1) Restriction
2) Mutagenesis and Repair: The Beauty of Mutations

These processes are essential to the survival of cells. They are also the source of most of the genetic variability in a population of cells or organisms, and along with gene rearrangements, form the basis for evolutionary change.

**Restriction**: One of the most important developments in biochemistry was the discovery of Restriction Endonucleases, enzymes that catalyze the double stranded cleavage of DNA at specific base sequences. This ability to cleave DNA at specific sites is the cornerstone of modern molecular biology.

Bacteria use site-specific DNA methylation to mark their own DNA, and cleave DNA at the same sites to inactivate the DNA of invaders, such as viruses (remember back to our discussion of phage), which lack these methylation patterns.
Studies have shown that different bacterial strains have different methylation patterns. Typical sites of methylation include the N6 position of adenine, the N4 position of cytosine, or the C5 position of cytosine. In addition, only a fraction of the bases are methylated and the sites of methylation occur at very specific sites in the DNA. A characteristic of these sites is that they involve palindromic DNA sequences (sequences that are identical when read from either DNA strand).

These specific nucleases (restriction enzymes), will not cleave at these specific sequences if the DNA if the DNA is methylated. Thus, this combination of a specific methylase and endonuclease functions as a primative immune system for individual bacterial strains, protecting them from infection by foreign DNA (e.g. viruses).
DNA Repair and Mutagenesis:

A **Mutation** is an inheritable change that occurs in the genome. Chemical changes occur in all biomolecules, due to environmental damage or errors in synthesis. For most of these molecules (RNA, protein, lipids) the effect of these changes is minimized by turnover and replacement of the altered molecules. DNA is distinct in that its informational content must be maintained and transferred. Thus, **DNA has a need for metabolic stability**. There is very good evidence suggesting a link between the accumulation of mutations and the process of aging and the development of cancer.

Genetic stability is accomplished in two ways:

1) **Highly accurate replication**

2) **Mechanisms to correct genetic information when DNA is damaged:** much of the damage can be repaired because genetic information is stored in a redundant fashion (DNA duplex).

**DNA Repair:** If replication of DNA proceeded without proof reading, DNA polymerase would make an error an average of once every 1000 base pairs. This level of mutation would be unacceptable, because too many genes would be rendered non-functional. Organisms have elaborate DNA proofreading and repair mechanisms, which can recognize false base-pairing and DNA damage, and repair it. The actual error rate is closer to one in a million to one in a billion.
Types of Mutations:

Chromosomal Mutations:

Mutations can occur at a fairly **macroscopic level**. Large sections of chromosomes can be altered or shifted, leading to changes in the way the genes on them are expressed.

- **Translocations** involve the interchange of large segments of DNA between two different chromosomes. This can change expression of genes if a gene is at the translocation breakpoint or if it finds itself reattached in a way that it is regulated by a new promoter region, which is not tailored to its expression needs.

  **Inversions** occur when a region of DNA flips its orientation with respect to the rest of the chromosome. This can lead to the same problems as translocations.

  Sometimes large regions of a chromosome are **deleted**. This can lead to a loss of important genes.

  Sometimes chromosomes can lose track of where they are supposed to go in cell division. One of the daughter cells will end up with more or less than its share of DNA. This is called a chromosome **nondisjunction**.
**Point Mutations:**

Point mutations are single base pair changes. What effect can these mutations have on the encoded protein?

A **nonsense mutation** creates a stop codon where none previously existed. This shortens the resulting protein, possibly removing essential regions.

A **missense mutation** changes the code of the mRNA and alters the information that is encoded by the nucleotide sequence. If an AGU codon is changed to an AGA codon, the protein will have an arginine where a serine was meant to go. This might alter the shape or properties of the protein.

A **silent mutation** has no effect on protein sequence. If an AGU were changed to an AGC, the protein would still have the appropriate serine at that position.

Within a gene, small deletions or insertions of a number of bases not divisible by 3 will result in a frame shift. For example, given the coding sequence:

```
AGA UCG ACG UUA AGC
```

The insertion of a C-G base pair between bases 6 and 7 would result in the following new coding sequence, which would result in a non-functional protein. Every amino acid after the insertion will be wrong.

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AGA UCG CAC GUU AAG C
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arginine - serine - **histidine** - valine - lysine

The frame shift might even generate a stop codon that would prematurely end protein synthesis (nonsense). Remember these examples when we discuss translation.
**Chemical Modification of the Bases:**

**Deamination:** Loss of exocyclic amino group.

![Chemical Modification of the Bases Diagram](image)
Cytosine --to-- Uracil

Deamination of C (in DNA) to U will occur ~1/107 cytosines/24 hr. This is ~ 100 spontaneous events/day/cell. U can pair with A and cause to a GC -to- AT transition.
UV radiation: Can cause adjacent pyrimidine bases to form cyclobutane dimers: Thymine dimers.
**Excision Repair:** In this process a damaged section of DNA is cut out and then a DNA polymerase and DNA ligase are used to regenerate a closed duplex at the site of the original damage.

In bacteria a three subunit enzyme recognizes the structural distortion caused by the thymine dimer and cleaves the damaged strand at two sites surrounding the damaged site. This enzymatic activity is not classical because it cleaves at two distinct sites and is called an **Exinuclease**.

Excision repair occurs in mammalian cells. A rare genetic disease called **xeroderma pigmentosa (XP)** involves mutational loss of this repair system and causes patients with the disease to be extremely sensitive to sunlight and prone to skin cancer.
Mismatch Repair:

Most non-Watson/Crick base pairs are removed by 3' exonucleolytic proofreading during strand replication. If DNA polymerase introduces an incorrect nucleotide, the fully replicated DNA will contain an error. Mismatch repair allows the removal of inappropriate but "normal" (the bases themselves are not chemically altered, just in the wrong basepair).
MutS and MutL bind to the site of the mismatch.

MutH binds to a hemimethylated site.

DNA is reeled in through MutS, creating a large loop of DNA.

A MutS-MutL-MutH complex forms. The hemimethylated site is now adjacent to the mismatch.
In bacteria these mismatches can be removed by an enzyme system that looks for mismatched bases, and single-base insertions or deletions on newly made DNA. When a mismatch is found the region is cut out and replaced. To determine which strand is the "new" one, the enzymes looks for the sequence -GATC- which is not methylated. These sequences are rapidly methylated on A residues shortly after synthesis. Once the methylation system has worked on all the GATC sequences, the chance to correct the mistake is lost forever.

A very common form of inheritable disorder called hereditary nonpolyposis colorectal cancer (HPCC, Lynch syndrome) affecting approximately 1 in 200 people results from mutations which inactive the human counterparts (MutS and/or MutL) of the Mismatch Repair System in bacteria. These mutations cause a hereditary predisposition to cancer.
Why are mutations beautiful?

Our environment is constantly changing, as ecosystems change. We must change along with them, or we as a species will become obsolete and die. One mechanism of change is at the DNA level. Mutations can often result in beneficial genes and functions, which enable an organism to adapt to a changing world. Thus, an organism with perfect DNA replication would almost certainly be doomed to extinction.

However, most mutations are deleterious, and cause many of the genetic diseases that we are discovering using modern biochemical techniques. These changes may lead to defective behavior in the affected somatic cells in the individual (e.g. cancer) or an inherited disease (if germ cells (egg or sperm) are affected). It is therefore important that while an organism (particularly one as complex as mammals) balance the need for genetic variability against the problems associated with too many mutations.