

COMPLEMENTATION TEST IN BACTERIOPHAGE

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COMPLEMENTATION TEST

- Term given by **Seymour Benzer**.
 - It is used to detect **two mutants** are in **same gene** or **not**.
 - Two different mutation or gene of interest occur in same chromosome/ gene or not that can be studied with the help of complementation test.
 - Its name saying that it's **complementary** to each other.
 - If genes are **complement** with each other that means **gene present in different chromosome or gene**.
 - If it is **not complement** that means **gene present in same chromosome**.
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- When the two mutations are on the same chromosome, the arrangement is called the coupling or cis configuration, and a heterozygote with this genotype is called a cis heterozygote.
 - When the two mutations are on different chromosomes, the arrangement is called the repulsion or trans configuration. An organism with this genotype is a trans heterozygote.

Apart from the gene transfer, one most important goal is to find out the gene location and the mutation if it exists. Most commonly used techniques in genetics are recombination test and complementation test, in which first one is used for finding out the distance between the genes and later one is used to find out, whether two mutations are on the same or different gene. Bacteriophage is frequently used as model to understand these mentioned tests. Benzer demonstrated the experiment for gene fine structure and location of mutations as complementation test which is also known as **cis-trans test**.

Bacteriophages or phages are viruses which infect bacteria. They are of two kinds with respect to their infective life style; virulent and temperate. The former, as the name suggests, always kill and lyse the infected cells. Examples of this class are the T-odd phages (T1, T3, etc.) and T-even phages (T2, T4, etc.). Phages are identified by what are called 'plaques'. This is called the wild type or

r⁺ morphology and mutation in this, forms r type morphology. Wild types of T4/λ phage infect *E. coli* strain B and K12, whereas mutant infects only B strain.

Benzer utilized the T4 rII mutants in two ways; the first was to carry out complementation test to see how many genes define the rII (mutation loci) function. For this, he co-infected E. coli K12 (λ) with two mutant, search of which does not develop in the host and observed whether the cultures lysed or not.

If they lysed, he examined the type of progeny phage (rII or r⁺). With some pairs of mutants, the infected cultures lysed and all the progeny phages were of the rII type, showing complementation between the mutants. With some other pairs there was no lysis and therefore, the mutants belonged to the same complementation group.

In this way, he was able to group all the rII mutants (over 2000) in two groups: A and B. All mutants in group A mapped at one locus and those in group B mapped at a locus very close. Therefore, the rII locus consists of two functional units, rIIA and rIIB, which Benzer called cistrons. The rII gene region with many individual mutations was accurately mapped using complementation test.

- If there is just **one gene**, then no two mutations will complement each other. When **two mutant phages coinfect the same cell**, no rII gene product (an enzyme or other protein) is produced by either mutant. So the plaque formation that was observed showed **no complementation**.
- If there are **two genes**, then mutations in different genes will complement each other when **two mutant phages coinfect the same cell**. Each mutant is deficient with respect to one product, but when combined they will provide all the products necessary. So the plaque formation was observed. This phenomena found is **complementation** instead of the recombination, because the progeny phages are the original mutants.

Moreover, co-infection of same mutant discovery aids many hidden phenomena of the genetic fine structure and helps in developing various techniques.

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