Development of a cDNA display method for discovery of substrate peptide sequences for E3 ligases

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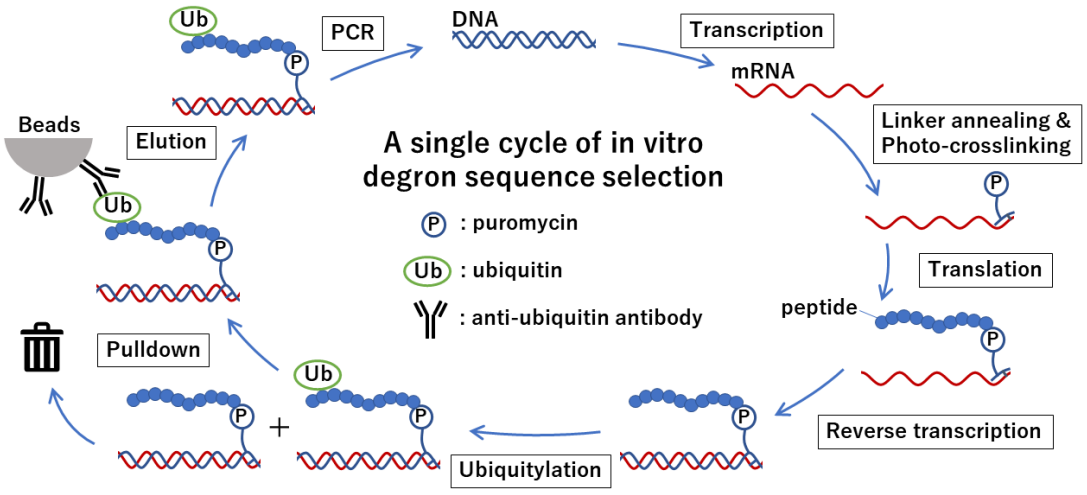
Keywords: cDNA display method, E3 ligase, degron, in vitro screening, substrate recognition

The cDNA display method is a technology to form covalent conjugates of peptides and their corresponding cDNA sequences.[1] In this technology, a special DNA linker containing puromycin is used to achieve covalent connection between a peptide and its cDNA. Randomized DNA libraries with molecular diversity on the order of $10^{13\text{-}14}$ can be converted to their corresponding peptide-cDNA conjugates in vitro and the obtained libraries can be used for in vitro binding-based screenings.[2]

Cells utilize ubiquitin as a posttranslational protein modifier to conduct various signals such as protein degradation signal via the proteasome system. E3 ubiquitin ligases recognize their specific substrate polypeptides, called degrons, and catalyze their ubiquitylation. More than 600 E3 ligases have been discovered but few of them are well characterized.[3] In this study, we aimed to construct a screening system which can identify the substrate peptide sequence of a target E3 from a randomized peptide-cDNA library. We are using the MDM2 E3 ligase, and its substrate, p53, as a model system. Briefly, the cDNA displayed substrate library is subjected to MDM2-catalyzed ubiquitylation. Then, ubiquitylated display molecules are pulled down with an anti-ubiquitin antibody (Figure). After several rounds of selection, the amplified substrate sequences are read out by the next-generation sequencing. So far, we have successfully validated the peptide-cDNA conjugate construction using the degron of p53 and we confirmed that the degron was properly ubiquitylation by MDM2.



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