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(1) What are complex haploinsufficient (CHI) interactions and why might they be important for human health?

Complex haplinsufficient interactions are genetic interactions which produce phenotypic alterations in growth between null alleles (heterozygous) of two different genes. These might be important to human health because statistically CHIs will arise multiple times in each individual due to the complexity and size of the human genome. These can be linked to diseases as well as many other morphogenic traits.

(3) Describe the experimental approach to map CHI interactions with *act1-* mutations in yeast.

A haploid yeast strain with a deletion in its ACT1 gene was grown with a low-copy-number plasmid containing carrying $ACT1^{wt}$ and was then mated to an array of other haploid strains containing a deletion in an unessential gene. This produced diploids containing a single wild type copy for the ACT1 gene and the query gene. These combinatorial diploids were then grown and observed for growth deficit. Those identified as having growth deficit in the initial screen and the manual screen would then be classified and quantified as having a CHI.

(6) What results suggested actin-related functional specificity with ribosomes? What explanations were offered? What others might there be? How might one test these ideas?
MORE SPECIFICALLY:
Most yeast ribosomal protein genes are encoded by two separate, unlinked genes. The two proteins encoded by these gene pairs are not usually identical, but differ by only 1-3 amino acids. The two genes in each pair may not necessarily be expressed at the same level (how would you figure this out?). Curiously *act1* deletions exhibit CHI with a knockout of one but not the other copy of some of these gene pairs....but not with either copy of other *RPL* gene pairs. Provide three different models to account for these observations and describe in detail experiments to test your models.

Ribosomal proteins were identified as being CHI with the actin gene. However, after testing each paralog of the ribosomal proteins, it was found that in almost every case that only one of the paralogs displayed CHI with the actin null. This suggests that there might be actin-related functional specificity to each paralog as they did not show identical actin arrangement or growth patterns. The offered explanation was that the differences in expression between paralogs would be correlated to the CHI, however this was not confirmed by the authors' experiments. To determine the expression level, one could roughly measure it with GFP tagging such as the authors

did, however to do so in a more quantitative way, one could employ Western Blotting.

One model by which a paralog might show actin-specific functional difference over its cognate paralog might be that the small difference in sequence provides a target site for regulatory modification. This model suggests that some other agent in the cell controls the actin-related activity of this paralog by chemically modifying the target. To test this hypothesis, I could compare mass spectroscopic data between the two paralogs during their same stages in expression and look for chemical modifcations to the amino acid change.

Another model might be that the amino acid change provides a better binding site for another protein or RNA to interact with the ribosomal protein paralog. This binding might introduce additional activity, or it might inhibit the native activity of the protein, and thus serve as a regulatory mechanism with differential influence over the two paralogs. To test this, we could do co-IP to try to pull out the associated interacting agent. Additionally, we could use mass spec to identify it and assay its activity.

One more possible model by which the small alterations in the amino acid sequence could introduce a noticeable difference in the activity of the paralogs might be that the amino acid alteration introduces a charged area on the surface of the protein. In this model, it can be hypothesized that the ribosomal protein interacts electrostatically with the charged regions on the surface of actin. Thus one paralog might interact more ably with the actin than the other. To test this hypothesis, one could do an experiment by GFP labeling the paralogs and staining the actin with rhodamine. If this hypothesis has validity, one would expect to see differences in the colocalization patterns for each paralog with the actin structure within the cell.

(8) How did the authors test whether loss of distinct actin functions recapitulated CHI interactions that were observed? What *act1* mutants were used? How were these *act1* mutants generated? What was the rationale for constructing these mutations in *ACT1?*

The authors sought to address the hypothesis that some of the CHIs observed might be due to the loss of specific actin function rather than the general decrease in actin concentration. One possible reason for this could be the specific loss of protein binding capacity in the low-actin environment. In order to generate these mutants, alanine replacement was conducted in six unique positions in the wild type version of the actin gene. The six mutants were act1-102,105,111,120,124,129. These mutants were screened with all the alleles identified to be CHI to the *act1Δ.*