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Genetic Interaction Networks: Toward an Understanding of Heritability

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Abstract

Understanding the relationship between the genotypes and phenotypes of individuals is key for identifying genetic variants responsible for disease and developing successful therapeutic strategies. Mapping the phenotypic effects of individual genetic variants and their combinations in human populations presents numerous practical and statistical challenges. However, model organisms, such as the budding yeast *Saccharomyces cerevisiae*, provide an incredible set of molecular tools and advanced technologies that should be able to efficiently perform this task. In particular, large-scale genetic interaction screens in yeast and other model systems have revealed common properties of genetic interaction networks, many of which appear to be maintained over extensive evolutionary distances. Indeed, despite relatively low conservation of individual genes and their pairwise interactions, the overall topology of genetic interaction networks and the connections between broad biological processes may be similar in most organisms. Taking advantage of these general principles should provide a fundamental basis for mapping and predicting genetic interaction networks in humans.

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INTRODUCTION

In an era of high-throughput whole-genome sequencing, one of the biggest challenges in genetics remains our understanding of the relationship between the genotypes and phenotypes of individuals. Mendelian disorders, caused by single-gene mutations, account for only a small fraction of rare human diseases (see OMIM; **http://www.ncbi.nlm.nih.gov/omim**), and the genetic causes of more common and complex conditions remain largely unknown. The difficulty in understanding common diseases stems from the complexity of the human genome: Each individual carries ∼4 million genetic variants and polymorphisms (37, 52), the overwhelming majority of which cannot be pinpointed as the single cause for a given phenotype. Instead, the effects of genetic variants may combine with one another both additively and synergistically, and each variant's contribution to a quantitative trait or disease risk could depend on the genotypes of dozens of other variants. Interactions between genetic variants, along with the environmental conditions, are likely to play a major role in determining the phenotype that arises from a given genotype and may provide the key to explaining the missing heritability of complex traits (104).

The study of genetic interactions in human populations is extremely challenging owing to the difficulty of isolating the effect of each individual variant from the effects of thousands of other variants carried by the same genome. As noted by Lewontin (61) almost 40 years ago, "there is simply no way to make a large number of individuals identically homozygous or heterozygous at one locus while keeping the rest of the genome segregating at random" (p. 42). Highly controlled genetic analysis, however, can be performed in model organisms such as the budding yeast *Saccharomyces cerevisiae*, in which systematic mutagenesis projects and automated genetic techniques have enabled researchers to analyze the effects of alleles alone and in combination. Here, we summarize the state of the art of genetic interaction research in various experimental systems and describe how the lessons learned from model organisms grown in defined laboratory environments can help disentangle the complexity of genetic networks in natural populations, including humans.

DEFINING GENETIC INTERACTIONS

A genetic interaction occurs when an unexpected phenotype arises from the combination of two or more genetic variants. For example, two mutations that cause no fitness defect individually can produce an inviable double mutant, resulting in a genetic interaction known as synthetic lethality (14, 73, 92) (see below). More generally, a genetic interaction can be defined as the difference between an experimentally measured double-mutant phenotype and an expected double-mutant phenotype, the latter of which is predicted from the combination of the single-mutant effects, assuming the mutations act independently. However, predicting how independent mutations combine is not straightforward. For example, in an additive model, each mutation would be expected to contribute additively to the phenotype, such that, in the absence of genetic interaction, the double-mutant phenotype would equal the sum of the two single-mutant phenotypes (**Figure 1*a***). Alternatively, a multiplicative model would suggest that each mutation alters the phenotype by a specific fraction, such that, in the absence of genetic interaction, the double-mutant phenotype would equal the product of the two single-mutant phenotypes (**Figure 1*a***).

Which of these two models is more appropriate to describe gene-gene relationships has been long debated among geneticists and remains an unresolved question (66, 77). In principle, the choice between them should depend on the phenotype under consideration and the specific measurement scale, because, for example, two phenotypes may combine multiplicatively when measured on a linear scale but additively after a logarithmic transformation. In the case of fitness, for instance, the multiplicative model is preferred owing to evolutionary considerations: When a population has reached equilibrium, such that the frequencies of a set of alleles have stabilized

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and do not change over time, it will remain at equilibrium only if the fitness effects of these alleles combine multiplicatively (34, 99). Furthermore, the additive model would often predict negative fitness values for mutant combinations with fitness defects greater than 50%, and therefore it may not be relevant for some fitness-based measurement of genetic interactions. In practice, however, the two models often yield similar results when examining relatively healthy mutants that exhibit subtle fitness defects, which represent the vast majority of single mutants in yeast and likely in other organisms as well (7, 13, 21, 25, 46, 100). As a result, although we must be attentive to potential exceptions, the most extreme genetic interactions would be detected using either model, and the general topology of the genetic interaction network would likely remain largely unaffected.

NEGATIVE AND POSITIVE GENETIC INTERACTIONS

Based on the difference between the observed and expected double-mutant phenotypes, genetic interactions can be broadly divided into two major classes, which we refer to as negative and positive genetic interactions. Negative genetic interactions describe double mutants whose phenotype is stronger than expected (66, 77) (**Figure 1*b***). The most extreme example of a negative genetic interaction for fitness is synthetic lethality, in which the combination of two mutations, each of which causes little or no growth defect on its own, results in an inviable phenotype (14, 73, 92). Synthetic lethality and its milder variant, synthetic sickness (slow growth), often involve genes with at least partially overlapping functions that can compensate for each other's absence to support cell viability (11, 42, 73, 92) (**Figure 1*b***). For example, many members of the DNA damage-sensing and repair pathways are synthetic lethal with one another (33, 43, 75), possibly reflecting the importance of compensatory systems for maintaining the integrity of the genetic material.

Genes acting in the same linear pathway may also exhibit synthetic lethality if the pathway is essential and each mutation has a partial inhibitory (hypomorphic) effect on pathway activity (3, 7, 42) (**Figure 1*b***). For example, the discovery of synthetic lethal interactions among conditional partial loss-of-function mutations in a group of essential *SEC* genes helped to elucidate the structure of the post-Golgi secretory pathway and its relationship to other secretion-related processes (36).

Positive genetic interactions define double mutants whose phenotype is less severe than expected based on single-mutant phenotypes (30, 66, 87) (**Figure 1*c*,*d***). For example, genetic suppression is a positive genetic interaction that occurs when a mutation in one gene rescues the fitness defect associated with a mutation in another gene, such that the double mutant's fitness is greater than that of the sickest single mutant (**Figure 1*c***). Genetic suppression between loss-of-function mutations can link components of a given pathway to genes encoding negative regulators of the same pathway (7) (**Figure 1*c***). For example, growth defects associated with mutations in the *GCD1* locus, which encodes a negative regulator of amino acid biosynthesis upon starvation, are suppressed by mutations in the *GCN4* locus, a tightly regulated transcriptional activator acting downstream of Gcd1 (47). The suppressor may also carry a gain-of-function mutation that renders the pathway independent of an upstream component (**Figure 1*c***). An example is the yeast pheromone response pathway, which is normally triggered by the binding of a pheromone to a cell surface receptor, leading to activation of the coupled heterotrimeric G protein composed of Gpa1, Ste18, and Ste4 (68). Constitutive pathway inactivation, observed in *ste4* null mutants, can be suppressed by dominant mutations in *STE11*, which encodes a protein kinase acting in downstream signal transmission (86).

Another type of positive interaction, known as a coequal interaction, often connects genes encoding members of the same nonessential protein complex or linear pathway (53, 55, 83, 87, 88, 96) (**Figure 1*d***). In a coequal interaction, the phenotypes of the single mutants and the corresponding double mutant are quantitatively indistinguishable, presumably because the observed

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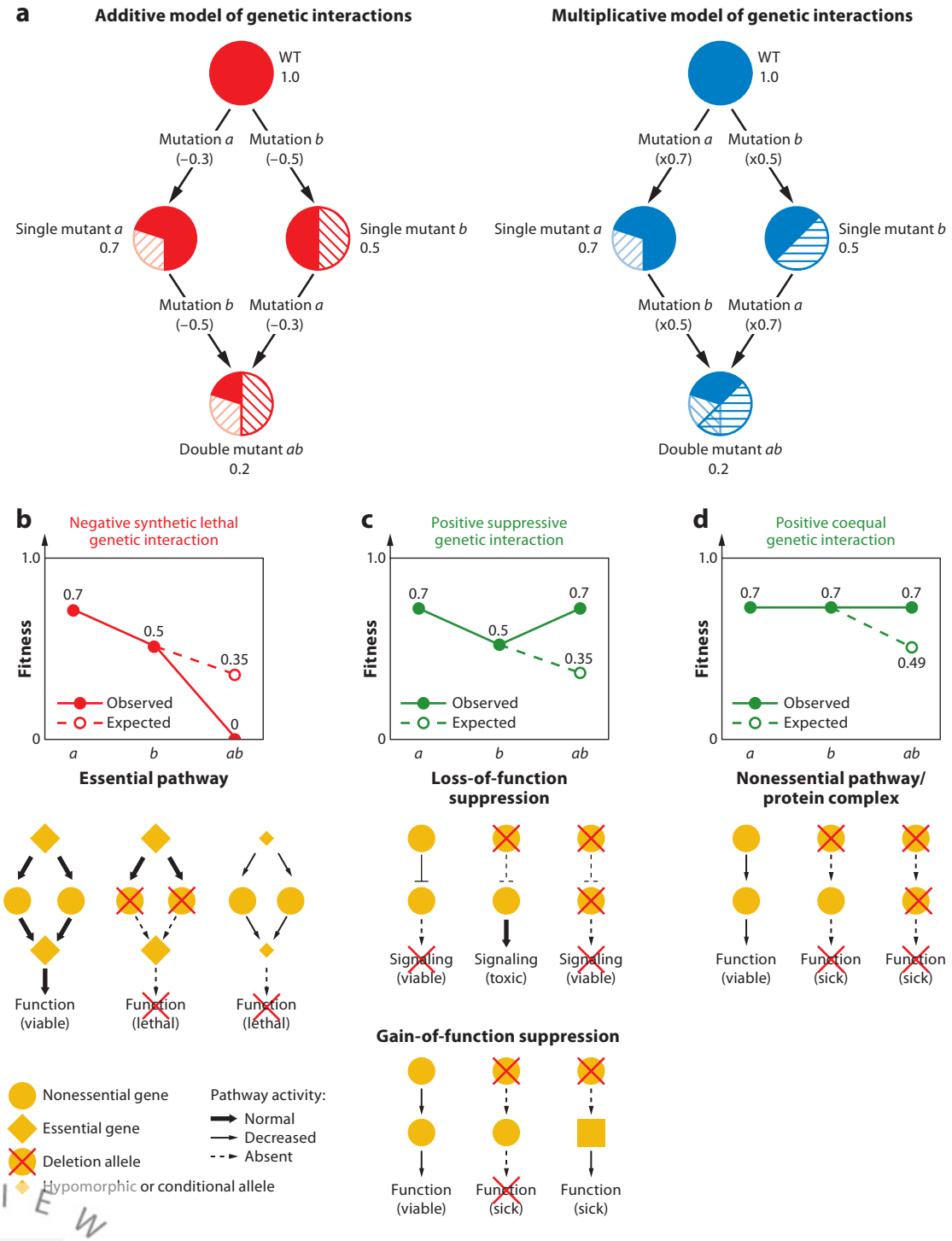

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phenotype is due to the complete loss of function of the pathway or protein complex, which can be achieved by removing any of its components, individually or in pairs (**Figure 1*d***). A systematic analysis of 26 genes involved in DNA damage repair showed that 9 out of 10 coequal interactions corresponded to physical interactions among the encoded proteins, including all 4 members of the well-characterized SHU complex (87).

MAPPING GENETIC INTERACTIONS IN YEAST

Owing to its facile genetics, the budding yeast *S. cerevisiae* has catalyzed the development of numerous genomic technologies, including methods for large-scale mapping of genetic interactions, and has played a primary role in deciphering the basic functional wiring diagram of the eukaryotic cell (12). Specifically, genome-scale mapping of genetic interactions requires three fundamental tools. First, large collections of mutant strains, carrying either gain- or loss-of-function alleles, are required to systematically perturb gene activity. Second, high-throughput methodologies must be available for combining mutations in a rapid, accurate, and comprehensive manner. Third, a phenotypic readout that is easily assayed in a high-throughput and quantitative manner is necessary to identify and measure genetic interactions. Below, we describe all three of these experimental components as they pertain to mapping genetic interactions in *S. cerevisiae*.

Mutant Strain Libraries

The yeast deletion collection is a library of mutant strains in which each known or suspected open reading frame has been deleted and replaced with a dominant drug-resistance marker (39) (**Figure 2*a***). This collection contains deletion strains for ~4,800 nonessential genes, available as haploids or homozygous diploids, as well as ~1,000 essential genes, which are required for viability under regular laboratory growth conditions and are maintained as heterozygous diploids. Molecular tags or bar codes (i.e., strain-specific 20-base-pair DNA sequences) were introduced at both ends of the deletion cassette and act as unique mutant strain identifiers (**Figure 2*a***). The presence of molecular bar codes enables assessment of mutant fitness within a pooled population using a bar-code microarray (40) or, more recently, high-throughput bar-code sequencing (85).

To enable the analysis of essential genes, the yeast community has also developed additional libraries of conditional mutants that complement the nonessential deletion collection. For

Figure 1

Defining genetic interactions. (*a*) Additive and multiplicative models of genetic interactions. According to the additive model, each of the two mutations (*a* and *b*) subtracts a certain quantity from the phenotype of the wild-type strain (WT = 1, $a = 1 - 0.3$, $b = 1 - 0.5$), such that, in the absence of genetic interaction, the expected double-mutant phenotype equals the sum of the two single-mutant phenotypes ($ab = 1 - 0.3 - 0.5 = 0.2$). The multiplicative model, in contrast, predicts that each mutation changes the phenotype by a specific fraction (WT = 1, $a = 1 \times 0.7$, $b = 1 \times 0.5$), such that, in the absence of genetic interaction, the expected double-mutant phenotype equals the product of the two single-mutant phenotypes ($ab = 1 \times 0.7 \times 0.5 = 0.35$). (*b*) Example of a negative synthetic lethal genetic interaction, whereby the observed fitness of the double mutant ($ab = 0.0$) is lower than expected ($ab = 0.35$). Synthetic lethal/sick genetic interactions often connect nonessential genes sharing compensating or partially overlapping roles in an essential biological pathway. In addition, synthetic lethality can occur between hypomorphic (partially functional) mutations of essential pathway components. (*c*) Example of a positive suppressive genetic interaction, whereby the observed fitness of the double mutant ($ab = 0.7$) is greater than expected ($ab = 0.35$) and greater than the sickest of the single mutants ($b = 0.5$). Genetic suppression among loss-of-function mutations can link genes to their negative regulators. Gain-of-function suppression can also occur between upstream and downstream components of the same pathway. (*d*) Example of a positive coequal genetic interaction, whereby the fitness phenotypes of the two single mutants and the double mutant are quantitatively indistinguishable ($a = b = ab = 0.7$). Coequal genetic interactions can occur between members of the same nonessential pathway or protein complex, such that the loss of one or two components of the pathway has the same effect on pathway activity.

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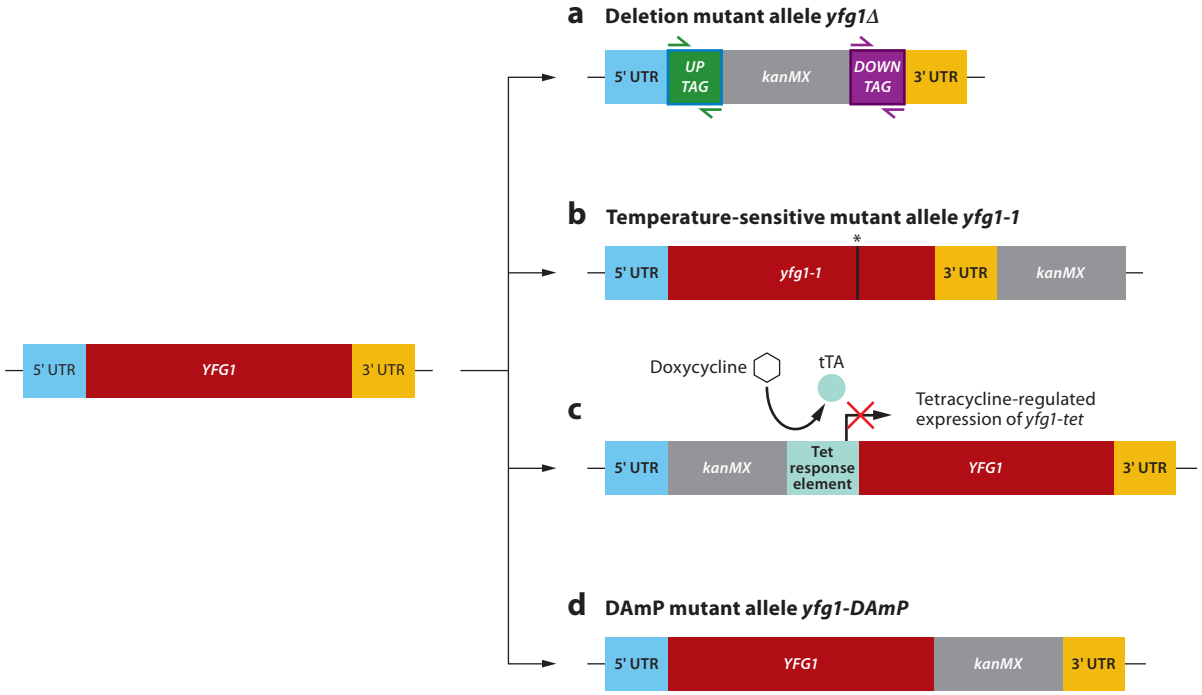

Figure 2

Yeast mutant strain libraries for large-scale mapping of genetic interactions. (*a*) The genome-wide deletion mutant collection contains ~6,000 mutant strains in which every open reading frame (e.g., *YFG1*) has been deleted and replaced with a dominant drug-resistance cassette (*kanMX*). Molecular tags or bar codes (*UPTAG* and *DOWNTAG*), consisting of strain-specific 20-base-pair DNA sequences, flank the deletion cassette and act as unique mutant strain identifiers. (*b*) In the collection of temperature-sensitive alleles of essential genes, each strain carries a point mutation (*asterisk*) linked to a selectable marker (*kanMX*). Point mutations alter protein stability at high temperatures and reduce protein function. (*c*) In the Tet collection, essential genes are expressed under the control of a tetracycline-regulated promoter (Tet response element), which normally activates gene expression in response to binding of the tetracycline transactivator protein (tTA). Tetracycline and its analogs (e.g., doxycycline), however, sequester tTA and prevent its binding to the Tet response element, thus turning off the expression of the downstream gene. (*d*) In the DAmP (decreased abundance by mRNA perturbation) collection, the selectable marker (*kanMX*) has been inserted in the 3′ untranslated region (UTR) of the gene, thus destabilizing its mRNA transcript and generating a hypomorphic phenotype.

example, temperature-sensitive alleles, in which a point mutation in the gene coding sequence alters protein stability at high temperatures and substantially reduces protein function, are available for ~65% of all essential genes (10, 62) (**Figure 2*b***). Conditional alleles of essential genes have also been generated by expressing the genes from a tetracycline-regulated promoter, which turns off gene expression in the presence of the tetracycline analog doxycycline (22, 71) (**Figure 2*c***). In addition, hypomorphic (partially functional) alleles with destabilized mRNA transcripts have been constructed systematically through the insertion of a selectable marker to displace the 3′ untranslated region of each gene (13, 81) (**Figure 2*d***).

Genetic Interaction Mapping Technologies

The availability of large-scale mutant strain collections prompted the development of high-throughput technologies for combining mutations and studying genetic interactions. The

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synthetic genetic array (SGA) was the first approach to automate classical yeast genetics and enable systematic construction of double mutants from ordered arrays of single mutants (6, 92) (see **Figure 3**). Methods complementary to SGA have also been developed; for example, diploid synthetic lethal analysis by microarray (dSLAM) takes advantage of the unique molecular bar codes associated with each deletion mutant to map synthetic lethal interactions within a mixed population of double mutants (64, 75, 76) (**Figure 3**). In this method, a pooled set of heterozygous deletion strains is mass transformed with a marked query mutation. Through the same selection steps as in SGA, double-mutant haploids are selected and their relative abundance is quantified by amplifying strain-specific bar codes and measuring their microarray hybridization intensities compared with a nonselected control pool (**Figure 3**).

In an SGA/dSLAM hybrid approach called genetic interaction mapping (GIM), double mutants are generated by mating and sporulation in a manner analogous to SGA; however, similarly to dSLAM, every step is performed in a pooled culture containing all yeast nonessential deletion strains (23) (**Figure 3**). Competitive growth of double-mutant meiotic progeny is followed by identification of quantitative genetic interactions via comparison of bar-code hybridization intensities between double mutants and a reference population.

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Phenotypes

All three approaches for mapping genetic interactions in yeast (SGA, dSLAM, and GIM) use cellular fitness as the primary phenotype. Fitness, defined broadly as growth relative to a reference strain in a given laboratory environment, is an excellent phenotypic readout because it can be measured easily and quantitatively and is amenable to high-throughput applications. Different methodologies measure fitness in different ways. In SGA, single- and double-mutant fitness estimates are obtained from measurements of colony sizes. High-density arrays of double mutants grown on agar plates are digitally photographed and analyzed using an image-processing algorithm that identifies the colonies and measures their areas in pixel units (6). dSLAM and GIM estimate fitness by measuring the hybridization intensities of mutant-associated bar codes, which are indicative of the relative abundance of double mutants in a population (23, 64, 75). The relative abundance of differently labeled mutant strains has also been quantified using fluorescence-activated cell sorting (13, 24). On a smaller scale, fitness has been measured by monitoring the optical density of a growing yeast culture over time and calculating its exponential growth rate, duration of lag phase, and saturation level (87).

Despite all the available measures of fitness, it is becoming increasingly evident that more-specific phenotypes are also necessary to obtain a complete picture of the cell's functional organization. An early indication came from the fact that ~50% of yeast deletion mutants with no visible fitness defect show abnormal cell morphology (74), suggesting that growth data alone are not sufficient to capture every aspect of a gene's functional role in the cell. Fortunately, technological advances in high-throughput microscopy and image-processing tools have made the analysis of cellular phenotypes achievable on a genome-wide scale and supported their integration with combinatorial genetic methods (51, 72, 74, 98). For example, high-content screening coupled with SGA enabled the investigation of mitotic spindle morphogenesis in single and double mutants and generated a genetic interaction network four times larger than the one derived from a similar fitness-based analysis (98).

THE YEAST GENETIC INTERACTION NETWORK

Despite being a relatively general phenotype, fitness has proved to be a powerful tool for mapping functional connections between genes. In one of its first applications, SGA was used to cross

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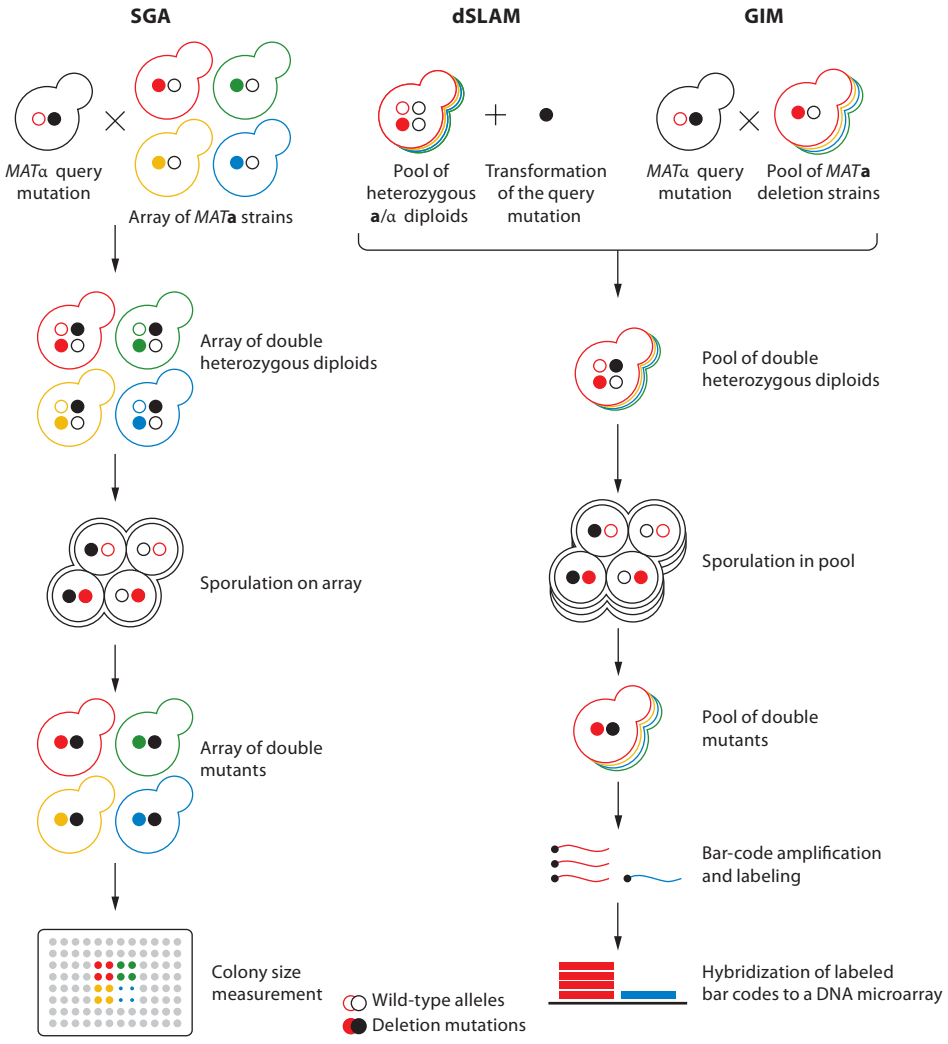
132 query strains to the complete array of ~4,800 haploid deletion mutants, resulting in a large-scale genetic interaction network consisting of ~1,000 genes and ~4,000 synthetic lethal/sick interactions (93). This network showed that synthetic lethality, despite being generally rare, tends to occur between genes that share similar biological functions and thus can be used to uncover novel functional relationships on a global scale (93).

To exploit this property, investigators have applied SGA to subsets of functionally related genes in order to optimize the recovery of genetic interactions in a smaller number of experiments. In this targeted approach, select query strains were crossed to arrays composed of several hundred deletion mutants known to be involved in specific biological processes, such as vesicle-mediated transport (81), chromosome biology (20), RNA processing (101), phosphorylation-mediated signaling (35), transcription (103), plasma membrane–related processes (1), and mitochondrial functions (48). Although this approach is useful for dissecting the inner workings of well-defined processes, targeted genetic interaction studies fail to uncover connections between diverse biological processes and result in a potentially biased view of the global topology of the genetic interaction network.



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An unbiased examination of genetic interactions was made feasible by the development of next-generation robotics, which dramatically increased the speed and throughput of SGA experiments. These technological advances were coupled with novel computational methods that enabled accurate processing of colony size data and extraction of high-quality quantitative genetic interactions (7, 21). As a result, a large-scale SGA study tested 1,712 query genes, including 334 conditional or hypomorphic alleles of essential genes, against the complete nonessential deletion collection, resulting in a total of ∼5.4 million gene pairs spanning all biological processes. This global genetic interaction data set, covering ∼30% of all possible pairwise gene combinations in yeast, generated ∼170,000 interactions (∼110,000 negative and ∼60,000 positive), providing an unprecedented view of the cell's functional organization (21) (**Figure 4**).

Early analyses of genetic interactions showed that a gene's genetic interaction profile (i.e., the set of genetic interactions associated with that gene) represents an informative phenotypic signature that can be used to assign function to uncharacterized genes and identify members of functional modules and specific pathways (93, 102) (**Figure 5*a***). For example, members of a pathway or protein complex tend to be synthetic lethal with the same genes from other pathways or protein complexes (93). Furthermore, common patterns of synthetic lethality are often more predictive of comembership in a protein complex than the direct genetic interactions themselves (102).

The availability of a large genome-wide data set has allowed the expansion of genetic interaction profile analysis to a global scale. Using the collection of ∼5.4 million gene pairs, Costanzo et al. (21) constructed a genetic profile similarity network in which genes with similar genetic interaction profiles were tightly connected and positioned close to one another, and genes sharing fewer genetic interactions were positioned farther away from one other (**Figure 4**). The resulting network revealed that genes acting within the same broad biological process are organized into large functional clusters and that the relative spatial positioning of the clusters is indicative of the functional interdependencies between the corresponding biological processes (21) (**Figure 4*a***). More-specific functional modules, such as individual molecular pathways and protein complexes, could also be discerned through inspection of the global genetic network at higher magnification (21) (**Figure 4*b–d*)**. This analysis showed that the wealth of functional information concealed

Figure 3

Genetic interaction mapping technologies. In a typical synthetic genetic array (SGA) screen, a *MAT*α mutant strain carrying a query mutation marked with the dominant drug-resistance marker *natMX4* (*filled black circle*) is crossed to an array of ∼4,800 viable *MAT***a** deletion mutants or conditional alleles of essential genes. Each array mutation is marked with a *kanMX4* drug-resistance cassette (*filled red*, *green*, *yellow*, *and blue circles*). Following mating, diploid selection, and sporulation, the meiotic progeny is robotically replica-pinned onto a series of selective media, enabling precise stepwise selection of double-mutant cells (6). As a result, an SGA screen produces an ordered array of double mutants, which can be scored for fitness through measurement of their colony size or assessed for a variety of other quantitative phenotypes. The diploid synthetic lethal analysis by microarray (dSLAM) and genetic interaction mapping (GIM) methods differ in their approaches to constructing a pool of double heterozygous diploid mutants, but are similar in the subsequent selection steps and phenotypic readout. In dSLAM, a query mutation (*filled black circle*) linked to the *URA3* selectable marker is introduced into the pool of haploid-convertible heterozygous diploid strains by high-efficiency integrative transformation. In GIM, a *MAT*α haploid query strain in which a specific genomic locus is replaced with a *natMX4* marker (*filled black circle*) is mated with a pool of viable *MAT***a** deletion mutants. Haploid double-mutant pools are selected after sporulation, and genomic DNA samples are isolated and used as templates for polymerase chain reaction amplification of the tags, during which they are labeled with fluorescent dyes. Competitive microarray hybridization of double-mutant and control single-mutant tags enables quantitative assessment of the abundance of double mutants in the pooled culture and therefore allows the identification of synthetic lethal/sick genetic interactions.

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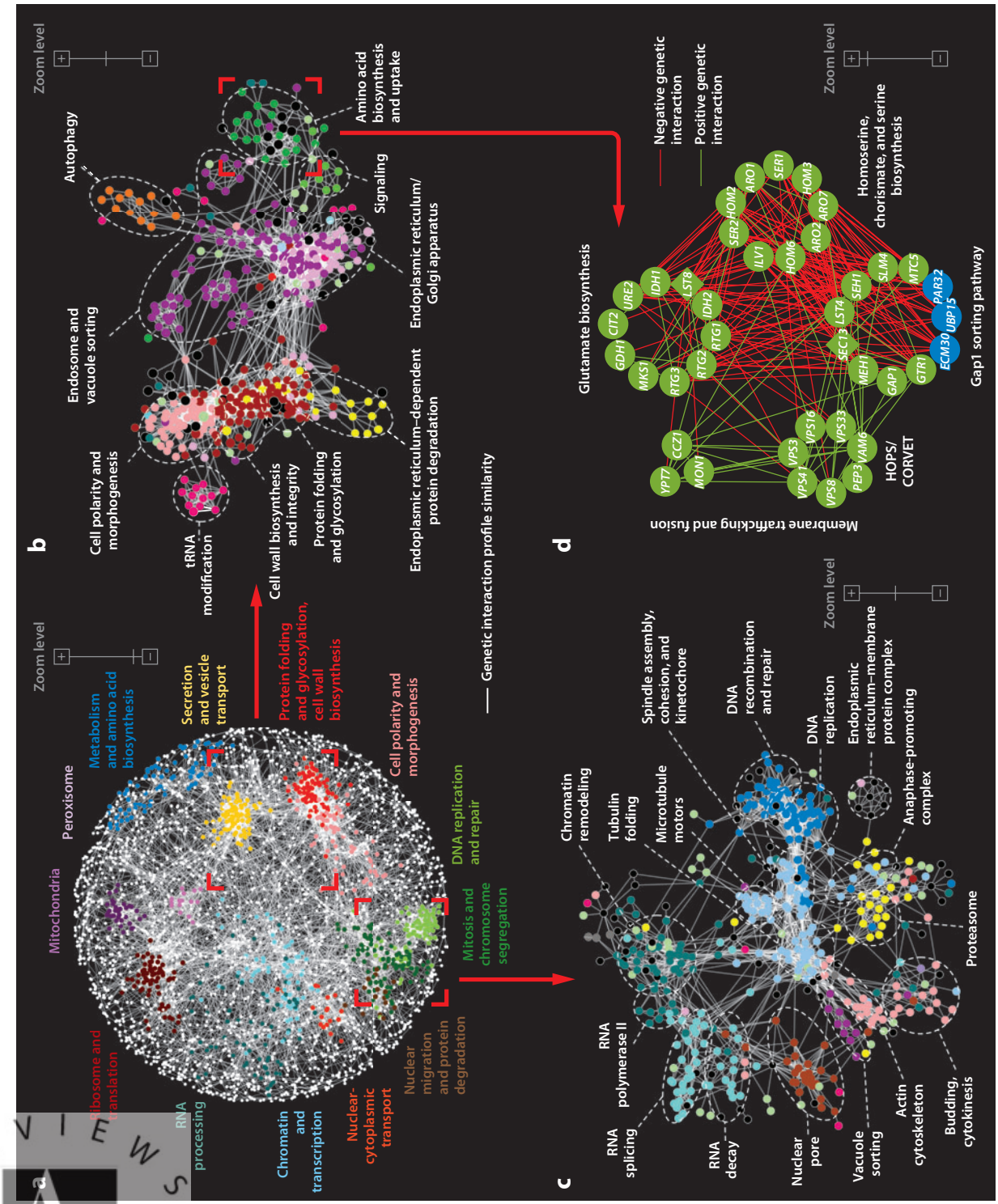

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within genetic networks at varying levels of resolution has the potential to assign a functional role to every gene in the genome as long as the gene responds to the tested experimental condition by showing a fitness defect in a range of genetic backgrounds.

YEAST AS A TOOL FOR UNDERSTANDING GENETIC INTERACTIONS IN COMPLEX SYSTEMS

The global yeast genetic network has revealed both the organization and the complexity of the constellation of genetic interactions (**Figure 4**). Estimates based on the current genetic interaction network indicate that a complete *S. cerevisiae* genetic interaction network may consist of as many as ∼200,000 extreme synthetic lethal/sick gene pairs (22, 93) and a smaller but comparable number of positive genetic interactions (21). These numbers clearly indicate that genetic interactions may significantly complicate the task of mapping genotypes to phenotypes in natural populations (45). For example, although in yeast there are ∼1,000 single-gene perturbations that result in a lethal phenotype (i.e., ∼1,000 essential genes), there seem to be at least ∼200-fold more digenic mutant combinations that result in the same phenotypic outcome. Moreover, the genetic structure of most phenotypes likely extends well beyond single-gene contributions or pairwise interactions and is governed by intricate networks involving multiple genomic loci. For example, most cases of conditional essentiality, in which a gene is essential in one yeast laboratory strain but completely dispensable in another, appear to be modulated by at least four modifiers (29). Similarly, yeast chemical-resistance traits are regulated by as many as 40–50 loci (32), with variation depending on the strain's genetic background and the trait under examination (31). These findings indicate that even an organism as simple as yeast has an intricate genetic architecture, and systematic mapping of pairwise genetic interactions may be revealing only the tip of the iceberg of its complex genetics.

Because the genetic variability of an organism increases with genome size, understanding the contribution of genetic interactions to the genotype-phenotype problem in higher eukaryotes represents an even more daunting task. Nevertheless, accomplishing this task should be facilitated by detailed examination of the general properties of the yeast genetic interaction network. For example, by integrating genetic interactions with other types of molecular data, we may identify rules governing the interdependence of genes in various cellular contexts and be able to predict which types of genes are more likely to exhibit genetic interactions in other organisms. Similarly, predictions can be made by comparing partial genetic networks in different model organisms and identifying rules governing conservation of genetic interactions. In the following sections, we review some of the properties of the yeast genetic interaction network that may facilitate the construction of similar maps in other organisms.

Figure 4

A functional map of the cell. (*a*) Correlation-based network connecting genes with similar genetic interaction profiles. Genetic interaction profile similarities were measured for all tested gene pairs using Pearson correlation coefficients. Genes (*nodes*) whose profile similarity exceeded a Pearson correlation coefficient of 0.2 were connected (*lines*) and positioned proximal to each other in two-dimensional space, using an automatic force-directed network layout. (*b*,*c*) Magnifications of the functional map, resolving cellular processes with increased specificity. Node colors correspond to specific biological processes; note that the color schemes are unique to each panel. (*d*) A further magnification of panel *b*, revealing modules corresponding to specific pathways and complexes connected by negative and positive genetic interactions. Subsets of genes belonging to the amino acid biosynthesis and uptake regions of the network were selected. Nonessential and essential genes (*circles* and *diamonds*, respectively) are represented as nodes grouped according to profile similarity; lines represent negative (*red*) and positive (*green*) genetic interactions. Characterized genes are in green, and genes with previously unknown function are in blue. Adapted from Reference 21.

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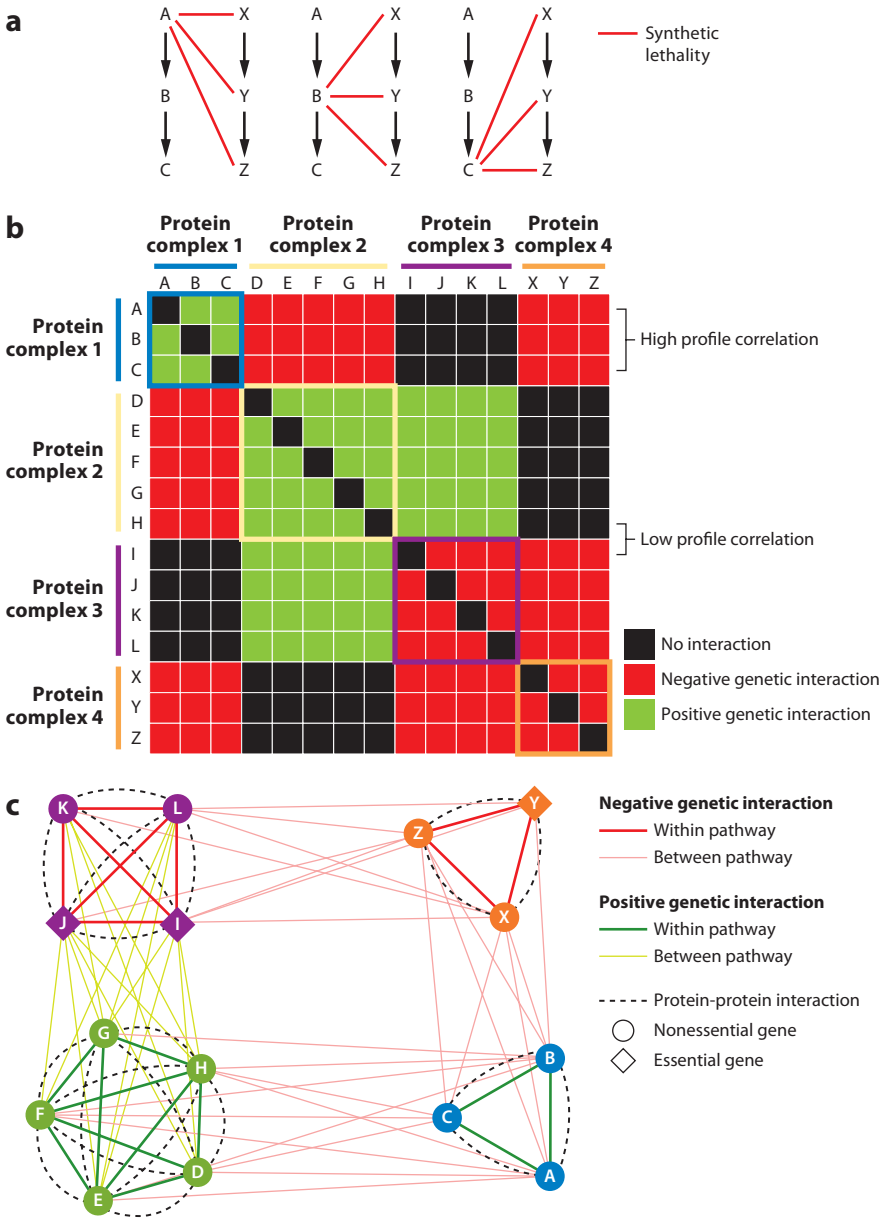

Figure 5

Modularity of genetic interaction networks. (*a*) Diagram illustrating that genes encoding members of the same pathway or protein complex tend to have the same genetic interaction partners. In this example, genes A, B, and C, acting in the same nonessential pathway, are consistently synthetic lethal with genes X, Y, and Z, acting in a parallel pathway. (*b*) Patterns of negative (*red*) and positive (*green*) genetic interactions, revealing an ordered yet incredibly complex structure. Negative and positive genetic interactions occur both within and between functional modules, such as molecular pathways and protein complexes. The similarity of the genetic interaction profiles, which measures the degree to which two genes share genetic interaction partners, represents a powerful tool for grouping genes according to their functional roles. (*c*) Network representation of genetic interactions from panel *b*, illustrating the modularity of genetic interactions and their relationship to protein-protein interactions (*dashed lines*). Adapted from Reference 26.

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PROPERTIES OF THE YEAST GENETIC INTERACTION NETWORK

Monochromaticity

The modular behavior of the genetic interaction network stems from the fact that genetic interactions tend to be shared by all members of a given functional module. As observed in early data on synthetic lethality, genes acting in the same pathway or protein complex tend to be synthetic lethal with the same set of genes in a parallel or compensating pathway/protein complex (93) (**Figure 5*a***). Similarly, all members of a given nonessential protein complex often share positive genetic interactions with one another, whereas members of essential protein complexes share negative genetic interactions (3, 7) (**Figure 5*b–c***). These properties of genetic interaction networks are referred to as between-pathway and within-pathway monochromaticity, respectively, to indicate that the same type or "color" of genetic interactions connects members of the same functional group (**Figure 5*b–c***). Since its original introduction in a theoretical study (82), monochromaticity has been extensively observed in experimental data (7, 21, 50, 70).

A systematic analysis of the yeast genetic interaction network showed that at least 70% of negative genetic interactions belong to monochromatic modules composed of nine or more gene pairs (8). Most of these interactions (∼85%) span different sets of genes, consistent with the between-pathway model of negative genetic interactions (8) (**Figure 5**). A smaller fraction (∼15%) connect genes within the same set and form clique-like structures. These within-pathway negative genetic interactions often correspond to groups of coregulated genes (8) or essential protein complexes (3, 7, 56), presumably because perturbation of at least two nonessential components is necessary to destabilize the function of an essential protein complex.

Among positive genetic interactions, only ∼20% can be assigned to monochromatic modules composed of nine or more gene pairs (8), which possibly reflects a greater challenge associated with the experimental detection of positive interactions and the resulting higher false-positive and false-negative rates (7, 21). As was also observed in more focused studies (20, 87), a subset (∼20%) of positive interactions assigned to monochromatic modules connected genes within the same module and were enriched for members of nonessential protein complexes (8) (**Figure 5*b–c***). However, large-scale genetic interaction studies revealed that the vast majority of positive interactions (∼80%) spanned between-pathway structures, suggesting that their role in connecting functionally distant processes is highly prevalent (8). Consistent with this hypothesis, analysis of a particular subtype of positive interaction, genetic suppression, showed that mutations in apparently very distant pathways can rescue one another's phenotypes (7). For example, fitness defects and sensitivity to UV radiation associated with DNA polymerase delta (Polδ) mutants can be suppressed by disrupting members of the conserved oligomeric Golgi (COG) complex, which is involved in maintaining the integrity and function of the Golgi apparatus (7). Understanding the mechanistic link between seemingly distant biological processes connected by positive genetic interactions will likely open a new perspective on the functional organization of the eukaryotic cell.

The Relationship Between Physical and Genetic Networks

Negative and positive within-pathway modules are enriched for protein complex structures containing essential and nonessential components, respectively (7, 8). However, the general overlap between the genetic and physical networks is fairly modest: Only 10–20% of protein-protein interaction pairs share a genetic interaction, and, vice versa, 0.9% of genetic interactions connect protein pairs that also physically interact (21). This relatively low overlap is consistent with the idea that protein-protein interactions form local connections between members of the same pathway or protein complex, whereas genetic interactions reflect the consequences of perturbing gene

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function and uncover much broader relationships between diverse functional modules. Although the relationship between the physical and genetic networks is not yet fully understood, these networks appear to be largely orthogonal to one another and contribute complementary information toward the mapping of gene-gene functional interconnections. As a result, several mathematical methods have been proposed to integrate physical and genetic networks in order to achieve more accurate functional predictions for uncharacterized genes (3, 56, 95).

Importantly, large-scale protein-protein interaction networks are being mapped for mammalian cells and other metazoan systems (9). Given the relationship between physical structures and genetic interaction networks in yeast, especially in the context of monochromatic modules, protein-protein interactions can be used as a skeleton for designing effective experimental strategies for the discovery of genetic interactions in complex systems.

Conservation of Genetic Interaction Networks

Cross-species comparison of genetic interaction networks has recently become possible thanks to the development of genetic interaction mapping technologies in several eukaryotic and prokaryotic organisms, including the fission yeast *Schizosaccharomyces pombe* (27, 38, 78, 79), the gram-negative bacterium *Escherichia coli* (16, 94), the nematode worm *Caenorhabditis elegans* (17, 60), and the fruit fly *Drosophila melanogaster* (49). Similarly to the methods used to analyze *S. cerevisiae*, these techniques emerged from the availability of large-scale collections of strains in which gene activity has been abolished or significantly reduced, as well as a variety of genetic tools for their manipulation. Strategies for systematic perturbation of gene function in mammalian cell cultures are also being developed (58) and have already been successful in mapping cell line–specific essentiality, which can be used for targeted cancer therapies (19, 65, 67, 84). However, combinatorial analysis of mammalian gene perturbations is still in its early days and has only recently enabled genome-wide profiling of pairwise genetic interactions (63).

Analysis of genetic interaction networks in *S. pombe* showed both differences and similarities compared with the *S. cerevisiae* network (27, 78). Despite hundreds of millions of years of evolutionary divergence, *S. cerevisiae* and *S. pombe* seem to share ∼30% of their genetic interactions, with the remaining 70% being species specific (27, 78) (**Figure 6*a***). At least some of the differences can be explained by unique gene functions, such as the RNA interference (RNAi) machinery, that are encoded in the *S. pombe* genome but not in the *S. cerevisiae* genome (89). In addition, a fraction of genes conserved in both genomes appear to have undergone functional repurposing, such that two orthologs, despite maintaining a high degree of sequence similarity, serve different or only partially overlapping roles in the two organisms (38).

In addition to examining individual gene pairs, comparative studies have assessed the conservation of broader patterns of genetic interactions, for example, at the level of functional modules and more general biological processes (79). A recent genome-wide study in *S. pombe* reported that, although only ∼30% of individual genetic interactions are conserved relative to *S. cerevisiae*, genetic interaction crosstalk between functional neighborhoods showed a much higher level of similarity (79) (**Figure 6*b***). Specifically, this study reported a significant correlation (Spearman rank $r = 0.72$) in the frequency of *S. pombe* and *S. cerevisiae* genetic interactions across pairs of biological processes, such as chromatin/transcription-related functions and chromosome segregation (79) (**Figure 6*b***). This level of agreement between the two species suggests that the major topological features of genetic interaction networks may be generally conserved and provide important insights into the functional organization of other organisms.

Similar experimental approaches have taken advantage of a deletion mutant collection in the bacterium *E. coli* (2) to uncover genetic interactions in a model prokaryote (16, 94). Despite the

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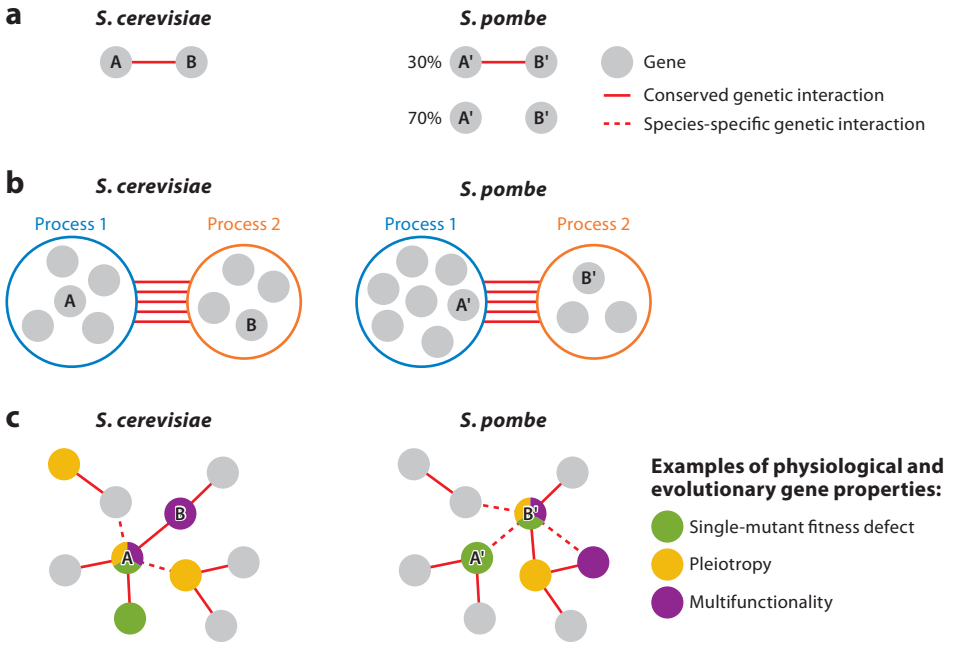

Figure 6

Conservation of genetic interaction networks. (*a*) Only 30% of genetic interactions involving orthologous gene pairs appear to be conserved between *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. (*b*) Despite relatively low conservation at the level of individual gene pairs, interactions between functional modules are more highly conserved. (*c*) Genetic interaction hubs in *S. cerevisiae* and *S. pombe*, although often involving nonorthologous genes, tend to have similar physiological and evolutionary properties; knowledge about one organism can thus be used to predict the degree of genetic interaction in the other.

paucity of available data and the lack of systematic comparisons to the *S. cerevisiae* network, preliminary results suggest that prokaryotic and eukaryotic genomes, despite their twofold difference in gene density, might have a similar degree of functional redundancy. For example, systematic interrogation of 39 nonessential genes in *E. coli* showed that each gene has ~20 synthetic lethal partners on average (16), which is comparable to the estimate of ~30 synthetic lethal interactions per nonessential gene in yeast (93). Furthermore, profiles of synthetic lethal interactions in *E. coli* are highly informative about a gene's biological function and, as in *S. cerevisiae*, enable grouping of genes into functional modules (16).

Multicellular organisms such as the nematode worm *C. elegans* (17, 60, 91) and cell lines from the fruit fly *D. melanogaster* (49) have also been assessed for genetic interactions on a large scale. High-throughput genetic analysis in worms and fly cells has been made possible by the availability of genome-wide RNAi libraries, which can reduce the abundance of specific transcripts (thus mimicking loss-of-function mutations) and which are scalable to high-level combinatorial approaches.

C. elegans is particularly suitable for RNAi-mediated genetic interaction studies because a gene's expression can be inhibited in the entire organism simply by feeding the worms on a bacterial lawn expressing the double-stranded RNA of interest. The first systematic study of genetic interactions in worms screened 37 query genes against ~1,750 individual RNAi molecules and identified 350 synthetic lethal interactions, many of which involved human disease orthologs (60). Although, similarly to *S. cerevisiae*, genes encoding chromatin regulators acted as network

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hubs in the *C. elegans* genetic interaction network, the overall degree to which individual genetic interactions are conserved between these two organisms is still unclear (17, 69, 90, 91).

Even though synthetic lethality was first discovered in *Drosophila* (14, 28), systematic combinatorial inhibition of gene function in the whole fly organism is much more challenging, and thus large-scale studies of genetic interactions have instead focused on cell cultures. In a recent report, an RNAi strategy was used to systematically map pairwise genetic interactions among 93 genes encoding signaling factors (49). Negative and positive genetic interactions were identified using several phenotypic readouts, including cell number, nuclear area, and fluorescence intensity of stained nuclei (49). Interestingly, different phenotypes seemed to uncover different functional relationships between genes, as only ~20% of the identified genetic interactions were common to all phenotypes under consideration. Consistent with yeast and bacterial studies, similar genetic interaction profiles were predictive of genes sharing similar biological functions, indicating that, as in all other organisms tested so far, genetic interactions will be instrumental for annotating uncharacterized *D. melanogaster*. Indeed, the analysis of genetic interaction profiles identified a novel activator of RAS-MAPK pathway signaling whose function is conserved from flies to humans (49).

The analysis of genetic interaction networks derived from a variety of organisms suggests that the general properties of genetic interactions, such as the connectivity of biological processes and the predictive power of genetic interaction profiles, are consistent across organisms, whereas the degree to which individual genetic interactions are conserved is much less clear. An important possibility is that, although individual genetic interactions are subject to evolution, the topological properties of genetic networks may be used to guide the discovery of genetic interactions in complex organisms.

The Structure and Topology of Genetic Interaction Networks

Topological analysis of genome-wide genetic interaction networks has shown that most genes have few genetic interactions, but a few genes are highly connected and act as network hubs (21, 93). This property is common to many biological networks—including, for example, the network of physical interactions between proteins—and seems to be a common rule of connectivity within the cell (5).

Interestingly, hubs in the yeast genetic interaction network share several fundamental properties. For example, there is a significant correlation between a gene's degrees of physical and genetic interaction, indicating that the same genes tend to be central in both networks (21). Being at the core of many physical and genetic connections, these genes are likely to be involved in primary cellular functions. Indeed, physical and genetic interaction hubs are overrepresented for essential genes as well as genes with severe fitness defects, and they show a wide range of phenotypes when mutated (21, 22, 54). Physical and genetic interaction hubs are also associated with many different functional annotations, indicating their tendency to be involved in a variety of cellular processes (21, 97). For example, many genetic interaction hubs act in chromatin remodeling and transcription-related functions, which are important for coordinating many cellular events and thus are expected to be genetically dependent on many different functions (21). Interestingly, transcription factors and chromatin regulators also appear to be central to the genetic interaction network in *C. elegans* (60) and *S. pombe* (79), suggesting that genetic interaction hubs may be generally conserved across organisms.

The clear association of genetic interaction hubs with a defined set of gene properties may be the key to discovering highly connected genes in other organisms and, consequently, to assessing the degree of conservation of genetic network topology (**Figure 6*c***). Using a combination of physiological and evolutionary gene properties, Koch et al. (57) built a computational model that successfully predicted genetic interaction degree for *S. cerevisiae* genes. Importantly, a model

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trained on *S. cerevisiae* gene features also accurately predicted genetic interaction degree in *S. pombe*, including the degree of genes with no *S. cerevisiae* orthologs, suggesting that many of the predictive relationships discovered in *S. cerevisiae* also hold true in an evolutionarily distant yeast (57) (**Figure 6*c***). Consistent with a previous study (78), this analysis also revealed that, although genetic network hubs in *S. cerevisiae* and *S. pombe* have similar properties, the hub genes themselves are not always conserved (57) (**Figure 6*c***). This finding supports the hypothesis that a substantial network rewiring occurred between *S. cerevisiae* and *S. pombe* (27, 38, 78, 79). Nonetheless, the ability to predict network hubs using a defined set of gene attributes supports the idea that network topology may be maintained across large evolutionary distances and suggests that a large-scale *S. cerevisiae* genetic interaction map may provide an orthology-independent reference to guide the study of similar interactions in more complex species.

GENETIC NETWORKS IN HUMAN HERITABILITY

One of the ultimate goals of studying genetic interaction networks is to shed light on the genetic basis of human phenotypic variation, identify genetic variants responsible for disease, and develop successful therapeutic strategies. This task is extremely challenging because, unlike in model organisms and cultured cell lines, standard genetic methods are not applicable in human populations; systematic genotype-to-phenotype mapping thus requires more sophisticated approaches.

Uncovering the genetic causes of human phenotypic variation is an extensive epidemiological and statistical endeavor, involving the design of sampling strategies, collection of health data, and development of new statistical analysis tools. The field has been dominated by genome-wide association studies (GWAS) in which genetic variants, identified by single-nucleotide polymorphism mapping and genome-wide sequencing efforts, are tested for statistical association with a particular phenotype. GWAS experiments have quickly and dramatically increased our knowledge of human genetics, linking more than 1,200 genetic variants to nearly 170 complex traits (59). However, only a few of these associations explain a substantial portion of trait heritability. For example, 71 genetic variants have been associated with Crohn's disease, a chronic inflammatory disorder of the gastrointestinal tract affecting ~0.1% of the North American population (104). However, only ~22% of Crohn's disease heritability is explained by the cumulative contribution of these 71 variants, indicating that our understanding of the genetic scaffold of this disorder is far from complete (104).

Several explanations for this missing heritability have been proposed, including environmental contributions, the influence of particularly rare and undiscovered genetic variants, and a failure to detect small genetic effects owing to the limited sample size of a typical GWAS (41). The penetrance of certain phenotypes may also be affected by stochastic changes in gene expression whereby the impact of a mutation is masked by a higher abundance of an ancestral duplicate or a promiscuous buffer gene (15, 18). Such stochastic variations in the abundance or activity of a cellular component have the potential to alter the phenotypic landscape associated with a particular genotype and might be comparable to the effect of environmental changes, which reorganize genetic networks in response to a physical or chemical perturbation (4).

Another potential cause of missing heritability is the presence of genetic interactions among the genetic variants that, when not accounted for, lead to an underestimate of the portion of heritability explained by the variants (104). Indeed, a model that incorporates the possibility for genetic interactions between the 71 genetic variants associated with Crohn's disease showed that these variants may explain as much as ~84% of the disease heritability, compared with ~22% when considering only additivity between variants (104). This possibility suggests that we might have already uncovered most of the variants necessary to explain heritable phenotypic variation, but more work is required to identify their genetic interactions.

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Evidence for the ubiquitous presence of genetic interactions in natural populations has been accumulating over the past several years. As mentioned above, in yeast, more than 3% of all gene pairs tested so far exhibit a detectable positive or negative genetic interaction for fitness (21), and this rate is substantially higher for more-specific phenotypes. Moreover, a comparative analysis of two closely related *S. cerevisiae* strains (S288C and Σ1278b) showed that conditional essentiality, in which a gene is essential in one strain's genetic background but not in the other, is almost always driven by two or more genetic modifiers (29). Being two variants of the same species, S288C and Σ1278b are >99% identical (80), just like two human individuals, suggesting that conditional essentiality and other strain-specific phenotypes are due to genetic interactions involving their individual sequence variants (29).

A simple conclusion from these observations is that genetic interactions must be taken into account when associating genotypes to phenotypes in natural populations. However, it has quickly become evident that this task presents enormous challenges. For example, analysis of the genetic architecture of Crohn's disease showed that as many as 500,000 subjects might be necessary to achieve sufficient statistical power to confidently detect nonadditive effects of genetic variants (104). Even if assembling such a large cohort of individuals were possible, it would enable only the detection of pairwise locus interactions, and traits dependent on three or more genetic variants would remain unexplained (104). We therefore must devise more elaborate strategies to overcome these statistical limitations.

An important tool at our disposal is the volume of knowledge accumulated through the extensive analysis of genetic interactions in yeast and other model organisms. For example, one of the most evident properties of genetic interactions is that they often connect genes encoding members of functional modules, such as molecular pathways or protein complexes (**Figures 4** and **5**). This information can be used to restrict the search space for significant genotype-phenotype associations and should result in the assignment of higher statistical confidence to the identified hits. Although the mathematical framework necessary to perform this kind of analysis is still being refined, preliminary results indicate that it is likely to be successful. In *S. cerevisiae*, for example, combinations of genetic markers responsible for specific patterns of gene expression were identified by clustering individual markers into coherent groups based on their genomic location and functional annotation of the corresponding loci (44). As a result, ∼4,700 marker pairs, originally identified as potentially affecting expression traits, were reduced to ∼200 interactions between protein complexes, which were biologically informative and appeared to regulate the expression of functionally coherent sets of genes (44). Given the promise of this and similar approaches to separate the true effectors of a particular phenotype from the rest of the potentially irrelevant genetic variation, the next important step is to apply similar strategies to human GWAS analyses.

In conclusion, given the importance of genetic interactions in determining the phenotype of an individual, understanding their general behavior is a fundamental goal in postgenomic biology. Although this task faces many experimental and statistical challenges in multicellular organisms, particularly in humans, it is theoretically and practically feasible in yeast, which will soon be the first organism with a complete pairwise genetic interaction network in at least one experimental condition. Taking advantage of the volume of knowledge obtained from decades of yeast genetics will provide a solid foundation for mapping genetic interaction networks in higher organisms.

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