**Lec 3: microbial genetics Ass.Prof.Dr.Lamees A.Razzaq**

**DNA Repair:-**

DNA repair is a collection of processes by which a [cell](https://en.wikipedia.org/wiki/Cell_%28biology%29) identifies and corrects damage to the [DNA](https://en.wikipedia.org/wiki/DNA) molecules that encode its [genome](https://en.wikipedia.org/wiki/Genome)

## Agents that Damage DNA

* Certain wavelengths of **radiation**
	+ ionizing radiation such as [gamma rays](http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/R/RadiantEnergy.html) and X-rays
	+ **ultraviolet rays**, especially the UV-C rays (~260 [nm](http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/U/Units.html)) that are absorbed strongly by DNA but also the longer-wavelength UV-B that penetrates the ozone shield.
* Highly-reactive **oxygen radicals** produced during normal cellular respiration as well as by other biochemical pathways.
* Chemicals in the **environment**
	+ many [hydrocarbons](http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/H/Hydrocarbons.html), including some found in cigarette smoke
	+ some plant and microbial products, e.g. the aflatoxins produced in moldy peanuts
* Chemicals used in [**chemotherapy**](http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/D/DNArepair.html#Cancer_Chemotherapy), especially chemotherapy of cancers

## Types of DNA Damage:-

1. All [four of the bases in DNA](http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/B/BasePairing.html) (**A, T, C, G**) can be covalently modified at various positions.
	* One of the most frequent is the loss of an [amino group](http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/G/Groups_5.gif) ("deamination") — resulting, for example, in a **C** being converted to a **U**.
2. **Mismatches** of the normal bases because of a failure of proofreading during [DNA replication](http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/D/DNAReplication.html).
	* Common example: incorporation of the [pyrimidine](http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/Nucleotides.html#purines) **U** (normally found only in RNA) instead of **T**.
3. **Breaks** in the backbone.
	* Can be limited to one of the two strands (a single-stranded break, **SSB**) or
	* on **both strands** (a **d**ouble-**s**tranded **b**reak (**DSB**).
	* Ionizing radiation is a frequent cause, but some chemicals produce breaks as well.
4. **Crosslinks** Covalent linkages can be formed between bases
	* on the same DNA strand ("intrastrand") or
	* on the opposite strand ("interstrand").

Several chemotherapeutic drugs used against cancers crosslink DNA

## Repairing Damaged Bases

Damaged or inappropriate bases can be repaired by several mechanisms:

**1-Direct chemical reversal** of the damage which occurs without breaking of the double helix.

**2- Excision Repair**, in which the damaged base or bases are removed and then replaced with the correct ones in a localized burst of DNA synthesis. There are three modes of excision repair, each of which employs specialized sets of enzymes.

* 1. **Base Excision Repair** (**BER**)
	2. **Nucleotide Excision Repair** (**NER**)
	3. **Mismatch Repair** (**MMR**)
* – Mismatch repair, which eliminates replication errors, some damaged DNA bases, as well as small loops.
* – Base excision repair, initiated by DNA glycosylases which cleave out the damaged base and initiate the synthesis step.
* – Nucleotide excision repair, in which a larger fragment of damaged DNA strand is removed (12-13 nucleotides in E. coli, 24-32 in eukaryote).

**Genetic engineering**:

Genetic engineering, also known as recombinant DNA technology, means altering the genes in a living organism to produce a Genetically Modified Organism (GMO) with a new genotype.

Various kinds of genetic modification are possible: inserting a foreign gene from one species into another, forming a transgenic organism; altering an existing gene so that its product is changed; or changing gene expression so that it is translated more often or not at all.

**Basic steps in genetic engineering:**

1. Isolate the gene
2. Insert it in a host using a vector
3. Produce as many copies of the host as possible
4. Separate and purify the product of the gene

# General outline of genetic engineering

* 1. DNA cleavage
	2. Production of recombinant DNA
	3. Cloning of the recombinant DNA
	4. Screening clones

# DNA cleavage and Production of Recombinant DNA

* 1. restriction enzymes and making recombinant DNA
		1. restriction enzymes are used to cut up DNA of interest and a “vector” into which you want to place the DNA, making **restriction fragments**
		2. particularly when sticky ends are involved, the target DNA restriction fragment can form base pairs with the vector

\* **Restriction enzymes** (RE) are endonucleases that will recognize specific nucleotide sequences in the DNA and break the DNA chain at those points. A variety of RE have been isolated and are commercially available. Most cut at specific palindromic sites in the DNA (sequence that is the same on both antiparallel DNA strands). These cuts can be a staggered which generate “sticky or overhanging ends” or a blunt

3.DNA ligase is then used to join the DNA strand backbones

# Cloning of recombinant DNA: using vectors

• **Gene Cloning**: isolating genes from one organism, manipulating purified DNA in vitro, and transferring to another organism

**A. cloning** is the process of making many genetically identical cells from cell containing recombinant DNA

1.the gene piece introduced in the recombinant DNA is said to be the DNA that is cloned

2.recombinant DNA is introduced to cells by a vector; the vector is usually maintained in the altered cell line

B. a **vector** is a means of delivering recombinant DNA to an organism

1.vectors must have a way of getting into the host organism (**transformation**)

2.vectors must have some way of being propagated

* + - * some types of vectors remain free but are copied and distributed in cell division
			* some type of vectors have the inserted DNA integrate all or in part with the host DNA

3.vector DNA sequence must be known enough so that restriction sites can be accurately predicted and used

 C. most commonly, vectors are either **plasmids**, viruses, or **yeast artificial chromosomes** (**YACs**)

# Screening

A. Often many clones are made with various DNA pieces inserted

* 1. Screening is used to find the DNA of interest; typically:
		1. a selectable marker is used to ensure that the vector is present
		2. a second type of selectable marker is tested to ensure that the vector contains inserted DNA (that is, make sure it is recombinant DNA)
		3. cells from cell colonies that pass the screens to this point are used as sources for making large numbers of cells; DNA from these cells is then subjected to other treatments to help identify cell lines containing the DNA of interest

### Medicine:-

Genetic engineering has resulted in a series of medical products. The first two commercially prepared products from recombinant DNA technology were insulin and human growth hormone, both of which were cultured in the E. coli bacteria. Since then a plethora of products have appeared on the market, including the following abbreviated list, all made in E. coli:

* **Tumor necrosis factor**. Treatment for certain tumor cells
* **Interleukin-2 (IL-2)**. Cancer treatment, immune deficiency, and HIV infection treatment
* **Prourokinase**. Treatment for heart attacks
* **Taxol**. Treatment for ovarian cancer
* **Interferon**. Treatment for cancer and viral infections

 In addition, a number of *vaccines* are now commercially prepared from recombinant hosts. At one time vaccines were made by denaturing the disease and then injecting it into humans with the hope that it would activate their immune system to fight future intrusions by that invader. Unfortunately, the patient sometimes still ended up with the disease.

 With DNA technology, only the identifiable outside shell of the microorganism is needed, copied, and injected into a harmless host to create the vaccine. This method is likely to be much safer because the actual disease-causing microbe is not transferred to the host. The immune system is activated by specific proteins on the surface of the microorganism. DNA technology takes that into account and only utilizes identifying surface features for the vaccine. Currently vaccines for the hepatitis B virus, herpes type 2 viruses, and malaria are in development for trial use in the near future.



**Polymerase chain reaction** (**PCR**):-

 The polymerase chain reaction (PCR) is a technique used in [molecular biology](https://en.wikipedia.org/wiki/Molecular_biology) to [amplify](https://en.wikipedia.org/wiki/DNA_replication) a single copy or a few copies of a piece of [DNA](https://en.wikipedia.org/wiki/DNA) across several orders of magnitude, generating thousands to millions of copies of a particular [DNA sequence](https://en.wikipedia.org/wiki/DNA_sequence). It is an easy and cheap tool to [amplify](https://en.wikipedia.org/wiki/Amplification_%28molecular_biology%29) a focused segment of DNA, useful in the [diagnosis](https://en.wikipedia.org/wiki/Medical_diagnosis) and [monitoring](https://en.wikipedia.org/wiki/Monitoring_%28medicine%29) of genetic diseases, identification of criminals (under the field of forensics), studying the function of targeted segment, etc

 PCR permits early diagnosis of [malignant](https://en.wikipedia.org/wiki/Malignant) diseases such as [leukemia](https://en.wikipedia.org/wiki/Leukemia) and [lymphomas](https://en.wikipedia.org/wiki/Lymphoma), which is currently the highest-developed in cancer research and is already being used routinely. PCR assays can be performed directly on genomic DNA samples to detect translocation-specific malignant cells at a sensitivity that is at least 10,000 fold higher than that of other methods. PCR allows for rapid and highly specific diagnosis of infectious diseases, including those caused by bacteria or viruses. PCR also permits identification of non-cultivatable or slow-growing microorganisms such as [mycobacteria](https://en.wikipedia.org/wiki/Mycobacterium), [anaerobic bacteria](https://en.wikipedia.org/wiki/Anaerobic_organism), or [viruses](https://en.wikipedia.org/wiki/Virus) from [tissue culture](https://en.wikipedia.org/wiki/Tissue_culture) assays and [animal models](https://en.wikipedia.org/wiki/Animal_model). The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes