Genetic interactions - modifiers of mutant phenotypes

Papers to read for this section:

1. Carlson, M., Osmond, B.C., Neigeborn, L. and Botstein D. (1984) A suppressor of *SNF1* mutations causes constitutive high-level invertase synthesis in yeast *Genetics* 107:19-32.

The previous sections have concentrated on identifying specific genes and cloning them. In particular, we spent a long time on how to isolate mutations that affect some kind of process, whether by looking for phenotypes, or by reverse genetics. In the next part of the course, we will examine how mutations interact with one another.

This chapter describes how we can use the phenotype of a mutation in one gene involved in a process to devise selections and screens to find other genes involved in the same process. We've already seen how selections and screens can be devised based environmental conditions that affect whether or not an organism will live or die. For example, the 8-azaguanine and 5-FOA select for loss of function mutations in the genes that encode enzymes that convert them to toxic compounds. The presence or absence of various nutrients in the medium allow us to screen for mutations that affect their synthesis or utilization.

Mutant phenotypes give us another whole set of things to select or screen for - the presence or absence of a mutation is no different from the presence or absence of a compound in the medium. For example, if a mutation prevents growth under some condition, you can select for a second mutation that restores growth. The selected cells that grow under the selective condition are called **revertants**. If the second mutation simply removes the first mutation by changing the sequence of the affected gene back to wild-type, the second mutation is a **true reversion**. However, in many cases, the second mutation is not at the same site as the first mutation; in these cases, the second mutation is a pseudoreversion. Pseudorevertants can be intragenic or extragenic. Extragenic pseudorevertants are also often called **suppressors**.

In the most general sense, there are two kinds of mutations one can look for that affect the phenotype of a mutation. In the example above, the secondary mutation suppresses the mutant phenotype; if the first mutation made the cells sick, then the suppressors made them better. The other kind of mutation

that can affect the phenotype of the first mutation would be one that made the phenotype more extreme. If the first mutation makes the cells sick, then the second mutation makes the cells sicker. The change in phenotype when the two mutations are combined is a called a **synthetic** effect. In the extreme case, two mutations that are viable on their own can kill the cell. This kind of relationship is called **synthetic lethality**.

Suppressors and synthetic lethals provide us with two powerful classes of selections and screens to find new genes and interesting alleles of previously identified genes. The next two sections describe suppression and synthetic lethality in more detail.

Suppression

A suppressor is any kind of mutation that decreases the intensity of the phenotype of another mutation. For example, we have already discussed nonsense suppressor mutations in tRNA genes. These suppressors relieve the loss of expression caused by mutations that prematurely terminate translation due to the introduction of stop codons within the reading frame of a gene. In the case of nonsense suppressors, the primary mutation decreases the synthesis of a gene, and the suppressor restores synthesis.

Just as loss of function mutations can affect the synthesis, degradation, folding or activity of a gene product, suppressors can exert their effects at any of these levels. If the effect of the primary mutation is to decrease the amount of the active gene product, anything that increases the amount of the protein will show up as a suppressor (Figure 9-1). For example, many missense mutations decrease the stability of the protein encoded by a gene of interest. Mutations that affect intracellular proteases or chaperones are often found as suppressors.

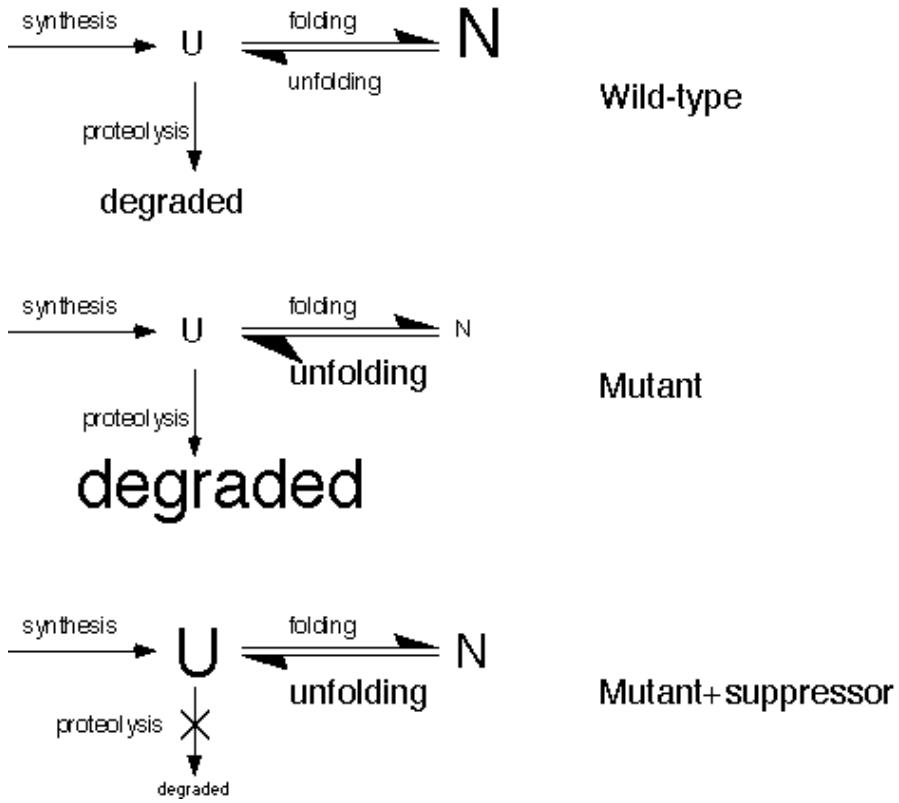

Figure 9-1. Suppression by eliminating proteolysis

Suppressors that act on many different primary mutations in many different genes often act by changing synthesis, folding or degradation. Nonsense suppressors will suppress nonsense mutations in any gene, as long as the amino acid placed at the position of the mutation is tolerated by the protein. Mutations that affect a proteolysis pathway will suppress mutations that affect the stability of any protein that can be degraded by that pathway. Mutations that affect the synthesis of an mRNA can be suppressed by mutations that affect chromatin structure or DNA supercoiling.

Note that the suppressor does not have to act on the same ste as the primary mutation. A mutation that destabilizes the protein structure might be suppressed by mutations that increase the initiation rate at the promoter for the gene that encodes the protein. By making more mRNA, the cell will make more protein. Even though the specific activity of the protein is still decreased by the primary mutation, the increase in concentration can restore the total intracellular activity.

Consider how this might happen for a heterodimeric protein (Figure 9-2). If a primary mutation affects the stability of subunit A, the equilibrium between the folded and unfolded forms of A will

shift toward the unfolded state. Since the assembly of AB heterodimers is dependent on the concentrations of A and B, this will decrease the level of heterodimer observed. A suppressor could work by shifting A back to the folded state. However, a different way to suppress this defect would be to increase the concentration of B. By mass action, B will pull A into heterodimers, and the observed activity of AB heterodimers will be restored.

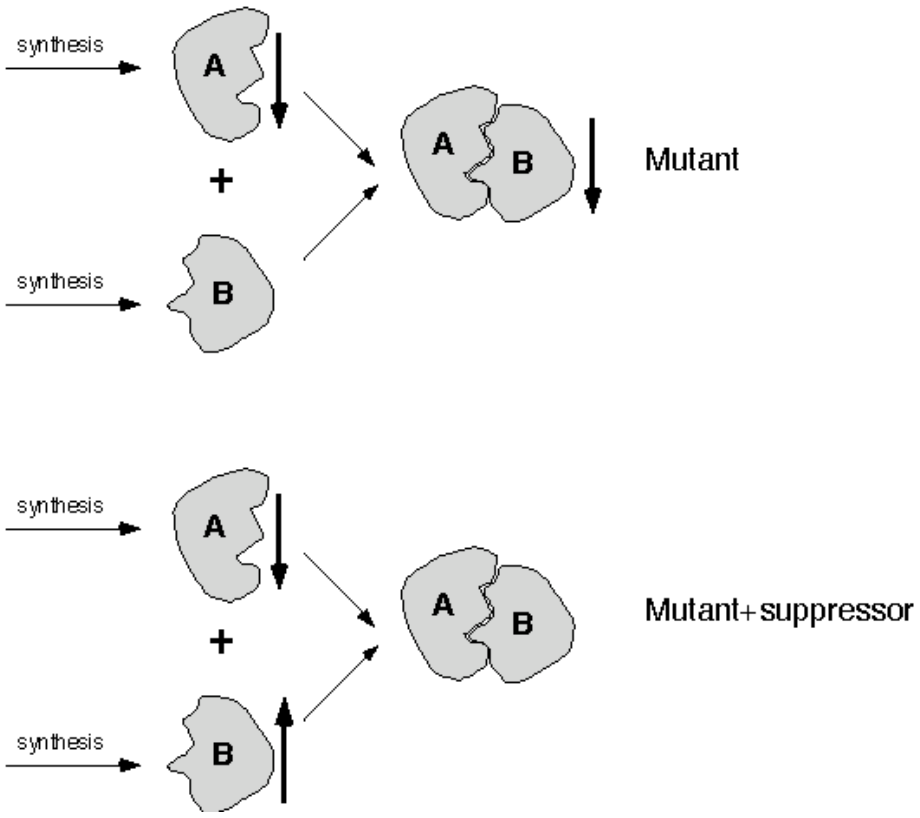

Figure 9-2. Increasing the expression of one subunit can suppressa defect in the stability of another subunit.

Multicopy suppression

One way to increase the concentration of B protein is to increase the dosage of the B gene. In yeast, suppressors are often found by transforming a mutant with a library of genomic fragments cloned into a multicopy plasmid. If suppression is observed, this is called multicopy suppression.

Note that the protocol for isolating multicopy suppressors is indistinguishable from the protocol for cloning by complementation. In fact, the first multicopy suppressors were probably found as

anomalous results from attempts to clone genes by complementation. If you are trying to clone *YFG* from a library by complementing a *yfg* mutant, you would expect that only the real *YFG* gene would be able to complement the defect. If you get two classes of clones with different genes on them, this is a clue that something else is going on!

As usual, it is important to note that there are almost always different mechanisms that can explain the same genetic phenomena. Above, I've illustrated how multicopy suppression can occur by overexpressing a protein that physically interacts with a mutant protein. However, it is also possible to imagine models where the protein encoded by the suppressor does not physically interact with the protein affected by the mutated gene. In fact, if the multicopy suppressor suppresses a deletion of *YFG*, then there is nothing in the cell for the suppressor protein to interact with. In these cases, the model shown in Figure 9-2 cannot explain the phenomenon of multicopy suppression.

How could suppression of a knockout work? There are several possible mechanisms. For example, the suppressor might encode an enzyme with an activity related to the activity of *YFG*. If the concentration of the suppressor enzyme is high enough, it might be able to catalyze enough of the reaction normally catalyzed by *YFG* to support growth. Alternatively, the suppressor might allow a different structure or reaction that bypasses the need for whatever *YFG* does.

In addition, it is important to remember that the failure to isolate a multicopy suppressor does not tell you anything. It is absolutely not justifiable to conclude that there are no interaction partners for protein A in the cell based on the failure to isolate B as a multicopy suppressor. Aside from the simple issue of whether or not B is represented in the library, increasing the dosage of a gene does not necessarily increase the concentration of the gene product. Many genes have negative autoregulatory feedback mechanisms to control the levels of their products. For these genes, increasing the copy number of the gene will not increase the expression of the gene product.

Allele specific suppression

Despite the caveats described above,suppressor mutations have been useful for identifying protein-protein interactions. Interactions identified by suppressor analysis have often been shown to reflect physical interactions by other methods. In cases where two proteins interact, a special kind of

suppressor analysis is often attempted in order to map where on the proteins the interaction surface lies. The basic idea is that certain kinds of mutations in the interface between two proteins will be mutually suppressing due to the creation of a new complementary interaction surface (Figure 9-3). This mutual suppression should be **allele specific**, since mutations in other parts of the protein should not be able to create the same complementarity with either of the two mutations.

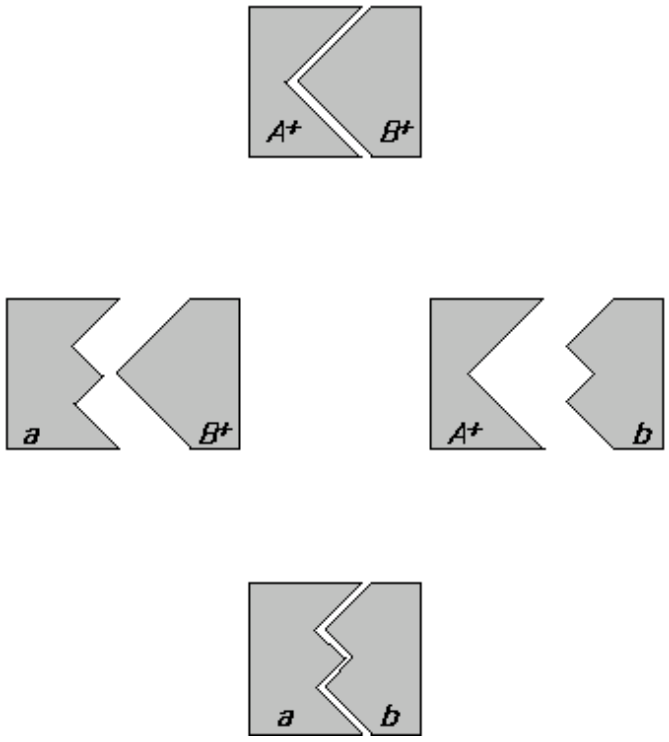

Figure 9-3. Bumps and holes modelfor allele specific suppression

The idea behind mapping contacts by allele specific suppression is often describes as a "bumps and holes" model, as illustrated in the figure. A mutation in subunit A makes a bump that prevents the A-B dimer from forming. A mutation in subunit B makes a hole in the interface that removes too much of the surface contacts to form a stable interface. If the bump in A fits into the hole in B, then the two mutant proteins are able to interact, although neither can interact with a wild-type partner.

As an example of how this might work at a more detailed level, imagine a buried ion pair in a dimer interface where a glutamate (E) in subunit A interacts with a lysine (K) in subunit B. A mutation that changes the E in the A subunit to a K will create a K-K pair. Repulsive coulombic interactions

between the two positively charged side chains will drive the subunits apart. Similarly, if a mutation changes the K in the B subunit to an E, the two negatively charged side chains will repel one another. When the two mutant proteins are combined, subunit A will have a K and subunit B will have an E. The orientation of the charged groups is reversed relative to the wild-type heterodimer, but the charges are complementary again and the interaction may be stabilized, as long as other factors such as the stereochemistry of the side chains are accomodated. Similar models based on hydrogen bond donor or acceptors or the sizes and shapes of side chains involved in hydrophobic or van der Waals interactions can be constructed.

NEXT PART ISN'T WRITTEN YET (only extremely rough outline)

- Alternative - Mass action
- Idea
- XY complex binds Z
- assembly is a coupled equilibrium
- less X, get less binding
- because there is less XY complex
- keep the lower amount of X and raise Y
- mass action drives formation of XY
- Hypothetical example
- Write out coupled equilibria
- Real examples
- Predictions of the alternative models
- Bumps and holes model predicts allele specificity
- Mass action model predicts relative allele independence
- Both models presuppose physical interaction between X andY
- Alternative to either model
- More on this later

Synthetic Lethality

× Classical and Mass Action can work the other way

× Bumps and holes

× OVERHEAD

× Mass action

• How do we start with a crippled version?

× Lets limit the discussion to yeast

× For suppression, need to start with situation where mut1 is lethal

× Need conditional

× ts or cs

× plasmid loss

× mutation *yfg1* and *ura3* on chromosome

× plasmid 1 has *YFG* and *URA3*

× mutagenize and select for FOA resistance

× Should get mutations that suppress yfg - syg

× What else will you get?

× ura3 on plasmid

× For synthetic lethality, need to start with a condition where the mutation is not lethal

× NOTE need to screen for synthetic lethals, can't select

× WHY?

× Can't work with the dead.

