Leading Edge
Essay

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Quantitative Genetic Interactions Reveal Biological Modularity

Pedro Beltrao,[1] Gerard Cagney,[1,2] and Nevan J. Krogan[1,*]
[1]Department of Cellular and Molecular Pharmacology, California Institute for Quantitative Biomedical Research, University of California, San Francisco, San Francisco, CA 94158, USA
[2]Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Ireland
*Correspondence: krogan@cmp.ucsf.edu
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Traditionally, research has been reductionist, characterizing the individual components of biological systems. But new technologies have increased the size and scope of biological data, and systems approaches have broadened the view of how these components are interconnected. Here, we discuss how quantitative mapping of genetic interactions enhances our view of biological systems, allowing their deeper interrogation across different biological scales.

In 1977, Charles and Ray Eames produced a short movie entitled "Powers of Ten," taking viewers on a journey through space that spanned many orders of magnitude, from the atom to the outer universe (http://www.powersof10.com). The journey can be a humbling experience. Quoting Carl Sagan: "We find that we inhabit an insignificant planet of a humdrum star lost in a galaxy tucked away in some forgotten corner of a universe in which there are far more galaxies than people."

Biomedical research has focused on a subset of the orders of magnitude explored by Charles and Ray Eames, from ecosystems (10^6 meters) to the atomic structure of biomolecules (10^{-10} meters). Although each of these orders of magnitude is typically explored with different sets of experimental tools, in nature they are intricately connected. For example, point mutations in proteins can lead to changes in signaling circuitry that can change species behavior (de Bono and Bargmann, 1998) with a potential impact on interspecies interactions. Meanwhile behaviors like algal blooms that create phenotypes visible from space are likely to be under genetic control (Erdner and Anderson, 2006). Still, biological research has largely focused on characterizing the components that make up systems of interest. Only recently, with the advent of systems biology, has the emphasis shifted toward integrative studies that aim to describe how observed biological phenomena depend on the interplay of these components. An increase in quantitative data and improvements in computational methods have led to the rise of models that, to some extent, can predict the nonintuitive behavior of biological systems at different scales. Examples of these include models of protein-binding affinities (Chen et al., 2008), signaling events in cell decision making (Santos et al., 2007), development (Bergmann et al., 2007), and homeostasis (Novák and Tyson, 2008).

In this Essay, we discuss one such method, quantitative genetic interaction mapping, and its application to the study of different scales of biology. In a tribute to "Powers of Ten," we journey from the whole organism to the atomic resolution of single amino acids.

Defining Genetic Interactions

The study of genetic interactions (or epistasis) has a strong theoretical basis in genetic linkage studies (Phillips, 2008). A genetic interaction between two genes implies that they impact each other's functions. Genetic interactions between two loci can be mapped by measuring how the phenotype of an organism lacking both genes (double mutant) differs from that expected when the phenotypes of the single mutations are combined (Figure 1A) (Mani et al., 2008; Phillips, 2008). The most commonly used neutral model assumes that the fitness of the double mutant is equal to the product of individual single mutant fitness. For example, if loss of gene A results in a growth rate 0.9 times the wild-type growth rate, whereas loss of gene B results in a growth rate of 0.8, then the expected growth rate of the double mutant (lacking genes A and B) would be 0.72 times that of the wild-type (Figure 1A). This neutral model assumes that two genes do not normally impact each other, and in fact, experimental observations support the intuitive idea that most genes do not interact (i.e., strong genetic interactions are rare) (Tong et al., 2001; Pan et al., 2004; Schuldiner et al., 2005). Cases where knocking out two genes causes a more deleterious effect than the fitness reduction expected from the combined loss of individual genes are referred to as *negative* or *aggravating* interactions (e.g., synthetic sickness) (Figure 1A) and often identify proteins that function in distinct but parallel pathways in a given process (Figure 1B). Alternatively, a double mutation can have a smaller than expected impact on fitness, and these cases represent *positive* or *alleviating* interactions (e.g., suppression) (Figure 1A).

We have shown that pairs of yeast mutants that display positive genetic interactions often indicate two proteins that act in the same pathway or are physically associated (Figure 1B) (Roguev et al., 2008; Collins et al., 2007). A possible explanation is that if removal of a component of a complex disables that complex, then deleting a second component would have no additional effect, resulting in an epistatic (i.e., positive) interaction (Figure 1A). Alternatively, deletion of one

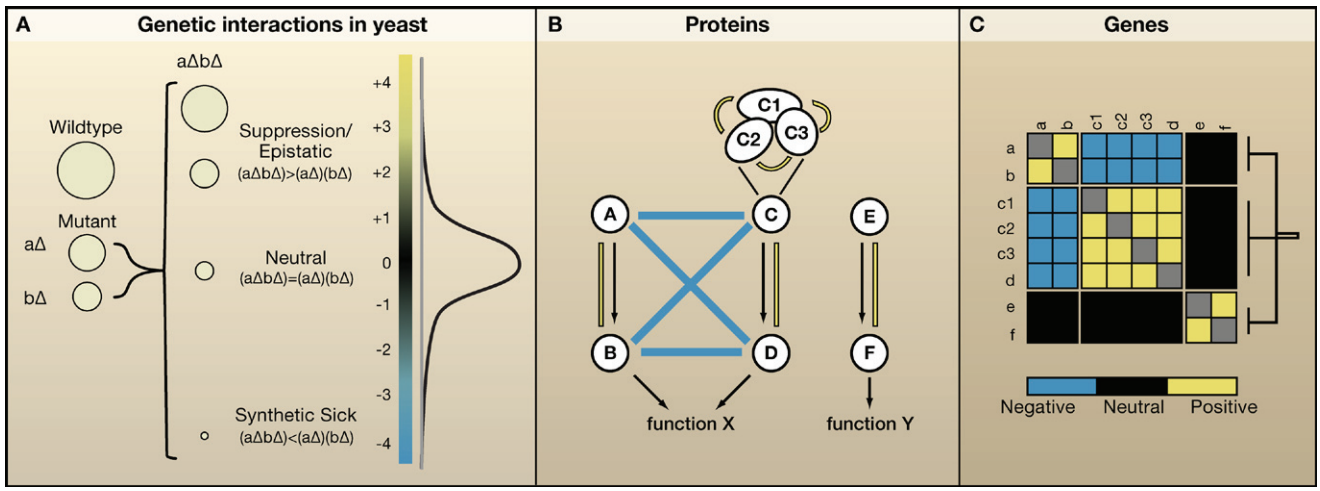

Figure 1. Genetic Interactions

(A) Genetic interaction scores in budding yeast determined by E-MAP screens. Scores range from negative (e.g., synthetic sickness) when fitness of the double mutant is less than expected, to positive (e.g., suppression) when the fitness of the double mutant is higher than expected. Most gene pairs have genetic interaction scores close to zero (i.e., neutral). Circles represent yeast colony size, a measure of fitness.

(B) In this hypothetical example, the pathway components E and F are required for function Y. Components A through D are important for function X, although the AB branch is redundant with the CD branch; C is a three-subunit complex. Both X and Y functions are important for yeast viability, but they are independent, and consequently no epistatic interactions exist between them.

(C) A matrix of genetic interactions for the pathway in (B). The branches AB, CD, and EF are enriched for positive interactions within each cluster. Additionally, the AB and CD branches are redundant and show an enrichment of negative interactions between them. Neutral genetic interactions are expected between the EF branch and the ABCD module given their independent contributions to fitness. The genetic interaction scores of each gene with all others form a phenotypic vector that can be analyzed using clustering methods. Hierarchical clustering of the expected genetic interaction scores for this example pathway is expected to result in three clearly distinct clusters (AB, CD, and EF). Genetic interactions alone would not distinguish between the C complex subunits (C1, C2, and C3) and D.

component of a complex could result in partial dysfunction of that complex with a detrimental effect on cell viability. If the removal of an additional component completely disabled this detrimental function, then the result would be a suppressive relationship, another type of positive interaction. Furthermore, if enough genetic interactions are collected for a set of genes, then each mutant engenders a genetic interaction profile, or phenotypic signature, representing how it genetically interacts with all other mutants tested. Comparison of these profiles is a powerful and unbiased way to identify genes that act in the same biochemical pathway (Figure 1C) (Pan et al., 2004; Schuldiner et al., 2005; Collins et al., 2007; Tong et al., 2004).

This multiplicative model is useful for quantitative measures such as growth rate but less so for complex phenotypes like cell morphology, necessitating alternative models of epistatic behavior (Mani et al., 2008). Here, our focus is on high-throughput quantification of genetic interactions, analysis methods, and their applications across different species and scales of biological organization.

Generating Genetic Interaction Maps

Genetic studies are traditionally subdivided into forward and reverse genetics. Forward genetics often defines a phenotype of interest and then identifies mutants that contribute to this phenotype. In contrast, reverse genetics starts with genes of interest and attempts to define their function through mutational analysis. In this context, genetic interaction screening can be defined as a form of reverse genetics.

The development of high-throughput genetic interaction screening was made possible by the creation of deletion libraries for single nonessential genes in the budding yeast *Saccharomyces cerevisiae* (reviewed in Boone et al., 2007). An important landmark was the first implementation, termed synthetic genetic array (SGA), where each *S. cerevisiae* single gene deletion strain was mated to produce arrays of double-mutant strains (Tong et al., 2001). This enabled the rapid qualitative assessment of synthetic lethal interactions for many thousands of gene pair combinations. An alternative approach, dSLAM (diploid-based synthetic lethal analysis with microarrays), detects genetic interactions using pools of barcoded yeast mutants (Pan et al., 2004). In this approach, genetic interactions are determined by the differential enrichment of double mutants growing in competitive culture as measured using barcode microarrays. Although in principle both methods can measure a range of epistatic effects, in practice they were used to identify synthetic sick or lethal (i.e., negative) interactions. The E-MAP (epistatic mini-array profile) strategy enabled colony size to be measured in an array format, thus quantifying genetic interactions in a high-throughput fashion (Collins et al., 2006; Schuldiner et al., 2005). The barcode approach has been adapted to provide a quantitative genetic score (Decourty et al., 2008), and a flow cytometry device has been developed that can quantify precisely very small epistatic effects (Breslow et al., 2008).

In parallel with genetic interaction screening for *S. cerevisiae*, screening methods using knock down of gene expression by RNA interference (RNAi) have been developed for the worm *Caenorhabditis elegans*. In this case, worm strains carrying a specific muta-