

# Allelic or Non-Allelic? Complementation Studies with Bacteriophage T4 rII Mutations

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## Abstract

An easy-to-perform exercise to demonstrate complementation is presented. Complementation tests are used to determine whether mutations affecting the same phenotype are within the same gene (allelic) or in different genes (non-allelic). In this exercise, mutations in the rII locus of bacteriophage T4 are studied. Host *E. coli* bacteria are co-infected with different T4 mutant phage. If two mutations are in different genes, the mutations will complement each other allowing phage to grow and produce a plaque on top agar. If two mutations are in the same gene, the mutations will not complement each other and phage will not grow to form a plaque. This miniworkshop presents the rII locus of T4 that provides a simple system for students to test complementation.

## Introduction

This complementation exercise is used in Biology 242, a sophomore level biology laboratory course for Biology Majors at Purdue University.

## Student Outline

### Objectives of this laboratory exercise

- Study the *cis-trans* test using T<sub>4</sub> rII mutants.
- Understand the concept of a gene and gene function.

### After this laboratory, you should be able to:

- Explain the experiments of Benzer to perform a *cis-trans* test.

### Materials per Pair

- 2 known T<sub>4</sub> rII mutants, 1 unknown mutant
- 2 EHA agar plates
- *E. coli* K12λ
- 2 top agar tubes
- Bunsen burner and striker or matches

### Complementation

Historically, scientists considered the gene to be the smallest unit of recombination, the smallest unit of mutation and the smallest unit of function. According to this dictum, recombination occurred between genes only. In fact, the genes were likened to beads on a string, where each bead represents a gene. The chromosomes broke between the genes and recombined as a whole unit. Likewise, a mutation affected the entire gene. Consider the beads again. You place a single pink glass bead in the middle of a pearl necklace. The glass bead would represent a mutation and shows that the entire gene has mutated.

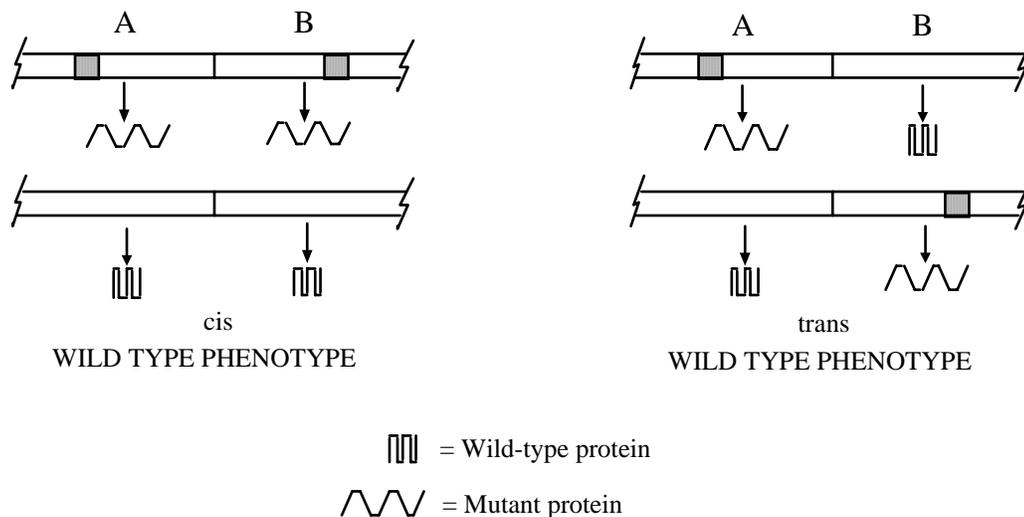
After Watson and Crick elucidated the structure of DNA scientists realized their concept of the gene had to change. Seymour Benzer was instrumental in altering the way people viewed the gene. Through his now famous experiments (which were done in the basement of Lilly Hall), he was able to demonstrate that mutations and recombination occurred within the gene. We now know the smallest unit of recombination and mutation is the nucleotide and not the gene. However, Benzer did confirm that the smallest unit of function was the gene.

After a new mutation is observed, the next task is to determine the nature of the mutation. Some questions that one might ask include 1) What function is affected? 2) Where does the mutation map? 3) Which gene has been mutated? Now, suppose two mutants occur that have the same phenotype. Then, the question that needs to be answered is whether two separate genes are responsible for the observed phenotype, or if one gene is responsible. If the mutations affect the same gene, then they are called **alleles**. When two or more genes affect the same phenotype, they are non-allelic.

These questions can be answered by performing a **complementation test**. Complementation tests are frequently used to assess whether two or more mutations affecting a particular phenotype are allelic (within the same gene) or are non-allelic (representing several different genes). These investigations provide valuable information when the mutations are in the *trans* configuration (when one mutation appears on one chromosome and the other mutation is on the other). In diploid organisms, mutations will be in *trans* when two homozygous individuals are crossed. In bacteria, a partial diploid condition can be produced by inserting DNA into these cells. (This will be discussed

in greater detail later in the course.) And in bacteriophages, partial diploids are produced by double infections.

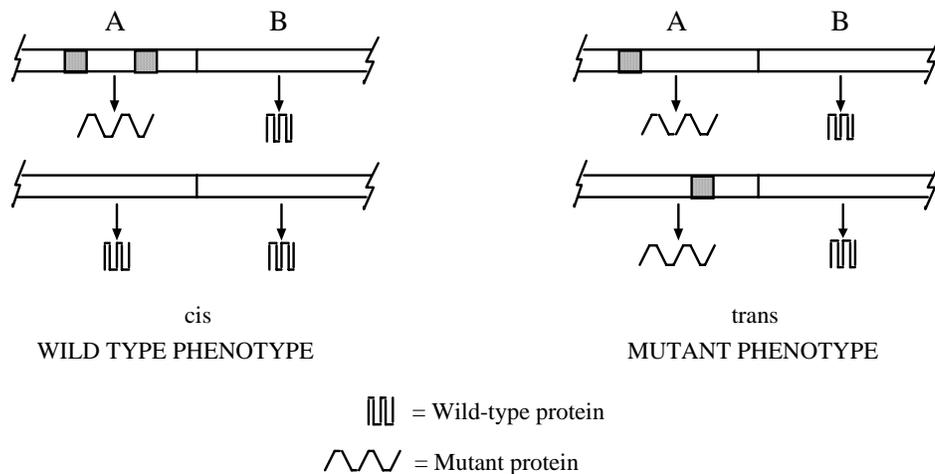
Consider when the two mutations arise in two separate genes as shown in Figure 1. Each mutation produces the same phenotype. Remember, each gene codes for a different protein which will diffuse throughout the cell once it is produced. When genes appear in the *cis* position (when the mutated genes of interest are carried on the same chromosome), the genes *A* and *B* found on chromosome 1 do not produce a functional, wild type protein. However, both *A* and *B* genes on Chromosome 2 do! Thus, the necessary proteins are found within the cells, causing the cells to exhibit a wild type phenotype. When the genes appear in the *trans* position, a good copy of gene *A* is carried on chromosome 2 and a good copy of gene *B* is carried on chromosome 1. Each good gene produces a functional protein that diffuses throughout the cell creating a wild type phenotype. In this sense, the gene on one chromosome complements or helps out the gene on its homolog. Because different genes are involved, the genes are not allelic. Recall, alleles are alternate forms of the same gene.



**Figure 1.** A diagram showing the phenotype produced when two genes are not allelic.

Now, think about the situation when the two mutations appear in the same gene (Figure 2). When the mutations are in the *cis* position and in the same gene, a wild type phenotype is expressed. Gene *A* of chromosome 1 produces an abnormal protein, however Gene *A* of chromosome 2 is intact and produces a normal protein capable of diffusing throughout the cytoplasm. But when the mutations occur in the *trans* position, a mutant phenotype is expressed. Why? Because the mutation appears in gene *A* of both chromosomes, it is impossible for a normal protein to be produced by either homolog. When two mutations occur in the same gene and produce a mutant phenotype in the *trans* position, they are said to be allelic.

The complementation or *cis-trans* test provides a useful means for determining whether two mutations affect the same gene or not. To summarize, mutations are non-allelic if a wild type phenotype results when the mutations occur in both the *cis* and *trans* position. When two mutations do not complement in the *trans* position, the genes are allelic. As alleles, the mutations are said to affect the same unit of function.



**Figure 2.** A diagram showing the phenotype produced when two genes are allelic.

As mentioned earlier, Benzer provided evidence that the gene was the smallest unit of function. He arrived at this conclusion by exploiting a characteristic of mutant T<sub>4</sub> phage. T<sub>4</sub> phage with a mutation at the rII locus will readily lyse the strain *E. coli* B but not *E. coli* K12λ. However, wild type T<sub>4</sub> will lyse both strains. Benzer produced partial diploids by doubly infecting both *E. coli* strains with two different rII mutants. He observed no cell lysis when he infected *E. coli* K12λ with each individual mutant. However, he saw cell lysis when two different mutants infected *E. coli* K12λ at the same time. How can this be explained?

Apparently, there are two subregions within the rII locus. Each region codes for a protein necessary for cell lysis. Therefore, each subregion is a unit of function, or as Benzer called it, a **cistron**. When one protein is defective, the phage are unable to lyse the cell. By doing a series of complementation tests, Benzer was able to segregate the different rII mutants to either cistron, which he called “A” and “B.” Remember, if the mutation is on the same gene and in the *trans* configuration, there will be no cell lysis.

You will perform the same experiment Benzer did and assign two unknown T<sub>4</sub> rII mutants to either the A cistron or the B cistron. A double infection of two different T<sub>4</sub> strains will be produced in a susceptible *E. coli* host. After an incubation period, you will examine the plates for plaque formation.

## Procedure

### A. Complementation in T<sub>4</sub>

#### Review the safety protocols for handling biological materials.

1. Obtain two EHA agar plates and tubes of two different known rII mutants. Obtain tester rII mutants. Label the plates with your name, tester ID and the unknown number. *Mark the bottom and lid so they can be aligned.*
2. Add one drop of *E. coli* K12λ and 0.1 ml of the known rII mutant to top agar tube. Shake the tube or flick with your finger to uniformly distribute the host bacteria throughout the

- top agar and quickly pour the top agar over the surface of the agar plate. Spread the top agar over the agar. Work quickly.
3. Allow the top agar to cool. Add an isolated drop about the size of a quarter of each tester strain which has a titer of  $10^8$  phage/ml. *Line up lid and mark each drop with the tester ID.*
  4. Allow the plate to sit for a few minutes before inverting the plate. If the plate is not allowed to dry before inverting, the drops may smear. Invert the plates and incubate them overnight at 37°C. Wipe the workbench with Rocal or alcohol after you complete your work.
  5. After incubating the plates for 6 to 8 hours (overnight incubation is all right, too), score each spot test for complementation. Complementation is indicated by complete clearing of the spotted area or by the existence of a large number of distinct plaques. Remember, plaques are clear spots, not colonies of bacterial growth. Some thinning of the indicator may occur if the rII mutants adsorb to and kill the K12 $\lambda$  cells even though they cannot produce progeny phage. Be careful not to call the thinned areas plaques. Plaques will be totally clear areas within the area where the tester strain was applied.
  6. Record your observations in your journal.
  7. Discard all plates in an orange autoclave bag.

### Report For Laboratory

1. Turn in all data for Parts A and B.
2. Discuss the experiment. Take into consideration the following thoughts.
  - a. What can you conclude about the unknown T4 mutants you were given? Which cistron do they map to? How do you know? Support your conclusion with your data.
  - b. What is the significance of Benzer's experiment determining cistron designation?

### Reference

Karam, J.D. 1993. Molecular Biology of Bacteriophage T4. American Society for Microbiology, Washington DC. ISBN 1-55581-064-0

## Notes to the Instructor

**A. Sources of bacteriophage T4 and *E. coli* B** (permissive for growth of wild type T4 and rII mutants) and *E. coli* K12 (for the complementation testing):

Carolina Biological Catalog number BA-12-4530 Genetic Recombination Culture Set \$43.10.

Or

Ward's Catalog number 85 W 0048 Genetic Recombination/complementation set \$42.75.

**B. To prepare a stock culture of each rII T4 mutant strain:**

Day 1. Inoculate an overnight culture of *E. coli* B in 50 ml Luria broth in a 250-ml flask. Incubate on a shaker at 37°C overnight. Both wild type bacteriophage T4 and rII mutants of T4 can grow in strain *E. coli* B.

- Day 2.
1. Inoculate three 250-ml flasks containing 50 ml Luria broth with 0.25 ml *E. coli* B overnight culture. Grow at 37°C for 1 to 1 1/2 hr to  $\sim 1 \times 10^8$  titer. Cells **must** be in log phase. Culture will look **slightly** turbid compared to an uninoculated flask.
  2. Add 1 M CaCl<sub>2</sub> to each flask to a final concentration of  $5 \times 10^{-3}$  M. Infect each labeled flask with a **T4 rII mutant strain** to get an MOI (multiplicity of infection) = 0.1 to 0.25. Prepare a control flask without bacteria for comparison of cell density.
  3. Incubate at 37°C in a shaking (90 oscillations/min.) water bath for 1 1/2 to 2 1/2 hours until the culture is **almost** but not quite cleared. Infection is complete at this time. Compare to control and note the difference. Add 0.5 ml of 1 M EDTA and several drops of chloroform to each flask. Mix and pour into *pre-cooled 15-mL Corex centrifuge tubes*. Keep the lysates on ice. Centrifuge at 8000 RPM at 5°C for 10 min. Save the supernates (lysates) in sterile snap cap tubes and discard the pellets (cell debris).
  4. Titer the lysate, label #1 through #8 and refrigerate.  
For the complementation tests, dilute the phage stocks about 100-fold in H broth to around  $10^8$  plaque forming units per mL.

**C. Recipes**

H broth: per liter: 10 g tryptone, 8 g NaCl,

EHA agar: per liter: 13 g typtone, 8 g NaCl, 2 g sodium citrate, 1.3 g glucose, 10 g agar

EHA top agar: per liter: 13 g tryptone, 8 g NaCl, 2 g sodium citrate, 3 g glucose, 6.5 g agar