The mechanistic implications of additive versus synthetic double mutant phenotypes

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1 Definitions

Two mutations can be called *additive* with respect to a phenotype if the double mutant phenotype is the expected phenotype if the mutations are independent. They might more accurately be called *independent* with respect to a phenotype, since the double mutant expectation depends on the phenotype measured. Since the most common ways to measure phenotypes are fold-change of a product or percent penetrance of a mutation, the effect of independent mutations will actually be multiplicitive most of the time, making the name "additive" misleading.

Synthetic mutations have no observed effect (with respect to the phenotype of interest) unless combined. More generally, *synergistic* mutations deviate from independent mutations in that the combined phenotype is more severe than the expectation. From a probability perspective, the two mutations in this case can be seen as random variables that have a covariance greater than zero.

2 Interpreting independent mutations

Mutations that appear to have an additive effect are often seen as being involved in two separate points in the same linear pathway. For example, if one measures an mRNA level and the two mutations affect transcription initiation and the mRNA stabiliy, one expects the phenotypes to be independent and additive.

However, this need not be the case. The two mutations could affect parallel entry points into a single pathway: if, for example, two genes antagonized the same repressor, there were no feedback between the two antagonists, and they operated under saturation in physiological conditions, mutations to the two genes would have an additive effect.

Likewise, parallel pathways feeding into the same phenotypic readout can yield additive independent mutations *if there is no crosstalk or feedback*

downstream of the two mutations between the two pathways. Although this seems at first glance like a tautology, the two pathways need not be independent upstream of the mutations. This may also seem unlikely because biology has many examples of feedback loops whereby the final product of a pathway influences compenents far upstream of it. However, given the diversity of possible methods for pathway control in biology, it is reasonable to assume that the above scenario appears in at least several instances.

3 Interpreting synergistic mutations

In contrast, synthetic or synergistic mutations are often used as evidence of the involvement of parallel pathways. This makes sense because biological systems are probably selected for robustness of phenotype in case of temporary or permanent single-gene knockout and functionally redundant pathways are an obvious solution. One example is the *de novo* amino acid synthesis mechanism, which can compensate when amino acid absorption fails. In the case of loss-of-function mutations in redundant pathways, one should always expect a synergistic phenotype.

Synergistic phenotypes could easily happen in a single pathway, as well. If the two gene products act at the same step in a pathway, they will each decrease function in that step. However, if the pathway step can tolerate some noise, no phenotype might be observed until the funciton falls below a certain threshold. For example, if there is a kinetic bottleneck downstream in a biochemical synthesis pathway, no phenotype will be observed until the upstream event becomes slower than the existing bottleneck. In another case, a positive feedback loop involving a downstream product of a pathway could mask an upstream decrease in function until the threshold of the feedback loop is no longer met. One important point is that in these noise control scenarios, these mutations might be independent with respect to their direct action, i.e. they may be synergistic with respect to the downstream measured phenotype even though they are additive with respect to an upstream product.

4 Conclusions

The observation of an interaction between two mutations is the first step towards uncovering the mechanism by which the genes of interest affect the measured phenotype. However, it is difficult to make any strong conclusions about the greater role of the two genes without considerable further information. The scenarios listed above could hold for any number of mechanisms, whether the mutations affect protein folding, binding, biogenesis, or other features. One must keep in mind that "synthetic", "synergistic", or "additive" are context-specific terms and may change with respect to

the phenotype studied or the experimental conditions used. In the end, one must apply as much extra knowledge from a systems level (including overall complexity, conservation, known pathways, and other datasets) as is available in order to have a hope of distinguishing between different gene interaction scenarios.