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Improved Intracellular Protein Degradation System for Rapid Screening and Quantitative Study of Essential Fungal Proteins in Biopharmaceutical Development

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Abstract : The selection of appropriate biomolecular targets is a crucial aspect of biopharmaceutical development. The Auxin-Inducible Degron Degradation (AID) technology has demonstrated remarkable potential in efficiently and rapidly degrading target proteins, thereby enabling the identification and acquisition of drug targets. The AID system also offers a viable method to deplete specific proteins, particularly in cases where the degradation pathway has not been exploited or when the adaptation of proteins, including the cell environment, occurs to compensate for the mutation or gene knockout. In this study, we have engineered an improved AID system tailored to deplete proteins of interest. This AID construct combines the auxin-responsive E3 ubiquitin ligase binding domain, AFB2, and the substrate degron, IAA17, fused to the target genes. Essential genes of fungi with the lowest percent amino acid similarity to human and plant orthologs, according to the Basic Local Alignment Search Tool (BLAST), were cloned into the AID construct in S. cerevisiae (AID-tagged strains) using a modular yeast cloning toolkit for multipart assembly and direct genetic modification. Each E3 ubiquitin ligase and IAA17 degron was fused to a fluorescence protein, allowing for real-time monitoring of protein levels in response to different auxin doses via cytometry. Our AID system exhibited high sensitivity, with an EC50 value of 0.040 µM (SE = 0.016) for AFB2, enabling the specific promotion of IAA17::target protein degradation. Furthermore, we demonstrate how this improved AID system enhances quantitative functional studies of various proteins in fungi. The advancements made in auxin-inducible protein degradation in this study offer a powerful approach to investigating critical target protein viability in fungi, screening protein targets for drugs, and regulating intracellular protein abundance, thus revolutionizing the study of protein function underlying a diverse range of biological processes.

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