Dude, Where’s My Phenotype? Dealing with Redundancy in Signaling Networks

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In the post-genomic era, our understanding of signal transduction networks necessarily entails the use of genetics analysis. Nowhere is this revealed more clearly than the use of publicly available knockout collections, which are plundered daily by plant researchers in search of mutants to test their latest signaling fantasies (Rhee et al., 2003). The lust for knockouts underscores the power of defined genotypes to dissect signal transduction, yet knockout lines often lead to frustration, as many mutants have no obvious phenotype. To the initiated geneticist, this fact is far from surprising since many signaling components are functionally redundant. The trivial explanation for functional redundancy stems from the rich sequence redundancy that eukaryotic genomes are built from—systematic analysis of *Saccharomyces cerevisiae* deletion strains suggests that about one-quarter of functional redundancy can be explained by compensation by duplicate genes (Gu et al., 2003). While sequence redundancy explains some functional redundancy, a “deeper” explanation stems from the pesky ability of networks to buffer the effects of perturbations in neighboring nodes and related pathways. This property, sometimes described as homeostasis, is difficult to predict from first principles (unlike the redundancy caused by sequence redundancy).

This problem does not mean traditional genetic analysis is waning. It simply means we need to squeeze more out of the classic equation Phenotype = Genotype + Environment. On one side of the equation, Phenotype is being refined through improvements in molecular analysis. In this context, this means that phenotype is in the eye of the beholder and that global transcript profiling of a mutant with “no observable phenotype” can yield enough information to place a gene into a signaling pathway or reveal the compensatory changes in related pathways that enable buffering. Again, we turn to *S. cerevisiae* for hard numbers—here greater than 95% of 300 gene deletions strains examined by whole-genome transcript profiling displayed effects on the transcription of at least one other gene besides the deleted gene (Hughes et al., 2000). Molecular fingerprinting as a phenotyping tool will undoubtedly continue to grow as high-throughput plant proteomics and metabolomics come on line (Zhu and Snyder, 2002).

On the other side of the equation, Genotype, which for most researchers encompasses single gene variation, needs to be prodded with a bit more gusto. In *S. cerevisiae*, for example, systematic genetic analysis has been attempted in which a mutation of interest is crossed to a large collection of deletion mutants to produce a synthetic genetic array (SGA) in which the phenotypic consequences of double mutants can be accessed (Tong et al., 2001). Plant biologists are beginning to construct lines containing multiple mutations in related genes to probe the functional redundancy caused by sequence redundancy, but the hurdles of plant breeding systems and diploid genetics make the scaling of this approach daunting (To et al., 2004). However, compared to other higher eukaryotes, the efficiency of Arabidopsis (*Arabidopsis thaliana*) transformation may be able to substitute for our inability to do mass mating on the scale of a yeast experiment. One productive strategy might be to use RNAi-based systems to create random assemblies of genes “stitched” together to inhibit disparate pathways simultaneously—think of SAGE meets RNAi. Ten or more genes could be stitched together randomly, transformed en masse into Arabidopsis, and the novel phenotypes generated could then dissected by deconvolution of the complex transgenes. Misexpressing multiple genes concurrently will similarly increase the phenotypic space available to probe signaling networks.

Manipulation of the third term, Environment, is seldom thought of by signaling researchers, although under duress we admit complex, natural, “real-world” environments may uncover previously hidden phenotypes. Another way to systematically prod the Environment term is through the application of chemical genetics. This technology normally involves phenotype-based screens of an organism against libraries of defined small molecules to identify compounds that perturb specific gene products (Stockwell, 2000; Blackwell and Zhao, 2003). Each compound in the library, however, can be thought of as a unique environment. Thus, unlike complex natural environments, 10,000 chemicals define 10,000 experimentally tractable environments in which to probe your genotype for novel phenotypes. Thus, an “aphenotypic” mutation

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may reveal an obvious phenotype in the right chemical context, which can be identified using a chemical genetic screen. Although we considered initially the case of an aphenotypic mutant isolated by reverse genetics, the application of this logic more broadly may lead to the identification of compounds that enable the Mendelization of loci that are otherwise quantitative in nature or difficult to score due to weak penetrance. Whatever the nature of the genetic variation probed using chemical genetics, the fruits of such an endeavor make small-molecule reagents useful for both exposing phenotype and unraveling the pathways that normally buffer a gene of interest. In essence, this strategy is a form of synthetic chemical genetics, used analogously to SGA in *S. cerevisiae*. Since the application of SGA in plants is hampered by technical difficulties, small molecules offer a powerful mechanism for sidestepping the problems of strain construction and add another weapon to the arsenal used by signal transduction researchers to expose phenotype and ultimately infer gene function using genetic analysis.

**LITERATURE CITED**


