Mutations –II

Conditional lethal mutations: powerful tools for genetic studies

Conditional lethal mutationsare lethal in onethe permissive condition. These allow genetic*ist*s to identify and study mutations in essential genes that result in complete loss of gene-product activity even in haploid organisms. Mutants carrying conditional lethals can be propagated under permissive conditions, and information about the functions of the gene products can be inferred by studying the consequences of their absence under the restrictive conditions.

The three major classes of mutants with conditional lethal phenotypes are (1) auxotrophic mutants, (2) temperature-sensitive mutants, and (3) suppressorsensitive mutants.

Auxotrophs are mutants that are unable to synthesize an essential metabolite (amino acid, purine, pyrimidine, vitamin, and so forth) that is synthesized by wild-type or prototrophic organisms of the same species. The auxotrophswill grow and reproduce when the metabolite is supplied in the medium (the permissive condition); they will not grow when the essential metabolite is absent (the restrictive condition).

Temperature-sensitive mutants will grow atone temperature but not at another. Most temperature-sensitive mutants are heat-sensitive; however, some are cold-sensitive. The temperature sensitivity usually results from the increased heat or cold lability of the mutant gene product—for example, an enzyme that is active at low temperature but partially or totally inactive at higher temperatures. Occasionally, only the synthesis of the gene product is sensitive to temperature, and once synthesized, the mutant gene product may be as stable as the wild-type gene product.

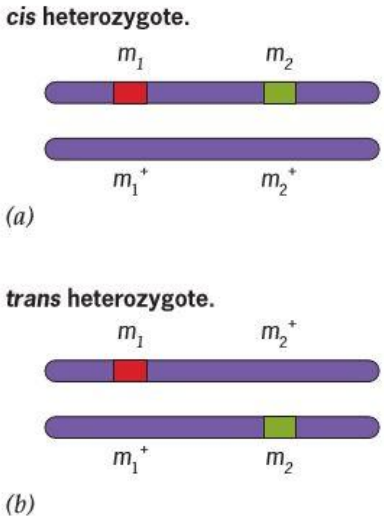
Suppressor-sensitive mutants are viable when a second genetic factor, a suppressor, is present, but they are nonviable in the absence of the suppressor. The suppressor gene may correct or compensate for the defect in phenotype that is caused by the suppressor-sensitive mutation, or it may cause the gene product altered by the mutation to be nonessential.

Conditional lethal mutations can be used to investigate biological processes. For example,

Intermediate Y is produced from precursor X by the action of enzyme A, the product of gene A, but intermediate Y may be rapidly converted to product Z by enzyme B, the product of gene B. If so, intermediate Y may be present in minute quantities and be difficult to isolate and characterize. However, in a mutant organism that has a mutation in gene B, resulting in the synthesis of either an inactive form of enzymeB or no enzyme B, intermediate Y may accumulate to much higher concentrations, facilitating its isolation and characterization. Similarly, a mutation in gene A may aid in the identification of precursor X. In this way, the sequence of steps in a given metabolic pathway can often be determined.

Edward Lewis developed the complementation test for functional allelism, to determine whether two mutations were in the same or different genes.A double heterozygote, which carries two mutations and their wild-typealleles, i.e. m_1 and m_1^+ along with m_2 and m_2^+, can exist in either of two arrangements:

- 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.



In the 1940s and 1950s, Lewis observed that fruit flies carrying certain mutants in the *cis*and *trans* configurations had different phenotypes. Flieshomozygous for two X-linked mutations recessive eye color mutations white (w) and apricot (apr) have apricot-colored eyes and white eyes, respectively, whereas wild-type had red eyes.

Heterozygotes	*cis*	*trans*
Genotype	apr w/apr$^+$ w$^+$	apr w$^+$/apr$^+$ w
Phenotype	red eyes like wildtype flies	light apricot-colored eyes

Both genotypes contained the same mutant and wild-type genetic information but in different arrangements. When organisms that contain the same genetic markers, but in different arrangements, have different phenotypes, the markers are said to exhibit position effects. The type of position effect that Lewis observed is called a *cis-trans* position effect. Lewis's discovery of *cis-trans* position effects led to the development of the complementation test or *trans*test for functional allelism. This test allows geneti*cis*ts to determine whether mutations that produce the same or similar phenotypes are in the same gene or in different genes. The mutations must be tested pairwise by determining the phenotypes of *trans* heterozygotes. That is, *trans* heterozygotes must be constructed for each pair of mutations and examined to determine whether they have mutant or wild-type phenotypes. Ideally, the complementation or *trans* test should be done in conjunction with the *cis*test—a control that is often omitted. *Cis* tests are performed by constructing *cis*heterozygotes with each pair of mutations being studied and determining whether the heterozygotes have mutant or wild-type phenotypes. Together, the complementation or *trans* test and the *cis* test are referred to as the *cis-trans* test. Each *cis* heterozygote, which contains one wild-type chromosome, should have the wild-type phenotype whether the mutations are in the same gene or in two different genes. Indeed, the *cis* heterozygote must have the wild-type phenotype for the results of the *trans* test to be valid. If the *cis* heterozygote has the mutant phenotype,the *trans* test cannot be used to determine whether the two mutations are in the samegene. Thus, the *trans* test cannot be used to assign dominant mutations to genes. With diploid organisms, *trans* heterozygotes are produced simply by crossing organisms that are homozygous for each of the mutations of interest. With viruses, *trans* heterozygotes are produced by simultaneously infecting host cells with two different mutants. Regardless of how

the *trans*heterozygotes are constructed, the results of the *trans* or complementation tests provide the same information. 1. If a *trans* heterozygote has the mutant phenotype (the phenotype of organisms or cells homozygous for either one of the two mutations), then the two mutations are in the sameunit of function, the same gene. 2. If the *trans* heterozygote has the wild-type phenotype, then the two mutations are in two different units of function, two different genes.