Published in final edited form as:

Chembiochem. 2005 January ; 6(1): 40–46. doi:10.1002/cbic.200400274.

Chemical Approaches to Controlling Intracellular Protein Degradation

John S. Schneekloth Jr.^a and Craig M. Crews^{*,a}

*a*Departments of Molecular, Cellular, and Developmental Biology, Chemistry, and Pharmacology, Yale University, New Haven, CT 06520 (USA), Fax: (+1) 20-432-6161

Keywords

bioorganic chemistry; chemical genetics; protein modifications; RNA; signal transduction

Introduction

A major goal of post-genomic research is to understand and control the function of specific proteins within the proteome. The fields of genetics, genomics, and chemical genetics attempt to study this problem from two fundamentally different approaches. The traditional genetic approach or genomic analysis involves the observation of a particular phenotype, identification of the molecule(s) responsible for that phenotype, and subsequent analysis and manipulation of the corresponding genetic sequence. In contrast, chemical genetics attempts to use small molecules as probes to perturb signaling pathways at the molecular level in the hopes of identifying novel proteins responsible for a particular phenotype. These two distinct approaches (among others) have yielded huge advances in the understanding of protein function. Despite their differences, these fields share the same strategy of inactivation of a protein to study its function.

Regulation of protein expression can be described as occurring on three basic levels. First, at the genetic level, the strength of a promoter determines the level of a particular gene product. Second, at the post-transcriptional level, stability of mRNA levels lead to increased production of a protein. Finally, at the post-translational level, modifications such as gly-cosylation, phosphorylation, or degradation significantly affect both intracellular levels and the activity of a protein. The most effective way to study the function of a protein has traditionally been to observe the phenotypic change in its absence. Therefore, methods at each of these three levels have been developed to disrupt protein expression. Many ways to control protein function are known, including inducible transcription[1] and methods that affect post-translational modifications[2–4] or inhibit degradation.[5] Although these methods are effective, they are fundamentally different from strategies that lower protein levels post-translationally. As genetic knockout methodology is well understood, this review focuses on recent advances in protein inactivation at the post-translational level, specifically comparing novel chemical and biochemical methods to the post-transcriptional method of inactivation, RNA interference (RNAi).

Post-transcriptional Inactivation (RNAi)

RNAi, also known as post-transcriptional gene silencing, has become a widely used method to inactivate a gene of interest. The phenomenon of RNAi was first observed when it was found that only a few molecules of double-stranded RNA (dsRNA) could largely suppress specific gene expression, as first seen in *C. elegans*, [6] then later in plants and mammalian cells. [7–13] Believed to be a conserved evolutionary method of gene silencing, the technique has garnered widespread interest in the biological community due to its myriad applications. [7–13] (For reviews, see refs. [14–17].)

As it has been studied extensively, the mechanism of RNAi is relatively well understood (Scheme 1). RNAi is initiated when dsRNA is recognized by the Dicer enzyme, an RNase III-like enzyme that processes the dsRNA into duplex short interfering RNA (siRNA) of approximately 22 nucleotides. [18] The siRNA is then separated into single strands and bound by the RNA-induced silencing complex (RISC). [19] RISCs activated with the antisense siRNA strand can bind to complementary mRNA from the gene of interest and cleave it, thus silencing its expression. After cleavage, the antisense RNA:RISC complex is then free to degrade other copies of the mRNA. RNAi is therefore catalytic, and only a small number of molecules are necessary to knock down a gene.

A number of advantages of RNAi stem from its specificity and potency. In practice, programming a RISC with the appropriate antisense mRNA can silence almost any gene of interest. The effects of RNAi spread throughout an organism to parts that were not originally treated; this limits the amount of dsRNA that is necessary. RNAi has the advantage that it is relatively easy to perform as compared to genetic knockout studies, and often results in an identical phenotype. RNAi has been shown to be effective in many in vitro assays and in vivo animal studies, and is currently being evaluated for its clinical efficacy in human disease. Through impressive amounts of research, it has become clear that RNAi is an extraordinarily powerful tool for cell biology, and has potential for use in the clinic.

While these advantages have made RNAi a boon to cell biologists, it falls short in several areas. First, RNAi does not necessarily result in a complete knockout of the gene of interest as a genetic knockout would. Second, highly stable proteins that have already been synthesized are not effectively silenced by this technique—RNAi only prevents further synthesis of a protein, not destruction of existing copies. Third, RNAi does not allow for fine temporal control over protein expression levels. Once introduced into a cell, the RNAi constitutively depresses levels of the target mRNA. Fourth, it is often necessary to test a number of target templates for any particular RNAi experiment. Because it is not completely understood how RISC associates with siRNA and recognizes mRNA, not all the sequences chosen are effective in silencing a gene. In addition to these biochemical disadvantages, a number of “off-target” phenotypic effects of RNAi have also been discovered. Among these effects are the nonspecific activation and suppression of a number of genes, including the interferon pathway. These shortcomings indicate that other methods of protein inactivation could prove useful in areas where RNAi is not applicable.

Post-translational Inactivation

A number of techniques have been developed to disrupt proteins in vivo at the post-translational level. These other techniques complement RNAi, as they can potentially be used to study proteins that are inaccessible by RNAi analysis. Post-translational approaches to protein inactivation, in contrast to RNAi, destroy a protein after it has been synthesized. These approaches fundamentally differ from RNAi by destroying existing copies of the protein of interest, rather than precluding new protein synthesis. For example, proteins with a long half-life may not necessarily be vulnerable to RNAi, because preventing new synthesis of a protein

would not affect the function of existing copies already present within the cell. On the other hand, such proteins could be studied by means of post-translational degradation (knock down).

Most reported post-translational approaches to protein inactivation utilize the cell's own regulated degradation pathway, the ubiquitin-proteasome pathway. The ubiquitin-proteasome pathway (UPP) is the main pathway for ATP-dependent protein degradation within the cell. [20] A cascade of enzymes results in the covalent attachment of ubiquitin, a 76-amino-acid polypeptide, to the amine functionality of lysine residues on a target protein. Following attachment of the initial ubiquitin, additional ubiquitins are added to lysine residues of the ubiquitin molecule itself resulting in a multiubiquitinated target protein. A protein that is labeled with at least four ubiquitin molecules is recognized by the 26S proteasome, unfolded, and threaded into the proteolytic chamber of the proteasome where it is proteolyzed. As the central role of the UPP is the controlled degradation of intracellular proteins, a number of groups have attempted to use this pathway to induce the degradation of normally stable proteins. Approaches to use the UPP to induce selective protein degradation include use of fusion proteins to artificially ubiquitinate target proteins as well as synthetic small-molecule probes to induce proteasome-dependent degradation.

Biochemical Approaches to Protein Degradation

One of the first attempts to induce selected protein degradation *in vivo* took advantage of chimeric proteins that were capable of inducing the degradation of protein targets that are normally stable *in vivo*. [21–23] This approach entailed the use of an F box protein engineered to contain a binding domain for the target protein. F-box-domain-containing proteins are known to exist as complexes with E3 ubiquitin ligases. Thus, once expressed in the cell, the chimeric F Box protein would recruit the target protein to the E3 ligase complex, ultimately leading to ubiquitination and degradation of the target (Scheme 2).

Initially, the system chosen to study chimeric-protein-induced degradation involved the retinoblastoma protein (RB). RB is a protein that is crucial to cell-cycle regulation and known to have a long half-life; these make it an excellent system to study for degradation. In order to target RB for degradation by a chimeric-protein approach, an appropriate RB-binding domain needed to be selected. It is known that the 35 N-terminal residues of the E7 protein encoded by human papillomavirus type 16 (E7N) bind selectively to RB. [24–26] E7N was therefore chosen as the binding domain for RB in the chimeric protein. The E3 ligase SCF is actually a multicomponent complex comprised of Skp1, cullin, and F-box proteins. [27] It was hypothesized that E7N could be fused to the F box/WD40 repeat of a known component of SCF, resulting in a heterobifunctional chimeric protein. When expressed, the E7N domain of the chimeric F-box protein would complex with RB. This complex would mimic a wild-type protein associating with the E3 ligase and so result in the ubiquitination and degradation of RB.

Both in yeast and mammalian cells, the constitutively expressed construct successfully degraded RB *in vivo*. [21–23] This effect was measured by direct observation of a decrease in the level of RB in human osteosarcoma SAOS-2 cells. Additionally, SAOS-2 cells cotransfected with RB and the chimeric F-box protein were observed to enter the cell cycle, while growth arrest was observed in cells transfected only with RB. It was clear that RB degradation induced by the chimeric protein effectively inhibits RB function. These results confirm that chimeric F-box proteins can effectively be used to induce the degradation of a normally stable target protein.

Since the binding domain of the engineered F-box protein could, in principle, be varied, stably expressed chimeric proteins could be useful as a method to degrade other proteins of interest. While this method has potential, it involves significant biochemical manipulation of the cells

of interest. Additionally, the chimeric proteins need to be constitutively expressed to observe degradation, thus eliminating the possibility of fine temporal control over protein expression. Although proven useful in model studies, this method may not necessarily be as useful with poorly understood protein systems or animal studies, since disruption of endogenous SCF complexes could also lead to undesired toxic effects.

Chemical-Genetics Approaches to Protein Degradation

Chemical-genetics approaches to protein degradation to date have used small molecules as biological probes to induce protein ubiquitination and degradation. These small-molecule probes are designed in a way similar to chemical inducers of dimerization[28,29] and consist of a ligand for the target protein connected to another ligand via a linker. Chemical probes can then induce complexation between two proteins to result in a desired biological effect. These probes are prepared by using traditional synthetic methods and used by means of addition to cells as drugs.

The use of small molecules serves as an alternative to biochemical strategies and has several advantages over other approaches. Although production of these probes requires synthetic preparation, minimal biochemical manipulation of the cells is necessary. This decreases the chance that an undesired effect could result from the introduction of a fusion protein. Additionally, drug-like molecules that induce protein degradation offer the possibility of temporal control over protein expression, while RNAi and biochemical methods only allow for the constitutive depression of protein levels. Such control would be an asset to the study of proteins, for example within a particular phase of the cell cycle or during certain stages of early embryonic development. A drug-like small molecule capable of inducing the inactivation of a protein of interest upon addition to cells or administration to an animal could be very useful as a biochemical reagent. In addition, such molecules could potentially lead to a new paradigm for the treatment of diseases by removing pathogenic or oncogenic proteins. As such, a number of approaches to using small molecules to induce the degradation of a targeted protein in vitro and in vivo have been reported.

Geldanamycin Derivatives

Geldanamycin (GM), an ansamycin natural product, was originally identified as an antiproliferative agent[30] inhibiting cells transformed by the v-src oncogene.[31,32] Its structural complexity was of significant interest to the synthetic community, and, as such, the total synthesis of GM[33] and several closely related alkaloids[34–37] were reported. This synthetic work opened the possibility of synthesizing a number of analogues. Although GM was originally proposed to be a src-kinase inhibitor, it was eventually found to bind specifically to the heat shock protein, Hsp90.[38]

Hsp90 has a number of biological functions, mainly acting as a chaperone that assists in the refolding of damaged proteins in response to cellular stress. Inhibitors of Hsp90, including the ansamycin class of natural products, have been shown to exert their cytotoxicity by inducing the proteasome-dependent degradation of several Hsp90 substrates.[39–43] Among these substrates are members of the src kinase family and the HER (Human Epidermal growth factor Receptor) -related family kinases. It had been determined that simply inhibiting Hsp90 resulted in the nonspecific degradation of many Hsp90 substrates. With this in mind, it was hypothesized that an appropriately functionalized GM derivative could initiate specific degradation by associating with only one of the Hsp90 substrates.

In order to test this hypothesis, a number of derivatives were synthesized, including geldanamycin dimers[44] with linkers of varying lengths, bifunctional geldanamycin–estradiol [45] hybrids, and geldanamycin–testosterone hybrids.[46] Geldanamycin dimers were

predicted to specifically degrade HER-family kinases. Synthetic geldanamycin–estradiol hybrids were predicted to selectively degrade the estrogen receptor (ER) via the UPP, while geldanamycin–testosterone hybrids were similarly predicted to be selectively cytotoxic to androgen receptor (AR) dependent cells.

Despite a surprising dependence on the nature of the linker connecting the two molecules, [44] GM derivatives were largely successful in conferring specific degradation. A GM dimer comprised of two GM units and a 1,4-diaminobutane linker was found to be the most effective. This dimer displayed an IC_{50} for HER-2 degradation comparable to the natural product, while showing a tenfold decrease in degradation of Raf-1 (another Hsp90 substrate) and a fourfold decrease in activity in growth-inhibition assays in the breast-cancer cell line, MCF-7. Other derivatives were similarly active, while negative controls, such as a chemically deactivated analogue, effectively displayed no activity.

Geldanamycin R = OMe

Geldanamycin dimer

Geldanamycin--estradiol

Geldanamycin--testosterone

A GM–estradiol hybrid was similarly effective in selective degradation of ER.[45] Results indicated that, in MCF-7 cells, a GM–estradiol hybrid consisting of a *trans*-but-2-ene linker selectively induced the degradation of ER while leaving other Hsp90 substrates largely intact. Encouragingly, the GM–testosterone hybrid also behaved as expected. The compound displayed an IC_{50} value comparable to the natural product GM against the LAPC4 and LNCaP androgen-dependent cell lines, while having a tenfold increase in IC_{50} against non-androgen-dependent cells as compared to GM itself. Taken together, these data suggest that geldanamycin derivatives are quite effective in selectively inducing the degradation of certain specific Hsp90 substrates over other Hsp90 substrates.

These results were particularly encouraging because they represent an advance toward an improved therapeutic index for breast-cancer and prostate-cancer treatments. It has been well established that, in certain types of breast and prostate cancers, ER and AR, respectively, are overexpressed and that abrogation of ER/AR activity is a valid approach for the treatment of these diseases.[47] Drugs that selectively inactivate these proteins could be very useful and would represent novel approaches for the treatment of prostate and breast cancers.

Although the GM-hybrid results were promising, the approach still left room for improvement. Geldanamycin is a highly complex natural product requiring a large number of synthetic steps to produce. Even with efficient access to the geldanamycin core, the GM hybrids only allow for the degradation of specific, targeted HSP90 substrates and probably would not be effective in degrading proteins that are not normally degraded in an Hsp90-dependent fashion. Despite speculative evidence provided by the authors, details about the specific mechanism of action of these bifunctional molecules are elusive. Without a discrete mechanism of action, it is difficult to ascertain the true scope of this technology. However, the obvious successes in this area indicate that new attempts to specifically degrade proteins of interest are still warranted.

Proteolysis-Targeting Chimeric Molecules (PROTACS)

Our group has recently reported the design and synthesis of several molecules that directly induce the proteasome-mediated degradation of targeted proteins within cells.[48–50] Proteolysis-targeting chimeric molecules (PROTACS) function by forming a complex between the target protein and an E3 ubiquitin ligase. The heterobifunctional molecule is comprised of a recognition element for the target, a linker, and a recognition element for an E3 ligase. Upon treatment, the target protein can then be artificially induced to become polyubiquitinated, and subsequently degraded by the proteasome (Scheme 3). In contrast to the geldanamycin approach, these molecules directly induce the ubiquitination of the target protein, and are not dependent on a chaperone protein.

The PROTAC approach has been shown to be effective in a number of systems. Because it was unclear whether or not this approach would be effective in vivo, attempts were first made to induce selective protein degradation in vitro. Initially, methionine aminopeptidase (MetAP-2)[51] was chosen as a target. Having previously identified MetAP-2 as the cellular target of fumagillin,[52] an antiangiogenic natural product, we elected to use fumagillin as the recognition element for MetAP-2 (Scheme 4). The I κ B α phosphopeptide[53,54] was chosen as a recognition element for the E3 ubiquitin ligase SCF,[55] similar to the E3 ligase studied by Howley in his biochemical approach to protein degradation,[21–23] Upon treatment of *Xenopus* frog extracts with the fumagillin-based PROTAC, we were able to observe, by immunoblot analysis, the covalent attachment of the PROTAC to the target and the subsequent proteasome-mediated degradation of the protein–PROTAC adduct over a 30 minute time course.

Having shown that recruitment of a target protein to the proteasome could effectively induce its degradation in vitro, we next tested whether a PROTAC would be effective in vivo. Inhibition (and therefore degradation) of MetAP-2 was known to result in cytotoxicity, therefore a separate in vivo system was necessary. We consequently studied two other target–ligand pairs: dihydrotestosterone (DHT)–AR and estradiol–ER. As these were both well-understood systems with implications in prostate[47] and breast[56] cancers, respectively, we anticipated that they would be excellent models to study the action of a PROTAC molecule. DHT–I κ B α and estradiol–I κ B α PROTACS were synthesized,[49] and subsequent testing clearly indicated that upon microinjection into HEK 293 cells, the PROTAC molecules initiated the degradation of green fluorescent protein (GFP) fusions of the estrogen receptor and androgen receptor, respectively.

Although these results were encouraging, the PROTAC design containing a diphosphate was unlikely to be cell permeable, and therefore not broadly useful. In addition, recognition of the E3 ligase is dependent on the phosphorylation state of the I κ B α peptide, and phosphatases could therefore render the PROTAC inactive. With these concerns in mind, we next developed a PROTAC containing all the elements necessary for in vivo activity upon incubation with cells.

The new design included a polyarginine molecular transporter,[57] which mimics the HIV-Tat [58] and antennapedia[59] proteins to ensure membrane permeability. Additionally, a seven-residue polypeptide fragment of the hypoxia-inducible factor 1 α (HIF1 α)[60] was used as the E3 ubiquitin ligase-recognition element. Under normoxic conditions, proline 564[61] (the central proline in our sequence) of HIF1 α is oxidized by a proline hydroxylase. The E3 ligase complex β TrCP selectively binds the oxidized form of HIF1 α , and induces its degradation. [62,63] To incorporate these new design elements, we synthesized two new PROTAC molecules. Having successfully shown that a DHT-I κ B α PROTAC could degrade a GFP-AR fusion protein upon microinjection, we synthesized a DHT-HIF-based cell-permeable PROTAC.[48] Additionally, a target-ligand pair of AP21998-FKBP12 was chosen. FKBP12, an immunophilin originally identified as the binding protein for the natural product FK506, has been studied extensively as a drug target. As a result of these studies, a number of small-molecule ligands for FKBP12 have been developed. AP21998 has been developed as a small-molecule ligand that binds exclusively to a mutant form of FKBP12 with no appreciable binding to the wild-type protein.[64,65] The well-understood AP21998-mutant FKBP12 system provided an opportunity to study a protein that is orthogonal to all other proteins within the cell. Thus, degradation of the mutant FKBP should have no effect on wild-type protein function.

We were pleased to find that both the DHT- and AP21998-HIF1 α -based PROTACS showed significant degradation of GFP-AR and GFP-FKBP (mutant) simply upon addition to cells (Scheme 5). Importantly, fusion proteins were chosen as initial targets for ease of analysis, but the approach does not require any biochemical manipulation of the cells. Along these lines, similar molecules consisting of fumagillin- and estrogen-derived “small-molecule proteolysis inducers” have been reported to be capable of inducing the degradation of MetAP-2 and ER, respectively.[66] Interestingly, these compounds, nearly identical to the PROTAC molecules described above, were shown to be cell permeable without a polyarginine molecular transporter. These results represent an important advance, as we now have access to molecules that can “chemically knock out” a protein of interest, simply upon addition to cells.

While the PROTAC approach has proven to be successful, the potential for improvement remains. The presence of a peptide region of the molecule suggests that it would not be easy to produce on a large scale, neither would it necessarily be stable within the cell. The most desirable solution to this problem would be to invoke a small-molecule ligand for an E3 ubiquitin ligase, although currently there are few, if any, examples in the literature.

Issues of design improvement aside, small molecules that induce the degradation of a selected protein in vivo could have significant use in a chemical-genetic screen. A screen could be organized such that cells would be treated with a library of PROTAC molecules comprised of a common E3 ligase recognition domain (such as the HIF1 α peptide) with a chemical-diversity element introduced in the target-ligand region. This library could then be screened for activity in a cell-based assay, such as selective cytotoxicity to carcinomas or intracellular degradation of a fluorescently tagged target. Once a hit is obtained, it would be trivial to use the target ligand in an affinity column to identify the protein and/or pathway that is being disrupted by degradation. A chemical-genetic screen could potentially help identify new therapeutically vulnerable protein targets, a major challenge of post-genomic research.

Conclusion

A wide number of techniques have been explored to study protein function by means of inactivation. Approaches have ranged from more traditional genetic knockout studies to small molecules that specifically degrade a target protein upon treatment. Although genetic knockout and RNAi are more established techniques, novel chemical approaches potentially offer solutions to problems that other methods cannot overcome. Chemical approaches to degrade selectively proteins of interest validate a successfully degraded protein as pharmacologically vulnerable, while screens could yield novel protein targets for the study of signal transduction or the treatment of disease. It is important to note that post-translational methods of controlling protein levels complement other techniques such as RNAi. Early experiments have indicated that chemical techniques can be useful, but not much is known about the true potential for this approach. It is clear from these early efforts that more work is needed to determine the true scope and applicability of chemical approaches to protein degradation.

Acknowledgements

J.S.S. thanks the American Chemical Society, Division of Medicinal Chemistry, and Aventis Pharmaceuticals for a predoctoral fellowship. C.M.C. acknowledges the generous support of the National Institutes of Health (GM062120).

References

1. Harvey DM, Caskey CT. *Curr. Opin. Chem. Biol* 1998;2:512. [PubMed: 9736925]
2. Dwek MV, Brooks SA. *Curr. Cancer Drug Targets* 2004;4:425. [PubMed: 15320718]
3. Bishop AC, Buzko O, Shokat KM. *Trends Cell. Biol* 2001;11:167. [PubMed: 11306297]
4. Shokat K, Velleca M. *Drug Discovery Today* 2002;7:872. [PubMed: 12546954]
5. Adams J. *Curr. Opin. Oncol* 2002;14:628. [PubMed: 12409653]
6. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. *Nature* 1998;391:806. [PubMed: 9486653]
7. Jorgensen R. *Trends Biotechnol* 1990;8:340. [PubMed: 1366894]
8. Napoli C, Lemieux C, Jorgensen R. *Plant Cell* 1990;2:279. [PubMed: 12354959]
9. van der Krol AR, Mur LA, de Lange P, Mol JN, Stuitje AR. *Plant Mol. Biol* 1990;14:457. [PubMed: 2102827]
10. Pal-Bhadra M, Bhadra U, Birchler JA. *Cell* 1997;90:479. [PubMed: 9267028]
11. Romano N, Macino G. *Mol. Microbiol* 1992;6:3343. [PubMed: 1484489]
12. Fire A, Albertson D, Harrison SW, Moerman DG. *Development* 1991;113:503. [PubMed: 1782862]
13. Dernburg AF, Zalevsky J, Colaiacovo MP, Villeneuve AM. *Genes Dev* 2000;14:1578. [PubMed: 10887151]
14. Hannon GJ. *Nature* 2002;418:244. [PubMed: 12110901]
15. Eddy SR. *Nat. Rev. Genet* 2001;2:919. [PubMed: 11733745]
16. Lai EC. *Curr. Biol* 2003;13:R925. [PubMed: 14654021]
17. Grewal SI, Moazed D. *Science* 2003;301:798. [PubMed: 12907790]
18. Blaszczyk, J.; Tropea, JE.; Bubunencko, M.; Routzahn, KM.; Waugh, DS.; Court, DL.; Ji, X. *Structure*. 9. Cambridge, MA, USA: 2001. p. 1225
19. Hammond SM, Bernstein E, Beach D, Hannon GJ. *Nature* 2000;404:293. [PubMed: 10749213]
20. Crews CM. *Curr. Opin. Chem. Biol* 2003;7:534. [PubMed: 14580555]
21. Scheffner M, Munger K, Huibregtse JM, Howley PM. *EMBO J* 1992;11:2425. [PubMed: 1321031]
22. Zhou P, Bogacki R, McReynolds L, Howley PM. *Mol. Cell* 2000;6:751. [PubMed: 11030355]
23. Zhou P, Howley PM. *Mol. Cell* 1998;2:571. [PubMed: 9844630]
24. Dyson N, Howley PM, Munger K, Harlow E. *Science* 1989;243:934. [PubMed: 2537532]
25. Dyson N, Guida P, Munger K, Harlow E. *J. Virol* 1992;66:6893. [PubMed: 1331501]

26. Munger K, Werness BA, Dyson N, Phelps WC, Harlow E, Howley PM. EMBO J 1989;8:4099. [PubMed: 2556261]
27. Bai C, Sen P, Hofmann K, Ma L, Goebel M, Harper JW, Elledge SJ. Cell 1996;86:263. [PubMed: 8706131]
28. Spencer DM, Wandless TJ, Schreiber SL, Crabtree GR. Science 1993;262:1019. [PubMed: 7694365]
29. Belshaw P, Ho S, Crabtree G, Schreiber S. Proc. Natl. Acad. Sci. USA 1996;93:4604. [PubMed: 8643450]
30. DeBoer C, Meulman PA, Wnuk RJ, Peterson DH. J. Antibiot 1970;23:442. [PubMed: 5459626]
31. Sasaki K, Yasuda H, Onodera K. J. Antibiot 1979;32:849. [PubMed: 500504]
32. Miller P, DiOrio C, Moyer M, Schnur RC, Bruskin A, Cullen W, Moyer JD. Cancer Res 1994;54:2724. [PubMed: 7909494]
33. Andrus MB, Meredith EL, Simmons BL, Soma Sekhar BB, Hicken EJ. Org. Lett 2002;4:3549. [PubMed: 12323066]
34. Nakata M, Osumi T, Ueno A, Kimura T, Tamai T, Tatsuta K. Bull. Chem. Soc. Jpn 1992;65:2974.
35. Carter KD, Panek JS. Org. Lett 2004;6:55. [PubMed: 14703349]
36. Martin SF, Dodge JA, Burgess LE, Limberakis C, Hartmann M. Tetrahedron 1996;52:3229.
37. Panek JS, Xu F, Rondon AC. J. Am. Chem. Soc 1998;120:4113.
38. Whitesell L, Mimnaugh EG, De Costa B, Myers CE, Neckers LM. Proc. Natl. Acad. Sci. USA 1994;91:8324. [PubMed: 8078881]
39. Schneider C, Sepp-Lorenzino L, Nimmesgern E, Ouerfelli O, Danishefsky S, Rosen N, Hartl FU. Proc. Natl. Acad. Sci. USA 1996;93:14536. [PubMed: 8962087]
40. Stancato LF, Silverstein AM, Owens-Grillo JK, Chow YH, Jove R, Pratt WB. J. Biol. Chem 1997;272:4013. [PubMed: 9020108]
41. Wartmann M, Davis RJ. J. Biol. Chem 1994;269:6695. [PubMed: 8120027]
42. Pratt WB. Proc. Soc. Exp. Biol. Med 1998;217:420. [PubMed: 9521088]
43. Pratt WB, Toft DO. Endocr. Rev 1997;18:306. [PubMed: 9183567]
44. Zheng FF, Kuduk SD, Chiosis G, Munster PN, Sepp-Lorenzino L, Danishefsky SJ, Rosen N. Cancer Res 2000;60:2090. [PubMed: 10786665]
45. Kuduk SD, Zheng FF, Sepp-Lorenzino L, Rosen N, Danishefsky SJ. Bioorg. Med. Chem. Lett 1999;9:1233. [PubMed: 10340605]
46. Kuduk SD, Harris TC, Zheng FF, Sepp-Lorenzino L, Ouerfelli Q, Rosen N, Danishefsky SJ. Bioorg. Med. Chem. Lett 2000;10:1303. [PubMed: 10866406]
47. Debes JD, Tindall DJ. Cancer Lett 2002;187:1. [PubMed: 12359344]
48. Schneekloth JS Jr, Fonseca FN, Koldobskiy M, Mandal A, Deshaies R, Sakamoto K, Crews CM. J. Am. Chem. Soc 2004;126:3748. [PubMed: 15038727]
49. Sakamoto KM, Kim KB, Verma R, Ransick A, Stein B, Crews CM, Deshaies RJ. Mol. Cell. Proteomics 2003;2:1350. [PubMed: 14525958]
50. Sakamoto KM, Kim KB, Kumagai A, Mercurio F, Crews CM, Deshaies RJ. Proc. Natl. Acad. Sci. USA 2001;98:8554. [PubMed: 11438690]
51. Li X, Chang Y-H. Proc. Natl. Acad. Sci. USA 1995;92:12357. [PubMed: 8618900]
52. Sin N, Meng L, Wang MQW, Wen JJ, Bornmann WG, Crews CM. Proc. Natl. Acad. Sci. USA 1997;94:6099. [PubMed: 9177176]
53. Yaron A, Hatzubai A, Davis M, Lavon I, Amit S, Manning AM, Andersen JS, Mann M, Mercurio F, Ben-Neriah Y. Nature 1998;396:590. [PubMed: 9859996]
54. Yaron A, Gonen H, Alkalay I, Hatzubai A, Jung S, Beyth S, Mercurio F, Manning AM, Ciechanover A, Ben-Neriah Y. EMBO J 1997;16:6486. [PubMed: 9351830]
55. Deshaies RJ. Annu. Rev. Cell Dev. Biol 1999;15:435. [PubMed: 10611969]
56. Howell A, Howell SJ, Evans DG. Cancer Chemother. Pharmacol 2003;52:S39. [PubMed: 12819938]
57. Yanai Y, Irie K, Ohigashi H, Wender PA. Bioorg. Med. Chem 1997;5:117.
58. Derossi D, Joliot AH, Chassaing G, Prochiantz A. J. Biol. Chem 1994;269:10444. [PubMed: 8144628]

59. Fawell S, Seery J, Daikh Y, Moore C, Chen LL, Pepinsky B, Barsoum J. *Proc. Natl. Acad. Sci. USA* 1994;91:664. [PubMed: 8290579]
60. Hon WC, Wilson MI, Harlos K, Claridge TD, Schofield CJ, Pugh CW, Maxwell PH, Ratcliffe PJ, Stuart DI, Jones EY. *Nature* 2002;417:975. [PubMed: 12050673]
61. Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhanda A, Tian YM, Masson N, Hamilton DL, Jaakkola P, Barstead R, Hodgkin J, Maxwell PH, Pugh CW, Schofield CJ, Ratcliffe PJ. *Cell* 2001;107:43. [PubMed: 11595184]
62. Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, Huang LE, Pavletich N, Chau V, Kaelin WG. *Nat. Cell Biol* 2000;2:423. [PubMed: 10878807]
63. Tanimoto K, Makino Y, Pereira T, Poellinger L. *EMBO J* 2000;19:4298. [PubMed: 10944113]
64. Yang W, Rozamus LW, Narula S, Rollins CT, Yuan R, Andrade LJ, Ram MK, Phillips TB, van Schravendijk MR, Dalgarno D, Clackson T, Holt DA. *J. Med. Chem* 2000;43:1135. [PubMed: 10737745]
65. Rollins CT, Rivera VM, Woolfson DN, Keenan T, Hatada M, Adams SE, Andrade LJ, Yaeger D, van Schravendijk MR, Holt DA, Gilman M, Clackson T. *Proc. Natl. Acad. Sci. USA* 2000;97:7096. [PubMed: 10852943]
66. Zhang D, Baek SH, Ho A, Kim K. *Bioorg. Med. Chem. Lett* 2004;14:645. [PubMed: 14741260]

Scheme 1.

Mechanism of RNA interference (RNAi). Double-stranded RNA is recognized by the Dicer enzyme and cleaved into 22-nucleotide siRNA fragments. These fragments are then separated by RISC, which associates with the antisense mRNA for the gene of interest to make an active complex. The RISC: mRNA complex cleaves the mRNA, resulting in gene silencing.

A) Wild-type E3 complex

B) Chimeric F-box E3 complex

Scheme 2.

Chimeric F-box approach to biochemical protein degradation. In the wild-type E3 ligase, the F box recognizes the target protein, which is then ubiquitinated and ultimately degraded. If a binding domain for a target protein is engineered into the F box, a protein that is normally stable may be artificially ubiquitinated and degraded. E3=E3 ubiquitin ligase complex; Ub=ubiquitin; F=F-box-containing protein; BP=binding protein, recognition domain for the target protein.

Scheme 3.

Function of a PROTAC molecule. The PROTAC molecule induces complexation between the target protein and the E3 ubiquitin ligase. Once in a complex, the target protein is ubiquitinated and degraded. This approach requires no biochemical manipulation of the E3 ligase.

Scheme 4.

Structures of PROTAC molecules. A) fumagillin-based PROTAC. B) DHT-based PROTAC. C) AP21998-based PROTAC. Each PROTAC contains a target ligand, a linker, and an E3 ligase-recognition domain. Polyarginine tags are included for cell permeability. S*=phosphorylated serine.

Scheme 5.

Use of a PROTAC molecule to degrade a fluorescent protein. HEK 293 cells stably expressing GFP–androgen receptor were treated with 50 μm DHT–HIF–(Arg)₈ PROTAC. After 1 h, the PROTAC induces ubiquitination and degradation of GFP–androgen receptor; this results in loss of fluorescence.