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NEWS AND VIEWS

Systematic genetics swims forward elegantly

Yo Suzuki and Frederick P Roth

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA

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Sometimes when two (or more) genes are perturbed simultaneously, the organism exhibits a surprising property. This property may be a phenotype—either qualitative or quantitative—or the penetrance of a phenotype. This phenomenon, called genetic interaction, suggests that the interacting genes have related functions. Genetic interactions have shaped our understanding of almost every known biological pathway (see Lu and Horvitz, 1998, for example).

The genomic era has brought with it the systematic study of genetic interaction. In yeast, over 3000 genetic interactions have been identified from over 700 000 systematic crosses of precise gene deletion mutants (Tong *et al*, 2004). A similar interaction screen in metazoans would be a daunting challenge because both targeted deletion and genetic crosses are more labor intensive. RNAi, however, has provided an essential breakthrough, allowing for 'knockdown' of specific genes at the transcript level. *Caenorhabditis elegans* is particularly amenable to RNAi, and the requisite double-stranded RNA reagents can be delivered either by microinjection or soaking methods, or by feeding of a specific 'library' strain of *Escherichia coli* to the worm.

Using the feeding approach in *C. elegans*, Ahringer and co-workers (Kamath *et al*, 2003) performed the first genome-scale RNAi screen. Large-scale RNAi screens have since been performed in cultured fly and mammalian cells. Genetic interactions can be tested by performing such an RNAi screen in the presence and absence of mutation in a 'query' gene. This approach has been used on successively increasing scales, and has recently approached a genome scale with studies of ~17 000 gene pairs (Van Haaften *et al*, 2004; Suzuki and Han, 2006).

Fraser and co-workers (Lehner *et al*, 2006) have now scaled up. Using a 96-well format liquid culture method (Van Haaften *et al*, 2004), they combined query mutations in 31 genes with each of ~1750 genes involved in signal transduction and transcription. Thus, they tested ~65 000 allele pairs (~54 000 unique gene pairs) in a single study. Focusing on gross phenotypes such as lethality and sterility, they found ~350 interactions. These interactions implicated many new genes as modulators, for example, of the EGF signaling pathway. It will be interesting to explore the molecular and cellular mechanisms behind these interactions in careful follow-up studies. Of particular interest, six chromatin remodeling genes were found to interact with more than 25% of the query genes. Furthermore, several of these highly connected 'hub' genes appear to be conserved both in flies and mammals. With this in mind, we examined the *Saccharomyces cerevisiae* interaction data (Tong *et al*, 2004) and, intriguingly, found that three chromatin modification genes—*MRC1*, *ARP6* and *HTZ1*—were also among the most connected 'prey' genes in yeast. Fraser and colleagues suggest that such 'genetic hubs' represent global modifier genes, whose loss enhances the consequences of mutations affecting a wide range of functionally unrelated pathways, and, as such, may have a significant impact on the susceptibility to a variety of human genetic diseases.

The number of gene pairs (~200 million in *C. elegans*) together with variation in their alleles means that there is an astronomical number of potential interactions to test. The scope of inquiry can be limited in a number of ways. Testing pairs among genes already implicated in a particular process will yield a higher hit rate, but limits the chance of a truly novel discovery. Lehner *et al* quite reasonably focused their attention on regulatory genes, which cut across many processes. An alternative approach—testing pairs predicted to interact—has shown some promise in yeast (Wong *et al*, 2004) and *C. elegans* (Zhong and Sternberg, 2006). However, fully realizing this promise will require improved computational models and larger systematically collected data sets like those of Lehner *et al*.

Although RNAi screening already offers the best chance of tackling a large network of genetic interactions in metazoans, there are many opportunities for improvement. More detailed and dynamic cellular, subcellular and molecular phenotyping would reveal more interactions and clues about function (reviewed by Gunsalus and Piano, 2005; Neumann *et al*, 2006). Using deletion mutants or more sensitive RNAi methods would provide additional confidence. Double RNAi is a potential alternative, especially where deletion mutants are unavailable (J Tischler and A Fraser, personal communication). RNAi can have a high false-negative rate, which can be reduced by repeating the initial screen and by using RNAi hypersensitive strains (Simmer *et al*, 2003). Further automation could also be helpful: for example, the automatic scoring of phenotypes using image analysis could allow for more sensitive determination of interaction. Quantitative approaches for defining interaction given the observed single- and double-mutant phenotypes have been described (Baugh *et al*, 2005), but further refinements are possible. Finally, whereas previous large-scale interaction screens have focused on aggravating (also called enhancing or synthetic) interactions, much can be learned from alleviating (also called suppressing or masking) interactions.

Although estimating the impact of genetic interaction in an outbred population (of human or *C. elegans*) is not as

straightforward as Lehner *et al* suggest, it is already clear that many human disorders are caused by defects in multiple genes (Nadeau, 2003). A candidate gene approach—the educated guessing of potential disease genes—is required when the size of a human linkage or association study limits the statistical power to detect disease genes. Likewise, a candidate gene pair approach may be required to detect multiple loci that together cause a disease. Model organisms can provide specific knowledge of molecular pathways and of general principles that allow us to predict genetic interactions, which in turn can increase our power to detect the genes behind complex human diseases (Carlson *et al*, 2004).

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