Hijacking protein degradation

Targeted protein degradation provides a powerful complement to small-molecule inhibition in modulating protein activity and allows access to otherwise intractable drug targets.

The field of targeted protein degradation (TPD) began with initial work from Ray Deshaies and Craig Crews (*Proc. Natl Acad. Sci. USA*. **98**, 8554–8559, 2001), who identified heterobifunctional small molecules termed 'proteolysis-targeting chimeras' (PROTACs) that bind a protein of interest (POI) and enable its selective degradation by simultaneously recruiting a ubiquitin ligase complex. Since then, the evolution of TPD technology (*Nat. Chem. Biol.* **11**, 634–635, 2015) has yielded robust chemical biology tools for probing biological mechanisms and promising therapeutic approaches by facilitating turnover of disease-relevant proteins. In this issue, we highlight recent research studies that report extensions of TPD to broader classes of protein targets, design innovations that improve target degradation efficiency, and applications that reveal new biological insights and advance the clinical potential of TPD.

The ubiquitin (Ub)-proteasome system (UPS) that directs proteins for degradation, which forms the basis for TPD, was uncovered in the Nobel Prize-winning work of Aaron Ciechanover, Avram Hershko, and Irwin Rose. Briefly, an ATP-dependent enzyme (E1) activates Ub, transferring it to the catalytic cysteine of an ubiquitin-conjugating enzyme (E2). The resulting thioester-linked intermediate (E2~Ub) is recruited by one of hundreds of ubiquitin ligases (E3s) that catalyze the transfer of Ub to substrate proteins, targeting the tagged protein for proteosomal degradation. Although the basic mechanisms of the UPS are known, even now there remain mechanistic gaps to be addressed. For example, recent work featured in this issue identified structural rearrangements that occur during E2–E3 Ub transfer and enable Ub relay mechanisms and a stapled peptide that targets an unidentified pocket in E1 to disrupt Ub transfer, which may serve as a useful probe of this step.

In early TPD systems, POIs were targeted to the UPS machinery by selective tagging of the protein with a peptide sequence that induces degradation (degron), the activity of which can be controlled by the presence of a small molecule. Such examples include the auxin-induced degradation system and the ligand-directed affinity-directed protein missile (L-AdPROM) system. However, these ligand-based approaches require transfection and expression of a fusion protein and can be hindered by slow turnover kinetics and system leakiness.

The possibility of selective protein turnover using a bifunctional compound or molecular 'glue' that induces stable protein–protein interactions between an endogenous POI and the E3 ligase complex was envisioned to address these limitations. The field of toxicology provided a major assist in the identification of a molecular glue with the discovery that the teratogen thalidomide directly interacts with cereblon (CRBN), a substrate-recognition component of the Cul4 E3 ubiquitin ligase complex, and promotes the ubiquitin-dependent degradation of zinc-finger transcription factors (*Science* **327**, 1345-1350, 2010; *Nat. Chem. Biol.* **14**, 981–987, 2018). The number of proteins that interact with thalidomide and its analogs has since been expanded, ranging from p63 (*Nat. Chem. Biol.* **15**, 1077–1084, 2019) to ARID2, a component of the PBAF chromatin-remodeling complex. The potential of altering CRBN target profiles to particular substrates can now be achieved, for instance, by screening a combinatorial library composed of a CRBN modulator fused to heterocyclic scaffolds.

Recent studies have also revealed that the biological activities of some natural products and synthetic compounds can be explained by a molecular-glue mechanism. For instance, target identification studies mapped compound activity to specific E3 ligase effectors: nimbolide, which recruits the E3 ligase RNF114 (*Nat. Chem. Biol.* **15**, 747–755, 2019), a class of polyketides that interact with the E3 UBR7, and sulfonamide compounds with previously unknown targets that bind to E3 substrate receptor DCAF15 (*Nat. Chem. Biol.* **13**, 675–680, 2017). Molecular glues have been proposed to remodel the E3 ligase–target protein interface to mediate their interaction. X-ray and cryo-electron microscopy structures of the DCAF15 E3 ligase complex containing aryl-sulfonamide and a substrate RBM39 have confirmed the binding mechanisms of these types of glues (*Nat. Chem. Biol.* **16**, 7–14, 2020; *Nat. Chem. Biol.* **16**, 15–23, 2020). Beyond these serendipitously identified glues, chemical-profiling approaches can identify compounds that induce ubiquitylation and degradation of cyclin K through interactions with a CRL4B ligase complex, suggesting that molecular-glue-based mechanisms may define a new 'mode of action' category for small-molecule inhibitors.

In the absence of a molecular glue that mediates the direct interaction between the target and the E3 ligase complex, many PROTACs use linkers to connect a known small-molecule inhibitor to an E3 complex. Although bifunctional compound design is conceptually simple, so far it appears that substantial compound and linker optimization is required to maximize target degradation activity (*Nat. Chem. Biol.* **15**, 937–944, 2019). The recent structural elucidation of PROTAC ternary complexes has revealed insights that may inform degrader design and has highlighted the importance of positive cooperativity in active complex formation (*Nat. Chem. Biol.* **13**, 514–521, 2017; *Nat. Chem. Biol.* **14**, 706–714, 2018).

TPD strategies offer an orthogonal approach for probing biological systems that complement genetic knockout, knockdown, or small-molecule inhibitor-based methods. For instance, TPD may be useful for probing non-catalytic roles of target proteins, as the development of an AURORA-A degrader revealed a kinase-independent role in DNA replication due to S-phase arrest. Degrader-based strategies may also overcome small-molecule-mediated resistance mechanisms, as shown with the selective inhibitory effects of disease variants of BRAF in cell lines.

TPD provides yet another example of the ingenuity and molecular know-how of chemical biologists and has spurred community engagement leading to recent innovations including optical PROTACs (PHOTACs, *Sci. Adv.* **6**, eaay5064, 2020) and lysosome-targeted PROTACs (LYTACs)). In addition, the advancement of ARV-110, a PROTAC targeting the androgen receptor, into phase 1 clinical trials (https://clinicaltrials.gov/ct2/show/NCT03888612) suggests the potential of TPD-based therapeutics as a new targeting modality in pharmaceutical development. At *Nature Chemical Biology*, we look forward to communicating future innovations in TPD research and the application of these techniques to uncover new biological insights and advance biomedical research. ❐

Published online: 16 October 2020
https://doi.org/10.1038/s41589-020-00685-3