

Suppressors

Although epistasis is useful in determining the order of gene products in a pathway, as we have seen, the architecture of a signalling pathway often does not allow for simple epistatic interactions. Divergence or convergence of pathways (e.g. furca paper) may not lead to clear phenotypes or clear interpretations. A potentially more powerful approach is to identify suppressor mutations.

A suppressor mutation: either partially or completely restores the phenotype of a mutation to wild type. Suppressor mutations may have no phenotype on their own. Suppressor screens are often initiated with dominant mutations, unlike with epistasis analyses. So, in *Arabidopsis*, is *cal* a suppressor of *ap1*?

Major types:

1) Intragenic.

A second mutation in the same gene. Screens that produce intragenic suppressors are most commonly carried out starting with a dominant mutation. By screening for a loss of the dominant phenotype it is often possible to identify new recessive alleles which can be used to better interpret the normal function of the gene. An example was the Gosti et al paper.

2) Intergenic.

These mutations often uncover new genes in a pathway that change the signal 'flux' through a pathway. If the initial mutation is a negative regulator, causing excess flux, a suppressor might identify a mutation in a positive regulator, which could reduce flux back to a level closer to wild type. An example is the identification of spindly (*spy1*) as a suppressor of *ga1-3* (*spy* was also identified by other means but we'll stick with this example here). *Ga1* encodes an essential enzyme in gibberellin biosynthesis, and shows typical dwarf, dark green phenotype. A screen for mutations that reversed this phenotype uncovered the *spy1* mutation that partially suppresses these phenotypes. *spy* alone in a wild type background has a slightly reduced requirement for GA in germination and increased internode length. Since *spy* is recessive, the data suggest that it is a negative regulator of GA biosynthesis. Yes, *spy* may have been identified in a general screen for increased internode length, but the phenotype is subtle enough that it may also have been missed.

Another possibility is that a compensatory mutation occurs in a protein downstream of the original defect, so that, say if \diamond normally interacts with \triangleright , the mutant protein \rightarrow could no longer carry out its function. However, if the downstream protein mutated to \ominus , function may be restored, i.e. [$\diamond\triangleright$] changes to [$\rightarrow\ominus$]. Generally these would be *allele-specific*, and would prove a molecular interaction. These may exist in plants, but I haven't found any in the literature.

Other expectations from suppressor screens

Often get mutations that only suppress a portion of the overall phenotype of the initial mutation. Such an example was recovered in a suppressor screen starting with a recessive mutation called *ctr*, which causes a constitutive expression of the ethylene 'triple response' (reduced root and hypocotyl elongation, radial swelling of hypocotyl, and exaggerated apical hook growth). A mutation called *hookless* was recovered. *Hookless* acts downstream of *Ctr* (of course – why must that be true?), and only affects the exaggerated hook aspect of the triple response.

A suppressor mutation can show an additive interaction with the original mutation and not be in the same pathway. But new additive interactions can be very interesting. Recall that ABA helps to establish seed dormancy and GA helps to break it. ABI-1, found in a screen for plants resistant to high levels of ABA during germination, is insensitive to the presence of ABA. This means it is defective in signal recognition, which is the equivalent to an absence of ABA production. Hence ABI-1 seeds do not establish strong dormancy and are wilted, because they cannot close their stomata in response to drought. In a screen for suppressors ABI-1, the first recessive GA-insensitive mutant (*sly1*) was uncovered. Importantly, *sly1* cannot germinate unless there is defect in ABA-acquired dormancy, such as is present in a double mutant. *sly1* would never have been identified as a mutant unless suppressor screens were used!

wild type seeds

Make ABA → receive ABA → dormancy..... Make GA → receive GA → break dormancy

With excess ABA:

Wild type: high ABA establishes over-dormancy

excess ABA → receive ABA → over-dormancy..... Make GA → receive GA → can't break dormancy

ABI-1: resistant to high ABA

excess ABA → partially ignore ABA → dormancy... Make GA → receive GA → break dormancy

ABI-1 + *sly1*: like wild type, can't germinate

excess ABA → partially ignore ABA → dormancy.... Make GA → poorly receive GA → can't break dormancy

with normal ABA:

ABA-1 + *sly1*

Make ABA → partially ignore ABA → weak dormancy..... Make GA → poorly receive GA → break dormancy

sly1 only

Make ABA → receive ABA → dormancy..... Make GA → poorly receive GA → can't break dormancy