Gene Mutations and Repair Mechanisms of the Human Cell

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Mutations are associated with diseases and defective genes. Mutated genes are actually the cause of many diseases, although they are also necessary. Without a permanent change in the genome, evolution is not possible, and purely static DNA would prevent any development. DNA is also the only molecule of the cell that is repaired. The information stored in the DNA is so important that the energy expended by repairing it is worthwhile. The cell must find the correct balance between the loss of evolution by mutation, and evolution by mutation.

Standard Genetic Code

nonpolar polar basic acidic (stop codon)

Standard genetic code

1st base	2nd base U		2nd base C		2nd base A		2nd base G		3rd base
U	UUU	(Phe/F) Phenylalanine	UCU	(Ser/S) Serine	UAU	(Tyr/Y) Tyrosine	UGU	(Cys/C) Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	(Leu/L) Leucine	UCA		UAA	Stop (*Ochre*)	UGA	Stop (*Opal*)	A
	UUG		UCG		UAG	Stop (*Amber*)	UGG	(Trp/W) Tryptophan	G
C	CUU		CCU	(Pro/P) Proline	CAU	(His/H) Histidine	CGU	(Arg/R) Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	(Ile/I) Isoleucine	ACU	(Thr/T) Threonine	AAU	(Asn/N) Asparagine	AGU	(Ser/S) Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine	A
	AUG[A]	(Met/M) Methionine	ACG		AAG		AGG		G
G	GUU	(Val/V) Valine	GCU	(Ala/A) Alanine	GAU	(Asp/D) Aspartic acid	GGU	(Gly/G) Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	(Glu/E) Glutamic acid	GGA		A
	GUG		GCG		GAG		GGG		G

Classification of Gene Mutations

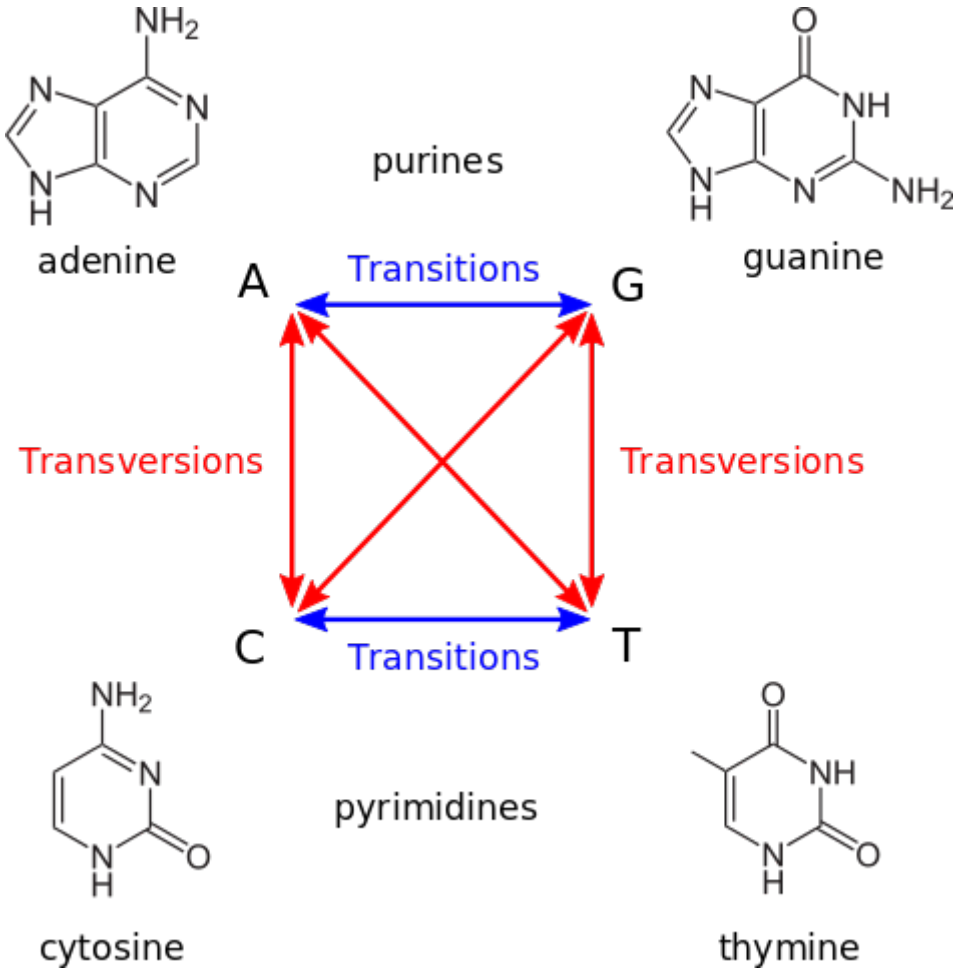

Image: “Definition of transitions and transversions“ by Petulda. License: CC BY-SA 4.0

The simplest types of mutation switch one type of base to another. These are called **transitions** or **transversions**. Transitions are pyrimidine-to-pyrimidine and purine-to-purine substitutions, such as T to C and A to G; and transversions are pyrimidine-to-purine and purine-to-pyrimidine substitutions, such as T to G or A and A to C or T. Mutations that alter a single nucleotide are called **point mutations**.

More drastic changes to the DNA sequence are caused by **extensive insertions and deletions**, or **large rearrangements of chromosome structure**. These can be caused by the insertion of a transposon, inserts thousands of nucleotides of foreign DNA into the coding or regulatory sequences genes, as well as cellular recombination processes.

The 3’à5’ exonuclease component of the replisome has a **proofreading mechanism**, which accounts for its high degree of accuracy during DNA replication, allowing it to remove incorrectly placed nucleotides. This proofreading improves the fidelity of DNA replication by a factor of 100, but is not foolproof, as some misincorporated nucleotides escape detection and become a mismatched between the newly synthesized strand and the template strand.

Three different nucleotides can be misincorporated opposite each of the four kinds of nucleotides in the template strand (for example, T, G, or C opposite a T in the template) for a total of 12 possible mismatches (T:T, T:G, T:C, etc.).

If the misincorporated nucleotide is not subsequently detected and replaced, the sequence change will become permanent. During the second round of replication, the

misincorporated nucleotide will direct the incorporation of its complementary nucleotide into the newly synthesized strand. The mismatch will no longer exist; instead, it will have resulted in a **permanent change (mutation) in the DNA sequence**.

Mismatches are generally detected and repaired, fortunately. The mismatch repair system increases the accuracy of DNA synthesis by an additional two to three orders of magnitude. The mismatch repair system scans the genome for mismatches.

Because mismatches are transient (they are eliminated following the second round of replication when they result in mutations), the mismatch repair system must rapidly find and repair mismatches. Second, the system must correct the mismatch accurately; in other words, it must replace the misincorporated nucleotide in the newly synthesized strand and not the correct nucleotide in the parental strand.

Causes of Genetic Mutation

Mutations arise not only from **errors in replication** but also from **damage to the DNA** itself. Environmental factors, such as **radiation** and **chemical agents** also cause DNA damage, as well as increase the frequency of mutations.

The most frequent and important kind of hydrolytic damage is **deamination of cytosine**. Under normal physiological conditions, cytosine undergoes spontaneous deamination, to create uracil in DNA. Uracil preferentially pairs with adenine and thus introduces that base in the opposite strand upon replication, rather than the G that would have been directed by C.

Adenine and guanine are also subject to spontaneous deamination. Deamination converts adenine to hypoxanthine, which hydrogen bonds to cytosine rather than to thymine; guanine is converted to xanthine, which continues to pair with cytosine, although with only two hydrogen bonds. DNA also undergoes depurination by spontaneous hydrolysis of the N-glycosyl linkage, and this produces an **abasic site** (deoxyribose lacking a base) in the DNA.

Notice that all of these hydrolytic reactions result in **unnatural alterations to the DNA**. Aprinic sites are unnatural, and each of the deamination reactions generates an unnatural base. This situation allows changes to be recognized by the repair systems. This also explains why DNA has thymine instead of uracil. If DNA naturally contained uracil instead of thymine, then deamination of cytosine would generate a natural base, which the repair systems could not easily recognize.

The hazard of having deamination generate a naturally occurring base is illustrated by the problem caused by the presence of 5-methylcytosine. Human DNA frequently contains 5-methylcytosine in place of cytosine as a result of the action of methyltransferases. This modified base has a role in transcriptional silencing.

Deamination of 5-methylcytosine generates thymine, which obviously will not be recognized as an abnormal base and, following a round of DNA replication, can become fixed as a C-to-T transition. **Methylated Cs are hot spots for spontaneous mutations in human DNA.**

DNA is also vulnerable to **damage from alkylation, oxidation, and radiation**. In alkylation, methyl or ethyl groups are transferred to reactive sites on the bases and to phosphates in the DNA backbone. Alkylating chemicals include nitrosamines and N-methyl-N1-nitro-N- nitrosoguanidine. One of the most vulnerable sites of alkylation is the

keto group at carbon atom 6 of guanine. The product of this methylation, O6-methylguanine, often mispairs with thymine, resulting in the change of a G:C base pair into an A:T base pair when the damaged DNA is replicated.

DNA is also subject to attack from **reactive oxygen species** (for example, O_2 , H_2O_2, and OH^+). These potent oxidizing agents are generated by ionizing radiation and by chemical agents that generate free radicals. Oxidation of guanine, for example, generates 7,8-dihydro-8-oxoguanine. This adduct is highly mutagenic because it can base-pair with adenine as well as with cytosine.

If it base-pairs with adenine during replication, it gives rise to a G:C to T:A transversion, which is one of the most common mutations found in human cancers. Thus, perhaps the carcinogenic effects of ionizing radiation and oxidizing agents are partly caused by free radicals that convert guanine to this adduct.

Another type of damage to bases is caused by **UV light**. Radiation with a wavelength of 260 nm is strongly absorbed by bases, one consequence of which is the fusion of two pyrimidines that occupy adjacent positions on the same polynucleotide chain. In the case of two thymines, the fusion is called a **thymine dimer**, which comprises a cyclobutane ring generated by links between carbon atoms 5 and 6 of adjacent thymines.

In the case of thymine adjacent to cytosine, the resulting fusion is a thymine-cytosine adduct in which the thymine is linked via its carbon atom 6 to the carbon atom 4 of cytosine. These linked bases are incapable of base pairing and cause the DNA polymerase to stop during replication.

Lastly, compounds called **base analogs** and **intercalating agents** cause replication errors and thus cause mutations. Base analogs are structurally similar to natural bases but differ in ways that make them treacherous to the cell. Thus, base analogs are similar enough to the natural bases to get taken up by cells, converted into nucleoside triphosphates, and incorporated into DNA during replication. Because of the structural differences between these analogs and the natural bases, the analogs base-pair inaccurately, leading to frequent mistakes during the replication process.

One of the most mutagenic base analogs is 5-bromouracil, an analog of thymine. The presence of the bromo substituent allows the base to mispair with guanine via the enol tautomer (the keto tautomer is strongly favored over the enol tautomer, but more so for thymine than for 5-bromouracil).

Intercalating agents are flat molecules that containing several polycyclic rings that bind to the equally flat purine or pyrimidine bases of DNA. This occurs just as the bases bind or stack with each other in the double helix. Example compounds are proflavin, acridine, and ethidium; which cause the deletion or addition of a base pair or even a few base pairs. When such deletions or additions arise in a gene they can have profound consequences on the translation of its mRNA, because they shift the coding sequence out of its proper reading frame.

Repair Mechanisms

Damage to DNA can have two consequences. Some kinds of damage, such as thymine dimers or nicks and breaks in the DNA backbone, create **impediments to replication or transcription**. Other kinds of DNA damage create **altered bases** that have no immediate structural consequence on replication but instead **cause mispairings**; which can result in a **permanent alteration to the DNA sequence** after replication.

For example, the conversion of cytosine to uracil by deamination creates a U:G mismatch, which, after a round of replication becomes a C:G to T:A transition mutation on one daughter chromosome. Cells possess elaborate mechanisms to identify and repair DNA damage before it blocks replication or causes a mutation. Cells would not endure long without such mechanisms.

The most direct of these systems occurs when a **repair enzyme simply reverses and undoes the damage**. A more elaborate system is **excision repair**, in which the damaged nucleotide is not repaired but removed from the DNA. In excision repair, the undamaged strand serves as a template for reincorporation of the correct nucleotide by DNA polymerase. Two kinds of excision repair systems exist, one involving the removal of only the damaged nucleotide and the other involving the removal of a short stretch of single-stranded DNA that contains the lesion.

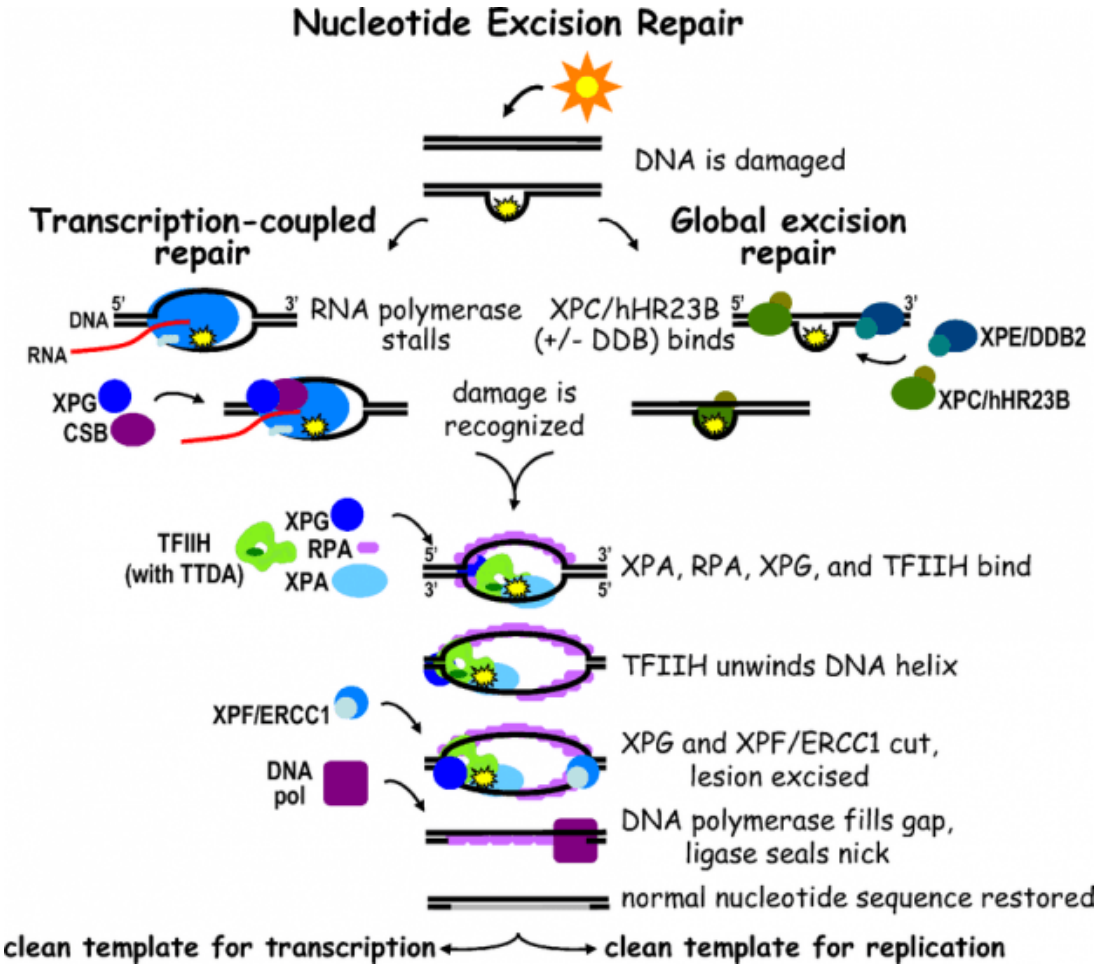

Image: “When DNA is damaged by sunlight, the damage is recognized differently depending on whether the DNA is transcriptionally active (transcription-coupled repair) or not (global excision repair). After the initial recognition step, the damage is repaired in a similar manner with the final outcome being the restoration of the normal nucleotide sequence. A more detailed description is provided in the text.“ by Jill O. Fuss, Priscilla K. Cooper. License: CC BY 2.5

An even more elaborate system is **recombinational repair**, which is used when both strands are damaged. In this case the DNA is broken. One strand cannot serve as a template for the repair of the other. Hence, in recombinational repair (also called **double-strand break repair**), the sequence information is retrieved from a second undamaged copy of the chromosome.

Finally, when damaged bases block the progression of a replicating DNA polymerase, a special translesion polymerase copies across the site of the damage in a manner that

does not depend on base pairing between the templates and newly synthesized DNA strands.

Photoreactivation directly reverses the formation of pyrimidine dimers that result from ultraviolet irradiation. In photoreactivation, the enzyme DNA photolyase captures energy from light and uses it to break the covalent bonds linking adjacent pyrimidines

Another example of direct reversal is the removal of the methyl group from the methylated base O6-methylguanine. In this example, a methyltransferase removes the methyl group from the guanine residue by transferring it to one of its own cysteine residues. This is costly to the cell because the methyltransferase is not catalytic; having once accepted a methyl group, it cannot be used again.

Base excision repair and **nucleotide excision repair** are the most prevalent ways in which DNA is repaired of damaged bases.

In base excision repair, an enzyme called a glycosylase recognizes and removes the damaged base by hydrolyzing the glycosidic bond. The resulting abasic sugar is removed from the DNA backbone in a further endonucleolytic step. Endonucleolytic cleavage also removes apurinic and apyrimidinic sugars that arise by spontaneous hydrolysis. After the damaged nucleotide has been entirely removed from the backbone, a repair DNA polymerase and DNA ligase restore an intact strand using the undamaged strand as a template.

DNA glycosylases are lesion-specific and cells have multiple DNA glycosylases with different specificities. Thus, a specific glycosylase recognizes uracil (generated as a consequence of deamination of cytosine), and another is responsible for removing those generated as a consequence of oxidation of guanine. There are a total of 11 different DNA glycosylases that have been identified in human cells.

Cloning and Detection of Gene Mutations

Cloning in natural biological function is the process of producing similar populations of genetically identical individuals that occurs in nature when organisms reproduce asexually.

Cloning in biotechnology refers to processes used to create copies of either piece of DNA, whole cells (cell cloning) or complete organisms.

Cloning a single-celled organism to derive a population of cells from a single cell. **Cloning stem cells** used to create cloned human embryos in what is termed ‘therapeutic cloning’. **Organism cloning** (reproductive cloning) creates a new multicellular organism, genetically identical to another, e.g. Dolly the sheep in 1996.

Review Questions

The correct answers can be found below the references.

1. Which of the following is true about tautomeric shifts?

A. They are typically induced by high-energy radiation.
B. They occur only in prokaryotes.
C. They may lead to a transversion mutation.
D. They may produce a transition mutation.

2. How do human cells typically repair DNA double-strand breaks?

A. Mismatch repair
B. Base excision repair
C. Nucleotide excision repair
D. Nonhomologous end-joining
E. All of the above

3. In humans, thymine dimers may be repaired by…

A. …photoreactivation.
B. …excision repair.
C. …oxidative damage.
D. …telomerase.
E. All of the above.

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Correct answers: 1D, 2E, 3B

Notes