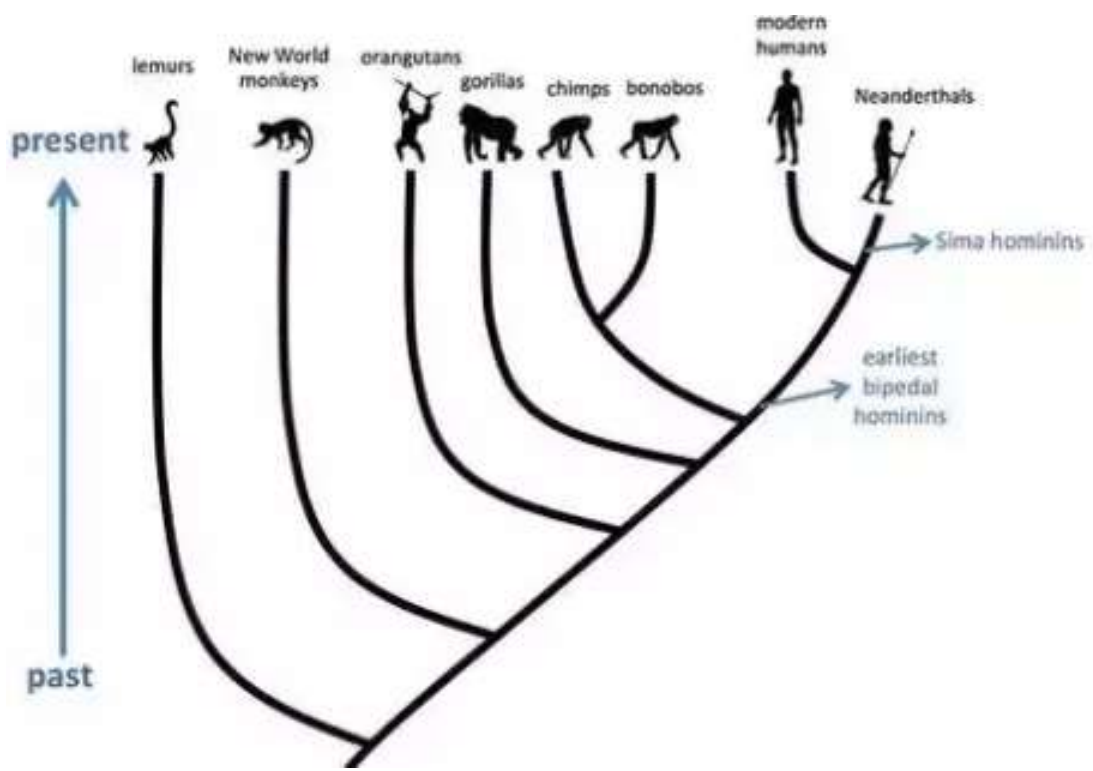
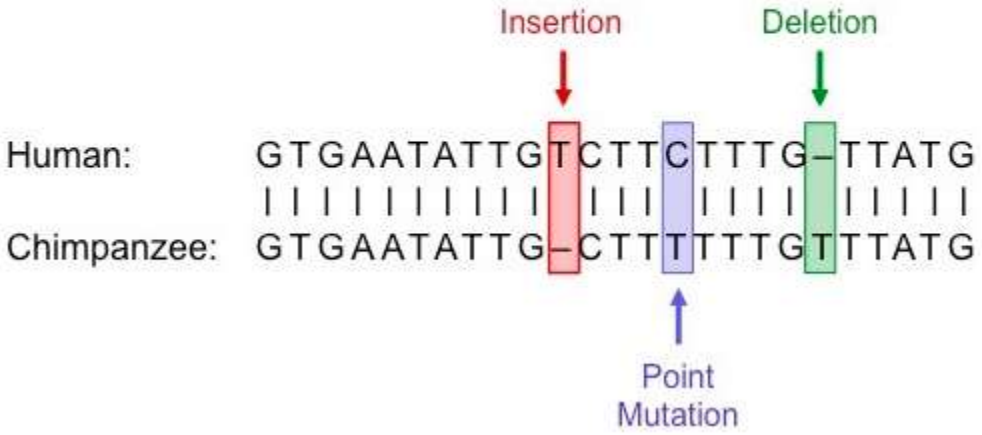
Genetic Variation



Sequence Alignment of DNA from Two Species

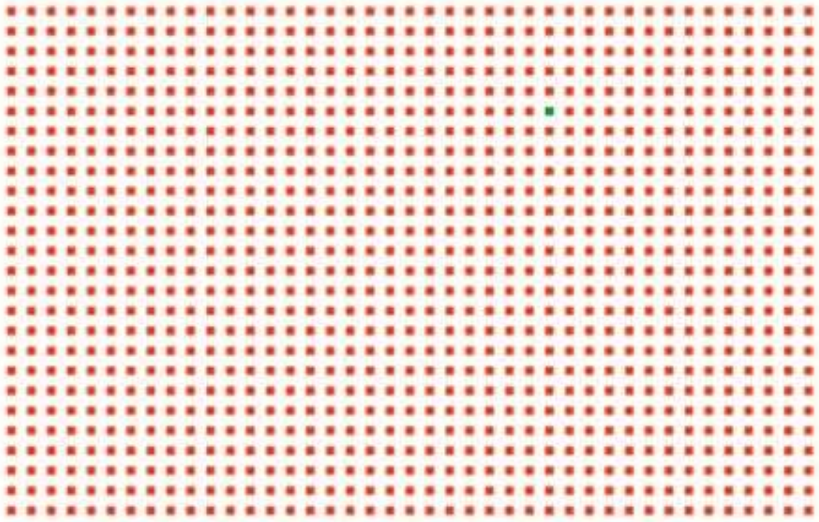


	Gene Sequences That Codes for Proteins	Random DNA Segments Between Genes
Chimpanzee	100%	98%
Dog	99%	52%
Mouse	99%	40%
Chicken	75%	4%
Fruit Fly	60%	~0%
Roundworm	35%	~0%

Likelihood of Finding Similar DNA Sequences Between Human and Other Organisms

Similarity of DNA

- The human genome is over 3 billion base pairs long
- Two random people are 99.9% identical
- However, that still leaves 3 MILLION base pairs that can be different



All DNA sequence variation arises via mutation of an ancestral sequence

	< 1%	≥ 1%
"Normal"	Rare variant or "private" polymorphism	polymorphism
"Disease"	Disease mutation	*Example: Factor V Leiden (thrombosis) 5% allele frequency*

Common but incorrect usage:

"a disease-causing mutation" **OR** *"a polymorphism"*

Genetic variation

- **Mutation:** A change in DNA sequence
  - Mutation ≠ deleterious change
  - Pathogenic mutation: DNA sequence changes responsible for causing disease or susceptibility to disease

- **Polymorphism:** Existence of two or more alleles of at least 1% frequency
  - Polymorphism ≠ neutral change
  - Alleles at a polymorphic locus can be pathogenic

    (e.g. GJB2 c.35delG - ~2% frequency)

GJB2:c.109G>A

chr13-20763612 C>T | p.Val37Ile | NM_004004.6 |

Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
East Asian	1665	19952	96	0.08345
Ashkenazi Jewish	83	10342	0	0.008026
Other	31	7212	0	0.004298
Latino/Admixed American	95	35428	1	0.002681
European (Finnish)	42	25104	0	0.001673
European (non-Finnish)	179	128578	1	0.001392
African/African-American	25	24964	1	0.001001
South Asian	12	30584	0	0.0003924
XX	1083	129104	53	0.008389
XY	1049	153060	46	0.006854
Total	**2132**	**282164**	**99**	**0.007556**

Mutation, polymorphism and variant

"A mutation is defined as a permanent change in the nucleotide sequence with a frequency below 1%

polymorphism is defined as a variant with a frequency above 1%

The terms "mutation" and "polymorphism," however, which have been used widely, often lead to confusion because of incorrect assumptions of pathogenic and benign effects, respectively.

Thus, it is recommended that both terms be replaced by the term "variant"" ACMG 2015 guidelines

Categories of variation and their estimated frequencies

Table 9-1

Types of Mutation and Their Estimated Frequencies

Class of Mutation	Mechanism	Frequency (Approximate)	Examples
Genome mutation	Chromosome missegregation	$2\text{-}4 \times 10^{-2}$/cell division	Aneuploidy
Chromosome mutation	Chromosome rearrangement	6×10^{-4}/cell division	Translocations
Gene mutation	Base pair mutation	10^{-10}/base pair/cell division $10^{-5}\text{-}10^{-6}$/locus/generation	Point mutations

Based on Vogel F, Motulsky AG: Human Genetics, 3rd ed. Berlin, Springer-Verlag, 1997; and Crow JF: The origins, patterns and implications of human spontaneous mutation. Nat Rev Genet 1:40-47, 2000.

- Genome mutations: affect the number of chromosomes in the cell, arising from errors in chromosome segregation during meiosis or mitosis.
- A genome mutation that deletes or duplicates an entire chromosome alters the dosage and thus the expression levels of hundreds or thousands of genes.
- Missegregation of a chromosome pair during meiosis causes genome mutations responsible for conditions such as trisomy 21 (Down syndrome).
- Genome mutations produce chromosomal aneuploidy and are the most common mutations seen in humans, with a rate of one missegregation event per 25 to 50 meiotic cell divisions.
- This estimate is clearly a minimal one because the developmental consequences of many such events may be so severe that the resulting aneuploid fetuses are spontaneously aborted shortly after conception without being detected.
- Genome mutations are also common in cancer cells

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- Chromosome mutations: mutations that alter the structure of individual chromosomes. The changes involve only a part of a chromosome, such as partial duplications or triplications, deletions, inversions, and translocations, which can occur spontaneously or may result from abnormal segregation of translocated chromosomes during meiosis.

- Chromosome mutations, occurring at a rate of approximately one rearrangement per 1700 cell divisions, happen much less frequently than genome mutations.

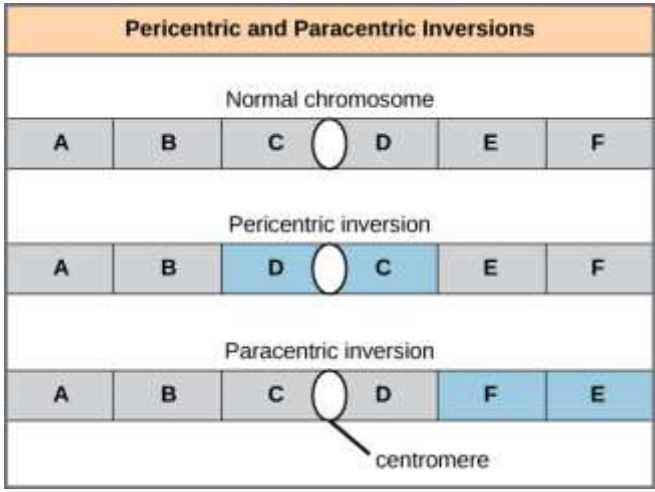
- Although the frequencies of genome and chromosome mutations may seem high, these mutations are rarely perpetuated from one generation to the next because they are usually incompatible with survival or normal reproduction.

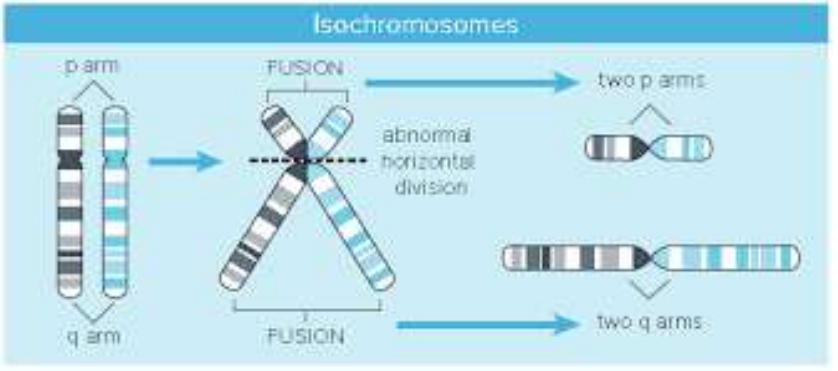
- Chromosome mutations are also frequently seen in cancer cells

TYPES OF CHROMOSOME ANOMALIES

A chrmosomal anomaly can be:

- **Numerical**
  - **Aneuploidy** (autosomal / Sex chromosomal)
    - Monosomy (loss of 1 chromosome)
    - Trisomy (gain of 1 chromosome)
    - Tetrasomy (gain of 2 chromosome)
  - **Polyploidy**
    - Triploidy
    - Tetraploidy
- **Structural** (gross / micro)
  - Translocation (t):
    - Reciprocal
    - Robertonian
  - Inversions:
    - Paracentric
    - Pericentric
  - Deletions (del)
  - Insertions
  - Rings
  - Isochromosomes
- **Others**
  - e.g. Mosaicism





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- Gene mutations: mutations that alter individual genes.

- Gene mutations are changes in DNA sequence of the nuclear or mitochondrial genomes, ranging from a change in as little as a single nucleotide to changes that may affect many millions of base pairs.

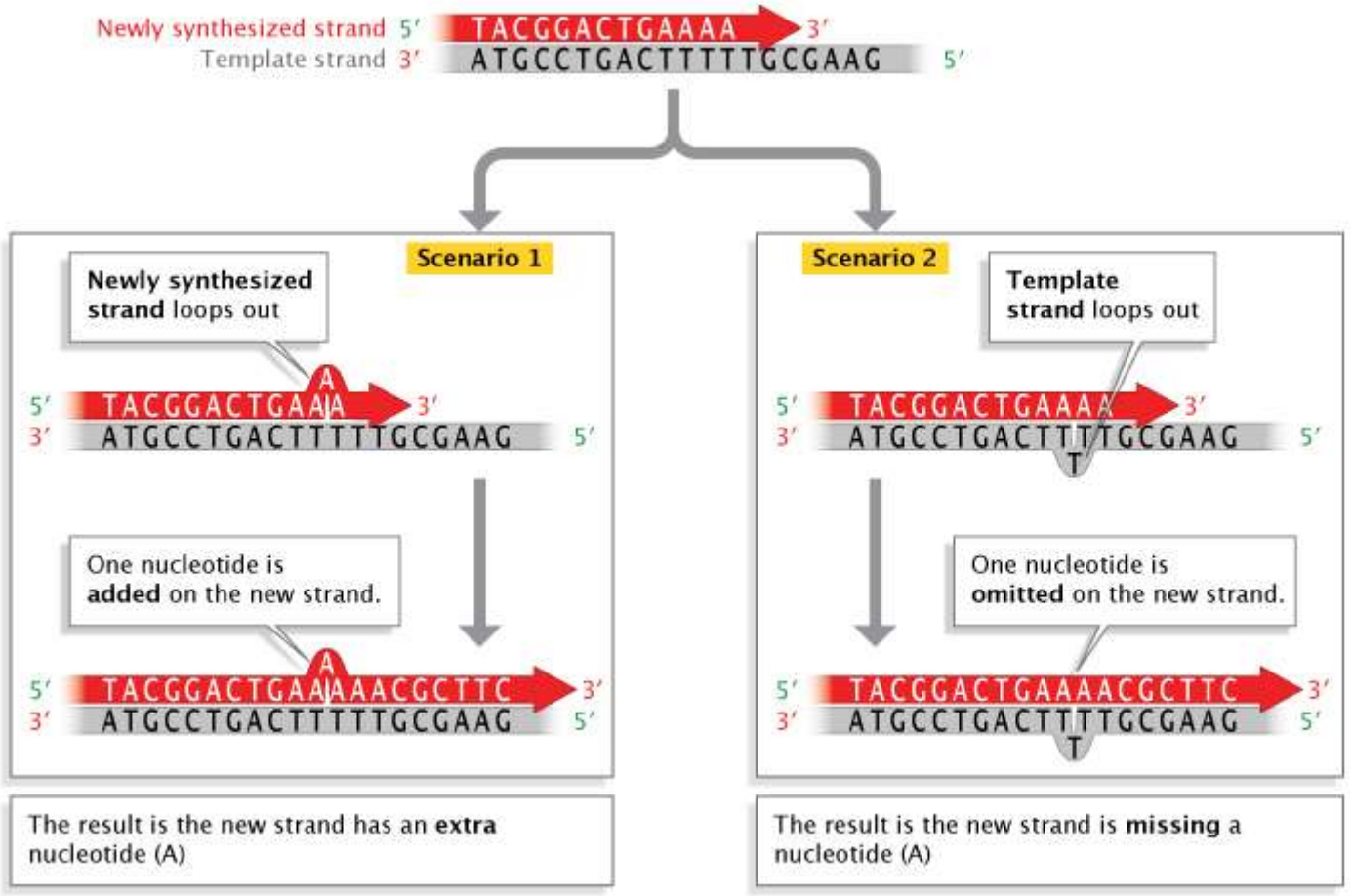
- Gene mutations, including base pair substitutions, insertions, and deletions, can originate by either of two basic mechanisms:
    - errors introduced during the normal process of DNA replication, or
    - mutations arising from a failure to repair DNA after damage and to return its sequence to what it was before the damage.

- Some mutations are spontaneous, whereas others are induced by physical or chemical agents called mutagens, because they greatly enhance the frequency of mutations.

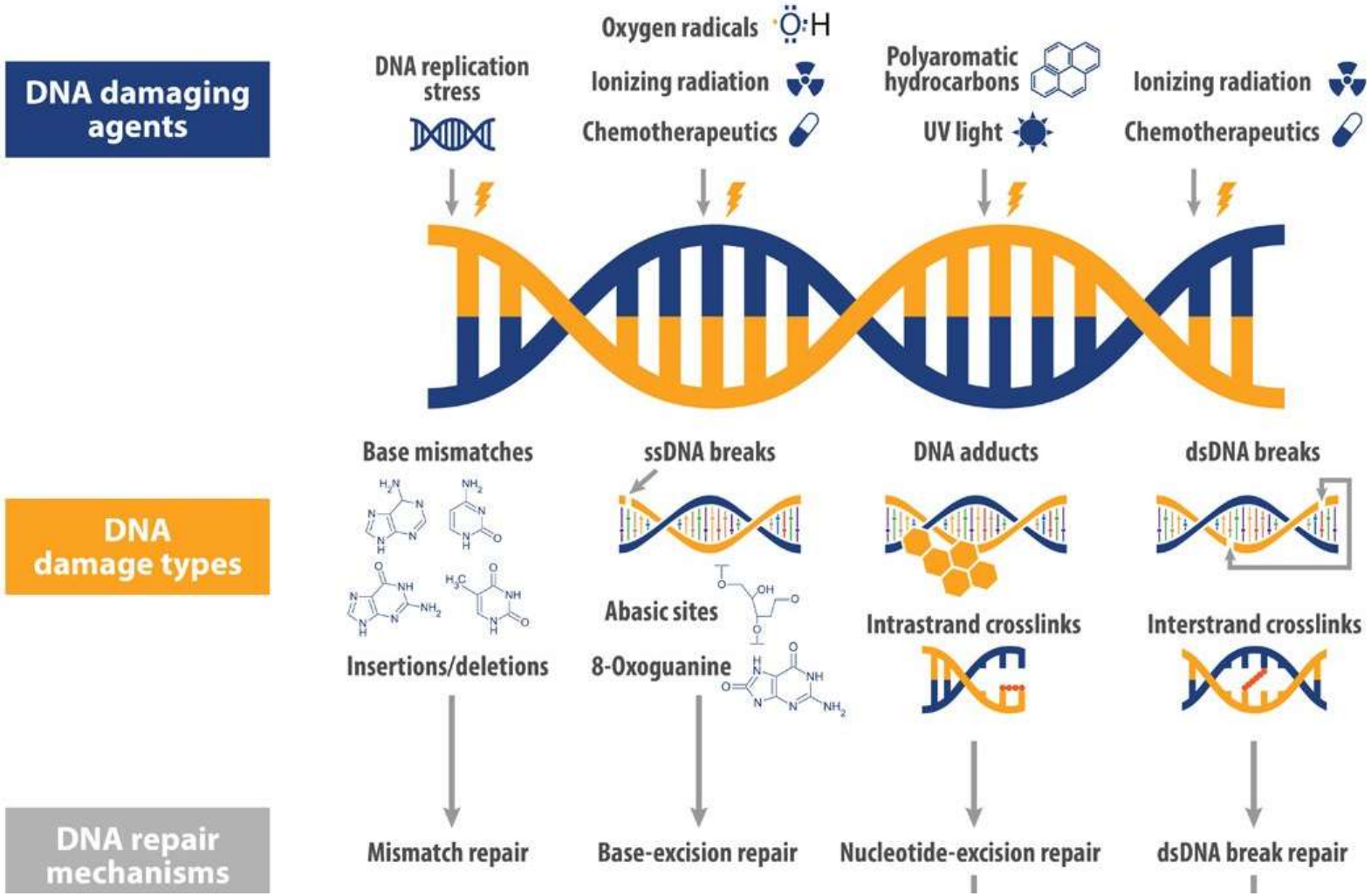
Replication Error

An incorrect nucleotide is introduced into one of the growing daughter strands only once every 10^{-10} million base pairs.

Additional replication error checking corrects more than 99.9% of errors of DNA replication.

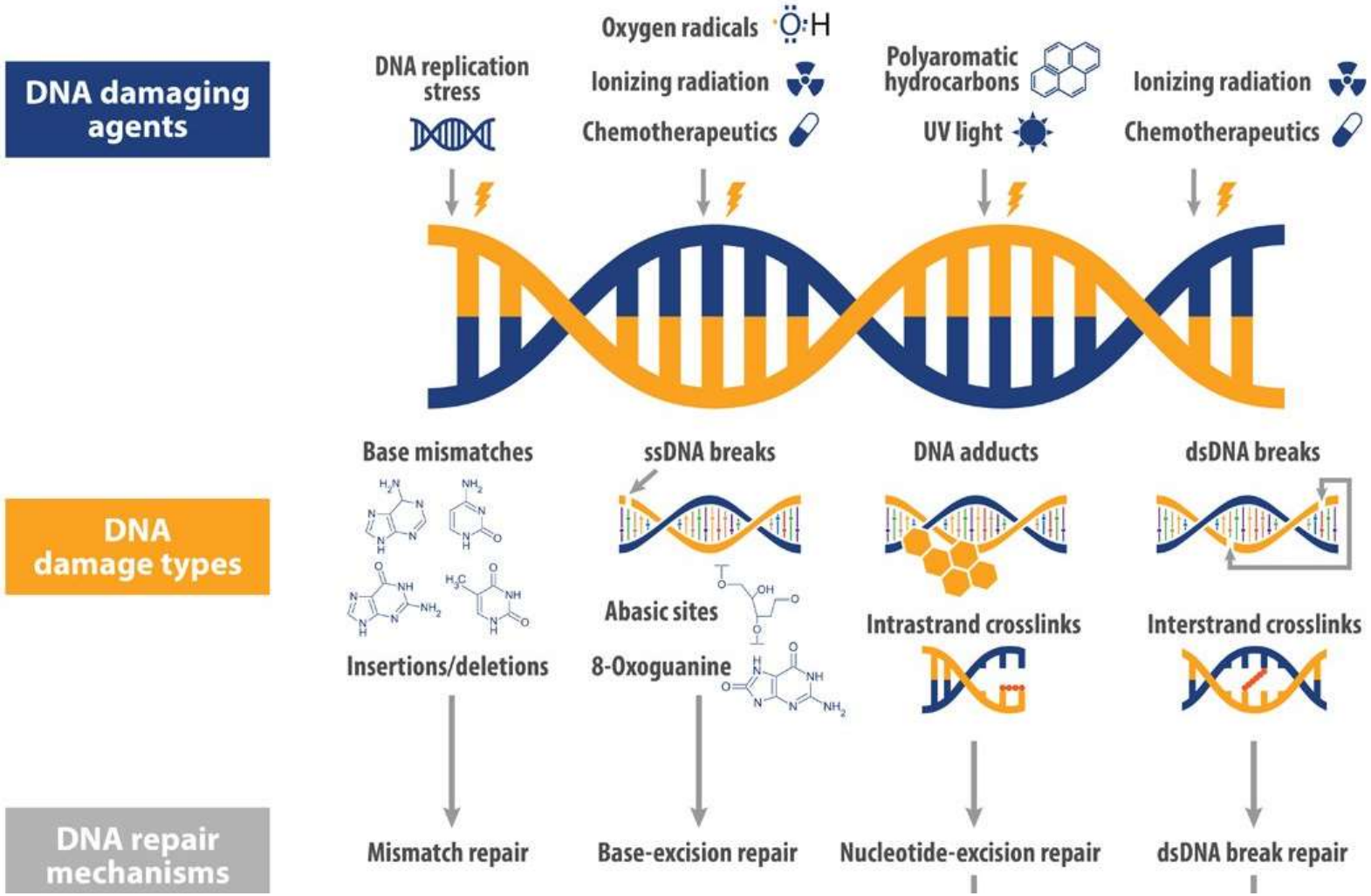


Because the human diploid genome contains approximately 6×10^9 base pairs of DNA, replication errors introduce less than one new base pair mutation per cell division.



10,000 and 1,000,000 nucleotides are damaged per human cell per day by spontaneous chemical processes such as depurination, demethylation, or deamination; by reaction with chemical mutagens (natural or otherwise) in the environment; and by exposure to ultraviolet or ionizing radiation.

Some but not all of this damage is repaired.



Even if the damage is recognized and excised, the repair machinery may not read the complementary strand accurately and, as a consequence, will create mutations by introducing incorrect bases.

Thus, in contrast to replication-related DNA changes, which are usually corrected through proofreading mechanisms,

nucleotide changes introduced by DNA damage and repair often result in permanent mutations.

Factors influencing mutation rates

- Chromosomal abnormalities are more likely with increasing maternal age due to meiotic arrest

  Down's Syndrome

- Point mutation frequency increases with paternal age due to increased germ-cell divisions

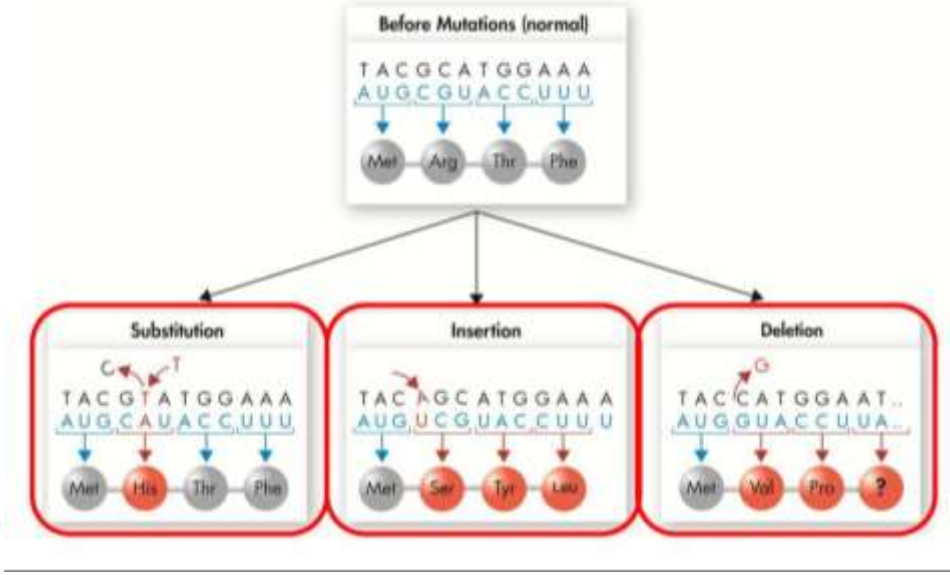
  Achondroplasia: 80% *de novo* – fathers tend to be older

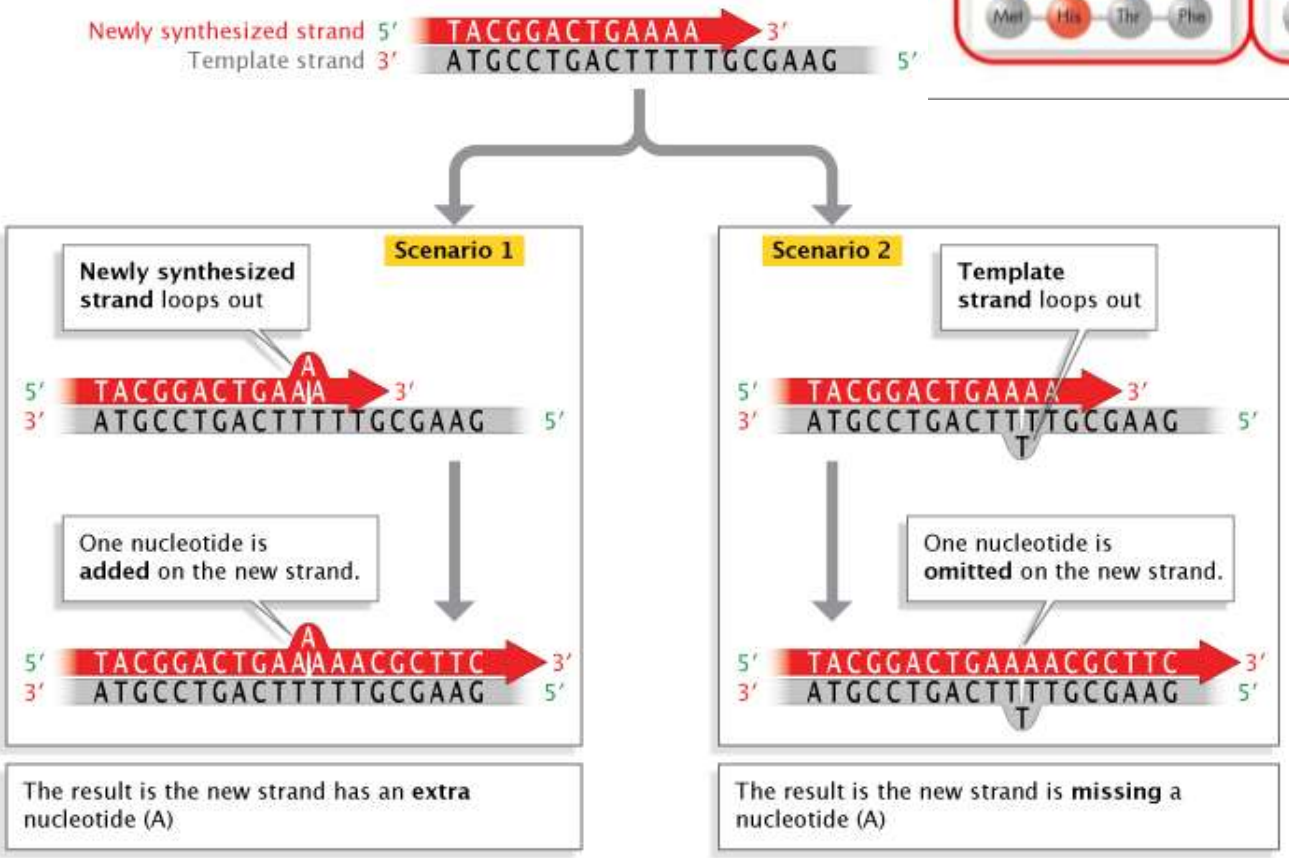
- Mitochondria have much increased mutation rates due to lack of repair systems

Gene Mutations: Point Mutations

A point mutation is a change in a single nucleotide.

There are three types of point mutations:





Gene and Variant nomenclature

Genes: https://www.genenames.org/

HGNC
HUGO Gene Nomenclature Committee

HGVS
HUMAN GENOME VARIATION SOCIETY

THE HUMAN VARIOME PROJECT

HUGO

Variant: https://varnomen.hgvs.org/

Sequence Variant Nomenclature

This site covers **HGVS-nomenclature**, the recommendations for the description of sequence variants. It is used to report and exchange information of variants found in DNA, RNA and protein sequences and serves as an international standard. When using the recommendations please cite: *Den Dunnen et al. 2016, Hum.Mutat. 37:564-569*. HGVS-nomenclature is authorised by the Human Genome Variation Society (HGVS), the Human Variome Project (HVP) and the Human Genome Organization (HUGO).

Reference Sequence Types

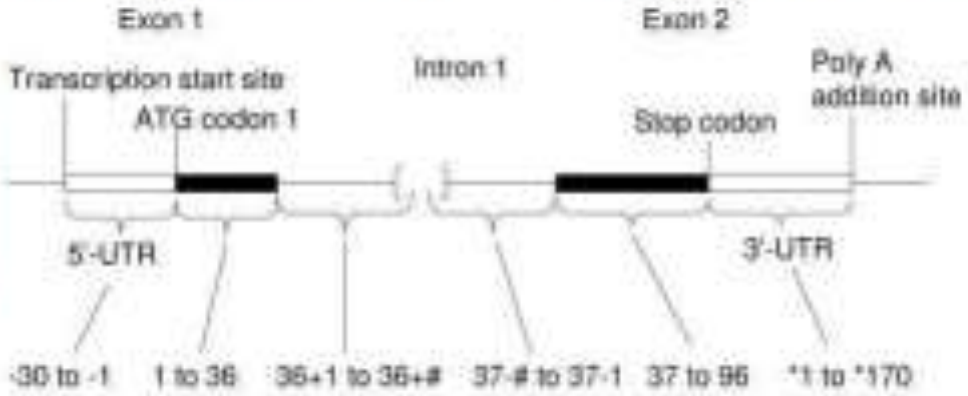
Depending on the variants to be reported, different reference sequence files are used at the DNA, RNA or protein level. It is mandatory to indicate the type of reference sequence file using a **prefix** preceding the variant description. Approved reference sequence types are **c.**, **g.**, **m.**, **n.**, **o.**, **p.** and **r.**:

- DNA
  - **g.** = linear genomic reference sequence
  - **o.** = circular genomic reference sequence
  - **m.** = mitochondrial reference (special case of a circular genomic reference sequence)
  - **c.** = coding DNA reference sequence (based on a protein coding transcript)
  - **n.** = non-coding DNA reference sequence (based on a transcript not coding for a protein)

Variant nomenclature: cDNA

- Nucleotide 1 is the A of the ATG initiation codon
- The nucleotide 5' of the ATG initiation codon is -1, the previous -2, etc.
- The nucleotide 3' of the stop codon is *1, the next *2, etc.
- Intronic nucleotides
  - **5' end of the intron**: the number of the last nucleotide of the preceding exon, a plus sign and the position in the intron, e.g., c.36+1G, c. 36+2T, etc.
  - **3' end of the intron**: the number of the first nucleotide of the following exon, a minus sign and the position upstream in the intron, e.g., c. 37-1G, c.37-2A

**Dividing the nucleotide number by 3 gives the number of the amino acid residue affected, in the example amino acid 26 (*36/3 = 12*)

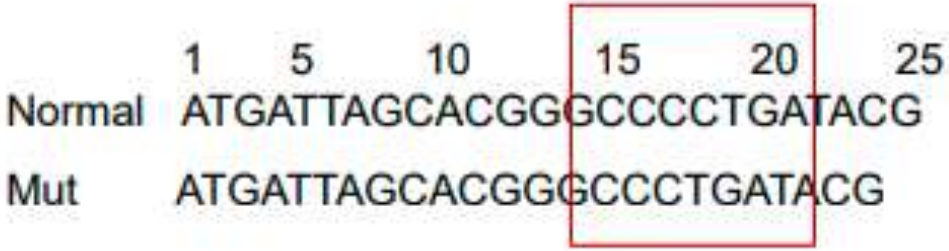


# indicates any positive integer number

Ogino et al. (2007), *JMD*

Symbols for specific variation types

- ">" indicates a **substitution** at DNA level: c.76A>T
- "_" (underscore) indicates a **range** of affected residues, separating the first and last residue affected: c.76_78delACT
- "dup" indicates a **duplication**: c.90_92dupACC
- "del" indicates a **deletion**: c.127delA
- "ins" indicates a **insertion**: c.76_77insG
- "delins" indications a **deletion and insertion:** c.56_58delinsCATG
  - ***For all descriptions the **most 3' position** possible is arbitrarily assigned to have been changed



We cannot know which C is deleted, so assign the most 3' position (c.18delC)

Variant nomenclature: Protein

- 3-letter amino acid code is preferred to describe the amino acid residues (Lys vs. K for lysine)
- For all descriptions the **most C-terminal position possible** is arbitrarily assigned to have been changed
- Methionine encoded by the translation initiation site (*start codon*) is numbered as residue 1 ("**Met1**" or "**M1**")
- "**Ter**" or "*****" designating a translation termination codon

Variant nomenclature: Protein

- **Silent changes:** p.Leu54Leu or p.=
- **Substitutions:** p.Trp26Cys
- **Nonsense variant:** p.Trp26Ter or p.Trp26*
- **No-stop change:** p.Ter110GlnextTer17 or p.*110Glnext*17
- **In-frame deletions:** p.Gln8del or p.Cys28_Met30del
- **Duplications:** p.Gly4_Gln6dup
- **Insertions:** p.Lys2_Met3insGlnSerLys
- **Frameshifts:** short description: p.Arg97fs

  long description: p.Arg97Profs*23

where the "Arg97Pro" describes the substitution of Arg for Pro at position 97, "fs" indicating the frameshift and the "*23" describes the position of the translational termination (stop) codon in the new reading frame (starting with proline as amino acid #1)

Major types of gene mutations: definitions

Silent (synonymous) – does not result in amino acid change

Missense (nonsynonymous) – changes a codon specific for one amino acid to specify another amino acid

Deletion – loss of DNA, single bp to kb

Duplication – gain of DNA, single bp to kb

Nonsense – single base substitution resulting in a stop codon

Frameshift – involves a deletion, insertion, or indel that changes the reading frame (and usually leads to a premature stop codon)

Splice site – typically affect splice donor or acceptor

Regulatory mutations – affect promoter, enhancer or UTR

Dynamic mutations –amplification of repeat sequences (Fragile X, Huntington's)