Chemical Genetics–Based Target Identification in Drug Discovery

Feng Cong,[1] Atwood K. Cheung,[2] and Shih-Min A. Huang[1,3]

[1]Developmental and Molecular Pathways, Novartis Institutes for BioMedical Research, Cambridge, Massachusetts 02139; email: feng.cong@novartis.com

[2]Global Discovery Chemistry – Chemogenetics and Proteomics, Novartis Institutes for BioMedical Research, Cambridge, Massachusetts 02139

[3]Current address: Sanofi-Aventis Oncology, Cambridge, Massachusetts 02139

Annu. Rev. Pharmacol. Toxicol. 2012. 52:57–78

First published online as a Review in Advance on August 2, 2011

The *Annual Review of Pharmacology and Toxicology* is online at pharmtox.annualreviews.org

This article's doi: 10.1146/annurev-pharmtox-010611-134639

0362-1642/12/0210-0057$20.00

Keywords

cell-based screen, target deconvolution, chemical proteomics, HIP/HOP

Abstract

Identification of novel, validated targets remains a top priority in modern drug discovery. Chemical genetics represents a powerful approach to the discovery of new targets. Unlike the traditional target-based screen that relies on a predefined, sometimes poorly validated target, a chemical genetics–based phenotypic screen probes the entire molecular signaling pathway in an efficient and unbiased manner for the most drug-sensitive node. The most significant obstacle associated with this approach is identification of the efficacy targets of small-molecule probes. The huge potential of chemical genetics cannot be realized without the establishment of reliable mechanisms for target identification. In this article, we describe each essential element of the chemical genetics process, discuss common challenges that the field is facing, and critically review various biochemical and genetics approaches recently developed for target deconvolution. We also attempt to summarize lessons that we have collectively learned and provide a practical perspective to facilitate the advancement of chemical genetics.

INTRODUCTION

A decade ago, encouraged by the massive amount of data that emerged from the fully sequenced human genome, pharmaceutical and academic researchers collectively predicted a rapid increase in treatments for all human diseases. However, the number of new molecular entities approved per year has steadily declined over the past decade. In the meantime, the discovery and validation of novel disease-relevant targets continue to be low, and many disease-relevant targets and pathways have remained "undruggable." It may be fair to say that the recent decline in innovative drugs is largely due to exhaustion of validated and tractable targets, but a counterargument can be made that the traditional approach to drug and target discovery no longer works. To maintain a healthy pipeline of novel validated targets for drug discovery, pharmaceutical companies must apply new and innovative approaches.

Genetics has been the traditional tool for discovering novel drug targets. Forward and reverse genetics screens in model organisms have been used to uncover the functions of many genes. With the recent advance in RNA interference (RNAi) technology, one can perform loss-of-function screens on a genome-wide scale without generating mutations (1). However, interpreting the functions of a given gene by eliminating its expression is an oversimplified approach, especially in the context of identifying pharmacological tractable mechanisms. The functions of a protein can be regulated through a variety of mechanisms, such as phosphorylation and dephosphorylation, which are not recapitulated with deletion. The formation and disassembly of protein complexes are under spatial and temporal regulations and thus cannot be understood with deletion. Also, the tractability (ability for small-molecule manipulation) of targets identified from genetics screens is often unclear. Thus, it is insufficient to rely solely on traditional genetics approaches to identify targets for pharmacological intervention in a complex biological system.

As an alternative approach for target discovery, chemical genetics, the study of genes through small-molecule perturbation, holds many advantages over traditional genetics (2–4). Disease can be caused by an imbalance in molecular signaling pathways; thus, chemicals that rebalance these pathways should have therapeutic potential (5). From a disease-relevant cellular pathway screen, chemical genetics can reveal tractable targets within a molecular signaling pathway and provide small-molecule tools with which to probe the target's function and understand its mechanism of action. Unlike the traditional target-based screen that relies on a predefined, often poorly validated target, the chemical genetics–based phenotypic screen probes the entire pathway for the most "druggable" node (**Figure 1**). With a chemical genetics approach, the biological activities and off-target potential of a drug candidate are assessed early in the discovery process, which should improve the efficiency of drug discovery. As perturbation through a small molecule is conditional and tunable, the approach enables a temporal analysis of the biological consequence and minimizes the complication of compensatory mechanisms often seen in genetics studies (4). Finally, because a small molecule can inhibit the activity of functional homologs, chemical genetics screens can overcome gene redundancy, which is an inherent problem posed by genetics screens.

The concept of chemical genetics has been practiced for centuries through the use of natural product compounds. For example, as early as the fifth century BCE, extracts from the bark of willows were found to reduce ache, fever, and inflammation. Around 1826, the active ingredient of willow bark was identified as salicylic acid, from which aspirin was derived. However, it was not until 1970 that John Vane was able to show that aspirin achieves its effects by inhibiting production of prostaglandins. Another well-known example is the discovery of rapamycin. Rapamycin is an immunosuppressive agent first extracted from *Streptomyces hygroscopicus* in the 1970s. Years later, rapamycin was discovered to function as an allosteric inhibitor of mammalian target of rapamycin (mTOR) (6). In 1968, cyclopamine was identified as the cause of a birth defect in lambs: sheep that

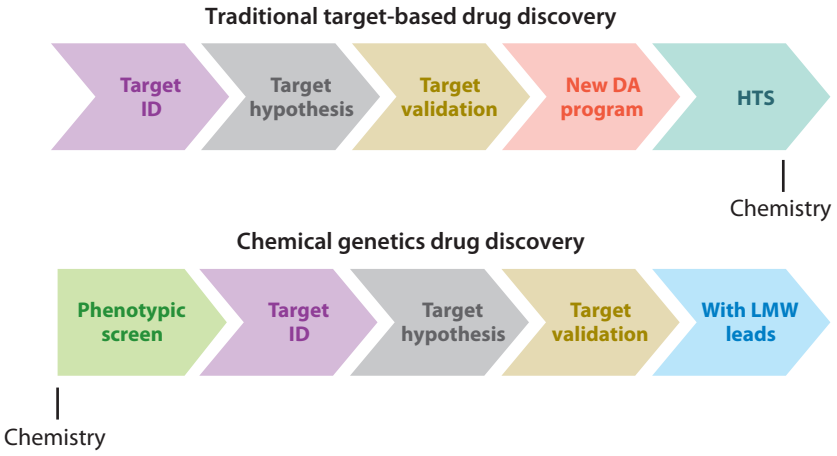

Figure 1

Conventional target-based drug discovery approach versus chemical genetics drug discovery approach. The target-based drug discovery process begins with a known protein target. The functions of a given target are then studied under a defined disease setting. Once the correlation and/or causal relationship between protein activity and disease state is established, a high-throughput screening (HTS) campaign is executed to seek modulators of a given target. The chemistry effort usually starts after the screen is accomplished. In the case of the chemical genetics drug discovery approach, the first step involves deployment of disease-relevant cell-based assays to identify bioactive small molecules. The chemists engage at this stage of the process to assess structure-activity relationships. The potency and physical properties of a given compound are then optimized on the basis of the cellular readout without knowledge of the exact target. A chemical probe is then used to identify possible cellular targets that are validated through additional genetic manipulations. This approach provides opportunities to discover novel mechanisms or targets that are otherwise unobvious. Once the target is validated, a new target with a small-molecule tool is then ready to move into the next stage of the drug discovery process. Abbreviations: DA, disease area; LMW, low-molecular-weight.

consumed wild corn lily had offspring with only one eye. In 1998, by observing the single cyclopic eye phenotype in the Sonic Hedgehog knockout mouse, Beachy's group made a connection between cyclopamine and Hedgehog signaling pathway (7). To this day, we still benefit from the use of natural products to uncover some of the most exciting discoveries in biology and diseases.

The discovery of the clinical uses and cellular targets of aspirin, rapamycin, and cyclopamine involves elements of serendipity and years of studies on mechanisms of action. The question remains whether success can be replicated in a predictable and timely fashion by incorporating advances in technology. In the chemical genetics approach, finding compounds with desired properties from pathway-based or phenotypic assays is relatively straightforward, but identifying the efficacy targets of these biological active compounds has been the bottleneck. The huge potential of chemical genetics in identifying novel targets cannot be realized without establishment of a robust mechanism to identify the cellular targets of bioactive compounds. In this review, we describe the process of chemical genetics–based target identification, examine the scientific challenges associated with target identification, discuss various approaches for target deconvolution, and highlight issues that need careful consideration.

COMPOUND SCREEN AND HIT SELECTION

The workflow of chemical genetics–based target identification is depicted in **Figure 2**. The first step is to perform cellular phenotypic screening to identify compounds that elicit a desired

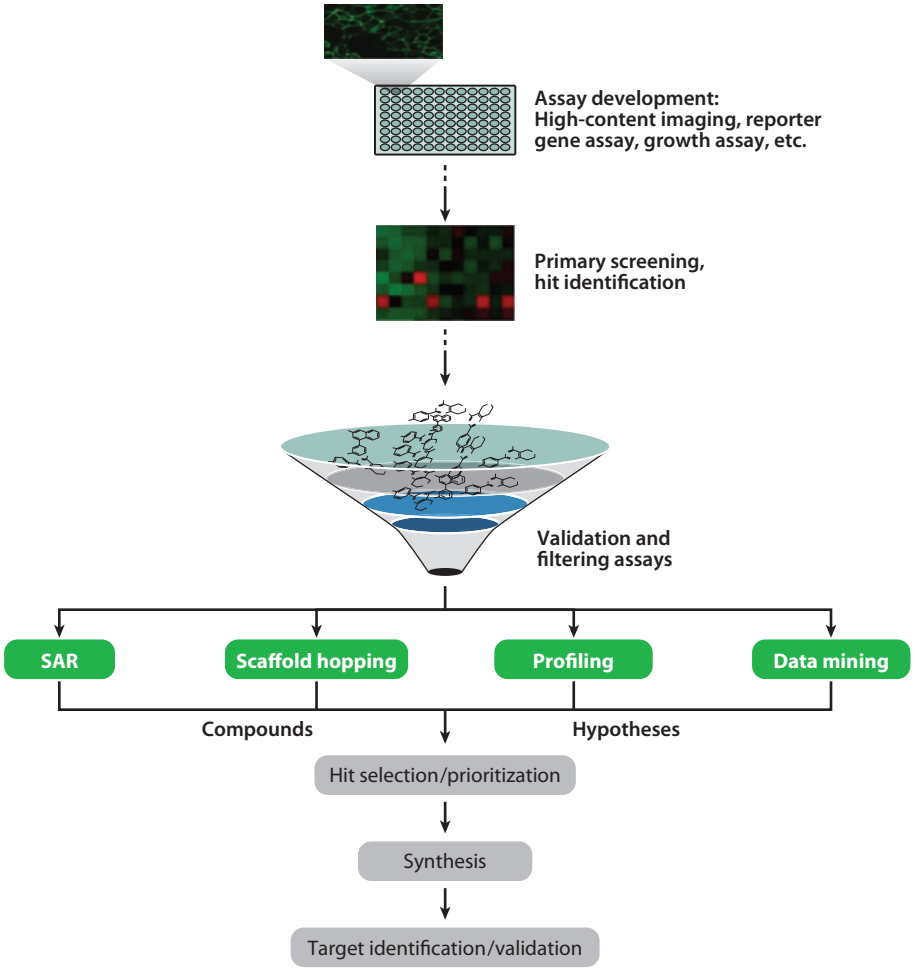

Figure 2

Workflow of the chemical genetics screening approach. A primary cell-based assay that captures pathways or phenotypic readouts is established and validated to screen a compound library. Owing to the frequent off-target effects of primary screen compounds, it is essential to implement counter screens and secondary screens to filter nonspecific hits in order to arrive at a group of high-confidence hit compounds. In silico methods for scaffold hopping and compound similarity searching can be utilized to select groups of similar molecules to generate structure-activity relationships (SAR) data to better understand the relevant "war head." In parallel, profiling and data mining can also arrive at hypotheses and facilitate hit selection and prioritization. Next, chemistry is initiated to expand the SAR for the hit and to identify sites for linker modification or prepare chemical probes. Target identification is conducted with the compound-linked beads by affinity purification of interacting proteins. This is followed by protein identification and quantification through the use of liquid chromatography–tandem mass spectrometry (LC-MS/MS) or other chemical probes. The final step is target validation through genetic, biochemical, or biophysical means.

phenotype. Then, an array of orthogonal secondary assays is performed to remove nonspecific compounds and identify compounds with desired properties for mechanistic studies. Because target deconvolution is a challenging and time-consuming process, one should carefully examine whether the compound of interest is truly worth the investment. For example, a compound affecting general cellular processes could suppress reporter expression. Unfortunately, such general

cellular toxicities could be subtle and hard to discern. Some cellular targets are quite sensitive to small-molecule modulation, and hitting these targets often leads to general toxicities. For example, many compounds with tubulin-destabilizing activities are frequent hitters in cell-based screens (8). There also exist assay-dependent nonspecific compounds. In a luciferase reporter screen aiming for pathway activators, histone deacetylase inhibitors and compounds that relieve general transcriptional repression often appear. Furthermore, in a screen for inhibitors of FOXO1a nuclear export, many inhibitors of nuclear exporter factor CRM1 were identified (9). Carefully designed secondary assays can remove these nonspecific compounds.

After nonselective compounds are removed, compounds with the desired activity profile need to be prioritized for target deconvolution. The performance of the compounds in past assays (proprietary or literature assay data) can provide clues as to the target class of the hit (e.g., kinase inhibitor) or mechanism of action (e.g., microtubule stabilizer for taxol-like hit). In silico target prediction tools can be applied to provide early target hypotheses (10, 11). Profiling in an array of biochemical assays through external vendors such as Ricerca (**http://www.ricerca.com**), Ambit (**http://www.ambitbio.com**), and Invitrogen (**http://www.invitrogen.com**) can provide clues regarding mechanism of action. Physiochemical properties of the hit compounds are important factors for hit prioritization. Solubility and permeability affect downstream activities, including generation of structure-activity relationships (SAR), and hinder future mechanism of action studies. For example, a compound with low solubility or low permeability can provide false-negative data and confound the development of SAR. Thus, prioritization of hits with good physiochemical properties focuses the chemistry efforts on establishing SAR, improving potency, and identifying the best target identification tool compound, and not preparing compounds with inconsistent or uninterpretable data owing to lack of solubility and permeability.

During target deconvolution, investigators first should consider known targets in the pathway or targets suggested by prediction tools. Once these target hypotheses are eliminated, profiling experiments need to be performed to identify the efficacy targets. Target deconvolution remains the most challenging step of chemical genetics–based target finding. Current target identification strategies can be roughly grouped into three categories: affinity-based (such as the chemical proteomics approach), genetics-based, and transcriptional profiling–based. These strategies are described in detail below.

CHEMICAL PROTEOMICS

Chemical proteomics represents a key approach for target identification (12–15). It consists of the classical drug-affinity chromatography and modern high-resolution mass spectrometry (MS) analysis for protein identification (**Figure 3**). This approach has emerged as the most popular strategy for target identification because it is an unbiased, large-scale method that enables the discovery of targets from a complex protein mixture. The procedure of drug-affinity chromatography involves immobilization of the compound of interest on a solid support through a spacer arm, and the affinity matrix is then used to purify specific interacting proteins from cellular lysate. Unlike other methods, chemical proteomics does not rely on inference to generate a target hypothesis as it identifies the direct binding targets of a compound. This approach probes the entire proteome for targets instead of relying on a predefined set of recombinant proteins, as is the case with biochemical profiling, and it can also be used on any cell and tissue of interest to study disease-relevant mechanisms.

In the 1990s, several classical examples of affinity chromatography–based target identification were published. FKBP12, a previously unknown *cis-trans* proline isomerase, was found as the binding partner of the immunosuppressive agent FK506 (16). The FK506-FKBP12 complex was

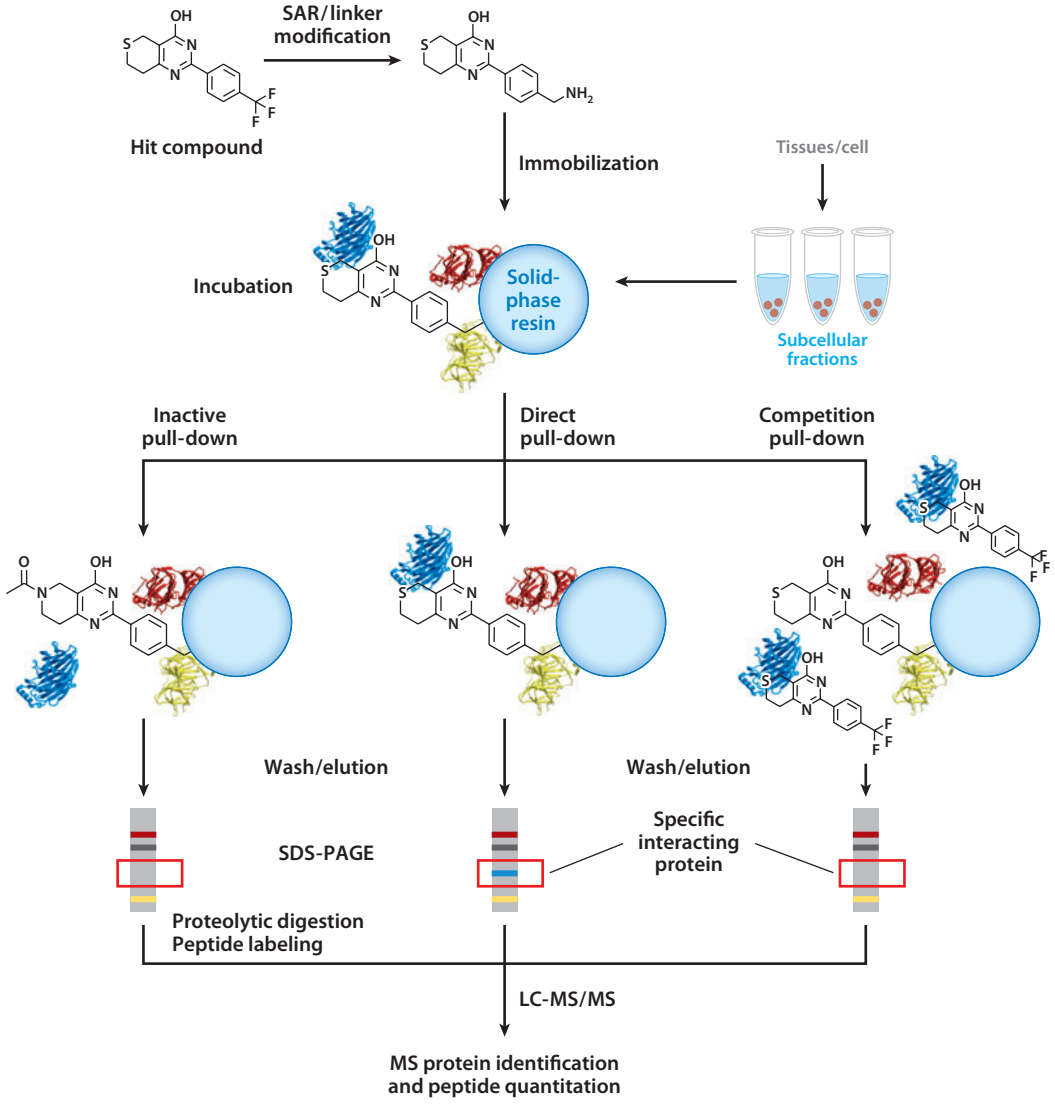

Figure 3

Workflow for target identification by chemical proteomics. From validated hit compounds, a structure-activity relationships (SAR) analysis is performed to identify positions amenable to linker attachment. A linker derivative that retains biological activity is prepared and then immobilized onto a solid-phase resin. The compound-immobilized resin is incubated with lysate prepared from whole cells/tissues or subcellular fractions. In direct pull-down mode (*middle*), the resin-enriched proteins are separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The specific bands are then proteolytically digested, and the proteins are identified by liquid chromatography–tandem mass spectrometry (LC-MS/MS). To better discern specific binding proteins from nonspecific binders, parallel experiments are performed: incubation with an immobilized inactive compound (*left*) or incubation with a free active compound (*right*). In both of these pull-down modes, the specific binding proteins are removed while the nonspecific binding proteins remain. Quantitative comparison of these experiments can be performed using isobaric tags for relative and absolute quantitation (iTRAQ) labeling of the peptides after separation and proteolytic digestion of the proteins through MS/MS. For quantification using stable isotope labeling by amino acids in cell culture (SILAC), the affinity purifications with light and heavy labeled cell lysates are performed in parallel (not shown) and combined immediately before protein separation by SDS-PAGE.

later shown to inhibit T cell activation through binding and inhibiting calcineurin (17). Trapoxin is a microbially derived cyclotetrapeptide that inhibits histone deacetylation and induces cell cycle arrest. Affinity purification using a trapoxin affinity matrix led to identification of the first human histone deacetylase (HDAC) (18), which established the field of epigenetics in drug discovery with the clinical HDAC inhibitors, including SAHA and FK-228 (19).

The power of classical drug-affinity chromatography in target identification is dramatically enhanced by the tremendous technological developments in the field of MS (20). The first breakthrough in MS technology for proteomics was MS identification of gel-separated proteins; this essentially replaced the classical technique of Edman degradation. The sequencing of the human genome brought about another key breakthrough: the creation of a look-up table of proteins, based on peptide sequences, that can be used to correlate mass of the de novo sequenced protein. The emergence of nanoelectrospray ionization methods for liquid chromatography–tandem mass spectrometry (LC-MS/MS)—along with highly sensitive, high-resolution instrumentation such as linear ion trap/Fourier transform ion cyclotron resonance and linear ion trap/orbitrap mass spectrometers—has enabled rapid analysis of complex protein mixtures with much higher throughput and sensitivity (21). Recent development of quantitative MS methods is critical for distinguishing specific and nonspecific binders. Stable isotope labeling by amino acids in cell culture (SILAC) (22) and chemical labeling of tryptic peptides with isobaric tags for relative and absolute quantitation (iTRAQ) (23) are the most commonly used quantitative MS methods at this time. In the SILAC approach, cells are grown in a medium containing stable-isotope-labeled amino acid such as [$^{13}C_6$]arginine and are mixed with unlabeled cells, and samples are processed and analyzed by MS. Mixing of intact cells significantly decreased the variations during sample handling. A commercially available iTRAQ reagent is based on the principle of isobaric mass tag (24). In the iTRAQ approach, peptides are labeled postproteolysis to produce isobarically labeled peptides that precisely comigrate in LC separation, and reporter ions for isobaric mass tag are then detected and quantified after peptide fragmentation (25). Multiplexed quantification of up to eight samples is now possible, which enables concentration-response experiments.

The most significant limitation for chemical proteomics is the specificity challenge. The cellular mixture is extremely complex, with a dynamic range of protein expression of 10^6. When a small molecule enters cells that are densely packed with proteins, it interacts with a plethora of proteins that have greatly varying affinities. The high-affinity interaction between a compound and its efficacy target, often a low-abundance protein, is the most physiologically relevant interaction. However, a low-affinity interaction between the drug and highly abundant proteins often creates significant noise during purification. In general, the higher the affinity and the more abundant the target, the more likely it is to be identified. Therefore, a small molecule with high affinity for its target should be selected for affinity purification, as low-affinity molecules result in loss of target proteins during washing steps. One can use the effective concentration of the compound in cellular assays, such as EC_{50} or IC_{50}, to estimate the binding affinity. However, potency could also be misleading, as some promiscuous compounds might interact with multiple targets and produce a strong cellular effect. In practice, compounds with EC_{50} or IC_{50} values in the nanomolar or low micromolar range are used for affinity purification.

Multiple strategies to reduce background binding have been tried. "Sticky" proteins tend to have low affinity for the hydrophobic surfaces of linker-drug complexes or drug-binding proteins. It has been realized that the hydrophobicity of the linker has a profound effect on background binding. Introduction of hydrophilic linkers such as tartaric acid derivatives (26) and polyethylene glycol (27) significantly decreases nonspecific binding and improves sensitivity of proteomics identifications. Various forms of cleavable linkers to improve the specific elution of target proteins are also being developed (28–31). Furthermore, novel matrices such as poly(methacrylate)-based

Toyopearl® (26) and latex-based nanobeads (32) offer lower background binding and greater compatibility with various chemistry methods for compound coupling. Another approach to reduce background contamination is determining the subcellular localization of the active molecule and its target to reduce sample complexity. If one can use a fluorescent derivative of the active molecule to determine that the compound's target is in the nucleus or mitochondria, extracts of the nucleus or the mitochondria could be used for affinity purification. For example, Kotake et al. (33) found that fluorescence-tagged pladienolide was concentrated in the nuclear speckles, suggesting that splicing-related proteins could serve as potential binding proteins for pladienolide. Using a partially purified nuclear extract, they identified spliceosome protein SAP130 as the direct target of pladienolide.

Even when background binding is minimized, methods to distinguish specific and nonspecific binders are still needed. One way to identify nonspecific binders is to generate a "frequent hitter" list through aggregating data from a large number of compound pull-down experiments. Furthermore, generation of a list of the most abundant proteins from unfractionated cell lysates also provides valuable information for data interpretation (34). However, this "blacklist" approach has its caveats. For instance, geldanamycin targets the high-abundance protein HSP90, a highly relevant cancer target (35). Elution of compound-associated proteins using the free active molecule is an option (36), but the utility of this method is limited if compounds are not soluble at high concentrations, leading to low efficiency of elution. A serial-affinity chromatography strategy has also been described (37). Cell extracts were first incubated with an affinity matrix. After removal of the resin, the same lysates were then incubated with a fresh affinity matrix. The idea is that the first batch of affinity matrix preferentially enriches specific binders, whereas both batches should capture the same amount of nonspecific binders.

One commonly used approach to discriminate specific and nonspecific binding proteins is to do a parallel pull-down using chemically analogous molecules that lack cellular activity. Proteins isolated by active molecules but not inactive control molecules are considered candidate targets. Using this approach, Sato et al. (38) identified mitochondria protein prohibitin 1 (PHB1) as the target of aurilide. Aurilide is a potent, cytotoxic marine natural product that induces apoptosis in cultured cells. To isolate the efficacy target of aurilide, the compound was conjugated with a biotin molecule through a protease-cleavable polyproline linker. The resulting molecule was bound to avidin beads and incubated with cellular extracts. Bound proteins were eluted with HRV-3C protease. The major protein enriched by beads with aurilide, but not its inactive analog 6-epi-aurilide, was identified as PHB1 by microsequencing. Interestingly, fluorescein-labeled aurilide is localized in the mitochondria. To validate the finding, Sato et al. (38) show that partial knockdown of PHB1 sensitized cells to aurilide, whereas overexpression of PHB1 rendered cells resistant. Follow-up experiments suggest that binding of PHB1 by aurilide activates the proteolytic processing of optic atrophy 1, leading to mitochondria-induced apoptosis. In addition, the identifications of multifunctional protein 2 as a target protein of chromeceptin (39), cytosolic malate dehydrogenase as the target of E7070 (an anticancer drug under clinical evaluation) (40), and ornithine δ-aminotransferase as the target of diazonamide A (41) were successfully carried out utilizing active and inactive linker molecules to help distinguish the targets of interest from nonspecific binding proteins. Some inactive molecules lack cellular activity owing to their poor permeability or solubility, and they might bind to the target protein in cell lysates. Furthermore, some control or inactive compounds only partially lose the binding affinity to the target protein. All of these factors might confound data interpretation.

An in-solution competition approach represents another popular strategy to differentiate specific and nonspecific binders. In this approach, cell lysates are pretreated with active molecules or vehicle before the affinity matrix is added. Capture of specific binders by the affinity matrix is

effectively blocked by active molecules in solution, so comparative analysis of parallel purifications through the use of MS should reveal specific binders (42, 43). Using this competition approach, we recently identified tankyrase as a novel therapeutic target for the Wnt pathway (44). The stability of Wnt pathway transcription factor β-catenin is regulated by the multisubunit destruction complex, in which axin is the key scaffolding protein. Deregulated Wnt pathway activity has been implicated in many cancers. However, the development of a small-molecule Wnt inhibitor has been hampered by a lack of tractable targets in the pathway. Using a Wnt-responsive luciferase reporter assay, we identified XAV939 as a potent and specific inhibitor for Wnt signaling. Biochemical analysis suggested that XAV939 blocked Wnt signaling by stabilizing axin. More than 50 analogs of XAV939 were synthesized, leading to the generation of an inactive analog (LDW643) and an active linker analog (LDW639). Immobilized active compound was incubated with cellular lysates spiked with an excess amount of XAV939, inactive analogs, or dimethyl sulfoxide. A three-channel iTRAQ quantitative MS analysis identified 18 proteins that were specifically competed off with XAV939, including the poly(ADP-ribose) polymerases PARP1, PARP2, tankyrase 1 (TNKS1), tankyrase 2 (TNKS2), and several known PARP1 substrates. Validation experiments demonstrated that XAV939 potently inhibited enzymatic activity of tankyrase, and that codepletion of TNKS1 and TNKS2 phenocopied XAV939. Further experiments suggest that tankyrase binds and modifies axin and promotes its ubiquitination and degradation. PARP1/2 and tankyrase binding proteins are copurified with direct interactors and represent physiological contaminants. These indirect interactors can be distinguished from direct interactors by searching proteins with a "druggable" domain (45) or mining protein-protein interaction databases (46). Identification of the axin-tankyrase link through classical genetics would be impossible as TNKS1 and TNKS2 have highly redundant function.

Identification of the cellular target of CB30865 represents another successful example of using the competition approach to eliminate nonspecific binders (47). CB30865 is a potent and selective cytotoxic compound with unknown mechanism of action. To identify the efficacy target of this compound, an active linker compound was immobilized on beads and incubated with cellular extracts in the presence or absence of excess free compound. The only protein significantly outcompeted by free compound was identified as nicotinamide phosphoribosyltransferase (Nampt), an enzyme present in the nicotinamide adenine dinucleotide (NAD) biosynthetic pathway. CB30865 potently inhibits the enzymatic activity of Nampt both in vitro and in vivo. Significantly, the cellular toxicity of CB30685 can be prevented by exogenous nicotinic acid, which cells convert into NAD via a Nampt-independent pathway. These results strongly suggest that cytotoxicity of CB30865 results from reduction in NAD through Nampt inhibition. In addition, the competition approach was used to identify Class Ia PI3K regulatory subunits p85a and p85b as the molecular targets of quinostatin (48) and KEAP-1 as the molecular target of antioxidant response element activator AI-1(49).

Chemical proteomics has also been used to discover new targets for known drugs, allowing the expansion into new disease indications and the understanding of off-target liabilities (50, 51). Protein kinases are well-established drug targets for oncology and other disease areas (52, 53). Given that the human genome encodes for 518 protein kinases and more than 2,000 ATP-binding proteins, small molecules competitive with ATP tend to be promiscuous (54, 55). Chemical profiling of p38 kinase inhibitor SB 203580 identified GAK, CK1, and RICK as previously unknown kinase targets (56). Similarly, the selective CDK2 inhibitor roscovitine (CYC202) was found to bind pyridoxal kinase (27), and the PDGFRb inhibitor SU6668 purified Aurora kinase A and TBK1, implicating these targets in observed antiproliferative and anti-inflammatory effects of the small molecule (57). Chemical profiling of clinical BCR-ABL inhibitors imatinib, nilotinib, and dasatinib revealed novel kinase and nonkinase targets (58), which might contribute to their clinical

activities. Another valuable approach to kinase inhibitor profiling is the use of affinity matrices that contain unspecific kinase inhibitors to purify a significant portion of the kinome for competition experiments (59). This "kinobead" approach has been used to assess target profiles of clinical kinase inhibitors.

Various affinity-based approaches have been developed to complement drug-affinity chromatography. The drug-affinity chromatography approach requires the generation of active linker compounds, which is often time consuming. This issue can be overcome by performing a screening using a tagged library (60). However, this imposes significant constraints on chemistry. Utilizing a photoaffinity reaction, Kanoh and coworkers (61) developed a nonselective method to attach small molecules to a solid surface for target enrichment without the need to modify the molecules. Drug affinity responsive target stability (DARTS) is a recently developed target identification method that relies on drug-protein interaction but does not require modification of small molecules (62). This method is based on the observation that binding to a drug often stabilizes the target protein and prevents the protein's flexibility and movement, thereby reducing the protease sensitivity of the target protein. Through the use of this method, eIF4A was identified as the target of resveratrol. It is often hard to identify low-abundance targets by chemical proteomics. Several strategies have been developed to circumvent this problem by artificially increasing the concentration of target proteins (63), including three-hybrid systems (64, 65), mRNA display (66), phage display (67–69), and protein microarray (70). Although these methods have their unique advantages, whether they will find widespread application in target deconvolution remains to be seen.

GENETICS-BASED APPROACH

Genetics screening has proven to be a successful strategy for target identification (**Figure 4**). The budding yeast *Saccharomyces cerevisiae* has dominated this field owing to its genetic tractability (71, 72). The yeast deletion collection consists of precise start-to-stop deletion strains of all 6,000 *S. cerevisiae* genes (homozygous for inessential genes and heterozygous for essential genes) (73, 74). A key feature of this collection is that each deletion strain is bar-coded with two unique 20-base-pair oligonucleotides that serve as strain identifiers. Molecular bar codes enable assessment of competitive growth of the entire collection of yeast deletion mutants in a single culture.

Drug-induced haploinsufficient profiling (HIP) is one of the assays that takes advantage of this competitive growth strategy. The HIP assay is based on the observation that decreased dosage of a drug target gene in a heterozygous mutant can result in increased drug sensitivity (75). It is unbiased and does not require prior knowledge of a compound's mechanism of action, but it requires the compound to inhibit cell growth. Because the HIP assay is based on cell growth, it is particularly suitable for identification of targets relevant in oncology and antifungal indications. The robustness of this assay has been demonstrated through identification of the targets of well-characterized compounds as well as novel compounds (76–80). The HIP assay reveals both the direct target and other components in the same pathway. As an unbiased genetics assay, HIP often yields unexpected findings. For example, although 5-fluorouracil was thought to exert its cytotoxic activity through inhibiting thymidylate synthase, HIP profiling suggests that 5-fluorouracil inhibits cell growth through inhibiting rRNA-processing exosomes (76, 77). HIP assays have also been conducted in *Candida albicans*, an opportunistic pathogen that is the leading cause of fungal infections in hospitals worldwide. These studies have led to the identification of guanine monophosphate synthase, δ-9 fatty acid desaturase, and poly(A) polymerase as targets of several compounds (81–84). Homozygous profiling (HOP) is analogous to HIP, except that the strains are completely deleted for nonessential genes. The HOP assay can occasionally reveal the direct target of the compound. One elegant example illustrating this principle is the identification

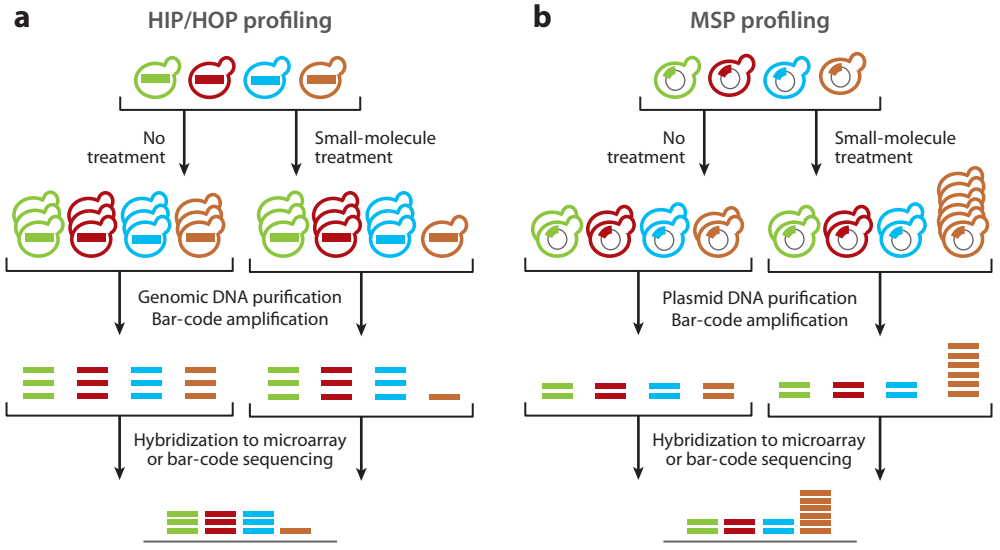

Figure 4

Chemogenomics approaches in yeast. (*a*) Haploinsufficiency profiling/homozygous profiling (HIP/HOP) assay. The yeast deletion strain is pooled, and the pool is grown competitively in the presence of the compound of interest. Genomic DNA is isolated from the compound-treated pool. Bar codes are PCR amplified and then hybridized to a TAG4 bar-code microarray or directly sequenced to assess the relative abundance of each strain. (*b*) Multicopy suppression profiling (MSP). Yeast cells sensitive to compound treatment are transformed with a high-copy yeast DNA library, and transformants are grown competitively in a compound such that only strains that confer resistance are selected from the population. Plasmids are isolated from resistant cells, and inserts are PCR amplified and hybridized to a DNA TAG4 microarray. Resistance is scored by comparing strain abundance between an untreated reference pool and a drug-treated pool.

of FKBP12 and TOR as targets of rapamycin (6). Yeast cells treated with rapamycin arrest in G1. Heitman et al. (6) isolated mutants that are resistant to rapamycin, mapped mutations to the drug-binding protein FKBP12, and evolutionally conserved TOR kinases. It is now accepted that rapamycin inhibits cell growth through forming a complex with FKBP12 and blocking the activity of TOR kinases. In most cases, the HOP assay identifies genes that buffer the drug target pathway, but not the direct target of the drug. The HOP assay mimics a double-deletion mutant because one gene is deleted and the function of the other gene is inhibited by the compound. Potential drug targets can be inferred by integrating chemical genetic profiles from the HOP assay with genetic interaction profiles. A proof-of-concept study demonstrated that a set of mutants sensitive to a given drug also shows synthetic lethal interactions with the gene encoding the drug target (Parson AB 62). In a more recent study, ERO1 was identified as the efficacy target of a previously uncharacterized compound through a genome-wide comparison of genetic and chemical genetic profiles (85).

Multicopy suppression profiling (MSP) is essentially the reverse of HIP and HOP. It is based on the idea that increasing the dosage of a drug target can confer resistance to drug-mediated growth inhibition. This concept is not new; the molecular targets of various antifungals such as tunicamycin (86), ketoconazole (49, 87), and soraphen (88) have been identified in this fashion. Current parallel screening technologies that use systematic collections of molecular bar-coded yeast open reading frame clones and genome fragments should provide genome-wide information with much higher resolution (89–91). Using an MSP assay, Hoon et al. (92) correctly identified Dfr1, Erg11, and Tor1 as the targets of methotrexate, fluconazole, and rapamycin, respectively.

The same approach has also been used for target identification in other model organisms. Pathania et al. (93) assembled an ordered, high-expression clone set of all essential genes from *Escherichia coli* and used it to systemically screen for suppressors of growth inhibitory compounds. In this study, efficacy targets of multiple well-known antibiotics were identified. Furthermore, the target of a compound with promising activity against multidrug-resistant *Pseudomonas aeruginosa* was identified; the compound exerts its toxic effect by inhibiting the function of the LolA protein and blocking lipoprotein targeting.

HIP, HOP, and MSP are complementary assays. For example, HIP can identify a target even if the target is part of a multisubunit complex, but such a target would not score in MSP if its function is under stoichiometric constraints. Conversely, a target with a functional paralog cannot be identified in HIP, but it would score in MSP. Another example is that a gene involved in drug detoxification confers sensitivity in HIP and resistance in MSP. However, if the gene also confers sensitivity in HOP, it is unlikely to be a direct target of the drug. Although HIP, HOP, and MSP assays are based on the same principle, each assay provides a distinct set of information. Integration of the results from each of these assays reveals a more complete picture of drug action in cells and significantly improves the sensitivity and specificity of drug target identification (92, 94).

Gene dosage–based screens have proven to be successful in identification of drug targets in microorganisms. In principle, a similar concept can be used in mammalian cells. Investigators are generating systematic collections of human cDNA clones, which can potentially be used in dosage-suppression studies (95, 96). In addition, a genome-wide RNAi study can potentially be used for target deconvolution in the same way that an HIP assay can be used (1, 97).

TRANSCRIPTIONAL PROFILING

Transcriptional profiling has been demonstrated as a useful method for target deconvolution (98–100). This approach relies on the generation of databases of transcriptional profiles that document the transcriptional effects of various compounds and genetic perturbations. Global transcriptional profiling provides a picture of the physiological status of cells. Perturbations targeting the same gene product or pathway are likely to generate similar transcriptional profiles; therefore, they would cluster together on the basis of the statistical analysis of their transcriptional signatures' similarity. Correlation of the profiles of novel compounds with profiles in the database provides insight into the molecular mechanisms of these compounds. On the basis of the premise that drug treatment and deletion of a drug target gene should have the same transcriptional profile, Hughes et al. (101) created a compendium of gene expression profiles of 300 yeast deletion strains and cells treated with dozens of drugs. Using this method, they identified ERG2 as the target of the anesthetic dyclonine. Boshoff et al. (102) generated a data set of transcriptional profiles of the response of *Mycobacterium tuberculosis* to 75 drugs and various growth inhibitory conditions and identified novel mechanisms of drugs by clustering these profiles. For instance, although the natural product ascididemin is thought to target DNA topoisomerase, its profile is clustered with profiles of known iron-scavenging agents. Further experiments indicate that ascididemin inhibits the growth of *M. tuberculosis* through iron depletion. Transcriptional profiling can be used to study compounds that do not affect growth and viability, whereas fitness-based methods cannot be used to study such compounds. Global transcriptional profiling has also been used in mammalian cells for target identification. Lam et al. (103) generated a collection of expression profiles of human cells treated with bioactive molecules and established a connectivity map using pattern-matching software. Using this approach, they identified the target of drugs of unknown mechanism of action.

Currently, the cost of full-genome transcriptional profiling has limited its use in large-scale compound profiling. Analysis of cDNA microarray data suggests that a small number of gene

signature profiles can be used to classify compounds with different mechanisms of action (100, 104). Recently developed gene signature assays such as bead-based LMF (ligation-mediated amplification with Luminex®-bar-coded microsphere and flow cytometry detection) technology significantly drive down the cost of transcriptional profiling and are more suitable for large-scale compound profiling (105, 106).

TARGET VALIDATION

Various target identification strategies can only generate hypotheses, and target-specific experiments need to be performed to validate these hypotheses. The first step in target validation is to determine whether the drug target mediates the biological activity of the compound in cellular assays. One should test whether depletion of the target using RNAi can phenocopy compound effects or sensitize cells to compound treatment. Many proteins have functional homologs, which may be targeted by a single small molecule. For this reason, the homolog of the candidate target needs to be considered even if it is not identified from the initial screen. Furthermore, a compound may have gain-of-function activity, and elimination of its target would suppress, instead of phenocopy, the compound's activity (107). In addition to RNAi, one can also use cDNA overexpression to establish compound-target relationships. Analogous to copy-number suppression in yeast, overexpression of the drug target might suppress the activity of the compound. This strategy is especially important for validating membrane targets, which are often blind to approaches such as chemical proteomics (108).

Once the putative target is validated in a functional assay, it is important to have a quantitative measurement of the binding affinity between the small molecule and the target. This can be done through various techniques such as surface plasmon resonance or isothermal calorimetry using recombinant or purified proteins (109, 110). If the putative target is an enzyme, an enzymatic assay can be set up to measure the effect of the compound on the enzyme activity. Structurally related compounds with various degrees of cellular activities should be tested in the binding assay or enzymatic assay to establish the SAR. Ultimately, rigorous validation by NMR or cocrystallization experiments should be performed to determine the three-dimensional structure of the compound-target complex. Such information not only validates the physical association but also provides critical information on the binding mode for future compound optimization.

CONSIDERATIONS FOR HIT CHARACTERIZATION AND TARGET DECONVOLUTION

Before launching a cell-based screen, one needs to examine carefully the molecular signaling pathway being interrogated and identify various molecular mechanisms that could potentially modulate the assay. A pilot screen using a collection of compounds (Tocriscreen™ Total collection: **http://www.tocris.com/screeningLibraries.php**) with known mechanisms of action can determine the mechanisms and/or compounds that could score as false positives in the assay or serve as possible targets for further validation. After prioritizing through the use of secondary assays, one can select compounds with the desired selectivity and activity profile for detailed mechanistic studies, including epistasis experiments to identify the level of compound intervention within a molecular pathway.

During target validation, the effect of the compound on known components of the molecular pathway should be examined carefully. Additionally, identification of a biological readout proximal to the compound's immediate target can help piece together the compound's mechanism of action. For example, in our discovery of novel Wnt pathway antagonists, our initial observation that

XAV939 stabilized axin led to axin-centric activities for target identification. An axin pull-down experiment was performed, and tankyrases were identified as axin binders. Furthermore, an axin Western blot assay, which is a more specific readout of compound activity than the Wnt pathway reporter assay, was used for siRNA validation of candidate targets derived from compound pull-down experiments (44).

Not only are cell-based pathway screens useful to discover novel targets, but these screens also can be employed to identify compounds that modulate known components of a pathway. This is exemplified by the identification of Smoothened (Smo) modulators and Porcupine inhibitors from cell-based screens. In the Hedgehog (Hh) pathway, Hh directly interacts with the multipass transmembrane protein Patched (Ptc) and activates Smo through an unknown mechanism. Several groups performed small-molecule, Hh-luciferase reporter screens to identify multiple classes of Hh agonists and Hh antagonists. Significantly, all of these compounds directly interact with Smo (111, 112). Porcupine is a membrane-bound O-acyltransferase essential for Wnt protein secretion (113). Chen et al. (108) performed a Wnt luciferase reporter screen using cells secreting Wnt, followed by a counterscreen using cells treated with Wnt-conditioned medium, and they identified compounds that selectively block Wnt secretion. These compounds were determined to be Porcupine inhibitors, and their activity can be suppressed by overexpression of Porcupine. The pairing of Hh modulators with Smo or Porcupine inhibitors with Porcupine is not based on affinity-based target identification. Although Smo and Porcupine are known components of Hh and Wnt signaling pathways, developing biochemical screens for these two membrane proteins can be challenging and time consuming as compared with development of cell-based pathway assays. With thorough pathway knowledge and proper screening strategies, using cell-based screens to identify compounds that hit known targets of a pathway is often straightforward.

A cell-based pathway screen should, in theory, identify compounds that hit various nodes in a pathway. However, at least three factors determine how frequent a node can be highlighted in a cell-based screen. The first is the functional requirement of the node. Different nodes contribute to the signaling output to different degrees. Even when a node is completely inhibited, the absolute effect of this inhibition on signaling output is very different for different nodes. The second factor is the tractability of the node or the susceptibility of the node to compound intervention. Different nodes have different tractability, so enzymes are likely to be more amenable to small-molecule modulation than transcription factors are. Of course, tractability is a relative term, and it is affected by the small-molecule library used for screening, which is often biased toward historical targets used in the pharmaceutical industry. The third is the effect of node inhibition on cellular processes. In cell-based screens, a specificity filter is often needed to remove nonspecific compounds such as luciferase inhibitors or compounds with general toxicity. The setup of this specificity filter can be tricky as it may lead to the exclusion of on-target compounds. If a node is important in a fundamental cellular process or involved in multiple biological processes, compounds affecting such a node may cause pleiotropic effects, leading to its removal by the specificity filter. Additionally, weak compounds act weakly on the target of interest but also interact with multiple other proteins at high concentrations, again leading to the node's removal by the specificity filter. If these factors are taken into account at the outset of the screen, the selection of follow-up compounds and targets along with the selectivity filter can be done judiciously to obtain the best results.

For reasons discussed above, a tractable target essential for signaling is the most likely target to be identified from a cell-based screen. Most Hh modulators from cell-based screens directly interact with Smo, although they could potentially hit other nodes of the Hh pathway. This observation can be explained as follows. First, Smo is the activator of the Hh pathway and is capable of constitutive activation in the absence of Ptc. Second, Smo is a molecule akin to a G protein-coupled receptor, readily tractable to small-molecule modulation. Last, Smo is absolutely

required for Hh signaling, and no functional paralog for Smo exists. This point is also illustrated by the identification of tankyrase inhibitors as inhibitors of Wnt/β-catenin signaling. Biochemical analysis has suggested that the concentration of axin is much lower than that of other components, thus representing the concentration-limiting factor for the assembly of the β-catenin degradation complex (114). Mathematic modeling suggests that increasing the concentration of axin is the most efficient way to inhibit Wnt signaling (115). The most potent and specific inhibitors from Wnt reporter screens performed by different labs are eventually deemed axin stabilizers (44, 108, 116), and our work suggests that all of these axin stabilizers are tankyrase inhibitors. Thus, the mechanism comprising tankyrase inhibition and axin stabilization represents the most robust and tractable mechanism for inhibiting Wnt signaling. All of this suggests that new screening and triage strategies might be required to identify additional targets in these pathways.

CONCLUSION AND PERSPECTIVES

The renaissance of phenotypic screening has brought drug discovery full circle. The field originally started from pharmacological screening in vivo; progressed to target-based, in vitro biochemical screening; and eventually found its way back to organism-based phenotypic screening (117). Historically, primary screens were done by administering small molecules to animals and monitoring direct pharmacological responses. Many therapeutic agents were developed without knowledge of their exact molecular targets. Recently, rapid expansion of genomic information and advances in high-throughput screening technology have led to a more reductionist strategy, which relies on performing drug discovery on poorly validated targets in overly simplified model systems. Phenotypic assays reintroduce biological complexity back into the model system and allow the study of disease-relevant pathways in a more physiological setting. In such assays, the collection of targets is interrogated in an efficient and unbiased manner. Instead of focusing on a predefined target in a biochemical screen, investigators can examine the entire pathway of interest for all possible intervention points, which significantly increases the repertoire of available targets and the efficiency of target discovery.

Using small molecules to perturb gene products conditionally, chemical genetics promises to complement classical genetics for target discovery. To realize the full potential of chemical genetics for target discovery, several factors need to be considered. First, it is important to set up complex and physiologically relevant cellular assays. Of importance are cell types differentiated from stem cells, especially patient-derived induced pluripotent stem cells to recapitulate the genetic drivers of disease; these stem cells can now be used for small-molecule screening (118). Three-dimensional cell culture with different cell types has been developed to mimic the tissue environment (119, 120). Further development of genetic-coded probes, an expanded collection of antibodies to read different cellular states, and improved methods of multiplexing probes will allow us to study many previously unexplored biological processes with increased precision. Second, it is important to establish a screening facility that allows high-throughput pharmacological interrogations of many cellular assays with different biological readouts. The adaptability and throughput of the screening facility will become more important as the complexity of cell-based assays and the number of compounds increase in the future. Currently, the establishment of the Chemical Genomics Center at the National Institutes of Health is enabling more researchers to gain access to an industrial-scale screening facility with a variety of assays (121). Third, a large and diverse compound library provides the advantage of saturating all possible cellular targets to identify a desirable cellular phenotype. Current compound libraries are biased toward known target classes, so more diverse compound libraries are needed to explore novel biology space (122). Fourth, it is essential to establish a robust target deconvolution platform that employs a

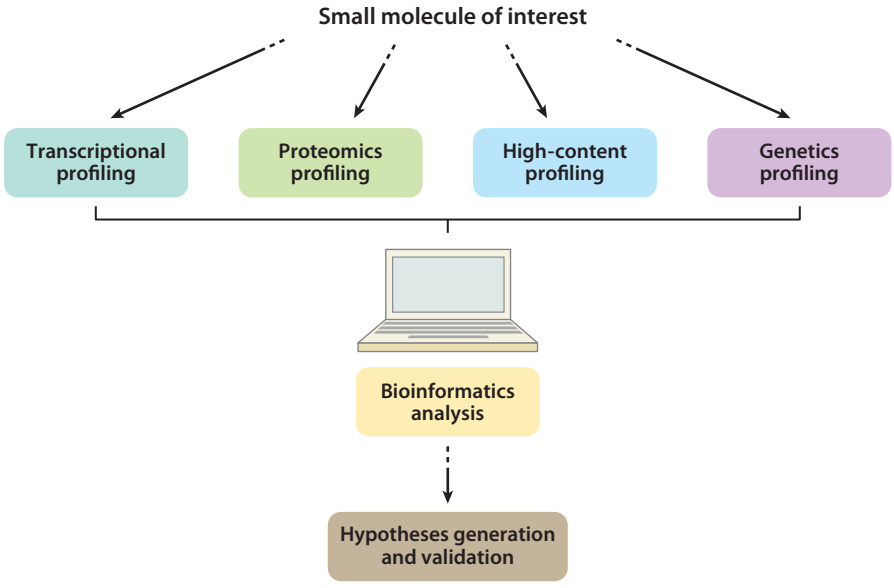

Figure 5

Integrated approach for target identification. The identification of an efficacy target represents a significant bottleneck of the chemical genetics drug discovery approach. Ideally, the compound of interest should go through various profiling platforms, e.g., transcription, proteomics, and genetics. The acquired information then can be analyzed and integrated with bioinformatics technology to generate hypotheses for experimental validation.

comprehensive collection of technologies such as those described above. Finally, target discovery requires a holistic approach that combines all available experimental strategies such as proteomics, genetics, and transcriptional profiling (**Figure 5**). Therefore, the power of bioinformatics needs to be fully exploited in this scenario. By intersecting data sets and applying statistical analysis, one should arrive at the most promising hypotheses.

Understanding the mechanism of action of bioactive compounds, along with identifying their efficacy targets, remains the most important topic in chemical genetics. With continued advances in this field, the discovery of novel drug targets will begin to increase, thus providing new lifeblood for pharmaceutical companies, and ultimately improve patient outcomes across many diseases.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors apologize to investigators whose work could not be referenced because of space limitations. The authors thank John A. Tallarico, Vic Myer, Andreas Bauer, Tewis Bouwmeester, Peter M. Finan, and Jeffery A. Porter for comments, advice, and continued support.

LITERATURE CITED

1. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391(6669):806–11
2. Smukste I, Stockwell BR. 2005. Advances in chemical genetics. *Annu. Rev. Genomics Hum. Genet.* 6:261–86
3. Bredel M, Jacoby E. 2004. Chemogenomics: an emerging strategy for rapid target and drug discovery. *Nat. Rev. Genet.* 5(4):262–75
4. Knight ZA, Shokat KM. 2007. Chemical genetics: where genetics and pharmacology meet. *Cell* 128(3):425–30
5. Fishman MC, Porter JA. 2005. Pharmaceuticals: a new grammar for drug discovery. *Nature* 437(7058):491–93
6. Heitman J, Movva NR, Hall MN. 1991. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* 253(5022):905–9
7. Cooper MK, Porter JA, Young KE, Beachy PA. 1998. Teratogen-mediated inhibition of target tissue response to *Shh* signaling. *Science* 280(5369):1603–7
8. Haggarty SJ, Mayer TU, Miyamoto DT, Fathi R, King RW, et al. 2000. Dissecting cellular processes using small molecules: identification of colchicine-like, taxol-like and other small molecules that perturb mitosis. *Chem. Biol.* 7(4):275–86
9. Kau TR, Schroeder F, Ramaswamy S, Wojciechowski CL, Zhao JJ, et al. 2003. A chemical genetic screen identifies inhibitors of regulated nuclear export of a Forkhead transcription factor in PTEN-deficient tumor cells. *Cancer Cell* 4(6):463–76
10. Bender A, Young DW, Jenkins JL, Serrano M, Mikhailov D, et al. 2007. Chemogenomic data analysis: prediction of small-molecule targets and the advent of biological fingerprint. *Comb. Chem. High Throughput Screen.* 10(8):719–31
11. Bender A, Mikhailov D, Glick M, Scheiber J, Davies JW, et al. 2009. Use of ligand based models for protein domains to predict novel molecular targets and applications to triage affinity chromatography data. *J. Proteome. Res.* 8(5):2575–85
12. Rix U, Superti-Furga G. 2009. Target profiling of small molecules by chemical proteomics. *Nat. Chem. Biol.* 5(9):616–24
13. Sato S, Murata A, Shirakawa T, Uesugi M. 2010. Biochemical target isolation for novices: affinity-based strategies. *Chem. Biol.* 17(6):616–23
14. Sleno L, Emili A. 2008. Proteomic methods for drug target discovery. *Curr. Opin. Chem. Biol.* 12(1):46–54
15. Cheung AK, Jain RK. 2010. Accelerating the discovery of new drug targets with chemical proteomics. *IDrugs* 13(12):862–68
16. Harding MW, Galat A, Uehling DE, Schreiber SL. 1989. A receptor for the immunosuppressant FK506 is a *cis–trans* peptidyl-prolyl isomerase. *Nature* 341(6244):758–60
17. Liu J, Farmer JD Jr, Lane WS, Friedman J, Weissman I, Schreiber SL. 1991. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* 66(4):807–15
18. Taunton J, Hassig CA, Schreiber SL. 1996. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science* 272(5260):408–11
19. Bradner JE, West N, Grachan ML, Greenberg EF, Haggarty SJ, et al. 2010. Chemical phylogenetics of histone deacetylases. *Nat. Chem. Biol.* 6(3):238–43
20. Han X, Aslanian A, Yates JR III. 2008. Mass spectrometry for proteomics. *Curr. Opin. Chem. Biol.* 12(5):483–90
21. Domon B, Aebersold R. 2006. Mass spectrometry and protein analysis. *Science* 312(5771):212–17
22. Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, et al. 2002. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Mol. Cell. Proteomics* 1(5):376–86
23. Ross PL, Huang YN, Marchese JN, Williamson B, Parker K, et al. 2004. Multiplexed protein quantitation in *Saccharomyces cerevisiae* using amine-reactive isobaric tagging reagents. *Mol. Cell. Proteomics* 3(12):1154–69

24. Thompson A, Schafer J, Kuhn K, Kienle S, Schwarz J, et al. 2003. Tandem mass tags: a novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. *Anal. Chem.* 75(8):1895–904
25. Wiese S, Reidegeld KA, Meyer HE, Warscheid B. 2007. Protein labeling by iTRAQ: a new tool for quantitative mass spectrometry in proteome research. *Proteomics* 7(3):340–50
26. Shiyama T, Furuya M, Yamazaki A, Terada T, Tanaka A. 2004. Design and synthesis of novel hydrophilic spacers for the reduction of nonspecific binding proteins on affinity resins. *Bioorg. Med. Chem.* 12(11):2831–41
27. Bach S, Knockaert M, Reinhardt J, Lozach O, Schmitt S, et al. 2005. Roscovitine targets, protein kinases and pyridoxal kinase. *J. Biol. Chem.* 280(35):31208–19
28. Speers AE, Cravatt BF. 2005. A tandem orthogonal proteolysis strategy for high-content chemical proteomics. *J. Am. Chem. Soc.* 127(28):10018–19
29. Fauq AH, Kache R, Khan MA, Vega IE. 2006. Synthesis of acid-cleavable light isotope-coded affinity tags (ICAT-L) for potential use in proteomic expression profiling analysis. *Bioconjug. Chem.* 17(1):248–54
30. Verhelst SH, Fonovic M, Bogyo M. 2007. A mild chemically cleavable linker system for functional proteomic applications. *Angew. Chem. Int. Ed. Engl.* 46(8):1284–86
31. Fonovic M, Verhelst SH, Sorum MT, Bogyo M. 2007. Proteomics evaluation of chemically cleavable activity-based probes. *Mol. Cell. Proteomics* 6(10):1761–70
32. Shimizu N, Sugimoto K, Tang J, Nishi T, Sato I, et al. 2000. High-performance affinity beads for identifying drug receptors. *Nat. Biotechnol.* 18(8):877–81
33. Kotake Y, Sagane K, Owa T, Mimori-Kiyosue Y, Shimizu H, et al. 2007. Splicing factor SF3b as a target of the antitumor natural product pladienolide. *Nat. Chem. Biol.* 3(9):570–75
34. Schirle M, Heurtier MA, Kuster B. 2003. Profiling core proteomes of human cell lines by one-dimensional PAGE and liquid chromatography-tandem mass spectrometry. *Mol. Cell. Proteomics* 2(12):1297–305
35. Trepel J, Mollapour M, Giaccone G, Neckers L. 2010. Targeting the dynamic HSP90 complex in cancer. *Nat. Rev. Cancer* 10(8):537–49
36. Emami KH, Nguyen C, Ma H, Kim DH, Jeong KW, et al. 2004. A small molecule inhibitor of β-catenin/CREB-binding protein transcription [corrected]. *Proc. Natl. Acad. Sci. USA* 101(34):12682–87
37. Yamamoto K, Yamazaki A, Takeuchi M, Tanaka A. 2006. A versatile method of identifying specific binding proteins on affinity resins. *Anal. Biochem.* 352(1):15–23
38. Sato S, Murata A, Orihara T, Shirakawa T, Suenaga K, et al. 2011. Marine natural product aurilide activates the OPA1-mediated apoptosis by binding to prohibitin. *Chem. Biol.* 18(1):131–39
39. Choi Y, Kawazoe Y, Murakami K, Misawa H, Uesugi M. 2003. Identification of bioactive molecules by adipogenesis profiling of organic compounds. *J. Biol. Chem.* 278(9):7320–24
40. Oda Y, Owa T, Sato T, Boucher B, Daniels S, et al. 2003. Quantitative chemical proteomics for identifying candidate drug targets. *Anal. Chem.* 75(9):2159–65
41. Wang G, Shang L, Burgett AW, Harran PG, Wang X. 2007. Diazonamide toxins reveal an unexpected function for ornithine δ-amino transferase in mitotic cell division. *Proc. Natl. Acad. Sci. USA* 104(7):2068–73
42. Ong SE, Schenone M, Margolin AA, Li X, Do K, et al. 2009. Identifying the proteins to which small-molecule probes and drugs bind in cells. *Proc. Natl. Acad. Sci. USA* 106(12):4617–22
43. Bantscheff M, Eberhard D, Abraham Y, Bastuck S, Boesche M, et al. 2007. Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. *Nat. Biotechnol.* 25(9):1035–44
44. Huang SM, Mishina YM, Liu S, Cheung A, Stegmeier F, et al. 2009. Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. *Nature* 461(7264):614–20
45. Overington JP, Al-Lazikani B, Hopkins AL. 2006. How many drug targets are there? *Nat. Rev. Drug Discov.* 5(12):993–96
46. Shoemaker BA, Panchenko AR. 2007. Deciphering protein-protein interactions: Part I. Experimental techniques and databases. *PLoS Comput. Biol.* 3(3):e42
47. Fleischer TC, Murphy BR, Flick JS, Terry-Lorenzo RT, Gao ZH, et al. 2010. Chemical proteomics identifies Nampt as the target of CB30865, an orphan cytotoxic compound. *Chem. Biol.* 17(6):659–64

48. Yang J, Shamji A, Matchacheep S, Schreiber SL. 2007. Identification of a small-molecule inhibitor of Class Ia PI3Ks with cell-based screening. *Chem. Biol.* 14(4):371–77
49. Hur W, Sun Z, Jiang T, Mason DE, Peters EC, et al. 2010. A small-molecule inducer of the antioxidant response element. *Chem. Biol.* 17(5):537–47
50. Brown D, Superti-Furga G. 2003. Rediscovering the sweet spot in drug discovery. *Drug Discov. Today* 8(23):1067–77
51. Bantscheff M, Scholten A, Heck AJ. 2009. Revealing promiscuous drug-target interactions by chemical proteomics. *Drug Discov. Today* 14(21–22):1021–29
52. Agrawal M, Garg RJ, Cortes J, Quintas-Cardama A. 2010. Tyrosine kinase inhibitors: the first decade. *Curr. Hematol. Malig. Rep.* 5(2):70–80
53. Catapano LA, Manji HK. 2008. Kinases as drug targets in the treatment of bipolar disorder. *Drug Discov. Today* 13(7–8):295–302
54. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. 2002. The protein kinase complement of the human genome. *Science* 298(5600):1912–34
55. Haystead TA. 2006. The purinome, a complex mix of drug and toxicity targets. *Curr. Top. Med. Chem.* 6(11):1117–27
56. Godl K, Wissing J, Kurtenbach A, Habenberger P, Blencke S, et al. 2003. An efficient proteomics method to identify the cellular targets of protein kinase inhibitors. *Proc. Natl. Acad. Sci. USA* 100(26):15434–39
57. Brehmer D, Greff Z, Godl K, Blencke S, Kurtenbach A, et al. 2005. Cellular targets of gefitinib. *Cancer Res.* 65(2):379–82
58. Rix U, Hantschel O, Dürnberger G, Remsing Rix LL, Planyavsky M, et al. 2007. Chemical proteomic profiles of the BCR-ABL inhibitors imatinib, nilotinib, and dasatinib reveal novel kinase and nonkinase targets. *Blood* 110(12):4055–63
59. Bantscheff M, Eberhard D, Abraham Y, Bastuck S, Boesche M, et al. 2007. Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. *Nat. Biotechnol.* 25(9):1035–44
60. Kim YK, Chang YT. 2007. Tagged library approach facilitates forward chemical genetics. *Mol. Biosyst.* 3(6):392–97
61. Kanoh N, Honda K, Simizu S, Muroi M, Osada H. 2005. Photo-cross-linked small-molecule affinity matrix for facilitating forward and reverse chemical genetics. *Angew. Chem. Int. Ed. Engl.* 44(23):3559–62
62. Lomenick B, Hao R, Jonai N, Chin RM, Aghajan M, et al. 2009. Target identification using drug affinity responsive target stability (DARTS). *Proc. Natl. Acad. Sci. USA* 106(51):21984–89
63. Terstappen GC, Schlupen C, Raggiaschi R, Gaviraghi G. 2007. Target deconvolution strategies in drug discovery. *Nat. Rev. Drug Discov.* 6(11):891–903
64. Becker F, Murthi K, Smith C, Come J, Costa-Roldan N, et al. 2004. A three-hybrid approach to scanning the proteome for targets of small molecule kinase inhibitors. *Chem. Biol.* 11(2):211–23
65. Caligiuri M, Molz L, Liu Q, Kaplan F, Xu JP, et al. 2006. MASPIT: three-hybrid trap for quantitative proteome fingerprinting of small molecule-protein interactions in mammalian cells. *Chem. Biol.* 13(7):711–22
66. McPherson M, Yang Y, Hammond PW, Kreider BL. 2002. Drug receptor identification from multiple tissues using cellular-derived mRNA display libraries. *Chem. Biol.* 9(6):691–98
67. Shim JS, Lee J, Park HJ, Park SJ, Kwon HJ. 2004. A new curcumin derivative, HBC, interferes with the cell cycle progression of colon cancer cells via antagonization of the Ca^{2+}/calmodulin function. *Chem. Biol.* 11(10):1455–63
68. Kim H, Deng L, Xiong X, Hunter WD, Long MC, Pirrung MC. 2007. Glyceraldehyde 3-phosphate dehydrogenase is a cellular target of the insulin mimic demethylasterriquinone B1. *J. Med. Chem.* 50(15):3423–26
69. Boehmerle W, Splittgerber U, Lazarus MB, McKenzie KM, Johnston DG, et al. 2006. Paclitaxel induces calcium oscillations via an inositol 1,4,5-trisphosphate receptor and neuronal calcium sensor 1-dependent mechanism. *Proc. Natl. Acad. Sci. USA* 103(48):18356–61
70. Huang J, Zhu H, Haggarty SJ, Spring DR, Hwang H, et al. 2004. Finding new components of the target of rapamycin (TOR) signaling network through chemical genetics and proteome chips. *Proc. Natl. Acad. Sci. USA* 101(47):16594–99

71. Smith AM, Ammar R, Nislow C, Giaever G. 2010. A survey of yeast genomic assays for drug and target discovery. *Pharmacol. Ther.* 127(2):156–64
72. Hoon S, St. Onge RP, Giaever G, Nislow C. 2008. Yeast chemical genomics and drug discovery: an update. *Trends Pharmacol. Sci.* 29(10):499–504
73. Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, et al. 1999. Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 285(5429):901–6
74. Giaever G, Chu AM, Ni L, Connelly C, Riles L, et al. 2002. Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* 418(6896):387–91
75. Giaever G, Shoemaker DD, Jones TW, Liang H, Winzeler EA, et al. 1999. Genomic profiling of drug sensitivities via induced haploinsufficiency. *Nat. Genet.* 21(3):278–83
76. Giaever G, Flaherty P, Kumm J, Proctor M, Nislow C, et al. 2004. Chemogenomic profiling: identifying the functional interactions of small molecules in yeast. *Proc. Natl. Acad. Sci. USA* 101(3):793–98
77. Lum PY, Armour CD, Stepaniants SB, Cavet G, Wolf MK, et al. 2004. Discovering modes of action for therapeutic compounds using a genome-wide screen of yeast heterozygotes. *Cell* 116(1):121–37
78. St. Onge RP, Mani R, Oh J, Proctor M, Fung E, et al. 2007. Systematic pathway analysis using high-resolution fitness profiling of combinatorial gene deletions. *Nat. Genet.* 39(2):199–206
79. Hillenmeyer ME, Fung E, Wildenhain J, Pierce SE, Hoon S, et al. 2008. The chemical genomic portrait of yeast: uncovering a phenotype for all genes. *Science* 320(5874):362–65
80. Yan Z, Costanzo M, Heisler LE, Paw J, Kaper F, et al. 2008. Yeast Barcoders: a chemogenomic application of a universal donor-strain collection carrying bar-code identifiers. *Nat. Methods* 5(8):719–25
81. Rodriguez-Suarez R, Xu D, Veillette K, Davison J, Sillaots S, et al. 2007. Mechanism-of-action determination of GMP synthase inhibitors and target validation in *Candida albicans* and *Aspergillus fumigatus*. *Chem. Biol.* 14(10):1163–75
82. Xu D, Sillaots S, Davison J, Hu W, Jiang B, et al. 2009. Chemical genetic profiling and characterization of small-molecule compounds that affect the biosynthesis of unsaturated fatty acids in *Candida albicans*. *J. Biol. Chem.* 284(29):19754–64
83. Jiang B, Xu D, Allocco J, Parish C, Davison J, et al. 2008. PAP inhibitor with in vivo efficacy identified by *Candida albicans* genetic profiling of natural products. *Chem. Biol.* 15(4):363–74
84. Oh J, Fung E, Schlecht U, Davis RW, Giaever G, et al. 2010. Gene annotation and drug target discovery in *Candida albicans* with a tagged transposon mutant collection. *PLoS Pathogens* 6(10):e1001140
85. Costanzo M, Baryshnikova A, Bellay J, Kim Y, Spear ED, et al. 2010. The genetic landscape of a cell. *Science* 327(5964):425–31
86. Rine J, Hansen W, Hardeman E, Davis RW. 1983. Targeted selection of recombinant clones through gene dosage effects. *Proc. Natl. Acad. Sci. USA* 80(22):6750–54
87. Launhardt H, Hinnen A, Munder T. 1998. Drug-induced phenotypes provide a tool for the functional analysis of yeast genes. *Yeast* 14(10):935–42
88. Vahlensieck HF, Pridzun L, Reichenbach H, Hinnen A. 1994. Identification of the yeast ACC1 gene product (acetyl-CoA carboxylase) as the target of the polyketide fungicide soraphen A. *Curr. Genet.* 25(2):95–100
89. Butcher RA, Schreiber SL. 2006. A microarray-based protocol for monitoring the growth of yeast overexpression strains. *Nat. Protoc.* 1(2):569–76
90. Ho CH, Magtanong L, Barker SL, Gresham D, Nishimura S, et al. 2009. A molecular barcoded yeast ORF library enables mode-of-action analysis of bioactive compounds. *Nat. Biotechnol.* 27(4):369–77
91. Jones GM, Stalker J, Humphray S, West A, Cox T, et al. 2008. A systematic library for comprehensive overexpression screens in *Saccharomyces cerevisiae*. *Nat. Methods* 5(3):239–41
92. Hoon S, Smith AM, Wallace IM, Suresh S, Miranda M, et al. 2008. An integrated platform of genomic assays reveals small-molecule bioactivities. *Nat. Chem. Biol.* 4(8):498–506
93. Pathania R, Zlitni S, Barker C, Das R, Gerritsma DA, et al. 2009. Chemical genomics in *Escherichia coli* identifies an inhibitor of bacterial lipoprotein targeting. *Nat. Chem. Biol.* 5(11):849–56
94. Kemmer D, McHardy LM, Hoon S, Reberioux D, Giaever G, et al. 2009. Combining chemical genomics screens in yeast to reveal spectrum of effects of chemical inhibition of sphingolipid biosynthesis. *BMC Microbiol.* 9:9

95. Gerhard DS, Wagner L, Feingold EA, Shenmen CM, Grouse LH, et al. 2004. The status, quality, and expansion of the NIH full-length cDNA project: the Mammalian Gene Collection (MGC). *Genome Res.* 14(10B):2121–27
96. Baross A, Butterfield YS, Coughlin SM, Zeng T, Griffith M, et al. 2004. Systematic recovery and analysis of full-ORF human cDNA clones. *Genome Res.* 14(10B):2083–92
97. Sachse C, Echeverri CJ. 2004. Oncology studies using siRNA libraries: the dawn of RNAi-based genomics. *Oncogene* 23(51):8384–91
98. Butcher RA, Schreiber SL. 2005. Using genome-wide transcriptional profiling to elucidate small-molecule mechanism. *Curr. Opin. Chem. Biol.* 9(1):25–30
99. Stoughton RB, Friend SH. 2005. How molecular profiling could revolutionize drug discovery. *Nat. Rev. Drug Discov.* 4(4):345–50
100. Gunther EC, Stone DJ, Rothberg JM, Gerwien RW. 2005. A quantitative genomic expression analysis platform for multiplexed in vitro prediction of drug action. *Pharmacogenomics J.* 5(2):126–34
101. Hughes TR, Marton MJ, Jones AR, Roberts CJ, Stoughton R, et al. 2000. Functional discovery via a compendium of expression profiles. *Cell* 102(1):109–26
102. Boshoff HI, Myers TG, Copp BR, McNeil MR, Wilson MA, Barry CE III. 2004. The transcriptional responses of *Mycobacterium tuberculosis* to inhibitors of metabolism: novel insights into drug mechanisms of action. *J. Biol. Chem.* 279(38):40174–84
103. Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, et al. 2006. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. *Science* 313(5795):1929–35
104. Gunther EC, Stone DJ, Gerwien RW, Bento P, Heyes MP. 2003. Prediction of clinical drug efficacy by classification of drug-induced genomic expression profiles in vitro. *Proc. Natl. Acad. Sci. USA* 100(16):9608–13
105. Peck D, Crawford ED, Ross KN, Stegmaier K, Golub TR, Lamb J. 2006. A method for high-throughput gene expression signature analysis. *Genome Biol.* 7(7):R61
106. Stegmaier K, Wong JS, Ross KN, Chow KT, Peck D, et al. 2007. Signature-based small molecule screening identifies cytosine arabinoside as an EWS/FLI modulator in Ewing sarcoma. *PLoS Med.* 4(4):e122
107. Weiss WA, Taylor SS, Shokat KM. 2007. Recognizing and exploiting differences between RNAi and small-molecule inhibitors. *Nat. Chem. Biol.* 3(12):739–44
108. Chen B, Dodge ME, Tang W, Lu J, Ma Z, et al. 2009. Small molecule–mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. *Nat. Chem. Biol.* 5(2):100–7
109. Morohashi K, Yoshino A, Yoshimori A, Saito S, Tanuma S, et al. 2005. Identification of a drug target motif: an anti-tumor drug NK109 interacts with a PNxxxxP. *Biochem. Pharmacol.* 70(1):37–46
110. Kuettel S, Mosimann M, Maser P, Kaiser M, Brun R, et al. 2009. Adenosine Kinase of *T. b. rhodesiense* identified as the putative target of 4-[5-(4-phenoxyphenyl)-2*H*-pyrazol-3-yl]morpholine using chemical proteomics. *PLoS Negl. Trop. Dis.* 3(8):e506
111. Chen JK, Taipale J, Young KE, Maiti T, Beachy PA. 2002. Small molecule modulation of Smoothened activity. *Proc. Natl. Acad. Sci. USA* 99(22):14071–76
112. Frank-Kamenetsky M, Zhang XM, Bottega S, Guicherit O, Wichterle H, et al. 2002. Small-molecule modulators of Hedgehog signaling: identification and characterization of Smoothened agonists and antagonists. *J. Biol.* 1(2):10
113. Tanaka K, Okabayashi K, Asashima M, Perrimon N, Kadowaki T. 2000. The evolutionarily conserved *porcupine* gene family is involved in the processing of the Wnt family. *Eur. J. Biochem.* 267(13):4300–11
114. Salic A, Lee E, Mayer L, Kirschner MW. 2000. Control of β-catenin stability: reconstitution of the cytoplasmic steps of the Wnt pathway in *Xenopus* egg extracts. *Mol. Cell* 5(3):523–32
115. Lee E, Salic A, Krüger R, Heinrich R, Kirschner MW. 2003. The roles of APC and axin derived from experimental and theoretical analysis of the Wnt pathway. *PLoS Biol.* 1(1):E10
116. Waaler J, Machon O, von Kries JP, Wilson SR, Lundenes E, et al. 2011. Novel synthetic antagonists of canonical Wnt signaling inhibit colorectal cancer cell growth. *Cancer Res.* 71(1):197–205
117. Drews J. 2000. Drug discovery: a historical perspective. *Science* 287(5460):1960–64
118. Csete M. 2010. Translational prospects for human induced pluripotent stem cells. *Regen. Med.* 5(4):509–19

119. Fischbach C, Chen R, Matsumoto T, Schmelzle T, Brugge JS, et al. 2007. Engineering tumors with 3D scaffolds. *Nat. Methods* 4(10):855–60
120. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, et al. 2009. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 459(7244):262–65
121. Austin CP, Brady LS, Insel TR, Collins FS. 2004. NIH Molecular Libraries Initiative. *Science* 306(5699):1138–39
122. Wilk W, Zimmermann TJ, Kaiser M, Waldmann H. 2010. Principles, implementation, and application of biology-oriented synthesis (BIOS). *Biol. Chem.* 391(5):491–97

Annual Review of Pharmacology and Toxicology

Volume 52, 2012

Contents

Indexes

Errata

An online log of corrections to *Annual Review of Pharmacology and Toxicology* articles may be found at http://pharmtox.annualreviews.org/errata.shtml