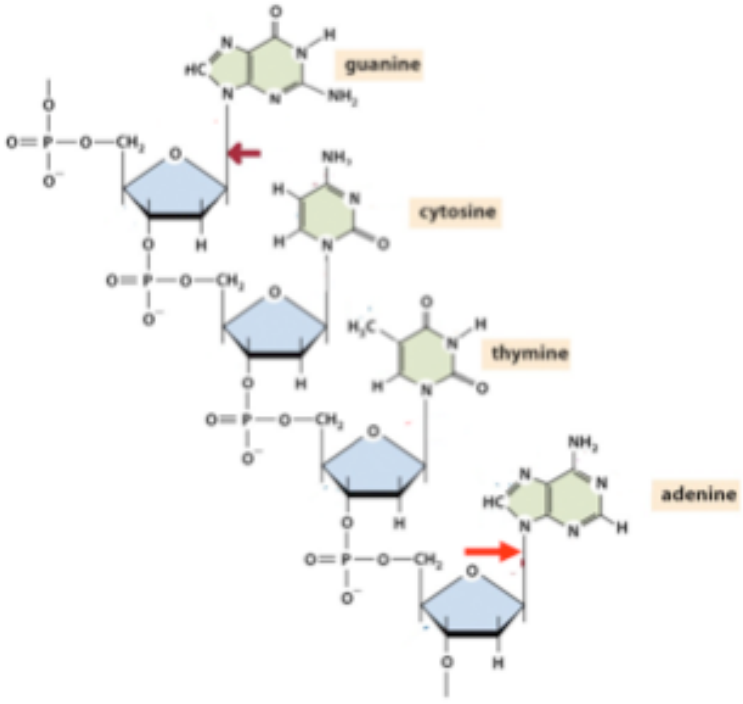

guanine
cytosine
thymine
adenine

Question 4.22

On the diagram below, identify the covalent bonds that break when hydrolytic attack results in depurination.



Answer 4.22



Question 4.23

List the major health consequences that arise as our DNA damage response and DNA repair systems become defective.

Answer 4.23

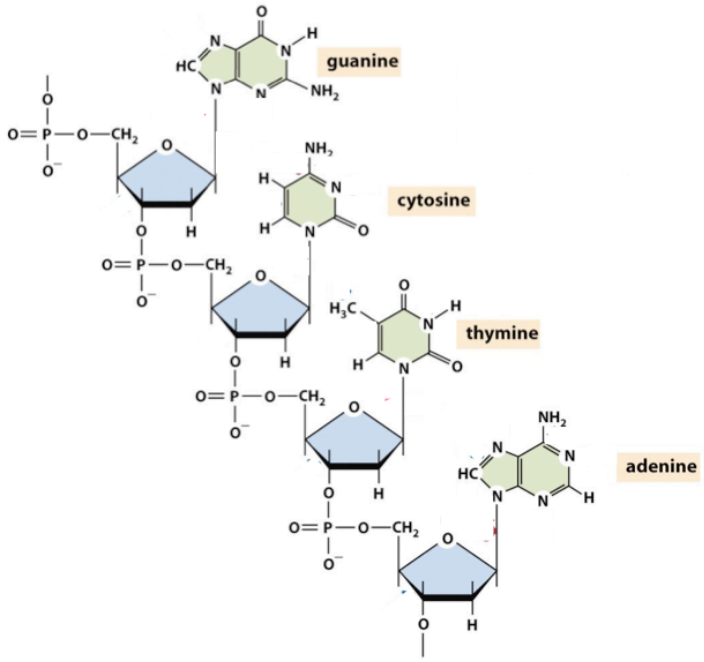
DNA damage accumulates in all of us throughout our lives. Inevitably, as we grow older, the incidence of somatic mutations increases and genes involved in DNA damage responses and DNA repair can be inactivated. Studies of cancers show that somatic mutations in genes that work in DNA damage responses and DNA repair are commonly implicated. We can get ideas about which other bodily systems are affected by looking at the effects of germline mutations in the DNA damage response/DNA repair genes. They often result in inherited disorders with increased susceptibility to cancer and also accelerated aging, but a significant number have developmental abnormalities, and neurological phenotypes are common (the table in Box 4.1 on page 90 gives examples).

Neurological phenotypes are common because although many cell types are regularly replaced, neurons are especially vulnerable. They have high oxygen and energy needs (with a resulting high frequency of oxidative damage), and they accumulate DNA damage over very long periods (because they are not replaced regularly by new cells).

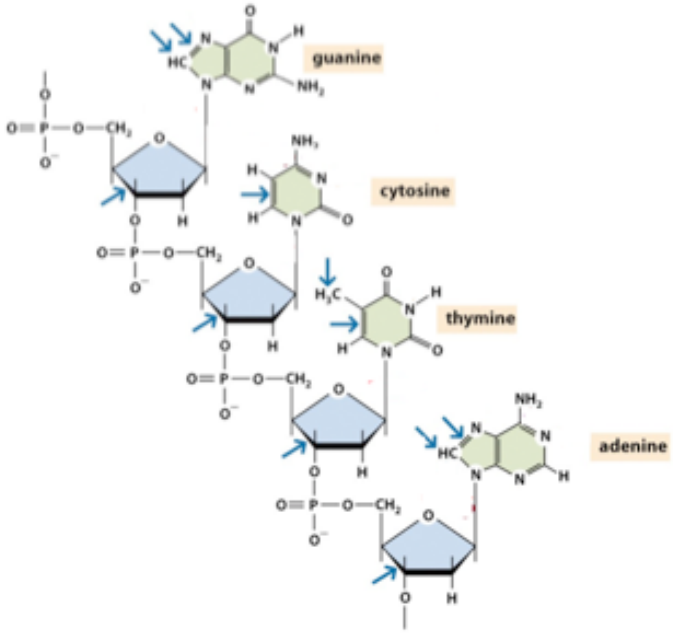
Another class of disorders associated with mutations in some of the genes that work in DNA repair are immunodeficiencies. Some of the proteins that work in DNA repair also function in specialized genetic mechanisms that occur exclusively in B and T lymphocytes. For example, the production of immunoglobulin and T-cell receptors requires components of the nonhomologous end joining DNA repair pathway, and deficiency of these components typically results in hypogammaglobulinemia and lymphopenia or severe combined immunodeficiency.

Question 4.24

On the diagram below, identify the covalent bonds that are susceptible to breakage as a result of oxidative damage.



Answer 4.24



Question 4.25

Most types of DNA damage are repaired by mechanisms that typically involves excising bases or nucleotides and then resynthesizing DNA. But certain types of DNA damage are directly reversible. Give two examples.

Answer 4.25

1) A DNA nick occurs when a single phosphodiester bond is cleaved on one DNA strand. It is easily repaired by using a cellular DNA ligase to restore the phosphodiester bond.
2) Guanine is occasionally methylated to give *O*-6-methylguanine which base pairs with thymine rather than with cytidine, effectively causing a G→A transition. But the reaction is directly reversible using a cellular enzyme, *O*-6-methylguanine DNA methyltransferase.

Question 4.26

What are the essential differences between base excision repair and nucleotide excision repair and what types of DNA damage are they dedicated to repairing?

Answer 4.26

Base excision repair is generally dedicated to repairing lesions where a single base has been modified or where an abasic site has been produced by hydrolytic damage (which cleaves the *N*-glycosidic bond linking the base to its sugar).

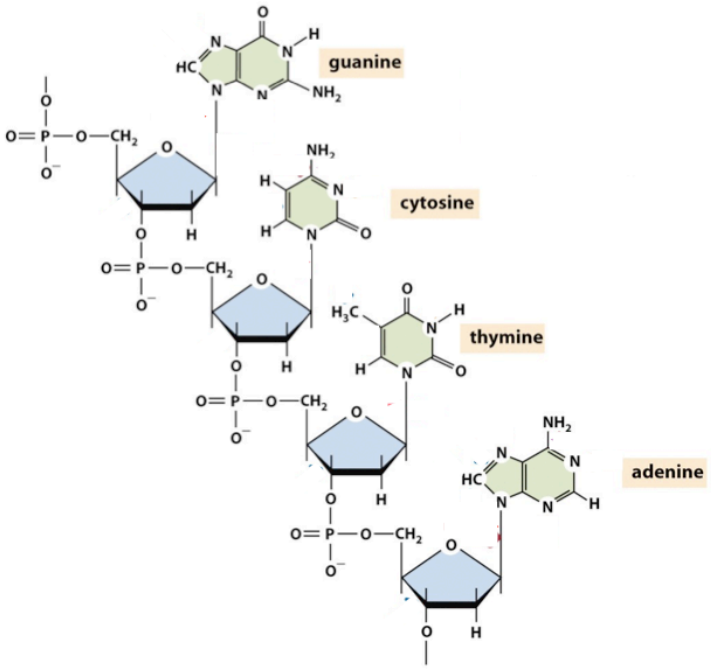
For a modified base the repair mechanism uses a specific DNA glycosylase to cleave the modified base to create an abasic site. For this type of abasic site, or one created by hydrolysis, the repair involves an endonuclease to cleave the residual sugar-phosphate residue. After that, the gap is filled by first a DNA polymerase (which inserts the correct nucleotide) and then a DNA ligase that links the nucleotide using a phosphodiester bond.

Nucleotide excision repair (NER) is generally dedicated to repairing bulky, helix-distorting lesions which can be particularly damaging when they block actively transcribed regions of DNA. After the lesion is detected, the damaged site is opened out and the DNA is cleaved some distance away on either side of the lesion, generating an oligonucleotide of about 30 nucleotides containing the damaged site, which is discarded. Resynthesis of DNA is performed using the opposite strand as a template.

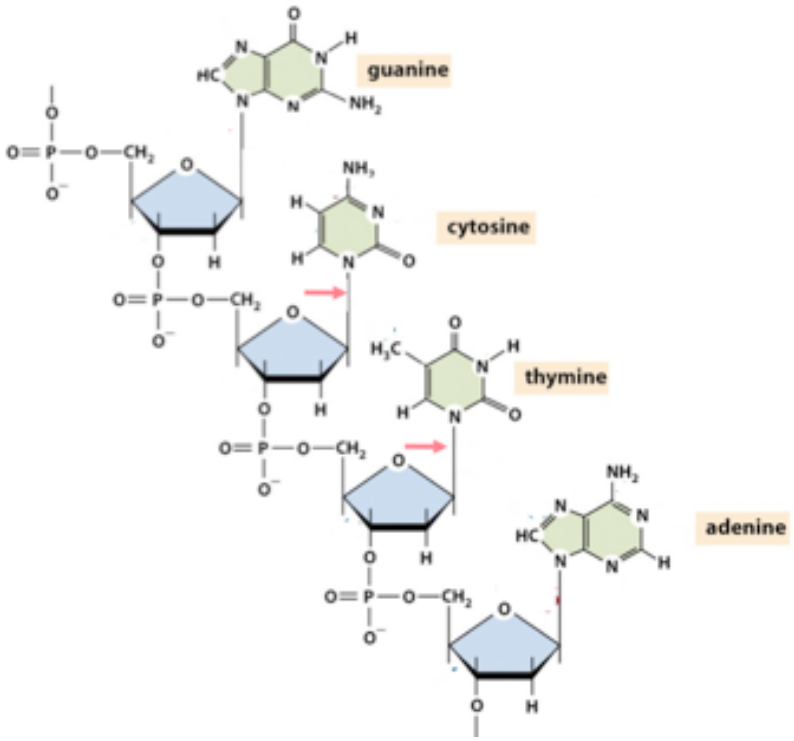
A global NER pathway is generally used across the genome, but when the lesions actively block actively transcribed regions of DNA, a specialized subpathway, *transcription-coupled repair*, initiates this type of repair after it detects RNA polymerases that have stalled at the damaged site.

Question 4.27

On the diagram below, identify the covalent bonds that are susceptible to breakage as a result of hydrolytic damage causing depyrimidination.


guanine
cytosine
thymine
adenine

Answer 4.27


guanine
cytosine
thymine
adenine

Question 4.28

The C→T transition is, by some distance, the most common base substitution in human (and vertebrate) DNA. What makes it so common?

Answer 4.28

Deamination of cytosine is a very common reaction in our cells and normally produces uracil, a base that is usually found in RNA, not DNA. Our cells are equipped with a special enzyme, uracil DNA glycosylase, that recognizes uracil residues in DNA and removes them as part of the base excision DNA repair pathway. However, as in the DNA of other vertebrates, many of our cytosines are methylated at the carbon atom 5.

Deamination of 5-methylcytosine produces thymine, a base normally found in DNA. Although a stable CG base pair has been replaced by a TG base mismatch, the base mismatch may often escape detection by the base mismatch repair system (which focuses on DNA replication events). At the subsequent round of DNA replication the thymine will form a T-A base pair, effectively producing a C→T mutation.

Question 4.29

What is meant by non-classical DNA-dependent DNA polymerases? What roles do they play in our cells?

Answer 4.29

Non-classical DNA-dependent DNA polymerases use a DNA template to synthesize new DNA strands but have a low fidelity of DNA replication compared to classical DNA polymerases such as those that that are responsible for genome-wide DNA synthesis. A list is given in Table 4.2 on page 92.

Most of them are involved in *translesion synthesis* which is a cellular mechanism for by-passing harmful lesions in DNA or in specialized mechanisms in B and T cells: VDJ and VJ recombination (used to diversify immunoglobulins and T cell receptors), and somatic hypermutation in B cells (used to diversify antibodies).

Question 4.30

What are single nucleotide polymorphisms and why do they occur at only certain nucleotides in our genome?

Answer 4.30

A single nucleotide polymorphism refers to any position in the nuclear genome where there is at least one minority variant nucleotide (or inserted nucleotide/deleted nucleotide) that occurs at a frequency of 1% or more of within the population. They occur at only certain nucleotides in our genome that represent ancestral variants which were contributed relatively recently from just a

few human ancestors (humans have limited genetic diversity because within the last 70,000 years or so, the human population underwent at least one dramatic bottleneck when the global population was reduced to just 10,000 or so individuals).

Question 4.31

Longer microsatellites are very prone to being polymorphic. What are microsatellites, why should long microsatellites be so prone to being polymorphic, and what is the mechanism that is responsible for the polymorphism?

Answer 4.31

A microsatellite is any sequence of tandem repeats of a short oligonucleotide that occurs in our DNA, such as CACACACACACACACACA, or tandem repeats of a single nucleotide such as AAAAAAAAAA (but usually the term is taken to mean a sequence of repeats that consist of from one to four nucleotides).

For any array of tandem oligonucleotide/mononucleotide repeats there is an increased chance of a replication error in which the parent strand and the newly replicated DNA strand pair up with their repeats out of register (*slipped strand mispairing*). The mispairing is stabilized by the remaining high degree of hydrogen bonding between mispaired repeats. Compare the situation where there are no repeats, where, if the two strands are out of register by one or two nucleotides. there would be few stable base pairs in the region of mismatching.

If the change is not detected by the cell, the result is a *replication slippage* which results in the newly synthesized strand having one or more extra repeats more than the parent DNA strand, or one or more fewer repeats. That is, there is variation in the number of tandem repeats. The longer is the microsatellite (that is, the greater is the number of repeat units), the higher chance of replication slippage.

The variation in the number of tandem repeats may occasionally cause problems in coding DNA (especially when the repeat unit is one, two or four nucleotides long, so that loss or gain of a repeat produces a translational frameshift). Most of the time, however, there are no important consequences (about 99% of our DNA is noncoding DNA) but the frequency of the variation results in a type of polymorphism (*short tandem repeat polymorphism*).

Question 4.32

What is meant by balanced and unbalanced structural variation?

Answer 4.32

Structural variation typically refers to moderate to large-scale changes to DNA sequences. Balanced structural variation arises when DNA sequences of this type are moved to a different position within the genome without any net gain or loss of DNA. This category includes balanced translocations and inversions.

Unbalanced structural variation arises in different ways. It can involve very large structural changes, as when, for example, a person with a balanced translocation passes one of the translocation chromosomes to a child, but not the other. It may involve very small net losses or gains in DNA sequence when chromosomes break and re-join, as in the case of many apparently balanced translocations. Another example includes some kinds of *copy number variation* in which there are variable numbers of copies of a moderately large to large DNA sequence (often in the range of several hundred bp to many hundreds of kb).

Question 4.33

With reference to positive selection, what is meant by a *selective sweep*?

Answer 4.33

Occasionally, a DNA variant confers some advantage that allows its bearer to have a higher biological *fitness* than persons who lack the variant. If the advantage is a strong one, the allele carrying the advantageous variant will rapidly increase in the population over a few generations. Other variants in the chromosomal region surrounding the advantageous variant will be carried along with the advantageous variant and will also increase in frequency, so that a particular haplotype containing the advantageous variant increases in frequency, a *selective sweep*. Over several generations, however, recombination will ensure that the haplotype is reduced in size. Nevertheless there will be a short region surrounding the advantageous variant where there is very limited genetic diversity because there has not been enough time for recombination to occur within such a short region. (Variants at loci that are extremely close to the advantageous variant locus are the same ones as on the haplotype in which newly emerged advantageous variant arose).

Question 4.34

A human zygote has three immunoglobulin loci, one that specifies the heavy chain and two that specify the light chain. Taking into account differences between maternal and paternal alleles a B cell might be expected to have the potential of making a total of two different heavy chains and four different light chains, and therefore eight different immunoglobulins. Instead, each mature B cell makes just a single type of immunoglobulin. How does that happen?

Answer 4.34

The V-J and V-D-J recombination mechanisms at the immunoglobulin loci work through a regulatory feedback-loop mechanism such that once a productive recombination works, a message is conveyed that inhibits the other allele from embarking on a similar recombination (*allelic exclusion*). Additionally, when one of the two light chain loci embarks on V-J recombination the feedback loop mechanism works to inhibit V-J recombination at not just the other allele but also at both alleles of the other light chain locus (*light chain exclusion*).

Question 4.35

Our immunoglobulins, T-cell receptors and HLA proteins are thought to belong to one large superfamily of proteins based on their structures as well as their functions. In what ways do the structures of these three sets of proteins resemble each other?

Answer 4.35

Each of these proteins is a heterodimer consisting of two chains that can work as a transmembrane protein (initially B cells make immunoglobulins that work as membrane receptors before class switching occurs to make the soluble form of immunoglobulins, known as antibodies). In each case the structure of the proteins is organized so that they have short cytoplasmic tails and large extracellular domains. The latter are made up domains with conserved sequence located close to the membrane and more distal domains that are more variable in sequence. The more distal domains are the ones that are responsible for recognizing and binding foreign antigen and the most variable sites in these sequences are the amino acid positions that are directly involved in recognizing foreign antigen. In each protein many of the domains are shaped by intrachain disulphide bonds. See Figure 4.13 on page 105.

Question 4.36

The HLA system is important in medicine for two major reasons. What are they?

Answer 4.36

First, HLA proteins are the most polymorphic human proteins. During organ or cell transplantation between individuals the grafted donor tissue/cells frequently has different HLA alleles to that of the recipient of the transplant. That often results in immune rejection of a transplant (the graft cells are attacked by the host's immune system) and sometimes if there are immune system cells within the graft they can attack host cells.

To minimize the chance of harmful immune responses following transplantation, attempts are made to minimize HLA differences between donor and recipient tissue (occasionally by finding donors that are close relatives of recipients, but usually by scanning for prospective donors that are closely HLA-matched to the recipient), and through administration of immunosuppressant drugs to the recipient.

A second reason for HLA's importance in medicine is that HLA proteins are the most important genetic factors in conferring susceptibility to a wide range of autoimmune disorders, such as rheumatoid arthritis, type I diabetes and so on. In these disorders the normal ability to distinguish between self and non-self has broken down, and a person's T cells launch attacks against certain types of host cell (such as pancreatic beta cells in the case of type I diabetes).

Question 4.37

The programmed rearrangements at immunoglobulin loci in B cells and in T-cell receptor loci in T cells that are required for antibody and T-cell receptor diversity are cell-specific. What precisely does that mean?

Answer 4.37

It means that although the rearrangements are programmed to occur at these loci, the *precise* rearrangement nevertheless shows a high degree of randomness. For example, in V-D-J recombination during the production of an immunoglobulin heavy chain, the recombination always involves first selecting a specific V gene segment from many V gene segments with slightly different sequences, and then joining to it a D segment, again from several similar D gene segments to form a unique VD segment, and then joining to that a specific J segment again from multiple similar J gene segments to form a unique VDJ combination. But the choice of gene segments that are selected from each of the duplicated V, D and J gene segments is made randomly. Similarly, somatic hypermutation occurs with a degree of randomness. The end result is that each maturing B cell (and its progeny, following cell division) makes an immunoglobulin of a unique type, and each maturing T cell makes a T-cell receptor of a unique type (but one that will also be expressed by its progeny).

Question 4.38

"The huge variation in immunoglobulins and T cell receptors is manifest at the level of the individual, but the huge variation in HLA proteins is expressed at the level of the population". What is meant by this statement?

Answer 4.38

Because of programmed post-zygotic rearrangements in B and T cells, each of the maturing B cells goes on to make a cell-specific immunoglobulin, and each of the maturing T cells goes on to make a cell-specific T cell receptor. As a result, each of us is capable of making millions of different immunoglobulins and T cell receptors, even although we inherited just six immunoglobulin genes (three from each parent) and just eight T-cell receptor genes.
In the case, of the classical HLA proteins, however, there are no post-zygotic rearrangements. Instead the diversity is created by mutation at the level of germline genomic DNA (we often inherit different HLA alleles from our parents). However, we have only three classical class I HLA loci and six classical class II HLA loci. That means any one individual can only make a limited number of different HLA proteins. However, HLA heterozygosity is advantageous because individuals who are heterozygous at multiple HLA loci are expected to have increased biological fitness (by being more resistant to infectious diseases. As a result of selection for HLA heterozygosity the classical HLA proteins are, by a long distance, the most highly polymorphic of our proteins. While a single individual shows a limited number of HLA proteins, the number of alleles at each classical HLA locus can be large.

Question 4.39

The human *HLA-DRB1* and the chimpanzee Patr-*DRB1* gene are orthologs. At the protein level, the human HLA-DRB1*0701 and HLA-DRB1*0302 alleles show 31 amino acid differences out of 270 amino acid positions. But human HLA-DRB1*0702 and the chimpanzee Patr-DRB1*0702 proteins are so closely related that they differ at only 2 positions out of the 270. What does the difference between these two pairwise comparisons tell us about the origins of HLA polymorphism?

Answer 4.39

It tells us that HLA polymorphism is comparatively ancient. By having a HLA protein more distantly related to anther allele than to a chimpanzee protein, selection for heterozygosity is ancient and pre-dated evolutionary divergence of the human and chimpanzee lineages (which has been estimated to have occurred about 5-7 million years ago.