Combining functional genomics and chemical biology to identify targets of bioactive compounds
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Genome sequencing projects have revealed thousands of suspected genes, challenging researchers to develop efficient large-scale functional analysis methodologies. Determining the function of a gene product generally requires a means to alter its function. Genetically tractable model organisms have been widely exploited for the isolation and characterization of activating and inactivating mutations in genes encoding proteins of interest. Chemical genetics represents a complementary approach involving the use of small molecules capable of either inactivating or activating their targets. Saccharomyces cerevisiae has been an important test bed for the development and application of chemical genomic assays aimed at identifying targets and modes of action of known and uncharacterized compounds. Here we review yeast chemical genomic assays strategies for drug target identification.

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Introduction
Linking bioactive small molecules, natural products, and other compounds to their cellular targets is a crucial and challenging goal of chemical biology. Bioactive compounds are increasingly valuable, not only as potential therapeutic agents, but also as bioprobes, allowing temporal, and spatial modulation of target function [1]. A pressing need of the chemical biology community is a means to rapidly and accurately identify the molecular targets and mechanism of action of newly discovered compounds. This need is being met partly through the application of several different functional genomic approaches developed first for the budding yeast, Saccharomyces cerevisiae. These new reagents and methodologies can be directed towards a chemical biology program and provide us with powerful tactics for discovering compound mode-of-action (MOA) and its corresponding target.

Yeast as a model for small molecule discovery
Since its genome sequence was completed almost 15 years ago [2], the model yeast S. cerevisiae has been a common test bed for pioneering genome-wide technologies and reagent sets. Most notable of which is the yeast gene deletion collection consisting of precise start-to-stop deletions where all ~6000 known and suspected S. cerevisiae genes are individually replaced with a kanMX dominant drug resistance marker (Figure 1a). This systematic effort resulted in ~6000 heterozygous diploid and ~5000 viable haploid gene deletion mutants revealing that ~1000 genes are indispensable for yeast viability under standard laboratory conditions [3]. An important feature of the collection is that each deletion strain is ‘barcoded’ with two unique 20 base-pairs oligonucleotides and flanking universal priming sequences, which surround each kanMX drug-resistance marker (Figure 1a). These barcodes allow measurement of individual strain abundance from a mixed population of mutant strains [4]. In fact, molecular barcodes enable parallel fitness assessment of the entire collection of yeast deletion mutants because the abundance of individual barcodes reflects the relative abundance of individual strains that are grown competitively as part of a pool (Figure 1b). Thus, this approach is ideal for systematic identification of gene deletion mutants that confer a growth advantage or disadvantage by subjecting mutant strain pools to specific conditions or drug treatments [4–8].

The competitive growth assay approach is ideal for chemical genomic analysis for several reasons. First, it requires a minimal amount of compound, which is often costly and limited by availability. Second, pooled screening is much more rapid than the testing of individual strains. Finally, pooled screening results in a more controlled experiment because all strains are tested simultaneously under identical conditions using the same batch of compound. A set of donor strains, called Barcoders, was recently constructed that allows...
unique barcode sequences to be transferred to any *S. cerevisiae* strain collection in a rapid and cost-effective manner using yeast mating and simple genetic manipulations, thereby offering the potential to expand and apply parallel pooled approaches to a wide variety of complex bioassays [9*].

**Drug-induced haploinsufficiency profiling (HIP)**

The competitive growth assay approach has been extensively applied to examine drug-induced haploinsufficiency profiling (HIP). Haploinsufficiency occurs when lowering the dosage of a gene from two copies to one copy
Figure 2

Two-dimensional hierarchical clustering analysis of chemical–genetic profiles. Chemical–genetic profiles associated with eighty-two conditions, including 75 compounds and 7 crude extracts, were clustered. 3418 genes are plotted on the horizontal axis and compounds are plotted on the vertical axis. Chemical–genetic interactions are represented as red lines. Compounds with similar MOAs tend to exhibit similar chemical–genetic profiles and thus cluster together. Distinct clusters are indicated by roman numerals: (i) actin binding agents (latrunculin B, and cytochalasin A); (ii) cell wall synthesis inhibitors (staurosporine, and caspofungin); (iii) nystatin and amphotericin, both increasing the permeability of the fungal cell membrane;
in a diploid organism results in a growth phenotype [10,11]. Giaever et al. demonstrated that decreased dosage of a drug target gene in a heterozygous mutant background can result in increased drug sensitivity [12]. Using the molecular barcode microarray as a readout, Giaever et al. identified ALG7 as one of the three strains from a pool of 223 heterozygous deletion mutants that are hypersensitive to tunicamycin, the drug that targets Alg7 [12]. More recently, the HIP assay was applied to the complete heterozygous deletion strain collection [7,13,14]. HIP profiling can reveal not only the direct drug target but also components that belong to the relevant signaling or metabolic pathway. For example, HIP profiling of methotrexate identifies its known target, dihydrofolate reductase (DFR1), as well as the FOL1 and FOL2 genes that encode upstream components of the same folic acid biosynthesis pathway [14]. Importantly, genome-wide approaches like the HIP assay are unbiased and do not require prior knowledge of the compound’s MOA, as long as the compound inhibits growth. For example, while the anti-cancer drug 5-flourouracil (5-FU) was thought to exert its cytotoxic effects by inhibiting thymidylate synthetase [15], two independent HIP profiling experiments both identified the rRNA processing exosome as a potentially new 5-FU target [13,14]. In another example, HIP profiling revealed that a wide range of yeast cellular processes are affected by exposure to human psychoactive compounds [16]. Despite lacking orthologs to the human target genes, many essential yeast genes involved in the establishment of cell polarity were haploinsufficient to fluoxetine (Prozac) while decreased dosage of RNA metabolic genes conferred sensitivity to paroxetine (Paxil) [16]. These findings suggest an important role for yeast cell-based studies in uncovering secondary drug targets that may contribute to the drug’s anti-psychotic effects. Alternatively, functional genomics studies, such as the yeast HIP assay, may also identify off-targets providing insight into potential side effects associated with a particular drug treatment [16].

Although pioneered in S. cerevisiae, haploinsufficiency profiling has also been applied to other yeast species. Recently, a collection of ~2300 uniquely barcoded heterozygous deletion mutants covering ~45% of the Candida albicans genome were constructed, pooled, and screened in the presence of variety of different compounds [17–20]. This approach, called fitness test (FT), identified GMP synthase, poly(A) polymerase, and Delta(9) fatty acid desaturase as targets of several different chemical compounds [18–20].

Despite the usefulness of this assay, there are limitations associated with HIP profiling assays. First, the HIP assay may not be able to identify the direct target, if the drug does not directly affect protein function. However, the pathway components involved in the activity of the drug targets may still be identified. For example, HIP profiling of papuamide A identified several hundred genes that are sensitive to exposure to this natural product (Parsons and Boone, unpublished data), but failed to identify phosphatidylerine as its direct target [21]. Second, the HIP assay will not work in its simplest form if the drug target is a non-essential gene or if the target has a highly redundant partner, such as a paralog.

Another limitation of the HIP assay is that not all target genes will lead to drug hypersensitivity when only one of two diploid copies is deleted. A more severe reduction in target gene expression may be required to sensitize a strain to a given drug. The use of DAmP (decreased abundance by mRNA perturbation) alleles where the native 3’UTR of the gene is disrupted resulting in decreased stability of the corresponding mRNA transcript may circumvent this problem [22]. Indeed, a study employing DAmP alleles of essential genes carrying two unique molecular barcodes integrated at the HO locus, demonstrated that DAmP mutants can be more sensitive to drug than heterozygous deletion mutants [9*].

**Chemical–genetic profiling using haploid or homozygous deletion strain collections**

A variation of HIP profiling involves screening of haploid or homozygous diploid deletion mutant collections [7,21]. Similar to screens using heterozygous diploid mutants, haploid and homozygous deletion mutants can be screened simultaneously in liquid [7,21], with the strain abundance monitored via barcode microarray hybridization or next-generation sequencing [3,23*]. Alternatively, drug sensitive mutants can also be identified by assessing colony growth of individual mutants on agar plates [24,25].

Unlike HIP assays using heterozygous diploids that usually identify fewer than 10 candidate genes per screen, chemical–genetic profiling of homozygous or haploid deletion mutants can often identify hundreds of drug sensitive mutants [14,21]. Chemical–genetic profiling of antifungal compounds with the viable haploid or homozygous deletion mutants does not identify drug targets directly because the target gene is absent from the strain collection. However, the genes identified

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(Figure 2 Legend Continued) (iv) clotrimazole and fluconazole (chemical analogs, and antifungal agents that target Erg11); (v) radicicol and geldanamycin, both highly selective inhibitors of Hsp90 function; (vi) microtubule poisons benomyl and nocodazole; (vii) ERG2 inhibitors haloperidol, fenpipomorph, and dyclonine; (viii) DNA damaging agents MMS, camptothecin, cisplatin, hydroxyurea, and mitomycin C; (ix) tamoxifen and amiodarone; (x) extract 00-192, extract 00-132, theopalaauamide and stichloroside; (xi) membrane pore-forming peptide alamethicin and papuamide B. Adapted from [25].
in this assay may include those that belong to the target pathway, genes whose pathways buffer the target pathway and are important for its cellular function when the target pathway is compromised, or genes involved in general stress response, drug metabolism, and transport [7,14,16,21,25,26]. For example, chemical–genetic profiling of the immunosuppressant rapamycin that binds to FKBP12 and in turn inhibits TOR kinase [27] identified 73 mutants that were rapamycin-hypersensitive and 27 mutants that were rapamycin-resistant [28]. These genes covered a wide range of cellular processes, from nutrient signaling to transcriptional regulation. In another study, Lee et al. screened the homozygous deletion collection to identify mutants sensitive to a set of 12 DNA-damaging agents [29]. Interestingly, each compound identified different DNA repair pathway components suggesting that different DNA damage stress can illicit unique cellular responses [29]. Therefore, although the actual drug targets were not identified in this study, chemical–genetic profiling provided insights into the cellular components required to deal with DNA damaging stress.

Compounds with similar biological activity should also share similar chemical–genetic profiles. Thus, although the drug target may not be identified directly in this assay, it can be inferred by global comparison of chemical–genetic profiles associated with well-characterized compounds with known MOAs (Figure 2) [7,21,25,29]. Based on this hypothesis, Parsons et al. constructed a compendium of 82 chemical–genetic profiles and systematically compared them using two-dimensional clustering [21]. This analysis revealed that chemicals with similar MOAs also share similar chemical–genetic profiles and as a result cluster together [21]. For example, a set of haploid deletion mutants sensitive to the membrane pore-forming peptide, alamethicin, were also sensitive to the uncharacterized natural product, papuamide B, suggesting that papuamide B may also act as a pore-forming compound [21]. Similar results were obtained by clustering drug-sensitivity and condition-sensitivity profiles associated with the homozygous deletion collection [7].

**Integrating genetic and chemical–genetic networks for drug target discovery**

Because drug-mediated perturbations mimic genetic perturbations to a certain extent (Figure 3a), integrating chemical–genetic with synthetic genetic interaction profiles represents another key method for uncovering targets of bioactive molecules [25,30*]. Genetic interactions, such as synthetic lethality, identify genes that impinge on a common essential function [31]. As described above, drug-sensitive mutants often belong to pathways or complexes that function in parallel to the target of a specific compound [14,21,25,32]. A proof-of-concept study demonstrated that a set of mutants, which are sensitive to a given drug, should also share synthetic lethal interactions with the gene encoding the drug target [25] (Figure 3b). This concept was recently put into practice through a genome-wide comparison of genetic and chemical–genetic profiles [30*] (Figure 3c). Combining genetic and chemical–genetic interaction profiles showed that the anti-fungal activity of a previously uncharacterized small molecule (subsequently named erodoxin) is mediated through inhibition of the ERO1 gene product, which is involved in oxidative protein folding [30*] (Figure 3d). Thus, although chemical–genetic profiles can be difficult to interpret owing to the large number of genes that may be sensitive to a single drug, global comparisons of chemical–genetic profiles alone or integrated with genetic–interaction profiles can provide a powerful way to decipher drug targets.

Chemical–genetic profiling of deletion mutants has yet to be applied extensively to model organisms other than budding yeast. However, genome-wide deletion sets have been reported for *E. coli* [33], fission yeast [34], and pathogenic models such as *Candida albicans* and *Cryptococcus neoformans* [35*,36*]. Moreover, a universal molecular barcoded vector collection called TagModule was recently developed and used to barcode *Shewanella oneidensis* and *Candida albicans* transposon mutagenesis libraries [37*].

The availability of genome-wide RNAi libraries enables analogous loss-of-function genetic screens in mammalian cells [38,39]. Recently, a haploid loss-of-function human cell line collection was generated using insertion mutagenesis and applied to identify host factors required for influenza infection [40*]. This collection should complement the RNAi knockdown collection and may be more suitable for chemical–genetic profiling. Importantly, several studies have shown that shRNA-mediated loss-of-function screens can also be conducted in a pooled format since shRNA hairpins can serve as a unique identifier sequence analogous to a molecular barcode [41–43]. Thus, chemical–genetic studies pioneered in budding yeast should soon be expanded to a wide range of different species and cell types.

**Dosage suppression**

In contrast to haploinsufficiency and chemical–genetic profiling, increasing the dosage of a drug target or components of a target pathway can confer resistance to drug-mediated growth inhibition. Dosage-dependent suppression of drug toxicity has long been used to identify drug targets in yeast [44]. Traditionally, a random genomic DNA library is introduced into a wild-type strain and individual transformants are subsequently screened for drug resistance. Advances in functional genomics have now provided several new technologies to allow faster and less labor-intensive gene dosage
screens while minimizing the amount of drug used. For example, arrays of yeast strains transformed with a genomic DNA (gDNA) library have been assembled [45,46] and dosage-dependent suppression screens can be carried out easily by pinning the arrays onto drug-containing media. Moreover, different plasmid libraries have been developed such that genomic DNA fragments or molecular barcodes can be used to monitor

(a) The relationship between genetic and chemical–genetic interactions. In a chemical–genetic interaction (above), a deletion mutant, lacking the product of gene X, is hypersensitive to a normally sublethal concentration of a growth-inhibitory compound. In a synthetic lethal genetic interaction (below), single gene deletions lead to viable mutants but result in a lethal phenotype when combined in the same double-mutant strain. Gene deletion alleles that show chemical–genetic interactions with a particular compound should also be synthetically lethal or sick with a mutation in the compound target gene. Adapted from [25]. (b) Comparison of a chemical–genetic profile to a compendium of genetic interaction (synthetic lethal) profiles should identify the pathways and targets inhibited by drug treatment. In this hypothetical figure, chemical–genetic and genetic interactions are both designated by red squares. The set of deletion mutants hypersensitive to compound X overlaps the set of deletion mutants showing a severe fitness defect when combined with deletion of gene B. Therefore, the product of gene B is likely to be a target of compound X. Adapted from [25]. (c) A chemical genetic interaction map is shown where colored triangles represent chemical compounds and white circles correspond to genes. Genetic profile similarities were measured for all gene pairs by computing Pearson correlation coefficients (PCC) from the complete genetic interaction matrix [62]. Gene pairs whose profile similarity exceeded a PCC > 0.2 threshold were connected in the network and laid out using an edge-weighted, spring-embedded, network layout algorithm. Genes sharing similar patterns of genetic interactions are proximal to each other in two-dimensional space, while less-similar genes are positioned further apart. Colored regions indicate sets of genes enriched for a specific GO biological process. Compounds were positioned on the map by highlighting the gene node whose genetic interaction profile most closely resembles the chemical genetic profile of the compound. Compounds tightly correlated to genes positioned within functional clusters were colored accordingly to the color of the cluster. The chemical–genetic profile of hydroxyurea clustered with genes involved in DNA replication and repair, whereas that of Erodoxin clustered with genes involved in protein folding, glycosylation, and cell wall biosynthesis. Compounds positioned outside functional clusters are colored in light blue. The chemical structures of Hydroxyurea and Erodoxin are shown. (d) Erodoxin inhibits Ero1-dependent oxidation of Trx1 in vitro. Adapted from [62].
the relative abundance of the individual transformants, allowing parallel analysis in pooled liquid cultures [47,48,49*].

Dosage-dependent suppression has also been used to identify drug targets in other model organisms. Pathania et al. used a multi-copy plasmid library to carry out dosage-suppression studies in E. coli. Specifically, a compound was identified that mediates its toxic effects by blocking lipoprotein targeting and showed antibacterial activity against multidrug-resistant gram-positive bacteria [50*].

As described above, dosage-dependent suppression is commonly used to find genes that confer drug resistance when overexpressed. A reverse strategy can also be informative for uncovering compounds that suppress growth defects associated with specific genetic perturbations. For example, a chemical library was screened to identify compounds capable of suppressing toxic effects associated with overexpression of the Pseudomonas aruginosa ADP-Ribosylate Exoenzyme S (ExoS) protein in budding yeast. ExoS is a protein required for infection of human cells and a chemical exosin was identified and shown to target the ExoS protein [51]. A strategy analogous to this can be used for proteins from other organisms [51].

With respect to methodological limitations, dosage suppression often suffers from the same problem as chemical–genetic profiling because a large number of candidate suppressors are often identified [45,47]. To help overcome this limitation a strategy was developed that integrates genome-wide haploinsufficiency, chemical–genetic profiling, and dosage-suppression [49*]. The integration of these three methods improved the sensitivity and specificity of drug target identification [49*]. Using this strategy, Kemmer et al. examined the biological effect of a tumor cell invasion inhibitor, dihydromotuporamine C (dhMotC) [52]. Dosage suppression analysis determined that mitochondrial respiration and cytochrome c heme lyase were required for growth inhibition mediated by dhMotC while chemical–genetic profiling suggested that dhMotC exposure affected vacuolar pH and vesicle-mediated transport [52]. These biological effects were attributed to dhMotC-dependent defects in sphingolipid biosynthesis, which were revealed in a haploinsufficiency assay [52]. This example shows that different assays provide different information regarding drug inhibition, ranging from drug target, components of drug target pathways and physiological effects of the drug [52].

In addition to identifying direct drug targets, dosage suppressors can be used as a signature profile for a compound, similar to chemical–genetic profiling. Using fission yeast as a model, Nishimura and colleagues generated dosage suppression as well as dosage sensitivity profiles for several drugs with known modes-of-action [53]. In their study, a galactose-inducible promoter is introduced in front of every gene, which is then integrated into the genome rather than on a plasmid [53]. Using this profiling method and several follow-up experiments, theonelladimide was shown to bind to ergosterol resulting in the induction of membrane damage and activation of Rho1-dependent cell wall biosynthesis [53].

Identification of spontaneous drug-resistant mutants
Most of the functional genomics strategies discussed so far involve reverse-genetic approaches. Forward-genetic approaches have also been used for small-molecule and drug target discovery. This is usually accomplished by random mutagenesis followed by selection for mutants that show a phenotype of interest. In chemical–genetic research, this phenotype usually involves increased drug resistance, but this strategy is amenable to any phenotype that can be easily monitored. A forward-genetic approach has several advantages. First, it enables isolation of mutations that produce a desired phenotype. Second, although not saturating or systematic, this approach is not restricted to a single type of genetic mutation (i.e. complete gene deletion) and a much broader spectrum of spontaneous mutations including partial gene deletion and point mutations can be surveyed for a given phenotype.

Spontaneous drug-resistant mutants have been used routinely to identify the MOA of bioactive chemicals and, in some cases, the mutations identify the primary drug target of the chemical. These mutations often occur in the binding site of a target protein thereby preventing interaction with the drug. For example, Rock et al. identified leucyl-tRNA synthetase as the target of 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole by isolating a dominant resistant mutation in the CDC60 gene, which encodes leucyl-tRNA synthetase [54]. Sometimes the mutation does not identify the primary target of the drug but rather components implicated in the same biological process and thus provide valuable clues about the actual target. For example, Parsons et al. identified phosphatidylserine as the target of papaumide B by isolating a recessive resistant mutant in the CHO1 gene, which encodes phosphatidylserine synthase [21]. This mutation prevents the cell from producing phosphatidylserine and renders the antifungal natural product ineffective [21]. In some cases, all of the major components of the MOA of the drug can be identified in a single forward-genetic screen. For example, rapamycin works by binding to FKBP12 and forms a toxic complex that inhibits the Tor1 and Tor2 kinases. In a screen to identify rapamycin resistant mutants Heitman and colleagues identified mutations belonging to three complementation groups FPR1, TOR1, and TOR2. FPR1 encodes yeast FKBP12 while TOR1 and TOR2 encode the yeast Tor kinases.
Strategies for mapping spontaneous drug resistant mutants

Genetic strategies for isolating drug-resistant mutants are well established [21,27,54,55]. Wild-type cells are usually plated on an agar plate containing a high concentration of drug and resistant mutants usually arise several days after incubation (Figure 4a). Traditionally, gene mapping is used to identify spontaneous drug resistant mutations and the methods used depend on the nature of the mutations. There are two major types of resistant mutants: dominant or recessive. To map a dominant drug resistant mutant, a random genomic DNA library is typically generated from the resistant mutant and transformed into a wild-type strain. Transformants are then tested for drug resistance by replica plating onto agar plate containing drug and colonies exhibiting drug resistance are considered candidates that harbor the drug-resistant mutation [54].

The majority of drug-resistant mutants identified in yeast are recessive and complementation cloning is usually used to identify these types of mutations [21,27,55–59]. Typically, a resistant mutant is transformed with a wild-type yeast genomic DNA library. Transformants are then tested for drug resistance by replica plating onto agar plates containing drug. Because the mutation is recessive and can be complemented by the wild-type copy of the gene, colonies exhibiting wild-type drug susceptibility are candidates that harbor the wild-type copy of the drug-resistant gene [21,60**].

A method named synthetic genetic array mapping (SGAM) that uses the haploid yeast deletion collection and SGA technology can be applied to map both dominant and recessive drug-resistant mutants [61,62]. The systematic deletion mutant collection represents markers covering almost every kilobase of the *Saccharomyces cerevisiae* genome. SGA is used to construct double mutants by mating, selection, and meiotic recombination. In SGAM, a drug-resistant mutant is mated to the deletion mutant array and a haploid mutant containing both the deletion and the drug-resistant mutation is then isolated through a series of robotic-assisted selection steps. The general chromosomal location of the suppressor allele(s) is then identified by failure to observe colony growth for a linked set of double-deletion strains [61,62].

With a major aim of increasing the efficiency of mapping drug-resistant mutants, a molecular barcoded yeast ORF library (MoBY-ORF) was developed for high-throughput complementation assays [60**] (Figure 4b). Each yeast gene in the MoBY-ORF library is under the control of its own promoter and transcriptional termination sequences. In addition, this collection is barcoded and, therefore, allows simultaneous analysis of the fitness of all transformants with barcode microarrays or barseq analysis [60**] (Figure 4c). Using this method, a partial loss-of-function mutation that conferred resistance to two different natural products, theopalaumamide and stichloroside, was identified in *MVD1* [60**]. Previously, the results of chemical–genetic profiling had suggested that these two different natural products worked through the same pathway and acted as membrane-disrupting agents; however, their primary target remained elusive [21]. *MVD1* encodes the enzyme pyrophosphate decarboxylase involved in ergosterol biosynthesis. Biochemical experiments identified ergosterol as the primary target of both theopalaumamide and stichloroside [60**].

The main limitation of the MoBY-ORF complementation approach is that cloning by complementation requires the drug-resistant mutant to be at least partially recessive. Recently, it has become clear that whole genome polymorphism analysis, either by tiling array or high-throughput sequencing, provides a straightforward approach for identifying candidate polymorphisms associated with a drug-resistant phenotype [63,64]. Nevertheless, the genes associated with candidate polymorphisms must be confirmed and the MoBY-ORF library makes this a relatively simple process because the library is ordered and covers most of the yeast genome.

Chemical–chemical profiling

Chemical–chemical profiling is a novel method of linking compounds to targets and modes of action using the phenomena of drug synergy and antagonism [65,66*]. Chemical–chemical profiling can loosely be defined as two bioactive drugs that when applied together at sub-active doses cause a phenotypic response not observed by either compound alone. For a complete review of the various models of drug synergy and antagonism see [67]. In a systematic application of chemical–chemical profiling 14 well-characterized antibacterial drugs were tested in combination with 186 unknown bioactive compounds to identify 255 synergistic drug–drug interactions in *E. coli* [66*]. The authors clustered these chemical–chemical interaction profiles to predict the MOA of two unknown bioactive compounds that may act as putative inhibitors of DNA gyrase and dihydrofolate reductase. Like other methods aimed at drug target identification, detailed biochemical and biophysical analysis was required to validate predictions. Nonetheless, this study represents the first instance where simple synergy screening was successfully used to characterize unknown compounds and highlights chemical–chemical profiling as an effective method for drug target identification in microbial systems. As such, chemical–chemical profiling may provide a way to develop high-throughput screens for bioactive compounds against genetically uncharacterized and emerging infectious diseases.
(a) Isolation of drug-resistant mutants. Wild-type yeast cells are plated on solid media containing a high dose of a growth inhibitory compound and incubated for several days. Cells harboring a mutation that confers drug resistance form colonies (shown in red).

(b) MoBY-ORF library. Each yeast ORF, flanked by its upstream and downstream sequences, is cloned into a vector containing selectable markers (URA3 and kanMX) and a yeast centromere sequence (CEN). The kanMX cassette is flanked by two different molecular barcodes, labeled UPTAG (UP) and DOWNTAG (DN) and common primer sites indicated in dark blue and brown colors. The vector backbone was designed to be compatible with the MAGIC system for manipulating plasmid inserts by homologous recombination in *Escherichia coli*. Filled yellow circles represent MAGIC recombination sites flanking the MoBY-ORF inserts. Adapted from [60**].

(c) A drug-resistant yeast strain, carrying a mutation in gene B, is transformed with the MoBY-ORF library.
While chemical–chemical profiling has yet to be applied in a high-throughput manner, this method provides certain advantages compared to chemical–genetic. For example, genome-wide resources such as gene deletion strain libraries are not required, thus making chemical-chemical profiling applicable to any organism of interest. Nevertheless, chemical–chemical profiling also has a number of limitations. First, the number of compounds with specifically defined targets is limited and, thus, far less data is available for clustering and ‘triangulating’ the potential target of novel, uncharacterized compounds. Second, as mentioned above, the great advantage of \textit{S. cerevisiae} (and \textit{S. pombe}) chemical-genomics is the ability to cross reference chemical genetic profiles with genetic interaction data. However, the amount of genetic interaction data available for organisms other than yeast is sparse and far more limited. Finally, chemical–chemical profiling is more compound intensive than chemical–genetic methods described above.

**Perspective**

Advances in yeast functional genomic resources have allowed different genome-wide technologies to be developed for chemical biology, and led the way for development of similar tools in other model organisms. These technologies allow systematic testing of all genes for roles in response to bioactive compounds. Applications of these technologies have led to characterization of a number of novel small molecules and their MOAs. Additionally, chemical–genetic profiling has proved valuable to related fields, such as discovery of drug synergy relationships \[68\]. Lessons learned in yeast can be transferred to other model organisms and should prove valuable for identifying targets conserved across taxa. In particular, the application of similar technologies in mammalian cell models will enable researchers to expand beyond yeast.

Drug-induced, genome-wide haploinsufficiency screens have not yet been performed in mammalian cells because analogous genome-wide heterozygous cell line collections do not exist. One surrogate, however, is to use RNA interference (RNAi) to silence the expression of individual genes. Currently, the majority of genome-wide RNAi screens in drug discovery research has focused on identifying druggable target genes in a disease model. Several studies have used RNAi coupled with anti-cancer compounds to identify genes that lead to increased efficacy of anti-cancer compounds \[43,69–72\]. Although the primary aim did not involve characterization of drug targets, these studies suggest that application of genome-wide RNAi assays for drug target identification in mammalian cells holds much promise.

What is the future for yeast in small molecule discovery research if it can be done in other models? In our opinion, while \textit{S. cerevisiae} will probably remain a widely used model for chemical genomics in the near term, more and more deletion collections are being created that will probably have other benefits owing to different homologies with mammalian cells. A suite of barcoded, fungal deletion collections (e.g. \textit{S. cerevisiae}, \textit{S. pombe}, \textit{C. albicans}, \textit{C. neoformans}) would be very valuable to defining conserved fungal networks and identifying novel targets for antifungal drugs. Additionally, these could be used to compare orthologous genes and their response to drug treatment providing insight into the rewiring of genetic networks over evolutionary time. Finally, as high-throughput experimentation and chemical-genomics studies are scaled up to comprehensive screening of large drug libraries, data archiving will become increasingly important and thus the chemical biology community would greatly benefit from a centralized database of these interactions, the compounds, and species involved.

It must be noted that chemical-genomic methods, while extremely powerful in predicting cellular targets, all require detailed validation of the physical ligand–protein interaction. This process can be a difficult task, requiring sophisticated biophysical and immunological methodologies. This is a new bottleneck in the practice of chemical biology and will probably remain so as chemical genomics predicts more and more targets. Thus, truly high-throughput chemical biology will depend on advancements in compound labeling, mass spectrometry, and protein/drug arrays, where known bioactive compounds with defined chemical–genetic profiles can be assessed in a high-density manner to confirm the target protein.

As powerful as the discussed strategies are, each provides different information and each has its own limitations. Integration of different methods should provide a clearer picture of the MOAs of chemical compounds. Current studies tend to focus on identification of the primary drug target while secondary targets, the off-targets, are usually not characterized. However, a comprehensive understanding of all biological consequences and physiological responses related to drug inhibition is crucial to fully understand a drug’s MOA and ultimately design improved drugs or chemical probes. To achieve this goal, chemical genomic, chemical proteomic, and hypothesis-driven biological studies must be combined in any studying of any small molecules.
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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest


This study describes a universal barcode collection for transferring unique barcode sequences to any budding yeast strain collection.


The first study to measure the relative strain abundance by barcode sequencing.


This study describes the largest genome-wide genetic interaction map for yeast and illustrates integration of genetic and chemical-genetic networks to identify a drug target.


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43. Brummelkamp TR, Fabius AWM, Mullenders J, Madiredjo M, C. Combining functional genomics and chemical biology to identify targets of bioactive compounds.


This paper is the first to describe a high-throughput, whole cell approach to drug target identification that is independent of model systems.


