ANNUAL REVIEWS

*Annual Review of Genetics*

# Conditional Degrons for Controlling Protein Expression at the Protein Level

Toyoaki Natsume and Masato T. Kanemaki

Division of Molecular Cell Engineering, National Institute of Genetics, Research Organization of Information and Systems (ROIS), and Department of Genetics, The Graduate University for Advanced Studies (SOKENDAI), Mishima, Shizuoka 411-8540, Japan; email: mkanemak@nig.ac.jp

Annu. Rev. Genet. 2017. 51:83–102

The *Annual Review of Genetics* is online at genet.annualreviews.org

https://doi.org/10.1146/annurev-genet-120116-024656





## Keywords

conditional degron, protein knockdown, proteolysis, ubiquitin–proteasome pathway

## Abstract

The conditional depletion of a protein of interest (POI) is useful not only for loss-of-function studies, but also for the modulation of biological pathways. Technologies that work at the level of DNA, mRNA, and protein are available for temporal protein depletion. Compared with technologies targeting the pretranslation steps, direct protein depletion (or protein knockdown approaches) is advantageous in terms of specificity, reversibility, and time required for depletion, which can be achieved by fusing a POI with a protein domain called a degron that induces rapid proteolysis of the fusion protein. Conditional degrons can be activated or inhibited by temperature, small molecules, light, or the expression of another protein. The conditional degron-based technologies currently available are described and discussed.

## INTRODUCTION

Studies of protein function in vivo are greatly helped by loss-of-function experiments, in which the expression of a protein of interest (POI) is constitutively or conditionally suppressed for phenotypic analyses. The recent explosion of genome-editing technologies, such as CRISPR–Cas9, transcription activator-like effector nuclease (TALEN), and Zn finger nuclease, has facilitated the generation of gene knockouts (KOs) with constitutive loss of a POI (20, 33, 66, 67, 83). However, it is impossible to generate gene KO mutants for the approximately 1,500 core-essential genes required for the viability of human cells (8, 40, 118). The proteins encoded by these genes must play important roles in cellular homeostasis or proliferation. To study the roles of these essential proteins in vivo, it is crucial to employ a method that can conditionally inactivate or deplete a POI. Even in the case of nonessential proteins, mutants generated by gene KOs might adopt changes caused by the constitutive loss of a POI (110). Therefore, there might be a phenotypic difference between constitutive and conditional KO mutants. Technologies enabling the conditional inactivation or depletion of POIs thus represent the next step in in vivo analyses of protein function, beyond simple gene KOs.

Protein expression can be conditionally perturbed at the DNA, mRNA, or protein level (**Figure 1*a***). Readers can refer to the following papers to learn about conditional technologies that work at the level of DNA and mRNA (27, 29, 34, 36, 44, 61, 95). Because all of these technologies function at the pretranslational level, the time required for POI depletion depends on the half-life of the POI in cells. This can be a problem when studying highly dynamic processes, such as the cell cycle, differentiation, or neural activity. For example, POI depletion using small interfering RNA (siRNA) in mammalian cells typically requires 2 to 3 days, which can be a major limitation to the use of this conventional depletion technology for studying the role of proteins involved

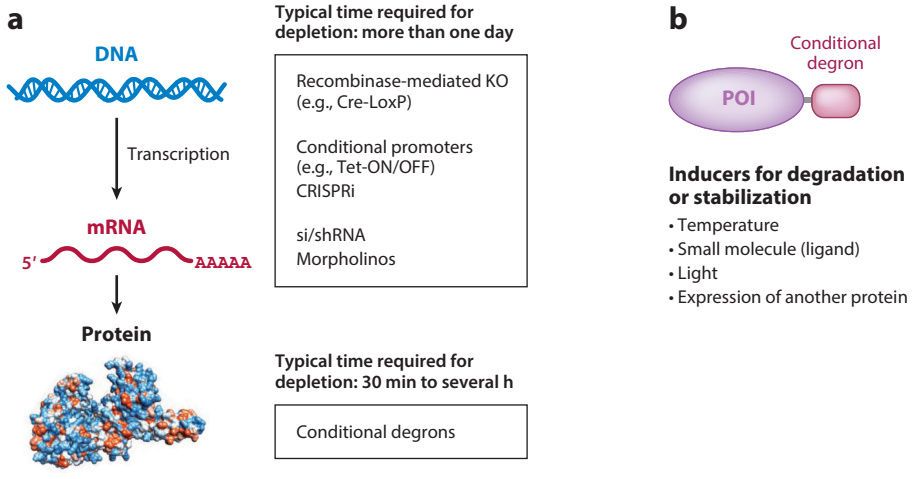


**Figure 1**

Technologies that allow the control of conditional expression. (*a*) Recombinase-mediated KOs, conditional promoters, CRISPRi, si/shRNAs, and morpholinos work at the pretranslation level. Conditional degrons affect protein stability directly. (*b*) A POI fused with a conditional degron. The stability of the degron is controlled by temperature, a small molecule (ligand), light, or the expression of another protein. Abbreviations: CRISPRi, CRISPR interference; KO, knockout; POI, protein of interest; shRNA, short hairpin RNA; siRNA, small interfering RNA; Tet-OFF, tetracycline-controlled transcriptional inactivation; Tet-ON, tetracycline-controlled transcriptional activation.

in cell-cycle progression. Because the depletion time is usually longer than the time required for one round of the cell cycle in mammalian cells, in some cases the initial defect was obscured or complemented by secondary effects caused by the loss of the POI (119, 121). It was also difficult to analyze proteins with pleiotropic functions by siRNA because the phenotype is complicated by multiple defects caused by slow depletion (91). Moreover, the off-target effects and depletion efficiency of siRNA are variable (46, 50).

These problems can be overcome by controlling protein levels directly. All eukaryotic cells are equipped with protein quality control systems, via which misfolded or damaged proteins are led to autophagy or rapid degradation by the 26S proteasome (12). In the ubiquitin–proteasome pathway, which is one such system, polyubiquitylated proteins are specifically degraded in a short time (35). This degradation pathway also contributes to the timely removal of signaling proteins that are involved in cell-cycle control, development, and homeostasis. Using endogenous degradation pathways, it is possible to artificially control protein levels. The proteolysis-targeting chimera (PROTAC) and F-box fusion methods were developed by exploiting endogenous degradation pathways (92, 96, 124, 125). However, these technologies cannot be applied to general POIs because specific ligands or binding domains must be found for each. To control protein levels, researchers have used a domain known as a degron, which confers instability to general POIs (116). By exerting timed, conditional control over the activity of a degron fused to a POI, the fusion protein can be targeted for rapid proteolysis by the 26S proteasome. Furthermore, a depleted POI can be reexpressed by inactivating the degron, and its expression level can be tuned in many cases by adopting a semipermissive condition. Degron-based protein knockdown is a relatively new approach that is now receiving more attention because of the advantages described above. Conditional degrons attached to a POI can be activated or inactivated by one of four factors: temperature, a small molecule (ligand), light, or the expression of another protein (**Figure 1*b***). In this review, we discuss currently available conditional degron-based technologies for protein knockdown.

## TEMPERATURE-INDUCED SYSTEMS

Varshavsky and colleagues (25) developed the first conditional degron using temperature to induce proteolysis in a POI. This technology remains a powerful tool in studies of the budding yeast *Saccharomyces cerevisiae*.

### Temperature-Sensitive and Low-Temperature Degrons

A protein exposing a non-Met amino acid residue, such as Arg, Lys, Tyr, or Phe, at the N terminus is efficiently degraded through an ubiquitin–proteasome pathway known as the N-end rule (107, 117). Such destabilizing residues located at the N terminus are recognized by an E3 ubiquitin ligase [Ubr1 in budding yeast (5) and PRT1 in *Arabidopsis thaliana* (84)] for rapid polyubiquitylation of the proteins, which leads to degradation by the 26S proteasome. Varshavsky and colleagues (104) initially constructed constitutive N-degrons, which comprise a destabilizing N-terminal residue and internal Lys residues for ubiquitylation. To generate an N-degron that works conditionally, they developed the heat-inducible or temperature-sensitive degron (ts-degron) system for budding yeast, via which a POI fused to a temperature-sensitive dihydrofolate reductase (ts-DHFR) protein with an Arg residue exposed at the N terminus (R-DHFR$^{ts}$) can be targeted for rapid proteolysis by shifting the temperature of the yeast culture (**Figure 2**) (**Table 1**) (24, 25). Placement of the Ub–R-DHFR$^{ts}$ cassette, which comprises the *ubiquitin* and *R-DHFR$^{ts}$* genes, at the 5′ end of a gene of interest leads to the expression of the fusion protein (Ub–R-DHFR$^{ts}$–POI). Ubiquitin is

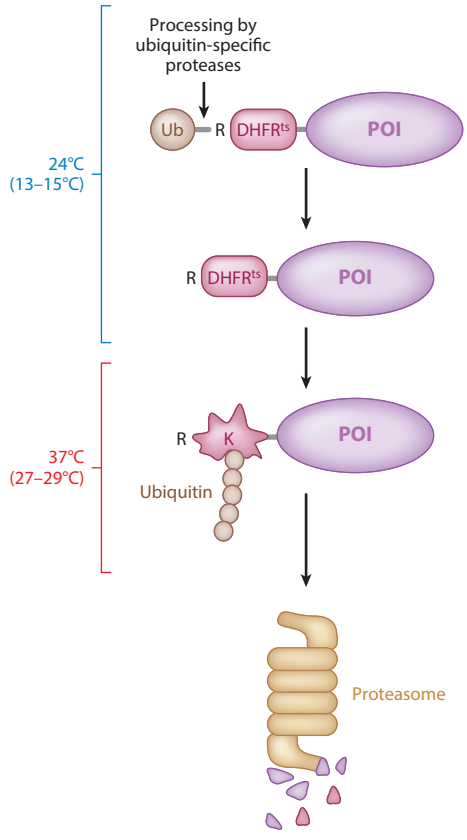


**Figure 2**

Schematic of temperature-induced conditional degron technologies (ts- and lt-degrons). The N-terminal ubiquitin moiety is cleaved by deubiquitinating enzymes, exposing the destabilizing Arg residue at the N terminus of DHFR$^{ts}$. A temperature shift causes rapid proteolysis of R-DHFR$^{ts}$–POI by the 26S proteasome. Temperatures are shown for the ts-degron (*no parentheses*) and for the lt-degron (*in parentheses*). Abbreviations: DHFR, dihydrofolate reductase; lt, low temperature; POI, protein of interest; ts, temperature sensitive; Ub, ubiquitin.

cleaved by ubiquitin-specific proteases, resulting in the exposure of the destabilizing Arg residue at the N terminus of R-DHFR$^{ts}$–POI. Even though the Arg residue can be recognized by the Ubr1 E3 ubiquitin ligase, the fusion protein is stable within the permissive temperature range (23–25°C). However, a shift to a higher temperature (approximately 37°C) leads to the unfolding of R-DHFR$^{ts}$, which results in rapid polyubiquitylation of R-DHFR$^{ts}$–POI and its proteolysis by the 26S proteasome ($t_{1/2}$ < 30 min). This ubiquitylation reaction is possibly induced by increased exposure of the Arg residue at the N terminus, exposure of internal Lys residues of R-DHFR$^{ts}$, or both.

Recently, molecular dynamics studies have demonstrated that, indeed, internal Lys residues acquire a higher conformational flexibility in a DHFR$^{ts}$ variant (30). To achieve efficient degradation in budding yeast, the ts-degron was further improved by overexpressing the Ubr1 E3 enzyme under the conditional *GAL1–10* promoter before the temperature shift (58, 93). The improved ts-degron system has been used in a functional proteomics screening to identify proteins that are required for cell-cycle progression in budding yeast (51, 94).

## Table 1 A comparison of conditional degron-based technologies

| Degron-based technology | Inducer | Nature of degron | Number of protein component(s) | Half-life ($t_{1/2}$) | Works in yeast? | Works in mammalian cells? | Multicellular organisms tested? | Comments |
|---|---|---|---|---|---|---|---|---|
| ts-degron and lt-degron | Temperature shift | $DHFR^{ts}$ (25 kDa) | 1 | Less than 30 min | Yes | Limited | Plants, *Drosophila* | Works only at the N terminus |
| AID | Auxin (e.g., IAA, NAA) | AID/IAA17 (25 kDa), mAID (7 kDa) | 2 | Less than 30 min | Yes | Yes | *Caenorhabditis elegans*, *Drosophila* | NA |
| DD-based systems | Shield-1 ($DD_{FKBP}$) | $DD_{FKBP}$ (12 kDa) | 1 | More than 45 min | Limited ($DD_{ecDHFR}$) | Yes | Mouse, rat, and *C. elegans* | Ligand binding induces stabilization |
| LID | Shield-1 | FKBP12–degron (13 kDa) | 1 | ~1 h | Not tested | Yes | Not tested | Works only at the C terminus |
| HaloTag-based systems and dTAG | HyT13, 36, HaloPROTACs, dTAGs (e.g., dTAG-7, -13) | HaloTag (34 kDa), FKBP12(F36V) (12 kDa) | 1 | More than 1.5 h (HaloPROTAC), ~1 h (dTAG) | Not tested | Yes | Mouse, zebrafish | NA |
| PSD and B-LID | Blue light | LOV2–degron 20 kDa) | 1 | ~30 min | Yes (PSD) | Yes (B-LID) | Zebrafish, *C. elegans* | NA |
| TIPI-degron | TEV protease expression | GFP–TEV site–N-degron (30 kDa) | 2 | ~45 min (yeast) | Yes | Not tested | *Arabidopsis* | NA |
| deGradFP | NSlmb–vhhGFP expression | GFP (25 kDa) | 2 | More than 45 min | Not tested | Yes | *Drosophila*, zebrafish | NA |
| SMASh | HCV protease inhibitor (e.g., asunaprevir) | SMASh tag (34 kDa) | 1 | POI's half-life | Yes | Yes | Not tested | NA |
| SURF | Rapamycin | 3xFRB-degron–Ub-C (58 kDa) | 2 | POI's half-life | Not tested | Yes | Not tested | Untagged POI is released in the presence of rapamycin |

Abbreviations: AID, auxin-inducible degron; B-LID, blue-light–inducible degron; DD, destabilizing domain; DHFR, dihydrofolate reductase; ec, *Escherichia coli*; FKBP, FK506 binding protein; FRB, FKBP rapamycin binding; GFP, green fluorescent protein; HCV, hepatitis C virus; IAA, indole-3-acetic acid; LID, ligand-induced degradation; LOV2, light oxygen voltage 2; lt, low temperature; mAID, mini auxin-inducible degron; NA, not applicable; NAA, 1-naphthaleneacetic acid; POI, protein of interest; PROTAC, proteolysis-targeting chimera; PSD, photosensitive degron; SMASh, small molecule-associated shutoff; SURF, split ubiquitin for the rescue of function; TEV, tobacco etch virus; TIPI, tobacco etch virus protease-induced protein inactivation; ts, temperature sensitive; vhhGFP, an anti-green fluorescent protein nanobody.

The fact that the degradation can be induced within 30 min after the temperature shift is a great advantage of this technology. We reported that the ts-degron system was advantageous for observing the direct effect caused by the loss of a POI (51). A subunit in a large complex can also be depleted without affecting the remaining interacting subunits (58). Because the Ub–R-DHFR$^{ts}$ cassette can be easily inserted into an endogenous gene of interest in yeast, this technology has become a powerful tool for studying the function of yeast proteins.

Even though the ts-degron has been used for the characterization of many proteins in budding yeast and, in part, in fission yeast (37, 53, 63, 86), the use of this technology in other species has been limited (with the exception of chicken DT40 cells) (6, 103). This is partly because mammalian cells are sensitive to drastic temperature-shift and the temperatures used in the ts-degron system are too high for plants and poikilothermic animals. To lower the restrictive temperature of the original ts-degron system, Dissmeyer and colleagues (30) recently isolated a modified version of R-DHFR$^{ts}$ termed the low-temperature degron (lt-degron), which is stable at 13–16°C but becomes unstable at 27–29°C (**Table 1**). Those authors successfully applied the lt-degron not only to budding yeast but also to multicellular organisms such as plants and *Drosophila*. By employing the ts- or lt-degron, it is now possible to analyze unicellular and poikilothermic multicellular organisms that can survive at temperatures ranging from 13 to 37°C. However, its application to mammalian cells remains difficult because these are usually cultured at a higher temperature than that used for inducing destabilization. It should also be noted that these degrons function only at the N terminus, and not at the C terminus, of a POI.

## SMALL-MOLECULE-INDUCED SYSTEMS

To avoid a drastic temperature shift that causes heat-shock responses, others have exploited the use of small molecules for controlling the activity of a conditional degron. Use of this type of conditional degron now predominates (**Figure 3**).

### Destabilizing Domain and Ligand-Induced Degradation

Rapamycin, an antibiotic produced by *Streptomyces hygroscopicus*, forms a ternary complex with the FK506 binding protein (FKBP12) and the FKBP rapamycin binding (FRB) domain of mTOR kinase (18). Stankunas et al. (101) noted that a mutant of FRB showed constitutive instability when fused to GSK-3β, and that the fusion was stabilized in the presence of FKBP12 and a rapamycin analog. Inspired by this finding, Wandless and colleagues (3) set out to develop a single-ligand–single-domain system, via which a constitutive degron or destabilizing domain (DD) is stabilized in the presence of its ligand. They isolated FKBP12 mutants that were stabilized in the presence of Shield-1, a derivative of rapamycin (**Figure 3*a***) (**Table 1**). In mammalian cell lines, a POI fused with an FKBP-based DD ($DD_{FKBP}$) was depleted in several hours after the removal of Shield-1 and was re-expressed by adding this ligand back to the culture medium. Moreover, $DD_{FKBP}$–POI fusions can be expressed in a dose-dependent manner. The $DD_{FKBP}$ technology has been successfully applied to protozoan parasites and mice (1, 4, 41, 90). However, it is not applicable to budding yeast and plants (87, 102). Recently, in combination with CRISPR–Cas9-mediated knock-in, the endogenous Treacher Collins–Franceschetti syndrome 1 protein was fused with $DD_{FKBP}$ for expression control in human 293T cells (80).

The development of $DD_{FKBP}$ paved a new way for the generation of additional DDs using a combination of another binding protein and its ligand. The Wandless group (45, 68) developed similar DDs based on *Escherichia coli* (ec) DHFR and the human estrogen receptor ligand-binding domain, together with their specific binding molecules, trimethoprim, and tamoxifen, respectively.

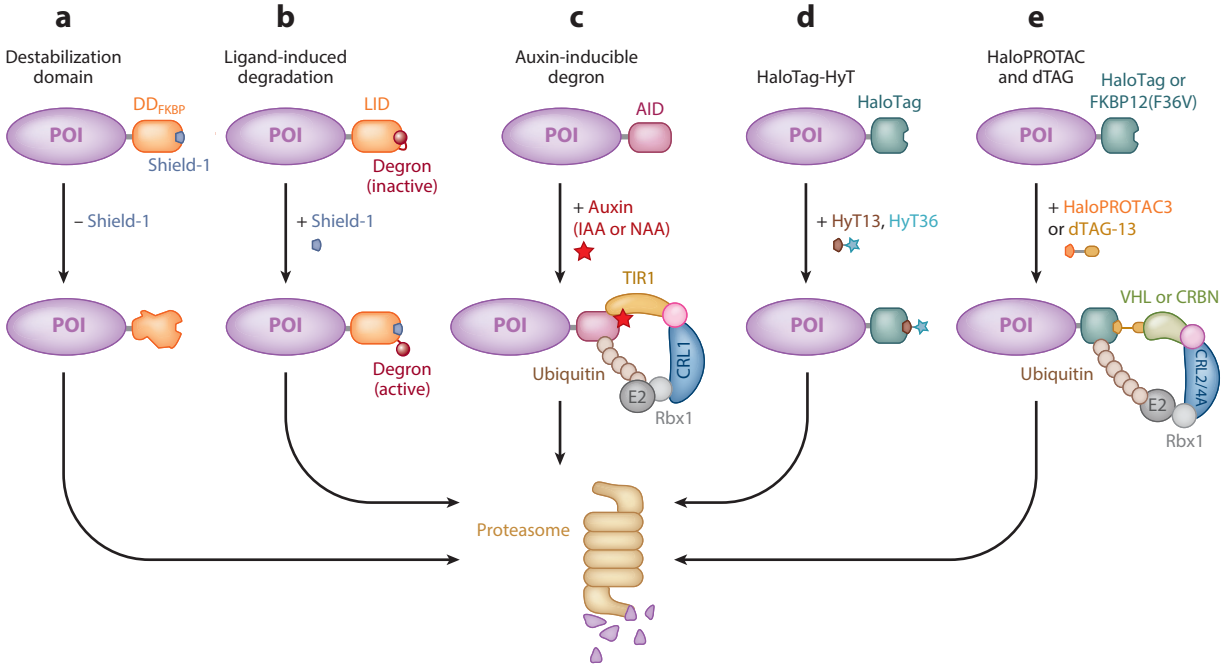


**Figure 3**

Schematic of small-molecule-induced conditional degron technologies. (*a*) A DD-fused protein is stabilized in the presence of its ligand (e.g., Shield-1), but it is degraded in its absence. (*b*) A synthetic degron in LID is hidden normally, but Shield-1 causes a conformational change that leads to the exposure of the degron for degradation. (*c*) An AID-tagged protein is rapidly degraded by the addition of auxin. (*d*) The HaloTag forms a covalent association with HyT13 or 36, each of which has a hydrophobic moiety. When bound, the POI–HaloTag fusion is degraded. (*e*) The HaloTag forms a covalent association with HaloPROTAC3, which brings the fusion protein to CRL2–VHL E3 ubiquitin ligase for rapid ubiquitylation. Similarly, FKBP12(F36V) binds to dTAG-13, which brings the fusion protein to CRL4A–CRBN E3 ubiquitin ligase. Abbreviations: AID, auxin-inducible degron; CRBN, cereblon; CRL, CUL–Ring ubiquitin ligase; DD, destabilization domain; LID, ligand-induced degradation; PROTAC, proteolysis-targeting chimera; POI, protein of interest; VHL, von Hippel-Lindau.

Using $DD_{FKBP}$ and $DD_{ecDHFR}$, the two proteins were controlled independently in NIH3T3 cells. Recently, $DD_{ecDHFR}$ was used for C-terminal tagging of endogenous human proteins via CRISPR–Cas9 genome editing (98). A modified $DD_{ecDHFR}$ was also used in nematodes (17). The same group also developed an UnaG protein-based DD (which becomes stable and fluorescent when bound to bilirubin) for the construction of a fluorescent bilirubin-dependent DD (73). Other than the involvement of the 26S proteasome, which plays a key role in the protein quality control systems, the proteolytic pathway of DDs is not well defined. However, the instability of DDs is related to their unfolding in the absence of the ligand (26).

All DDs are degraded in the absence of their specific ligand. To reverse this situation, the Wandless group (9) developed a new system in which a degron-fused POI is normally stable but is degraded in the presence of Shield-1. They fused a synthetic 19-aa (amino acid) degron at the C terminus of an FKBP12 variant to construct a ligand-induced degradation (LID) domain (**Figure 3*b***) (**Table 1**). The 19-aa degron is hidden in the ligand-binding pocket of FKBP12, which protects the degron from degradation. Shield-1 strongly binds to the binding pocket, which leads to the exposure of the degron and induction of the degradation of the POI–LID. Unlike $DD_{FKBP}$, which works at either the N or C terminus of the POI, LID works only at the C terminus of

the POI. The control of two proteins using LID and $DD_{FKBP}$ is an intriguing possibility. In the absence of Shield-1, POI1 fused with LID would be expressed, whereas POI2 fused with $DD_{FKBP}$ would be constantly degraded. The addition of Shield-1 should reverse this situation such that POI1 is targeted for degradation and POI2 is expressed. This might be a good strategy to study the function of a mutant protein: Wild-type protein would be expressed before the administration of Shield-1; after its administration, expression would switch instantly to the mutant protein.

## Auxin-Inducible Degron

Plants have a unique degradation pathway that is controlled by the auxin-family plant hormones, such as indole-3-acetic acid (IAA; a natural auxin) and 1-naphthaleneacetic acid (NAA; a synthetic auxin). These small molecules induce rapid degradation of the AUX/IAA proteins, which bind to auxin-response factors to inhibit the transcription of auxin-responsive genes in plants (16). Auxin binds to an auxin-receptor F-box protein, TIR1, which forms a CUL1–Ring ubiquitin ligase (CRL1) (106). Upon association of auxin with TIR1, CRL1–TIR1 binds to AUX/IAAs for polyubiquitylation, leading to rapid proteolysis by the 26S proteasome (23, 54). TIR1 is found only in plants, whereas the other CRL1 components are well conserved in all eukaryotes. Therefore, we aimed to transfer the auxin-dependent degradation pathway to other eukaryotic cells via introduction of the *TIR1* gene (75). Initially, we found that TIR1 derived from *Arabidopsis thaliana* (AtTIR1) formed a CRL1 complex with endogenous Cdc53 (a CUL1 homolog) in budding yeast. Subsequently, we used the *Arabidopsis* IAA17 protein as an auxin-inducible degron (AID). By fusing AID to POIs in budding yeast, we showed that the fusion proteins were rapidly degraded within 30–60 min after the addition of IAA or NAA (**Figure 3*c***) (**Table 1**) (75). Subsequently, we found that TIR1 derived from *Oryza sativa* (OsTIR1) worked better than AtTIR1, even at the high temperature of 37°C. Using OsTIR1, a POI fused with AID was depleted within 45 min after auxin addition in chicken DT40 and mammalian cells ($t_{1/2} < 15$ min) (75). Holland et al. (43) also reported the successful use of AID in mammalian cells by combining it with siRNA for the suppression of endogenous target proteins; they demonstrated that POI–AID fusions were mostly depleted in less than 60 min ($t_{1/2} = 10$–20 min).

A recent study using DT40 cells showed clear advantages of the AID over the Tet-OFF system with regard to the detailed analysis of the formation of the kinetochore complex and its maintenance, because the AID is capable of degrading an existing POI (121). Importantly, an AID can be attached to either the N or C terminus of a POI, and it works in both the nucleus and the cytoplasm. This technology has been successfully applied to control POIs in other unicellular (fission yeast, *Plasmodium*) and multicellular (nematode, *Drosophila*) organisms (52, 56, 82, 113, 122). We expect that the AID technology will be applicable to all nonplant eukaryotes.

The original AID/IAA17 tag was 25 kDa (229 aa) and contained dimerization domains (known as the AUX/IAA domains III and IV), which were not responsible for the interaction with TIR1 (79, 88). Therefore, we and other groups generated truncated AIDs lacking the dimerization domains. We reported the miniAID (mAID) tag (65–132 aa), which works in a manner similar to that of the original AID (57, 76). Other groups reported other versions of a truncated AID, termed $AID^{*}$ (71–114 aa) and $AID^{47}$ (63–109 aa) (11, 69). We found that multiple copies of the mAID fused to POIs induced tighter phenotypic defects in budding yeast for some proteins (57).

Before CRISPR-based genome-editing technology was available, it was difficult to control endogenous proteins in mammalian cells using degron-based approaches because gene tagging was a challenge. Initially, Holland and colleagues (60) used the adeno-associated virus for gene tagging with AID to control Plk4 in human retinal hTERT-RPE1 cells. We recently developed an efficient CRISPR–Cas9-based AID tagging approach using short-homology donors in human colorectal

HCT116 and mouse embryonic stem (ES) cells (72). In that study, we generated parental HCT116 cells that expressed OsTIR1 under the control of a constitutive cytomegalovirus (CMV) or a conditional tetracycline-controlled activation (Tet-ON) promoter (CMV- or Tet-OsTIR1 cells, respectively). The RAD21 cohesin subunit tagged with mAID in the CMV-OsTIR1 background exhibited a half-life of 17 min after auxin addition, before cell death from acute growth defects. In some cases, an endogenous POI fused with mAID was reduced in cells expressing OsTIR1, even without adding auxin (72; T. Natsume & M. Kanemaki, unpublished data). This may have been caused by the activation of OsTIR1 by contaminating auxin, because the bovine serum used for culture medium contains a small amount of auxin-like chemicals derived from intestinal microbes or grass (81, 123). Using Tet-OsTIR1 cells, it was possible to generate AID mutants that maintained the endogenous expression level of the POI–AID fusion in the absence of tetracycline (or doxycycline) (72). However, the expression level of OsTIR1 was not sufficient for inducing degradation until more than 4 h after tetracycline addition. This notion and our previous work in yeast (75) support the idea that OsTIR1 expression needed to be high for efficient degradation of AID-fused POIs. Technical improvements are expected for applications involving Tet-OsTIR1 cell lines.

Even though there have been few applications of the AID technology to stem cells to date (2, 11), we expect that it will be used increasingly in studies using ES cells and induced pluripotent stem cells in the future. Rapid degradation of a POI in stem cells before, during, or after differentiation will be advantageous for many studies. The application of the AID technology to relatively large animals, such as mice, will represent a challenge, because a method for the administration of auxin (IAA or NAA) has not been established. In yeast and mammalian cells, relatively high doses of IAA (100–500 μM) are usually added to the culture medium to induce degradation (75). In the case of nematodes and *Drosophila*, even higher doses of IAA (1–2 mM) are applied (113, 122). IAA causes a defect in the kidney when converted to indoxyl sulfate in the liver (77). It is therefore likely that high doses of IAA cannot be applied to mice. A new auxin-related inducer based on NAA or related chemicals that functions at lower concentrations might overcome this problem. Chemical and pharmacological improvements are expected for applications involving mice.

In addition to auxin, plants generate other phytohormones that induce rapid proteolysis of transcription regulatory factors via the ubiquitin–proteasome pathway (100). Jasmonic acid–isoleucine and its related compound produced by *Pseudomonas syringae*, coronatine (COR), induce the degradation of JAZ-family proteins by activating the F-box protein coronatine-insensitive 1 (COI1), which is structurally related to TIR1 (97, 111). Lemischka and colleagues (11) recently developed a JAZ degron system using a modified COI1 protein and a 23-aa degron derived from the JAZ1 protein. Importantly, they used the AID and JAZ degrons successfully to control two proteins independently using IAA and COR in mammalian cells.

## HaloTag-Hydrophobic Tag, HaloPROTAC, and dTAG

The bacterial dehalogenase-derived HaloTag is a versatile tag that can be used for protein purification, cell imaging, and other purposes because of its ability to form a specific covalent bond with compounds that contain alkyl chloride (64). Crews and colleagues (74) aimed to induce degradation using HaloTag and a small molecule containing alkyl chloride. They focused on hydrophobic compounds, such as adamantane, as they expected that a HaloTag bound by such a compound mimicked a denatured or misfolded protein, which would be degraded through protein quality control systems (12). They developed a hydrophobic tag (HyT13) that induced the degradation of a POI–HaloTag fusion in mammalian cells, zebrafish embryos, and mice (74) (**Figure 3*d***) (**Table 1**). Subsequently, they developed an improved inducer, HyT36, which induced stronger

degradation when bound to the original HaloTag and to the improved HaloTag7 (78, 105). It is now possible to convert HaloTag, one of the most commonly used tags, into a degron by adding HyT13 or 36. The shortcomings of this technology are that the time required for degradation is relatively long ($t_{1/2}$ = 1.5 h) and that depletion may not be very efficient, especially when HaloTag7 is used (a maximum of 70% of the initial expression level).

To overcome these problems, the same group developed other HaloTag-binding compounds that contained a moiety recruited to the CRL2–von Hippel-Lindau (VHL) E3 ubiquitin ligase (HaloPROTACs) (**Figure 3*e***) (**Table 1**) (13). Among the various HaloPROTACs tested by those authors, HaloPROTAC3 was the most potent for inducing the degradation of a POI–HaloTag7 fusion. This relatively new approach showed high efficiency of degradation within 24 h (90% of the initial expression level), but the degradation kinetics was rather slow ($t_{1/2}$ = 5 h). Further improvement of the system is expected in the future. A similar strategy using a HaloTag-reactive compound that binds to the cellular inhibitor of apoptosis protein 1 (cIAP-1) E3 ubiquitin ligase was also reported (112).

Recently, Bradner and colleagues (28, 120) synthesized dTAG ligands that induce degradation of FKBP12(F36V) (**Figure 3*e***) (**Table 1**). dTAGs are cell permeable ligands based on Shield-1 and thalidomide with high affinity, respectively, to FKBP12(F36V) and cereblon (CRBN). The latter is a component of the CRL4A–CRBN E3 ubiquitin ligase. In the presence of dTAGs, FKBP12(F36V)-fused proteins are recruited to the E3 ligase. Indeed, the ENL protein fused with FKBP12(F36V) was rapidly degraded within a few hours after the addition of dTAG-13. This simple single-component system looks promising for studies in cell biology. There are various types of protein tags that form a covalent bond or specifically associate with a small molecule (38). Therefore, it should be possible to convert them to degrons following ideas similar to those used for HaloPROTAC and dTAG.

## LIGHT-INDUCED SYSTEMS

Conditional degrons that are controlled by temperature or small molecules allow the temporal control of protein expression. However, they are not suited for spatial control because both factors are diffusive. The use of light-activated ligands can offer spatial control to some extent, but it might not be very tight (22). To control POI expression in a tight spatial and temporal manner, light-induced degrons were developed.

### Photosensitive Degron and Blue-Light-Inducible Degron

The light oxygen voltage 2 (LOV2) domain of phototropin is well studied for its conformational change upon blue-light irradiation (39). Taxis and colleagues (89) aimed to generate a blue-light-activated degron using the LOV2 domain of phototropin from *Arabidopsis thaliana*. LOV2 was fused with a constitutive degron derived from murine ornithine decarboxylase (ODC), which induced ubiquitin-independent degradation by the 26S proteasome (47). Among the various combinations tested in budding yeast, the authors identified a LOV2–ODC protein that was stable in the dark but became unstable upon blue-light irradiation; they named it a photosensitive degron (PSD) (**Figure 4*a***) (**Table 1**) (89, 115). Similarly, Wandless and colleagues (10) used LOV2 fused with a short synthetic degron to develop another LOV2 degron termed blue-light-inducible degron (B-LID), which induced proteolysis in mammalian cells and zebrafish upon blue-light irradiation. In both cases, it is supposed that LOV2 hides the active degron in the dark condition. Blue light induces a conformational change in LOV2, thus exposing the degron moiety (**Figure 4*a***). The time required for POI depletion is relatively short ($t_{1/2}$ = 30 min), and these new degrons

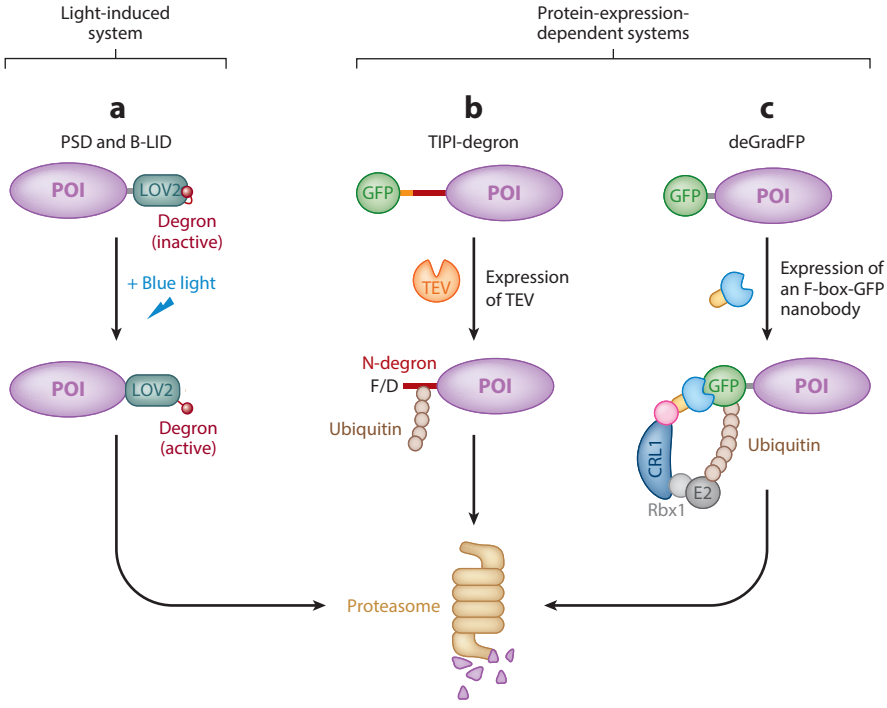


**Figure 4**

Schematic of light-induced and protein-expression-dependent conditional degron technologies. (*a*) A degron is hidden in LOV2 in the dark condition. Blue light causes a conformational change that leads to the exposure of the degron for degradation. (*b*) The N-degron in the fusion protein is protected by GFP. When GFP is removed by TEV protease, the N-degron is exposed for degradation. (*c*) The expression of an F-box–GFP nanobody fusion protein leads to the recruitment of a GFP-fused POI to CRL1 E3 ubiquitin ligase for degradation. Abbreviations: B-LID, blue-light-inducible degron; CRL, CUL–Ring ubiquitin ligase; deGradFP, degrade green fluorescent protein; GFP, green fluorescent protein; LOV2, light oxygen voltage 2; POI, protein of interest; PSD, photosensitive degron; TEV, tobacco etch virus; TIPI, tobacco etch virus protease-induced protein inactivation.

are expected to be useful in other organisms. In fact, PSD has already been used in nematodes (42). Because optogenetic tools are important in neuroscience, it is likely that light-controllable degrons will become particularly useful in this field.

## PROTEIN-EXPRESSION-DEPENDENT SYSTEMS

Conditional degrons can be activated by the expression of another protein, such as a degron activator or an ubiquitin ligase that recognizes the degron. Compared with the small-molecule-induced and light-induced systems, it is inevitable that POI depletion using this strategy is slower because the inducer must reach a level sufficient for proteolysis.

### Tobacco Etch Virus Protease-Induced Protein Inactivation-Degron

The tobacco etch virus (TEV) protease-induced protein inactivation (TIPI)-degron system is based on the N-end rule and was developed in budding yeast (**Table 1**) (108, 109). To achieve

conditional exposure, green fluorescent protein (GFP) was placed as a blocker at the N terminus of an N-degron (**Figure 4*b***). The expression of TEV protease leads to cleavage at a recognition site placed between GFP and the N-degron, resulting in deprotection of the N-degron for degradation via the N-end rule pathway. To enhance cleavage by TEV protease, two modifications were introduced. In the first, a domain derived from the human SF3b155 protein was placed close to the TEV protease-recognition site. TEV protease fused with p14, which binds to the domain, was efficiently recruited to the target site. The other modification was the use of $TEV^{+}$, a hyperactive TEV protease mutant. In budding yeast, a POI fused with GFP–N-degron was depleted within 1–2 h. The TIPI-degron has been shown to work in *Arabidopsis thaliana* (21).

Because this system relies on the N-end rule, the original TIPI-degron can be placed only at the N terminus of a POI, as is the case with the ts- and lt-degrons. Taxis and colleagues (49) subsequently generated an improved TIPI-degron that works at the C terminus of a POI using a C-terminal ODC-degron. The authors generated a bidirectional degron cassette [GFP–(ODC-degron)–(N-degron)–red fluorescent protein]. When placed at the C terminus of a POI, TEV protease cleaves the recognition site located between the ODC- and N-degrons, resulting in the activation of both degrons for codegradation.

### Degrade Green Fluorescent Protein

Nanobodies, which are single-domain antibodies derived from camelids, have been used in research tools because a nanobody expressed in living cells binds to its antigen (e.g., GFP) with high specificity (70). Caussinus et al. (14, 15) aimed to deplete GFP-fused proteins by expressing an anti-GFP nanobody (vhhGFP4) that forms a CRL1 E3 ubiquitin ligase; they named this technology deGradFP (degrade green fluorescent protein) (**Figure 4*c***) (**Table 1**). For this purpose, they prepared a chimeric F-box protein termed NSlmb-vhhGFP4 that was composed of an N terminal F-box domain derived from *Drosophila* Slmb (NSlmb) and vhhGFP4. The expression of NSlmb-vhhGFP4 induced the proteolysis of GFP-fused POIs in mammalian cells and *Drosophila*. It depleted both nuclear and cytoplasmic proteins within several hours. Shin et al. (99) tested other cullin ligases and found that vhhGFP4, when fused with the BTB domain of SPOP (an adaptor domain for CUL3), functioned better than the original NSlmb-vhhGFP4 in depleting nuclear proteins in mammalian cells and zebrafish embryos. Similarly, the VHL protein (an adaptor for CUL2) was fused with another GFP nanobody and used for depletion of GFP-fused proteins in mammalian cells (32). The original NSlmb-vhhGFP4 has been successfully used in many studies performed using *Drosophila* (7, 62, 114), possibly because NSlmb-vhhGFP4 is constructed using the *Drosophila* Slmb F-box protein. Because many GFP-trapped *Drosophila* and zebrafish lines are available, deGradFP might be an easy way to deplete POIs in these established lines.

## OTHER SYSTEMS

Thus far, we have described conditional degron technologies that enable the depletion of a POI that had already been expressed in living cells. Even though the technologies described below are based on a degron, they are different from the others because they do not actively degrade an existing POI. Instead, they are advantageous for titrating the level of a POI under semipermissive conditions. This feature is useful because a POI can continue functioning without a change to its half-life.

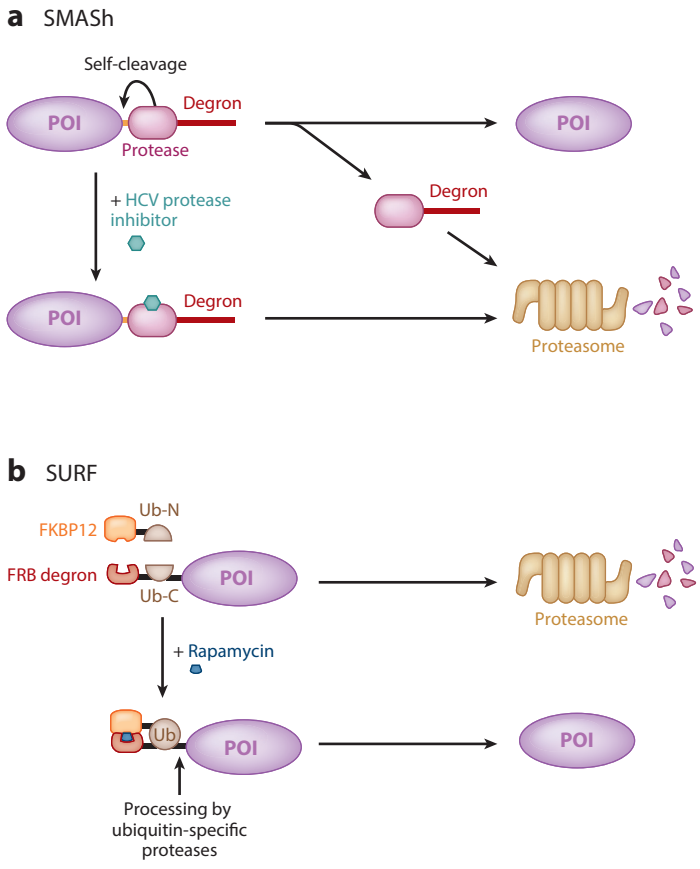


**Figure 5**

Schematic of the (*a*) SMASh and (*b*) SURF technologies. (*a*) The SMASh tag is self-cleaved to release the untagged POI. In the presence of an HCV protease inhibitor, the self-processing is attenuated for degradation of the fusion protein. (*b*) An FRB-degron–Ub-C–POI fusion protein is continuously degraded by the activity of the FRB degron. Rapamycin induces the association between FRB and FKBP, which brings Ub-C and Ub-N into proximity for the formation of ubiquitin. The C terminus of ubiquitin is cleaved for the release of the POI. Abbreviations: FKBP, FK506 binding protein; FRB, FKBP rapamycin binding; HCV, hepatitis C virus; POI, protein of interest; SMASh, small molecule-associated shutoff; SURF, split ubiquitin for the rescue of function, Ub-C, C-terminal fragment of ubiquitin; Ub-N, N-terminal fragment of ubiquitin.

## Small Molecule-Associated Shutoff

Lin and colleagues (19) developed the small molecule-associated shutoff (SMASh) technology (**Table 1**). The SMASh tag is a fusion polypeptide composed of the NS3-cleavage site, the NS3 protease, and a degron from NS4A, all of which are derived from the hepatitis C virus (HCV) (55). The expression of a POI fused with the SMASh tag led to the autocleavage of the SMASh tag and the release of an untagged POI soon after translation (**Figure 5*a***). The addition of an HCV protease inhibitor, such as asunaprevir, led to the expression of a POI–SMASh fusion, which was thus degraded by the activity of the degron. The SMASh tag works at the N or C terminus of a POI, and in mammalian and yeast cells. An advantage of this system is that a POI expressed

under a permissive condition is untagged. The time required for POI depletion depends on the half-life of the POI itself, because this technology does not actively induce the proteolysis of an existing POI. It is an interesting strategy to combine with another conditional degron technology for tight conditional control because one can stop protein synthesis and at the same time induce the proteolysis of an existing POI.

### Split Ubiquitin for the Rescue of Function

Split ubiquitin for the rescue of function (SURF), which was developed by Muir and colleagues (85), works in an opposite manner from that described for SMASh. This technology is a combination of an unstable FRB (FRB degron) and an ubiquitin-based split-protein sensor (48, 101). Ubiquitin can be split into N- and C-terminal fragments (Ub-N and Ub-C, respectively). Two components are needed for the technology's introduction into cells: FKBP12–Ub-N and a POI fused with FRB-degron–Ub-C, the latter of which is constitutively degraded by the activity of FRB degron (**Figure 5*b***). Rapamycin induces the formation of the ternary complex of FKBP–rapamycin–FRB, which brings Ub-N and Ub-C into proximity. As a result, ubiquitin is restored for processing by ubiquitin-specific proteases and releases an untagged POI. This POI cannot be controlled.

## DISCUSSION AND FUTURE PROSPECTS

All conditional degron technologies discussed in this review, with the exception of SMASh and SURF, induce temporal degradation of an existing POI in living cells. This is the main advantage of protein knockdown approaches using conditional degrons. These technologies can be used not only for the functional analysis of a POI, but also for artificially perturbing or controlling a system in cells. For example, if a key protein that drives the cell cycle (e.g., cyclin-dependent kinase) can be temporally degraded, it might be possible to artificially control the cell cycle. Other features, such as reversibility and tunability, add flexibility to the system.

The expression level of a POI is determined by a balance between protein synthesis and degradation. Although all conditional degron systems, with the exception of SMASh and SURF, greatly accelerate degradation, protein synthesis is ongoing. Depending on the POI and degron used in the experiment, depletion can be partial under restrictive conditions, leading to a leaky phenotype. It should be noted that in some cases it might not be possible to generate a null-like mutant using only degron-based technologies.

The most important advantage of a degron-based system, with the exception of SMASh and SURF, is that one can control protein expression within a short time. In this respect, the ts-degron, lt-degron, and AID systems deplete POIs the fastest ($t_{1/2} < 30$ min) and have been successfully used to control endogenous proteins that are essential for cell viability. However, to date, successes in the use of ts- or lt-degrons in nonyeast organisms are still few (6, 30, 103). Even though AID has been successfully used in many functional analyses within yeasts and mammalian cells, this is a two-component system, making its introduction more complicated compared with other systems. DD-based technologies are simpler single-component systems and have successfully been used in many studies with mammalian cells. However, one needs to continuously administer the stabilizing ligand to maintain the expression of a DD-fused POI, a feature which can be costly. Other small-molecule-based degrons have been shown to work in principle, but they have not been extensively used to control endogenous proteins in functional studies to date. Among them, HaloPROTAC and dTAG are interesting because these are single-component systems and they work efficiently in mammalian cells. We await applications to

functional studies by controlling endogenous proteins. Light-induced degrons are a new, promising approach, but this system requires a special illumination device. Expression-induced degrons, such as deGradFP, are advantageous because they use GFP-trapped zebrafish or *Drosophila* lines, which are available from a bioresource center; however, protein knockdown might not be very fast.

All conditional degron systems have both advantages and disadvantages. Depending on the organism, the depletion time and efficiency requirements, the availability of existing resources, and the feasibility of genetic manipulation, one needs to choose a suitable system. Because most degron-based technologies are relatively new, it is likely that they will be improved in the near future or that a new degron-based technology will be developed.

Currently, small-molecule-based degrons have reached the mainstream, and these are used primarily in single-celled organisms and cultured mammalian cells because the administration of an inducing or inhibiting small molecule is simple. Conversely, their application in animals remains limited with the exception of DD-based systems (4, 45, 64). Light-induced degrons might be a powerful technology for the control of POI expression, especially in the brain. Illumination systems are already available for optogenetic studies in which a channel rhodopsin is expressed in the brain to activate neurons (31). A similar experimental setup can be used to control protein expression using a light-induced degron in the brain.

Because all degron-based technologies require the attachment of a degron moiety to a POI, at least one genetic modification is essential. This used to represent a limitation when controlling an endogenous protein and was the reason why conditional degrons were mostly used in yeasts, where gene targeting is feasible. The advent of CRISPR–Cas9-based genome editing completely changed this situation. It is now possible to generate conditional cells in which a POI–degron fusion can be controlled at the protein level either by knocking out an endogenous gene and complementing it with a transgene expressing a POI–degron fusion or by directly fusing a conditional degron to an endogenous protein. Endogenous gene tagging using CRISPR–Cas9 is being improved. Bi-allelic tagging can be efficiently achieved using two donor plasmids containing different selection markers for double selection (80). We showed that donors harboring short-homology arms worked efficiently for homologous-recombination-mediated knock-in (72). Other authors exploited the development of nonhomologous end-joining– and microhomology-mediated gene tagging (59, 71). Improvements to both genome-editing and conditional degron technologies will facilitate the generation of degron-based mutants. We believe that protein knockdown via a conditional degron will be used more frequently and will become an important approach for future detailed analyses in cell biology and developmental biology.

## 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

We thank Dr. T. Kiyomitsu for discussion and Dr. N. Dissmeyer for his critical reading of the manuscript. This work was supported by Japan Society for the Promotion of Science KAKENHI Grants 15K18482 and 16K15095 and by research grants from the Mochida Memorial Foundation for Medical and Pharmaceutical Research, the SGH Foundation, the Sumitomo Foundation, and the Canon Foundation.

## LITERATURE CITED


1. Armstrong CM, Goldberg DE. 2007. An FKBP destabilization domain modulates protein levels in *Plasmodium falciparum*. *Nat. Methods* 4:1007–9
2. Baker O, Gupta A, Obst M, Zhang Y, Anastassiadis K, et al. 2016. RAC-tagging: recombineering and Cas9-assisted targeting for protein tagging and conditional analyses. *Sci. Rep.* 6:25529
3. **Banaszynski LA, Chen LC, Maynard-Smith LA, Ooi AGL, Wandless TJ. 2006. A rapid, reversible, and tunable method to regulate protein function in living cells using synthetic small molecules. *Cell* 126:995–1004**
4. Banaszynski LA, Sellmyer MA, Contag CH, Wandless TJ, Thorne SH. 2008. Chemical control of protein stability and function in living mice. *Nat. Med.* 14:1123–27
5. Bartel B, Wünning I, Varshavsky A. 1990. The recognition component of the N-end rule pathway. *EMBO J.* 9:3179–89
6. Bernal JA, Venkitaraman AR. 2011. A vertebrate N-end rule degron reveals that Orc6 is required in mitosis for daughter cell abscission. *J. Cell Biol.* 192:969–78
7. Blattner AC, Chaurasia S, McKee BD, Lehner CF. 2016. Separase is required for homolog and sister disjunction during *Drosophila melanogaster* male meiosis, but not for biorientation of sister centromeres. *PLOS Genet.* 12:e1005996
8. Blomen VA, Májek P, Jae LT, Bigenzahn JW, Nieuwenhuis J, et al. 2015. Gene essentiality and synthetic lethality in haploid human cells. *Science* 350:1092–96
9. Bonger KM, Chen LC, Liu CW, Wandless TJ. 2011. Small-molecule displacement of a cryptic degron causes conditional protein degradation. *Nat. Chem. Biol.* 7:531–37
10. **Bonger KM, Rakhit R, Payumo AY, Chen JK, Wandless TJ. 2014. General method for regulating protein stability with light. *ACS Chem. Biol.* 9:111–15**
11. Brosh R, Hrynyk I, Shen J, Waghray A, Zheng N, Lemischka IR. 2016. A dual molecular analogue tuner for dissecting protein function in mammalian cells. *Nat. Commun.* 7:11742
12. Buchberger A, Bukau B, Sommer T. 2010. Protein quality control in the cytosol and the endoplasmic reticulum: brothers in arms. *Mol. Cell* 40:238–52
13. Buckley DL, Raina K, Darricarrere N, Hines J, Gustafson JL, et al. 2015. HaloPROTACS: use of small molecule PROTACs to induce degradation of HaloTag fusion proteins. *ACS Chem. Biol.* 10:1831–37
14. Caussinus E, Affolter M. 2016. deGradFP: a system to knockdown GFP-tagged proteins. *Methods Mol. Biol.* 1478:177–87
15. **Caussinus E, Kanca O, Affolter M. 2012. Fluorescent fusion protein knockout mediated by anti-GFP nanobody. *Nat. Struct. Mol. Biol.* 19:117–21**
16. Chapman EJ, Estelle M. 2009. Mechanism of auxin-regulated gene expression in plants. *Annu. Rev. Genet.* 43:265–85
17. Cho U, Zimmerman SM, Chen LC, Owen E, Kim JV, et al. 2013. Rapid and tunable control of protein stability in *Caenorhabditis elegans* using a small molecule. *PLOS ONE* 8:e72393
18. Choi J, Chen J, Schreiber SL, Clardy J. 1996. Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. *Science* 273:239–42
19. Chung HK, Jacobs CL, Huo Y, Yang J, Krumm SA, et al. 2015. Tunable and reversible drug control of protein production via a self-excising degron. *Nat. Chem. Biol.* 11:713–20
20. Cong L, Ran FA, Cox D, Lin S, Barretto R, et al. 2013. Multiplex genome engineering using CRISPR/Cas systems. *Science* 339:819–23
21. Daum G, Medzihradszky A, Suzaki T, Lohmann JU. 2014. A mechanistic framework for noncell autonomous stem cell induction in *Arabidopsis*. *PNAS* 111:14619–24
22. Delacour Q, Li C, Plamont MA, Billon-Denis E, Aujard I, et al. 2015. Light-activated proteolysis for the spatiotemporal control of proteins. *ACS Chem. Biol.* 10:1643–47
23. Dharmasiri N, Dharmasiri S, Estelle M. 2005. The F-box protein TIR1 is an auxin receptor. *Nature* 435:441–45
24. Dohmen RJ, Varshavsky A. 2005. Heat-inducible degron and the making of conditional mutants. *Methods Enzymol.* 399:799–822


**3. This article described the first DD-based system using FKBP12 and its ligand, Shield-1.**

**10. The Wandless laboratory developed a light-induced degron system using the LOV2 domain and a synthetic degron.**

**15. This article described F-box fusion protein, the first system targeting GFP using a GFP-nanobody.**

**25. Dohmen RJ, Wu P, Varshavsky A. 1994. Heat-inducible degron: a method for constructing temperature-sensitive mutants. *Science* 263:1273–76**

26. Egeler EL, Urner LM, Rakhit R, Liu CW, Wandless TJ. 2011. Ligand-switchable substrates for a ubiquitin-proteasome system. *J. Biol. Chem.* 286:31328–36

27. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. 2001. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 411:494–98

28. Erb MA, Scott TG, Li BE, Xie H, Paulk J, et al. 2017. Transcription control by the ENL YEATS domain in acute leukaemia. *Nature* 543:270–74

29. Faden F, Mielke S, Lange D, Dissmeyer N. 2014. Generic tools for conditionally altering protein abundance and phenotypes on demand. *Biol. Chem.* 395:737–62

**30. Faden F, Ramezani T, Mielke S, Almudi I, Nairz K, et al. 2016. Phenotypes on demand via switchable target protein degradation in multicellular organisms. *Nat. Commun.* 7:12202**

31. Fenno L, Yizhar O, Deisseroth K. 2011. The development and application of optogenetics. *Annu. Rev. Neurosci.* 34:389–412

32. Fulcher LJ, Macartney T, Bozatzi P, Hornberger A, Rojas-Fernandez A, Sapkota GP. 2016. An affinity-directed protein missile system for targeted proteolysis. *Open Biol.* 6:160255

33. Gaj T, Sirk SJ, Shui SL, Liu J. 2016. Genome-editing technologies: principles and applications. *Cold Spring Harb. Perspect. Biol.* 8:a023754

34. Gilbert LA, Larson MH, Morsut L, Liu Z, Brar GA, et al. 2013. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell* 154:442–51

35. Glickman MH, Ciechanover A. 2002. The ubiquitin–proteasome proteolytic pathway: destruction for the sake of construction. *Physiol. Rev.* 82:373–428

36. Gossen M, Bujard H. 1992. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *PNAS* 89:5547–51

37. Gregan J, Lindner K, Brimage L, Franklin R, Namdar M, et al. 2003. Fission yeast Cdc23/Mcm10 functions after pre-replicative complex formation to promote Cdc45 chromatin binding. *Mol. Biol. Cell* 14:3876–87

38. Gronemeyer T, Godin G, Johnsson K. 2005. Adding value to fusion proteins through covalent labelling. *Curr. Opin. Biotechnol.* 16:453–58

39. Harper SM, Neil LC, Gardner KH. 2003. Structural basis of a phototropin light switch. *Science* 301:1541–44

40. Hart T, Chandrashekhar M, Aregger M, Steinhart Z, Brown KR, et al. 2015. High-resolution CRISPR screens reveal fitness genes and genotype-specific cancer liabilities. *Cell* 163:1515–26

41. Herm-Götz A, Agop-Nersesian C, Münter S, Grimley JS, Wandless TJ, et al. 2007. Rapid control of protein level in the apicomplexan *Toxoplasma gondii*. *Nat. Methods* 4:1003–5

42. Hermann A, Liewald JF, Gottschalk A. 2015. A photosensitive degron enables acute light-induced protein degradation in the nervous system. *Curr. Biol.* 25:R749–50

43. Holland AJ, Fachinetti D, Han JS, Cleveland DW. 2012. Inducible, reversible system for the rapid and complete degradation of proteins in mammalian cells. *PNAS* 109:E3350–57

44. Housden BE, Muhar M, Gemberling M, Gersbach CA, Stainier DY, et al. 2017. Loss-of-function genetic tools for animal models: cross-species and cross-platform differences. *Nat. Rev. Genet.* 18:24–40

45. Iwamoto M, Björklund T, Lundberg C, Kirik D, Wandless TJ. 2010. A general chemical method to regulate protein stability in the mammalian central nervous system. *Chem. Biol.* 17:981–88

46. Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, et al. 2003. Expression profiling reveals off-target gene regulation by RNAi. *Nat Biotech.* 6:635–37

47. Jariel-Encontre I, Bossis G, Piechaczyk M. 2008. Ubiquitin-independent degradation of proteins by the proteasome. *Biochim. Biophys. Acta* 1786:153–77

48. Johnsson N, Varshavsky A. 1994. Split ubiquitin as a sensor of protein interactions in vivo. *PNAS* 91:10340–44

49. Jungbluth M, Renicke C, Taxis C. 2010. Targeted protein depletion in *Saccharomyces cerevisiae* by activation of a bidirectional degron. *BMC Syst. Biol.* 4:176

50. Kaelin WG Jr. 2012. Use and abuse of RNAi to study mammalian gene function. *Science* 337:421–22


**25. This article reported the first conditional degron system, the ts-degron, in budding yeast.**

**30. This article reported the development and use of the lt-degron in yeast, plants, and *Drosophila*.**

Erratum

51. Kanemaki M, Sanchez-Diaz A, Gambus A, Labib K. 2003. Functional proteomic identification of DNA replication proteins by induced proteolysis *in vivo*. *Nature* 423:720–24
52. Kanke M, Nishimura K, Kanemaki M, Kakimoto T, Takahashi TS, et al. 2011. Auxin-inducible protein depletion system in fission yeast. *BMC Cell Biol.* 12:8
53. Kearsey SE, Gregan J. 2009. Using the DHFR heat-inducible degron for protein inactivation in *Schizosaccharomyces pombe*. *Methods Mol. Biol.* 521:483–92
54. Kepinski S, Leyser O. 2005. The *Arabidopsis* F-box protein TIR1 is an auxin receptor. *Nature* 435:446–51
55. Kim JL, Morgenstern KA, Lin C, Fox T, Dwyer MD, et al. 1996. Crystal structure of the hepatitis C virus NS3 protease domain complexed with a synthetic NS4A cofactor peptide. *Cell* 87:343–55
56. Kreidenweiss A, Hopkins AV, Mordmüller B. 2013. 2A and the auxin-based degron system facilitate control of protein levels in *Plasmodium falciparum*. *PLOS ONE* 8:e78661
57. Kubota T, Nishimura K, Kanemaki MT, Donaldson AD. 2013. The Elg1 replication factor C-like complex functions in PCNA unloading during DNA replication. *Mol. Cell* 50:273–80
58. Labib K, Tercero JA, Diffley JFX. 2000. Uninterrupted MCM2–7 function required for DNA replication fork progression. *Science* 288:1643–47
59. Lackner DH, Carré A, Guzzardo PM, Banning C, Mangena R, et al. 2015. A generic strategy for CRISPR-Cas9-mediated gene tagging. *Nat. Commun.* 6:10237
60. Lambrus BG, Uetake Y, Clutario KM, Daggubati V, Snyder M, et al. 2015. p53 protects against genome instability following centriole duplication failure. *J. Cell Biol.* 210:63–77
61. Le Y, Sauer B. 2000. Conditional gene knockout using Cre recombinase. *Methods Mol. Biol.* 136:477–85
62. Lee KH, Zhang P, Kim HJ, Mitrea DM, Sarkar M, et al. 2016. C9orf72 dipeptide repeats impair the assembly, dynamics, and function of membrane-less organelles. *Cell* 167:774–88.e17

**72. This article reported the combined use of AID and CRISPR–Cas9 to control endogenous proteins in human cells.**

63. Lindner K, Gregán J, Montgomery S, Kearsey SE. 2002. Essential role of MCM proteins in premeiotic DNA replication. *Mol. Biol. Cell* 13:435–44
64. Liu KJ, Arron JR, Stankunas K, Crabtree GR, Longaker MT. 2007. Chemical rescue of cleft palate and midline defects in conditional GSK-3β mice. *Nature* 446:79–82
65. Los GV, Encell LP, McDougall MG, Hartzell DD, Karassina N, et al. 2008. HaloTag: a novel protein labeling technology for cell imaging and protein analysis. *ACS Chem. Biol.* 3:373–82
66. Mali P, Yang L, Esvelt KM, Aach J, Guell M, et al. 2013. RNA-guided human genome engineering via Cas9. *Science* 339:823–26
67. Miller JC, Tan S, Qiao G, Barlow KA, Wang J, et al. 2011. A TALE nuclease architecture for efficient genome editing. *Nat. Biotechnol.* 29:143–48
68. Miyazaki Y, Imoto H, Chen LC, Wandless TJ. 2012. Destabilizing domains derived from the human estrogen receptor. *J. Am. Chem. Soc.* 134:3942–45

**74. This article reported the first use of the HaloTag for targeted proteolysis.**

69. Morawska M, Ulrich HD. 2013. An expanded tool kit for the auxin-inducible degron system in budding yeast. *Yeast* 30:341–51
70. Muyldermans S. 2013. Nanobodies: natural single-domain antibodies. *Annu. Rev. Biochem.* 82:775–97
71. Nakade S, Tsubota T, Sakane Y, Kume S, Sakamoto N, et al. 2014. Microhomology-mediated end-joining-dependent integration of donor DNA in cells and animals using TALENs and CRISPR/Cas9. *Nat. Commun.* 5:5560
**72. Natsume T, Kiyomitsu T, Saga Y, Kanemaki MT. 2016. Rapid protein depletion in human cells by auxin-inducible degron tagging with short homology donors. *Cell Rep.* 15:210–18**
73. Navarro R, Chen LC, Rakhit R, Wandless TJ. 2016. A novel destabilizing domain based on a small-molecule dependent fluorophore. *ACS Chem. Biol.* 11:2101–4
**74. Neklesa TK, Tae HS, Schneekloth AR, Stulberg MJ, Corson TW, et al. 2011. Small-molecule hydrophobic tagging-induced degradation of HaloTag fusion proteins. *Nat. Chem. Biol.* 7:538–43**
75. Nishimura K, Fukagawa T, Takisawa H, Kakimoto T, Kanemaki M. 2009. An auxin-based degron system for the rapid depletion of proteins in nonplant cells. *Nat. Methods* 6:917–22

**76. This article was the first to describe AID technology and its use in yeast and mammalian cells.**

**76. Nishimura K, Kanemaki MT. 2014. Rapid depletion of budding yeast proteins via the fusion of an auxin-inducible degron (AID). *Curr. Protoc. Cell Biol.* 64:20.9.1–16**
77. Niwa T, Ise M, Miyazaki T. 1994. Progression of glomerular sclerosis in experimental uremic rats by administration of indole, a precursor of indoxyl sulfate. *Am. J. Nephrol.* 14:207–12

78. Ohana RF, Encell LP, Zhao K, Simpson D, Slater MR, et al. 2009. HaloTag7: a genetically engineered tag that enhances bacterial expression of soluble proteins and improves protein purification. *Protein Expr. Purif.* 68:110–20
79. Ouellet F, Overvoorde PJ, Theologis A. 2001. IAA17/AXR3: biochemical insight into an auxin mutant phenotype. *Plant Cell* 13:829–41
80. Park A, Won ST, Pentecost M, Bartkowski W, Lee B. 2014. CRISPR/Cas9 allows efficient and complete knock-in of a destabilization domain-tagged essential protein in a human cell line, allowing rapid knockdown of protein function. *PLOS ONE* 9:e95101
81. Patten CL, Glick BR. 1996. Bacterial biosynthesis of indole-3-acetic acid. *Can. J. Microbiol.* 42:207–20
82. Philip N, Waters AP. 2015. Conditional degradation of *Plasmodium* calcineurin reveals functions in parasite colonization of both host and vector. *Cell Host Microbe* 18:122–31
83. Porteus M. 2008. Design and testing of zinc finger nucleases for use in mammalian cells. *Methods Mol. Biol.* 435:47–61
84. Potuschak T, Stary S, Schlögelhofer P, Becker F, Nejinskaia V, Bachmair A. 1998. *PRT1* of *Arabidopsis thaliana* encodes a component of the plant N-end rule pathway. *PNAS* 95:7904–8
85. Pratt MR, Schwartz EC, Muir TW. 2007. Small-molecule-mediated rescue of protein function by an inducible proteolytic shunt. *PNAS* 104:11209–14
86. Rajagopalan S, Liling Z, Liu J, Balasubramanian M. 2004. The N-degron approach to create temperature-sensitive mutants in *Schizosaccharomyces pombe*. *Methods* 33:206–12
87. Rakhit R, Edwards SR, Iwamoto M, Wandless TJ. 2011. Evaluation of FKBP and DHFR based destabilizing domains in *Saccharomyces cerevisiae*. *Bioorg. Med. Chem. Lett.* 21:4965–68
88. Reed JW. 2001. Roles and activities of Aux/IAA proteins in *Arabidopsis*. *Trends Plant Sci.* 6:420–25
**89. Renicke C, Schuster D, Usherenko S, Essen LO, Taxis C. 2013. A LOV2 domain-based optogenetic tool to control protein degradation and cellular function. *Chem. Biol.* 20:619–26**
90. Rodriguez S, Wolfgang MJ. 2012. Targeted chemical-genetic regulation of protein stability in vivo. *Chem. Biol.* 19:391–98
91. Sacher R, Stergiou L, Pelkmans L. 2008. Lessons from genetics: interpreting complex phenotypes in RNAi screens. *Curr. Opin. Cell Biol.* 20:483–89
92. Sakamoto KM. 2010. Protacs for treatment of cancer. *Pediatr. Res.* 67:505–8
93. Sanchez-Diaz A, Kanemaki M, Marchesi V, Labib K. 2004. Rapid depletion of budding yeast proteins by fusion to a heat-inducible degron. *Sci. STKE* 2004:PL8
94. Sanchez-Diaz A, Marchesi V, Murray S, Jones R, Pereira G, et al. 2008. Inn1 couples contraction of the actomyosin ring to membrane ingression during cytokinesis in budding yeast. *Nat. Cell Biol.* 10:395–406
95. Sauer B, Henderson N. 1988. Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1. *PNAS* 85:5166–70
96. Schneekloth JS Jr., Fonseca FN, Koldobskiy M, Mandal A, Deshaies R, et al. 2004. Chemical genetic control of protein levels: selective in vivo targeted degradation. *J. Am. Chem. Soc.* 126:3748–54
97. Sheard LB, Tan X, Mao H, Withers J, Ben-Nissan G, et al. 2010. Jasmonate perception by inositol-phosphate-potentiated COI1–JAZ co-receptor. *Nature* 468:400–5
98. Sheridan RM, Bentley DL. 2016. Selectable one-step PCR-mediated integration of a degron for rapid depletion of endogenous human proteins. *Biotechniques* 60:69–74
99. Shin YJ, Park SK, Jung YJ, Kim YN, Kim KS, et al. 2015. Nanobody-targeted E3–ubiquitin ligase complex degrades nuclear proteins. *Sci. Rep.* 5:14269
100. Spartz AK, Gray WM. 2008. Plant hormone receptors: new perceptions. *Genes Dev.* 22:2139–48
101. Stankunas K, Bayle JH, Gestwicki JE, Lin YM, Wandless TJ, Crabtree GR. 2003. Conditional protein alleles using knockin mice and a chemical inducer of dimerization. *Mol. Cell* 12:1615–24
102. Su L, Li A, Li H, Chu C, Qiu JL. 2013. Direct modulation of protein level in *Arabidopsis*. *Mol. Plant* 6:1711–14
103. Su X, Bernal JA, Venkitaraman AR. 2008. Cell-cycle coordination between DNA replication and recombination revealed by a vertebrate N-end rule degron-Rad51. *Nat. Struct. Mol. Biol.* 15:1049–58
104. Suzuki T, Varshavsky A. 1999. Degradation signals in the lysine–asparagine sequence space. *EMBO J.* 18:6017–26

**89. The Taxis laboratory developed a light-induced degron system using the LOV2 domain and an ODC-degron.**

105. Tae HS, Sundberg TB, Neklesa TK, Noblin DJ, Gustafson JL, et al. 2012. Identification of hydrophobic tags for the degradation of stabilized proteins. *ChemBioChem*. 13:538–41
106. Tan X, Calderon-Villalobos LIA, Sharon M, Zheng C, Robinson CV, et al. 2007. Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature* 446:640–45
107. Tasaki T, Sriram SM, Park KS, Kwon YT. 2012. The N-end rule pathway. *Annu. Rev. Biochem.* 81:261–89
108. Taxis C, Knop M. 2012. TIPI: TEV protease-mediated induction of protein instability. *Methods Mol. Biol.* 832:611–26
**109. Taxis C, Stier G, Spadaccini R, Knop M. 2009. Efficient protein depletion by genetically controlled deprotection of a dormant N-degron. *Mol. Syst. Biol.* 5:267**
110. Teng X, Dayhoff-Brannigan M, Cheng WC, Gilbert CE, Sing CN, et al. 2013. Genome-wide consequences of deleting any single gene. *Mol. Cell* 52:485–94
111. Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, et al. 2007. JAZ repressor proteins are targets of the $SCF^{COI1}$ complex during jasmonate signalling. *Nature* 448:661–65
112. Tomoshige S, Naito M, Hashimoto Y, Ishikawa M. 2015. Degradation of HaloTag-fused nuclear proteins using bestatin–HaloTag ligand hybrid molecules. *Org. Biomol. Chem.* 13:9746–50
113. Trost M, Blattner AC, Lehner CF. 2016. Regulated protein depletion by the auxin-inducible degradation system in *Drosophila melanogaster*. *Fly* 10:35–46
114. Urban E, Nagarkar-Jaiswal S, Lehner CF, Heidmann SK. 2014. The cohesin subunit Rad21 is required for synaptonemal complex maintenance, but not sister chromatid cohesion, during Drosophila female meiosis. *PLOS Genet*. 10:e1004540
115. Usherenko S, Stibbe H, Muscó M, Essen LO, Kostina EA, Taxis C. 2014. Photo-sensitive degron variants for tuning protein stability by light. *BMC Syst. Biol.* 8:128
116. Varshavsky A. 1991. Naming a targeting signal. *Cell* 64:13–15
117. Varshavsky A. 2011. The N-end rule pathway and regulation by proteolysis. *Protein Sci*. 20:1298–345
118. Wang T, Wei JJ, Sabatini DM, Lander ES. 2014. Genetic screens in human cells using the CRISPR–Cas9 system. *Science* 343:80–84
119. Weiss WA, Taylor SS, Shokat KM. 2007. Recognizing and exploiting differences between RNAi and small-molecule inhibitors. *Nat. Chem. Biol.* 3:739–44
120. Winter GE, Buckley DL, Paulk J, Roberts JM, Souza A, et al. 2015. Phthalimide conjugation as a strategy for in vivo target protein degradation. *Science* 348:1376–81
121. Wood L, Booth DG, Vargiu G, Ohta S, deLima Alves F, et al. 2016. Auxin/AID versus conventional knockouts: distinguishing the roles of CENP-T/W in mitotic kinetochore assembly and stability. *Open Biol*. 6:150230
122. Zhang L, Ward JD, Cheng Z, Dernburg AF. 2015. The auxin-inducible degradation (AID) system enables versatile conditional protein depletion in *C. elegans*. *Development* 142:4374–84
123. Zhao Y. 2010. Auxin biosynthesis and its role in plant development. *Annu. Rev. Plant Biol.* 61:49–64
124. Zhou P. 2005. Targeted protein degradation. *Curr. Opin. Chem. Biol.* 9:51–55
125. Zhou P, Bogacki R, McReynolds L, Howley PM. 2000. Harnessing the ubiquitination machinery to target the degradation of specific cellular proteins. *Mol. Cell* 6:751–56

**109. This article was the first to describe TIPI-degron, used in yeast.**