Changing the Genetic Message
Although the processes of DNA replication and RNA transcription are remarkable in their fidelity, sometimes mistakes are made that alter the nucleotide sequence. Each chromosome has a distinct pattern, size and shape. Each gene is a precise sequence of DNA. Anything that affects the structure of a chromosome or a region of DNA, the nucleotide sequence of DNA, the number of chromosomes typical for a species, or that affects the ability of DNA to be transcribed accurately, is known as a mutation. More simply said, a mutation is a change in the nucleotide sequence of the DNA. Physical damage and chemical damage can also induce mutations, and are used by researchers to study mutations.

Mutation is an important source of variation among individuals in populations, particularly those that reproduce asexually. The rate of mutation is highly variable, and depends in part of the ability of repair enzymes such as DNA polymerase and DNA ligase to fix mistakes, as mentioned earlier. Some genes mutate at much greater rates than others. Mutations can be passed from cell to cell by mitosis and from generation to generation. Mutations that occur in gamete formation (the germ-line cells) will be passed on to subsequent generations. Mutations in the somatic (or body cells) are not passed on to subsequent generations, but can have dramatic effects on the individual in whom they occur, particularly if they occur during development. We will begin our discussion with chromosome mutations.

Phenotypic Effects of Mutation
The phenotypic effects of mutation are also variable, and not surprisingly, are also defined. Mutations may enhance cell activity, be neutral or cause the cell to not function properly.

A silent mutation is one that does not affect the function of the gene product, which is typically the protein for which the gene codes. Mutations may occur in non-coding portions of the DNA (DNA organization is discussed in our genome section), or can be in a coded region that does not impact the gene product function.

A mutation that results in loss of function will affect the gene product's function. Structural proteins or enzymes may not function. In diploid organisms, as discussed, a mutation may occur in one allele, but the alternative allele codes correctly and enough gene product is produced for the cell to function. Many of our recessive alleles are the result of loss of function mutations.

A mutation that results in gain of function will enhance the gene product's function. Such mutations will typically be expressed as phenotypic dominant alleles.

Conditional mutations affect the phenotypic expression only under certain conditions. Genes controlled by environmental conditions, such as temperature-sensitive genes can be the result of mutations that result in an unstable functional protein in certain environmental conditions.
Mutation and Gene Alteration

Phenotypic Effects of Mutations

Mutation Categories
We further divide mutations into molecular categories. Changes that involve entire chromosomes or groups of genes are known as chromosome mutations. Mutations involving a portion of a single gene or nucleotides are known as point mutations. We shall discuss a number of examples of both chromosomal mutations and point mutations in this section.

Chromosome Mutations
A chromosome mutation will involve whole chromosomes or portions of chromosomes. Chromosome mutations involving whole chromosomes include nondisjunction and polyploidy. Mutations involving groups of genes including deletions, duplications, and gene transfers such as inversions and translocations are also considered chromosome mutations.

Nondisjunction Chromosome Mutations
In earlier discussions of meiosis and inheritance we mentioned aneuploidy, a chromosome number that differs from "normal" as a result of a meiotic nondisjunction, and results in either one more than normal (trisomy) or one less than normal (Monosomy) chromosome number in the affected individual. One cause of non-disjunctions may be a malfunction of the cohesins that normally breakdown in division.

Non-disjunctions are fairly common in human zygotes, in the range of 10 – 30%, but few develop. About 20% of miscarriages in the first two months of pregnancy have non-disjunctions. Most often, a nondisjunction results in a gamete that does not survive; in some cases, however, gametes and zygotes do survive, producing individuals with atypical chromosome numbers. Most of these nondisjunctions have genetic consequences.

A nondisjunction can affect either the sex-determining chromosomes or autosomes. In addition, translocations, which are discussed in a bit, may have the same impact on the phenotype as a non-disjunction because we have additional gene information on the translocation chromosome.
Autosome Nondisjunction

Survival with an autosomal nondisjunction is rare; embryos with monosomies or trisomies generally do not survive. There are few exceptions, and survival with an autosomal monosomy is unknown.

The most common human nondisjunction is Trisomy 21, or Down Syndrome. As many as 1 in 20 eggs produced after the age of 40 may have this nondisjunction. Older fathers are also more likely to produce sperm with two number 21 chromosomes.

Down syndrome characteristics may include poor muscle tone, including cardiac muscle, jaw and tongue proportion (which affects speech), possible mental retardation and a weakened immune system.

Trisomy of the four other smallest chromosomes, numbers 13, 15, 18 and 22, also occur, but the developmental problems are so severe that children rarely survive past early childhood.
Sex Chromosome Nondisjunction
There are a number of human sex chromosome nondisjunctions, both monosomy and trisomy.

Sex-Chromosome Nondisjunction Effects

Monosomy X0
- Turner syndrome
- Symptoms include absence of secondary sexual development and sterility, but when treated with estrogen replacement drugs develop some secondary sexual characteristics.
- Turner syndrome is more often the result of nondisjunction in sperm formation (75%) than in egg formation.
- They do not produce Barr bodies.

Monosomy Y0
- Lethal in embryonic development

Trisomy XXX (female)
- No detectable problems.
- Females are usually fertile and bear normal XX or XY children.
- XXX females likely produce two Barr bodies.

Trisomy XXY (male) (and other multiples with both X and Y, except XYY)
- Kleinfelter syndrome
- Mixed secondary sexual development at puberty and low sperm production leading to sterility. The testes and prostate gland are smaller than normal.
- Generally taller and heavier than average males.
- Two-thirds of the extra Xs come from the egg
- The extra "Xs" appear to form Barr bodies.

Trisomy XYY (male)
- Jacob Syndrome
- Generally fertile
- Increased vertical stature
Polyploidy
Polyploidy is defined as an increase in the number of sets of chromosomes, usually resulting from the formation of diploid gametes (from complete non-disjunction).

- If a diploid gamete unites with a normal haploid gamete, the triploid hybrid is sterile (no homologous matches at meiosis).
- If both gametes are diploid, the individuals are often fertile.
- Polyploidy is used extensively in developing agricultural plant varieties, because of their increased hybrid vigor.

Polyploidy occurs naturally in many plants and may produce larger, hardier individuals. Although less common in animals, a naturally occurring tetraploid rat is found in Argentina. Plants we consume are often hybrid varieties selected for their enhanced productivity. Agricultural companies must also grow the parental stock for each year's seed sources that farmers plant. Polyploid hybrids are particularly productive. In some cases polyploids are fertile and form instant new species, since they cannot cross back to their parents. Wheat grown today is a polyploid composite of several ancestral species.

Ongoing research with triploid animals, and in particular, fish, is perceived as beneficial, particularly with genetically modified fish, because the modified animals cannot breed with "natural" stock.

In addition to nondisjunctions and aneuploidy, a number of changes can occur within individual chromosomes altering their structure and the ability to read the genetic instructions correctly.
**Mutations Involving Structurally Altered Chromosomes**

**Deletions**

A chromosomal deletion is a loss of a portion of the chromosome (generally a fragment without a centromere). The remainder of the chromosome, with the centromere intact, contains the deletion. Many deletions cause serious problems and can be lethal, since critical genes are missing. A deletion in a non-coding region of DNA may have no impact in somatic cells, and in some cases, the alternative allele is functional. Pairing of homologous chromosomes may be affected when deletions are present in germ-cell lines.

- A chromosome deletion on chromosome 5 causes the Cri du Chat syndrome, characterized by a small head and unusual facial features and respiratory problems, along with mental impairment. Most born with this deletion die in childhood.
- A variant of Down Syndrome is caused by a translocation, which is unrelated to the parents' age.
- Some phenotypic women are genetically XY but exhibit no male traits because the sex-determining region of the Y chromosome (SRY) is missing (a chromosomal deletion mutation).

**Duplications**

Duplications of portions of chromosomes also occur. A duplication within a chromosome may or may not have an effect depending on where the duplication occurs. If the duplication aligns adjacent to the original gene sequence, it is called a tandem duplication. Tandem repeats are common in our DNA as we shall discuss later and are important in related gene families. In some genes, a segment of the gene or chromosome undergoes multiple repetitions, so that several copies are located on the chromosome.

Deletions and duplications occur more frequently in meiosis, particularly with crossing over and recombination. Non-sister chromatids don't do an even exchange so one gets more genes and the second fewer. This is called a non-reciprocal crossover.

**Gene Transfers – Changes in Gene Position**

We have discussed how recombination between homologous chromosomes changes the specific alleles on a given chromosome and increases variation in populations. Recombination can also involve gene transfer. Gene transfer is when genes from one chromosome (or even an organism) are transferred to a different chromosome or rearranged within a chromosome. There are two common gene transfers: inversions and translocations (or transpositions).
Gene Inversions
For an inversion, a small group of genes can have their order reversed on the chromosome so that a gene sequence that should be \(A-B-C-D-E-F-G-H\) is changed to \(A-B-F-E-D-C-G-H\) instead. This change in sequence is effectively the reverse of the original DNA sequence, and if transcribed, results in a different amino acid sequence and non-functional protein.

During meiosis, inversions may not pair properly and form uneven, internal loops that cannot do crossing-over without losing genes or duplicating genes on chromatids. Many gametes are not viable after inversions.

Gene Translocation
A gene can be transposed or translocated (moved) to a different location along the gene, so that the sequence might read \(A-B-C-E-F-G-D\) rather than \(A-B-C-D-E-F-G\). Since many genes are read in sequence, altering the sequence may affect the ability to read a gene. Translocation can also involve transferring a part of a chromosome to a different, non-homologous chromosome.

Reciprocal translocations involve exchanging genes between non-homologous chromosomes. Some XX genotypes have a SRY (sex-determining region of the Y chromosome) translocation. These individuals are phenotypically male.

The reciprocal translocation of a piece of the human #22 chromosome to the #9 chromosome causes a form of leukemia (chronic myelogenous leukemia) when it occurs in the formation of some white blood cells because it interferes with a gene that controls cell division. This abnormal chromosome is called the Philadelphia chromosome, from the city in which the researchers who discovered this abnormality lived.
Recombination, Unequal Crossing Over and Mismatches

Crossing over during prophase I of meiosis isn’t always reciprocal. When multiple copies of a nucleotide sequence (see duplications above) exist near each other on a chromosome, mistakes are more likely to happen during synapsis. One nucleotide sequence may align with one of the duplicate copy sequences putting the homologous chromosomes out of alignment. This can result in mis-pairing that can lead to deletions, duplications and/or frameshift mutations (change in reading the DNA nucleotide triplet instructions). Chromosomes of different lengths can result; some with many repeats of certain nucleotide sequences, and some much shorter, and missing genes.

In addition transposons, DNA sequences that move routinely from chromosome to chromosome affect gene organization, but aren’t really considered mutations. Transposons may also copy themselves and move the copies to new locations scattered throughout the eukaryotic chromosome. Transposons are as likely to insert in the middle of a gene as not, and when they do, they may cause the gene to be unreadable, hence, inactivating the gene. This is called insertional inactivation. (We shall discuss transposons later.)

Gene Pair Differences at Synapsis

Since homologous chromosomes are not identical, not all gene pairs in synapsis are complementary. Alteration of gene pairs at the crossover points is another source of gene change. Mismatched pairs (non-complements) will be "fixed" by DNA’s correcting enzymes, but the manner in which the mismatch is decided is variable so the "corrected nucleotide pairs" on one of the homologues may differ from its original DNA but will be identical to its homologue. This is called gene conversion. Gene conversion makes homologous chromosomes more similar to each other.
Point Mutations – Mutations Within a Single Gene

A mutation that affects a single nucleotide, or just a few nucleotides, is known as a point mutation. Point mutations are identified by type: substitution, deletion or insertion. Deletions and insertions are also referred to as frameshift mutations if they alter the reading sequence of the nucleotide triplets.

Point mutations are also "classified" by the impact of the mutation: silent, nonsense or missense. Point mutations affect just the gene in which they occur, and most often affect protein structure and function.

- A point mutation in a non-coding region of DNA is a silent mutation since there is no impact on the cell. Point mutations that occur in coding DNA may still code for functional proteins. In somatic cells, a point mutation may have no impact if the alternative allele is functional.
- Missense mutations affect coding genes and alter their gene product (protein) function.
- Nonsense mutations result in a stop codon, terminating the transcribed DNA, so that a non-functional truncated protein is formed.

Base Substitutions

In a base substitution mutation, a single base pair is incorrectly matched, so that an adenine will bond to cytosine or guanine, rather than to thymine. The DNA correcting enzymes may find the incorrectly matched pair, but might make the wrong correction, so that a different base pair results. This affects the "reading" of the gene, and may result in DNA instructions that cannot be followed.

- In many cases, DNA with a base substitution will code with no problem, other than a substitute amino acid may be found in the polypeptide, resulting in a silent mutation and no impact on the cell or organism.
  - If the substitution occurs in the third nucleotide of a redundant DNA triplet, there will be no impact on the cell or the organism; the correct amino acid will still be coded.
  - If the substitute amino acid is in a non-functional part of the protein, it will also not matter.
• If the substitution (or any point mutation) results in coding for one of the stop codons, transcription will be halted at that point, and no protein can be synthesized resulting in a **nonsense mutation**.

![Nonsense mutation diagram](image)

• A base substitution may code for a protein with an altered amino acid sequence that can have a dramatic impact, either negative, such as is the case with abnormal hemoglobin, or it can result in a protein with an enhanced function. Such mutations are **missense mutations**.

![Missense mutation diagram](image)

Some proto-oncogenes (genes that can become oncogenes or cancer-promoting genes) become oncogenes through missense mutations that change the gene product’s activity. For example, p53 mutation results in a protein that no longer inhibits damaged DNA from being duplicated. This results in enhanced cancer-promoting activity.

The DNA for Hemoglobin normally codes for glutamic acid as the #6 amino acid of the 146-amino acid β-globin polypeptide. A common abnormal hemoglobin codes for valine in this position. The difference is Normal DNA code = CTT and Abnormal code = CAT. The result of this base substitution is the genetic disorder sickle cell anemia. Of note, this is just one of 700 mutations in the two globin genes (α and β)
Frameshift Mutations

Mutations that change the reading of the triplet code by inserting (insertions) or deleting (deletions) one or two nucleotides are known as frameshift mutations. Generally, the amino acids coded for after the point of deletion or insertion are different from those intended, resulting in a missense mutation. If the frameshift results in stop code, the mutation is a nonsense mutation. Some three-nucleotide insertions or deletions may not have an impact on the protein being synthesized.
Nucleotide Repeat (Expanding Triplet Repeat) Sequences
An unusual duplication mutation is the multiple repeats of specific DNA triplets, called trinucleotide repeats (or expanding triplet repeats). They are a type of tandem repeats (see genomics section later) because they occur in sequence along the chromosome and often expand in the number of repeats from generation to generation. The trinucleotide repeat occurs within some abnormal genes, and is responsible for several genetic disorders.

Trinucleotide repeats were first reported in the genetic disease, the fragile X syndrome, a developmental disorder leading to mental retardation. In fragile X, the leader sequence (CGG repeats) is repeated hundreds of times. As the number of CGGs increases, cytosine methylation occurs, inactivating a gene involved in nerve cell translation. Without translation, proteins aren’t synthesized and nerve cells die.

In spinal muscular dystrophy, the repeat is CAG. Huntington's is also related to expanding tandem trinucleotide sequences. In Huntington's, hundreds of CAGs get translated forming a protein with many, many glutamines. Friedreich's ataxia is a trinucleotide repeat of GAA. Trinucleotide repeats can be on any chromosome, not just the X.

Trinucleotide repeat disorders vary in severity by number of repeats and age of onset, becoming more unstable in successive generations, as the number of repeats increases. Repeats may be in a codable exon, in the leader sequence, or in an intron.

Trinucleotide repeats are found throughout DNA beyond those causing genetic problems. The initiating mechanism unknown, although unequal crossing over can lead to repeat sequences. "Diplet" or "tetraplet" repeats are unknown. Interestingly, triplet repeat sequences to date have been found only in mammals.
Promoting Mutations – Spontaneous or Induced
Mutations that result from errors in DNA duplication, crossing-over and recombination, non-disjunction or DNA repair are naturally occurring and said to be spontaneous. Spontaneous mutations can also result from alterations in the chemical structure of the nucleotide nitrogen bases. Spontaneous mutations occur randomly in cells about once in a billion DNA replications. If DNA proofreading or repair fails, the change will be perpetuated.

A common spontaneous mutation is the substitution of a different form of the nitrogen base (a tautomer or base analog)

![Cytosine Tautomer](image)

The tautomer typically pairs with a different nucleotide that results in a mis-matched pair in DNA duplication. The cytosine tautomer, which is the most common of the tautomer mutations, pairs with adenine rather than guanine. The impact can be perpetuated in DNA replication on the mis-matched strand.

Correcting enzymes may fix the tautomer prior to DNA replication, so that the original DNA template with the tautomer will pair correctly. It's the template with the adenine substitution that perpetuates the mutation by replicating a thymine.

Induced mutations, those which involve an outside agent, are used to study the impacts of mutation. Substances that promote mutations are known as mutagens. There are a number of different kinds of mutagens, both physical and chemical; many are used in research.
Chemical mutagen examples include:

- Cytosine deamination, which can occur naturally or be induced, results in substituting uracil in RNA or thymine in DNA rather than cytosine. Cytosine deamination is readily induced by nitrous acid, nitrosamines and similar substances. Nitrites, commonly used to preserve meats, are converted in smooth endoplasmic reticulum to nitrosamines.

![Cytosine Deamination](image)

- Benzopyrene, a combustion by-product, adds chemical groups to guanine so the base is not recognized. DNA polymerase adds a base at random in the modified guanine spot. About 75% of the time, the substitution will not be guanine.

- Aflatoxin, a product of the common fungus, Aspergillus, is converted in ER to a product that alters guanine to an unreadable nucleotide.

- 5-Bromouracil mimics thymine and can substitute for thymine. A 5-bromouracil with a different structure base pairs to guanine rather than adenine. Such chemicals are called base analogs (a different structure that has same function).

![Base Analogs](image)

- Other chemical mutagens cause a nucleotide's nitrogen base to be changed into a new substance. For example, adenine can be converted to hypoxanthine, a nitrogen base that bonds to cytosine rather than to thymine. Other chemicals distort the DNA so it cannot replicate.

![Nitrous Acid Changing Adenine into Hypoxanthine](image)
Radiation Mutations  
Some mutagens, such as radiation, physically damage the DNA by causing structural changes. Hermann Muller noted in the 1920’s that ionizing radiation in X-rays caused mutations in fruit flies. The X-rays fragmented chromosomes. UV radiation also damages chromosomes.

- Ionizing radiation that produces free radicals can cause breaks in the phosphodiester bonds of the DNA helix. If the breaks occur on both sides of the DNA helix, repair enzymes may lack a template nucleotide for repair, and DNA fragmentation occurs. This is unrepairable in prokaryotes, with their single chromosome. Eukaryotes can often repair DNA breaks when homologous chromosomes pair and synapse during meiosis.

- One example of the impact of UV radiation is the thymine dimer, mentioned earlier with DNA repair mechanisms. When two thymine bases are adjacent to each other on a DNA single strand, UV radiation may promote breaking the hydrogen bonds that pair thymine to adenine on its complement strand, and promote a covalent bond between the two thymine pyrimidines instead. This puts a kink in the DNA helix. Generally DNA repair enzymes can find the dimer, and with photolase and other enzymes, catalyze the excision and replacement of the excised nucleotides using the unaffected complement DNA strand template (but not always).

![Image of DNA damage by UV radiation](image)

We also use mutations to our advantage, both for research knowledge and to interfere with pathogen reproduction. By substituting certain nitrogen bases in viral and host DNA we can block the synthesis of viruses, including HIV.

Frequency of Mutation  
The natural instability of the nitrogen bases in DNA leads to mutations. DNA sequencing has helped researchers locate certain nucleotide sequences that are more prone to mutation than others. Such frequent mutation sequences are known as "hot spots" in the DNA.

Cytosine methylation, in particular, which causes cytosine to lose its amino group in spontaneous or chemically induced mutations, forms thymine, resulting in a DNA mismatch pair of G-T. During DNA replication, one of the daughter chromosomes will have the correct G-C pair, but the second daughter chromosome will have a mutated A-T pair. As mentioned earlier, deaminated cytosine can also produce uracil in RNA.
Adding methyl groups to DNA bases is an effective way to regulate genes, as we will discuss later. Methylated DNA can't be transcribed.

**Reviewing the Effects of Mutations**

A mutation may have no effect if a point mutation occurs at a place in the DNA coding that is redundant or if the mutation codes for an amino acid that doesn't change the gene product's function. Substituting an amino acid that does not alter the shape or the chemical nature of the protein can still produce a functional protein.

In diploid organisms, many mutations in somatic cells are undetected, just as recessive alleles in heterozygotes are not phenotypically detected. A mutation that is expressed is one in which the protein coded for by that gene will not be synthesized, or, if synthesized, cannot function normally, as we shall explore below. When the alternative allele is not "normal" or codable, some biochemical activity in the affected cell will not occur, or some structural component of the cell will be missing. Depending on the specific activity not occurring, the mutation may or may not prove fatal to the cell, and in some cases, the organism.

In addition, if a mutated cell divides, any cells formed from that cell will perpetuate the mutation. If a mutation occurs in the germ-line cells, when the mutated gamete unites with another gamete, all cells of the new individual will have the mutation. The effect on the individual depends entirely on the specific mutation. As we will discuss later, mutagens that become cancer promoting (hence carcinogens) are of worldwide health interest.

While we often give examples of DNA alternations that produce harmful effects in the individual, it is by the act of mutation and gene alterations that many **good** variations also arise and can be passed on within populations. Without such variation, populations would not be able to respond to changing environments. Without such changes in the DNA over the millions of years of life on earth, we would not have the remarkable array of proteins, and hence, the remarkable array of life processes we have today. Mutation is truly the starting point of evolution. Without change in DNA that is passed on, there would be no change in populations through time.