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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – I – SBC3102 – GENETICS

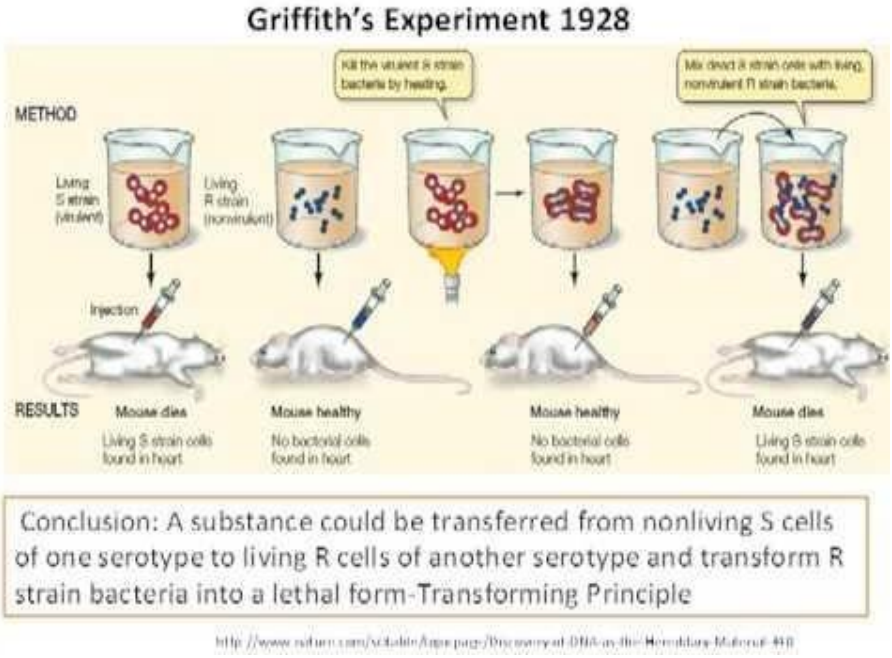
MENDELIAN PRINCIPLE

The physical nature of the gene fascinated scientists for many years. A series of experiments beginning in the 1920s finally revealed that DNA was the genetic material.

Discovery of transformation

A puzzling observation was made by Frederick Griffith in the course of experiments on the bacterium *Streptococcus pneumoniae* in 1928. This bacterium, which causes pneumonia in humans, is normally lethal in mice. However, different strains of this bacterial species have evolved that differ in virulence (in the ability to cause disease or death). In his experiments, Griffith used two strains that are distinguishable by the appearance of their colonies when grown in laboratory cultures. In onestrain, a normazl virulent type, the cells are enclosed in a polysaccharide capsule, giving colonies a smooth appearance; hence, this strain is labeled *S.* In Griffith's other strain, a mutant nonvirulent type that grows in mice but is not lethal, the polysaccharide coat is absent, giving colonies a rough appearance; this strain is called *R.*

Griffith killed some virulent cells by boiling them and injected the heat-killed cells into mice. The mice survived, showing that the carcasses of the cells do not cause death. However, mice injected with a mixture of heat-killed virulent cells and live nonvirulent cells did die. Furthermore, live cells could be recovered from the dead mice; these cells gave smooth colonies and were virulent on subsequent injection. Somehow, the cell debris of the boiled S cells had converted the live R cells into live S cells. The process is called transformation. Griffith's experiment is summarized in Figure .



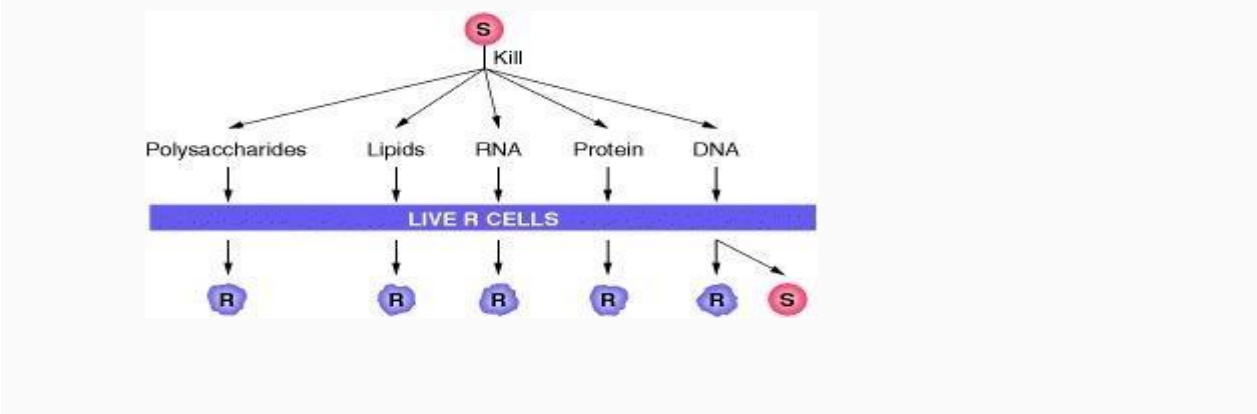
The first demonstration of bacterial transformation. (a) Mouse dies after injection with the virulent S strain. (b) Mouse survives after injection with the R strain. (c) Mouse survives after injection with heat-killed S strain. (d) Mouse dies after injection with a mixture of heat-killed S strain and live R strain. The heat-killed S strain somehow transforms the R strain into virulence. Parts a, b, and c act as control experiments for this demonstration.

This same basic technique was then used to determine the nature of the *transforming principle*— the agent in the cell debris that is specifically responsible for transformation. In 1944, Oswald Avery, C. M. MacLeod, and M. McCarty separated the classes of molecules found in the debris of the dead S cells and tested them for transforming ability, one at a time. These tests showed that the polysaccharides themselves do not transform the rough cells. Therefore, the polysaccharide coat, although undoubtedly concerned with the pathogenic reaction, is only the phenotypic expression of virulence. In screening the different groups, Avery and his colleagues found that only one class of molecules, DNA, induced the transformation of R cells (Figure given below). They deduced that DNA is the agent that determines the polysaccharide character and hence the pathogenic character (see pages 219–220 for a description of

the mechanism of transformation). Furthermore, it seemed that providing R cells with S DNA was tantamount to providing these cells with S genes.

Demonstration that DNA is the transforming agent. DNA is the only agent that produces smooth

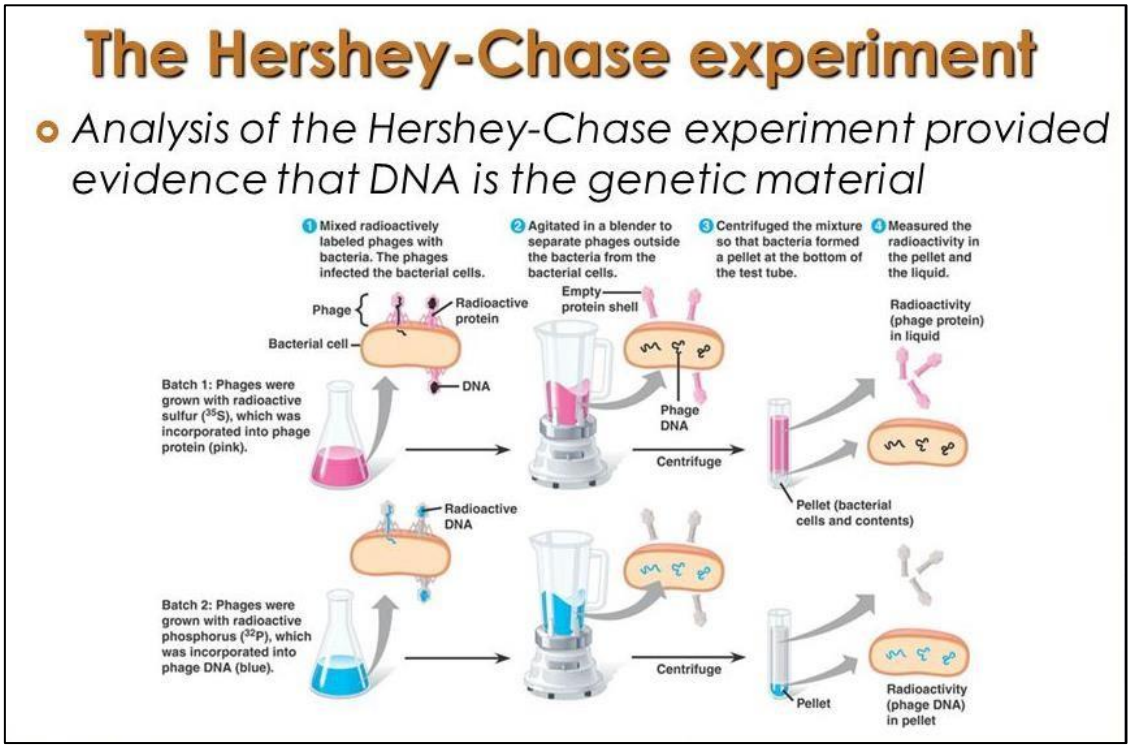
(S) colonies when added to live rough (R) cells.

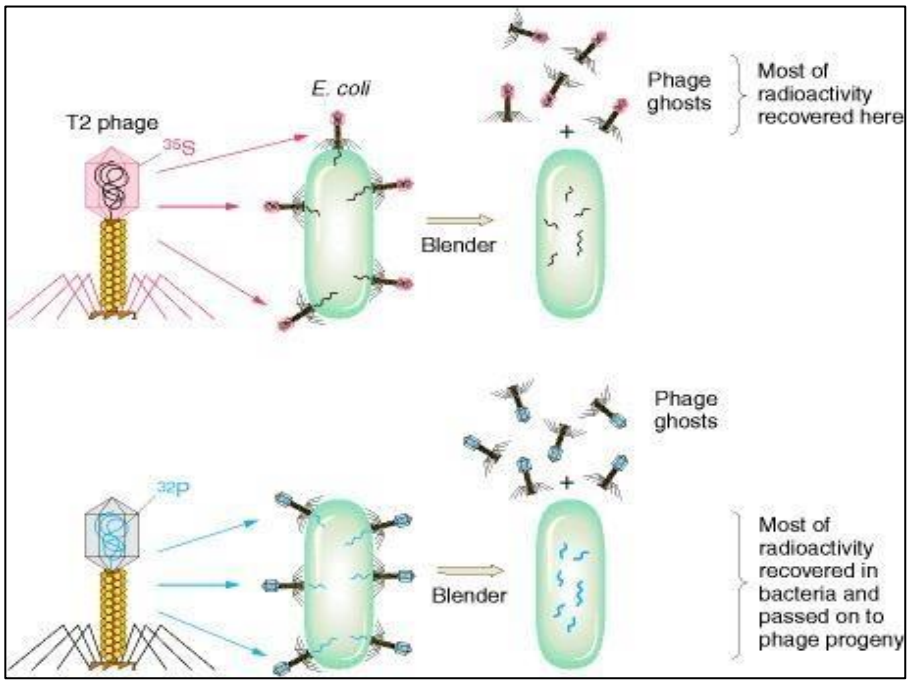


Hershey-Chase experiment

The experiments conducted by Avery and his colleagues were definitive, but many scientists were very reluctant to acceptDNA (rather than proteins) as the genetic material. The clincher was provided in 1952 by Alfred Hershey and Martha Chase with the use of the phage (virus) T2. They reasoned that phage infection must entail the introduction (injection) into the bacterium of the specific information that dictates viral reproduction. The phage is relatively simple in molecular constitution. Most of its structure is protein, with DNA contained inside the protein sheath of its "head." Phosphorus is not found in proteins but is an integral part of DNA; conversely, sulfur is present in proteins but never in DNA. Hershey and Chase incorporated the radioisotope of phosphorus (^{32}P) into phage DNA and that of sulfur (^{35}S) into the proteins of a separate phage culture. They then used each phage culture independently to infect *E. coli* with many virus particles per cell. After sufficient time for injection to take place, they sheared the empty phage carcasses (called *ghosts*) off the bacterial cells by agitation in a kitchen blender. They used centrifugation to separate the bacterial cells from the phage ghosts and then measured the radioactivity in the two fractions. When the ^{32}P-labeled phages were used, most of the radioactivity ended up inside the bacterial cells, indicating that the phage DNA entered the cells. ^{32}P can also be recovered from phage progeny. When the ^{35}S-labeled phages were used, most of the radioactive material ended

up in the phage ghosts, indicating that the phage protein never entered the bacterial cell. The conclusion is inescapable: DNA is the hereditary material; the phage proteins are mere structural packaging that is discarded after delivering the viral DNA to the bacterial cell.





The Hershey-Chase experiment, which demonstrated that the genetic material of phage is DNA, not protein. The experiment uses two sets of T2 bacteriophages. In one set, the protein coat is labeled with radioactive sulfur (^{35}S), not found in DNA. ther set, the DNA is labeled with radioactive phosphorus (^{32}P), not found in protein. Only the ^{32}P is injected into the *E. coli*, indicating that DNA is the agent necessary for the production of new phages.

Mendelian Inheritance

Mendelian inheritance is inheritance of biological features that follows the laws proposed by Gregor Johann Mendel in 1865 and 1866 and re-discovered in 1900. It was initially very controversial. When Mendel's theories were integrated with the Boveri–Sutton chromosome theory of inheritance by Thomas Hunt Morgan in 1915, they became the core of classical genetics while Ronald Fishercombined them with the theory of natural selection in his 1930 book *The Genetical Theory of Natural Selection*, putting evolutiononto a mathematical footing and forming the basis for Population genetics and the modern evolutionary synthesis.

History

The laws of inheritance were derived by Gregor Mendel, a nineteenth-century Austrian monk conducting hybridization experiments in garden peas (*Pisum sativum*) he planted in the backyard of the church. Between 1856 and 1863, he cultivated and tested some 5,000 pea plants. From these experiments, he induced two generalizations which later became known as *Mendel's Principles of Heredity* or *Mendelian inheritance*. He described these principles in a two-part paper, *Versuche über Pflanzen-Hybriden*(*Experiments on Plant Hybridization*), that he read to the Natural History Society of Brno on February 8 and March 8, 1865, and which was published in 1866.

Mendel's conclusions were largely ignored. Although they were not completely unknown to biologists of the time, they were not seen as generally applicable, even by

Mendel himself, who thought they only applied to certain categories of species or traits. A major block to understanding their significance was the importance attached by 19th-century biologists to the apparent blending of inherited traits in the overall appearance of the progeny, now known to be due to multigene interactions, in contrast to the organ-specific binary characters studied by Mendel. In 1900, however, his work was "re-discovered" by three European scientists, Hugo de Vries, Carl Correns, and Erich von Tschermak. The exact nature of the "re-discovery" has been somewhat debated: De Vries published first on the subject, mentioning Mendel in a footnote, while Correns pointed out Mendel's priority after having read De Vries' paper and realizing that he himself did not have priority. De Vries may not have acknowledged truthfully how much of his knowledge of the laws came from his own work, or came only after reading Mendel's paper. Later scholars have accused Von Tschermak of not truly understanding the results at all.

Regardless, the "re-discovery" made Mendelism an important but controversial theory. Its most vigorous promoter in Europe wasWilliam Bateson, who coined the terms "genetics" and "allele" to describe many of its tenets. The model of heredity was highly contested by other biologists because it implied that heredity was discontinuous, in opposition to the apparently continuous variation observable for many traits. Many biologists also dismissed the theory because they were not sure it would apply to all species. However, later work by biologists and statisticians such as Ronald Fisher showed that if multiple Mendelian factors were involved in the expression of an individual trait, they could produce the diverse results observed, and thus showed that Mendelian genetics is compatible with natural selection. Thomas Hunt Morgan and his assistants later integrated the theoretical model of Mendel with the chromosome theory of inheritance, in which the chromosomes of cells were thought to hold the actual hereditary material, and created what is now known as classical genetics, which was extremely successful and cemented Mendel's place in history.

Mendel's findings allowed scientists such as Fisher and J.B.S. Haldane to predict the expression of traits on the basis of mathematical probabilities. A large contribution to Mendel's success can be traced to his decision to start his crosses only with plants he demonstrated were true-breeding. He also only measured absolute (binary)

characteristics, such as color, shape, and position of the offspring, rather than quantitative characteristics. He expressed his results numerically and subjected them to statistical analysis. His method of data analysis and his large sample size gave credibility to his data. He also had the foresight to follow several successive generations (f2, f3) of pea plants and record their variations. Finally, he performed "test crosses" (back-crossing descendants of the initial hybridization to the initial true-breeding lines) to reveal the presence and proportion of recessive characters.

Mendel's laws

Mendel discovered that, when he crossed purebred white flower and purple flower pea plants (the parental or P generation), the result was not a blend. Rather than being a mix of the two, the offspring (known as the F_1 generation) was purple-flowered. When Mendel self-fertilized the F_1 generation pea plants, he obtained a purple flower to white flower ratio in the F_2 generation of 3 to 1.

He then conceived the idea of heredity units, which he called "factors". Mendel found that there are alternative forms of factors—now called genes—that account for variations in inherited characteristics. For example, the gene for flower color in pea plants exists in two forms, one for purple and the other for white. The alternative "forms" are now called alleles. For each biological trait, an organism inherits two alleles, one from each parent. These alleles may be the same or different. An organism that has two identical alleles for a gene is said to be homozygous for that gene (and is called a homozygote). An organism that has two different alleles for a gene is said be heterozygous for that gene (and is called a heterozygote).

Mendel also hypothesized that allele pairs separate randomly, or segregate, from each other during the production of gametes: egg and sperm. Because allele pairs separate during gamete production, a sperm or egg carries only one allele for each inherited trait. When sperm and egg unite at fertilization, each contributes its allele, restoring the paired condition in the offspring. This is called the**Law of Segregation**. Mendel also found that each pair of alleles segregates independently of the other pairs of alleles during gamete formation. This is known as the **Law of Independent Assortment**.

The genotype of an individual is made up of the many alleles it possesses. An individual's physical appearance, or phenotype, is determined by its alleles as well as by its environment. The presence of an allele does not mean that the trait will be expressed in the individual that possesses it. If the two alleles of an inherited pair differ (the heterozygous condition), then one determines the organism's appearance and is called the dominant allele; the other has no noticeable effect on the organism's appearance and is called the recessive allele. Thus, in the example above dominant purple flower allele will hide the phenotypic effects of the recessive white flower allele. This is known as the **Law of Dominance** but it is not a transmission law, dominance has to do with the expression of the genotype and not its transmission. The upper case letters are used to represent dominant alleles whereas the lowercase letters are used to represent recessive alleles.

Mendel's laws of inheritance

Law	**Definition**
Law of segregation	During gamete formation, the alleles for each gene segregate from each other so that each gamete carries only one allele for each gene.
Law of independent assortment	Genes for different traits can segregate independently during the formation of gametes.
Law of dominance	Some alleles are dominant while others are recessive; an organism with at least one dominant allele will display the effect of the dominant allele.

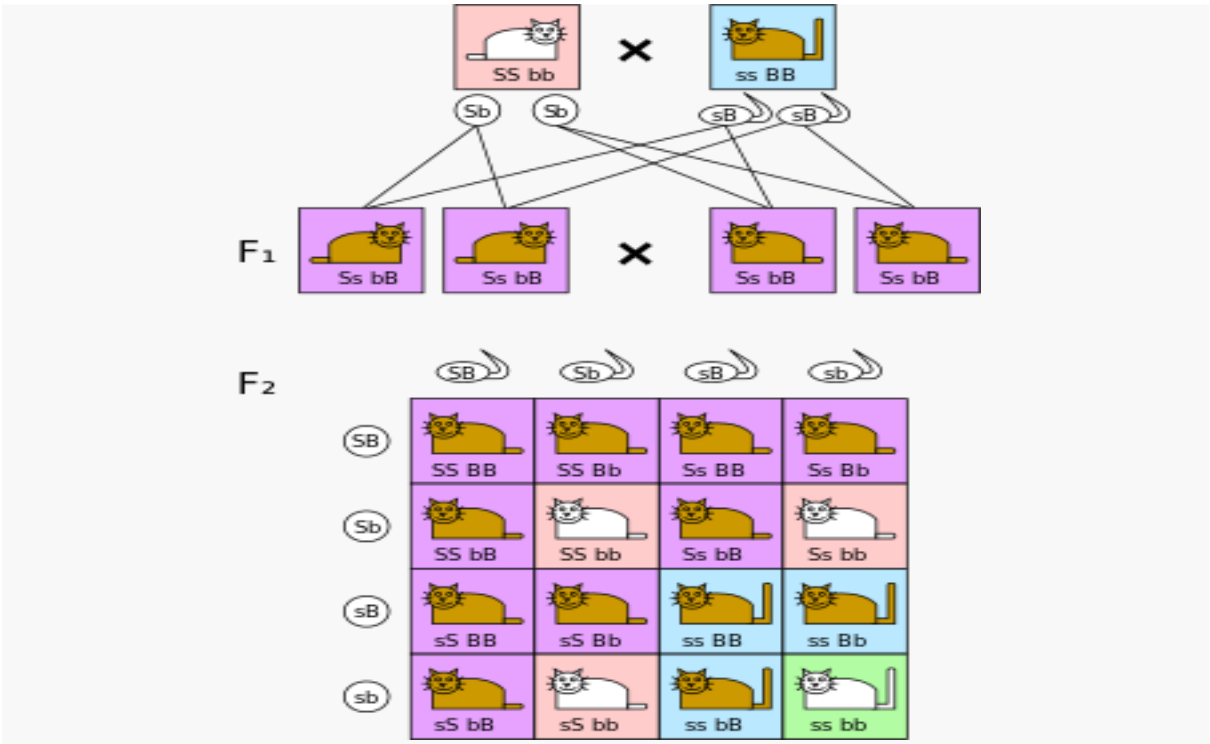
In the pea plant example above, the capital "P" represents the dominant allele for purple flowers and lowercase "p" represents the recessive allele for white flowers. Both parental plants were true-breeding, and one parental variety had two alleles for purple flowers (*PP*) while the other had two alleles for white flowers (*pp*). As a result of fertilization, the F_1 hybrids each inherited one allele for purple flowers and one for white. All the F_1 hybrids (*Pp*) had purple flowers, because the dominant *P* allele has its full effect in the heterozygote, while the recessive *p* allele has no effect on flower color. For the F_2 plants, the ratio of plants with purple flowers to those with white flowers (3:1) is called the phenotypic ratio. The genotypic ratio, as seen in the Punnett square, is 1 *PP* : 2 *Pp* : 1 *pp*.

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.

Molecular proof of this principle was subsequently found through observation of meiosis by two scientists independently, the German botanist Oscar Hertwig in 1876, and the Belgian zoologist Edouard Van Beneden in 1883. Paternal and maternal chromosomes get separated in meiosis and the alleles with the traits of a character are segregated into two different gametes. Each parent contributes a single gamete, and thus a single, randomly successful allele copy to their offspring and fertilization.

Law of Independent Assortment (the "Second Law")

The Law of Independent Assortment states that alleles for separate traits are passed independently of one another from parents to offspring. That is, the biological selection of an allele for one trait has nothing to do with the selection of an allele for any other trait. Mendel found support for this law in his dihybrid cross



Dihybrid cross. The phenotypes of two independent traits show a 9:3:3:1 ratio in the F_2 generation. In this example, coat color is indicated by **B** (brown, dominant) or **b** (white), while tail length is indicated by **S** (short, dominant) or **s** (long). When parents are homozygous for each trait (**SSbb** and **ssBB**), their children in the F_1 generation are heterozygous at both loci and only show the dominant phenotypes (**SsbB**). If the children mate with each other, in the F_2 generation all combinations of coat color and tail length occur: 9 are brown/short (purple boxes), 3 are white/short (pink boxes), 3 are brown/long (blue boxes) and 1 is

experiments. In his monohybrid crosses, an idealized 3:1 ratio between dominant and recessive phenotypes resulted. In dihybrid crosses, however, he found a 9:3:3:1 ratio. This shows that each of the two alleles is inherited independently from the other, with a 3:1 phenotypic ratio for each.

Independent assortment occurs in eukaryotic organisms during meiotic prophase I, and produces a gamete with a mixture of the organism's chromosomes. The physical basis of the independent assortment of chromosomes is the random orientation of each bivalent chromosome along the metaphase plate with respect to the other bivalent chromosomes. Along with crossing over, independent assortment increases genetic diversity by producing novel genetic combinations. The are many violations of independent assortment due to genetic linkage.

Of the 46 chromosomes in a normal diploid human cell, half are maternally derived (from the mother's egg) and half are paternally derived (from the father's sperm). This occurs as sexual reproduction involves the fusion of two haploid gametes (the egg and sperm) to produce a new organism having the full complement of chromosomes. During gametogenesis—the production of new gametes by an adult—the normal complement of 46 chromosomes needs to be halved to 23 to ensure that the resulting haploid gamete can join with another gamete to produce a diploid organism. An error in the number of chromosomes, such as those caused by a diploid gamete joining with a haploid gamete, is termed aneuploidy.

In independent assortment, the chromosomes that result are randomly sorted from all possible combinations of maternal and paternal chromosomes. Because gametes end up with a random mix instead of a pre-defined "set" from either parent, gametes are therefore considered assorted independently. As such, the gamete can end up with any combination of paternal or maternal chromosomes. Any of the possible combinations of gametes formed from maternal and paternal chromosomes will occur with equal frequency. For human gametes, with 23 pairs of chromosomes, the number of possibilities is 2^{23} or 8,388,608 possible combinations.[4] The gametes will normally end up with 23 chromosomes, but the origin of any particular one will be randomly selected from paternal or maternal chromosomes. This contributes to the genetic variability of progeny.

Law of Dominance (the "Third Law")

Mendel's Law of Dominance states that recessive alleles will always be masked by dominant alleles. Therefore, a cross between a homozygous dominant and a homozygous recessive will always express the dominant phenotype, while still having a heterozygous genotype. Law of Dominance can be explained easily with the help of a mono hybrid cross experiment:- In a cross between two organisms pure for any pair (or pairs) of contrasting traits (characters), the character that appears in the F1 generation is called "dominant" and the one which is suppressed (not expressed) is called

"recessive." Each character is controlled by a pair of dissimilar factors. Only one of the characters expresses. The one which expresses in the F1 generation is called Dominant. It is important to note however, that the law of dominance is significant and true but is not universally applicable.

According to the latest revisions, only two of these rules are considered to be laws. The third one is considered as a basic principle but not a genetic law of Mendel.

Mendelian trait

A Mendelian trait is one that is controlled by a single locus in an inheritance pattern. In such cases, a mutation in a single gene can cause a disease that is inherited according to Mendel's laws. Examples include sickle-cell anemia, Tay-Sachs disease, cystic fibrosis and xeroderma pigmentosa. A disease controlled by a single gene contrasts with a multi-factorial disease, like arthritis, which is affected by several loci (and the environment) as well as those diseases inherited in a non-Mendelian fashion.

Non-Mendelian inheritance

Non-Mendelian inheritance

Mendel explained inheritance in terms of discrete factors—genes—that are passed along from generation to generation according to the rules of probability. Mendel's laws are valid for all sexually reproducing organisms, including garden peas and human beings. However, Mendel's laws stop short of explaining some patterns of genetic inheritance. For most sexually reproducing organisms, cases where Mendel's laws can strictly account for the patterns of inheritance are relatively rare. Often, the inheritance patterns are more complex.

The F1 offspring of Mendel's pea crosses always looked like one of the two parental varieties. In this situation of "complete dominance," the dominant allele had the same phenotypic effect whether present in one or two copies. But for some characteristics, the F1 hybrids have an appearance *in between* the phenotypes of the two parental

varieties. A cross between two four o'clock (*Mirabilis jalapa*) plants shows this common exception to Mendel's principles. Some alleles are neither dominant nor recessive. The F1 generation produced by a cross between red-flowered (RR) and white flowered (WW) *Mirabilis jalapa* plants consists of pink-colored flowers (RW). Which allele is dominant in this case? Neither one. This third phenotype results from flowers of the heterzygote having less red pigment than the red homozygotes. Cases in which one allele is not completely dominant over another are called **incomplete dominance**. In incomplete dominance, the heterozygous phenotype lies somewhere between the two homozygous phenotypes.

A similar situation arises from **codominance**, in which the phenotypes produced by both alleles are clearly expressed. For example, in certain varieties of chicken, the allele for black feathers is codominant with the allele for white feathers. Heterozygous chickens have a color described as "erminette," speckled with black and white feathers. Unlike the blending of red and white colors in heterozygous four o'clocks, black and white colors appear separately in chickens. Many human genes, including one for a protein that controls cholesterol levels in the blood, show codominance, too. People with the heterozygous form of this gene produce two different forms of the protein, each with a different effect on cholesterol levels.

In Mendelian inheritance, genes have only two alleles, such as *a* and *A*. In nature, such genes exist in several different forms and are therefore said to have **multiple alleles**. A gene with more than two alleles is said to have multiple alleles. An individual, of course, usually has only two copies of each gene, but many different alleles are often found within a population. One of the best-known examples is coat color in rabbits. A rabbit's coat color is determined by a single gene that has at least four different alleles. The four known alleles display a pattern of simple dominance that can produce four coat colors. Many other genes have multiple alleles, including the human genes for ABO blood type.

Furthermore, many traits are produced by the interaction of several genes. Traits controlled by two or more genes are said to be **polygenic traits**. *Polygenic* means "many genes." For example, at least three genes are involved in making the reddish-brown pigment in the eyes of fruit flies. Polygenic traits often show a wide range of

phenotypes. The variety of skin color in humans comes about partly because more than four different genes probably controls this trait.

Variations to Mendelian Genetics

- Not all genetic traits strictly follow the laws discovered by Gregor Mendel.
- Some variations can be observed in all animals including humans.
- Four types:
  - Incomplete dominance
  - Codominance
  - Polygenic inheritance
  - Sex linked traits and/or Sex Influenced

Incomplete & Codominance

Incomplete Dominance

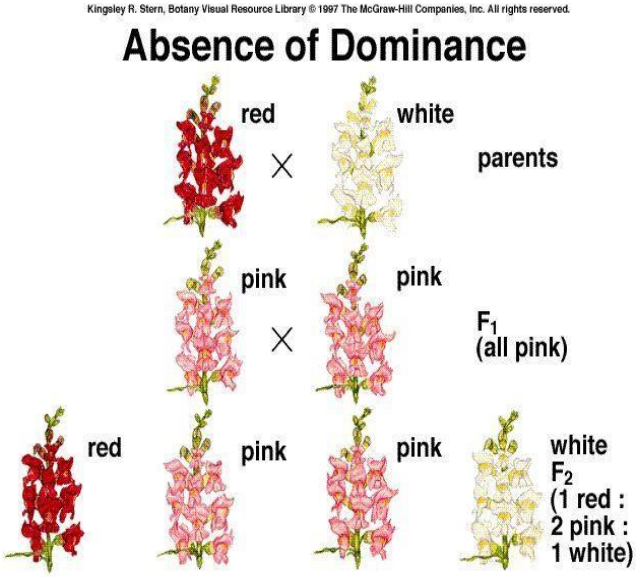
In many ways Gregor Mendel was quite lucky in discovering his genetic laws. He happened to use pea plants, which happened to have a number of easily observable traits that were determined by just two alleles. And for the traits he studied in his peas, one allele happened to be dominant for the trait & the other was a recessive form. Things aren't always so clear-cut & "simple" in the world of genetics, but luckily for Mendel (& the science world) he happened to work with an organism whose genetic make-up was fairly clear-cut & simple.
If Mendel were given a mommy black mouse & a daddy white mouse & asked what their offspring would look like, he would've said that a certain percent would be black & the others would be white. He would never have even considered that a white mouse & a black mouse could produce a *GREY* mouse! For Mendel, the phenotype of the offspring from parents with different phenotypes always resembled the phenotype of at least one of the parents. In other words, Mendel was unaware of the phenomenon of INCOMPLETE DOMINANCE.

With incomplete dominance, a cross between organisms with two different phenotypes produces offspring with a third phenotype that is a blending of the parental traits.

It's like mixing paints, red + white will make pink. Red doesn't totally block (dominate) the pink, instead there is *incomplete*dominance, and we end up with something in- between.

We can still use the Punnett Square to solve problems involving incomplete dominance. The only difference is that instead of using a capital letter for the dominant trait & a lowercase letter for the recessive trait, the letters we use are both going to be capital (because neither trait dominates the other). So the cross I used up above would look like this:

	R	r
R	R R	R r
r	R r	r r



Codominance

The genetic gist to codominance is pretty much the same as incomplete dominance. A hybrid organism shows a third phenotype --- not the usual "dominant" one & not the "recessive" one... but a third, *different* phenotype. With incomplete dominance we get a <u>blending</u> of the dominant & recessive traits so that the third phenotype is something in the middle (red x white = pink).

In <u>CO</u>dominance, the "recessive" & "dominant" traits appear <u>together</u> in the phenotype of hybrid organisms.

A very common phenotype used in questions about codominance is roan fur in cattle. Cattle can be red (RR = all red hairs), white (WW = all white hairs), or roan (RW = red & white hairs <u>together</u>). A good example of codominance.

Another example of codominance is human blood type AB, in which two types of protein ("A" & "B") appear <u>together</u> on the surface of blood cells.

Multiple Alleles

It makes absolutely no sense whatsoever to continue if we don't know what the word "allele" means.

allele = (*n)* a form of a gene which codes
for one possible outcome of a phenotype

If there are only two alleles involved in determining the phenotype of a certain trait, but there are *three* possible phenotypes, then the inheritance of the trait illustrates either incomplete dominance or codominance.

In these situations a heterozygous (hybrid) genotype produces a 3rd phenotype that is either a blend of the other two phenotypes (incomplete dominance) or a mixing of the other phenotypes with both appearing at the same time (codominance).

Here's an example with Incomplete Dominance:

GENOTYPES	RESULTING PHENOTYPE
BB = Homozygous Black	Black Fur
BW = Heterozygous	Grey Fur
WW = Homozygous White	White Fur

where
B = allele for black &
W = allele for white

And here's an example with Codominance:

GENOTYPES	RESULTING PHENOTYPE
BB = Homozygous Black	Black Fur
BW = Heterozygous	Black & White Fur
WW = Homozygous White	White Fur

where
B = allele for black &
W = allele for white

Now, if there are *4* or more possible phenotypes for a particular trait, then more than 2 alleles for that trait must exist in the population. We call this "MULTIPLE ALLELES". Because individuals have only two biological parents. We inherit half of our genes (alleles) from ma, & the other half from pa, so we end up with two alleles for every trait in our phenotype.

An excellent example of multiple allele inheritance is human blood type. Blood type exists as four possible phenotypes: A, B, AB, & O.

There are 3 alleles for the gene that determines blood type.

ALLELE	CODES FOR
I^A	Type "A" Blood
I^B	Type "B" Blood
i	Type "O" Blood

GENOTYPES	RESULTING PHENOTYPES
I^AI^A	Type A
I^Ai	Type A
I^BI^B	Type B
I^Bi	Type B
I^AI^B	Type AB
ii	Type O

- Note that there are two genotypes for both "A" & "B" blood --- either homozygous (I^AI^A or I^BI^B) or heterozygous with one recessive allele for "O" (I^Ai or I^Bi).
- Note too that the only genotype for "O" blood is homozygous recessive (ii).

Gene Interactions

Definition

With the help of lot of experiments it was found that most of the characters of living organisms are controlled / influenced / governed by a collaboration of several different genes.

This condition where a single character is governed by two or more genes and every gene affect the expression of other genes involved (means these genes affect each others expression) is known as gene interaction.

In simple way we could say that, in gene interaction, expression of one gene depends on expression (presence or absence) of another gene.

As we know, gene interactions may involve two or more pairs of genes. But all the gene interactions we have described below have the two pairs of non-allelic genes, affecting the phenotypic expression of same character. These interactions produce modified dihybrid ratios.

Types of Gene Interactions

Gene interactions can be classified as

- Allelic gene interaction
- Non-allelic gene interaction

Non-allelic gene interaction

Expression of character is produced by interaction between two or more genes. The interactions we have listed below, as inter and intra allelic are of this type.

- Inter-allelic
- Intra-allelic

Inter-allelic

- Without modification of normal F_2 ratio
- With modification of normal F_2 ratio

Such kinds of interactions modify the normal F_2 ratio (9:3:3:1). Various types of

such interactions are as below.

Gene Interaction	F2 Ratio	Test Cross Ratio
Complementary Gene Interaction	9:7	1:3
Supplementary Gene Interaction	9:3:4	1:1:2
Epistasis	12:3:1	2:1:1
Duplicate Factor	15:1	3:1
Inhibitory Factor	13:3	1:3
Polymerism or Additive Factor	9:6:1	1:2:1

Intra-allelic

- Lethal Gene

Allelic gene interaction

Expression of character is produced by interaction between alleles of a single gene.

- Complete dominance
- Incomplete dominance
- Co-dominance

Epistasis

Definition

- Involves two pairs of non-allelic genes
- Both the dominant genes affect the same character
- One of them, when present alone or along with other dominant gene expresses itself
- The other gene expresses itself only when it is alone.
- The recessive forms of both the genes give rise to different phenotype
- The phenomenon of masking of effect of one dominant gene by the other gene is

called as “epistasis”, and hence the interaction is named as epistasis. The masking gene is epistatic gene and the masked one is hypostatic gene.

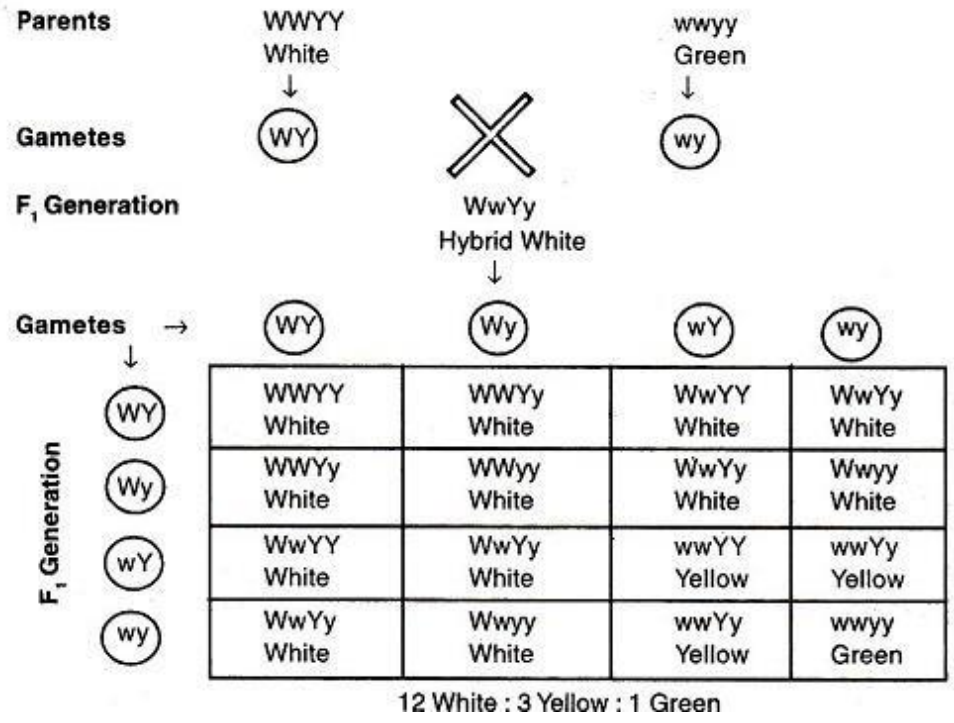
Dominant Epistasis

- Epistasis is observed in fruit color of summer squash (white, yellow and green)

White and yellow fruit colors are monogenic dominant over green color. When plant with white fruit is crossed with that with green fruit, progeny had white fruits.

But, in F2 segregation in the ratio of 12white: 3yellow: 1green was observed. This behavior is based on ratio of dihybrid cross, so the trait must be governed by two pairs of genes.

Suppose, gene W, gene Y and gene w produce white, yellow and green colors resp. Now, the genotype of pure white fruit plant must be WWYY as yellow fruited plants are produced in F2. The gene for yellow color compulsorily should be present there. Both the recessive genes in homozygous condition produce green phenotype. So, genotype of pure breeding green plant must be wwyy. The F2 segregation shows that the effect of gene Y is masked by gene W, when both are present together.



Recessive Episatsis

Recessive alleles at one locus (aa) mask the phenotypic expression of other gene locus (BB, Bb or bb) such epistasis is called recessive epistasis. The alleles of 'B' locus express themselves only when epistatic locus 'A' has dominant allele like AA or Aa. The phenotypic ratio is 9: 3: 4. Example:

Complete dominance at both gene pairs, but one gene, when homozygous recessive, is epistatic to the other.

In Mouse coat colour.

Gene pair A: colour dominant over albino.

Gene pair 'B' agouti colour dominant over black.

Interaction: homozygous albino is epistatic to aguoti

and black. Agouti 9/16

Black

3/16

Albino

4/16

Agouti (gray) × white albino
AABB ↓ aabb
Aa Bb Agouti × Aa Bb Agouti

AB = 9 Agouti
Ab = 3 Albino
Ba = 3 Black
ab = 1 Albino

	AB	Ab	aB	ab
AB	AABB Agouti	AABb Agouti	AaBB Agouti	Aa Bb Agouti
Ab	AABb Agouti	AAbb Albino	AaBb Agouti	Aabb Albino
aB	AaBB Agouti	AaBb Agouti	aaBB Black	aaBb Black
ab	AaBb Agouti	Aabb Albino	aa Bb Black	aabb Albino

9/16 : 3/16 : 4/16
Agouti : Black : Albino

Duplicate Factor

Definition

- Involves two pairs of non-allelic genes
- Affect the same character
- Dominant form of both genes, either alone or in combination produce same phenotypic expression.
- Recessive forms of both the genes when together produce different phenotypic effect.
- Duplicate factor interaction is observed in awned and awnless condition in rice.

In case of rice when pure breeding awned plant is crossed with awnless, the progeny was awned. In F_2 segregation was observed in the ratio of 15 awned: 1 awnless. This behavior is based on ratio of dihybrid cross, so the trait must be governed by two pairs of genes.
Suppose, A1 and A2 are two duplicate factors.

Genotype of pure breeding awned plant must be $A_1A_1A_2A_2$, as it is dominant and genotype of awnless plant must be a1a1a2a2 as it is recessive.

Cross between	F1	F2
$A_1A_1A_2A_2$ X $a_1a_1a_2a_2$ awned X awnless	$A_1a_1A_2a_2$ awned	15 awned : 1 awnless

Complementary Gene Interaction

Definition

- Involves two pairs of non-allelic genes
- When dominant forms of both the genes involved in complementary gene interaction are alone have same phenotypic expression

- But, if they are present in combination, yield different phenotypic effect.
- Flower color in garden pea follow this type of gene interaction.

We have considered garden pea for the explanation of this type of gene interaction, in which it was noted for the first time. Two different varieties of garden pea produce same color flowers – white. But on crossing they yield purple color flowers. Again in F_2, 9 purple : 7 white segregation was observed. How this happened? The answer is complementary gene interaction. For the sake of understanding we will name the two varieties as variety I and II.

Suppose, Gene W in variety I and gene C in variety II produces white flowers. But, the progeny resulting from cross between these two has purple flowers. This means both the dominant genes, W and C together produce purple color flowers.

In the variety I, dominant C gene is absent but it posses recessive c gene. Similarly, variety II contains recessive w gene. Both recessive genes produce white flowers, when present together. As in this type of interaction, the two recessive genes com

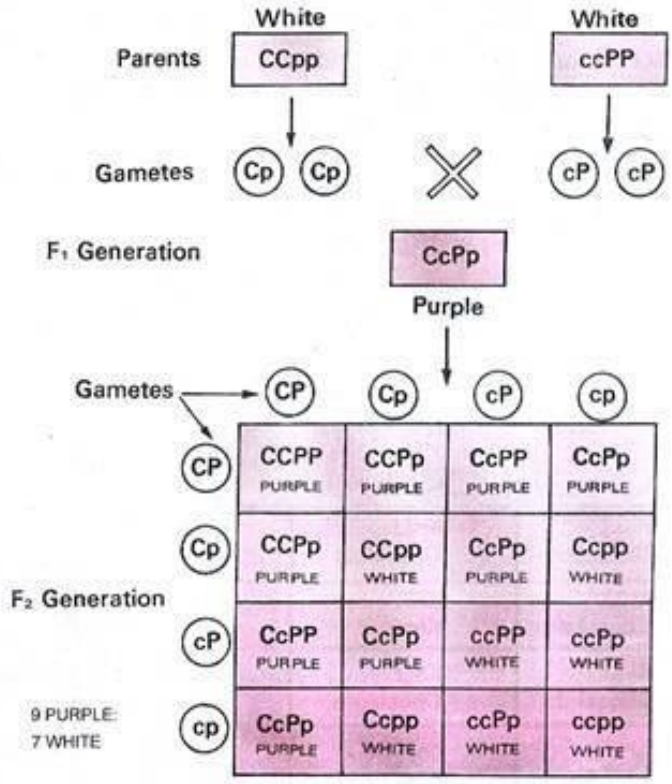


Fig. 5. 32. Inheritance of purple colour in Sweet Pea (*Lathyrus odoratus*) due to complementary and epistatic interactions of two nonallelic gene pairs.

Supplementary Gene Interaction

Definition

- Involves two pairs of non-allelic genes
- Affect the same character
- One of the dominant gene has visible effect itself
- Second dominant gene expresses itself when supplemented by the other dominant gene of a pair
- Coat color (black, albino and agouti) of mice follows supplementary gene interaction.

  In mice, black coat color is monogenically dominant over albino and agouti. The offspring resulting from the cross between black and albino has agouti coat color. F_2 generation shows segregation in the ratio 9 agouti: 3 black: 4 albino. This behavior is based on ratio of dihybrid cross, so the trait must be governed by two pairs of genes.

  Suppose, gene C is essential for the development of black coat color, so present in black mice and absent in albino mice. Albino mice contains only gene A, so produces albino phenotype. But, when gene A is present along with gene C, produces agouti phenotype. Both the genes in recessive form produce albino phenotype.

Cross between	F1	F2
CCaa X ccAA black X albino	CcAa agouti	9 agouti : 3 black: 4albino

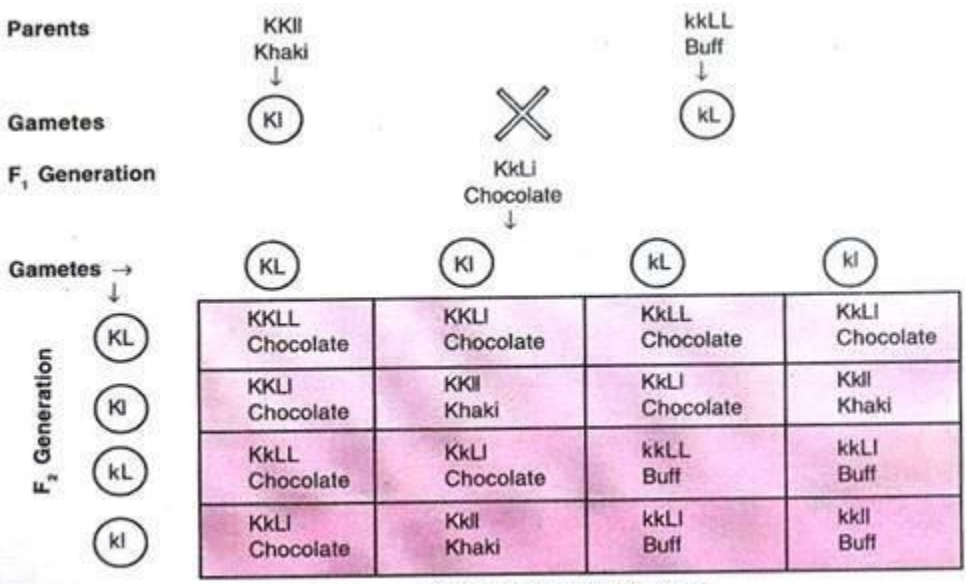


Fig. 5.33. Inheritance of seed coat colour in Lablab showing effect of supplementary gene.

Lethal Genes

Definition

Genes which result in viability reduction of individual or become a cause for death of individuals carrying them are called as lethal genes.

Phenomenon of action of lethal genes is called as **lethality**.

Some lethal genes cause death of zygote or the early embryonic stage while some express their effect in later stages of development.

Individuals carrying dominant lethal will die. Even though the dominant lethal is eliminated from the genotype of population previously carrying it, the recessive lethals are still carried in heterozygous condition. The recessive lethal in heterozygous condition reduce the viability and when they occur in homozygous condition produce lethal effect.

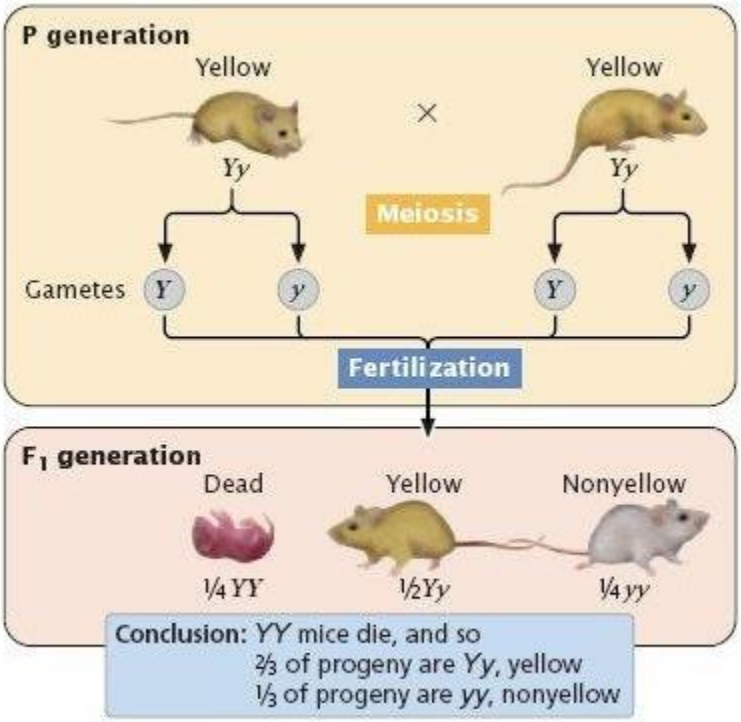
Usually lethal genes are dominant with respect to the phenotype they control and recessive for their lethal actions.

Example of Lethal Genes

In case of **mice** (By Cuenot)

In a cross of a yellow mouse with another yellow, yellow and brown mice are obtained in 2:1 ratio. True breeding yellow mice never obtained. In 1917, Stiegseder reported that 1/4th of the offspring die in embryonic condition in such crosses.
These premature dead forms are dominant homozygous.

According to Cuenot gene Y has multiple effects. It controls the yellow color of the body and affects to individual viability. It means the gene is dominant for body color and recessive with respect to lethality. The living yellow forms are heterozygous and somehow manage to survive.



In case of **maize**, albinism is example of lethal factor.

The lethal factor in heterozygous condition has no lethal effect but in homozygous condition it makes plant to die. Lethal factor in homozygous condition produces albino plant, which is unable to synthesize food due to lack of chlorophyll. Thus, the lethal factor modifies normal ratio from 3:1 to 2:1.

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UNIT – III – SBC3102 – GENETICS

Chromosome

A **chromosome** (*chromo-* + *-some*) is a packaged and organized structure containing most of the DNA of a living organism. It is not usually found on its own, but rather is structured by being wrapped around protein complexes called nucleosomes, which consist of proteins called histones. The DNA in chromosomes is also associated with transcription (copying of genetic sequences) factors and several other macromolecules. During most of the duration of the Cell cycle, a chromosome consists of one long double- stranded DNA molecule (with associated proteins). During S phase, the chromosome gets replicated, resulting in an 'X'-shaped structure called a metaphase chromosome. Both the original and the newly copied DNA are now called chromatids. The two "sister" chromatids join together at a protein junction called a centromere. Chromosomes are normally visible under a light microscope only when the cell is undergoing mitosis (cell division). Even then, the full chromosome containing both joined sister chromatids becomes visible only during a sequence of mitosis known as metaphase (when chromosomes align together, attached to the mitotic spindle and prepare to divide). This DNA and its associated proteins and macromolecules is collectively known as chromatin, which is further packaged along with its associated molecules into a discrete structure called a nucleosome. Chromatin is present in most cells, with a few exceptions - erythrocytes for example. Occurring only in the nucleus of eukaryotic cells, chromatin composes the vast majority of all DNA, except for a small amount inherited maternally which is found in mitochondria. In prokaryotic cells, chromatin occurs free-floating in cytoplasm, as these cells lack organelles and a defined nucleus. Bacteria also lack histones. The main information-carrying macromolecule is a single piece of coiled double-stranded DNA, containing many genes, regulatory elements and other noncoding DNA. The DNA-bound macromolecules are proteins, which serve to package the DNA and control its functions. Chromosomes vary widely between different organisms. Some species such as certain bacteria also contain plasmids or other extrachromosomal DNA. These are circular structures in the cytoplasm which contain cellular DNA and play a role in horizontal gene transfer.

Compaction of the duplicated chromosomes during cell division (mitosis or meiosis) results either in a four-arm structure (pictured to the right) if the centromere is located in the middle of the chromosome or a two-arm structure if the centromere is located near one of the ends. Chromosomal recombination during meiosis and subsequent sexual reproduction plays a vital role in genetic diversity. If these structures are manipulated incorrectly, through processes known as chromosomal instability and translocation, the cell may undergo mitotic catastrophe

and die, or it may unexpectedly evade apoptosis leading to the progression of cancer.

In prokaryotes (see nucleoids) and viruses, the DNA is often densely packed and organized: in the case of archaea, by homologs to eukaryotic histones, and in the case of bacteria, by histone-like proteins. Small circular genomes called plasmids are often found in bacteria and also in mitochondria and chloroplasts, reflecting their bacterial origins.

Walter Sutton and Theodor Boveri independently developed the chromosome theory of inheritance in 1902. The word *chromosome* comes from the Greek χρῶμα (*chroma*, "colour") and σῶμα (*soma*, "body"). Chromatin and chromosomes are both very strongly stained by particular dyes. Schleiden, Virchow and Bütschli were among the first scientists who recognized the structures now so familiar to everyone as chromosomes. The term was coined by von Waldeyer-Hartz, referring to the term chromatin, which was introduced by Walther Flemming.

Prokaryotes

The prokaryotes – bacteria and archaea – typically have a single circular chromosome, but many variations exist. The chromosomes of most bacteria can range in size from only 130,000 base pairs in the endosymbiotic bacteria *Candidatus Hodgkinia cicadicola*[10] and *Candidatus Tremblaya princeps*, to over 14,000,000 base pairs in the soil-dwelling bacterium *Sorangium cellulosum*. Spirochaetes of the genus *Borrelia* are a notable exception to this arrangement, with bacteria such as *Borrelia burgdorferi*, the cause of Lyme disease, containing a single *linear* chromosome.

Structure in sequences

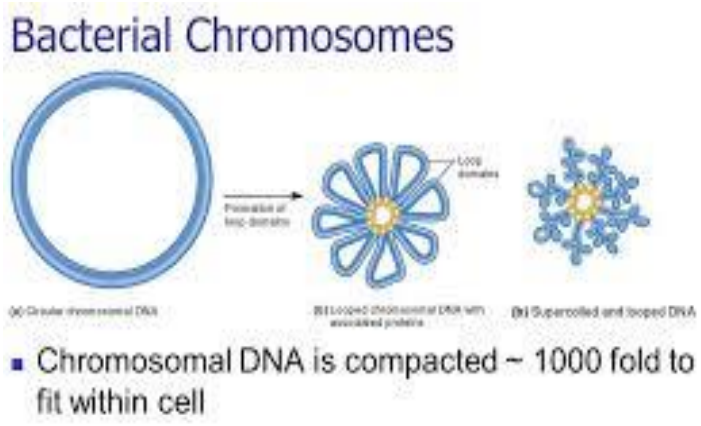
Prokaryotic chromosomes have less sequence-based structure than eukaryotes. Bacteria typically have a single point (the origin of replication) from which replication starts, whereas some archaea contain multiple replication origins. The genes in prokaryotes are often organized in operons, and do not usually contain introns, unlike eukaryotes.

DNA packaging

Prokaryotes do not possess nuclei. Instead, their DNA is organized into a structure called the nucleoid. The nucleoid is a distinct structure and occupies a defined region of the bacterial cell. This structure is, however, dynamic and is maintained and remodeled by the actions of a range of histone-like proteins, which associate with the bacterial chromosome. In archaea, the DNA in chromosomes is even more organized, with the DNA packaged within structures

similar to eukaryotic nucleosomes.

Bacterial chromosomes tend to be tethered to the plasma membrane of the bacteria. In molecular biology application, this allows for its isolation from plasmid DNA by centrifugation of lysed bacteria and pelleting of the membranes (and the attached DNA).



Prokaryotic chromosomes and plasmids are, like eukaryotic DNA, generally supercoiled. The DNA must first be released into its relaxed state for access for transcription, regulation, and replication.

In eukaryotes, nuclear chromosomes are packaged by proteins into a condensed structure called chromatin. This allows the very long DNA molecules to fit into the cell nucleus. The structure of chromosomes and chromatin varies through the cell cycle. Chromosomes are even more condensed than chromatin and are an essential unit for cellular division. Chromosomes must be replicated, divided, and passed successfully to their daughter cells so as to ensure the genetic diversity and survival of theirprogeny. Chromosomes may exist as either duplicated or unduplicated. Unduplicated chromosomes are single linear strands, whereas duplicated chromosomes contain two identical copies (called chromatids or sister chromatids) joined by a centromere.

Eukaryotes (cells with nuclei such as those found in plants, yeast, and animals) possess multiple large linear chromosomes contained in the cell's nucleus. Each chromosome has one centromere, with one or two arms projecting from the centromere, although, under most circumstances, these arms are not visible as such. In addition, most eukaryotes have a small circular mitochondrial genome, and some eukaryotes may have additional small circular or

linear cytoplasmic chromosomes.

In the nuclear chromosomes of eukaryotes, the uncondensed DNA exists in a semi-ordered structure, where it is wrapped around histones (structural proteins), forming a composite material called chromatin.

Chromatin

Chromatin is the complex of DNA and protein found in the eukaryotic nucleus, which packages chromosomes. The structure of chromatin varies significantly between different stages of the cell cycle, according to the requirements of the DNA.

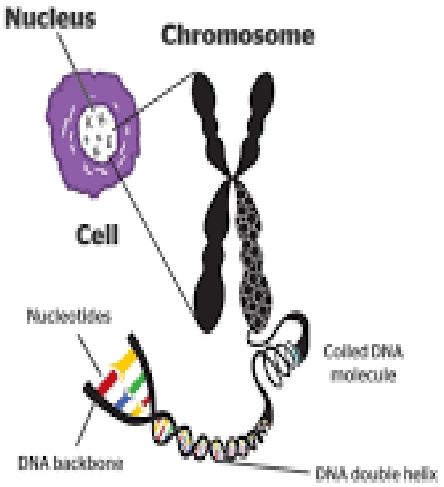
Interphase chromatin

During interphase (the period of the cell cycle where the cell is not dividing), two types of chromatin can be distinguished:

Euchromatin, which consists of DNA that is active, e.g., being expressed as protein.

Heterochromatin, which consists of mostly inactive DNA. It seems to serve structural purposes during the chromosomal stages. Heterochromatin can be further distinguished into two types:

- *Constitutive heterochromatin*, which is never expressed. It is located around the centromere and usually contains repetitive sequences.
- *Facultative heterochromatin*, which is sometimes expressed.



Metaphase chromatin and division

In the early stages of mitosis or meiosis (cell division), the chromatin strands become more and more condensed. They cease to function as accessible genetic material (transcription stops) and become a compact transportable form. This compact form makes the individual chromosomes visible, and they form the classic four arm structure, a pair of sister chromatids attached to each other at the centromere. The shorter arms are called *p arms* (from the French *petit*, small) and the longer arms are called *q arms* (*q* follows *p* in the Latin alphabet; q-g "grande"; alternatively it is sometimes said q is short for *queue* meaning tail in French[19]). This is the only natural context in which individual chromosomes are visible with an optical microscope.

During mitosis, microtubules grow from centrosomes located at opposite ends of the cell and also attach to the centromere at specialized structures called kinetochores, one of which is present on each sister chromatid. A special DNA base sequence in the region of the kinetochores provides, along with special proteins, longer-lasting attachment in this region. The microtubules then pull the chromatids apart toward the centrosomes, so that each daughter cell inherits one set of chromatids. Once the cells have divided, the chromatids are uncoiled and DNA can again be transcribed. In spite of their appearance, chromosomes are structurally highly condensed, which enables these giant DNA structures to be contained within a cell nucleus .

Human chromosomes

Chromosomes in humans can be divided into two types: autosomes and sex chromosomes. Certain genetic traits are linked to a person's sex and are passed on through the sex chromosomes. The autosomes contain the rest of the genetic hereditary information. All act in the same way during cell division. Human cells have 23 pairs of chromosomes (22 pairs of autosomes and one pair of sex chromosomes), giving a total of 46 per cell.

VARIATION IN CHROMOSOME NUMBER & STRUCTURE

Chromosome Number in Different Species

In "higher" organisms (diploids), members of same species typically have identical numbers of chromosomes in each somatic cell. Diploid chromosome number (2n). Nearly all chromosomes will exist in pairs (identical wrt length and centromere placement) except the sex chromosomes. Members of pair are homologous chromosomes. Haploid number (n) isthe number of chromosome pairs.

	2n	n
Human	46	23
Horse	64	32
Cat	38	19
Geometrid moth	224	112
Tomato	24	12
Pink bread mold	14	7

Autosomal monosomy and trisomy

Occasionally, one finds an organism that has an extra copy of a particular chromosome. This is known as trisomy--because there are now 3 copies of an autosome. Some trisomies are viable in animals, but the condition usually has severe effects. These effects are presumably related to the fact that there are 3 copies of every gene on the trisomic chromosome, but only 2 copies of all the genes on the other chromosomes. We will see later, that organisms with three or more copies of all the chromosomes are often perfectly viable.

Trisomy of human chromosome 21 is the cause of the disorder known as Down syndrome. (Remember, humans have 23 pairs of chromosomes--the pairs are numbered 1 through 22, plus the X and Y). It is characterized by multiple physical defects, including epicanthal fold, furrowed tongue, characteristic palm and finger print patterns, and lowered IQ. About 1 in 750 live births produces a child with this condition. It results from the non-disjunction of chromosome 21 during meiotic anaphase I or anaphase II, when the paired homologs (or paired chromatids) normally migrate to opposite poles of the cell.

Nondisjuction: The members of a chromosome pair (homologs) line up at the metaphase plate during meiotic metaphase I, then separate to opposite poles of the cell during anaphase I--review this material in Klug and Cummings or any introductory Genetics text if you are not thoroughly familiar with it!). If the pair fails to separate, and both migrate to the same pole, half of the resulting gametes will have two copies of chromosome 21, rather than one. When this gamete unites with a normal gamete (bearing one copy of chromosome 21) during

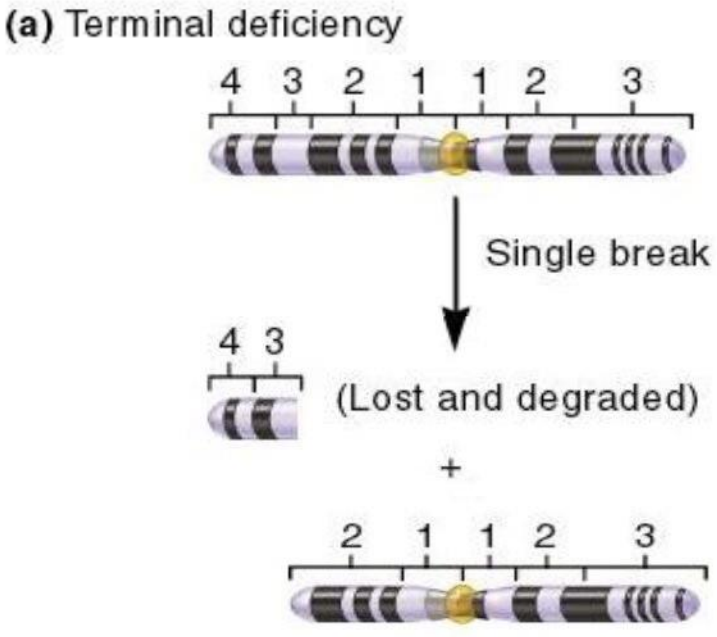
fertilization, the resulting gamete has 3 copies of chromosome 21, rather than the normal 2. Nondisjunction of chromosome 21 seems to occur more often in the production of eggs than sperm, and the frequency increases with the age of the parent. Older individuals are often encouraged to test for trisomy 21 by amniocentesis at 15 to 16 weeks after conception.

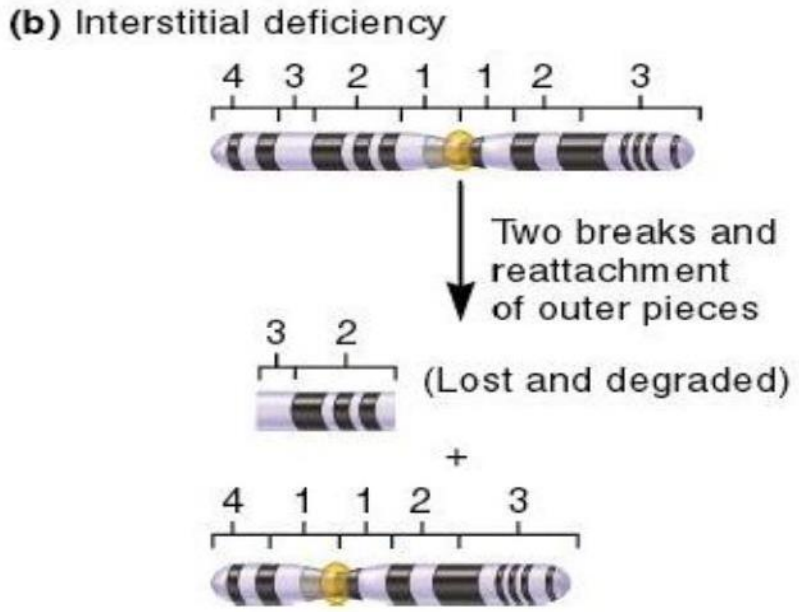
Nondisjunction can happen to other chromosomes in addition to chromosome 21. But human embryos that are trisomic for any other chromosome do not survive to birth. It should be obvious that the other half of the gametes resulting from a non-disjunction event at anaphase 1 will have 0 copies of the chromosome. When a gamete with 0 copies of a chromosome unites with a normal gamete, the result is a zygote that has only one copy of that chromosome. This is monosomy. Monosomy is not well tolerated in animals—usually lethal. Some plants can survive (observed in maize, tomato, Oenothera, and Datura) but they have low viability and are usually sterile.

Chromosomal Rearrangements:

Deletions

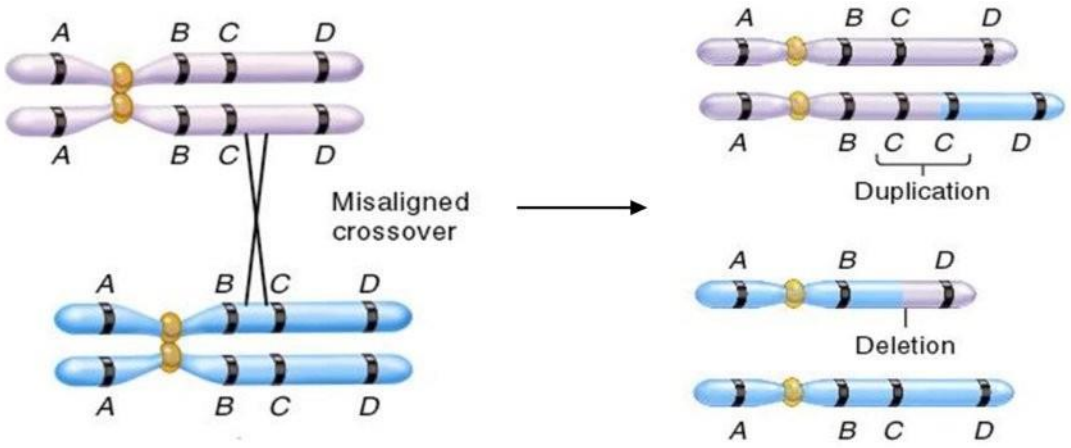
Sometimes a chromosome will arise in which a segment is missing. These chromosomes are said to have **deletions.** Deletions are generally harmful, and typically, the larger the deletion, the more harmful it is. Small deletions are often viable if the deletion is heterozygous, ecause the other chromosome contains copies of the genes missing in the chromosome with the deletion. But even small deletions are usually lethal if they are homozygous. A deletion on one homologue can "unmask" recessive alleles on the other homologue--this effect is called pseudo-dominace.





Duplications

Duplication often results in reduction of viability, but in general duplications are less severe in their effects than are deletions. Duplication may be important in generating multiple copies of genes that require very high levels of expression (**gene amplification**)--**rDNA** codes for genes producing RNA for construction of ribosomes. Also may be source of evolutionary origin and divergence of genes coding for similar (but not identical) proteins (**alpha and beta globin components of hemoglobin**)



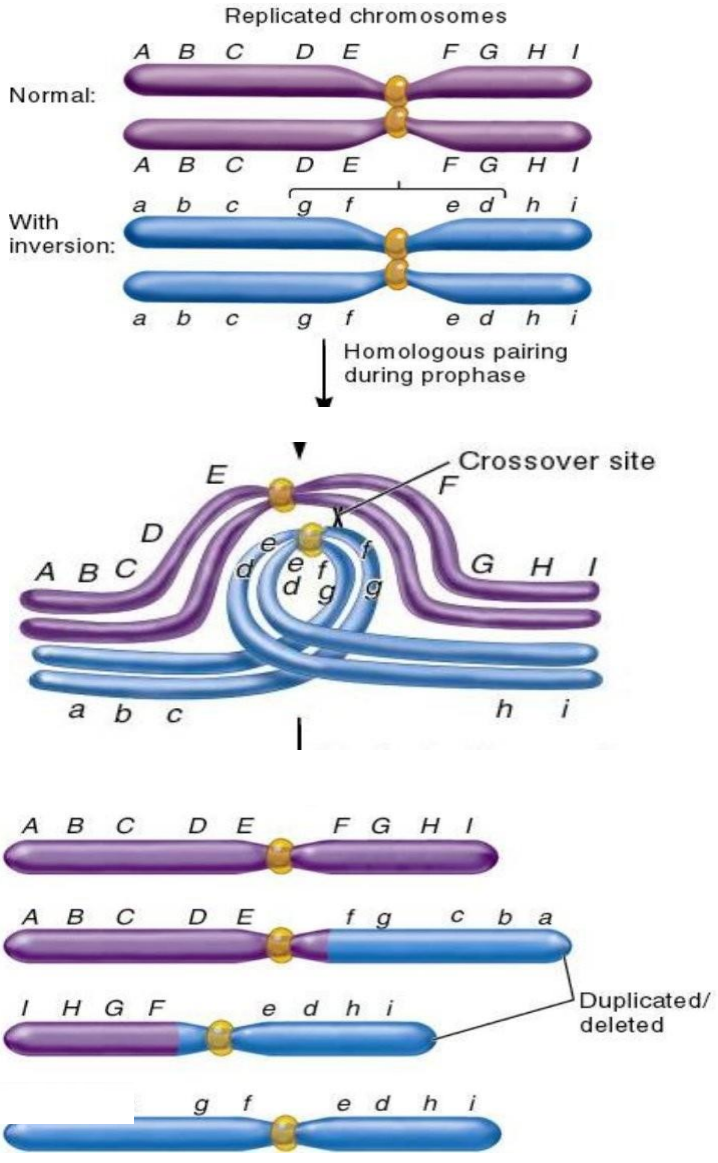
Inversions

Chromosomes can sometimes break and rejoin in a different orientation. If this happens in a germ cell, the gametes will contain a **rearrangement or inversion.**

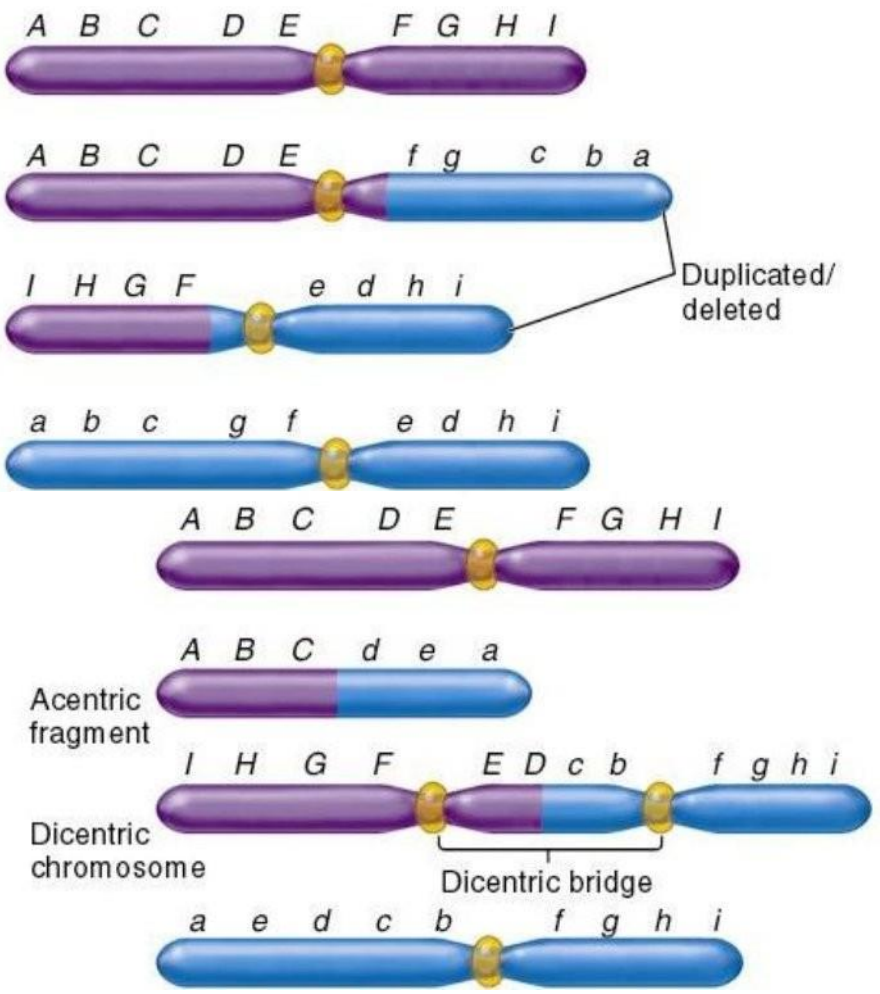
Crossing-over within an inversion

Paracentric Inversion. If the inversion does not includes the centromere, then the chromatids resulting from crossing-over may have no centromere (acentric) or two centromeres (dicentric).

Pericentric Inversion. If the inversion does includes the centromere, then the chromatids resulting from crossing-over all have centromeres, but some will contain duplications or deletions for parts of the chromosome.



(a) Pericentric inversion



Translocations (Reciprocal)--Two nonhomologous chromosomes

Other variations in numbers of ch romosomes—Polyploidy

POLYPLOIDY results from having additional whole haploid sets of chromosomes. If a normal diploid individual is said to have 2n chromosomes, and a normal gamete is said to have n chromosomes, then polyploid individuals can be represented as: 3n, 4n, 6n, 8n, etc. Polyploidy is rare in many groups of animals, but we do observe it in fish, amphibians, and lizards.

Polyploidy is much more common in plants (plants hybridize more easily than animals many are self-fertile). Nearly half of all flowering plants (including many important crops) are polyploids! Can result from nondisjunction of entire chromosomes sets, or from hybridization between different species with chromosomes that don't "match up".

Human karyotype

The normal human karyotypes contain 22 pairs of autosomal chromosomes and one pair of sex chromosomes (allosomes). Normal karyotypes for females contain two X chromosomes and are denoted 46,XX; males have both an X and a Y chromosome denoted 46,XY. Any variation from the standard karyotype may lead to developmental abnormalities

Ploidy

Ploidy is the number of complete sets of chromosomes in a cell.

Polyploidy, where there are more than two sets of homologous chromosomes in the cells, occurs mainly in plants. It has been of major significance in plant evolution according to Stebbins. The proportion of flowering plants which are polyploid was estimated by Stebbins to be 30–35%, but in grasses the average is much higher, about 70%. Polyploidy in lower plants (ferns, horsetails and psilotales) is also common, and some species of ferns have reached levels of polyploidy far in excess of the highest levels known in flowering plants.

Polyploidy in animals is much less common, but it has been significant in some groups.Polyploid series in related species which consist entirely of multiples of a single basic number are known as euploid. Haplo-diploidy, where one sex is diploid, and the other haploid. It is a common arrangement in the Hymenoptera, and in some other groups.

Endopolyploidy occurs when in adult differentiated tissues the cells have ceased to divide by mitosis, but the nuclei contain more than the original somatic number of chromosomes. In the *endocycle* (endomitosis or endoreduplication) chromosomes in a 'resting' nucleus undergo reduplication, the daughter chromosomes separating from each other inside an *intact* nuclear membrane.

Chromosomal Theory of Inheritance

The Chromosomal Theory of Inheritance identified chromosomes as the genetic material responsible for Mendelian inheritance

KEY POINTS

- Homologous chromosome pairs are independent of other chromosome pairs.
- Chromosomes from each homologous pair are sorted randomly into pre-gametes.
- Parents synthesize gametes that contain only half of their chromosomes; eggs and sperm have the same number of chromosomes.
- Gametic chromosomes combine during fertilization to produce offspring with the same chromosome number as their parents.
- Eye color in fruit flies was the first X-linked trait to be discovered; thus, Morgan's experiments with fruit flies solidified the Chromosomal Theory of Inheritance.

TERMS

∑ wild type
the typical form of an organism, strain, gene or characteristic as it occurs in nature

∑ hemizygous
having some single copies of genes in an otherwise diploid cell or organism

The Chromosomal Theory of Inheritance was consistent with Mendel's laws and was supported by the following observations:

∑ During meiosis, homologous chromosome pairs migrate as discrete structures that are independent of other chromosome pairs.

∑ The sorting of chromosomes from each homologous pair into pre-gametes appears to be random.

∑ Each parent synthesizes gametes that contain only half of their chromosomal complement.

∑ Even though male and female gametes (sperm and egg) differ in size and morphology, they have the same number of chromosomes, suggesting equal genetic contributions from each parent.

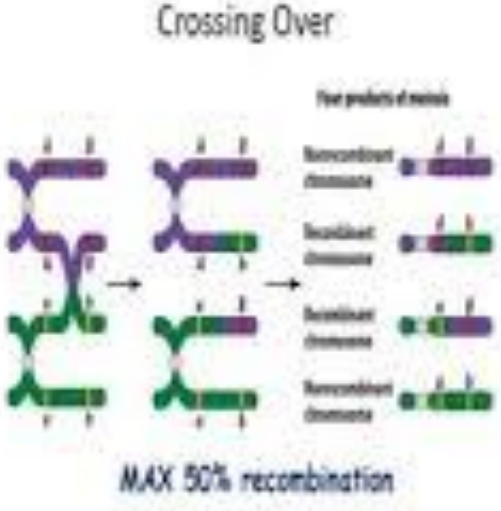
∑ The gametic chromosomes combine during fertilization to produce offspring with the same chromosome number as their parents.

Parent	Blue flower long pollen BBLL						Red flower round pollen bbll
Gametes	BL				×		bl
F_1 generation	BbLl (Blue long)						
Dihybrid test cross	BbLl				×		bbll
Gametes	BL		B l		bL		bl
	BbLl		Bbll		bbLl		bbll
Phenotype	Blue long		Blue round		Red long		Red round
Observed percentage frequency	44		6		6		44
Observed ratio	7	:	1	:	1	:	7
Expected ratio	1	:	1	:	1	:	1

Mechanism of linkage - coupling in Lathyrus odoratus

Crossing Over

- A random exchange of DNA between two non-sister chromatids of homologous chromosomes.
- Results in recombination of genetic material



Linkage	Crossing over
1. The genes present on chromosome stay close together	It leads to separation of linked genes
2. It involves same chromosome of homologous chromosome	It involves exchange of segments between non-sister chromatids of homologous chromosome.
3. It reduces new gene combinations	It increases variability by forming new gene combinations. lead to formation of new organism

Table 3.4: Differences between linkage and crossing over

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UNIT – II – SBC3102 – GENETICS

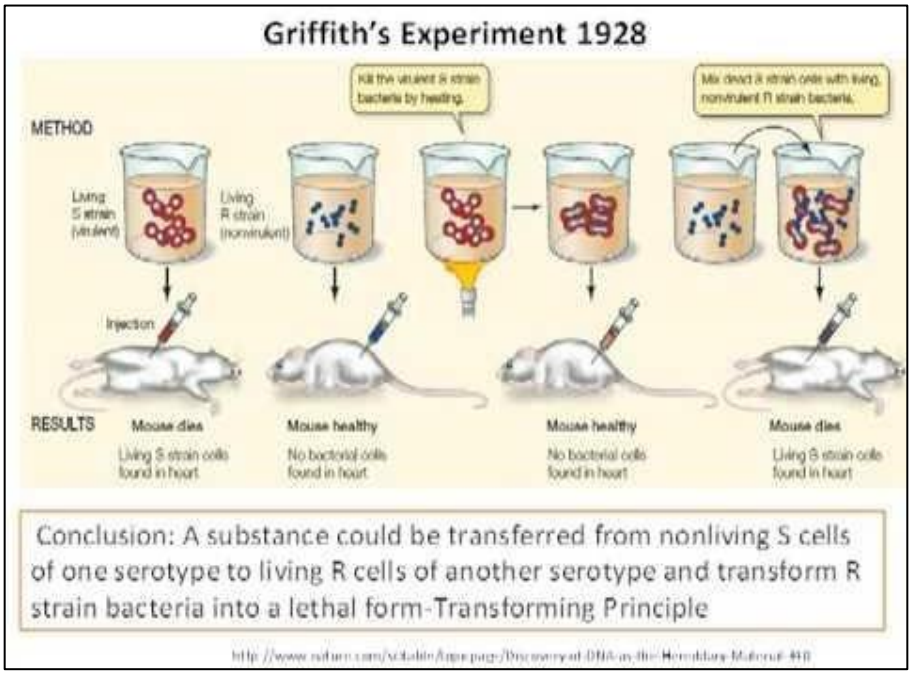
Genetic material and Mutation

The physical nature of the gene fascinated scientists for many years. A series of experiments beginning in the 1920s finally revealed that DNA was the genetic material.

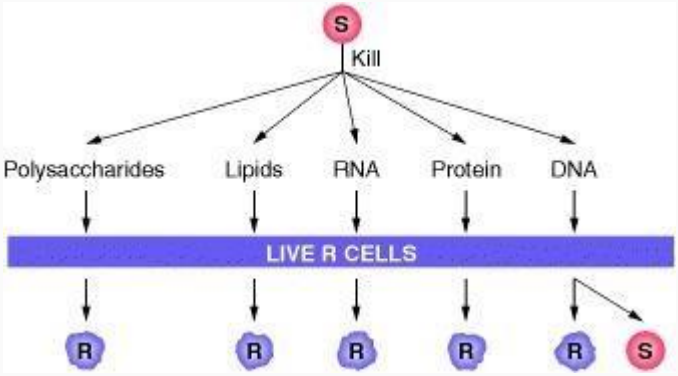
Discovery of transformation

A puzzling observation was made by Frederick Griffith in the course of experiments on the bacterium *Streptococcus pneumoniae* in 1928. This bacterium, which causes pneumonia in humans, is normally lethal in mice. However, different strains of this bacterial species have evolved that differ in virulence (in the ability to cause disease or death). In his experiments, Griffith used two strains that are distinguishable by the appearance of their colonies when grown in laboratory cultures. In onestrain, a normazl virulent type, the cells are enclosed in a polysaccharide capsule, giving colonies a smooth appearance; hence, this strain is labeled *S.* In Griffith's other strain, a mutant nonvirulent type that grows in mice but is not lethal, the polysaccharide coat is absent, giving colonies a rough appearance; this strain is called *R.*

Griffith killed some virulent cells by boiling them and injected the heat-killed cells into mice. The mice survived, showing that the carcasses of the cells do not cause death. However, mice injected with a mixture of heat-killed virulent cells and live nonvirulent cells did die. Furthermore, live cells could be recovered from the dead mice; these cells gave smooth colonies and were virulent on subsequent injection. Somehow, the cell debris of the boiled S cells had converted the live R cells into live S cells. The process is called transformation. Griffith's experiment is summarized in Figure .

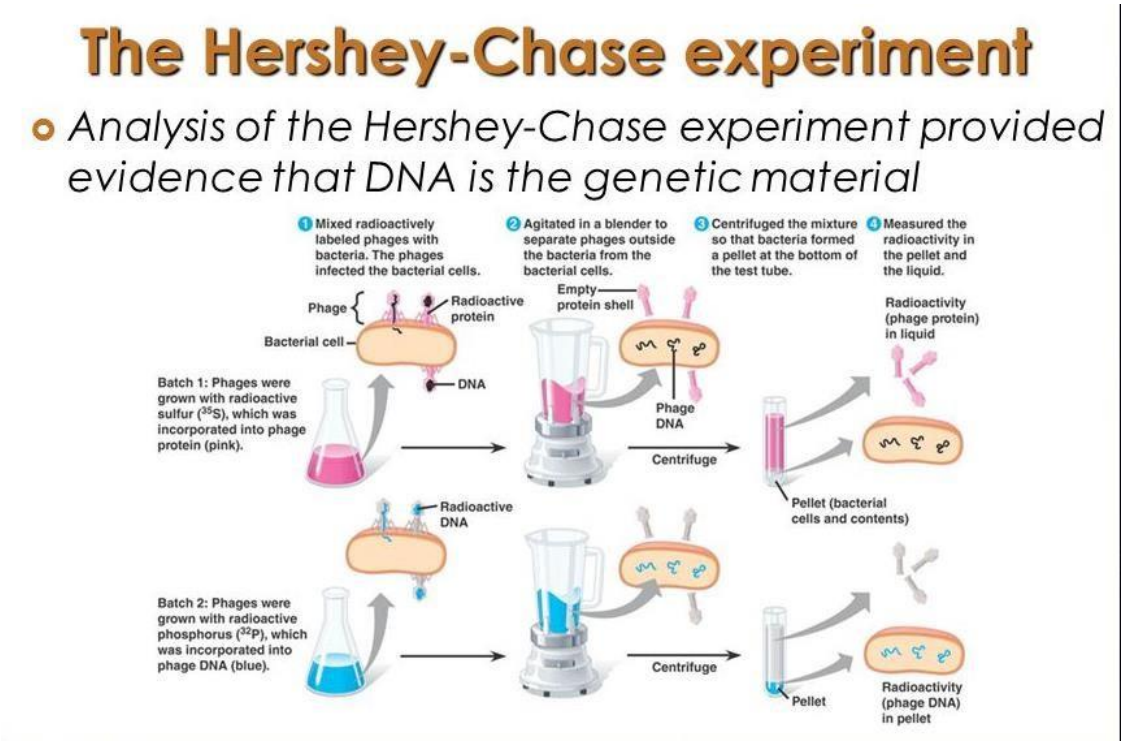


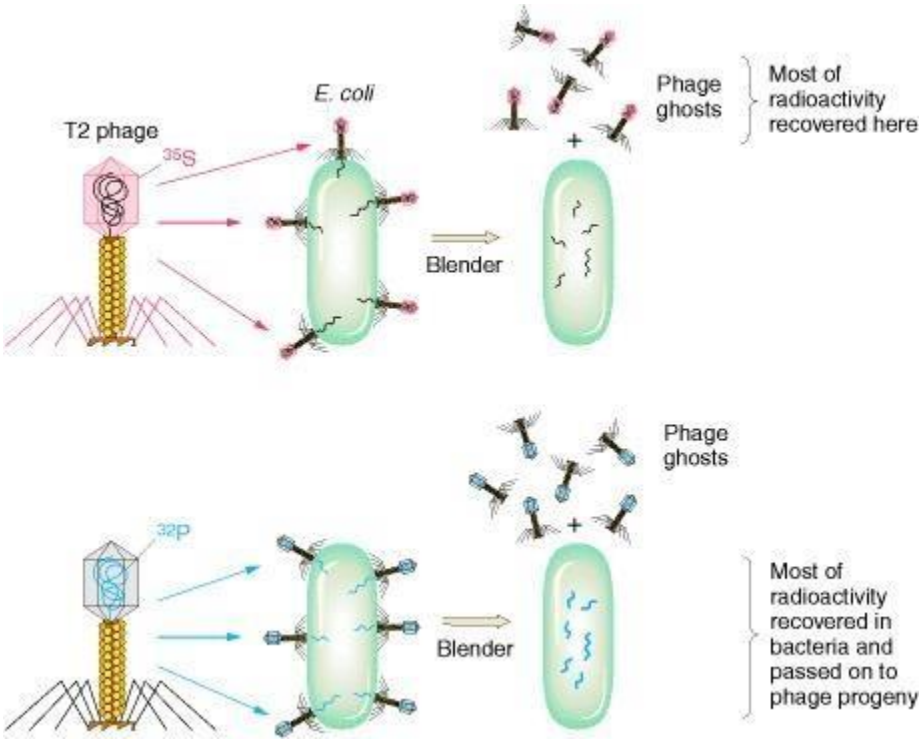
This same basic technique was then used to determine the nature of the *transforming principle*—the agent in the cell debris that is specifically responsible for transformation. In 1944, Oswald Avery, C. M. MacLeod, and M. McCarty separated the classes of molecules found in the debris of the dead S cells and tested them for transforming ability, one at a time. These tests showed that the polysaccharides themselves do not transform the rough cells. Therefore, the polysaccharide coat, although undoubtedly concerned with the pathogenic reaction, is only the phenotypic expression of virulence. In screening the different groups, Avery and his colleagues found that only one class of molecules, DNA, induced the transformation of R cells (Figure given below). They deduced that DNA is the agent that determines the polysaccharide character and hence the pathogenic character (see pages 219–220 for a description of the mechanism of transformation). Furthermore, it seemed that providing R cells with S DNA was tantamount to providing these cells with S genes.



MESSAGE

The demonstration that DNA is the transforming principle was the first demonstration that genes are composed of DNA.





The Hershey-Chase experiment, which demonstrated that the genetic material of phage is DNA, not protein. The experiment uses two sets of T2 bacteriophages. In one set, the protein coat is labeled with radioactive sulfur (^{35}S), not found in DNA. ther set, the DNA is labeled with radioactive phosphorus (^{32}P), not found in protein. Only the ^{32}P is injected into the *E. coli*, indicating that DNA is the agent necessary for the production of new phages.

RNA as genetic material

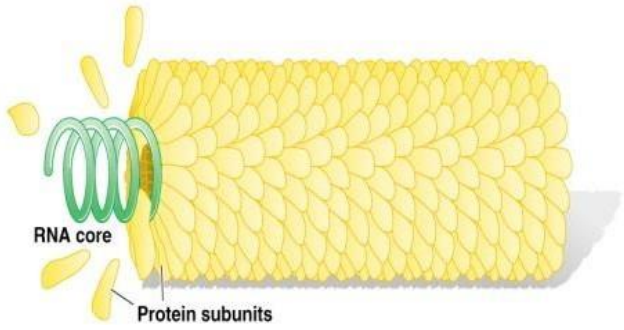
RNA is the genetic material in viruses was demonstrated in 1956 with the experiments conducted on tobacco plant by A.Gierer and G.Schramm.

All viruses are not limited to bacterial hosts. Viruses that infect and parasite plant cells, some animal cells contain RNA only. In these viruses RNA act as genetic material. One plant virus, Tobacco mosaic virus(TMV), that ontains RNA, not DNA was an important tool for genetic Experiments. TMV infects tobacco, causing the infected regions on leaves to become discoloured and bristled. Different strains of TMV produce clearly different inherited lesions on the infected leaves.The common virus produces a green mosaic disease, but a variant Holmes rib grass(TMV-

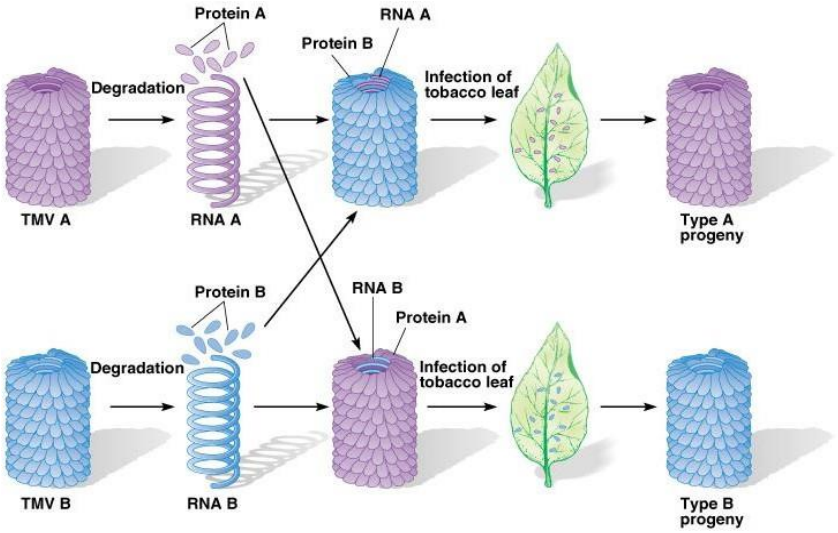
HR), produces ring spot lesions. Moreover , the amino acid compositions of the proteins of these two strains differ.

H.Fraenkel-Conrat and B.Singer first developed the techniques for separating TMV particles into RNA and protein. They found that virus could be broken into component parts and they could again be reassembled or reconsitituted to form functional virus.From the two strains of TMV they were able to reconstitute viruses with the RNA from TMV common enclosed in TMV-HR protein and TMV-HR RNA with TMV common protein. When these reassembled viruses were used to infect tobacco leaves, the progency viruses produced were always found to be phenotypically and genotypically identical to the parent strain from which the RNA had been obtained. The reassembled viruses with the TMV- common RNA and TMV-HR protein produced a green mosaic disease characteristic of TMV-common. Recovered virus had protein characteristic of TMV common.This proved that specificity of virus proteins was determined by RNA alone and that proteins carried no genetic information. Hence RNA carries genetic information not proteins.

The genetic RNA is usually found to be single stranded but in some it is double stranded as in reovirus, wound tumor virus.



Demonstration that RNA is the genetic material in tobacco mosaic virus (TMV)



MUTATION AND REPAIR OF DNA

Most biological molecules have a limited lifetime. Many proteins, lipids and RNAs are degraded when they are no longer needed or damaged, and smaller molecules such as sugars are metabolized to compounds to make or store energy. In contrast, DNA is the most stable biological molecule known, befitting its role in storage of genetic information. The DNA is passed from one generation to another, and it is degraded only when cells die. However, it can change, i.e. it is mutable. **Mutations**, or changes in the nucleotide sequence, can result from errors during DNA replication, from covalent changes in structure because of reaction with chemical or physical agents in the environment, or from transposition. Most of the sequence alterations are **repaired** in cells.

Sequence alteration in the genomic DNA is the fuel driving the course of evolution. Without such mutations, no changes would occur in populations of species to allow them to adapt to changes in the environment. Mutations in the DNA of germline cells fall into three categories with respect to their impact on evolution. Most have no effect on phenotype; these include sequence changes in the large portion of the genome that neither codes for protein, or is involved in gene regulation or any other process. Some of these **neutral** mutations will become prevalent in a population of organisms (or **fixed**) over long periods of time by stochastic processes. Other mutations do have a phenotype, one that is advantageous to the individuals carrying it. These mutations are fixed in populations rapidly (i.e. they are subject to **positive selection**). Other mutations have a detrimental

phenotype, and these are cleared from the population quickly. They are subject to **negative** or **purifying selection**.

Whether a mutation is neutral, disadvantageous or useful is determined by where it is in the genome, what the type of change is, and the particulars of the environmental forces operating on the locus. For our purposes, it is important to realize that sequence changes are a natural part of DNA metabolism. However, the amount and types of mutations that accumulate in a genome are determined by the types and concentrations of mutagens to which a cell or organism is exposed, the efficiency of relevant repair processes, and the effect on phenotype in the organism.

Mutations and mutagens

Types of mutations

Mutations commonly are **substitutions**, in which a single nucleotide is changed into a different nucleotide. Other mutations result in the loss (**deletion**) or addition (**insertion**) of one or more nucleotides. These insertions or deletions can range from one to tens of thousands of nucleotides. Often an insertion or deletion is inferred from comparison of two homologous sequences, and it may be impossible to ascertain from the data given whether the presence of a segment in one sequence but not another resulted from an insertion of a deletion. In this case, it can be referred to as an **indel**. One mechanism for large insertions is the **transposition** of a sequence from one place in a genome to another.

Nucleotide substitutions are one of two classes. In a **transition**, a purine nucleotide is replaced with a purine nucleotide, or a pyrimidine nucleotide is replaced with a pyrimidine nucleotide. In other words, the base in the new nucleotide is in the same chemical class as that of the original nucleotide. In a **transversion**, the chemical class of the base changes, i.e. a purine nucleotide is replaced with a pyrimidine nucleotide, or a pyrimidine nucleotide is replaced with a purine nucleotide.

Errors in Replication

Despite effective proofreading functions in many DNA polymerases, occasionally the wrong nucleotide is incorporated. It is estimated that *E. coli* DNA polymerase III holoenzyme (with a

fully functional proofreading activity) uses the wrong nucleotide during elongation about 1 in 10^8 times. It is more likely for an incorrect pyrimidine nucleotide to be incorporated opposite a purine nucleotide in the template strand, and for a purine nucleotide to be incorporated opposite a pyrimidine nucleotide. Thus these misincorporations resulting in a transition substitution are more common. However, incorporation of a pyrimidine nucleotide opposite another pyrimidine nucleotide, or a purine nucleotide opposite another purine nucleotide, can occur, albeit at progressively lower frequencies. These rarer misincorporations lead to transversions.

The *enol* tautomers of the normal deoxynucleotides guanidylate and thymidylate are rare, meaning that a single molecule is in the *keto* form most of the time, or within a population of molecules, most of them are in the *keto* form. However, certain nucleoside and base analogs adopt these alternative isomers more readily. For instance 5-bromo-deoxyuridine (or 5-BrdU) is an analog of deoxythymidine (dT) that is in the *enol* tautomer more frequently than dT is (although most of the time it is in the *keto* tautomer).

Thus the frequency of misincorporation can be increased by growth in the presence of base and nucleoside analogs. For example, growth in the presence of 5-BrdU results in an increase in the incorporation of G opposite a T in the DNA, as illustrated in Fig. 3. After cells take up the nucleoside 5-BrdU, it is converted to 5-BrdUTP by nucleotide salvage enzymes that add phosphates to its 5' end. During replication, 5-BrdUTP (in the *keto* tautomer) will incorporate opposite an A in DNA. The 5-BrdU can shift into the *enol* form while in DNA, so that when it serves as a template during the next round of replication (arrow 1 in the diagram below), it will direct incorporation of a G in the complementary strand. This G will in turn direct incorporation of a C in the top strand in the next round of replication (arrow 2). This leaves a C:G base pair where there was a T:A base pair in the parental DNA. Once the pyrimidine shifts back to the favored *keto* tautomer, it can direct incorporation of an A, to give the second product in the diagram below (with a BrU-A base pair).

Misincorporation during replication is the major pathway for introducing *transversions* into DNA. Normally, DNA is a series of purine:pyrimidine base pairs, but in order to have a transversion, a pyrimidine has to be paired with another pyrimidine, or a purine with a purine. The DNA has to undergo local structural changes to accommodate these unusual base pairs. One way this can happen for a purine-purine base pair is for one of the purine nucleotides to shift from the preferred

anti conformation to the *syn* conformation. Atoms on the "back side" of the purine nucleotide in the *syn*-isomer can form hydrogen bonds with atoms in the rare tautomer of the purine nucleotide, still in the preferred *anti* conformation. For example, an A nucleotide in the *syn-*, *amino-* isomer can pair with an A nucleotide in the *anti-*, *imino-* form. Thus the transversion required a shift in the tautomeric form of the base in one nucleotide as well as a change in the base-sugar conformation (*anti* to *syn*) of the other nucleotide.

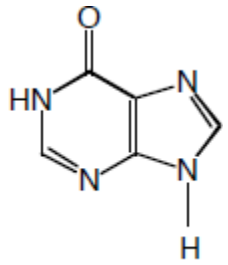
Errors in replication are not limited to substitutions. **Slippage errors** during replication will add or delete nucleotides. A DNA polymerase can insert additional nucleotides, more commonly when tandem short repeats are the template (e.g. repeating CA dinucleotides). Sometimes the template strand can loop out and form a secondary structure that the DNA polymerase does not read. In this case, a deletion in the nascent strand will result. The ability of intercalating agents to increase the frequency of such deletions is illustrated in Fig. 10.B. (see below).

Reaction with mutagens

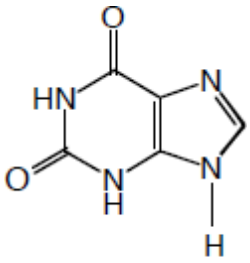
Many mutations do not result from errors in replication. Chemical reagents can oxidize and alkylate the bases in DNA, sometimes changing their base-pairing properties. Radiation can also damage DNA. Examples of these mutagenic reactions will be discussed in this section.

Chemical modification by oxidation

When the amino bases, adenine and cytosine, are oxidized, they also lose an amino group. Thus the amine is replaced by a keto group in the product of this oxidative deamination reaction. For instance, oxidation of cytosine produces uracil, which base pairs with adenine. Likewise, oxidation of adenine yields hypoxanthine, which base pairs with cytosine . Thus the products of these chemical reactions will be mutations in the DNA, if not repaired. Oxidation of guanine yields xanthine (Fig. 7.B). In DNA, xanthine will pair with cytosine, as does the original guanine, so this particular alteration is not mutagenic.

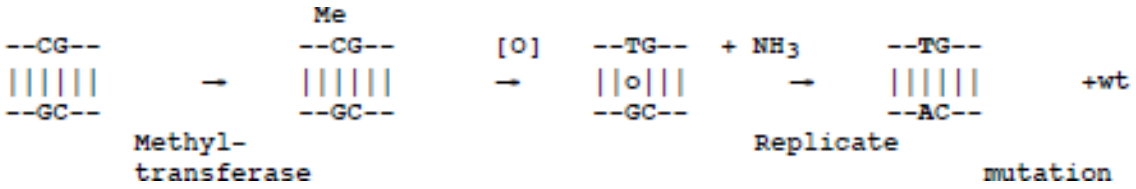


hypoxanthine



xanthine

Methylation of C prior to its oxidative deamination will effectively mask it from the repair processes to remove U's from DNA. This has a substantial impact on the genomes of organisms that methylate C. In many eukaryotes, including vertebrates and plants (but not yeast or *Drosophila*), the principal DNA methyl transferase recognizes the dinucleotide CpG in DNA as the substrate, forming 5-methyl-CpG (Fig. 8). When 5-methyl cytosine undergoes oxidative deamination, the result is 5-methyl uracil, which is the same as thymine. The surveillance system that recognizes U's in DNA does nothing to the T, since it is a normal component of DNA. Hence the oxidation of 5-methyl CpG to TpG, followed by a round of replication, results in a C:G to T:A transition at former CpG sites. This spontaneous deamination is quite frequent; indeed, C to T transitions at CpG dinucleotides are the most common mutations in humans. Since this transition is not repaired, over time the number of CpG dinucleotides is greatly diminished in the genomes of vertebrates and plants.



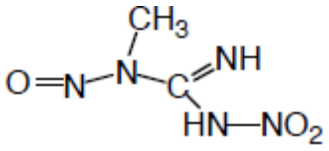
Methylation of CpG dinucleotides followed by oxidative deamination results in TpG dinucleotides.

Chemical modification by alkylation

Many mutagens are **alkylating agents**. This means that they will add an alkyl group, such as methyl or ethyl, to a base in DNA. Examples of commonly used alkylating agents in laboratory work are N-methyl-nitrosoguanidine and N-methyl-N'-nitro-nitrosoguanidine. The chemical warfare agents sulfur mustard and nitrogen mustard are also alkylating agents.

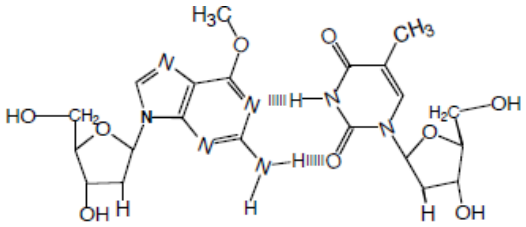
N-methyl-nitrosoguanidine and MNNG transfer a methyl group to guanine (e.g. to the O^6 position) and other bases (e.g. forming 3-methyladenine from adenine). The additional methyl (or other alkyl group) causes a distortion in the helix. The distorted helix can alter the base pairing properties. For instance, O^6-methylguanine will sometimes base pair with thymine

N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)



MNNG

6-O-methyl-G will pair with T



Chemicals that cause deletions

Some compounds cause a loss of nucleotides from DNA. If these deletions occur in a protein-coding region of the genomic DNA, and are not an integral multiple of 3, they result in a frameshift mutation. These are commonly more severe loss-of-function mutations than are simple substitutions. Frameshift mutagens such as proflavin or ethidium bromide have flat, polycyclic ring structures. They may bind to and **intercalate** within the DNA, i.e. they can insert between stacked base pairs. If a segment of the template DNA is the looped out, DNA polymerase can replicate past it, thereby generating a deletion. Intercalating agents can stabilize secondary structures in the loop, thereby increasing the chance that this segment stays in the loop and is not copied during replication .Thus growth of cells in the presence of such intercalating agents increase the probability of generating a deletion.

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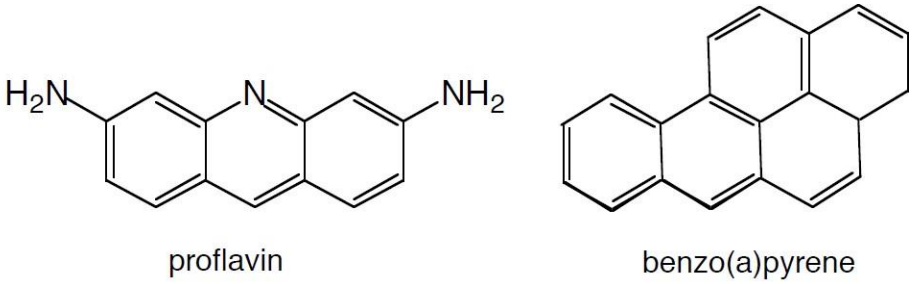

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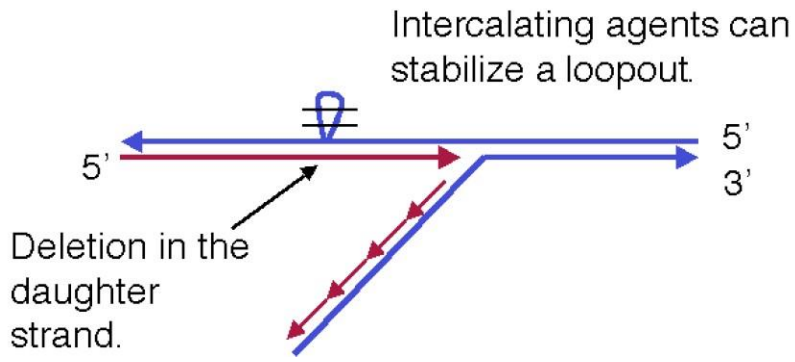


Ionizing radiation

High energy radiation, such as X-rays, γ-rays, and β particles (or electrons) are powerful mutagens. Since they can change the number of electrons on an atom, converting a compound to an ionized form, they are referred to as **ionizing radiation**. They can cause a number of chemical changes in DNA, including directly break phosphodiester backbone of DNA, leading to deletions. Ionizing radiation can also break open the imidazole ring of purines. Subsequent removal of the damaged purine from DNA by a glycosylase generates an apurinic site.

Ultraviolet radiation

Ultraviolet radiation with a wavelength of 260 nm will form pyrimidine dimers between adjacent pyrimidines in the DNA. The dimers can be one of two types (Fig. 11). The major product is a cytobutane-containing thymine dimer (between C5 and C6 of adjacent T's). The other product has a covalent bond between position 6 on one pyrimidine and position 4 on the adjacent pyrimidine, hence it is called the "6-4" photoproduct.

Repair mechanisms

The second part of this chapter examines the major classes of DNA repair processes. These are:

- reversal of damage,
- nucleotide excision repair,
- base excision
- repair, mismatch repair,
- recombinational repair, and
- error-prone repair.

Many of these processes were first studies in bacteria such as *E. coli*, however only a few are limited to this species. For instance, nucleotide excision repair and base excision repair are found in virtually all organisms, and they have been well characterized in bacteria, yeast, and mammals. Like DNA replication itself, repair of damage and misincorporation is a very old process.

Reversal of damage

Some kinds of covalent alteration to bases in DNA can be directly reversed. This occurs by specific enzyme systems recognizing the altered base and breaking bonds to remove the adduct or change the base back to its normal structure.

Photoreactivation is a light-dependent process used by bacteria to reverse pyrimidine dimers formed by UV radiation. The enzyme photolyase binds to a pyrimidine dimer and catalyzes a second photochemical reaction (this time using visible light) that breaks the cyclobutane ring and reforms the two adjacent thymidylates in DNA. Note that this is not formally the reverse of the reaction that formed the pyrimidine dimers, since energy from visible light is used to break the bonds between the pyrimidines, and no UV radiation is released. However, the result is that the DNA structure has been returned to its state prior to damage by UV. The photolyase enzyme has two subunits, which are encoded by the *phrA* and *phrB* genes in
E. coli.

A second example of the reversal of damage is the **removal of methyl groups**. For instance, the enzyme O^6-methylguanine methyltransferase, encoded by the *ada* gene in *E. coli*, recognizes O^6-methylguanine in duplex DNA. It then removes the methyl group, transferring it to an amino acid of the enzyme. The methylated enzyme is no longer active, hence this has been referred to as a suicide mechanism for the enzyme.

Excision repair

The most common means of repairing damage or a mismatch is to cut it out of the duplex DNA and recopy the remaining complementary strand of DNA, as outlined in Fig. 7.12. Three different types of excision repair have been characterized: nucleotide excision repair, base excision repair, and mismatch repair. All utilize a **cut, copy, and paste** mechanism. In the **cutting** stage, an enzyme or complex removes a damaged base or a string of nucleotides from the DNA. For the **copying**, a DNA polymerase (DNA polymerase I in *E. coli*) will copy the template to replace the excised, damaged strand. The DNA polymerase can initiate synthesis from 3' OH at the single-strand break (nick) or gap in the DNA remaining at the site of damage after excision. Finally, in the **pasting** stage, DNA ligase seals the remaining nick to give an intact, repaired DNA.

Nucleotide excision repair

In **nucleotide excision repair** (**NER**), damaged bases are cut out within a string of nucleotides, and replaced with DNA as directed by the undamaged template strand. This repair system is used to remove pyrimidine dimers formed by UV radiation as well as nucleotides modified by bulky chemical adducts. The common feature of damage that is repaired by nucleotide excision is that the modified nucleotides cause a significant distortion in the DNA helix. NER occurs in almost all organisms examined.

Some of the best-characterized enzymes catalyzing this process are the UvrABC excinuclease and the UvrD helicase in *E. coli*. The genes encoding this repair function were discovered as mutants that are highly sensitive to UV damage, indicating that the mutants are defective in UV repair.

The enzymes encoded by the *uvr* genes have been studied in detail. The polypeptide products of the *uvrA, uvrB,* and *uvrC* genes are subunits of a multisubunit enzyme called the **UvrABC excinuclease**. UvrA is the protein encoded by *uvrA,* UvrB is encoded by *uvrB,* and so on. The UvrABC complex recognizes damage-induced structural distortions in the DNA, such as pyrimidine dimers. It then cleaves on both sides of the damage. Then UvrD (also called helicase II), the product of the *uvrD* gene, unwinds the DNA, releasing the damaged segment. Thus for this system, the UvrABC and UvrD proteins carry out a series of steps in the cutting phase of excision repair. This leaves a gapped substrate for copying by DNA polymerase and pasting by DNA ligase.

The UvrABC proteins form a dynamic complex that recognizes damage and makes endonucleolytic cuts on both sides. The two cuts around the damage allow the single-stranded segment containing the damage to be excised by the helicase activity of UvrD. Thus the UvrABC dynamic complex and the UvrBC complex can be called **excinucleases**. After the damaged segment has been excised, a gap of 12 to 13 nucleotides remains in the DNA. This can be filled in by DNA polymerase and the remaining nick sealed by DNA ligase. Since the undamaged template directs the synthesis by DNA polymerase, the resulting duplex DNA is no longer damaged.

In more detail, the process goes as follows (Fig. 14). $UvrA_2$ (a dimer) and Uvr B recognize the damaged site as a $(UvrA)_2$UvrB complex. $UvrA_2$ then dissociates, in a step that requires ATP

hydrolysis. This is an autocatalytic reaction, since it is catalyzed by UvrA, which is itself an ATPase. After UvrA has dissociated, UvrB (at the damaged site) forms a complex with UvrC. The UvrBC complex is the active nuclease. It makes the incisions on each side of the damage, in another step that requires ATP. The phosphodiester backbone is cleaved 8 nucleotides to the 5' side of the damage and 4-5 nucleotides on the 3' side. Finally, the UvrD helicase then unwinds DNA so the damaged segment is removed. The damaged DNA segment dissociates attached to the UvrBC complex. Like all helicase reactions, the unwinding requires ATP hydrolysis to disrupt the ase pairs. Thus ATP hydrolysis is required at three steps of this series of reactions.The Uvr(A)BC excinuclease of *E. coli* recognizes AP sites, thymine dimers, and other structural distortions and makes nicks on both sides of the damaged region. The 12-13 nucleotide-long fragment is released together with the excinuclease by helicase II action.

Mismatch repair

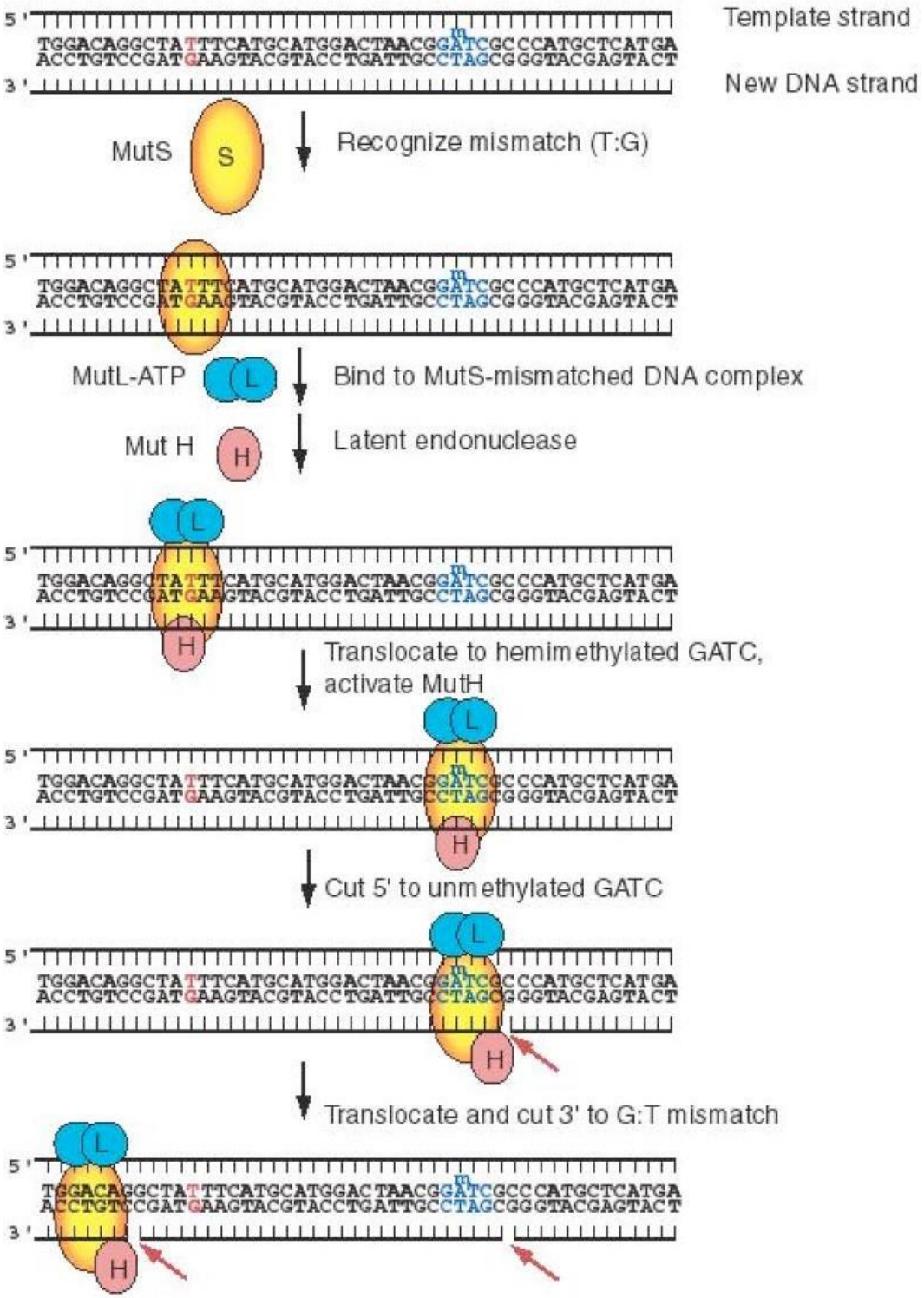
The third type of excision repair we will consider is **mismatch repair**, which is used to repair errors that occur during DNA synthesis. Proofreading during replication is good but not perfect. Even with a functional ε subunit, DNA polymerase III allows the wrong nucleotide to be incorporated about once in every 10^8 bp synthesized in *E. coli*. However, the measured mutation rate in bacteria is as low as one mistake per 10^{10} or 10^{11} bp. The enzymes that catalyze **mismatch repair** are responsible for this final degree of accuracy. They recognize misincorporated nucleotides, excise them and replace them with the correct nucleotides. In contrast to nucleotide excision repair, mismatch repair does not operate on bulky adducts or major distortions to the DNA helix. Most of the mismatches are substitutes within a chemical class, e.g. a C incorporated instead of a T. This causes only a subtle helical distortions in the DNA, and the misincorporated nucleotide is a normal component of DNA. The ability of a cell to recognize a mismatch reflects the exquisite specificity of **MutS**, which can distinguish normal base pairs from those resulting from misincorporation. Of course, the repair machinery needs to know which of the nucleotides at a mismatch pair is the correct one and which was misincorporated. It does this by determining which strand was more recently synthesized, and repairing the mismatch on the nascent strand.

In *E. coli*, the methylation of A in a GATC motif provides a covalent marker for the parental strand, thus methylation of DNA is used to discriminate parental from progeny strands.

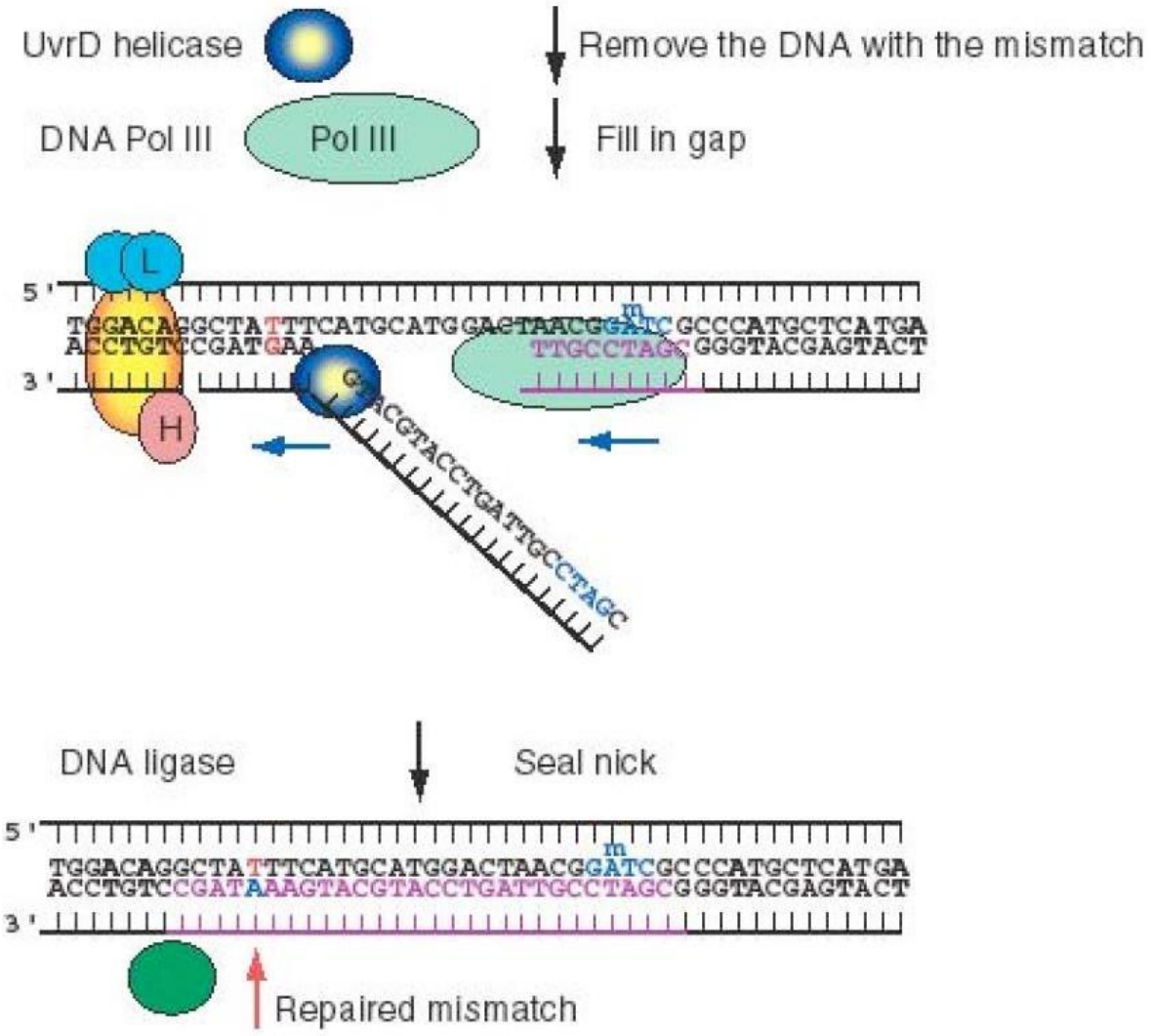
Recall that the ***dam* methylase** catalyzes the transfer of a methyl group to the A of the pseudopalindromic sequence GATC in duplex DNA. Methylation is delayed for several minutes after replication. IN this interval before methylation of the new DNA strand, the mismatch repair system can find mismatches and direct its repair activity to nucleotides on the unmethylated, newly replicated strand. Thus replication errors are removed preferentially.

The enzyme complex MutH-MutL-MutS , or MutHLS, catalyzes mismatch repair in *E. coli*. The genes that encode these enzymes, *mutH*, *mutL* and *mutS*, were discovered because strains carrying mutations in them have a high frequency of new mutations. This is called a **mutator phenotype**, and hence the name *mut* was given to these genes. Not all mutator genes are involved in mismatch repair; e.g., mutations in the gene encoding the proofreading enzyme of DNA polymerase III also have a mutator phenotype. This gene was independently discovered in screens for defects in DNA replication (*dnaQ*) and mutator genes (*mutD*). Three complementation groups within the set of mutator alleles have been implicated primarily in mismatch repair; these are *mutH*, *mutL* and *mutS*.

MutS will recognize seven of the eight possible mismatched base pairs (except for C:C) and bind at that site in the duplex DNA (Fig. 16). **MutH** and **MutL** (with ATP bound) then join the complex, which then moves along the DNA in either direction until it finds a hemimethylated GATC motif, which can be as far a few thousand base pairs away. Until this point, the nuclease function of MutH has been dormant, but it is activated in the presence of ATP at a hemimethylated GATC. It cleaves the unmethylated DNA strand, leaving a nick 5' to the G on the strand containing the unmethylated GATC (i.e. the new DNA strand). The same strand is nicked on the other side of the mismatch. Enzymes involved in other processes of repair and replication catalyze the remaining steps. The segment of single-stranded DNA containing the incorrect nucleotide is to be excised by UvrD, also known as helicase II and MutU. SSB and exonuclease I are also involved in the excision. As the excision process forms the gap, it is filled in by the concerted



action of DNA polymerase III .



Mismatch repair is highly conserved, and investigation of this process in mice and humans is providing new clues about mutations that cause cancer. Homologs to the *E. coli* genes *mutL* and *mutS* have been identified in many other species, including mammals. The key breakthrough came from analysis of mutations that cause one of the most common hereditary cancers, *hereditary nonpolyposis colon cancer* (HNPCC). Some of the genes that, when mutated, cause this disease encode proteins whose amino acid sequences are significantly similar to those of two of the *E. coli* mismatch repair enzymes. The human genes are called *hMLH1* (for human *mutL* homolog 1), *hMSH1*, and *hMSH2* (for human *mutS* homolog 1 and 2, respectively). Subsequent work has shown that these enzymes in humans are involved in mismatch repair. Presumably the increased frequency of mutation in cells deficient in mismatch repair leads to the accumulation of mutations

in proto-oncogenes, resulting in dysregulation of the cell cycle and loss of normal control over the rate of cell division.

The SOS response

A coordinated battery of responses to DNA damage in *E. coli* is referred to as the SOS response. This name is derived from the maritime distress call, “SOS” for "Save Our Ship".

Accumulating damage to DNA, e.g. from high doses of radiation that break the DNA backbone, will generate single-stranded regions in DNA. The increasing amounts of single-stranded DNA induce SOS functions, which stimulate both the recombination repair and the translesional synthesis just discussed.

Key proteins in the SOS response are **RecA** and **LexA**. RecA binds to single stranded regions in DNA, which activates new functions in the protein. One of these is a capacity to further activate a latent proteolytic activity found in several proteins, including the LexA repressor, the **UmuD** protein and the repressor encoded by bacteriophage lambda.

RecA activated by binding to single-stranded DNA is not itself a protease, but rather it serves as a co-protease, activating the latent proteolytic function in LexA, UmuD and some other proteins.

In the absence of appreciable DNA damage, the LexA protein represses many operons, including several genes needed for DNA repair: *recA, lexA, uvrA, uvrB, and umuC.* When the activated RecA stimulates its proteolytic activity, it cleaves itself (and other proteins), leading to coordinate induction of the SOS regulated operons.

organism will ever synthesize. In eucaryotes, DNA is contained in the cell nucleus.

CHEMICAL AND PHYSICAL PROPERTIES OF DNA

I. ABSORPTION

A. The bases in DNA absorb ultraviolet light at the wavelength of 260 nm

1. This absorption can be monitored using a spectrophotometer
2. This is one method used to figure the concentration of DNA in solution

B. The less ordered the bases the more ultraviolet light is absorbed

1. Free bases absorb 1.60 units at 260 nm
2. Single stranded DNA absorb 1.37 units at 260 nm
3. Double stranded DNA absorb 1.00 units at 260 nm

II. DENSITY

A. Density can be measured by CsCl-density ultracentrifugation

1. CsCl, upon ultracentrifugation, will form a density gradient, with the most dense solution at the bottom
2. Macromolecules, such as DNA, will concentrate in the area of CsCl that has the same density as themselves

a. Hence, more dense DNA will migrate downward and less dense DNA upwards forming bands

B. Density can be used to estimate G+C content

1. GC base pairs are more dense than AT base pairs
2. Therefore, DNA with more GC base pairs will form bands lower down than an equal number of base pairs with high AT content

C. Density studies show the existence of satellite DNA

1. If chromosomal DNA is cut into about equal size pieces and subjected to CsCl-density ultracentrifugation two bands are formed

a. One band contains most of the DNA from the genome

b. The second band (the satellite) contains about 5% of the DNA from the genome and has a highly repetitive sequence

III. DENATURATION

A. Definition

1. DNA is considered denatured when the double stranded DNA molecule is converted into two single stranded molecules
2. This can be monitored by noting the increase in absorption of ultraviolet light

B. Temperature

1. As thermal energy increases, the frequency of hydrogen bonds breaking between the molecules increases

a. As temperature increases, the two molecules will separate into single-stranded molecules

2. The *Tm* (melting temperature) of a DNA molecule is the temperature in which half the DNA molecules are denatures

a. The *Tm* is used to estimate the G+C content of a DNA molecule

1. G-C base pairs are held together by three hydrogen bonds (A-Ts by two) and it therefore takes more energy (higher temperatures) to separate molecules with high GC contents

C. Hydrophobicity of solvent

1. Substances that are hydrophobic tend to decrease the *Tm* of DNA molecules

a. Hydrophobic substances will allow the bases in DNA to dissolve into the solvent

b. Hence, the bases are not constricted to being stacked upon one another

1. This will make it easier to disrupt the hydrogen bonding between DNA molecules

2. Substances that are hydrophilic tend to increase the *Tm* of DNA molecules

a. These will keep the bases of DNA stacked upon one another in the orientation that most favors hydrogen bonding between DNA strands

D. pH

1. Acids

a. pHs lower then one result in the breakage of phosphodiester bonds between nucleotides and breakage of the N-glycosidic bond between the sugar and purine bases

b. pH of around 4 results in the selective breakage of N-glycosidic bonds between the sugar and purines

1. DNA treated this way is referred to a apurinic acid, since the purines have been removed

2. Alkali

a. Base tends to change the polarity of groups involved in hydrogen bonds

1. Above pH 11.3, all hydrogen bonds are disrupted and the DNA is totally denatured

b. DNA is resistant to hydrolysis to about pH 13

1. Unless it is apurinic, then it is hydrolyzed

c. RNA is hydrolyzed into ribonucleotides around pH 11

E. Ionic strength

1. The phosphates of the DNA sugar-phosphate backbones are negatively charged

a. Like charges repel each other

b. DNA in distilled water will spontaneously denature into single stranded DNA

2. Salts that dissociate into ions will neutralize the charges of the phosphate groups

a. Salts will stabilize the DNA double helix resulting in a higher *Tm*

F. G+C content

1. Variation

a. Most higher organisms have a G+C content of about 0.5 (0.49 - 0.51 for primates)

b. Lower organisms range widely from 0.27 to 0.76 for some bacteria

2. Estimating G+C content

a. G+C content of a DNA molecule can be estimated from its thermal melting temperature (*Tm*)

IV. SOLUBILITY

A. RNA is more soluble in aqueous solutions then DNA

B. RNA is less stable then DNA

1. The hydroxyl group on the 2' carbon of ribose is more reactive the hydrogen found in deoxyribose

V. SIZE

A. Electrophoresis

1. DNA has a negative charge that is proportional to its size

a. This is due to the negatively charged phosphates in the sugar- phosphate backbone

b. If DNA is placed in an electrical field it will migrate towards the positive electrode (the cathode)

2. If DNA is electrophoresed through a gel, smaller pieces will migrate faster than larger pieces

a. Larger pieces have trouble squeezing through the gel matrix and are hence retarded while smaller pieces migrate easier

b. Type of gels

1. Agarose is used to separate fairly large DNA molecules

a. 5 million to a few thousands base pairs

2. Polyacrylamide is used to separate small pieces of DNA

a. 2 to several hundred base pairs

3. The size of DNA is estimated by comparing its migration through the gel to DNA molecules of

known size

B. Velocity sedimentation

1. Sedimentation velocity is dependent upon two variables: density and shape

a. The more dense the DNA the quicker it will sediment upon centrifugation

b. Globular (more compact) molecules will sediment faster than linear molecules

C. Electron microscopy

1. The size of DNA molecules can be determined by electron microscopy

a. The DNA is visualized on a grid of known size so that the size of the DNA molecule can be estimated

VI. DNA CONCENTRATION

A. Absorption

1. DNA absorbs ultraviolet light at 260 nm

2. The more DNA present, the higher the absorption

a. DNA concentrations can be estimated by comparing its absorption to known concentrations of DNA

b. DNA most be fairly pure, since many contaminating substances (e.g., proteins) also absorb around this wavelength

VII. RENATURATION STUDIES

A. DNA that has been denatured will often come back together when condition are met

1. This is referred to as renaturation

2. Renaturation occurs because hydrogen bonds of complimentary base pairs reform

a. Slowly lowering the temperature or adding ions to solution may lead to renaturation

B. Renaturation rates are dependent on DNA concentration

1. The rate limiting step in renaturation is the collision of complimentary DNA molecules

a. The more molecules of complimentary DNA molecules present, the faster they can find each other and renature

b. DNA molecules in low concentration in solution will take awhile to find a complimentary partner, and will therefore renature slower

C. In eukaryotes, three major drops in absorbance occur in renaturation studies

1. The first drop in absorption is when the highly repetitive DNA sequence renatures

a. Since these are repeated so often, they are in the highest concentration

2. The second drop in absorption occurs when the moderately repetitive DNA renatures
3. Unique DNA sequences are the last to renature

a. These are in the lowest concentration and take the longest time to find each other

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – IV – SBC3102 – GENETICS

BACTERIAL GENETICS

BACTERIAL GENE TRANSFER

Bacterial gene exchange differs from eukaryotes:

- Bacteria do not exchange genes by meiosis. (Why not?) They rarely exchange two entire genomes.
- Bacteria commonly exchange small pieces of genome, a few genes at a time, through transformation, transduction, or conjugation.
- Transfer between species, even kingdoms, is common; less common in eukaryotes, though it does occur.

Bacteria reproduce by the process of binary fission. In this process, the chromosome in the mother cell is replicated and a copy is allocated to each of the daughter cells. As a result, the two daughter cells are genetically identical. If the daughter cells are always identical to the mother, how are different strains of the same bacterial species created? The answer lies in certain events that change the bacterial chromosome and then these changes are passed on to future generations by binary fission. In this chapter, you will explore some of the events that result in heritable changes in the genome: genetic transfer and recombination, plasmids and transposons.

Genetic Transfer

- Genetic transfer is the mechanism by which DNA is transferred from a donor to a recipient.
- Once donor DNA is inside the recipient, crossing over can occur.

1. The result is a recombinant cell that has a genome different from

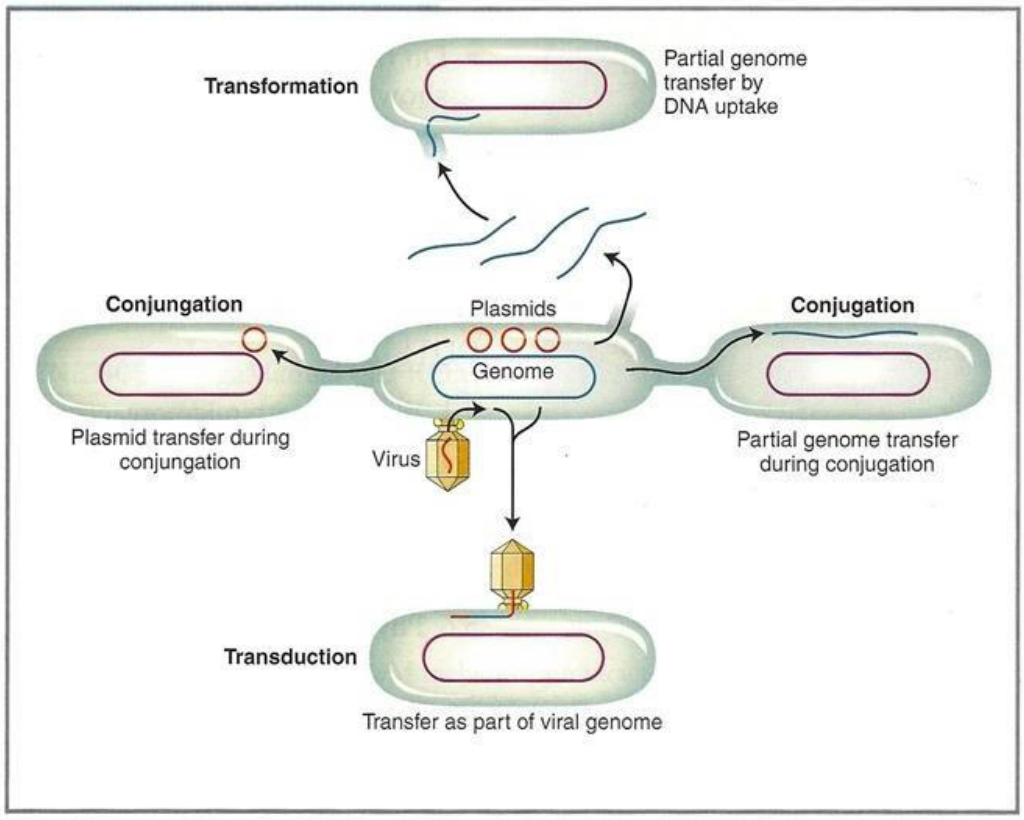
- either the donor or the recipient.

- In bacteria genetic transfer can happen three ways:

1. Conjugation
2. Transformation
3. Transduction

Remember that a recombination event must occur after transfer in order that the change in the genome be heritable (passed on to the next generation).

DNA transfer between bacterial cells



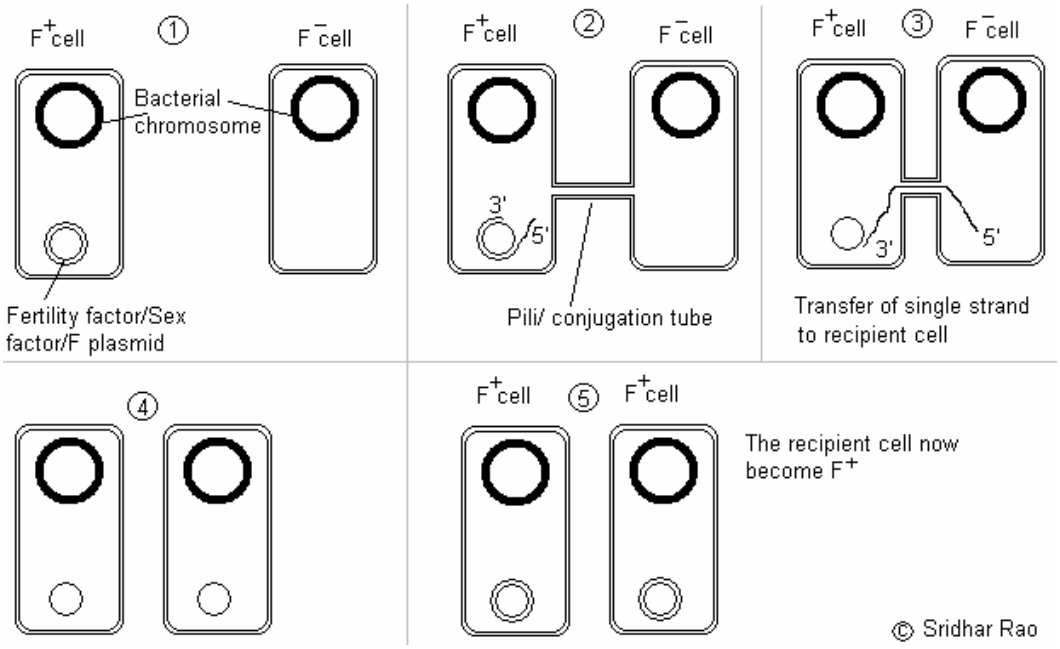
CONJUGATION:

In 1946 Joshua Lederberg and Tatum discovered that some bacteria can transfer genetic information to other bacteria through a process known as conjugation. Bacterial conjugation is the transfer of DNA from a living donor bacterium to a recipient bacterium. Plasmids are small autonomously replicating circular pieces of double-stranded circular DNA. Conjugation involves the transfer of plasmids from donor bacterium to recipient bacterium. Plasmid transfer in Gram-negative bacteria occurs only between strains of the same species or closely related species.Some plasmids are designated as F factor (F plasmid, fertility factor or sex factor) because they carry genes that mediate their own transfer. The F factor can replicate autonomously in the cell. These genes code for the production of the sex pilus and enzymes necessary for conjugation. Cells possessing F plasmids are F+ (male) and act as donors. Those cells lacking this plasmid are F- (female) and act as recipient. All those plasmids, which confer on their host cells to act as donors in conjugation are called transfer factor.

Each Gram negative F+ bacterium has 1 to 3 sex pili that bind to a specific outer membrane protein on recipient bacteria to initiate mating. The sex pilus then retracts, bringing the two bacteria in contact and the two cells become bound together at a point of direct envelope-to- envelope contact. In Gram-positive bacteria sticky surface molecules are produced which bring the two bacteria into contact. Gram-positive donor bacteria produce adhesins that cause them to aggregate with recipient cells, but sex pili are not involved. DNA is then transferred from the donor to the recipient. Plasmid-mediated conjugation occurs in *Bacillus subtilis*, *Streptococcus lactis*, and *Enterococcus faecalis* but is not found as commonly in the Gram-positive bacteria as compared to the Gram-negative bacteria.

1. F+ conjugation:

This results in the transfer of an F+ plasmid (coding only for a sex pilus) but not chromosomal DNA from a male donor bacterium to a female recipient bacterium. The two strands of the plasmid separate. One strand enters the recipient bacterium progressing in the 5' to 3' direction while one strand remains in the donor. The complementary strands are synthesized in both donor and recipient cells. The recipient then becomes an F+ male and can make a sex pilus. During conjugation, no cytoplasm or cell material except DNA passes from donor to recipient. The mating pairs can be separated by shear forces and conjugation can be interrupted. Consequently, the mating pairs remain associated for only a short time. After conjugation, the cells break apart. Following successful conjugation the recipient becomes F+ and the donor remains F+.



2. Resistance plasmid conjugation:

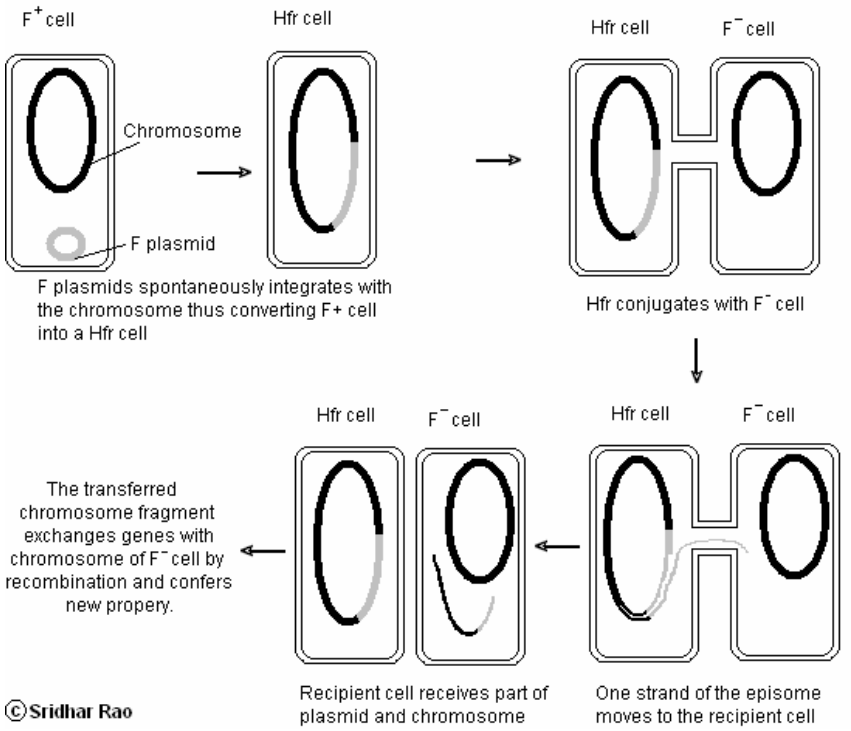
Some Gram-negative bacteria harbor plasmids that contain antibiotic resistance genes, such plasmids are called R factors. The R factor has two components, one that codes for self transfer (like F factor) called RTF (resistance transfer factor) and the other R determinant that contains genes coding for antibiotic resistance. R plasmids may confer resistance to as many as five different antibiotics at once upon the cell and by conjugation; they can be rapidly disseminated through the bacterial population. The difference between F factor and R factor is that the latter has additional genes coding for drug resistance. During conjugation there is transfer of resistance plasmid (Rplasmid) from a donor bacterium to a recipient. One plasmid strand enters the recipient bacterium while one strand remains in the donor. Each strand then makes a complementary copy. R-plasmid has genes coding for multiple antibiotic resistance as well as sex pilus formation. The recipient becomes multiple antibiotic resistant and male, and is now able to transfer R-plasmids to other bacteria. When the recipient cells acquire entire R factor, it too expresses antibiotic resistance. Sometimes RTF may disassociate from the R determinant and the two components may exist as separate entities. In such cases though the host cell remains resistant to antibiotics, it can not transfer this resistance to other cells. Sometimes RTF can have other genes (such as those coding for hemolysin, enterotoxin) apart from R determinants attached to it.

3. Hfr (high frequency recombinant) conjugation:

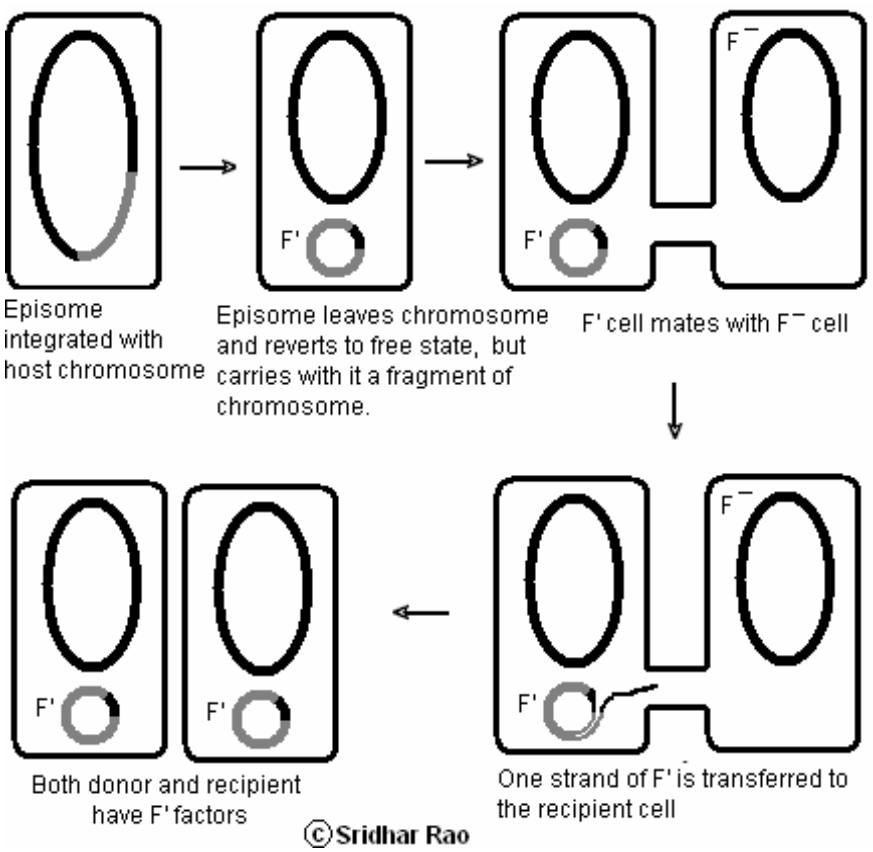
Plasmids may integrate into the bacterial chromosome by a recombination event depending upon the extent of DNA homology between the two. After integration, both plasmid and chromosome will replicate as a single unit. A plasmid that is capable of integrating into the chromosome is called an episome. If the F plasmid is integrated into the chromosome it is called an Hfr cell. After integration, both chromosome and plasmid can be conjugally transferred to a recipient cell. Hfr cells are called so because they are able to transfer chromosomal genes to recipient cells with high frequency.

The DNA is nicked at the origin of transfer and is replicated. One DNA strand begins to passes Through a cytoplasmic bridge to the F- cell, where its complementary strand is synthesized. Along with the portion of integrated plasmid, the chromosome is also transmitted to the F- cell. The

bacterial connection usually breaks before the transfer of the entire chromosome is completed so the remainder of the F+ plasmid rarely enters the recipient. Usually only a part of the Hfr chromosome as well as the plasmid is transferred during conjugation and the recipient cell does not receive complete F factor. After conjugation the Hfr cell remains Hfr but the F- cell does not become F+ and continues to remain F-. However the transferred chromosome fragment recombines with the chromosome of F- cell thereby transferring some new property to the recipient cell.



The integration of episome into the chromosome is not stable and the episomes are known to revert back to free state. While doing so, the episomes sometimes carry fragments of chromosomal genes along with it. Such an F factor that incorporates some chromosomal genes is called F prime (F') factor. When such a F' cell mates with F- recipient cell, it not only transfers the F factor but also the host genes that it carried with it. This process of transfer of chromosomal genes along with F factor is known is **sexduction**.

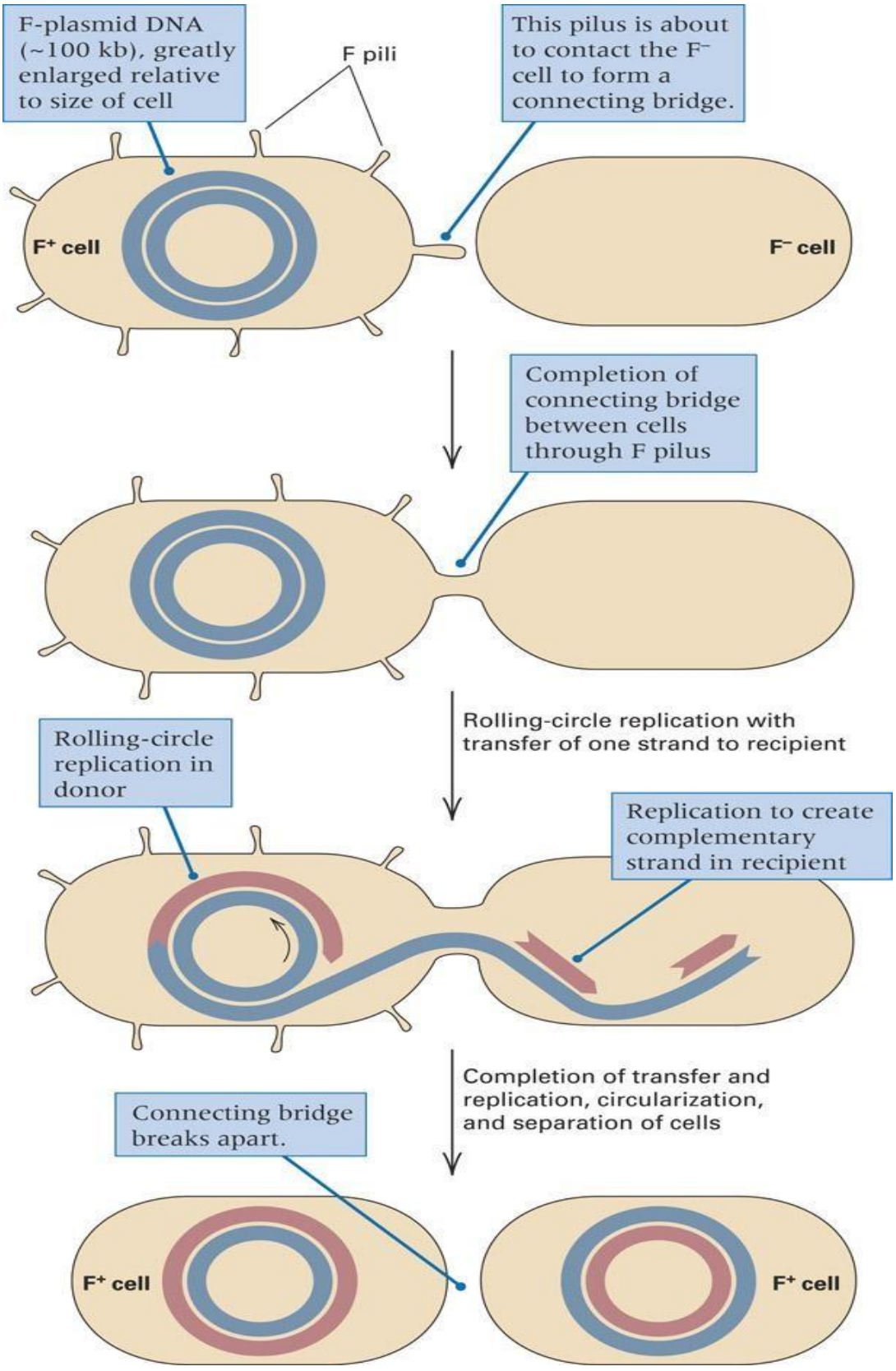


Significance: Among the Gram negative bacteria this is the major way that bacterial genes are transferred. Transfer can occur between different species of bacteria. Transfer of multiple antibiotic resistance by conjugation has become a major problem in the treatment of certain bacterial diseases. Since the recipient cell becomes a donor after transfer of a plasmid, an antibiotic resistance gene carried on a plasmid can quickly convert a sensitive population of cells to a resistant one.

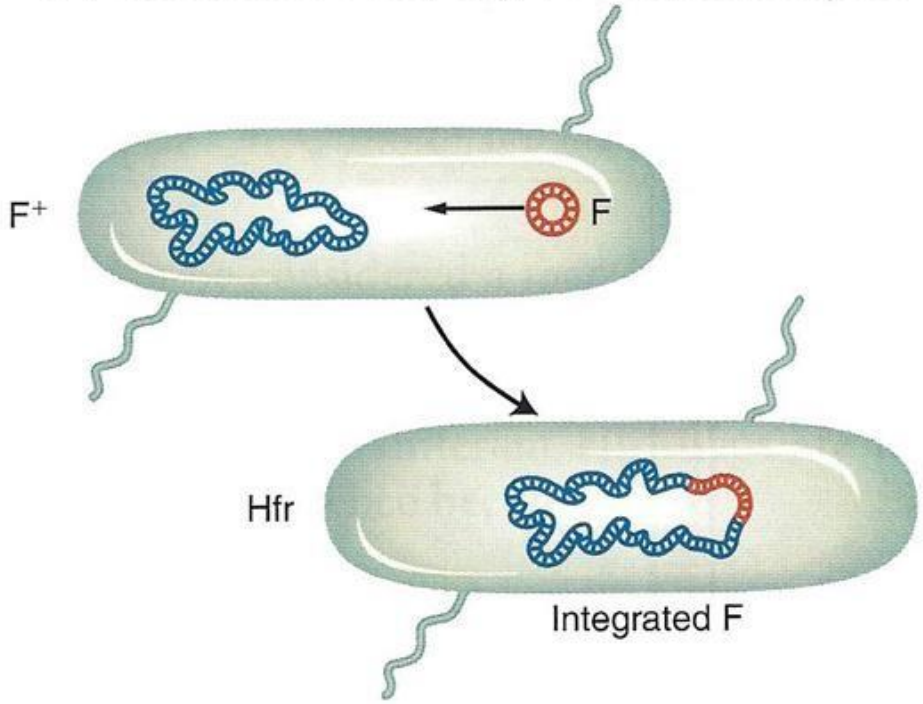
IN SUMMARY

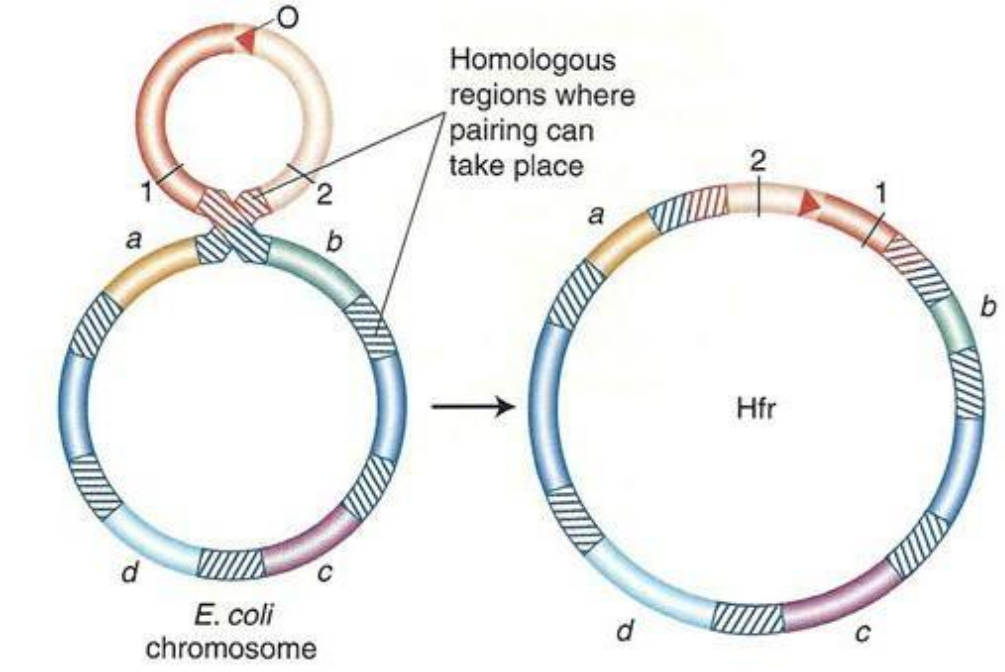
- Conjugation mechanism requires the presence of a special plasmid called the F plasmid.
- Therefore, we will briefly review plamid structure before continuing.
  - Plasmids are small, circular pieces of DNA that are separate and replicate indepentently from the bacterial chromosome.
  - Plasmids contain only a few genes that are usually not needed for growth and reproduction of the cell.
  - However, in stressful situations, plasmids can be crucial for survial.
  - The F plasmid, for example, facilites conjugation.

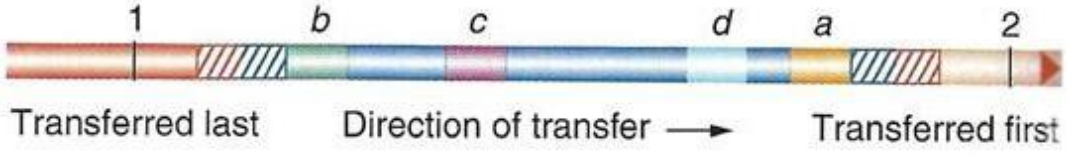
- This can give a bacterium new genes that may help it survive in a changing environment.
  - Some plasmids can integrate reversibly into the bacterial chromosome.
  - An integrated plasmid is called an episome.
- Bacteria that have a F plasmid are referred to as as F+ or male.
  - Those that do not have an F plasmid are F- of female.
- The F plasmid consists of 25 genes that mostly code for production of sex pilli.
- A conjugation event occurs when the male cell extends his sex pili and one attaches to the female.
  - This attached pilus is a temporary cytoplasmic bridge through which a replicating F plasmid is transferred from the male to the female.
  - When transfer is complete, the result is two male cells.
- The F plasmid can behave as an episome.
  - When the F+ plasmid is integrated within the bacterial chromosome, the cell is called an Hfr cell (high frequency of recombination cell).
  - The F plasmid always insetrs at the same spot for a bacterial species.
- The Hfr cell still behaves as a F+ cell, transferring F genes to a F-cell, but now it can take some of the bacterial chromosome with it.
- Replication of the Hfr chromosome begins at a fixed point within the F episome and the chromosome is transferred to the female as it replicates.
- Movement of the bacteria usually disrupts conjugation before the entire chromosome, including the tail of the F episome can be transferred.
  - Therefore, the recipient remains F- because the F plasmid is not entirely transferred.
- A cross over event can occur between homologous genes on the Hfr fragment and the F- DNA.
- Pieces of DNA not recombined will be degraded or lost in cell division.
- Now the recombinant genome can be passed on to future generations.


F-plasmid DNA (~100 kb), greatly enlarged relative to size of cell
F pili
This pilus is about to contact the F⁻ cell to form a connecting bridge.
F⁺ cell
F⁻ cell
Completion of connecting bridge between cells through F pilus
Rolling-circle replication with transfer of one strand to recipient
Rolling-circle replication in donor
Replication to create complementary strand in recipient
Completion of transfer and replication, circularization, and separation of cells
Connecting bridge breaks apart.
F⁺ cell
F⁺ cell

F+ becomes Hfr by recombination







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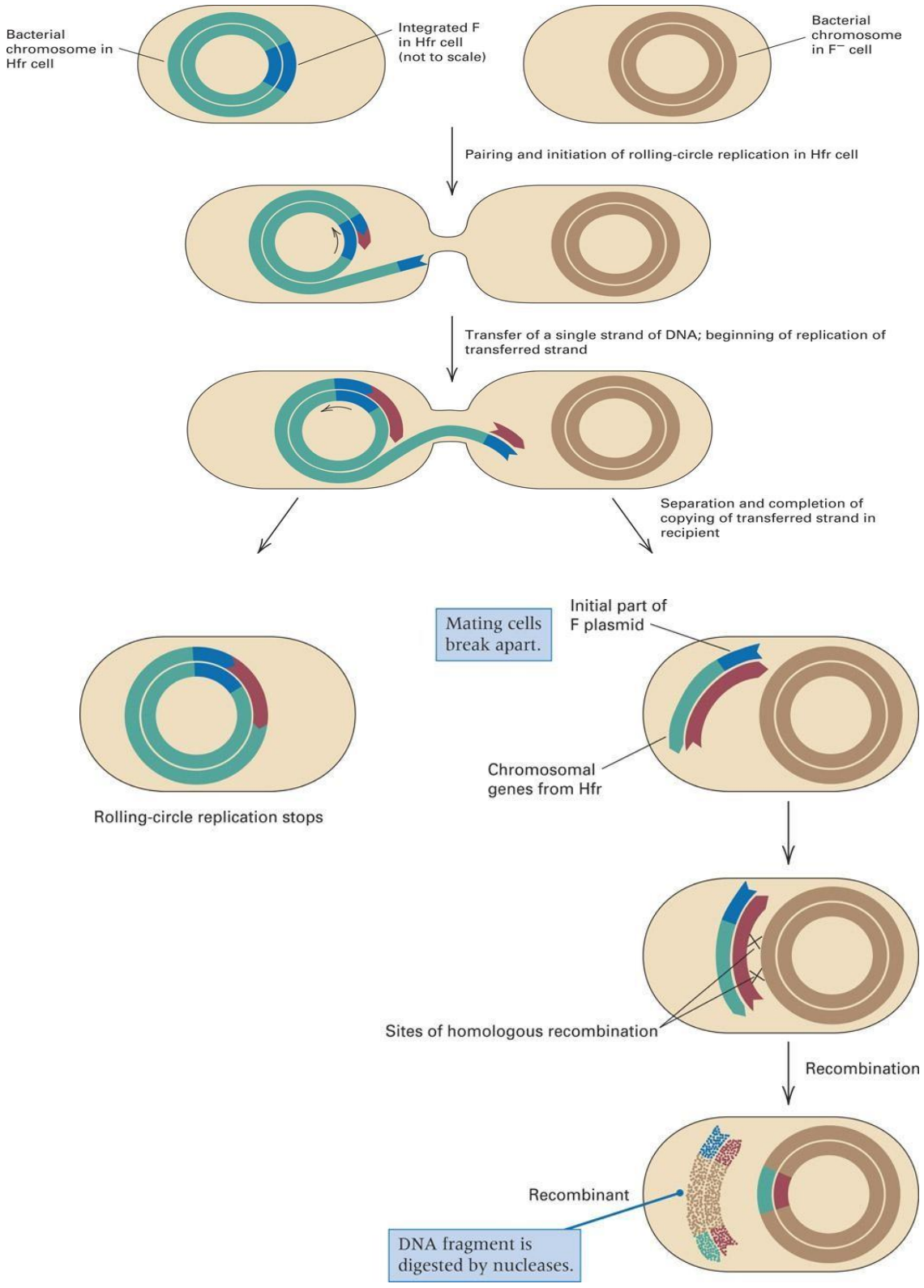
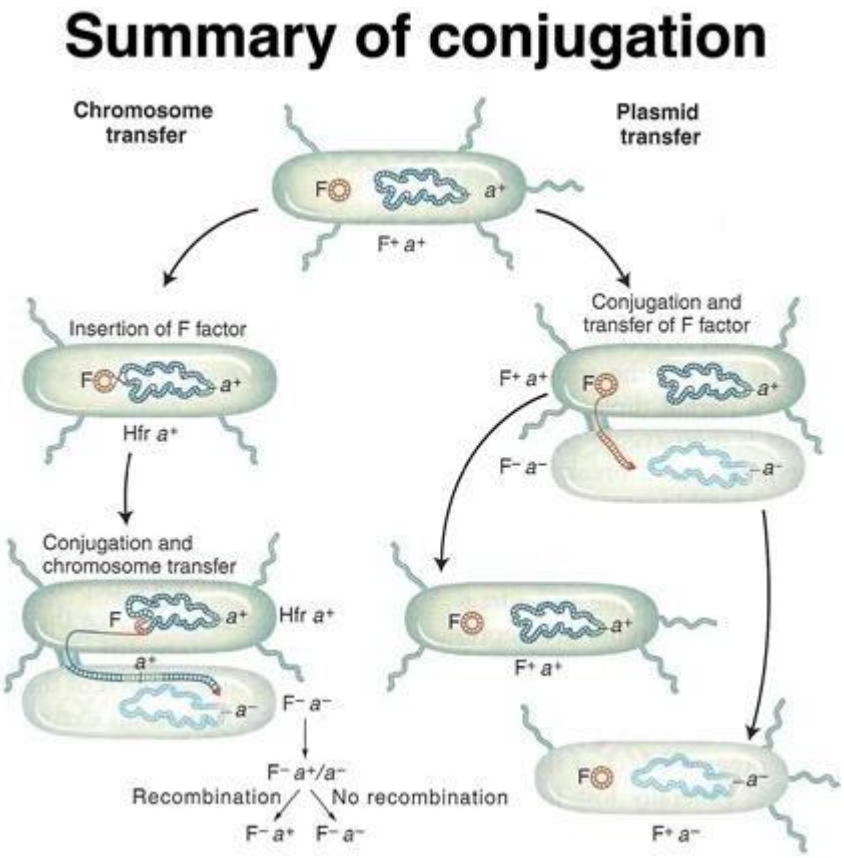

Bacterial chromosome in Hfr cell
Integrated F in Hfr cell (not to scale)
Bacterial chromosome in F⁻ cell
Pairing and initiation of rolling-circle replication in Hfr cell
Transfer of a single strand of DNA; beginning of replication of transferred strand
Separation and completion of copying of transferred strand in recipient
Rolling-circle replication stops
Mating cells break apart.
Initial part of F plasmid
Chromosomal genes from Hfr
Sites of homologous recombination
Recombination
Recombinant
DNA fragment is digested by nucleases.



TRANSFORMATION:

Transformation involves the uptake of free or naked DNA released by donor by a recipient. It was the first example of genetic exchange in bacteria to have been discovered. This was first demonstrated in an experiment conducted by Griffith in 1928. The presence of a capsule around some strains of pneumococci gives the colonies a glistening, smooth (S) appearance while pneumococci lacking capsules have produce rough (R) colonies. Strains of pneumococci with a capsule (type I) are virulent and can kill a mouse whereas strains lacking it (type II) are harmless. Griffith found that mice died when they were injected with a mixture of live non capsulated (R, type II) strains and heat killed capsulated (S, type I) strains. Neither of these two when injected alone could kill the mice, only the mixture of two proved fatal. Live S strains with capsule were

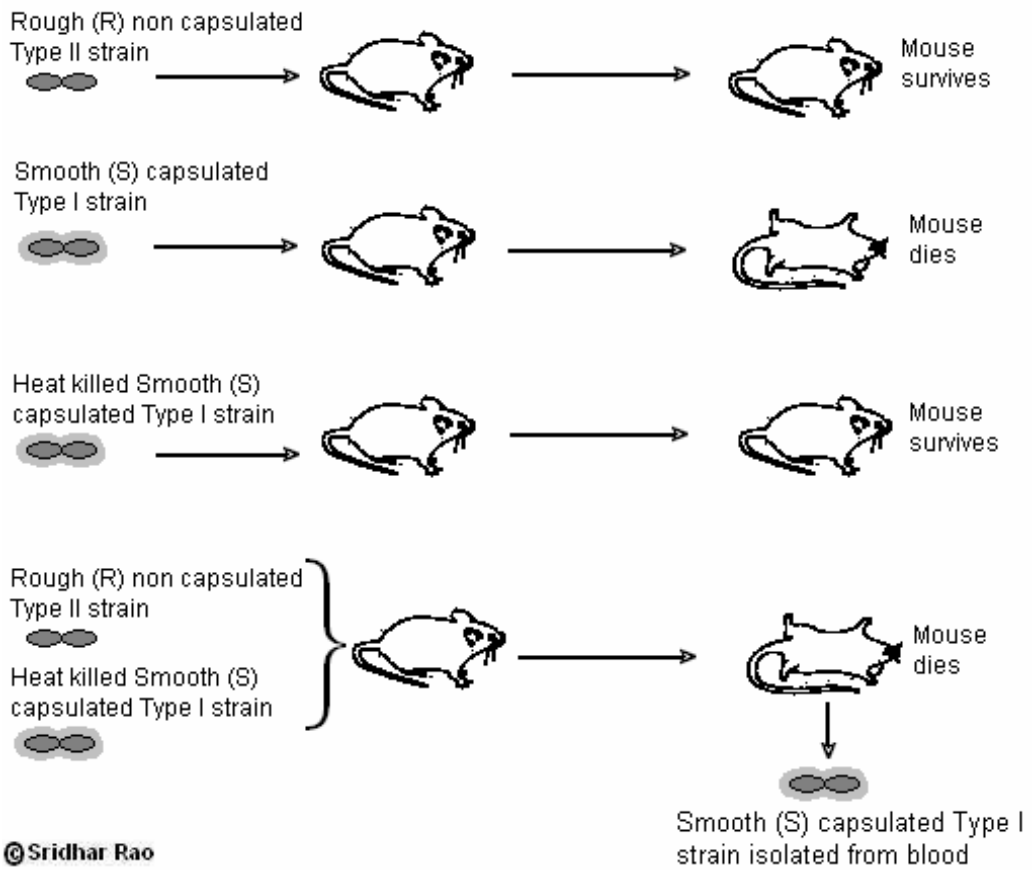
isolated from the blood of the animal suggesting that some factor from the dead S cells converted the R strains into S type. The factor that transformed the other strain was found to be DNA by Avery, McLeod and McCarty in 1944.

Transformation is gene transfer resulting from the uptake by a recipient cell of naked DNA from a donor cell. Certain bacteria (e.g. Bacillus, Haemophilus, Neisseria, Pneumococcus) can take up DNA from the environment and the DNA that is taken up can be incorporated into the recipient's chromosome.

- The transformation process was first demonstrated in 1928 by Frederick Griffith.
- Griffith experimented on *Streptococcus pneumoniae*, a bacteria that causes pneumonia in mammals.
- When he examined colonies of the bacteria on petri plates, he could tell that there were two different strains.
  - The colonies of one strain appeared smooth.
    - Later analysis revealed that this strain has a polysaccharide capsule and is virulent, that it, it causes pneumonia.
  - The colonies of the other strain appeared rough.
    - This strain has no capsules and is avirulent.
  - When Griffith injected living encapsulated cells into a mouse, the mouse died of pneumonia and the colonies of encapsulated cells were isolated from the blood of the mouse.
  - When living nonencapsulated cells were injected into a mouse, the mouse remained healthy and the colonies of nonencapsulated cells were isolated from the blood of the mouse.
  - Griffith then heat killed the encapsulated cells and injected them into a mouse.
    - The mouse remained healthy and no colonies were isolated.
    - The encapsulated cells lost the ability to cause the disease.
  - However, a combination of heat-killed encapsulated cells and living nonencapsulated cells did cause pneumonia and colonies of living encapsulated cells were isolated from the mouse.
    - How can a combination of these two strains cause pneumonia when either strand alone does not cause the disease?
    - If you guessed the process of transformation you are right!
    - The living nonencapsulated cells came into contact with DNA fragments of the dead capsulated

cells.

- The genes that code for the capsule entered some of the living cells and a crossing over event occurred.
- The recombinant cell now has the ability to form a capsule and cause pneumonia.
- All of the recombinant's offspring have the same ability.
- That is why the mouse developed pneumonia and died.



The steps involved in transformation are:

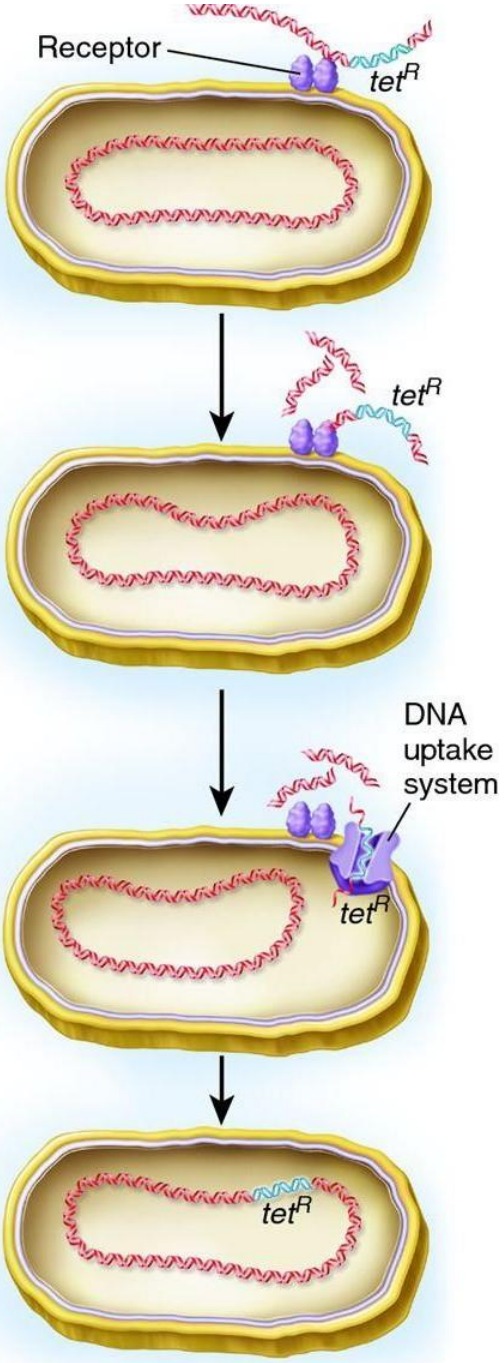
1. A donor bacterium dies and is degraded.
2. A fragment of DNA (usually about 20 genes long) from the dead donor bacterium binds to DNA binding proteins on the cell wall of a competent, living recipient bacterium.
3. Nuclease enzymes then cut the bound DNA into fragments.
4. One strand is destroyed and the other penetrates the recipient bacterium.
5. The Rec A protein promotes genetic exchange (recombination) between a fragment of the donor's DNA and the recipient's DNA.

1 A DNA fragment containing the *tet*^{R} gene binds to a cell surface receptor.

2 Bacterial enzymes cut the DNA into smaller fragments.

3 One strand is degraded, and a single strand is imported into the cell by a DNA uptake system.

4 The imported DNA is incorporated into the bacterial chromosome.



Transformed cell that is resistant to the antibiotic tetracycline

Some bacteria are able to take up DNA naturally. However, these bacteria only take up DNA a particular time in their growth cycle (log phase) when they produce a specific protein called a competence factor. Uptake of DNA by Gram positive and Gram negative bacteria differs. In Gram positive bacteria the DNA is taken up as a single stranded molecule and the complementary strand is made in the recipient. In contrast, Gram negative bacteria take up double stranded DNA.

Natural transformation

Natural transformation is a bacterial adaptation for DNA transfer that depends on the expression of numerous bacterial genes whose products appear to be responsible for this process. In general, transformation is a complex, energy-requiring developmental process. In order for a bacterium to bind, take up and recombine exogenous DNA into its chromosome, it must become competent, that is, enter a special physiological state. Competence development in *Bacillus subtilis* requires expression of about 40 genes. The DNA integrated into the host chromosome is usually (but with rare exceptions) derived from another bacterium of the same species, and is thus homologous to the resident chromosome. he capacity for natural transformation appears to occur in a number of prokaryotes, and thus far 67 prokaryotic species (in seven different phyla) are known to undergo this process.

Competence for transformation is typically induced by high cell density and/or nutritional limitation, conditions associated with the stationary phase of bacterial growth. Transformation in *Haemophilus influenzae* occurs most efficiently at the end of exponential growth as bacterial growth approaches stationary phase. Transformation in *Streptococcus mutans*, as well as in many other streptococci, occurs at high cell density and is associated with biofilm formation. Competence in *B. subtilis* is induced toward the end of logarithmic growth, especially under conditions of amino acid limitation

Natural competence

About 1% of bacterial species are capable of naturally taking up DNA under laboratory conditions; more may be able to take it up in their natural environments. DNA material can be transferred between different strains of bacteria, in a process that is called horizontal gene transfer. Some species upon cell death release their DNA to be taken up by other cells, however transformation works best with DNA from closely related species. These naturally competent bacteria carry sets

of genes that provide the protein machinery to bring DNA across the cell membrane(s). The transport of the exogeneous DNA into the cells may require proteins that are involved in the assembly of type IV pili and type II secretion system, as well as DNA translocase complex at the cytoplasmic membrane.

Due to the differences in structure of the cell envelope between Gram-positive and Gram- negative bacteria, there are some differences in the mechanisms of DNA uptake in these cells, however most of them share common features that involve related proteins. The DNA first binds to the surface of the competent cells on a DNA receptor, and passes through the cytoplasmic membrane via DNA translocase. Only single-stranded DNA may pass through, the other strand being degraded by nucleases in the process. The translocated single-stranded DNA may then be integrated into the bacterial chromosomes by a RecA-dependent process. In Gram-negative cells, due to the presence of an extra membrane, the DNA requires the presence of a channel formed by secretins on the outer membrane. Pilin may be required for competence, but its role is uncertain. The uptake of DNA is generally non-sequence specific, although in some species the presence of specific DNA uptake sequences may facilitate efficient DNA uptake.

Artificial competence

Artificial competence can be induced in laboratory procedures that involve making the cell passively permeable to DNA by exposing it to conditions that do not normally occur in nature. Typically the cells are incubated in a solution containing divalent cations (often calcium chloride) under cold conditions, before being exposed to a heat pulse (heat shock).

It has been found that growth of Gram negative bacteria in 20 mM Mg reduces the number of protein-to-lipopolysaccharide bonds by increasing the ratio of ionic to covalent bonds, which increases membrane fluidity, facilitating transformation. The role of lipopolysaccharides here are verified from the observation that shorter O-side chains are more effectively transformed — perhaps because of improved DNA accessibility.

The surface of bacteria such as *E. coli* is negatively charged due to phospholipids and lipopolysaccharides on its cell surface, and the DNA is also negatively charged. One function of the divalent cation therefore would be to shield the charges by coordinating the phosphate groups and other negative charges, thereby allowing a DNA molecule to adhere to the cell surface.

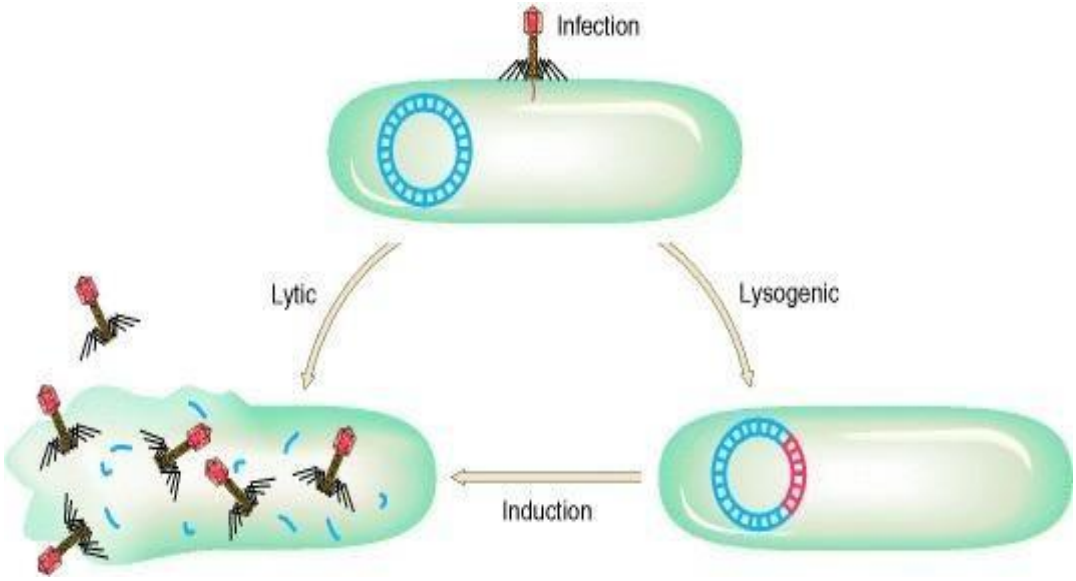
DNA entry into *E. coli* cells is through channels known as zones of adhesion or Bayer's junction, with a typical cell carrying as many as 400 such zones. Their role was established when cobalamine (which also uses these channels) was found to competitively inhibit DNA uptake. Another type of channel implicated in DNA uptake consists of poly (HB):poly P:Ca. In this poly (HB) is envisioned to wrap around DNA (itself a polyphosphate), and is carried in a shield formed by Ca ions.

It is suggested that exposing the cells to divalent cations in cold condition may also change or weaken the cell surface structure, making it more permeable to DNA. The heat-pulse is thought to create a thermal imbalance across the cell membrane, which forces the DNA to enter the cells through either cell pores or the damaged cell wall.

Electroporation is another method of promoting competence. In this method the cells are briefly shocked with an electric field of 10-20 kV/cm, which is thought to create holes in the cell membrane through which the plasmid DNA may enter. After the electric shock, the holes are rapidly closed by the cell's membrane-repair mechanisms.

Significance: Transformation occurs in nature and it can lead to increased virulence. In addition transformation is widely used in recombinant DNA technology.

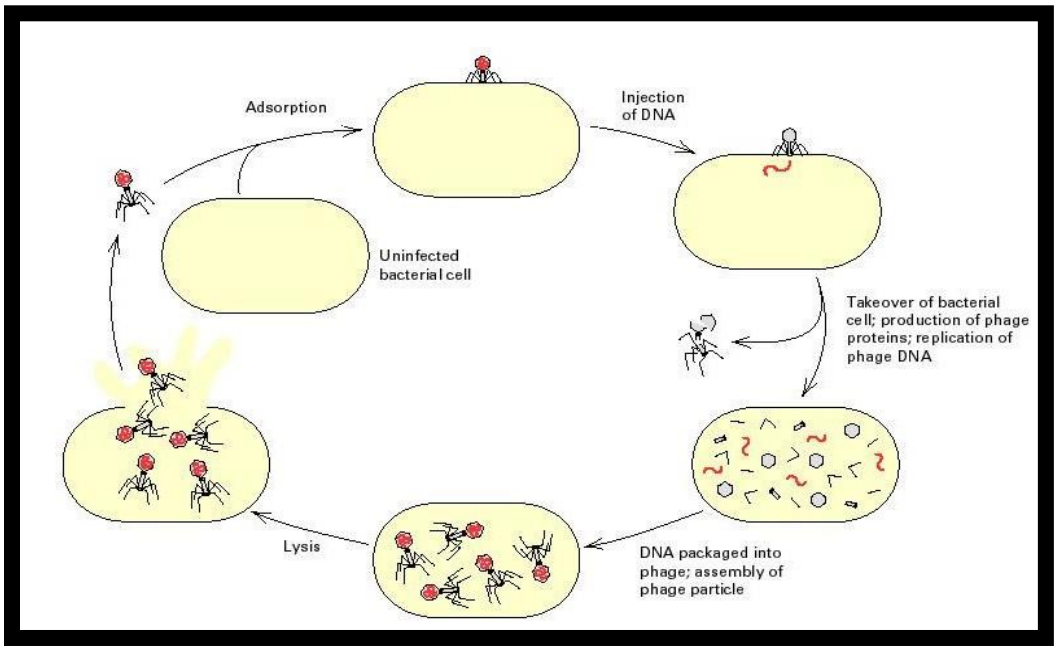
TRANSDUCTION:



- Another method of genetic transfer and recombination is transduction.
- This method involves the transfer of DNA from one bacterium to another with the use of a bacteriophage (phage).
  - A phage is a virus that infects bacteria.
  - The phage T4 and the phage lambda, for example, both infect *E. coli*.
- Because the phage reproductive system is important to understanding transduction, we will briefly review phage lifecycle.
- Phages are obligatory intracellular parasites and must invade a host cell in order to reproduce.
  - T4 multiplies by the lytic cycle which kills the host and lamba multiplies by the lysogenic cycle which does not cause the death of the host cell.
  - In lysogeny, the phage DNA remains latent in the host until it breaks out in a lytic cycle.

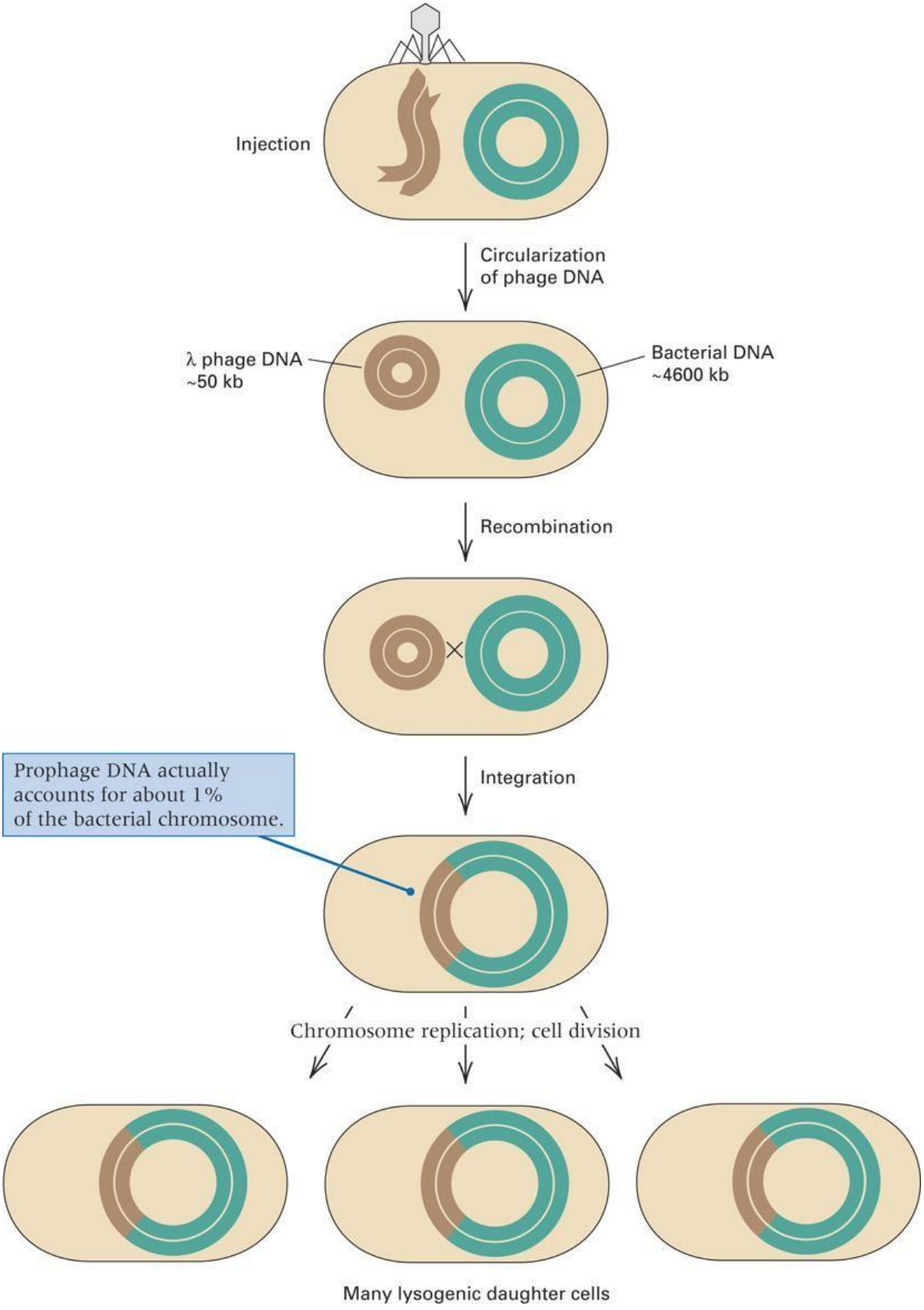
- General Steps Of The Lytic Cycle:

1. Attachment of T4 to receptors on *E. coli* cell wall.
2. Penetration of the cell wall by tail core. Inject DNA into host.
3. *E. coli* DNA is hydrolyzed. Phage DNA directs biosynthesis of viral parts using the host cell's machinery.
4. The phages mature as the parts are assembled.
5. Lyses of *E. coli* and release of the new phages.



- General Steps Of The Lysogenic Cycle:

1. Phage attaches to *E. coli* and injects DNA.
2. Phage circularizes and can enter either the lytic or the lysogenic cycle.
3. The lytic cycle would occur as previously described.
4. In the lysogenic cycle the circular phage DNA recombines with *E. coli* DNA and the phage DNA is now called prophage.
5. *E. coli* undergoes cell division, copying prophage and passing to daughter.
   - With more divisions there are more cells with the prophage.
6. The prophage may exit the chromosome and start a lytic cycle at any time.


Injection
Circularization of phage DNA
λ phage DNA ~50 kb
Bacterial DNA ~4600 kb
Recombination
Integration
Prophage DNA actually accounts for about 1% of the bacterial chromosome.
Chromosome replication; cell division
Many lysogenic daughter cells

Transduction can be generalized or specialized.

The Steps Of General Transduction:

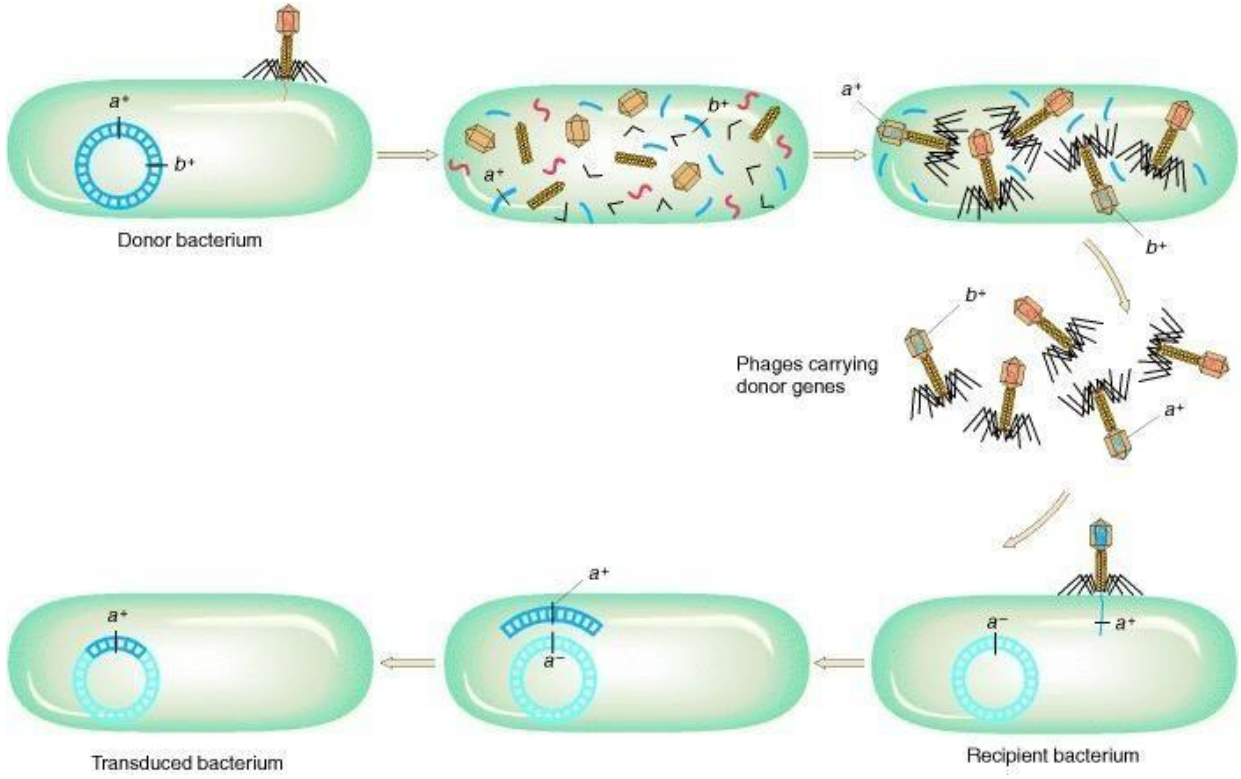
1. A phage attaches to cell wall of bacterium and injects DNA.
2. The bacterial chromosome is broken down and biosynthesis of phage DNA and protein occurs.
3. Sometimes bacterial DNA can be packaged into the virus instead of phage DNA.

- This phage is defective (can't destroy another host cell) because it does not carry its own genetic material.

4. The cell lyses, releasing viruses.
5. The phage carrying bacterial DNA infects another cell.
6. Crossing over between donor and recipient DNA can occur producing a recombinat cell.

In **generalized transduction,** any bacterial genes can be transferred bacause the host's chromosome is broken down into fragments.

- Whatever piece of bacterial DNA happens to get packaged within the phage is the genetic material



that will be transferred between cells.

Complete Transduction vs Abortive Transduction

In **complete transduction**, the exogeneote or the transduced DNA fragment gets integrated within the recipient bacterial chromosome (endogenote), forming a recombinant chromosome. This DNA fragment, replicate along with recipient bacterial chromosome replication and passed on to the daughter cells.

Abortive transduction

Transduction in which the genetic fragment from the donor bacterium is not integrated in the genome of the recipient bacterium. When the recipient bacterium divides, the genetic fragment from the donor bacterium is transmitted to only one of the daughter cells.

In **specialized (restricted) transduction,** on the other hand, only certain bacterial genes can be transferred.

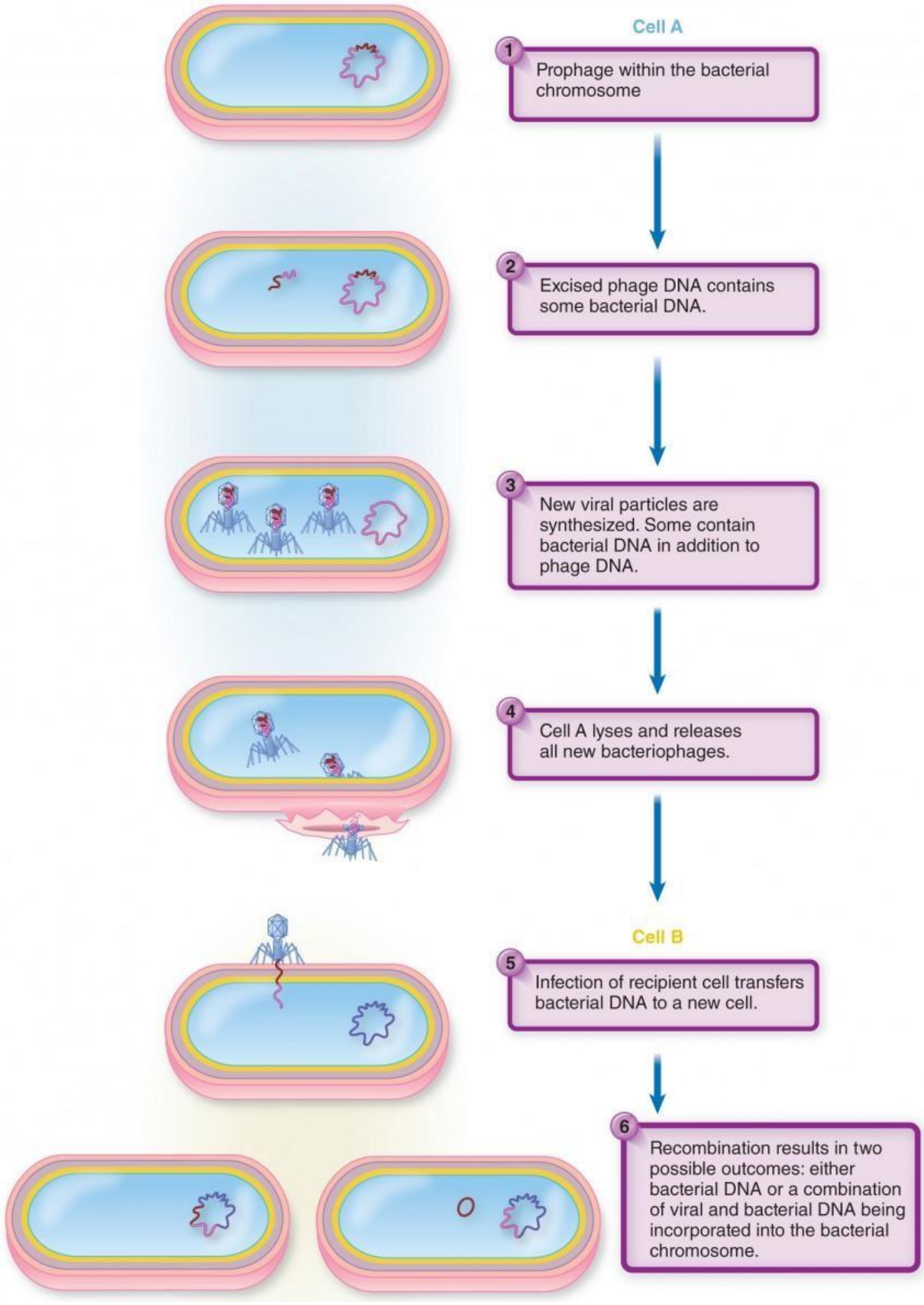
- These genes, as you will see, must exist on either side of the prophage.
- Specialized transduction requires a phage that uses the lysogenic cycle for reproduction.
- The Steps In Specialized Transduction:

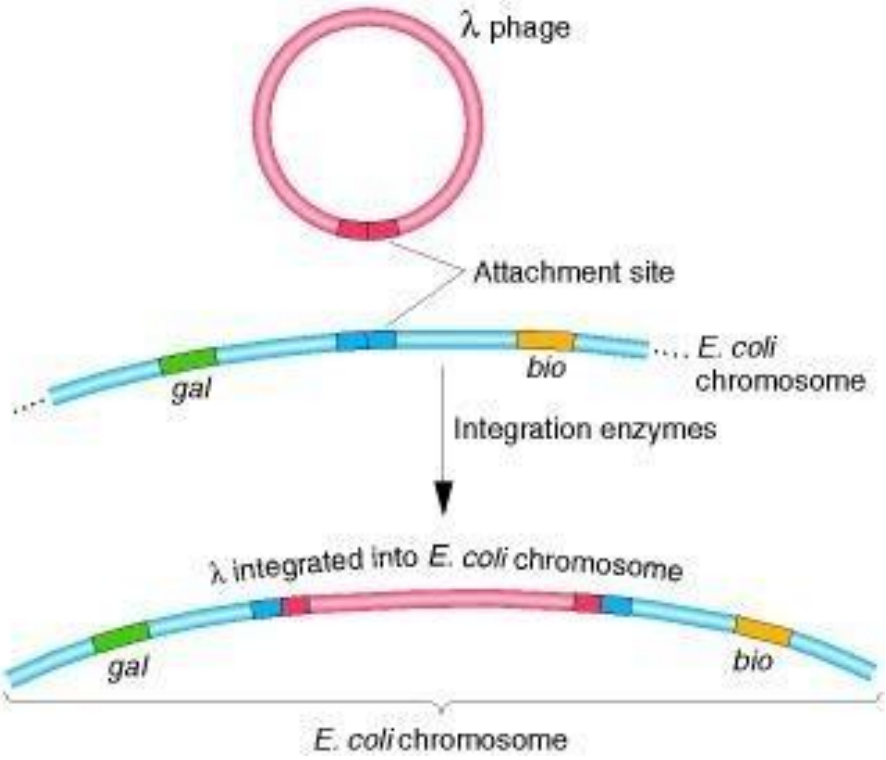
1. Remember that in the lysogenic cycle, phage DNA can exist as a prophage (integrated in the bacterial chromosome)
2. Occasionally when the prophage exits it can take adjacent bacterial genes with it.
3. The phage DNA directs synthesis of new phages.

- The phage particles carry phage DNA and bacterial DNA.

4. The cell lyses, releasing the phages.
5. A phage carrying bacterial DNA infects another cell.
6. The joined phage and bacterial DNA circularize.
7. Along with the prophage, bacterial DNA integrayes with the recipient chromosome by a cross over event.

- This forms a recombinant cell.




λ phage
Attachment site
gal
bio
E. coli chromosome
Integration enzymes
λ integrated into E. coli chromosome
gal
bio
E. coli chromosome

The recombination between regions of λ and the bacterial chromosome is catalyzed by a specific enzyme system. This system normally ensures that λ integrates at the same point in the chromosome and, when the lytic cycle is induced (for instance, by ultraviolet light), it ensures that the λ prophage excises at precisely the correct point to produce a normal circular λ chromosome. Very rarely, excision is abnormal and can result in phage particles that now carry a nearby gene and leave behind some phage genes. In λ, the nearby genes are gal on one side and bio on the other. The resulting particles are defective due to the genes left behind and are referred to as **λdgal** (λ-defective gal), or **λdbio.** These defective particles carrying nearby genes can be packaged into phage heads and can infect other bacteria. In the presence of a second, normal phage particle in a double infection, the λdgal can integrate into the chromosome at the λ- attachment site. In this manner, the gal genes in this case are transduced into the second host. Because this transduction mechanism is limited to genes very near the original integrated prophage, it is called specialized transduction.

PLASMIDS:

Plasmids are extrachromosomal elements found inside a bacterium. These are not essential for the survival of the bacterium but they confer certain extra advantages to the cell. A plasmid is a circular, self-replicating DNA molecule carrying a few, useful but non necessary genes.

Number and size: A bacterium can have no plasmids at all or have many plasmids (20-30) or multiple copies of a plasmid. Usually they are closed circular molecules; however they occur as linear molecule in *Borrelia burgdorferi*. Their size can vary from 1 Kb to 400 Kb.

Multiplication: Plasmids multiply independently of the chromosome and are inherited regularly by the daughter cells.

Plasmids are classified

I. By their ability to be transferred to other bacteria

1. Conjugative

   The sexual transfer of plasmids to another bacterium through a pilus. those plasmids possess the 25 genes required for transfert
2. Non-conjugative

Non-conjugative plasmids don’t initiate conjugaison. They can only be transferred with the help of conjugative plasmids.

3. mobilisable

   An intermediate class of plasmids are mobilisable, and carry only a subset of the genes required for transfer. These plasmids can 'parasitise' another plasmid, transferring at high frequency in the presence of a conjugative plasmid

4. incompatibility groups:

II. By function

1. Fertility-(F) plasmids,

   They are capable of conjugation (they contains the genes for the pili).

2. Resistance-(R) plasmids,

   contain gene (s) that can build resistance against one or several antibiotics or poisons.

3. Col-plasmids,

   contain genes coding for colicines, proteins that can kill other bacteria.

4. Degradative plasmids,

   able to digest unusual substances, e.g., toluene or salicylic acid.

5. Virulence plasmids,

   turn a bacterium into a pathogen.

6. addiction system.

   These plasmids produce both a long-lived poison and a short-lived antidote. Daughter cells that retain a copy of the plasmid survive, while a daughter cell that fails to inherit the plasmid dies or suffers a reduced growth-rate because of the lingering poison from the parent cell.

III. By number of plasmid in the bacterial cell

1. High copy number plasmids, also called relaxed plasmids (~1000 copies/cell)
2. Low copy number plasmids, also called stringent plasmids (~20 copies/cell).

IV. Based on conformation

Plasmid DNA may appear in one of five conformations,

1. Nicked open-circular DNA has one strand cut.
2. Relaxed circular DNA is fully intact with both strands uncut, but has been enzymatically relaxed (supercoils removed). This can be modeled by letting a twisted extension cord unwind and relax

and then plugging it into itself.

3. Linear DNA has free ends, either because both strands have been cut or because the DNA was linear in vivo. This can be modeled with an electrical extension cord that is not plugged into itself.
4. Supercoiled (or covalently closed-circular) DNA is fully intact with both strands uncut, and with an integral twist, resulting in a compact form. This can be modeled by twisting an extension cord and then plugging it into itself.
5. Supercoiled denatured DNA is like supercoiled DNA, but has unpaired regions that make it slightly less compact; this can result from excessive alkalinity during plasmid preparation.

V. Based on compatibility

1. Compatible Group: Several types of plasmids could coexist in a single cell.
2. Incompatible Group: On the other hand, related plasmids are often 'incompatible', resulting in the loss of one of them from the cell line.

Significance of plasmids:

1. Codes for resistance to several antibiotics. Gram-negative bacteria carry plasmids that give resistance to antibiotics such as neomycin, kanamycin, streptomycin, chloramphenicol, tetracycline, penicillins and sulfonamides.
2. Codes for the production of bacteriocines.
3. Codes for the production of toxins (such as Enterotoxins by *Escherichia coli, Vibrio cholerae,* exfoliative toxin by *Staphylococcus aureus* and neurotoxin of *Clostridium tetani*).
4. Codes for resistance to heavy metals (such as Hg, Ag, Cd, Pb etc.).
5. Plasmids carry virulence determinant genes. Eg, the plasmid Col V of *Escherichia coli* contains genes for iron sequestering compounds.
6. Codes resistance to uv light (DNA repair enzymes are coded in the plasmid).
7. Codes for colonization factors that is necessary for their attachment. Eg, as produced by the plasmids of *Yersinia enterocolitica*, *Shigella flexneri*, Enteroinvasive *Escherichia coli*.
8. Contains genes coding for enzymes that allow bacteria unique or unusual materials for carbon or energy sources. Some strains are used for clearing oil spillage.

Genetic Determinants Borne by Plasmids

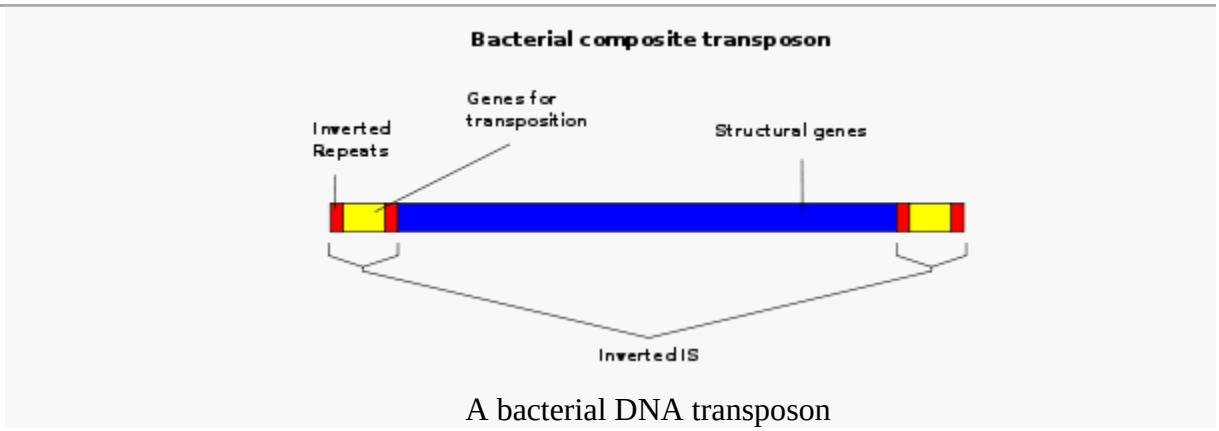
Characteristic	Plasmid examples
Fertility	F, R1, Col
Bacteriocin production	Col E1
Heavy-metal resistance	R6
Enterotoxin production	Ent
Metabolism of camphor	Cam
Tumorigenicity in plants	T1 (in *Agrobacterium tumefaciens)*

Application of plasmids:

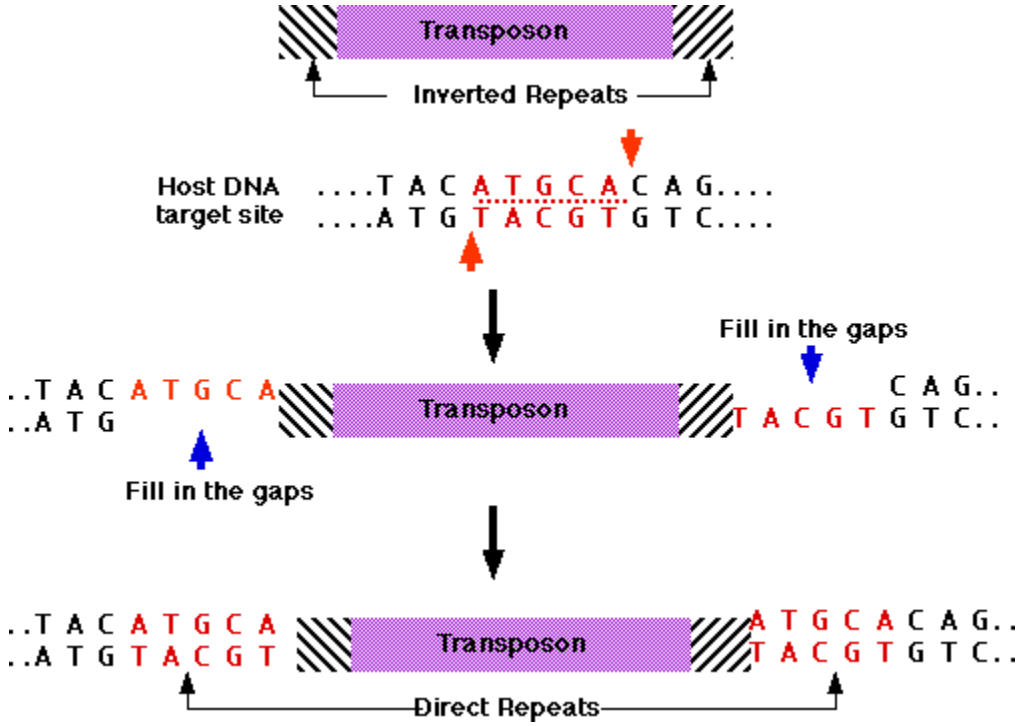
1. Used in genetic engineering as vectors.
2. Plasmid profiling is a useful genotyping method.

Episomes: Jacob and Wollman coined the term episome. Previously, it was considered synonymous with plasmids. F factors are those plasmids that can code for self transfer to other bacteria. Occasionally such plasmids get spontaneously integrated into chromosome. Plasmids with this capability are called episomes and such bacterial cells are called Hfr cells i.e. high frequency of recombination.

TRANSPOSABLE ELEMENT



A bacterial DNA transposon



A **transposable element** (**TE** or **transposon**) is a DNA sequence that can change its position within a genome, sometimes creating or reversing mutations and altering the cell's genome size. Transposition often results in duplication of the TE. Barbara McClintock's discovery of these **jumping genes** earned her a Nobel Prize in 1983.

Transposable elements make up a large fraction of the C-value of eukaryotic cells. There are at least two classes of TEs: Class I TEs generally function via reverse transcription, while Class II TEs encode the protein transposase, which they require for insertion and excision, and some of these TEs also encode other proteins. It has been shown that TEs are important in genome function and evolution. In *Oxytricha*, which has a unique genetic system, they play a critical role in development. They are also very useful to researchers as a means to alter DNA inside a living organism.

Discovery

Barbara McClintock discovered the first TEs in maize (*Zea mays*) at the Cold Spring Harbor Laboratory in New York. McClintock was experimenting with maize plants that had broken chromosomes.

In the winter of 1944–1945, McClintock planted corn kernels that were self-pollinated, meaning that the flowers were pollinated by the silk of their own plant. These kernels came from

a long line of plants that had been self-pollinated, causing broken arms on the end of their ninth chromosomes. As the maize plants began to grow, McClintock noted unusual color patterns on the leaves. For example, one leaf had two albino patches of almost identical size, located side by side on the leaf. McClintock hypothesized that during cell division certain cells lost genetic material, while others gained what they had lost. However, when comparing the chromosomes of the current generation of plants with the parent generation, she found certain parts of the chromosomes had switched positions on the chromosome. This refuted the popular genetic theory of the time that genes were fixed in their position on a chromosome. McClintock found that genes could not only move, but they could also be turned on or off due to certain environmental conditions or during different stages of cell development.

McClintock also showed that gene mutations could be reversed. She presented her report on her findings in 1951, and published an article on her discoveries in *Genetics* in November 1953 entitled "Induction of Instability at Selected Loci in Maize.

Her work would be largely dismissed and ignored until the late 1960s-1970s when it would be rediscovered after TEs were found in bacteria. She was awarded a Nobel Prize in Physiology or Medicine in 1983 for her discovery of TEs, more than thirty years after her initial research.

Approximately 90% of the maize genome is made up of TEs, as is 44% of the human genome.

Classification

Transposable elements represent one of several types of mobile genetic elements. TEs are assigned to one of two classes according to their mechanism of transposition, which can be described as either *copy and paste* (Class I TEs) or *cut and paste* (Class II TEs).

Class I (retrotransposons)

Class I TEs are copied in two stages: first, they are transcribed from DNA to RNA, and the RNA produced is then reverse transcribed to DNA. This copied DNA is then inserted back into the genome at a new position. The reverse transcription step is catalyzed by a reverse transcriptase, which is often encoded by the TE itself. The characteristics of retrotransposons are similar to retroviruses, such as HIV.

Retrotransposons are commonly grouped into three main orders:

- TEs with long terminal repeats (LTRs), which encode reverse transcriptase, similar to retroviruses

- Long interspersed nuclear elements (LINEs, LINE-1s, or L1s), which encode reverse transcriptase but lack LTRs, and are transcribed by RNA polymerase II
- Short interspersed nuclear elements do not encode reverse transcriptase and are transcribed by RNA polymerase III

Retroviruses can also be considered TEs. For example, after conversion of retroviral RNA into DNA inside a host cell, the newly produced retroviral DNA is integrated into the genome of the host cell. These integrated DNAs are termed *proviruses*. The provirus is a specialized form of eukaryotic retrotransposon, which can produce RNA intermediates that may leave the host cell and infect other cells. The transposition cycle of retroviruses has similarities to that of prokaryotic TEs, suggesting a distant relationship between the two.

Class II (DNA transposons)

The cut-and-paste transposition mechanism of class II TEs does not involve an RNA intermediate. The transpositions are catalyzed by several transposase enzymes. Some transposases non-specifically bind to any target site in DNA, whereas others bind to specific target sequences. The transposase makes a staggered cut at the target site resulting in single- strand 5' or 3' DNA overhangs, so-called "sticky ends". This step cuts out the DNA transposon, which is then ligated into a new target site; the process involves activity of a DNA polymerase that fills in gaps and of a DNA ligase that closes the sugar-phosphate backbone. This results in duplication of the target site. The insertion sites of DNA transposons may be identified by short direct repeats (created by the staggered cut in the target DNA and filling in by DNA polymerase) followed by a series of inverted repeats important for the TE excision by transposase. Cut-and-paste TEs may be duplicated if their transposition takes place during S phase of the cell cycle, when a donor site has already been replicated but a target site has not yet been replicated. Such duplications at the target site can result in gene duplication, which plays an important role in genomic evolution. Not all DNA transposons transpose through the cut-and- paste mechanism. In some cases, a replicative transposition is observed in which a transposon replicates itself to a new target site.

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DEPARTMENT OF BIOTECHNOLOGY

UNIT – V – SBC3102 – GENETICS

Human Genetics

Human chromosomes

Chromosomes in humans can be divided into two types: autosomes and sex chromosomes. Certain genetic traits are linked to a person's sex and are passed on through the sex chromosomes. The autosomes contain the rest of the genetic hereditary information. All act in the same way during cell division. Human cells have 23 pairs of chromosomes (22 pairs of autosomes and one pair of sex chromosomes), giving a total of 46 per cell. In addition to these, human cells have many hundreds of copies of the mitochondrial genome.Sequencing of the human genome has provided a great deal of information about each of the chromosomes. Below is a table compiling statistics for the chromosomes, based on the Sanger Institute's human genome information in the Vertebrate Genome Annotation (VEGA) database. Number of genes is an estimate as it is in part based on gene predictions. Total chromosome length is an estimate as well, based on the estimated size of unsequenced heterochromatin regions.

Development of Human Cytogenetics

Cytogenetics, the study of chromosomes, originated more than a century ago. However, not until the last 30 years or so have human chromosome studies become a major field in the biomedical sciences. Chromosome banding methods, for example, are today’s vital tools in clinical genetics and evolutionary studies. Furthermore, human cytogenetics coupled with molecular techniques has revolutionized the field of molecular genetics, including gene mapping and recombinant DNA technology.

Prior to 1956 the number of human chromosomes was estimated to be between 37 and 48. The difficulty of determining the exact chromosome number was due to the lack of proper techniques for cell culturing and chromosome spreading for microscope observation. In the 1950s two major breakthroughs led to rapid advances in human cytogenetics: (1) the use of phytohemagglutinin (PHA), a substance from plants, to stimulate cell division of lymphocytes (white blood cells) so that the number of cells in metaphase can be obtained, and (2) the use of hypotonic (low salt) solution to cause cells to absorb water and swell, resulting in the spreading and separating of the individual chromosomes to make them readily distinguishable. In 1956 researchers in Sweden systematically counted 46 chromosomes in human cells. Three years later, the discovery of an extra chromosome 21 in patients with Down syndrome marked the beginning of clinical application of chromosome studies. The identification of other chromosome disorders soon followed.

Conventional dyes such as aceto-orcein used for direct chromosome staining could only distinguish chromosomes according to their sizes and centromere positions. On the basis of these two criteria, human chromosomes were classified into seven groups: A through G. Accurate chromosome identification started in the late 1960s when quinocrine mustard (QM) was used to stain human chromosomes. With this technique chromosomes were differentiated under the fluorescence microscope into bright and dark regions called Q-bands. Since then, numerous banding techniques have been developed, of which G-banding is the most widely used technique for chromosome analysis. Banding techniques are extremely useful for the detection of structural changes. Clinical applications include the association of chromosome disorders with numerous diseases, including cancers.

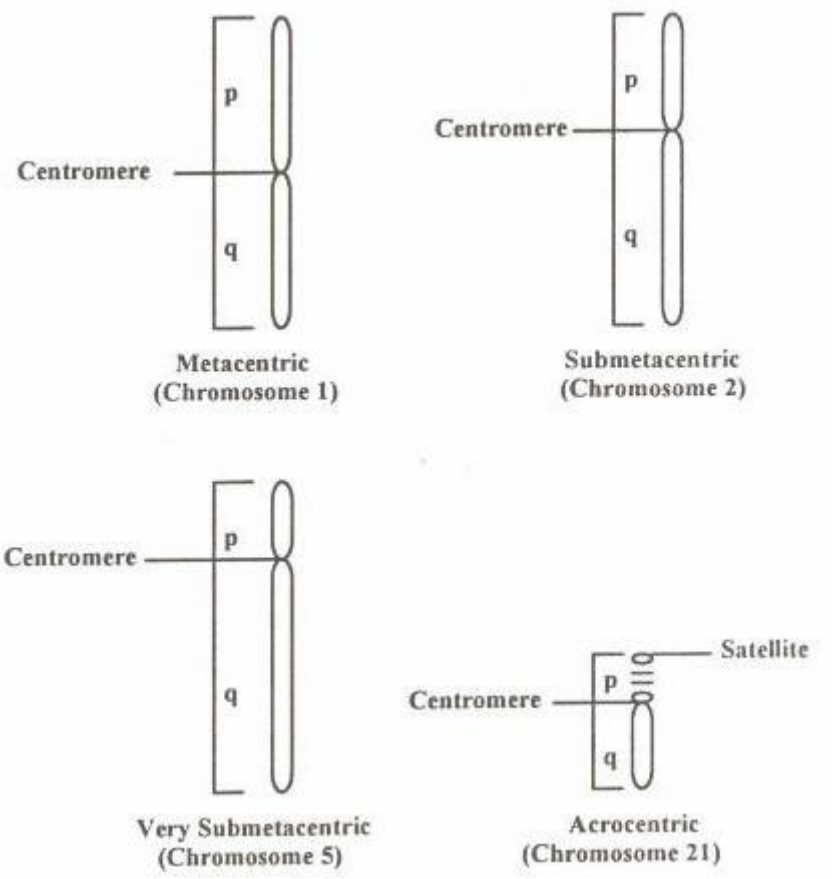
Today, chromosome analysis of cultured amniotic fluid cells is routinely performed in medical genetics laboratories for prenatal diagnosis. For structural changes too small to be detected by routine cytogenetic techniques, other approaches, such as the use of a known DNA sequence (DNA probe) to hybridize the homologous DNA of the chromosome (*in situ* hybridization), are available. The integration of cytogenetics with molecular technology has thus opened up an exciting field in modern biology. For further reading see Barch (1991) and Verma and Babu (1989).

Chromosome Structure

At metaphase the chromosomes are at their most condensed state, with spindle fibers attaching to the area of the centromere called the kinetochore, forming pole-chromosome fibers. The fibers which bypass the centromeres are called pole-pole fibers. Anaphase begins with the division of the centromere and the separation of chromatids. Once separated, each chromatid is known as a chromosome. Telophase marks the final stage of mitosis, during which the cell undergoes a reversal of prophase, including uncoiling of the chromosomes, disappearance of the spindle fibers, and reappearance of the nuclear envelope and nucleolus. At the same time the cytoplasm is divided (cytokinesis) into two parts, forming two daughter cells.

The best mitotic stage for chromosome analysis is prometaphase or metaphase. A typical metaphase chromosome consists of two arms separated by a primary constriction or centromere. Each of the two sister-chromatids contains a highly coiled double helix of DNA. Often the sister-chromatids are so close to each other that the whole chromosome appears as a single rod-like structure (see Worksheets 2 and 3). A chromosome may be characterized by its total length and the position of its centromere. A chromosome with the centromere at or near

the middle is called metacentric. A submetacentric chromosome has a centromere somewhat displaced from the middle point. If the centromere is obviously off center (e.g., halfway between the middle and the tip of the chromosome), the term very submetacentric may be used. Acrocentric chromosomes have their centromeres very near one end. Telocentric chromosomes, which are absent in human cells, have their centromeres at the very tip of one end. The short chromosome arm is designated p (petite) and the long arm q (one letter after p). Certain human chromosomes may also contain a secondary constriction, which appears as an unstained gap (also called a satellite stalk) near the tip. The chromosomal segment distal to this gap appears as a satellite. Chromosome numbers 13–15 (D group) and 21–22 (G group) have satellites .



Chromosome Number

The diploid chromosome number is the number of chromosomes in the somatic cell and is designated by the symbol 2N. The gametes, which have one-half the diploid number, have the haploid number N. In humans the diploid number is 46, with 23 inherited from each parent through the sperm or egg. Same (homologous) chromosomes form a pair with one member from each parent. Thus, there are 23 pairs of chromosomes in human cells. Of these, 22 pairs are not directly involved in sex determination, and are known as autosomes. The remaining

chromosome pair consists of the sex chromosomes, and is directly involved in sex determination. In females the two sex chromosomes are identical (XX), whereas in males the two sex chromosomes are not identical (XY). The Y chromosome is smaller than the X chromosome. A gene which is responsible for switching the embryo to male characteristics was recently discovered on the Y chromosome.

Giemsa Banding

The purpose of learning banding techniques is to uniquely identify chromosomes or portions of chromosomes, both normal and abnormal. Most laboratories in North America use a form of Giemsa banding (G-banding) as a general-purpose banding method. Giemsa reagent is a DNA stain that consists of a mixture of dyes including the basic aminophenothiazine dyes (azure A, azure B, azure C, thionin, and methylene blue) and the acidic dye eosin. Prior to staining, some form of protease (an enzyme which digests proteins) treatment is needed. In this laboratory exercise students examine G-banded chromosomes by a standard pancreatin-Giemsa procedure. This technique produces patterns of light-staining (G-light) regions and dark-staining (G-dark) regions. The pattern is unique to each chromosome, and therefore serves as a landmark for chromosome identification.

The G-light bands are thought to be relatively GC-rich (rich in the DNA bases guanine and cytosine), and the G-dark bands relatively AT-rich (rich in the DNA bases adenine and thymine). Furthermore, the light bands represent the regions which are relatively open and which contain most of the genes, including housekeeping genes (genes active in every cell type). On the other hand, the G-dark bands represent regions which are relatively compact and contain few genes. The genes in the dark regions are mainly tissue-specific.

It should be noted that the pattern and number of light and dark bands changes with the stage of mitosis, ranging from 300 bands or fewer in late metaphase to more than 1000 bands in early prophase. As mitosis progresses, bands coalesce, accounting for the reduction of number of visible bands. Most routine lab work is done at the 350–400 band level. For critical analysis, special techniques, known as high-resolution analysis, must be employed to produce 550 or more bands.

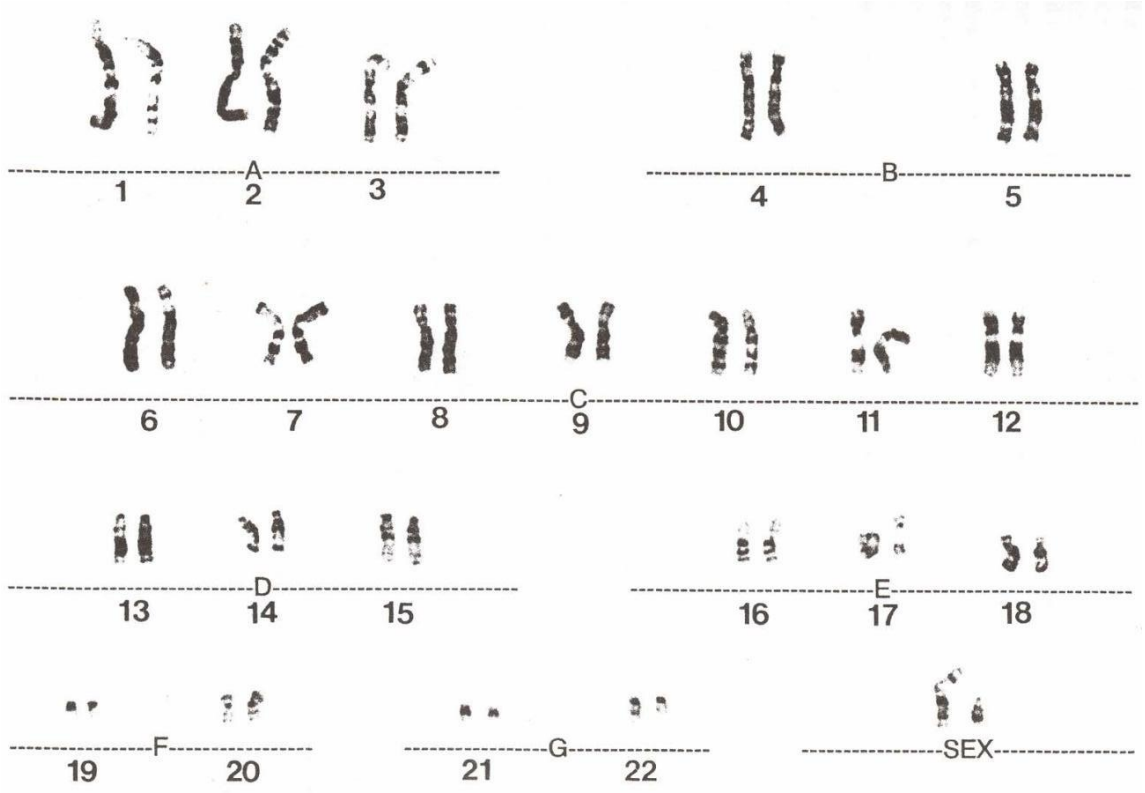
Karyotype

The term karyotype refers to a display of the chromosomes of a cell by lining them up, beginning with the largest and with the short arm oriented toward the top of the karyotyping sheet. In humans seven (A–G) groups of autosomes are recognized. Sex chromosomes (X,Y)

are placed last. A diagram of the karyotype based on chromosome measurements in many cells is called an idiogram.

Nomenclature

Human chromosome nomenclature systems were developed during six international conferences on chromosome nomenclature. To write a karyotype description, work from left to right and leave no spaces. Separate each item with a comma unless otherwise specified. The karyotype formula begins with the total number of chromosomes in the cell, followed by



the notation of the sex chromosomes (first the Xs and then the Ys). For example, the formula for a normal male is ‒46,XY‖ and for a normal female ‒46,XX‖. An extra or a missing chromosome is designated with a ‒+‖ or ‒□‖ sign, respectively, before the number of chromosome. Thus, a male with trisomy (three chromosomes) for chromosome 18 is 47,XY,+18, and a female with a monosomy (one chromosome) for 22 is 45,XX,□22. Addition or deletion of a chromosome segment is denoted with a plus ‒+‖ or minus ‒□‖ sign after the symbol of the chromosome arm, respectively. For example, the formula for a female with the cri du chat syndrome (resemblance of the cry of an affected newborn to cat mewing) is 46,XX,5p□ (missing a piece of the short arm of chromosome 5). The formula for a male with a translocation (exchange of chromosome segments) between chromosome 14 and 21 is 46,XY,t(14;21).

Description of Human Chromosomes

Chromosome 1 (Group A): The longest chromosome, metacentric; p arm fading at distal end, with at least two dark bands above centromere; q arm with a dark area just below centromere, a light band below, followed by four dark bands of which the middle two are often more intense.

Chromosome 2 (Group A): Almost as long as chromosome 1, submetacentric; p arm without light distal end, with four dark bands; q arm with more than four dark bands.

Chromosome 3 (Group A): Metacentric; both p and q arms with a large light band in the middle; dark area (consisting of two or three close dark bands) distal to the light band of q arm thicker than its counterpart in p arm.

Chromosome 4 (Group B): Very submetacentric; p arm with a thick dark band which may sometimes be resolved as double; q arm with a distinct, prominent dark band below centromere.

Chromosome 5 (Group B): Very submetacentric; p arm with a single dark band; q arm without a prominent dark band below centromere, with three close dark bands in the middle.

Chromosome 6 (Group C): Submetacentric; p arm with a distinct, large light band in the middle; q arm with several dark bands.

Chromosome 7 (Group C): Submetacentric; p arm with a prominent flat dark band at the top; q arm with two distinct dark bands followed by a much less intense dark band.

Chromosome 8 (Group C): Very submetacentric; p arm with two dark bands (sometimes unclear); q arm with two (sometimes three) dark bands, the distal dark band characteristically more intense.

Chromosome 9 (Group C): Varying from slightly submetacentric to very submetacentric; p arm with a broad (often square in shape) dark band (which may be resolved as double); q arm with a variable, proximal light area (from as long as the p arm to almost non-existent) followed by two distinct, equally intense broad dark bands with a light band between them; these three bands (two dark and one light) equal in thickness.

Chromosome 10 (Group C): Submetacentric; p arm with a dark band; q arm with three distinct dark bands, the proximal dark band most intense.

Chromosome 11 (Group C): Submetacentric; p arm with one or occasionally two dark bands; q arm with a very large light area followed by a dark band which may be resolved into two dark bands.

Chromosome 12 (Group C): Very submetacentric; p arm with a dark band; q arm with a proximal light band (which is relatively thinner than the counterpart of chromosome 11) followed by a dark area in the middle which may be resolved into two, or more often three, bands.

Chromosome 13 (Group D): Acrocentric; p arm with satellites (visible or invisible); q arm with several dark bands.

Chromosome 14 (Group D): Acrocentric; p arm with satellites (visible or invisible); q arm with a distal dark band which is more intense than other dark bands.

Chromosome 15 (Group D): Acrocentric; p arm with satellites (visible or invisible); q arm with dark proximal half and light distal half.

Chromosome 16 (Group E): Metacentric or occasionally submetacentric; p arm light or slightly dark; q arm with a prominent dark band just below centromere, followed by one or two lesser dark bands.

Chromosome 17 (Group E): Submetacentric or very submetacentric; p arm with a thin dark band (may be invisible); centromere dark followed by a distinct, large light band occupying a large part of q arm, q arm with one or two distinct dark bands near the terminal end.

Chromosome 18 (Group E): Submetacentric or very submetacentric; p arm often uniformly dark; q arm with two distinct dark bands, one proximal and one distal; the light band between these two dark bands slightly smaller than the counterpart of chromosome 17.

Chromosome 19 (Group F): Metacentric; p and q arms both light; centromere dark.

Chromosome 20 (Group F): Metacentric; p arm dark; q arm light, with two narrow, less intense dark bands.

Chromosome 21 (Group G): Acrocentric; p arm with satellites (visible or invisible); q arm with intense dark area proximally, fading out toward distal end.

Chromosome 22 (Group G): Acrocentric; p arm with satellites (visible or invisible); centromere dark; q arm with a narrow, less intense dark band.

X Chromosome (sex chromosome): Submetacentric; p arm with distinct, strong dark band at the middle; q arm with a proximal dark band about the same distance from the centromere as the p arm dark band, with two less intense dark bands near distal end.
Y Chromosome (sex chromosome): Very submetacentric; p arm very short or invisible; q arm dark throughout.

DOSAGE COMPENSATION

Dosage Compensation is the equalization of gene expression between the males and females of a species. Because sex chromosomes contain different numbers of genes, different species of organisms have developed different mechanisms to cope with this inequality. Replicating the actual *gene* is impossible; thus organisms instead equalize the*expression* from each gene. In humans, the females (XX) silence the transcription of one X chromosome of each pair, and transcribe all information from the other, expressed X chromosome. Thus, human females have the same number of expressed X-linked genes as do human males (XY), both genders having essentially one X chromosome per cell, from which to transcribe and express genes.

AUTOSOMAL ABNORMALITIES

AUTOSOME RELATED CHROMOSOMAL ABNORMALITIES

TRISOMY 21- (DOWN'S SYNDROME)

Down's Syndrome English physician John Langdon Down first characterized Down syndrome as a separate form of mental disability in 1862.

• Genetic disorder caused by the presence of all or part of a third copy of chromosome 21.

• Most common chromosomal abnormality in humans, occurring in about 1 per 1000 babies born each year.

Signs And Symptoms

Characteristics Percentage: Mental impairment 99% Abnormal teeth 60% Stunted growth 90% Slanted eyes 60% Umbilical hernia 90% Shortened hands 60% Increased

skin back of neck 80% Short neck 60% Low muscle tone 80% Obstructive sleep apnea 60% Narrow roof of mouth 76% Bent fifth finger tip 57% Flat head 75% Brushfield spots in the iris 56% Flexible ligaments 75% Single transverse palmar crease 53% Large tongue 75% Protruding tongue 47% Abnormal outer ears 70% Congenital heart disease 40% Flattened nose 68% Starbismus ~35% Separation of 1st and 2nd toes 68% Undescended testicles 20%

Brushfield spots, visible in the irises of a baby with Down syndrome.

Neurology • Have mild (IQ: 50–70) or moderate (IQ: 35– 50) intellectual disability with some cases having severe (IQ: 20–35) difficulties. • As they age, they perform less well compared to their same-age peers. • After 30 years of age may lose their ability to speak. • Causes about a third of cases of intellectual disability. • Developmental milestones are delayed with the ability to crawl typically occurring around 8 months rather than 5 months and the ability to walk independently typically occurring around 21 months rather than 14 months. • Better language understanding than ability to speak. • Between 10-45% have either a stutter or rapid irregular speech. • Behavior problems are not generally as great an issue as in other syndromes associated with intellectual disability. • Mental illness occurs in nearly 30% with autism occurring in 5–10%. • Wide range of emotions; generally happy, symptoms of depression and anxiety may develop in early adulthood.

Fertility • Males usually do not father children. • Females have lower rates of fertility relative those who are unaffected. • Menopause typically occurs at an earlier age. • As of 2006, there have been three recorded instances of males with Down syndrome fathering children and 26 cases of women having children. • Without assisted reproductive technologies, approximately half of the pregnancies of someone with Down syndrome will also have the syndrome.

Genetics • Down syndrome is caused by having three copies of the genes on chromosome 21, rather than the usual two. • Those who have one child with Down syndrome have about a 1% risk of having a second child with the syndrome, if both parents are found to have normal karyotypes.

Karyotype

- Trisomy 21 is caused by a failure of the 21st chromosome to separate during egg or sperm development. • About 88% of cases of trisomy 21 result from non separation of the

chromosomes in the mother, 8% from non-separation in the father, and 3% after the egg and sperm have merged.

Screening Guidelines recommend that screening for Down's syndrome be offered to all pregnant women, regardless of age. Screen Week of pregnancy when performed Detection rate False positive Combined test 10–13.5 wks 82–87% 5% Quad screen 15–20
wks 81% 5% Integrated test 15–20 wks 94–96% 5% Cell-free fetal DNA From 10 wks

96-100% 0.3%

Ultrasound of a fetus with down's syndrome having a large bladder.

Prognosis • Have a higher risk of early death than the general population; often from heart problems or infections. • Currently between 4 and 12% die in the first year of life. • In those without heart problems 85% survive to 10 years and 80% survive to 30 years of age. • About 10% live to 70 years of age.

TRISOMY 13 (PATAU SYNDROME)

- Some or all of the cells of the body contain extra genetic material from chromosome 13. This can occur either because each cell contains a full extra copy of chromosome 13 (a disorder known as trisomy 13 or trisomy D), or because each cell contains an extra partial copy of the chromosome (i.e., Robertsonian translocation) or because of mosaic Patau syndrome.

Trisomy 13 was first observed by Thomas Bartholin in 1657, but the chromosomal nature of the disease was ascertained by Dr. Klaus Patau in 1960.

- A partial trisomy - Part of chromosome 13 becomes attached to another chromosome in a Robertsonian translocation. • Not inherited, but occur as random events during the formation of reproductive cells. • Balanced translocation

Ultrasound (after 14 weeks) Normal baby Trisomy 13

TRISOMY 18 – (EDWARD'S SYNDROME)

Edward's Syndrome. Named after John Hilton Edwards, who first described the syndrome in 1960.

- A chromosomal disorder caused by the presence of all, or part of, an extra 18th chromosome. • Second-most common autosomal trisomy, after Down's syndrome, that carries

to term.

• One in 6,000 live births, since many of the fetuses with the syndrome die before birth. • Very low rate of survival, resulting from heart abnormalities, kidney malformations, and other internal organ disorders.

• Has a lot to do with the mother's age just like down's syndrome. • Average maternal age for conceiving a child with this disorder is 32½.

Signs and symptoms

• Kidney malformations. • Structural heart defects at birth (i.e., ventricular septal defect, atrial septal defect, patent ductus arteriosus). • Intestines protruding outside the body (omphalocele), esophagial atresia, intellectual disability, developmental delays, growth deficiency, feeding difficulties, breathing difficulties. • Arthrogryposis (a muscle disorder that causes multiple joint contractures at birth).

Physical Malformations. • Small head (microcephaly). • A prominent back portion of the head (occiput). • Low-set, malformed ears. • Abnormally small jaw (micrognathia). • Cleft lip/cleft palate, upturned nose.

• Narrow eyelid folds (palpebral fissures), widely spaced eyes (ocular hypertelorism), drooping of the upper eyelids (ptosis). • A short breast bone. • Clenched hands • Underdeveloped thumbs and or nails. • In males, undescended testicles.

Genetics • Chromosomal abnormality characterized by the presence of an extra copy of genetic material on the 18th chromosome. • Either in whole (trisomy 18). • Or in part (such as due to translocations).

Prognosis • It is impossible to predict an exact prognosis during pregnancy or the neonatal period. • Half of infants with this condition do not survive beyond the first week of life. • The median lifespan is five to 15 days. • About 8% of infants survive longer than 1 year. • One percent of children live to age 10, typically in less severe cases of the mosaic Edwards syndrome.

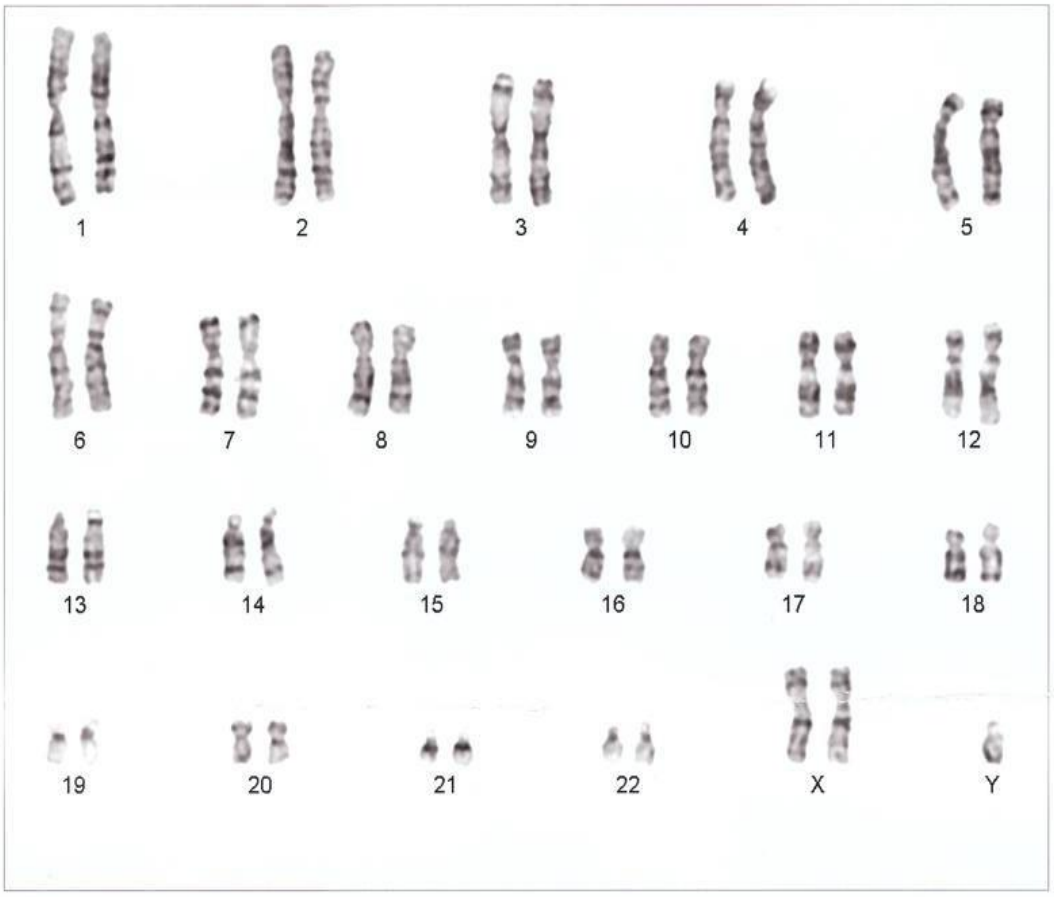
SEX CHROMOSOME DISORDERS

Sex chromosome disorders belong to a group of genetic conditions that are caused or affected by the loss or damage of sex chromosomes (gonosomes).

In humans this may refer to:

- Klinefelter's syndrome, XXY
- Turner syndrome, X
- XYY syndrome
- **Klinefelter syndrome** or **Klinefelter's syndrome** (**KS**)- **47,~~XXY~~ or ~~XXY~~**, is the set of symptoms that result from two or more X chromosomes in males. The primary feature is sterility. Often symptoms may be subtle and many people do not realize they are affected. Sometimes symptoms are more prominent and may include weaker muscles, greater height, poor coordination, less body hair, smaller genitals, breast growth, and less interest in sex. Often it is only at puberty that these symptoms are noticed. Intelligence is usually normal; however, reading difficulties and problems with speech are more common. Symptoms are typically more severe if three or more X chromosomes are present.

  Klinefelter syndrome usually occurs randomly. An older mother might increase the risk slightly. The condition is not inherited from one's parents. The underlying mechanisms involves at least one extra X chromosome in addition to a Y chromosome such that there is a



total of 47 or more chromosomes rather than usual 46. KS is diagnosed by the genetic test known as a karyotype. 47, XXY karyotype, showing an unpairedextra X

While there is no cure, a number of treatments may help. Physical therapy, speech and language therapy, counselling, and adjustments of teaching methods may be useful. Testosterone replacement may be used in those who have significantly low levels. Enlarged breasts may be removed by surgery. About half of males affected with the help of assisted reproductive technology have a chance of having children; however, this is expensive and carries risks. The condition has a nearly normal life expectancy.

Klinefelter syndrome is one of the most common chromosomal disorders, occurring in 1:500 to 1:1000 live male births. It is named after Harry Klinefelter who identified the condition in the 1940s. 1956 saw the identification of the extra X chromosome. Mice also can have the XXY syndrome, making them a useful research model.

Turner syndrome (**TS**) also known as **Ullrich–Turner syndrome**, **gonadal dysgenesis**, and **45,X**, is a condition in which a female is partly or completely missing an X chromosome. Signs and symptoms vary among those affected. Often, a short and webbed neck, low-set ears, low hairline at the back of the neck, short stature, and swollen hands and feet are seen at birth. Typically they are without menstrual periods, do not develop breasts, and are unable to have children. Heart defects, diabetes, and low thyroid hormone occur more frequently. Most people with TS have normal intelligence. Many, however, have troubles with spatial visualization such as that needed for mathematics. Vision and hearing problems occur more often.

Turner syndrome is not usually inherited from a person's parents. No environmental risks are known and the mother's age does not play a role. Turner syndrome is due to a chromosomal abnormality in which all or part of one of the X chromosomes is missing or altered. While most people have 46 chromosomes, people with TS usually have 45. The chromosomal abnormality may be present in just some cells in which case it is known as TS with mosaicism. In these cases, the symptoms are usually fewer and possibly none occur at all. Diagnosis is based on physical signs and genetic testing.

No cure for Turner syndrome is known. Treatment, however, may help with symptoms. Human growth hormone injections during childhood may increase adult height. Estrogen replacement therapy can promote development of the breasts and hips. Medical care is often required to manage other health problems with which TS is associated.

Turner syndrome occurs in between one in 2000 and one in 5000 females at birth. All regions

of the world and cultures are affected about equally. People with TS have a shorter life expectancy, mostly due to heart problems and diabetes. Henry Turner first described the condition in 1938. In 1964, it was determined to be due to a chromosomal abnormality. Of the following common symptoms of Turner syndrome, an individual may have any combination of symptoms and is unlikely to have all symptoms.

- Short stature
- Lymphedema (swelling) of the hands and feet of a newborn
- Broad chest (shield chest) and widely spaced nipples
- Low hairline
- Low-set ears
- Reproductive sterility
- Rudimentary ovaries gonadal streak (underdeveloped gonadal structures that later become fibrotic)
- Amenorrhoea, the absence of a menstrual period
- Increased weight, obesity
- Shortened metacarpal IV
- Small fingernails
- Characteristic facial features
- Webbed neck from cystic hygroma in infancy
- Aortic valve stenosis
- Coarctation of the aorta
- Bicuspid aortic valve

- Horseshoe kidney
- Visual impairments - sclera, cornea, glaucoma, etc.
- Ear infections and hearing loss
- High waist-to-hip ratio (the hips are not much bigger than the waist)
- Attention deficit hyperactivity disorder (problems with concentration, memory, attention with hyperactivity seen mostly in childhood and adolescence)
- Nonverbal learning disability (problems with math, social skills, and spatial relations)

Other features may include a small lower jaw (micrognathia), cubitus valgus, soft upturned nails, palmar crease, and drooping eyelids. Less common are pigmented moles, hearing loss, and a high-arch palate (narrow maxilla). Turner syndrome manifests itself differently in each female affected by the condition; therefore, no two individuals share the same features.

While most of the physical findings are harmless, significant medical problems can be associated with the syndrome.

GENETIC DISORDER (INHERITED DISORDER)

A **genetic disorder** is a genetic problem caused by one or more abnormalities in the genome, especially a condition that is present from birth (congenital). Most genetic disorders are quite rare and affect one person in every several thousands or millions.

Genetic disorders may or may not be heritable, i.e., passed down from the parents' genes. In non-heritable genetic disorders, defects may be caused by new mutations or changes to the DNA. In such cases, the defect will only be heritable if it occurs in the germ line. The same disease, such as some forms of cancer, may be caused by an inherited genetic condition in some people, by new mutations in other people, and mainly by environmental causes in still other people. Whether, when and to what extent a person with the genetic defect or abnormality will actually suffer from the disease is almost always affected by the environmental factors and events in the person's development.

Single-gene

A **single-gene disorder** is the result of a single mutated gene. Over 4000 human diseases are caused by single-gene defects. Single-gene disorders can be passed on to subsequent generations in several ways. Genomic imprinting and uniparental disomy, however, may affect inheritance patterns. The divisions between recessive and dominant types are not "hard and fast", although the divisions between autosomal and X-linked types are (since the latter types

are distinguished purely based on the chromosomal location of the gene). For example, achondroplasia is typically considered a dominant disorder, but children with two genes for achondroplasia have a severe skeletal disorder of which achondroplasics could be viewed as carriers. Sickle-cell anemia is also considered a recessive condition, but heterozygous carriers have increased resistance to malaria in early childhood, which could be described as a related dominant condition. When a couple where one partner or both are sufferers or carriers of a single-gene disorder wish to have a child, they can do so through *in vitro* fertilization, which means they can then have a preimplantation genetic diagnosis to check whether the embryo has the genetic disorder.

1. **Autosomal dominant**

   Only one mutated copy of the gene will be necessary for a person to be affected by an autosomal dominant disorder. Each affected person usually has one affected parent. The chance a child will inherit the mutated gene is 50%. Autosomal dominant conditions sometimes have reduced penetrance, which means although only one mutated copy is needed, not all individuals who inherit that mutation go on to develop the disease. Examples of this type of disorder are Huntington's disease, neurofibromatosis type 1, neurofibromatosis type 2, Marfan syndrome, hereditary nonpolyposis colorectal cancer, hereditary multiple exostoses (a highly penetrant autosomal dominant disorder),Tuberous sclerosis, Von Willebrand disease, and acute intermittent porphyria. Birth defects are also called congenital anomalies.

2. **Autosomal recessive**

   Two copies of the gene must be mutated for a person to be affected by an autosomal recessive disorder. An affected person usually has unaffected parents who each carry a single copy of the mutated gene (and are referred to as carriers). Two unaffected people who each carry one copy of the mutated gene have a 25% risk with each pregnancy of having a child affected by the disorder. Examples of this type of disorder are Albinism, Medium-chain acyl- CoA dehydrogenase deficiency,cystic fibrosis, sickle-cell disease, Tay-Sachs disease, Niemann-Pick disease, spinal muscular atrophy, and Roberts syndrome. Certain other phenotypes, such as wet versus dry earwax, are also determined in an autosomal recessive fashion.

3. **X-linked dominant**

   X-linked dominant disorders are caused by mutations in genes on the X chromosome. Only a few disorders have this inheritance pattern, with a prime example being X-linked hypophosphatemic rickets. Males and females are both affected in these disorders, with males

typically being more severely affected than females. Some X-linked dominant conditions, such as Rett syndrome, incontinentia pigmenti type 2, and Aicardi syndrome, are usually fatal in males either *in utero* or shortly after birth, and are therefore predominantly seen in females. Exceptions to this finding are extremely rare cases in which boys with Klinefelter syndrome (47,XXY) also inherit an X-linked dominant condition and exhibit symptoms more similar to those of a female in terms of disease severity. The chance of passing on an X- linked dominant disorder differs between men and women. The sons of a man with an X-linked dominant disorder will all be unaffected (since they receive their father's Y chromosome), and his daughters will all inherit the condition. A woman with an X-linked dominant disorder has a 50% chance of having an affected fetus with each pregnancy, although it should be noted that in cases such as incontinentia pigmenti, only female offspring are generally viable. In addition, although these conditions do not alter fertility *per se*, individuals with Rett syndrome or Aicardi syndrome rarely reproduce.

4. X-linked recessive

X-linked recessive conditions are also caused by mutations in genes on the X chromosome. Males are more frequently affected than females, and the chance of passing on the disorder differs between men and women. The sons of a man with an X-linked recessive disorder will not be affected, and his daughters will carry one copy of the mutated gene. A woman who is a carrier of an X-linked recessive disorder (X^RX^r) has a 50% chance of having sons who are affected and a 50% chance of having daughters who carry one copy of the mutated gene and are therefore carriers. X-linked recessive conditions include the serious diseases hemophilia A, Duchenne muscular dystrophy, and Lesch-Nyhan syndrome, as well as common and less serious conditions such as male pattern baldness and red-green color blindness. X-linked recessive conditions can sometimes manifest in females due to skewed X- inactivation or monosomy X (Turner syndrome).

5. Y-linked

Y-linked disorders, also called holandric disorders, are caused by mutations on the Y chromosome. These conditions display may only be transmitted from the heterogametic sex (e.g. male humans) to offspring of the same sex. More simply, this means that Y-linked disorders in humans can only be passed from men to their sons; females can never be affected because they do not possess Y-allosomes.

Y-linked disorders are exceedingly rare but the most well-known examples typically cause infertility. Reproduction in such conditions is only possible through the circumvention of

infertility by medical intervention.

6. **Mitochondrial**

   This type of inheritance, also known as maternal inheritance, applies to genes in mitochondrial DNA. Because only egg cells contribute mitochondria to the developing embryo, only mothers can pass on mitochondrial conditions to their children. An example of this type of disorder is Leber's hereditary optic neuropathy.

Many genes

Genetic disorders may also be complex, multifactorial, or polygenic, meaning they are likely associated with the effects of multiple genes in combination with lifestyles and environmental factors. Multifactorial disorders include heart disease and diabetes. Although complex disorders often cluster in families, they do not have a clear-cut pattern of inheritance. This makes it difficult to determine a person’s risk of inheriting or passing on these disorders. Complex disorders are also difficult to study and treat, because the specific factors that cause most of these disorders have not yet been identified. Studies which aim to identify the cause of complex disorders can use several methodological approaches to determine genotype-phenotype associations. One method, the genotype-first approach, starts by identifying genetic variants within patients and then determining the associated clinical manifestations. This is opposed to the more traditional phenotype-first approach, and may identify causal factors that have previously been obscured by clinical heterogeneity, penetrance, and expressivity.

On a pedigree, polygenic diseases do tend to "run in families", but the inheritance does not fit simple patterns as with Mendelian diseases. But this does not mean that the genes cannot eventually be located and studied. There is also a strong environmental component to many of them (e.g., blood pressure).

- asthma
- autoimmune diseases such as multiple sclerosis
- cancers
- ciliopathies
- cleft palate
- diabetes

- heart disease
- hypertension
- inflammatory bowel disease
- intellectual disability
- mood disorder
- obesity
- refractive error
- infertility

FEW COMMON INHERITED DISEASES

Haemophilia (also spelled hemophilia) is a group of hereditary genetic disorders that impairs the body's ability to control blood clotting, which is used to stop bleeding when a blood vessel is broken. Haemophilia A (clotting factor VIII deficiency) is the most common form of the disorder, present in about 1 in 5,000–10,000 male births. Haemophilia B (factor IX deficiency) occurs in around 1 in about 20,000–34,000 male births**.**

Like other recessive sex-linked, X chromosome disorders, haemophilia is more likely to occur in males than females. This is because females have two X chromosomes while males have only one, so the defective gene is guaranteed to manifest in any male who carries it. Because females have two X chromosomes and haemophilia is rare, the chance of a female having two defective copies of the gene is very remote, so females are almost exclusively asymptomatic carriers of the disorder. Female carriers can inherit the defective gene from either their mother or father, or it may be a new mutation. Although it is not impossible for a female to have haemophilia, it is unusual: daughters which are the product of both a male with haemophilia A or B and a female carrier will possess a 50% chance of having haemophilia, while the non-sex-linked haemophilia C due to coagulant factor XI deficiency, which can affect either sex, is more common in Jews of Ashkenazi (east European) descent but rare in other population groups.

People with haemophilia have lower clotting factor level of blood plasma or impaired activity of the coagulation factors needed for a normal clotting process. Thus when a blood vessel is injured, a temporary scab does form, but the missing coagulation factors prevent fibrin formation, which is necessary to maintain the blood clot. A haemophiliac does not bleed

more intensely than a person without it, but can bleed for a much longer time. In severe haemophiliacs even a minor injury can result in blood loss lasting days or weeks, or even never healing completely. In areas such as the brain or inside joints, this can be fatal or permanently debilitating.

Females possess two X-chromosomes, males have one X and one Y- chromosome. Since the mutations causing the disease are X-linked, a woman carrying the defect on one of her X-chromosomes may not be affected by it, as the equivalent allele on her other chromosome should express itself to produce the necessary clotting factors, due to X inactivation. However, the Y-chromosome in men has no gene for factors VIII or IX. If the genes responsible for production of factor VIII or factor IX present on a male's X- chromosome are deficient there is no equivalent on the Y-chromosome to cancel it out, so the deficient gene is not masked and he will develop the illness.

Since a male receives his single X-chromosome from his mother, the son of a healthy female silently carrying the deficient gene will have a 50% chance of inheriting that gene from her and with it the disease; and if his mother is affected with haemophilia, he will have a 100% chance of being a haemophiliac. In contrast, for a female to inherit the disease, she must receive two deficient X-chromosomes, one from her mother and the other from her father (who must therefore be a haemophiliac himself). Hence haemophilia is far more common among males than females. However, it is possible for female carriers to become mild haemophiliacs due to lyonisation (inactivation) of the X-chromosomes. Haemophiliac daughters are more common than they once were, as improved treatments for the disease have allowed more haemophiliac males to survive to adulthood and become parents. Adult females may experience menorrhagia (heavy periods) due to the bleeding tendency. The pattern of inheritance is criss-cross type. This type of pattern is also seen in colour blindness.

A mother who is a carrier has a 50% chance of passing the faulty X-chromosome to her daughter, while an affected father will always pass on the affected gene to his daughters. A son cannot inherit the defective gene from his father. This is a recessive trait and can be passed on if cases are more severe with carrier.

Genetic testing and genetic counselling is recommended for families with haemophilia. Prenatal testing, such as amniocentesis, is available to pregnant women who may be carriers of the condition.

Sickle-cell disease (**SCD**), also known as **sickle-cell anaemia** (**SCA**), is a group of genetically passed down blood disorders.[1] It results in an abnormality in the oxygen- carrying protein haemoglobin found in red blood cells. This leads to a rigid, sickle-like shape under certain circumstances. Problems in sickle cell disease typically begin around 5 to 6 months of age. A number of health problems may develop, such as attacks of pain ("sickle- cell crisis"), anemia, bacterial infections, and stroke. Long term pain may develop as people get older. The average life expectancy in the developed world is 50 years.

Sickle-cell disease occurs when a person inherits two abnormal copies of the haemoglobin gene, one from each parent. Several subtypes exist, depending on the exact mutation in each haemoglobin gene. An attack can be set off by temperature changes, stress, dehydration, and high altitude. A person with a single abnormal copy does not usually have symptoms and is said to have sickle-cell trait. Such people are also referred to as carriers. Diagnosis is by a blood test and some countries test all babies at birth for the disease. Testing is also possible during pregnancy.

The complications of sickle-cell disease can be managed to a large extent with vaccination, preventive antibiotics, high fluid intake,folic acid supplementation, and pain medication. Other measures may include blood transfusion, and the medicationhydroxycarbamide (hydroxyurea). A small proportion of people can be cured by a transplant of bone marrow cells.

As of 2013 about 3.2 million people have sickle-cell disease while an additional 43 million have sickle-cell trait. About 80% of sickle-cell disease cases are believed to occur in sub-Saharan Africa. It also occurs relatively frequently in parts of India, the Arabian peninsula, and among people of African origin living in other parts of the world. In 2013, it resulted in 176,000 deaths, up from 113,000 deaths in 1990. The condition was first described in the medical literature by the American physician James B. Herrick in 1910. In 1949 the genetic transmission was determined by E. A. Beet and J. V. Neel. In 1954 the protective effect againstmalaria of sickle-cell trait was determined.

Color blindness, or **color vision deficiency**, is the inability or decreased ability to see color, or perceive color differences, under normal lighting conditions. Color blindness affects a significant percentage of the population. There is no actual blindness but there is a deficiency of color vision. The most usual cause is a fault in the development of one or more sets of retinal cones that perceive color in light and transmit that information to the optic nerve. This type of color blindness is usually a sex-linked condition. The genes that produce

photopigments are carried on the X chromosome; if some of these genes are missing or damaged, color blindness will be expressed in males with a higher probability than in females because males only have one X chromosome, whereas females have two and a functional gene on only one of the X chromosomes is sufficient to yield the necessary photopigments.

Color blindness can also be produced by physical or chemical damage to the eye, the optic nerve, or parts of the brain. For example, people with achromatopsia suffer from a completely different disorder, but are nevertheless unable to see colors.

Color blindness is usually classified as a mild disability. There are occasional circumstances where it is an advantage: some studies conclude that color blind people are better at penetrating certain color camouflages. Such findings may give an evolutionary reason for the high prevalence of red–green color blindness. There is also a study suggesting that people with some types of color blindness can distinguish colors that people with normal color vision are not able to distinguish.

Color blindness can be inherited. It is most commonly inherited from mutations on the X chromosome but the mapping of the human genome has shown there are many causative mutations—mutations capable of causing color blindness originate from at least 19 different chromosomes and 56 different genes (as shown online at the Online Mendelian Inheritance in Man (OMIM) database at Johns Hopkins University). Two of the most common inherited forms of color blindness are protanopia and deuteranopia. One of the common color vision defects is red-green deficiency which is present in about 8 percent of males and 0.5 percent of females of Northern European ancestry.

About 8 percent of males, but only 0.5 percent of females, are color blind in some way or another, whether it is one color, a color combination, or another mutation. The reason males are at a greater risk of inheriting an X linked mutation is that males only have one X chromosome (XY, with the Y chromosome carrying altogether different genes than the X chromosome), and females have two (XX); if a woman inherits a normal X chromosome in addition to the one that carries the mutation, she will not display the mutation. Men do not have a second X chromosome to override the chromosome that carries the mutation. If 5% of variants of a given gene are defective, the probability of a single copy being defective is 5%, but the probability that two copies are both defective is $0.05 \times 0.05 = 0.0025$, or just 0.25%.

GENE THERAPY

Introduction

Gene therapy is a novel treatment method which utilizes genes or short oligonucleotide sequences as therapeutic molecules, instead of conventional drug compounds. This technique is widely used to treat those defective genes which contribute to disease development. Gene therapy involves the introduction of one or more foreign genes into an organism to treat hereditary or acquired genetic defects. In gene therapy, DNA encoding a therapeutic protein is packaged within a "vector", which transports the DNA inside cells within the body. The disease is treated with minimal toxicity, by the expression of the inserted DNA by the cell machinery. In 1990 FDA for the first time approved a gene therapy experiment on ADA-SCID in the United States after the treatment of Ashanti DeSilva. After that, approximately 1700 clinical trials on patients have been performed with various techniques and genes for numerous diseases. Many diseases such as ADA-SCID, X-linked SCID, Leber's congenital amaurosis (a retinal disease), Parkinson's disease, multiple myeloma, chronic and acute lymphocytic leukemia, adrenoleukodystrophy have reported of successful clinical trials. But these are still not approved by FDA. Some other diseases on which gene therapy-based research is going on are Haemophilia, Tyrosinemia, Hyperbilirubinemia (Criglar-Nijjar Syndrom), Cystic Fibrosis and many other cancers. After 30 years of research and clinical trials, only one product called Glybera got approval in November 2012 which may be available in market in late 2013. It has the ability to cure lipoprotein lipase deficiency (LPLD) a very rare disease.

Types of gene therapy

There are several approaches for correcting faulty genes; the most common being the insertion of a normal gene into a specific location within the genome to replace a non-functional gene. Gene therapy is classified into the following two types:

1. Somatic gene therapy
2. Germ line gene therapy

Somatic Gene Therapy

In somatic gene therapy, the somatic cells of a patient are targeted for foreign gene transfer. In this case the effects caused by the foreign gene is restricted to the individual patient only, and not inherited by the patient's offspring or later generations.

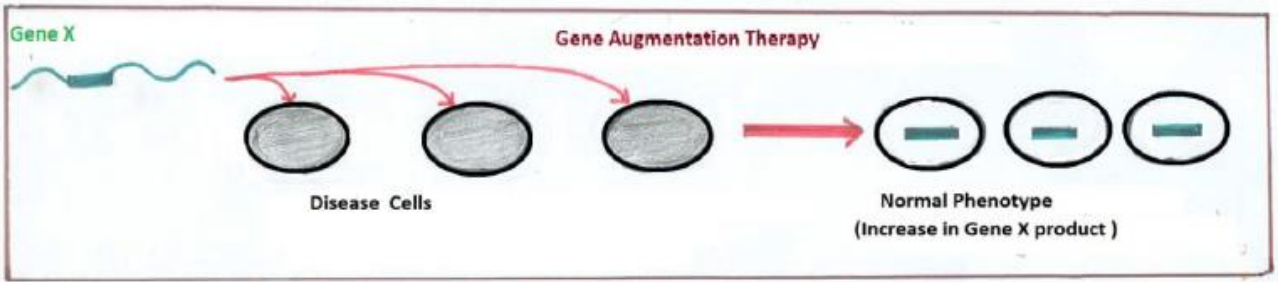
Germ Line Gene Therapy

Here, the functional genes, which are to be integrated into the genomes, are inserted in the germ cells, i.e., sperm or eggs. Targeting of germ cells makes the therapy heritable.

Gene Therapy Strategies

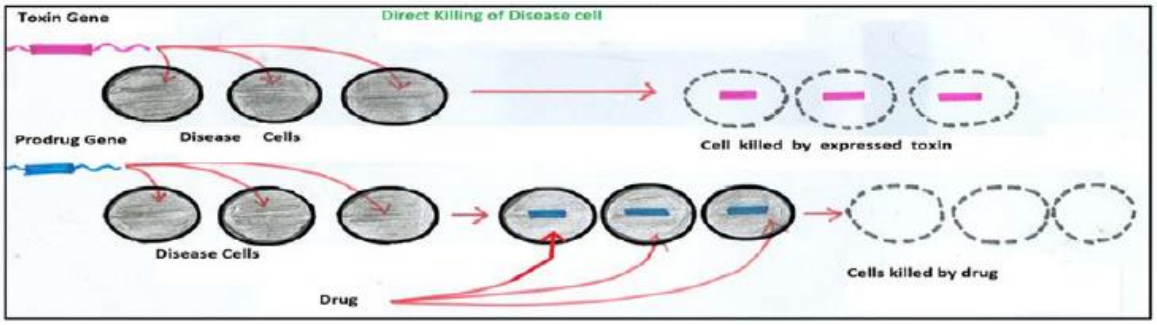
Gene Augmentation Therapy (GAT)

In GAT, simple addition of functional alleles is used to treat inherited disorders caused by genetic deficiency of a gene product, e.g. GAT has been applied to autosomal recessive disorders. Dominantly inherited disorders are much less amenable to GAT.

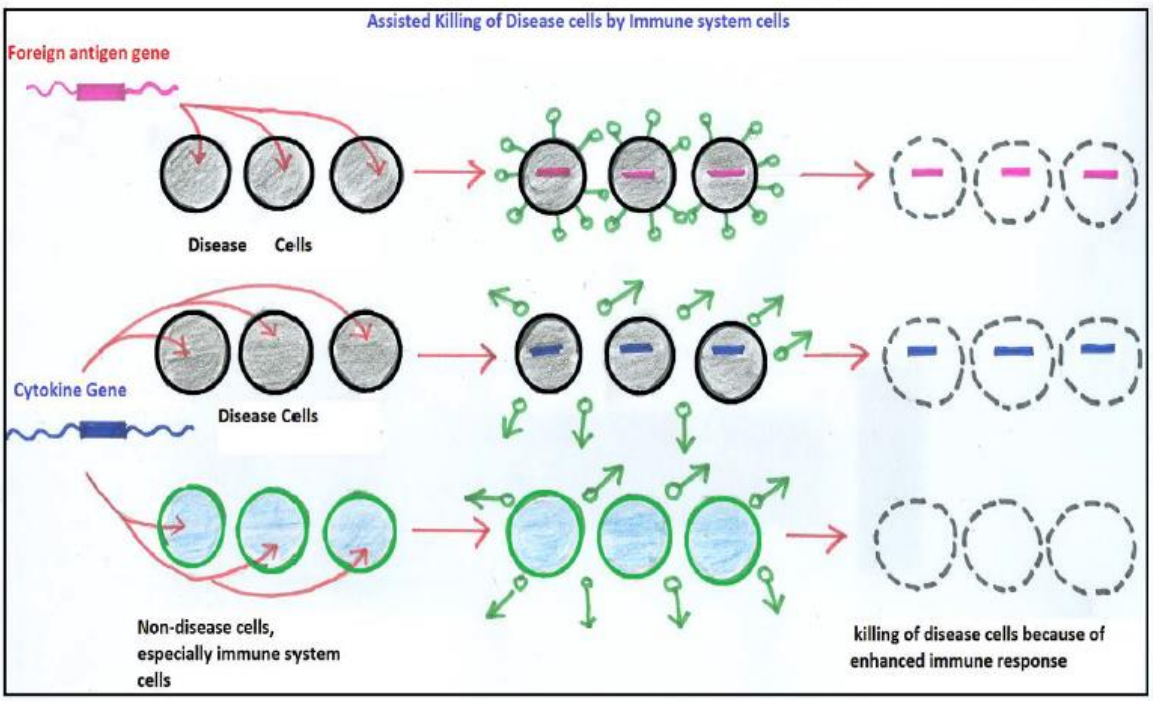


Targeted Killing of Specific Cells

It involves utilizing genes encoding toxic compounds (**suicide genes**), or **prodrugs** (reagents which confer sensitivity to subsequent treatment with a drug) to kill the transfected/ transformed cells. This general approach is popular in cancer gene therapies.

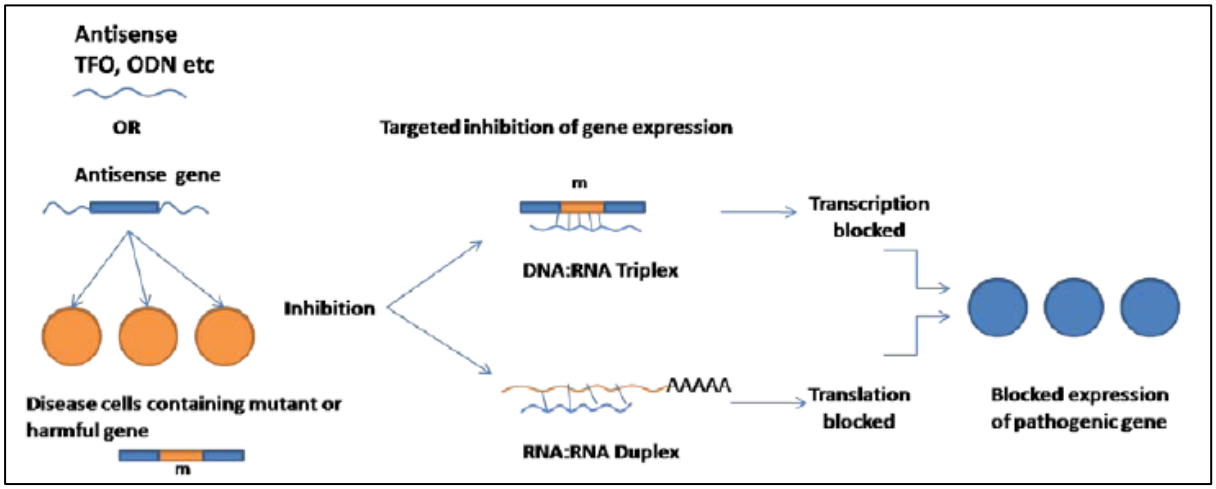


Thymidine kinase (TK) phosphorylates the introduced prodrug ganciclovir which is further phosphorylated by endokinases to form ganciclovir triphosphate, an competitive inhibitor of deoxyguanosine triphosphate. Ganciclovir triphosphate causes chain termination when incorporated into DNA.



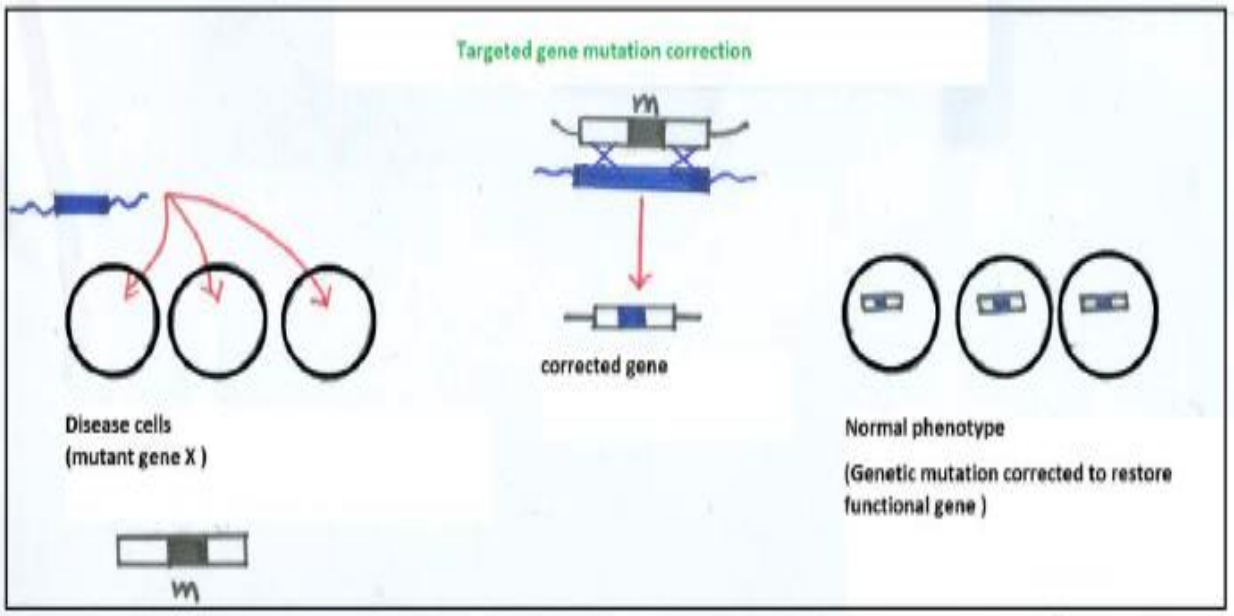
Targeted Inhibition of Gene Expression

This is to block the expression of any diseased gene or a new gene expressing a protein which is harmful for a cell. This is particularly suitable for treating infectious diseases and some cancers.



Targeted Gene Mutation Correction

It is used to correct a defective gene to restore its function which can be done at genetic level by homologous recombination or at mRNA level by using therapeutic ribozymes or therapeutic RNA editing.



Gene Therapy Approaches

Classical Gene Therapy

It involves therapeutic gene delivery and their optimum expression once inside the target cell. The foreign genes carry out following functions.

- Produce a product (protein) that the patient lacks;
- Produces toxin so that diseased cell is killed.
- Activate cells of the immune system so as to help in killing of diseased cells.

Non-classical gene therapy

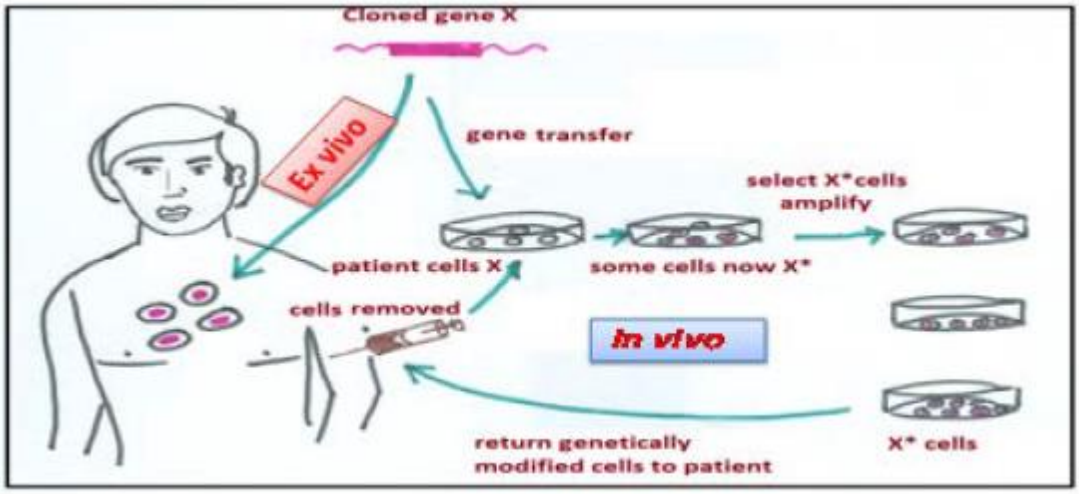
It involves the inhibition of expression of genes associated with the pathogenesis, or to correct a genetic defect and restore the normal gene expression.

Methods of gene therapy

There are mainly two approaches for the transfer of genes in gene therapy:

1**.** Transfer of genes into patient cells outside the body (*ex vivo gene therapy*)

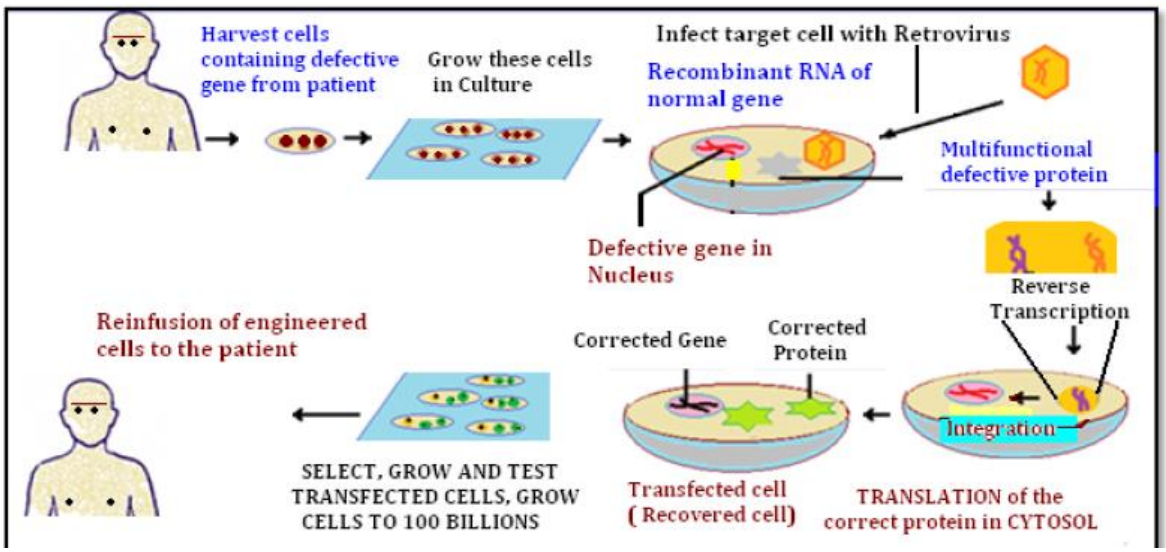
2. Transfer of genes directly to cells inside the body (*in vivo*).



***Ex vivo* gene therapy**

- In this mode of gene therapy genes are transferred to the cells grown in culture, transformed cells are selected, multiplied and then introduced into the patient.
- The use of autologous cells avoids immune system rejection of the introduced cells.
- The cells are sourced initially from the patient to be treated and grown in culture before being reintroduced into the same individual.

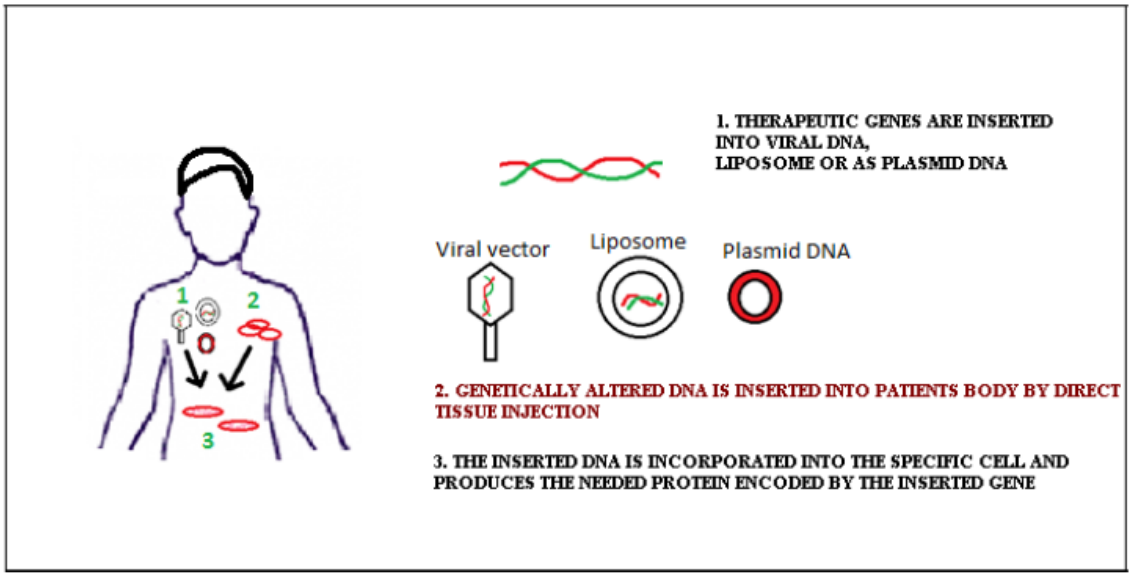
- This approach can be applied to the tissues like hematopoietic cells and skin cells which can be removed from the body, genetically corrected outside the body and reintroduced into the patient body where they become engrafted and survive for a long period of time.



***In Vivo* Gene Therapy**

- *In vivo* method of gene transfer involves the transfer of cloned genes directly into the tissues of the patient.

- This is done in case of tissues whose individual cells cannot be cultured *in vitro* in sufficient numbers (like brain cells) and/or where re-implantation of the cultured cells in the patient is not efficient.
- Liposomes and certain viral vectors are employed for this purpose because of lack of any other mode of selection.
- In case of viral vectors such type of cultured cells were often used which have been infected with the recombinant retrovirus *in vitro* to produce modified viral vectors regularly. These cultured cells will be called as vector-producing cells (VPCs)). The VPCs transfer the gene to surrounding disease cells.
- The efficiency of gene transfer and expression determines the success of this approach, because of the lack of any way for selection and amplification of cells which take up and express the foreign gene.



Differences between In Vivo and Ex Vivo gene therapy Difference Between *in vivo* and *ex vivo* Gene Delivery Systems	
In vivo	***Ex vivo***
Less invasive	More invasive
Technically simple	Technically complex
Vectors introduced directly	No vectors introduced directly
Safety check not possible	Safety check possible
Decreased control over target cells	Close control possible

Target sites for Gene Therapy

Therapeutic genes have to be delivered to specific target sites for a specific type of disease. This table describes the list of such disease and their target sites for gene therapy.

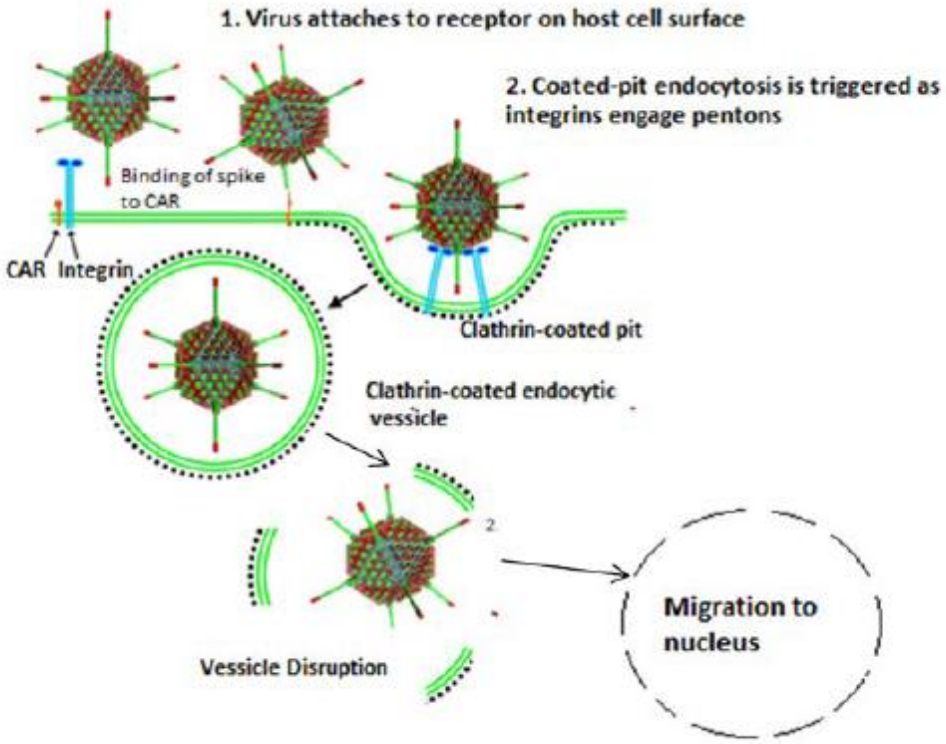
Target cells for gene transfer Target cells for gene transfer	
Disease	**Target Cells**
Cancer	Tumor cells, antigen presenting cells (APCs), blood progenitor cells, T cells, fibroblasts, muscle cells
Inherited monogenic disease	Lung epithelial cells, macrophages, T cells, blood progenitor cells, hepatocytes, muscle cells
Infectious disease	T cells, blood progenitor cells, antigen presenting cells (APCs), muscle cells
Cardiovascular disease	Endothelial cells, muscle cells
Rheumatoid arthiritis	Sinovial lining cells
Cubital tunnel Syndrome	Nerve cells

Viral vectors

Retroviruses, adenoviruses and adeno-associated viruses (AAV) some commonly used viral vectors whereas some less commonly used viral vectors are derived from the Herpes simplex virus (HSV-1), the baculovirus etc.

Adenoviral vectors

Adenoviruses are large linear double-stranded DNA viruses that are commonly used for preparing gene transfer vectors. Adenovirus vectors are known to be the second most popular gene delivery vector for gene therapy of various diseases like cystic fibrosis and certain types of cancer. Figure shows how the adenoviruses enter cells by receptor-mediated endocytosis. A primary cellular receptor binds to viral fibre then the virus interacts with secondary receptors which are responsible for its internalization. Coxsackie and Adenovirus Receptor (CAR), Heparan sulphate glycosaminoglycans, sialic acid, CD46, CD80, CD86, alpha domain of MHC I are the primary receptors which are specific for specific strains of adenovirus. Integrins are the secondary receptors which helps in the internalization of viral particles. Some adenovirus directly interacts with integrins like in the case of fibre deficient Ad2 virions.



The adenoviral DNA has inverted terminal repeats (ITRs) and a terminal protein (TP) is attached covalently to 5' termini. The adenoviral genome is classified as early and late regions based on the proteins they express. Proteins encoded by early region (E1, E2, E3, E4) genes are involved in viral DNA replication, cell cycle modulation and defense system. The late region genes (L1, L2, L3, L4, L5) encodes the viral structural proteins. Three classes of adenoviral vectors namely first, second and third generation viral vectors are developed for gene therapy purpose.

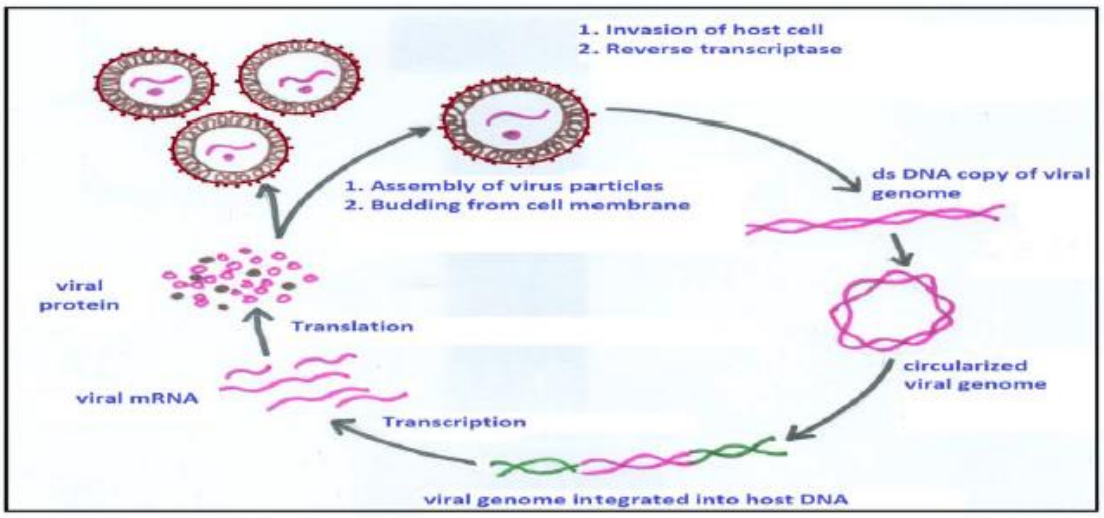
Retroviral Vectors

Retroviruses are RNA viruses which possess a reverse transcriptase activity, enabling them to synthesize a complementary DNA. Following infection (transduction), retroviruses deliver a nucleoprotein complex (pre-integration complex) into the cytoplasm of infected cells. The viral RNA genome is reverse transcribed first and then integrates into a single site of the hromosome.

• **Tumor retroviruses**, example Moloney's murine leukemia virus (MoMuLV), is widely used for the generation of recombinant vectors. these are produced at low titers as all the viral genes are deleted.

• **Oncoretroviruses:** The cells that divide shortly after infection can only be transduced by oncoretrovirus. The preinitiation complex is excluded and their entry is restricted in to the nucleus as they can only enter when nuclear membrane dissolves during cell division the target cells for this viral vector is limited

• **Recombinant lentiviruses** are being developed that are non- pathogenic to humans and have the ability to transduce stationary cells.



Some non viral methods

Direct injection/particle bombardment:

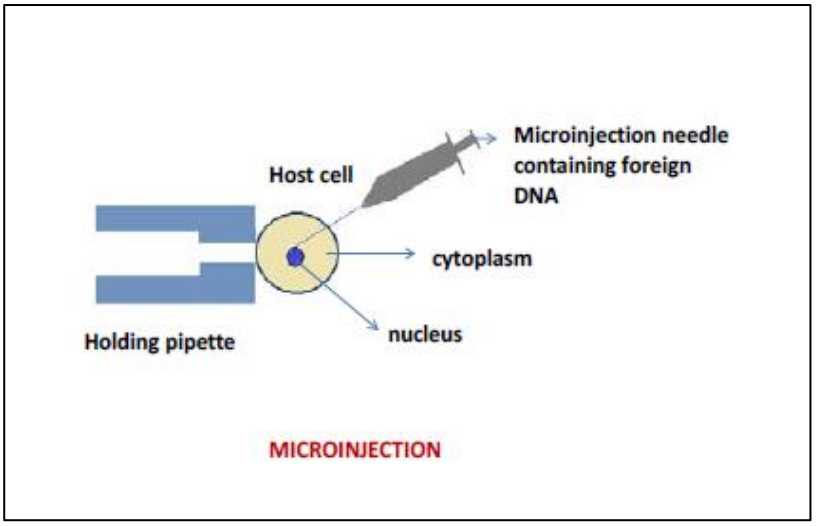
DNA can be injected parenterally which can be considered for Duchenne muscular dystrophy (DMD). An alternative approach uses particle bombardment (‘gene gun’) technique, in which DNA is coated on to metal micro particles and fired from a ballistic gun into cells/tissues. This technique is used to transfer the foreign DNA and its transient expression in mammalian cells *in vitro* and *in vivo* as well. It can cross the physical barriers like skin, muscle layer for which it is used for vaccination. Particle bombardment is used to deliver drugs, fluorescent dyes, antigenic proteins etc.

Advantage: Simple and comparatively safe.

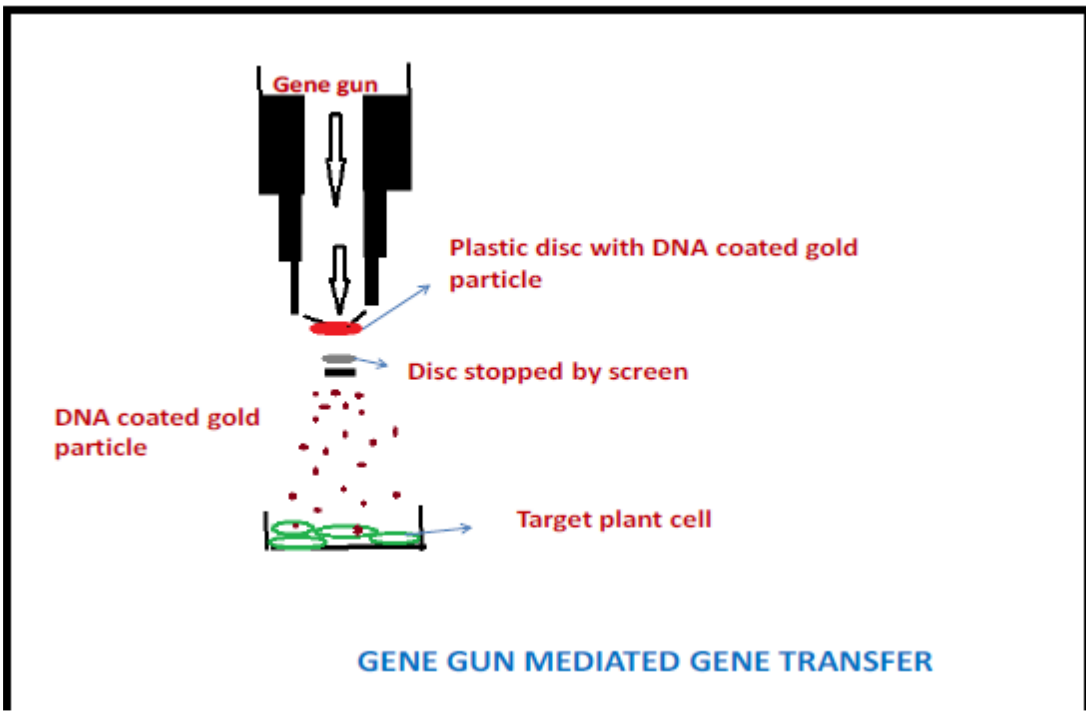
Disadvantage:

• Poor efficiency of gene transfer.

• A low level of stable integration of the injected DNA. Repeated injection may cause damage in the proliferating cells.



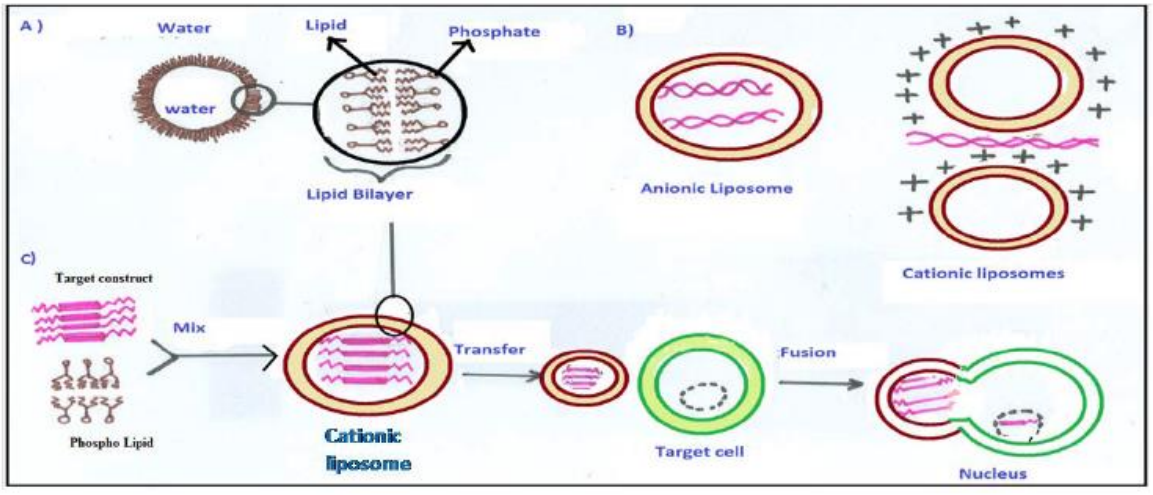
Microinjection involves the delivery of foreign DNA, by the help of glass micropipette into a living cell. The cell is held against a solid support or holding pipette and micro neeedle containing the desired DNA is inserted into the cell. The tip of the pipette used is about 0.5 to 5 micro meter diameter which resembles an injection needle. For this, glass micropipette is heated until the glass becomes somewhat liquefied and is quickly stretched to ressemble a injection needle. The delivery of foreign DNA is done under a powerful microscope (micromanupulator).



In **particle bombardment** method, the tungsten or gold particles (micro projectiles) are coated with the foreign DNA. Micro-projectile bombardment uses high-velocity metal particles to deliver biologically active DNA into the target cells. The macroprojectile is coated with the coated particles and is accelerated with air pressure and shot into plant the target tissue. A perforated plate is used, which allows the micro-projectiles to pass through to the cells on the other side of the plate and stops the macropojectile. Particle coated with the foreign gene releases the foreign gene when enters into the target cell and integrates into the chromosomal DNA. This technique is also used to transfer genes in mammalian cells. Mammalian cell lines like HEK 293, MCF7 showed gene expression when transfected with luciferase and green fluorescent genes and their gene expression was dependent on helium pressure, size and amount of gold particle and DNA load on each particle. Cell viability depends on helium pressure.

Liposomes Mediated

Liposomes are spherical vesicles which are made up of synthetic lipid bilayers which imitate the structure of biological membranes. DNA to be transferred is packaged into the liposome *in vitro* and transferred to to the targeted tissue. The lipid coating helps the DNA to survives *in vivo* and enters into the cell by endocytosis. Cationic liposomes, where the positive charge on liposomes is stabilized by binding of negatively charged DNA, are popular vehicles for gene transfer *in vivo.*



Advantage:

• The liposomes with the foreign DNA are easy to prepare.

• There is no restriction in the size of DNA that is to be transferred.

Disadvantage:

• Efficiency of gene transfer is low and transient expression of the foreign gene is obtained as they are not designed to integrate into the chromosomal DNA.

Electroporation

In electroporation, the external electric field is applied to the protoplast, which changes the electrical conductivity and the permeability of cell membrane; and thus the exogenous molecules found in the medium are taken up to either the cytoplasm (transient transfection) or into the nucleus (stable transfection).The efficiency of electroporation can be increased by giving the cell a heat shock, prior to the application of electric field or by using small quantity of PEG while doing electroporation. See lecture 3 of module 5 for detailed explanation.

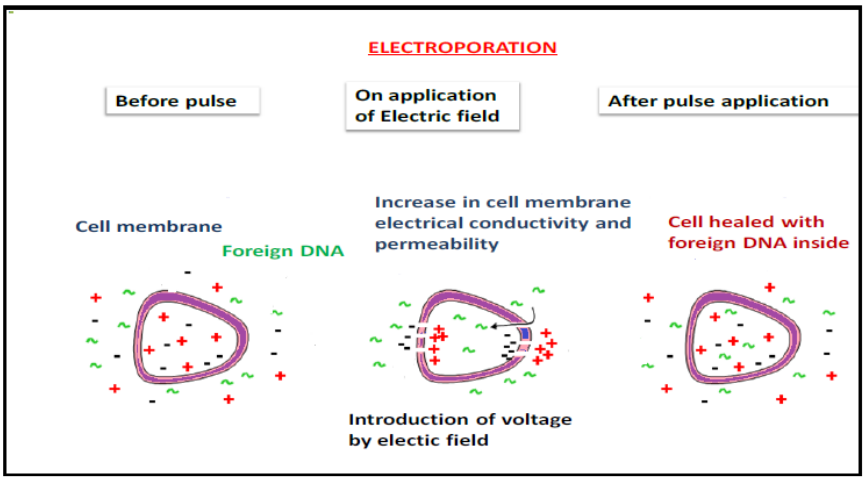
Advantage:

• By electroporation large numbers of cells can be processed at once, and thus the amount of time spent processing cells can be cut down.

Disadvantages:

• If the voltage applied is not calculated properly, the cells may damage.

• If electroporation does not occur in controlled environment, the potentially harmful substances can enter the cell or the impurities from solution may enter. This is because there is no way to control what enters the cell membrane.



Advantages of Gene Therapy

• Gene therapy can cure genetic diseases by addition of gene or by removal of gene or by replacing a mutated gene with corrected gene.

• Gene therapy can be used for cancer treatment to kill the cancerous cells.

• Gene expression can be controlled.

• Therapeutic protein is continuously produced inside the body which also reduces the cost of treatment in long term.

Gene Silencing

Gene silencing is a term of gene regulation used to describe the "switching off" of a gene by a mechanism without introducing any genetic modification. DNA is transcribed into mRNA but the mRNA is never translated into proteins. Gene regulation can be done either at the transcriptional level or at post-transcriptional level. In case of transcriptional level, it is done by inducing modification in histone protein, changing the environment for the binding of transcriptional machineries such as RNA polymerase, transcription factors, etc. However, in case of post-transcriptional level of gene regulations the transcribed mRNA by a particular gene is being blocked or destroyed. The post-transcriptional level of gene silencing is achieved by

1. Antisense Technology
2. RNA interference (RNAi)

Antisense Technology

Antisense technology talks about the production of complementary nucleic acid molecules against the mRNA molecule transcribed from the DNA in order to stop the translation into protein. These complementary molecules can be synthetically produced and delivered inside the cell to block the expression of diseased protein. It can be a short length of either RNA or DNA which commonly termed as Antisense oligonucleotides (AON). Here antisense refers the complementary nature of the synthetic molecule with respect to mRNA. When these AON inserted inside the cell it forms RNA duplex (i.e. double stranded RNA or RNA-DNA duplex). The formation of double stranded RNA inhibits gene expression at translation level as protein synthesis requires single stranded mRNA molecule as a template. This phenomenon is still not well understood but the current hypothesis about this is following-:

• blocking RNA splicing,

• accelerate the degradation process of the RNA and it also prevents the introns from splicing

• preventing the migration of mRNA from nucleolus to cytoplasm

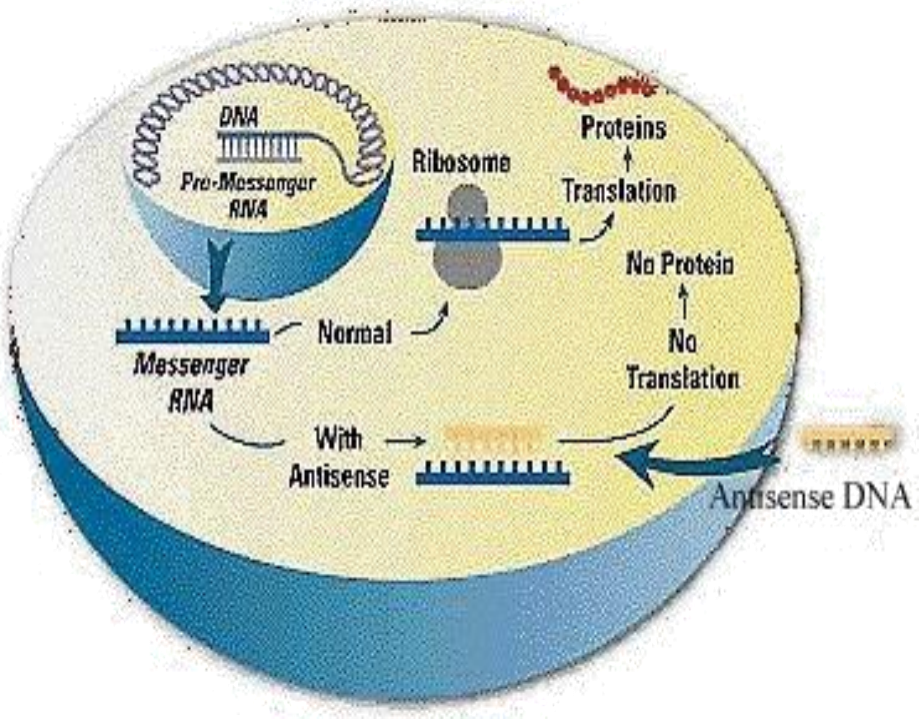
• stopping the translation of diseased protein, and

• If complementary DNA molecule is used there may be a formation of triplex in DNA template.

Mechanism of Antisense Technology

• The synthetic AON introduced inside the cell according to the gene of interest.

• If it is a DNA molecule it binds with the DNA inside the nucleus to form a triplex which inhibits the transcription and finally translation. Sometimes RNA-DNA heterodimer is also formed to stop the translation. In case of antisense RNA, it binds with mRNA to stop the translation.



Application of Antisense Technology

• In oncology antisense RNA has been used to inhibit many target proteins, such as growth factors receptors, growth factors, proteins responsible for invasion of cancerous cells and cell cycle proteins.

• If it is complementary to viral RNAs then may help in controlling various types of viral infections.

• Development of animal models for long-term normal blood pressure in hypertensive animals . • In January 2013 a drug called mipomersen (trade name Kynamro) got approval from the FDA for curing homozygous hypercholesterolemia.

• Fomivirsen, an antiviral drug developed for the treatment of cytomegalovirus retinitis is basically an antisense oligonucleotide

RNA interference

RNAi has shown its importance in the analysis of gene functions and silencing of gene for therapeutic purpose. It was first reported by Andrew Fire and his team in the year of 1997 while studying the introduction of dsRNA into *C. elegans* for silencing a gene unc-22 gene. RNA interference (RNAi) is basically a post-transcriptional phenomenon which may be triggered by providing a double-stranded RNA (dsRNA) which is known as double RNA activation. Two types of small RNA molecules – microRNA (miRNA) and small interfering RNA (siRNA) plays a central role in RNA interference-based gene silencing. RNAi looks very similar to plant posttranscriptional gene-silencing (i.e.PTGS) and quelling in case of fungi.

Functions of RNAi are as follows:

• Immunity: the immune response to viruses and other foreign genetic material

(In case of plants)

• Down regulation of genes through mi RNA (micro RNA)

• Up-regulation of genes by using both siRNA and miRNA complementary to parts of a promoter.

Micro RNA (miRNA) and Small Interfering RNA (siRNA)

Both are considered as interfering RNA. Historically miRNA was discovered in 1993 by Ambros and his co-workers whereas siRNAs concept came in 1999 from another discovery in which a dsRNA showed its role in post-transcriptional gene silencing (PTGS) in plants by David Baulcombe's group. During PTGS at one stage there is a role of ∼20 − 25 nt RNAs in silencing which was produced by the dsRNA. miRNAs is considered as regulators of cellular self-genes(i.e. endogenous genes), and siRNAs act as guards of foreign or invasive genes coming from viruses, transposons, and transgenes etc. which try to get integrated into host genome. The miRNA gene is always present in the host gnome which gets transcribed into primary-miRNA (pri-miRNA) first with the help of RNA polymerase II. This pri-miRNA is cleaved by an enzyme called Drosha which is a type of ribonuclease III enzyme. It liberates approximately 60 to 70 nt looped structure which is consider as precursor miRNA or pre-miRNA. This pre-miRNA is the transported with the help of Exportin 5 present in cytoplasm. Once the pre-miRNA is exported into cytoplasmic space another dsRNA specific enzyme called Dicer helps in duplexing with other miRNA. The unwinding of the duplexed miRNA is done by helicase. Now the both dsRNA-specific endonucleases enzymes (Drosha and Dicer) help to generate 2-nucleotide-long-3’ overhangs near the cleavage site. After unwinding of the double stranded miRNA, the generation of target specific Guide strand and the passenger stand. Now the miRNA (i.e. Guide strand) is considered as mature miRNA which is then incorporated

with the RNA-induced silencing complex (RISC). The target specific miRNA now binds with the mRNA and stops the translation. Finally, the gene is silenced with the help of miRNA and the cell undergo self-destruction pathway.

Mechanism of RNAi based Gene Silencing

A plasmid vector along with the target construct has to be delivered inside the diseased cell. This vector is able to transcribe a double stranded shRNA (short hairpin RNA). This shRNA is first processed into siRNA (small interfering RNA) and then siRNA inhibit the mRNA translation by sequence specific degradation process thereby silencing the gene. In first step of formation of siRNA the shRNA bind to a ribonuclease enzyme (similar to RNase III) and cleaved into 21 to 25 nucleotide siRNA. These siRNAs are complexed with RNA Interference Specificity Complex (RISC). RISC helps siRNA to find the mRNA complementary sequence and formation of the duplex.

The introduced plasmid has expressed a short hairpin RNA (shRNA). It requires Exportin 5 for the nuclear export.

2. Transactivating response (TAR) RNA-binding protein (TRBP) complexes with Dicer to form a dimer and then attaches to the shRNA.

3. Dicer generates 19-23 nucleotides siRNA and 2 nucleotides with 3’ overhangs in one step only from the shRNA attached to the complex

4. Argonaute 2 (Ago 2) is a RNase which belongs to AGO subfamily and binds to the 3’ overhang of siRNA in the RNA silencing complex resulting in unwinding of the dsRNA.,

5. The strand to which Ago 2 binds is called the guide strand and the other strand is known as “passenger strand”. The latter is cleaved by Ago2.

6. Now the “passenger strand” becomes free to leave the complex.

7. The integrated “guide strand” is now known as the active RISC (RNA Interference Specificity Complex). The RISC that contains various other argonautes and also few argonaute-associated proteins.

8. The siRNA sequence remained in the complex (i.e. guide strand) help the RISC to find the mRNA and bind at the correct location.

9. Now the RISC bring the mRNA to a processing bodies (i.e. P- bodies or GW-bodies). It is a cytoplasmic focus where mRNA decay factors are in high concentration which leads to the mRNA degradation.

10. The mRNA in the P-body is now cleaved by Argonaute 2 and then degraded.

Application of RNAi

1. Tool for studying gene expression and regulations

2. Medical applications: Treatment of viral infections, cardiovascular diseases, cancer and metabolic disorders.

Examples:

− Treatment of age-related macular degeneration (AMD)

− RNAi is used to block production of VEGF (Vascular endothelial growth factor).

− Treatment of Hypercholesterolemia

− To block the production of LDL particles.

Advantage of RNAi

• It is target specific

• Very small amount of small dsRNA is sufficient for silencing gene expression

• A natural method of gene regulation

Limitations of RNAi

• Less is known regarding machinery and mechanism of RNA.

• Uses of siRNA to therapeutic purposes is now more concerns about the subject safety as there may be a chance of disturbances in natural regulation of the immune system.

• Problem of-target effects is not clear at present

• Not much is known regarding Dosage requirement, stabilization & synthesis of tailored/engineered mRNA.