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Rapid protein degradation systems to determine gene function in vivo

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The functional characterization of proteins during temporally constrained periods of mammalian development and disease is largely limited by the inability to rapidly and reversibly perturb their function. A new study addresses this challenge by directly comparing two targeted protein degradation systems in mice. These systems enable precise temporal degradation of proteins, offering unprecedented opportunities to study dynamic biological processes. Several technical components remain to be optimized, but these technologies promise to provide novel insights in vivo.

Adapting the Cre-loxP system for use in mice revolutionized the study of tissue-specific gene function *in vivo*[1]. While this technology remains indispensable for mammalian genetic research, the slow and irreversible kinetics of gene knockout using inducible Cre recombinase presents a substantial limitation[2]. Studying gene function in its native context requires methods enabling normal development, as well as rapid gene disruption and phenotyping, which can be challenging with knockout gene models. In cell line models, targeted protein degradation systems provide rapid and reversible control of protein function by using synthetic ligands to induce proximity between target proteins and E3 ubiquitin ligase complexes, facilitating acute protein degradation[3,4]. These systems involve tagging the target gene with an inducible degron at its endogenous locus using CRISPR editing and expressing an exogenous adapter protein that links the degron-tagged protein to the E3 ubiquitin ligase complex. Conveniently, mouse lines expressing these adapter proteins already exist and can be crossed with degron-tagged gene lines. The systemic administration of small molecule synthetic ligands facilitates the interaction between the degron-tagged protein and the E3 ligase through the adapter protein. Because the adapter proteins selectively recognize the degron tag, tissue specificity is achieved by regulating expression of the adapter genes with tissue- or temporally specific Cre drivers.

Recently, Yamashita and colleagues applied two of these systems, *Oryza sativa* $TIR1^{F74G}$ (OsTIR1)-auxin-inducible degron 2 (AID2) and human cereblon (hCRBN)-SALL4 degron (S4D), to mice[5]. While not the first report of degron use in mammals[6,7], in this study the investigators conducted direct comparisons of these two systems, offering valuable insights to guide the choice of system for future studies. They directly compared the efficiency and kinetics of the two degron systems for depleting the transcription factor Satb1 in T cells. While both systems efficiently reduced Satb1 protein expression by over 90%, the S4D system was more rapid, with maximum effects in peripheral blood T cells achieved at 2 hours and a return to baseline by 24 hours after wash out. The AID2 system took 6 hours to reach a maximum effect and 72 hours to recover. Although the researchers did not directly compare these kinetics to an inducible *Satb1* knockout mouse model, these degron systems are likely much faster and have the added advantage of reversibility. With regards to choosing one of these degron systems to apply to future studies, AID2 may be more convenient for repeated dosing for sustained depletion, while S4D may be more useful for studying the effects of reversible depletion. However, our work showed that basal degradation levels, inducible degradation kinetics and recovery rates can vary significantly between target proteins, even when using the same degron system[8,9].

In addition to recapitulating the published phenotypic effects of *Satb1* knockout in mice, Yamashita and colleagues demonstrated that these degron systems can effectively reach tissues beyond the blood–brain barrier, as well as induce protein of interest degradation in gestating fetuses and nursing neonates via treatment of the mothers with the ligands. They next directly compared the substrate specificity of the two degron systems. As has been previously demonstrated, thalidomide and its derivatives, such as pomalidomide, used with the S4D system induce hCRBN to interact with neosubstrates such as the Ikaros and Aiolos transcription factors that are expressed in lymphocytes. As such, care should be taken when using the S4D system in the hematopoietic niche, including by using a control condition of treatment with the thalidomide-based ligand in mice without a degron-tagged protein of interest. Of note, though highly conserved with the human protein, mouse CRBN does not recognize these two neosubstrates when treated with pomalidomide[10]. Therefore, limiting heterologous hCRBN expression to a non-blood tissue should minimize the cell-nonautonomous thalidomide-dependent effects of the immune system on the target tissue of interest.

While these two degron systems provide faster kinetics than Cre-loxP based systems, they are not as rapid as their *in vitro* counterparts. Slower kinetics could be due to the non-instantaneous bioavailability of the depletion-inducing ligands administered via intraperitoneal injection or oral gavage. At the 16-hour time point in the proteomics experiment shown in Figure 4[5], secondary effects of Satb1 depletion are evident through expression changes in known Satb1-target genes and proteins commonly affected by both degron systems. These kinetics are likely sufficient to investigate the effects of target protein depletion on a days-long process of tissue differentiation. However, if attempting to isolate the primary effects of transcription factor depletion on target gene expression *in vivo*, the more rapid S4D system would be preferable to the AID2 system. Although not explored in this study, future investigations into tail–vein injection of degradation-inducing ligands are warranted, as this approach could enable more rapid and efficient compound uptake.

Another potential limitation of these degron systems that was underexplored in this study is the basal degradation of degron-tagged proteins of interest in the absence of treatment with the depletion-inducing ligand. A key advantage of studying an endogenously tagged gene, as opposed to

an exogenous copy, is that its expression before perturbation should be comparable to the wildtype gene. Unfortunately, basal OsTIR1-dependent degradation of AID-tagged proteins has been observed in the original AID system, with multiple iterations of the AID system designed to reduce the effect, including the AID2 system used in this study[6]. In an experiment focusing on pomalidomide-induced depletion of neosubstrates, shown in Figure 3D[5], the authors did provide an immunoblot directly comparing expression of untagged and S4D-tagged Satb1 in thymocytes expressing hCRBN, with no obvious qualitative basal degradation in the absence of pomalidomide. These immunoblots are an essential early quality control experiment when establishing these degron systems in a new context. While the investigators did perform an immunoblot and a flow cytometry experiment to compare the expression of AID-tagged and untagged proteins to confirm that the AID tag did not affect expression in Supplementary Figure 1[5], these experiments were done in the absence of OsTIR1, which is the heterologous E3 ligase.

As it stands, a current practical limitation of these degron systems is the cumbersome number of crosses needed to produce a desired mouse strain. Assuming a target tissue-specific Cre line already exists, a mouse with tissue-specific E3 ligase expression must be generated in addition to the degron-tagged target gene allele. However, as the technology becomes more widely adopted, an increasing number of both of the above resources will likely become available. New combinations of tissues and genes will be only two generations away, as is currently the status quo using the Cre-loxP system.

Despite these technical challenges, the application of degron systems to *in vivo* study promises to provide novel insights into mammalian development and disease. These tools allow for previously unachievable temporal precision in targeted protein perturbation. Studies like this are essential for establishing a robust foundation to guide the selection of the most suitable degron system for specific research objectives in future projects.

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References

1. Gu, H., Marth, J. D., Orban, P. C., Mossmann, H. & Rajewsky, K. *Science* **265**, 103–106 (1994).
2. Donocoff, R. S., Teteloshvili, N., Chung, H., Shoulson, R. & Creusot, R. J. *Sci. Rep.* **10**, 15244 (2020).
3. Nishimura, K., Fukagawa, T., Takisawa, H., Kakimoto, T. & Kanemaki, M. *Nat. Methods* **6**, 917–922 (2009).
4. Bondeson, D. P. et al. *Nat. Chem. Biol.* **11**, 611–617 (2015).
5. Yamashita, M. et al. *Nat. Commun.* **15**, 10129 (2024).
6. Yesbolatova, A. et al. *Nat. Commun.* **11**, 5701 (2020).
7. Abuhashem, A., Lee, A. S., Joyner, A. L. & Hadjantonakis, A.-K. *Dev. Cell* **57**, 1068–1080.e6 (2022).
8. Scott, T. G., Sathyan, K. M., Gioeli, D. & Guertin, M. J. *PLoS Genet.* **20**, e1011159 (2024).
9. Sathyan, K. M. et al. *Genes Dev.* **33**, 1441–1455 (2019).
10. Chamberlain, P. P. et al. *Nat. Struct. Mol. Biol.* **21**, 803–809 (2014).

Competing interests

The authors declare no competing interests.