Bioorganic & Medicinal Chemistry 20 (2012) 1952–1960

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Chemical-genomic profiling: Systematic analysis of the cellular targets of bioactive molecules

Kerry Andrusiak [a], Jeff S. Piotrowski [b], Charles Boone [a,b,*]

[a] Banting and Best Department of Medical Research and Department of Molecular Genetics, Donnelly Centre, University of Toronto, 160 College St., Toronto, ON, Canada M5S 3E1
[b] Chemical-Genomic Research Group, RIKEN Advance Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

ARTICLE INFO

Article history:
Available online 21 December 2011

Keywords:
Chemical-genomics
Target identification
Genetic interactions
Yeast mutants
Genetic networks

ABSTRACT

Chemical-genomic (CG) profiling of bioactive compounds is a powerful approach for drug target identification and mode of action studies. Within the last decade, research focused largely on the development and application of CG approaches in the model yeast *Saccharomyces cerevisiae*. The success of these methods has sparked interest in transitioning CG profiling to other biological systems to extend clinical and evolutionary relevance. Additionally, CG profiling has proven to enhance drug-synergy screens for developing combinatorial therapies. Herein, we briefly review CG profiling, focusing on emerging cross-species technologies and novel drug-synergy applications, as well as outlining needs within the field.

1. Introduction

Drug discovery endeavors are continually challenged to keep pace with market demands for novel, effective therapeutics. There is also a general need for compound tools that allow us to modulate cellular function to probe diverse biological processes. The path between discovering small molecules to obtaining a detailed description of their target(s) and mode of action (MOA) is critical but remains a significant bottleneck in the drug/tool compound development pipeline. Given the current scope of small molecule chemical space (>10^{60} compounds)[1] and the increasing rate of compound synthesis via combinational chemistry, identifying specific drug leads is a massive undertaking requiring a multifaceted approach. High throughput screening (HTS) strategies have been successful in identifying lead compounds from large libraries[2]; however these approaches are disadvantaged by their design around a relatively small subset of specific targets. There is a clear need for methodologies that rapidly characterize compounds in an unbiased fashion and provide rich, functional information to elucidate their MOAs and targets. Combining chemical biology and functional genomics methodologies and reagents provides a powerful new way of achieving this goal.

In particular, CG profiling takes advantage of genome-wide reagent sets that modulate gene function to probe the cellular roles of bioactive compounds. While the chemical-genomics platform centered on the *Saccharomyces cerevisiae* deletion mutant collection[3] has been used for nearly a decade, the past few years have seen a rapid increase in novel applications and successes extending across different biological systems. In this review, we will summarize existing CG methods, focusing on newly evolved cross-taxa approaches and novel applications for drug synergy screening, which may set a course for chemical-genomics analysis in the coming years.

2. Yeast chemical-genomic profiling

Chemical-genomics seeks to identify functional relationships between specific genes and chemical compounds through systematic analysis of all genes in a genome. To accomplish this, the most common approach is to genetically alter each gene and assess resulting mutants for a phenotypic response (such as a change in growth rate) in the presence of a bioactive molecule (Fig. 1a). As the first eukaryotic organism fully sequenced, *S. cerevisiae* has been the basis of many functional genomics resources, including a genome-wide deletion mutant collection[3] and gene-overexpression libraries. Also, budding yeast has long been favored as a model organism given its homology to other eukaryotes and its genetic and experimental tractability. Therefore, not surprisingly, some of the first CG methods developed were pioneered in *S. cerevisiae*.

The general CG approaches based in *S. cerevisiae* have been recently reviewed[4–7] and representative studies are presented in Table 1. Apart from the identification of spontaneous drug-resistant mutants, the majority of these methods take advantage of analyzing the chemical sensitivity of mutants with altered gene dosages. Different gene dosages range from 0% gene dose with homozygous/haploid deletion profiling (HOP), to increased gene dosage (~5- to 10-fold) with multi-copy suppression analysis using gene-overexpression libraries. In the middle, haploid insufficiency profiling (HIP) screens diploid mutants with ~50% gene

* Corresponding author. Tel.: +1 416 946 7260; fax: +1 416 978 8287.
E-mail address: charlie.boone@utoronto.ca (C. Boone).

0968-0896/$ - see front matter © 2012 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2011.12.023

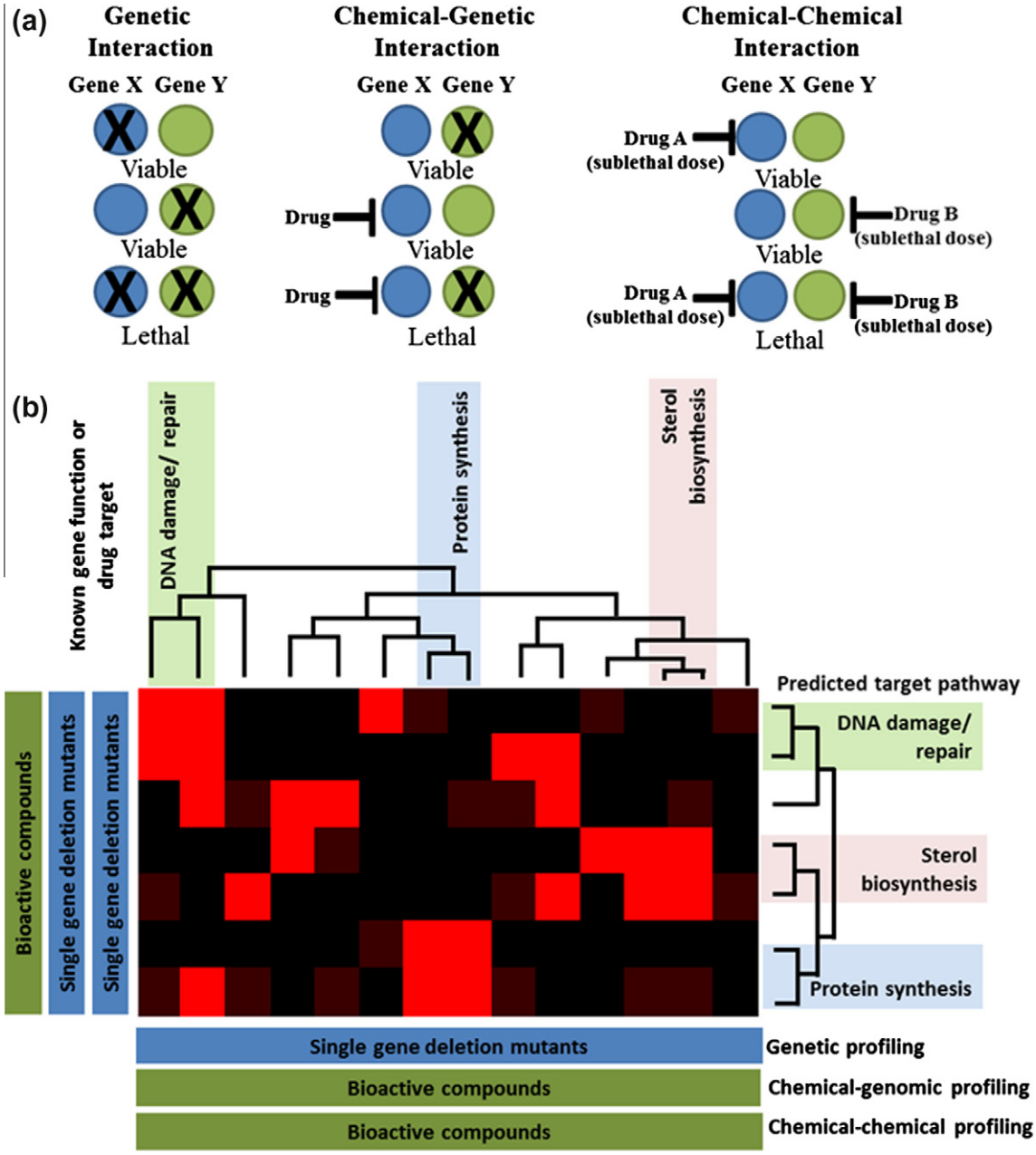

Figure 1. *Comparison of genetic, chemical–genetic, and chemical–chemical profiling.* Two interacting genes are inactivated via targeted deletion and the resultant double mutant assessed for viability (a). In chemical–genetic interactions, one gene product is inactivated via gene deletion and the other is inactivated by chemical inhibition. In a chemical–chemical interaction, the activities of two independent compounds are synergistic. Three methods of compound profiling are presented in (b).The X and Y axes may be either an array of single gene deletion mutants (genetic profiling), a combination of gene deletion mutants and compounds (chemical–genetic profiling) or chemical compounds only (chemical–chemical profiling). Red indicates a significant gene–gene, drug–gene, or drug–drug interaction, and can to predict the target process of unknown agents. Here, known compounds or genes on the X-axis are clustered based on known mode of action or function, and the Y-axis shows clustering of other unknown compounds or genes with similar profiles.

dose while a more extreme reduction in gene dose, in the range of ~5–50%, can be achieved through the DaMP (decreased abundance by mRNA perturbation) mutant collection (Table 1).

Because most of these yeast mutant collections were created with a set of molecular barcodes tagging each gene perturbation, the relative-abundance and thus the chemical-sensitivity of individual mutant strains can be assayed in parallel and tracked via a barcode microarray or barcode sequencing. This generates a CG profile that details the performance of each strain in the presence of a specific compound (Fig. 1). This individual CG profile can be compared to the CG profiles produced by other drugs, and those that are highly similar likely function through similar MOAs. As a first pass for compound characterization, the CG approach is technically accessible because in addition to compounds, only a library of yeast strains and a method to measure individual strain performance is required.

Chemical-genomic profiles can be further analyzed by comparing to the growing *S. cerevisiae* genetic interaction knowledgebase, comprised of profiles produced by testing all possible gene pairs for digenic interactions.[8] These digenic interactions are scored when the fitness of a double mutant deviates from the expected multiplicative effect of the two single mutants.[9] If the genetic interaction profile produced by a given query gene resembles the CG profile of a bioactive compound, then the query gene likely

Table 1
Variant chemical-genomic profiling methodologies and their use in *S. cerevisiae* and other organism

Profiling method	*S. cerevisiae* studies	Alternative systems presented in this review
(HIP) Haploid insufficiency profiling • One gene copy deleted in a diploid strain; gene dose reduced to 50%. • Abundance of barcoded heterozygous deletion mutants can be monitored in pooled growth. • Sensitive strains are heterozygous for the gene encoding the drug target.	Giaever et al. (1999)[16] Lum et al. (2004)[18] Giaever et al. (2004)[17] Baetz et al. (2004)[73] Parsons et al. (2006)[11] Ericson et al. (2008)[69] Hoon et al. (2008)[62] Hillenmeyer et al. (2008)[19]	*C. albicans*: Xu et al. (2007)[20] Rodriguez-Suarez et al. (2007)[22] Jiang et al. (2008)[44] Xu et al. (2009)[23] *A. fumigatus*: Hu et al. (2007)[26]
DAmP (decreased abundance by mRNA perturbation) • Disruption of the 3'UTR of each gene reduces mRNA resulting in a gene dose of 5∼50%. • Strains screened similar to HIP.	Yan et al. (2008)[74]	
HOP (homozygous deletion profiling) • Both gene copies deleted in a diploid, or single gene in a haploid; gene dose 0%. • Abundance of barcoded deletion mutants can be monitored in pooled growth. • Sensitive strains are deleted for genes required to buffer the cell against drug toxicity.	Giaever et al. (2004)[17] Parsons et al. (2004)[10] Parsons et al. (2006)[11] Hillenmeyer et al. (2008)[19] Ericson et al. (2008)[69] Hoon et al. (2008)[62] Costanzo et al. (2010)[8] Kapinzky et al. (2011)[31]	*S. pombe*: Kapinzky et al. (2011)[31] Han et al. (2010)[33]
Overexpression profiling (dosage suppression) • Strain abundance monitored when gene dosage is >100%. Two strategies: 1) Monitor genes that confer resistance to a drug when overexpressed. 2) Monitor drugs that recover the inhibitory effects of toxic gene overdose. • Variation: expression and overexpression of non-yeast genes in a yeast model & look for drugs that recover gene-dosage growth inhibition.	Luesch et al. (2005)[75] Butcher et al. (2006)[76] Hoon et al. (2008)[62] Kemmer et al. (2009)[77]	*S. pombe*: Nishimura et al. (2010)[38] Arita et al. (2011)[39] *H. sapiens* via *S. pombe*:Yashiroda et al. (2010)[41]

functions in the targeted pathway, providing important insight into the compounds biological function (Fig. 1).[8,10,11]

3. Emergent chemical-genomic profiling systems

While the CG methods described above continue to prove valuable for compound characterization, they were originally limited in their application to the model yeast *S. cerevisiae*. In recent years, new technologies have emerged that may increase clinical relevance by characterizing compounds in pathogenic fungi and mammalian cells. CG profiling in these alternative systems will not only aid in finding novel antibiotics and anti-cancer therapeutics, it will also allow for studying conserved genetic networks. Moreover, by increasing the number of diverse organisms amenable to CG profiling, a larger portion of the druggable metagenome can be explored to define both conserved and unique targets. This will aid in prioritizing newly synthesized or discovered compounds based on their therapeutic potential. Finally, CG approaches may allow us to more efficiently identify and characterize synergistic drug interactions as a potential means for developing combinatorial therapeutics and extending the clinical life of approved drugs.

3.1. *Candida albicans*

The pathogenic yeast *C. albicans* is responsible for approximately 50% of all human life-threatening nosocomial fungal infections.[12] With the *C. albicans* genome sequence completed,[13] research has focused on understanding the biology and pathogenicity of this fungus to develop novel antifungal therapeutics. *S. cerevisiae* has often been used as a proxy for studying *C. albicans*, despite their obvious biological differences (e.g., pathogenicity and lifecycle). Genomic studies have also revealed significant fundamental differences between *C. albicans* and *S. cerevisiae* with regards to genomic organization[13,14] and gene essentiality.[15] As such, biological insights gained and potential antifungal compounds identified in budding yeast may not be directly transferable, underscoring the need for direct study of *C. albicans*.

Haploinsufficiency profiling in *S. cerevisiae* has been a valuable tool for directly identifying drug targets and the components of relevant signaling or metabolic pathways.[16–19] Transferring the HIP assay to *C. albicans* has been facilitated by the availability of large-scale collections of barcoded heterozygous deletion mutants. One of these widely used collections covers 45% of the genome; the genes selected for deletion were chosen for their potential to yield informative biological insights based on their essentially and conservation across yeast species, as well as their conservation in higher eukaryotes.[20] More recently, a second *C. albicans* deletion collection was created with greater genome coverage (∼78%) to alleviate any potential biases resulting from analyzing only eukaryotic conserved genes.[21] As with *S. cerevisiae* HIP analysis, barcoded *C. albicans* heterozygous mutants can be pooled and assayed in parallel to enable high-throughput drug profiling. This particular CG assay has been coined the *C. albicans* fitness test, or CaFT (Table 1).[20]

Xu et al. (2007) benchmarked CaFT by analyzing 35 known and novel compounds, demonstrating drug induced haploinsufficiency was specific and restricted to a small subset of deletion strains corresponding to drug targets, other factors directly associated with the targets, or aspects of drug resistance.[20] For instance, the *ERG11*, *NCP1* and *CDR1* heterozygotes were identified as sensitive to fluconazole; *ERG11* encodes the target of fluconazole, *NCP1* encodes the Erg11p co-factor and *CDR1* encodes a drug efflux pump. Importantly, the authors were also able to characterize two novel bioactive compounds as microtubule inhibitors through identifying the *TUB1* heterozygote as hypersensitive and performing requisite biochemical follow-up experiments. This assay therefore provides a valuable tool for rapidly characterizing compounds using *C. albicans*. The results obtained from CaFT screens can also be used to gain insight into the function of unannotated *C. albicans* genes. The results obtained by Xu et al. (2007) enabled the addition of first-level

annotations to ~100 *C. albicans* genes, many of which had only previously been characterized based on homology to *S. cerevisiae*.[20]

There are variations of CG profiling in which the CaFT approach can be applied, including focused screens seeking to characterize specific bioactive compounds of interest. For example, Rodriguez-Suarez et al. (2007) used CaFT to identify the *GUA1* heterozygote as uniquely sensitive to the synthetic compound ECC138, which possesses potent activity against several pathogenic fungi species (Table 1).[22] The *GUA1* gene encodes a GMP synthase, which is essential and required for virulence, suggesting ECC1385 constitutes a novel class of antifungal compounds. In contrast to this compound-motivated analysis, CaFT can also be used to characterize large collections of chemicals, identifying agents eliciting a desired response or targeting important cellular processes. Xu et al. (2009) screened a collection of compounds to identify a subset of molecules that affect unsaturated fatty acid biosynthesis through inhibiting Ole1p, the 9Δ fatty acid desaturase.[23] A follow-up analysis of all strains sensitive to these compounds also revealed novel genetic interactions between fatty acids and other cellular processes, providing insight into the fine-tuned regulation of the unsaturated fatty acid composition of cell membranes.

CaFT can also be used to characterize natural products through directly screening crude natural extracts. Natural products provide an ideal source of specific small molecule inhibitors and have served as the basis of many therapeutic agents.[24] However, analysis of these compounds is confounded by the time-consuming and often difficult task of purifying active constituents. Jiang et al. (2008) demonstrated CaFT analysis of fermentation extracts could produce unique and mechanistically informative CG profiles,[25] reaffirming the previous observation that the CG analysis of crude natural product extracts in *S. cerevisiae* can provide valuable insight into the compounds targets (Table 1).[11] Purification of the compound corresponding to the unique profile revealed a mixture of diasteromers that intriguingly, contained an isoxazolidinone ring not previously reported in natural products. Identification and characterization of this unique class of molecules, coined parnafungin, demonstrates the power of analyzing crude fermentation extracts using CaFT for natural-product-based drug discovery in the clinically relevant yeast *C. albicans*.

3.2. *Aspergillus fumigatus*

A. fumigatus is the most prevalent airborne, filamentous fungal pathogen that causes severe and often fatal infections in immunocompromised patients. Despite the current therapeutic options available, the mortality rate associated with invasive aspergillosis remains high and as such, there is a need to develop more efficient therapeutics with novel targets, a goal that may very well be best achieved by developing drug screening techniques specific to *A. fumigatus*.

Chemical-genomic analysis in *A. fumigatus* is presently limited by the absence of a genome-wide deletion collection. However, a series of *A. fumigatus* conditional alleles were created recently using a promoter replacement strategy, whereby native gene promoters were replaced with the ammonium sensitive *A. fumigatus* p*NiiA* promoter. Growing the p*NiiA* mutants in the appropriate concentration of ammonium can reduce activity of the regulated gene products to ~50%, making them amenable to target-based drug analysis using chemical-induced haploinsufficiency (Table 1). Hu et al. (2007) demonstrated the potential of this method in showing the mutant strains p*NiiA-ALG7* and p*NiiA-ERG11A* were uniquely sensitive to the drugs tunicamycin and fluconazole, respectively.[26] This approach is currently limited by the small subset of p*NiiA* mutants available and would ultimately benefit from expanding the collection for genome-wide analysis. Nevertheless, the preliminary analysis by Hu et al. (2007) shows the feasibility of this approach to directly confirm the targets of bioactive compounds.[26]

3.3. *Schizosaccharomyces pombe*

The fission yeast *S. pombe* is a widely used eukaryotic model that differs from *S. cerevisiae* in a number of aspects, including cell cycle organization, centromere complexity, heterochromatin and the prevalence of introns.[27] Therefore, studies in *S. pombe* can expand upon information learnt in *S. cerevisiae* to define genes and process required more generally across yeast species, potentially identifying conserved antifungal targets. Additionally, *S. pombe* possess a number of genes with homology to those in higher eukaryotes, including more than 100 genes related to human diseases,[27] highlighting the value of this yeast for drug target characterization. The strength of *S. pombe* as a tool for CG analysis will continue increase with the growing genetic interaction data generated in *S. pombe* using synthetic genetic array technology.[28,29]

Genome-wide *S. pombe* deletion collections are available, in which each protein coding open reading frame has been replaced with a dual-barcoded *kanMX* deletion cassette (covering 98.4% of ORFs in heterozygous diploid strains & 73.9% in haploid strains).[30] These collections can be used to gain insights into the mode of action and molecular targets of bioactive compounds using protocols that are analogous to those pioneered in *S. cerevisiae* (Table 1). Kapitzky et al. (2010) used the *S. pombe* and *S. cerevisiae* homozygous haploid deletion collections to identify a novel DNA damaging agent through characterizing a subset of bioactive compounds from a library of National Cancer Institute chemicals.[31] Of note, the authors demonstrated that drug target prediction could be improved significantly through comparing *S. cerevisiae* and *S. pombe* CG data. Alternatively, the *S. pombe* homozygous deletion collection can be used to profile bioactive compounds using the *S. cerevisiae* barcode sequencing, or Bar-seq approach.[32] In lieu of the traditional DNA microarray, Bar-seq is a cost-effective alternative to quantify the relative fitness of each drug-sensitive or resistant strain in pooled growth experiments. Han et al. (2010) revealed the Bar-seq approach is also relevant in *S. pombe*, through reproducibly identifying mutants deleted for genes involved in nuclear division and the DNA damage response as sensitive to a microtubule-depolymerizing drug and genotoxic agents, respectively.[33]

Another valuable resource for fission yeast chemical-genomics is the *S. pombe* ORFeome.[34] This collection covers 99.2% of predicted protein coding ORFs, with each of the ~5000 strains containing an ORF inserted at the chromosomal *leu1* locus under the control of *nmt1* inducible promoter. While comprehensive drug-profiling approaches have focused largely on the analysis of deletion mutants, multicopy suppression screens for genes that confer resistance to a drug of interest when overexpressed have allowed for characterizing many bioactive compounds.[35–37] As such, screening the *S. pombe* ORFeome for changes in drug sensitivity can identify strains required for drug resistance, thus providing insights into the drug MOA. The chemical sensitivity of each ORFeome mutant can be evaluated with one of many approaches (Table 1). Each strain can be grown individually in liquid culture and monitored for changes in cell viability due to drug treatment using a colorimetric assay. This methodology was employed to help identify 3β-hydroxysterols as the target of theonellamides based on a GO enrichment of the genes conferring drug sensitivity or resistance when overexpressed.[38] The chemical sensitivity of each strain present in the ORFeome collection can also be evaluated in a high-throughput pooled growth assay, despite the absence of a molecular barcoded tagging each ORFeome strain. After growing the ORFeome pool in the presence of a compound of interest, the relative fitness of each strain can be measured by harvesting the genomic DNA, PCR amplifying each ORF and

hybridizing the amplified ORFs to a custom DNA microarray containing ORF-specific probes. In the study that benchmarked this approach, the ORFeome strains screened were first regenerated in a drug hypersensitive background lacking genes encoding drug efflux pumps.[39] This feature reduced the amount of compound required for analysis and made the assay applicable to a broader spectrum of drugs, including cancer therapies. Arita et al. (2011) used this system to characterize the drug etoposide, a widely used anti-cancer drug whose mode of action was not fully understood.[39]

An alternative application of gene overexpression analysis in *S. pombe* is the analysis of yeast strains overexpressing human ORFs to characterize compounds specific to human disease-causing genes (Table 1**)**. For instance, the telomere-associated protein tankyrase 1 is considered to be an ideal target for cancer therapy, as it is synthetically lethal when combined with a breast cancer associated (BRCA) deficiency.[40] To identify tankyrase 1 inhibitors, a *S. pombe* strain overexpressing the human tankyrase 1 gene (*TNKS1*) was created and screened against a large library of natural products.[41] Because *TNKS1* expression caused growth retardation in *S. pombe*, the authors were able to identify flavone as a tankyrase 1 inhibitor based on its ability to restore wild-type growth. This 'humanized' drug-screening technique has great potential for rapidly identifying inhibitors of human proteins, like the PARP family of enzymes important in cancer physiology.

3.4. CG profiling of mammalian cell lines

Moving from simple eukaryotes to mammalian systems is a major goal for CG profiling. Mammalian cells are less genetically tractable than yeast, but with the advent of RNAi technologies it is now possible to create loss of gene function (or reduced function) mutants in mammalian cells to permit CG analysis. The diversity of mammalian RNAi-based chemical-genomics has been recently reviewed.[42] Furthermore, with the mouse conditional KO collection a predicted 3 years from completion,[43] mammalian CG profiling will likely become a more straightforward assay.

A streamlined HTS CGs approach should be possible in mammalian cells. Jiang et al. (2011) used mouse cancer cells lines to profile anti-cancer drugs involved in apoptosis.[44] Rather than screening the whole genome, the researchers focused on a set of 29 genes involved in cell death and created cell lines expressing corresponding GFP tagged shRNAs. Using a cell competition assay, changes in chemical sensitivity corresponding to a shRNA-induced reduction in gene function were measured by assessing GFP signal intensity relative to a control. For instance, the GFP signal measured for the shRNA cell pool would increase relative to control cells if the reduced gene function conferred resistance to a drug, while an increase in drug-sensitivity would result in a decrease in GFP signal. This analysis generated a GFP index of strain sensitivity, and the resultant profiles resemble those produced by yeast CG assays. Despite using only 29 genes and 15 compounds, compounds with similar modes of action clustered together.[44] Although this methodology would be difficult to employ on a genome-wide scale, the authors demonstrate the potential for analyzing proxy genes specific to different cellular process. A possible pipeline for characterizing novel compounds could therefore be to first estimate a drug's target pathway in yeast at the genome scale, then transfer the results into a mammalian system by analyzing a subset of genes representative of the predicted pathway. Small proxy gene sets covering different functional groups could also be arrayed and analyzed for broader screening capacity.

3.5. System independent chemical–chemical profiling

Chemical–chemical profiling is an application of drug synergy that can be used to characterize novel compounds (Fig. 1b). Drug synergy occurs when the combined effect of two drugs exceeds the expected sum of the individual agents. Identifying synergistic interactions between well-characterized drugs and unknown compounds of interest can provide insight their targets, as compounds exhibiting synergy likely function through related MOAs and the synergy profile may represent a diagnostic pattern reflective of the MOA. The logic behind this concept is borrowed from large-scale genetic interaction analyses, in which genes that show synthetic lethal genetic interactions are often functionally related and genes with similar genetic interaction patterns often encode proteins in the same pathway.[8] In a systematic application of chemical–chemical profiling, Farha and Brown (2010) tested 186 uncharacterized bioactive compounds in combination with 14 well-characterized antibacterial drugs to identify 255 synergistic drug-drug interactions in *Escherichia coli*.[45] By clustering the chemical–chemical interaction profiles generated, the authors were able to predict the MOA of two unknown bioactive compounds—putative inhibitors of folate biosynthesis and the enzyme DNA gyrase. Similar to other methods aimed at characterizing bioactive compounds, traditional biochemical and biophysical follow-up experiments were required to validate predicted targets. In addition, this approach is restricted by the availability of well-characterized compounds targeting diverse molecules and cellular pathways. However, one resounding advantage of chemical–chemical profiling is this method it is not reliant on the availability of a genome-wide deletion collection and is therefore not specific to any model organism. As such, this approach can be used to develop high throughput drug screens for other relevant systems, including genetically uncharacterized organisms and emerging disease-causing microbes.

3.6. Enhanced drug synergy screening using CG profiling

The phenomenon of drug synergy has been extensively explored in the fields of pharmacology and toxicology and has long been recognized for its potential in developing combinatorial drug therapies.[46–48] Medical treatments reliant on drugs functioning through individual targets are often insufficient to combat drug-resistant pathogens and treat multigenic diseases such as cancer.[48–50] By simultaneously targeting redundant pathways or intervening at various points in a common pathway, combinatorial drugs therapies are often more efficient and less prone to the development of drug resistance.[46,51] Additionally, this treatment approach requires a lower dosing of the individual drug components and therefore affords reduced drug toxicity. The characterization of drug synergy is also a valuable tool for querying biological systems. Combined perturbations have been used extensively in *S. cerevisiae* to study the connectivity of cellular components and gain insight into the complex networks of signaling pathways.[8] Recent work has demonstrated that identifying and understanding synergistic drug combinations can also be used to predict connectivity in biological systems, complementing the information gained from genetic studies.[52–54] Importantly, chemical combinations can be used to probe different cellular components that are inaccessible by genetic studies and unaffected by treatment with an individual drug. This approach can also be used to study relevant disease models that are not amenable to mutagenesis and traditional genetic analysis.

Synergistic drug combinations have been traditionally identified using the sensitive, yet low-throughput dose-matrix response assay. By testing serial dilutions of two compounds at all possible permutations, the dose-matrix samples maximal chemical space and can uncover rare synergistic interactions that can be missed by more conservative approaches. To identify synergistic interactions from dose-matrix response assay data, one of the prevailing models of drug synergy can be used for comparison. While the

models of Bliss independence and Gaddum's non-interaction have well-used counterparts in the genetic interaction models of multiplicativity and masking, respectively, Loewe additivity is the most relevant and frequently used model for medical applications.[54] Each synergy model defines a baseline efficacy level for any two compounds screened; Loewe additively states the baseline is the efficacy level that would be expected if a compound were tested against itself.[55] Drug synergy is defined when the efficacy exceeds the baseline measurement, while a decrease in efficacy defines drug antagonism. The utility of any model used to predict drug synergy depends on the comprehensiveness of the data obtained with the dose-matrix assay. Covering all possible chemical combinations is difficult to achieve in a cost-effective and timely manner. For instance, a collection of only 1000 compounds would yield ~500,000 pairwise experiments and many more higher-order combinations. To alleviate this issue, there has been extensive focus on the development of high-throughput experimental approaches for identifying drug synergy.

One of the first automated, high-throughput methods for screening drug synergy was developed to identify effective combinations of therapeutic compounds in fungal and mammalian cells.[56] To minimize the number of pairwise combinations screened, Borisy et al. (2003) demonstrated compounds classified as non-bioactive could be screened in pools of four and later deconvoluted to identify the pairwise combination producing a synergistic interaction.[56] Using a custom robotic screening and informatics platform, the authors were able to assess over 120,000 different two-drug combinations at six separate concentrations. This resulted in the identification of 22 *C. albicans* fungicidal pairwise combinations, 13 novel inhibitors of tumor proliferation and a series of glucorticoid and antiplatelet agents capable of suppressing the production of TNF-α, with potential for use as combinatorial anti-inflammatory therapies. Many of these combinations included drugs whose intrinsic activity was distinct from their synergistic function, highlighting the potential of chemical combinations to repurpose existing drugs.

A modified high-throughput approach for identifying drug synergy within a broad spectrum of compounds has been the foundation of many published studies.[52,57] However, this methodology can also be used to identify synergistic enhancers of currently used antifungal drugs to expand the spectrum of available antifungal therapies. For example, Spitzer et al. (2011) undertook a systematic screen of 1120 off-patent drugs and other bioactive agents to identify synergistic enhancers of fluconazole in three clinically relevant yeast species, *C. albicans*, *Cryptococcus neoformans* and *Cryptococcus gattii*, as well as the model organism *S. cerevisiae*.[58] Fluconazole has favorable pharmokinetic and toxicological properties and is therefore an ideal constituent compound for developing combinatorial antifungal therapies. In total, the authors identified 148 structurally diverse compounds capable of potentiating fluconazole, often improving activity from fungistatic to fungicidal. These drugs had a range of intrinsic therapeutic activities, including antiparasitic drugs, cardiovascular protectants and hormone modulators. Interestingly, only six of the 148 compounds were found to elicit synergy in all four yeast species; the remainder exhibited either species or genus specific drug synergy. To gain insight into mechanisms through which drug synergy disseminates, CG profiles for the constitutive compounds can be analyzed for genetic interactions. Spitzer et al. (2011) identified there was a significant number of genetic interactions between the top fluconazole sensitive strains and the strains sensitive to the enhancer drugs, and these strains were enriched for mutants involved in sphingolipid biosynthesis and membrane integrity.[58] Identifying genetic interactions between drug sensitive strains can ultimately be used predict additional synergistic drug combinations to further expand the antifungal chemical space.

Despite the development of high-throughput drug synergy screening methods, the ability to sample the vast number of chemical combinations possible remains a primary challenge for identifying this phenomenon. Through integrating chemical–genetic profiles[11,19] with a comprehensive genetic interaction network,[8] synergistic drug pairs can be predicted computationally.[53,59,60] This approach is an extension of using similarities in CG profiles to predict drug responses[10] and can be used effectively to reduce testing space to a manageable number of chemical combinations enriched for synergistic interactions. By comparing the CG profiles of ~1300 compounds to the profile produced by fluconazole, Jansen et al. (2009) identified 8 unique drugs with significant profile similarity.[61] An additional 10 potential synergistic interactions were identified when comparing these 8 profiles against each other. Using a standard dose-matrix response assay, 56% of these total interactions were validated in *S. cerevisiae* and 69% were validated in *C. albicans*. Given that the observed rate of drug synergy is around 3.6% when testing random drug combinations,[56] the computational predictions resulted in at best, a 20-fold increase in identifying true drug synergies. Importantly, identifying these drug synergies required analysis of only 18 pairwise combinations, instead of the potential ~844,000 combinations that would require testing if a dose-matrix assay were used to analyze pairwise combinations for each of the 1300 compounds. Unfortunately, drug synergy computational predictions are limited by the availability of CG profiles—drugs cannot be analyzed for synergy if a CG profile for the drug does not exist. As the compendium of chemical–genetic profiles continues to expand, the scope of the computational-based drug synergy prediction methods will therefore also increase.

4. Assay selection

The diversity of drug-profiling strategies available can complicate selecting the appropriate assay. When seeking to characterize a small set of chemicals or a single compound of interest, an integrated approach using several profiling strategies is ideal.[62] For instance, using a combined HIP/HOP assay in *S. cerevisiae* provides a rich source of data for predicting the specific targets, as well more broadly, the affected cellular pathways. As described above, the predictive power of these assays can be further enhanced using a cross-species CG approach.[31] By extending the CGs analysis to additional yeast species, drugs can be evaluated for their potential as broad-spectrum antifungals by analyzing the conservation of drug targets across divergent yeast species. If a researcher instead seeks to extend the life of existing pharmaceuticals by identifying new combinational therapies, drug-synergy predictions would be the optimal strategy.[61] Finally, to characterize one of the many large-scale synthetic or natural product collection libraries available, a high-throughput chemical-genomics strategy is desirable. This would likely require selecting a single profiling method (e.g., HIP or HOP) to greatly reduce the time required to screen a complete library. Once compounds eliciting a desired cellular response are highlighted from the results of a high-throughput profiling strategy, an integrated set of assays can be employed for validation.

Many of the CG profiling methods rely on the growth-inhibitory properties of a compound and as such, one should first test whether the compounds of interest inhibit yeast growth. However, bioactivity in yeast is a flexible condition and drug-hypersensitive yeast strains can often be used in instances where compounds do not exhibit activity in wild-type yeast.[39,63] These strains have been rendered up to 250 times more sensitive to a variety of compounds by systematically deleting genes involved in facilitating multi-drug resistance (e.g., *PDR1* & *SNQ2*). Chemical-genomic profiling with these sensitized yeast strains can dramatically increase the bioactivity of various compounds and thus reduce compound usage, an

important characteristic for analyzing valuable natural products often available in very limited quantities. One concern surrounding the use of drug-sensitized strains for CG analysis is the applicability of results to broader contexts, as most systems possess intact drug-response machinery. While this concern is valid for directly translating putative drugs to relevant systems, the use of drug-sensitized strains permits the analysis of compounds normally inaccessible for screening and yields CG profiles predictive of their targets and MOAs. Compounds found to target desired cellular processes could then be used as templates for further chemical modifications to improve bioactivity in wild-type cells for use as potential therapeutics or probes for biological research.

There are instances where bioactivity in yeast is not an absolute prerequisite for yielding informative biological information with CG profiling, as some deletion mutants may show increased drug sensitivity or resistance while wild-type yeast is unaffected. For example, despite Padanamide A exhibiting very slight growth inhibition in rich media, a deletion strain (*MET32*) lacking an enzyme involved in sulfur amino acid biosynthesis was identified as sensitive.[64] Follow up analysis on this observation demonstrated the drug's bioactivity was much greater in minimal media lacking sulfur amino acids and that inhibition was partially recovered when media was supplemented with methionine.

5. Perspective

Chemical-genomic approaches for drug-target identification will continue to grow and diversify as new functional genomic tools are available in a variety of taxa. Hundreds of organisms have already been fully sequenced and this will increase exponentially with the application of next-generation and next–next generation sequencing technologies. Novel high-throughput strain creation techniques will allow for the rapid development of new deletion collections and other biological tools that future chemical-genomics approaches will take full advantage of. However, as illustrated by the relative advantages and weaknesses of extant CG assays, researchers must choose the most appropriate method to maximize resource-to-result ratio. In addition to requiring both genetic and organism specific knowledge, selection of the best screening approach requires an understanding of chemical space. To facilitate this, two of the most immediate needs of the field are a centralized CG profile database and greater details into the chemical space accessible by assay organisms.

As the number of profiled bioactive compounds increases, data access and management will become a significant limitation. The creation of a centralized, open source data depository could benefit CG researchers across all biological systems. Given the structural redundancy of compounds available in different chemical libraries, a database could allow researchers to prioritize resources to screen compounds that either yielded desired responses in other systems or are completely new to CG analysis. In addition to compound information, this database could provide detailed assay conditions to enable standardization of results across experiments. Finally, wide-spread access to CG data could enhance in silico drug-synergy screening efforts and aid in computationally identifying conserved chemical–genetic networks across various organisms.[65]

Creating an open sourced CG database may be very difficult without significant statistical forethought. Even now as researchers try to merge developing CG data with that obtained by previous studies, much statistical power is lost due to differences in experimental conditions across profiling experiments collectively known as 'batch effects'. For instance, the large available datasets of Hillenmeyer et al. (2008) and Parsons et al. (2006) would provide an ideal external comparison for analyzing new CG profiles to give insight into compound MOAs through cluster analysis.[11,19] While these comparisons are possible to an extent, methodological differences between studies limit correlation-based analysis and decrease signal to noise ratio. Standardization for yeast-based studies should be relatively easy to achieve given the organism's growth requirements. However, the profiles developed in other emergent model systems will have their own unique 'assay signatures' that will have to be accounted for cross-systems analysis. To ensure the ease of comparisons across taxa and datasets, experimental procedures must be completely transparent and a level of standardization is required. For instance, most CG profile data in yeast is normalized to a DMSO solvent control and cultures are generally grown in rich media. Changing the solvent control or media conditions can dramatically alter resulting CG profiles,[19] and identifying true drug-gene interactions across experiments becomes a complicated endeavor. Additionally, as CG profiling is extended into less common systems and clinical isolates, detailed descriptions of the genotypes and fitness of strain collections will be necessary to disentangle true drug-gene interactions from fitness defects. Despite these obstacles and the massive undertaking required to create a CG database at the level of Genbank or Pubchem, this resource will ultimately help unify bourgeoning CG research groups and make CG accessible to broader disciplines.

Finally, as advances in combinatorial and diversity-oriented chemistry continue to yield torrents of diverse compounds with varied structural and chemical properties, an understanding of the chemical space accessible by various organisms is essential. While a compound can be fully characterized in one organism, there is no guarantee the same compound will even cross the cell membrane of another organism. Therefore, understanding the chemical space accessible by organisms used in CG profiling is germane to the translation of approaches to diverse taxa.[66] The physiochemical properties of compounds as they relate to bioavailability have long been considered during drug discovery efforts,[67] most notably with the advent of the Lipinski 'Rule of 5'.[67] Recently, Burns et al. (2010) used high-throughput HPLC to define the optimal structural and chemical properties of a compound for *Caenorhabditis elegans* permeability, suggesting ranking compound libraries based on predicted bioavailability could maximize the efficiency of drug-lead discovery in contrast to shotgun library screening.[68] Before embarking on high-throughput CG profiling of a large compound library, it is therefore worth understanding the chemical space of the organism in which the drug will be screened and the ultimate cell type in which the desired drug would be functioning.

6. Conclusion

As highlighted in this review, the integration of functional genomics with chemical biology provides a powerful tool for characterizing bioactive molecules. The successes afforded by *S. cerevisiae*-based CG approaches have heightened interest in adapting this technology for broader application across different biological systems to identify novel therapeutics and provide valuable insights into the cellular processes constituting less-studied organisms. As discussed, the search for drug synergy can also be enhanced by CG approaches and the results from CG studies to expand available antifungal and cancer treatments using combinatorial drug therapies. While this review has focused primarily on CG profiling efforts in eukaryotic systems, substantial advances in prokaryotic systems have been made using similar approaches.[69–72] The merging of eukaryotic and prokaryotic CG data will be invaluable for defining conserved drug targets and identifying unique bacterial and fungal targets as present day antibiotics reach obsolescence. Novel applications of CGs will likely remain a heavy focus of drug-discovery research in the near future in the hopes of addressing outstanding limitations, and transitioning approaches to additional organisms of biological interest.

References and notes

1. Kirkpatrick, P.; Ellis, C. *Nature* **2004**, *32*, 823.
2. Macarron, R.; Banks, M. N.; Bojanic, D.; Burns, D. J.; Cirovic, D. A.; Garyantes, T.; Green, D. V. S.; Hertzberg, R. P.; Janzen, W. P.; Paslay, J. W.; Schopfer, U.; Sittampalam, G. S. *Nat. Rev.* **2011**, *10*, 188.
3. Winzeler, E. A.; Shoemaker, D. D.; Astromoff, A.; Liang, H.; Anderson, K.; Andre, B.; Bangham, R.; Benito, R.; Boeke, J. D.; Bussey, H.; Chu, A. M.; Connelly, C.; Davis, K.; Dietrich, F.; Dow, S. W.; EL Bakkoury, M.; Foury, F.; Friend, S. H.; Gentalen, E.; Giaever, G.; Hegemann, J. H.; Jones, T.; Laub, M.; Liao, H.; Liebundguth, N.; Lockhart, D. J.; Lucau-Danila, A.; Lussier, M.; M'Rabet, N.; Menard, P.; Mittmann, M.; Pai, C.; Rebischung, C.; Revuelta, J. L.; Riles, L.; Roberts, C. J.; Ross-MacDonald, P.; Scherens, B.; Snyder, M.; Sookhai-Mahadeo, S.; Storms, R. K.; Veronneau, S.; Voet, M.; Volckaert, G.; Ward, T. R.; Wysocki, R.; Yen, G. S.; Yu, K. X.; Zimmermann, K.; Philippsen, P.; Johnston, M.; Davis, R. W. *Science* **1999**, *285*, 901.
4. Chan, J. N. Y.; Nislow, C.; Emili, A. *Trends Pharmacol. Sci.* **2009**, *31*, 82.
5. Hoon, S.; St Onge, R. P.; Giaever, G.; Nislow, C. *Trends Pharmacol. Sci.* **2008**, *29*, 499.
6. Smith, A. M.; Ammar, R.; Nislow, C.; Giaever, G. *Pharmacol. Ther.* **2010**, *127*, 156.
7. Ho, C. H.; Piotrowski, J.; Dixon, S. J.; Baryshnikova, A.; Costanzo, M.; Boone, C. *Curr. Opin. Chem. Biol.* **2011**, *15*, 66.
8. Costanzo, M.; Baryshnikova, A.; Bellay, J.; Kim, Y.; Spear, E. D.; Sevier, C. S.; Ding, H.; Koh, J. L. Y.; Toufighi, K.; Mostafavi, S.; Prinz, J.; Onge, R. P. S.; VanderSluis, B.; Makhnevych, T.; Vizeacoumar, F. J.; Alizadeh, S.; Bahr, S.; Brost, R. L.; Chen, Y.; Cokol, M.; Deshpande, R.; Li, Z.; Lin, Z.-Y.; Liang, W.; Marback, M.; Paw, J.; Luis, B.-J. S.; Shuteriqi, E.; Tong, A. H. Y.; van Dyk, N.; Wallace, I. M.; Whitney, J. A.; Weirauch, M. T.; Zhong, G.; Zhu, H.; Houry, W. A.; Brudno, M.; Ragibizadeh, S.; Papp, B.; Pal, C.; Roth, F. P.; Giaever, G.; Nislow, C.; Troyanskaya, O. G.; Bussey, H.; Bader, G. D.; Gingras, A.-C.; Morris, Q. D.; Kim, P.; M. Kaiser, C. A.; Myers, C. L.; Andrews, B. J.; Boone, C. *Science* **2010**, *327*, 425.
9. Baryshnikova, A.; Costanzo, M.; Kim, Y.; Ding, H.; Koh, J.; Toufighi, K.; Youn, J.-I.; Ou, J.; San Luis, B.-J.; Bandyopadhyay, S.; Hibbs, M.; Hess, D.; Gingras, A.-C.; Bader, G. D.; Troyanskaya, O. G.; Brown, G. W.; Andrews, B.; Boone, C.; Meyers, C. L. *Nat. Methods* **2010**, *7*, 1017.
10. Parsons, A. B.; Brost, R. L.; Ding, H.; Li, Z.; Zhang, C.; Sheikh, B.; Brown, G. W.; Kane, P. M.; Hughes, T. R.; Boone, C. *Nat. Biotechnol.* **2004**, *22*, 62.
11. Parsons, A. B.; Lopez, A.; Givoni, I. E.; Williams, D. E.; Gray, C. A.; Porter, J.; Chua, G.; Sopko, R.; Brost, R. L.; Ho, C. H.; Wang, J.; Ketela, T.; Brenner, C.; Brill, J. A.; Fernandez, G. E.; Lorenz, T. C.; Payne, G. S.; Ishihara, S.; Ohya, Y.; Andrews, B.; Hughes, T. R.; Frey, B. J.; Graham, T. R.; Andersen, R. J.; Boone, C. *Cell* **2006**, *126*, 611.
12. Wisplinghoff, H.; Bischoff, T.; Tallent, S. M.; Seifert, H.; Wenzel, R. P.; Edmond, M. B. In *Clinical Infectious Diseases*; Virginia Commonwealth Univ., Dept. Internal Med.: Richmond, VA USA, 2004; Vol. 39, pp 309–317.
13. Jones, T.; Federspiel, N. A.; Chibana, H.; Dungan, J.; Kalman, S.; Magee, B. B.; Newport, G.; Thorstenson, Y. R.; Agabian, N.; Magee, P. T.; Davis, R. W.; Scherer, S. *PNAS* **2004**, *101*, 7329.
14. Braun, B. R.; Hoog, M. V.; d' Enfert, C.; Martchenko, M.; Dungan, J.; Kuo, A.; Inglis, D. O.; Uhl, M. A.; Hogues, H.; Berriman, M.; Lorenz, M.; Levitin, A.; Oberholzer, U.; Bachewich, C.; Harcus, D.; Marcil, A.; Dignard, D.; Iouk, T.; Zito, R.; Frangeul, L.; Tekaia, F.; Rutherford, K.; Wang, E.; Munro, C. A.; Bates, S.; Gow, N. A.; Hoyer, L. L.; Kohler, G.; Morschhauser, J.; Newport, G.; Znaidi, S.; Raymond, M.; Turcotte, B.; Sherlock, G.; Costanzo, M.; Ihmels, J.; Berman, J.; Sanglard, D.; Agabian, N.; Mitchell, A. P.; Johnson, A. D.; Whiteway, M.; Nantel, A. *PLoS Genet* **2005**, *1*, 36.
15. Roemer, T.; Jiang, B.; Davison, J.; Ketela, T.; Veillette, K.; Breton, A.; Tandia, F.; Linteau, A.; Sillaots, S.; Marta, C.; Martel, N.; Veronneau, S.; Lemieux, S.; Kauffman, S.; Becker, J.; Storms, R.; Boone, C.; Bussey, H. C. *Mol. Microbiol.* **2003**, *50*, 167.
16. Giaever, G.; Shoemaker, D. D.; Jones, T. W.; Liang, H.; Winzeler, E. A.; Astromoff, A.; Davis, R. W. *Nat. Genet.* **1999**, *21*, 278.
17. Giaever, G.; Flaherty, P.; Kumm, J.; Proctor, M.; Nislow, C.; Jaramillo, D. F.; Chu, A. M.; Jordan, M. I.; Arkin, A. P.; Davis, R. W. *PNAS* **2004**, *101*, 793.
18. Lum, P. Y.; Armour, C. D.; Stepaniants, S. B.; Cavet, G.; Wolf, M. K.; Butler, J. S.; Hinshaw, J. C.; Garnier, P.; Prestwich, G. D.; Leonardson, A.; Garrett-Engele, P.; Rush, C. M.; Bard, M.; Schimmack, G.; Phillips, J. W.; Roberts, C. J.; Shoemaker, D. D. *Cell* **2004**, *116*, 121.
19. Hillenmeyer, M. E.; Fung, E.; Wildenhain, J.; Pierce, S. E.; Hoon, S.; Lee, W.; Proctor, M.; St Onge, R. P.; Tyers, M.; Koller, D.; Altman, R. B.; Davis, R. W.; Nislow, C.; Giaever, G. *Science* **2008**, *320*, 362.
20. Xu, D.; Jiang, B.; Ketela, T.; Lemieux, S.; Veillette, K.; Martel, N.; Davison, J.; Sillaots, S.; Trosok, S.; Bachewich, C.; Bussey, H.; Youngman, P.; Roemer, T. *PLoS Pathog.* **2007**, *3*, 835.
21. Oh, J.; Fung, E.; Schlecht, U.; Davis, R. W.; Giaever, G.; St Onge, R. P.; Deutschbauer, A.; Nislow, C. *PLoS Pathog.* **2010**, *6*, 1.
22. Rodriguez-Suarez, R.; Xu, D.; Veillette, K.; Davison, J.; Sillaots, S.; Kauffman, S.; Hu, W.; Bowman, J.; Martel, N.; Trosok, S.; Wang, H.; Zhang, L.; Huang, L.-Y.; Li, Y.; Rahkhoodaee, F.; Ransom, T.; Gauvin, D.; Douglas, C.; Youngman, P.; Becker, J.; Jiang, B.; Roemer, T. *Chem. Biol.* **2007**, *14*, 1163.
23. Xu, D.; Sillaots, S.; Davison, J.; Hu, W.; Jiang, B.; Kauffman, S.; Martel, N.; Ocampo, P.; Oh, C.; Trosok, S.; Veillette, K.; Wang, H.; Yang, M.; Zhang, L.; Becker, J.; Martin, C. E.; Roemer, T. J. *Biol. Chem.* **2009**, *284*, 19754.
24. Newman, D. J.; Cragg, G. M.; Snader, K. M. J. *Nat. Prod.* **2003**, *66*, 1022.
25. Jiang, B.; Xu, D.; Allocco, J.; Parish, C.; Davison, J.; Veillette, K.; Sillaots, S.; Hu, W.; Rodriguez-Suarez, R.; Trosok, S. *Chem. Biol.* **2008**, *15*, 363.
26. Hu, W.; Sillaots, S.; Lemieux, S.; Davison, J.; Kauffman, S.; Breton, A.; Linteau, A.; Xin, C.; Bowman, J.; Becker, J.; Jiang, B.; Roemer, T. *PLoS Pathog.* **2007**, *3*, 19754.
27. Wood, V.; Gwilliam, R.; Rajandream, M.-A.; Lyne, M.; Lyne, R.; Stewart, A.; Sgouros, J.; Peat, N.; Hayles, J.; Baker, S.; Basham, D.; Bowman, S.; Brooks, K.; Brown, D.; Brown, S.; Chillingworth, T.; Churcher, C.; Collins, M.; Connor, R.; Cronin, A.; Davis, P.; Feltwell, T.; Fraser, A.; Gentles, S.; Goble, A.; Hamlin, N.; Harris, D.; Hidalgo, J.; Hodgson, G.; Holroyd, S.; Hornsby, T.; Howarth, S.; Huckle, E. J.; Hunt, S.; Jagels, K.; James, K.; Jones, L.; Jones, M.; Leather, S.; McDonald, S.; McLean, J.; Mooney, P.; Moule, S.; Mungall, K.; Murphy, L.; Niblett, D.; Odell, C.; Oliver, K.; ONeil, S.; Pearson, D.; Quail, M. A.; Rabbinowitsch, E.; Rutherford, K.; Rutter, S.; Saunders, D.; Seeger, K.; Sharp, S.; Skelton, J.; Simmonds, M.; Squares, R.; Squares, S.; Stevens, K.; Taylor, K.; Taylor, R. G.; Tivey, A.; Walsh, S.; Warren, T.; Whitehead, S.; Woodward, J.; Volckaert, G.; Aert, R.; Robben, J.; Grymonprez, B.; Weltjens, I.; Vanstreels, E.; Rieger, M.; Schaefer, M.; MuEller-Auer, S.; Gabel, C.; Fuchs, M.; Fritzc, C.; Holzer, E.; Moestl, D.; Hilbert, H.; Borzym, K.; Langer, I.; Beck, A.; Lehrach, H.; Reinhardt, R.; Pohl, T. M.; Eger, P.; Zimmermann, W.; Wedler, H.; Wambutt, R.; Purnelle, B.; Goffeau, A.; Cadieu, E.; DreAano, S.; Gloux, S.; Lelaure, V.; Mottier, S.; Galibert, F.; Aves, S. J.; Xiang, Z.; Hunt, C.; Moore, K.; Hurst, S. M.; Lucas, M.; Rochet, M.; Gaillardin, C.; Tallada, V. A.; Garzon, A.; Thode, G.; Daga, R. R.; Cruzado, L.; Jimenez, J.; Sanchez, M. S.; del Rey, F.; Benito, J.; Dominguez, A.; Revuelta, J. L.; Moreno, S.; Armstrong, J.; Forsburg, S. L.; Cerrutti, L.; Lowe, T.; McCombie, W. R.; Paulsen, I.; Potashkin, J.; Shpakovski, G. V.; Ussery, D.; Nurse, B. G. B. P. *Nature* **2002**, *415*, 871.
28. Baryshnikova, A.; Costanzo, M.; Dixon, S.; Vizeacoumar, F. J.; Myers, C. L.; Andrews, B.; Boone, C. *Methods Enzymol.* **2010**, *470*, 145.
29. Dixon, S. J.; Fedyshyn, Y.; Koh, J. L. Y.; Prasad, T. S. K.; Chahwan, C.; Chua, G.; Toufighi, K.; Baryshnikova, A.; Hayles, J.; Hoe, K.-L.; Kim, D.-U.; Park, H.-O.; Myers, C. L.; Pandey, A.; Durocher, D.; Andrews, B. J.; Boone, C. *PNAS* **2008**, *105*, 16653.
30. Kim, D.-U.; Hayles, J.; Kim, D.; Wood, V.; Park, H.-O.; Won, M.; Yoo, H.-S.; Duhig, T.; Nam, M.; Palmer, G.; Han, S.; Jeffery, L.; Baek, S.-T.; Lee, H.; Shim, Y. S.; Lee, M.; Kim, L.; Heo, K.-S.; Noh, E. J.; Lee, A.-R.; Jang, Y.-J.; Chung, K.-S.; Choi, S.-J.; Park, J.-Y.; Park, Y.; Kim, H. M.; Park, S.-K.; Park, H.-J.; Kang, E.-J.; Kim, H. B.; Kang, H.-S.; Park, H.-M.; Kim, K.; Song, K.; Song, K. B.; Nurse, P.; Hoe, K.-L. *Nat. Biotechnol.* **2010**, *28*, 617.
31. Kapitzky, L.; Beltrao, P.; Berens, T. J.; Gassner, N.; Zhou, C.; Wuster, A. W.; Wu, J.; Babu, M. M.; Elledge, S. J.; Toczyski, D.; Lokey, R. S.; Krogan, N. J. *Mol. Syst. Biol.* **2010**, *6*, 1.
32. Smith, A. M.; Heisler, L. E.; Mellor, J.; Kaper, F.; Thompson, M. J.; Chee, M.; Roth, F. P.; Giaever, G.; Nislow, C. *Genome Res.* **2009**, *19*, 1836.
33. Han, T. X.; Xu, X.-Y.; Zhang, M.-J.; Peng, X.; Du, L.-L. *Genome Biol.* **2010**, *11*, 1.
34. Matsuyama, A.; Arai, R.; Yashiroda, Y.; Shirai, A.; Kamata, A.; Sekido, S.; Kobayashi, Y.; Hashimoto, A.; Hamamoto, M.; Hiraoka, Y.; Horinouchi, S.; Yoshida, M. *Nat. Biotechnol.* **2006**, *24*, 841.
35. Miyamoto, Y.; Machida, K.; Mizunuma, M.; Emoto, Y.; Sato, N.; Miyahara, K.; Hirata, D.; Usui, T.; Takahashi, H.; Osada, H.; Miyakawa, T. J. *Biol. Chem.* **2002**, *277*, 28810.
36. Vahlensieck, H. F.; Pridzun, H. F.; Reichenbach, H.; Hinnen, A. *Curr. Genet.* **1994**, *25*, 95.
37. Nishi, K.; Yoshida, M.; Fujiwara, D.; Nishikawa, M.; Horinouchi, S.; Beppu, T. J. *Biol. Chem.* **1994**, *269*, 6320.
38. Nishimura, S.; Arita, Y.; Honda, M.; Iwamoto, K.; Matsuyama, A.; Shirai, A.; Kawasaki, H.; Kakeya, H.; Kobayashi, T.; Matsunaga, S.; Yoshida, M. *Nat. Chem. Biol.* **2010**, *6*, 519.
39. Arita, Y.; Nishimura, S.; Matsuyama, A.; Yashiroda, Y.; Usui, T.; Boone, C.; Yoshida, M. *Mol. Biosyst.* **2011**, *7*, 1463.
40. McCabe, N.; Cerone, M. A.; Ohishi, T.; Seimiya, H.; Lord, C. J.; Ashworth, A. *Oncogene* **2009**, *28*, 1465.
41. Yashiroda, Y.; Okamoto, R.; Hatsugai, K.; Takemoto, Y.; Goshima, N.; Saito, T.; Hamamoto, M.; Sugimoto, Y.; Osada, H.; Seimiya, H.; Yoshida, M. *Biochem. Biophys. Res. Commun.* **2010**, *394*, 569.
42. Iorns, E.; Lord, C. J.; Turner, N.; Ashworth, A. *NRD* **2007**, *6*, 556.
43. Skarnes, W. C.; Rosen, B.; West, A. P.; Koutsourakis, M.; Bushell, W.; Iyer, V.; Mujica, A. O.; Thomas, M.; Harrow, J.; Cox, T.; Jackson, D.; Severin, J.; Biggs, P.; Fu, J.; Nefedov, M.; de Jong, P. J.; Stewart, A. F.; Bradley, A. *Nature* **2011**, *474*, 337.
44. Jiang, H.; Pritchard, J. R.; Williams, R. T.; Lauffenburger, D. A.; Hemann, M. T. *Nat. Chem. Biol.* **2011**, *7*, 92.
45. Farha, M. A.; Brown, E. D. *Chem. Biol.* **2010**, *17*, 852.
46. Walsh, C. *Nature* **2000**, *406*, 775.
47. Dancey, J. E.; Chen, H. X. *NRD* **2006**, *5*, 649.
48. Zimmermann, G. R.; Lehár, J.; Keith, C. T. *Drug Discovery Today* **2007**, *12*, 34.
49. Sams-Dodd, F. *Drug Discovery Today* **2005**, *10*, 139.
50. Onyewu, C.; Heitman, J. *Anti-Infect. Agents Med. Chem.* **2007**, *6*, 3.
51. Groll, A. H.; Walsh, T. J. *SMW* **2002**, *132*, 303.
52. Yeh, P.; Tschumi, A. I.; Kishony, R. *Nat. Genet.* **2006**, *38*, 489.
53. Lehár, J.; Zimmermann, G. R.; Krueger, A. S.; Molnar, R. A.; Ledell, J. T.; Heilbut, A. M.; Short, G. F., III; Giusti, L. C.; Nolan, G. P.; Magid, O. A.; Lee, M. S.; Borisy, A. A.; Stockwell, B. R.; Keith, C. T. *Mol. Syst. Biol.* **2007**, *3*, 1.
54. Lehár, J.; Stockwell, B. R.; Giaever, G.; Nislow, C. *Nat. Chem. Biol.* **2008**, *4*, 674.

55. Loewe, S. *Arzneimittel-Forsch* **1953**, *3*, 285.
56. Borisy, A. A.; Elliott, P. J.; Hurst, N. W.; Lee, M. S.; Lehar, J.; Price, E. R.; Serbedzija, G.; Zimmermann, G. R.; Foley, M. A.; Stockwell, B. R.; Keith, C. T. *PNAS* **2003**, *100*, 7977.
57. Zhang, L.; Yan, K.; Zhang, Y.; Huang, R.; Bian, J.; Zheng, C.; Sun, H.; Chen, Z.; Sun, N.; An, R.; Min, F.; Zhao, W.; Zhuo, Y.; You, J.; Song, Y.; Yu, Z.; Liu, Z.; Yang, K.; Gao, H.; Dai, H.; Zhang, X.; Wang, J.; Fu, C.; Pei, G.; Liu, J.; Zhang, S.; Goodfellow, M.; Jiang, Y.; Kuai, J.; Zhou, G.; Chen, X. *PNAS* **2007**, *104*, 4606.
58. Spitzer, M.; Griffiths, E.; Blakely, K. M.; Wildenhain, J.; Ejim, L.; Rossi, L.; De Pascale, G.; Curak, J.; Brown, E.; Tyers, M.; Wright, G. D. *Mol. Syst. Biol.* **2011**, *7*, 1.
59. Lehár, J.; Krueger, A.; Zimmermann, G.; Borisy, A. *Mol. Syst. Biol.* **2008**, *4*, 1.
60. Nelander, S.; Wang, W.; Nilsson, B.; She, Q.-B.; Pratilas, C.; Rosen, N.; Gennemark, P.; Sander, C. *Mol. Syst. Biol.* **2008**, *4*, 1.
61. Jansen, G.; Lee, A. Y.; Epp, E.; Fredette, A.; Surprenant, J.; Harcus, D.; Scott, M.; Tan, E.; Nishimura, T.; Whiteway, M.; Hallett, M.; Thomas, D. Y. *Mol. Syst. Biol.* **2009**, *5*, 1.
62. Hoon, S.; Smith, A. M.; Wallace, I. M.; Suresh, S.; Miranda, M.; Fung, E.; Proctor, M.; Shokat, K. M.; Zhang, C.; Davis, R. W.; Giaever, G.; St Onge, R. P.; Nislow, C. *Nat. Chem. Biol.* **2008**, *4*, 498.
63. Rogers, B.; Decottignies, A.; Kolaczkowski, M.; Carvajal, E.; Balzi, E.; Goffeau, A. J. *Mol. Microbiol. Biotechnol.* **2001**, *3*, 207.
64. Williams, D. E.; Dalisay, D. S.; Patrick, B. O.; Matainaho, T.; Andrusiak, K.; Deshpande, R.; Myers, C. L.; Piotrowski, J. S.; Boone, C.; Yoshida, M.; Andersen, R. J. *Org. Lett.* **2011**, *13*, 3936.
65. Deshpande, R.; Sharma, S.; Verfaillie, C. M.; Hu, W.-S.; Myers, C. M. *PLoS Comput. Biol.* **2010**, *6*, 1.
66. Hopkins, A. L.; Bickerton, G. R. *Nat. Methods* **2010**, *6*, 482.
67. Lipinski, C.; Hopkins, A. *Nature* **2004**, *432*, 855.
68. Burns, A. R.; Wallace, I. M.; Wildenhain, J.; Tyers, M.; Giaever, G.; Bader, G. D.; Nislow, C.; Cutler, S. R.; Roy, P. J. *Nat. Methods* **2010**, *6*, 549.
69. Pathania, R.; Zlitni, S.; Barker, C.; Das, R.; Gerritsma, D. A.; Lebert, J.; Awuah, E.; Melacini, G.; Capretta, F. A.; Brown, E. D. *Nat. Chem. Biol.* **2009**, *5*, 849.
70. Nichols, R. J.; Sen, S.; Choo, Y. J.; Beltrao, P.; Zietek, M.; Chaba, R.; Lee, S.; Kazmierczak, K. M.; Lee, K. J.; Wong, A.; Shales, M.; Lovett, S.; Winkler, M. E.; Krogan, N. J.; Typas, A.; Gross, C. A. *Cell* **2011**, *144*, 143.
71. Donald, R. G. K.; Skwish, S.; Forsyth, R. A.; Anderson, J. W.; Zhong, T.; Burns, C.; Lee, S.; Meng, X.; LoCastro, L.; Jarantow, L. W.; Martin, J.; Lee, S. H.; Taylor, I.; Robbins, D.; Malone, C.; Wang, L.; Zamudio, C. S.; Youngman, P. J.; Phillips, J. W. *Chem. Biol.* **2009**, *16*, 826.
72. (a) Huber, J.; Donald, R. G. K.; Lee, S. H.; Jarantow, L. W.; Salvatore, M. J.; Meng, X.; Painter, R.; Onishi, R. H.; Occi, J.; Dorso, K.; Young, K.; Park, Y. W.; Skwish, S.; Szymonifka, M. J.; Waddell, T. S.; Miesel, L.; Phillips, J. W.; Roemer, T. *Chem. Biol.* **2009**, *16*, 837; (b) Ericson, E.; Gebbia, M.; Heisler, L. E.; Wildenhain, J.; Tyers, M.; Giaever, G.; Nislow, C. *PloS Gen.* **2008**, *4*, 1.
73. Baetz, K.; McHardy, L.; Gable, K.; Tarling, T.; Rebérioux, D.; Bryan, J.; Andersen, R. J.; Dunn, T.; Hieter, P.; Roberge, M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 4525.
74. Yan, Z.; Costanzo, M.; Heisler, L. E.; Paw, J.; Kaper, F.; Andrews, B. J.; Boone, C.; Giaever, G.; Nislow, C. *Nat. Methods* **2008**, *5*, 719.
75. Luesch, H.; Wu, T. Y. H.; Ren, P.; Gray, N. S.; Schultz, P. G.; Supek, F. *Chem. Biol.* **2005**, *12*, 55.
76. Butcher, R.; Schreiber, S. *Nat. Protoc.* **2006**, *1*, 569.
77. Kemmer, D.; McHardy, L. M.; Hoon, S.; Rebérioux, D.; Giaever, G.; Nislow, C.; Roskelley, C. D.; Roberge, M. *BMC Microbiol.* **2009**, *9*, 1.