Complementation Test

M.Sc. Microbiology, 2nd Semester
MCB 202 : Genetics and Gene regulation
Gr. A: Fundamental Genetics

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Introduction

- **G. Mendel** (1866) - One factor - one character

  Inheritance is governed by "characters" or "constant factors" that each controls a phenotypic trait such as flower colour.

- **A.E. Garrod** (1909) - one mutant gene – one metabolic block
  - *Inborn errors of Metabolism*
  - Individual genes can mutate to cause a specific metabolic block.
  - Concept later elaborated as "one gene-one enzyme".

Mutant screening is one of the beginning steps geneticists use to investigate biological processes. When geneticists obtain two independently derived mutants with similar phenotypes, an immediate question is whether or not the mutant phenotype is due to a loss of function in the same gene, or are they mutant in different genes that both affect the same phenotype (e.g., in the same pathway). That is, are they allelic mutations, or non-allelic mutations.

Allelic Complementation

- In genetics, complementation occurs when two strains of an organism with different homozygous recessive mutations that produce the same mutant phenotype produce offspring with the wild-type phenotype when mated or crossed.

- Complementation will occur only if the mutations are in different genes. In this case, each strain's genome supplies the wild-type allele to "complement" the mutated allele of the other strain's genome.

- Since the mutations are recessive, the offspring will display the wild-type phenotype. A complementation test (sometimes called a "cis-trans" test) can be used to test whether the mutations in two strains are in different genes. Complementation will not occur if the mutations are in the same gene. The convenience and essence of this test is that the mutations that produce a phenotype can be assigned to different genes without the exact knowledge of what the gene product is doing on a molecular level.

Complementation test

- A complementation test (sometimes called a "cis-trans" test) can be used to test whether the mutations in two strains are in different genes.
- Complementation will not occur if the mutations are in the same gene.
- The convenience and essence of this test is that the mutations that produce a phenotype can be assigned to different genes without the exact knowledge of what the gene product is doing on a molecular level.
- The complementation test was developed by American geneticist Edward B. Lewis.

Edward B. Lewis

Example of complementation test

Drosophila melanogaster (fruit fly)

Wild type flies = red eyes

Eye color is related to two genes: A and B.

Each one of the genes has 2 alleles:

dominant one that codes for a working protein (A and B)

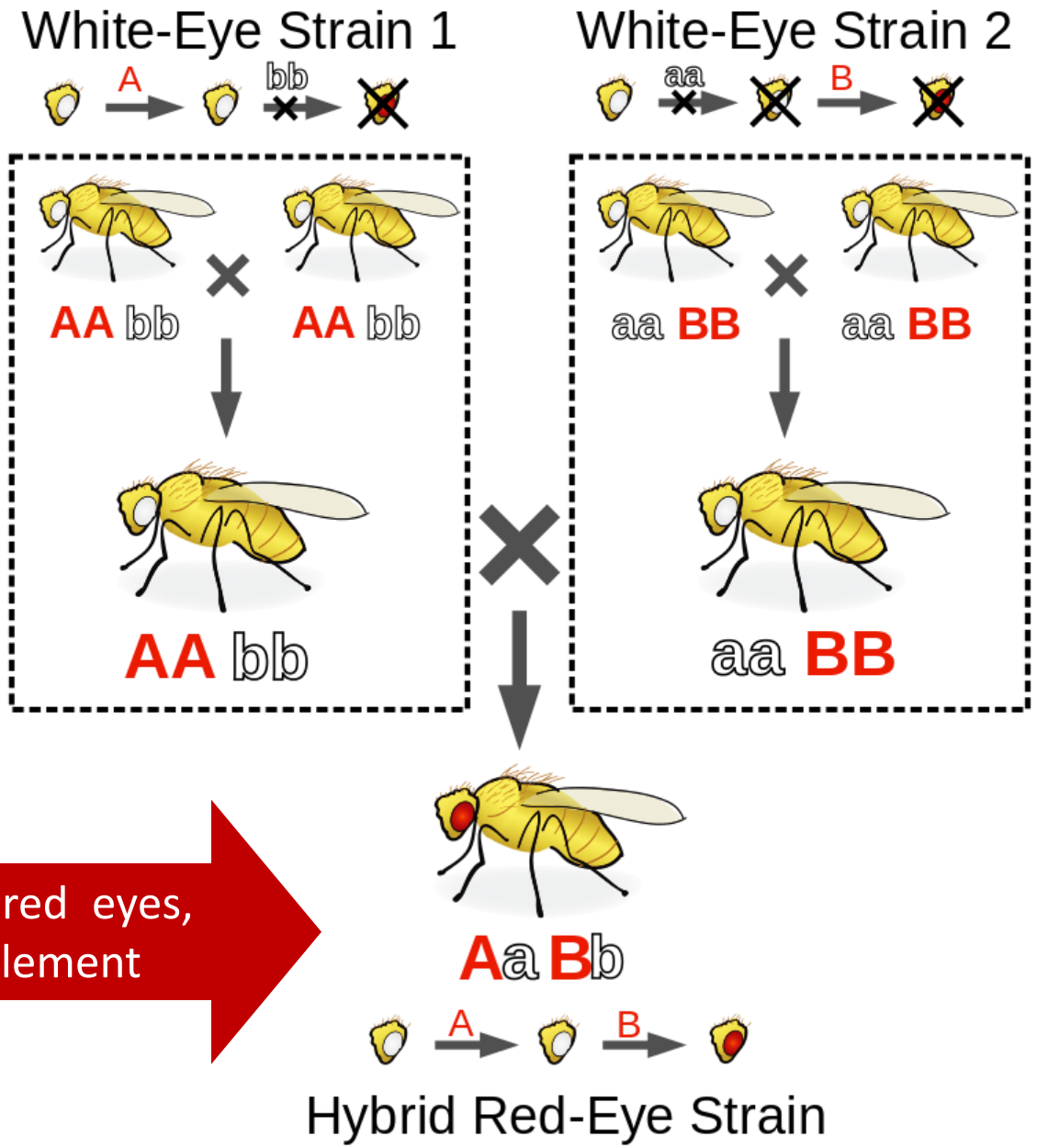
recessive one that codes for a malfunctioning protein (a and b)

Both proteins are necessary for the synthesis of red pigmentation in the eyes, if a given fly is homozygous for either a or b, it will have white eyes.

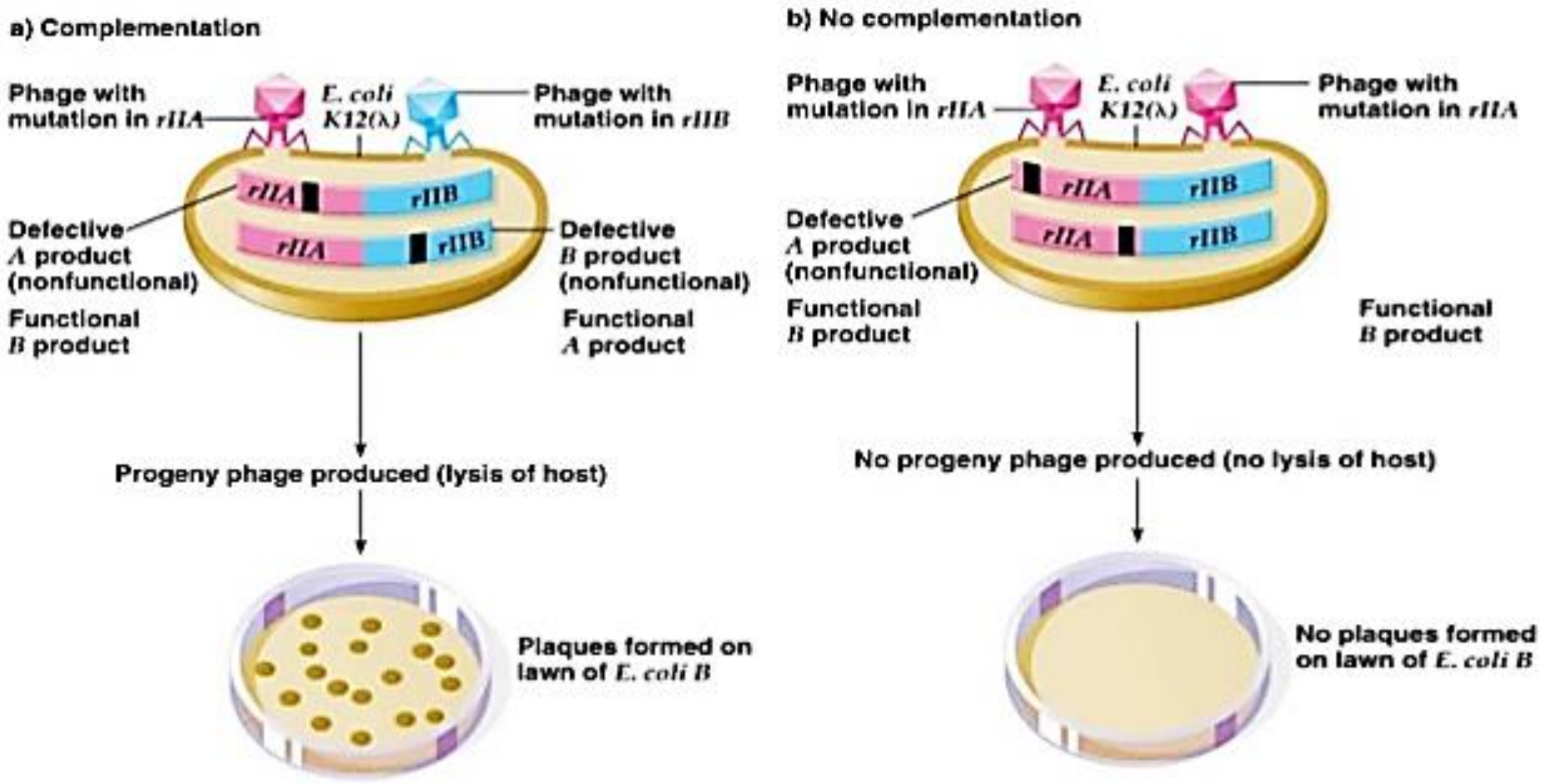
AABB = red ***A__bb = white*** ***aaB__ = white***

Knowing this, the geneticist may perform a complementation test on two separately obtained strains of pure-breeding white-eyed flies.

The test is performed by crossing two flies, one from each strain.

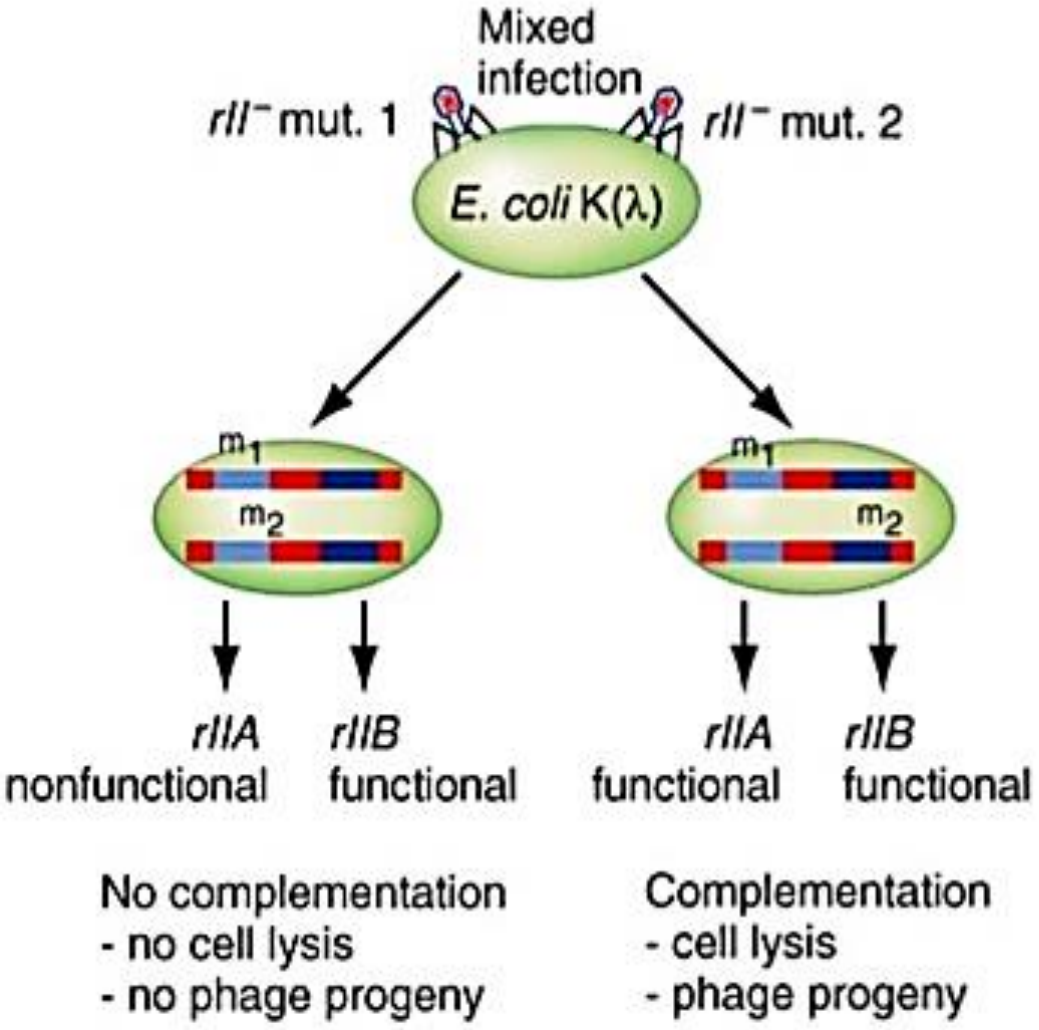


If the resulting progeny have red eyes, the two strains are said to complement



☞ **Cistron:** Term coined by Benzer for the smallest genetic unit that does NOT show genetic complementation when two different mutations are in trans position; but shows wild-type phenotype when the same mutations are in cis.

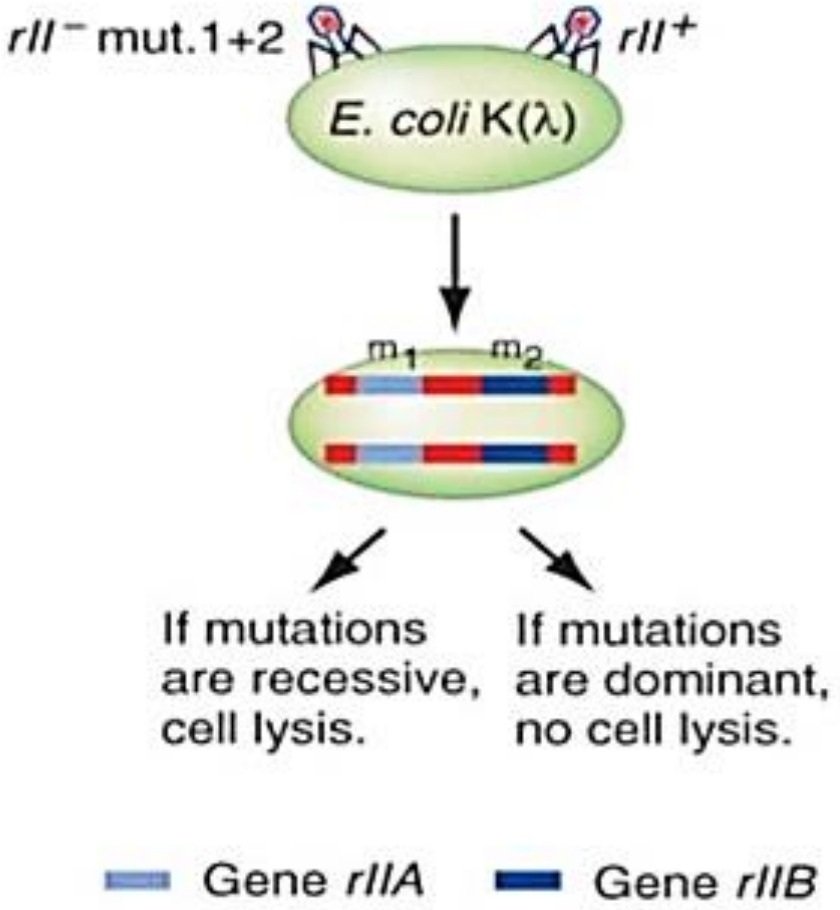
(*trans* configuration)



No complementation
- no cell lysis
- no phage progeny

Complementation
- cell lysis
- phage progeny

(*cis* configuration)



Other example

- Complementation test was one of the main tools used in the early Neurospora work, because it was easy to do, and allowed the investigator to determine whether any two nutritional mutants were defective in the same, or different genes.
- The complementation test was also used in the early development of molecular genetics when bacteriophage T4 was one of the main objects of study.
- Blue-white screening of pUC vector.

Conclusion

- The number of units of functions (genes) is determined by complementation tests.
- Given a set of mutations expressing the same mutant phenotype, two mutants are combined and the phenotype is determined.
- If the phenotype is wild-type, the two mutations have complemented and must be in different units of function.
- If the phenotype is mutant, the two mutations have not complemented and must be in the same unit of function.