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Integrating genetic and protein-protein interaction networks maps a functional wiring diagram of a cell

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Abstract

Systematic experimental approaches have led to construction of comprehensive genetic and protein-protein interaction networks for the budding yeast, *Saccharomyces cerevisiae*. Genetic interactions capture functional relationships between genes using phenotypic readouts, while protein-protein interactions identify physical connections between gene products. These complementary, and largely non-overlapping, networks provide a global view of the functional architecture of a cell, revealing general organizing principles, many of which appear to be evolutionarily conserved. Here, we focus on insights derived from the integration of large-scale genetic and protein-protein interaction networks, highlighting principles that apply to both unicellular and more complex systems, including human cells. Network integration reveals fundamental connections involving key functional modules of eukaryotic cells, defining a core network of cellular function, which could be elaborated to explore cell-type specificity in metazoans.

Mapping the global yeast genetic interaction network

Genetic interactions (GIs) appear to be important for understanding the relationship between genotype and phenotype. Although technological advances have enabled large-scale, whole genome sequencing, our ability to use this growing wealth of data to predict most inherited phenotypes, remains limited. Several explanations for this underlying “missing heritability” have been proposed, and evidence suggests that a failure to account for *epistasis* or GIs may be a significant component [1,2]. Originally, the term epistasis was coined to describe how the presence of a mutation can mask the effects of an allele at another locus. The term

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subsequently evolved to include GIs, in which a combination of two or more mutations yields an unexpected phenotype. In particular, GI analysis has been used productively in model system biology to provide a functional context for interpretation of biochemical experiments (reviewed in [3]).

The approach to exploration of GI networks in a particular model system depends on the ease of genetic manipulation, availability of mutant collections in a defined genetic background, and the ability to assay a phenotype in a systematic and scalable manner. GIs have been explored experimentally in several model microbial systems, including the budding yeast *Saccharomyces cerevisiae* (ref), *Escherichia coli* [4,5] and the fission yeast *Schizosaccharomyces pombe* [6,7], as well as in the *Caenorhabditis elegans* metazoan model system [8,9], cultured *Drosophila melanogaster* [10] and human cell lines [11–15]. However, the most extensive genetic network mapping experiments have used the *S. cerevisiae* model system, which has numerous large-scale collections of gene-specific mutants as well as high-throughput genetic methodologies that enable systematic exploration of GIs (reviewed in [16]). Indeed, a global yeast network provides a general view of the vast extent to which complex GIs can influence phenotypes and the relationship between genotype and phenotype (Fig. 1)[17,18].

A widely used method for assaying yeast GIs is Synthetic Genetic Array (SGA) analysis [19]. SGA automates yeast genetics and enables large-scale construction and selection of yeast double-mutant strains carrying precise mutations, including double-mutant combinations of deletion alleles involving nonessential genes or hypomorphic, or partially functional, alleles of essential genes. GIs are subsequently identified by measuring double mutant fitness from high-density arrays of yeast colonies [20], and quantitative analysis enables the discovery of both positive and negative GIs. Negative GIs correspond to synthetic lethal or sick interactions, where a double mutant shows a fitness defect greater than the expected multiplicative effect of the combined single mutant fitness phenotypes (Fig. 2A). Alternatively, positive GIs, which include masking or suppression interactions, are scored for double mutants that grow better than the expected model (Fig. 2A)(reviewed in [16]). Large-scale SGA analysis of the majority of all possible yeast gene pairs (~18 million) enabled the construction of the first comprehensive GI network for any organism, a global network consisting of nearly one million GIs (~550,000 negative and ~350,000 positive) [18,21].

A global genetic profile similarity network defines a functional map of a yeast cell

The set of negative and positive GIs for a given gene, called a GI profile, provides a quantitative phenotypic signature that is indicative of gene function. Genes belonging to similar biological processes tend to share numerous GIs in common, and genes encoding proteins that function together in the same pathway or protein complex often display highly similar GI profiles. A comprehensive network of genes connected by edges reflecting the similarity of their GI profiles predicts gene function and serves as a powerful, unbiased data-driven resource for organizing genes into functional modules (Fig. 3) [17,18,22]. For

example, at the most detailed level of network resolution, genes sharing many GIs in common are grouped together into relatively small, densely connected modules, which correspond to known protein complexes and biological pathways. At an intermediate level of network resolution, functionally-related pathway and protein complex modules are grouped together to highlight distinct biological processes. At the most general level of network resolution, bioprocess gene clusters group together into larger modules corresponding to specific cellular compartments. Thus, a global profile similarity network derived from fitness-based GIs can be used to infer protein-protein interactions (PPIs) and functional relationships between protein complexes to reveal a hierarchical model of yeast cell function (Fig. 3)[18].

General overlap between global genetic and physical interaction networks

The genetic accessibility of the budding yeast enables not only construction of arrays of yeast mutants, but also arrays of strains carrying tagged alleles, which allowed for systematic analysis of physical interactions, including protein-protein interactions (PPIs) [23–26] or protein-DNA interactions [27]. Because GIs capture the functional consequences of combining genetic perturbations, they are highly complementary to information derived from PPIs, which identify physical interactions among gene products. However, since the phenotypic consequence of a mutation is not constrained by physical connections, not only is the global GI network much larger than the global PPI network but the direct overlap between the two networks is modest. In fact, less than 0.4% of directly interacting pairs from the global GI network also show a corresponding PPI [17,18]. Nonetheless, as described below, examining the genetic networks connecting protein complexes and pathways has revealed fundamental, composite network principles and functional information that could not have been deduced from either network alone.

Gene essentiality impacts the relationship between genetic and physical interactions

Essential genes provide important insight into the relationship between GIs and PPIs [17,18,28]. The GIs for essential yeast genes were largely mapped using conditional, temperature-sensitive (TS) alleles, scoring double mutants for GIs at a semi-permissive temperature [17,18]. The essential gene GI network displays a number of unique features. For example, essential genes participate in ~5-fold more negative and positive GIs than nonessential genes, and GIs tend to occur most frequently between pairs of essential genes [18]. Thus, just as essential proteins are often hubs in PPI networks [29], essential genes tend to be hubs in the GI network [18]. Importantly, since essential genes are more conserved than non-essential genes [30], and participate in a large fraction of the interactions on the global yeast genetic network, they may define a GI network that is generally conserved.

Negative GIs involving essential genes often occur among genes within a specific protein complex or pathway (Fig. 4)[18]. In fact, ~50% (~10-fold enrichment) of essential gene pairs that encode physically interacting proteins are connected by a negative GI (Fig. 4A). In contrast, only ~5% (~2-fold enrichment) of nonessential gene pairs whose products

physically interact also show a negative GI (Fig. 4A) [18]. This enrichment for negative GIs among essential gene pairs that overlap PPIs is even more striking when only interactions between genes encoding proteins within the same essential protein complex are considered (Fig. 4B). In fact, ~80% of essential protein complexes are enriched for negative GIs among their members [18]. This remarkable relationship suggests that PPIs encoded by essential gene pairs and essential protein complex membership in yeast should be highly predictive of GIs among orthologous genes in other cells.

Unlike negative GIs, positive GIs associated with yeast essential gene pairs appear to capture more general regulatory relationships related to cell cycle progression, or mRNA and protein turnover [18]. For example, TS alleles, which may lower protein levels or reduce protein activity, often show positive GIs with genes involved in protein degradation [18]. In addition, so-called DAmP alleles of essential genes, which contain an insertion in 3'UTR regions that can lower mRNA levels [31], tend to share many of their positive GIs with genes involved in mRNA turnover. Moreover, a recent comprehensive study of the yeast chaperone system found a significant bias toward positive GIs between essential genes and a specific subset of chaperone genes [32]. These more general regulatory connections translate to relatively few overlapping connections between PPIs and the positive GIs among essential gene pairs (Fig. 4).

However, while a positive GI involving essential genes does not typically overlap with a PPI (Fig. 4) [18], a relatively small subclass of the strongest positive GIs, known as genetic suppression interactions, are enriched for genes encoding interacting proteins. Genetic suppression occurs when the phenotype associated with one mutation is rescued by mutation of a second gene (Fig. 2A). Importantly, these specific suppression interactions often involve precise alleles rather than relatively severe loss-of-function alleles, although these interactions can happen and represent a few of the extreme SGA positive interactions. Systematic analysis of suppressors of yeast fitness defects identified a subset of strong positive genetic suppressor interactions that was highly enriched for genes encoding proteins in the same pathway or complex [33]. In fact, apparent causal suppressive mutations occurred at a PPI interface nearly half the time [33].

Unlike essential gene pairs, nonessential gene pairs whose products exhibit a PPI often exhibit a positive GI (Figs. 4–5)[18]. However, the underpinnings of this relationship are quite distinct - the enrichment of PPIs among non-essential gene pairs that have a positive GI reflects the fact that deleting a gene within a nonessential complex usually leads to a null phenotype for that complex. As a result, a double mutant that is deleted for two genes within the same complex fails to show a more exaggerated phenotype than the corresponding single mutants and is scored as a positive GI under the multiplicative model (Fig. 2B)[16]. Notably, while PPIs between nonessential proteins are significantly enriched for overlap with positive GIs, these account for only a small fraction of all positive GIs in the nonessential gene network [17,18].

Genetic interactions occur within and between functional modules

GIs are often organized into clear coherent subsets of interactions, either all positive or all negative, that connect gene pairs within functional units. In particular, genes encoding proteins within the same complex tend to be connected to one another by a single type of GI, positive or negative, forming a network structure referred to as a “within pathway” model or module (WPM) (Fig. 5A)[18]. Alternatively, genes within the same pathway or complex can also be connected to genes belonging to another complex or pathway by a single type of GI, positive or negative, a network structure referred to as a “between pathway” model or module (BPM) (Fig. 5B). These general observations validate theoretical studies predicting that GIs within the same functional module or biological process would commonly be connected by coherent subsets of GIs, a network property termed “monochromaticity” [34]. Indeed, within the global yeast genetic network, genes encoding hundreds of different pairs of protein complexes are connected by coherent sets of negative or positive GIs organized into WPMs and BPMs [18]. Similar to the relationship observed with protein complexes, the type of genetic interactions that comprise a WPM depends, in part, on whether genes in the functional module are essential or nonessential [17,18,20,28]. Indeed, members of a WPM comprised of mostly essential genes tend to be connected by negative GIs, emphasizing a cell’s inability to tolerate multiple perturbations in the same essential functional module [17,18]. Importantly, because the structure and essentiality of fundamental functional modules (e.g. the proteasome) is highly conserved, WPM negative GIs will often be conserved from yeast to human cells [35].

A network comprised of both negative and positive BPMs maps a highly organized functional wiring diagram of a cell or organism. A BPM connected by negative GIs identifies pairs of modules that are functionally connected and impinge on a common essential cellular role. A BPM connected by positive GIs often identifies sets of masking or suppressor interactions, in which mutations in genes in one module bypass the growth defect associated with mutations in genes in another module [18,33]. BPMs are observed among functional modules that can be composed of both essential and nonessential genes, and a global network of BPMs maps biologically relevant functional connections between these units. From an integrated network perspective, the BPM structure identifies GIs that do not overlap with PPIs, which encompass the vast majority of GIs on the global yeast genetic network (Fig. 5).

The WPMs and BPMs associated with the 19S proteasome provide clear examples. Genes encoding the 19S proteasome components are essential and connected to each other by a coherent set of WPM negative GIs. Moreover, the 19S proteasome genes are connected through BPM negative GIs to the APC (anaphase promoting complex), and through BPM positive GIs with the COG (conserved oligomeric Golgi) complex genes (Fig. 5C)[18]. Across the global yeast genetic network, one study estimated that as many as ~70% of negative GIs occur as WPMs or BPMs, suggesting that this type of local clustering of GIs is the rule rather than the exception [36]. Another probabilistic approach that integrated PPI data assigned ~40% of negative interactions to WPM and BPM structures [37]. Taken together, these observations support the idea that PPIs capture specific biochemical

relationships that define the physical architecture of functional modules, while GIs identify functional relationships between them [38].

Intriguingly, the general WPM and BPM genetic network structure may be relevant to the study of GIs underlying genotype to phenotype relationships in human population data, including genome-wide association studies. For example, the prevalence of GIs occurring in WPM or BPM structures can be used as prior knowledge to improve the statistical sensitivity of approaches for measuring potential GI maps from human population genetic data [39]. Thus, with the knowledge of pathways and complexes in human cells, often largely defined as PPIs and other biochemical connections, it may be possible to trace natural genetic variation bridging subsets of genes, corresponding to a specific WPMs or BPMs, to disease risk or protection.

The conserved relationships between genetic and physical interactions

Comparative analyses have uncovered some GIs that are conserved from yeast to higher organisms [6,7,9,35], typically in highly conserved WPM or BPM structures, such as those associated with DNA replication and chromosome integrity [40,41]. However, the global extent of this conservation of individual GIs remains unclear, and phenotypes assayed for interactions in higher organisms may not be directly comparable to those used in single-celled organisms and in cell culture systems.

However, both computational and experimental studies have revealed that the general properties of GIs are conserved at the level of network structure and topology. For example, several physiological and evolutionary properties associated with gene connectivity in the global budding yeast genetic network were predictive of GIs in the fission yeast genetic network [42]. Extensive mapping of GIs in *S. pombe* revealed that GIs found in WPMs and BPMs are more highly conserved from budding to fission yeast [43,44]. Similarly, a comparison of functional networks derived from *S. cerevisiae*, *S. pombe*, *C. elegans* and *D. melanogaster* demonstrated that conservation of biological interactions detected between species is more significant at the level of WPMs and BPMs, as compared to conservation at the level of individual genes or proteins [45].

The ability to predict functional relationships and protein complex membership based on GI profile similarity also appears to be a general feature of genetic networks. For example, GI analysis involving ~1,400 *Drosophila* genes spanning core cellular processes such as transcription, translation, protein degradation and cell cycle control showed that GI profile similarity between gene pairs encoding physically products was higher than between gene pairs encoding non-interacting proteins [10]. Another study examined GIs between ~80 human genes implicated in ricin resistance and found that, similar to studies in yeast, GI profiles were predictive of PPIs [12]. Finally, genes within the same module corresponding to a stimulated cell communication pathway appear to share more similar GI profiles than genes belonging to different modules [46]; however, more systematic comparisons with physical interactions requires more complete PPI data.

Integrating genetic and protein-protein interaction networks in human cells

Recent technological developments, including transposon-mutagenesis combined with human haploid cell lines [47] and CRISPR (clustered regularly interspaced short palindromic repeats)-based gene editing methods [48], have enabled large-scale, systematic GI studies in a number of different organisms and human cell model systems, which could only have been imagined a few years ago. At the level of a single genetic perturbation, genome-wide, loss-of-function genetic screens have defined both a “core” set of ~1500 genes that are each essential for viability across all human cell lines tested as well as genes specifically required for viability in only a few cancer cell lines [11,49–53]. These “context-specific” essential genes may result from negative GIs that illuminate cancer cell line-specific dependencies on certain functional modules, signaling pathways or biological processes. Thus, due to cell line-specific GIs, genes belonging to the same pathway may show similar patterns of essentiality in the same subset of cell lines [49,50,53]. For example, comparison of gene essentiality profiles showed that three genes, *NFE2L1*, *NGLY1* and *DDI2*, were required for viability of the same set of cancer cell lines, suggesting that these genes share similar genetic interactions and may work together as part of the same functional module [52]. Similarly, studies in *C. elegans* showed that *NGLY1*, *NRF1* and *DDI2* work together as part of a proteasome recovery response (PRR) pathway that functions to transcriptionally upregulate the bounce-back response of genes encoding proteasome components in response to proteotoxic stress [54].

More recently, strategies for CRISPR-based combinatorial loss-of-function screens have also been developed to facilitate GI analysis in human cells by targeting pairs of genes simultaneously. While genome-scale application remains technically challenging, most analyses to date have focused on relatively small subsets of genes to: [1] identify GIs among panels of cancer genes revealing potential targets for synthetic lethal-based cancer therapy [13,15]; [2] explore genes encoding known drug targets to identify opportunities for combinatorial therapies [12] and; [3] chromatin regulatory factors, which provided preliminary evidence to suggest that, similar to yeast, human protein complexes also appear to be connected by coherent sets of either negative or positive interactions [14].

Genome-scale CRISPR-Cas9-based GI mapping strategies will ultimately enable the integration of comprehensive GI and PPI networks in human cells. While there are many different human cell types, and their specific biology will undoubtedly lead to cell-type specific GIs, we anticipate that an extensive core GI network will be common to all cell types, just as there appears to be a common set of core essential genes [11,49–51]. Thus, at the GI network level, cell-type specificity could be viewed as an elaboration of a core genetic network. The comprehensive mapping of the core GI network and the integration of this information with large-scale proteomic [55,56] and other biologically relevant networks [57,58] should reveal the most basic functional wiring diagram of a human cell, organizing the fundamental connections among key functional modules, which could be further expanded upon for a detailed analysis of cell-type specificity.

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Figure 1. Global yeast genetic interaction profiles similarity network.

(A) A global genetic profile similarity network encompassing all nonessential and essential genes was constructed by computing Pearson correlation coefficients (PCCs) for genetic interaction profiles of all pairs of genes (nodes). Gene pairs whose profile similarity exceeded a $PCC > 0.2$ were connected and graphed using a spring-embedded layout algorithm. Genes sharing similar genetic interactions profiles map proximal to each other, whereas genes with less similar genetic interaction profiles are positioned further apart. (B) Network regions enriched for specific GO biological process terms are colored. Adapted from [18].

Figure 2. Quantitative definition of a genetic interaction

Under a multiplicative model, the expected fitness of a double mutant (relative to wild-type) is the product of the fitness of each single mutant. If the observed fitness of the double mutant falls below this expectation, genes X and Y share a negative GI (blue), while if double mutant fitness exceeds expectation, the interaction is positive GI (yellow). (A) Negative GIs include synthetic lethal or synthetic sick interactions. Positive GIs include masking or suppression GIs. (B) If the two single mutants (X and Y) exhibit the same defect in and the resultant double mutant shows the same fitness defect as the two single mutants, it is scored as a positive genetic interaction, which is often the case for nonessential genes in the same protein complex.

Figure 3. A functional map of a yeast cell

The global yeast genetic interaction profile similarity network, in which genes are linked closely to one another if they share a highly similar genetic interaction profile is organized as a hierarchy of functional modules, enriched for specific cellular compartments, biological processes or protein complexes.

A

B

Figure 4. Relationship between GIs and protein complexes

(A) Fold enrichment for physically interacting proteins (PPIs) among negative (blue) and positive (yellow) GIs connecting pairs of nonessential or essential genes. (B) (Left panel) The percentage of nonessential and essential complexes enriched for GIs within a complex. GIs are biased towards negative (blue) or positive (yellow) interactions. Black dashed lines indicate the background rate of coherent genetic interaction enrichment within individual complexes or between pairs of complexes. (Center panel). GI enrichment evaluated on complex-complex pairs. (Right panel) Schematic summary of GIs occurring within and between essential and nonessential protein complexes. Adapted from [18].

Figure 5. Within- and Between-Pathway Models of GIs

(A) Within-pathway model (WPM). (i) A single mutation in a pathway (or complex) does not result in an extensive phenotype, but simultaneous mutation of two genes within the same pathway results in an extreme GI. Perturbation of any pair of genes within the same pathway results in a similar GI phenotype. (ii) Negative WPMs identify genes that belong to the same essential pathway or complex while positive WPMs often identify nonessential pathways or complexes. (B) Between-pathway model (BPM). (i) Two biological pathways (or protein complexes) impinge on the same cellular function. Mutations in individual

pathways do not result in a phenotype, but mutations in both pathways result in a GI. (ii) Any combination of mutations in both pathways will result in the same type of GI, either negative or positive. (C) Coherent WPM and BPM genetic interactions involving the 19S proteasome. (i) Protein complexes that show coherent negative or positive WPM or BPM GIs with the 19S proteasome are placed on a schematic representation of the global GI profile similarity network based on the average genetic interaction profile similarity of the complex and connected with negative (blue) or positive (yellow) edges, respectively. (ii) Genes belonging to a subset of protein complexes that showed coherent negative (blue) or positive (yellow) GIs with genes encoding the 19S proteasome. Adapted from [18].

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