CHI-SQUARE TEST

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- **We need an objective procedure to compare the results of the experiment with the predictions of the underlying hypothesis.**
- **To take into account how chance might affect the outcome of the experiment. Even if the hypothesis is correct, we do not anticipate that the results of the experiment will exactly match the predictions of the hypothesis. If they deviate a bit, as mendel's data did, we would ascribe the deviations to chance variation in the outcome of the experiment. However, if they deviate grossly, we would suspect that something was amiss. The experiment might have been executed poorly—for example, the crosses might have been improperly carried out, or the data might have been incorrectly recorded—or, perhaps, the hypothesis is simply wrong. The possible discrepancies between observations and expectations obviously lie on a continuum from small to large, and we must decide how large they need to be for us to entertain doubts about the execution of the experiment or the acceptability of the hypothesis.**

- **uses a statistic called chi-square**
- **A statistic is a number calculated from data—for example, the mean of a set of examination scores. The statistic allows a researcher to compare data, such as the numbers we get from a breeding experiment, with their predicted values.**
- **If the data are not in line with the predicted values, the statistic will exceed a critical number and we will decide either to reevaluate the experiment—that is, look for a mistake in technique—or reject the underlying hypothesis.**
- **If the statistic is below this number, we tentatively conclude that the results of the experiment are consistent with the predictions of the hypothesis. The statistic therefore reduces hypothesis testing to a simple, objective procedure. As an example, let's consider the data from the experiments of mendel and devries.**
- **Mendel's F2 data seemed to be consistent with the underlying hypothesis, whereas devries's F2 data showed some troubling discrepancies.**

	F_2 Phenotype		Observed Number	Expected Number
Mendel's dihybrid cross	Yellow, round		315	313
	Green, round		108	104
	Yellow, wrinkled		101	104
	Green, wrinkled		32	35
	Total:		**556**	**556**
DeVries's dihybrid cross	Red, hairy		70	88.9
	White, hairy		23	29.6
	Red, smooth		46	29.6
	White, smooth		19	9.9
	Total:		**158**	**158**

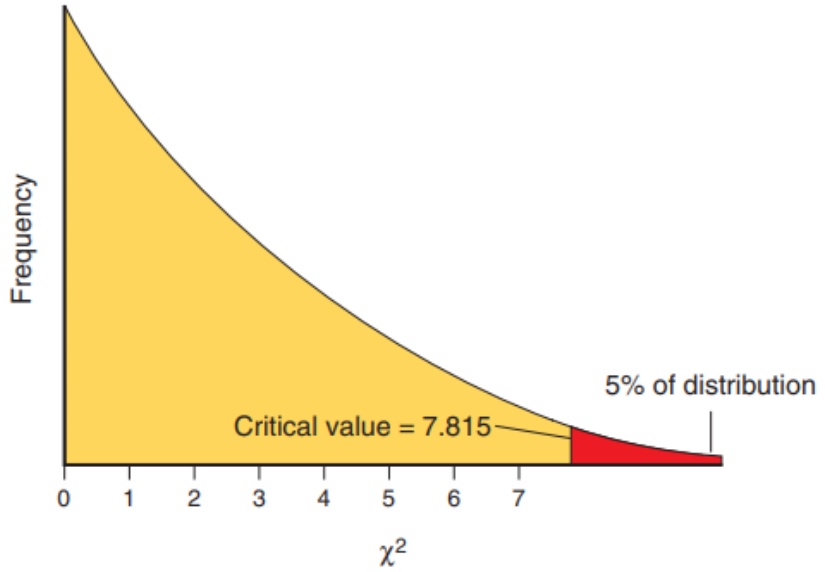

Table of Chi-Square (χ^2) 5% Critical Values[a]

Degrees of Freedom	5% Critical Value
1	3.841
2	5.991
3	7.815
4	9.488
5	11.070
6	12.592
7	14.067
8	15.507
9	16.919
10	18.307
15	24.996
20	31.410
25	37.652
30	43.773

[a]Selected entries from R. A. Fisher and Yates, 1943, *Statistical Tables for Biological, Agricultural, and Medical Research*. Oliver and Boyd, London.

Q: Two true-breeding strains of peas, one with tall vines and violet fl owers and the other with dwarf vines and white fl owers, were crossed. All the F1 plants were tall and produced violet fl owers. When these plants were backcrossed to the dwarf, white parent strain, the following offspring were obtained: 53 tall, violet; 48 tall, white; 47 dwarf, violet; 52 dwarf, white. Do the genes that control vine length and flower color assort independently?

- **Answer: The hypothesis of independent assortment of the vine length and fl ower color genes must be evaluated by calculating a chi-square test statistic from the experimental results. To obtain this statistic, the results must be compared to the predictions of the genetic hypothesis. Under the assumption that the two genes assort independently, the four phenotypic classes in the F2 should each be 25 percent of the total (200); that is, each should contain 50 individuals. To compute the chi-square statistic, we must obtain the difference between each observation and its predicted value, square these differences, divide each squared difference by the predicted value, and then sum the results: $x^2 = (53- 50)^2/50 + (48- 50)^2/50 + (47- 50)^2/50 + (52-50)^2/50 = 0.52$ this statistic must then be compared to the critical value of the chi-square frequency distribution for 3 degrees of freedom (calculated as the number of phenotypic classes minus one). Because the computed value of the chi-square statistic (0.52) is much less than the critical value, there is no evidence to reject the hypothesis of independent assortment of the vine length and flower color genes. Thus, we may tentatively accept the idea that these genes assort independently**

MUTATION

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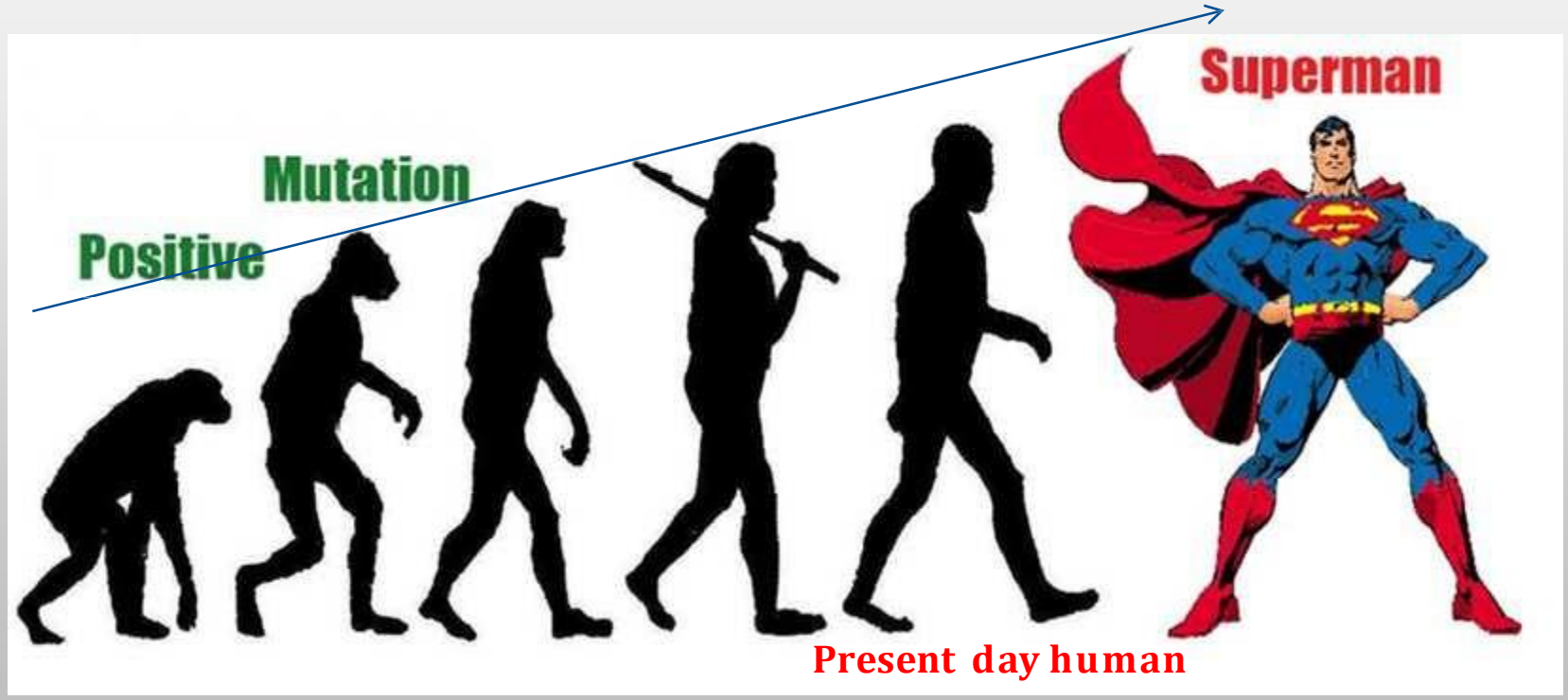
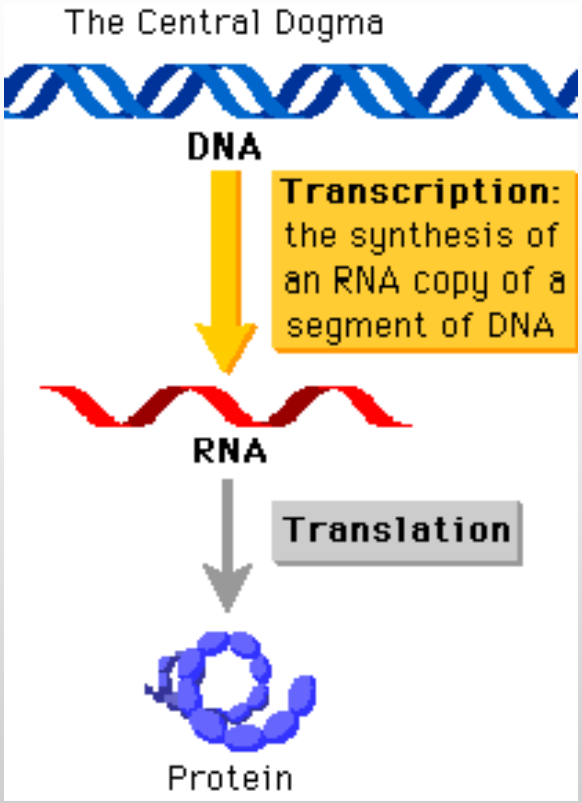
SIGNIFICANCE OF MUTATIONS

According to one hypothesis mutation is responsible for the separation of existing human beings from our ancestor primates.





Mutation is an abrupt qualitative or quantitative change in the genetic material/ Any change in the nucleotide sequence of DNA, which gives rise to a mutant genotype

The organism which undergoes mutation is known as mutant

HISTORY

THE EARLIEST RECORD OF POINT MUTATION DATES BACK TO 1791, WHEN SETH WRIGHT NOTICED A LAMB WITH EXCEPTIONALLY SHORT LEGS IN HIS FLOCK OF SHEEP.

Mutation - Coined by Hugo de Vris in1900 to explain the heritable changes in evening primrose *Oenothera lamrckiana*

Mutagenesis - Process of producing mutations

Mutagen - A physical/chemical/biological agent that causes mutations

The first scientific study of mutation started in 1910, when morgan started his work on fruit fly *drosophila melanogaster* after he observed white eyed male individuals among red eyed male individuals.

CLASSIFICATION

Based on the origin: Natural / Artificial (induced)

Based on the type of cells: Somatic/Gametic

Based on type of chromosomes: Autosomal / Allosomal

Based on direction: Forward/Reverse

Based on size: Point/Gross

Silent Mutations:- has no detectable effect of phenotype

Leaky mutations:- Causes amino acid substitution, eventually it reduces the activity of an enzyme.

Nonsense Mutations or Chain termination Mutation:- Causes premature termination of polypeptide chain.

etc.,

NATURAL MUTATION

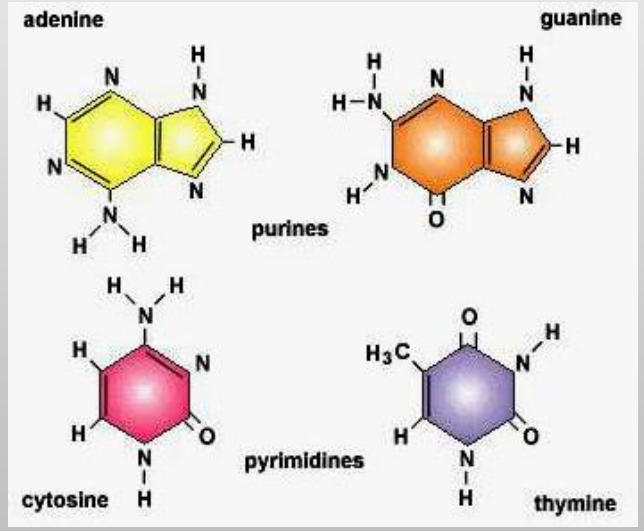
Spontaneous mutations occur suddenly in the nature and their origin is unknown. They are also called as background mutations and have been reported in many organisms such as Oenothera, Maize, Drosophila, Mice, Man *etc*.,

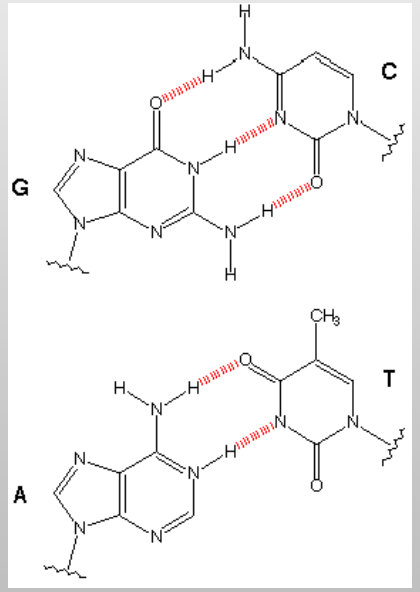
For example TAUTOMERISM

Frequency of total mutations - 10^{-7} to 10^{-12}/organism
(*i.e.*, 0.0000001- 0.000000000001)
Frequency of detectable mutations - 1 in 10^{6} (*i.e.*, 0.000001)

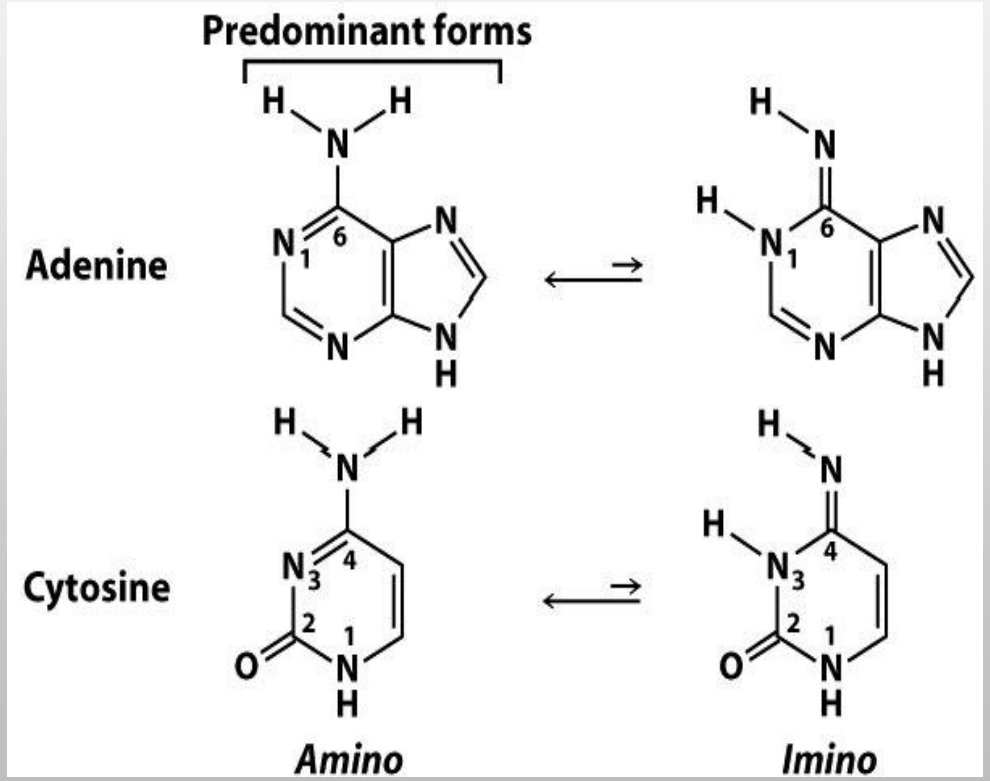
TAUTOMERISM:-THE ABILITY OF A MOLECULE TO EXIST IN MORE THAN ONE CHEMICAL FORM

Normal DNA bases and their pairing patter





REGULAR AND TAUTOMERIC FORMS OF DNA BASES



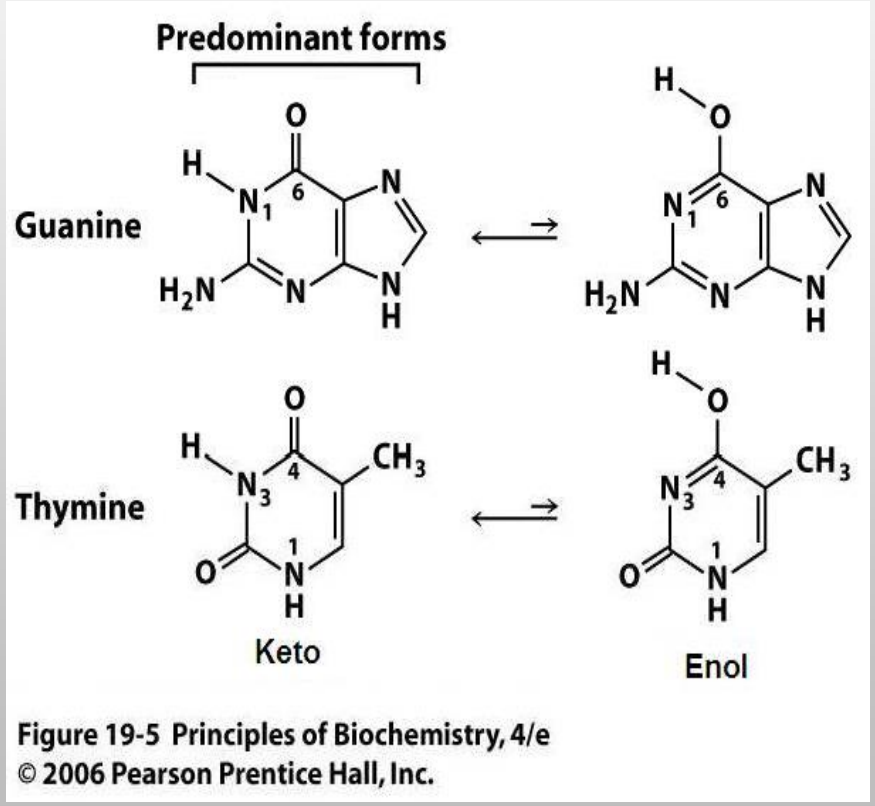
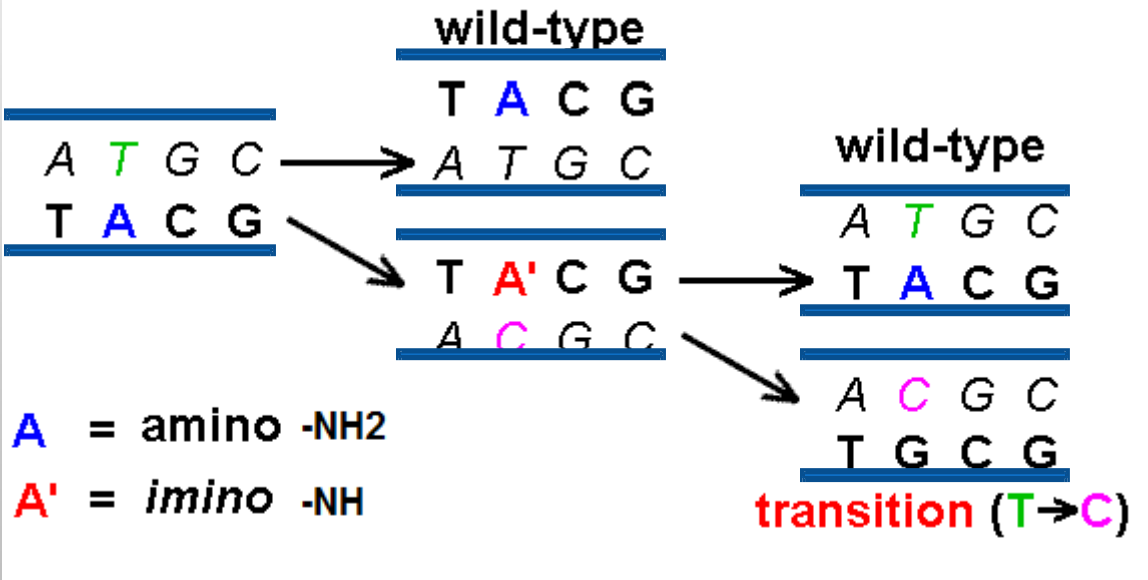


Figure 19-5 Principles of Biochemistry, 4/e

All the four common bases of DNA (adenine, guanine, cytosine and thymine) have unusual tautomeric forms, which are however rare. A tautomeric shift is believed to occur when an amino (NH_2) form of adenine is changed to an imino (NH) form.



Base change from AT to G C

MUTATION

Similarly a tautomeric shift may occur in thymine changing from keto (C=O) form to rare enol (COH) form. Natural base pairing in DNA is A=T and G≡C. The taustomeric forms are however capable of unusual (forbidden) base pairing like T≡G, G≡ T, C=A and A=C.

INDUCED MUTATION

Mutation can be induced artificially in the living organisms by exposing them to abnormal environment such as radiations, certain physical conditions (*i.e*. temperature) and chemicals.

MUTAGENS

The substances or agents which induce mutations are called mutagens or mutagenic agents. They may be physical, chemical or biological.

1. Physical –Radiations: i. Ionizing -X-rays, gamma, Alpha, Beeta, protons, nutrons *etc.*,

ii. Non Ionizing -Uv

Temperature-

2.CHEMICAL

Base Analogues: 2,aminopurine, 5-bromourasil *etc.*,

Base Modifying agents: Nitrous acid, Hydroxylamine *etc*.,

Distortion producing agents: Proflavin, acridine orange *etc*.,

3.Biological Mutagens: They may be viral or bacterial.

H. Pylori- implicated in stomach cancer

Hepatitis B virus- implicated in liver cancer

H. papiloma virus-implicated in cervical cancer

Human T-cell lymphocytic virus implicated in lymphoma

POINT MUTATION / GENE MUTATION

A mutation that changes only one small area or one nucleotide in a gene

Earliest record of point mutation dates back to 1791 by Seth Wright.

Since then mutations have been reported in *E. coli*, Neurospora, Pea, Maize, Rodents, Fowls, Man, *etc*.,

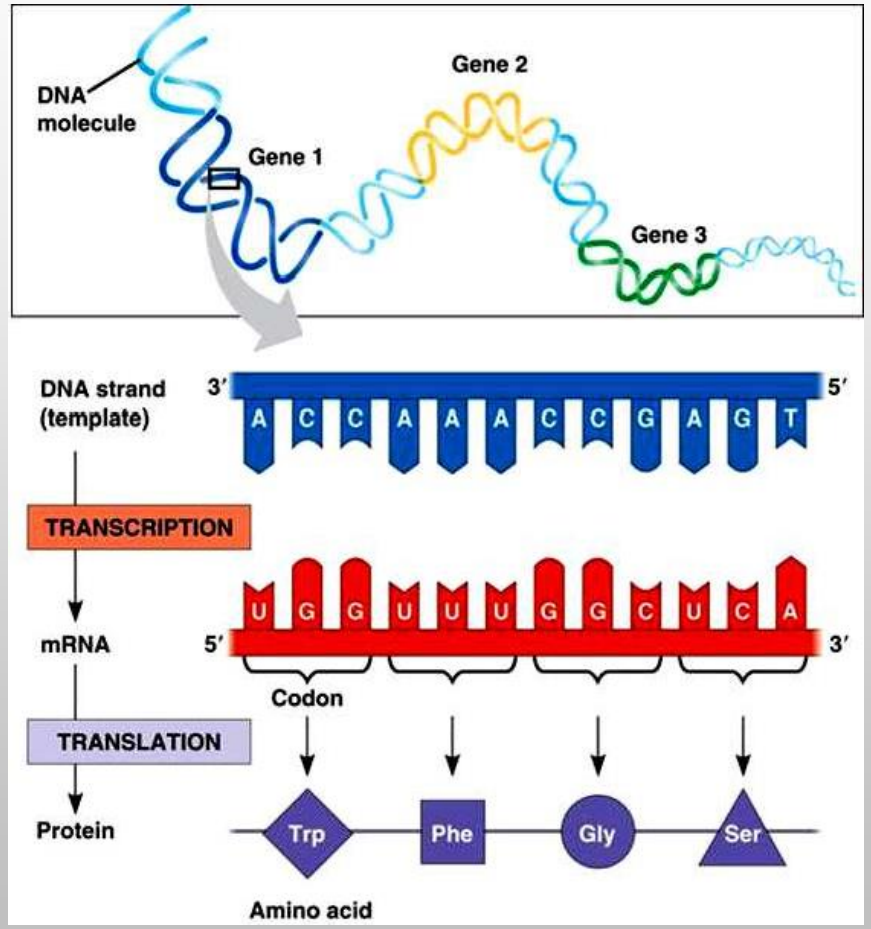
FRAME SHIFT MUTATION

A mutation that inserts or deletes a single base (normally single rarely more) will change the reading frame for the entire subsequent sequence.

A change of reading frame is called Frame shift mutation.

Central Dogma of Molecular Biology

Shows the connection between DNA, RNA & Protein

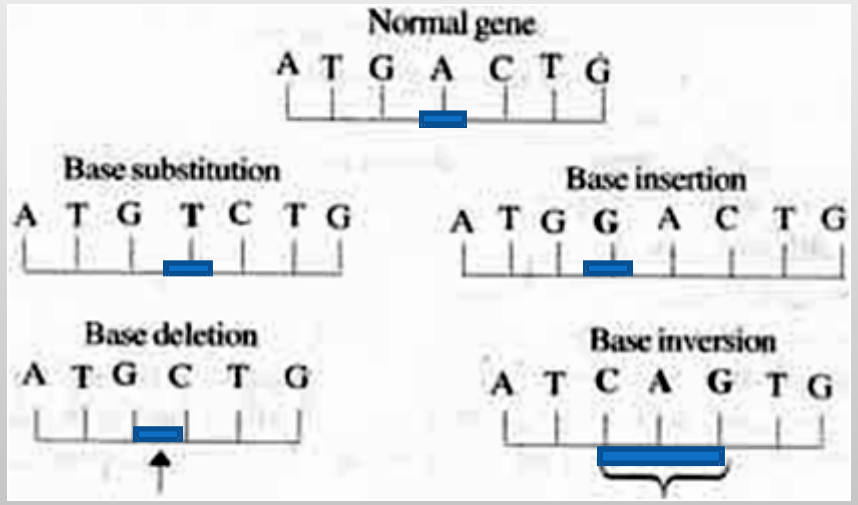


As shown in this picture,
If any change occurs in DNA Molecule; that alters the reading frame during Transcription and translation. Hence, it is called as Frame shift mutation

Frame Shift Mutation may be

i.Deletion: Removal of one or few bases from a nucleotide chain is called a deletion.

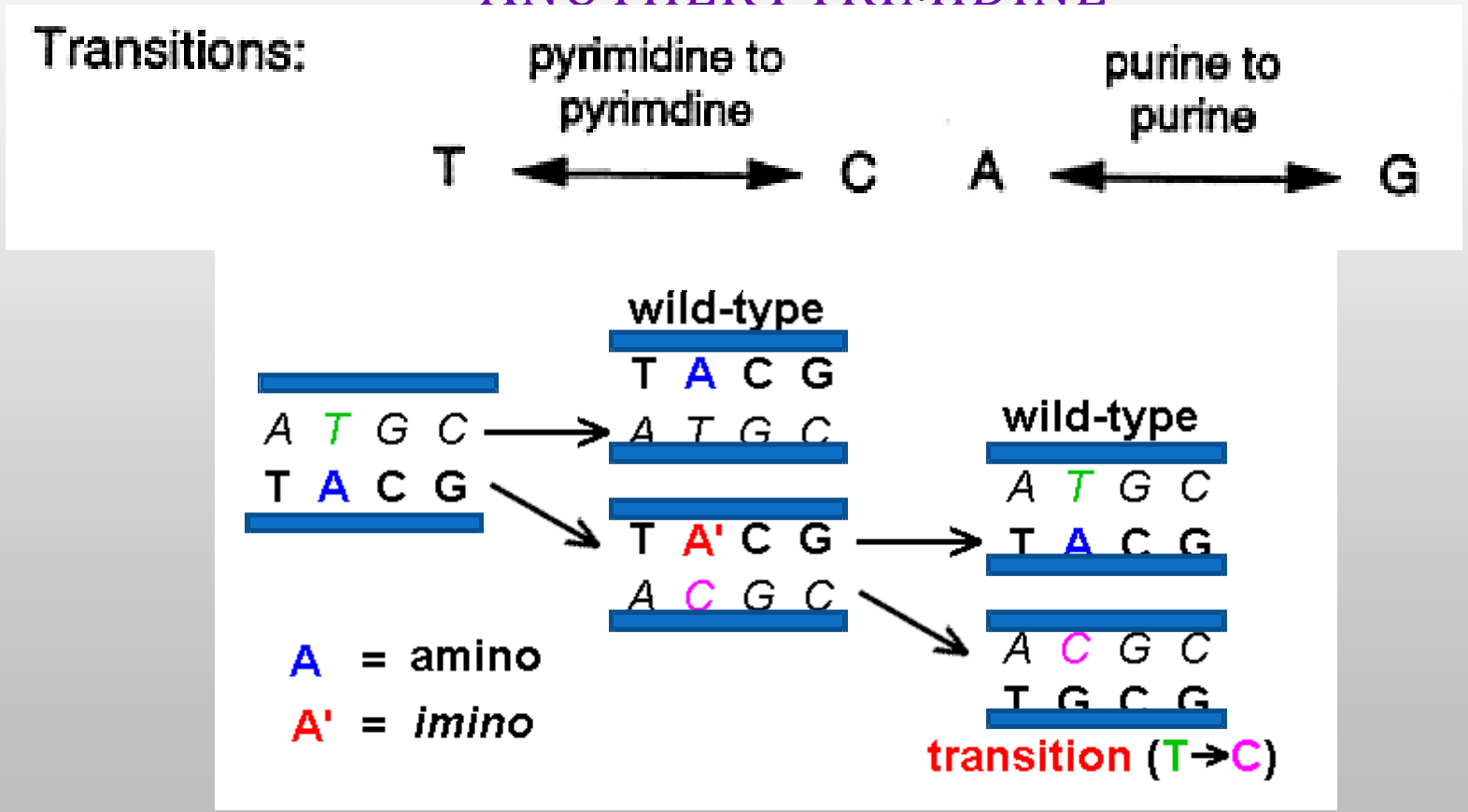
ii.Insertion: This is due to the addition of one or more (few) nucleotides to the DNA.



Substitution:- A nitrogenous base of a codon is replaced by another base is called substitution mutation.They maybe

1. **Transition**
2. **Transversion**

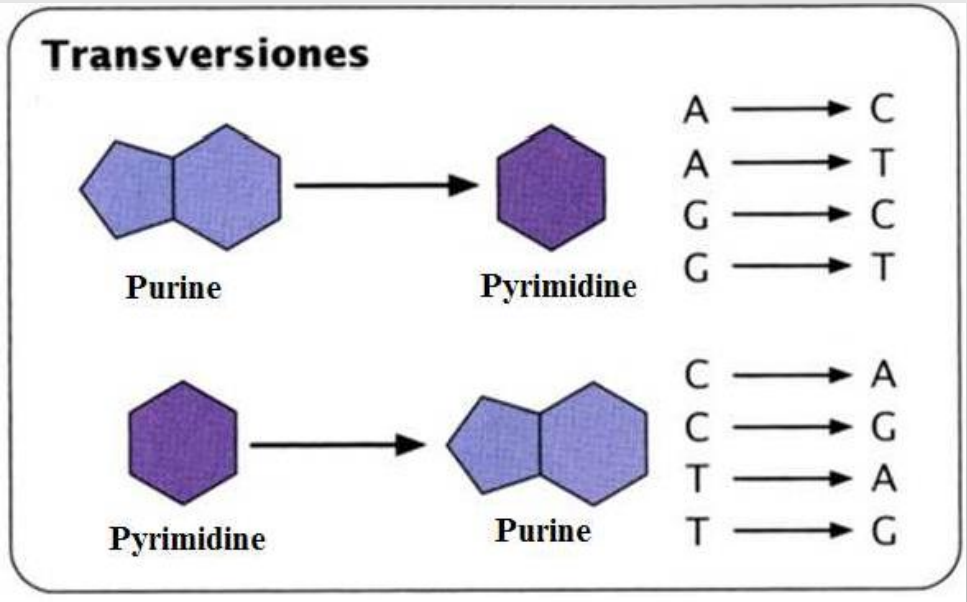
1. TRANSITION: WHEN A PURINE BASE OF A TRIPLET CODON IS SUBSTITUTED BY ANOTHER PURINE BASE OR A PYRIMIDINE BASE IS SUBSTITUTED BY ANOTHER PYRIMIDINE



Base change from AT to G C

MUTATION

Transversion: The substitution mutation when involves the substitution or replacement of a purine with a pyrimidine or vice versa, then such type of substitution mutation is called transversion mutation.



CHEMICAL MUTAGENESIS

Test for mutagenic effects of chemical agents are almost as old as modern genetics. In 1934, Morgan tried to produce mutations in Drosophila by treating with alcohol and ether, but without success. After a number of attempts by many workers the search for chemical agents met with only during Second World War.

Types:

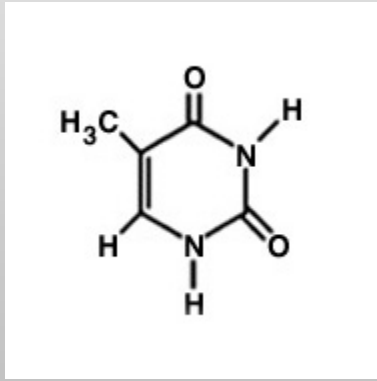
Copy Errors by Base Analogues

Direct Effect on DNA

Agents Producing Distorsions in DNA

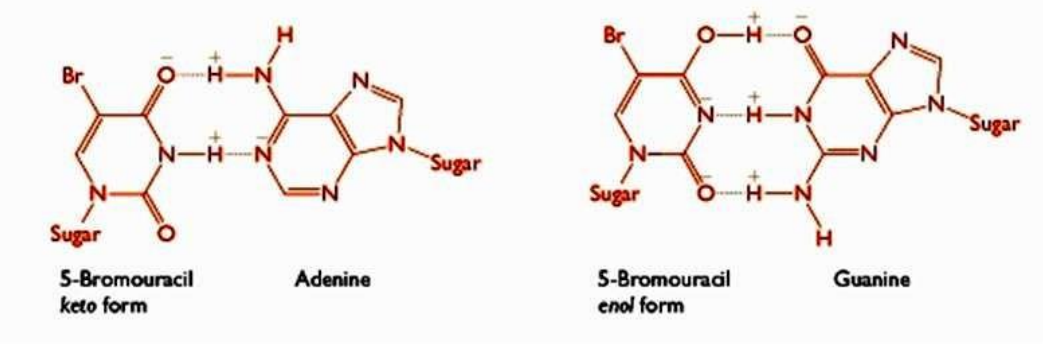
COPY ERRORS BY BASE ANALOGUES: THESE BASE ANALOGUES HAVE THE MOLECULAR STRUCTURE SIMILAR TO THAT OF NUCLEIC ACID BASES, WHICH ARE INCORPORATED IN TO DNA WITHOUT destroying its capacity for replication. However, because the analogue differs from the normal base in the distribution of hydrogen atoms, it has greater tendency for improper pairing and causes mutation.

Eg.,

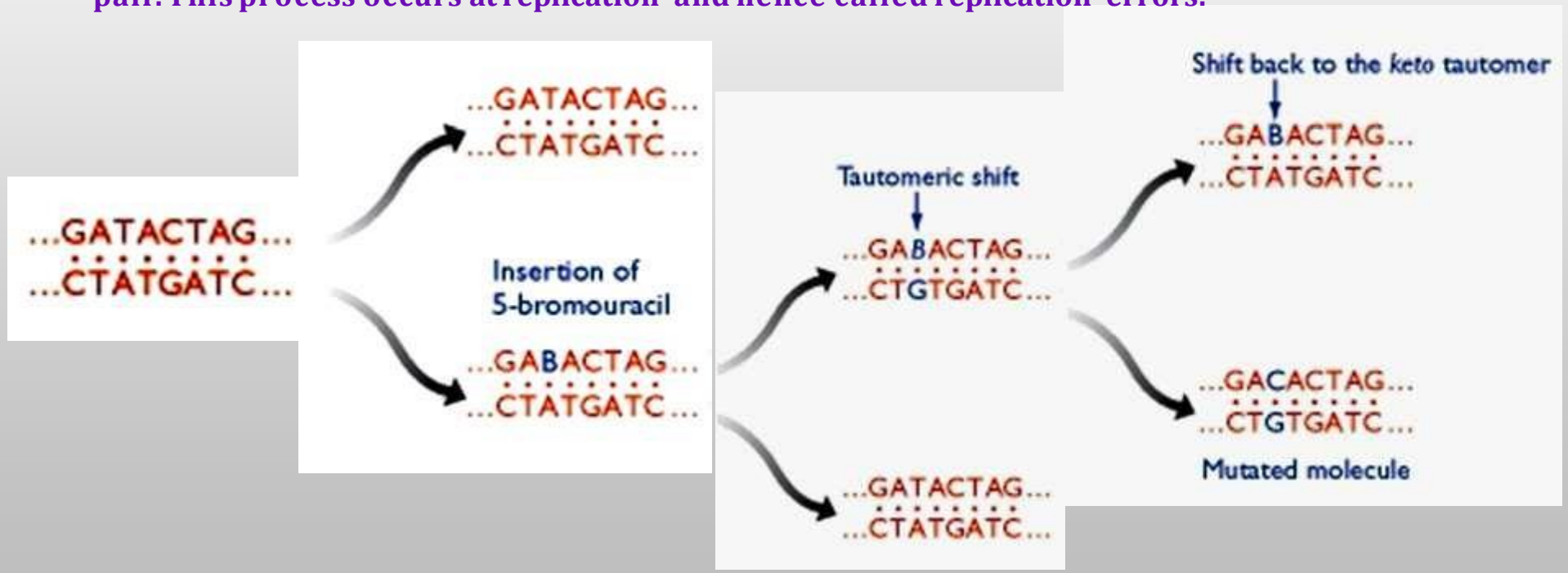


Thymine

5 Bromouracil

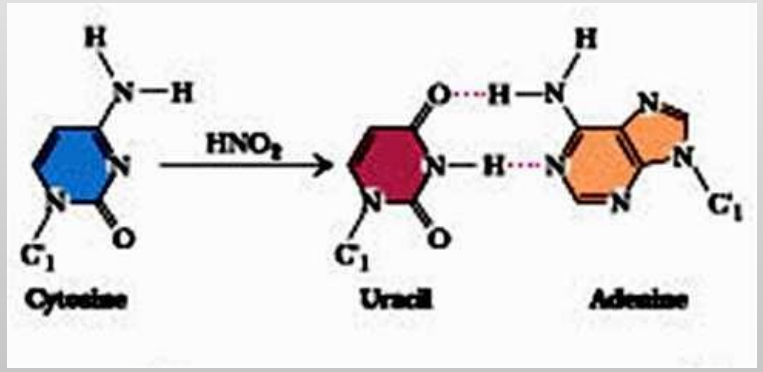


If 5-bu in enol state is incorporated in to DNA, in enol state it pairs with guanine and in the next generation in keto state it pairs with adenine. Thus the G ≡ C pair is replaced by A=T pair. This process occurs at replication and hence called replication errors.



DIRECT EFFECT ON DNA

AGENTS MODIFYING PURINES OR PYRIMIDINE INCLUDE NITROUS ACID (HNO_2), HYDROXYLAMINE (NH_2OH), ALKYLATING AGENTS



Guanine → Uracil =Adenine

Nitrous acid (HNO_2): it is a very powerful mutagen becauses it acts directly on the nucleic acid, replacing amino groups (NH_2) by hydroxyl groups (OH).

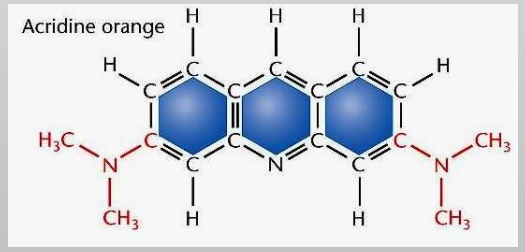
Hydroxylamine NH_2OH: It reacts specifically with cytosine and converts it to a modified base that pairs only with adenine so that a G ≡ C pair ultimately becomes an A = T pair

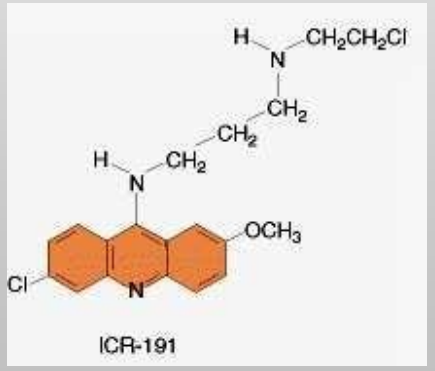
Cytocin $-(NH_2OH)->$Modified Base pairs with Adenine

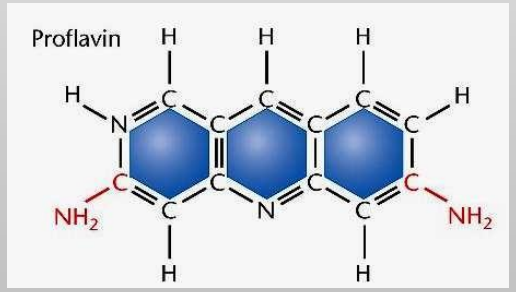
Alkylating Agents: Like ethyl methane sulfonate (EMS), ethyl ethane sulfonate (EES) have been used extensively in genetic engineering research. These alkylating agents impair the normal hydrogen bonding of the bases causing mispairing of G with T, leading to transition of A=T > G≡C and G≡C to A=T. also induces recessive lethal mutations, specific locus mutation, translocations, dominant lethal and partial and complete chromosomal loss in *Drosophila melanogaster*. EMS causes lethal mutations, deletion, translocation, dominant lethal in silkworm *Bombyx mori*.

AGENTS PRODUCING DISTORTIONS IN DNA

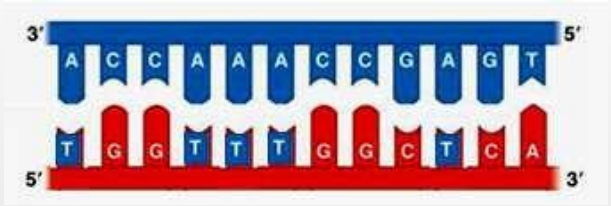
Certain fluorescent acridine dyes like proflavin and acridine orange causes mutations by insertion or deletion of bases. The acridines are planer (flat) molecules like purine bases and can be intercalated between the bases of the DNA helics. This distorts the structure of DNA. Result in deletion or insertion of bases during replication.





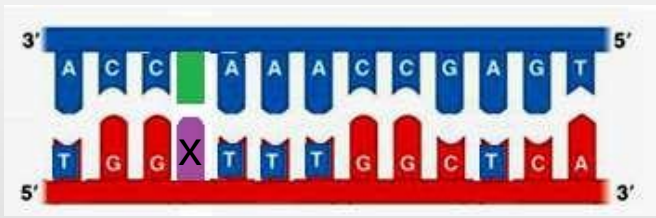


INTERCALATION RESULTING IN ADDITION OF THE BASE



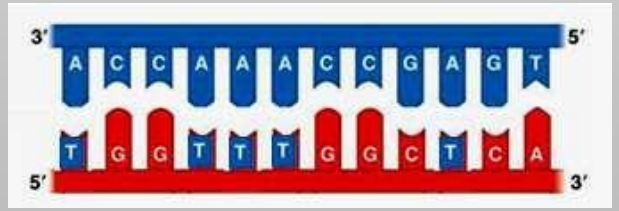
Acridine

Intercalation of the acridine molecule



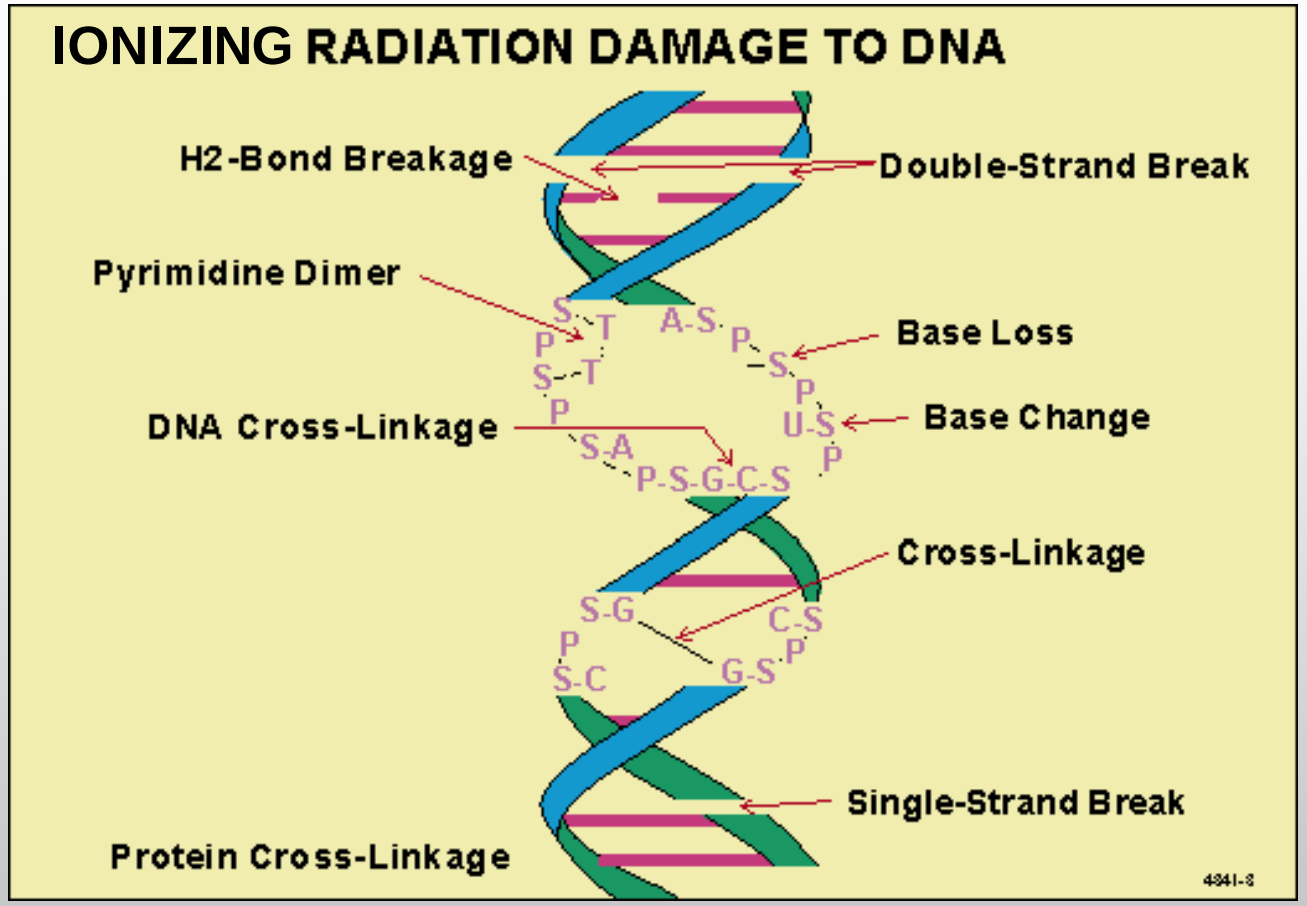
X-Unknown Base

a base (x') is inserted at random opposite the acridine molecule in the new chain.



RADIATION MUTAGENESIS

- **IONIZING RADIATIONS:- HIGH-ENERGY RADIATION CAPABLE OF PRODUCING IONIZATION IN SUBSTANCES THROUGH WHICH IT PASSES.**
- **IONIZATION:- ANY PROCESS THAT LEADS TO THE DISSOCIATION OF A NEUTRAL ATOM, MOLECULE OR OTHER SPECIES INTO IONS; THE STATE OF BEING IONIZED.**
- ***EG*., X-RAYS, A, B, GAMMA RAYS, FAST MOVING**
- **PARTICLES ETC.,**
- **NON IONIZING RADIATIONS:- UV, RADIO WAVES,**
- **VISIBLE LIGHT**



Besides ionizing radiations causes chromosome type as well as chromatid type aberrations namely terminal deletion, interstitial deletion, translocation, inversion etc., this can be seen under a compound microscope.

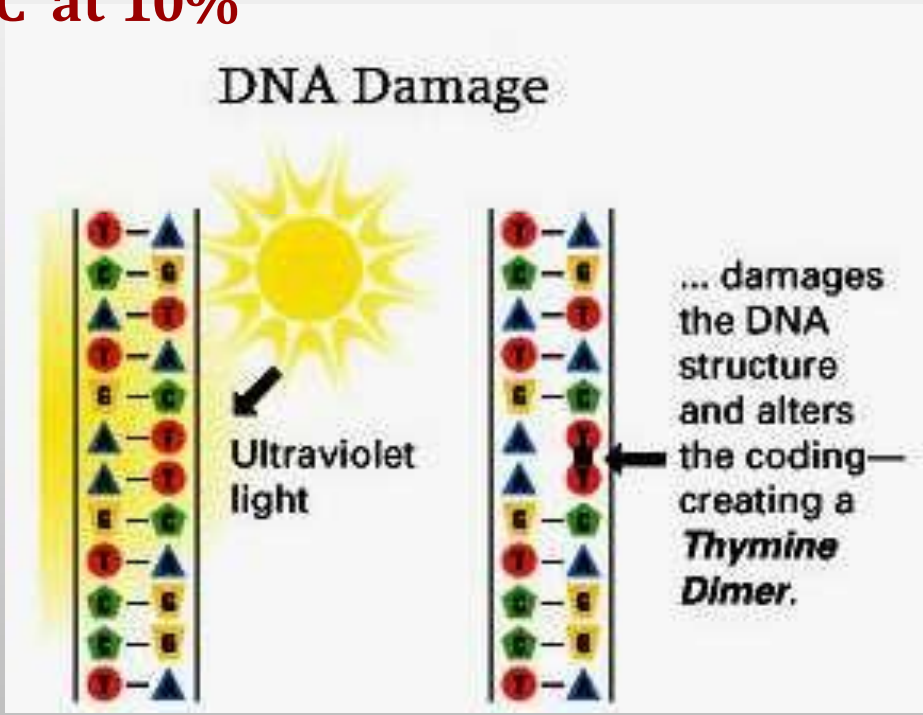
Effect of Non Ionizing radiation on DNA molecule

UV light causing nitrogen bases to become highly reactive free radicals. The resulting unstability causes conversion of one base to another (a purine to another purine or a pyrimidine to another pyrimidine).

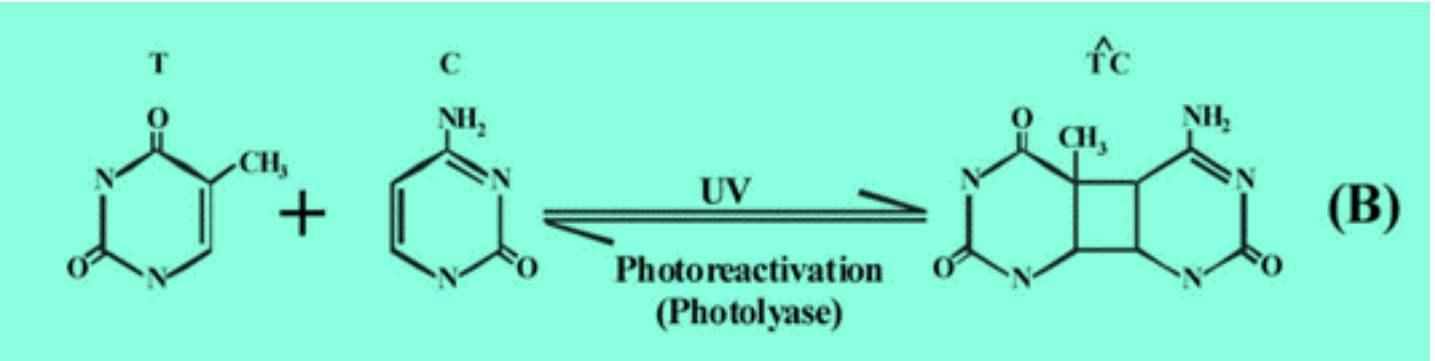
THE PRIMARY MUTAGENIC EFFECT OF UV LIGHT IS THE PRODUCTION OF DIMMERS. IRRADIATION OF A BACTERIAL CULTURE

and subsequent extraction of DNA yields three possible types of pyrimidine dimmers in DNA. That is T=T at 50%; T=C at 40% C=C at 10%

Thimine Dimers



Thymine cytosine Dimer formation and Repair mechanism



CIB TECHNIQUE

- This method was invented by **Muller** and used for the unequivocal **demonstration of mutagenic action of X rays**. In this method, females containing one normal x-chromosome and **another x-chromosome (C*I*B) containing extra 3 genes** are used for the analysis. Out of the 3 extra genes, **one gene suppresses crossover (c)**, the **other is a recessive lethal (L)** in heterozygous condition, and the **last gene is semidominant marker, bar (B) gene.**

• Females containing C*l*B chromosome are called as C*l*B stock drosophila. The normal males are exposed to mutagenic source for a fixed period and then mated to the C*l*B stock drosophila. **Males containing C*l*B chromosome will die** due to the effect of lethal genes, **whereas norm ill males and females both normal and with C*l*B will survive**.

• Females with c*l*b chromosomes and identified by **barred phenotype** are selected and crossed to normal males. **In this next generation 50% of males (which have received the C*l*B gene) will die.**

- If mutation has occurred in normal x chromosome then **even the normal male (without cib gene) will die**. If no mutation has occurred all the other **50% of males will survive**. The frequency of lethal mutations can be accurately scored in large samples. This technique is **simple, rapid and there is little chance of an error in scoring**. However, it is suitable for the scoring of sex linked recessive lethal only.