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Targeting the Undruggable Proteome: The Small Molecules of My Dreams

Craig M. Crews[1,2,3,*]
[1]Department of Molecular, Cellular, and Developmental Biology
[2]Department of Chemistry
[3]Department of Pharmacology
Yale University, New Haven, CT 06511, USA
*Correspondence: craig.crews@yale.edu
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Biologically active small molecules have long proven useful in the exploration of cell biology. Although many early compounds were by-products of drug development efforts, recent increased small molecule screening efforts in academia have expanded the repertoire of biological processes investigated to include areas of biology that are not of immediate pharmaceutical interest. Many of these new bioassays score for small molecule–induced phenotypic changes at the cellular or even organismal level and thus have been described as "chemical genetic" screens. However, this analogy with traditional genetic screens is misleading; although each gene has roughly an equivalent chance of being mutated in a traditional genetic screen, the amount of "proteomic space" that a chemical genetics approach can reach using current small molecule libraries is considerably smaller. Thus, new chemical biology methodologies are needed to target the remaining "undruggable proteome" with small druglike molecules.

Magic Bullets

Over the last 40 years, the development of new antiviral, antitumor, antibiotic, and central nervous system–targeted drugs has had an immense impact on life expectancy and quality of life (Munos, 2009). In addition to these direct benefits, drug development over the past four decades has indirectly benefited the basic research community by generating new small molecule probes for basic biological studies. As detailed in the other reviews in this special issue of *Chemistry & Biology*, these small molecules have had a profound impact on many basic biological investigations and are among the impetuses for the burgeoning field of chemical biology. For example, although the natural product phosphoinositide 3–kinase (PI3K) inhibitor wortmannin has played a key role in identifying contributions for PI3K in biological processes as diverse as cell survival, histamine release, glucose uptake, and phagocytosis (Lennartz, 1999; Nakanishi et al., 1995; Ui et al., 1995), this natural product lacks PI3K isoform specificity. Fortunately, recent medicinal chemistry efforts have yielded more isoform-selective inhibitors that are helping to define PI3K function in specific cellular contexts (Siragusa et al., 2010; Soond et al., 2010; Sturgeon et al., 2008; Wang et al., 2008a). Other examples of useful biological probes that resulted from drug development efforts include inhibitors for the serine/threonine kinases MEK, JNK, and GSK-3β (Katsanakis et al., 2002; Saporito et al., 2002; Takahashi-Yanaga and Sasaguri, 2009; Wang et al., 2004).

Despite these many successes, there still are many instances where the right small molecule probe is lacking. As a chemical biologist who approaches the chemistry-biology interface from the biological side, there have been several occasions when I have wished for a "magic bullet" to allow for specific regulation of a biological process of interest. Here, I discuss the impact of academic screening efforts on new probe development, the shortcomings of "chemical genetic" approaches, and the currently unfulfilled need for novel libraries of small molecules capable of controlling intracellular protein function independently of protein class.

Developing Small Molecule Biological Probes: Design versus Serendipity

The availability and diversity of new bioactive probes for research has exploded in the past two decades, primarily because of the increase in academic small molecule screening facilities (Wu and Schultz, 2009). Previously, biologically active compounds were either direct by-products of the pharmaceutical industry's efforts to develop novel drugs or an indirect consequence of these efforts (e.g., natural product screens for drug target inhibitors). Thus, not surprisingly, these early small molecule probes had biological properties (e.g., anticancer, antiinflammatory, and antiangiogenic) that were of interest to the pharmaceutical industry. Despite their pharmaceutical origins, many of these compounds have proven extremely useful as probes in basic research, such as the immunosuppressive natural products FK506 and rapamycin that were instrumental in the exploration of immune cell signaling pathways (Cardenas et al., 1998). In addition, the use of these probes in basic research studies has generated new leads for novel drug targets, thus generating renewed interest in their therapeutic potential. For example, the identification of methionine aminopeptidase–2 (METAP2) as the target of the antiangiogenic microbial metabolite fumagillin has led to the development of novel antiangiogenic METAP2 inhibitors (Kallander et al., 2005; Marino et al., 2007; Sin et al., 1997; Wang et al., 2008b). These early probes, however useful, failed to span the breadth of cell biology, thus leaving many areas that lacked small molecule-based research tools.

Although small molecule–powered cell biology research proved very successful, such as in the use of novel histone

deacetylase inhibitors to explore the role of chromatin structure in gene regulation (Yoshida et al., 2001), most of the early chemical biology probes were limited to those research areas of interest to the pharmaceutical industry. However, once academic laboratories began to acquire small molecule screening capabilities, new compounds could be identified possessing biological activities unrelated to drug development. This second generation of small molecule probe development relies more on targeted screens looking for compounds with highly specific biological activities, as opposed to more general phenotypes such as decreased proliferation (Kawasumi and Nghiem, 2007; Schlueter and Peterson, 2009). Moreover, it has allowed for increased input from the basic biology research community to custom design and select compounds with particular biological characteristics. For example, a high-content cell-based assay for perturbation of mitotic spindle formation yielded monastrol, an inhibitor of the kinesin KIF11 (Mayer et al., 1999). Subsequent studies using monastrol demonstrated the importance of KIF11 in normal spindle body formation (Kapoor et al., 2000; Kapoor and Mitchison, 2001). Blebbistatin is another small molecule probe that resulted from a basic biology-driven screen. Identified as a nonmuscle myosin II inhibitor, blebbistatin has been critical in the investigation of cleavage furrow formation during mitosis and cytokinetic contractile ring assembly (Straight et al., 2003). Likewise, new assays have been developed to screen for inhibitors of the Wnt/β-catenin pathway, which is a major developmental biology signaling pathway. In a creative fusion of a small molecule and RNA interference (RNAi) screening, the Moon laboratory recently identified Bruton's tyrosine kinase (BTK) as a Wnt/β-catenin pathway inhibitor (James et al., 2009). These targeted proactive approaches to small molecule probe identification contrast with the more serendipitous nature of how traditional chemical biology probes were discovered and continues to have a major impact on biology through the identification of useful research reagents.

Chemical Genetics: An Unrealized Dream

This growth in novel bioassays has generated many new research tools, as well as excitement about the potential for small molecule–based biological discovery, in general. Indeed, the use of a combination of new bioassay development and compound library screening to identify novel bioactive molecules has become commonplace on campuses today (Sachinidis et al., 2008; Soderholm et al., 2006; Specht and Shokat, 2002; Wheeler and Brandli, 2009). Many of these new screens are phenotype-based—that is, assays that screen for small molecule–induced changes in a cellular context or even in whole organisms such as zebrafish, *Drosophila*, or nematodes. Because these screens score for a change in phenotype without regard a priori to a given target protein, this approach has been compared to a traditional "forward" genetic screen, leading to the sobriquet "chemical genetics" to describe these small molecule screens. However semantically appealing this analogy with traditional genetic screening may be, it is grossly misleading; although each gene has an equivalent chance of being mutated in a traditional genetic screen (ignoring mutagenic hotspots for the sake of argument), the amount of "proteomic space" that a chemical genetics approach can reach using small molecule perturbagens is considerably smaller. Put another way, the oft-stated goal of "a small molecule inhibitor for every protein" has yet to be realized.

Today's Challenge: Targeting the Undruggable Proteome

Both academic and pharmaceutical screening efforts have been inherently limited in the types of proteins that are targeted using small molecules—that is, the segment of proteome that is characterized by the presence of well-defined small molecule binding pockets, such as ion channels, nuclear receptors, GPCRs, or enzymes (Overington et al., 2006). Collectively, these protein families are but a fraction of the entire proteome and, thus, this exclusive focus leaves as "undruggable" many other types of proteins that cannot be controlled using small molecules, such as transcription factors, nonenzymatic proteins, regulatory or scaffolding proteins, and so forth. (Arakaki et al., 2006; Verdine and Walensky, 2007). The challenge, therefore, is how can one develop a methodology that targets this undruggable proteome? Is it possible to make every protein equally susceptible to small molecule control? I argue that a true chemical genetic screen will require a small molecule library that targets both traditional drug targets as well as the 80% of the proteome lacking a catalytic site or a small molecule binding site that controls protein function when occupied.

Wanted: Controlling Protein Function Irrespective of Protein Class

Given the incomplete coverage of the proteome by current compound libraries, new methods are needed to control protein function using small molecules. One possible solution is to use the cell's own quality control mechanisms to induce the degradation of targeted proteins and thus modulate intracellular protein concentrations. For example, a recent report described the use of heat shock cognate protein HSC70 peptide-binding motifs to recruit proteins to the lysosome for degradation (Figure 1A) (Bauer et al., 2010). By harnessing HSC70, a chaperonin protein responsible for either the refolding or targeted degradation of misfolded proteins, this approach selectively induced the degradation of mutant huntingtin, the protein responsible for Huntington's disease and, moreover, ameliorated disease in an animal model.

A similar approach to use cellular protein degradation machinery to control intracellular protein levels was developed in my laboratory, in collaboration with Ray Deshaies (CalTech). Although the approach described above recruits targeted proteins to the lysosome for degradation, *Pro*teolysis *T*argeting *C*himeras (PROTACs) recruit targeted proteins to E3 ubiquitin ligases (Rodriguez-Gonzalez et al., 2008; Schneekloth and Crews, 2005) as a first step in their induced degradation. E3 ubiquitin ligases, together with E2-conjugating enzymes, are responsible for coupling the 76 amino acid tag ubiquitin to lysine ε-amino groups on the surface of proteins, thus targeting them for degradation by the major intracellular proteolytic complex, the 26S proteasome. As heterobifunctional compounds composed of a target protein-binding ligand and an E3 ubiquitin ligase ligand (Figure 1), PROTACs induce proteasome-mediated degradation of selected proteins via their recruitment to E3 ubiquitin ligase and subsequent

ubiquitination (Bargagna-Mohan et al., 2005). In several proof-of-concept studies, this method has been shown to induce intracellular protein degradation with greater temporal and dosage control than that offered by RNAi-mediated gene knockdown (Puppala et al., 2008; Sakamoto et al., 2003; Schneekloth et al., 2004). In addition, unlike the lysosomal targeting approach using peptidic HSC70 binding motifs, it is possible to target proteins for degradation using a nonpeptidic, all-small-molecule–based PROTAC (Itoh et al., 2010; Schneekloth et al., 2008).

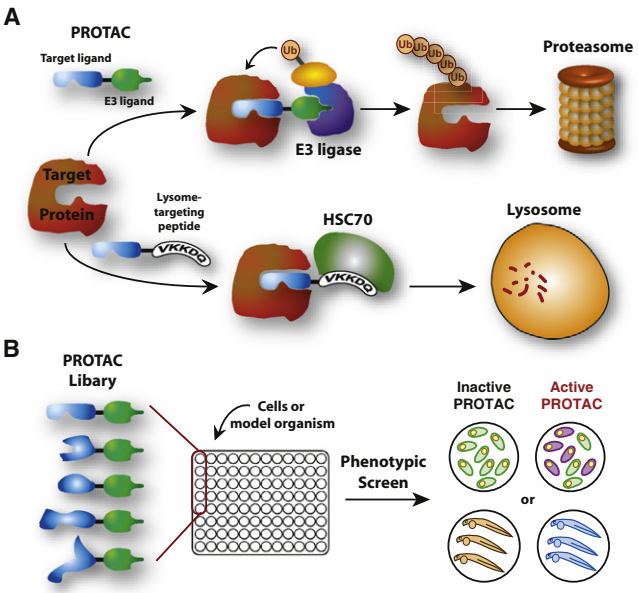

Figure 1. Strategies for Using Cellular Protein Degradation Machinery to Control Intracellular Protein Levels

(A) Schematic of inducing protein degradation via recruitment to the proteasome or lysosome.

(B) Design of a PROTAC-based library targeting protein function independently of protein class.

Affinity-Based HTS screens: An Unbiased Approach to Identify a Ligand for Every Protein

Whether targeting proteins for proteasomal- or lysosomal-mediated degradation, these approaches for controlling intracellular protein levels face the same major challenge, namely, the identification of ligands for proteins to be targeted for degradation. Fortunately, new advances in high-throughput affinity-based screening are helping to address this challenge (Zhu and Cuozzo, 2009). Unlike common functional assay–based HTS, which identifies compounds according to their ability to elicit a biological consequence upon binding, affinity-based HTS instead focuses only on identifying compounds that bind their protein targets, irrespective of their protein class. Although several low- and medium-throughput affinity strategies are available (e.g., calorimetry-based, surface plasmon resonance–based, NMR/X-ray structure-based, mass spectrometry-based, small molecule microarrays), newer methods offer the ability to screen readily hundreds of thousands to millions of compounds (Zhu and Cuozzo, 2009). For example, several related DNA-tagged small molecule libraries (Gartner et al., 2004; Melkko et al., 2007) have been used to identify protein ligands from libraries as large as 10^8 compounds (Clark et al., 2009). As these technologies continue to mature, low- and medium-throughput affinity-based approaches should become more amenable to HTS. Hence, functionally unbiased affinity-based HTS screens hold the promise of identifying a ligand to each protein in the proteome. Although ligands identified for each protein by these methods may not have an inherent biological activity, when they are coupled to an approach such as PROTACs, they will ultimately allow for the elusive all-encompassing chemical genetic screen.

The Molecules of My Dreams: A Truly Comprehensive Small Molecule Library

In theory, coupling protein ligands identified in affinity-based screens to an HSC70 ligand or an E3 ligase ligand (e.g., the MDM2-binding compound nutlin) would generate compounds capable of targeting any desired protein for intracellular degradation (Schneekloth et al., 2008). Although all of the technology is in place to generate a degradation-inducing compound for every protein, I question whether this "reverse" genetic approach is the best. Instead of starting with individual proteins and identifying novel targeting ligands to them for incorporation into such molecules, why not take a forward genetic approach to score a cellular phenotype on the basis of loss of protein function? A library of PROTACs could be generated in which all compounds possess the same E3 ubiquitin ligase ligand, but each is coupled to a different chemical diversity element (Figure 1B). Such a naive PROTAC library of sufficient size might be capable of binding to (and inducing the degradation of) every protein within the proteome irrespective of protein function or class. However, there are several limitations to this strategy. First, some proteins are naturally unstable and, thus, would be difficult to control via a PROTAC. Likewise, it may be difficult to find a small molecule ligand capable of binding to a target protein with the requisite affinity and specificity for use in a PROTAC. Despite these potential limitations, the generation of a PROTAC-based compound collection would represent the first step toward a comprehensive small molecule library that could be used to perform proteomewide chemical genetic screens for induction or modulation of a given cellular phenotype.

The development of new research strategies has permitted small molecule bioassays and screens to evolve significantly in the past two decades. This has enabled researchers to investigate more basic biological phenomena beyond those areas mandated by clinical needs. Nevertheless, much remains to be done before the dream of a small molecule perturbagen for every protein is realized. In my opinion, the largest unmet need in bioprobe development today is the ability to modulate protein function independently of protein class. Although several current technologies offer possible solutions to this challenge, chemical genetics will not truly be on par with traditional genetics until this challenge is overcome.

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