The synthetic genetic interaction spectrum of essential genes

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The nature of synthetic genetic interactions involving essential genes (those required for viability) has not been previously examined in a broad and unbiased manner. We crossed yeast strains carrying promoter-replacement alleles for more than half of all essential yeast genes[1] to a panel of 30 different mutants with defects in diverse cellular processes. The resulting genetic network is biased toward interactions between functionally related genes, enabling identification of a previously uncharacterized essential gene (*PGA1*) required for specific functions of the endoplasmic reticulum. But there are also many interactions between genes with dissimilar functions, suggesting that individual essential genes are required for buffering many cellular processes. The most notable feature of the essential synthetic genetic network is that it has an interaction density five times that of nonessential synthetic genetic networks[2,3], indicating that most yeast genetic interactions involve at least one essential gene.

The complete analysis of gene-deletion mutations for each of the ~6,000 known or predicted genes in the budding yeast *Saccharomyces cerevisiae* identified ~5,000 viable deletion mutants and ~1,000 essential genes[4]. Many (if not most) genes are nonessential because multiple pathways of eukaryotic cells buffer one another to create systems that are robust to environmental and genetic perturbation[5,6]. Large-scale identification of 'synthetic lethal' phenotypes among nonessential genes, in which the combination of mutations in two genes causes cell death or reduced fitness, provides a means for

mapping these genetic interactions[2,3]. Essential gene function is also buffered by both nonessential and other essential genes because hypomorphic (partially functional) alleles of essential genes often have synthetic lethal interactions with deletion alleles of nonessential genes and hypomorphic alleles of other essential genes[2,3,7]. Genetic interactions among essential genes have not been examined systematically and objectively because of the inherent difficulty in creating and working with hypomorphic alleles.

Figure 1 Matrix display of SGA data. Left, synthetic genetic interactions between a query and array strain are represented as red rectangles; black indicates no interaction. Right, Gene Ontology classifications of genes mutated in the array strains are indicated; black rectangles indicate that the gene mutated in the array strain has the given annotation. We determined row and column order by hierarchical clustering analysis followed by rearrangement of higher-level nodes to achieve a diagonal appearance. Full spreadsheets are posted on our project website.
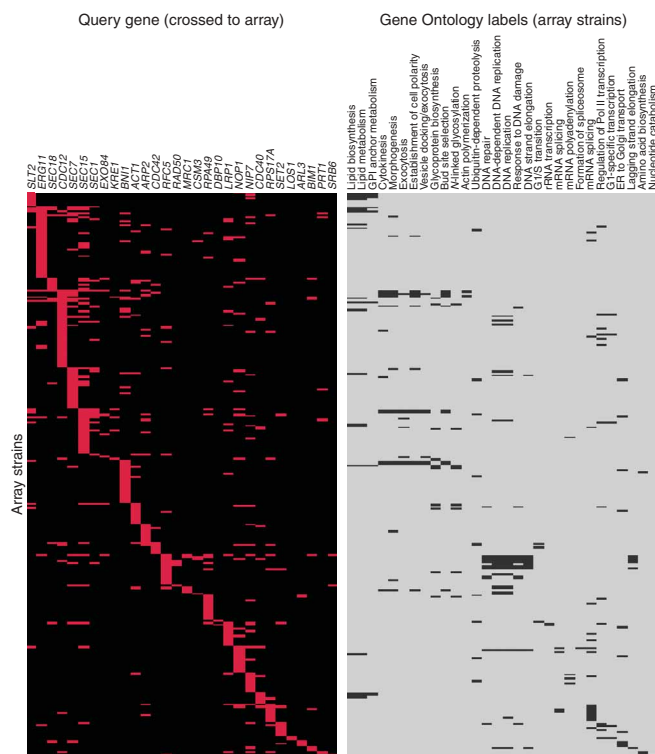
To examine systematically the basic principles of genetic networks with essential genes, we used synthetic genetic array (SGA) technology[2] and carried out large-scale analysis of synthetic genetic interactions among temperature-sensitive conditional alleles and conditional expression alleles (using the tetracycline (tet) promoter) of essential yeast genes[1]. In the tet-promoter strains, the expression of the gene of interest is controlled by a promoter that can be shut off by adding doxycycline (an analog of tetracycline) to the growth medium. In SGA analysis, a mutation in a particular query gene is first crossed to an input array of single mutants and then a series of robotic pinning steps is used to generate an output array of double mutants, which is scored for fitness defects relative to either of the single mutants[2]. We carried out 30 SGA screens using an array of tet-promoter mutants in 575 essential genes[1]. The set of query genes represents diverse biological processes and the query mutations include 14 temperature-sensitive and 2 promoter mutation alleles of essential genes and 14 deletion alleles of nonessential genes (**Fig. 1** and **Supplementary Table 1** online). We examined both the tet-promoter strains and the temperature-sensitive strains at a semipermissive condition: we scored interactions at the semipermissive temperature for temperature-sensitive (*i.e.*, query) strains, and because of a delay of several hours in the onset of growth cessation in tet-promoter strains, virtually all of the tet-promoter (*i.e.*, array) strains formed at least a small colony. We evaluated growth phenotypes both with and without doxycycline in the medium, because tet-promoter strains may show abnormal phenotypes even with the promoter 'on'[1]. We identified instances of smaller colony size for a double mutant versus either single mutant grown under the same conditions and confirmed them by random spore analysis (RSA). Although there were variations between strains with respect to growth rate as single mutants (in the case of tet-promoter strains, this is presumably a result of the varying response of cells to depletion of different transcripts[1]), we obtained interactions at roughly comparable frequencies from strains with both mild and severe growth phenotypes (**Supplementary Methods** online). This analysis resulted in a genetic network of 567 interactions, 386 of which occur between two essential genes, encompassing 286 essential genes (**Fig. 1**). Only two interactions (between *RFC5* and *RFC2* and between *SEC15* and *MYO2*) were found among the 4,922 known yeast genetic interactions cataloged by the GRID database[8]. All the data, including RSA images, are available from our project website (see URL in Methods).

To determine whether the interactions we obtained were general or allele specific, we created a second array of strains carrying both tet-promoter and temperature-sensitive alleles of 42 essential genes for which temperature-sensitive alleles could be obtained. We then crossed this array to five query strains that interacted with these genes (**Fig. 2**). We detected a total of 40 interactions by at least one of the two allele types (tet-promoter or temperature-sensitive alleles), of which 22 were common to both allele types, showing that there is a strong tendency for genetic interactions between two genes to be observed independently of a particular allele ($P < 10^{-12}$, hypergeometric distribution). Most of the interactions that were specific to either tet-promoter or temperature-sensitive strains seemed to stem from technical aspects, such as mismatched semipermissive temperatures. These results show that most of the interactions detected in our larger tet-promoter network were not allele specific.

Large-scale mapping of genetic interactions among nonessential yeast genes[3] showed that synthetic interactions are highly biased toward genes that have related functions. The essential SGA network was also biased toward interactions between genes that share at least one Gene Ontology Biological Process annotation ($P \leq 10^{-4}$
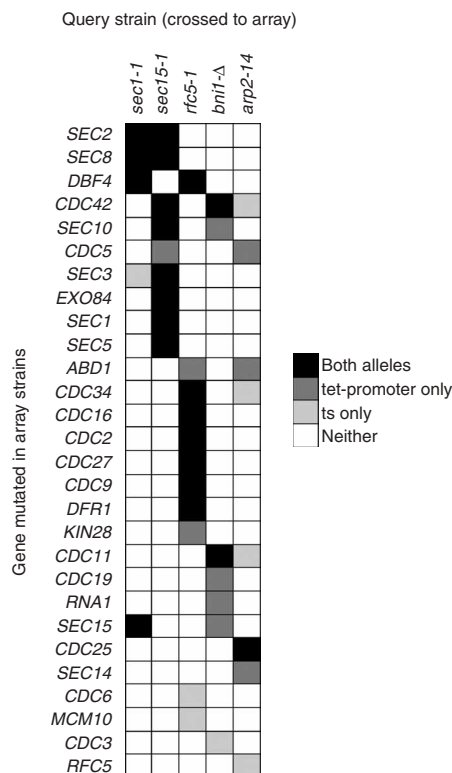

Figure 2 Allele specificity of essential gene interactions. Genetic interactions obtained by crossing five query strains into an array containing strains with both tet-promoter and temperature-sensitive (ts) alleles of 42 essential genes are shown.

compared with distribution of number of interactions for randomly reshuffled row and column labels; **Fig. 1**). In some screens, these relationships were easily rationalized. For instance, most interactions with *RFC5*, which encodes a DNA replication–repair–chromatid cohesion factor[9–11], occurred with components of the DNA replication ($P = 6.4 \times 10^{-11}$) or chromosome segregation machinery (chromosome cycle, $P = 4.2 \times 10^{-10}$; **Fig. 3**). Synthetic interactions with *LRP1*, which encodes an accessory component of the exosome that processes and degrades many types of RNA[12,13], were enriched for genes involved in RNA processing ($P = 9.1 \times 10^{-7}$) and ribosome biogenesis ($P = 1.0 \times 10^{-5}$; **Fig. 3**). Synthetic interactions with *SLT2*, a gene involved in cell-wall organization and biogenesis[14], were biased toward lipid biosynthesis ($P = 7.1 \times 10^{-10}$), exocytosis ($P = 2.1 \times 10^{-5}$), ergosterol biosynthesis ($P = 8.2 \times 10^{-5}$) and protein amino acid glycosylation ($P = 0.0013$; **Fig. 3**). We selected the query genes to represent diverse cellular functions, and consequently, their interaction spectra were largely nonoverlapping (**Fig. 1**); however, we also obtained an interconnected network among genes involved in secretion (**Fig. 3**), consistent with previous genetic analysis of this pathway[7].

There was also a strong tendency for essential genes that share Gene Ontology annotations to show a similar spectrum of interactions in our network ($P < 10^{-6}$, rank sum test), even if the interactions were not suggestive of a direct functional relationship. In one example, among 19 interactions obtained with *RPS17A*, 8 were with genes involved in RNA splicing ($P = 3.3 \times 10^{-7}$; **Figs. 1** and **3**). *RPS17B*, which is nearly identical to *RPS17A* and presumably compensates for its deletion, is one of only 222 yeast genes with an intron[15]; consequently, it is plausible that the *rps17a-Δ* mutant would be sensitive to
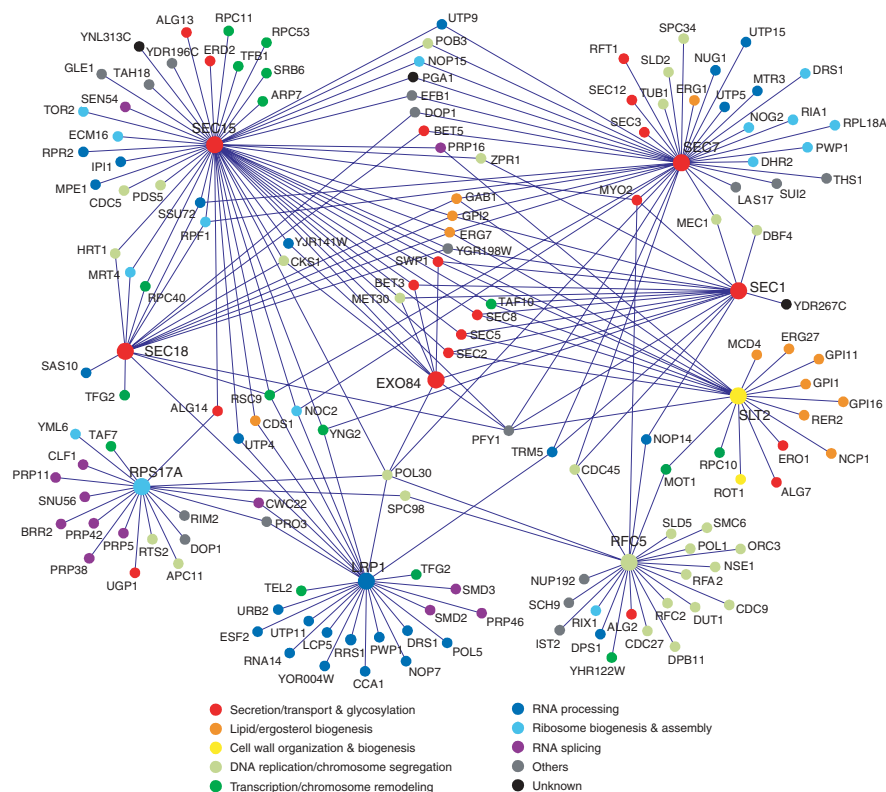
Figure 3 Network diagram summarizing 229 synthetic genetic interactions between nine different queries (*sec1-1*ts, *sec7-1*ts, *sec15-1*ts, *sec18-1*ts, *exo84-102*ts, *rfc5-1*ts, *lrp1-Δ*, *rps17a-Δ* and *slt2-Δ*) and 147 strains in the TetO7-promoter array. Genes are represented as nodes and interactions are represented as edges. The nodes are colored according to their Gene Ontology Biological Process; some were manually annotated from the literature.

mutants, like wild-type yeast, contained largely 2C DNA (*i.e.*, they progressed through S phase more rapidly than *rfc5-1*ts; **Fig. 4a**). In CHEF gel analysis of the double mutants, the chromosome bands had a reduced intensity at 40 min (**Fig. 4b**), indicative of the presence of DNA structures, perhaps replication intermediates, that prevented the chromosomes from entering the gel[17,18]. This reduction was less evident in the *rfc5-1*ts single mutant and was not observed in TetO7-*NSE1* or TetO7-*SMC6*. Accelerated S-phase progression combined with the presence of abnormal DNA structures specifically observed in the double mutants may explain the synthetic genetic interaction of *rfc5-1*ts with TetO7-*NSE1* and TetO7-*SMC6*.

The functional specificity associated with genetic interactions presents an opportunity to assign roles to previously uncharacterized essential genes. For example, the uncharacterized essential gene *YNL158W*, which we now call *PGA1*, interacted genetically with *SEC7*, *SEC15* and *ERG11* (in addition to *BNI1* and *PRT1*), suggesting that it is involved in secretion or membrane biosynthesis (**Figs. 1** and **3**). *YNL158W* encodes Ynl158wp, which localizes to the nuclear periphery[19,20], the site of extrusion of the endoplasmic reticulum (ER) from the nuclear envelope. In a previous analysis[1], TetO7-*YNL158W* showed a phenotypic profile resembling that of genes that function in glycosylation and lipidation. Furthermore, two-hybrid analysis showed that Ynl158wp interacts with Zrg17p, an ER protein that also interacts with several other ER proteins, including Gpi14p (involved in glycosylphosphatidylinositol anchor biosynthesis), Yif1p (involved in ER-to-Golgi protein transport) and Ynl146wp and Yjl097wp (uncharacterized ER proteins[20]). We examined TetO7-*YNL158W* for defects in protein trafficking or associated post-translational modifications using three different glycosylated marker proteins[21–23] (**Fig. 5**). TetO7-*YNL158W* showed no defects in processing of carboxypeptidase Y (CPY), indicating that it is not central to

perturbation of mRNA splicing. Another notable example is the interaction of *RFC5* with *NSE1* and *SMC6* (**Fig. 3**), whose products are both members of a large nuclear complex required for diverse functions in DNA replication and mitosis[16]. To examine these interactions in more detail, we analyzed the DNA content of single and double mutants by flow cytometry (**Fig. 4a**) and by contour-clamped homogeneous electric field (CHEF) gel electrophoresis (**Fig. 4b**). Whereas ~60% of *rfc5-1*ts mutant cells possessed 1C DNA content at 60 min (**Fig. 4a**), indicating a DNA replication block, the double

Figure 4 Analysis of DNA replication in the *rfc5-1* mutants. WT, wild-type. (**a**) Analysis of DNA contents using flow cytometry. Fluorescence-activated cell sorting profiles of *rfc5-1*ts, TetO7-*NSE1*, TetO7-*SMC6* or double-mutant cells in asynchronous culture before G1 arrest (A), arrested in G1 (0′) and at the indicated times after release into the cell cycle are shown. The positions of cells with 1C and 2C DNA contents are indicated. (**b**) CHEF gel analysis of chromosomal DNA. *rfc5-1*ts, TetO7-*NSE1*, TetO7-*SMC6* or double-mutant cells were cultured as in **a** and processed for CHEF gel electrophoresis. Samples were taken 0, 40, 80 and 120 min after release from G1 arrest as indicated. Arrows show chromosomes from the double mutants failing to enter the gel in the 40-min samples.
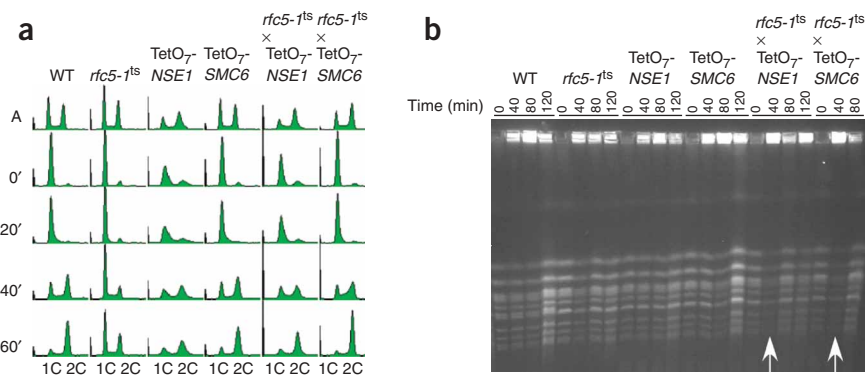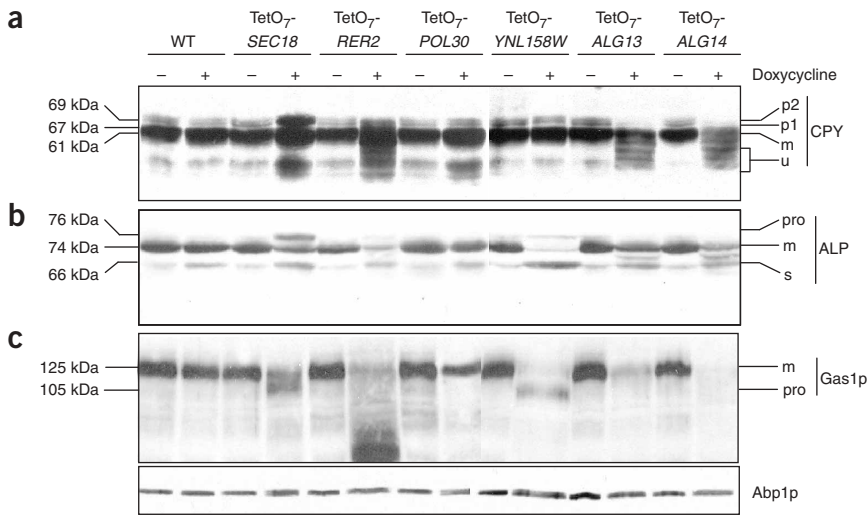
Figure 5 *PGA1* (also known as *YNL158W*) is required for normal protein processing of ALP and Gas1p. We prepared total protein extracts and analyzed them by SDS-PAGE and western blotting. TetO$_7$-*POL30* is shown as a negative control. WT, wild-type. (**a**) CPY-specific western blot. The ER precursor (p1), the Golgi precursor (p2), the mature vacuolar (m) and the unglycosylated (u) forms of CPY are indicated. TetO$_7$-*RER2*, TetO$_7$-*ALG13* and TetO$_7$-*ALG14* are shown as positive controls. (**b**) ALP-specific western blot. The ER (pro), mature vacuolar (m) and soluble aberrant (s) forms of ALP are indicated. TetO$_7$-*ALG13* and TetO$_7$-*ALG14* are shown as positive controls. (**c**) Gas1p-specific western blot. The blot was first probed with Gas1p-specific antibody and then stripped and reprobed with Abp1-specific antibody as a loading control. The ER (pro) and mature (m) forms of Gas1p are indicated. TetO$_7$-*RER2*, TetO$_7$-*ALG13* and TetO$_7$-*ALG14* are included as positive controls.

ER-to-Golgi transport, *N*-linked glycosylation or vacuolar signal peptide cleavage (**Fig. 5a**); however, it accumulated an immature form of Gas1p that comigrated with pro-Gas1p seen in the TetO$_7$-*SEC18* mutant (**Fig. 5c**). TetO$_7$-*YNL158W* also showed incomplete processing of alkaline phosphatase (ALP), predominantly yielding a soluble aberrant 66-kDa form seen in mutants that affect direct targeting of ALP from Golgi to vacuole[24,25] (**Fig. 5b**) and also in *sec7-1*[ts] and *sec18-1*[ts] mutants, which have defects at earlier stages of vesicle trafficking[21,22]. These results strongly support the idea that Ynl158wp has a role in protein sorting or modification in the ER. We named the gene *PGA1*, reflecting its role in processing of Gas1p and ALP.

Our essential synthetic genetic interaction network resembles the nonessential genetic network in that it has a scale-free topology (**Fig. 6a**) and most of the interactions are nonoverlapping with protein-protein interactions (11 of 567 interactions overlap with a set of ~15,000 known physical interactions[3]; data not shown). But there are also distinctions between the synthetic essential network and previously described protein- or genetic-interaction networks[2,3,26]. Despite a statistically strong tendency toward interactions among functionally related genes, the relationship between cataloged gene function and genetic interaction does not seem to be as strong as it is for nonessential genetic interactions[3]: 26% of genetic interactions in the nonessential network (previously described nonessential query and

Figure 6 Characteristics of the essential SGA network. (**a**) Degree distribution. The number of array strains (vertical axis) that interact with any given number of the 30 query strains (horizontal axis) is plotted. (**b**) Cumulative distribution of the number of interactions per query strain, for this study (blue) and a previously published study[3] (red), separated according to essential, nonessential slow-growing and nonessential normal-growing query strains. For this study, the proportion is with respect to the 575 strains on the tet-promoter array; for the other study[3], the proportion is with respect to 4,700 nonessential genes. Each line was drawn by first separating the types of interactions and then sorting the queries by their percent of interactions with the array. The vertical axis plots the proportion of queries exceeding a given percent threshold on the horizontal axis. The higher proportion of essential versus nonessential interactions obtained in this study is presumably the result of scoring at the semipermissive temperature, rather than permissive temperature as previously described[3]. (**c**) Estimated total number of genetic interactions in yeast, categorized by growth phenotypes of interacting genes. The data from this study (three fields at top; *i.e.*, essential versus essential, essential versus nonessential slow growers and essential versus nonessential normal growers) and another study[3] (the lower three fields; *i.e.*, nonessential slow growers versus nonessential normal growers, nonessential slow growers versus nonessential slow growers and nonessential normal growers versus nonessential normal growers) were partitioned into tested interactions of the indicated type (with slow-growing nonessentials as previously defined[4]). The density of interactions in each of the partitioned blocks is shown as a percent of all possible pairs (*e.g.*, if all queries were on the horizontal axis and all array strains on the vertical axis). The total number of expected interactions, based on these percentages, is shown. The size of each field is scaled to the number of potential interactions.
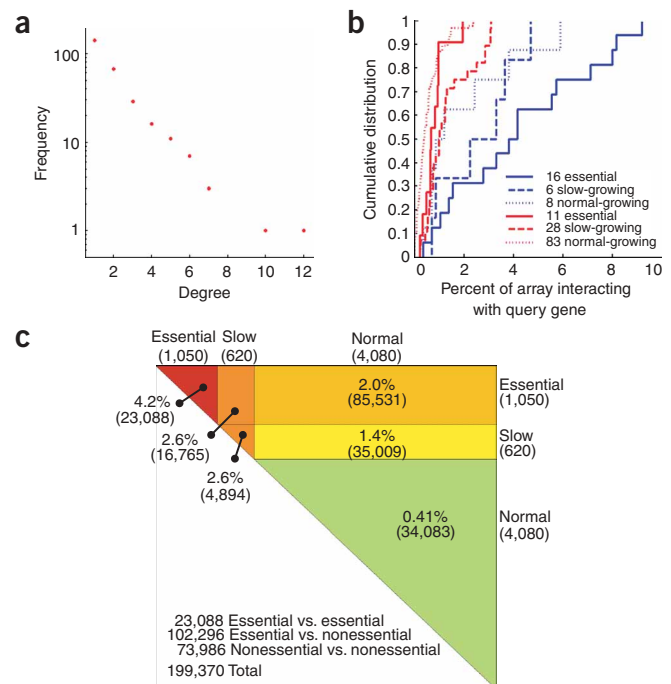
interactor[3]) also share a Gene Ontology Biological Processes annotation, whereas only 17% of genetic interactions in the essential network (essential query and interactor) share the same biological process ($P = 4.0 \times 10^{-5}$, difference in proportions test). Nevertheless, the bias toward functionally relevant interactions indicates that many interactions reflect *bona fide* functional relationships rather than nonspecific additive effects. The interactions that occur among genes of disparate functions also seem to be specific to particular query genes: a proportion of array strains that were sick and thus sensitive to random perturbation would have appeared as horizontal stripes in the matrix display (**Fig. 1**). Moreover, interactions often occur with tet-promoter strains that have little or no growth defect when scored by manual inspection of plates[1].

Perhaps the most notable property of the essential genetic network is its density. The median frequency of interactions was 3% (**Fig. 6b,c**); that is, crossing a query strain to the array of 575 tet-promoter strains resulted in 18 or more interactions for half of the screens (average of 3.28%; 4.19% for essential queries; 2.24% for nonessential queries). This is five times the frequency obtained in a previously described network[3], which used essentially the same screening protocol but consisted primarily of interactions among deletion mutants in non-essential genes. Thus, our results indicate that essential genes are highly connected hubs on the genetic interaction network, and essential pathways, which are presumably under continuous selective pressure toward optimization, are also highly buffered, serve to buffer many other processes, or both. This observation has a profound practical consequence: although only ∼18% of all yeast genes are essential[4,15], the fact that essential genes show about five times the number of genetic interactions as nonessential genes indicates that the full yeast genetic-interaction network may be twice as extensive as previously estimated[3] and that most yeast genetic interactions will involve at least one essential gene (**Fig. 6c**). Furthermore, we obtained many interactions with alleles of essential genes that have little or no impact on the yeast growth rate as single mutants; this type of allele can presumably accumulate in outbred populations[3,27]. Mammalian genomes contain roughly the same proportion of essential genes as yeast, and in laboratory mice the most common phenotype of randomly selected mutants is lethality[28]. Provided that genetic interactions among essential genes in mammals follow the same general trends as in yeast[5,6], genetic interactions involving otherwise silent alleles of essential genes will be key contributors to complex traits in humans and may underlie many of the ∼50% of early catastrophic developmental phenotypes in humans that are not accounted for by mendelian inheritance or chromosomal abnormalities[29].

METHODS

SGA and RSA. For SGA analysis, we crossed 30 nat[R]-marked query strains constructed according to a previously published study[3] to the TetO$_7$ promoter array containing strains corresponding to 575 essential genes. We sporulated three replicates of each of the 30 sets of matings, selected haploid double-mutant progeny on medium with and without 10 μg ml^{-1} doxycycline and incubated them at the semipermissive temperature of the query strain as described[1]. We scored double mutants using a computer-based system and confirmed interactors by RSA as described[3]. We scored the interactions after colonies appeared on the plates, usually after 3 d. A full protocol for SGA analysis and RSA is given in **Supplementary Methods** online. Because we confirmed every reported interaction by RSA, the false-positive rate is zero by our scoring criteria. The false-negative rate is ≥20% (8 of 40) on the basis of gene interaction results (**Fig. 2**; as a result of allele dependency of interactions). In the tet-promoter versus temperature-sensitive allele experiments, of the ten cases in which the tet-promoter allele detected an interaction that the temperature-sensitive allele did not, seven could be attributed to the observation that the semipermissive temperature of either the query or array allele was substantially lower than the other, precluding analysis of the double mutant under conditions where the activity of both genes was compromised. Similarly, in five of the eight instances in which the temperature-sensitive allele detected an interaction that was missed by the tet-promoter allele, the tet-promoter strain had a severe growth defect that may have complicated the scoring of a synthetic interaction.

Flow cytometry. We grew cells at 26 °C in the presence of 10 μg ml^{-1} doxycycline for 14 h until mid-log phase and arrested growth with 2 μg ml^{-1} α-factor for 1 h at 26 °C and then for 1 h at 30 °C. We pelleted G1-arrested cells, washed them and released them into fresh YPD medium (1% yeast extract, 2% peptone and 2% dextrose) at 30 °C. We collected 1×10^7 cells at the indicated times, fixed them with 70% ethanol, washed them with water, resuspended them in 500 μl of 2 mg ml^{-1} RNaseA in 50 mM Tris (pH 8.0) and incubated them for 4 h at 37 °C. We then pelleted treated cells, resuspended them in 500 μl of 50 mM Tris (pH 7.5), 2 mg ml^{-1} proteinase K and incubated them for 1 h at 50 °C. We collected cells and resuspended them in 500 μl of a mixture of 200 mM Tris (pH 7.5), 200 mM NaCl and 78 mM MgCl$_2$. We stained 100 μl of cells with 1 ml of 50 mM Tris (pH 7.5) and 1 μM Sytox Green (Molecular Probes) and sonicated the volume for 5 s before analysis.

CHEF gels. We released cells arrested in G1 as above into fresh YPD medium. We collected 2×10^8 cells at the indicated times and fixed them with 70% ethanol overnight. We washed these cells with 0.5 M EDTA, 1.2 M sorbitol and 1 M Tris (pH 7.5), resuspended them in 125 μl SEMZ (1 M sorbitol, 50 mM EDTA, 28 mM β-mercaptoethanol and 1 mg ml^{-1} zymolyase) and rotated them at 37 °C for 1 h. We added 125 μl of molten 1% InCert agarose (BioWhittaker) to the cells and used 100 μl of the mixture to make one plug. We incubated hardened plugs in 1 ml of SEMZ overnight at 37 °C and incubated them overnight again in 1 ml of 100 mM EDTA, 1% Sarkosyl and 10 mM Tris (pH 8.0) at 37 °C with rotation. We carried out electrophoresis for 17 h in 1.2% agarose (Bio-Rad MegaBase), 0.5× TBE, at 200 V. Pulse times were 90 s for 6 h, 105 s for 5 h and 125 s for 6 h. We stained gels with ethidium bromide.

Western blotting. We grew strains in YPD in the presence or absence of doxycycline (10 μg ml^{-1}) for 12 h (TetO$_7$-SEC18, TetO$_7$-RER2, TetO$_7$-POL30, TetO$_7$-YNL158W) or for 18 h (wild-type, TetO$_7$-ALG13, TetO$_7$-ALG14) at 30 °C to an A_{600} of 0.5. We then collected and centrifuged cultures (25 ml) and resuspended the cell pellets in 1.6 ml fresh YPD. We prepared protein extracts by lysing cells with 200 μl of 1.85 M NaOH for 10 min on ice. We precipitated proteins by incubation with 10% TCA for 30 min on ice and then pelleted them by centrifugation for 5 min at 16,000g. We resuspended protein pellets in 280 μl 1× sample buffer (50 mM Tris-HCl (pH 6.8), 2 mM EDTA, 2% SDS, 10% glycerol, 0.001% bromophenol blue, 48% 1 M Tris-base and 2% β-mercaptoethanol), heated them for 10 min at 95 °C, centrifuged them to pellet debris and transferred the supernatant to a new tube. We separated proteins by 8% SDS-PAGE and transferred them to nitrocellulose membranes. We detected proteins by western blotting with monoclonal CPY-specific antibody or ALP-specific antibody (Molecular Probes), polyclonal Gas1p-specific antibody (gift from H. Riezman, Biozentrum of the University of Basel, Switzerland) or polyclonal Abp1p-specific antibody (gift from B. Goode, Brandeis University, Waltham, Massachusetts), and detected them using enhanced chemiluminescence.

URL. Additional information is available on our project website at http://hugheslab.med.utoronto.ca/Davierwala.

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Note: Supplementary information is available on the Nature Genetics website.

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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