**Bypass and interaction suppressors; pathway analysis**

The isolation of extragenic suppressors is a powerful tool for identifying genes that encode proteins that function in the same process as a gene of interest.
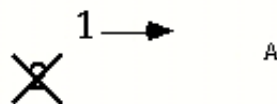
Bypass suppressors suppress all mutant alleles of a gene, including null alleles, and hence bypass the requirement for a gene. These suppressors are gene specific, allele nonspecific. There are three types of bypass suppressors that we will consider here.

Often bypass suppressors can be generated by up-regulation of a gene in a parallel pathway. One good example of bypass suppression by up-regulation of a parallel pathway is suppression of yeast *cyc1* loss-of-function mutants by mutations that increase the expression of the *CYC7* gene. *CYC1* encodes the major form of cytochrome c, and *CYC7* encodes a minor form. The mutant phenotype of *CYC1* can be suppressed by a mutation in *CYC7* that increases its expression. This mutation is caused by the transposable element *Ty1* inserting into the promoter of the *CYC7* gene.

Pathway 2 is primary

1 → A
2 ——→
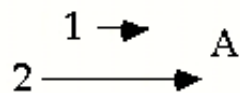
Mutation in 2, little B accumulates

1 →
✗   A

Suppressor mutation in 1 increases B to normal levels

1*——→
✗   B

Bypass suppression by mutation in a gene in a parallel pathway.

Bypass suppressors can also result by altering the function of a gene that acts in a distinct process. Bypass suppressors of mutations in the *E. coli* maltose permease gene, which encodes a protein that transports maltose into the cell, alter the specificity of the lactose permease gene. Lactose permease normally transports lactose into the cell, but the bypass suppressors now cause it to transport maltose.

Epistatic interactions
Bypass suppressors can also lie in genes that act downstream in a regulatory pathway, and in this case can referred to as epistatic suppressors. (Some do not consider epistatic interactions to be suppression. This is a semantic issue that we won't worry about, and we will consider an epistatic suppressor to be a type of bypass suppressor.)

Positive regulatory pathway; signal turns pathway ON

Signal ⟶ 1 ⟶ 2 ON

Mutation in 1 inactivates pathway

Signal ⟶ ✗ OFF

Suppressor mutation in 2 turns pathway ON

Signal ⟶ ✗ ⟶ 2* ON

Bypass suppression by mutation in a gene downstream in the same pathway.

Positive regulation
Let's consider an example of epistatic interactions between genes that are involved in determining sex in the nematode *C. elegans*. As in *Drosophila*, the X chromosome to autosome ratio determines sex in *C. elegans*. The ratio is 1.0 in XX animals, causing them to develop as self-fertilizing hermaphrodites (hermaphrodites are basically females that produce sperm for a short time). The ratio is 0.5 in X0 animals (there is no Y

chromosome in *C. elegans*), causing them to develop as males. The *tra-1* and *tra-2* genes are part of a pathway that determines sex in *C. elegans*. In this pathway, *tra-2* encodes a cell surface receptor that is upstream of *tra-1*, which encodes a transcription factor that regulates genes involved in sexual differentiation. In XX animals, the X to autosome signal activates the pathway that contains the *tra-2* and *tra-1* genes to produce the hermaphrodite fate. Loss-of-function mutations in *tra-2* or *tra-1* transform XX animals into males. The transformation by *tra-2* mutations can be suppressed by certain mutations in the *tra-1* gene: XX animals that are doubly mutant for a loss-of-function *tra-2* mutation and a gain-of-function *tra-1* mutation develop as hermaphrodites.

As with the *CYC7* suppressors described above, the *tra-1* suppressors bypass the requirement for another gene. The difference is that *tra-2* and *tra-1* act in the same pathway, whereas *CYC1* and *CYC7* act in parallel pathways. How can you distinguish between bypass suppressors in the same or in parallel pathways. Analysis of double loss-of-function mutants can sometimes be used to distinguish between the two. In the case of parallel pathways, the double mutants will result in a more severe phenotype. In the *cyc1; cyc2* double mutant, for example, no cytochrome c is produced, whereas some cytochrome c is produced in either single loss-of-function mutant. The *tra-2; tra-1* double mutant, by contrast, has the identical phenotype to either single mutant.

Negative regulation
A third example of bypass suppression is in a pathway containing a negative regulatory interaction. In this case a mutation in a negative regulatory gene produces a phenotype because the gene downstream is activated inappropriately. But a loss-of-function mutation in the downstream gene suppresses this phenotype. It is worth noting that inactivating the downstream gene usually leads not only to suppression, but to an additional and often opposite phenotype. One good example of this type of suppression is in *C. elegans* sexual development. One of the genes that the transcription factor TRA-1 negatively regulates in XX animals is the cell death gene *egl-1*, which is required for programmed cell death in *C. elegans*. A sexually dimorphic cell in *C. elegans* is the HSN motor neuron, which innervates vulval muscles and stimulates egg laying by hermaphrodites. Males don't lay eggs and hence don't need HSNs; the HSNs undergo programmed cell death in males. In XX animals lacking *tra-1* function, the HSNs adopt the male fate and die because the cell death gene *egl-1* is expressed and activates the cell death pathway. But eliminating *egl-1* function in a *tra-1* XX animal suppresses the cell death phenotype caused by the *tra-1* mutation. This suppression occurs only for the HSNs. Cells other than the HSNs still adopt male fates in XX animals

containing mutations in *tra-1* and *egl-1* because TRA-1 regulates other genes in these cells.

Gene 1 inhibits gene 2

1 ———| 2   2 is inactive

Mutation in 1 causes 2 to be active

✗———| 2   2 is active

Suppressor mutation in 2 inactivates 2

✗———|✗   2 is inactive

Bypass suppression by mutation in a gene downstream in the same pathway (negative regulation).

Interaction suppressors are compensating mutations in physically interacting components. A mutation in one protein disrupts the interaction, but a mutation in the second protein restores the interaction. These mutations are gene specific and allele specific.

The actin and fimbrin proteins inteact in budding yeast, and this interaction is essential for viability. Many proteins bind to actin to regulate actin assembly in cells. In order to find proteins that regulate actin assembly *in vivo*, suppressors of a temperature-sensitive allele of the yeast actin gene, *ACT1*, were isolated. A diploid homozygous *act1/act1* mutant was mutagenized, and hence only dominant suppressors were isolated. The investigators assumed that the original actin mutation impaired the interaction of an actin-binding protein with actin. They reasoned that isolation of dominant suppressors was more likely to yield mutations in genes that encode interacting proteins because such mutants would be mutations that restored interactions that were disrupted by the actin mutation. Some suppressors were in the gene *SAC6*, which was later shown to encode the actin-binding protein fimbrin. When the *sac6* mutations were separated from the *act1* mutation, it was found that the *sac6* mutants were also inviable at higher temperatures. Thus, the two

mutants, *act1* and *sac6*, were on their own very sick, but the *act1 sac6* double mutant was healthy. This phenomenon, called reciprocal suppression (see diagram), provides strong evidence that two gene products interact functionally.

   The *sac6* mutants could also be suppressed. Not surprisingly, the mutations that suppressed the *sac6* mutants mapped to *ACT1*. When the sequences of the new *act1* mutants were determined, and the positions of these mutations on the atomic model of actin were located, they defined a single surface that turned out to be the surface that binds to fimbrin. Thus, suppressor analysis can also provide information about the molecular contacts between interacting proteins. Biochemical binding experiments confirmed most of the conclusions of the studies modeled below with one twist: Mutant Sac6 protein binds too <u>tightly</u> to wild-type actin, not too weakly. The actin mutations weaken this interaction to suppress the *sac6* mutation. Thus, the reason *sac6* mutant result in a lethal phenotype is because to high an affinity between Act1p and Sac6p is deleterious. This is an example of the importance of biochemical experiments to fine-tune and test models based on genetics alone.

Model for interactions between wild-type and mutant Sac6p (fimbrin) and Act1p (actin).

ACT1 SAC6	*act1* *SAC6*	*act1* *sac6*	*ACT1* *sac*
Ts $^+$	Ts $^-$	Ts $^+$	Ts $^-$
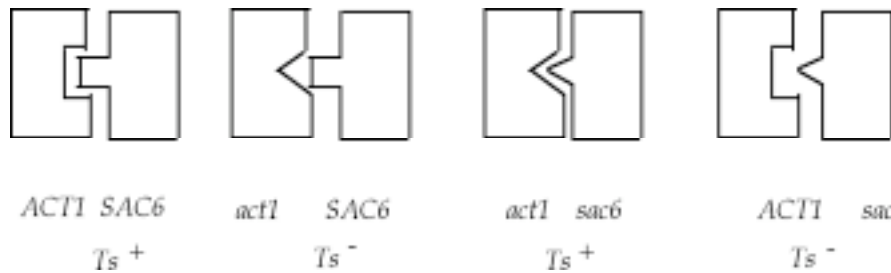
Reciprocal suppression is one example of a criterion that supports the possibility that a suppressor mutation has identified a gene that encodes an interacting protein.