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Opinion

droTAG: Adapting dTAG Toolkit to *Drosophila Melanogaster*

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Targeted protein degradation (TPD) allows an acute and reversible knockdown of protein of interest (POI) so that the direct effects of protein depletion can be studied and distinguished from secondary effects or adaptive responses [1]. Therefore, protein degradation techniques apply in studying the function of gene products in a short time frame, and rapid effectiveness can be exploited to downregulate POI in a stage-specific manner or when time is a relevant factor, including cell division.

In the last decade, several strategies have been developed to obtain an accurate and efficient protein degradation, such as deGradFP, Auxin-inducible Degradation (AID) and degradation TAG (dTAG) which aim to achieve proteolysis of POI exploiting the powerful of degradation signal peptide sequences (tags) to hijack POI to E3 ubiquitin ligases for ubiquitylation and consequentially proteasomal degradation by recruitment of the ubiquitin-proteasome system [2].

deGradFP exploits the proteasome-based pathway to achieve direct depletion of GFP-tagged proteins, while AID needs of Auxin and transgenic OsTIR1 adapter to trigger POI depletion.

Instead, the dTAG system developed by Nabet et al. [3] induces a rapid and selective degradation of POI exploiting the heterobifunctional activity of degrader (dTAG-13). This molecule binds both $FKBP12^{F36V}$-fused POI and Cereblon (CRBN), the recognition unit of CRL4-CRBN E3 ubiquitin ligase complex leading to exclusive POI degradation by the proteasome.

Most of these tools are versatile and have been adapted to work in different model organisms spanning yeast (*S. cerevisiae*) to humans (*H. sapiens*). Indeed, deGradFP strategy has been firstly developed in non-vertebrates (*Drosophila*) by Caussinus et al. [4], and later tailored in humans [5], while the AID system evolved in the exact opposite way arising from humans and later adapted in *Drosophila* by Trost et al. [6].

On the other hand, dTAG system have been well established in vitro and in vivo in different species, but to date, no attempt has been made to extend it to *Drosophila*, as a matter of fact, Yesbolatova et al. [7] claim that CRBN is not evolutionarily conserved in non-vertebrates, therefore dTAG is likely not functional in these organisms.

In contrast, here we would like to focus the attention on the conservation of all components of the mammalian CRL4 complex in *Drosophila*, including the Cullin 4 homolog and the DDB1 homolog PIC [8]. In particular, it must be emphasized that there is a 44% of aminoacidic identity in thalidomide binding domain (TBD) between human CRBN and *Drosophila* OHGT (Figure 1A) [9]. This data strongly supports the idea that the function of the ubiquitin ligase complex is evolutionary conserved in *Drosophila melanogaster*, making the hypothetical applicability of dTAG in *Drosophila* (droTAG), a powerful strategy that is worth to be developed (Figure 1B).

In conclusion, drawing up droTAG could be indispensable for all the worldwide fly researchers, including us, who have the possibility to highlight phenotypes that otherwise they cannot see by using conventional RNA interference approaches [10-14].

A. Schemes of human Cereblon (CRBN) and its *Drosophila* orthologue, Ohgata (OHGT). The evolutionary conserved

thalidomide binding domains (TBD) are indicated as filled boxes (blue). Percentage of identity at the amino acid level is noted.

B. Cartoon showing a putative mechanism of droTAG system in *Drosophila melanogaster.* Heterobifunctional dTAG-13 molecules bring together OHGT and FKBP12^{F36V}-fused POI, hijacking it towards endogenous proteasome machinery for rapid degradation. CRL4–OHGT E3 ubiquitin ligase include cullin scaffold (CUL4A), adaptor protein (PIC), substrate receptor (OHGT), N8 ubiquitin-like protein (NEDD8) and the RING protein (Roc1a) recruiting an E2 ligase.

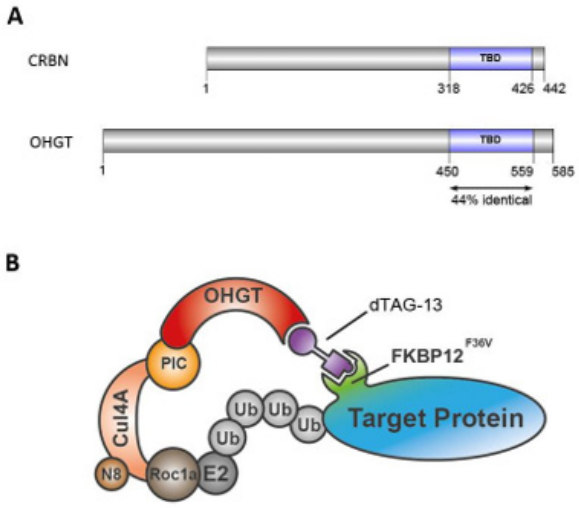

Figure 1: droTAG system.

Conflict of Interest

There is no conflict of interest.

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