Exploring genetic interactions and networks with yeast

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Abstract | The development and application of genetic tools and resources has enabled a partial genetic-interaction network for the yeast *Saccharomyces cerevisiae* to be compiled. Analysis of the network, which is ongoing, has already provided a clear picture of the nature and scale of the genetic interactions that robustly sustain biological systems, and how cellular buffering is achieved at the molecular level. Recent studies in yeast have begun to define general principles of genetic networks, and also pave the way for similar studies in metazoan model systems. A comparative understanding of genetic-interaction networks promises insights into some long-standing genetic problems, such as the nature of quantitative traits and the basis of complex inherited disease.

Synthetic enhancement
The situation in which a mutation in one gene exacerbates the phenotypic severity of a mutation in a second gene.

Synthetic lethality
The situation in which two genes that are non-essential when individually mutated cause lethality when they are combined as a double mutant.

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Genome sequencing and large-scale genetic analyses have unmasked the enormous scale of genetic interactions in biological systems[1,2]. A key challenge now is to understand how genes function as networks to carry out and regulate cellular processes. Many recent insights into genetic interactions and networks have emerged from studies using the yeast *Saccharomyces cerevisiae*, in which powerful functional genomic tools allow systematic analyses[3,4], revealing both novel interacting components and key properties of the genetic networks in which they participate. A general understanding of the topology of genetic-interaction networks, which is rapidly being gained for yeast, has a wider importance, because similar networks are expected to underlie the relationship between genotype and phenotype in outbred populations in which combinations of specific alleles determine the fitness of individuals. In terms of human disease, numerous modifiers and enhancers contribute to complex genetic disorders, but the topology of the underlying networks is largely unknown. Thus, mapping genetic networks in model organisms such as yeast provides an important framework for studying genetic interactions in more complex systems.

Here we provide a detailed discussion of the tools that have allowed genetic interactions to be so extensively mapped in *S. cerevisiae* and the insights that they provide into the structure and function of genetic networks in this organism. We then examine how this knowledge can be applied more widely to gain an understanding of gene networks in complex traits, including human disease.

Enhancement genetics: synthetic lethality

Large-scale genetic analyses reveal that mutations in most eukaryotic genes have little discernable effect. For example, systematic gene deletion in *S. cerevisiae*, discussed in detail below, produced a remarkable result: only ~20% of yeast genes are essential for viability when deleted individually in haploids growing in standard laboratory conditions[5,6]. Recent systematic analyses revealed a measurable growth phenotype under at least one condition for virtually every yeast gene deletion[7,8]. Nonetheless, the ability of most deletion mutants to grow under optimal conditions reflects the robustness of biological circuits and cellular buffering against genetic variation, underscoring a key property of biological networks: their resilience to attack at a single node[9,10].

Synthetic enhancement genetics can be used to examine how mutations in two genes interact to modulate a phenotype. Essentially, synthetic enhancement screens represent an application of Fisher's definition of epistasis (BOX 1) — in this case, a double mutant shows an unexpected, non-multiplicative phenotype, the most dramatic being inviability. Early genetic investigations using the fruitfly found that some pairwise combinations of mutant alleles were inviable, whereas singly, the same alleles were viable[11,12], a phenomenon termed synthetic lethality (BOX 2). Yeast geneticists embraced the tools of synthetic enhancement to assist in functional analyses[13] (reviewed in REF. 14). However, synthetic enhancement combinations are infrequent in the large combinatorial sea of possible pairs of genes, and finding interacting partners for a given gene has required the development of sensitive and selective screening methods[15,16].

Box 1 | **Epistasis**

A review by Philips from 1998 describes the early literature on epistasis, much of which refers to the fruitfly, *Drosophila melanogaster*[77]. The language of genetic interactions has been profoundly influenced by these early studies and has led to two related but distinct meanings of the term epistasis, both of which derive from the quantitative analysis of double-mutant phenotypes and are relevant to large-scale mapping and interpretation of genetic networks.

One view of epistasis derives from the world of statistical genetics. Fisher referred to deviations from the expected quantitative combination of independently functioning genes as 'epistacy'[78], a concept that has been adopted by quantitative geneticists to describe a range of genetic interactions. The Fisher definition is quite general and inclusive, and encompasses any phenotype of a given double mutant that cannot be anticipated by simply combining its component single-locus effects multiplicatively. In other words, in the absence of a genetic interaction, the fitness of a double-mutant is expected to be the product of the individual fitness of the corresponding single mutants. For example, consider a yeast strain that carries a mutation in gene A, conferring a defective response, and consequent increased sensitivity, to the DNA-damaging agent methyl methanesulphonate (MMS), with a 20% growth-rate reduction compared with a wild-type strain at the same dose of MMS. Likewise, mutant B shows an MMS sensitivity with a 10% growth-rate reduction. The double mutant, however, grows 90% slower than the wild type in the presence of MMS, such that the genetic combination causes a much more severe phenotype than expected for the combination of the mutant B allele within the mutant A genetic background ($0.8 \times 0.9 = 0.72$, or a 28%-reduced growth rate). One interpretation of this type of genetic interaction is that both genes might be involved in DNA repair but occur in separate pathways, such that the cell can tolerate loss-of-function mutations in either pathway but not both.

The second definition of epistasis derives from the pioneering work of Bateson, who coined the term to explain genetic interactions that alter single Mendelian gene effects. The Bateson definition is familiar to classical and molecular geneticists, who typically use epistasis to describe situations in which the activity of one gene masks effects at another locus, allowing inferences about the order of gene action. As a simple example, consider the yeast transcriptional activator *SWI5* — mutation of *SWI5* results in a failure to express the *HO* endonuclease gene that is required for mating-type switching. The *swi5* mutant phenotype is suppressed by loss-of-function mutations in the *SIN3* gene, which encodes a transcriptional repressor[79]. According to the Bateson definition, *SIN3* is epistatic to *SWI5*, because its mutation masks defects in *SWI5*. This observation allows an inference to be made about a pathway (in this case, that the *SIN3* product lies downstream of the *SWI5* product in a common pathway). Classical examples of Bateson-type epistasis analysis include studies of signalling pathways that control the yeast cell cycle[80] and pheromone responses (reviewed in REF. 81), development in the nematode worm *Caenorhabditis elegans* [82] and sex determination in *D. melanogaster*[83].

Haploinsufficiency
The situation in diploid cells in which heterozygous mutants that produce a reduced amount of functional gene product can be less robust than the wild type to perturbations that affect essential functions.

Tetrad analysis
The four haploid cells that are produced by an individual meiosis in budding yeast are referred to as a tetrad. The tetrad is enclosed in a sac called an ascus. Tetrad analysis involves the isolation and analysis of the haploid meiotic spores of individual asci for the segregation of genetic markers.

Functional genomic tools for systematic genetics

Compiling genetic interactions case by case as a by-product of directed biological studies is highly informative. However, genomics allows genetic networks to be built systematically. Only in this way can a complete genetic network be mapped (a goal that is still far from being achieved for any organism) and its full explanatory potential realized. Many technological platforms and tools have been created for large-scale functional analysis in *S. cerevisiae*[17].

Deletion-mutant collection. By 2001, a deletion allele was available for each yeast gene[5,6]. In these deletion strains, the entire target gene is replaced with a kanamycin-resistance marker[18] plus two unique 20-bp flanking barcodes (FIG. 1a). In this way, the abundance of each mutant can be quantified from a mixed population using a barcode microarray (FIG. 1b).

The yeast gene-deletion set is a key resource for large-scale and systematic genetics. The collection includes ~6,000 heterozygous diploid strains, each of which is deleted for a single copy of a specific gene in the S288c genetic background. Deletion alleles for all *S. cerevisiae* genes are represented and, apart from a few hundred haploinsufficient genes (~3%), the heterozygous mutants grow normally on a rich medium[8]. Tetrad analysis of the heterozygous strains identified ~1,000 deletion mutants that failed to grow as haploid meiotic progeny, thereby defining the *S. cerevisiae* essential gene set and creating a set of ~5,000 viable haploid deletion-mutant strains[5]. Mating of these mutants generated a set of ~5,000 homozygous diploid mutants, which carry a deletion of both alleles of each gene. As the roster of ORFs has been revised, largely through sequencing of evolutionarily related yeast species[19,20], the deletion-mutant set has been correspondingly updated[21].

Essential gene mutant collections. Conditional alleles of the ~1,000 essential *S. cerevisiae* genes are required to enable systematic genetic analysis. There is value in generating a variety of collections of essential gene alleles, as they are likely to provide complementary information in systematic function and genetic interactions of this important gene set. An extensive set of promoter-shutoff strains, in which an essential gene is placed under the control of a tetracycline (tet)-repressible promoter, has been constructed[22]. In these strains, the endogenous promoter of an essential gene is replaced with one that binds a tet-repressible transcriptional activator, which is expressed constitutively. Both the engineered essential gene and the tet-responsive activator are linked to selectable markers, for ease of use in genetic analysis.

Temperature-sensitive (ts) conditional alleles of essential genes have been used traditionally for studying essential processes such as cell-cycle control and secretion. The recently introduced 'heat-inducible degron system' provides a simple way to systematically generate ts alleles of essential genes[23]. An Arg-Dhfr(ts) protein, a ts variant of dihydrofolate reductase, carrying an amino (N)-terminal arginine (Arg) residue (a destabilizing residue according to the N-end rule), functions as a heat-activated degron, resulting in destruction of the tagged protein at 37°C. Large collections of degron alleles of essential genes have been made and subjected to phenotypic analysis[24]. In addition, ts alleles for ~50% of essential genes have been collected, and these are being integrated into the same strain background as the deletion collection (C.B. and B.J.A., unpublished observations).

Hypomorphic allele collections can also be constructed systematically. For example, replacing the 3′ UTR of an essential gene with a selectable marker often leads to lower transcript levels and a resultant phenotype[25] — a method known as DAmP (decreased abundance by mRNA perturbation).

Comprehensive gene-overexpression libraries. The complete set of yeast genes has been cloned into several yeast vectors that allow expression under the control of the strong galactose-inducible *GAL1* promoter,

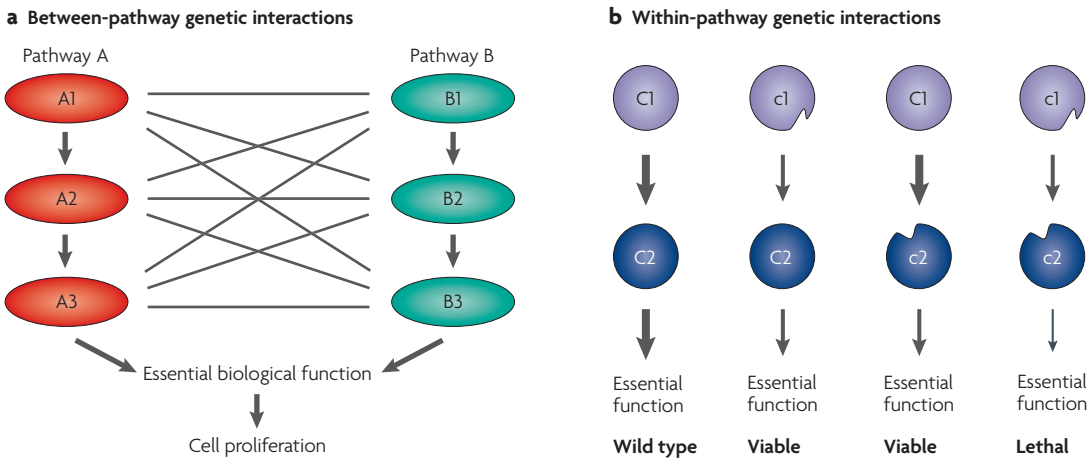
typically resulting in protein overproduction. Partial but significant collections of genes have been constructed encoding *GAL1*-regulated proteins, which are either untagged or carry a carboxy (C)-terminal Flag epitope[26,27]. Complete collections of genes have also been generated that encode proteins tagged with N-terminal glutathione S-transferase (GST)–histidine 6 (His6) or C-terminal His6–HA–ZZ[28,29]. Although overexpression and tagging of proteins is valuable, there can be limitations with such collections owing to dosage and functional issues. To obviate such limitations, ordered libraries of full-length genes under the control of their native promoters are under construction by our group and others.

Methods for systematic genetic analysis in yeast

Synthetic genetic array (SGA) analysis. In its simplest form, synthetic genetic array analysis[30] involves a series of replica-pinning procedures, in which mating and meiotic recombination are used to convert an input array of single mutants into an output array of double mutants (FIG. 2). SGA has been used extensively for synthetic-lethal screening of non-essential genes involved in many cellular functions[2]. The final transfer step (FIG. 2e–f) results in an ordered array of double-mutant haploid strains, the growth rates of which can be quantitatively assessed[25].

Essential-gene mutant collections can also be used both as queries and as input arrays in an SGA screen

Box 2 | **Mechanisms of synthetic-lethal interactions**



What is the mechanistic basis for synthetic-lethal interactions? Because our knowledge of cellular functions is incomplete, we often do not understand why particular double mutants show a synthetic-lethal phenotype. However, possible mechanisms depend on the characteristics of the interacting alleles. For example, if both mutations occur in non-essential genes and are null alleles, the common interpretation is that the genes function in parallel pathways that impinge on a shared essential function (part **a;** thin lines indicate potential genetic interactions). This is often referred to as the 'between-pathway' model and typically reflects bidirectional genetic redundancy, in that each pathway compensates for defects in the other[14,32,55]. More elaborate mechanisms can be understood from a more detailed knowledge of gene function and pathway circuitry, such as a synthetic-lethal interaction that reflects 'unidirectional compensation', whereby one pathway normally prevents a potentially harmful cellular event that can be corrected by another pathway[32]. A pertinent example involves the oxidative-stress response system, which precludes the accumulation of reactive oxygen species and protects the cell from DNA damage. By this mechanism, functional DNA repair pathways can compensate for defects in the oxidative-stress response system, but not the reverse.

The specific case of synthetic genetic interactions involving duplicated genes or paralogues is also of interest. Here recent systematic studies revealed that patterns of genetic interactions are divergent between duplicates, suggesting that paralogous genes maintain functional specificity[84]. Conversely, distant paralogues encoding metabolic genes can show synthetic interactions, indicating that the product of the evolved copy of the duplicated gene might retain sufficient activity to mask the loss of the conserved copy[85].

For essential genes, in which single null mutations are lethal, conditional or hypomorphic alleles can be used to evaluate synthetic phenotypes. In these cases, interpretation is more complex, because interactions can occur 'within pathways' as well as between pathways. In the within-pathway model (part **b**; conditional mutations are indicated by an altered protein shape and a lower-case 'c'), synthetic lethality indicates that both genes function in the same essential pathway, the function of which is diminished by each mutation[14,86]. In this context, synthetic lethality can result from mutations in genes that affect the same stage of the pathway; for instance, when mutations weaken interactions between subunits of a protein complex so that two mutations disrupt complex formation altogether, or render its function below the viability threshold. Biologically compelling examples of this type of interaction are seen in the yeast secretion system; most so-called *SEC* genes are essential, but synthetic-lethal interactions between *sec* mutants are highly specific for genes that are involved in the same stage of the system[87], and also occur among protein-complex subunits (for example, the exocyst complex[88]).

N-end rule
Relates the *in vivo* half-life of a protein to the identity of its N-terminal residue. In eukaryotes, the N-end rule pathway is part of the ubiquitin system.

Hypomorphic
Describes an allele that carries a mutation that causes a partial loss of gene function.

Synthetic genetic array analysis
A robotic procedure that is used to create, select and systematically examine the growth phenotypes of yeast double-mutant haploid strains.

Pinning
The use of hand-held or robotic tools, which are composed of small floating pinheads, to replicate yeast colonies to different media for genetic tests (typical formats include 96, 384, 768 and 1,536 pinheads per replica tool).

Suppression
The situation in which a mutation in one gene counteracts the effects of a mutation in another, so that the phenotype of the double mutant is more like that of the wild type.

to generate networks that focus on essential genes. A proof-of-principle study generated a network of 567 interactions, 386 of which occur between 286 essential genes[1]. The use of expanded collections should soon incorporate all essential genes in the global genetic-interaction map (see below).

Diploid-based synthetic lethality analysis with microarrays (dSLAM). As an alternative to visualizing colonies in an array format, the barcodes that are associated with each deletion mutant enable quantification of each double mutant in a mixed population. The dSLAM method takes advantage of this barcode approach[31] (FIG. 3). Analysis of the barcode representation in each population, by hybridization to a barcode microarray, provides a measure of the relative fitness of the double mutants and identifies potential synthetic interactions. dSLAM has been used to define a network of genes involved in maintaining genome integrity[32].

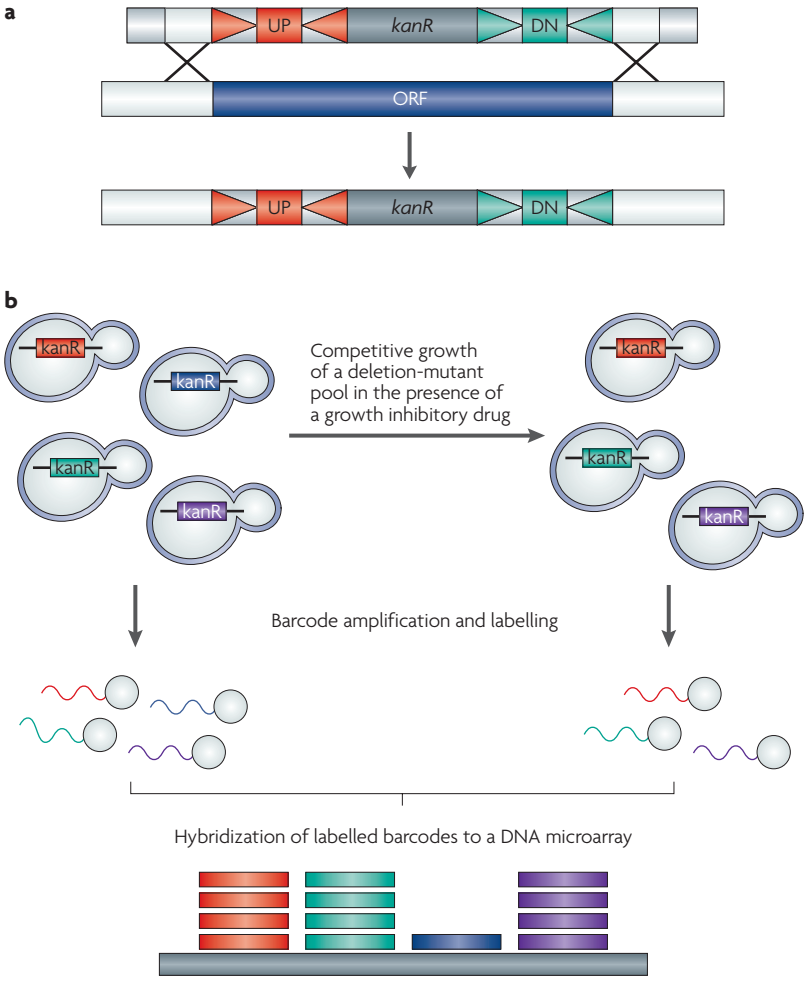


Figure 1 | **The yeast deletion collection and parallel analysis. a** | Construction strategy for the yeast deletion-mutant collection. Each yeast ORF is replaced with a 'deletion cassette' that consists of an antibiotic-resistance marker, *kanR* (which confers resistance to kanamycin), and two unique 20-nt molecular barcodes ('uptag' (UP) and 'downtag' (DN)). Each barcode is flanked by common primer sites (indicated by coloured half-arrows). Incorporation of the cassette into the yeast genome is accomplished through homologous recombination of 45-bp regions of homology upstream and downstream of the yeast ORF. **b** | Parallel analysis of large pools of deletion mutants. Populations of pooled mutant cells, each marked with unique molecular barcodes, are grown in the presence or absence of a growth-inhibitory drug. Genomic DNA is extracted from the pool of mutants, and barcodes that represent each strain are amplified by PCR using common primers that are labelled with fluorescent markers Cy3 or Cy5. Drug-sensitive mutants are identified by competitive hybridization of the barcode PCR products to a microarray that contains oligonucleotides corresponding to each barcode, giving a quantitative read-out of the representation of each mutant in a mixed population.

Synthetic dosage-suppression and lethality. Other types of synthetic genetic interaction are powerful for navigating genetic pathways, and have recently been incorporated into systematic platforms. Dosage-suppression analysis, in which mutants are screened for phenotypic suppression using a library of overexpressed genes, has augmented pathway analysis in yeast. In a typical dosage-suppression screen, a mutant that carries a ts allele of an essential gene is transformed with a genomic library, which is carried on a multicopy plasmid, at a growth-permissive temperature. The transformants are then screened for dosage suppressors at a restrictive temperature. For example, using a conditional allele of the cell-division cycle gene *CDC28* that is defective only at the G2–M transition of the cell cycle, a screen for dosage suppressors identified a set of G2-specific B-type cyclins[33]. Hundreds of such dosage suppressors are known and have broadly contributed to our understanding of functional pathways[34].

In a conceptually reciprocal approach, dosage-lethality screens exploit features of both dosage-suppression and synthetic-lethal screens to identify interacting proteins. Synthetic dosage lethality (SDL) derives from the idea that increasing levels of a protein might have no effect on the growth of an otherwise wild-type strain, but might cause a phenotype — such as lethality — in a mutant strain in which the activity of an interacting protein is reduced[35,36]. For example, SDL defined a broad range of interacting mutations involving components of the yeast kinetochore and the origin recognition complex (ORC)[35,37].

Current overexpression libraries have recently been arrayed so that SGA-based manipulations allow the introduction of any specific query mutation into a collection of ~6,000 yeast strains, each of which carries a unique gene-overexpression plasmid. This method allows rapid assessment of gene-overexpression phenotypes in any mutant background of interest. In addition to examining loss-of-function phenotypes associated with deletion-mutant alleles, overexpression alleles enable the exploration of gain-of-function phenotypes to augment gene-function analysis[38]. Conversely, SDL can be assessed by scoring for an enhanced-fitness defect that is due to gene overexpression in any mutant background. As proof-of-principle, a deletion allele of *PHO85*, which encodes a cyclin-dependent kinase, was crossed to a gene-overexpression array, revealing 65 SDL interactions[38], several of which involve *in vivo* substrates for the kinase (see below for more discussion).

Haploinsufficiency. Other genetic interactions that reflect gene-dosage effects can be crucial for cellular and developmental homeostasis. In diploids, haploinsufficiency can arise when a mutation in one copy of an allelic pair reduces the amount of functional gene product to a point at which a phenotype is produced. Classically, a heterozygote is viewed as the wild type (that is, the mutant phenotype is recessive), and this is the case for most enzyme-coding genes[39]. However, for human transcription factors, over 65% of disease-causing mutations are dominant, and might reflect a haploinsufficient phenotype[39]. Haploinsufficiency can be particularly significant in the context of environmental or chemical interactions and has been exploited extensively to link inhibitory bioactive molecules to their targets, as heterozygote target-gene deletion mutants are often hypersensitive when compared with wild-type cells owing to their reduced target-gene dosage[40,41].

The combination of two heterozygous mutations might lead to a genetic interaction in which the diploid hemizygote double mutant shows an extreme synergistic phenotype, such as synthetic lethality. This combinatorial double-mutant effect has been referred to as complex haploinsufficiency[42]. A screen of 4,800 complex hemizygote yeast strains, in which an actin-null allele was combined with the non-essential gene-deletion collection, identified 208 genes showing deleterious complex haploinsufficient (CHI) interactions and many of the double mutants showed actin-based morphology defects. Thus, CHI genetic-interaction screens can provide extensive functional information if carried out on a global scale.

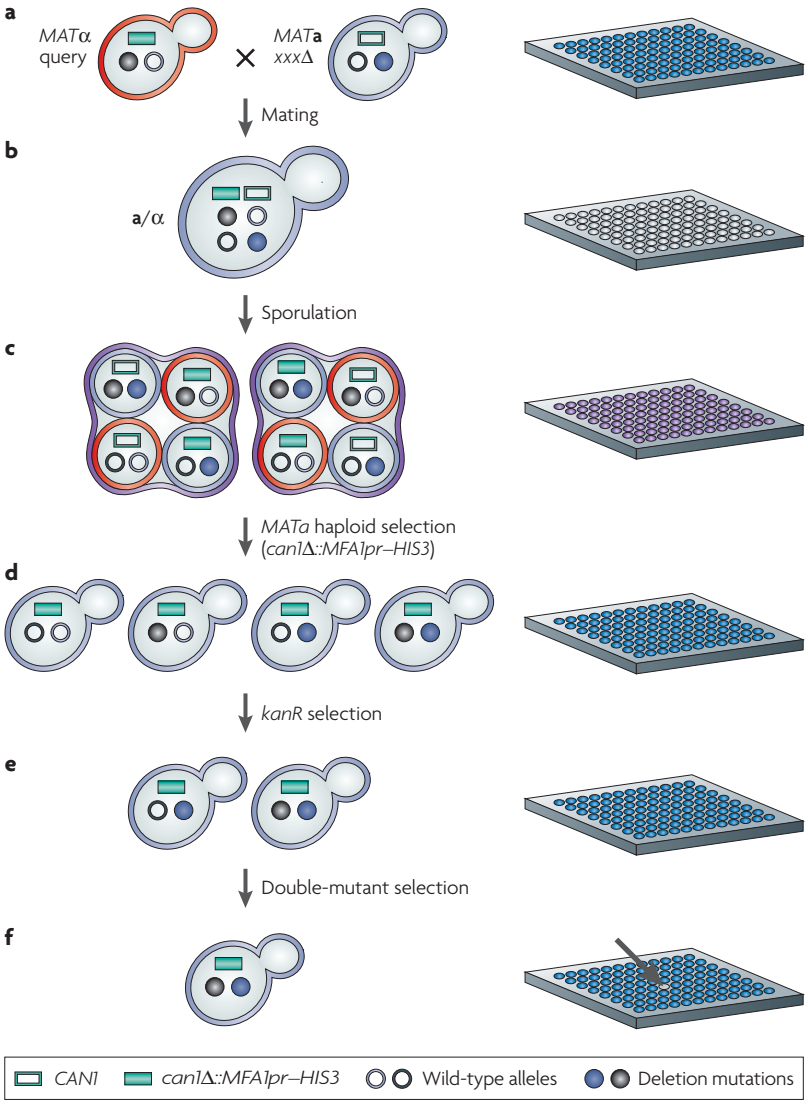


Figure 2 | **The synthetic genetic array (SGA) methodology. a** | A *MATα* strain carries a query mutation linked to a dominant selectable marker (represented as a filled black circle), such as the nourseothricin-resistance marker *natMX*, and the SGA reporter *can1Δ1::MFA1pr–HIS3* (in which *MFA1pr–HIS3* is integrated into the genome such that it deletes the ORF of the *CAN1* gene, which normally confers sensitivity to canavinine). This query strain is crossed to an ordered array of *MAT***a** deletion mutants (*xxx*Δ). In each of these deletion strains, a single gene is disrupted by the insertion of a dominant selectable marker, such as the kanamycin-resistance (*kanR*) module (the disrupted gene is represented as a filled blue circle). **b** | The resultant heterozygous diploids are transferred to a medium with reduced carbon and nitrogen to induce sporulation and the formation of haploid meiotic spore progeny. **c** | Spores are transferred to a synthetic medium that lacks histidine, which allows for selective germination of *MAT***a** meiotic progeny because these cells express the SGA reporter *can1Δ1::MFA1pr–HIS3*. To improve this selection, canavinine, which selects for *can1Δ1* and kills *CAN1* cells, is included in the selection medium. **d** | The *MAT***a** meiotic progeny are transferred to a medium that contains kanamycin, which selects for single mutants, equivalent to the original array mutants and double mutants. **e**,**f** | An array of double mutants is selected on a medium that contains both nourseothricin and kanamycin.

Quantitative mapping of epistatic relationships

Synthetic methodologies allow a quantitative assessment of the relative fitness of double-mutant meiotic progeny. This means that, in addition to Fisher's general idea of epistasis, other more specific ones, including Bateson's classical definition in which one allele masks the effects at another locus (BOX 1), can be examined globally. In the Fisher model, the double-mutant growth rate should deviate from the expected multiplicative value that is associated with the combined single-mutant phenotypes, and this can potentially be examined in detail. In particular, so-called aggravating interactions, in which the double-mutant fitness is lower than expected, might reflect separate but compensatory pathways. Synthetic-lethal double mutants obviously deviate from the multiplicative; however, synthetic slow-growing double mutants with fitness rates that are less than either single mutant but equal to the expected multiplicative double-mutant fitness would not be scored as showing a genetic interaction. Using Fisher's quantitative definition of epistasis may be important for identifying true interactions and thereby revise genetic networks that have not applied this model[43].

In contrast to aggravating interactions, so-called alleviating interactions occur when the double-mutant fitness is greater than expected, such as cases in which the fitness defect of a double mutant is no greater than for either of the single mutants. This often occurs when genes function in the same non-essential pathway or complex. Indeed, a quantitative analysis of an SGA interaction map[44] that focused on genes involved in endoplasmic reticulum (ER) to Golgi transport seems to support this idea, because genes in the same pathway deviated from the expected multiplicative double-mutant phenotype and displayed a level of fitness resembling the single-mutant phenotypes[25]. Thus, genes

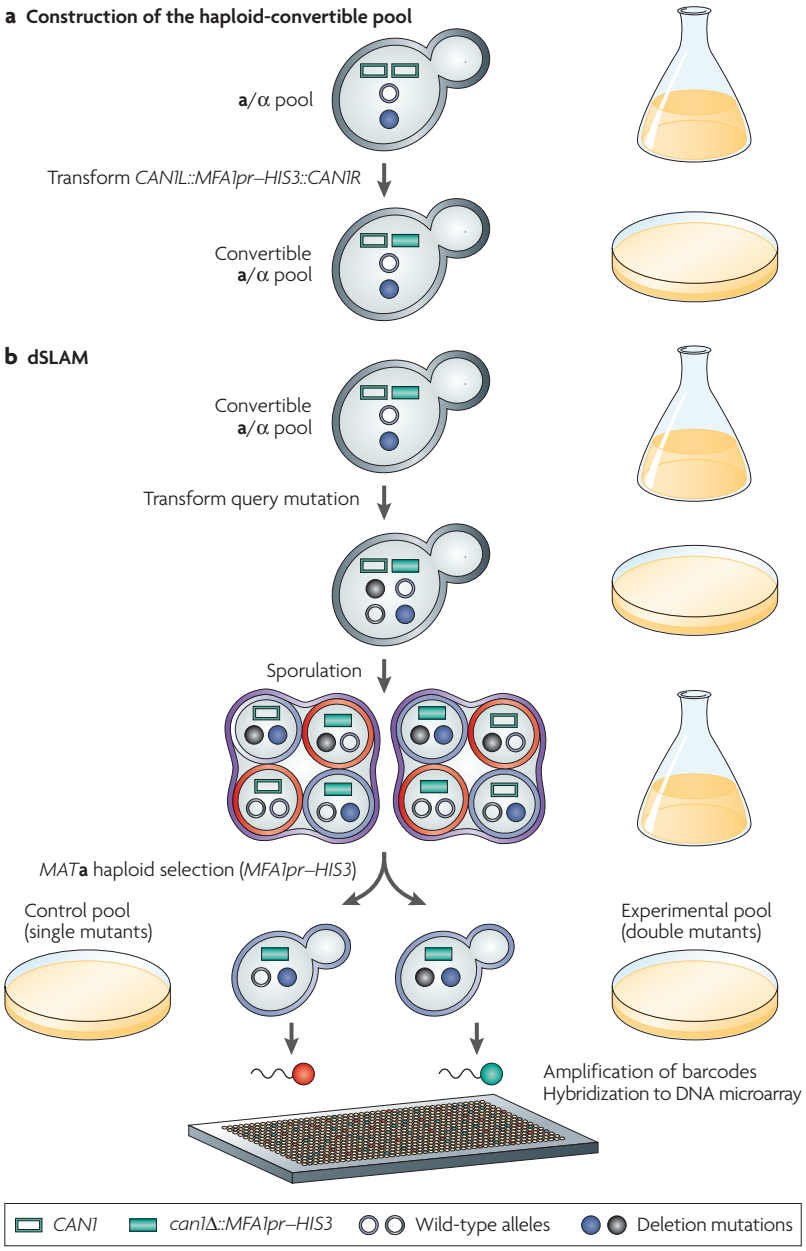


Figure 3 | **Diploid-based synthetic lethality analysis with microarrays (dSLAM).** **a** | The first step in this method is the construction of a haploid-convertible heterozygous diploid pool. A haploid selection synthetic genetic array (SGA) reporter, which includes sequences that flank the endogenous *CAN1* locus (*CAN1L–LEU2–MFA1pr–HIS3–CAN1R*), is transformed into a pool of heterozygous diploid deletion mutants to replace one copy of *CAN1* in each mutant. In each of these deletion strains, a single gene is disrupted by the insertion of a kanamycin-resistance (*kanR*) module (the disrupted gene is represented as a filled blue circle), which is tagged with unique barcodes, and a wild-type copy of the same gene. Transformants are selected on plates and then pooled for genetic-interaction screens. **b** | For dSLAM, a query mutation that is linked to the *URA3* selectable marker (represented as a filled black circle) is introduced into the pool of haploid-convertible heterozygous diploid strains by high-efficiency integrative transformation. Haploid single-mutant (control) or double-mutant (experimental) pools are selected after sporulation, through germination of spores on a medium that lacks histidine and selection for the relevant alleles. Genomic DNA samples are isolated from both pools and used as templates for PCR amplification of the tags, during which they are labelled with fluorescent dyes (Cy5 for the single-mutant pool and Cy3 for the double-mutant pool). Microarray analysis of these dye-labelled tags reveals the synthetic interaction between each of the corresponding deletion alleles with the query mutation.

in the same pathway share alleviating interactions with each other.

Exploring systems and pathways with quantitative and qualitative interaction maps. Several recent studies have widened the types of genetic interaction that can be identified in yeast. Drees[45] and colleagues defined a range of interactions by enumerating all possible 'greater than', 'less than' and 'equal to' relationships among single- and double-mutant invasive growth phenotypes. They also scored for nine general types of epistatic interaction, including aggravating and alleviating types, but also for less familiar ones that were not previously considered. For example, in an 'asynthetic' interaction, a double mutant and its corresponding single mutants all have the same deviant phenotype, which is a specific subset of alleviating interactions. This broad analysis revealed that genetic interactions can occur frequently and allowed construction of elaborate interaction networks.

In a theoretical analysis, Segre and colleagues examined the predicted fitness of a double mutant under a multiplicative model and showed that, in addition to aggravating (antagonistic) synthetic effects, alleviating (buffering) interactions that ameliorate the effects of a mutation in double-mutant combinations are common among the genes involved in intermediary metabolism[46]. As with synthetic interaction studies, they found that alleviating interactions tend to be the same for related groups of genes, revealing functional or modular clustering. Comparisons between aggravating and alleviating effects revealed that, for most functional groups, interactions were either largely aggravating or largely alleviating, but not mixed, an asymmetrical feature that they termed 'monochromatic'.

In another study, 650 double-deletion strains were made, corresponding to all possible pairings of 26 deletions that confer sensitivity to the DNA-damaging agent methyl methanesulphonate (MMS)[43]. The fitness of each strain was measured and examined with respect to the multiplicative neutral model. In the presence of MMS, approximately one-third of the unique double mutants that were tested were found to deviate from the multiplicative model, corresponding to both aggravating and alleviating combinations. Distinct forms of alleviating interactions were identified, and those that were asymmetrical were used to infer pathway order corresponding to the classical Bateson definition of epistasis.

Properties of genetic networks

Genetic networks are complex but functionally coherent. Analysis of the large but still incomplete yeast genetic network offers a glimpse at its size and structure. From a set of SGA screens, a network of ~1,000 genes and ~4,000 interactions was generated[2]. The number of genetic interactions averaged 34 in each screen for non-essential genes[2], with screens that were focused on essential genes exhibiting fivefold more interactions[1]. From these studies, we estimate that a global network will contain ~200,000 synthetic-lethal interactions. To put this number in context, there are ~1,000 essential genes in yeast, for which a single mutation leads to a lethal

phenotype, but there are 200-fold more ways to generate a similar phenotype through a digenic synthetic-lethal interaction. This finding indicates that digenic interactions might underlie many inherited phenotypes, and begins to explain why the analytical power of single-gene effects on many phenotypes has been so limited.

For both non-essential[2,32] and essential genes[1], genetic interactions tend to occur among functionally related genes (FIG. 4), although interactions of essential genes correspond to a broader functional range. So, the set of interactions that are observed for a particular query gene can be suggestive of its function, with the position of a gene in a genetic-interaction network being highly predictive of its molecular role. For example, when a deletion allele of *BNI1*, which functions in actin-based polarized secretion and spindle orientation[47], was screened against all viable gene-deletion mutants, most of the interacting genes had roles in cell polarity and spindle orientation (annotated as 'mitosis' in FIG. 4). By contrast, the genetic interactions for *SGS1*, which encodes a DNA helicase, were largely associated with roles in DNA synthesis and repair (FIG. 4).

The small world of genetic interactions. The current synthetic genetic network for yeast has two properties that are shared by networks as diverse as the World Wide Web and protein–protein interaction maps[48]. First, the connectivity distribution broadly follows a power-law distribution, containing many genes with few interactions and a few genes with many interactions[2]. Highly connected 'hub genes' are likely to be more important for fitness than less connected genes, because random mutations in organisms that lack these genes are more likely to be associated with a fitness defect. Indeed, yeast hub genes that are conserved in humans could be potential targets for anti-cancer drugs, because cancer cells often carry a large mutation load making them more susceptible to chemical perturbation, and therefore may be killed preferentially when network hubs are attacked[49].

Second, the genetic network seems to be an example of a small-world network in which the length of the shortest path between a pair of vertices or nodes tends to be small (that is, the network has a short characteristic path length) and local neighbourhoods tend to be densely connected. The genetic network that was mapped by Tong *et al.*[2] has a short characteristic path length of 3.3, which is consistent with a small-world network[48]. The topology of the genetic network also exhibits dense local neighbourhoods, as the immediate neighbours of a gene, its genetic-interaction partners, also tend to interact with one another[2]. The dense neighbourhood characteristic of small-world networks is of particular interest because it can be exploited to predict interactions, as previously shown for protein–protein interactions[50]. Thus, if all the yeast genes are placed on a relatively sparse genetic network — that is, a network that contains most or all the genes with a small subset of their interactions — most interactions should be efficiently identified by testing for interactions among genes that share interaction partners (in the same neighbourhood). Indeed, when the immediate neighbours of three query genes, *SGS1*, *RAD27* or *BIM1* were tested for interactions with one another, ~20% of the tested potential interactions were confirmed[2], and were highly enriched compared with the 1% observed for the average query gene against all SGA-tested gene pairs.

Genetic networks reveal gene functions

Relationship between the physical-interaction and the genetic-interaction maps. Large-scale analysis of genetic networks has revealed a relationship between the physical-interaction and the genetic-interaction networks. The physical-interaction map, generated by large-scale two-hybrid[51,52] or affinity purification followed by mass spectrometry identification[26,43,53,54], provides a view of the gene products that assemble into soluble protein complexes and function together as biochemical machines. Rather than physical information, the genetic-interaction map provides functional information, largely identifying gene products that operate in functionally related pathways. Although genetic interactions overlap with protein–protein interactions more often than expected by chance, such overlap is relatively rare, occurring at a frequency of less than 1% (REF. 2).

Neither the genetic- nor the physical-interaction map has been deeply sampled so far, and the overlap between the maps might increase. Nonetheless, a large overlap between the two is not expected as far as genes that encode components of non-essential pathways are concerned, because physical interactions should occur among the pathway components but synthetic-lethal interactions would be precluded by definition (BOX 2; FIG. 5a). However, synthetic-lethal interactions are expected among the components of essential pathways and, in this case, physical and genetic interactions might overlap (BOX 2; FIG. 5b) — these are so-called within-pathway interactions[55]. Regardless, essential genes often buffer numerous different pathways[1], and therefore most interactions for these genes occur between pathways and show no overlap with physical interactions (FIG. 5b).

Because most genetic interactions do not overlap with physical interactions, the two types of interaction are said to be largely orthogonal[55–57]. Nevertheless, the genetic-interaction map is rich in physical-interaction information. For example, the set of interacting genes that is associated with a particular query is often enriched for all of the genes encoding the components of a functionally related pathway or complex. This makes sense, because if the activity of a particular pathway or complex is required in the absence of function of the query gene, then genes encoding all of the important components of that pathway or complex should be identified in the synthetic-lethal screen. Because a given query gene often shows in the order of ~30 different interactions, and most pathways contain only a handful of genes, each gene seems to buffer numerous other pathways.

Precise biochemical functions can be deciphered from genetic-interaction maps because genes with products that function in the same pathway or complex often show a similar pattern of genetic interactions[2]. Indeed, clustering algorithms or other measures of shared genetic-interaction patterns, such as the congruency score[57], can be used to identify genes encoding components

Nodes
In typical network diagrams, genes or proteins are represented as nodes, whereas the connections between the nodes are termed edges.

Clustering algorithms
Algorithms that group together objects that are 'similar'; objects belonging to other clusters are 'dissimilar'. Clustering algorithms have been used extensively to view large collections of biological data, such as microarray expression profiles and genetic-interaction data.

Congruency score
A numerical ranking of the degree of partner sharing in a network.

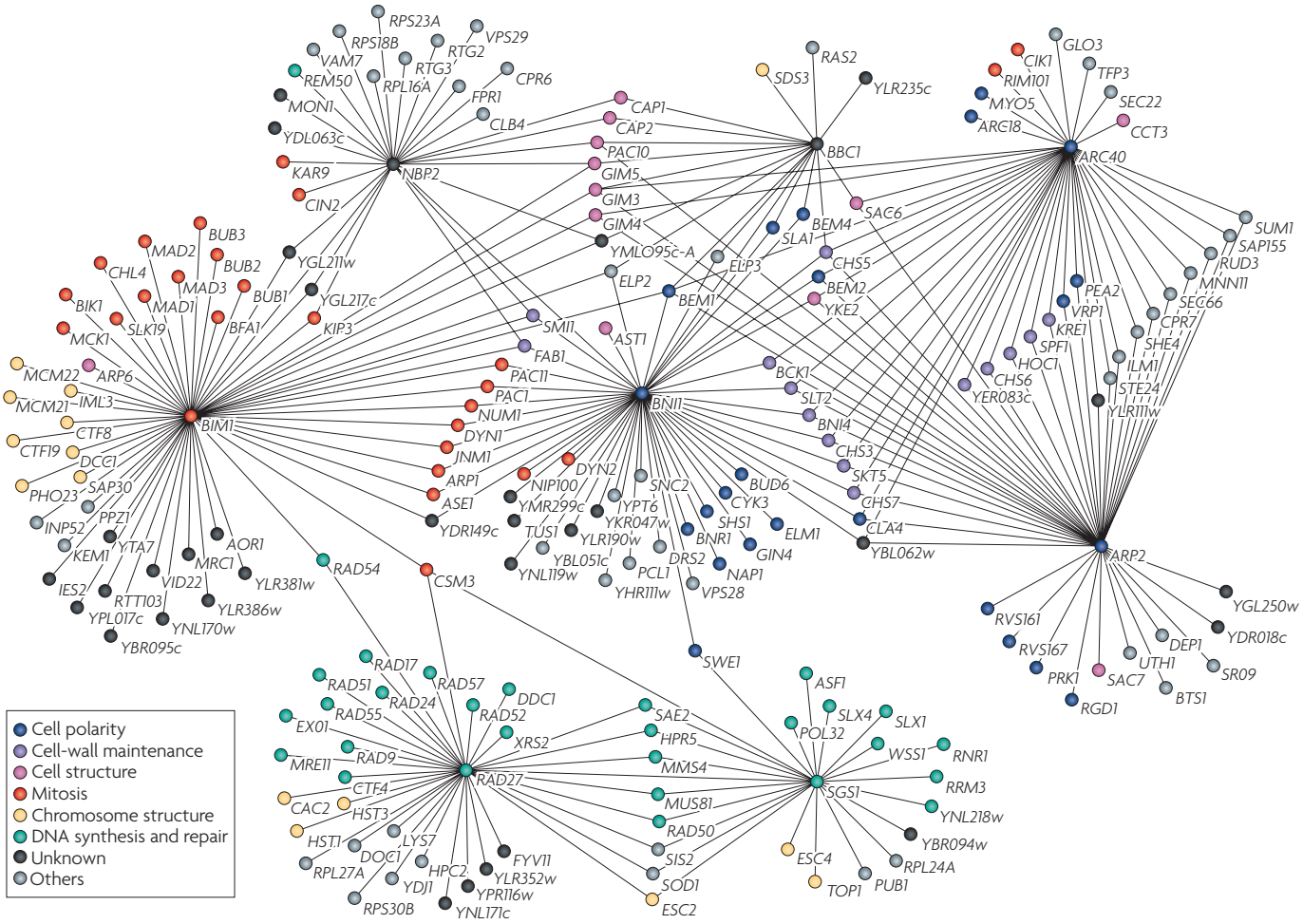


Figure 4 | **A yeast genetic-interaction network, as determined by synthetic genetic array (SGA) analysis.** A genetic-interaction network was obtained by identifying synthetic-lethal or synthetic-sick interactions using SGA analysis. Genes are represented as nodes (shown as circles), and interactions are represented as edges (shown as lines) that connect the nodes: 291 interactions and 204 genes from eight different SGA screens are shown. Deletion-mutant alleles of *BNI1*, *RAD27*, *SGS1*, *BBC1*, *NBP2*, *BIM1* and temperature-sensitive conditional alleles of *ARP2* and *ARP40* were crossed to the set of ~5,000 viable yeast deletion mutants and scored for synthetic-lethal or synthetic-sick double-mutant interactions. All interactions were confirmed by tetrad analysis, with 8–14 tetrads examined in each case. The genes are coloured according to their cellular roles as annotated by the Yeast Proteome Database (YPD) (see the BIOBASE web site). Modified with permission from REF. 30 © (2001) American Association for the Advancement of Science.

of that pathway or complex (FIG. 6a). For example, on the basis of genetic-interaction patterns, *CSM3* was linked to the S-phase replication checkpoint pathway and *DYN3* (also known as *YMR299c*) was linked to the dynein–dynactin pathway[2]. From an extensive analysis of the DNA-integrity network in yeast, 16 functional modules or mini-pathways were identified on the basis of global patterns of genetic interactions[32]. Ultimately, the combination of the global genetic-interaction map and the physical-interaction map can be simplified by representation as a higher-order network in which the nodes represent complexes and pathways rather than individual genes, and the edges represent a collection of numerous synthetic genetic interactions that are associated with the individual genes of the pathway or complex[55,57,58].

Deciphering enzyme target relationships from genetic networks. Because synthetic-lethal interactions often identify pathways that buffer one another, genetic-interaction maps are useful for predicting enzyme–substrate relationships. For example, if a gene encoding a kinase is identified in a synthetic-lethal screen, then genes encoding upstream activators and downstream targets of the kinase might also be found in the genetic-interaction profile from the same query. Indeed, a synthetic-lethal screen with a *CLA4* query mutation identified both the gene encoding a p21-activated kinase, *STE20*, and the formin gene *BNI1*, the product of which is postulated to be activated by the Ste20 kinase[59].

By contrast, SDL can be particularly useful for identifying proteins that are negatively regulated by specific

enzymes. For example, if a kinase normally negatively regulates a particular substrate, then overproduction of that substrate in the relevant kinase mutant background might overwhelm the ability of the cell to cope with inappropriate regulation of a significant biological pathway. Indeed, of the 65 synthetic dosage interactions that were observed for the kinase gene *PHO85* (REF. 38), four substrates of Pho85 (Pho4, Gsy1, Gsy2 and Gcn4) were identified, each of which is negatively regulated by Pho85 phosphorylation.

Challenges for the future

Synthetic lethality, population genetics and complex inherited human disease. Yeast genetic-interaction studies involve an inbred isogenic strain under a single set of growth conditions. However, in human populations the issues of an outbred population with high levels of genetic polymorphism and variable environmental conditions add considerable complexity. The Kruglyak group used yeast to address the issue of polymorphism in genetic interactions[60,61]. Using variations in transcript expression levels between two yeast strains as 'endophenotypes' for QTL analysis, they examined the polymorphic alleles that were involved in the variation. Having identified a primary locus that functioned as a modulator of a given transcript or set of transcripts, they carried out a second search to identify any interacting secondary loci. Such locus pairs were estimated to be responsible for the variation that is seen among some 57% of transcripts. Importantly, 67% of the secondary loci that they identified had effects that were undetectable when assessed singly, the detection of which required the two-step search[60,61]. Because this strategy requires the identification of a primary locus on the basis of its individual effect on transcript level, it cannot be used to examine the frequency of pairs of polymorphic alleles that are singly undetectable but interact to affect transcript levels. Identifying such interacting loci remains a huge problem in all systems, including humans.

To identify candidate interacting alleles in complex disease, it is useful to have a detailed understanding of the genetic polymorphisms in a population so that they can be assessed as contributing allelic components in gene association studies. For humans, this idea has led to the generation of the human HapMap (see the International HapMap Project web site), a database that includes most of the common polymorphisms that are present in the human population[62]. An extension of this idea would be to sequence and compare the genomes of affected and unaffected relatives for a given disease. Although this goal remains unattainable, recent work in yeast approaches it: hybridization of DNA from yeast strains to highly overlapping whole-genome DNA microarrays now allows the global detection of polymorphisms to a single nucleotide resolution[63]. The application of such technology in deciphering the genomic basis of complex phenotypes has been demonstrated[64] and, although challenging, the extension of such an approach to more complex systems, including humans, can be contemplated.

Isogenic
Strains or organisms that share identical genotypes.

Gene association studies
Studies that assess whether genotype frequencies are different between two groups that differ in phenotype.

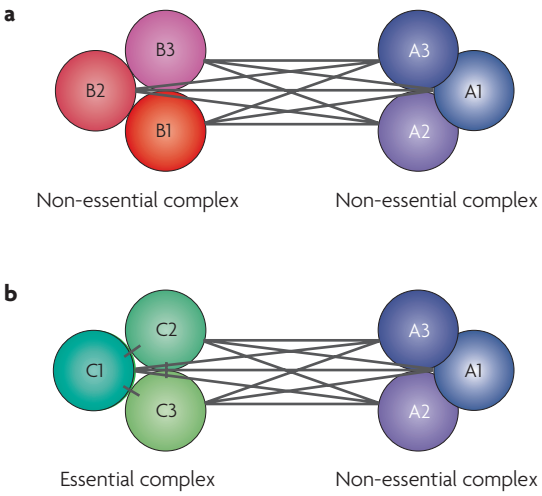


Figure 5 | **Relationships between genetic and protein interactions for complexes.** Interactions are shown for complexes, but the same principles apply to pathways. **a** | Genetic interactions between two non-essential complexes. Two complexes (A and B) comprising proteins that are encoded by non-essential genes are shown. Protein–protein interactions are indicated by contact between proteins (represented as coloured circles), whereas genetic interactions are indicated by black lines. Genetic interactions occur among the mutant alleles of the genes, but for representational purposes are shown here in the context of the proteins within the complexes. In this model, the two non-essential complexes impinge on the same essential pathway and buffer one another (as shown in FIG. 1a); therefore, genetic interactions occur between the two complexes, but do not occur for components within a particular complex. The genetic-interaction pattern that is associated with each component of the complex is identical; that is, the genes that encode B1, B2 and B3 each show genetic interactions with the genes encoding A1, A2 and A3, and the reverse is also true. **b** | Genetic interactions that occur within an essential complex, and between an essential and a non-essential complex. The proteins in complex C are each encoded by essential genes. In this model, complex C is buffered by the activity of complex A and thus genetic interactions occur between each component of the two complexes as well as between the genes that encode complex C components (within-pathway interactions).

Extrapolating from yeast: network conservation and prediction. Is the yeast genetic network likely to be a good comparative model for such networks in metazoans? The creation of RNAi libraries to target all predicted genes in metazoan models and human genomes offers the potential for genome-wide analysis in complex systems. RNAi screens have been used to systematically identify the genes involved in many biological processes in *Caenorhabditis elegans*, and in fly and mammalian cell lines[65,66], and screens to examine double-mutant interactions in metazoan systems are now underway. Focused analyses of interactions between genes involved in DNA repair and posterior patterning in the *C. elegans* embryo have already uncovered novel genes and genetic

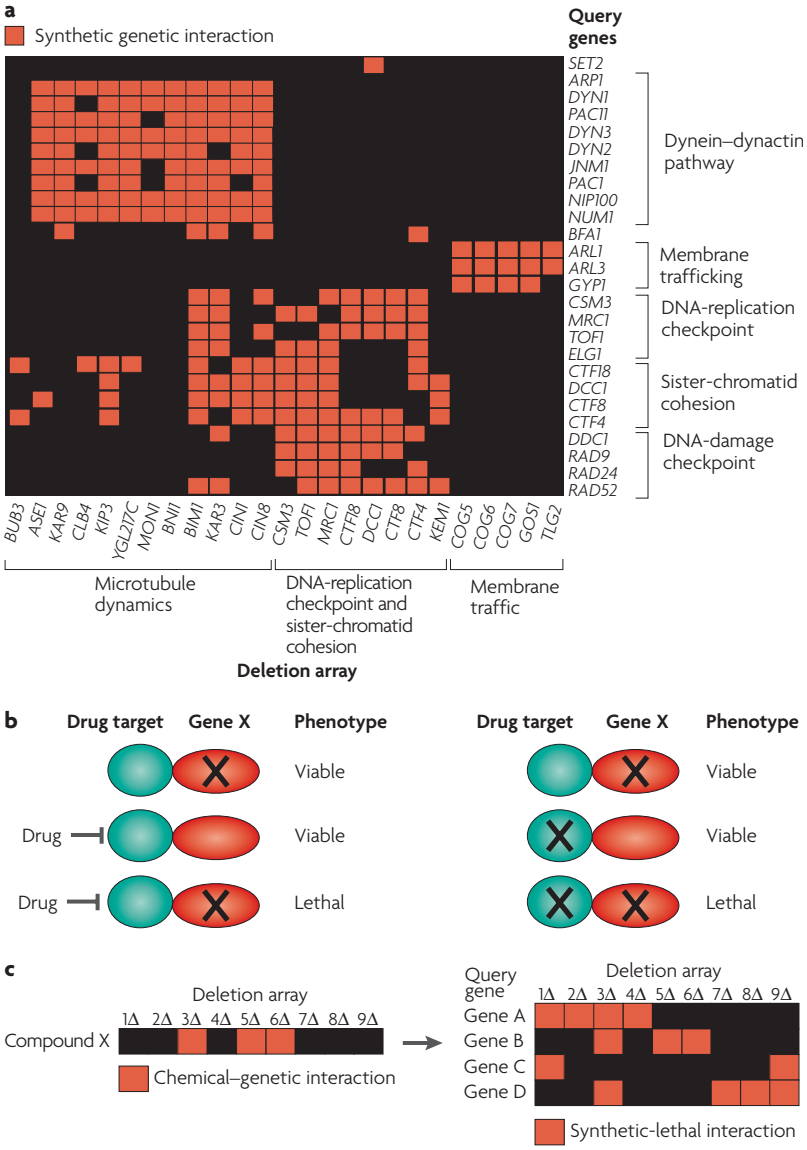


Figure 6 | **Hierarchical clustering of genetic and chemical-genetic interactions.** **a** | Two-dimensional hierarchical clustering of synthetic genetic interactions, as determined by synthetic genetic array (SGA) analysis. A small subset of the genetic interactions mapped by Tong *et al.*[2] is shown. The hierarchical clustering algorithm organizes the query and array genes into sets that show similar patterns of genetic interactions (shown as red squares), thereby grouping together components of specific functional pathways and complexes. Large-scale mapping of genetic interactions provides a genetic-interaction phenotype for each gene, and clustering analysis orders genes into pathways and complexes. **b** | The left panel shows a chemical–genetic interaction, in which a deletion mutant, which lacks the product of the deleted gene (represented by a black X), is hypersensitive to a normally sublethal concentration of a growth-inhibitory compound. The right panel shows a synthetic-lethal genetic interaction in which two single deletions individually lead to viable mutants but are not viable in a double-mutant combination. Gene-deletion alleles that show chemical–genetic interactions with a particular compound should also be synthetically lethal or sick when combined with a mutation in the compound target gene. **c** | Comparison of a chemical–genetic profile to a compendium of genetic interaction (synthetic lethal) profiles should identify the pathways and targets that are inhibited by drug treatment. A hypothetical example is shown. Deletion mutants *3*, *5*, and *6* are hypersensitive to compound X, and a mutation in query gene A leads to a fitness defect when combined with deletion alleles *1*, *2*, *3* and *4*. Here the chemical–genetic profile of compound X resembles the genetic profile of gene B, identifying the product of gene B as a putative target of compound X. Part **a** modified with permission from REF. 2 © (2004) American Association for the Advancement of Science.

interactions for both processes[67,68]. More recently, large-scale RNAi mapping of genetic interactions for signalling and transcriptional-regulatory pathways in *C. elegans* uncovered ~350 genetic interactions. Again, both known and novel signalling components were identified. Despite its currently modest size, the *C. elegans* genetic network recapitulates the topology of genetic networks in yeast[69], suggesting that a general network structure is conserved in eukaryotes. Indeed, some general principles of genetic networks for model organisms have already been shown to extend to human genetics, with individual anecdotal examples of complex inherited human diseases seeming to act through dense local neighbourhoods of interactions that resemble the yeast network topology. For example, the Bardet–Beidl syndrome is caused by interactive defects in genes involved in the assembly and function of the centrosome[70].

However, even in experimentally tractable organisms, the generation of genetic-interaction data is labour-intensive, and comprehensive interaction maps are some way off for most biological systems. It is therefore important to continue efforts to understand the topology of networks in simpler systems so that predictions about more complex organisms can be attempted. Prediction of genetic interactions in yeast following mapping of the local topology around gene pairs has provided candidate interacting pairs that are enriched for synthetic interactions[71]. Predictions that come from comparative genomics are also useful; for example, knowledge of interactions in both yeast and *Drosophila melanogaster* has assisted in identifying candidate interactions among orthologous genes in *C. elegans*[72]. Similar approaches should also provide useful predictions for candidate interactions in less accessible systems.

One implication of the high degree of interconnectedness of the yeast network is that, assuming that human gene networks show the same properties, uncovering the genetic basis of disease susceptibility in humans will be a huge challenge. Many mutant alleles that have no discernable individual effect could contribute to combinatorial synthetic effects that cause disease.

Chemical genomics and genetic-interaction networks. To help to gain a global understanding of complex biological processes, small molecules can serve as a powerful counterpart to gene mutations as rapid and reversible modulators of gene activity. The use of such chemical probes on a genome-wide scale is called 'chemical genomics' and is well suited for use in yeast, in which simple assays for cell fitness are available. In principle, deletion of a gene that encodes the target of an inhibitory compound should cause cellular effects that are similar to inhibition of the target by drug treatment. If so, crossing a target deletion mutation into the set of ~5,000 viable yeast deletion mutants by SGA, and scoring the resultant double mutants for reduced fitness, should generate a set of synthetic-lethal interactions for the gene target that resembles the chemical–genetic interaction profile of its inhibitory compound (FIG. 6b,c). In a proof-of-principle study[73], the chemical–genetic profiles of five different compounds were found to be highly similar to the genetic-interaction

profiles of the target gene or genes in the target pathway. In general, a comprehensive compendium of global genetic-interaction profiles should allow the targets of growth-inhibitory compounds to be identified.

Given that most genes are non-essential, and that proper cell function reflects an interconnected robustness, both gene–drug and drug–drug combinations that inhibit such cellular systems should be investigated for therapeutic intervention. For example, an understanding of synthetic-lethal genetic interactions might enable the identification of compounds that target specific pathways and selectively kill cells with defined mutant genotypes in cancer pathways[74]. In fact, it is well established that combinations of molecules can provide highly effective drug regimens[75,76]. A systematic way to identify drugs that could have synergistic effects is by selecting pairs of drugs affecting targets that are themselves synthetically lethal. For example, consider two drugs that target different essential gene products that show a synthetic genetic interaction. For essential genes, a synthetic-lethal interaction is often detected by assaying for the lethality of a double mutant that carries conditional alleles of the essential genes at what is normally a permissive temperature for each of the single mutants. In this scenario, synthetic drug combinations that target each of the essential genes should act synergistically by working together at lower minimum inhibitory concentrations (MICs) than if used singly. Most importantly, this type of combination therapy is not limited to essential gene products but, rather, encompasses the entire synthetic-lethal genetic-interaction network. Although there are only 1,000 essential target genes in *S. cerevisiae*, we estimate that there are ~200,000 synthetic digenic combinations. Thus, by using combinations of drugs that cut at the Achilles' heel of cell function, we can find a 200-fold-wider repertoire of drugs that work in a way that exploits a fundamental weakness of cellular networks.

Conclusion

Grasping an understanding of genetics through phenotype can be a slippery task, as phenotypes seldom reflect the function of just one gene. In modern genetics, an isogenic background allows a focus on the phenotype and function of individual genes, and this has been a useful initial strategy. However, lurking just below the surface is a complexity that we must face. For example, every intensively studied organism shows strain-background differences that everyone notices and almost everyone ignores. Indeed, the genetics we are taught is clearly a simplified and limited view of the nature of human variation. A hard reality, then, is that most phenotypes are not caused by alleles of a single gene. Even most phenotypes that are thought to have their basis in a single gene have, on further study, been found to vary under the influence of many modifying genes. Quantitative traits and most inherited human diseases fall into this abyss of complexity, and progress in our understanding of them has been difficult and slow.

Here we have examined how geneticists have begun to grapple with such genetic complexity. The advent of genomics and global gene catalogues, coupled with a growing understanding of the properties of biological networks, has facilitated the study of genetic interactions through double-mutant combinations in systems like yeast, for which there is the technology to manipulate and analyse large numbers of crosses. The resulting genetic networks strongly reflect function, with genetic interactions clustering as functional modules in dense local neighbourhoods. Furthermore, these networks emphasize the deep intrinsic buffering of cellular function through redundant or overlapping pathways. However, a minority of genes are essential, and these define hubs of activity that can in some cases extend beyond a given functional module to influence and even coordinate multiple cellular processes. It is no wonder, given this interactional complexity, that single genes rarely specify a phenotype in its entirety. The outlines of a yeast genetic network are now apparent, but a compelling case can be made for a deeper and more complete exploration of this model system as an exemplar for more complex eukaryotes.

As useful as it is in defining general genetic principles, yeast only pioneers the way by validating the usefulness of such genetic analysis. We anticipate a growing flood of genetic-interaction networks from model organisms, including chordates such as zebrafish and mice. Such studies should progressively sharpen the outlines of our own, human genetic-interaction network space, and move beyond a comprehension of single-gene effects to a deeper understanding of our inheritance, including our susceptibility to environmental insult, and the basis of collective inherited disorders.

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
BIM1 | *BNI1* | *CAN1* | *CDC28* | *CLA4* | *CSM3* | *DYN3* | *GAL1* | *HO* | *PHO85* | *RAD27* | *SGS1* | *SIN3* | *SWI5*
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