9 Enhancement and Synthetic Phenotypes

OVERVIEW

Enhancement is the opposite of suppression. In suppression the two mutations act together to produce a phenotype that is similar to the wild-type. In enhancement the two mutations act together to produce a mutant phenotype that is more severe than that exhibited by either mutation alone. Examples of enhancement are often discovered by chance. An investigator carries out a selection procedure for a particular mutant phenotype, but, when the mutant strains obtained are analyzed, it is discovered that the mutant phenotype is dependent on alterations in two genes. For example, a researcher carries out a selection for a particular mutant phenotype in a S. cerevisiae. A mutant is isolated and crossed to an otherwise isogenic strain of the opposite mating type. The diploid is sporulated, and the four haploid meiotic products (the tetrad) is analyzed for the segregation pattern of the mutant phenotype. If all of the tetrads exhibit 2 wild-type: 2 mutant segregation, the mutant phenotype is the result of a single altered gene. If 4 wild-type: 0 mutant, 3 wild-type: 1 mutant, or some other such pattern is seen, one should consider the possibility that the mutant phenotype results from the interaction of mutations in two different genes and either mutation alone is insufficient to produce a mutant phenotype.

Investigators with a goal of identifying functionally interacting genes often will carry out searches for enhancers as well as for suppressors. As discussed for suppressor hunts, it also is important to use the appropriate type of starting mutation in a search for enhancer genes. If one is interested in gene function, one should start the enhancer search using a mutation in the coding region of a gene that affects function. Often mutant alleles with a modest effect on a phenotype are used and the enhancer search is for second site mutations that increase the severity of the mutant phenotype.

Enhancers can be intragenic or intergenic. Researchers interested in gene function will want to focus on intergenic enhancers. Mutant strains isolated from an enhancer search must be crossed to an otherwise isogenic wild-type strain of the opposite mating type and tetrad analysis undertaken. If the original mutant phenotype is recovered from the haploid progeny, then the enhancer mutation is in another gene. The enhancer mutation alone may or may not exhibit a phenotype and, if it does, the phenotype may be similar to the original mutation or it may be novel. This can all be determined from the results of the tetrad analysis. For example, if the cross produces tetratype tetrads with 1 wild-type: 2 mutant: 1 enhanced mutant spores, then one can conclude that the phenotype of the enhancer mutation has a phenotype and it is similar to that of the original mutation.

MECHANISMS OF ENHANCEMENT

Enhancement interactions can be complex, but three possible models for such interactions are described below. First, it is possible that the two genes encode
components of parallel pathways with a common or overlapping function. The loss of one pathway can be tolerated, but not the loss of both. Therefore, a mutation blocking one pathway alone may have a slight mutant phenotype but two mutations each of which blocks one of the pathways will have a severe mutant phenotype. This is illustrated in Model 1 in Figure 9.1. Mutation of GEN1 or GEN4 may decrease the rate of function X only slightly but the double mutant is severely defective in the level of function X.

In the second model (shown in Figure 9.2), proteins Gen1p and Gen2p interact to form a heterodimer with a particular function. Non-null mutations in either GEN1 or GEN2 that alter residues involved in the interaction between Gen1p and Gen2p might slightly destabilize the interaction but have little or no effect on the function of the complex thus producing no phenotype or a modest phenotype. A combination of both of these mutations, that is a gen1 gen2 double mutant strain, is likely to more fully destabilize the interaction and be detrimental to the functional activity of the complex thereby producing a mutant phenotype.

The third model involves a pathway of reactions such as the one illustrated in Figure 9.3. If the capacity of the pathway is greater than required for the wild-type phenotype, then the rate of reactions 1 or 2 can be reduced slightly without the overall rate of the pathway falling below a critical threshold rate. If the rates of both reactions are decreased, then the effect is multiplied and the impact on the activity of the pathway is likely to be reduced sufficiently to produce a mutant phenotype.

SYNTHETIC ENHANCEMENT

One mechanism for exploring the functional relationship between two known mutant genes with similar phenotypes is to test for enhancement. The mutants are crossed and double mutants isolated. If the double mutant exhibits a more severe
Figure 9.2 Allele-specific enhancement

![Model 2 Diagram]

Figure 9.3 Enhancement within a common pathway

phenotype than either mutation alone, the mutations exhibit enhancement. This is referred to as synthetic enhancement because the researcher constructed the double mutant strain. One can then begin to explore the mechanism of enhancement, for example by testing null alleles of the genes for enhancement.

CONDITIONAL LETHAL MUTATIONS FOR THE ISOLATION OF ENHANCER MUTATIONS

Strains carrying conditional mutations, such as cold sensitive or temperature-sensitive mutations, are usually considered to exhibit the wild-type phenotype at the permissive temperature but, in reality, the function of these proteins is often somewhat compromised even at the permissive temperature. For example, if two proteins interact as in Model 2 above, a conditional mutation in either protein in the sites of interaction most likely will weaken the interaction even at the permissive temperature. The effect of either mutant gene alone may not be sufficient to disrupt function at the permissive temperature. However, if both proteins carry alterations at their sites of interaction, it is possible that the combination of the two mutant alleles will decrease the binding constant of the proteins even at the permissive temperature to a degree that is sufficient to disrupt interaction. In such a situation,
strains carrying both conditional mutations will have the mutant phenotype. If the proteins carry out an essential function, then the combination of these two mutations will be lethal even at the permissive temperature. This is referred to as **synthetic lethality**. Allele-specific synthetic lethality is strong evidence of physical interaction between two proteins.

### GENETIC INTERACTION

When genetic analysis of two or more genes with similar mutant phenotypes identifies mutations in these genes that suppress one another or exhibit synthetic enhancement with one another, the genes are said to **interact genetically**. The implication of this statement is that the encoded proteins function in the same, overlapping, or related pathways or that the proteins interact physically to form a multimeric complex. A thorough examination of the evidence from studies of genetic interaction like suppression analysis, enhancement studies, and other so-called **synthetic phenotypes** will provide information on the role of these proteins in the process under investigation.

### FURTHER READING