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Review

Chemical Genomic Approaches to Study Model Microbes

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Recent genome-scale analyses of genetic interactions in model microbes have revealed the inherent functional organization of the cell as a dense network of highly interconnected pathways. While classical one gene at a time paradigms offer limited insight into cellular systems, genome-scale approaches are making considerable headway. Indeed, where small organic compounds are ideal probes of biological complexity, systematic chemical genomic methods are emerging as requisite and powerful approaches to describing both the small molecule probe and network with which it interacts. Here, we highlight various chemical genomic approaches that are being pioneered in model microbes.

Introduction

In light of the explosion in sequencing efforts and the expansion in genome-scale approaches to chart genetic interactions, our view of the cell is changing. With this new wealth of information comes a new appreciation and understanding of complex biological systems. While classical genetics has played a pivotal role in elucidating biology by investigating relationships between genes and phenotypes, we are now increasingly turning to small molecules as modulators. The thesis is that small molecules are ideal probes of biological systems with advantages over genetic manipulation. It has been pointed out that genetic inactivation is effectively permanent and technically tedious, even in the most tractable systems, and that these limitations can be circumvented through the use of small molecules (Specht and Shokat, 2002).

Probing biological functions with small molecules has helped elucidate functional roles for enigmatic areas of biology in which conventional genetic and biochemical approaches have provided limited understanding. Insights gained over the last 80 years into microbial physiology have frequently come from efforts to understand the mechanisms of action of antibiotics discovered during this same period. Indeed, our understanding of basic processes of nucleic acid, protein, and cell wall synthesis has often been due to remarkable discoveries in model microbes, enabled by antibiotic compounds. Despite the advantages of small molecules as probes, efforts to discover and characterize their interactions within biological systems have been narrowly focused and limited for the most part to traditional nongenomic approaches. The new understanding of cellular complexity that has come from large-scale studies of protein and genetic interactions (Butland et al., 2005; Costanzo et al., 2010; Faith et al., 2007; Jeong et al., 2001), has sparked a demand for genome-scale techniques to characterize both new and old chemical probes. Such chemical genomic methodologies, it is reasoned, more fittingly describe complex biology by informing on how network components interact to produce physiological responses or maintain phenotypic stability under states of stress.

Along with providing a global view of the biological system under study, chemical genomics also provides remarkable new tools to understand the mechanism of action of small molecules of unknown function. This is a daunting hurdle in both new probe development and in drug discovery (Burdine and Kodadek, 2004). Classically, protein targets have been identified through biochemical screens using labeled or immobilized molecules. A growing repertoire of new approaches to study the mode of action of small molecules now includes transcriptional profiling (Shaw and Morrow, 2003), network inference models (Gardner et al., 2003), small-molecule (Bradner et al., 2006; Duffner et al., 2007), as well as protein microarrays (MacBeath and Schreiber, 2000). Further, breakthrough genome-scale approaches in *Saccaromyces cerevisiae*, which take advantage of its diploid nature, have emerged and been successful in identifying cellular targets of small molecules (Baetz et al., 2004; Giaever et al., 1999; Lum et al., 2004; Parsons et al., 2004). Chemical genomic strategies have also advanced the field of natural product research by facilitating the characterization of the ever-increasing repertoire of novel natural products (Jiang et al., 2008; Parsons et al., 2006). These studies in yeast were among the first to yield biological insights in response to chemical perturbants on a network level and have inspired the establishment of postgenomic tools in a variety of bacterial organisms. The present review emphasizes the successful use of these approaches in model microbes, principally bacteria and yeast. Chemical genomics in *S. cerevisiae* has been recently reviewed (Hoon et al., 2008b) and accordingly, we have emphasized principles and included work in bacterial systems here. The value of genome-wide approaches in tackling cellular complexity and exploiting the activity of small molecules is enabling biological investigations previously not deemed possible. Herein, we describe the emerging tools for chemical genomic studies (Figure 1), including genome-scale clone sets, microarray-based transcriptional profiling, chemical proteomics, and computational methodologies, highlighting success stories of intriguing biological and mechanistic findings.

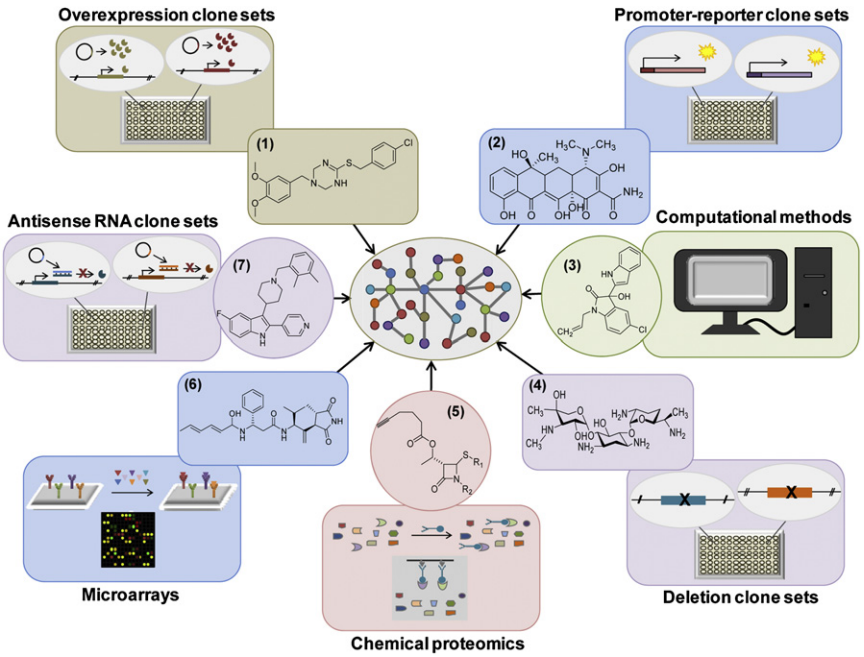

Figure 1. Experimental Approaches for the Global Investigation of Protein Function and Identification of Biological Probes

Schematically shown are various genomic methodologies, which when used in parallel with small molecules, aid in understanding the complex cellular network (middle). Depicted are small molecules that have been identified using the respective methods (1, 3, 7) or used to perturb the cellular system (2, 4, 5, 6). (1) MAC-13243 (Pathania et al., 2009), (2) tetracycline (Bollenbach et al., 2009), (3) ECi8 (Shen et al., 2010), (4) gentamicin (Kohanski et al., 2008), (5) β-lactam probe (Staub and Sieber, 2009), (6) moiramide B (Hughes et al., 2000), (7) DMPI (Donald et al., 2009).

Genome-Scale Clone Sets—Altering Gene Dosage to Infer Function

With the availability of comprehensive genome sequence information, it was inevitable that efforts would follow to construct elaborate genome-scale clone sets (Table 1) well suited to studying genetic and chemical-genetic interactions. Chemical genomics has been best established with extraordinary genomic tools available for the baker's yeast *Saccharomyces cerevisiae* (Hoon et al., 2008b). Among the most exciting developments in genome-wide approaches has been the creation of barcoded homozygous and heterozygous deletion clone sets (Giaever et al., 2002), where high-throughput competitive growth assays have allowed the parallel study of multiple *S. cerevisiae* strains (Giaever et al., 2002; Shoemaker et al., 1996; Winzeler et al., 1999). Thus, by exploiting the diploid nature of *S. cerevisiae*, the effect a small molecule has on the fitness of a particular strain can be examined when gene dosage is tuned from 0% (homozygous deletions) (Lee et al., 2005; Parsons et al., 2006) to 50% (heterozygous deletions) (Giaever et al., 2004; Parsons et al., 2006) to >100% (overexpressors) (Butcher et al., 2006; Gelperin et al., 2005; Luesch et al., 2005).

Using both the heterozygous and homozygous deletion collections, Hillenmeyer et al. looked to uncover a phenotype for all genes in *S. cerevisiae* (Hillenmeyer et al., 2008). In the presence of over 400 small molecules and diverse environmental stresses, the study revealed that 97% of the gene deletions exhibited a measurable growth defect, suggesting that nearly all genes are essential under at least one condition. The fact that only 20% of the *S. cerevisiae* genome was thought to be essential under rich media conditions highlights the power of this chemical genomic approach to uncover a phenotype for virtually all of the remaining genes, and provides additional insight into the role of so-called nonessential genes. Although these techniques are not completely transferrable to monoploid organisms, the work performed in *S. cerevisiae* nicely exemplifies the utility of altering gene dosage to identify chemical-genetic interactions and provided the groundwork to extend chemical genomics to bacteria.

Reducing Gene Dosage

Mutagenesis experiments have had a long-standing role in determining gene dispensability and investigating genetic interactions. Most recently, systematic gene knockout libraries of all possible deletion mutants have recently been completed in a variety of bacterial species (Table 1). These have particular utility in chemical genomics (Figure 2A). Decreasing the dosage of a given gene can lead to any of three possible outcomes with respect to the biological activity of a compound: no effect, enhancement, or suppression of phenotype. Systematic enhancement screens using the *E. coli* deletion collection have focused on known antibiotics to date and revealed signature chemical-genetic interactions that enhance the growth inhibition exerted by antibiotics (Tamae et al., 2008). These studies have huge potential in facilitating mechanism of action studies. Indeed, genetic enhancers of the action of gentamicin provided key insights that ultimately led to the understanding that aminoglycosides mediate cell death through the production of toxic hydroxyl radicals (Kohanski et al., 2007, 2008).

Other genome-scale chemical-genetic enhancement screens have proven useful in identifying genes implicated in intrinsic multidrug resistance (Breidenstein et al., 2008; Duo et al., 2008), as well as those responsible for maintaining a population of persister cells (Hansen et al., 2008). However, unlike deletion libraries in yeast, the bacterial counterparts do not contain molecular barcodes, limiting their ability to be used in pooled competitive assays. To this end, a set of barcoded deletions in *E. coli* is currently being generated, allowing for future parallel analysis of competitive growth assays similar to those in yeast (Mori et al., 2009). Moreover, two groups have recently reported methods for the high-throughput generation of double deletions in *E. coli* (Butland et al., 2008; Typas et al., 2008), further expanding the current tool set for microbial chemical genomics. Indeed, systematic studies of the interactions of double deletions with, e.g., growth inhibitory small molecules, would allow for higher-order studies of the cellular network and its capacity to buffer the effects of multiple perturbations.

Table 1. Genome-Scale Clone Sets in Model Microbes

Organism	Reference	Note
Gene knockdown		
Bacillus subtilis	Kobayashi et al. (2003)	
Candida albicans	Xu et al. (2007)	Heterozygous barcoded
Escherichia coli	Baba et al. (2006)	
Helicobacter pylori	Salama et al. (2004)	
Pseudomonas aeruginosa PA01	Jacobs et al. (2003)	
Pseudomonas aeruginosa PA14	Liberati et al. (2006)	
Saccharomyces cerevisiae	Giaever et al. (2002); Winzeler et al. (1999)	Homo/heterozygous barcoded
Staphylococcus aureus	Donald et al. (2009)	Antisense knockdown
Overexpression		
Escherichia coli	Kitagawa et al. (2005)	
Neisseria gonorrhoeae	Brettin et al. (2005)	
Pseudomonas aeruginosa	Labaer et al. (2004)	
Saccharomyces cerevisiae	Gelperin et al. (2005)	
Staphylococcus aureus	Brandner et al. (2008)	
Promoter-reporter		
Escherichia coli	Zaslaver et al. (2006)	*gfp*-promoter fusions
Salmonella typhimurium	Goh et al. (2002)	Promoter-*lux* reporter fusions

Along with knockout libraries, antisense technology has provided an additional means to explore small molecule effects by controlled reduction of gene expression (Figure 2B). Recently, Donald et al. arrayed xylose-inducible antisense RNA strains corresponding to 245 essential genes in *Staphylococcus aureus* (Donald et al., 2009). This antisense RNA system led to the discovery of new cell wall inhibitors, suggested to inhibit SAV1754, a previously uncharacterized cell surface transmembrane protein involved in cell wall assembly (Huber et al., 2009). SAV1754, structurally related to the *E. coli* peptidoglycan flippase, MurJ (Ruiz, 2008), is thus speculated to perform an analogous function in Gram positive bacteria. Profiles generated using antisense knockdown not only capture direct targets, but also pathway-related genes by exploring various strain sensitivities in the presence of the small molecule. A significant limitation of this array is the lack of genome coverage; it is limited to those genes essential for growth, excluding the possibility of charting chemical-genetic interactions with the dispensable gene set. A counter point to this concern argues that the targets of inhibitory small molecules should be essential for cell viability (Chalker and Lunsford, 2002). Thus, mechanism of action studies needn't explore chemical-genetic interactions with the dispensable fraction of the genome. Nevertheless, suppression and enhancement phenotypes can reveal interactions that reflect the underlying cellular network with which small molecules are interacting (Prelich, 1999), highlighting the important role of deletion clone sets in understanding mechanism of action.

Increasing Gene Dosage

Libraries designed for high expression of each gene through an inducible promoter have been increasingly exploited for small molecule target identification (Butcher et al., 2006; Li et al., 2004; Luesch et al., 2005; Pathania et al., 2009), where the presence of the target gene in multiple copies leads to suppression of growth inhibition by a particular small molecule (Figure 2C). Our group recently reported on the first systematic use of this methodology using the *E. coli* overexpression clone set (A Complete Set of E. coli K-12 ORF Archive [ASKA]) (Pathania et al., 2009). Proof of principle experiments employed a panel of antibiotics and revealed a unique set of chemical-genetic interactions for each compound. A stringency analysis of the concentration dependence of suppression revealed that known cellular targets were typically encoded by those genes that suppressed the highest doses of antibiotic. Of interest, the inhibitory action of a novel molecule, MAC13243, was suppressed at high stringency when the lipoprotein chaperone, LolA, was expressed at high copy. Further physiological and biochemical experiments suggested that MAC13243 is a new probe of lipoprotein trafficking in bacteria (Pathania et al., 2009). Nevertheless, target identification through suppression of growth inhibition is not always straightforward; the inherent complexity of the cellular network often leads to a variety of puzzling chemical-genetic interactions.

A particularly innovative use of high-copy suppression was that of Arnoldo and coworkers who used the methodology to identify *P. aeruginosa* virulence factors (Arnoldo et al., 2008). A yeast-based method was developed to screen for *P. aeruginosa* genes, which when overexpressed, were growth inhibitory to *S. cerevisiae.* Such genes were subsequently counter screened against a library of small molecules to uncover compounds which restored the growth of *S. cerevisiae*. An inhibitor of Exoenzyme S, a toxin of the *P. aeruginosa* type III secretion system, was identified and subsequently shown to prevent *P. aeruginosa* infection in mammalian cells. Integration of bacterial expression systems into *S. cerevisiae* provides a unique platform for the identification of novel virulence factors that can serve as potential antibacterial targets.

Like most techniques, diverse genome-scale chemical genomic approaches appear to benefit from integration. Of interest, several recent studies in yeast have combined efforts that involve multiple genome-scale clone sets to provide a more comprehensive and sensitive inference on small molecule action (Hoon et al., 2008a; Kemmer et al., 2009; Yan et al., 2009).

Microarray-Based Gene Expression Profiling

Chemical compounds lend themselves particularly well for gene-expression profiling studies, whereby transcriptional effects of small molecules can be documented using DNA microarrays. In fact, studying the effect of a small molecule on the regulation of gene expression has been at the forefront of recent chemical genomic studies. These approaches have proven vital in the global exploration of protein function, revealing novel insights

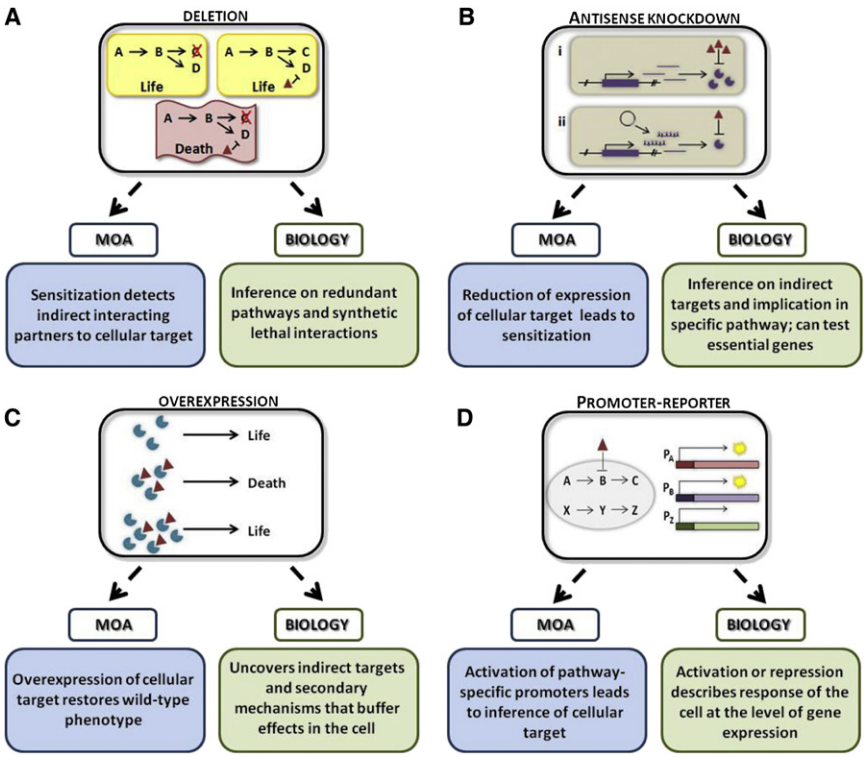

Figure 2. Genome-Scale Clone Sets Aid in Mode of Action (MOA) Determination and Provide Insights on Biological Processes (Biology) in Bacteria

Here, the red triangle represents the small molecule and the pacman, a protein target of interest.
(A) Mutagenesis using deletion collections in the presence of small molecules leads to death when perturbing a redundant pathway.
(B) Reducing gene expression through antisense RNA clone sets. (i) No antisense RNA expression such that the protein target is available at wild-type levels, requiring a certain amount of compound for inhibition. (ii) Antisense RNA expression from an inducible plasmid knocks down the amount of transcript, leading to reduced levels of protein targets, thus requiring a lower concentration of compound for inhibition than in (i).
(C) Suppression using high-expression libraries, whereby overexpression of a gene of interest in the presence of a small molecule can restore life.
(D) Promoter-reporter construct libraries allow for the detection of specific promoter activity (yellow star) in the affected pathway perturbed by a small molecule. For example, the small molecule targets protein B, such that promoter activity can be detected from both P_A and P_B, but not P_Z.

into many cellular pathways as well as mechanisms of action of uncharted small molecules.

Mode of action studies using microarrays have frequently involved comparative analyses with transcriptional profiles of known inhibitors or regulatory proteins (Freiberg et al., 2005; Hughes et al., 2000; Kung et al., 2005; Marton et al., 1998). Indeed, with gene-expression profile databases rapidly expanding, comparisons to available profiles can be readily made to identify relevant cellular pathways of unknown small molecules. Accordingly, the work by Kung et al. (2005) identified Cdk1 and Pho85, two cyclin-dependent kinases, as the targets of the novel inhibitor GW4000426 in *S. cerevisiae* through direct comparison to microarray transcriptional signatures elicited by specific pharmacological agents. Generation of drug reference profiles presents the advantage of identifying all targets that together lead to the cellular effects resulting from drug treatment. GW4000426 was shown to simultaneously inhibit both kinases, revealing the synthetic interaction of Cdk1 and Pho85. In this case, the novel protein kinase inhibitor aided in uncovering this unique cellular response through microarray-based transcriptional profiling. Another interesting study by Freiberg et al. generated a collection of genome-wide expression profiles of *Bacillus subtilis* in response to a panel of 14 diverse antibiotics (Freiberg et al., 2005). In addition, the authors extended their data sets with expression profiles from conditional mutants of four distinct essential genes coding for emerging antibacterial targets. As proof of principle, this reference compendium was used to pinpoint the molecular targets of two novel antibiotics and also identified a novel mode of action for moiramide B, based on inhibition of acetyl coenzyme A carboxylase.

In addition to mechanistic studies, the development of microarray-based technology has provided the prospect to gain a genome-wide understanding of changes within the organism's transcriptome in response to small molecules. In fact, chemical genomic studies using transcriptional profiling have revealed novel insights into a number of different cellular processes following treatment with chemical compounds, such as anticancer agents (Wu et al., 2004), metabolic analogs (Yu et al., 2008), and DNA-damaging agents (Birrell et al., 2002; Lee et al., 2005). An interesting study using the latter, aimed at further expanding our knowledge of DNA damage, made use of the collection of barcoded yeast deletion strains competitively grown in the presence of various DNA-damaging agents (Lee et al., 2005). This study shed light on the cell's complex response to DNA damage, uncovering 34 previously uncharacterized genes involved in DNA repair, as well as novel epistatic interactions between genes implicated in the defense mechanisms against DNA-damaging agents. This global analysis further revealed the genetic requirements important for resistance to the various compounds, overall advancing our understanding of the DNA-damage response. Indeed, powerful microarray-based chemical-genomic technologies in *S. cerevisiae* are well established, due to its robustness and ease of genetic modification, allowing a thorough functional characterization of the genome (Giaever et al., 1999; Hughes et al., 2000; Lum et al., 2004; Parsons et al., 2004).

Similarly, microarray-based studies of gene expression following treatment with small molecules have proven useful strategies for mode of action predictions and biological studies in prokaryotic organisms. A successful example was provided by the recent work of Mendez-Ortiz et al. who were interested

in the global response of *E. coli* to the biologically relevant small molecule, 3′,5′-cyclic diguanylic acid (c-di-GMP), an important second messenger that controls motility and adhesion in bacteria (Mendez-Ortiz et al., 2006). Expression profiles of *E. coli* in the presence of high levels of c-di-GMP, revealed its effects on the transcriptional regulation of certain genes in the cell, including several cell surface and membrane-bound proteins. Expectedly, genes involved in motility and cell division were altered, while elevated levels of c-di-GMP interestingly triggered the transcription of 50 genes of unknown function, highlighting the complex regulatory mechanisms where c-di-GMP takes part in bacterial metabolism.

Early genome-wide transcriptional profiling studies in *Staphylococcus aureus* increased our understanding of cell wall active antibiotics through the uncovering of a cell wall stress stimulon (Utaida et al., 2003). Identification of genes in the presence of oxacillin, D-cycloserine, or bacitracin, three antibiotics that inhibit different steps in peptidoglycan synthesis, all lead to the upregulation of a large number of common genes, including ones that code for proteins involved in cell wall metabolism and stress responses. This study provides insights into the molecular events that take place following inhibition of peptidoglycan synthesis in bacteria and deepens our understanding of the inhibitory mode of action of some of the most common antibiotics. Such studies foreshadow the future evaluation and comparison of the transcriptional responses to these antibiotics in strains resistant to these compounds. Similarly, Kaldalu et al. used transcriptional profiling in *E. coli* to show that, even with the most mechanistically unrelated antibiotics, a subset of genes is commonly affected in their transcriptional response, offering fundamental insight into the basis of antibacterial activity of antibiotics and mechanisms of bacterial death (Kaldalu et al., 2004).

Promoter-Reporter Construct Libraries

Bacterial promoter-reporter construct libraries (Table 1) have also found utility in chemical genomic studies in assessing transcription patterns on a global level in response to small molecules (Fischer et al., 2004; Goh et al., 2002; Yim et al., 2006) (Figure 2D). In contrast to microarray technology, these libraries provide high resolution, data-rich time courses of promoter response to bioactive small molecules. These advantages facilitated the investigation of the action of antibiotics in *Salmonella typhimurium* at subinhibitory concentrations (Davies et al., 2006; Goh et al., 2002; Yim et al., 2006). Here, significant transcriptional activation of various promoters revealed that antibiotics can have multiple effects on the cell by acting as chemical signals to control bacterial metabolic processes, suggesting a new role beyond therapeutic utility.

In 2006, Zaslaver et al. reported on the creation of a transcriptional fusion promoter library in *E. coli*, where ~2000 promoters were fused to *gfp* (Zaslaver et al., 2006). Using this library, Bollenbach et al. looked to shed light on the mechanism of suppressive drug interactions between DNA and protein synthesis inhibitors, whereby the combination of the two allows the cells to grow faster (Bollenbach et al., 2009). Examination of the expression profiles of ~200 *E. coli* promoters in response to different antibiotics revealed that ribosomal levels are not optimally regulated under conditions of DNA stress, causing an imbalance between DNA and protein levels. Reducing protein levels with protein synthesis inhibitors restores this imbalance allowing cells to grow faster; this same trend is observed when mutations in the ribosomal RNA operons impinge on ribosome synthesis (Bollenbach et al., 2009). Overall, promoter-reporter construct libraries provide a unique look at expression dynamics in cells and can reveal fascinating roles for even the most well-established antibiotics.

Chemical Proteomics

Proteomic studies have long played an integral part in assessing protein structure, function, and cellular interactions. Incorporating chemical strategies has provided efficient detection of select classes of proteins, as well as provided an effective means for subsequent isolation from the proteome. The realm of increasingly diverse chemical moieties needed for chemical proteomics has positioned synthetic chemistry as a crucial partner for the success of this field. Overall, the study of chemical proteomics to describe protein function, or uncover cellular targets of compounds, employs different methods, depending upon the nature of the study and the type of chemical probe used.

Activity-based probe profiling (ABPP) involves covalently modifying the active site of an enzyme with a chemical probe to allow for its isolation (Cravatt et al., 2008; Jeffery and Bogyo, 2003; Verhelst and Bogyo, 2005). This type of approach can yield information on enzymatic activities (such as function, mechanism, or active site properties) (Artavanis-Tsakonas et al., 2006; Barglow and Cravatt, 2007; Greenbaum et al., 2002; Hekmat et al., 2005; Misaghi et al., 2006; Staub and Sieber, 2008), groups of functionally related enzymes (Dalhoff et al., 2009; Misaghi et al., 2006), and has been employed as a tool to understand the virulence machinery of infectious bacterial and parasitic species (Bottcher and Sieber, 2008b; Greenbaum et al., 2002; Hang et al., 2006; Puri and Bogyo, 2009). As well, a competitive variation of ABPP can identify highly potent and selective inhibitors, which outcompete the chemical probe (Bottcher and Sieber, 2009; Leung et al., 2003). One interesting application of ABPP used various β-lactam modified chemical probes to profile the β-lactamase class of enzymes in both sensitive and resistant *S. aureus* strains (Staub and Sieber, 2009). Several unique enzyme activities were detected in the resistant strain including two novel enzymes, potentially implicated in resistance in methicillin-resistant *S. aureus* (MRSA). Chemical proteomics provides the advantage of studying enzymatic properties in the context of the proteome (not requiring recombinant protein); however, it is limited by the ability to selectively target the active site of a certain family of enzymes for covalent modification. As of yet, selective ABPP probes are only available for a few classes of enzymes and to increase the utility of this approach, new chemical probes for other enzymatic families must be created.

In contrast to ABPP, compound-centric chemical proteomics (CCCP) is largely about target discovery. Here mechanism of action of a bioactive compound is inferred through the identification of interacting cellular components, most often by affinity chromatography and advanced mass spectrometry techniques (Rix and Superti-Furga, 2009). CCCP has successfully identified cellular targets for β-lactones (Bottcher and Sieber, 2008a; Staub and Sieber, 2008), β-lactams (Staub and Sieber, 2008), anticancer agents (Bantscheff et al., 2007; Rix et al., 2007), and a variety of natural products (Piggott and Karuso, 2004;

Rix and Superti-Furga, 2009). Here, target identification deals with proteins in their natural environment, as they are expressed at physiological levels in the presence of binding partners and any posttranslational modifications. However, CCCP is only applicable to small molecules which can be chemically modified for immobilization and does not distinguish specific versus nonspecific interactions. Like other genomic approaches, tackling the proteome with small molecules as modulators is an emerging technology with much promise to characterize enzyme function and understand compound mode of action.

Computationally-Assisted Chemical Genomics

Significant advances in computational power have made it possible to streamline processes in the discovery of chemical probes of biology and leads for drugs. A variety of databases exist, from those that detail molecular structures (Klebe, 2000), generate virtual libraries of small molecules (Barone et al., 2001), and, most recently, provide gene expression profiles (Faith et al., 2008). Additionally, computer programs and algorithms are increasingly being developed to guide major steps in chemical genomic studies. Genome-scale technologies are yielding an overwhelming amount of valuable biological data and, as such, computational analyses that apply the data to generate and interpret network models, are expanding. In particular, with the growing challenges in microarray data analysis come models with predictive power to assess the expression profile of a compound of interest and assign its potential targets (Bansal et al., 2006; di Bernardo et al., 2005; Hallen et al., 2006). Recently, gene network inference algorithms have been thoroughly reviewed in Bansal et al. (2007). Such powerful computational analysis methods generally rely on integrating genetic perturbation outputs, such as microarray experimental data, with designed algorithms aimed at elucidating compound targets and providing insights into biological networks. For example, Hallén et al. designed an algorithm (CutTree) for genome-wide expression sets to identify the primary affected genes of a chemical compound of interest (Hallen et al., 2006). These were filtered out from genes that were indirectly affected and finally relatively ranked according to their importance in the microarray experiments. As a proof of principle, CutTree was capable of identifying four of the five known primary targets of galactose in yeast.

Another approach, mode of action by network identification (MNI), distinguishes direct cellular targets of a bioactive compound from other indirect gene products, which simply result from changes in the activity of the primary target (di Bernardo et al., 2005). Specifically, MNI computes the likelihood that gene products and related pathways are the targets of a small molecule. By first reverse engineering a network model from a set of expression profiles, MNI then analyzes drug-induced expression profiles to identify the genes targeted by the compound. This method overcomes the limitation of generating whole-genome expression profiles that inherently do not distinguish directly targeted genes, from those that are indirectly regulated. Following validation of their method with a variety of compound treatments, di Bernardo et al. uncovered thioredoxin reductase as the target of PTSB, a previously uncharacterized inhibitor of yeast growth (di Bernardo et al., 2005).

Other computational methods include studies of small molecule modeling and virtual screening (Eckert and Bajorath, 2007). Recently, Shen et al. proposed a strategy for antimicrobial discovery that begins solely with computational methods to identify chemical matter for novel targets (Shen et al., 2010). Following genome-scale metabolic reconstruction in *E. coli* and computational identification of essential metabolic reactions, the enzymes of interest are docked against a library of small molecules. Prediction through virtual screening leads to the identification of a subset of inhibitors, which in turn are experimentally validated for enzymatic and bacterial growth inhibition. Largely, efforts in computationally assisted chemical genomics are increasingly contributing to the prediction and understanding of the biological system following chemical perturbation. In fact, advanced statistical and computational analyses following genome-scale studies are becoming inevitable, due to their inherent overwhelming and complex nature.

Conclusions

Microbial systems, characterized by redundant and complex functional pathways typify the modern view of the cell and, as such, modern technologies, used to perturb, explore, and even reconstruct these systems, are increasingly touted for studying biological systems.

Chemical genomic approaches provide a platform that contends with the complexity inherent in this postgenomic view of cellular biology. Accordingly, our repertoire of chemical matter is expanding, providing new means to globally study biology, largely attributed to technological advances in synthetic chemistry and natural product research. The creation of genome-wide clone sets has been a technical feat that has enabled remarkable advances in chemical genomics in model microbes. The systematic modulation of gene dosage on a genome scale has been among the most important technological developments. Additionally, microarray- and promoter-based transcriptional profiling experiments have made it possible to thoroughly understand the cell's immediate and dynamic response to a perturbant on a genome-wide level. Studies of the proteome using small molecules of interest rely on direct physical interactions to infer compound mode of action and enzymatic activities. Overall, chemical genomic studies yield information-rich data sets, often requiring additional means of analysis. Hence, booming advances in computational power are becoming a vital necessity. A plethora of innovative algorithms make it possible to generate a wide array of outputs, from network connectivity maps to simple clustograms. It is important to note that a major caveat to all the aforementioned methodologies is the abundant detection of indirect interactions. Thus, more than ever, experimental validation is becoming a critical step in the pipeline for the discovery of biological probes. Accordingly, the field is increasingly dependent on large collaborative efforts whereby biology, chemistry, biochemistry, and computational sciences are at interplay. Overall, a better understanding can be obtained by integrating multiple genome-scale techniques to piece together the complete extent of chemical-genetic interactions. The assembly and chemical-genomic characterization of a repertoire of diverse and novel bioactives will provide an exceptional launching point for future studies pursuing the activity of small molecule probes within the cellular

network. Along with providing new and extraordinary information about the genetic networks of the model organism under study, the field of chemical genomics remains largely untapped as a paradigm for antibacterial drug discovery. Modern days in chemical genomics are embracing a new emerging theme in understanding biology, one that envisions the cell not as individual components, but as a dynamic unity of complex and robust pathways; a perspective that will largely benefit the study of fundamental biological processes, not to mention drug discovery.

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