Ubiquitination

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TRIM-away via Gln/C-degrons

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A combined structural and biochemical analysis reveals that TRIM7 E3 ligase targets viral proteins for degradation by recognizing their C-terminal glutamine (C-Gln) via its PRY-SPRY domain, providing mechanistic insight into the C-degron pathway.

Protein ubiquitination involving an E1–E2–E3 enzymatic cascade plays important roles in a wide spectrum of cellular events, including protein turnover, innate immunity responses, gene transcription and the cell cycle[1]. Proteins may contain various degradation signals (degrons) that are sufficient to cause them to be targeted by E3 ligase or its complex for subsequent protein degradation via the ubiquitin–proteasome system (UPS)[2]. Specifically, degrons at the N or C termini recruit E3 ligases to induce ubiquitin-dependent elimination, through processes termed the N-degron and C-degron pathways, respectively[2]. In contrast with the finding that all 20 natural amino acids act as the destabilizing signal for N-degron pathways, very few C-degrons have been discovered until recently, when several C-degron pathways including Arg/C-degron and Gly/C-degron were revealed[3,4]. Whether other C-degron pathways control protein turnover remains to be investigated. In this issue of *Nature Chemical Biology*, Liang et al.[5] reveal a TRIM7-mediated Gln/C-degron pathway and its important role in antiviral function.

Tripartite-motif-containing family (TRIM) proteins belong to a family of RING-type E3 ligases, most of whose members are characterized by an N-terminal RING/B-box/coiled-coil (RBCC) domain and a variant C-terminal region (Fig. 1). The expression of TRIM proteins is upregulated in response to interferons (IFNs), and they have critical roles in various innate-immunity-related pathways[6]. Recently, TRIM7 was reported to suppress enterovirus replication by provoking the lysine 48 (K48)-linked ubiquitination and degradation of the viral 2BC protein[7]. In addition, TRIM7 was found to stabilize RACO-1 by catalyzing its polyubiquitination with a K63-linked chain[8]. In this way, TRIM7 shows remarkable plasticity in ubiquitination by installing distinct linkage types of ubiquitin chains on different substrates. Other work indicated that TRIM7 is hijacked to promote the replication of virus[9]. Despite the complicated roles of TRIM7 in protein turnover and in viral infection, how the protein recognizes and ubiquitinates its substrates is largely unknown.

To unravel the recognition mechanism of TRIM7, Liang et al.[5] first used an isothermal titration calorimetry (ITC) assay to show that the C-terminal PRY-SPRY domain of TRIM7 (TRIM7$^{PRY\text{-}SPRY}$, also known as the B30.2 domain) specifically recognizes the C-terminal fragments of viral 2C proteins (Fig. 1)[5]. They further solved the 2C-bound structure of TRIM7, in which the C-terminal glutamine (C-Gln) of the peptide is snugly fitted into a positively charged pocket of TRIM7$^{PRY\text{-}SPRY}$. C-Gln (position −1) interacts with TRIM7$^{PRY\text{-}SPRY}$ via glutamine-specific hydrogen bonding and hydrophobic interactions. In addition, the main chain carbonyl groups of C-Gln form several hydrogen bonds with TRIM7 residues, making it intolerant of extra groups at the C terminus. Peptide

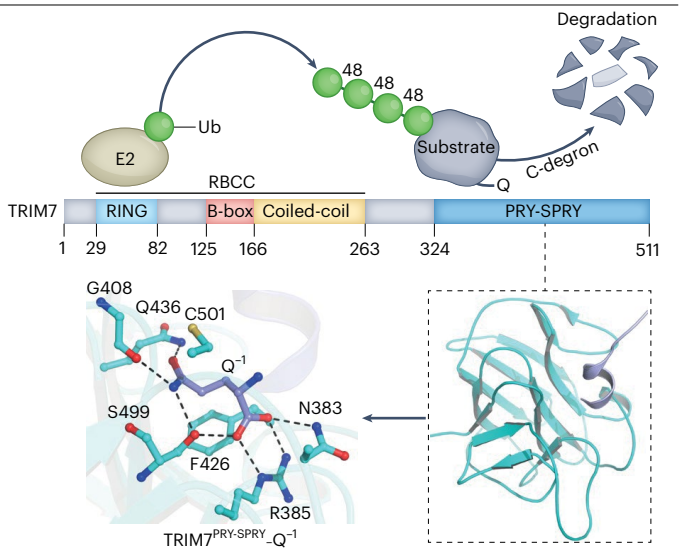

Fig. 1 | Mechanisms underlying TRIM6-mediated polyubiquitination. Top, TRIM7 recognizes and catalyzes K48-linked ubiquitination via its PRY-SPRY and RING domains, respectively, leading to proteasome-mediated degradation of substrate. Bottom, The structure of TRIM7$^{PRY\text{-}SPRY}$ (cyan) is bound to the Gln/C-degron peptide derived from viral 2C (purple), which adopts a helix configuration (right) (PDB code 7W0Q). The C-Gln of the degron is accommodated by a pocket on the surface of TRIM7$^{PRY\text{-}SPRY}$ primarily via glutamine-specific hydrogen bonding interactions (left).

residues upstream of C-Gln (positions −2 and −3) make additional hydrogen bonding and hydrophobic interactions with TRIM7$^{PRY\text{-}SPRY}$, implying that TRIM7 preferentially binds to substrates harboring a Gln and a bulky hydrophobic residue at the -1 and -2 positions, respectively[5]. In agreement with the structure analysis, they found that SARS-CoV-2 proteins and RWDD2B identified from the protein-protein interaction (PPI) database[10] are eliminated by TRIM7 in a C-Gln/degron-dependent manner, suggesting that they are potential substrates for TRIM7. By solving a set of structures of TRIM7$^{PRY\text{-}SPRY}$ domain bound with C-Gln/degron-containing peptides, they unveil the conserved C-Gln/degron recognition mode, which is validated by the mutagenesis and binding experiments. Further in vivo experiments indicate that overexpression and knockout of TRIM7 result in increased and reduced degradation of substrates, respectively[5]. Taken together, these results shed light on the Gln/C-degron-dependent protein turnover mediated by TRIM7 (Fig. 1).

Before this study, the known C-degrons were those targeted by substrate receptors within Cullin-RING E3 ubiquitin ligase (CRL) complexes[3,4]. This work indicates that an E3 ligase also acts as the receptor to target C-degrons, and the C-degron pathway has a key role in defense against viruses. The human genome encodes more than 60 putative TRIM family members, nearly half of which contain a PRY-SPRY domain. It would be intriguing to know whether other PRY-SPRY domains also act as substrate-binding modules. Further work is required to understand

how TRIM7 ubiquitinates substrates with different linkage types and to examine whether other TRIM proteins also display linkage plasticity in substrate ubiquitination. Analogous to PROTACs, degrons have the potential to target specific E3 ligases or complexes to degrade non-native protein substrates. Thus, the discovery of new degron pathway, as well as the newly uncovered mechanisms, will also contribute to the rational molecular design of PROTACs in the near future.

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Competing interests

The authors declare no competing interests.