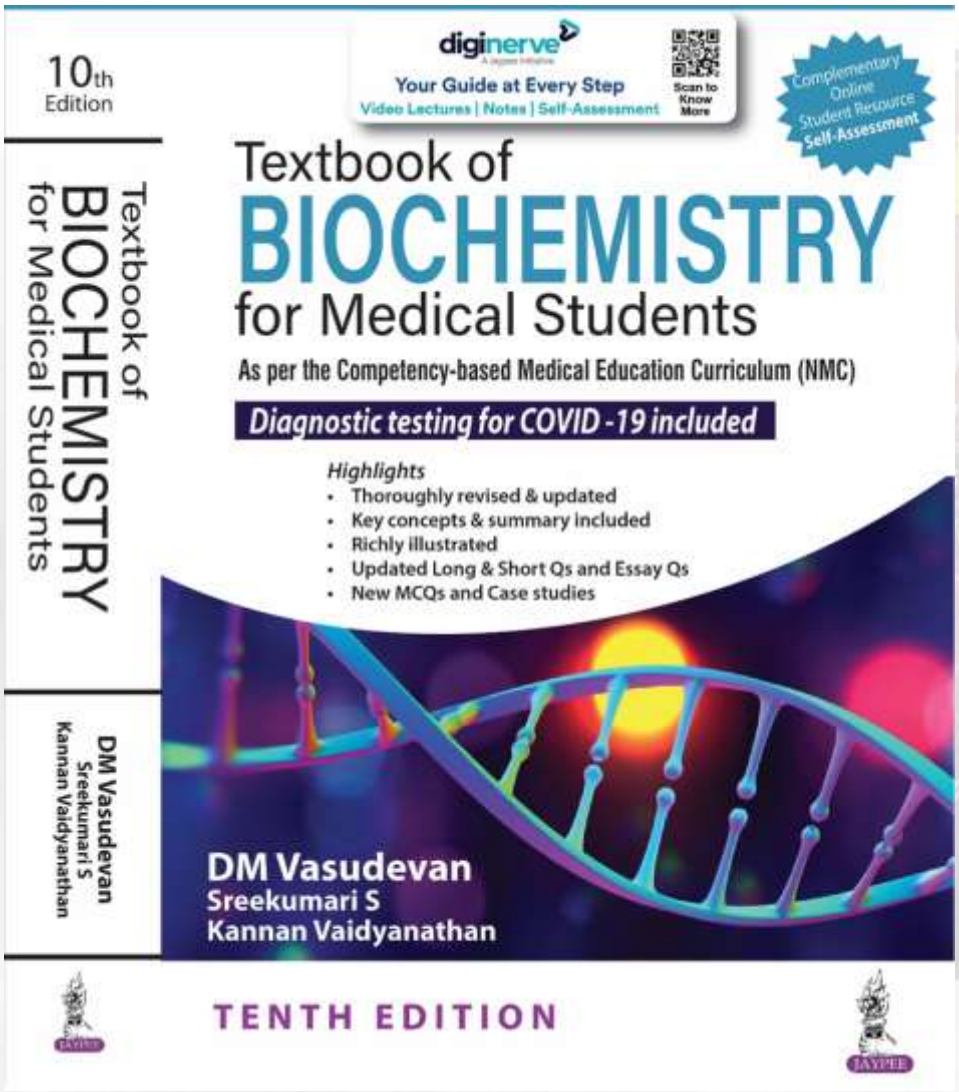


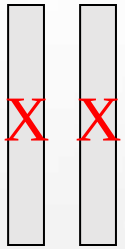
Chapter 36:

Mutations, Cell Cycle and Control of Gene Expression

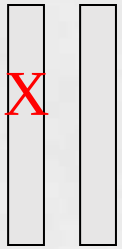
Textbook of
BIOCHEMISTRY
for Medical Students

By DM Vasudevan, *et al.*

TENTH EDITION



When both alleles carry
The same defect, it is
Called HOMOZYGOUS

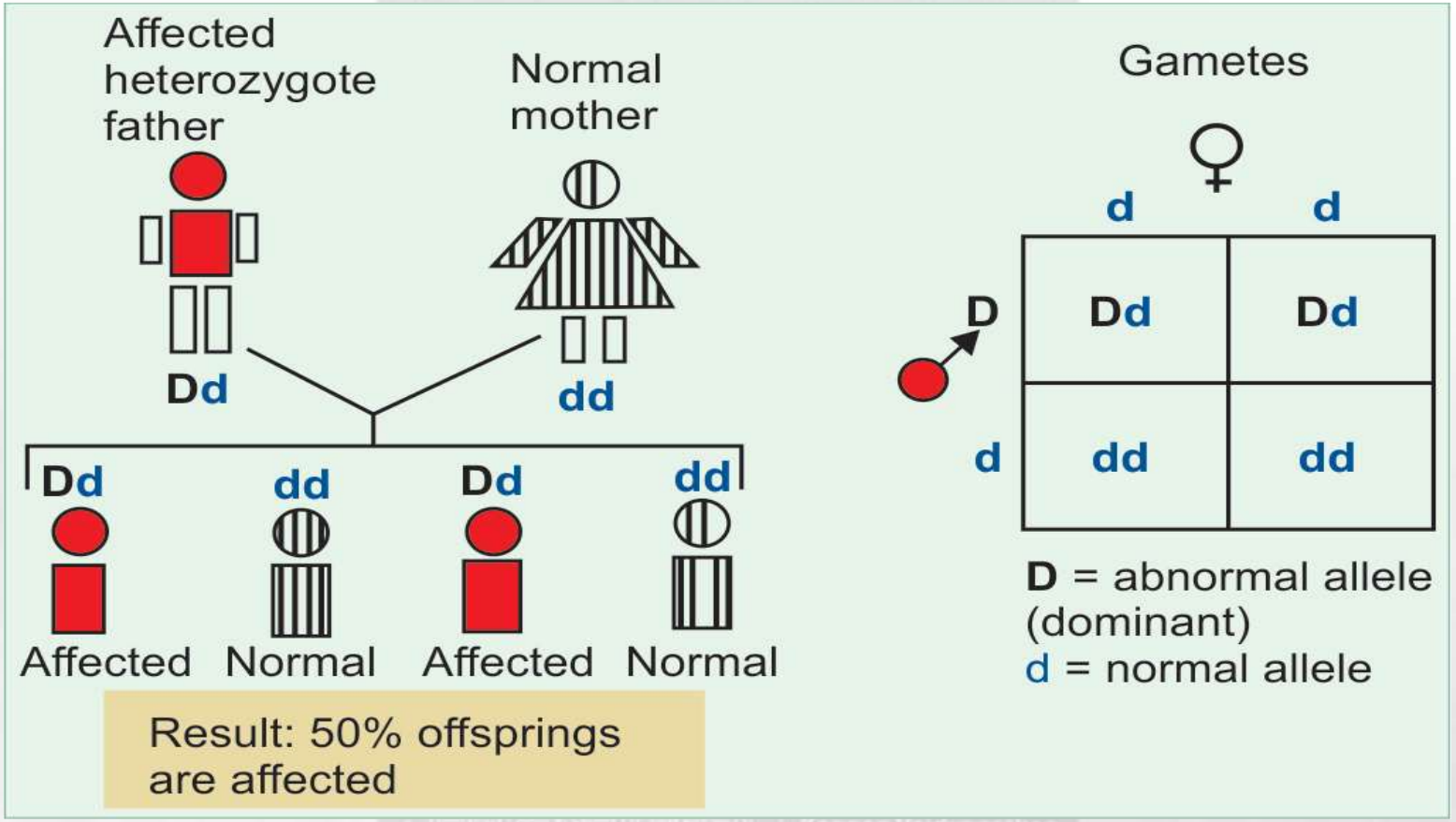


When one gene is normal,
And the counterpart is
Defective, it is called
HETEROZYGOUS

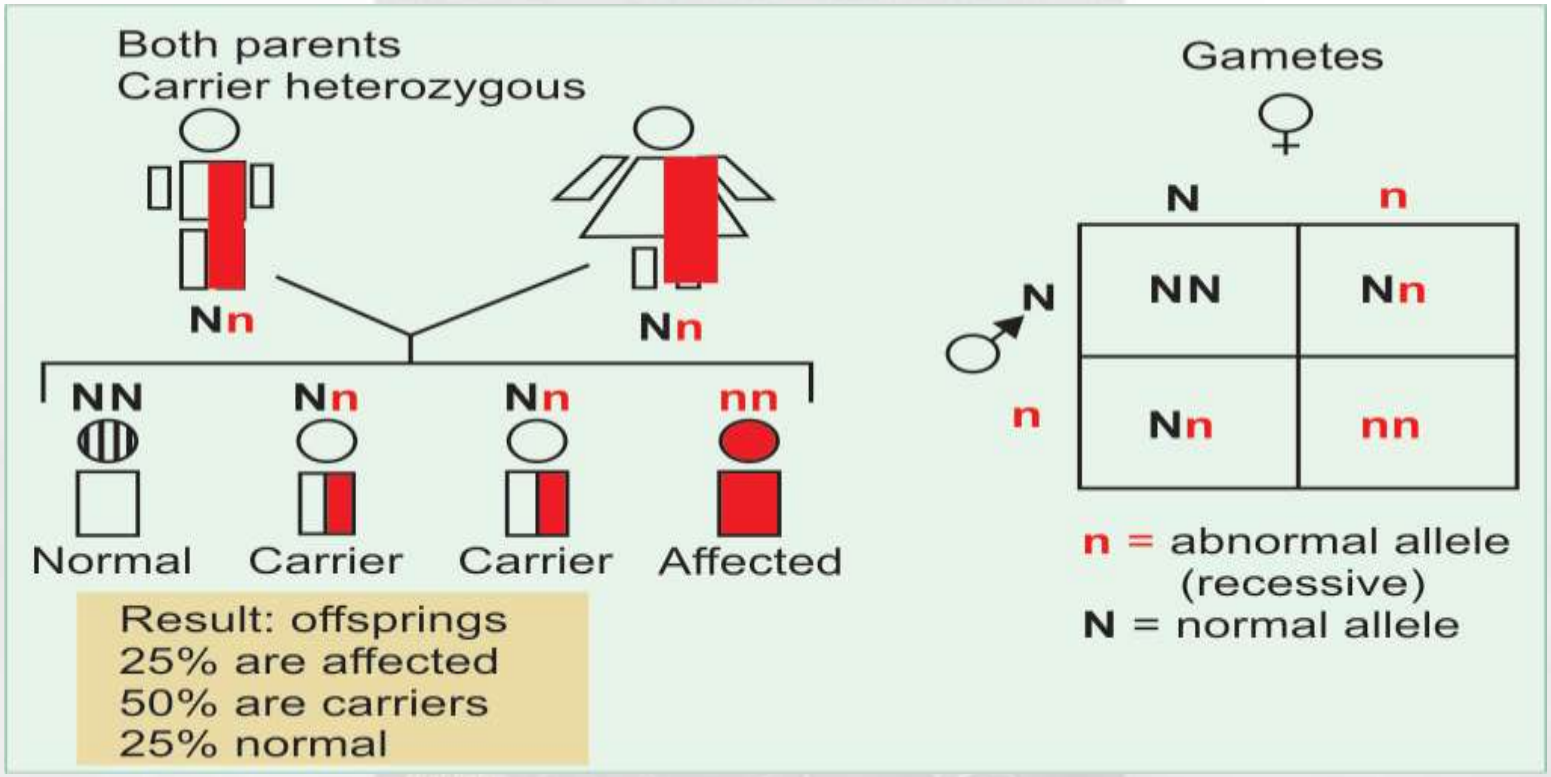
Carrier state
Trait of the disease

Basic Principles of Heredity

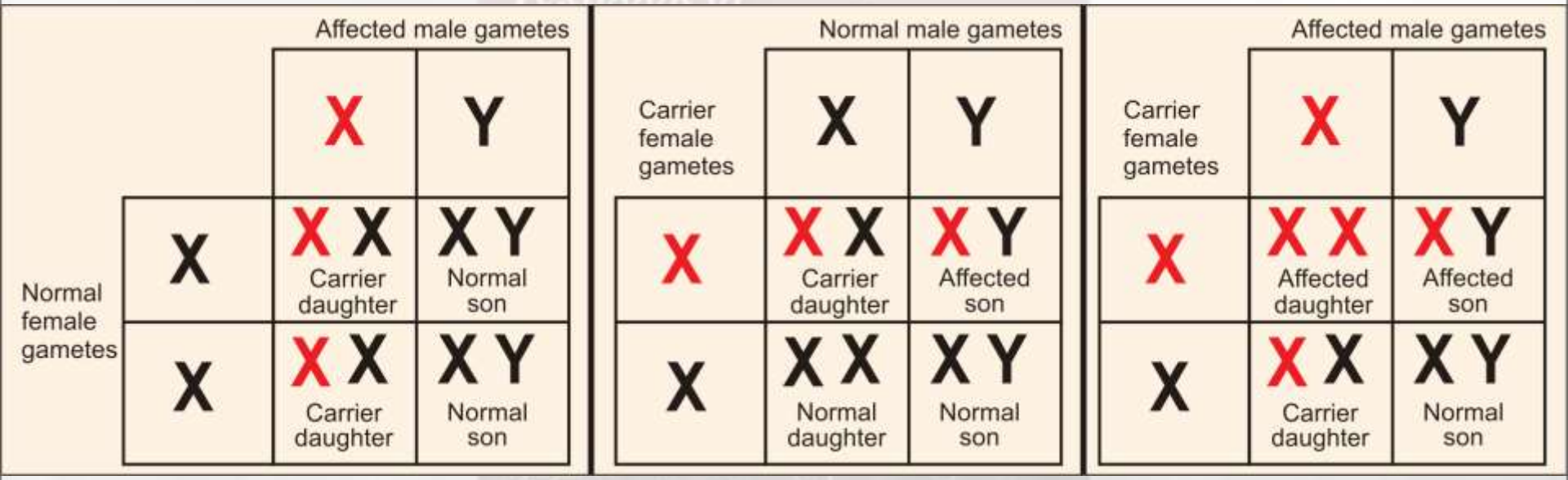
1. Heredity is transmitted from parent to offspring as individual characters.
2. The genes are linearly distributed on chromosomes at fixed positions (**loci**).
3. Genes that may replace one another at the same locus are called allelomorphic genes or **alleles**. Alleles are genes responsible for alternate or contrasting characters. Usually one allele is inherited from father and the other from mother.
4. When both alleles carry the same defect, it is said to be **homozygous**.
5. When one allele is normal, and the counterpart is defective; it is called **heterozygous**.
6. Genes on the same chromosome are **linked**; and the linkages are more pronounced in the nearby genes.
7. The observed character expressed by the gene is called **phenotype.**
8. The **genotype** represents the set pattern of genes present in the cell.



Autosomal dominant inheritance.



Autosomal recessive inheritance.



Sex-linked recessive inheritance

Sex-linked (X-linked) Recessive Inheritance

In the autosomal conditions, the disease occurs in both sexes with equal frequency. But in sex-linked conditions, X-chromosome carries the abnormal gene. In a wedding between a normal male and a carrier female, the probabilities are that one quarter is male with disease; one-quarter is female carrier; one-quarter normal male; and one-quarter normal female.

If an affected male marries a normal female, male children will be normal, but all female children will be carriers, because they all inherit the abnormal X from their father.

Hemophilia, glucose-6-phosphate dehydrogenase deficiency, pseudo-hypertrophic muscular dystrophy (Duchenne type), and red green color blindness are examples of sex-linked recessive inheritance.

Variable Phenotypic Expression and Penetrance

All genetic conditions are not congenital (present at birth). Even dominant characters may not show 100% penetrance in actual practice, so that an affected parent may not have an affected child, or the offspring may carry the mutant gene but will be phenotypically normal.

When the phenotypic expression of a mutant trait occurs only in adult life, it is referred to as age relate penetrance, e.g. triplet expansion defects.

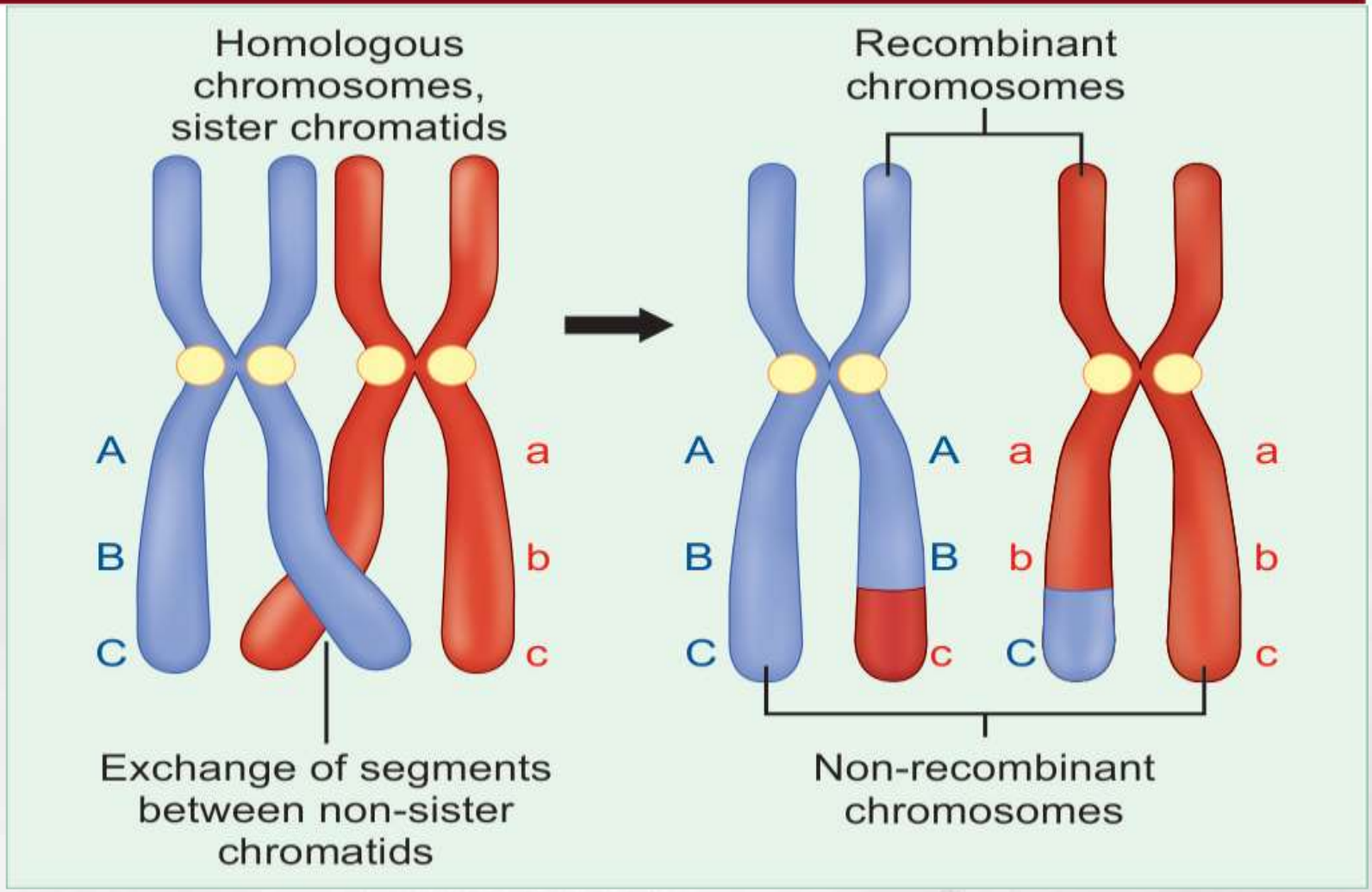
In dominant conditions, variable expression of clinical features may be seen in different family members.

Genetic Disorders

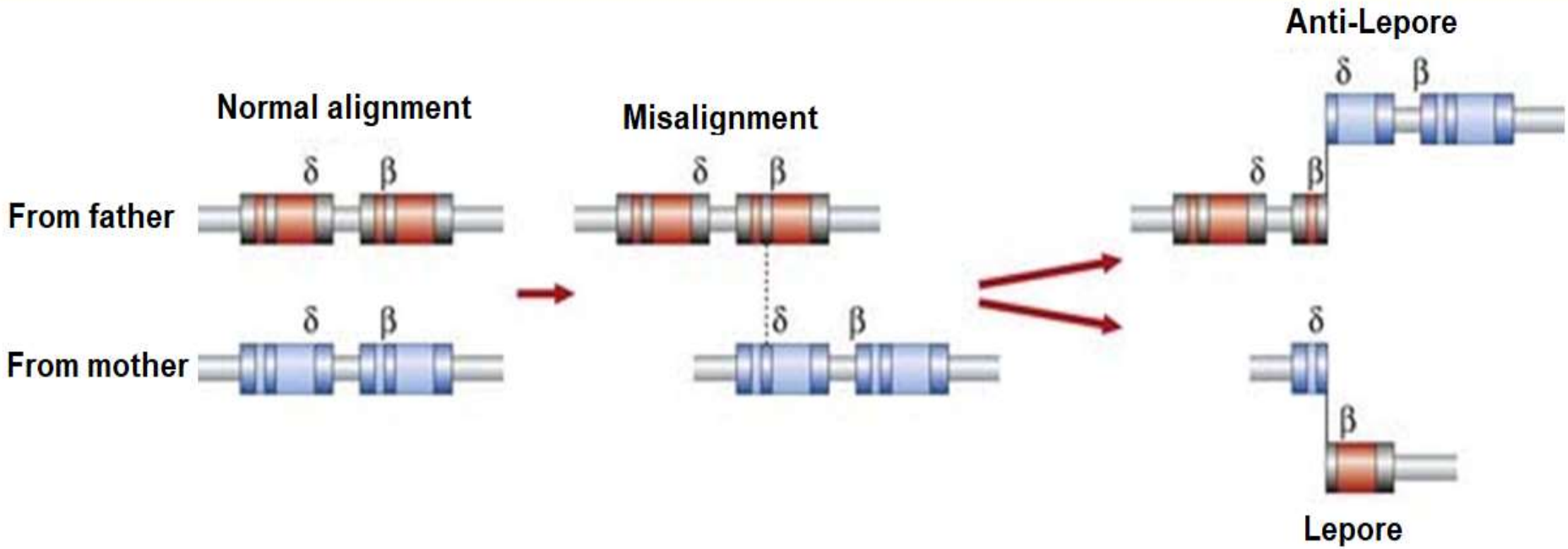
1. Chromosomal disorders Identified by karyotyping Eg, 21 trisomy – Mongolism

2. Single gene defect Identified by biochemical methods, eg, Phenylketonuria

3. Mitochondrial abnormalities Maternal inheritance Specific DNA



Chromosomal recombination



Unequal recombination produces Lepore hemoglobin.

Mutations

An alteration in the genetic material.

1 in million cell divisions

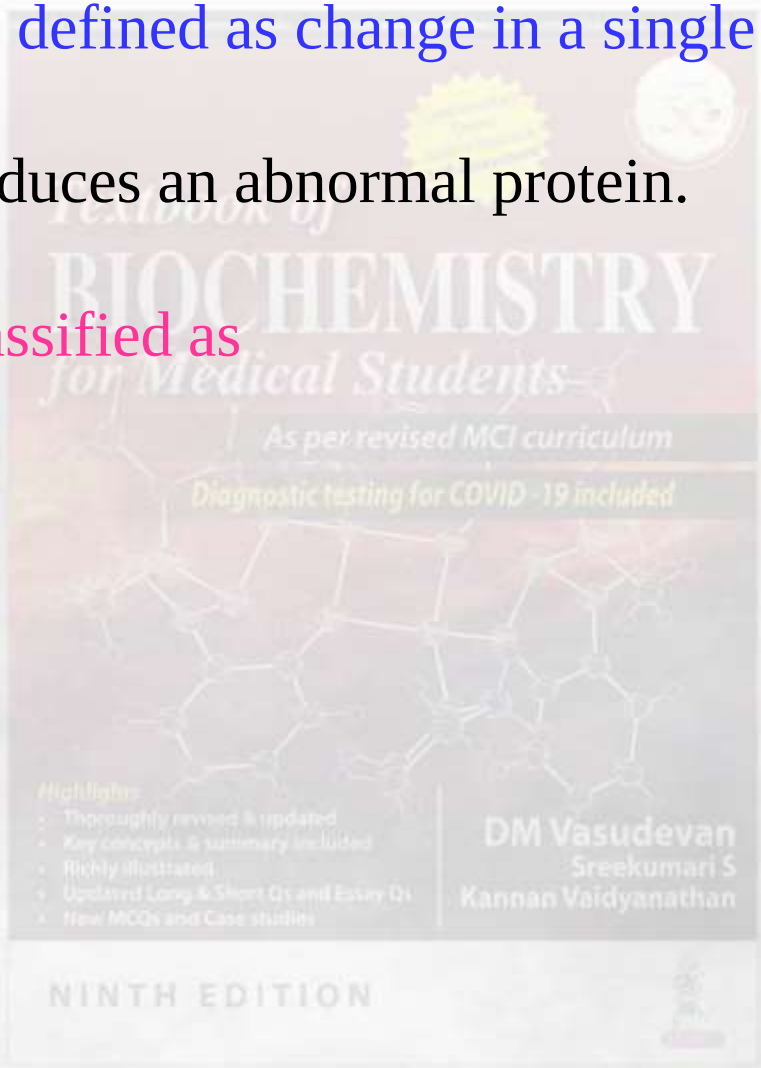
Mutation in germ line is transmitted to off-spring

Classification of Mutations

A point mutation is defined as change in a single nucleotide.

Defective gene produces an abnormal protein.

This may be subclassified as
(a) substitution;
(b) deletion and
(c) insertion.



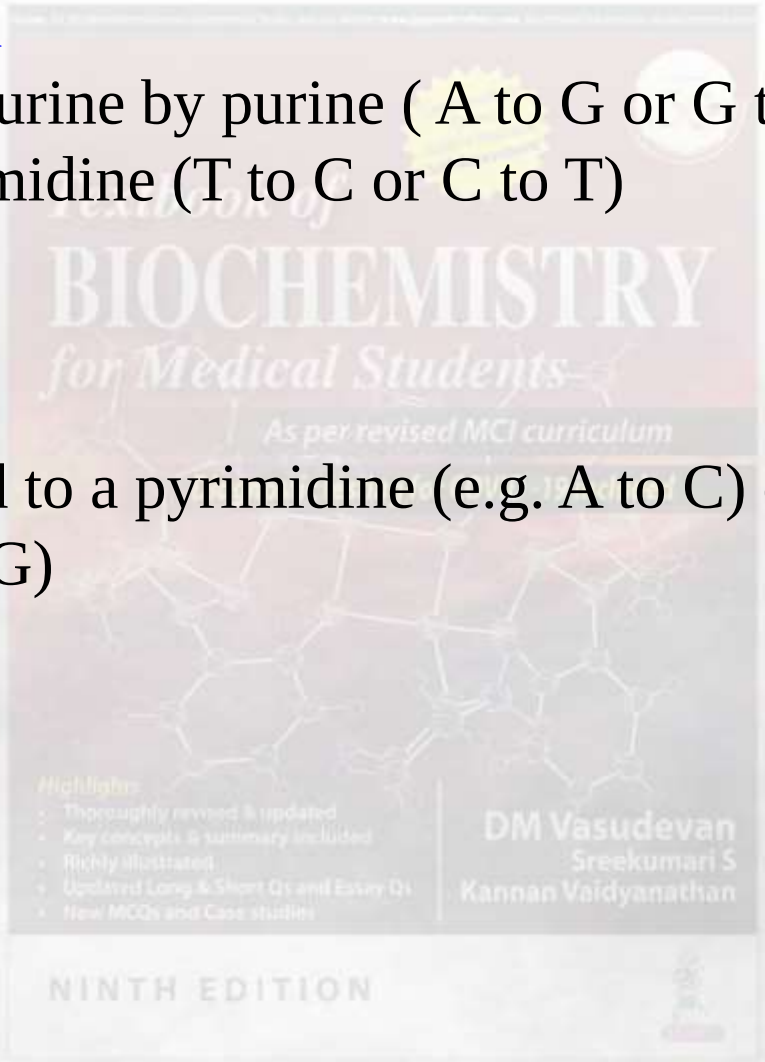
A. Substitution

Transition mutation

Replacement of a purine by purine (A to G or G to A) or pyrimidine by pyrimidine (T to C or C to T)

Transversion

A purine is changed to a pyrimidine (e.g. A to C) or a pyrimidine to a purine (e.g. T to G)

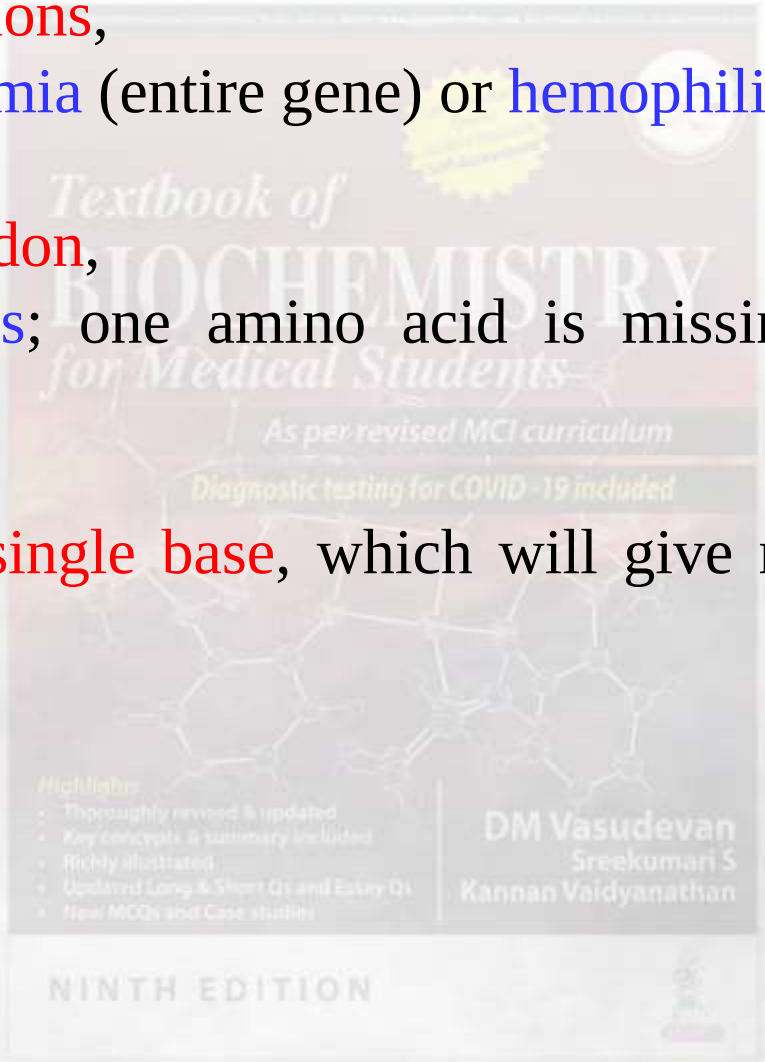


B. Deletion

i) Large gene deletions,
e.g., alpha thalassemia (entire gene) or hemophilia (partial)

ii) Deletion of a codon,
e.g., cystic fibrosis; one amino acid is missing in the CFTR protein.

iii) Deletion of a single base, which will give rise to frameshift effect.

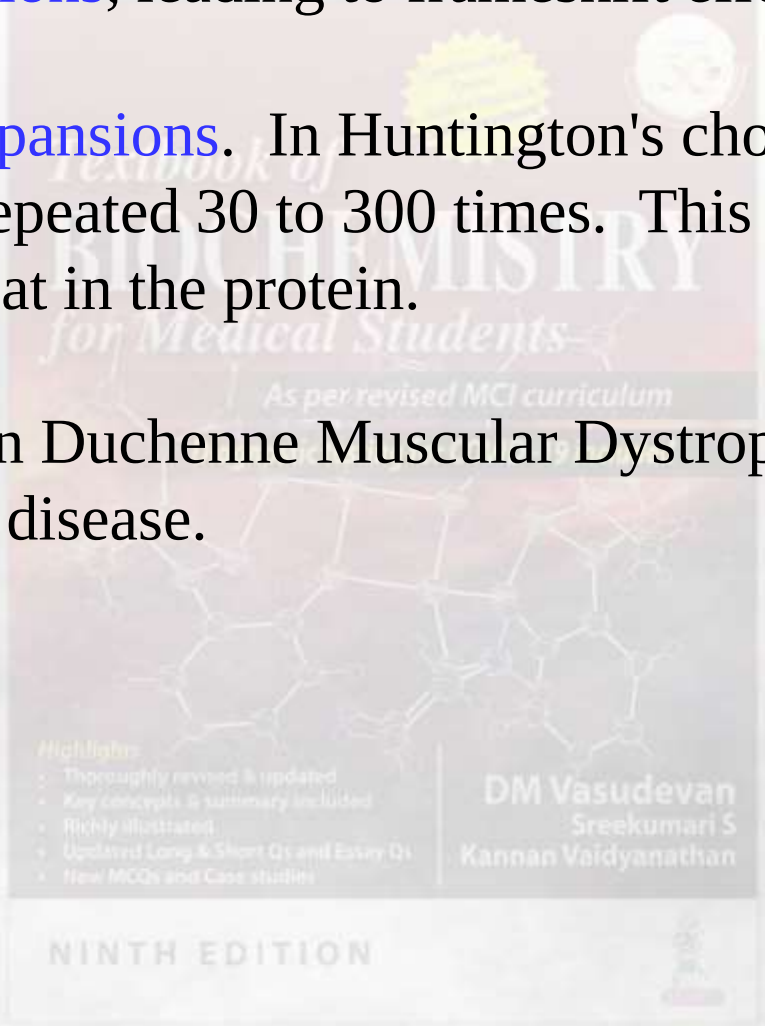


C. Insertion

i) Single base additions, leading to frameshift effect.

ii) Trinucleotide expansions. In Huntington's chorea, CAG trinucleotides are repeated 30 to 300 times. This leads to a polyglutamine repeat in the protein.

iii) Duplications. In Duchenne Muscular Dystrophy (DMD) gene is duplicated in the disease.



Effects of Mutations

i) Silent Mutation

ii) Mis-sense but Acceptable Mutation

iii) Mis-sense; Partially Acceptable

iv) Mis-sense; Unacceptable Mutation

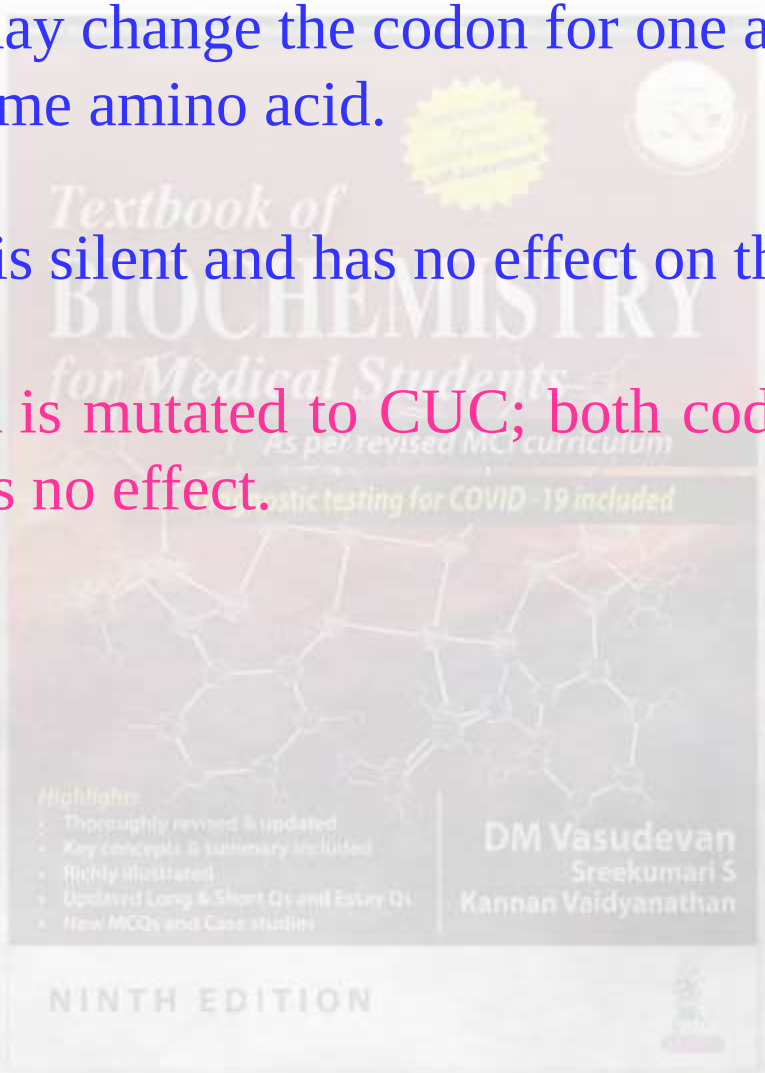
v) Nonsense; Terminator Codon Mutation

Silent Mutation

A point mutation may change the codon for one amino acid to a synonym for the same amino acid.

Then the mutation is silent and has no effect on the phenotype.

For example, CUA is mutated to CUC; both code for leucine, and so this mutation has no effect.



Mis-sense but Acceptable Mutation

A change in amino acid may be produced in the protein; but with no functional consequences.

For example, in the normal hemoglobin A molecule, (HbA beta-67) is valine.
The codon in mRNA is GUU.

(HbA beta-67) is valine.
The codon in mRNA is GUU.

If a point mutation changes it to GCU, the amino acid becomes alanine; this is called Hb Sydney.
This variant is functionally normal.

Mis-sense; Partially Acceptable Mutation

The amino acid substitution affects the functional properties of the protein.

HbS or sickle-cell hemoglobin is produced by a mutation of the beta chain in which the 6th position is changed to valine, instead of the normal glutamate.

HbS or sickle-cell hemoglobin.
Here, the normal codon GAG is changed to GUG (transversion).
HbS has abnormal electrophoretic mobility and subnormal function, leading to sickle-cell anemia.

Advantage in Heterozygous Status

Malaria endemicity
Heterozygous state: 50% HbS; 50% HbA
So malarial parasites will not grow
Selection pressure, advantage

Balanced polymorphism

Mis-sense; Unacceptable Mutation

The amino acid substitution alters the properties of the protein to such an extent that it becomes nonfunctional and the condition is incompatible with normal life.

For example, HbM results from histidine to tyrosine substitution (CAU to UAU) of the distal histidine residue of alpha chain.

HbM results from histidine to tyrosine substitution (CAU to UAU)

There is met-hemoglobinemia which considerably decreases the oxygen carrying capacity of hemoglobin.

Nonsense; Terminator Codon Mutation

A tyrosine (codon, UAC) may be mutated to a termination codon (UAA or UAG).

This leads to premature termination of the protein, and so functional activity may be destroyed, e.g. beta-thalassemia.

Or, a terminator codon is altered into a coding codon

(UAA to CAA). This results in elongation of the protein to produce

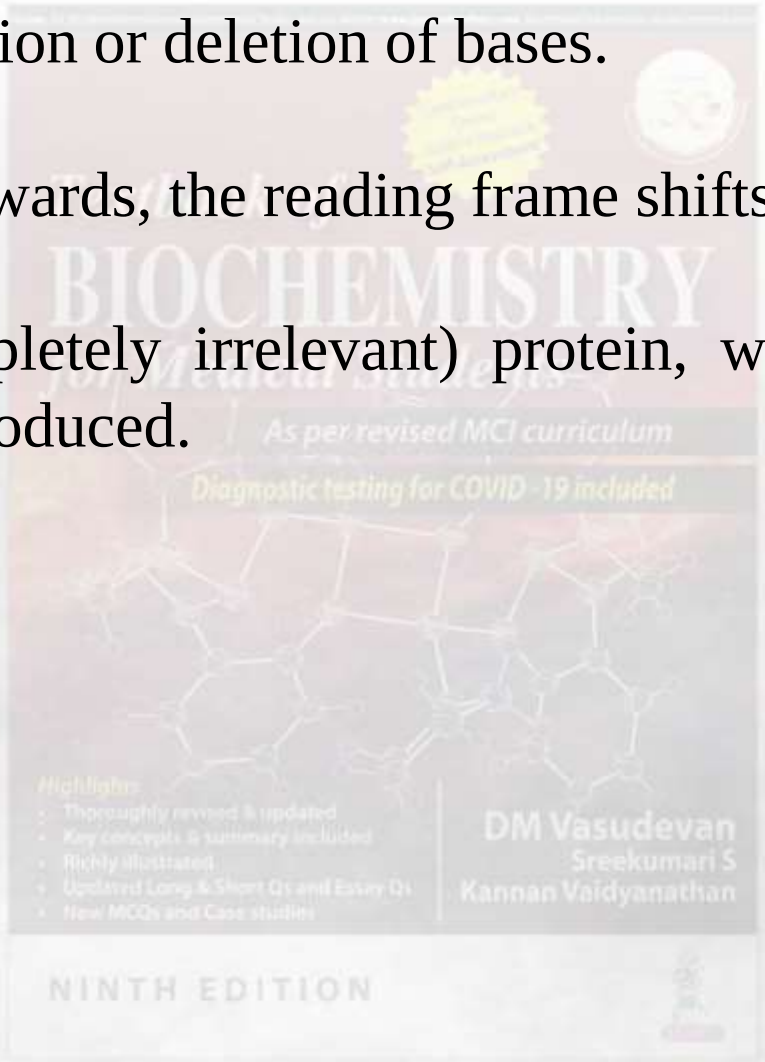
"run on polypeptide"

Frameshift Mutation

This is due to addition or deletion of bases.

From that point onwards, the reading frame shifts.

A "garbled" (completely irrelevant) protein, with altered amino acid sequence is produced.



Normal	mRNA	AUG	UCU UGC AAA....
Normal	protein	Met	Ser Cys Lys.....

DeletedU	mRNA	AUG	CUU GCA AA.....
Garbled	protein	Met	Leu Ala .....

Deletion of one uracil changes all the triplet codons thereafter. Therefore, a useless protein is produced.

Conditional Mutations

Most of the spontaneous mutations are *conditional*; they are manifested only when circumstances are appropriate.

Bacteria acquire resistance, if treated with antibiotics for a long time.

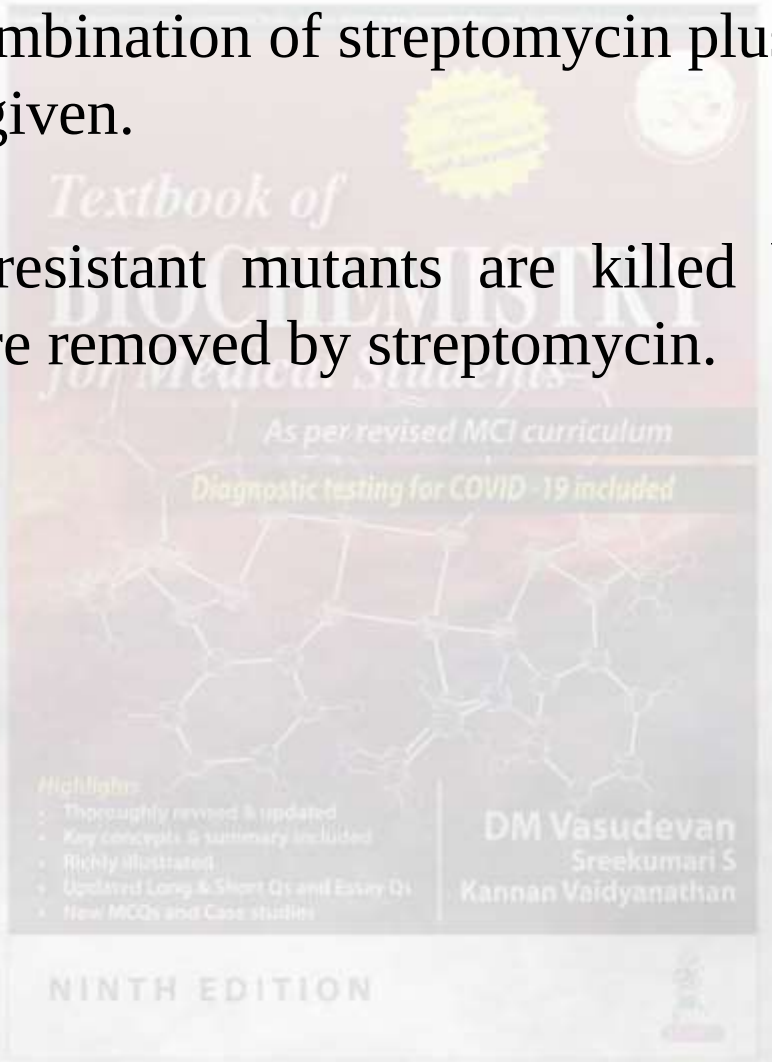
In the normal circumstances, wild bacilli will grow. In the medium containing antibiotic, the resistant bacilli are selected.

In a tuberculous lung cavity may harbor about 10^{12} bacilli. This may contain about 10^{6} mutations, out of which a few could be streptomycin resistant.

If the patient is given streptomycin alone, after sometime, there will be overgrowth of drug resistant bacilli.

To avoid this, a combination of streptomycin plus INH (isonicotinic acid hydrazide) is given.

So, streptomycin resistant mutants are killed by INH and INH resistant mutants are removed by streptomycin.

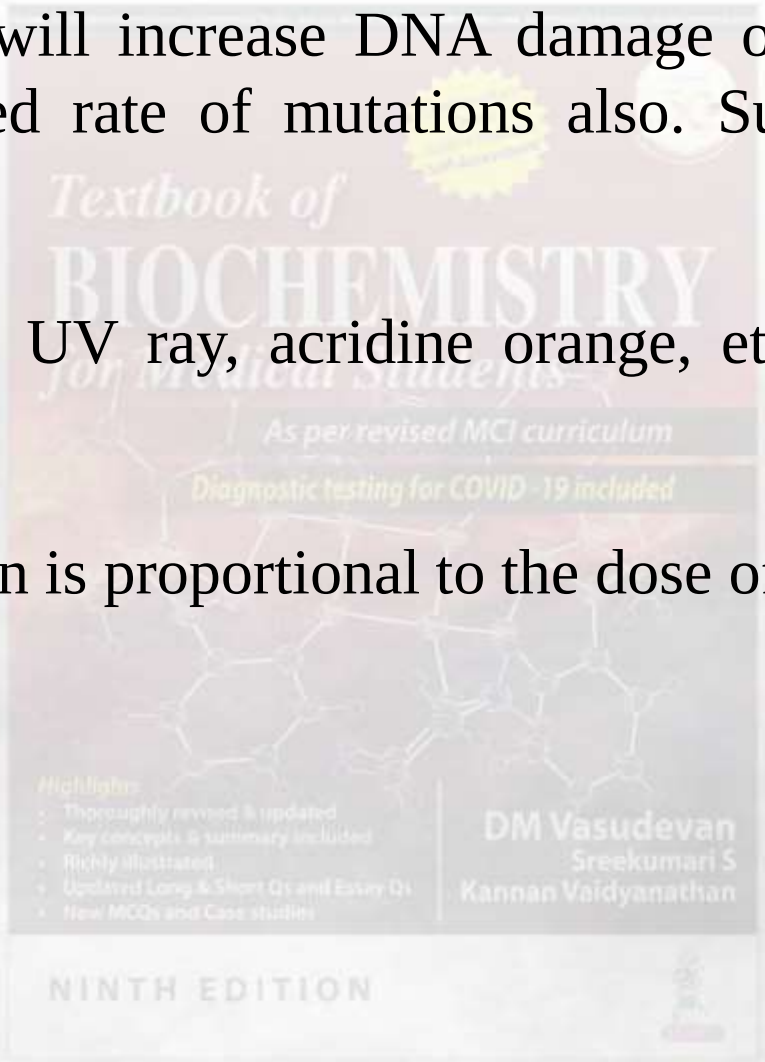


Mutagens and Mutagenesis

Any agent which will increase DNA damage or cell proliferation can cause increased rate of mutations also. Such substances are called mutagens.

X-ray, gamma-ray, UV ray, acridine orange, etc. are well known mutagens.

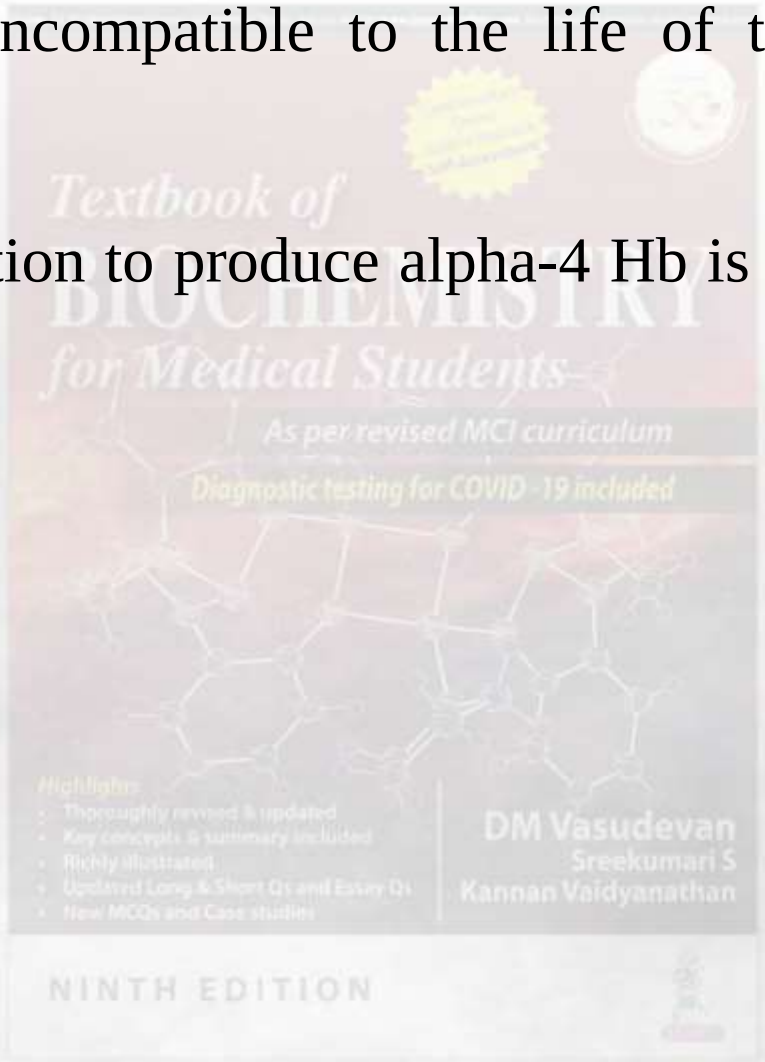
The rate of mutation is proportional to the dose of irradiation.



Lethal Mutations

The alteration is incompatible to the life of the cell or to the organism.

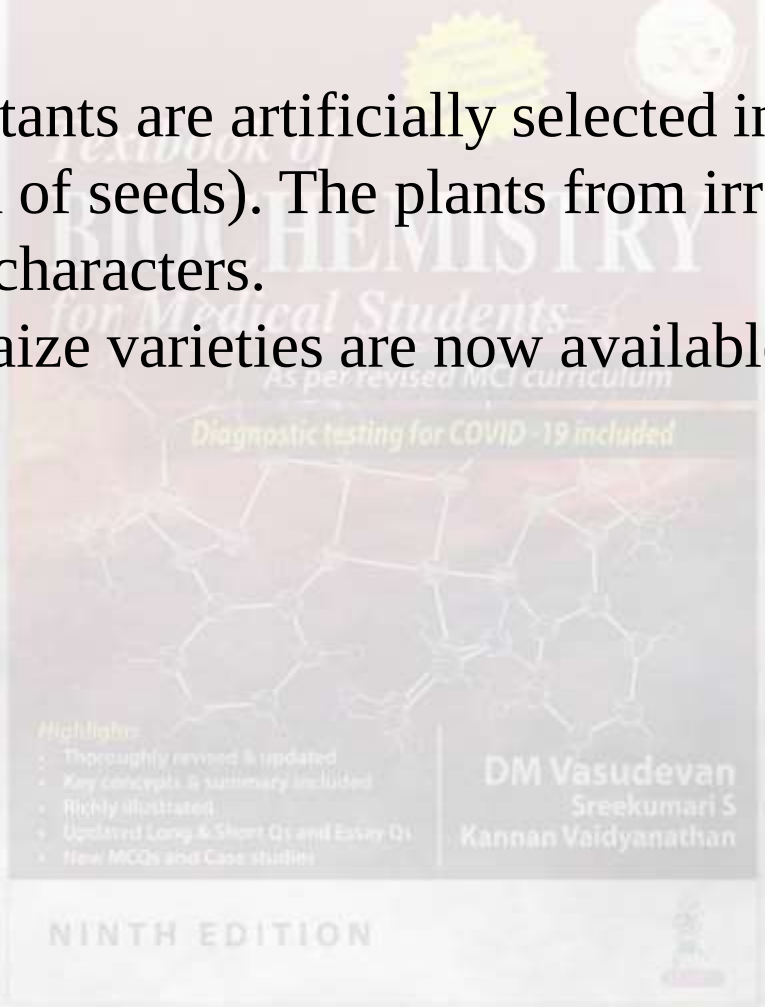
For example, mutation to produce alpha-4 Hb is lethal, and so the embryo dies.



Beneficial Mutations

Spontaneous mutations are the basis of evolution.

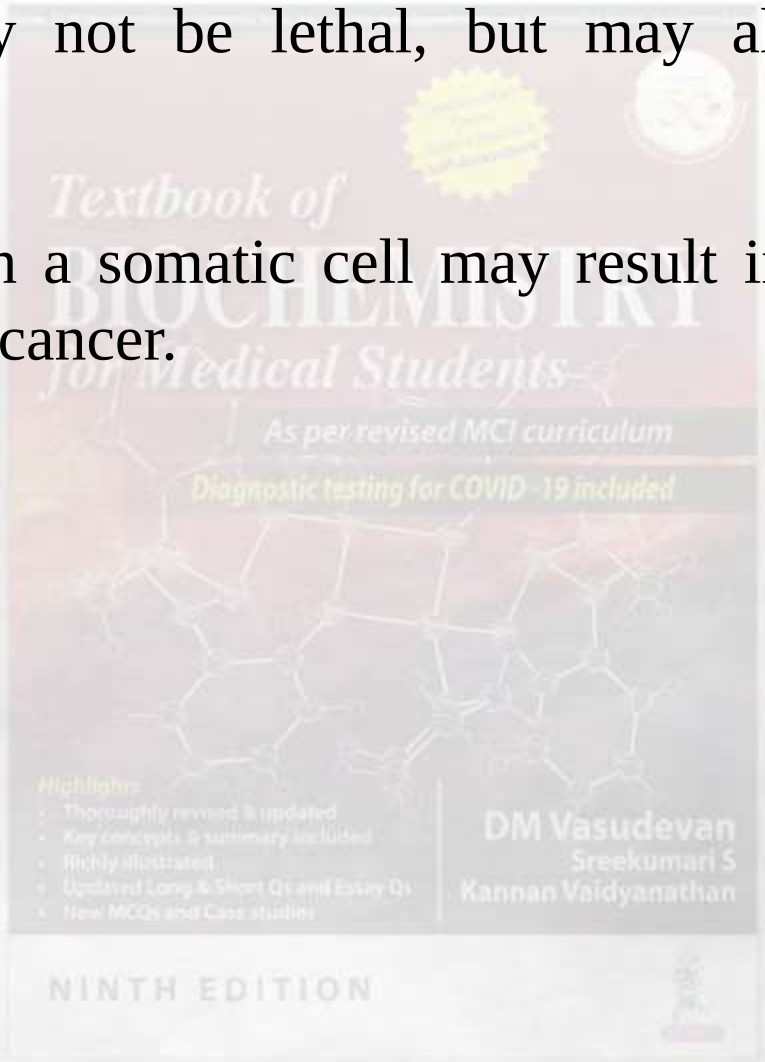
Such beneficial mutants are artificially selected in agriculture. (gamma-irradiation of seeds). The plants from irradiated seeds are selected for useful characters.
Tryptophan-rich maize varieties are now available for cultivation.

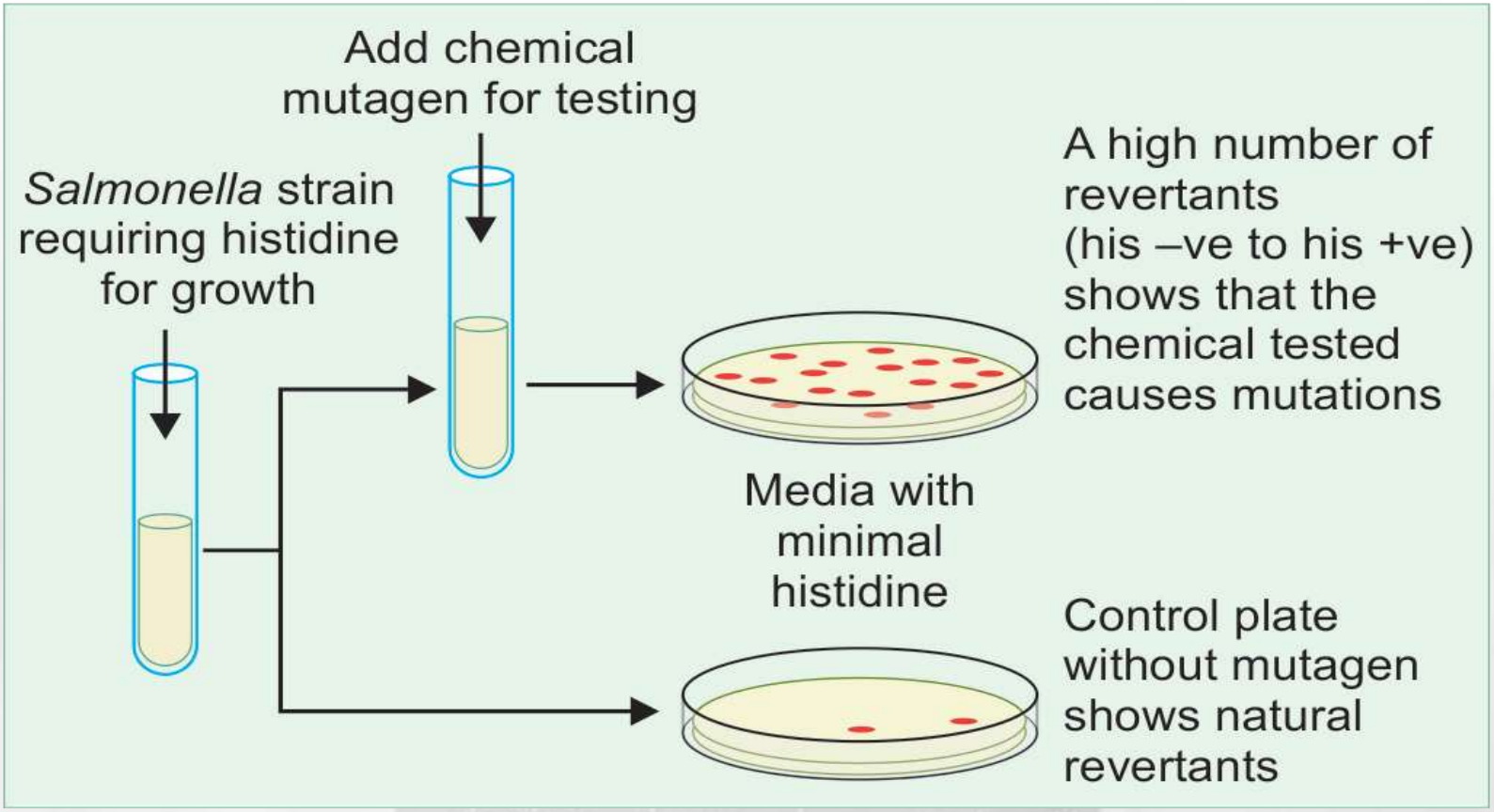


Carcinogenic Mutation

The mutation may not be lethal, but may alter the regulatory controls.

Such a mutation in a somatic cell may result in uncontrolled cell division leading to cancer.





Ames test to detect mutagenicity.

Triplet Repeat Disorders

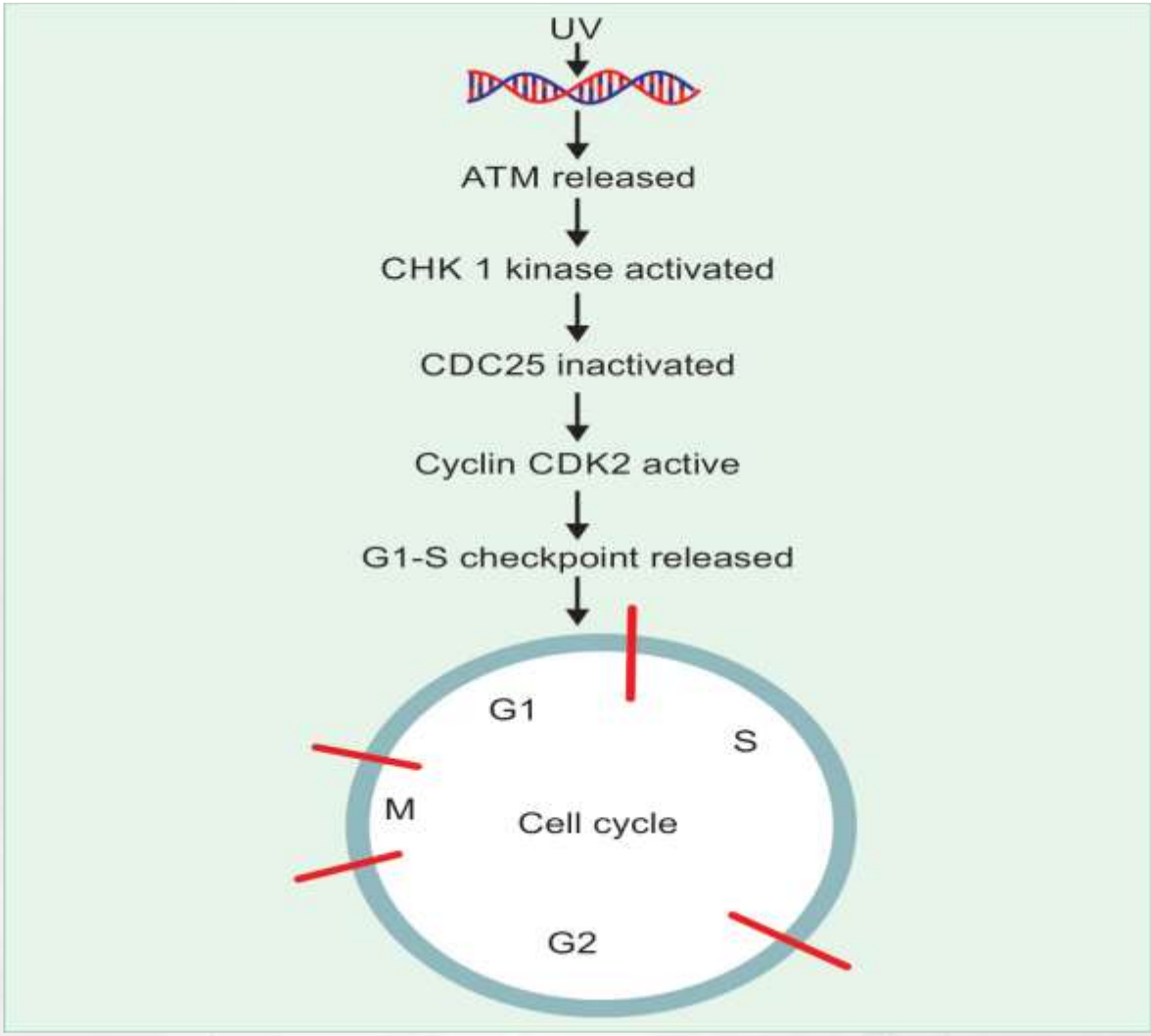
The genetic code contains regions of triplet repeats (CAG, CTG, CGG, or CAA). These regions of triplet repeats have a normal number of repetitions and can be located before, after, or within a gene. As the triplet repeat expands with successive generations there is increasing dysfunction of the gene and worsening of the clinical symptoms. A number of triplet repeat disorders have been described with autosomal dominant inheritance (myotonic dystrophy, Huntington's disease), autosomal recessive inheritance (Freidrich ataxia), and X-linked recessive inheritance (Fragile X syndrome).

Cell Cycle

The term cell cycle refers to the events occurring during the period between two mitotic divisions. It is divided into G1 (gap-1), S (synthesis), G2 (gap-2), and M (mitosis) phases. The cell division is taking place in **M phase**.

The daughter cells then either enter into G0 (undividing or dormant) phase or re-enter the cell cycle when there is necessity for growth and repair. In a normal cell population, most of the cells are in G0 phase. General metabolic events are taking place in G0 phase.

Cell Cycle



Cell cycle phases (total 20–22 hours).

Cell Cycle Controls or Checkpoints

The important checks occur in 3 stages; at G1-S transition, during S phase or at G2-M boundary. Of this, G1 phase checkpoint is more complex and is under strict control.

Four types of **cyclins** (A, B, D and E) and 5 different cyclin dependent **kinases** (CDK 1, 2, 4, 5 and 6) control the cycle. Cyclins are so named because they are synthesized throughout the cell cycle, and are abruptly destroyed during mitosis. Cyclins activate CDKs which phosphorylate specific substrates (regulatory proteins).

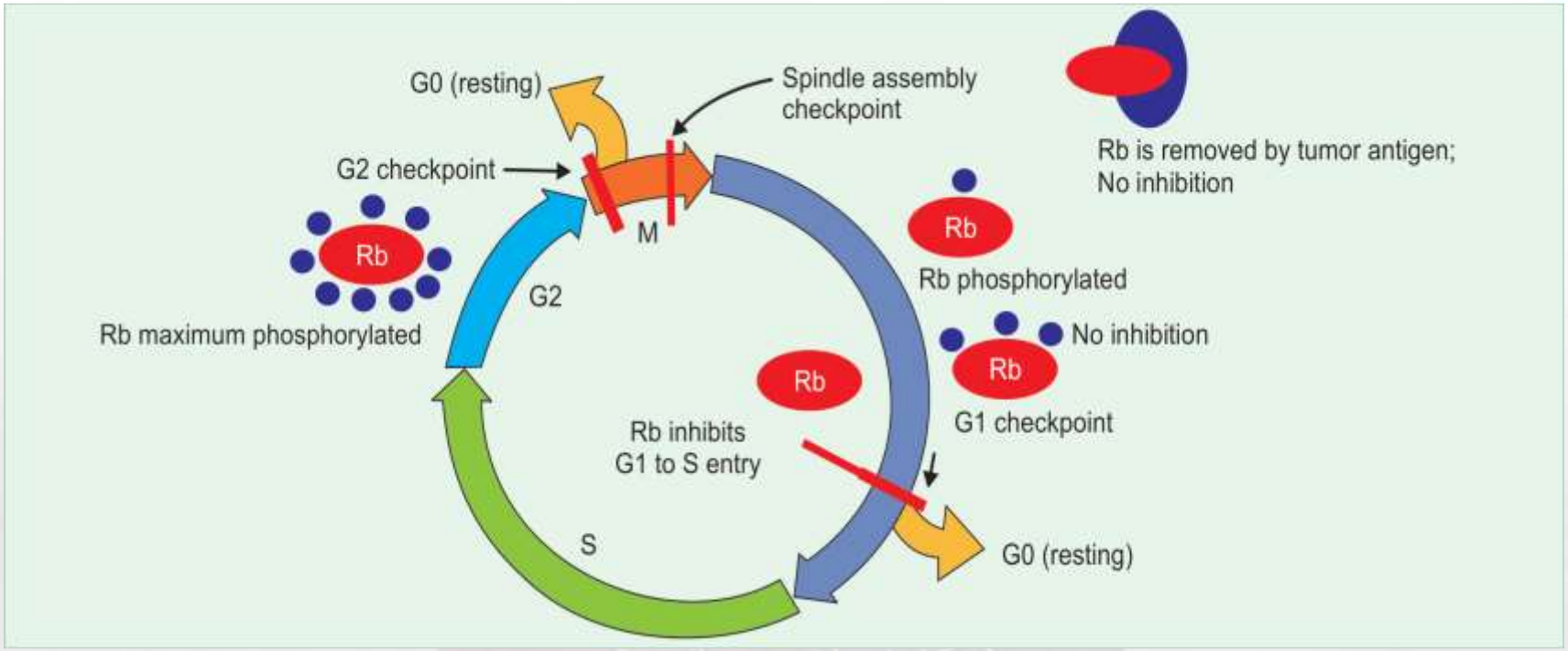
ATM is a protein kinase, which is associated with the DNA. If a break in DNA is produced (e.g. UV light), the ATM is dissociated, activated, and then initiates a series of cascade reaction. The CDK2-cyclin E complex directs the cells in G1 phase to enter into S phase.

Retinoblastoma Oncosuppressor Protein

Retinoblastoma (Rb) protein is the product of an oncosuppressor gene, so named because it was isolated from patients of retinoblastoma (cancer arising from retina).
It is a cell cycle regulator. It binds and inactivates the E2F, a transcription factor. Thus Rb inhibits cell cycle at G1 phase.

But in controlled cell cycles, cyclin D levels rise in the late G1 phase. The cyclin D inactivates Rb, which is separated from E2F. This is the normal mechanism to overcome the G1 arrest by the Rb.

Certain tumor antigens derived from viruses such as SV40, HSV, HPV may combine with the Rb. Then, the Rb cannot inhibit cell cycle, leading to continuous cell division and cancer.



Cell cycle controls or check points. Retinoblastoma (Rb) protein inhibits cell cycle at G1 checkpoint. Body circumvents this block by phosphorylation of Rb protein. This is done normally by cyclin D-CDK. Tumor antigens will attach with Rb protein, so Rb inhibition is lost; there will be uncontrolled cell division, leading to cancer.

The p53 Oncosuppressor Protein

It is so named because it is a protein with 53 kD in size, having 393 amino acids. The protein has a half-life of only 5–10 minutes and inhibits cell division, allowing them to repair. If damage is extensive and repair is not possible, then the *p53* directs the cell to apoptosis.

The PCNA gene encodes a nuclear protein that is a cofactor for DNA polymerase. The *p53* downregulates PCNA transcription blocks the DNA polymerase and causes arrest in G1 phase of the cell cycle.

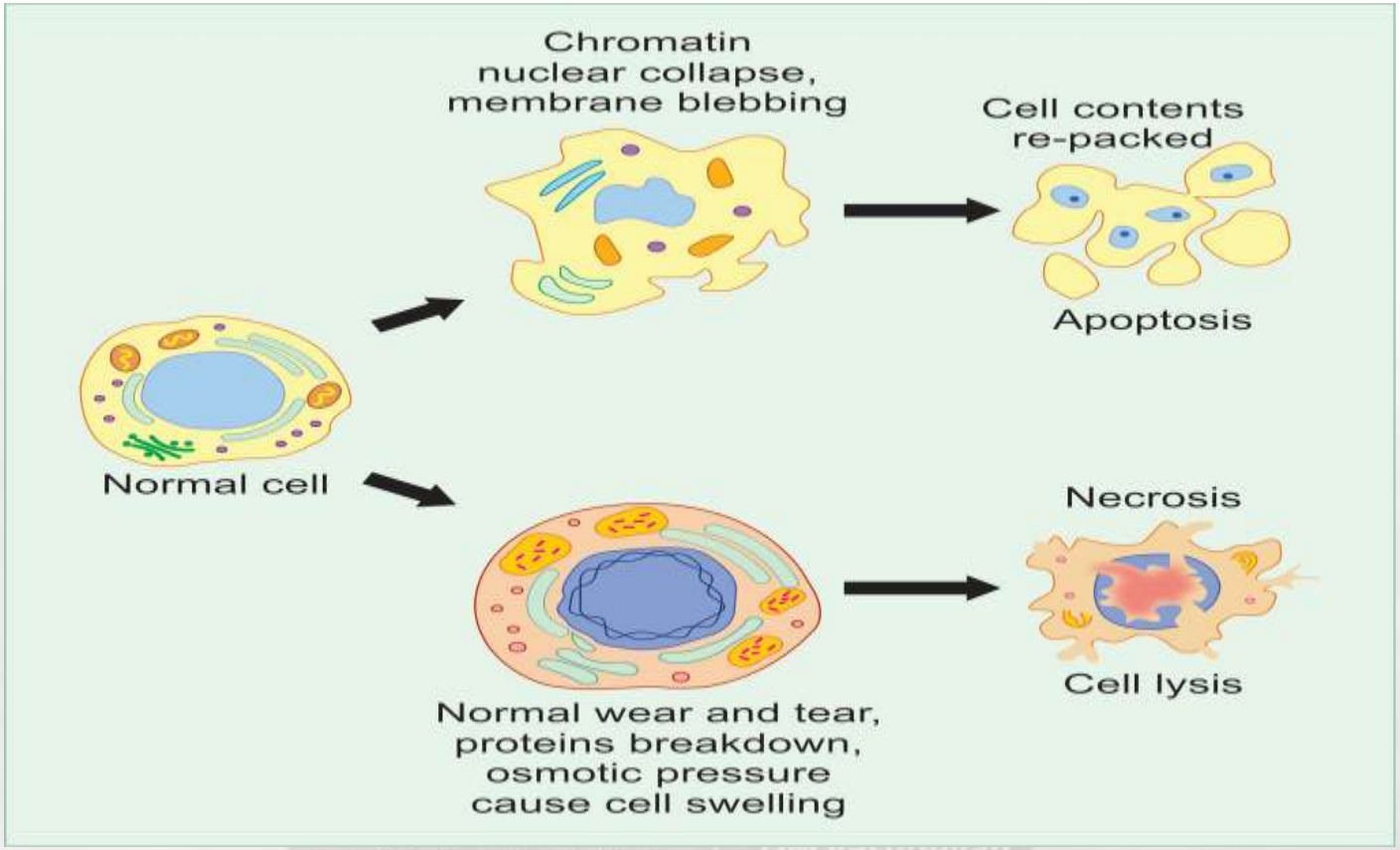
The *p53* is phosphorylated in a cell cycle dependent manner by the CDK4. Maximum level of *p53* phosphorylation is reached during mitosis.

Apoptosis (Programmed Cell Death)

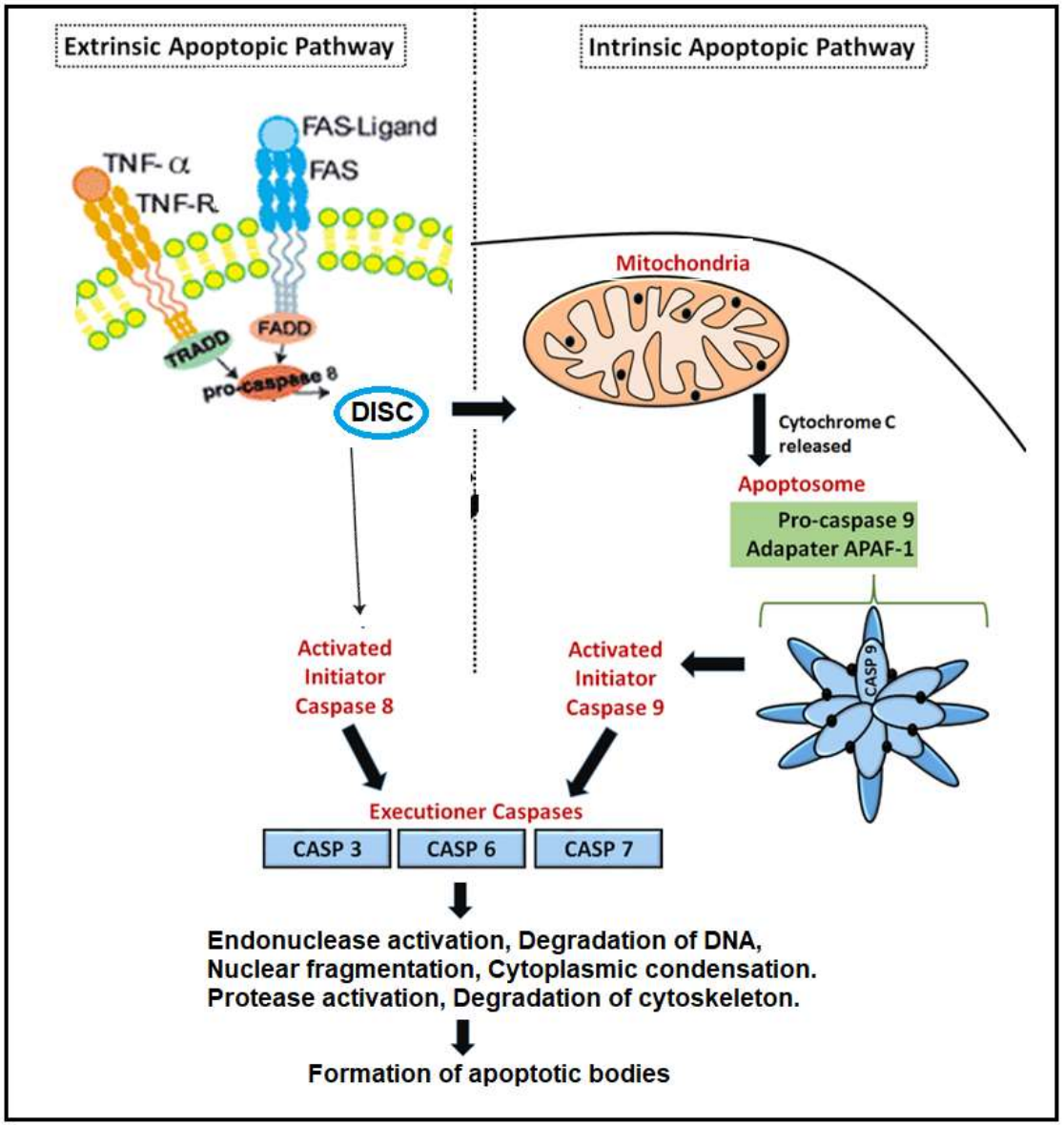
Removal of superfluous, aged or partially damaged cell is done by apoptosis. The term means "dropping off", similar to the old leaf falling from the tree. Nuclear shrinkage, chromatin condensation, membrane blebbing and stepladder pattern of DNA in electrophoresis are characteristic features of apoptosis.

Apoptosis mediating genes (suicidal genes) (oncosuppressor genes) are c-fos, p53, and Rb. Apoptosis-protecting genes are bcl2 and some oncogenes. Stress and other stimuli activate certain cell surface receptors, and a cascade of activation takes place.

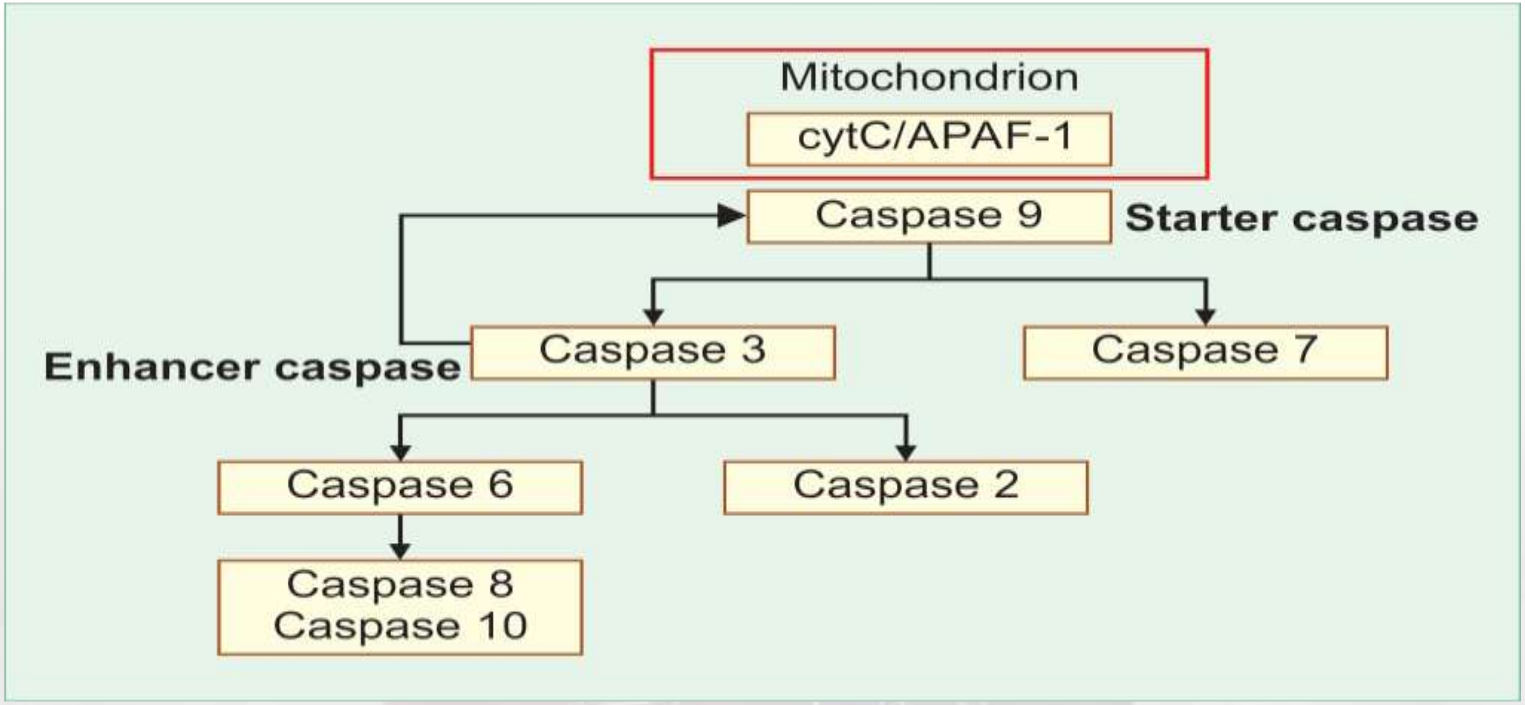
The final effector mechanism of cell death is through the activation of caspases.



Differences between apoptosis and necrosis. Apoptosis is a programmed cell death, while necrosis is the end stage of the day to day wear and tear.



Extrinsic and intrinsic apoptotic pathways.
TNF = Tumor necrosis factor.
TNFR = Receptor for TNF.
TRADD = TNF receptor-associated death domain.
FADD = Fas-associated death domain.
DISC = Death-inducing signalling complex.



When triggered by the apoptotic stimuli, the cytochrome c and apoptosis inducing factor (AIF) are released from the mitochondrial intermembrane space to the cytosol. These activate caspase 3 via the formation of a complex known as **apoptosome**, which is made up of cytochrome c, Apaf-1 and caspase 9. The caspase 3 then cleaves other caspases sequentially. Caspase 3 is the executor of death, which is officially named as "Yama".

Regulation of GENE Expression

Synthesis of proteins under the influence of gene is called gene expression.

Some genes are expressed almost always in all cells. For example, enzymes of glycolysis. Such genes are called constitutive genes or housekeeping genes.

Induction is the phenomenon of increased synthesis of protein or enzyme in response to certain signal. Such enzymes are said to be inducible; and the signals are called inducers.

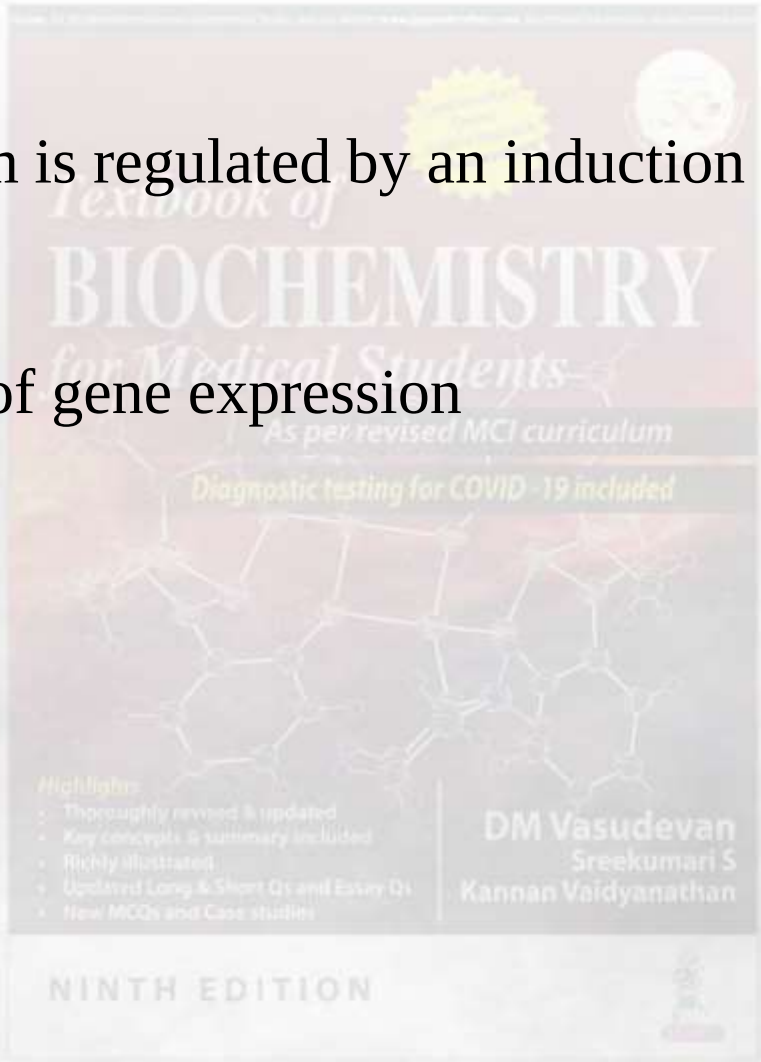
Induction is turning "on" the switch of the gene. Repression is turning "off" the gene expression.

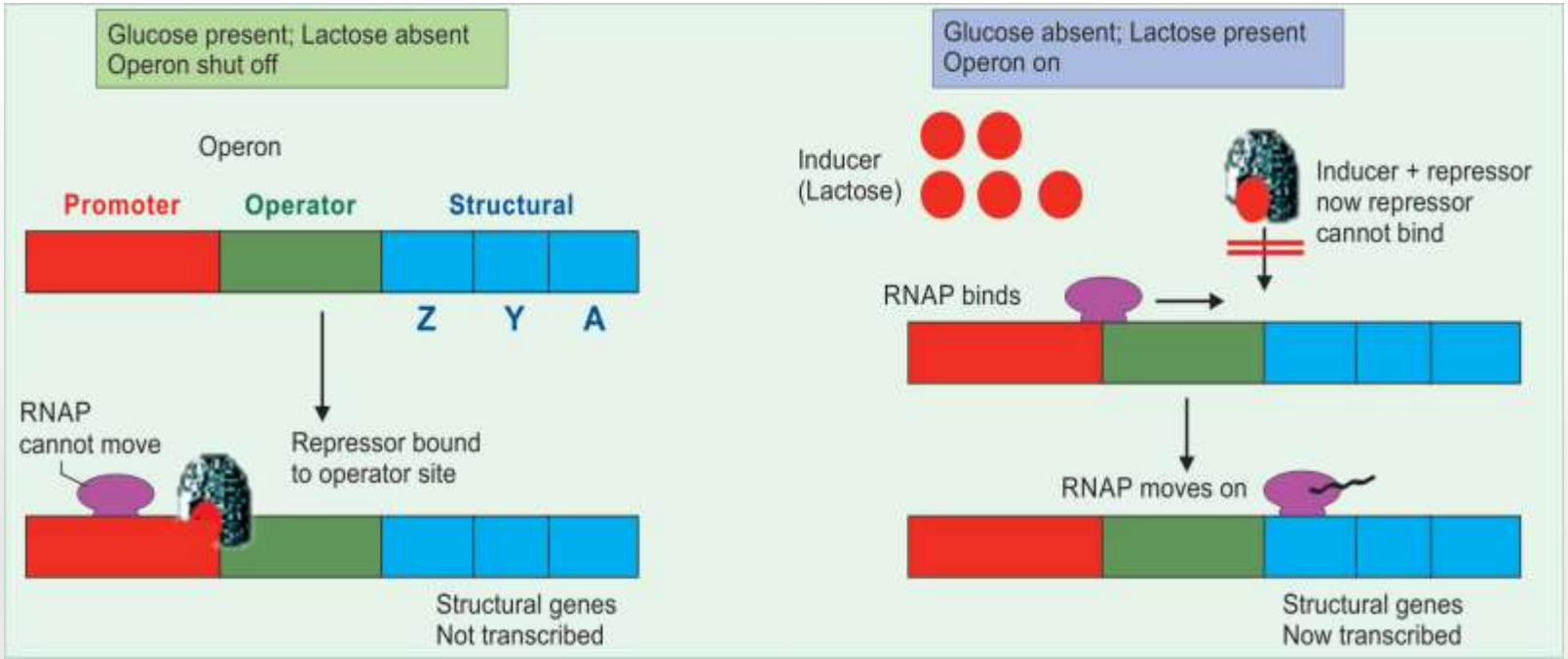
Operon Concept of Gene Regulation

The Lac Operon

Lactose metabolism is regulated by an induction or derepression process.

Operon is the unit of gene expression





Left side, Repression of the lac operon. When the lactose is absent, the repressor molecules bind to the operator site. So RNAP cannot work, and the genes are in "off" position.

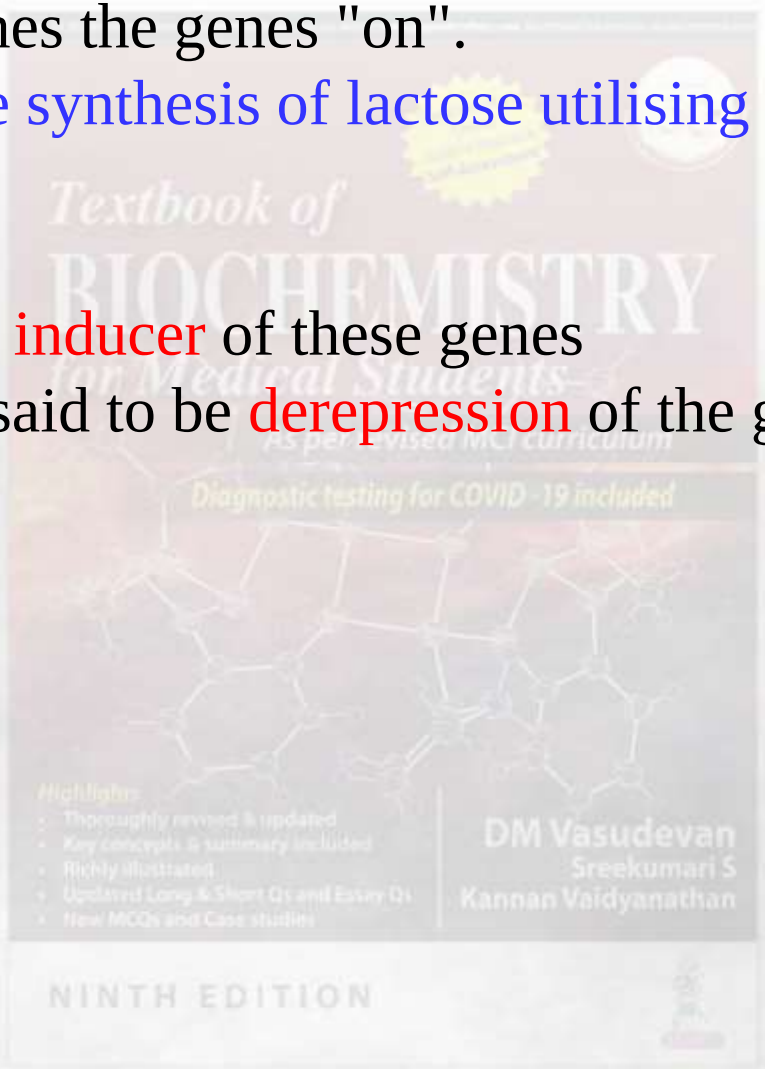
Right side, **Induction** or derepression of the lac operon. Lactose attaches to the repressor; so the repressor cannot bind to the operator site which is free; genes are in "on" position; protein is then synthesized.

Thus lactose switches the genes "on".

Lactose induces the synthesis of lactose utilising enzymes.

Hence lactose is an inducer of these genes

The mechanism is said to be derepression of the gene.

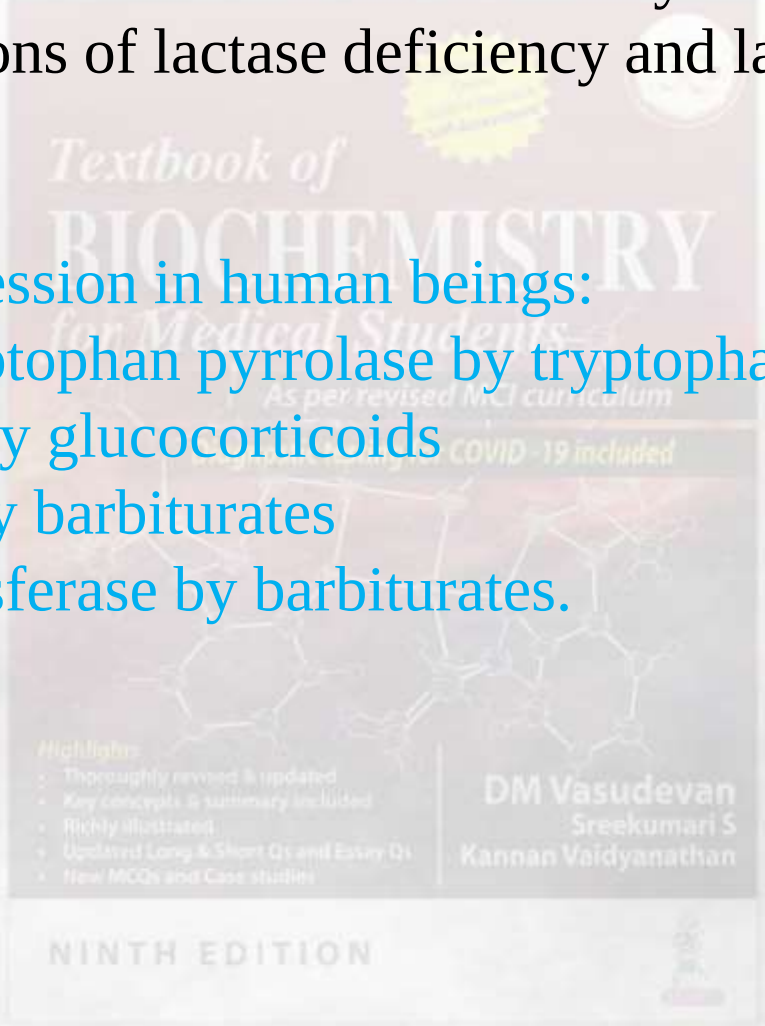


Clinical Applications of Derepression

Lactase in human intestine is an inducible enzyme.
Clinical manifestations of lactase deficiency and lactose intolerance are described.

Examples of derepression in human beings:

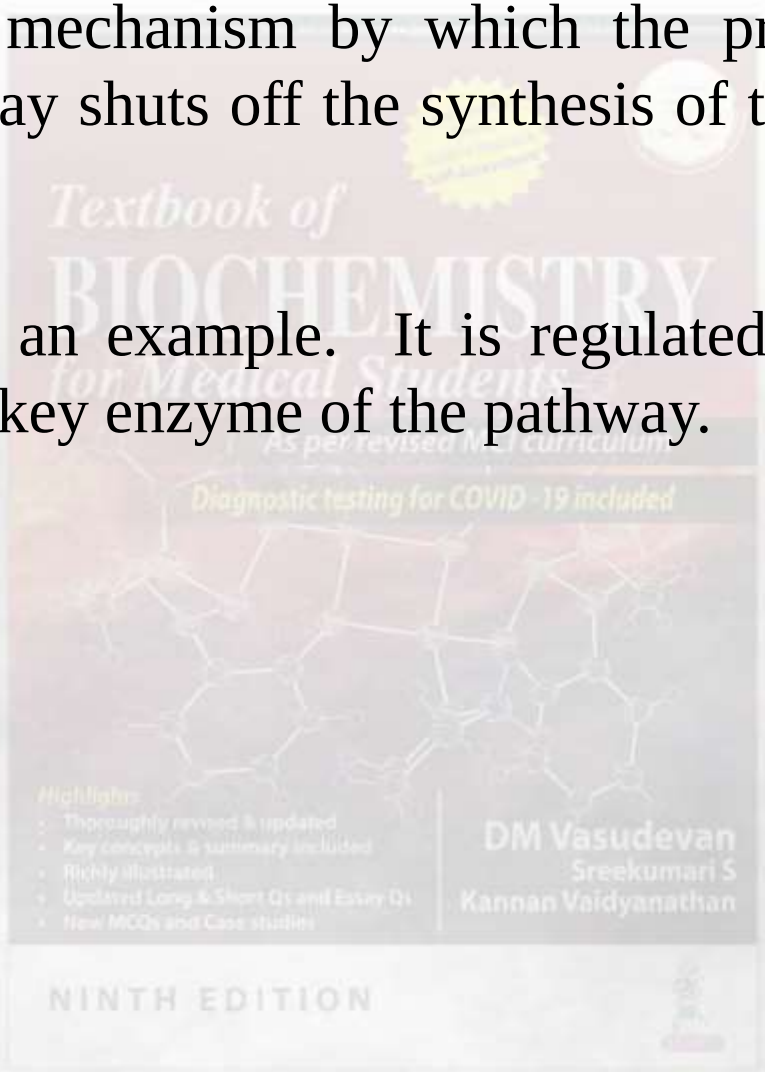
1. Induction of tryptophan pyrrolase by tryptophan
2. Transaminases by glucocorticoids
3. ALA synthase by barbiturates
4. Glucuronyl transferase by barbiturates.

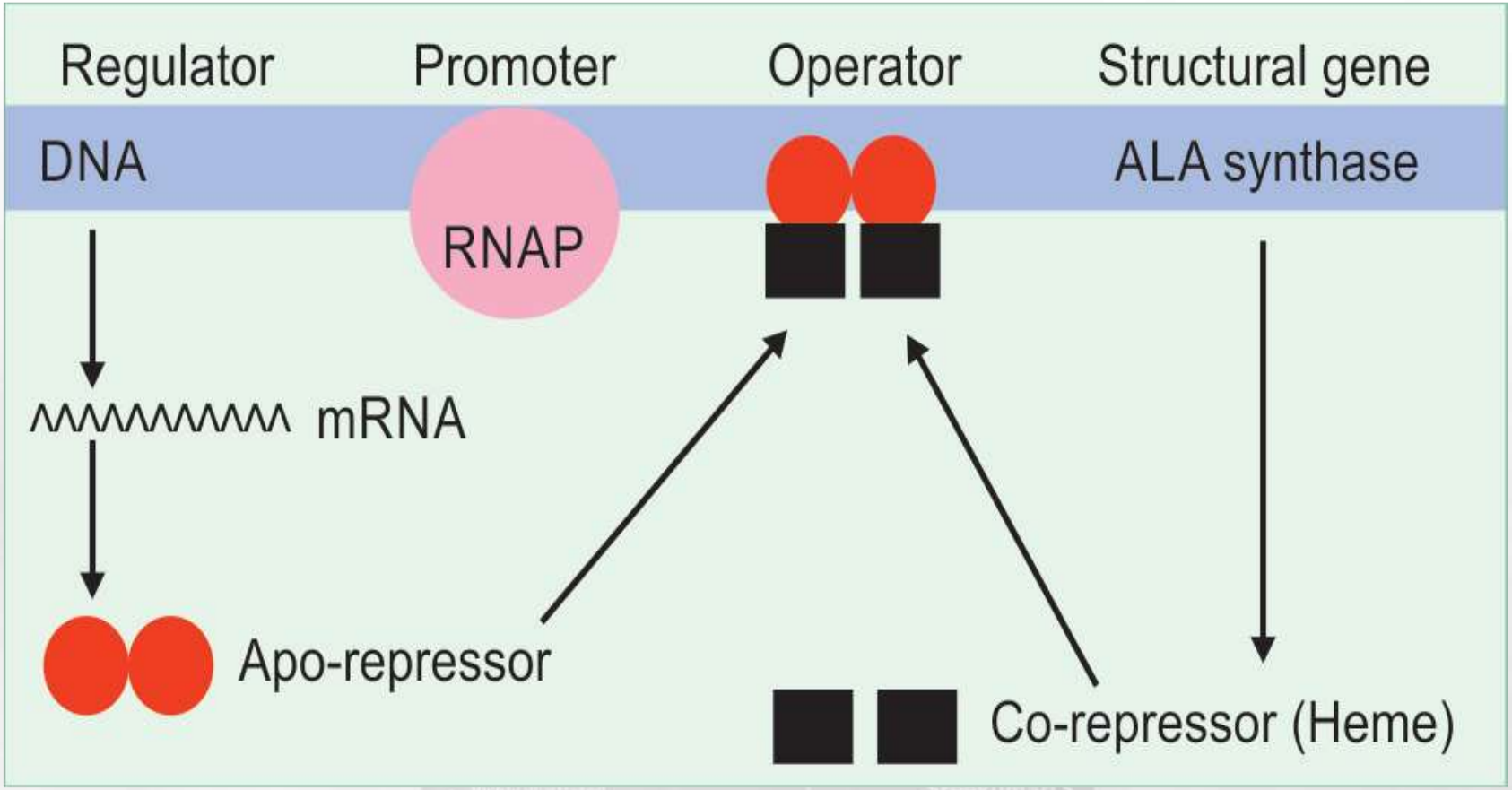


Regulation of Genes by Repression

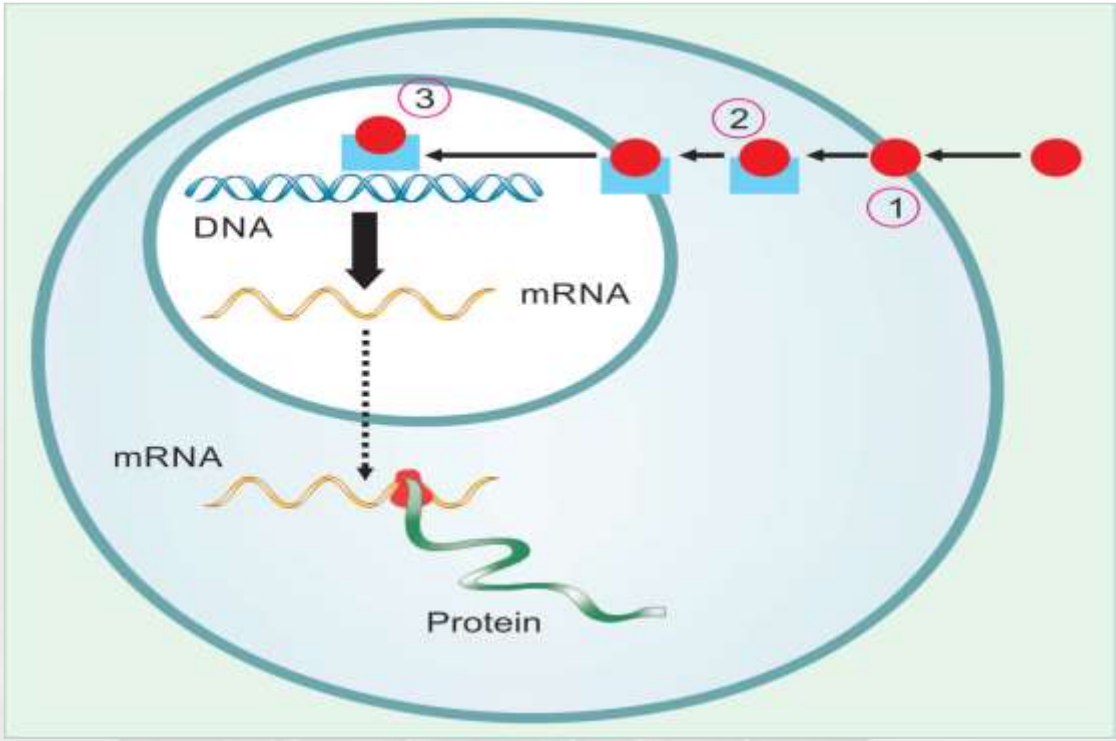
Repression is the mechanism by which the presence of excess product of a pathway shuts off the synthesis of the key enzyme of that pathway.

Heme synthesis is an example. It is regulated by repression of ALA synthase, the key enzyme of the pathway.

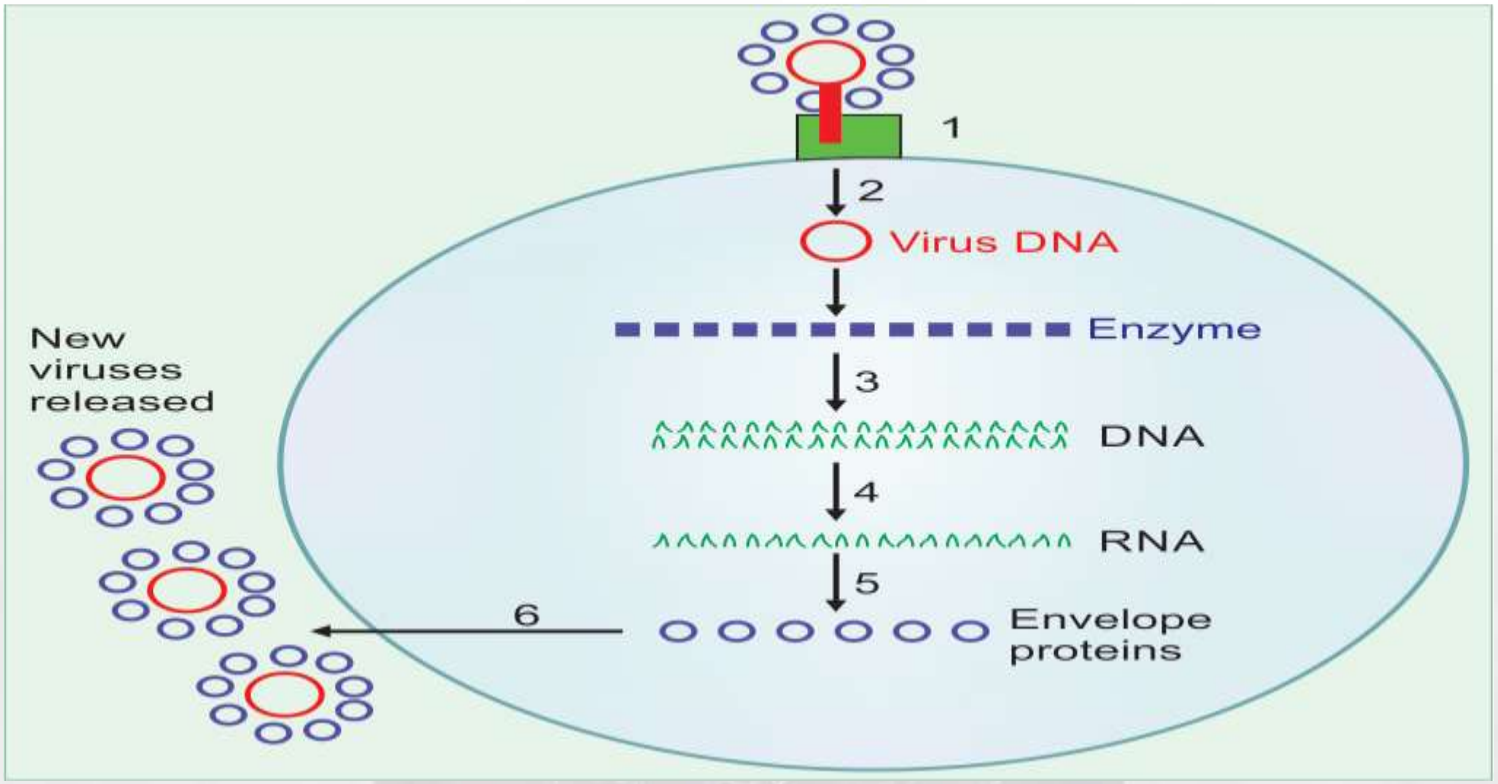




Repression of heme synthesis by heme.
(ALA: aminolevulinic acid; RNAP: RNA polymerase)



Steroid hormone binds to the HRE (hormone response element) region of DNA, leading to the gene activation. (1) Steroid hormone reaches cytoplasm. (2) Hormone binds with the cytoplasmic receptor. (3) Hormone binds the HRE.



Life cycle of viruses in general. The numbers show the site of action of antiviral drugs. (1) Virus entry through receptor. (2) Uncoating. (3) DNA synthesis. (4) RNA synthesis. (5) Late protein synthesis. (6) Packaging or assembly of new daughter viruses.

Antiviral Agents

Site 1: Adsorption and penetration of the virus into the host cell is inhibited by **antibodies**, either passive or active. Neuraminidase inhibitors can be used in the treatment of influenza virus infections, e.g. **Oseltamivir** (Tamiflu).

Site 2: **Amantadine** inhibits uncoating of viral nucleic acid in influenza virus.

Site 3: The synthesis of DNA is inhibited by purine or pyrimidine analogues. **Acyclovir** is an analogue of Guanosine. **Iododeoxyuridine** is the first antiviral drug marketed. **Ribavirin** is a guanosine analogue. It inhibits capping of viral mRNA. It is useful against respiratory syncytial virus and viral hemorrhagic fever.

Site 4: The synthesis of RNA is abolished by inhibitors of reverse transcriptase. **Zidovudine (**deoxythymidine analogue) and **Didanosine** (dideoxyinosine) inhibit reverse transcriptase and are used in HIV infection.

Site 5: Late protein synthesis is controlled by protease inhibitors, such as **Ritonavir,** Saquinavir and Indinavir. They are used against HIV infection.

Epigenetic Modifications

The epigenetic modifications include changes in histones and DNA methylation. Genetic code is comparable to writing in indelible ink using the sequence of 4 nucleotides. Information provided by epigenome is like a code written by a pencil which can be erased and rewritten. However, at times pencil writing leaves smudges even after erasing. Similarly, the **epimutations** may be transmitted to the next generation.

Epigenetic modifications referred to as genomic imprinting occurs very early in the embryo. Methylation of DNA leads to selective silencing of genes. This will explain genomic imprinting. In **Prader Willi syndrome**, a mutant gene is derived from father and in **Angelman syndrome**, the mutant gene is from mother.