GENETICS 1ST

- Genetics is the study of heredity and variation
- Heredity is the transmission of characters from one generation to another
- Variation refers to the differences among individuals in a population

Gregor John Mendel laid down the foundation of genetics and is called father of genetics.

He worked on the pea plant (***Pisum sativum)*** and published his work in **1866**

But his work remained unnoticed during his life time due to various reasons

His work was **rediscovered** during **1900** by 3 different scientists simultaneously

1. **Hugo De Vries** – a Dutch biologist
2. **Carl Correns** – a German botanist
3. **Erich Von Tshermark** – an Austrian botanist

BASIC TERMINOLOGY

Trait – Character or feature in an individual, e.g height of plant or an animal etc

Factor – Physical basis of heredity, which get expressed as trait. Factor term was coined by Mendel and later replaced by Johannsen as **gene**

Alleles– Different forms of a gene occupying same locus on the homologous chromosome,

Dominant allele – The allele which expresses itself when both types of allele are present in an individual is called dominat allele. It is denoted by capital letter

Recessive Allele – The allele which can't express itself when present with dominat allele is called recessive allele. It is denoted by small letter

Homozygous (pure) – The individual having same alleles for a trait is called homozygous individual

Heterozygous(Hybrid) – The individual having diffrenet alleles for a trait is called heterozygous individual

Example

- let us take the example of **pea plant** where **height** is a trait, this trait has two contrasting forms **tall(T)** and **dwarf(t)** form, each form is controlled by a gene or factor, these two factors for height are called alleles.
- As alleles are present on homologous chromosomes and generally almost each cell of an individual have a pair of homologous chromosomes therefore every individual contains a pair of alleles.
- When the pea plant contains both the alleles for tallness or dwarfness it is called **homozygous individual(TT or tt)** and when the one of the allele is for tallness and the other for dwarfness it is called **heterozygous individual(Tt)**
- But in case of heterozygous condition(Tt) pea plants are tall, therefore allele for tallness expresses itself in heterozygous condition and is called **dominat allele(T)** where as the allele for **dwarfness(t)** remains unexpressed and is called recessive allele

Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

Genotype – It is the genetic constitution of an individual with respect to the traits taken into consideration, e.g in case of height in pea plant genetic constitution or genotype may be TT(both alleles for tallness), Tt(one allele for tallness and the other for dwarfness) or tt(both alleles for dwarfness)

Phenotype – It is appearance or observable characteristics in an individual, e.g in case of a pea plant the phenotype is either dwarf or tall with respect to height which we can observe

- It is actually genotype in association with the environment which controls the phenotype of an individual

F1 Generation – It is the generation produced by crossing organisms with different genotypes with respect to the trait taken into consideration. The F1 generation is also called F1 hybrids., e.g generation produced by crossing pea plants with genotype TT and tt

Parental Generation – The individuals producing F1 generation is called parental generation or P generation

F2 Generation – It is the generation produced by selfing or interbreeding F1 generation

Phenotypic ratio – The ratio of different phenotypes in a generation. This ratio is taken from observing the phenotypes of a generation

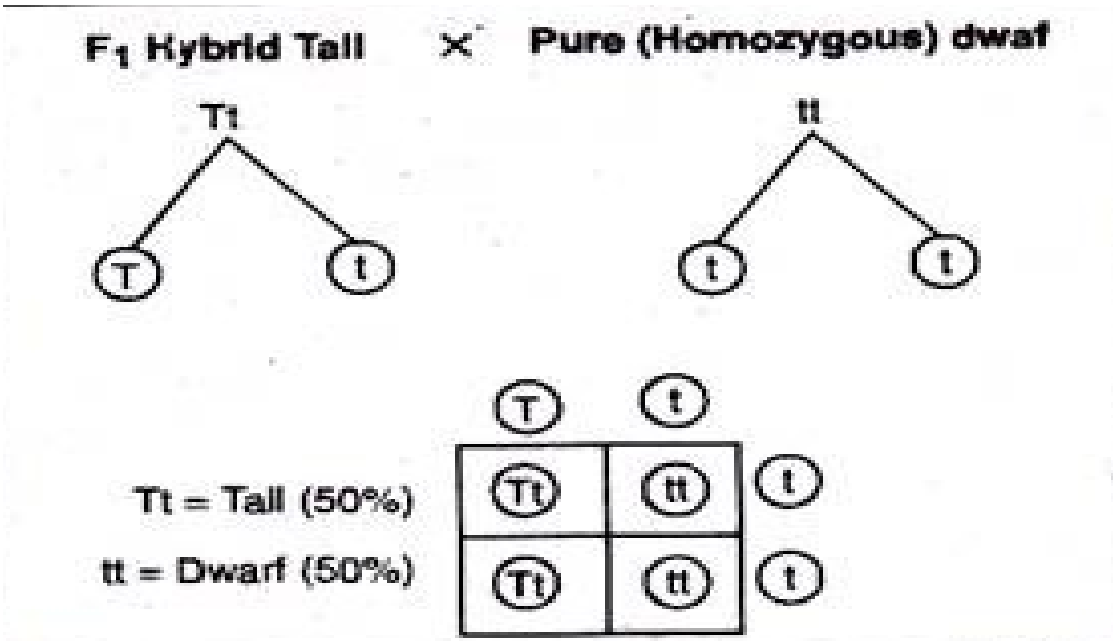
Genotypic ratio – The ratio of different genotypes in a generation. This ratio is obtained from punnet square

Back Cross –

It is the cross between **F1 hybrid and any of the parents**. It is used to get offspring with genetic identity closer to its parent

Test Cross –

It is the **back cross between F1 hybrid and the homozygous recessive parent**. It is used to know whether individual is homozygous or heterozygous for the dominant allele. If the test cross progeny comes out to have single type of phenotype then the F1 hybrid is homozygous , however if the progeny comes out to have more then one type of phenotype then the F1 hybrid is heterozygous



Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

Monohybrid cross – The cross in which single trait is taken into consideration at a time, e.g a cross between pea planta where only height is taken into account. Phenotypic ratio in monohybrid cross F2 generation is **3:1** and genotypic ratio is **1:2:1**

Dihybrid cross – The cross in which two traits are taken into consideration at a time, e.g cross between pea plant where the colour of the flower and seed shape is taken into consideration. The phenotypic ratio in F2 generation of dihybrid cross is **9:3:3:1**

Trihybrid cross – The cross in which 3 traits are taken into consideration at a time, e.g cross between pea plant where the colour of the flower and seed shape and seed colour is taken into consideration

Reciprocal cross - It is the second cross involving same genotypes as first cross but the sexes are reversed. If the first cross is Tall (female) x Dwarf (male), then the second or reciprocal cross will be Tall (male) x Dwarf(female)

Mendel And His Laws(Mendelism)…….1

Mendel worked on pea plant by selfing and crossing them in a controlled way. He studied the inheritance of seven different pairs of contrasting characters in this plant but mostly considered only one pair at a time.

List of seven pairs of contrasting characters in pea plant

Character	*Dominant*	*Recessive*
1. Stem length	Tall	Dwarf
2. Flower position	Axial	Terminal
3. Pod shape	Inflated	Constricted
4. Pod colour	Green	Yellow
5. Seed shape	Round	Wrinkled
6. Cotyledon colour	Yellow	Green
7. Seed coat colour	Grey	White

Mendel selected garden pea as his experimental material due to the following advantages:

1. Pea plant is easy to grow and interbreed.

2. Peas reproduce and grow to maturity in a season (an annual plant). Because of short life cycle several generations can be produced within a short period.

3 The flowers are bisexual containing both male and female parts. They are self-fertilizing in nature.

4. The pea plants have a number of contrasting characters.

5. It is easy to hybridize because pollen from one plant can be introduced into the stigma of another plant by removing anthers.

6. Because of easy self-fertilization, it is easy to get pure lines for several generations.

7. It produces fertile hybrids. This enabled Mendel to continue his experiments for generations.

From his study he concluded various assumptions which are summarized as Mendel's laws or principles

Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

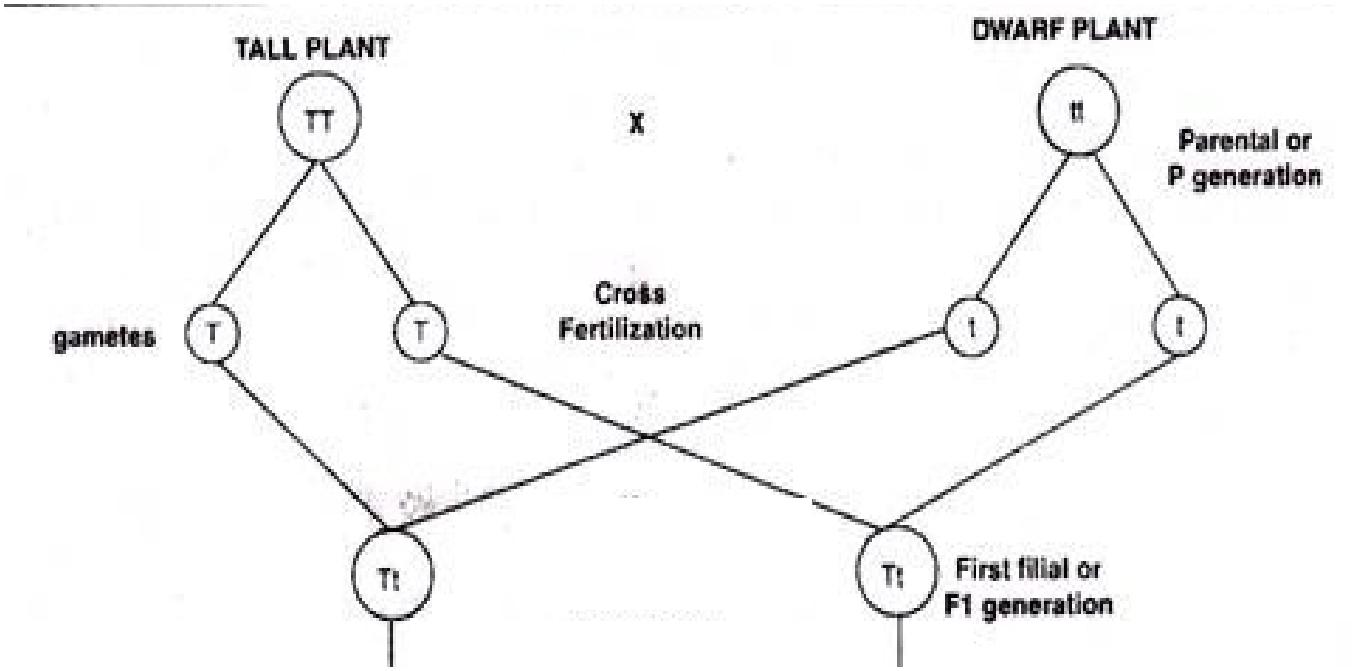
Law of Dominance

When a pair of contrasting characters (or allelomorphic characters or alleles) are present together, only one of them expresses itself and the other remains suppressed of hidden The character which is expressed (or is visible) is called as dominant and the character which remains hidden is termed as recessive.

Explanation

In a monohybrid cross experiment Mendel took a tall pea plant and crossed with a dwarf plant. He transferred the pollen grains of tall pea plants and placed them on the stigma of the dwarf pea plant and vice versa. To prevent self pollination he earlier removed all stamens from the flowers of the dwarf plant. Mendel noticed that all the progenies of F1 or first filial generation were tall plants. This gave him the clue to state the Law of dominance.

According to this law, out of a pair of contrasting characters (tallness and dwarfness) one character (tallness) appeared in the F1, generation and the other character (dwarfness) remained hidden or suppressed. The character which appeared in Ft generation is called dominant and the other character remained hidden is called recessive character. These two contrasting characters are known as allelomorphic characters or allelomorphs or alleles



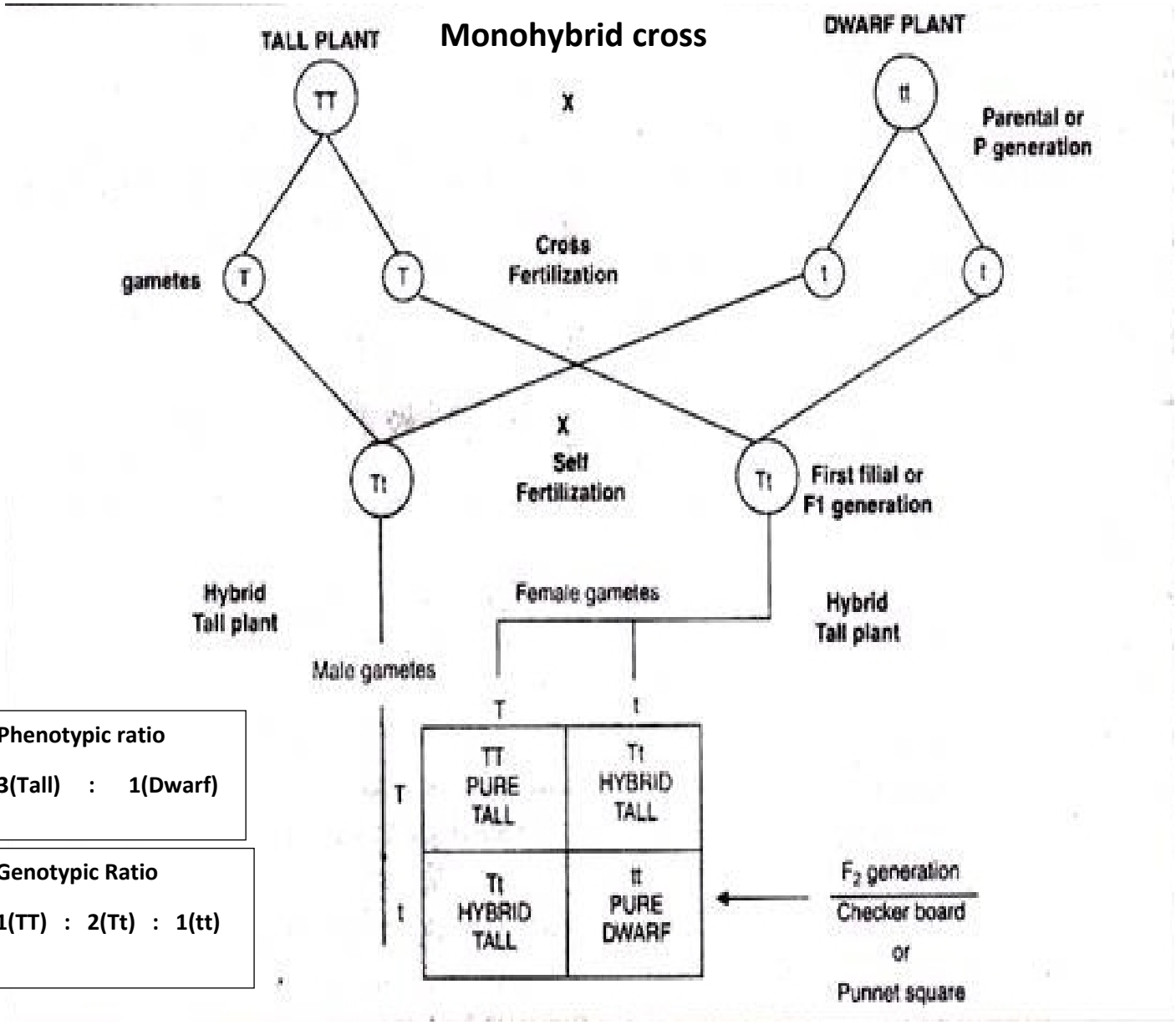
Law of segregation

The Law of Segregation states that every individual organism contains two alleles for each trait, and that these alleles segregate (separate) during meiosis such that each gamete contains only one of the alleles. An offspring thus receives a pair of alleles for a trait by inheriting homologous chromosomes from the parent organisms: one allele for each trait from each parent.

Explanation

According to this law the F1 plants have both the factor T and t which never fuse or mix. When such plants form gametes, the factors T and l separate from each other and enter separate zygotes. Thus, in the F2 generation dwarf plant appears, besides true tall and hybrid tall plants

Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag



Phenotypic ratio

3(Tall) : 1(Dwarf)

Genotypic Ratio

1(TT) : 2(Tt) : 1(tt)

Law of independent assortment

When two or more pairs of contrasting characters are taken into consideration in a cross, each factor, assort or place itself independently of the other (lining its passages from on; generation to the other.

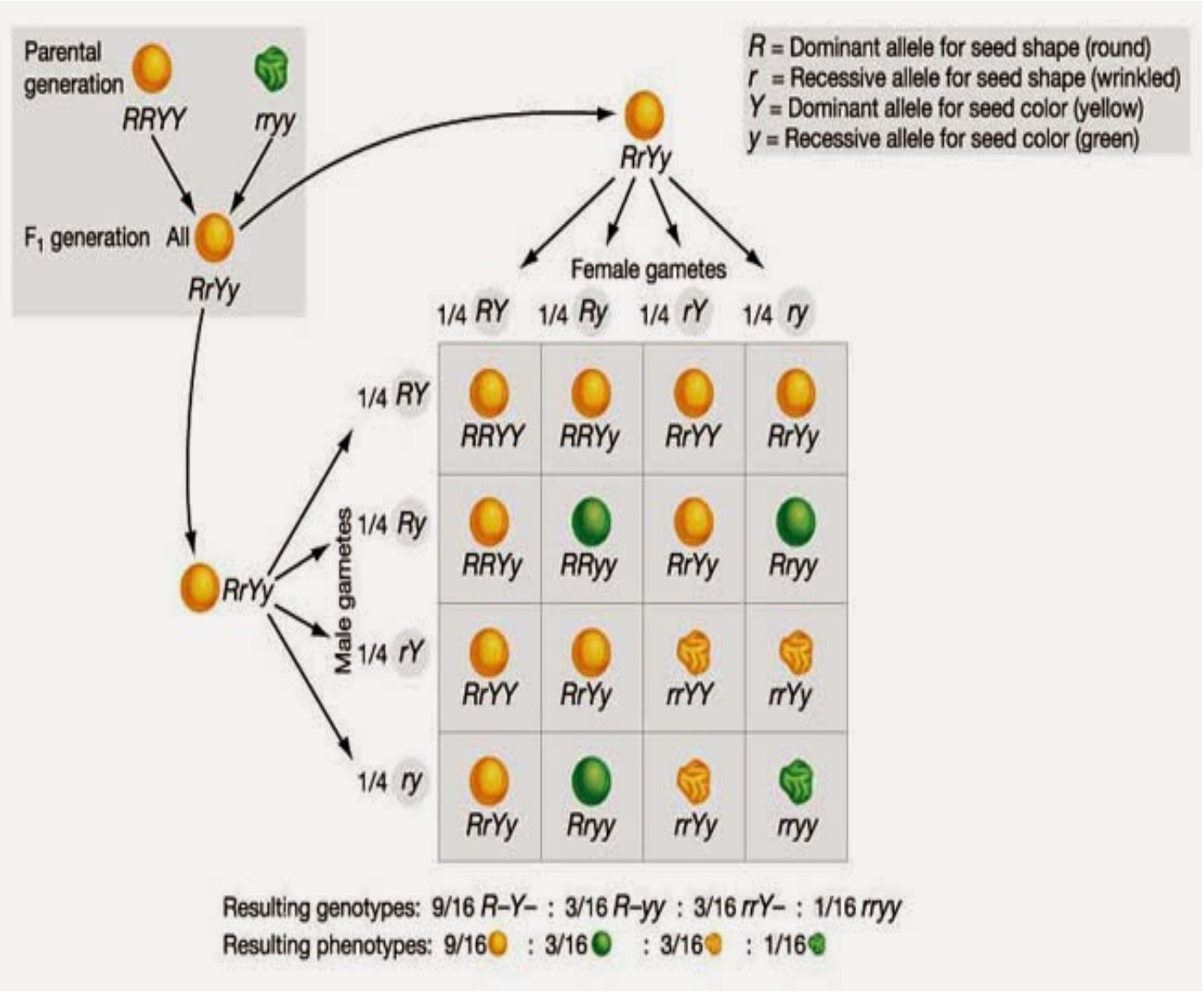
Explanation

In a di-hybrid cross the parents differ through two characters. Mendel conducted a cross between a true breeding Round Yellow plants (RRYY) with true breeding Wrinkled Green plant (rryy). Round and Wrinkled are the shapes of seed coat whereas Yellow and Green are the colours of the seed coat.

Mendel observed the seeds of the F1 plants were all Round and Yellow. This showed Round and Yellow were dominant over Wrinkled, Green. In the F2 generation four types of combinations were observed such as Round Yellow, Wrinkled Yellow, Round Green and Wrinkled Green. Thus the above types of offspring's of F2 generation were produced in the ratio of 9:3:3:1. This ratio is called Di-hybrid ratio

Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

In his dihybrid cross experiment Mendel observed round and yellow characters are dominant over wrinkled and green so that all the F1 offspring's are round and yellow. In the F2 generation, he noticed an allele (dominant or recessive) of a given character freely combines with either one (dominant or recessive) of the alleles of another character. Hence, a dominant allele of a character combines, not only with the dominant, but also with the recessive allele of another character.



Round – Yellow × Wrinkled – Green – P (Parental generation)

(Pure) (Pure)

Round Yellow × Round Yellow – F_1 (First filial generation)

(self fertilisation)

Round Yellow	Round Green	Wrinkled Yellow	Wrinkled Green	F_2 (Second filial generation)
9	3	3	1	

Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

Exceptions to Mendelism(Non Mendelian Genetics) OR Gene Interactions

Following the rediscovery of Mendelian principles of heredity in 1900, scientists all the over the world tried to verify these laws by conducting experiments on different species of plants and animals. To their surprise it was found that Mendelian principles did not come out true universally, rather there were many cases of exceptions to this.

Mendelian genetics does not explain all kinds of inheritance for which the phenotypic ratios in some cases are different from Mendelian ratios (3:1 for monohybrid, 9:3:3:1 for di-hybrid in F2). This is because sometimes a particular allele may be partially or equally dom-inant to the other or due to existence of more than two alleles or due to lethal alleles. These kinds of genetic interactions between the alleles of a single gene are referred to as allelic or intra- allelic interactions.

Non-allelic or inter-allelic interactions also occur where the development of single character is due to two or more genes affecting the expression of each other in various ways.

Thus, the expression of gene is not inde-pendent of each other and dependent on the presence or absence of other gene or genes; These kinds of deviations from Mendelian one gene-one trait concept is known as Factor Hypothesis or Interaction of Genes

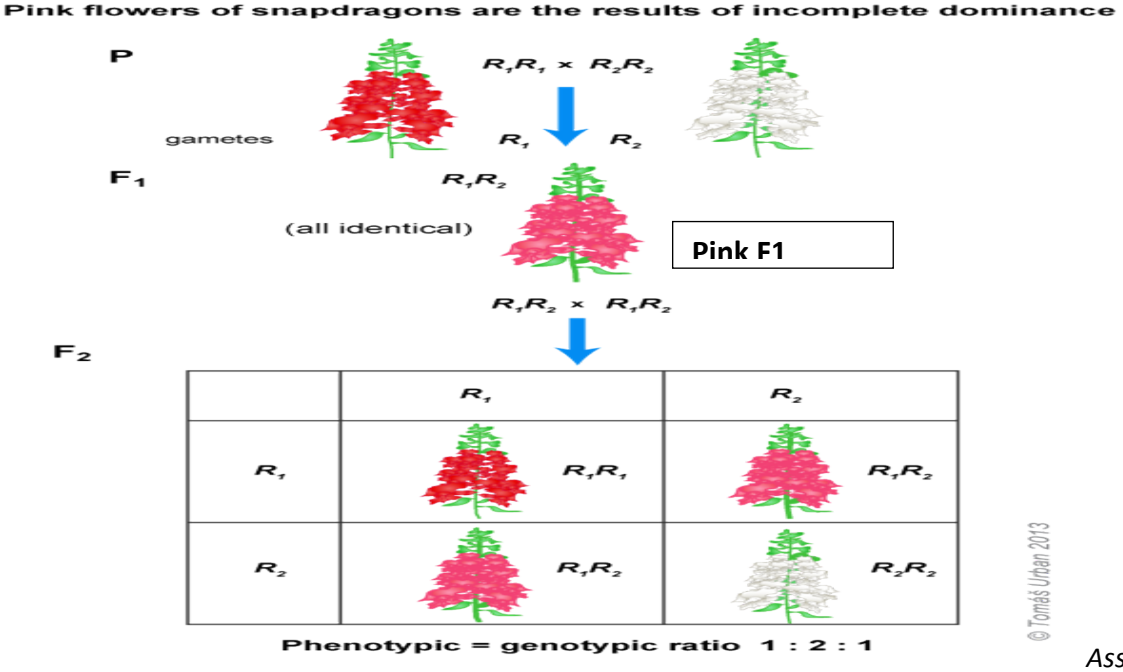
Allelic Gene Interactions

Incomplete Dominance

When none of the alleges show dominance and the F1 hybrid shows phenotype different from both the parents it is called incomplete Dominance

e.g, A monohybrid cross between a red-flowered snapdragon (*Antirrhinum majus*) and a white flowered variety does not produce red or white flowered plants in F1 as expected from mendelism. Instead the flowers are pink, i.e. intermediate between the two parents. This is because neither red flower colour nor white is dominant, but each allele has its influence in color development and the hybrid appears pink.

If the F1 pink flowers are self-pollinated, the F2 progeny shows red, pink and white flowered plants in the proportion 1: 2: 1. It may be recalled that this is the same genotypic ratio that Mendel obtained in garden peas. The difference is that in the present case the heterozygous progeny is distinct in appearance from the homozygotes**.**



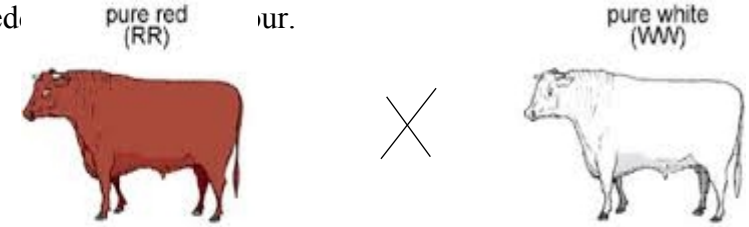
Asif Ahmed Kamgar
Asst. professor Zoology
G D C Boys Anantnag

Co Dominance

Codominance is a form of dominance wherein the alleles of a gene pair in a heterozygote are fully or co expressed. This results in offspring with a phenotype that is neither dominant nor recessive or we can say both the alleys express itself

e.g, "roan" (reddish grey) coat colour of short horn cattle. When homozygous red-haired cattle are crossed with homozygous white-haired type, the F1 has reddish grey hair and is designated "roan".It must be noted that there is no mixture of red and grey pigments in a roan. But some hair are all red, others all white, so that the final effect is a red colour.

P generation



Gametes. R. W

F1 generation

Multiple Allelism

The phenomenon of presence of more then two alleles of a trait in a population is known as multiple allelism

These alleles are called multiple alleles, Although individual humans (and all diploid organisms) can only have two alleles for a given gene, multiple alleles may exist in a population level, and different individuals in the population may have different pairs of these alleles. Multiple alleles are situated on homologous chromosomes at the same locus

e.g, Blood type is an example of a common multiple allele trait. There are 3 different alleles for blood type, IA, IB and i. IA is dominant to i. IB is also dominant to i. IA and IB are both co dominant. IA is a gene for the production of the anti-gin A. IB for antigen B, and i for neither antigen.Allele IA results in blood group A, allele IB give blood group B and Allele i gives blood group O. Therefore the various combinations of these 3 alleles result in blood group A, B and O as

Blood groups	Genotype
AB	I^AI^B
B	I^BI^B or $I^B i$
A	I^AI^A or $I^A i$
O	ii

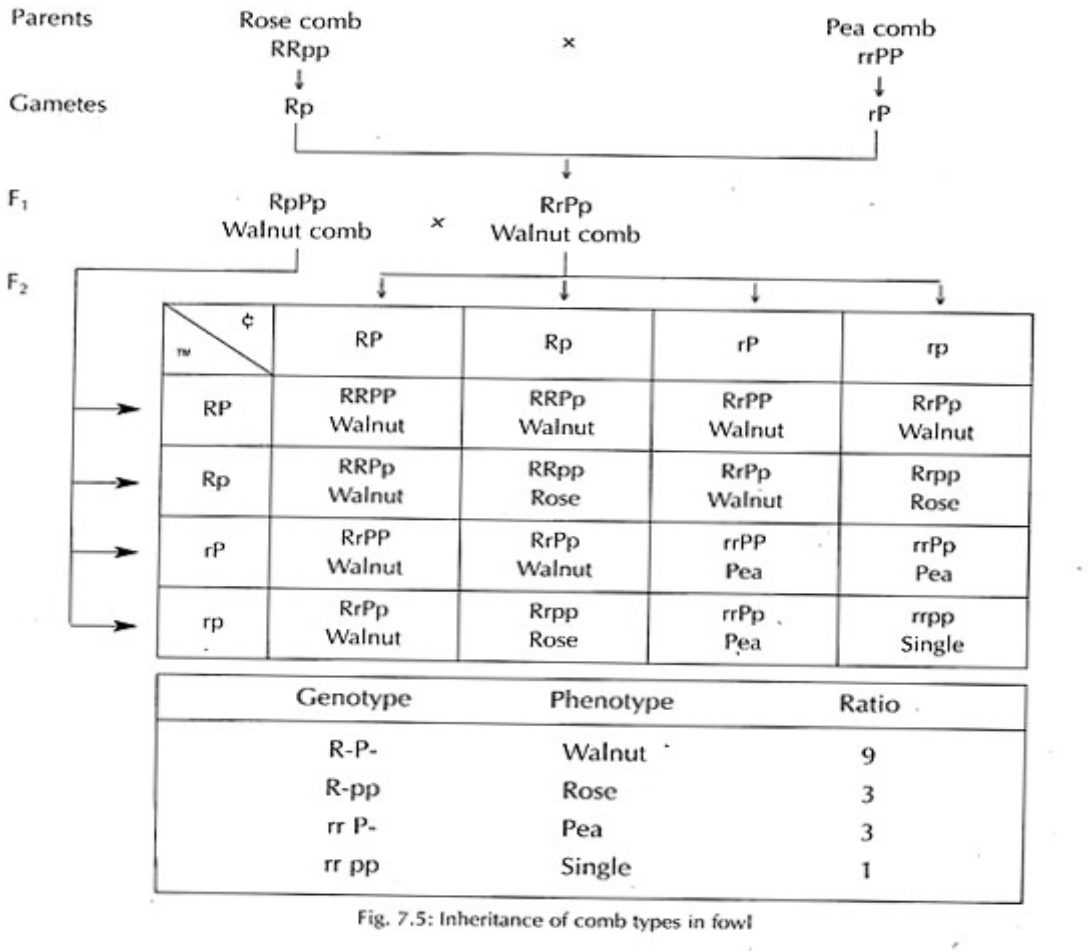
Asif Ahmed Kamgar
Asst. professor Zoology
G D C Boys Anantnag

Non Allelic Gene Interactions:

Simple Interaction (9:3:3:1):

In this case, two non-alleiic gene pairs affect the same character. The dominant allele of each of the two factors produces separate phenotypes when they are alone. When both the dominant alleles are present together, they produce a distinct new phenotype. The absence of both the dominant alleles gives rise to yet another phenotype.

The inheritance of comb types in fowls is the best example where R gene gives rise to rose comb and P gene gives rise to pea comb; both are dominant over single comb; the presence of both the dominant genes results in walnut comb (Fig. 7.5). Similar pattern of inheritance is found in Streptocarpus flower colour (Fig. 7.6).



♂ \ ♀	RP	Rp	rP	rp
RP	RRPP Walnut	RRPp Walnut	RrPP Walnut	RrPp Walnut
Rp	RRPp Walnut	RRpp Rose	RrPp Walnut	Rrpp Rose
rP	RrPP Walnut	RrPp Walnut	rrPP Pea	rrPp Pea
rp	RrPp Walnut	Rrpp Rose	rrPp Pea	rrpp Single

Genotype	Phenotype	Ratio
R-P-	Walnut	9
R-pp	Rose	3
rr P-	Pea	3
rr pp	Single	1

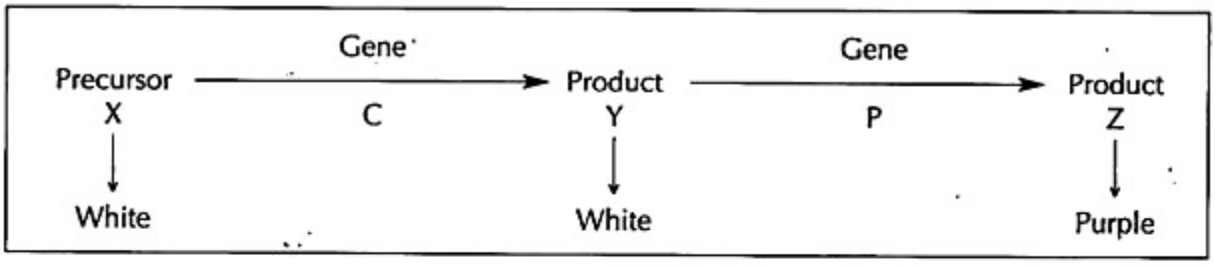
Fig. 7.5: Inheritance of comb types in fowl

Complementary Factor (9:7):

Certain characters are produced by the interaction between two or more genes occupying different loci inherited from different parents. These genes are complementary to one another, i.e., if present alone they remain unexpressed, only when they are brought together through suitable crossing will express.

In sweet pea (Lathyrus odoratus), both the genes C and P are required to synthesize anthocyanin pigment causing purple colour. But absence of any one cannot produce anthocyanin causing white flower. So C and P are complementary to each other for anthocyanin formation (Fig. 7.7).

Asif Ahmed Kamgar
Asst. professor Zoology
G D C Boys Anantnag



Parents: White CCpp × White ccPP

Gametes: Cp, cP

F_1: CcPp Purple

Selfing

F_2:

Genotype	Phenotype	Ratio
C-P-	Purple	9
C-pp	White	3
cc P-	White	3
cc pp	White	1

(3 + 3 + 1 = 7)

Fig. 7.7: Inheritance of flower colour in Lathyrus odoratus

Involvement of more than two complementary genes is possible, e.g., three complementary genes governing aleurone colour in maize.

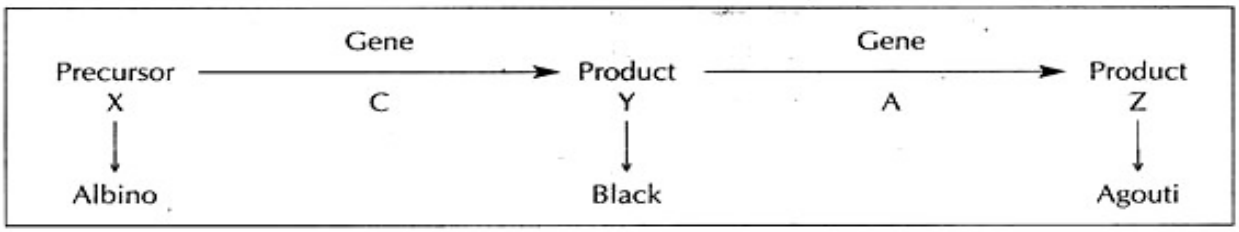
Epistasis:

When a gene or gene pair masks or prevents the expression of other non-allelic gene, called epistasis. The gene which produces the effect called epistatic gene and the gene whose expression is suppressed called hypostatic gene.

(a) Recessive Epistasis or Supplementary Factor (9:3:4):

In this case, homozygous recessive condition of a gene determines the phenotype irrespective of the alleles of other gene pairs, i.e., recessive allele hides the effect of the other gene. The coat colour of mice is controlled by two pairs of genes.

Dominant gene C produces black colour, absence of it causes albino. Gene A produces agouti colour in presence of C, but cannot express in absence of it (with cc) resulting in albino. Thus recessive allele c (cc) is epistatic to dominant allele A (Fig. 7.8).



Parents: Black CCaa × Albino ccAA

Gametes: Ca × cA

F_1: CcAa Agouti × CcAa Agouti

F_2:

Genotype	Phenotype	Ratio
C-A-	Agouti	9
C-aa	Black	3
cc A-	Albino	3 } 4
cc aa	Albino	1 }

Fig. 7.8: Inheritance of coat colour in mice

(b) Dominant Epistasis (12:3:1):

Sometimes a dominant gene does not allow the expression of other non-allelic gene called dominant epistasis. In summer squash, the fruit colour is governed by two genes. The dominant gene W for white colour, suppresses the expression of the gene Y which controls yellow colour. So yellow colour appears only in absence of W. Thus W is epistatic to Y. In absence of both W and Y, green colour develops (Fig. 7.10).

Parents: White WWYY × Green wwyy

Gametes: WY × wy

F_1: WwYy White

Selfing

F_2:

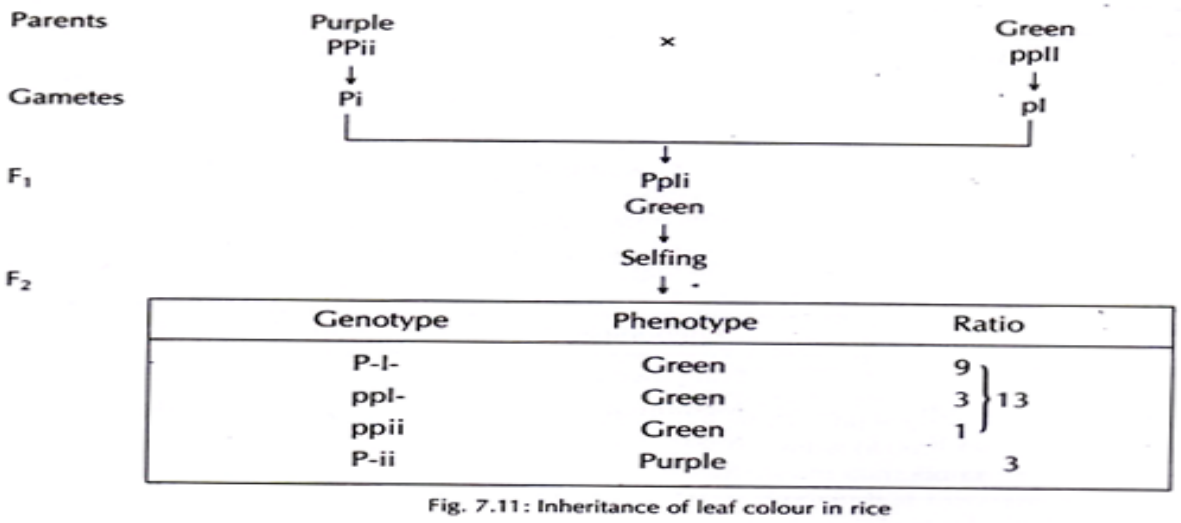
Genotype	Phenotype	Ratio
W-Y-	White	9 } 12
W-yy	White	3 }
ww Y-	Yellow	3
ww yy	Green	1

Fig. 7.10: Inheritance of fruit colour in summer squash

Asif Ahmed Kamgar
Asst. professor Zoology
G D C Boys Anantnag

Inhibitory Factor:

Inhibitory factor is such a gene which itself has no phenotypic effect but inhibits the expression of another non-allelic gene; in rice, purple leaf colour is due to gene P, and p causing green colour. Another non-allelic dominant gene I inhibits the expression of P but is ineffective in recessive form (ii). Thus the factor I has no visible effect of its own but inhibits the colour expression of P (Fig. 7.11).



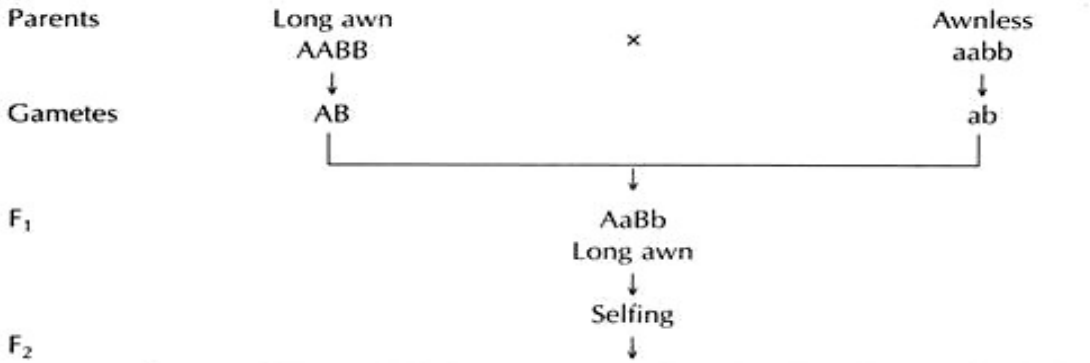
Genotype	Phenotype	Ratio
P-I-	Green	9 } 13
ppI-	Green	3 } 13
ppii	Green	1 } 13
P-ii	Purple	3

Fig. 7.11: Inheritance of leaf colour in rice

Polymorphic Gene (9:6:1):

Here two non-allelilc genes controlling a character produce identical phenotype when they are alone, but when both the genes are present together their phenotypes effect is enhanced due to cumulative effect. In barley, two genes A and B affect the length of awns.

Gene A or B alone gives rise to awns of medium length (the effect of A is same as B); but when both present, long awn is produced; absence of both results aweless (Fig. 7.13).



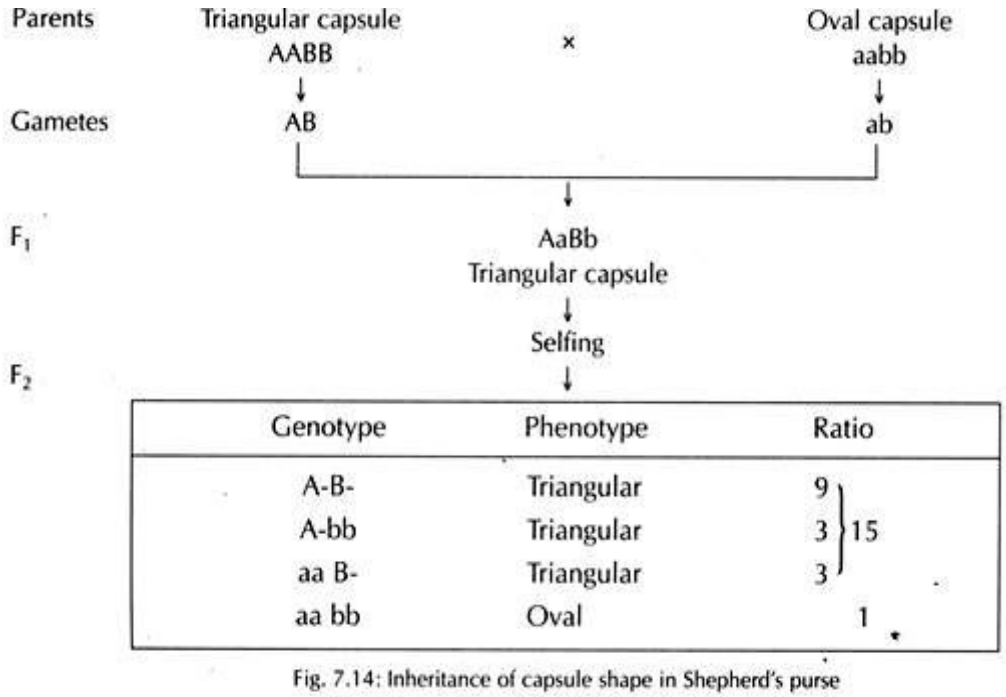
Genotype	Phenotype	Ratio
A-B-	Long awn	9
A-bb	Medium awn	3 } 6
aa B-	Medium awn	3 } 6
aa bb	Awnless	1

Fig. 7.13: Inheritance of awns in barley

Asif Ahmed Kamgar
Asst. professor Zoology
G D C Boys Anantnag

Duplicate Gene (15:1):

Sometimes a character is controlled by two non-allelic genes whose dominant alleles produce the same phenotype whether they are alone or together. In Shepherd's purse (Capsella bursa-pastoris), the presence of either gene A or gene B or both results in triangular capsules; when both these genes are in recessive forms, the oval capsules produced (Fig. 7.14).



Genotype	Phenotype	Ratio
A-B-	Triangular	9 } 15
A-bb	Triangular	3 } 15
aa B-	Triangular	3 } 15
aa bb	Oval	1

Fig. 7.14: Inheritance of capsule shape in Shepherd's purse

Asif Ahmed Kamgar
Asst. professor Zoology
G D C Boys Anantnag

linkage

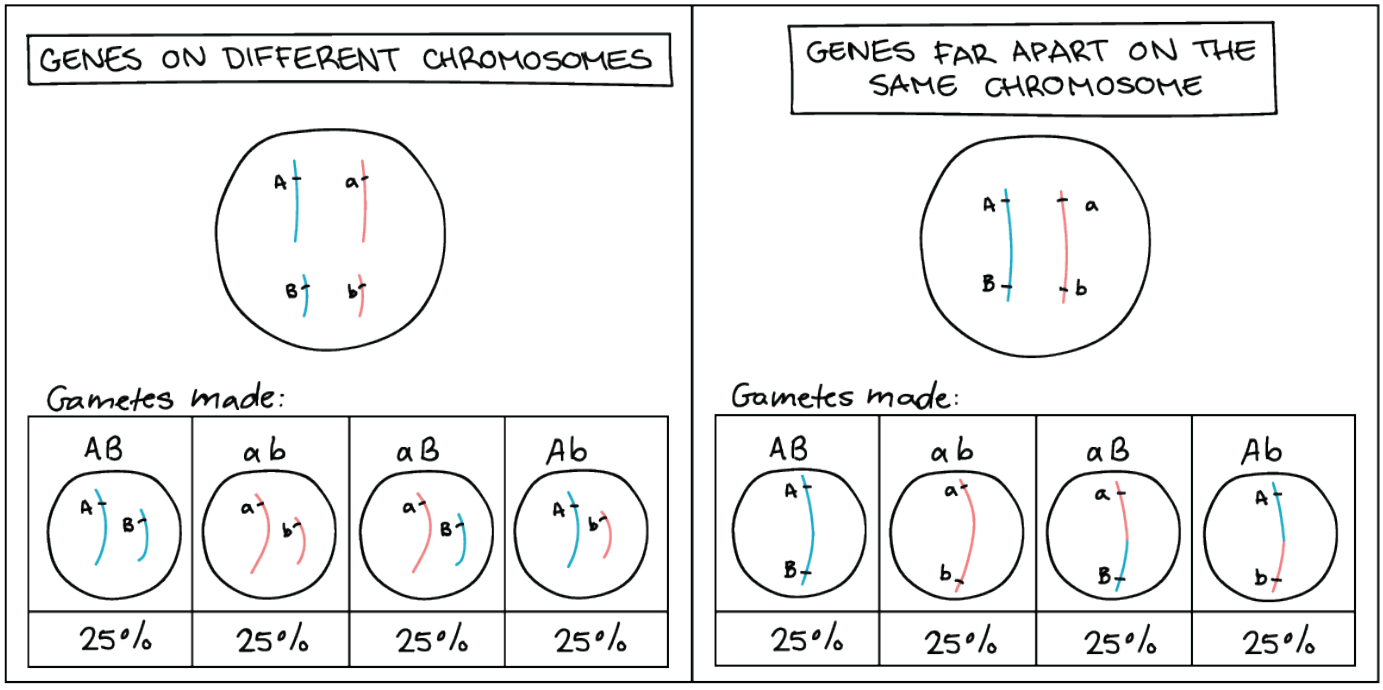
When two or more characters or genes from parents are transmitted to the offsprings of few generations such as F1, F2, F3 etc. without any recombination, they are called as the linked characters or genes and the phenomenon is called as linkage. Or the tendency of two or more genes of the same chromosome to remain together in the process of inheritance is called linkage

- Genetic linkage is actually the tendency of DNA sequences that are close together on a chromosome to be inherited together during the meiosis phase of sexual reproduction.
- When genes are close together on the same chromosome, they are said to be linked. That means the alleles, or gene versions, already together on one chromosome will be inherited as a unit more frequently than not.
- Mendel's law of independent assortment is applicable to the genes that are situated in separate chromosomes. When genes for different characters are located in the same chromosome, they are tied to one another and are said to be linked, therefore does not assort independently
- When genes are linked then the %age of non-parental offspring's is less then 50% in test cross but unlinked genes show 50% non-parental progeny

Explanation of the concept

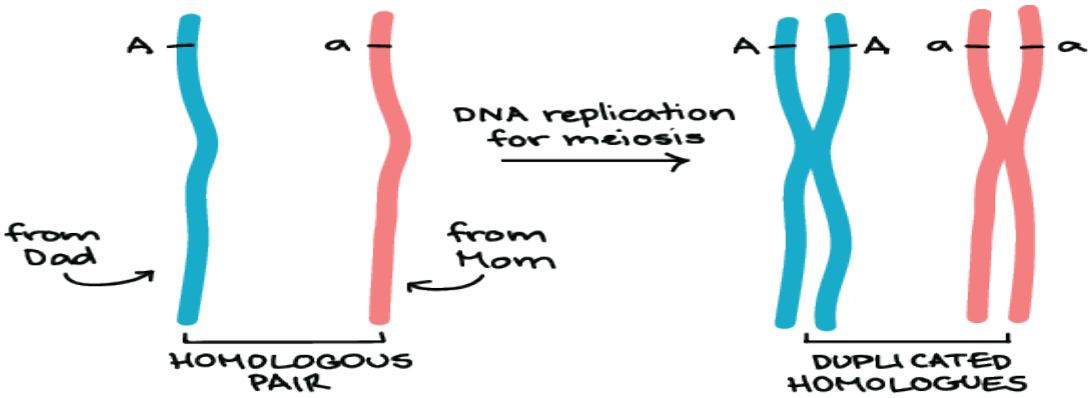
Traits are controlled by factors or genes and genes are present on chromosomes and in diploid organisms chromosomes are in pairs where each pair is known as homologous chromosomes. Different genes may be present on same or different chromosomes but alleles are present on homologous chromosomes with same position.

When genes are on separate chromosomes, or very far apart on the same chromosomes, they assort independently. That is, when the genes go into gametes, the allele received for one gene doesn't affect the allele received for the other. In a double heterozygous organism (AaBb), this results in the formation of all 444 possible types of gametes with equal, or 25\%25%25, percent, frequency.

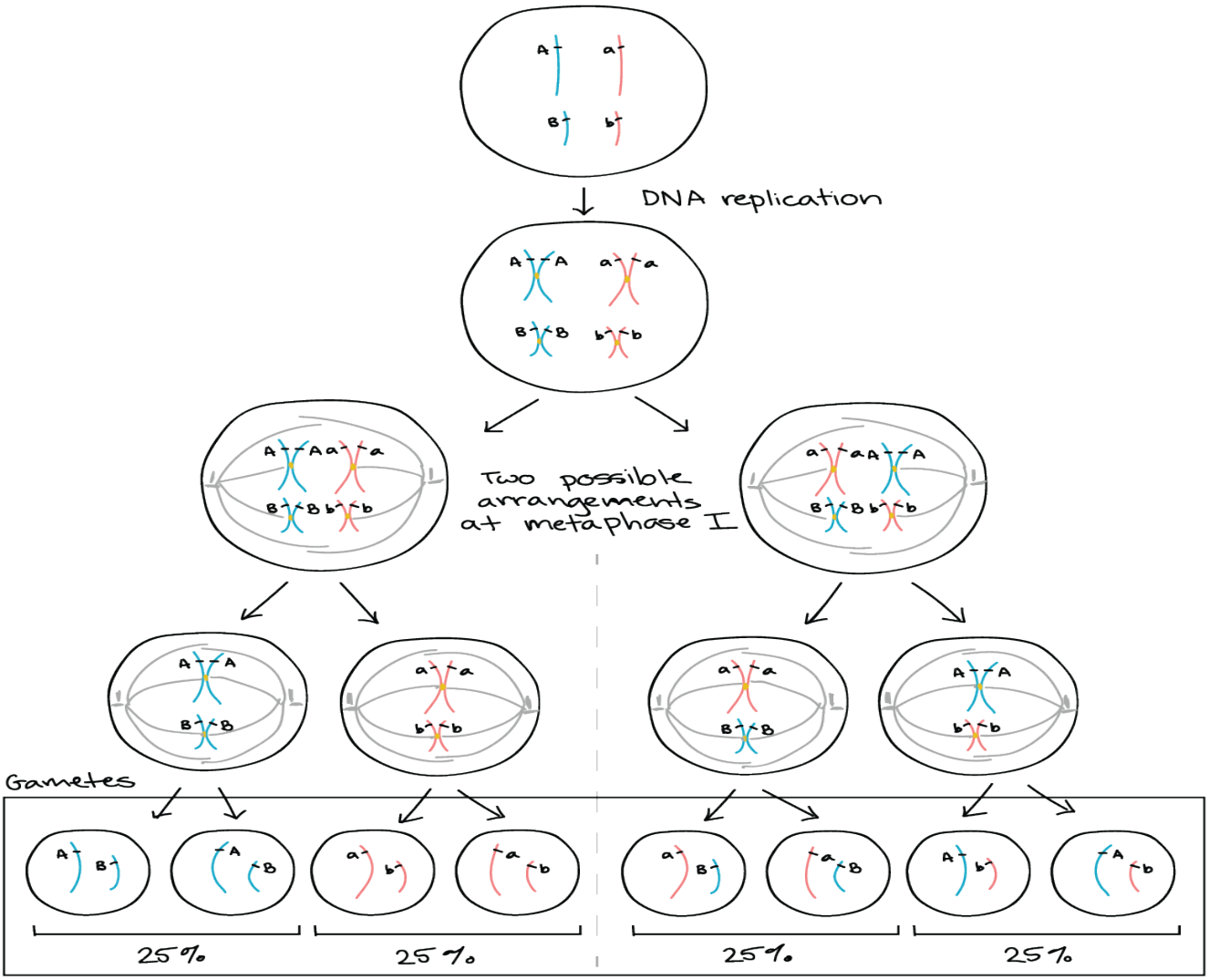


Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

Why is this the case? Genes on separate chromosomes assort independently because of the random orientation of homologous chromosome pairs during meiosis. Homologous chromosomes are paired chromosomes that carry the same genes, but may have different alleles of those genes. One member of each homologous pair comes from an organism's mom, the other from its dad.

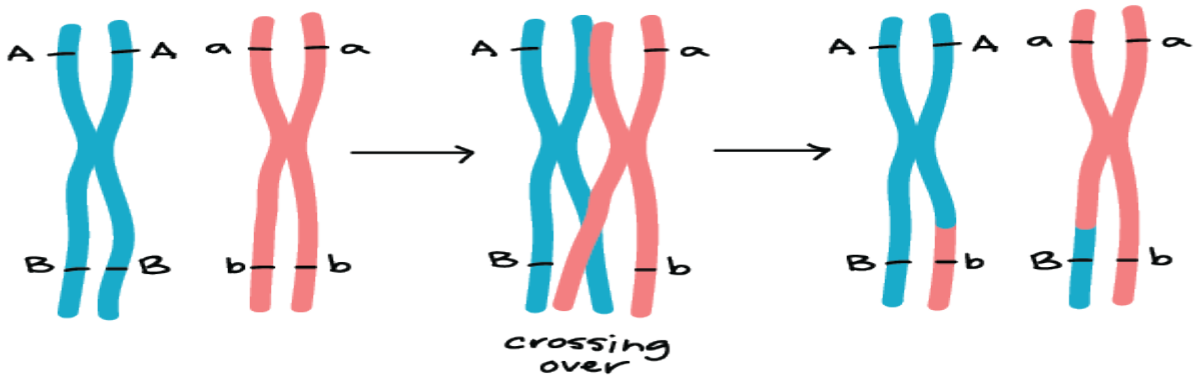


As illustrated in the diagram below, the homologues of each pair separate in the first stage of meiosis. In this process, which side the "dad" and "mom" chromosomes of each pair go to is random. When we are following two genes, this results in four types of gametes that are produced with equal frequency.

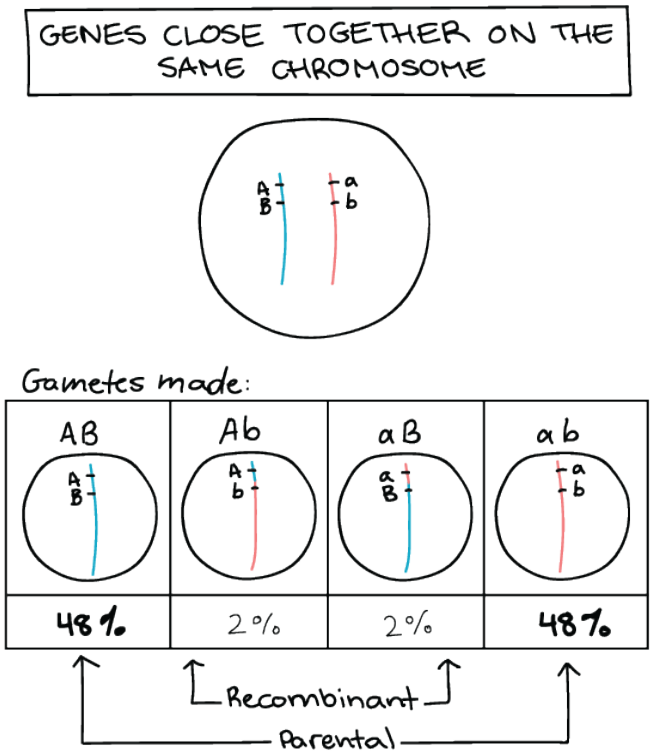


Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

When genes are on the same chromosome but very far apart, they assort independently due to crossing over (homologous recombination). This is a process that happens at the very beginning of meiosis, in which homologous chromosomes randomly exchange matching fragments. Crossing over can put new alleles together in combination on the same chromosome, causing them to go into the same gamete. When genes are far apart, crossing over happens often enough that all types of gametes are produced with 25 percent frequency.



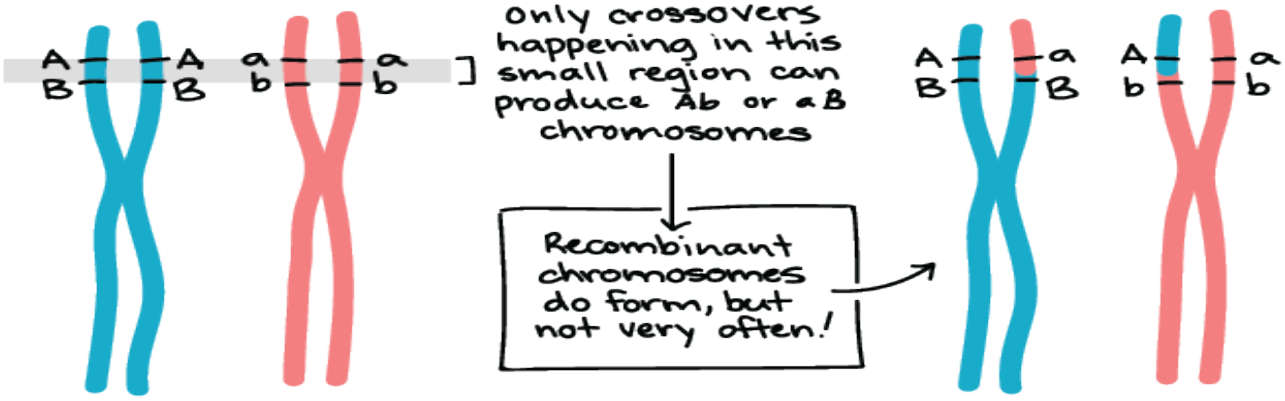
When genes are very close together on the same chromosome, crossing over still occurs, but the outcome (in terms of gamete types produced) is different. Instead of assorting independently, the genes tend to "stick together" during meiosis. That is, the alleles of the genes that are already together on a chromosome will tend to be passed as a unit to gametes. In this case, the genes are linked. For example, two linked genes might behave like this:



Now, we see gamete types that are present in very unequal proportions. The common types of gametes contain parental configurations of alleles—that is, the ones that were already together on the chromosome in the organism before meiosis (i.e, on the chromosome it got from its parents). The rare types of gametes contain recombinant configurations of alleles, that is, ones that can only form if a recombination event (crossover) occurs in between the genes.

Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

Why are the recombinant gamete types rare? The basic reason is that crossovers between two genes that are close together are not very common. Crossovers during meiosis happen at more or less random positions along the chromosome, so the frequency of crossovers between two genes depends on the distance between them. A very short distance is, effectively, a very small "target" for crossover events, meaning that few such events will take place (as compared to the number of events between two further-apart genes).



Thanks to this relationship, we can use the frequency of recombination events between two genes (i.e., their degree of genetic linkage) to estimate their relative distance apart on the chromosome. Two very close-together genes will have very few recombination events and be tightly linked, while two genes that are slightly further apart will have more recombination events and be less tightly linked.

Linkage Groups

Linkage group refers to a group of genes which are present in one chromosome. In other words, all those genes which are located in one Chromosome constitute one linkage group. The number of linkage groups is limited in each individual. The maximum number of linkage groups is equal to the haploid chromosome number of an organism.Eg- For example there are ten linkage groups in corn (2n = 20), seven in garden pea (2n = 14), seven in barley (2n =14), four in Drosophila melanogaster (2n = 8) and 23 in man (2n = 46).

Discovery

Bateson and Punnet (1906), were the first to report linkage , while working with sweet pea (*Lathyrus odoratus*) they observed that flower colour and pollen shape tend to remain together and do not assort independently as per Mendel's law of independent assortment.

When two different varieties of sweet pea—one having red flowers and round pollen grain and other having blue flower and long pollen grain were crossed, the F1 plants were blue flowered with long pollen (blue long characters were respectively dominant over red and round characters). When these blue long (heterozygous) hybrids were crossed with double recessive red and round (homozygous) individuals (test cross), they failed to produce expected 1:1:1:1 ratio in F2 generation. These actually produced following four combinations in the ratio of 7 : 1 : 1 : 7 (7 blue long : 1 blue round : 1 red long : 7 red round)

The above result of the test cross clearly indicates that the parental combinations (blue, long and red, round) are seven times more numerous than the non-parental combinations. Bateson and Punnet suggested that the genes (such as B and L) coming from the same parent (BBLL × bbll) tend to enter the same gamete and to be inherited together (coupling). Similarly, the genes (B and 1) coming from two different parents (such as BBLL x bbll), tend to enter different gametes and to be inherited separately and independently (repulsion). This is calle coupling and repulsion theory of

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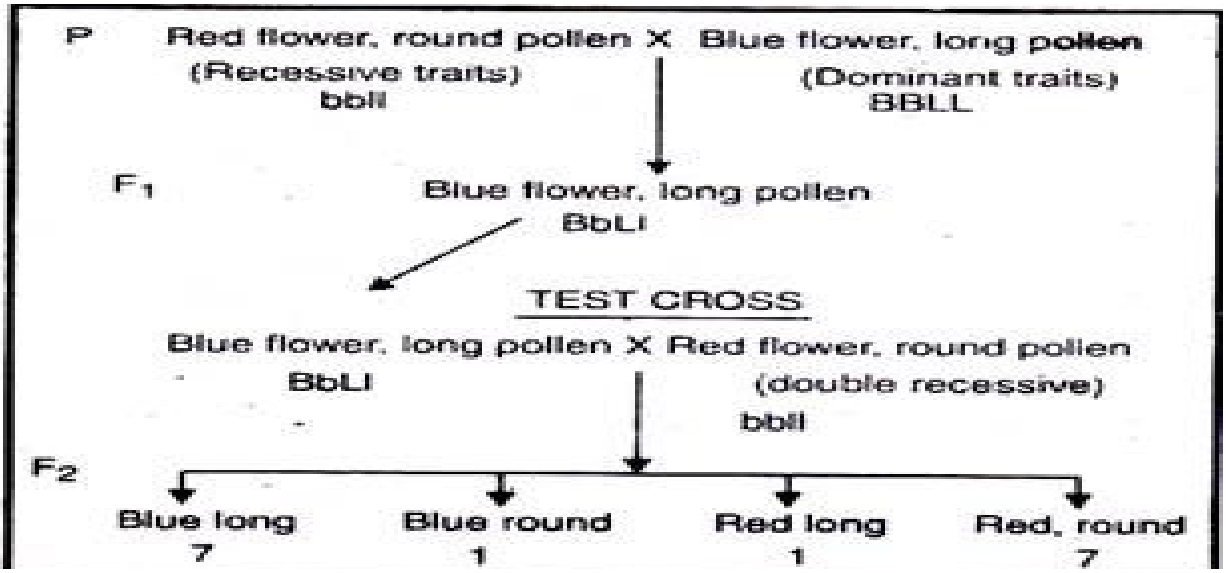
Asst. Professor Zoology
G D C Boys Anantnag



The concept of linkage was actually given by Morgan

Morgan (1910), while working on Drosophila stated that coupling and repulsion are two aspects of linkage. He defined linkage as the tendency of genes, present in the same chromosome, to remain in their original combination and to enter together in the same gamete.'

The genes located on the same chromosome and are being inherited together are known as linked genes, and the characters controlled by these are known as linked characters. Their recombination frequency is always less than 50%. All those genes which are located in the single chromosome form one linkage group. The total number of linkage group in an organism corresponds to the number of chromosome pairs. For example, there are 23 linkage groups in man, 7 in sweet pea and 4 in Drosophila melanogaster.

***Features of Theory of Linkage**:*

Morgan and Castle formulated 'The Chromosome Theory of Linkage'.

It has the following salient features:

1. Genes that show linkage are situated in the same chromosome.

2. Genes are arranged in a linear fashion in the chromosome i.e., linkage of genes is linear.

3. The distance between the linked genes is inversely proportional to the strength of linkage. The genes which are closely located show strong linkage, whereas those, which are widely separated, have more chance to get separated by crossing over (weak linkage).

4. Linked genes remain in their original combination during course of inheritance.

Examples of Linkage:

Morgan (1911) crossed an ordinary wild type Drosophila with grey body and long wings (BB VV) with another Drosophila (mutant type) with black body and vestigial wings (bbvv). All the hybrids in F1 generation are with grey bodies and long wings (BbVv) i.e., phenotypically like the wild type of parents. If now a male of F, generation (Bb Vv) is back crossed with a double recessive female (test cross) having black body and vestigial wings (bbvv) mostly parental combinations are formed in F2 generation with fewer appearance of new combinations (recombinants or non-parental combinations). The results indicate that grey body character is inherited together with long wings.

Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

It implies that these genes are linked together. Similarly, black body character is associated with vestigial wing. Since only parental combinations of character appear in the offspring of F2 generation and no new or non-parental combinations appear, this shows complete linkage. Complete linkage is seen in Drosophila males.

Incomplete linkage is exhibited by those genes which produce some percentage of non-parental combinations. Such genes are located distantly on the chromosome. It is due to accidental or occasional breakage of chromosomal segments during crossing over.

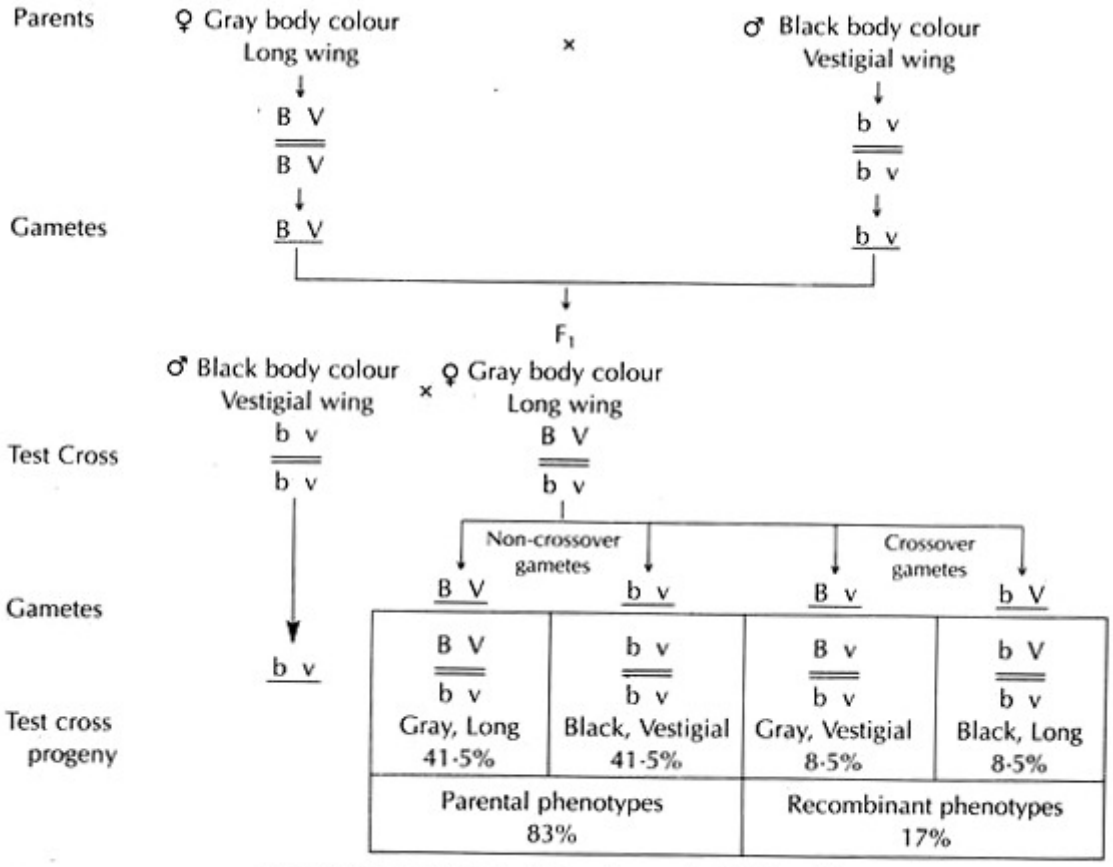


Fig. 8.2: Diagram illustrating linkage (incomplete) in Drosophila

Types of Linkage

1.**Based on crossing over:** Linkage may be classified into

(a) Complete linkage: If two or more characters are inherited together and consistently appear in two or more generations in their original or parental combinations, it is called complete linkage. These genes do not produce non-parental combinations. Genes showing complete linkage are closely located in the same chromosome. E.g, genes for grey body and long wings in male Drosophila show complete linkage.

(b) Incomplete / partial linkage: Incomplete linkage is exhibited by those genes which produce some percentage of non-parental combinations. Such genes are located distantly on the chromosome. It is due to accidental or occasional breakage of chromosomal segments during crossing over. e.g, Bateson and punnet experiment on sweet pea

Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

2. **Based on genes involved** : Depending on whether all dominant or some dominant and some recessive alleles are linked together, linkage can be categorized into (a) Coupling phase and (b) Repulsion phase

(a) Coupling phase: Dominant alleles present on the same chromosome and recessive alleles present on same chromosome shows coupling phase

TR tr

---- ---- Coupling phase

TR tr

(b) Repulsion phase: Dominant alleles of some genes are linked with recessive alleles of other genes on same chromosome shows repulsion phase

Tr tR

---- ---- Repulsion phase

Tr tR

3. **Based on chromosomes involved:** Based on the location of genes on the chromosomes, linkage is categorized into

(a) Autosomal linkage: It refers to linkage of those genes which are located in autosomes (other than sex chromosomes).

(b) Allosomal linkage / sex linkage: It refers to linkage of genes which are located in sex chromosomes i.e. either 'X' or 'Y' (generally 'X')

Linkage Groups

Linkage group refers to a group of genes which are present in one chromosome. In other words, all those genes which are located in one Chromosome constitute one linkage group. The number of linkage groups is limited in each individual. The maximum number of linkage groups is equal to the haploid chromosome number of an organism.

Eg- For example there are ten linkage groups in corn (2n = 20), seven in garden pea (2n = 14), seven in barley (2n =14), four in Drosophila melanogaster (2n = 8) and 23 in man (2n = 46).

Linkage Mapping

A genetic map is a linkage map of a species or experimental population that shows the position of its known genes and/or genetic markers relative to each other in terms of recombination frequency during crossover of homologous chromosomes

The greater the frequency of recombination (segregation) between two genetic markers or genes, the farther apart they are assumed to be. Conversely, the lower the frequency of recombination between the markers, the smaller the physical distance between them.

The observations by Thomas Hunt Morgan that the amount of crossing over between linked genes differs (partial linkage) led to the idea that crossover frequency might indicate the distance separating genes on the chromosome which leads into recombinant phenotypes. Morgan's student Alfred Sturtevant developed the first genetic map, also called a linkage map.

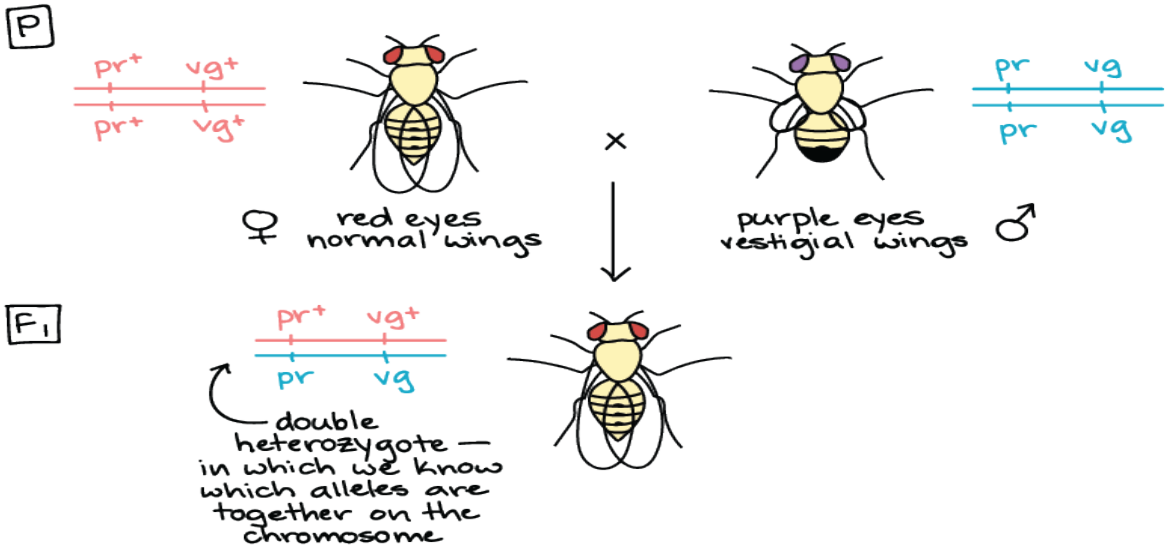
He found that by working out the number of recombinants it is possible to obtain a measure for the distance between the genes. This distance is called a genetic map unit (m.u.), or a centimorgan (cM)and is defined as the distance between genes for which one product of meiosis in 100 is recombinant or a recombinant frequency (RF) of 1% is equivalent to 1 m.u.(cM)

Recombination Frequency(R F)

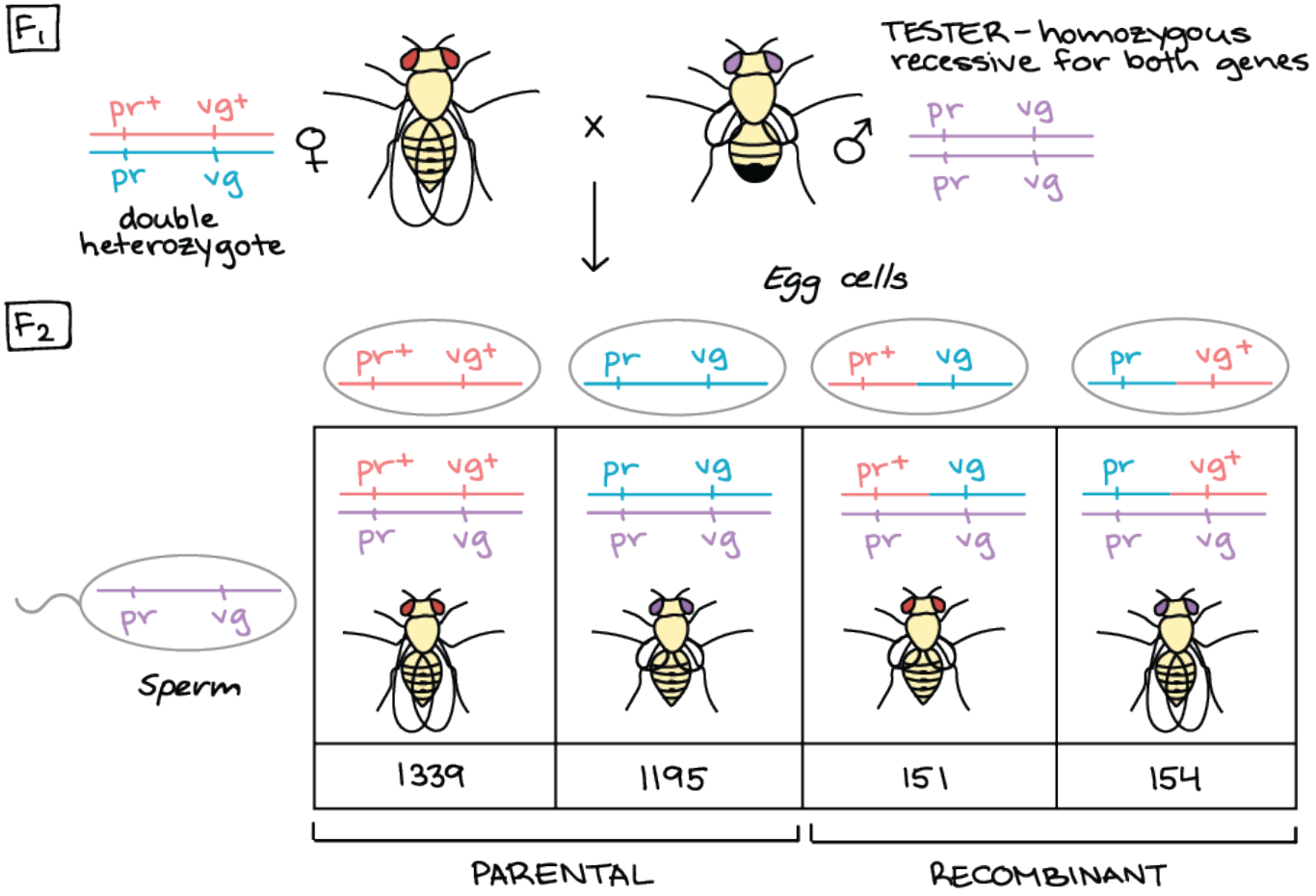
R F is the proportion of recombinant offspring produced in a genetic cross between two organisms, generally a test cross. Recombination frequency (θ) can also be defined the frequency with which a single chromosomal crossover will take place between two genes during meiosis, as the recombinant offspring's in case of linked genes are produced by the crossing over between homologous chromosomes in meiosis during gamete formation.

$$\text{Recombination frequency (RF)} = \frac{\text{Recombinants}}{\text{Total offspring}} \times 100\%$$

Calculation of RF from the inheritance data of *Drosophilla*



Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag



The four classes of offspring are not produced in equal numbers, which tells us that the purple and vestigial genes are linked. As we expect for linked genes, the parental chromosome configurations are over-represented in the offspring, while the recombinant chromosome configurations are under-represented. To measure linkage quantitatively, we can calculate the recombination frequency (RF) between the purple and vestigial genes:

$$\text{Rec. frequency (RF)} = \frac{\text{Recombinants}}{\text{Total offsprings}} \times 100\ \text{-}\% = \frac{151+154}{1195+1339+151+154} \times 100 = \frac{305}{2839} \times 100 = \mathbf{10\%}$$

The recombination frequency between the purple and vestigial genes is 10% and are hence are10cM apart

Construction of linkage map

Linkage maps are constructed by using the recombination frequency data .Let us take the example of three hypothetical genes A, B and C. On taking gene A and B into consideration we got 10% recombination frequency, and on taking gene A and C into account we found 15% recombination frequency and with respect to gene B and C it came out to be 5% recombinant individuals. Therefore the linkage map of these 3 genes can be represented as

A ---------10cM-------- B ----5cM--- C

---------- 15cM ----------

Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

The R C can be calculated from two point cross or three point cross data , which are discussed as

Two Point Cross

A two-point cross is a testcross used to determine the recombinant frequency between 2 linked genes. The classic Mendelian dihybrid cross ratio (round yellow and green wrinkled seeds) is 1:1:1:1, which shows that genes are not linked and located on different chromosomes. Any deviation from this ratio shows that genes are linked and located on same chromosomes, and the distance between them determines the recombinant frequency and chances of crossover during meiotic division. Three scenarios can be considered for two gene locuses A and B:

- A and B very close: crossover occurs outside the loci and inherited as linked genes; therefore, no recombinant classes and 100% parental.
- A and B very far: crossover occurs and 50% will be paental and 50% recombinant.
- A and B at an intermediate distance: crossover can occur and can generate 0–50% of recombinant forms and 50–100% parental.

Thus linkage mapping can be developed using a two-point testcross based on the recombinant frequency.

Example

A two-point testcross in Drosophila as shown above with following character can be observed as an example:

• Eye color: purple **(pr)** or red **(pr+)**

• Wing length: vestigial **(vg)** or normal **(vg+)**

• Wild type alleles are dominant

• Cross between wild type **(pr+vg+)** and double recessive **(pr vg)**

• Possible F1 **(prpr+ vgvg+)**

• Testcross of F1 with homozygous recessive: **prpr+ vgvg+ vs. prpr vgvg,.**

Following types of gametes or offsprings were obtained

Type	Number	
pr vg----------	**1195**	**Parental are in greater proportion**
pr+ vg+----------	**1339**	
pr+ vg ----------	**151**	**Non –Parental or Recombinants are in less proportion**
pr vg+----------	**154**	

Ratio deviating from 1:1:1:1 showing that alleles are on same chromosomes and are linked together.

$$\text{Rec. frequency (RF)} = \frac{\text{Recombinants}}{\text{----------}} \times 100 \text{ -\%} = \frac{151+154}{\text{----------}} \times 100 = \frac{305}{\text{---------}} \times 100 = \mathbf{10\%}$$

Thus the gnes related to eye color (pr) and wing length(VG) are 10cM apart

pr ---------10cM--------vg

Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

Total offsprings	1195 +1339+151+154	2839

Three Point cross

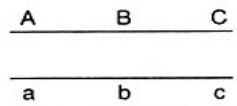
A three-point cross is a testcross used to determine the recombinant frequency between 3 linked genes or markers from a sigle cross. It can be used to order three loci on a chromosome, and map the distance between these loci in centimorgans (cM). This cross is also used to notice the double crossover between genes.

The three-point test cross involves a mating between a triple heterozygote and a triply recessive homozygote. Once offspring phenotypes have been identified and counted in classes, the procedure is as follows:

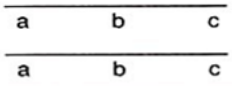
1. Sum the offspring to get a total number of progeny
2. Identify the offspring with parental genotypes (these will have highest frequency classes)
3. Identify the offspring with double recombinant genotypes or double crossover (these will have the lowest frequency classes)
4. Identify which locus has swapped position, relative to the other two, in the double recombinant genotypes compared to the parental genotypes - this locus is the middle locus on the chromosome, and the other two loci may be placed either side (in any order)
5. Draw a map of the chromosome, and divide the spaces between loci into two 'regions'
6. For the first region identify the two classes of single recombinant offspring where a recombination event has occured in this area. Sum the offspring of these classes and the offspring of the double recombinant classes to give you a recombinant value for that region
7. The map distance for this region is then (recombinant value)/(total number of progeny) x 100
8. Repeat steps 6 & 7 for the second 'region' to give this region a map distance

Example

Let us take the example of three linked genes A, B and C.



F1 Heterozygote



Homozygous recessive

In case of heterozygote genes will become unlinked by crossing over and recombinants will be produced as.

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Asst. Professor Zoology
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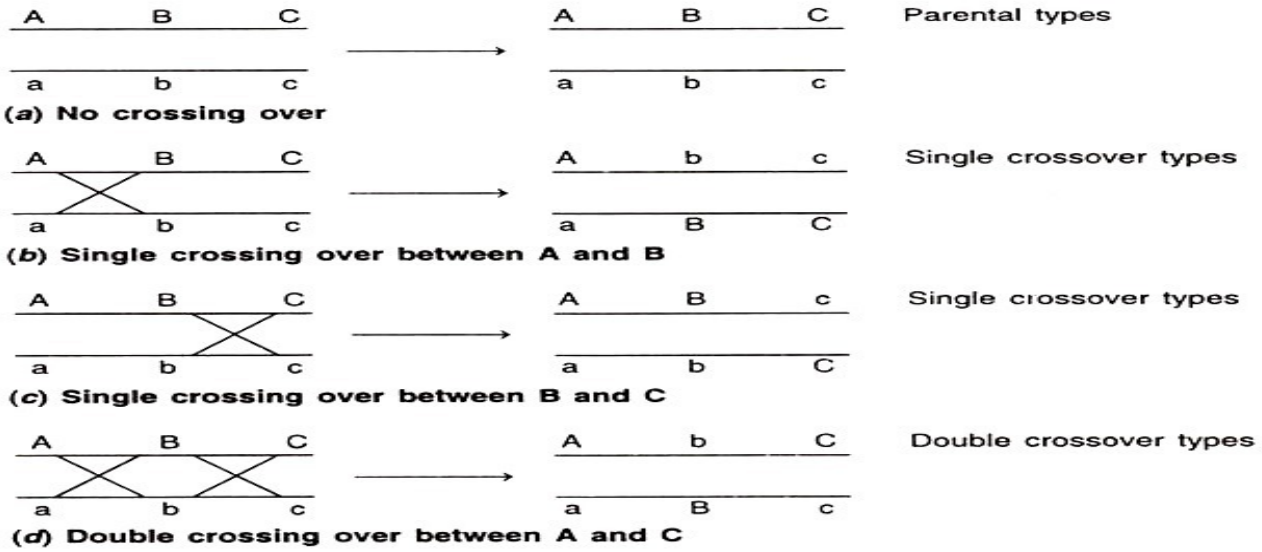

Thus eight types of gametes are produced by F1 and only one type of gamete is produced by homozygous recessive parent. Union of male and female gametes will produce eight different phenotypic classes

I. Summary of the results obtained from a three point test cross between *ABC/abc* × *abc/abc*

Genotypic classes	*Phenotypic classes*	*Assumed frequencies*	*Remarks*
ABC/abc	ABC	349	Parental types.
abc/abc	abc	360	
Abc/abc	Abc	114	Single crossover between A and B.
aBC/abc	aBC	116	
ABc/abc	ABc	128	Single crossover between B and C.
abC/abc	abC	124	
AbC/abc	AbC	5	Double crossover between A and C.
aBc/abc	aBc	4	
Total		1200	

Total offsprings (TO) –1200

Single crossover (SCO) between A and B offsprings —114 + 116 = 230

Single crossover (SCO) between B and C offsprings ---128 + 124 = 252

Double crossover (DCO) between A and C --- 5 + 4 = 9

As double crossover results in exchange of gene B allelrs therefore the gene B is in between A and C and the arrangement of genes is as

A----------B----------C

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Asst. Professor Zoology
G D C Boys Anantnag

Now the relative distance between these genes in cM can be calculated by RF

For Genes A and B

$$RF = \frac{\text{SCO for A and B + DCO}}{\text{TO}} \times 100 = \frac{230 + 9}{1200} \times 100 = 19.91 \text{ 0r } 20\%$$

The distance between A and B will be **20cM**

For Genes B and C

$$RF = \frac{\text{SCO for B and C + DCO}}{\text{TO}} \times 100 = \frac{252 + 9}{1200} \times 100 = 21.75 \text{ 0r } 22\%$$

The distance between B and C will be **22cM**

And for genes A and C the RF will be 20 + 22 = **42%**

The distance between A and C will be **42cM**

So the Linkage Map of genes A, B and C is as

------20 cM------ --------22cM---------

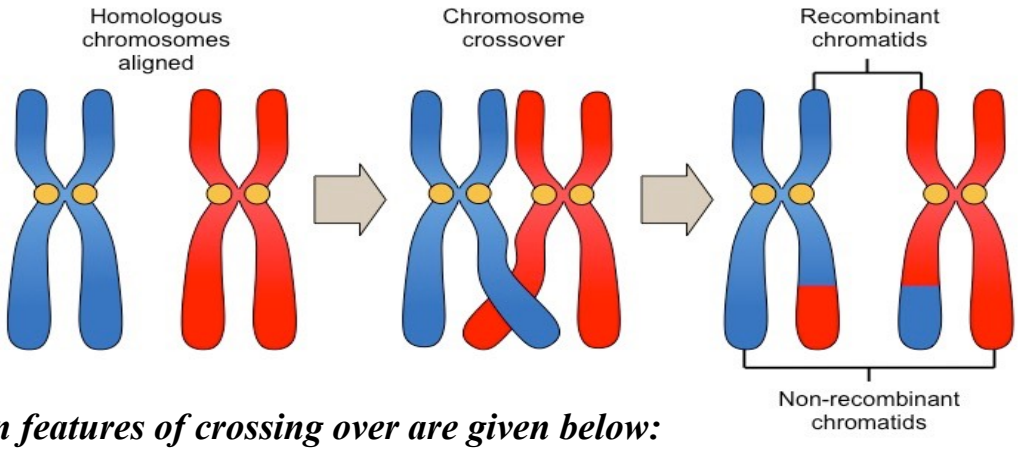
A----------B----------C

----------42cM----------

Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

Crossing Over

Crossing over refers to the interchange of parts between non-sister chromatids of homologus chromosomes during meiotic prophase (pachytene) resulting into the production of recombinant chromatids. In other words, crossing over results from exchange of genetic material between non-sister chromatids involving breakage and reunion at precise point. The term crossing over was first used by Morgan and Cattell in 1912.



The main features of crossing over are given below:

1. Crossing over takes place during meiotic prophase, i.e., during pachytene. Each pair of chromosome has four chromatids at that time.

2. Crossing over occurs between non-sister chromatids. Thus one chromatid from each of the two homologus chromosomes is involved in crossing over.

4.It is universally accepted that crossing over takes place at four strand stage.

4. Each crossing over involves only two of the four chromatids of two homologus chromosomes. However, double or multiple crossing over may involve all four, three or two of the four chromatids, which is very rare.

5. Crossing over leads to re-combinations or new combinations between linked genes. Crossing over generally yields two recombinant types or crossover types and two parental types or non-crossover types.

6.Crossing over generally leads to exchange of equal segments or genes and recombination is always reciprocal. However, unequal crossing over has also been reported.

Process or mechanism of recombination

The process involves Piring of homologous chromosomes, exchange of chromosome segemnts and terminalisation .

Pairing:-

During the zygotene stage of the first prophase of meiosis, the homologous maternal and paternal chromosomes start pairing and lie closely side by side. This phenomenon is called synapsis. This pairing of homologous chromosomes is brought about by the mutual attraction between the allelic genes. The paired chromosomes are known as bivalent. A recent study reveals that synapsis and chiasma formation is facilitated by a highly organised structure of filaments called synaptonemal complex. Synapsis is followed by the duplication of chromosomes which change the bivalent nature of chromosome pair into tetravalent..

Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

Exchange of Ch. Segments:-

During this each of the homologous chromosomes in a bivalent split longitudinally into two sister chromatids attached to the undivided centromere. Thus, four chromatids are formed which remain side by side as two pairs. Later, in pachytene stage crossing over takes place during which the non-sister chromatids of homologous pair twist over each other, the point of contact of cross over chromatids being called as chiasma (plural chiasmata). It is thought to be the place where crossing over takes place. Chiasma was first discovered by Janssens in 1909. Depending on the position, chiasma is of two types, viz., terminal and interstitial.When the chiasma is located at the end of the pairing chromatids, it is known as terminal chiasma and when it is located in middle part of no-nsister chromatids, it is referred to as interstitial chiasma. Later on interstitial chiasma is changed to terminal position by the process of chiasmaterminalization.The number of chiasma per bivalent may vary from one to more than one depending upon the length of chromatids. When two chiasmata are formed, they may involve two, three or all the four chromatids.

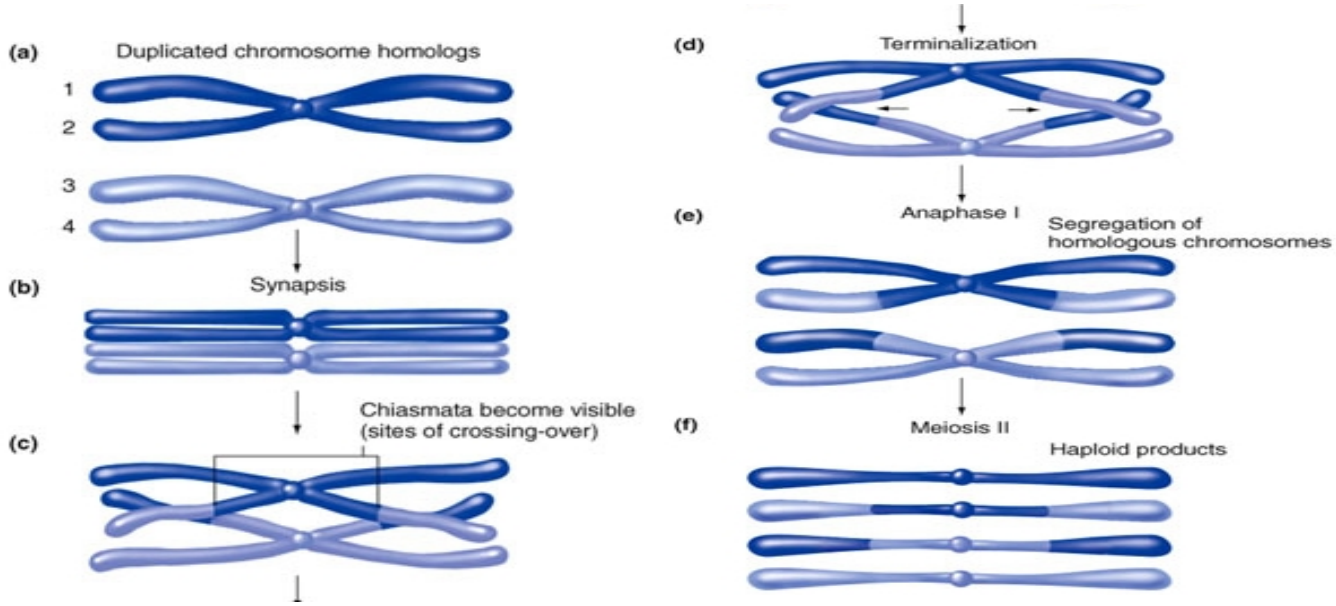
***Chiasma Terminalization*:**The movement of chiasma away from the centromere and towards the end of tetrads is called terminalization. The total number of chiasmata terminalized at any given stage or time is known as coefficient of terminalization. Generally, chiasma terminalization occurs between diplotene and metaphase I.

There are three theories to explain the mechanism of chiasma terminalization, viz:

i. Electrostatic Hypothesis: According to this hypothesis, terminalization takes place due to localized repulsion force in centromere and generalized repulsion force on chromosome surface during diplotene stage.

ii. Coiling Hypothesis: According to this hypothesis, terminalization takes place by mechanical tension developed within the chromosome due to coils. Thus tension force becomes greater than the force binding the chromatids at the point of exchange resulting in terminalization.

iii. Elastic Chromosome Repulsion: According to this theory, all bodies having a definite shape resist any change that leads to alter their shapes. Chiasma forces the chromosome out of shape by its binding force. This leads to the development of repulsion at the point of exchange resulting in terminalization of chiasma



Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

Molecular Mechanism of Crossing Over:

There are two important theories viz:

i. Copy Choice Theory:

This theory was proposed by Belling. This theory states that the entire recombinant section or part arises from the newly synthesised section. The non-sister chromatids when come in close contact they copy some section of each other resulting in recombination. According to this theory, physical exchange of preformed chromatids does not take place.

This theory has two objections:

1. According to this theory breakage and reunion does not occur, while it has been observed cytological.

2. Generally crossing over takes place after DNA replication but here it takes place at the same time.

ii. Breakage and Reunion Theory:

This theory states that crossing over takes place due to breakage and reunion of non-sister chromatids. The two segments of parental chromosomes which are present in recombinants arise from physical breaks in the parental chromosomes with subsequent exchange of broken segments (Fig. 9.2).

The breakage results due to mechanical strains that result from the separation of paired homologous chromosomes and chromatids in each chromosome during pachytene stage. The broken ends of non-sister chromatids unite to produce chiasmata resulting in crossing over.

Actually in most of the case of crossing over it is the breakage and reunion of chromatids segments by the formation of holiday junction at DNA level

Types of Crossing Over

Single crossing over -When the chiasma occurs only at one point of chromosome.It produces two crossover chromatids and two non sister chromatids

Fig 12 Single crossing over

Double crossing over -When the chiasmata occurs at two points in same chromosome.In this type two types of chiasma may formed-

- Reciprocal chiasma-In this the same two chromatids are involved in the second chiasma as in the first.So the second chiasma restore the order which was changed by first.It produces two non-cross over chromatids.
- Complimentary chiasma-In this two chromatids that are involved in the second chiasma are different from first.It produces four single cross overs but no non-cross over.

Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

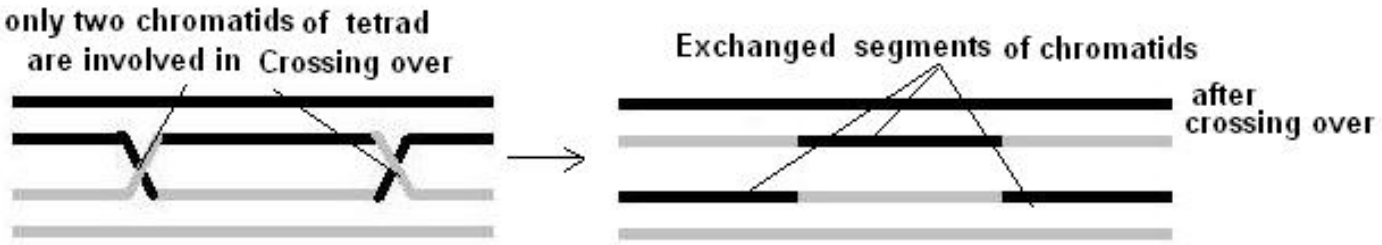


Fig 12 Double crossing over (Reciprocal chiasma)

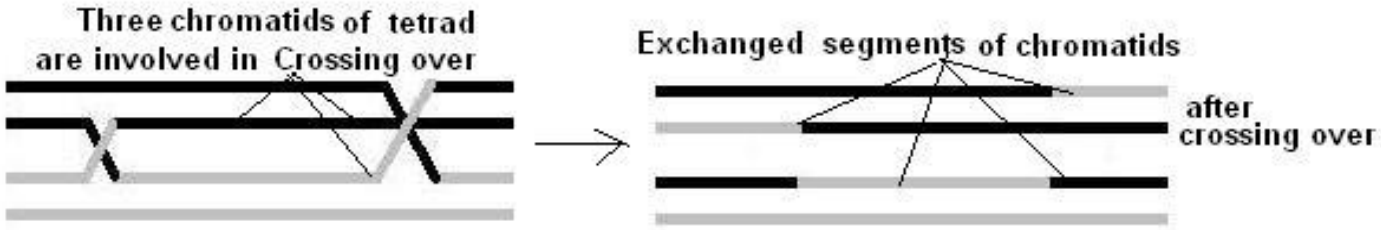


Fig 13 Double crossing over (Complimentary chiasma)

Multiple crossing over -When crossing over takes place at more than two places in the same chromosome pair.It occurs rarely.

Asif Ahmed Kamgar
Asst. Professor Zoology
G D C Boys Anantnag

Organistion of Genetic Material

Genetic material in prokaryotes and eukaryotes is the DNA.

It does not occur as such inside the cell but is organized in a proper way in association with proteins. However the level of organization is not same in both types of cells but differs.

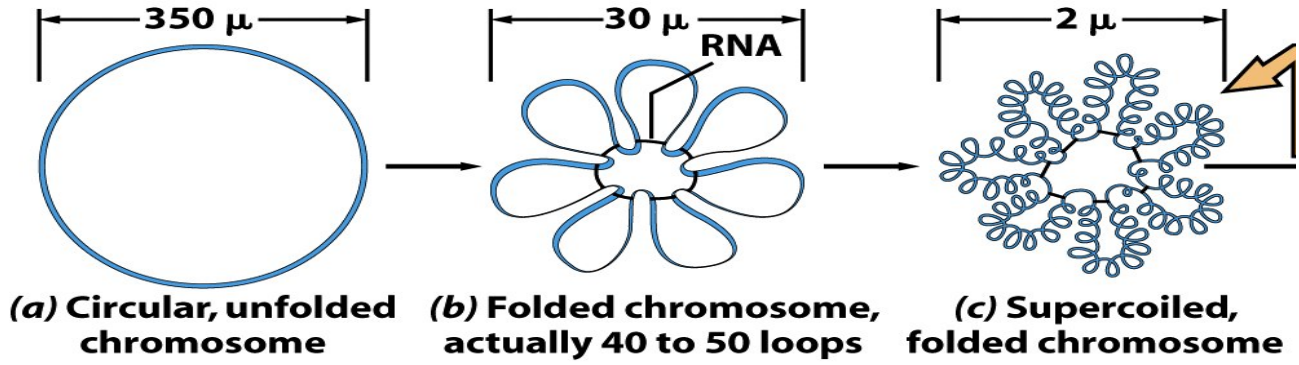
Organisation of genetic material in prokaryotes

In prokaryotes like E. coli, a well-defined nucleus is absent. The prokaryotic chromosome is circular and dispersed within the cell and is not enclosed by a separate membrane.Prokaryotes are monoploid = they have only one set of genes (one copy of the genome).In most prokaryotes, the single set of genes is stored in a single chromosome (single molecule either RNA or DNA).Prokaryotic genomes are exemplified by the *E. coli* chromosome. The bulk of the DNA in *E. coli* cells consists of a single closed-circular DNA molecule of length **4.6 million base pairs**.The DNA is packaged into a region of the cell known as the nucleoid.

Experiments in which DNA from E. coli is carefully isolated free of most of the attached proteins and observed under the electron microscope reveal one level of organization of the nucleoid. The DNA consists **of 50–100 domains or loops**, the ends of which are constrained by binding to a structure which probably consists of proteins attached to part of the cell membrane. **The loops are about 50–100 kb in size**

The *E. coli* chromosome as a whole is negatively supercoiled, although there is some evidence that individual domains may be supercoiled independently.

The DNA is associated with some proteins and themost abundant of these are **protein HU,** a small basic (positively charged) protein. It's binds DNA nonspecifically by the wrapping of the DNA around the protein, and **H-NS(histone-like nucleoid-structuring protein),** a monomeric neutral protein, which also binds DNA nonspecifically in terms of sequence. These proteins are sometimes known as histone-like proteins, and have the effect of compacting the DNA, which is essential for the packaging of the DNA into the nucleoid, and of stabilizing and constraining the supercoiling of the chromosome



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G D C Boys Anantnag

Organisation of genetic material in Eukaryotes

All the chromosomal DNA of an eukaryotic cell is embedded in a membranous cellular organelle called the nucleus. The eukaryotic DNA, in the nucleus is linear and not circular. In the non-dividing resting cell all the DNA of the cell forms a fine filamentous network in the nucleus called the chromatin. During cell division the chromatin network is subdivided into defined number and shaped chromosomes, their diploid number (pairs) depends upon the species of organism.

The normal chromosome num-ber in humans is 46 (23 pairs). Each chromosome has a central axis called the centromere, from which two arms of DNA project out and each is referred to as chromatid. Each chromosome differs in size and shape within a given organism.

In chromosomes Chemical composition of chromatin

- DNA= 20-40 %- most important chemical constituent of chromatin
- RNA=05-10 %-associated with chromatin as;
  Ribosomal RNA-(rRNA)
  Messenger RNA- (mRNA)
  Transfer RNA- (tRNA)
- PROTEINS=55-60%-associated with chromatin as;
  Histones: very basic proteins, constitute about 60% of total protein, almost 1:1 ratio with DNA.
  Non-Histones: They are 20% of total chromatin protein:
  Histones are asic proteins-positively charged at neutral pH called histones.
  Where as Non- Histones are heterogeneous largely acidic (negatively charged at neutral pH) group of proteins

The 2 metre long eukaryotic human cell DNA is to be packed in the cell of about 5-10 micrometer in diameter. Human chromosome 22 contains about 48 million nucleotide pairs. Stretched out end to end, its DNA extend about 1.5 cm.Chromosome 22 measures only about 2µm in length, giving end-to-end compaction ration of nearly 10,000 fold. In order to facilitate its package, the helical DNA molecule is bound, tightly around beads of basic proteins called histones, which are spaced at regular intervals.The DNA in association with proteins forms the chromatin. The basic unit of chromatin is the nucleosome

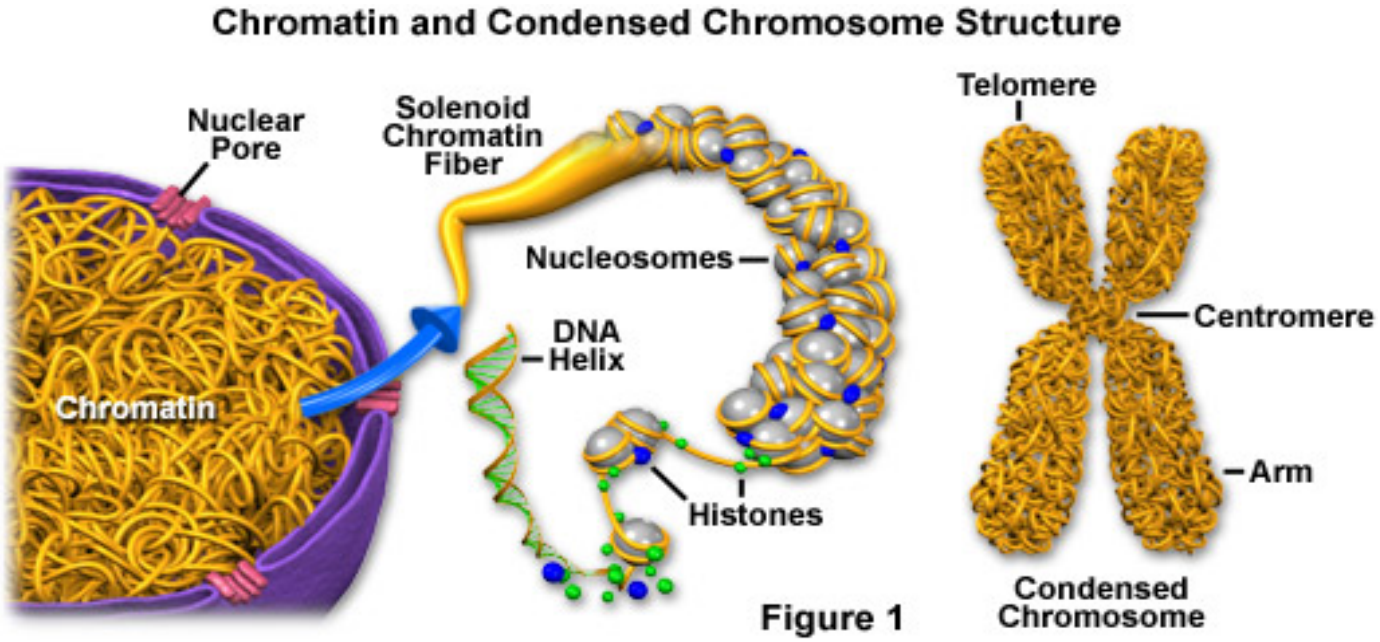

Figure 1

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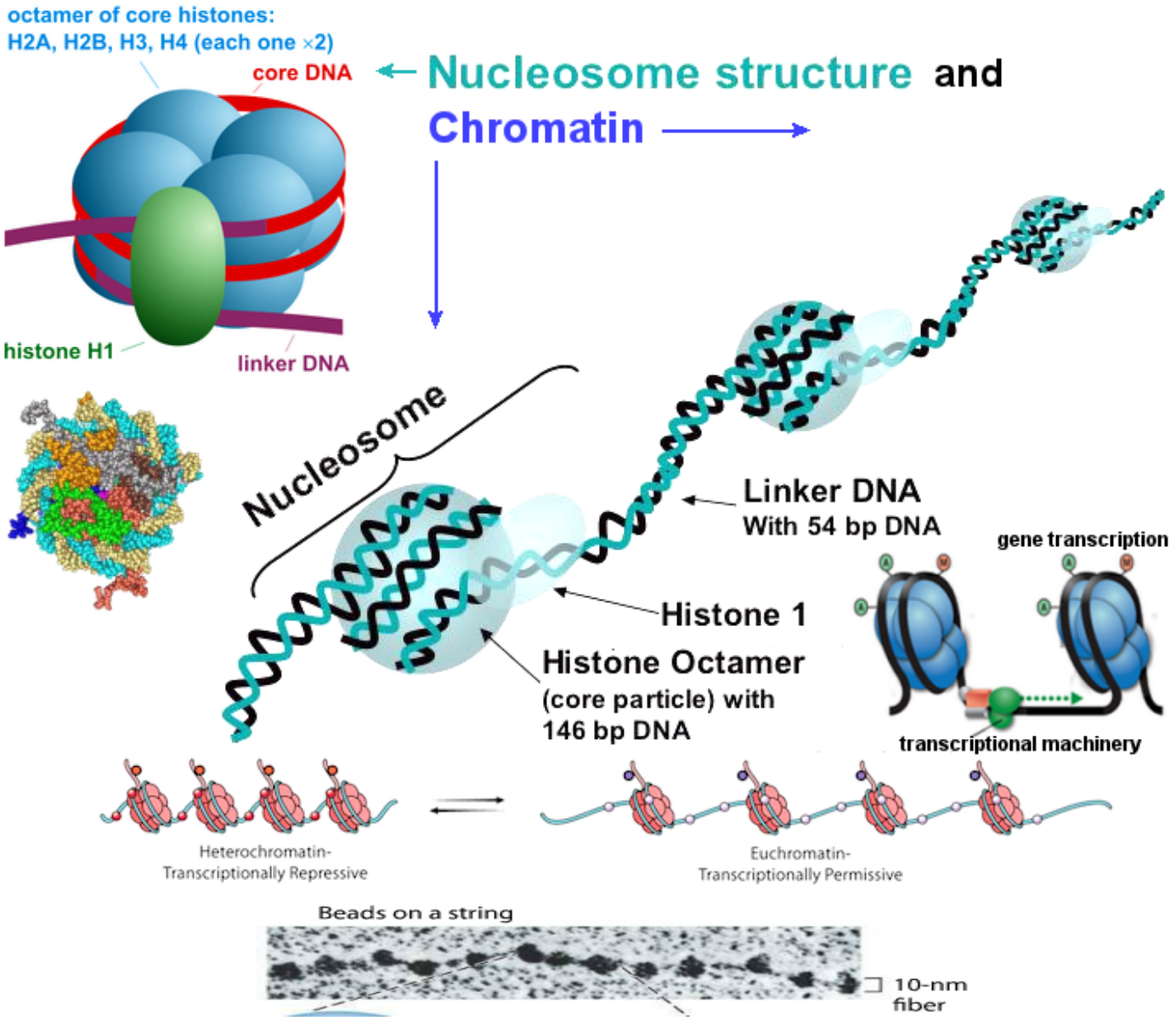
Asst. Professor Zoology
G D C Boys Anantnag

Nucleosome

- Simplest packaging structure of DNA that is found in all eukaryotic chromosomes.
- The nucleosome is composed of approximately 146 base pairs of DNA wrapped in 1.8 helical turns around an eight-unit structure called histone protein octamer.
- The length of DNA that is associated with the nucleosome unit varies between species.
- This histone octamer consists of two copies each of the histones H2a, H2b, H3, and H4, These are known as the core histones and together as octamer forms core particle.
- The DNA wrapped around the core particle in association with H1 forms chromatosome.
- The space in between individual nucleosomes is referred to as linker DNA, and can range in length from 8 to 114 base pairs, with 55 base pairs being the average ,this variation is species specific. Linker DNA interacts with the linker histone, called H1.
- Chromatosome in association with linker DNA forms Nucleosome.The nucleosomes give the DNA strand the appearance of a string of beads, and this arrangement of DNA wrapped around histones serves to package the DNA efficiently and protected from enzymatic degradation.
- Nuceosomes are further organized into higher levels.



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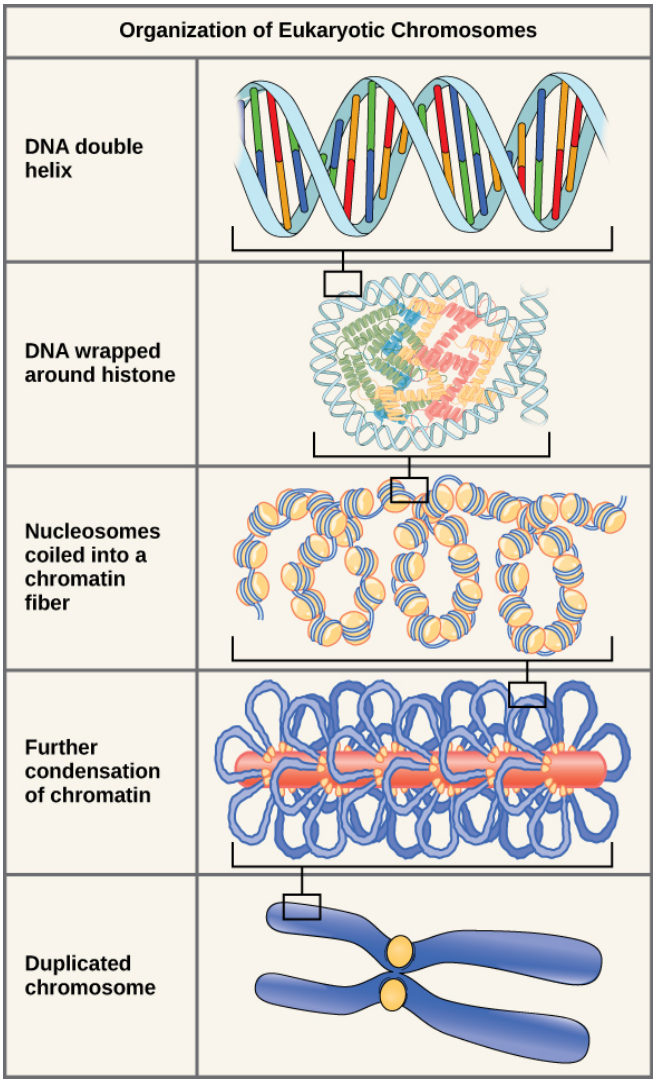
Higher Levels of organization

These nucleosomes are organized very close together to form a 30 nm fibre, called asolenoid structure.

This appears to be a solenoid structure with about 6 nucleosomes per turn.

This solenoid structure is further folded into-the loops, domains and scaffold structure to form the fully condensed Metaphase chromosome



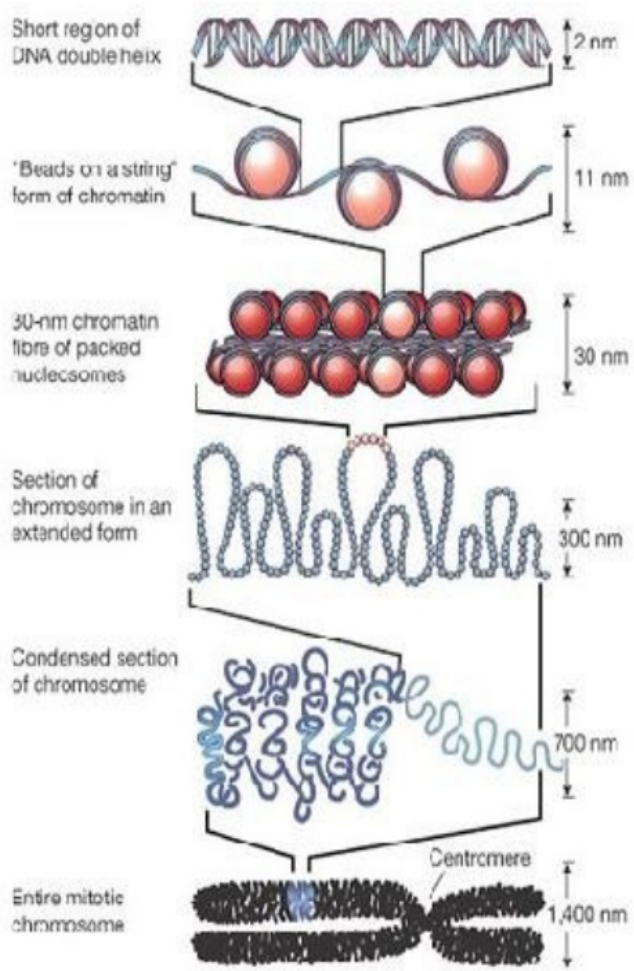


Fig.6. Organization of chromatin in chromosome

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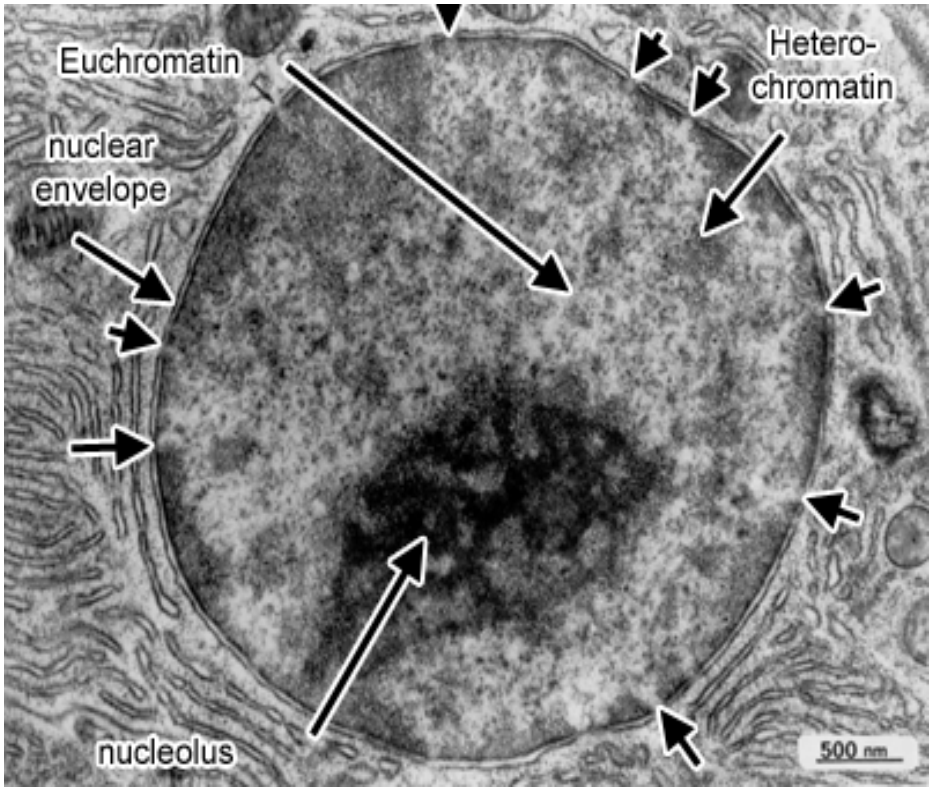
Nature of Chromatin

DNA is the main genetic constituent of cells, carrying information in a coded form from cell to cell and from organism to organism.

Within cell nucleus, DNA is not free but is complexed with proteins in a structure called chromatin.

Chromatin consists of double-stranded DNA (two hydrogen-bonded polynucleotide strands) to which large amounts of protein and a small amount of RNA are bound. The complex of DNA plus histones and other structural proteins is called chromatin.

Chromatin exists in two forms. One form, called euchromatin, is less condensed and can be transcribed. The second form, called heterochromatin, is highly condensed and is typically not transcribed.



Euchromatin

- Euchromatin is a form of chromatin that is lightly packed—as opposed to heterochromatin, which is densely packed.
- The presence of euchromatin usually reflects that cells are transcriptionally active, i.e. they are actively transcribing DNA to mRNA.
- Euchromatin is found in the nucleus of eukaryotes and represents more than 90% of the human genome.
- Lightly packed form of chromatin that is rich in gene concentration takes up light stain and represent most of the chromatin, that disperse after mitosis has completed.
- Consists of structural genes which replicate and transcribe during G1 and S phase of the interphase.
- Considered genetically active chromatin, since it has a role in their phenotypic expression of the genes.
- During metaphase it takes up dark stain.

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Asst. Professor Zoology
G D C Boys Anantnag

Heterochromatin

Heterochromatin (HC) refers to the chromosomal segments or chromatin part which appear extremely condensed and dark in colour in the interphase nucleus.

There are two types of heterochromatin, constitutive HC and facultative HC, which differ slightly, depending on the DNA that they contain.

1 Constitutive heterochromatin

Constitutive HC contains a particular type of DNA called satellite DNA, which consists of large numbers of short tandemly repeated sequences :

Constitutive HC is stable and conserves its heterochromatic properties during all stages of development and in all tissues.

In most organisms, constitutive heterochromatin occurs around the chromosome centromere and near telomeres.

2 Facultative heterochromatin

Facultative HC is characterised by the presence of LINE-type repeated sequences. These sequences, dispersed throughout the genome, Facultative HC is reversible, its heterochromatic state depending on the stage of development or the cell type examined.

The inactive X (Barr body) in the somatic cells females and the inactive sex vesicle at the pachytene stage of male meiosis provide two examples of facultative HC.

Facultative HC is not particularly rich in satellite DNA, and is therefore not polymorphic.

Properties of heterochromatin

Despite the differences described above, constitutive HC and facultative HC have very similar properties.

1 Heterochromatin is condensed

This is in fact what defines heterochromatin, and it is applicable to both constitutive HC and facultative HC. This high condensation renders it strongly chromophilic and inaccessible to DNAse 1 and to other restriction enzymes in general.

2 Heterochromatin DNA is late replicating

The incorporation of various nucleotide analogues shows that the DNA from both constitutive and facultative HC, is late replicating. HC late replication results, on the one hand, from its high degree of condensation, which prevents the replicating machinery from easily accessing the DNA, and, on the other hand, from its location in a peripheral nuclear domain that is poor in active elements.

3 Heterochromatin DNA is methylated

The DNA of constitutive HC is highly methylated on the cytosines. As regards facultative HC, the methylation of the DNA is more discrete

4.Heterochromatin is transcriptionally inactive

The facultative HC is relatively poor in genes, and its genes are not usually transcribed in a heterochromatic context.

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5.Heterochromatin does not participate in genetic recombination

It is generally accepted that constitutive HC does not participate in genetic recombination. As regards the facultative HC, it does not participate in meiotic recombination when it is in its inactive form.

6.Heterochromatin has a gregarious instinct

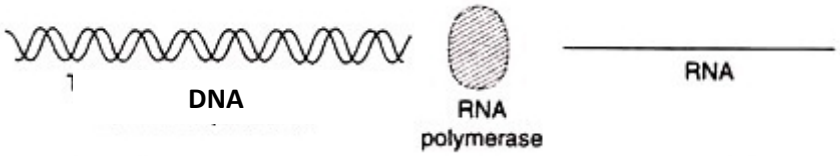
The study of various organisms has shown that constitutive HC has a genuine tendency to aggregate during interphase. In Drosophila larvae, the centromeres of polytene chromosomes, which are rich in heterochromatin, can aggregate to form the chromocentres during interphase. This tendency of the heterochromatin to aggregate appears to be strongly linked to the presence of satellite DNA sequences, but it may also involve other additional sequences.

Heterochromatin is thought to be involved in various processes like gene expression, chromatin organization and separation of chromosomes during cell division

Asif Ahmed kamgar
Asst. Professor Zoology
G D C Boys Anantnag

TRANSCRIPTION

- Transcription is the first step of gene expression, in which a particular segment of DNA is copied into RNA by the enzyme RNA polymerase
- Actually the formation of an RNA copy from a DNA template is called transcription

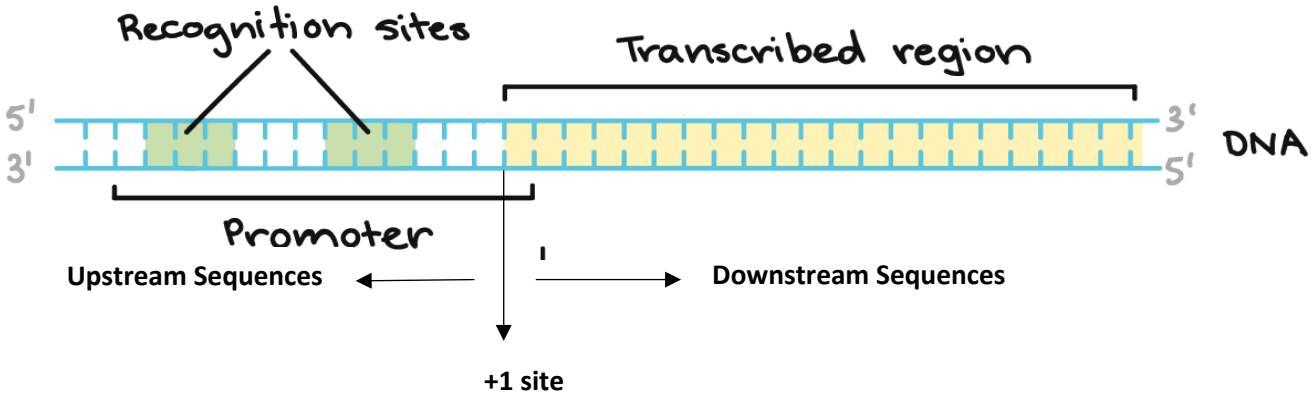


General characteristics of Transcription

- The RNA molecule produced in transcription is synthesised from a single strand of DNA, because in any particular stretch of DNA, usually only one strand of DNA serves as a template for RNA synthesis.
- The synthesis of RNA uses the four **ribonucleoside 5′-triphosphates (ATP, GTP, CTP, UTP)** as precursors. They differ from DNA precursors in having a ribose sugar and uracil, instead of deoxyribose and thymine
- The sequence of bases in the RNA molecule is complementary to the sequence of bases in the DNA template. Thus, the bases T, C, A and G in DNA template result in addition of A, G, U and C respectively to the growing chain of RNA.
- Nucleotides are added only to the 3′-OH end of the growing chain, with the result that the 5′ end of the growing RNA molecule contains a triphosphate group. The **5′ to 3′** direction of chain growth is identical to that in DNA synthesis.
- **RNA polymerase(RNAP)** does not require a preformed primer to initiate chain growth.
- Each transcribed region is preceeded by the **promoter region** which acts as a regulator of gene expression and binding site for RNAP.

Transcription in Prokaryotes

- General Structure of prokaryotic gene to be transcribed is as:
- The site on the DNA from which the first RNA nucleotide is transcribed is called **the +1,plus 1 site**, or the **initiation site**. Nucleotides that come before the initiation site are given negative numbers and said to be **upstream**. Nucleotides that come after the initiation site are marked with positive numbers and said to be **downstream**.



- Transcription involves three different stages—initiation, elongation and termination

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Asst. Professor (Zoology)
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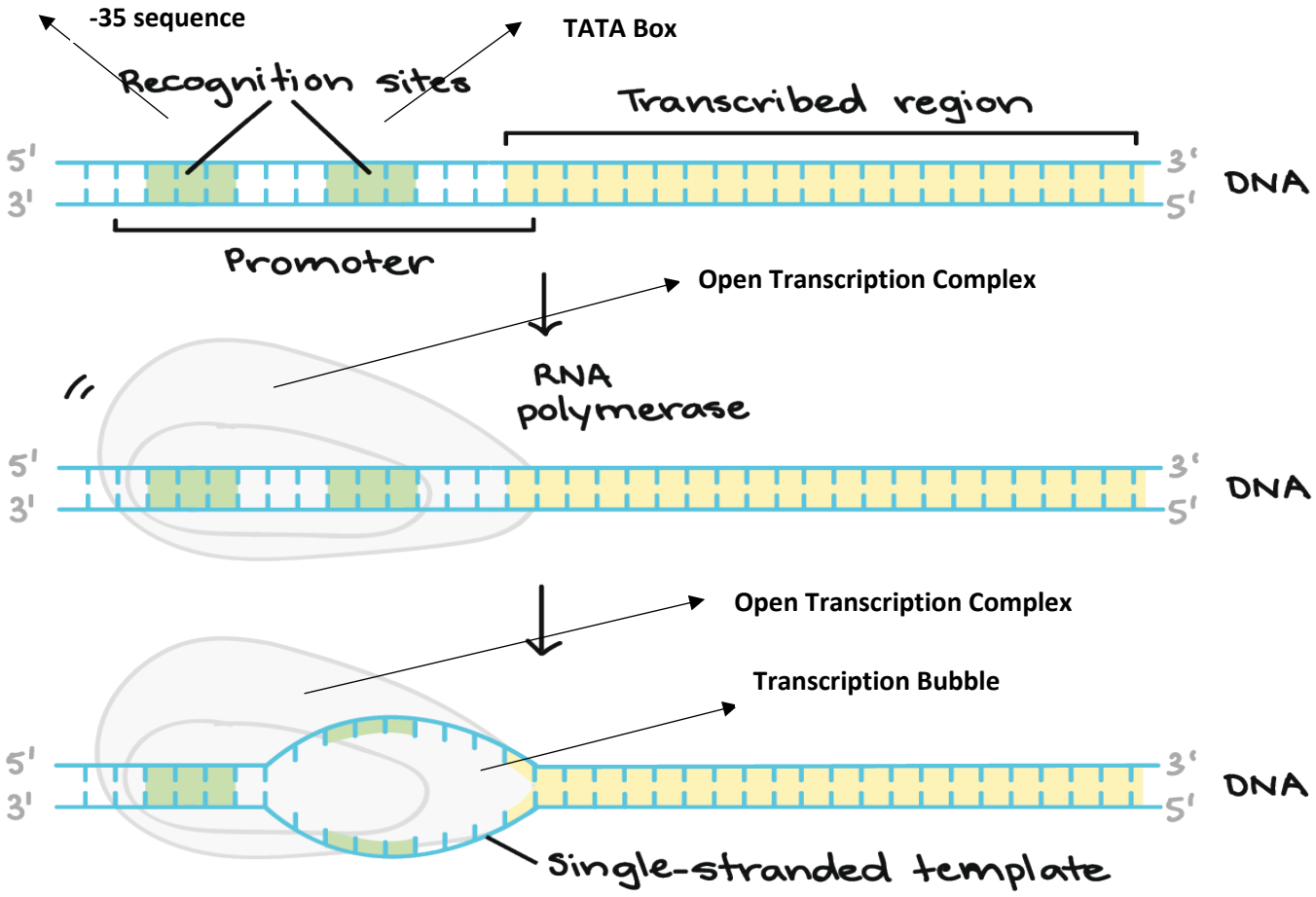
Initiation

- The binding of the enzyme RNA polymerase to DNA is the prerequisite for the transcription to start.
- The specific region on the DNA where the enzyme binds is known as promoter region.
- **Transcription factor sigma** helps the RNAP to bind the promoter region
- There are two base sequences on the coding DNA strand, one is **TATA Box** and the other one is **-35 sequence** which the sigma factor of RNA polymerase can recognize for initiation of transcription

1. **Pribnow box (TATA box):**
   This consists of 6 nucleotide bases (TATAAT), located on the left side about 10 bases away (upstream) from the starting point of transcription.
2. **The '-35' sequence**:
   This is the second recognition site in the promoter region of DNA. It contains a base sequence TTGACA, which is located about 35 bases (upstream, hence -35) away on the left side from the site of transcription start.

- The binding of RNA to promoter region forms **Closed transcription complex**.
- Each gene (or group of co-transcribed genes, in bacteria) has its own promoter. Once bound, RNA polymerase separates the DNA strands by using ATP, providing the single-stranded template needed for transcription.
- The Unwinding of DNA forms the **open transcription complex** with **transcription bubble**.



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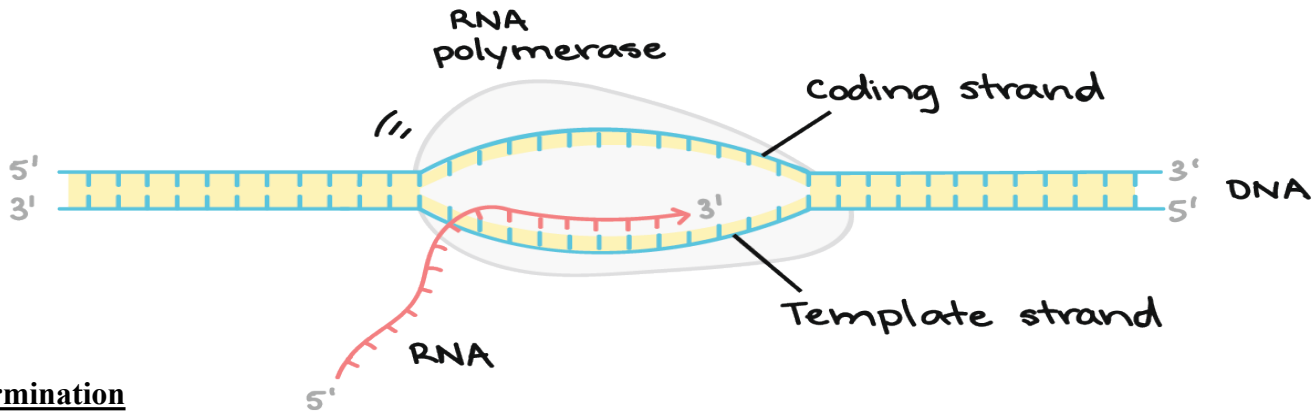
Elongation

- As the holoenzyme, RNA polymerase recognizes the promoter region, the sigma factor is released and transcription proceeds.
- RNA is synthesized from 5′ end to 3′ end (5'→3′) antiparallel to the DNA template.
- RNA polymerase utilizes ribo-nucleotide triphosphates (ATP, GTP, CTP and UTP) for the formation of RNA.
- For the addition of each nucleotide to the growing chain, a pyrophosphate moiety is released.
- The sequence of nucleotide bases in the mRNA is complementary to the template DNA strand. It is however, identical to that of coding strand except that RNA contains U in place of T in DNA
- RNA polymerase differs from DNA polymerase in two aspects. No primer is required for RNA polymerase and, further, this enzyme does not possess endo- or exonuclease activity. Due to lack of the latter function (proof-reading activity), RNA polymerase has no ability to repair the mistakes in the RNA synthesized.
- The strand from which RNA is copied is called **template or antisense strand** and the strand of DNA complementary to template strand is called **Coding or sense strand**.
- The double helical structure of DNA unwinds as the transcription goes on, resulting in supercoils. The problem of supercoils is overcome by **topoisomerases**

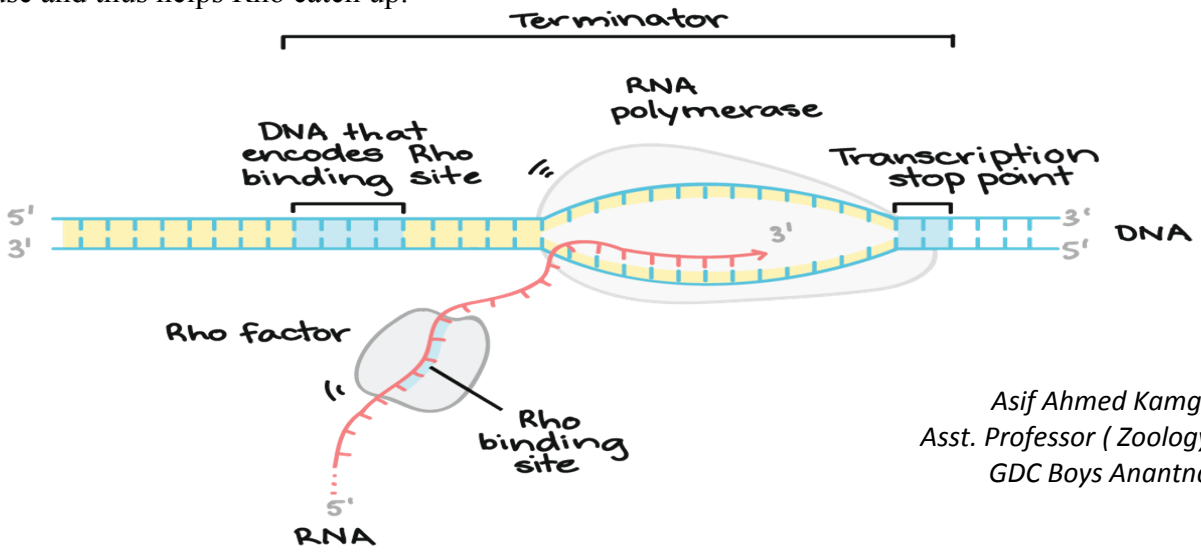


Termination

- The process of transcription stops by termination signals. Two types of termination are identified.

1. **Rho (p) dependent termination:**

In Rho-dependent termination, the nascent RNA contains a binding site for a protein called Rho factor. Rho factor binds to this sequence and starts "climbing" up the transcript towards RNA polymerase. When it catches up with the polymerase at the transcription bubble, Rho pulls the RNA transcript and the template DNA strand apart, releasing the RNA molecule and ending transcription. Another sequence found later in the DNA, called the transcription stop point, causes RNA polymerase to pause and thus helps Rho catch up.



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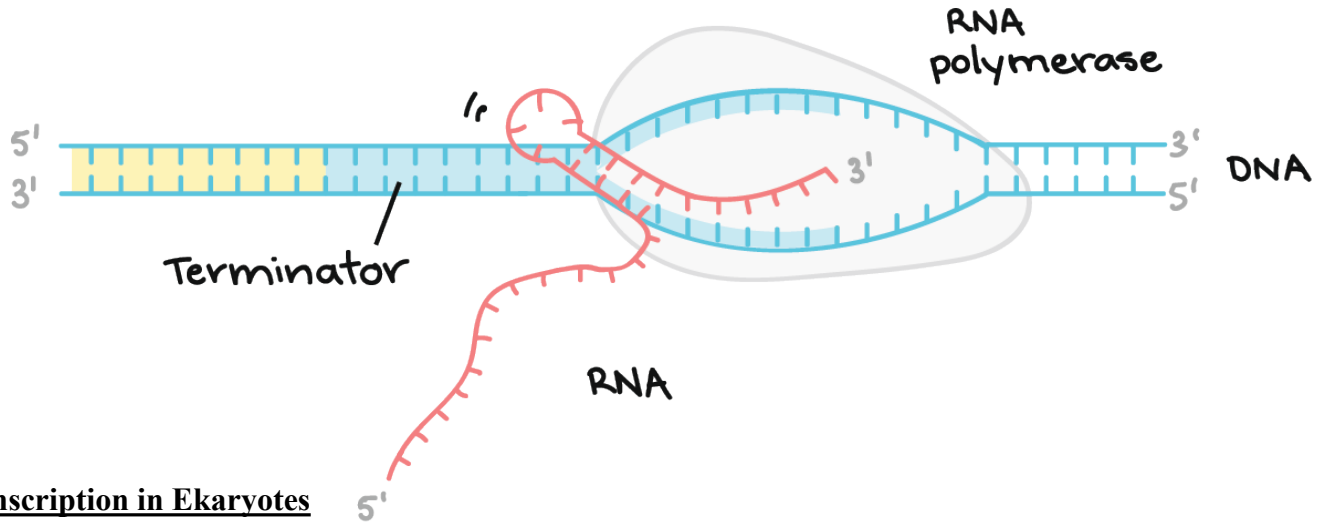
2. Rho (p) independent termination:

The termination in this case is brought about by the formation of hairpins of newly synthesized RNA. This occurs due to the presence of palindromes. The presence of palindromes in the base sequence of DNA template (same when read in opposite direction) in the termination region is known. As a result of this, the newly synthesized RNA folds to form hairpins (due to complementary base pairing) that cause termination of transcription



Transcription in Ekaryotes

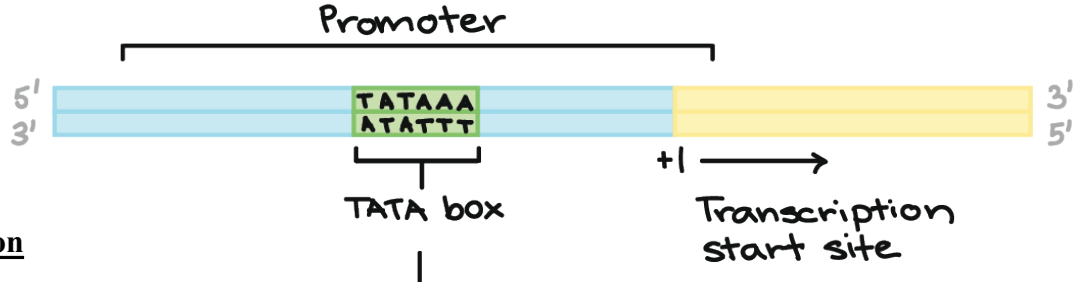
- RNA synthesis in eukaryotes is a much more complicated process than the transcription described above for prokaryotes. Its sailent features are as:

RNA Polymerases:

- The nuclei of eukaryotic cells possess three distinct RNA polymerases
- Transcription in Eukaryotes
- **RNA polymerase I** is responsible for the synthesis of precursors for the large ribosomal RNAs.
- **RNA polymerase II** synthesizes the precursors for mRNAs and small nuclear RNAs.
- **RNA polymerase III** participates in the formation of tRNAs and small ribosomal RNAs.
- Besides the three RNA polymerases found in the nucleus, there also exists a mitochondrial RNA polymerase in eukaryotes. The latter resembles prokaryotic RNA polymerase in structure and function.

Promoter Sites:

- There is variation in nucleotide sequence among the eukaryotic promoters
- But a sequence of DNA bases—which is almost identical to pribnow box of prokaryotes, this sequence, known as **Hogness box (or TATA box),** is located on the left about 30 nucleotides away (upstream) from the starting site of mRNA synthesis.



Initiation

- The initiation of gene transcription in eukaryotes occurs in specific steps

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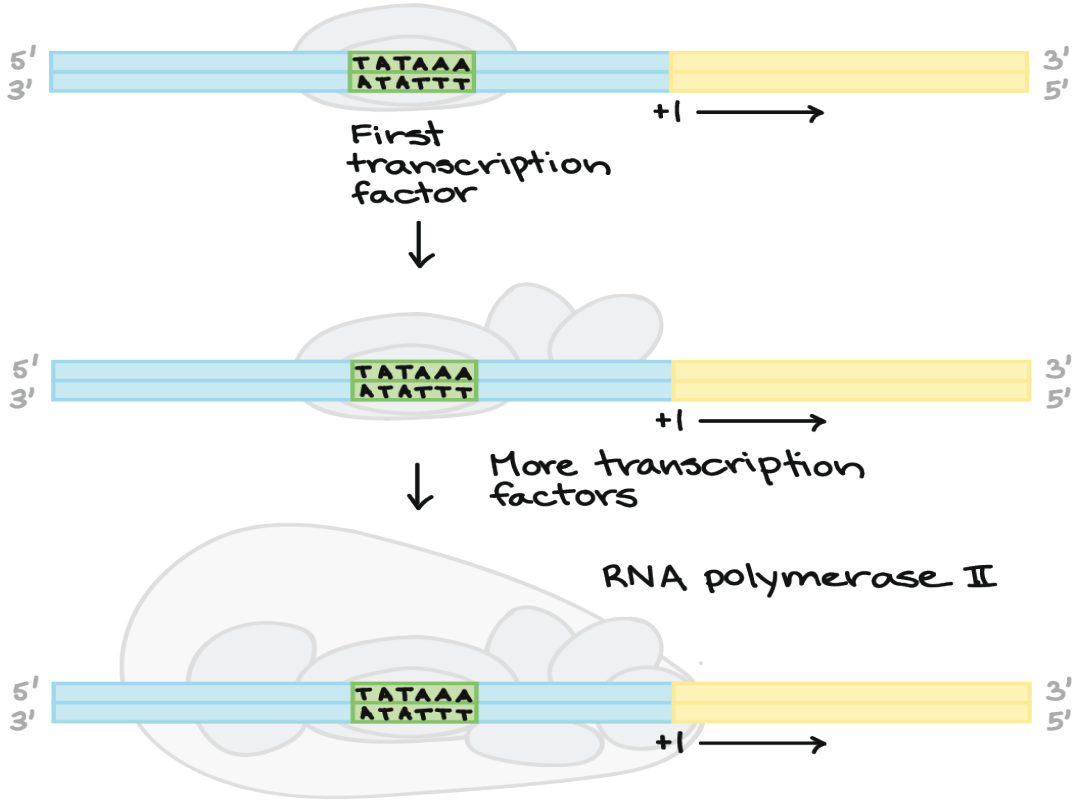
- The TATA box plays a role much like that of the -10element in bacteria. It's recognized by one of the general transcription factors, allowing other transcription factors and eventually RNA polymerase to bind. It also contains lots of As and Ts, which make it easy to pull the strands of DNA apart.
- First, an RNA polymerase along with general transcription factors binds to the promoter region of the gene to form a closed complex called the **preinitiation complex**. A large number of transcription factors interact with eukaryotic promoter regions. In humans, about six transcription factors have been identified (TFIID, TFIIA, TFIIB, TFIIF, TFIIE, TFIIH). It is postulated that the TFs bind to each other, and in turn to the enzyme RNA polymerase.
- The subsequent transition of the complex from the closed state to the open state results in the melting or separation of the two DNA strands and the positioning of the template strand to the active site of the RNA polymerase. Without the need of a primer, RNA polymerase can initiate the synthesis of a new RNA chain using the template DNA strand to guide ribonucleotide selection and polymerization chemistry



Elongation

- Elongation proceeds the same way as in case of prokaryotes with some differneces, like:
- TFIIF remains associated with Pol II throughout elongation. During this stage, the activity of the polymerase is greatly enhanced by proteins called **elongation factors.**
- The elongation factors suppress pausing during transcription and also coordinate interactions between protein complexes involved in the post-transcriptional processing of mRNAs.

Termination

- Once the RNA transcript is completed, transcription is terminated. Pol II is dephosphorylated and recycled, ready to initiate another transcript

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Asst. Professor (Zoology)
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Difference Between:

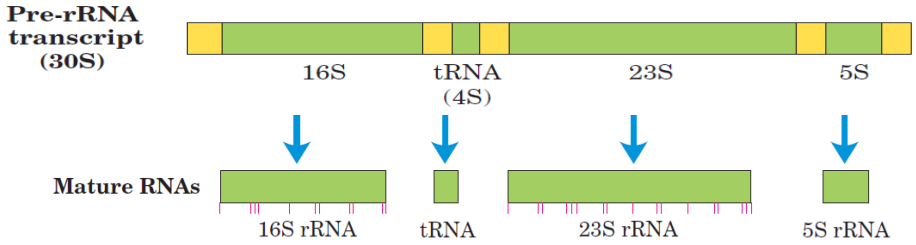
Prokaryotic Transcription	Eukaryotic Transcription
1. Transcription is coupled with translation inside cytoplasm	1. Transcription is separated from translation as these processes occur in Nucleus and Cytoplasm Respectively
2.Only one type of RNA polymerase is involved for each type of RNA synthesis	2. Different RNA Polymerase enzymes used for different RNA types
3. Initiation is assisted by the Sigma factor	3. Initiation is assisted by number of transcription factors
4. Promoter region contains Pribnow Box at -10 position	4. Promoter region contains TATA box located 25 to 35 upstream.
5. Termination occurs by Rho dependent or Rho indepependent Mechanism	5. Termination is not completely understood, may be due to Poly A tail or by the presence of termination sequences in DNA.
6. Usually there is no post transcriptional modification of nascent RNA	6. Primary transcript undergoes post transcriptional modification
7. Genes are usually polycistronic, i:e, single transcript codes for many polynuceotides	7. Genes are Monocistronic, i:e, Single transcript codes for single polynucleotide.

POST TRANSCRIPTIONAL MODIFICATION

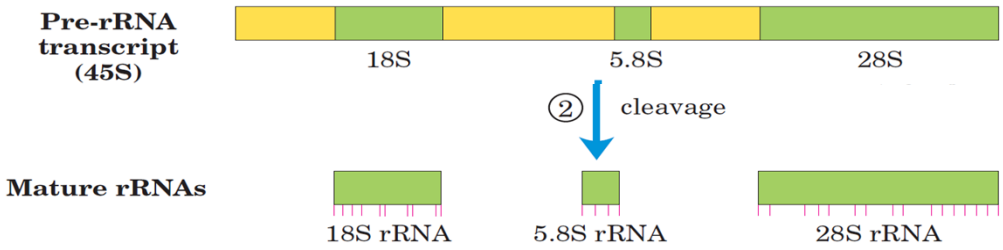
- Many of the RNA molecules in bacteria and virtually all RNA molecules in eukaryotes are processed to some degree after synthesis
- In case of prokaryotes m mRNA is translated as soon as it comes out of the transcription bubble however in case of eukaryotes before translation mRNA is modified in nucleus to make it able for protein synthesis
- In most of the cases the modification is done by the aid of **Ribozymes**
- A newly synthesized RNA molecule is called a **primary transcript** or **pre-RNA**
- The most extensive processing of primary transcripts occurs in eukaryotic mRNAs and in tRNAs of both bacteria and eukaryotes.
- The primary transcript for a eukaryotic mRNA typically contains sequences encompassing one gene, although the sequences encoding the polypeptide may not be contiguous. Noncoding tracts that break up the coding region of the transcript are called **introns**, and the coding segments are called **exons**
- The introns have to be removed and exons are joined together to form functional RNA
- The various post transcriptional modifications in primary transcript especially in pre mRNA are as

1.Cleavage

- In this process pre ribosomal RNA or **(pre-rRNA)** in both eukaryotes and prokaryotes is cleaved into different rRNA.
- This process is mostly carried out by **Ribonuclease** enzyme.
- In prokaryotes **the pre-rRNA is 30S**, which is cleaved into **16S, 23S, 5S rRNA** and **tRNA**



- In case of eukaryotes **pre-rRNA is 45S**, which is cleaved into **18S, 28S and 5.8S rRNA**



5-Capping

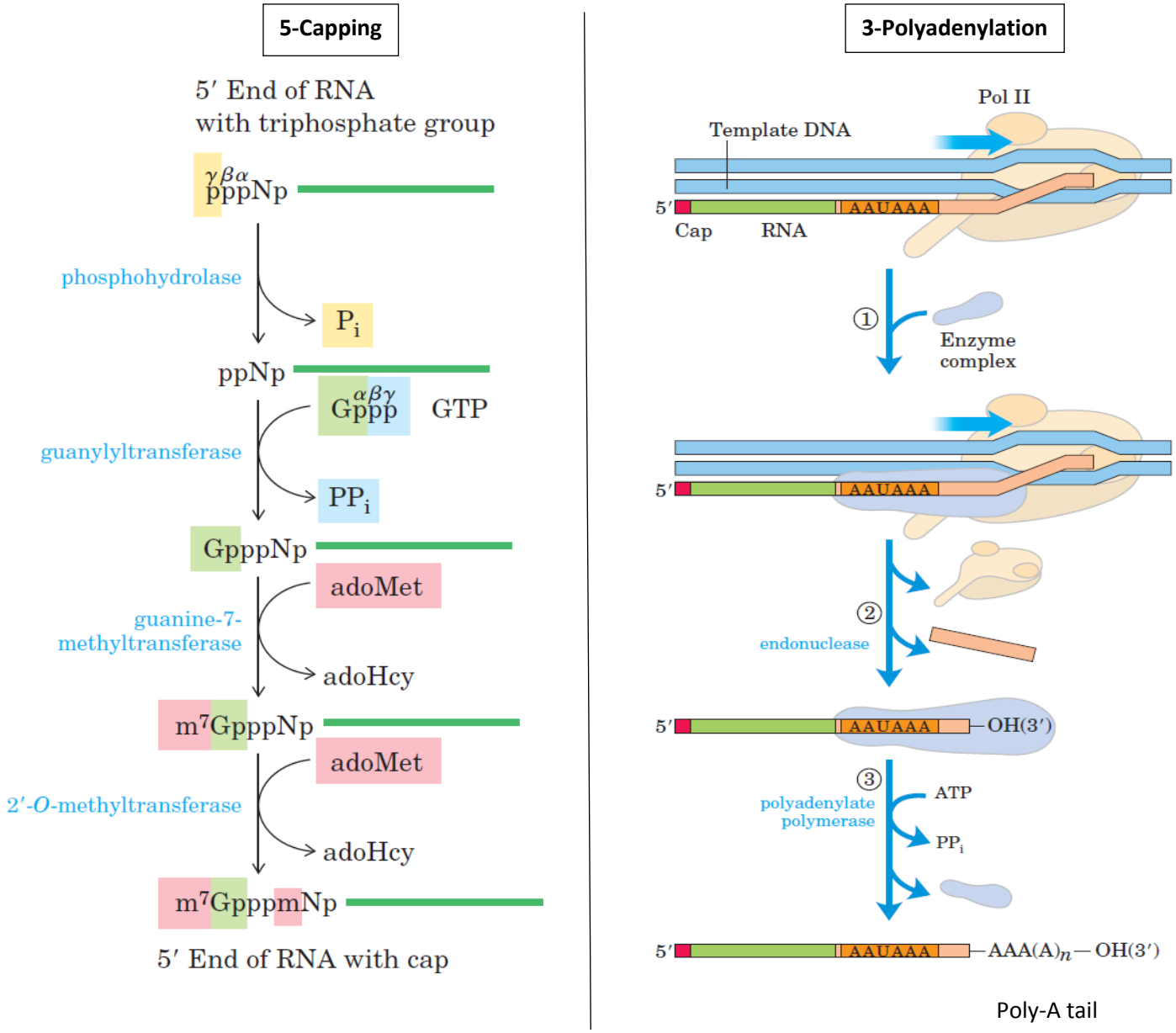
- Most eukaryotic mRNAs have a **5' cap**, a residue of **7-methylguanosine** linked to the 5'-terminal residue of the mRNA through an unusual **5',5'-triphosphate linkage**
- The 5' cap helps protect mRNA from **ribonucleases.**
- The cap also binds to a specific cap binding complex of proteins and participates in binding of the mRNA to the ribosome to initiate translation
- In 5-capping enzyme **phosphohydrolase** reomoves terminal 5' phosphate from the first nucleotideof mRNA.
- The enzyme **guanosyl transferase** then catalyses the reaction, which adds the guanine residue in a 5'5' triphosphate link.
- The enzyme **methyltransferase** transfers a methyl group from S-adenosyl methionine to the guanine ring

3-End Polyadenylation

- At their 3' end, most eukaryotic mRNAs have a string of 80 to 250 A residues, making up the polyadenine or **poly(A) tail.**
- This tail serves as a binding site for one or more specific proteins.
- The poly(A) tail and its associated proteins probably help protect mRNA from enzymatic destruction.
- Polyadenylation is preceeded by cleavge, The cleavage and adenylation reactions occur if a **polyadenylation signal sequence (5'- AAUAAA-3')** is located near the 3' end of the pre-mRNA molecule, which is followed by another sequence, which is usually (5'-CA-3') and is the site of

cleavage. A GU-rich sequence is also usually present further downstream on the pre-mRNA molecule.

- After the synthesis of the sequence elements. A protein complex forms and contains additional cleavage factors and the enzyme **Polyadenylate Polymerase (PAP).**
- This complex cleaves the RNA between the polyadenylation sequence and the GU-rich sequence at the cleavage site marked by the (5'-CA-3') sequences. Poly(A) polymerase then adds about 200 adenine units to the new 3' end of the RNA molecule using ATP as a precursor

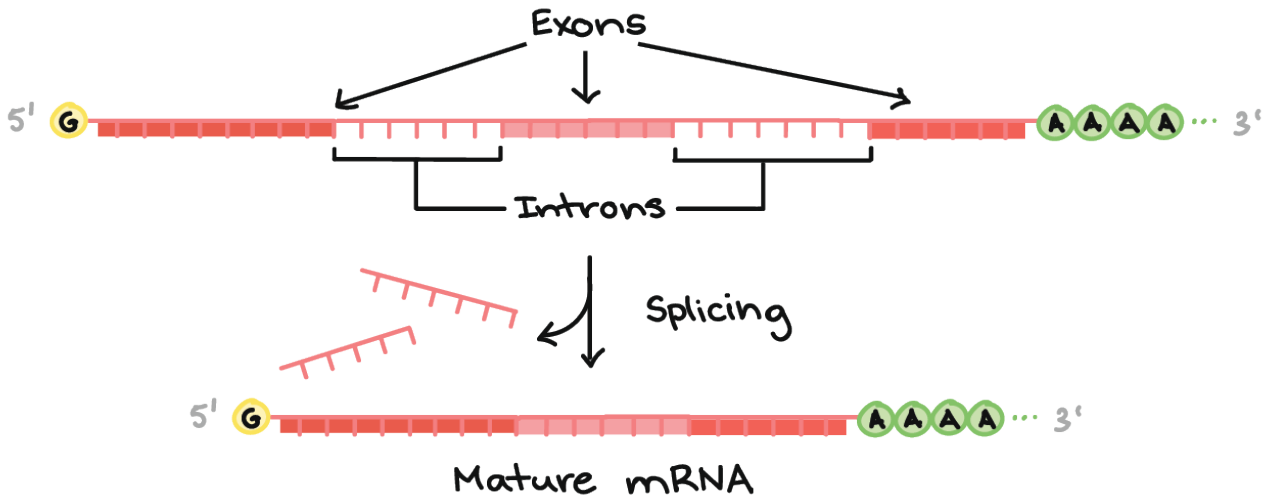


Splicing

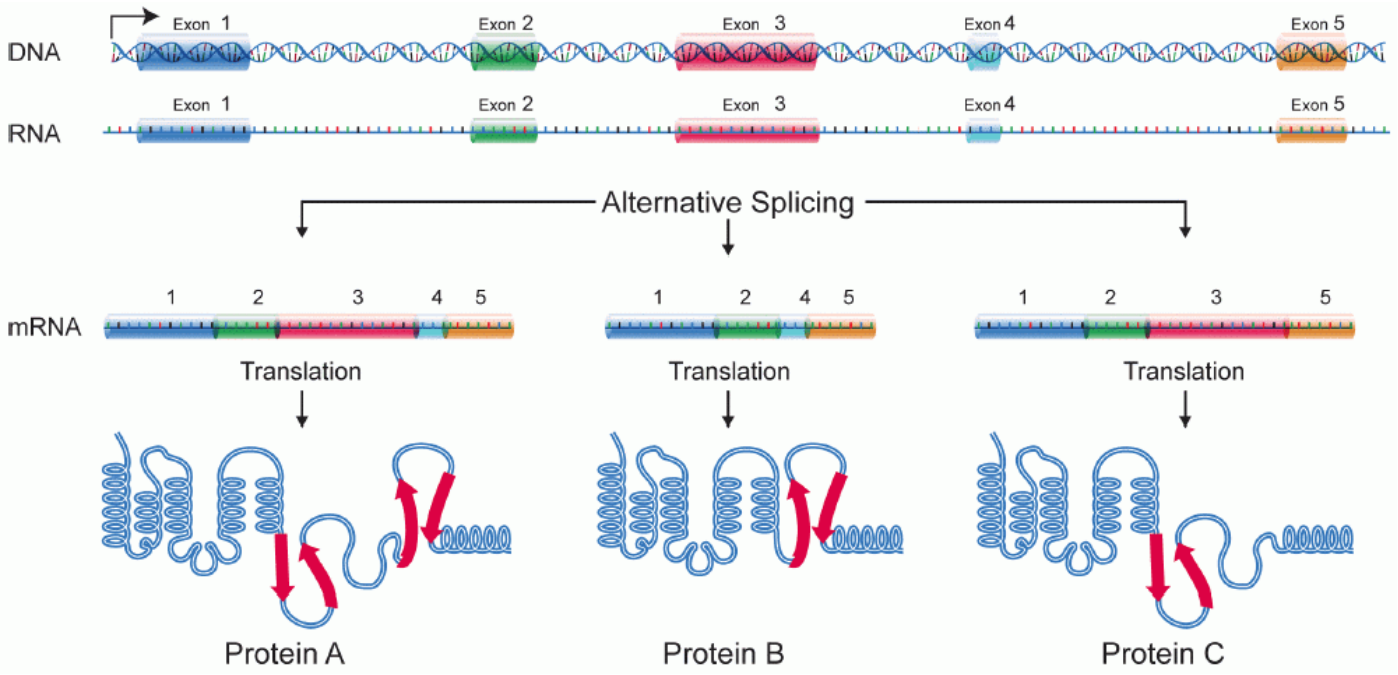
- Splicing removes non coding introns and joins coding introns
- In eukaryotic mRNAs, most exons are less than 1,000 nucleotides long, and Introns vary in size from 50 to 20,000 nucleotides
- There are four classes of introns. The first two, the group I and group II introns are self-splicing—no protein enzymes are involved. Group I introns are found in some nuclear, mitochondrial, and chloroplast genes coding for rRNAs, mRNAs, and tRNAs. Group II introns are generally found in

the primary transcripts of mitochondrial or chloroplast mRNAs in fungi, algae, and plants. Group I and group II introns are also found among the rarer examples of introns in bacteria.

- The third and largest class of introns includes those found in nuclear mRNA primary transcripts. These are **called spliceosomal introns,** because their removal occurs within and is catalyzed by a large protein complex called a s**pliceosome.**
- The spliceosome is made up of specialized RNA-protein complexes, **small nuclear ribonucleoproteins (snRNPs, often pronounced "snurps")**



- Many pre-mRNAs, including those encoding antibodies, can be spliced in multiple ways to produce different mature mRNAs that encode different protein sequences. This process is known as **alternative splicing,**

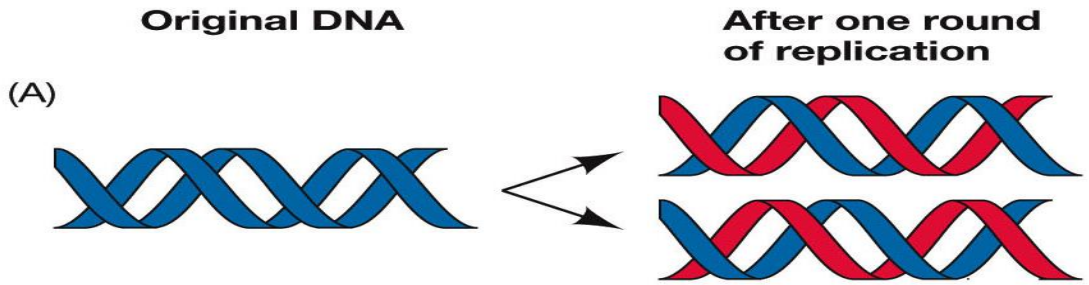


Asif Ahmed Kamgar
Asst. Professor (Zoology)
GDC Boys Anantnag

Genetics 2nd

DNA Replication

It is the biological, cellular process in which the 2 identical copies of DNA are formed from single DNA molecule.



General characcteristics of DNA replication

1.It is semiconservative.

Each DNA strand serves as a template for the synthesis of new strand producing two new DNA molecules, each with one new strand and one old strand. This is called semiconservative replication.

Watson and Crick proposed the hypothesis of semiconservative replication, but was proven by **Meselson and Stahl** in 1957.

- Meselson and Stahl grew *E. coli* cells for many generations in a medium in which the sole nitrogen source (**NH4Cl) contained 15N,** the "heavy" isotope of nitrogen, instead of the normal, more abundant "light" isotope, **14N.**
- The ***E. coli*** cells grown in the 15N medium were transferred to a fresh medium containing only the 14N isotope, where they were allowed to grow until the cell population had just doubled.
- The DNA isolated from these first-generation cells formed a single band in the cesium chloride **(CsCl)** gradient after centrifugation at a position indicating that the double helical DNA molecules of the daughter cells were hybrids containing one new 14N strand and one parent 15N. Cells were again allowed to double in number in the 14N medium.
- The isolated DNA product of this second cycle of replication exhibited two bands in the density gradient, one with a density equal to that of light DNA and the other with the density of the hybrid DNA observed after the first cell doubling. This proved that the DNA replication is semiconservative in nature.

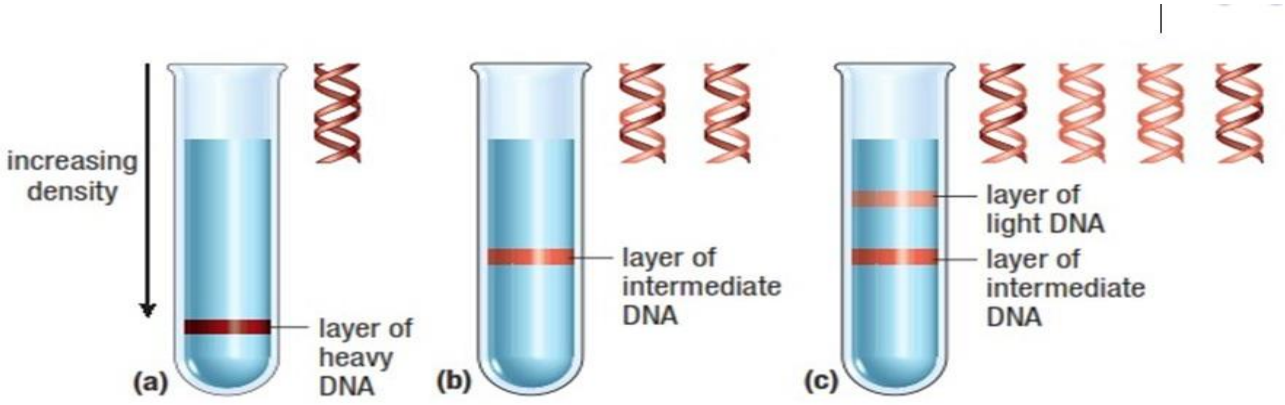
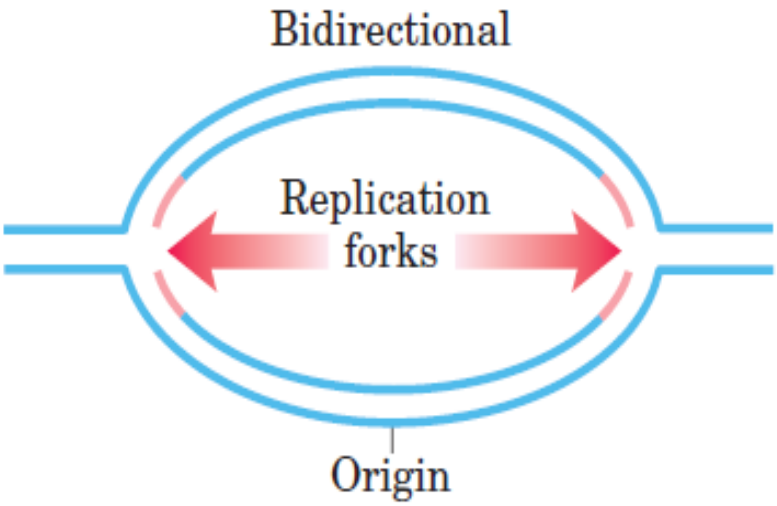


Figure 2 Results of Meselson and Stahl's experiment: (a) position of the original parent heavy ^{15}N DNA, (b) position of hybrid DNA produced after one round of DNA replication, and (c) positions of DNA formed after two rounds of replication

2. Replication Begins at an Origin and Usually Proceeds Bidirectionally.

The origin of replication is a particular sequence in a genome at which replication is initiated. DNA replication may proceed from this point bidirectionally or unidirectionally. The specific structure of the origin of replication varies somewhat from species to species, but all share some common characteristics such as high AT content.



3. DNA Synthesis Proceeds in a 5' to 3' Direction and Is Semidiscontinuous

A new strand of DNA is always synthesized in the 5' to 3' direction with reference to new strand. Because the two DNA strands are antiparallel, the strand serving as the template is read from its 3'end toward its 5'end.

4. DNA is synthesised by DNA polymerases.

The main enzyme responsibe for the polymerisation of new DNA strand is DNA polymerase which has many variants in both prokaryotes and eukaryotes.

5. Replication is very accurate.

The accuracy of replication can be understood from the fact that in *E. coli*, a mistake is made only once for every 10^9 to 10^{10} nucleotides added

6. Raw material for DNA replication is deoxyribonucleotides

Nuceotides are used in DNA replication as triphosphates

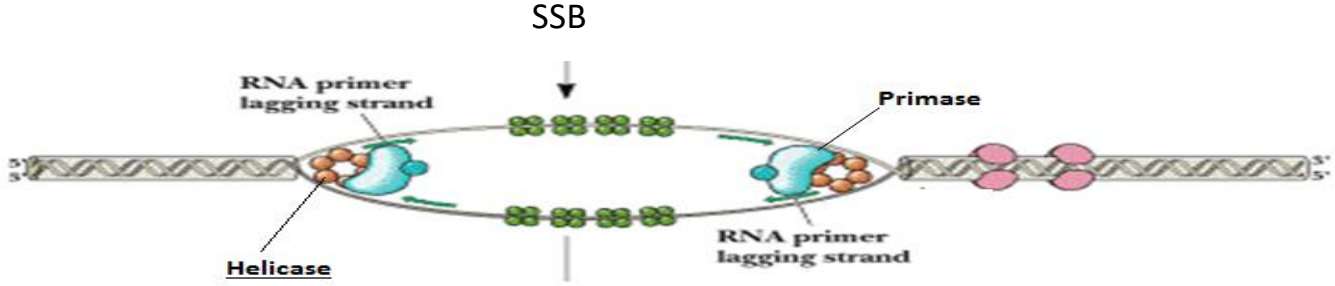
- Deoxyadenosine Triphosphate
- Deoxyguanosine Triphosphate
- Deoxythymidine Triphosphate
- Deoxycytidine Triphosphate

Process of Replication In Prokaryotes.

In *E. Coli* replication is completed in three steps

1. **Initiation:-**

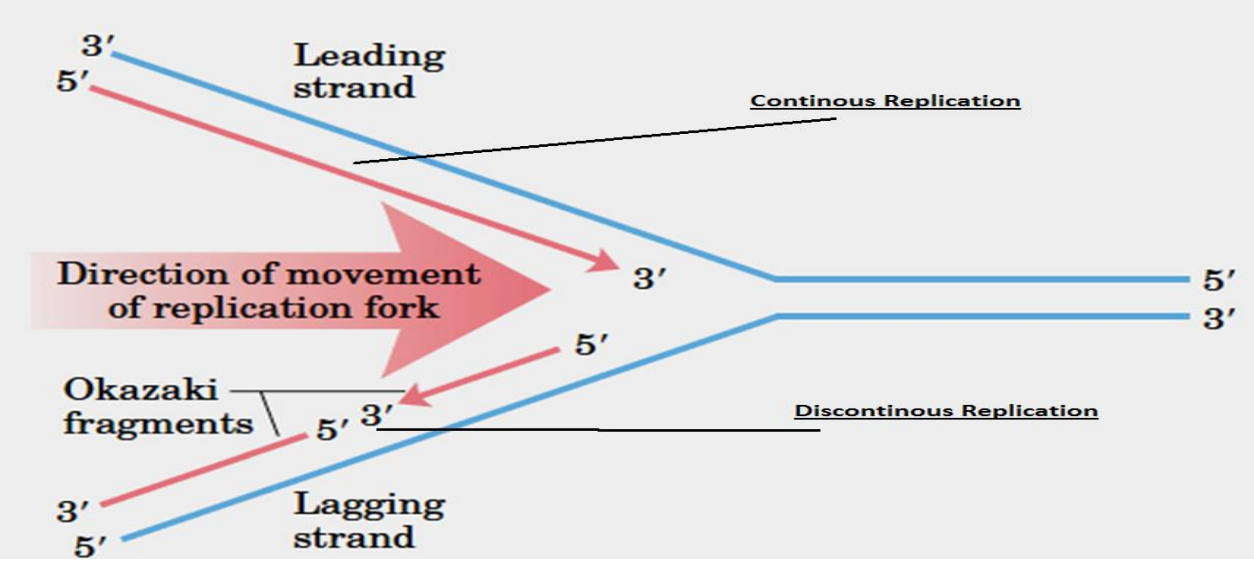
- Replication is initiated at origion of replication, rich in AT bases
- The *E. coli* replication origin***, oriC***, consists of 245 bp;
- It is acted upon by the enzyme **Helicase** to unwind the double strand utilizing ATP
- As the DNA opens, two Y-shaped structures called **replication forks** are formed, together making up what's called a **replication bubble**.
- Single strands are stabilized by **Single Strand Binding Protein (SSB)**
- Polymerase is not able to initiate daughter strand formation, but **Primase** does.
- Primase makes **an RNA primer**, or short stretch of nucleic acid complementary to the template, that provides a 3' end for DNA polymerase to work on.

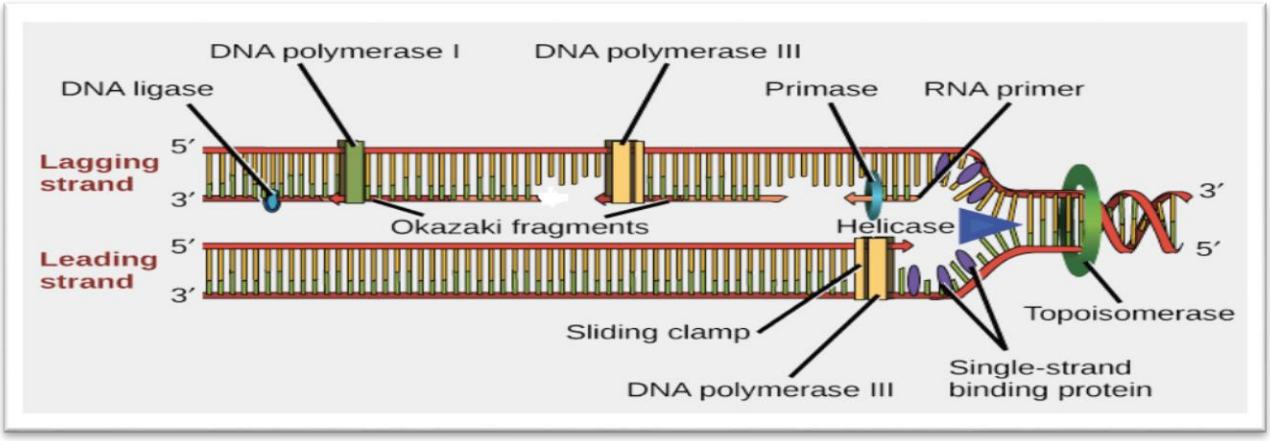


INITIATION

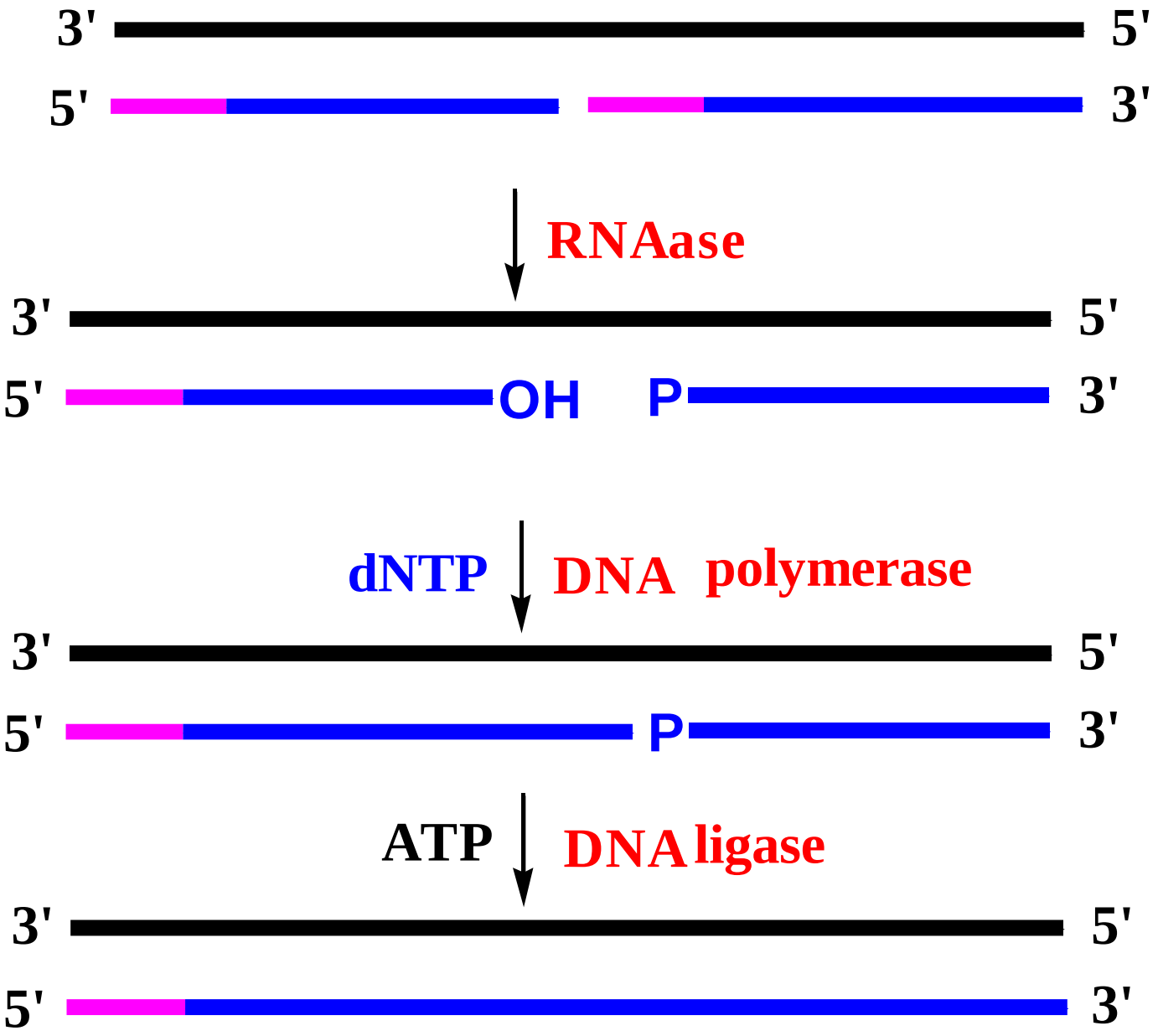
2. Elongation

- The main enzymes responsible for elongation of daughter strand is DNA polymerase
- In *E. coli*, the DNA polymerase that handles most of the synthesis is **DNA polymerase III,** this has 3' to 5' exonuclease activity but 5' to 3' polymerase activity
- Once the RNA primer is in place, DNA polymerase "extends" it, adding nucleotides one by one to make a new DNA strand that's complementary to the template strand.
- The deoxyribonucleotide triphosphates add to the 3 end of new strand releasing **pyrophosphate**, which in turn gets dissociated **into phosphate ion** releasing enegy
- This energy is used for hydrogen bonding between complementary nucleotides of parent and daughter strand.
- DNA Polymerase makes the DNA in **only 5' to 3' direction**. The nature of chain elongation is the series formation of the phosphodiester bonds.
- The torsion produced by continuous uncoiling of DNA by helicase is released by the enzyme **Gyrase (Topoisomerase 2)**
- The Complementary strands of DNA are antiparallel and DNA polymerase only catalyses polymerization in 5' to 3' direction
- So on one strand the polymerization is **continuous** but on the other strand the polymerization is **discontnous**
- The strand with continuous polymerization is called **leading strand** and the other one is called **Lagging strand**
- On lagging strand DNA is formed in fragments, each one with a short primer.
- These fragments are called **Okazaki fragments**. They are 1000 – 2000 nt long in Prokaryotes

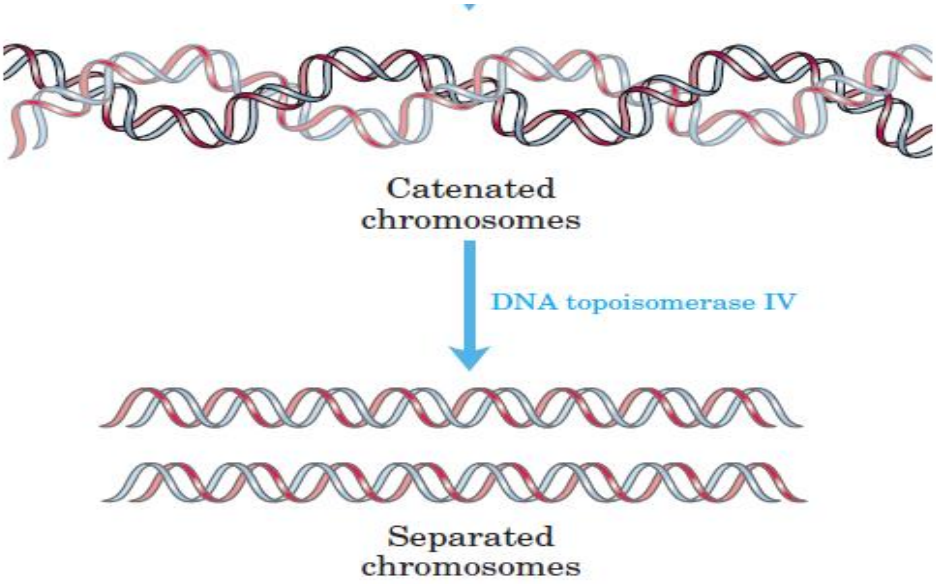




- Once an Okazaki fragment is completely formed ,its RNA primer is removed and replaced by deoxyribonucleotides by **DNA polymerase I.**
- The remaining nick is sealed by **DNA ligase using ATP.**



3. **Termination**
   - ▶ Eventually, the two replication forks of the circular *E. coli* chromosome meet at a terminus region containing multiple copies of a 20 bp sequence **called *Ter*.**
   - ▶ All the primers will be removed, and all the fragments will be connected by DNA-pol I and ligase.
   - ▶ Replication of the DNA separating the opposing replication forks leaves the completed chromosomes joined as **catenanes**, or topologically interlinked circles.
   - ▶ These catenanes are then separated from each other by **Topoisomerase iv**



Replication in Eukaryotes.

In eukaryotes replication occurs in the S (synthetic) phase of cell cycle.

The process involves same 3 steps as in eukaryotes.

1. **Initiation:-**
   - ▶ Replication starts from origion of replication called **autonomously replicating sequences (ARS) or replicators.**
   - ▶ **ARS** is short compared to **Ori C of *E. Coli.***
   - ▶ Each chromosome has many replicators.
   - ▶ During G1 phase the replicators are acted upon by a complex of proteins called **pre initiation complex**.
   - ▶ At the start of **S phase** the preinitiation complex gets converted into **Initiation Complex** by **kinases.**

- This initiation complex contains **Mini chromosome maintenance complex,** which acts as helicase.
- As the DNA opens, two Y-shaped structures called **replication forks** are formed, together making up what's called a **replication bubble**.
- **DNA Polymerase α**, initiates protein synthesis by forming a short **RNA Primer.**
- Formation of primer ends initiation.

2. Elongation.

- Elongation is same as for prokaryotes upto the okazaki fragment formation except the enzymes involved.
- **DNA polymerase δ** is used in elongation instead of **DNA polymerase.**
- The primer from fragments is cleaved by **RNAse H** , gap left is filled by **DNA Polymerase α** and then finally the fragments are joined together by **Ligase.**

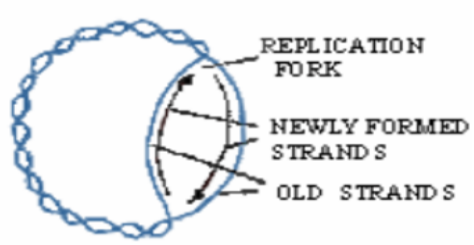
3. Termination

- When the replication forks from different replicators meet each other on chromosome replisome complex gets dissociated and the daughter strands are annealed together.
- However the ends of the DNA pose a problem solved by enzyme **Telomerase.**
- While the leading strand can use a single RNA primer to extend the 5' terminus of the replicating DNA strand, multiple RNA primers are responsible for lagging strand synthesis, creating Okazaki fragments.
- This leads to an issue due to the fact that DNA polymerase is only able to add to the 3' end of the DNA strand. The 3'-5' action of DNA polymerase along the parent strand leaves a short single-stranded DNA (ssDNA) region at the 3' end of the parent strand when the Okazaki fragments have been repaired.
- Since replication occurs in opposite directions at opposite ends of parent chromosomes, each strand is a lagging strand at one end. Over time this would result in progressive shortening of both daughter chromosomes. This is known as the end replication problem.
- The end replication problem is handled in eukaryotic cells by telomere regions and telomerase. Telomeres extend the 3' end of the parental chromosome beyond the 5' end of the daughter strand..
- Telomerase causes extension of the 3' end of the parental DNA molecule. This 3' addition provides a template for extension of the 5' end of the daughter strand by lagging strand DNA synthesis
- When the replication forks from different replicators meet each other on chromosome replisome complex gets dissociated and the daughter strands are annealed together.

Difference between:

DNA replication in Prokaryotes

1. It occurs inside the cytoplasm



2. There is only one origin of replication per DNA molecule

3. Origin of replication is formed of about 100-200 or more nucleotides

4. Replication of DNA occurs at one point in each prokaryotic DNA molecule

5. Only two replication fork is formed in each replicating prokaryotic chromosome, as DNA replication is bidirectional

6. Prokaryotic chromosome has one replicon

7. One replication bubble is formed during DNA replication

8. Initiation of DNA replication in prokaryotes is carried out by protein DnaA and DnaB

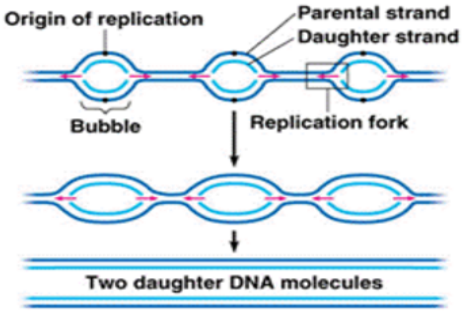
9. DNA gyrase is needed

10. Okazaki fragment are large, 1000-2000 nucleotides long.

11. Replication is very rapid, some 2000 bp per second are added.

DNA replication in Eukaryotes

1. It occurs inside the nucleus



2. Origin of replication are many(over 1000) in each eukaryotic chromosome

3. Each origin of replication is formed of about 150 nucleotides

4. Replication of DNA occurs at several points simultaneously in each chromosome.

5. A number of replication forks are formed simultaneously in each replicating DNA.

6. Eukaryotic DNA molecules have large number of replicons (50,000 and above), but replication does not occur simultaneously on all replicons

7. Numerous replication bubbles are formed in one replicating DNA molecule.

8. Initiation of DNA replication is carried out by multisubunit protein, origin recognition complex.

9. DNA gyrase is needed

10. Okazaki fragment are short, 100-200 nucleotides long.

11. Replication is slow, some 100 nucleotides per second are added